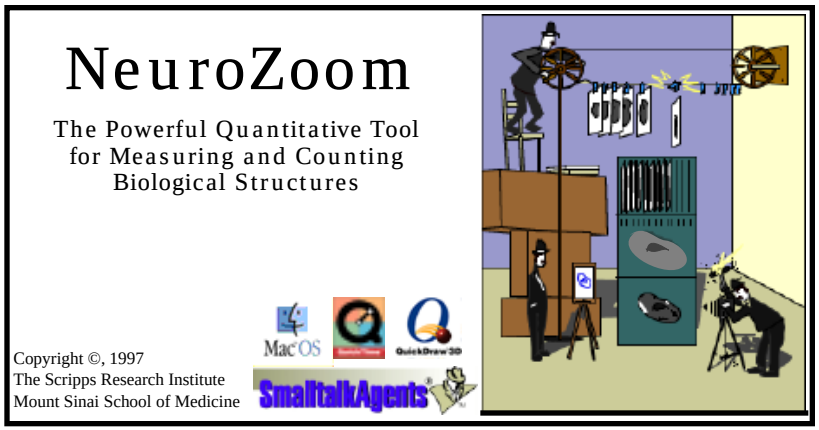
Reference



Macintosh and Power Macintosh Versions
Version 0.9b0

The Scripps Research Institute
San Diego, CA
Mount Sinai School of Medicine
New York, NY
1997

DISCLAIMER

The Scripps Research Institute (TSRI) and Mount Sinai School of Medicine (MSSM) makes no warranties, express or implied, including without limitation the implied warranties of merchantability and fitness for a particular purpose, regarding the contents of this manual or accompanying software. TSRI and MSSM does not warrant, guarantee or make any representations regarding the use or the results of the use of TSRI and MSSM software in terms of its correctness, accuracy, reliability, currentness or otherwise. The entire risk as to the results and performance of TSRI and MSSM software is assumed by you. The exclusion of implied warranties is not permitted by some states. The above exclusion may not apply to you. TSRI and MSSM further reserves the right to make changes to the specifications of the program and contents of the manual without obligation to notify any person or organization of such changes.

In no event will TSRI and MSSM, its directors, officers, employees or agents be liable to you for any consequential, incidental or indirect damages (including damages for loss of business profits, business interruption, loss of business information, and the like) arising out of the use or inability to use the TSRI and MSSM software even if TSRI and MSSM has been advised of the possibility of such damages. Because some states do not allow the exclusion or limitation of liability for consequential or incidental damages, the above limitations may not apply to you.

Contents

CHAPTER 1 *Introduction*

This reference manual is intended to be a comprehensive reference to all of the functions and features of NeuroZoom. This manual is not intended to introduce you to concepts or protocols. Please refer to the other manuals for that information.

This manual is organized by presenting the menus of the Menubar. Each menu item is thoroughly discussed. As every window is produced by some action on a menu item, or from a window control that is produced by a menu item, every function in NeuroZoom should be covered.

CHAPTER 2 *Menus*

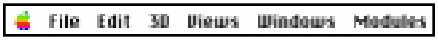
Menubar

All of the menus in NeuroZoom are presented by the Macintosh Menu Manager as a Menubar at the top of the primary monitor. If you have two or more monitors, the primary monitor is the one that presented all of the start-up windows and information when you booted the computer.

The menus are presented for NeuroZoom only when NeuroZoom is the Macintosh application that is running in the “foreground”. Multiple applications may be running simultaneously on the Macintosh, but only one can be the foreground application. This application is the one that controls the Menubar.

The following Menubar is available when a 2D Mapping window is open and is the front window.

The following Menubar is available when a 3D Mapping window is open and is the front window. The File, Edit, Windows, and Modules menus are the same as from the 2D Mapping window. The Views menu is different from the Mapping window.



The following Menubar is available when neither a 2D Mapping window nor a 3D Mapping window is open. Other windows, such as Text windows, may be opened. The File, Edit, Windows, and Modules menus are the same as from other two Menubars.

Each menu will be discussed, and all of the menu items it contains detailed. The menu items perform a command when the mouse is moved over onto it and then released. This command may open a window, execute some code, or perform some action that may not be visible to you. Some menu items also have a *Command key* equivalent, whereby you can activate the command without using the mouse by pressing its assigned Command key equivalent. For example, pressing *Command-S* is equivalent to pulling the **File** menu down to the **Save Document** menu item.

Many of the menu items operate on the current mapping window. See the chapter on ***Mapping Windows*** for more information on NeuroZoom windows and what the current mapping window is. Since NeuroZoom can have many mapping windows open, only one, the mapping window in the foreground, is considered the current mapping window, and it is the one that many of the menu items operate on. Each menu items will indicate if it is specific to the current mapping window.

Many of the menu item commands can be called from other NeuroZoom control objects, such as buttons or popup menus in other windows. For example, the

Grab Image menu item command can also be activated by the **Grab Image** button in the **Imaging** window. Each menu item command will indicate if there is another method to activate its command.

File Menu

The File menu is used to control actions surrounding the use of NeuroZoom documents, and of some aspects of NeuroZoom itself.

New Map

Command N Opens a new NeuroZoom document. The name starts with the word Untitled, and a number that increments from 1, and is unique among all the opened documents of NeuroZoom.

Open Map...

Command O A standard file dialog opens to choose a NeuroZoom document. Only NeuroZoom documents and folders are visible from this dialog. If a currently opened NeuroZoom document is opened, that document's mapping window is brought to the foreground as the active window in NeuroZoom.

Close Map

Command W Closes the current NeuroZoom mapping window and the document itself. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Close All Maps

Closes all NeuroZoom mapping windows and their documents. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Save Map

Command S Saves the current NeuroZoom mapping window and its document. If the document has not been previously saved, a standard file dialog opens in which to enter the name of the file for the document. If the document has been previously saved or the mapping window was opened from a document, the document is saved to the same file. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Save Map As...

Saves the current NeuroZoom mapping window and its document to a new file. A standard file dialog opens in which to enter the name of the file for the document. The name of the mapping window is changed to this new name, and all subsequent selections of Save Map saves to this new file. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Export

Pops up a hierarchical menu for exporting data to other files formats.

Export Map Image...

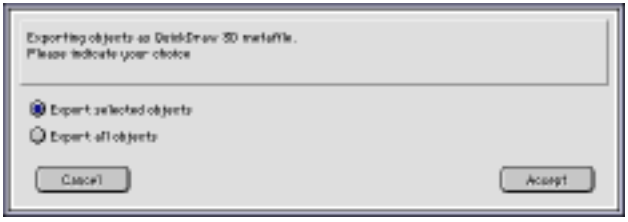
Saves the current NeuroZoom mapping window to a file containing an image of the map. A standard file dialog opens in which to enter the name of the file for the document. The default name of the file is the name of the current document. There is also a popup menu on this dialog to select either PICT or TIFF format.

The image that is saved represents exactly what is seen in the mapping window. If the grid is on, the grid is saved to the file as well. The only exception is live video. Live video will not be saved as an image to the file. However, it the image

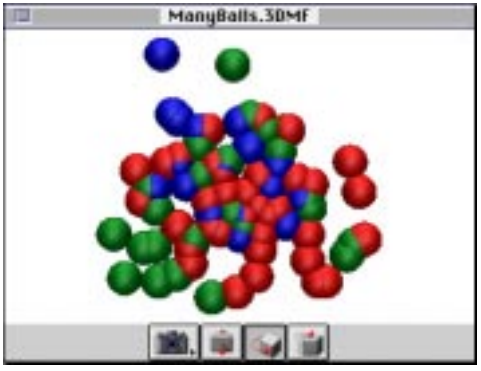
is required, simply grab the image once, and the image itself will also be saved to the file along with mapping data. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Export QuickDraw™ 3D Metafile...

Saves mapping data from the current NeuroZoom mapping window to a 3DMF file. This file can be opened by any 3D application that supports this format (such as NeuroZoom or SimpleText or any QuickDraw™ 3D application). A dialog appears asking for the objects to export.



Either the selected objects, if any, are exported, or all objects are exported. Choose by pressing on the appropriate selection and then press **Accept**. A standard file dialog opens in which to enter the name of the 3DMF file. The default name of the file is the name of the current document with.3DMF as the file extension. The creator of the file is ttxt (SimpleText) and is of type TEXT. Double clicking on this file will typically launch SimpleText. If QuickDraw™ 3D is installed on your Macintosh, the following browser window may appear (the data will look different, of course). This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).



Export Text File...

Saves mapping data from the current NeuroZoom mapping window to a TEXT file. This file is human readable and usable as a file exchange medium with other software applications (or with NeuroZoom itself). A standard file dialog opens in which to enter the name of the TEXT file. The default name of the file is the name of the current document with.TEXT as the file extension. The creator of the file is ttxt (SimpleText) and is of type TEXT. Double clicking on this file will typically launch SimpleText. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Alert: Images are not displayed to the text file.

Import

Pops up a hierarchical menu for importing data from other files.

Import Text File

Reads in a TEXT file containing mapping data and creates them in a new mapping window. A standard file dialog opens from which to choose a file. This should be a file that is produced by the **Export Text File..**. selection as described above. A new mapping window opens, and data, if within the bounds of the current mapping window coordinate system, is displayed.

New Text Document

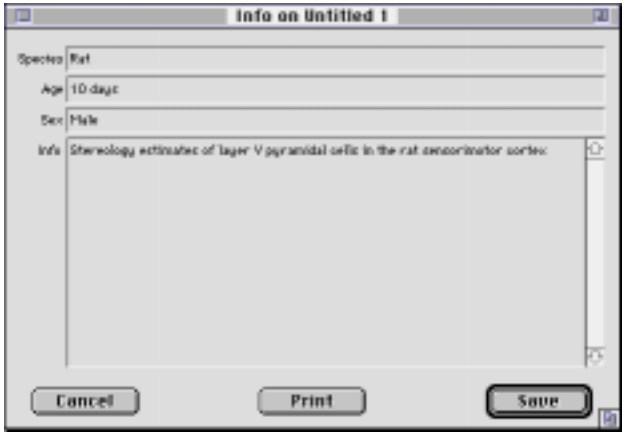
Opens a new text document window that can accept keyboard entry. Use this for any notes, or as s simple word processing window. The window can be printed by pressing the **Print** button, saved to a file by pressing the **Save** button, or closed by pressing the **Done** button.

Open Text Document...

Opens a text file and displays the contents in a text document window. A standard file dialog opens from which to choose a file of type TEXT.

Get Info...

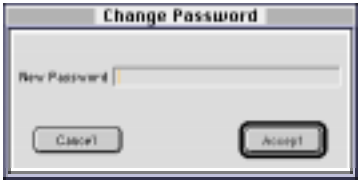
Opens a window in which general information can be entered about this document. The species, age, sex, and information can be entered. Pressing on the **Save** button will store this information into the document and close the window. Pressing the **Print** button will print a the information to the selected printer for this computer. Pressing **Cancel** will close the window without saving the information. Opening this window again by reselecting **Get Info...** will again display the information previously stored. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).



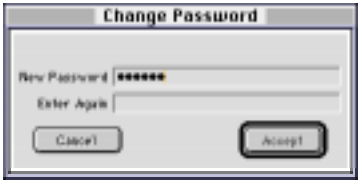
Password...

Opens a window in which a password for the document may be entered. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded). Any document may be protected with a password. The password is requested twice when opening a document. A window opens asking you to enter in the password twice.

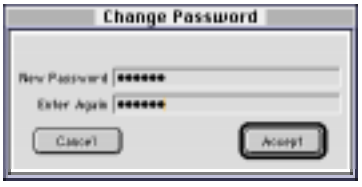
Enter the password in this window for the first time



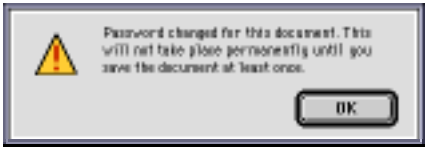
Second field appears in which you type the same password again



Click on Accept to install the password



Press **Accept** to accept the passwords. A message window opens indicating that the password has been changed. The password is permanently stored when the document is next saved.



Opening a protected document asks for the password.

Alert: The password is CASE SENSITIVE. If you forget the password, you cannot gain access to the contents of the document. Contact Technical Support if that happens.

Page Setup

Opens the printer setup window for the chosen printer. An example dialog displays. For NeuroZoom, the orientation as Landscape is typically more useful then Portrait. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Print...

Command P Prints the current window to the chosen printer. This item is not enabled if no documents are opened, or if the front window is not printable. What is printed depends on what is being displayed in the current window. If the mapping window is in the front, the map data, images, grid, counting frames, etc. are all printed. Only the visible contents of the mapping window are printed. If the map extends beyond the boundaries of the mapping window, and you want to print these invisible portions, select **Print Entire Map...** from the **File** menu.

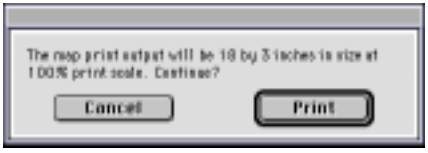
Several window dialogs may appear depending on what is being displayed in the mapping window. Should there be no data whatsoever, the following window dialog appears warning that no data are available, but that you can print the grid itself.

Press **Print Grid** to print the grid only, or press **Cancel** to cancel the printing.

If there is no data or grid displayed, the a window dialog indicates that there is nothing to print. Press **OK** to dismiss the dialog.

Print Entire Map...

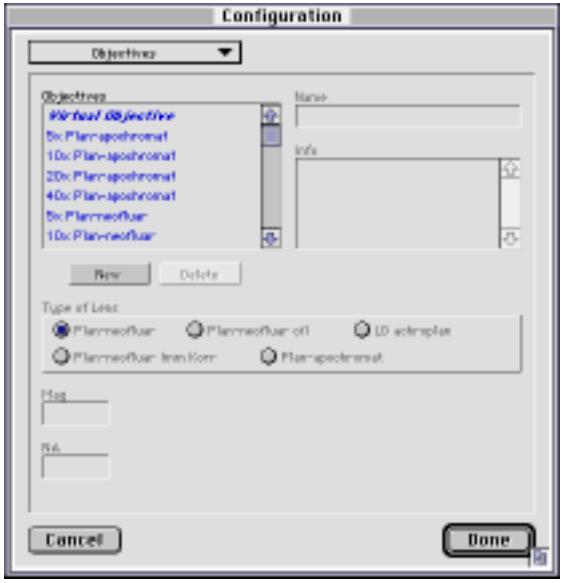
Prints the current mapping window to the chosen printer. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded). The document's entire map is printed. This includes the non-visible portions of the mapping window should the data exceed the visible boundaries. The same conditions are test and presented in dialogs as described in the section Print... from the **File** menu. However, if data are located beyond the boundaries of the mapping window, the size of the print output displays in a window dialog. This is useful in case there is a large map requiring a lot of pages.



Press **Print** to continue printing the entire document's map. Press **Cancel** to cancel the print request.

Configure Devices...

Opens the **Configuration** window for devices. See the next section for more information on how to use this window.



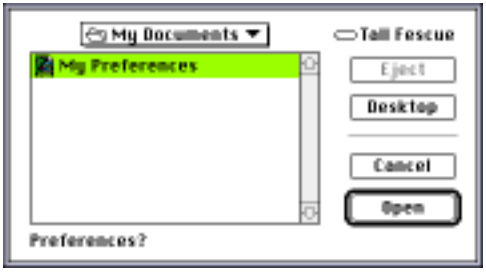
Preferences

Pops up a hierarchical menu for controlling preferences for NeuroZoom.

Preferences... Opens the **Preferences** window. See the next section for more information on how to use this window.

Restore from File...

Allows preferences stored in disk files to be opened and used in NeuroZoom. This is useful if several people are sharing the use of NeuroZoom on one computer. Personal preferences may be created and stored in a file, and restored individually for each person. A standard file dialog opens from which to choose a file containing the preferences. Only NeuroZoom preference files will be displayed.



Choose the correct preference file and press **Open** to read in the preferences.

> **Alert:** Preferences apply to the entire session of NeuroZoom. These preferences apply to all documents opened by NeuroZoom until the preference items are explicitly changed, or when a new preference file is read in.

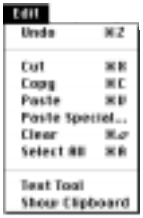
Save to File...

All preferences currently in place are written to a disk file. This file can be restored later as new preferences. A standard file dialog opens in which to enter the name of the Preference file. The default name of the file is *Preference*.

Quit

Command Q Quits NeuroZoom. Any opened and unsaved mapping windows are presented with an option to save the document to a disk file. If no windows are opened, or if no opened document has been modified, NeuroZoom will simply quit.

Edit Menu



The **Edit** menu is used to control actions surrounding the editing of objects in NeuroZoom. In general, this pertains to cutting and pasting operations found in most Macintosh applications.

Undo

Command Z Some commands in NeuroZoom can be undone. If so, this menu item is enabled. Selecting it will undo the last action. For example, if selected mapped cells were deleted, selecting Undo will undelete the cells and redisplay them again. The label of this menu item may also change to reflect the particular action that will be undone. In this deletion example, the menu would read **Undo Delete**, indicating that selecting it will undo the last deletion. This item is not enabled if there is nothing to undo.

Cut

Command X Selected objects are copied to the Macintosh clipboard and then cleared from the mapping window. Use this to move objects from one document to another. This item is not enabled if there are no selected objects. This action can be undone by the **Undo** menu selection.

Copy

Command C Selected objects are copied to the Macintosh clipboard with the command. They are not cleared from the current mapping window. This item is not enabled if there are no selected objects.

Alert: Objects copied to the clipboard may be pasted into other applications. How they paste out depends on the type of data that was copied, and how the NeuroZoom preferences are configured. For example, if you select a group of point locations, the copy buffer would contain a TEXT description of the selected cells and their locations. If the preference for **Copy Pict Object...**were set, an image of the objects would also be copied to the clipboard as a graphics clipping.

Paste

Command V Any copied NeuroZoom object can be pasted into a mapping document. The location of the objects are preserved in absolute microns. The current structure of the mapping window is used as the structure association for these data. See the section on the mapping window and current structure for more information on this. This item is not enabled if there are no NeuroZoom objects in the clipboard.

Paste can also be used for normal text pastes into text based windows. If a text based window, such as the **Text Document** window is at the front, selecting Paste will paste any text in the clipboard into the text field at the current cursor location.

Paste Special...

This is used for pasting in PICT objects that might contain QuickDraw data that may be represented by NeuroZoom objects. For example, if you use Canvas to create a polygon, select and copy that polygon, you can use this command in NeuroZoom to extract out all of the relevant data to recreate a contour in NeuroZoom. The object is always pasted into the current view of the mapping window, regardless of the stage location or the scale of the mapping window. The size of the object as described by QuickDraw screen coordinates are used. The object will also anchored to the top left of the mapping window. If multiple objects are in the clipboard, subsequent NeuroZoom objects will be created at the same offset from the first object.

Clear

Command Delete Selected objects are cleared from the mapping window. They are not copied to the clipboard. This item is not enabled if there are no selected objects. This action can be undone by the **Undo** menu selection.

Select All

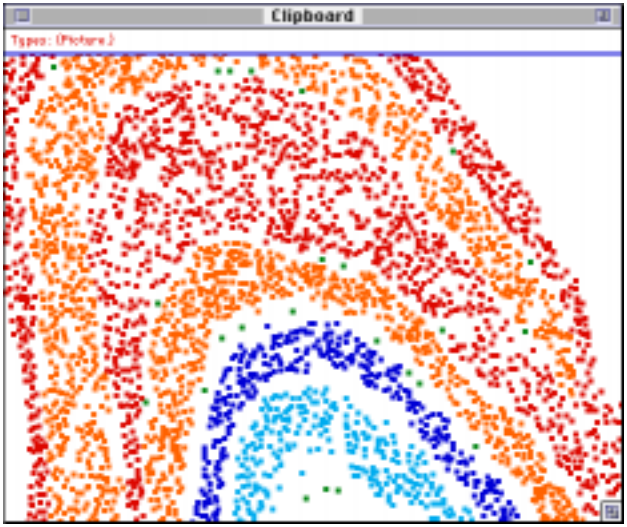
Command A All data objects in the current mapping window are copied to the clipboard.

Text Tool

Opens the **Text Tool** window. See the next section for more information on how to use this window.

Show Clipboard

Opens the **Clipboard** window. Shows what is currently in the clipboard. Only Text and Pictures are currently displayed.



Map Menu

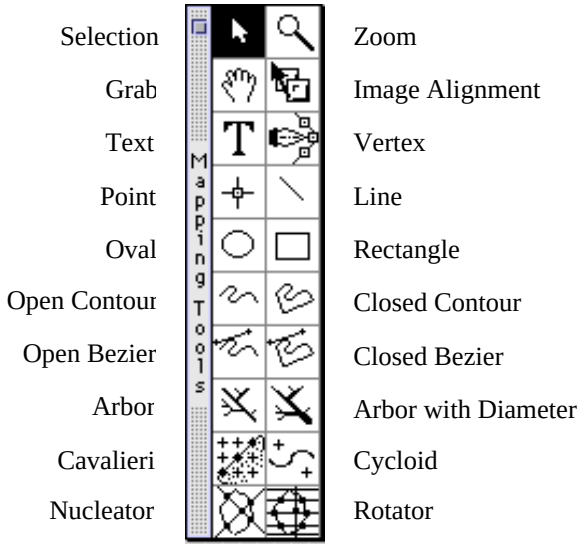
The **Map** menu is used to control actions surrounding the front mapping window. This menu is not available if no mapping windows are open of if the front window is a standard or dialog window other than a mapping window.

Refresh Window

Command R Redraws the content in the current mapping window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded). This menu action can be useful if NeuroZoom fails to draw a portion of the mapping window content properly.

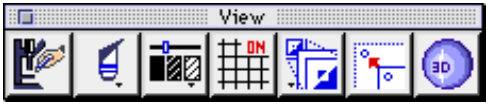
Mapping Tools Window

Command 1 Command can also be called from the **Window Togglers**. Opens the **Mapping Tools** window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded). This window contains the tools that are used for mapping data. All data acquisition tools that used for mapping structures are selected from this window. The currently selected tool is highlighted with black on white graphics. From this window, select the proper tools to begin data acquisition from the mapping window. See the next section on **Mapping Tools** window for more information on how to use this window. If the window is opened, there is a checkmark displayed before this menu item.



View Window

Command 2 Command can also be called from the **Window Togglers**. Opens the **View** window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded). This window contains functions that pertains to the Views. A view is a combination of a microscope, a camera, and the lens objective. Each view is scaled within NeuroZoom so that the precise ratio of real world units (microns) is know for every device unit (pixels). The presentation of these views, and other related functions are controlled from the View Window. See the next section on **View** window for more information on how to use this window. Also see NeuroZoom Basics for information on Views. If the window is opened, there is a checkmark displayed before this menu item.

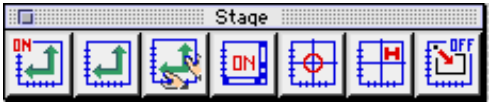


Imaging Window

Command 3 Command can also be called from the **Window Togglers**. Opens the **Imaging** window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded). This window contains functions that deal with video, imaging, or the use of files that contains images. See the next section on **Imaging** window for more information on how to use this window. If the window is opened, there is a checkmark displayed before this menu item.

Stage Window

Command 4 Command can also be called from the **Window Togglers**. Opens the **Stage** window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded). This window contains functions that deal with aspects of the stage. See the next section on **Stage** window for more information on how to use this window. If the window is opened, there is a checkmark displayed before this menu item.



Info Window

Command 5 Command can also be called from the **Window Togglers**. Opens the **Mapping Window Info** window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded). This window displays real time information pertaining to the configuration of NeuroZoom, the mapping window, tools, or data. See the next section on **Mapping Window Info** window for more information on how to use this window. If the window is opened, there is a checkmark displayed before this menu item.

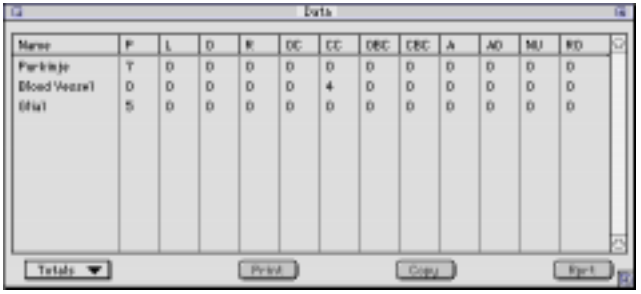
Data Type Window

Command 6 Command can also be called from the **Window Togglers**. Opens the **Data Type** window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded). This window displays all of the structures and their data type currently mapped in the current mapping window. The layer for each structure object is also displayed. This window is useful to control the visibility of specific kinds of structures. For example, turning off all data except for Purkinje cells that are mapped as a point locations. See the next section on **Data Type** window for more information on how to use this window and how to generate reports from it. If the window is opened, there is a checkmark displayed before this menu item.

Data Window

Command 7 Command can also be called from the **Window Togglers**. Opens the **Data** window. This item is not enabled if no documents are opened, or if the front

window is not a mapping window (floating palettes excluded). This window displays all structures currently mapped in the current mapping window, and lists their total count per type of data. Use this window to easily see how many points or boundaries (open contours) there may be of pial surface or Purkinje cells. See the next section on **Data** window for more information on how to use this window and how to generate reports from it. If the window is opened, there is a checkmark displayed before this menu item.



Microscope Setup...

Command can also be called from the **View** window. Opens the **Microscope Setup** window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded). Use this window to setup all of the devices used with the microscope for this mapping window. The microscope, camera, lens objective, and stage must be chosen before any data can be entered. The defaults are Virtual Microscope, Virtual Camera, Virtual Lens Objective, and Virtual Stage. See the next section on **Microscope Setup** window for more information on how to use this window, and the chapter on ***Configuring NeuroZoom Devices*** in the ***User Guide Manual*** on how to configure and use devices.

Select Microscope Objective...

Command can also be called from the **View** window. Pops up a hierarchical menu for selecting all configured lens objectives for the selected microscope and camera. See the next section on **Microscope Setup** window for more information on how to use this window, and the chapter on ***Configuring NeuroZoom Devices*** in the ***User Guide Manual*** on how to configure and use devices. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Select Microscope Filter...

Command can also be called from the **View** window. Pops up a hierarchical menu for selecting all configured microscope filters. See the chapter on ***Configuring NeuroZoom Devices*** in the ***User Guide Manual*** on how to configure and use

devices. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Grid

Command can also be called from the **View** window. Toggles the visibility of the grid on the current mapping window. The grid displays the current location of the stage location (as measured from the center of the field). The X and Y tics are in absolute microns from the last known zero point (origin) of the stage controller. This reflects the current mapping coordinate system. The X and Y axes on the origin are always displayed in solid lines, while the lines corresponding to the tic locations are displayed as dotted lines.

If the grid tic interval is determined to be too small and thus would clutter up the mapping window, NeuroZoom will turn it off automatically. You will probably see this if you zoom down from the current scale to a point where the grid is too dense.

The appearance of the grid can be altered with the **Map** menu command **Grid Setup....** See the section on ***Mapping Coordinate System*** in the ***User Guide Manual*** for more information. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded). If the grid is on, there is a checkmark displayed before this menu item.

Grid Setup...

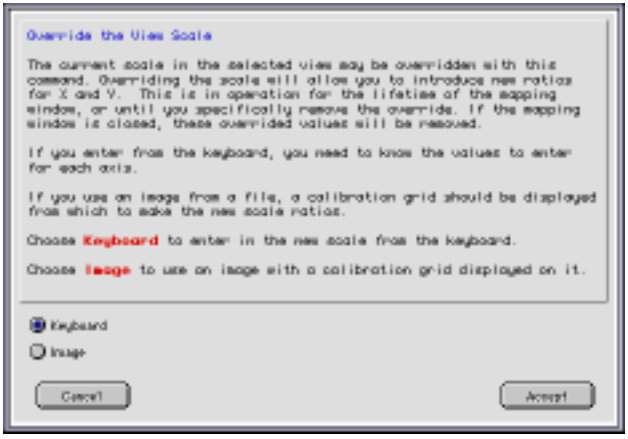
Command can also be called from the **View** window by option clicking on the **Grid** button. This item is not enabled if no documents are opened, or if the front

window is not a mapping window (floating palettes excluded). Opens the **Grid Setup** window so parameters controlling the appearance of the mapping window grid may be configured. See the next section on **Grid Setup** window for more information.

Override View Scale...

Allows the current scale in the X and Y dimensions to be overrided in the current mapping window. The new scale stays in effect until the override is manually turned off. This allows you to enter in any scale. The current scale for any view in place is then ignored. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Selecting this opens a window describing the command and the options to enter in the scale.



If the scale had not been previously overrided in the mapping window, there are only two options as displayed. **Keyboard** can be selected to enter in scale factors for X and Y from the keyboard as floating value. **Image** can be selected from which a live image or a digitized image of a calibration grid can be used to determine the scale. Press **Accept** to continue with the override process, or **Cancel** to cancel the override.

Keyboard - a window opens in which you enter the X and Y scale as floating values expressed as microns per pixel. Press **Accept** to accept the values, or **Cancel** to cancel the override.

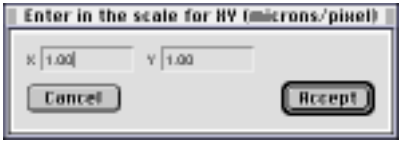
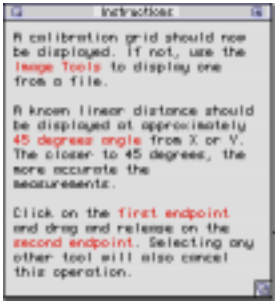


Image - If the preferences in NeuroZoom are set to display information windows, the following window opens.

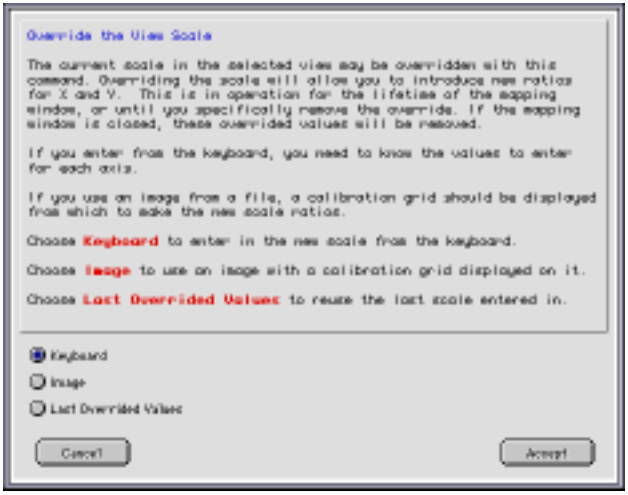


A calibration grid should already be displayed in the mapping window. This can be a live image of one on the microscope, or a digitized image displayed by using the **Open Image From File** command from the **Imaging** window or from the **Imaging** Menu. The grid is best displayed with a known linear distance at 45 degrees from the X or Y axis. Press and hold the mouse on one endpoint of the known distance. Drag the mouse to the other endpoint and release the mouse. The scale for the X and Y axes will be computed and used for the override values. Selecting any other tool without specifying the endpoints cancels this override process.

Once an override is in progress, a checkmark appears before the menu item **Override View Scale...** in the **Map** menu. Selecting the menu item opens a dialog asking if the override should be cleared.

Press **Clear** to clear the override, or **Cancel** to continue using the current override.

Once an override has been used in the mapping window, an additional option will appear in the options window when turning it back on.



Select **Last Overrided Values** to use the last scale values in effect. Press **Accept** to accept the values, or **Cancel** to cancel the override.

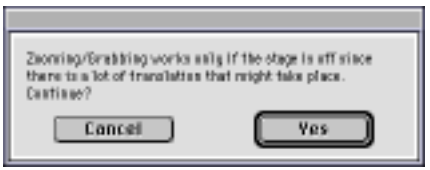
Objects Menu

The **Objects** menu is used to control actions surrounding the display, maintenance, or use of all objects in the current mapping window. This menu is not available if no mapping windows are open of if the front window is a standard or dialog window other than a mapping window.

Zoom In

***Command* =** Command can also be called from the **Zoom** tool of the **Mapping Tools** window. Zooms in on the current mapping window by a factor of 2. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

If a stage controller is on, a dialog window opens stating that the controller must be turned off. This is to prevent large movements of the stage on the microscope when zooming or grabbing the image in the window.

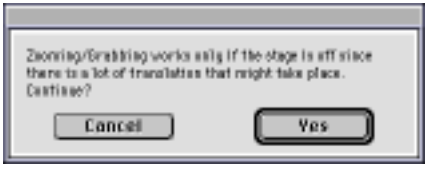


Press **Yes** to continue with the zooming and redraw the mapping window at the new zoom, or press **Cancel** to cancel the command. The current zoom value is displayed in the **Mapping Window Info** window.

Zoom Out

Command - Command can also be called from the **Zoom** tool of the **Mapping Tools** window. Zooms out on the current mapping window by a factor of 2. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

If a stage controller is on, a dialog window opens stating that the controller must be turned off. This is to prevent large movements of the stage on the microscope when zooming or grabbing the image in the window.

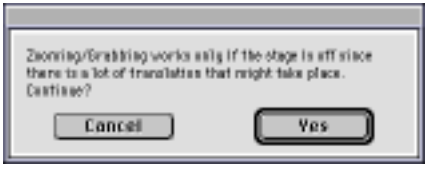


Press **Yes** to continue with the zooming and redraw the mapping window at the new zoom, or press **Cancel** to cancel the command. The current zoom value is displayed in the **Mapping Window Info** window.

Enter Zoom...

Opens a window in which the zoom factor may be entered. The zoom factor may be any floating point value. A zoom of 1.0 is the same size as the image from the microscope (assuming that the microscope, camera, and lens have all been scaled properly). This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

If a stage controller is on, a dialog window opens first stating that the controller must be turned off. This is to prevent large movements of the stage on the microscope when zooming or grabbing the image in the window.



Press **Yes** to continue with the entering of the zoom value or press **Cancel** to cancel the command. Another dialog window opens that allows you to enter in the value.

The default value displayed is the current zoom factor. Enter any floating point value. Zero and negative numbers are not permitted. Press **Accept** to continue with the zooming and redraw the mapping window at the new zoom, or press **Cancel** to cancel the command. The current zoom value is displayed in the **Mapping Window Info** window.

Full Scale

Command 0 Redraws the current mapping window at 1:1 scale. This item is not enabled if the stage controller is on, or if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Lock

Command L Locks all selected objects in the current mapping window. This item is not enabled if there are no selected objects, or if no documents are opened, or if the front window is not a mapping window (floating palettes excluded). Locking an object prevents it from being deleted, edited, or moved. Locked objects can still be selected for copying, dragging to other windows or to the desktop, generating information or reports, or hiding. A locked object appears with a dotted graphics representation when selected, otherwise it appears normally in the mapping window.

Unlock

Command U Unlocks all selected objects in the current mapping window. This item is not enabled if there are no selected objects, or if no documents are opened, or if the front window is not a mapping window (floating palettes excluded). Unlocking an object allows it to be deleted, edited, or moved.

Configure Layers...

Option-Command L Command can also be called from the **Window Togglers**. Opens the **Layers** window so parameters controlling the layers in the current mapping window can be configured.

Layers are used to organize the data into manageable parts. Data can go into containers known as *layers*. Each layer can be shown, hidden, activated for data entry, or inactivated to protect it against data entry (and thus change).

Layers can be used to hold different parts of an entity that you might be mapping. For example, boundaries of the cortical surface can go into a layer called *pial surface*. Different regions can go into a layer called regions. Cortical layers can go into a layer called cortical *layers*. Cell can go into a layer called *cells*, and fiber can go into a layer called *fibers*. See the next section on **Layers** window for more information on how to use this window.

Make Current Layer

Command can also be called from the **Layers** window. Pops up a hierarchical menu for selecting one of the layers as the current layer.

The current layer is the layer into which all new data objects are entered. There is always one current layer at all times. A checkmark appears before its name in the popup menu. See the next section on **Layers** window for more information. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Layers

Many of these commands can also be called from the **Layers** window. Pops up a hierarchical menu for controlling visibility of all layers, activity of all layers, and the visibility of the stereology layer. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

A checkmark appears before the menu item in the popup menu that indicates the currently selected mode.

Show All Layers will turn on the visibility of all layers.

Hide All Layers will turn off the visibility of all layers.

Activate All Layers will turn on the activity of all layers. The objects in all of the layers are then selectable.

Deactivate All Layers will turn off the activity of all layers. The objects in all of the layers are then not selectable.

Show Stereology Layer will turn on the visibility of only the stereology layer. Data that are acquired from a stereology protocol are displayed.

Hide Stereology Layer will turn off the visibility of only the stereology layer. Data that are acquired from a stereology protocol are not displayed.

Enable Data Rendering

Toggles the rendering of the data in the current mapping window. If this is off, data are not displayed. If this is on, data are displayed. A checkmark appears before the menu item **Enable Data Rendering** in the **Object** menu when rendering is enabled.

Use this when there is a lot of data in the mapping window, and you are performing some command that causes a lot of rendering to occur. An example is moving the stage using the software controller. Each time the stage is moved and

settled, data will be rendered. Disabling the rendering temporarily will speed up the process of moving the stage until you locate the field of interest.

Data Types

Many of these commands can also be called from the **Data Type** window. Pops up a hierarchical menu for controlling visibility of all data types. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

A checkmark appears before the menu item in the popup menu that indicates the currently selected mode.

Show All Data Types will turn on the visibility of data types.

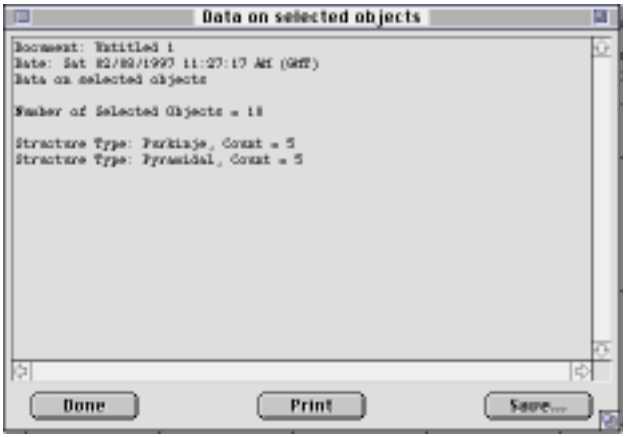
Hide All Data Types will turn off the visibility of data types.

Note that this is different from the **Enable Data Rendering** command because only data types from mapped data are affected. Stereology data, images, grids, etc. are not affected by this command.

Object Info...

Command I Shows information on the selected objects in the current mapping window. This item is not enabled if there are no selected objects, or if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

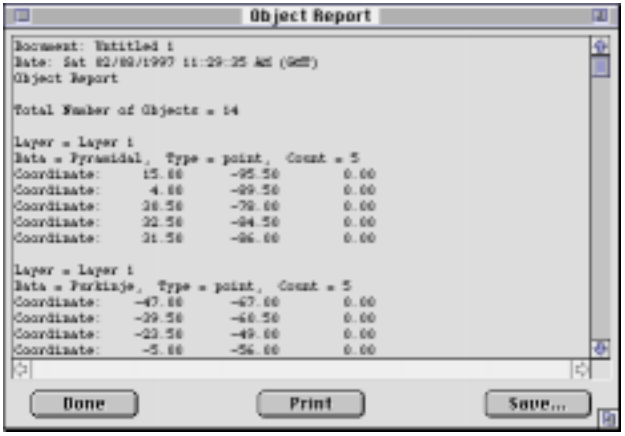
The information is displayed in a report window that summarizes the total count information for each structure in the selected objects. If a more complete report is needed, use **Object Report...**.



Object Report...

Option-Command R Shows full report on all objects in the current mapping window. Objects do not have to be selected to use this command. All objects in the mapping layer of the current mapping window are reported. Stereology objects are not included. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

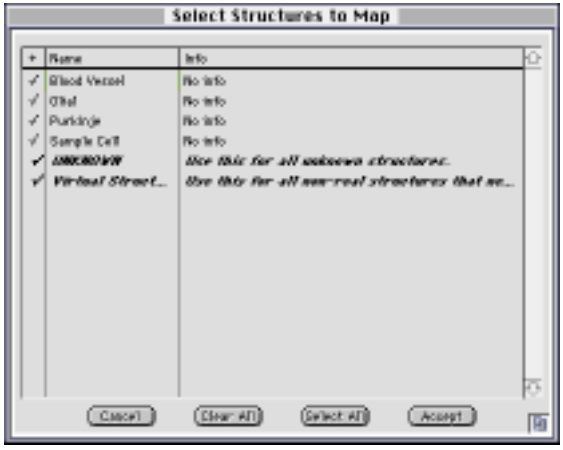
The report is displayed in a report window that details the information for each structure in the selected objects.



The format of the report is affected by the **Copy/Paste** preferences stored for NeuroZoom See the next section on **Preference** window for more information on how to use preferences.

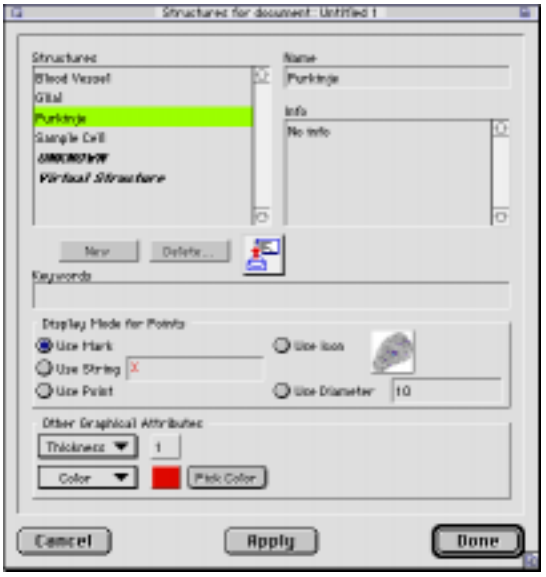
Select Structures to Map...

Option-Command S Opens the **Select Structures to Map** window so parameters controlling the currently selectable structures in the current mapping window can be configured.



Many structures may be defined for a document, but only a small subset may actually be used in one experiment of the document. See the next section on **Select Structures to Map** window for more information on how to use this window.

Configure Structures... Command can also be called from the **Window Togglers**. Opens the **Structure Configuration** window so parameters controlling the all structures of the document in the current mapping window can be configured.



The names, information, keywords, and graphics representation of all or any structures can all be controlled from this window. See the next section on **Structure Configuration** window for more information on how to use this window.

Special

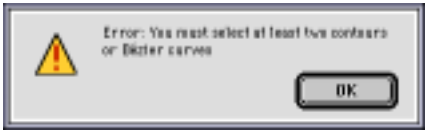
Pops up a hierarchical menu for special commands on objects. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Currently, there is only one command available.

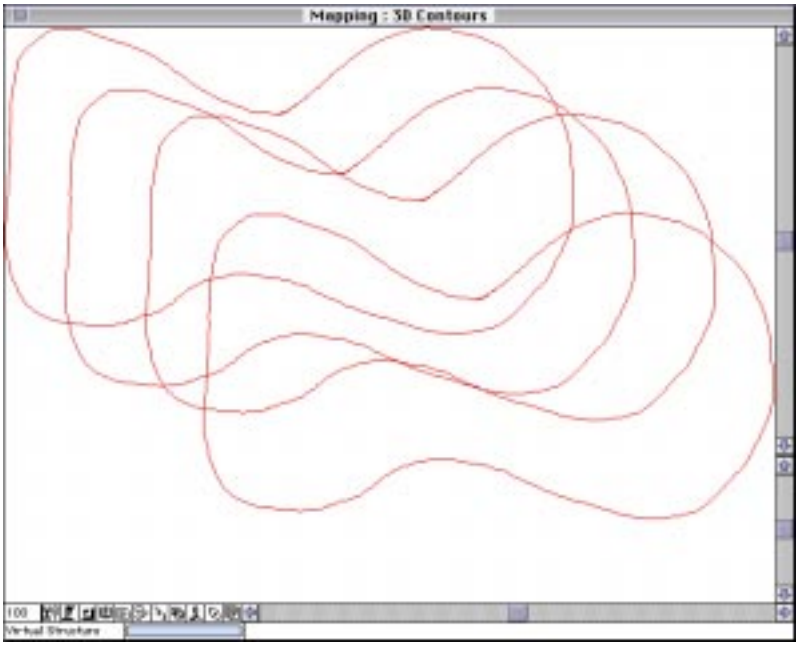
Create Mesh from Selected Objects

This creates a 3D surface mesh from selected contours or bezier curves. This is then used to create the 3D model for 3D visualization. Only contours or Bezier curves can be selected, otherwise an error will display.

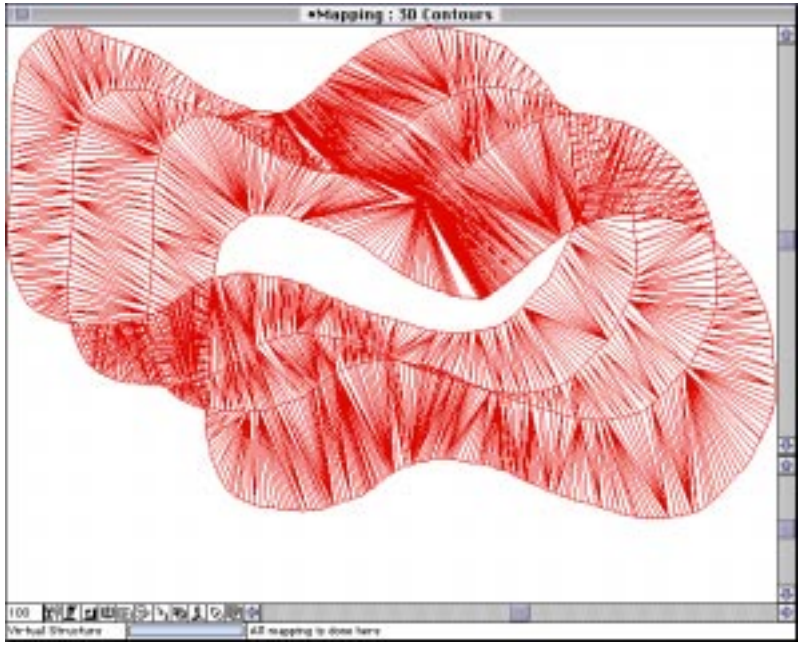
Also, at least two contours or Bezier curves must be selected, otherwise an error displays.



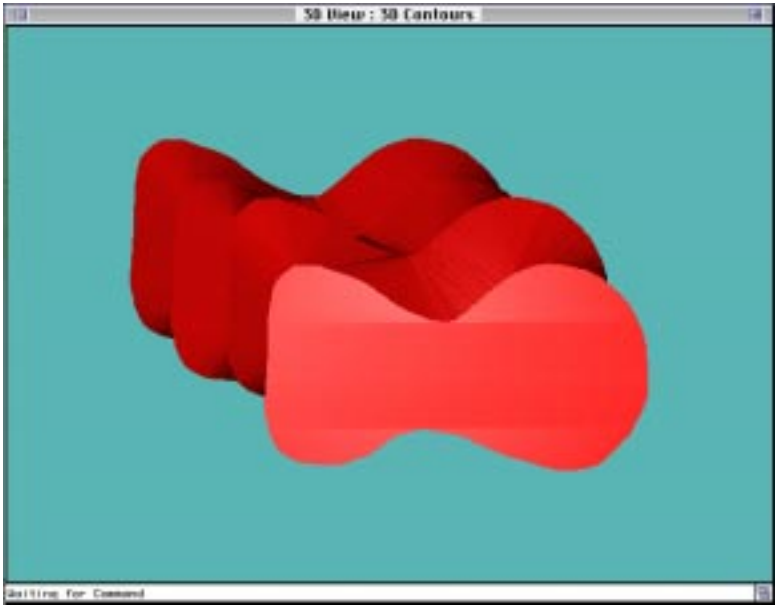
When the correct objects are selected, a confirmation window appears.

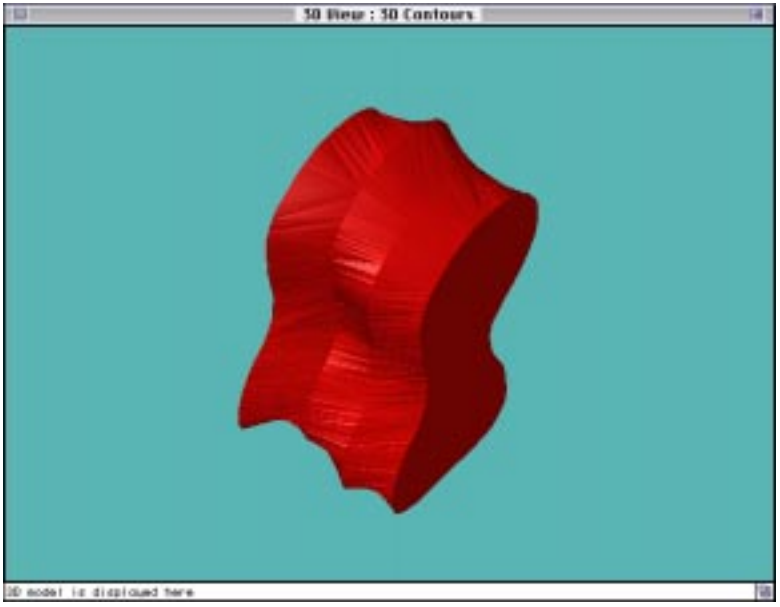


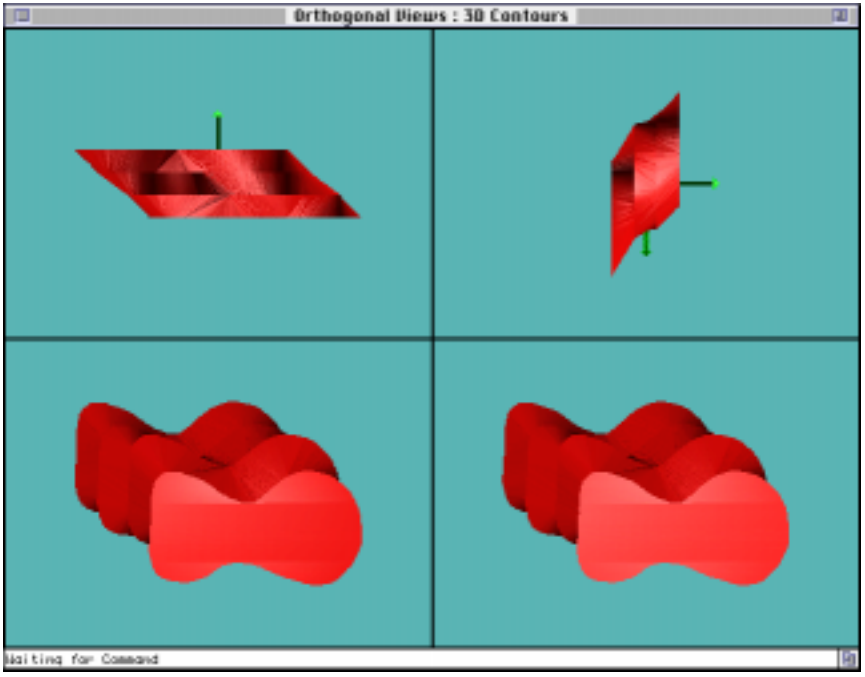
Press **Continue** to create the mesh, or **Cancel** to cancel the command. External spare disk space is used to hold data while the mesh is being created. If there is not enough disk space, NeuroZoom alerts you. When the mesh has been successfully created, the mesh object is selected as the only object.



You can then proceed with 3D visualization by created a 3D model.


3D View : 3D Contours
Waiting for Command


3D View : 3D Contours
3D model is displayed here



See the chapters on 3D ***Visualization*** for more information on how to create 3D models and how to use 3D visualization.

Imaging Menu

The **Imaging** menu is used to control actions surrounding the display, maintenance, or use of video and images in the current mapping window. This menu is not available if no mapping windows are open of if the front window is a standard or dialog window other than a mapping window.

Turn Video On/Off

This command can also be called from the **Imaging** window. If video is off, this turns on live video if video is possible. If video is on, this turns off live video. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Video is affected by a variety of factors, such as the Macintosh model, the amount of VRAM present, the video board if present, the type of electronic camera attached, and the digitizer configured for the current camera. See the appendix on ***Optimizing Video*** for more information on using and maintaining live video in NeuroZoom.

Grab Image

This command can also be called from the **Imaging** window. Grabs one frame from the video digitizer if video digitization is possible. If video is on, one frame is grabbed and video then turned off. If video is off, the video is momentarily turned on, a frame is grabbed, then video is turned off again. The image that is grabbed becomes one background image and is displayed at the location of the stage (current location). If the command **Spatially Map All Images** is on, the

image will *move* with the stage when the stage is moved. If not, the image will stay in the current mapping window at all times, unless it is obscured by a newer image grabbed, opened, or pasted on top of it. The images all appear in the **Background Images** window, and visibility may be controlled from there. See the next section on **Background Images** window for more information on how to use this window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Image Size

This command can also be called from the **Image Size Popup** menu of the **Mapping** window. Pops up a hierarchical menu for setting the percent size of the current mapping window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

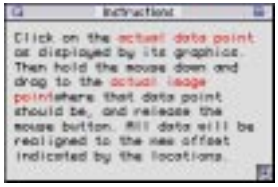
This is selectable from a minimum of 5% to a maximum of 100%. The value indicates the percent size of the mapping window relative to the pixel width and height of the displayed video, or the displayed image. Live video is scaled to match the size of the window. Therefore there is no loss of spatial context, only resolution. This command is useful if the monitor size is small.

Realign Data to Image...

This command can also be called from the **View** window. Allows data to be realigned to the image. Small errors may creep into the display of the data graphics over the live image from the microscope. This comes from

imperfections in the lens objections, camera, or even the microscope optics. The stage controller itself can sometimes be at fault if the precision is not high enough. This command makes it easy to realign all data when it displays improperly.

If the preferences in NeuroZoom are set to display information windows, the following window opens.



Position the cursor over any data graphics that can be visualized. While holding the mouse button down on that data graphics, move the cursor to the real object in the image. When the mouse button is released, the realignment factor is calculated and a confirmation window opens.

Press **Continue** to accept the realignment, or press **Cancel** to cancel the realignment command. You can also select any other tool before dragging the mouse to cancel this command. If you press **Continue**, all of the data will be offset by the realignment factor. All subsequent data entered into this mapping window will align itself to the new values.

Save Image to File...

This command can also be called from the **Imaging** window. Allows the current background image to be saved to a disk file. If there are no images currently in the background, an error window will open.

Otherwise, a standard file dialog opens in which to enter the name of the file for the image.

The default name of the file is the name of the document, with *.IMAGE* as the file extension. Either *PICT* or *TIFF* can be selected for the image format by pressing on the Format popup menu button.

Press **Save** to save the image to the file, or press **Cancel** to cancel this command.

If the image displayed is not the one you wish to save to a file, use the **Background Images** window to select the proper image. See the next section on **Background Images** window for more information on how to use this window.

Open Image From File...

This command can also be called from the **Imaging** window. Opens a PICT or TIFF file and reads the image into the current mapping window as a background

image. A standard file dialog opens to choose a file. Only PICT and TIFF files and folders are visible from this dialog.

The image that is opened becomes one background image and is displayed at the location of the stage (current location). If the command **Spatially Map All Images** is on, the image will *move* with the stage when the stage is moved. If not, the image will stay in the current mapping window at all times, unless it is obscured by a newer image grabbed, opened, or pasted on top of it. The images all appear in the **Background Images** window, and visibility may be controlled from there. See the next section on **Background Images** window for more information on how to use this window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

> **Alert:** Multi-file and compressed TIFFs cannot be displayed in NeuroZoom. Use another graphics application to convert it to single file, standard TIFF or a PICT file.

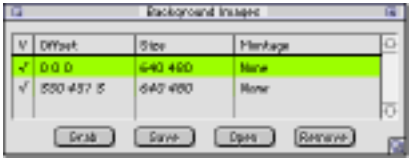
Remove Image From List...

Command can also be called from the **Imaging** window. The current background image is removed from the list of images for the current mapping window. If there are no background images for this window, the following error window opens.

Otherwise, a confirmation opens.

Press **Remove** to remove the background image from the current mapping window, or press **Cancel** to cancel this command. Images all appear in the **Background Images** window, and may be removed from there. See the next section on **Background Images** window for more information on how to use this window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Background Images... Command can also be called from the **Imaging** window. Opens the **Background Images** window.

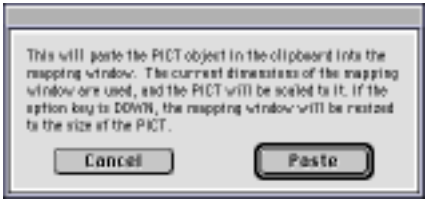


This window lists all of the background images for this current mapping window. These background images display behind the data, acting as a kind of template on which data are mapped. In this figure, the first image is positioned exactly on the origin. The second image is 350 microns to the right in the X axis, 451 microns down in the Y axis, and 3 microns up in the Z axis. See the next section on **Background Images** window for more information on how to use this window.

This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Paste Image from Clipboard...

Any copied PICT object can be pasted into a mapping document. This item is not enabled if there is no PICT object in the clipboard. A dialog window opens.



Press **Paste** to continue with the Paste command, or **Cancel** to cancel the command. The PICT image is automatically scaled to fit the size of the mapping window. A 640 by 480 image will fit perfectly in a standard NTSC camera, 640 by 480 mapping window. If the *option* key is held down when answering this dialog, the image will not be scaled, and the mapping window will resize to fit the size of the pasted image. The image that is pasted becomes one background image and is displayed at the location of the stage (current location). If the command **Spatially Map All Images** is on, the image will *move* with the stage when the stage is moved. If not, the image will stay in the current mapping window at all times, unless it is obscured by a newer image grabbed, opened, or pasted on top of it. The images all appear in the **Background Images** window, and visibility may be controlled from there. See the next section on **Background Images** window for more information on how to use this window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Spatially Map All Images

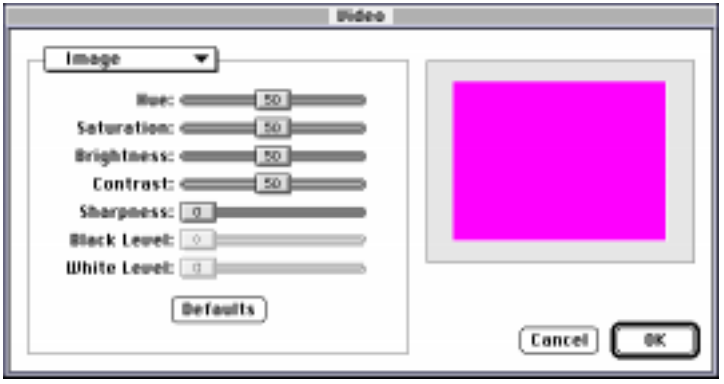
All background images may be displayed at their location stored with the image when the image was grabbed, opened, or pasted. This allows a montage of images to be created that all act as part of the background. If the microscope stage moves, the images will *move* with it. If this menu item **Spatially Map All Images** is on, all of the background images act in this manner. If it is not on, all images will display on top of each other in the mapping window regardless of the

location of the microscope stage. A checkmark appears before the menu item that indicates the currently selected mode.

The images all appear in the **Background Images** window, and visibility may be controlled from there. See the next section on **Background Images** window for more information on how to use this window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Video Settings...

The standard QuickTime Video Settings dialog window opens with this menu command. This item is not enabled if live video is not currently on.

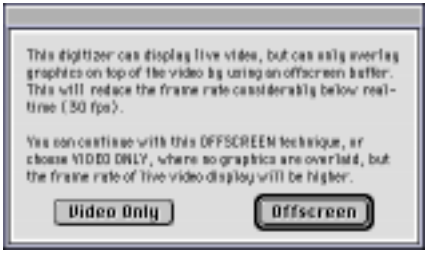


Some of the video parameters may be controlled from this dialog. Refer to the appendix on ***Optimizing Video*** for more information.

Check QuickTime VDIG...

Every Macintosh AV model and QuickTime compatible board has a specific video digitizer (VDIG) implementation. NeuroZoom uses different VDIGs in different ways because of different capabilities. If it detects that the VDIG is incapable of color keying live video, and if it is not capable of digitizing into offscreen memory, live video is the only available option. However, in some cases of offscreen digitization, the frame update, which is less than real time (30 frames per second), may not be optimal for focusing when using the Macintosh

monitor. So, depending on your needs, you can switch between different modes of using the VDIG, if more than one mode is available. By selecting this menu item, the VDIG is checked again by NeuroZoom, and any options are presented for selection. For example, an 8500AV Power Macintosh will display the following window.



Press **Offscreen** to force NeuroZoom to use offscreen digitization for live video, which is less than real time (30 frames per second). Press **Video Only** to force NeuroZoom to use Video Only for live video, which is real time but cannot display data graphics overlaid on top of the video. Refer to the appendix on ***Optimizing Video*** for more information.

Refresh Video...

Command T Redraws the video once in the current mapping window. This item is not enabled if no documents are opened, if the front window is not a mapping window (floating palettes excluded), or if live video is not on. This function is useful for some video cards that exhibit anomalies when other windows may be overlapping the mapping window.

Stage Menu

The **Stage** menu is used to control actions surrounding the maintenance or use of the stage in the current mapping window. This menu is not available if no mapping windows are open of if the front window is a standard or dialog window other than a mapping window.

Turn Stage On/Off

Command can also be called from the **Stage** window. If the stage controller is off, this turns on the stage controller. If the stage controller is on, this turns off the stage controller. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

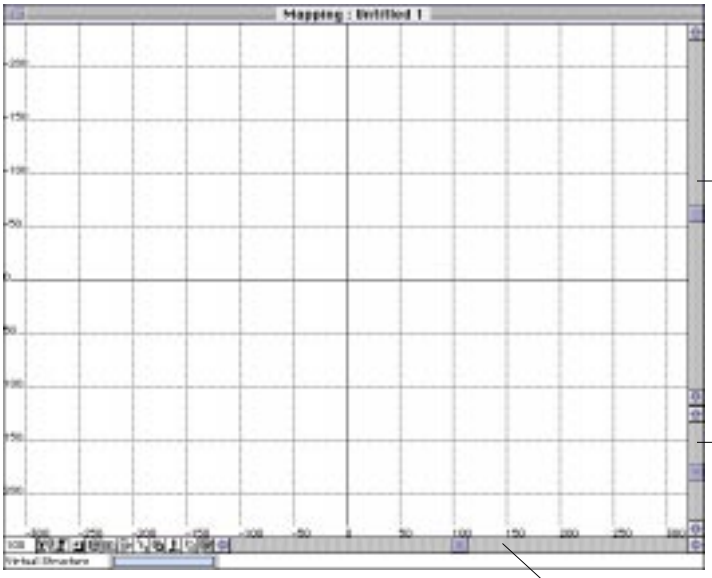
Turn Stage Joystick On/ Off

If the stage controller joystick is off, this turns on the stage controller joystick. If the stage controller joystick is on, this turns off the stage controller joystick. This item is not enabled if the stage controller does not support the toggling of the joystick enable, no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Turn Map Scrollers On/ Off

Command can also be called from the **Stage** window. If the scrollers in the current mapping window are off, this turns on the scrollers. If the scrollers in the current mapping window are on, this turns off the scrollers. This item is not

enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

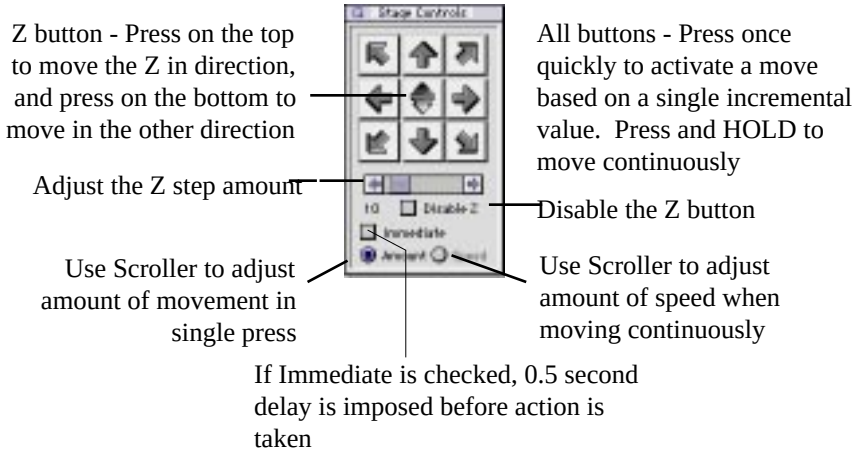


The scrollers will activate the computerized stage and move the stage in a particular axis. They are used to move the stage, not the image per se. See the next section on **Mapping** window for more information on how to use these scrollers.

Stage Controls...

Command can also be called from the **Stage** window. Opens the **Stage Controls** window. This window displays a software based controller for the stage controller that will work with any of the stages selected for the mapping window. This software joystick should not be used as a replacement for any hardware joystick or trackball supplied with your stage controller. Those will be much easier to use since this software joystick will have a certain amount of inherent non-responsiveness due to the nature of how the computer communicates with

the stage controllers. A checkmark appears before the menu item that indicates if the window is opened. Subsequent selections of this menu command toggles the window on and off. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).



Move Stage Manually

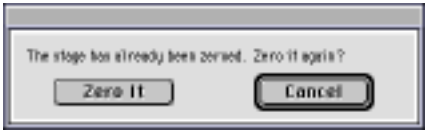
Command M Command can also be called from the **Stage** window. By default, NeuroZoom does not constantly communicate with the stage controller to determine if the stage was moved. Therefore, when you have moved the stage in any of the three axes, you need to call this command to have NeuroZoom update its internal coordinate information with that presented by the new location of the stage. The preference setting for Warnings (see the section on **Preferences** window for more information) determines if a confirmation window opens. If the menu item is followed by an ellipsis (**Move Stage Manually...**), this indicates that the preference setting is on for warnings. The following confirmation window then opens.

Press **OK** to indicate that you moved the stage. NeuroZoom updates its information with the current location of the stage. Press **Cancel** to cancel the command. If no preference is set for the warning, no confirmation window opens, and NeuroZoom will immediate update its information with the current location of the stage.

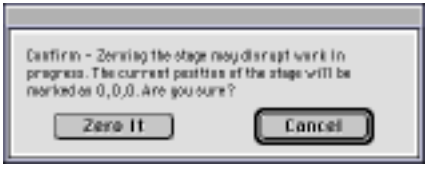
Alert: This function is needed when the Automatic Behavior preference for automatically updating the stage preference during mapping is off. If it is on, the stage position is updated when data are entered. This function would only be needed if an explicit update from the stage controller is required.

Zero the Stage

Command can also be called from the **Stage** window. Zeroes the stage controller to the current location of the microscope stage. If the stage controller has already been zeroed, a warning confirmation dialog appears.



The final confirmation dialog then appears before zeroing the stage controller.



A zeroed stage controller establishes an origin in the mapping coordinate system so that all subsequent data entry are relative to this origin. A stage controller is zeroed usually for new documents. A fiducial that can be located with ease is typically positioned over the origin. This becomes the origin of the mapping coordinate system when the stage is zeroed, and the grid if displayed will show the origin as two solid X and Y lines in the center of the mapping window. Once the stage controller is zeroed, all data are mapped relative to this origin. If there is data already mapped to another fiducial, the data will appear misaligned.

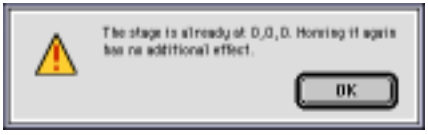
The indication of the zero point can be altered by holding down the *OPTION* key. In this case, you move the mouse to the desired zero location in the mapping window and *CLICK* on that location to be the origin. The origin of the grid is then moved to that location, rather than defaulting to the center of the mapping window.

Tip: Use this method when the structure you want to be the zero fiducial point is on the screen already. It really doesn't matter where on the mapping window the zero fiducial is, as long as the origin of the mapping system falls on top of it.

Home the Stage

Command can also be called from the **Stage** window. Home the stage controller back to 0,0,0. A confirmation dialog displays.

If the stage has already been homed, a warning dialog appears saying that this will have no effect.



Homing the stage does not destroy data. It only moves the stage back to the last known origin.

Ignore Stage Limits

NeuroZoom can check that the stage controllers do not exceed a limit in either of the three axes. This limit imposes an additional safety margin to protect microscope equipment and the microscope slides. **Ignore Stage Limits** toggles whether the limits are ignored or not. A checkmark appears before the menu item that indicates the currently selected mode. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

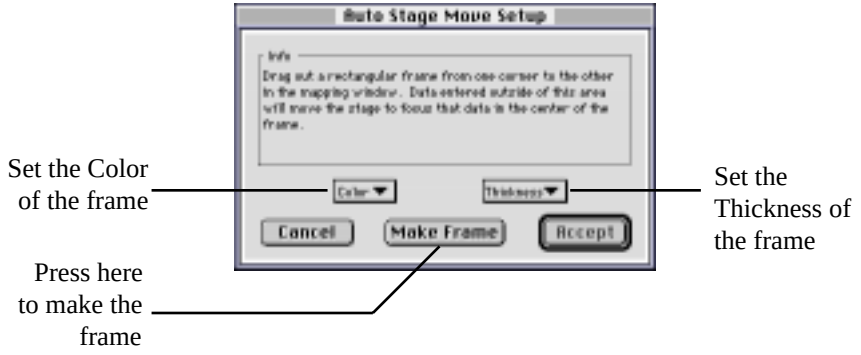
Set the Stage Limits...

The limits as mentioned above can be set by selecting this menu item. Opens the **Set Stage Limits** window.

This window can be used to configure in software based limits on the XYZ movement. This is useful if the stage controller does not have physical limit switches on the stage, or if you want to program in additional safety protecting the microscope equipment and the microscope slides. A checkmark appears before the menu item that indicates if the window is opened. Subsequent selections of this menu command toggles the window on and off. See the next section on **Set Stage Limits** window for more information on how to use this window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Auto Stage Move

Command can also be called from the **Stage** window. NeuroZoom can automatically move the stage when data are being entered, and the last data point is outside of a specified rectangular region in the current mapping window. This rectangular region is the auto move frame. This auto move frame must be initially configured. If this is the first time, the **Auto Move Stage** window opens where you can create or adjust the auto move frame



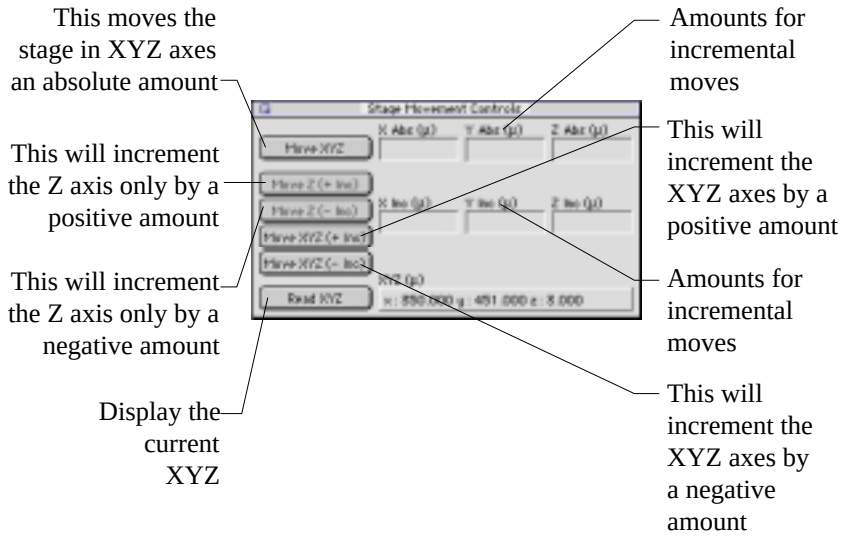
Subsequent selections of this menu command will toggle the frame on and off. Selecting the menu command with the *Option* key down will open the **Auto Stage Move Setup** window again. See the next section on **Auto Move Stage** window for more information on how to use this window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Auto Stage Move Setup...

Command can also be called from the **Stage** window. This opens the **Auto Stage Move Setup** window directly.

Stage Movement Controls...

Opens the **Stage Movement Controls** window. This window can be used to move the stage in certain prescribed amounts, for example, to perform systematic stepwise movements though the tissue section.



A checkmark appears before the menu item that indicates if the window is opened. Subsequent selections of this menu command toggles the window on and off. See the next section on **Stage Movement Controls** window for more information on how to use this window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

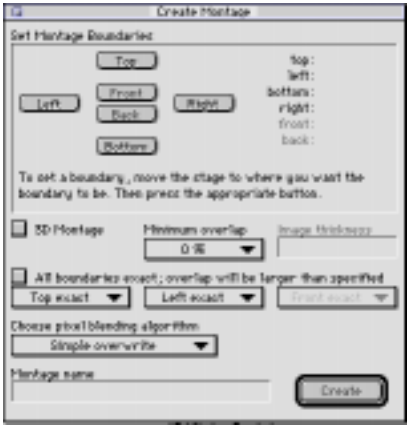
Stage Locations...

Opens the **Stage Locations** window. This window displays all stored stage locations for this document.

Stage locations are wherever the stage happens to be when the position is recorded. By using this window, those locations can be returned to immediately. A checkmark appears before the menu item that indicates if the window is opened. Subsequent selections of this menu command toggles the window on and off. See the next section on **Stage Locations** window for more information on how to use this window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Create Montage...

Opens the **Create Montage** window. This window contains all of the functions needed to create a montage across the microscope slide section. If a motorized stage is configured, the entire montage can be captured automatically. Because images act precisely the same way as live video, this montage can be treated as a *mounted* tissue section, and analyzed off-line from the microscope.



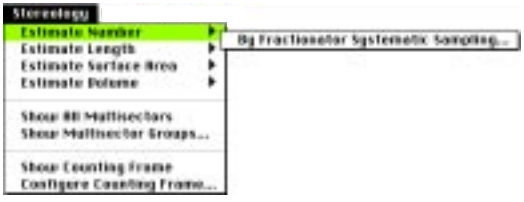
A checkmark appears before the menu item that indicates if the window is opened. Subsequent selections of this menu command toggles the window on and off. See the next section on **Create Montage** window for more information on how to use this window. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Stereology Menu

The **Stereology** menu is used to control actions surrounding the use of all stereology protocols and probes in the current mapping window. This menu is not available if no mapping windows are open of if the front window is a standard or dialog window other than a mapping window.

Estimate Number

Pops up a hierarchical menu to select a probe for estimating number of particles. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).



By Fractionator Systematic Sampling...

Uses the Fractionator protocol in a systematic random sampled fields. See the chapters on ***Stereology*** for more information on how to use this probe.

Estimate Length

Currently there are no probes for estimating length.

Estimate Surface Area

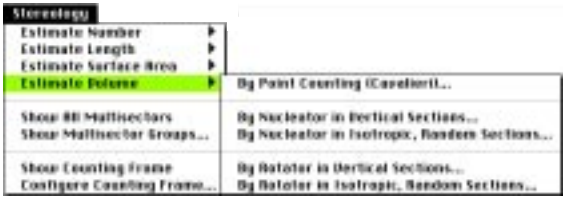
Pops up a hierarchical menu to select a probe for estimating surface area of any three dimensional object. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

By Cycloids and Point Counting in Vertical Sections...

Only vertically blocked samples should be estimated with this probe. Uses cycloids to estimate the surface area, and point counting (Cavalieri technique) for estimating the volume simultaneously. See the chapters on ***Stereology*** for more information on how to use this probe.

Estimate Volume

Pops up a hierarchical menu to select a probe for estimating volume of any three dimensional object. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).



By Point Counting (Cavalieri)...

This is a global estimator useful to estimate the singular volume of large objects, such as nuclei. See the chapters on ***Stereology*** for more information on how to use this probe.

By Nucleator in Vertical Sections...

This is a local estimator useful to estimate the mean volume of small objects, such as cells. Use this probe only if the sections are vertically blocked. The Nucleator requires a nucleolus, and is more efficient than the Rotator. See the chapters on ***Stereology*** for more information on how to use this probe.

By Nucleator in Isotropic, Random Sections...

This is a local estimator useful to estimate the mean volume of small objects, such as cells. Use this probe only if the sections are not vertically blocked, but are isotropic and randomly sectioned. The Nucleator requires a nucleolus, and is more efficient than the Rotator. See the chapters on ***Stereology*** for more information on how to use this probe.

By Rotator in Vertical Sections...

This is a local estimator useful to estimate the mean volume of small objects, such as cells. Use this probe only if the sections are vertically blocked. The Rotator requires a nucleolus, and is less efficient than the Nucleator. See the chapters on ***Stereology*** for more information on how to use this probe.

By Rotator in Isotropic, Random Sections...

This is a local estimator useful to estimate the mean volume of small objects, such as cells. Use this probe only if the sections are not vertically blocked, but are isotropic and randomly sectioned. The Rotator requires a nucleolus, and is less efficient than the Nucleator. See the chapters on ***Stereology*** for more information on how to use this probe.

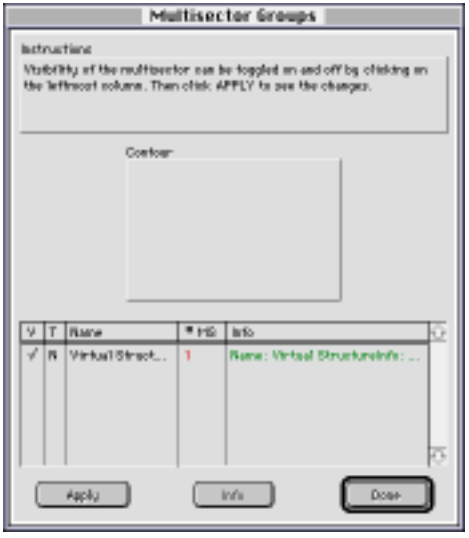
Show All Multisectors

If any stereological protocol has been created for the current mapping window, and if it uses multisectors, this menu command toggles the visibility of the multisector frame in the current mapping window. A checkmark appears before

the menu item that indicates the currently selected mode. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

Show Multisector Groups

If any stereological protocol has been created for the current mapping window, and if it uses multisectors, this menu command opens a window from which the multisector groups may be toggled on and off. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).



See the next section on **Multisector Groups** window and the chapters on ***Stereology*** for more information on how to use this window.

Configure Counting Frame...

Opens the Configure **Counting Frame** window to configure the stereological counting frame. This item is not enabled if no documents are opened, or if the front window is not a mapping window (floating palettes excluded).

See the next section on **Configure Counting Frame** window and the chapters on ***Stereology*** for more information on how to use this window.

Windows Menu

The **Windows** menu is used to select from the various open windows in NeuroZoom one window to be the frontmost active window. All of the windows are listed by name in the Windows menu. The current window has a • character before its name. Selecting any window brings that window to front. Note that palettes behave differently because they always appear to float in front of other standard windows.

Modules Menu

The **Modules** menu is used to display any loadable modules in NeuroZoom. Different modules perform different tasks. A module loaded into NeuroZoom displays its menu from the **Modules** menu.

3D

Pops up a hierarchical menu for various 3D functions.

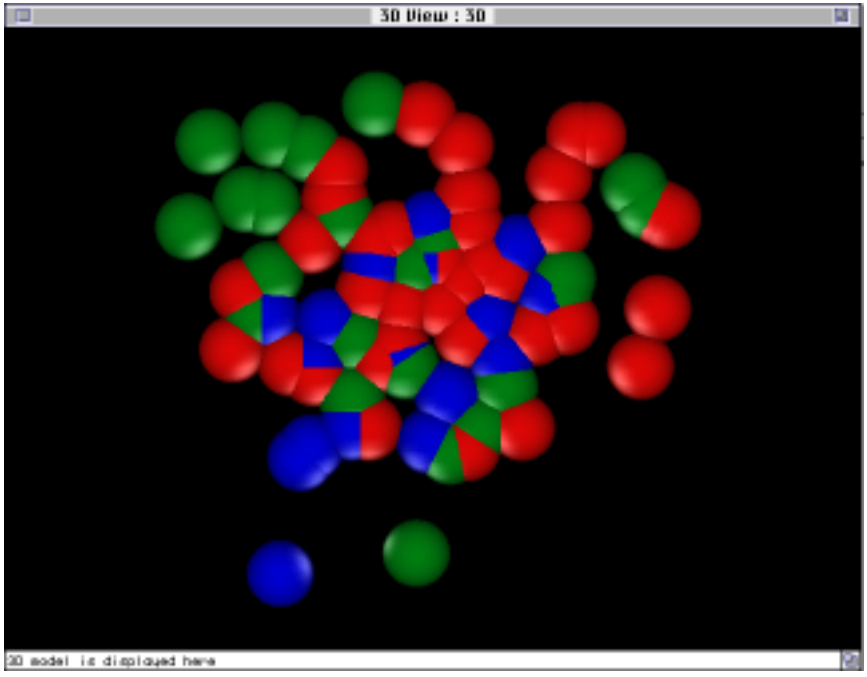
The functions open the mapping data into a **3D Mapping** window, or opens a 3D metafile into a **3D Viewer** window.

3D View

Opens the mapping data into a **3D Mapping** window. Before you open a 3D window you should decide if you want to visualize all the data objects from the mapping window or just a selection of them.

- If there are data objects selected, those selected objects will be visualized on the 3D window.
- If there are no data objects selected, all the objects in the mapping window will be visualized.

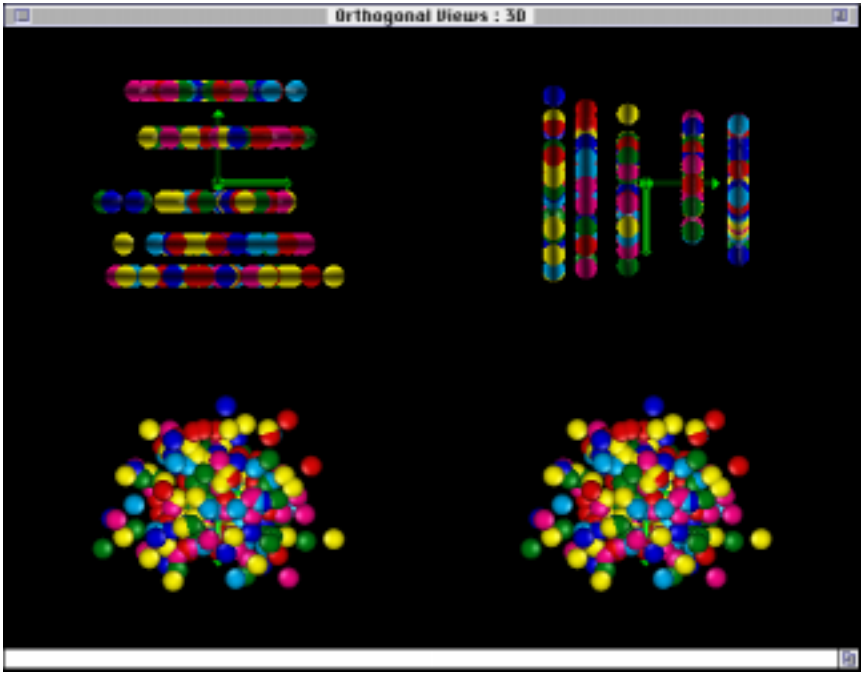
NeuroZoom takes all the necessary steps to create the QuickDraw™ 3D object, the 3D model, that represents the data to be visualized in the 3D window. A progress window displays the progress of creating the 3D model from the data. A new 3D window is then opened showing the 3D model associated with it. The name of the window is the same name of the mapping window, with *3D View:* prepended to it.



See later chapters on the 3D windows to learn how to work with these 3D models.

Orthogonal Views

Opens the mapping data into an orthogonal **3D Mapping** window divided in four views, where each view displays a different orthogonal view of the 3D model.



Top View	Side View
Front View	3D View

The **Top**, **Side** and **Front** Views show the orthographic projection of the 3D model into the plane perpendicular to the direction of the y, x and z axis respectively. The **3D View** shows either a one point perspective projection or an orthographic parallel projection of the 3D model onto the front plane (parallel to the z direction) that upon initialization will look exactly like the Front View if the type of camera selected is Orthographic. Note that the 3D View will change as the 3D Tools are used to move through the 3D model.

When the frontmost window is a 3D mapping window and you use the **3D** menu to open a new 3D window, the model displayed on the new 3D window will be a copy of the model displayed on the frontmost window. If the frontmost window is a mapping window, the data from that mapping window will be used to create the model of the new 3D window. This means you can have several 3D windows displaying either the same or different data sets.

See the chapters on ***3D Visualization*** to learn how to work with these 3D models.

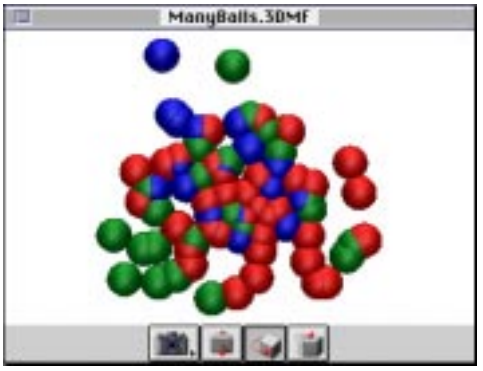
View QuickDraw 3D™ Metafile...

Opens a QuickDraw 3D™ metafile (3DMF) in a QuickDraw™ 3D Viewer window.

A standard file dialog opens asking for the 3DMF file. Press **Open** to accept the file.

A **QuickDraw™ 3D Viewer** window opens to display the model in that file. The Viewer window is provided as part of the *Apple QuickDraw™ 3D* library and

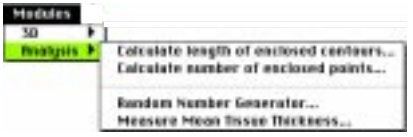
provides a very simple method for displaying 3D models with controls that permit limited interaction with the models.



Alert: To display a file in QuickDraw™ 3D Metafile format from NeuroZoom, the QuickDraw™ 3D Viewer extension and the QuickDraw™ 3D extensions must be installed in your system.

Analysis

Pops up a hierarchical menu for various functions for analyses.



Calculate length of enclosed contours

All opened contours falling within a specified closed contour, oval, or rectangle are summed for total length. This is useful when tracing a dendritic tree, and you just want a quick sum of all fiber branches as a quick estimate of terminal density.

Calculate number of enclosed points

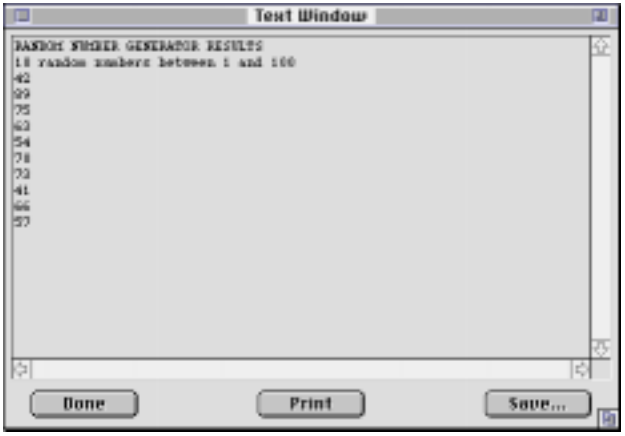
All points falling within a specified closed contour, oval, or rectangle are summed for total number of points. This is useful when mapping cells, and you just want a quick total of cells in a certain area.

Random Number Generator

Selecting this menu item opens a **Random Number Generator** window.

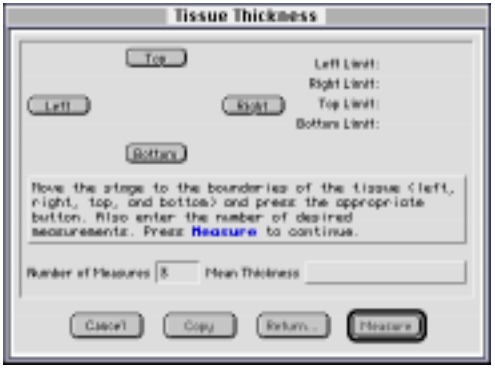
Any number of random numbers can be generated as *floats* or *integers* between a low and a high range. Enter the low and high values and select **Float** or **Integer**. Press **Generate** to make one or more random numbers. If only one is specified, it is displayed in the **Result** field. Press **Copy** to copy it to the clipboard. Alternatively, you can select the contents of the **Result** field and select the menu item **Copy** from the **Edit** menu.

If more than one random number are being generated, they will open in a separate text window.



Measure Mean Tissue Thickness

Selecting this menu item opens the **Tissue Thickness** window. The mean tissue thickness is needed for some stereology protocols.



Use the buttons labelled **Left**, **Top**, **Right**, **Bottom** to indicate the boundaries of the tissue from which to measure. Use the trackball, joystick, or other stage controller device that is connected to your stage controller. This window is modal and blocks out interaction with all other windows.

Indicate the number of measures by entering a number from 1 to 999 in the field labelled **Number of Measures**. The **Measure** button should enable. Press this button to continue.

NeuroZoom moves the stage to the first randomly calculated position on the tissue based on the boundaries. A dialog window opens asking you to focus on the *top* of the tissue section. Press the **Ready** button when focused. A second window opens asking you to focus on the *bottom* of the tissue section. Press the **Ready** button when focused. The thickness is stored for this location. This procedure repeats for the number of measures indicated.

If you press the **Cancel** button, you will be given the option of cancelling the entire measurement process, or generating a new random position for the current measure number. This is useful if a position happens to be off the tissue.

After the final measure, the mean tissue thickness is displayed in the field labelled **Mean Thickness**.

Pressing the **Copy** button copies the contents of the field (minus the units descriptor) to the clipboard for pasting into other windows, such as the stereology protocol windows.

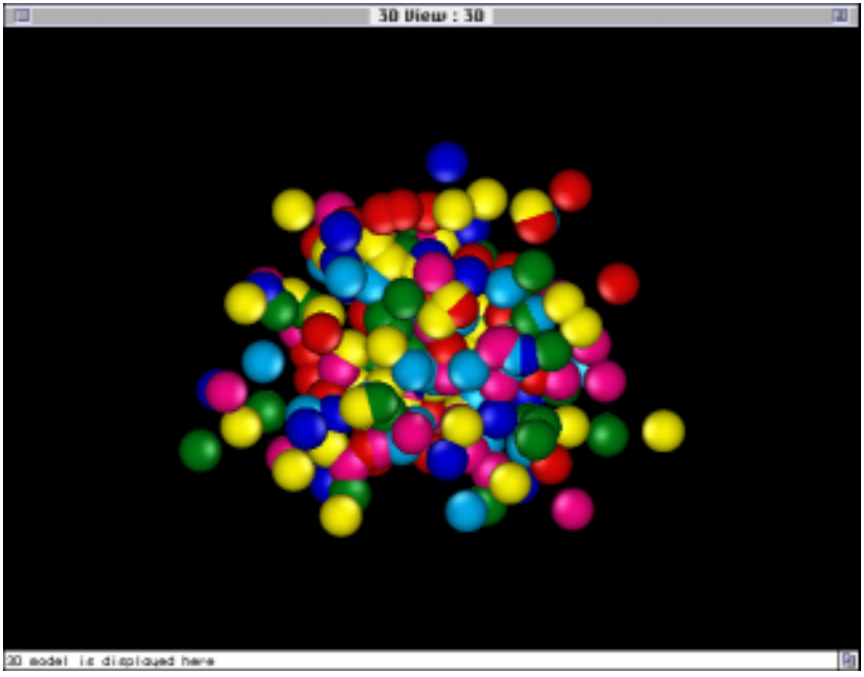
Pressing the **Return...** button prompts you for confirmation to return to the starting stage location when this window was originally opened.

3D Menu

The **3D** menu is available only when a 3D Mapping window is open and is the front window. The menu items in this menu pertain only to 3D. They are used to open the 3D model in new windows by reusing the current model in the 3D Mapping window. This menu can also be used to save the current model out to a QuickDraw™3D metafile.

3D View

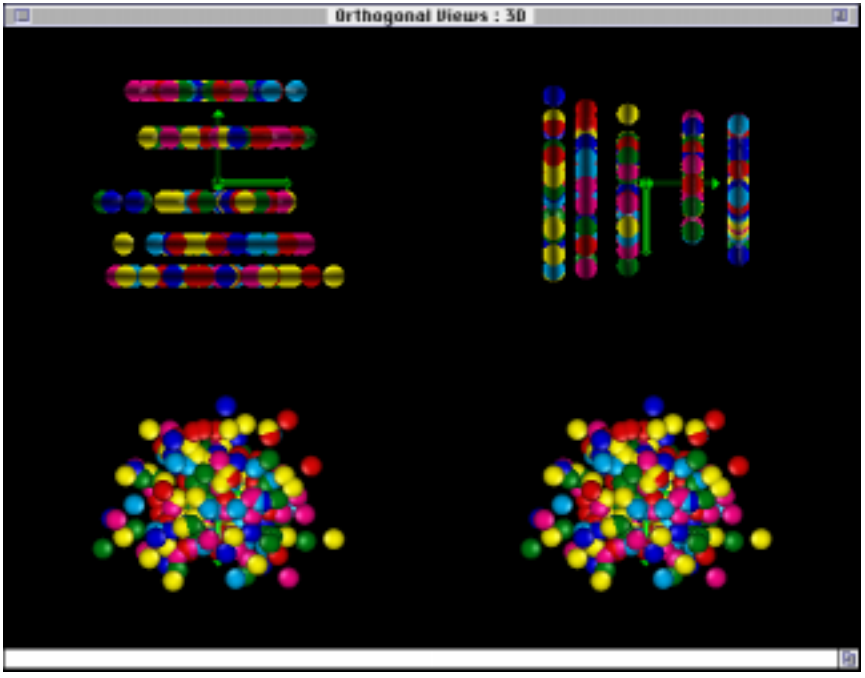
This menu item is checked if the current 3D Mapping window is a normal one-view model. This is the default view that is opened when the **3D** button is pressed in the **View** palette.



The 3D tools presented in the 3D palette can be control some affine transformations on this model (rotation, scaling, translation), as well as to pick certain objects for information.

Orthogonal Views

This menu item is checked if the current 3D Mapping window is an orthogonal four-view model. Each view displays a different orthogonal view of the 3D model.



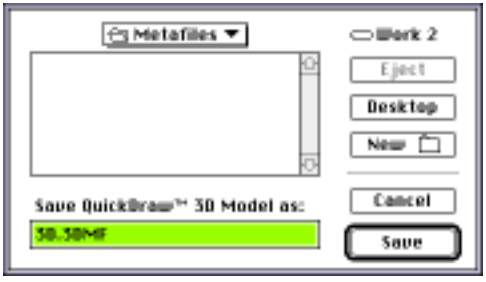
Top View	Side View
Front View	3D View

The **Top**, **Side** and **Front** Views show the orthographic projection of the 3D model into the plane perpendicular to the direction of the y, x and z axis respectively. The **3D View** shows either a one point perspective projection or an orthographic parallel projection of the 3D model onto the front plane (parallel to the z direction) that upon initialization will look exactly like the Front View if the type of camera selected is Orthographic. Note that the 3D View will change as the 3D Tools are used to move through the 3D model.

Save QuickDraw™ 3D Model as...

Use this to save the current model in the front 3D Mapping window to a QuickDraw™ 3D metafile.

A standard save file dialog opens asking for the name of the file for the model. Entering the filename and press the **Save** button to save the model to disk.

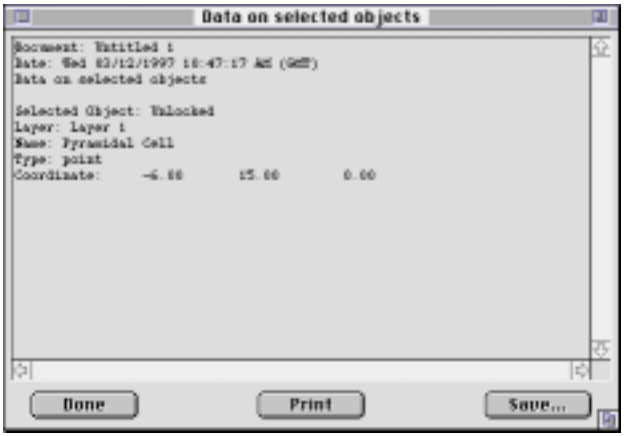


The 3D model, whose subobjects are the QuickDraw™ 3D objects that correspond to mapping data from NeuroZoom, is written to the file in QuickDraw™ 3D Metafile format (3DMF). The 3DMF file only contains the geometric data that defines the 3D model. Orientation, magnification, and other data that determine how the model is displayed on the screen are not currently written to the file.

By The Way: The 3DMF file can be read by any application that supports the QuickDraw™ 3D metafile format.

Object Info...

This is enabled only when an object has been picked using the **Anchor** tool. The 3D identity of the object is returned to NeuroZoom's associated 2D Mapping window, and the information on that object is displayed.



Views Menu

The **Views** menu is available only when a 3D Mapping window is open and is the front window. The menu items in this menu pertain only to 3D. They are used to control the appearance of the 3D model generated from the mapping data from the 2D Mapping window.

Show Bounding Box

Toggles the 3D model Bounding Box on the current 3D window. Depending of the Image mode selected, Wireframe or Realistic, the Bounding Box will be drawn showing only its edges (Wireframe Image) or showing its surfaces (Realistic Image). The appearance of the bounding box is determined, among other parameters, by the way polygons are drawn (Solid, Only Edges, Only Points).

Show Axis

Toggles the XYZ coordinate axes on the current 3D window. If the current rotation mode is **Rotate Relative to Model**, these axes correspond to the model coordinate system. If the current rotation mode is **Rotate Relative to Camera**, these axes correspond to the camera coordinate system. In either case, the axes are shown at the current center of rotation which is defined in the model coordinate system. The axes are provided for two reasons: to indicate where the center of rotation (or anchor) is and to indicate the direction of rotations.

Double Buffer

Toggles Double Buffering on the current 3D window. When Double Buffering is on, two frame buffers are used for smooth update of the screen, making objects appear to move naturally and at a speed that appears realistic. The image stored in one frame buffer is displayed while rendering occurs on the other buffer. When the updates are complete, the buffers are switched. Only complete images are displayed and the process of drawing is not shown. The result is the appearance of a smooth update. This can be appreciated better when the model is being rotated. When Double Buffering is off, only one frame buffer is used and the window flickers as it's being updated.

Set Background Color...

Select this item to change the background color in the current 3D window.

3D Tools

Toggles the **3D Tools** window. The **3D Tools** window contains a selection tool and a set of basic navigational tools used to change the ways the model is displayed.

Picking Tool

Zoom Tool

Rotate Tool

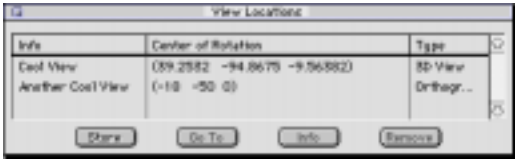
Translate Tool

Anchor Tool

See the chapter on the ***3D Tools Window*** in the ***Reference Manual*** for more detailed information on how to use this window.

View Locations Window

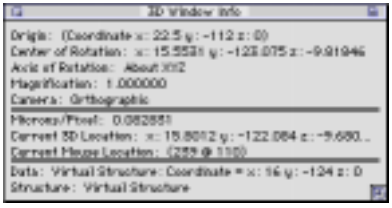
Toggles the **View Locations** window. The **View Locations** window is used to save and restore different views of the model. A view of the model is obtained after rotating, translating or zooming the model. The parameters that define a view can be saved and restored at any time, therefore storing a view means storing the necessary parameters (rotation angle, magnification, translation, center of rotation, etc.) that will recreate the view.



See the chapter on the ***View Locations Window*** in the ***Reference Manual*** for more detailed information on how to use this window.

3D Info Window

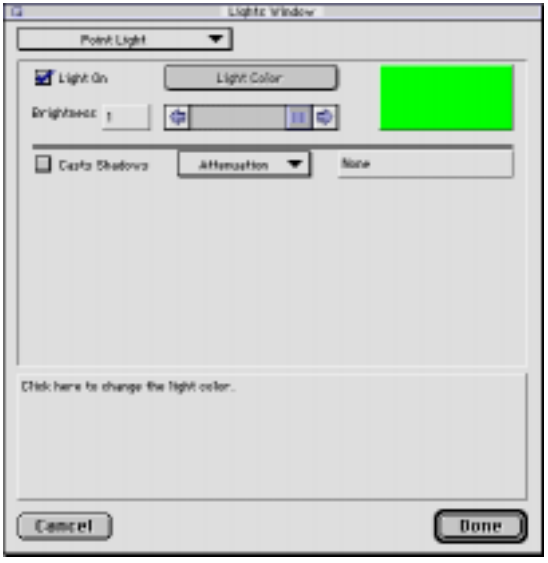
Toggles the **3D Window Info** window. The **3D Window Info** window shows the following information for the frontmost 3D window: the origin of the camera coordinate system, which is equal to the center of the data bounds, the current center of rotation which initially is equal to the origin of the camera coordinate system, the current axis of rotation, the view magnification or zoom factor, the camera type, the current scale in use as microns/pixel, the current 3D location of the cursor in microns in the model coordinate system, the current mouse location in pixels and data, and the structure of the object under the current mouse location.



See the chapter on the ***3D Indo Window*** in the ***Reference Manual*** for more detailed information on how to use this window.

Lights Window

Toggles the **Lights** window. This window can be used to edit some of the attributes of the light sources in the light group that provide illumination for the objects in the 3D model. For 3D windows with four views (Orthogonal View), the light group associated with each view is the same. Changing the light sources with orthogonal views affects all the views displayed in that window.



See the chapter on the ***Lights Window*** in the ***Reference Manual*** for more detailed information on how to use this window.

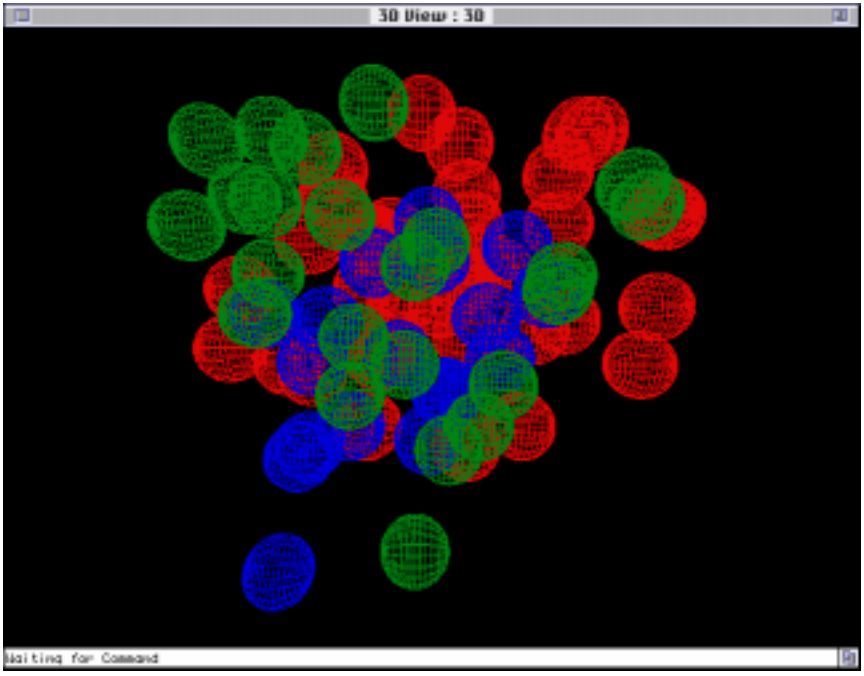
Camera

Use this menu item to select the camera type used in the current 3D window to display the 3D model. The image displayed on the current 3D window will be refreshed immediately after the camera type is changed.

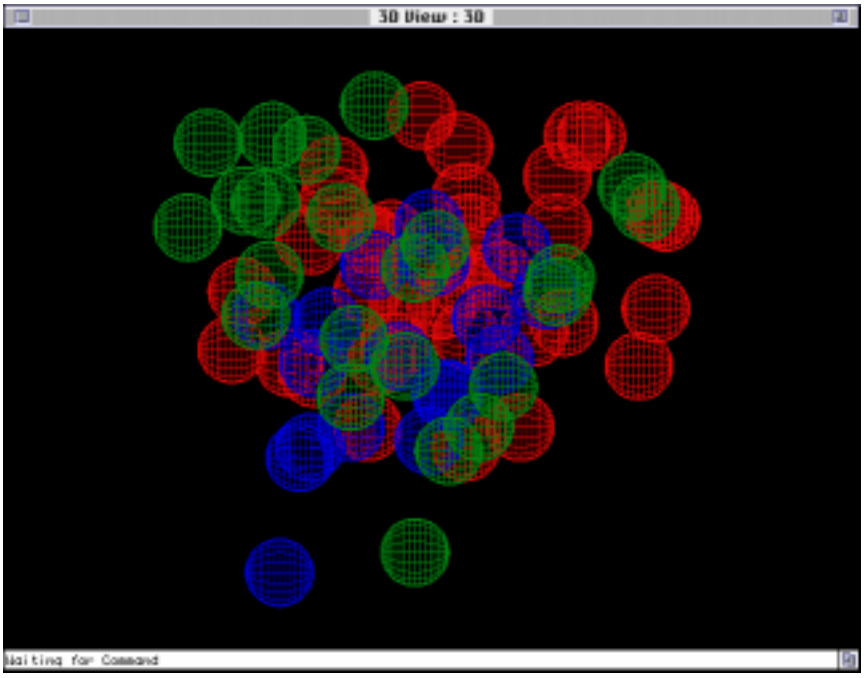
A camera is a type of QuickDraw™ 3D object that is used to define a point of view, a range of visible objects and a method of projection for generating a two-dimensional image of those objects from the 3D model. A camera type is defined by its method of projection.

- Perspective
- Orthographic

Select **Perspective** to use this type of camera. When the perspective camera is the current camera, a perspective projection is used. The visual effect of a perspective projection is similar to the human visual system where the size of an object varies inversely with the distance of that object from the observer (known as perspective foreshortening).



Select **Orthographic** to use this type of camera. When the orthographic camera is the current camera, an orthographic parallel projection is used. The resulting view is less realistic but parallel lines in the model remain parallel in the projection, and distances are not distorted by perspective foreshortening.



When the current 3D window contains four views, changing the camera type only affects the 3D view. The orthographic views, front, side and top are not affected by the camera selection. These views have a orthographic camera associated with it that can not be changed.

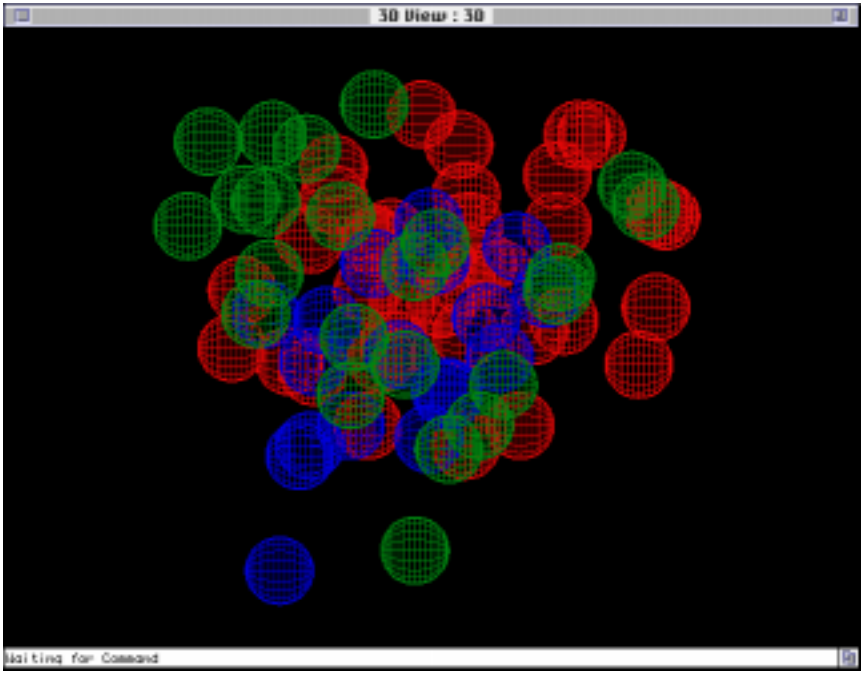
Image

Use this menu item to select the type of renderer for the current 3D window. The image displayed on the current 3D window will be refreshed immediately after the renderer type is changed.

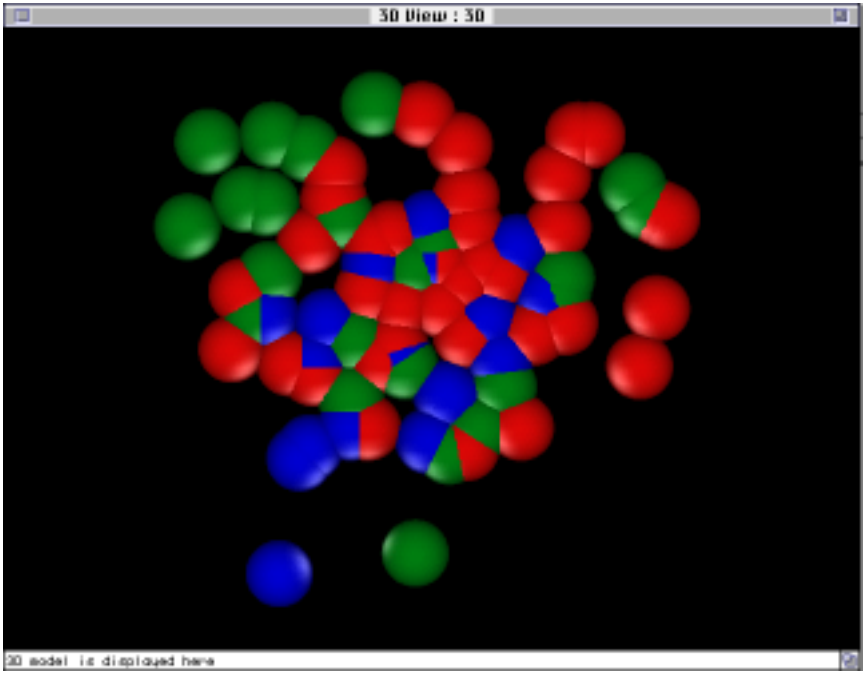
Rendering is the process of converting 3D geometries into pixels on the screen. The result is based on the information stored in the geometry, taking into account the lighting, surface attributes, shading, smoothness, and camera location. QuickDraw™ 3D provides two basic renderers: a wireframe and an interactive (or realistic) renderer.

- Wireframe
- Realistic

Select **Wireframe** to use QuickDraw™ 3D wireframe renderer. The wireframe renderer creates line drawings of models. It operates extremely quickly and used less memory than the interactive renderer (Realistic). The speed of this renderer is due to the fact that no surfaces need to be renderer or highlighted.



Select **Realistic** to use QuickDraw™ 3D interactive renderer. The interactive renderer uses a fast and accurate depth-sorting algorithm for drawing solid, shaded surfaces as well as vectors, producing a more realistic image of the model. It is usually slower and requires more memory than the wireframe renderer but provides an acceptable interactive performance for models of reasonable size.



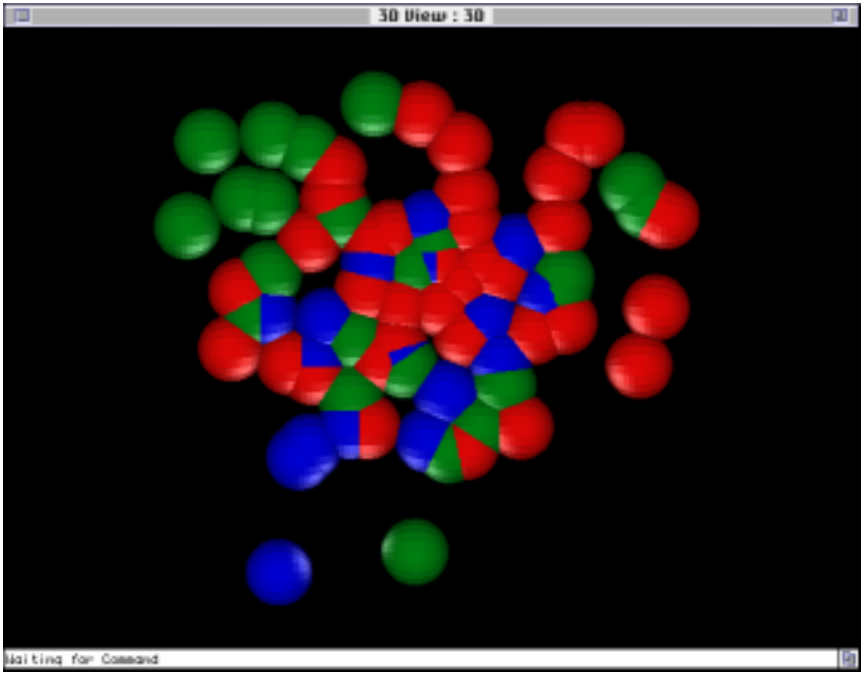
Smoothing

Use this menu item to select the interpolation style used by the Realistic or Interactive renderer in the current 3D window to produce smooth surfaces. The interpolation style determines the method of interpolation the renderer uses when applying lighting or other shading effects to surfaces. When the renderer type is *Wireframe*, this item is disabled.

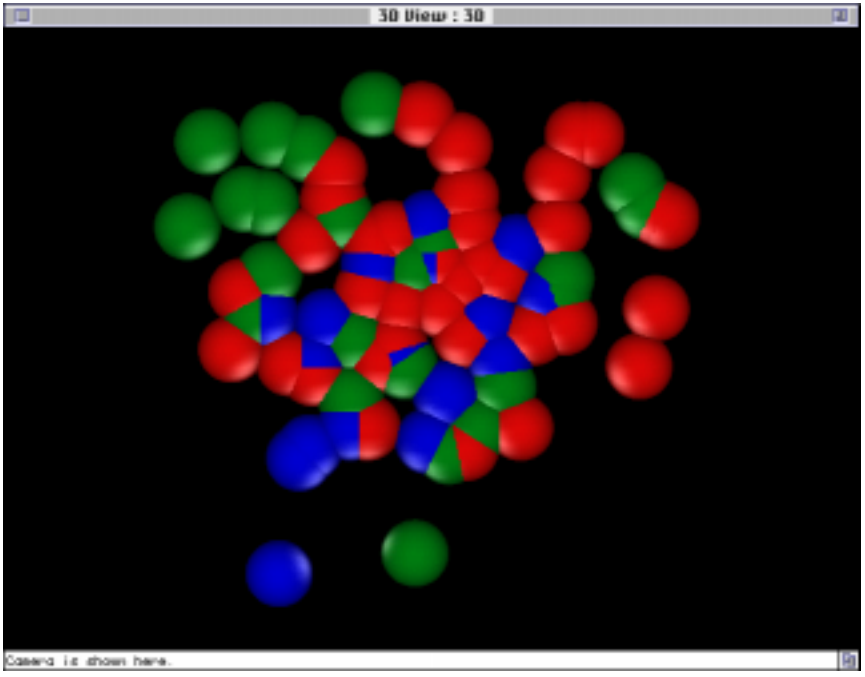
The image displayed on the current 3D window will be refreshed immediately after the interpolation style is changed. QuickDraw ™ 3D provides three interpolation styles: **None**, **Vertex,** and **Pixel**.

- None
- Smooth (Vertex)
- Very Smooth (Pixel)

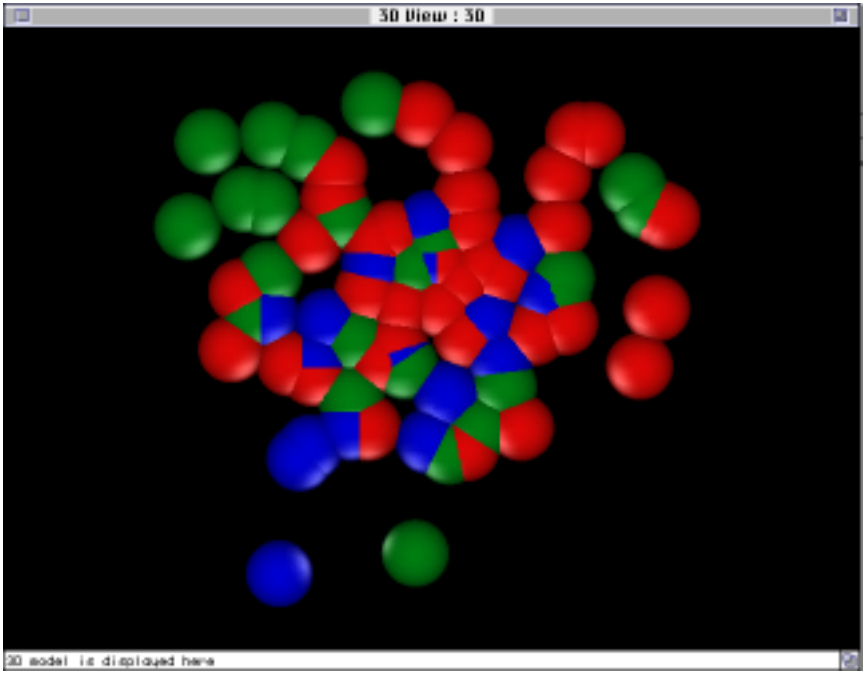
Select **None** to perform no interpolation at all. This results in a model's surfaces having a faceted appearance.



Select **Smooth** to select the Vertex interpolation style. When this style is selected, the renderer interpolates the values of the pixels linearly across a polygon, using the pixel values at the vertices.



Select **Very Smooth** to select the Pixel interpolation style. When this style is selected, the renderer applies an effect at every pixel in the image, resulting in a smoother appearance of the surfaces of the model.



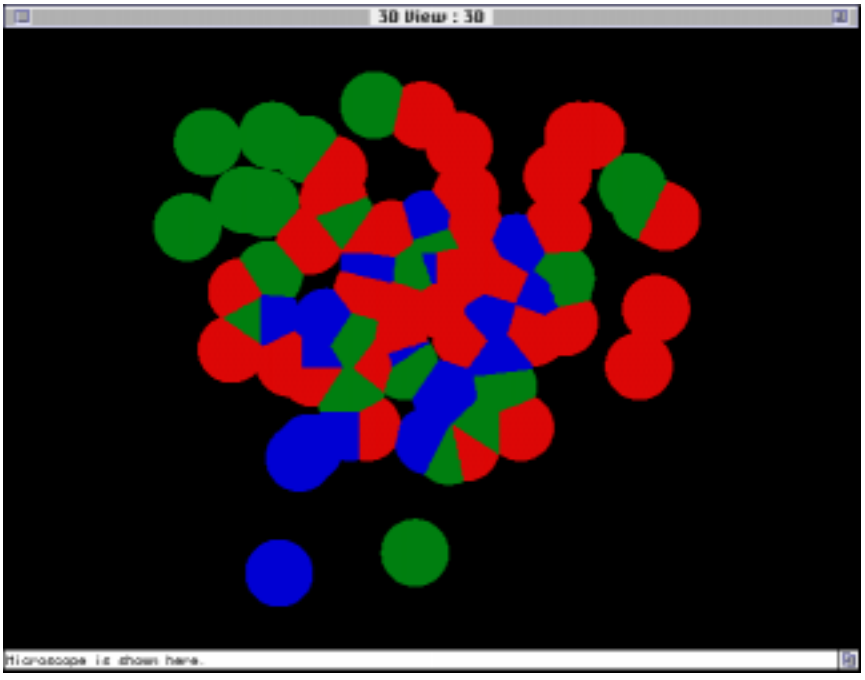
Shading

Use this menu item to select the type of illumination shader used by the Realistic or Interactive renderer in the current 3D window. When the renderer type is *Wireframe*, this item is disabled.

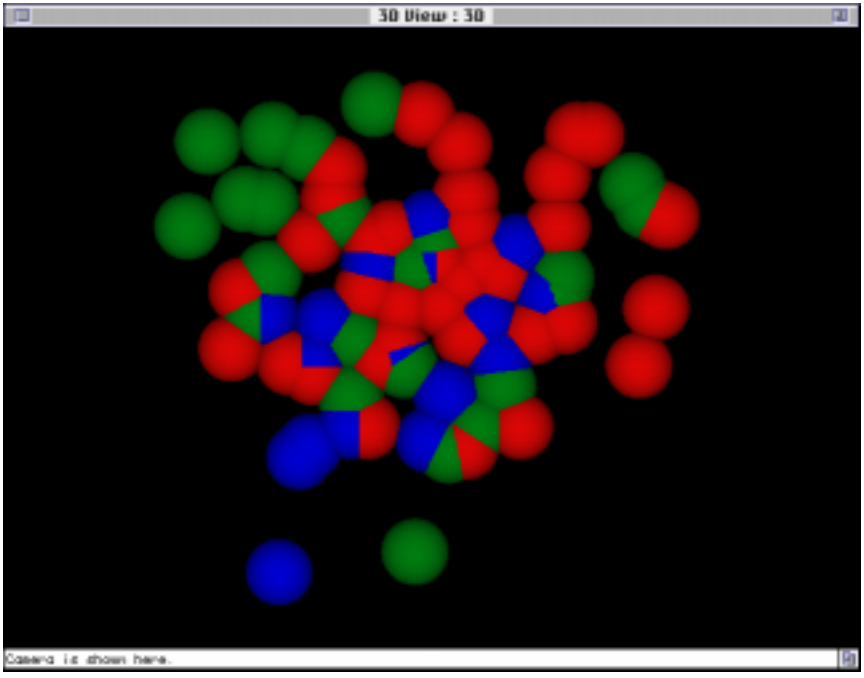
The image displayed on the current 3D window will be refreshed immediately after the illumination shader type is changed. Illumination shaders determine the effect of a group of lights on the objects in a model.The result is the identification of appropriate colors for the object's pixels. QuickDraw™ 3D provides three types illumination shaders: **Null**, **Lambert** and **Phong** illumination shader.

- None
- Some Shading
- Better Shading

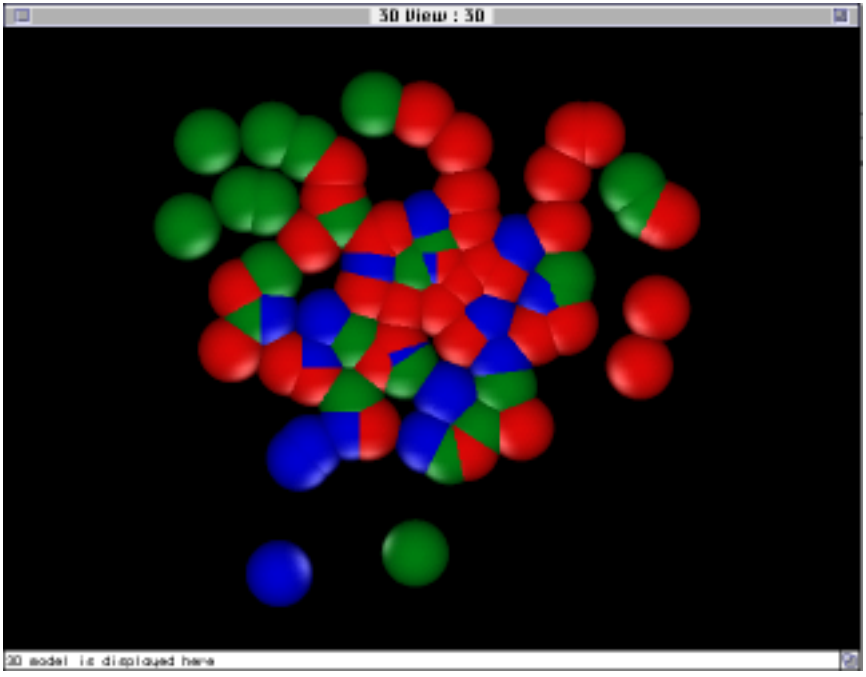
Select **None** to select the Null illumination shader. The null illumination shader draws objects using only the diffuse colors of those objects, ignoring the light sources for the model. When the null illumination shader is active, all the facets of an object are drawn the same color.



Select **Some Shading** to select the Lamber illumination shader. The Lambert illumination shader implements an illumination model based on the diffuse reflection of a surface. Diffuse reflection is characteristic of light reflected from a dull, nonshiny surface. With this shader, object surfaces reflect the light with the same intensity appearing equally bright from all viewing directions.



Select **Better Shading** to select the Phong illumination shader. The Phong illumination shader implements an illumination model based on both the diffuse reflection and specular reflection. Specular reflection is characteristic of light reflected from a shiny surface, where a bright highlight appears from certain viewing directions.The Phong method produces highly realistic effects by calculating the light at many points across an object surface.



Backfacing

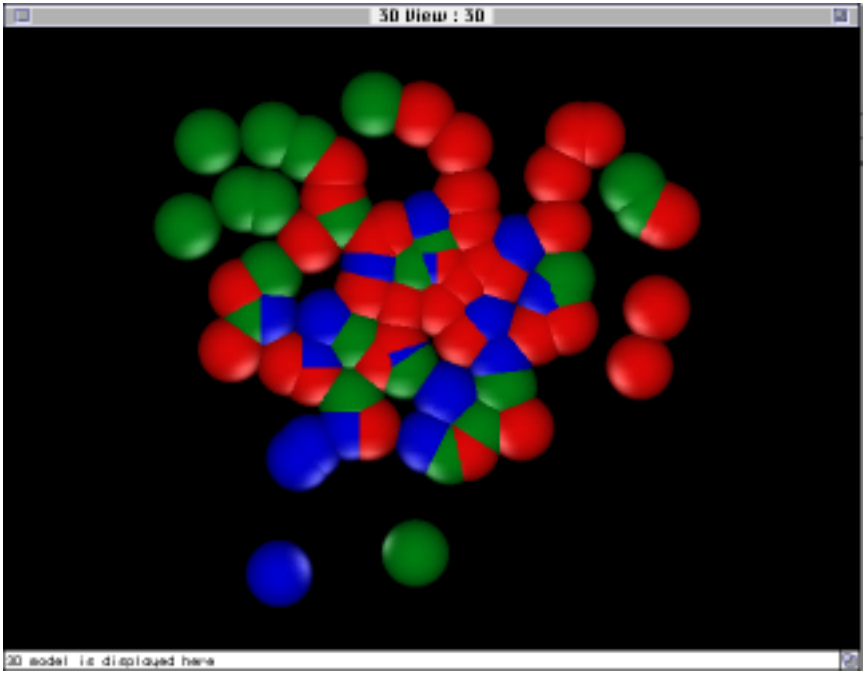
Use this menu item to select the backfacing style used to draw shapes in the current 3D window. A backfacing style determines whether or not a renderer draws shapes that face away from the observer or view's camera. The image displayed on the current 3D window will be refreshed immediately after the backfacing style method is changed.

- Visible: the renderer draws shapes that face either toward or away from the camera. The backfacing shapes may be illuminated only dimly or not at all, because their face normals point away from the camera.
- Removed: the backfacing shapes are not drawn.
- Flipped: Similar to visible but the normals of the backfacing shapes are flipped so they face toward the camera. Illumination of the flipped shapes might change if the current illumination model uses surface normals

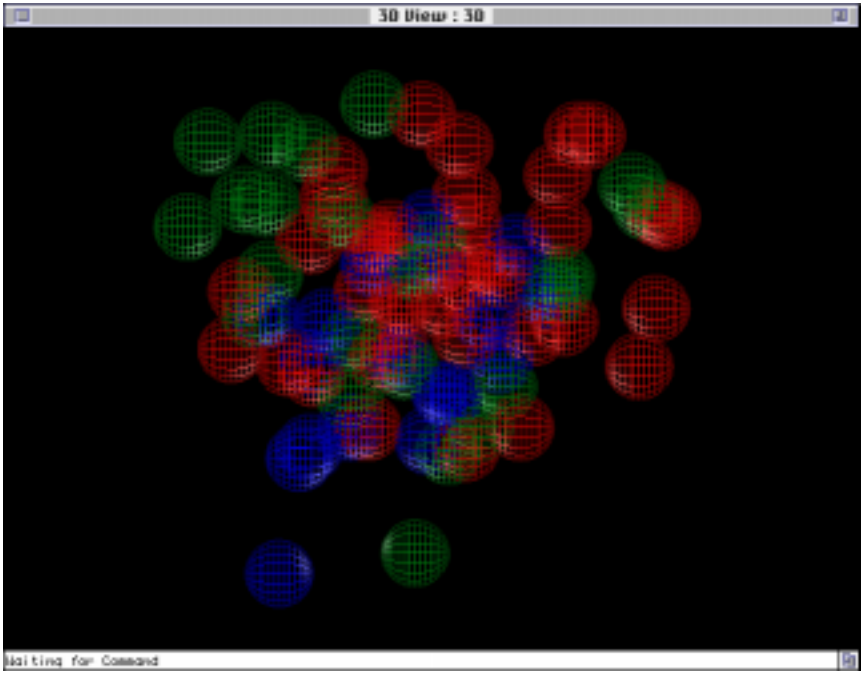
Polygons

Use this menu item to select the fill style used by the renderer to draw shapes (typically polygons) in the current 3D window. A fill style determines whether an object is drawn as solid filled object or is drawn as a set of edges or points. The image displayed on the current 3D window will be refreshed immediately after the fill style method is changed.

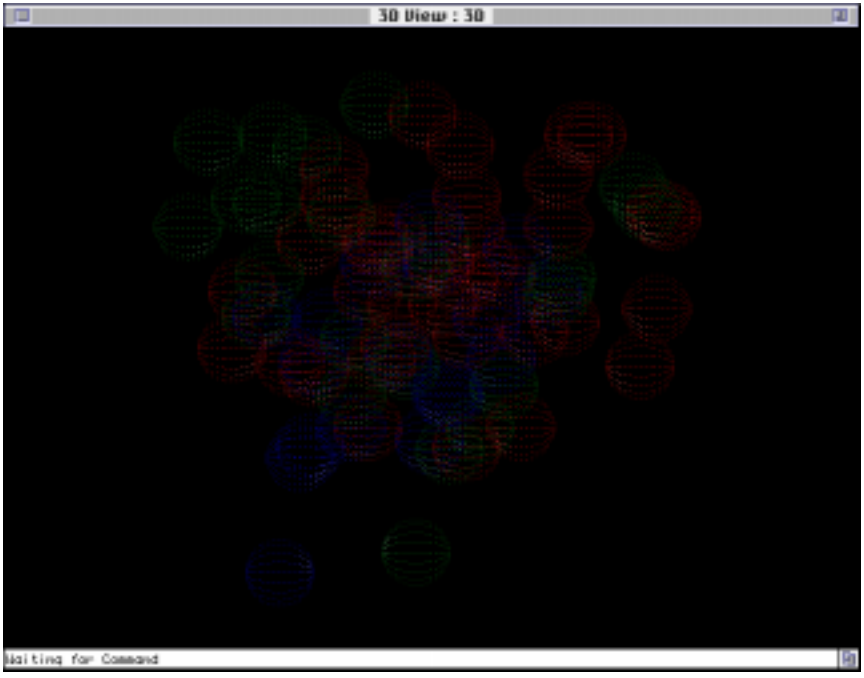
- Solid: Objects are drawn as solid filled objects.



- Only Edges: Objects are drawn as the set of lines that define the edges of the surfaces. The resulting image is similar to the one obtained with the Wireframe renderer with the addition of shading and lighting if the Interactive or Realistic renderer is the current renderer.



- Only Points: Objects are drawn as the set of points that define the vertices of the surface. This style is used to accelerate the rendering of very complex shapes.



Rotate Relative to Model

If the menu item **Rotate Relative to Model** is checked, rotating the model is equivalent to rotating an imaginary camera about the model. The scene represents the image that is viewed through that camera. This imaginary camera is also equivalent to an observer looking at the model from a certain distance. The axes and the model appears rotated.

Rotate Relative to Camera

If the menu item **Rotate Relative to Camera** is checked, rotating the model is equivalent to spinning the model about the axes in the camera coordinate system. The camera remains fixed while the model is rotated.

AutoRotate

If the menu item **Auto Rotate** is checked, the model rotates as long as the mouse button is depressed. The speed and direction of the rotation is proportional to the mouse distance and direction from the location where the button was initial depressed.

CHAPTER 3 *Mapping Windows*

All the windows in NeuroZoom are opened by activating a menu command. In this chapter, all of the mapping windows in NeuroZoom will be detailed in a mapping-centric manner. The mapping window will be detailed first, followed by all of its dependent windows. The modules and their windows will then be detailed.

- See the chapter on ***Stereology Windows*** for specific information on the stereology windows.
- See the chapter on ***3D Mapping Windows*** for specific information on the 3D windows.

Please be sure to read the previous chapter on **Menus** to understand fully how these windows are opened.

Macintosh Window Types

There are three basic types of windows in NeuroZoom as defined by the Macintosh operating system and User Interface Guidelines:

- Standard windows
- Dialog windows

- Palette windows.

Standard windows have a normal titlebar adorning the top of the window content area. The window may have a close box, a zoom box, and a resize box, or any combination of these three. The window is not modal, meaning that you can click the mouse on another window to bring that other window to the foreground. The current window will then become the second window in the window layer, and go *behind* the new one. Standard windows are usually the windows in which most of the work or the user attention is required.

Dialog windows may or may not have title bars. If they do, they are movable. If they do not, they are generally fixed to the monitor at some predetermined position. These windows are called dialog windows because they are generally requesting a dialog with the user. They are modal because they cannot be dismissed by clicking on another window, unless that other window is from another application. When a dialog window appears in a specific application, it is usually because the application requires some immediate attention from you. For example, print dialogs appear asking for the number of copies and the page range to print. This dialog must be answered before the application can proceed. A dialog window can present another dialog window. An example of this is the print dialog where the user clicks on the **Help** button. Another dialog presenting help is then opened directly over the print dialog window.

Palette windows have a small title bar adorning it. The title bar may be horizontal or vertical. Palettes float, and are often associated with a standard window. Palettes themselves are located in a special window layer so that palette windows can be layered one after another, but all palettes of one application always float (or appear on top of, or in front of) any standard windows. Palettes are used to present controls such as buttons that are often used. NeuroZoom uses palettes to present the mapping tools, for example. Palettes can also be used to present information. Since the palettes float, they do not generally get lost when there are many windows opened at one time on the monitor. Information that is needed often is almost always available and readily displayed. However, because they do float and never get obscured by standard windows, they can also clutter up the monitor.

Alert: Throughout this documentation, the term Palette may be used to refer to a window that contains multiple buttons from which a choice is made (category 1). Other windows may contain more textual or graphical information (category 2). In both of these cases, the formal window type

might be of type Palette, but may be called a palette or window, depending on whether it falls into category 1 or category 2.

Windows Menu

There is a special menu named **Windows** in the Menubar. All windows that are currently opened by NeuroZoom are listed in this menu. Use this to select a window immediately and to bring it to the foreground (on top of the other windows). Note that this will not work with dialog windows, because most menus in the Menubar are disabled when a dialog window is opened. The window that is currently in the foreground is preceded with a bullet •. The following figure shows **Mapping Tools** as the window in the foreground.

Current Mapping Window

Of all opened mapping windows, only one mapping window is the current mapping window. This is the frontmost mapping window. This is also true even if there is another window on top of the mapping window. The notion of the current mapping window is useful because many actions from other window or from the menus act on the current mapping window only.

Some windows appear only when a mapping window is opened. For example, almost all palettes showing the tools and functions will close when the last mapping window is closed. Furthermore, the information displayed in these windows will reflect the current mapping window that is in the foreground. For example, if one mapping window has the mapping grid on and another mapping window has it off, the **View** window will show the grid as *ON* when the first mapping window is in the foreground, and *OFF* when the second is in the front.

NeuroZoom Windows

The following mapping and basic windows will be discussed in detail in this chapter.

- Mapping Window
- Mapping Tools Window
- View Window
- Imaging Window
- Stage Window
- Mapping Window Info window
- Data Type Window
- Data Window
- Background Images Window
- Stage Controls Window
- Stage Movement Controls Window
- Stage Location Window
- Set Stage Limits Window
- Structure Configuration Window
- Select Structures To Map Window
- Layers Window
- Create Montage Window
- Configuration Window
- Preference Window

Mapping Window

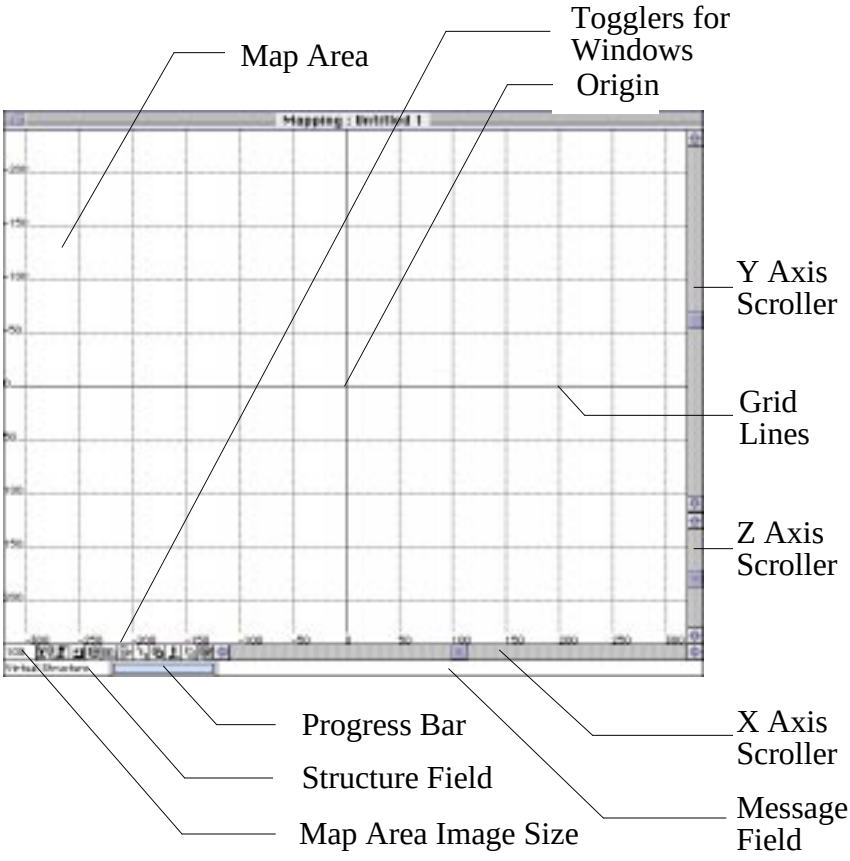
The main mapping window is where video is displayed, and acquired data are mapped into documents.

Opened by:

- New Map in File menu
- Open Map... in File menu
- Command - N
- Command - O
- Double clicking or opening a NeuroZoom file from the Finder
- Network transmission of object package to NeuroZoom client (if networking module is loaded)
- Holding down the Option key when NeuroZoom is launching suppresses the opening of a new window.

NeuroZoom, like any other Macintosh program, is document-centric. All of the data that you acquire from the microscope, and all of the data that you enter are stored in a single document that you specify. The document is linked to a single mapping window that is used to display all data two-dimensionally. The mapping window is the main window where you will spend most of your time.

All images, video, and data are all displayed in the mapping window. When NeuroZoom launches for the first time, an empty window is displayed.



Current Structure

There is always one current structure that is being mapped within the document. That is, if you selected a tool that is used to enter in data, the data that is entered is associated with the currently selected structure. For example, if **Purkinje** were the currently selected structure, and a **Point Tool** were the current tool, all data that are mapped using that tool are stored as Purkinje cell locations.

Tip: The current structure for a new document is always Virtual Structure. When opening a new document, select a new, real structure immediately as the current structure.

The Components of the Mapping Window - The mapping window contains several control and display fields.

Structure Field

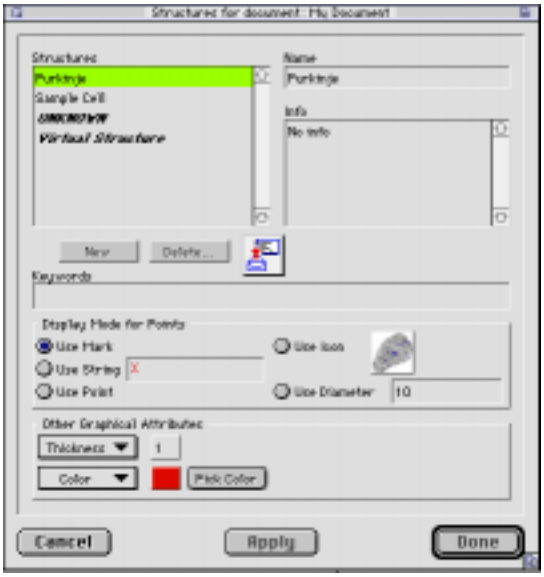
On the bottom left side is the **Structure** field where the currently selected structure is always displayed. This is the structure that is always used for mapping any data when using any data entry tool in the mapping window.

Current Structure

The field is actually a popup menu, and if you click and hold the mouse button down while in the field, a menu will pop up. There are several options from the top menu items in the menu, followed by structures that can be selected as the current structure.

The top menu items designate specific actions on structures.

- **Configure all structures...** opens the **Structure Configuration** window for the addition, deletion, and editing of existing structures.

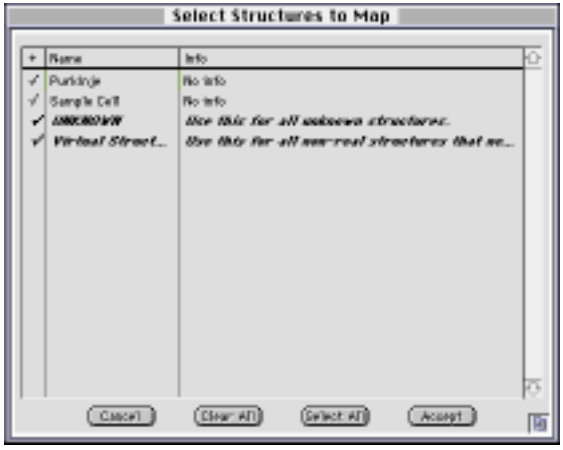


- **Configure current structure...** also opens the same window but immediately displays the attributes of the current structure for this mapping window.
- **Configure new structure...** also opens the same window but immediately creates a new structure and selects it for immediate configuration.

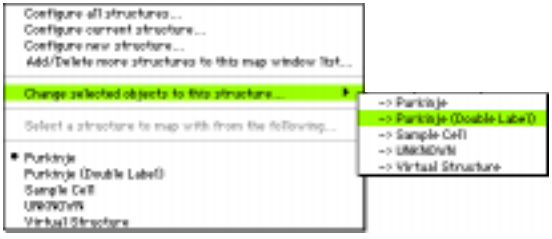
See the section on **Structure Configuration** window for more information on how to use these features.

- **Add/Delete more structures to this map window list...** displays the **Select Structures to Map** window that lets you narrow down the choices that are selectable from within the mapping window. Because a document can have many, many structures defined for its use, but only a handful are used at any one time, a subset of the structures can be stored for the mapping window that is opened. Certain keys are used to navigate this subset, making it easy to select for a particular experiment. For example, a double labeling study only requires two structures, Structure Label 1 and Structure Label 2. Out of many structures configured for the document, only these two structures are selected for mapping. Hitting the **TAB** key will then toggle each structure as the

current one, making it easy to switch back and forth when mapping these double labels. See the section on **Select Structures to Map** window for more information on how to use these features.



- The next part of the popup menu is named **Change selected objects to this structure**. This will display a hierarchical menu with all of the structures in the document. Selecting from any of these will change all selected objects to the new structure. Use this if you entered in data for the wrong structure. Be sure to have data selected from the mapping window or else the hierarchical menu will not be enabled.



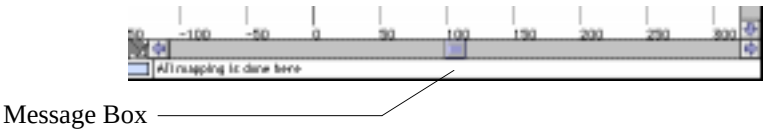
- The third section of the popup menu shows individually each structure for this document. Selecting any one of these will make it the current structure for the document, and for the mapping window.

Progress Bar

The box to its right is a progress bar. Occasionally when there are long operations, the bar will be used to show the amount of work remaining.

Message Field

The message accompanying the bar will be displayed in the message field to its right. This field also actively shows a synopsis of the function of various buttons or components as you pass the cursor over them.



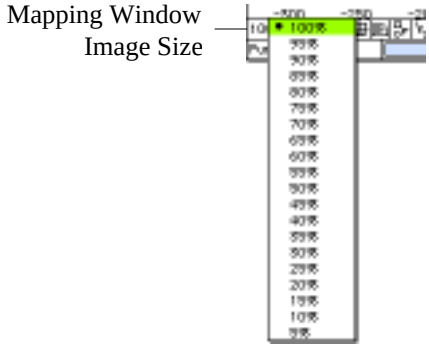
The buttons or components that are active to showing messages on their functions are those from the Mapping Tools, View, Imaging, Stage, and Mapping Window itself.

The amount of memory available for objects is also displayed. This is a constantly changing value, and as it gets lower, memory collection is automatically invoked to recovered released or unused memory.

Map Area Image Size

Above the current structure field is the **Image Size Popup Field** field that shows the percent size of the mapping window. This is selectable from a minimum of 5% to a maximum of 100%. The value indicates the percent size of the mapping window relative to the pixel width and height of the displayed video, or the

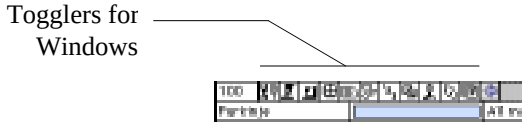
displayed image. Live video is scaled to match the size of the window. Therefore there is no loss of spatial context, only resolution. This command is useful if the monitor size is small. This field is also a popup menu. Pressing on this field will pop up a menu that allows you to select the percent size to use on the mapping window. This function is also accessible from the Imaging menu in the Menubar.



Alert: If a small size is chosen, some of the components of the mapping window will not be visible. This is normal, since it is not possible to scale the components down to a usable size. In that case, the Menubar or the controls from the other windows will have to be utilized in place of the controls on the mapping window.

Togglers for Window

The field to its right is a strip of small icons that control the visibility of various control windows in NeuroZoom. Clicking on a specific icon will toggle the display of its associate window. This is a fast way of getting to different windows, or hiding them when the monitor size is small. Keyboard equivalents are also available for most of these windows.



Here is what each toggler controls. A description of each window is found later this chapter.


Mapping Tools


View


Imaging


Stage


Information


Data Type


Data


Background images

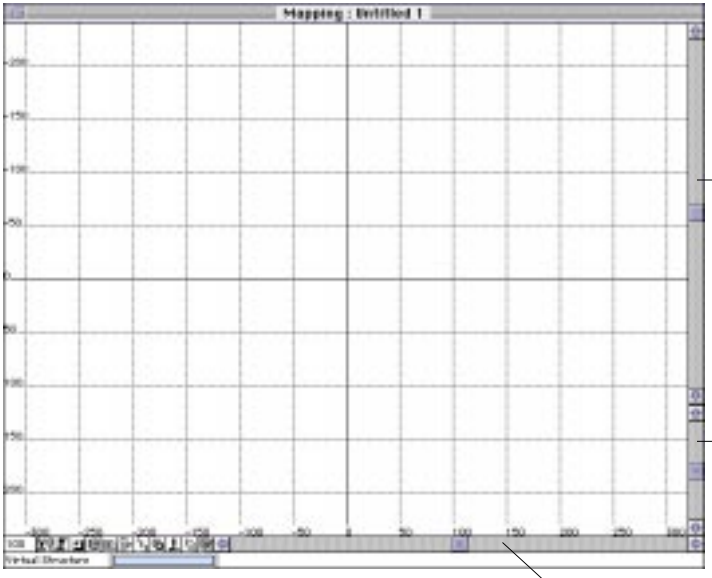

Stage Controller


Structure Configuration


Layers

Stage Scrollers

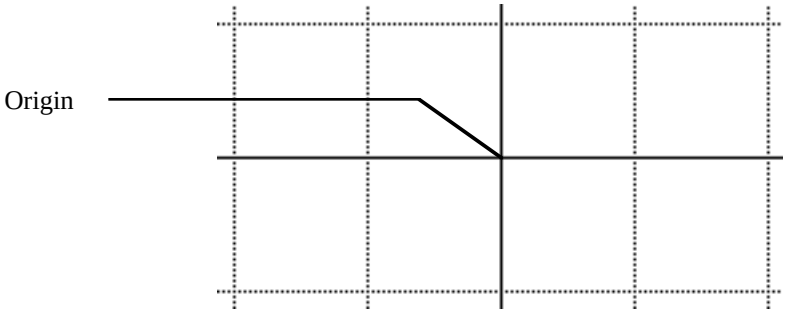
There are three scrollbars located on the mapping window. Each controls a specific axis on the microscope.



The scrollers activate the computerized stage and move the stage in a particular axis. They are used to move the stage, not the image per se. The scrollers may also be hidden by using the menu item **Turn Map Scrollers Off** in the **Stage** menu.

Grid Lines

The grid lines (grid) in the mapping window is a live representation of the current coordinate system that is in place due to the overall magnification of the microscope and location of the microscope stage. The origin of the coordinate system is indicated in the X and Y axes with a solid line, while the tics from the origin are displayed with dotted lines.



The appearance of the grid may be altered with the **Map** menu of the Menubar with the item **Grid Setup...**.

Grid Setup Window

You can also option-click on the **Grid** button in the View Window. A **Grid Setup** window opens. See the section on ***Grid Setup Window*** in the ***Reference Manual*** for more information.

Title Bar

The title bar of the window begins with the word *Mapping* to indicate that it is a mapping window, and then shows the name of the document currently opened for the mapping window, or else the name *Untitled* n is used, where n is a number from 1 to infinity, depending on the number of windows opened. If the document in memory has data that has not been written to the document on the disk, the name in the titlebar of the window is preceded with a '•' character. Once the document in memory is saved to the document on disk, the '•' will disappear.

Unsaved Document — •Mapping : Untitled 1

Saved Document — Mapping : Untitled 1

Using the Mapping Window

To effectively use live video in this window, please read the appendix on ***Optimizing Video***.

The mapping window should be thought of as the field of view into the microscope. Whatever the microscope camera sees, it is displayed in the mapping window (when the video is on, or if a image is digitized). NeuroZoom communicates with the microscope stage controller to determine where the field of view is. By knowing the scale relationship (microns per pixel) of X and Y for each lens, camera, and microscope (known collectively as a view) in place, the real dimensions of any structure in the field of view is known. If the microscope

stage moves, the XYZ offset from the origin is known, and computed to maintain the proper spatial relationships of all data and data graphics.

However, NeuroZoom does not constantly communicate with the microscope stage controller. Therefore, it is important to remember that whenever you manually move the stage, you must alert NeuroZoom that the stage position needs to be updated. See **Move Stage Manually** in the chapter on the **Stage** menu for more information on this.

Alert: It is important to remember that NeuroZoom must be alerted manually that the stage controller has been moved. NeuroZoom does not continually communicate with the controller, and thus cannot know if you move the stage to a new position.

It is equally important that you inform NeuroZoom that a lens objective has been switched into place. Again, NeuroZoom has no way of knowing if you switch to another lens objective. You must alert NeuroZoom by using the **Select Microscope Objective...** command of the **Map** menu, or use the **Lens Objective** button in the **View** window. See the section on **Map** menu, or the section on **View** window for more information on how to use this command.

Alert: It is important to remember that NeuroZoom must be alerted manually when you switch to another lens objective.

By remembering to do these maintenance items, NeuroZoom will remain synchronized with the field of view coming from the microscope, and all data mapped to that field of view will be precise and real with respect to the image.

Many of the other windows in NeuroZoom serve to support the mapping window. While many mapping windows may be opened simultaneously, some of these support windows show content information as it pertains to the current mapping window. That is, the mapping window that is above all other mapping windows. Therefore, if you perform some command from those windows (or from the Menubar), the current mapping window will be affected. The windows that subserve the mapping window are:

- Mapping Tools Window
- View Window
- Imaging Window

- Stage Window
- Mapping Window Info window
- Data Type Window
- Data Window
- Background Images Window
- Stage Controls Window
- Stage Movement Controls Window
- Stage Location Window
- Set Stage Limits Window
- Structure Configuration Window
- Select Structures To Map Window
- Layers Window
- Create Montage Window

The details of each window follows.

Mapping Tools Window

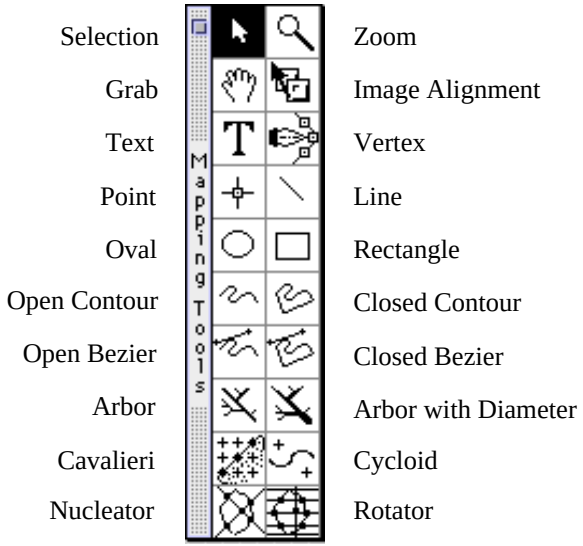
The **Mapping Tools** window is a vertical window. This window contains the tools that are used for mapping data.

Opened by:

- Mapping Tools Window in Map menu
- Command - 1
- Pressing Window Toggler in the Mapping window 
- Opening a new Mapping window if the preference is set to automatically open the Mapping Tools Window

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.

All data acquisition tools that used for mapping structures are selected from this window. The currently selected tool is highlighted with black on white graphics. The selection tool is currently selected in the following figure. To select another tool, click once on that tool to make it active.



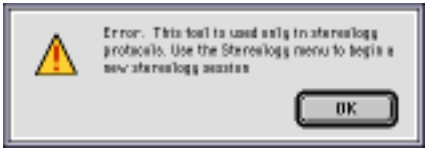
By The Way: The current tool is also displayed at all times in the Mapping Window Info Window.

Popup Menus

Some of the mapping tools also have a popup menu providing more functions pertaining to the tool or to the data that are created by the tool. To see the popup menu, press and hold the mouse on the tool for about a second. A menu will popup with additional functions.

Context Sensitivity

Some tools cannot be used in some situations. For example, the stereology tools cannot be used until a valid stereology protocol has been established by using the **Stereology** menu protocols. An error message opens when the Cavalieri, Cycloid, Nucleator, and Rotator tool are selected. After dismissing the error dialog, the Selection tool is made the current tool.



The details of each tool follows.

Selection Tool

Selection Tool

The **Selection** tool is used to select data objects in the mapping window. Once the objects are selected, further actions may be performed on them, such as moving, editing, deleting, getting information on, etc.

There are 12 basic kinds of data objects:

- Image
- Text
- Vertex
- Point
- Line
- Oval
- Rectangle
- Open Contour
- Closed Contour
- Open Bezier
- Closed Bezier
- Arbor
- Arbor with Diameter

By The Way: These basic object types correspond directly to the tools named for them. The other tools are more specialized tools for dealing with other mapping protocols (for example, stereology), or deal with the basic object types above.

To select an object, you should use one of two methods:

1. Click directly on any visible part of an object
2. Sweep a rectangular selection box around the desired objects.

Exceptions to methods are:

Bezier Curves - To select a Bezier curve, you need to click directly on a vertex. This is not always that easy, depending on the density of the data in the mapping

window, or the scale. If so, show the vertices on the Bezier curves. See the Vertex tool or Bezier tools for more information.

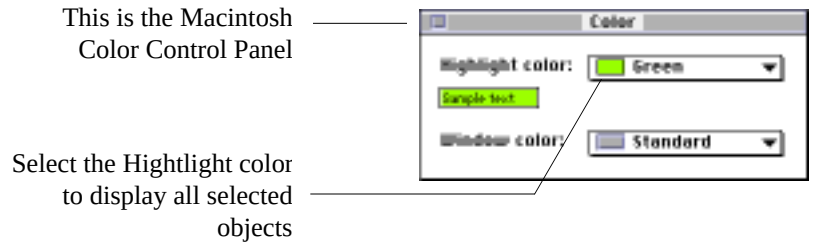
Images - Images are not selected with the Selection tool. Use the Image Alignment tool to select images.

Sweeping a rectangle is also useful for selecting objects. Simply press the mouse button in the map area of the window that is not occupied by an object. While keeping the button depressed, move the mouse to sweep a gray rectangle around the desired objects. When you release the mouse button, the objects will be selected.

Various modifier keys modify the mode of the selection.

- **Shift-Click** or **Shift-Drag** will add new objects to the selection without deselecting previous selections.
- **Command-Drag** will toggle the current selection in the selection rectangle to off, and add the new ones to the selection. Command-Click will not work to toggle an object because this is interpreted as an action on the selection itself.

All selected objects are highlighted in the selection color that is set for your Macintosh computer. This color is selected from the Control Panel named *Color*.



Once selection(s) are made, you can perform one of several actions on them.

- **Move** - click on any selected object (remember to keep the shift key down if you are starting trying to move more than one selected object) and while keeping the mouse button depressed, drag them to a new location. The coordinates associated with each data are updated to the new locations.

- **Delete** - hit the **Delete** key and they will be deleted from the document. This action can be undone with the **Undo** menu item in the **Edit** menu.
- **Copy** - select the **Copy** menu item from the **Edit** menu, or press **Command-C**. The selected objects will be copied to the copy buffer of the Macintosh. The copied objects can then be pasted back into the same or another NeuroZoom document. Pasting them into other applications will have different effects depending on the object type and the application in which they are being pasted. In most cases, a text representation will be pasted. If the paste is suitable as an image, a PICT representation of the objects will be pasted instead. The text copied for each object is typically TAB delimited data that is suitable for a spreadsheet. The text can be affected by certain preferences, for example, whether a verbose mode is used or not for longer reports. See the section on ***Preferences Window*** in the ***Reference Manual*** for more information.
- **Drag to Finder** - the objects may be dragged anywhere in the Finder. Various files representing the selected objects will be created. The contents of text files can be affected by certain preferences, for example, whether a verbose mode is used or not for longer reports. See the section on ***Preferences Window*** in the ***Reference Manual*** for more information. This will also create an object package if the Networking module is loaded.
- **Get Info** - select **Object Info...** in the **Objects** menu to get more information displayed in a text window. **Object Info** shows summary information on all selected objects.
- **Get Report** - select **Object Report...** in the **Objects** menu to get more information displayed in a text window. **Object Report** shows expanded information on all selected objects. The report can be affected by certain preferences, for example, whether a verbose mode is used or not for longer reports. See the section on ***Preferences Window*** in the ***Reference Manual*** for more information. If no objects are selected, a report is generated for all objects in the mapping window.
- **Change structure type** - using the **Current Structure** popup menu, select **Change Selected Objects to this Structure**. A hierarchical menu will popup up next to this selection displaying all of the structures that are available in this document. Choosing any one of them will change the current selections from their current structure association to the new one. This action can be undone with the **Undo** menu item in the **Edit** menu.

- **Edit** - some objects can be edited (text, lines, ovals, rectangles, open contours, closed contours, open Beziers, closed Beziers, arbors, and arbors with diameters). The popup menu from its tool must be used in most cases to enter into data edit mode. Alternatively, all objects with vertices may be edited directly with the **Vertex** tool.
- **Lock/Unlock** - select **Lock** or **Unlock** in the **Objects** menu to lock or unlock selected objects. Locking an object prevents it from being deleted, edited, or moved. Locked objects can still be selected for copying, dragging to other windows or to the desktop, generating information or reports, or hiding. A locked object appears with a dotted graphics representation when selected, otherwise it appears normally in the mapping window.
- **Double clicking** - double clicking on selected objects automatically opens an information window. This is the same as selecting **Object Info...** from the **Objects** menu.
- **Option-double clicking** - option-double clicking on a single selected objects automatically opens the Structure Configuration Window, and automatically selects the structure of the single object as the current structure to edit. This is the same as selecting **Configure current structure...** from the **Structure** field popup menu of the **Mapping** window. This makes it convenient to change the graphics presentation of all data of a structure by simply option-double clicking on it. If you have more than one object selected, option-double clicking will open an information window as described above.
- **Create a 3D Model** - selected objects may be viewed in the 3D mapping window by pressing on the **3D** button in the **View** window. This is available only on a Power Macintosh with QuickDraw™ 3D installed. See the chapters on ***3D Visualization*** in the ***Reference Manual*** for more information.
- **Export QuickDraw 3D Metafile** - select **Export QuickDraw 3D Metafile...** in the **File** menu to export selected objects to a file using QuickDraw™ 3D metafile format. This file can then be opened by any QuickDraw™ 3D application. A Power Macintosh is not needed to create the metafile. However, it is required for the visualization. See the chapters on ***3D Visualization*** in the ***Reference Manual*** for more information.
- **Special Create Mesh from Selected Objects** - select **Special Create Mesh from Selected Objects...** in the **Objects** menu to create a mesh object from selected objects. The selected objects must be open or closed contours, or open or closed Beziers, and there must be two or more selected objects. The mesh is created and selected automatically in the mapping window so that the 3D button in the **View** window may be pressed for 3D visualization. A Power

Macintosh is not needed to create the mesh. However, it is required for the visualization. See the chapters on ***3D Visualization*** in the ***Reference Manual*** for more information.

- Some of the stereology tools require selected objects before the stereology protocols can be set up properly. See the chapters on ***Stereology*** in the ***Reference*** Manual for more information.

Selection Tool Popup Menu

There is also a popup menu associated with the tool.

Select from one of the three options to control the sensitivity of all tools towards the data objects in the mapping window. When the sensitivity is low, a data object may be acted on (such as selected by the Selection tool) when the cursor is a larger distance from the data object. Conversely, when the sensitivity is high, the cursor must be closer to the data object. High sensitivity is useful when the scale of the window and the density of objects is such that it is difficult to select a specific object.

The sensitivity is based on pixels. At high sensitivity, the cursor must be within 2 pixel. Medium sensitivity requires 4 pixels. Low sensitivity requires only 6 pixels.

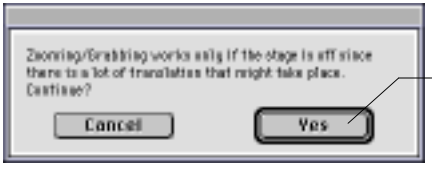
Zoom Tool

Zoom Tool

Zoom Up Cursor

Zoom Down Cursor

The **Zoom** tool is used to zoom the current data and image in the mapping window up and down. The cursor will change to a *Zoom Up* cursor when zooming up, and a *Zoom Down* cursor when zooming down. To enable zooming down, hold down the **Option Key** when clicking the mouse. When this tool is selected and the current zoom magnification is 1:1 and the stage device is ON, a warning will be displayed stating that the stage controller will be turned off from within NeuroZoom. This is to prevent large movements of the stage when zooming or grabbing the image to translate in the **XY** directions.



Press Yes to turn off the stage and to continue with Zooming and Grabbing

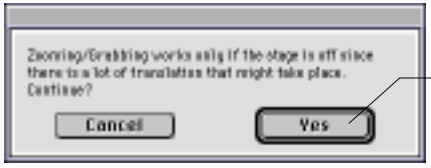
Alert: Displayed background images may disappear at a higher magnification due to a limitation in Apple's 16 bit QuickDraw toolbox commands that display the images in the window.

Grab Tool

Grab Tool

Grab Cursor

The **Grab** tool is used to hold and pan the data and image in the mapping window left/right and up/down. The cursor will change to a *Grab* cursor. Similar to the Zoom Tool, when this tool is selected and the current zoom magnification is 1:1 and the stage device is ON, a warning will be displayed stating that the stage controller will be turned off from within NeuroZoom. This is to prevent large movements of the stage when zooming or grabbing the image to translate in the **XY** directions.



Press Yes to turn off the stage and to continue with Zooming and Grabbing

To pan or translate the image, click and hold the mouse button any where in the mapping area. Move the mouse to a new location. A line will sweep out from the beginning point to the current mouse location. When you release the mouse button, all data will be translated by the amount and direction of the line from beginning to end point. There is no change in the data itself. The view into the mapping area coordinate system is changed.

Zooming and grabbing will preserve all objects in their coordinate system. This includes new objects as well that you might map in at a zoom magnification other than 1:1. Fine editing of position or shape can be done this way at higher zoom magnifications. Note that contours, which are vertex-based polylines will look more jagged as you go up in zoom magnification. Beziers will remain smooth. If images are displayed in the mapping window, these images will be scaled up and down to match the data. This does not apply to live video. Any live video that was currently on and displayed will be turned off when the Zoom or the Grab tool are selected.

By The Way: Grabbing the data and moving in this manner is convenient when you want to view other regions of the data population, but moving the stage would be too tedious or slow. Likewise, zooming the data is easier then switching objectives to see more or less of the data.

Zooming is also useful when trying to select Bezier curves whose vertices may be outside the mapping area. Zoom down to see more of the object. Then click on a vertex or sweep a rectangle around a vertex to select it.

Image Alignment Tool

Image Alignment Tool

Grab Cursor

The **Image Alignment** tool is used to move the selected image left/right and up/down. The cursor will change to a Grab cursor. This tool is useful when images have been imported from files, and need to be manually aligned to existing data that is displayed in the mapping window. When this tool is active, the keyboard arrow keys can also be used to nudge the images a certain amount. This is useful for precise location of the images. The data will not move when this tool is used, only the image. With precise control on each image using the arrow keys, it is possible to manually create montages from image files. The image files may be from some unsupported NeuroZoom imaging device, such as a scanner.

The current image will have a animated frame drawn around its borders. See also the section on ***Background Images Window*** in the ***Reference Manual*** for more information on selecting a current image.

Text Tool

Text Tool

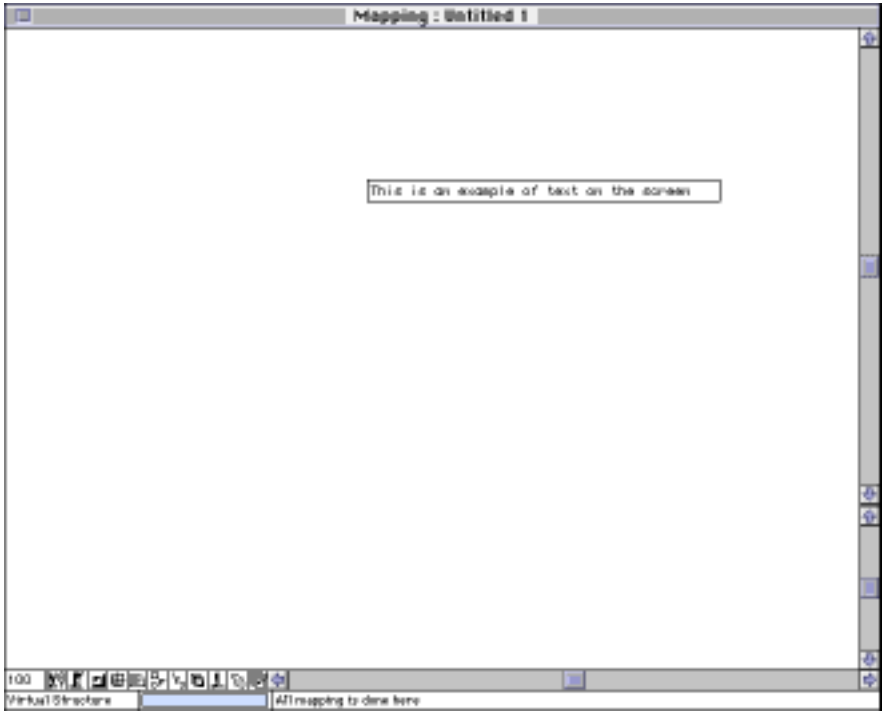
Text Cursor

The **Text** tool is used to enter in a floating label that is anchored to a particular location in the mapping window just like other mapping data. The cursor will change to a Text cursor.

To enter in text:

1. Select the Text tool
2. Click once anywhere in the mapping area to display a text insert cursor
3. Use the keyboard to type in the desired label

Any characters of any font can be used. The label supports multiple styles and fonts.



Use the **Text Tool** window selectable from the Edit menu to open a window that helps you choose fonts, sizes, and styles.

The Text tool is also used to reselect a label for more editing. To do this:

1. Select the label using the Selection Tool
2. Click on the Text Tool in the Mapping Tools Window to select it
3. Click on the selected label to display a text insert cursor
4. Position the text insert cursor where you want new text inserted, or select a range of characters
5. Use the keyboard to type in the changes

Vertex Tool

Vertex Tool

Vertex Cursor

Edit Vertex

The **Vertex** tool is used to edit the vertices of selected objects in the mapping window. The cursor will change to a Vertex cursor. As the cursor is moved over any selected object with a vertex, the cursor will changed to an Edit Vertex cursor. Depending on the data object, the vertex can now be moved away from its present position.

Popup Menu

There is also a popup menu associated with the tool.

1. **Show Selected Object Vertices** - Selecting this forces the *selected* data objects to *show* vertices if they have any. Data objects with vertices are lines, ovals, rectangles, contours, Beziers, and arbors.

2. **Hide Selected Object Vertices** - Selecting this forces the *selected* data objects to *hide* vertices if they have any.

3. **Show All Vertices** - Selecting this forces *all* data objects to *show* vertices if they have any.

4. **Hide All Vertices** - Selecting this forces *all* data objects to *hide* vertices if they have any.

The next three menu items control the editing of vertices. When selected, they operate only on selected data objects. The cursor actively changes to assist in determining whether a vertex of an data object can be edited. Data objects may be selected directly as if this tool were a **Selection** tool. This makes it easier to move from object to object when editing the vertices. A bullet character also appears before the selected mode in the menu.

5. **Edit Vertices** - This is the default mode when selecting this tool. In edit mode, the vertices of the selected data objects may be moved to new locations. The cursor changes to a **Vertex Edit** cursor when inside a selected vertex.

Data objects will respond slightly differently from one another.

- **Lines** - The two endpoints of the line may be moved. This simply relocates the line.
- **Ovals** - The bounding box of the oval has 4 corner and 4 edge vertices. Moving them will change the shape and size of the oval.
- **Rectangle** - The bounding box of the rectangle has 4 corner and 4 edge vertices. Moving them will change the shape and size of the rectangle.
- **Contours** - Each vertex of a contour can be moved independently of others.
- **Beziers** - Each vertex of a contour can be moved independently of others. In addition, the handle for the bezier vertex displays. Moving the cursor into the endpoints of the handle allows the shape of the vertex segments to be altered.
- **Arbors** - Each vertex of an arbor can be moved independently of others. Note however that if you move the vertex that serves as the bifurcation point of a branch, the branch will separate into two segments.

6. **Insert Vertices** - In insert mode, new vertices of the selected data objects may be added. The cursor changes to a **Vertex Add** cursor when on a segment that allows the addition of vertices.

Data objects will respond slightly differently from one another.

- **Lines** - This mode has no effect. Lines always have 2 vertices. The tool will act as in edit more.
- **Ovals** - This mode has no effect. Ovals always have 8 vertices. The tool will act as in edit more.
- **Rectangle** - This mode has no effect. Rectangles always have 8 vertices. The tool will act as in edit more.
- **Contours** - The vertex is added at the cursor location.
- **Beziers** - The cursor will not be active when moved onto a segment of a Bezier curve. Instead, move the cursor into a Bezier vertex. The new vertex is added at a point on the segment halfway between the vertex clicked on and the next vertex. If clicked on the last vertex of an open Bezier curve, the new vertex is added at a point on the segment halfway between this vertex and the previous vertex.
- **Arbors** - The vertex is added at the cursor location.

7. **Delete Vertices** - In delete mode, vertices of the selected data objects may be deleted. The cursor changes to a **Vertex Delete** cursor when on a vertex that can be deleted.

Data objects will respond slightly differently from one another.

- **Lines** - This mode has no effect. Lines always have 2 vertices. The tool will act as in edit more.
- **Ovals** - This mode has no effect. Ovals always have 8 vertices. The tool will act as in edit more.
- **Rectangle** - This mode has no effect. Rectangles always have 8 vertices. The tool will act as in edit more.
- **Contours** - The selected vertex is deleted. You cannot delete the vertex if only two are remaining in the contour. A closed contour is converted into an open contour.

- **Beziers** - The selected vertex is deleted. You cannot delete the vertex if only two are remaining in the Bezier curve. A closed Bezier curve is converted into an open Bezier curve.
- **Arbors** - The selected vertex is deleted. Note however that if you delete the vertex that serves as the bifurcation point of a branch, the orphaned branch will separate into a separate segment and become a primary branch.

Point Tool

Point Tool

Point Cursor

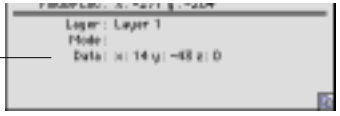
The **Point** tool is used to enter in 3D locations of data, associated with the current structure of the mapping window. The cursor will change to a *Point* cursor.

To record a location of an object:

1. Position the cursor on the object's center
2. Press the mouse button

For each press of the mouse button, a new **Point** object will be created and associated with the current structure. The location of the last object in real world coordinates (microns) will be displayed in the **Mapping Window Info** window.

Location of the last Point object entered



The location of the point is graphically displayed with whatever has been configured for the currently selected structure. For example, if the structure is configured to show a small red colored square, then that red square will be displayed at the point object location. The graphical attributes can be changed by selecting **Configure Structures...** of the **Objects** menu, or by pressing and holding the mouse button on the Current Structure field of the Mapping Window to get a pop up menu, from which you then select the menu item **Configure Current Structure...**.

Point objects would be the most used data object in NeuroZoom, useful to indicate the location of cells, blood vessels, nuclei, nearly anything that can be represented by a single dimensional point. The point is recorded in three dimensional (XYZ) and is the precise location of the object with respect to the stage coordinates of the microscope.

Tip: Any data object just created is stored in a special buffer. Hitting **Option-Delete** will delete that last object specifically. This is useful when a mistake was just made during data entry and you want to just delete the last object.

Line Tool

Line Tool

Line Cursor

The **Line** tool is used to enter in 3D line data, associated with the current structure of the mapping window. The cursor will change to a *Line* cursor. A line is a single, straight vector that has a beginning point and an ending point. It can be used to measure lengths, produce quick borders, separate data objects, etc.

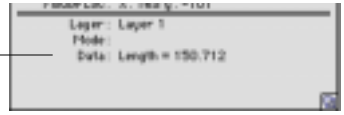
To create a line object:

1. Select the Line Tool
2. Position the cursor on the beginning point of the line
3. Press the mouse button, and while holding it down, move the mouse to the end point of the line. A line will sweep from the beginning to the end
4. Release the mouse button

A line object will be created with the beginning and end point specified. As with point objects, the line object's graphical attributes may be altered by configuring the structure associated with it. In this case, only the line width, color, and pattern may be changed.

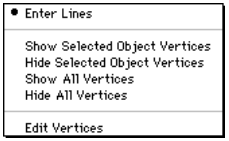
The length of the last line in real world coordinates (microns) will be displayed in the **Mapping Window Info** window.

Length of the last Line object entered



Popup Menu

There is also a popup menu associated with the tool.



1. **Enter Lines** - This is the default mode of the tool. New lines can be entered with this mode. A bullet character appears before this mode in the menu if selected.

2. **Show Selected Object Vertices** - Selecting this forces the *selected* lines to *show* their vertices.

3. **Hide Selected Object Vertices** - Selecting this forces the *selected* lines to *hide* their vertices.

4. **Show All Vertices** - Selecting this forces *all* lines to *show* their vertices.

5. **Hide All Vertices** - Selecting this forces *all* lines to *hide* their vertices.

6. **Edit Vertices** - One unlocked, selected line object must be selected before this menu item can be used. When selected, the vertices of the line object may be moved to new locations. This simply relocates the line. The cursor changes to a **Vertex Edit** cursor when inside a vertex of the line. There are two vertices to a line serving as the endpoints.

Oval Tool

Oval Tool

Oval Cursor

The **Oval** tool is used to enter in 3D oval data, associated with the current structure of the mapping window. The cursor will change to an *Oval* cursor. An oval has a major and minor axis, and is regularly shaped. It can be used to measure an area, highlight an area, etc.

To create an oval object:

1. Select the Oval Tool
2. Envision the bounding box of the oval
3. Position the cursor on one corner of the box
4. Press the mouse button, and while holding it down, move the mouse to the opposite corner. An oval will be drawn from corner to corner
5. Release the mouse button

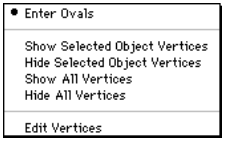
An oval object will be created that is bounded by the box specified by the beginning and end corners. As with other objects, the oval object's graphical attributes may be altered by configuring the structure associated with it. In this case, only the line width, color, and pattern may be changed.

The area of the last oval in real world coordinates (microns) will be displayed in the **Mapping Window** Info window

Area of the last Oval object entered

Popup Menu

There is also a popup menu associated with the tool.



1. **Enter Ovals** - This is the default mode of the tool. New ovals can be entered with this mode. A bullet character appears before this mode in the menu if selected.

2. **Show Selected Object Vertices** - Selecting this forces the *selected* ovals to *show* their vertices.

3. **Hide Selected Object Vertices** - Selecting this forces the *selected* ovals to *hide* their vertices.

4. **Show All Vertices** - Selecting this forces *all* ovals to *show* their vertices.

5. **Hide All Vertices** - Selecting this forces *all* ovals to *hide* their vertices.

6. **Edit Vertices** - One unlocked, selected oval object must be selected before this menu item can be used. When selected, the vertices of the oval object may be moved to new locations. This changes the shape and size of the oval. The cursor changes to a **Vertex Edit** cursor when inside a vertex of the oval. The bounding box of the oval has 4 corner and 4 edge vertices.

Rectangle Tool

Rectangle Tool

Rectangle Cursor

The **Rectangle** tool is used to enter in 3D rectangle data, associated with the current structure of the mapping window. The cursor will change to a *Rectangle* cursor. A rectangle has a width and a height. It can be used to measure an area, highlight an area, etc.

To create a rectangle object:

1. Select the Rectangle Tool
2. Envision the rectangle
3. Position the cursor on one corner of the rectangle
4. Press the mouse button, and while holding it down, move the mouse to the opposite corner. A rectangle will be drawn from corner to corner
5. Release the mouse button

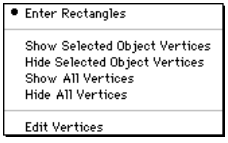
A rectangle object will be created that has the beginning and end corners specified. As with other objects, the rectangle object's graphical attributes may be altered by configuring the structure associated with it. In this case, only the line width, color, and pattern may be changed.

The area of the last oval in real world coordinates (microns) will be displayed in the **Mapping Window Info** window.

Area of the last Rectangle object entered

Popup Menu

There is also a popup menu associated with the tool.



1. **Enter Rectangles** - This is the default mode of the tool. New rectangles can be entered with this mode. A bullet character appears before this mode in the menu if selected.

2. **Show Selected Object Vertices** - Selecting this forces the *selected* rectangles to *show* their vertices.

3. **Hide Selected Object Vertices** - Selecting this forces the *selected* rectangles to *hide* their vertices.

4. **Show All Vertices** - Selecting this forces *all* rectangles to *show* their vertices.

5. **Hide All Vertices** - Selecting this forces *all* rectangles to *hide* their vertices.

6. **Edit Vertices** - One unlocked, selected rectangle object must be selected before this menu item can be used. When selected, the vertices of the rectangle object may be moved to new locations. This changes the shape and size of the rectangle. The cursor changes to a **Vertex Edit** cursor when inside a vertex of the rectangle. The rectangle has 4 corner and 4 edge vertices.

Open Contour Tool

Open Contour Tool

Open Contour Cursor

In Progress Cursor

The **Open Contour** tool is used to enter in 3D open contour data, associated with the current structure of the mapping window. The cursor will change to an *Open Contour* cursor. There is a little number 1 in the cursor indicating that it is waiting for a first point. An open contour is a polyline created by joining vertices. It can be used to measure a border, highlight an area, separate data objects, etc.

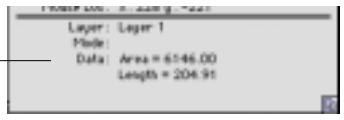
To create an open contour object:

1. Select the Open Contour Tool
2. Position the cursor on the first point of the contour
3. Press the mouse button once and release it. The cursor will change to a crosshair with an ellipsis (...) in it. This indicates that a contour creation is in progress
4. Without having to hold the mouse button down, move the mouse to the next vertex of the contour. A line will be drawn from the last vertex to the current mouse location as you move the mouse
5. Click the mouse button once to store the current mouse location as a vertex
6. Double-click the mouse button to end the open contour and to enter the last vertex. The cursor will change back to the Open Contour cursor

An open contour object will be created that has the specified vertices. As with other objects, the open contour object's graphical attributes may be altered by configuring the structure associated with it. In this case, only the line width, color, and pattern may be changed.

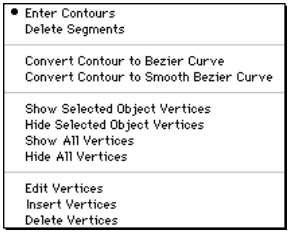
The area and length of the last open contour in real world coordinates (microns) will be displayed in the **Mapping Window Info** window.

Area and length of the last Open Contour object entered



Popup Menu

There is also a popup menu associated with the tool.



1. **Enter Contours** - This is the default mode of the tool. New open contours can be entered with this mode. A bullet character appears before this mode in the menu if selected.

- **Extending a contour** - This mode is also used to extend an existing open contour. Move the cursor onto an endpoint of an open contour. The cursor will change to the **In Progress** cursor. *Press and release* the mouse button to connect to the endpoint. Move the cursor to the desired location of the next vertex and press the mouse button. A new segment will be made.
- **Joining the endpoints of an open contour** - This mode is also used to join the endpoints of an open contour together to form a closed contour. Move the cursor onto an endpoint of an open contour. The cursor will change to the **In Progress** cursor. *Press and release* the mouse button to connect to the endpoint. Move the cursor to the other endpoint until it changes to a **Vertex Edit** cursor.

Press and release the mouse and the endpoints will be joined.

Alert: Note that the object will be a closed contour after joining.

---

2. **Delete Segments** - Selecting this allows deletion of segments of an open contour. A bullet character appears before this mode in the menu if selected. The cursor changes to a *black* colored **Segment Eraser** cursor.

Moving the cursor onto a segment of an open contour that can be deleted changes the color of the cursor to *red*. Click the mouse button while it is red will delete the segment under the cursor.

- If this is the last segment of an open contour, the open contour object will be completely removed.
- If this is a segment in the middle of an open contour, the original open contour will decompose into two separate open contours.

  An open contour does not have to be selected in order to use this tool mode. Any unlocked open contour can be acted on.

3. **Convert Contour to Bezier Curve -** One unlocked, selected open contour object must be selected before this menu item can be used. This converts the open contour object to an open Bezier object. A confirmation opens because this action is not undoable.

Press **Cancel** if you do not want to convert the contour. Press **Change** to convert the contour to a Bezier curve. Each vertex of the open contour is converted to the corresponding vertex of the open Bezier curve. Note that while the data object type is changed to Bezier, the selected tool remains as the **Open Contour** tool.

4. **Convert Contour to Smooth Bezier Curve -** One unlocked, selected open contour object must be selected before this menu item can be used. This converts the open contour object to an open Bezier object. Smoothing of the curve is attempted by automatically adjusting the handles of the vertices of the open Bezier curve. A confirmation opens because this action is not undoable.

Press **Cancel** if you do not want to convert the contour. Press **Change** to convert the contour to a Bezier curve. Each vertex of the open contour is converted to the corresponding vertex of the open Bezier curve. Note that while the data object type is changed to Bezier, the selected tool remains as the **Open Contour** tool.

5. **Show Selected Object Vertices** - Selecting this forces the *selected* open contours to *show* their vertices.

6. **Hide Selected Object Vertices** - Selecting this forces the *selected* open contours to *hide* their vertices.

7. **Show All Vertices** - Selecting this forces *all* open contours to *show* their vertices.

8. **Hide All Vertices** - Selecting this forces *all* open contours to *hide* their vertices.

9. **Edit Vertices** - One unlocked, selected open contour object must be selected before this menu item can be used. When selected, the vertices of the open contour object may be moved to new locations. A bullet character also appears before the selected mode in the menu. The cursor changes to a **Vertex Edit** cursor when inside a vertex of the open contour. Each vertex of an open contour can be moved independently of others.

10. **Insert Vertices** - One unlocked, selected open contour object must be selected before this menu item can be used. When selected, new vertices of the open contour object may be added. A bullet character also appears before

the selected mode in the menu. The cursor changes to a **Vertex Add** cursor when on a segment that allows the addition of vertices. The vertex is added at the cursor location.

11. **Delete Vertices -** One unlocked, selected open contour object must be selected before this menu item can be used. When selected, vertices of the open contour object may be deleted. A bullet character also appears before the selected mode in the menu. The cursor changes to a **Vertex Delete** cursor when on a vertex that can be deleted. The selected vertex is deleted. You cannot delete the vertex if only two are remaining in the open contour.

Closed Contour Tool

Closed Contour Tool

Closed Contour Cursor

In Progress Cursor

The **Closed Contour** tool is used to enter in 3D closed contour data, associated with the current structure of the mapping window. The cursor will change to a *Closed Contour* cursor. There is a little number 1 in the cursor indicating that it is waiting for a first point. A closed contour is a polyline created by joining vertices, with the beginning point the same as the end point. It can be used to measure a area, highlight an area, separate data objects, etc.

To create a closed contour object:

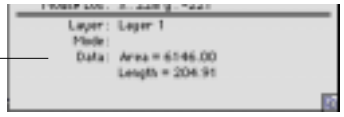
1. Select the Closed Contour Tool
2. Position the cursor on the first point of the contour
3. Press the mouse button once and release it. The cursor will change to a crosshair with an ellipsis (...) in it. This indicates that a contour creation is in progress
4. Without having to hold the mouse button down, move the mouse to the next vertex of the contour. A line will be drawn from the last vertex to the current mouse location as you move the mouse
5. Click the mouse button once to store the current mouse location as a vertex
6. Double-click the mouse button to end the closed contour. The contour will be closed automatically by joining the beginning and end points. The cursor will change back to the Closed Contour cursor

A closed contour object will be created that has the specified vertices. As with other objects, the closed contour object's graphical attributes may be altered by configuring the structure associated with it. In this case, only the line width, color, and pattern may be changed.

A closed contour object will be created that has the specified vertices. As with other objects, the closed contour object's graphical attributes may be altered by configuring the structure associated with it. In this case, only the line width, color, and pattern may be changed.

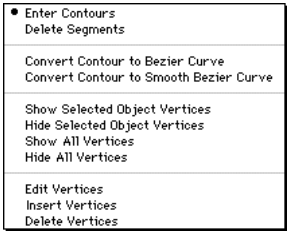
The area and length of the last closed contour in real world coordinates (microns) will be displayed in the **Mapping Window Info** window.

Area and length of the last Closed Contour object entered



Popup Menu

There is also a popup menu associated with the tool.



1. **Enter Contours** - This is the default mode of the tool. New closed contours can be entered with this mode. A bullet character appears before this mode in the menu if selected.

2. **Delete Segments** - Selecting this allows deletion of segments of a closed contour. A bullet character appears before this mode in the menu if selected. The cursor changes to a *black* colored **Segment Eraser** cursor.

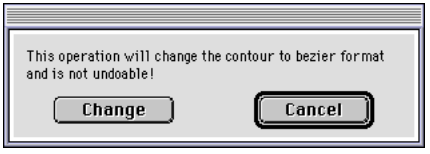
Moving the cursor onto a segment of an closed contour that can be deleted changes the color of the cursor to *red*. Click the mouse button while it is red will delete the segment under the cursor.

- If this is the last segment of a closed contour, the closed contour object will be completely removed.
- If this is a segment in the middle of a closed contour, the original closed contour will change into an open contour.

A closed contour does not have to be selected in order to use this tool mode. Any unlocked closed contour can be acted on.

Alert: As soon as one segment of a closed contour is deleted, that closed contour is converted into an open contour. Note that the selected tool remains as the **Closed Contour** tool.

3. **Convert Contour to Bezier Curve** - One unlocked, selected closed contour object must be selected before this menu item can be used. This converts the closed contour object to an closed Bezier object. A confirmation opens because this action is not undoable.



Press **Cancel** if you do not want to convert the contour. Press **Change** to convert the contour to a Bezier curve. Each vertex of the closed contour is converted to the corresponding vertex of the closed Bezier curve. Note that while the data object type is changed to Bezier, the selected tool remains as the **Closed Contour** tool.

4. **Convert Contour to Smooth Bezier Curve** - One unlocked, selected open contour object must be selected before this menu item can be used. This converts the closed contour object to an closed Bezier object. Smoothing of the curve is attempted by automatically adjusting the handles of the vertices of the closed Bezier curve. A confirmation opens because this action is not undoable.

Press **Cancel** if you do not want to convert the contour. Press **Change** to convert the contour to a Bezier curve. Each vertex of the closed contour is converted to the corresponding vertex of the closed Bezier curve. Note that while the data object type is changed to Bezier, the selected tool remains as the **Closed Contour** tool.

5. **Show Selected Object Vertices** - Selecting this forces the *selected* closed contours to *show* their vertices.

6. **Hide Selected Object Vertices** - Selecting this forces the *selected* closed contours to *hide* their vertices.

7. **Show All Vertices** - Selecting this forces *all* closed contours to *show* their vertices.

8. **Hide All Vertices** - Selecting this forces *all* closed contours to *hide* their vertices.

9. **Edit Vertices** - One unlocked, selected closed contour object must be selected before this menu item can be used. When selected, the vertices of the closed contour object may be moved to new locations. A bullet character also appears before the selected mode in the menu. The cursor changes to a **Vertex Edit** cursor when inside a vertex of the closed contour. Each vertex of an closed contour can be moved independently of others.

10. **Insert Vertices** - One unlocked, selected closed contour object must be selected before this menu item can be used. When selected, new vertices of the closed contour object may be added. A bullet character also appears

before the selected mode in the menu. The cursor changes to a **Vertex Add** cursor when on a segment that allows the addition of vertices. The vertex is added at the cursor location.

11. **Delete Vertices** - One unlocked, selected closed contour object must be selected before this menu item can be used. When selected, vertices of the closed contour object may be deleted. A bullet character also appears before the selected mode in the menu. The cursor changes to a **Vertex Delete** cursor when on a vertex that can be deleted. The selected vertex is deleted. You cannot delete the vertex if only two are remaining in the closed contour.

Open Bezier Tool

Open Bezier Tool

Open Bezier Cursor

In Progress Cursor

The **Open Bezier** tool is used to enter in 3D open Bezier data, associated with the current structure of the mapping window. The cursor will change to an *Open Bezier* cursor. There is a little number 1 in the cursor indicating that it is waiting for a first point. An open Bezier curve is a smooth curve created by using curves. It can be used to measure a area, highlight an area, separate data objects, etc. Since it is a smooth curve and not a polyline using connected vertices, the Bezier curve will remain smooth at all levels of magnification.

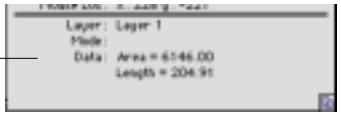
To create an open Bezier curve object:

1. Select the Open Bezier Tool
2. Position the cursor on the first point of the curve
3. Press and hold the mouse button down. The cursor will change to a crosshair with an ellipsis (...) in it. This indicates that a Bezier curve creation is in progress. This first point is the first vertex
4. Drag the mouse down along the curve you are trying to fit. A straight line will be created
5. Release the mouse button
6. At a point further down the curve, press and hold the mouse button down. This point will become the next vertex
7. Drag the mouse down further along the curve. Move the mouse up and down. A smooth curve will be displayed. Try to fit the curve as close to the edge that you are trying to trace
8. Continue this process until done
9. Double-click the mouse button to end the open Bezier curve and to enter the last vertex. The cursor will change back to the Open Bezier cursor

An open Bezier curve will be created that has a curve smoothed around the specified vertices. As with other objects, the open Bezier curve object's graphical attributes may be altered by configuring the structure associated with it. In this case, only the line width, color, and pattern may be changed.

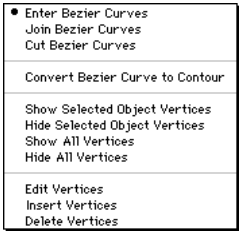
The area and length of the last open Bezier curve in real world coordinates (microns) will be displayed in the Mapping Window Info window.

Area and length of the last Open Bezier Curve object entered



Popup Menu

There is also a popup menu associated with the tool.



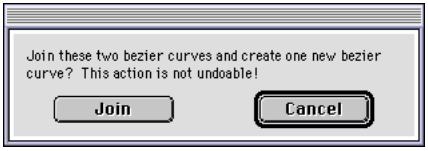
1. **Enter Bezier Curves** - This is the default mode of the tool. New open Bezier curves can be entered with this mode. A bullet character appears before this mode in the menu if selected.

2. **Join Bezier Curves** - Selecting this allows the joining of a two unlocked, selected open Bezier curves into one closed Bezier curve. A bullet character appears before this mode in the menu if selected. Move the cursor into one of the endpoints of a selected open Bezier curve. The cursor changes to an **Open Forceps** cursor.

Press and hold the mouse button and move the cursor to an endpoint of the other Bezier curve until the cursor changes to a **Closed Forceps** cursor.

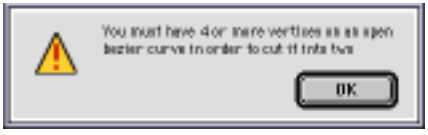
If the vertex is not valid, the cursor would be a **Forceps** with an **X** across it. Internal vertices are not legitimate because it would result in branches being formed.

Release the mouse and a confirmation window opens.



Press the **Join** button and the endpoints will be joined together. The other pair of endpoints will join automatically to form one closed Bezier curve. Press the **Cancel** button to cancel any changes.

3. **Cut Bezier Curves** - Selecting this allows the cutting of a one unlocked, selected open Bezier curve into two open Bezier curves. A bullet character appears before this mode in the menu if selected. If the selected open Bezier curve does not have 4 or more vertices when you select this menu item, an error window opens.

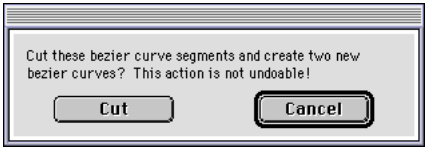


Move the cursor into one of the vertices of a selected open Bezier curve that can be cut. The cursor changes to an **Scalpel** cursor with a number **1** displayed on it, indicating that this is the first part of the cut.

Press and hold the mouse button and move the cursor to another vertex of the same Bezier curve until the cursor changes to a **Scalpel** cursor with a number **2** on it, indicating that this is the second part of the cut.

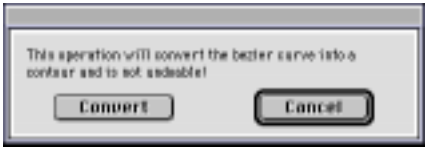
If the vertex is not valid, the cursor would be a **Scalpel** with a **X** across it. For example, an endpoint is not a legitimate vertex is include in the cut because it would result in only one contour, not a split into two contours.

Release the mouse and a confirmation window opens.



Press the **Cut** button and the selected Bezier curve will be cut into two separate Bezier curves with the segment between the indicated vertices removed. Press the **Cancel** button to cancel any changes. The first part of the Bezier curve remains selected, while the second half is deselected.

4. **Convert Bezier Curve to Contour** - One unlocked, selected open Bezier curve object must be selected before this menu item can be used. If not, an error window opens. This converts the open Bezier curve object to an open contour object. A confirmation opens because this action is not undoable.



Press **Cancel** if you do not want to convert the Bezier curve. Press **Change** to convert the Bezier curve to a contour. As many vertices are created in the contour object to maintain smoothness at the current scale of the window. Note that while the data object type is changed to open contour, the selected tool remains as the **Open Bezier** tool.

5. **Show Selected Object Vertices** - Selecting this forces the *selected* open Bezier curves to *show* their vertices.

6. **Hide Selected Object Vertices** - Selecting this forces the *selected* open Bezier curves to *hide* their vertices.

7. **Show All Vertices** - Selecting this forces *all* open Bezier curves to *show* their vertices.

8. **Hide All Vertices** - Selecting this forces *all* open Bezier curves to *hide* their vertices.

9. **Edit Vertices** - One unlocked, selected open Bezier curve object must be selected before this menu item can be used. When selected, the vertices of the open Bezier curve object may be moved to new locations. A bullet character also appears before the selected mode in the menu. The cursor changes to a **Vertex Edit** cursor when inside a vertex of the open Bezier curve. Each vertex of an open Bezier curve can be moved independently of others. In addition, the handle for the bezier vertex displays. Moving the cursor into the endpoints of the handle allows the shape of the vertex segments to be altered.

10. **Insert Vertices** - One unlocked, selected open Bezier curve object must be selected before this menu item can be used. When selected, new vertices of the open Bezier curve object may be added. A bullet character also appears before the selected mode in the menu. Move the cursor into a Bezier vertex and the cursor changes to a **Vertex Add** cursor.

Clicking the mouse button adds the new vertex at a point on the segment halfway between the vertex clicked on and the next vertex. If clicked on the last vertex, the new vertex is added at a point on the segment halfway between this vertex and the previous vertex.

11. **Delete Vertices** - One unlocked, selected open Bezier curve object must be selected before this menu item can be used. When selected, vertices of the open Bezier curve object may be deleted. A bullet character also appears before the selected mode in the menu. The cursor changes to a **Vertex Delete** cursor when on a vertex that can be deleted. The selected vertex is deleted. You cannot delete the vertex if only two are remaining in the open Bezier curve.

Closed Bezier Tool

Closed Bezier Tool

Closed Bezier Cursor

In Progress Cursor

The **Closed Bezier** tool is used to enter in 3D closed Bezier contour data, associated with the current structure of the mapping window. The cursor will change to a *Closed Bezier* cursor. There is a little number 1 in the cursor indicating that it is waiting for a first point. A closed Bezier curve is a smooth curve created by using curves. It can be used to measure a area, highlight an area, separate data objects, etc. Since it is a smooth curve and not a polyline using connected vertices, the Bezier curve will remain smooth at all levels of magnification.

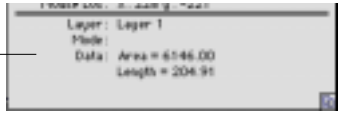
To create a closed Bezier curve object:

1. Select the Closed Bezier Tool
2. Position the cursor on the first point of the curve
3. Press and hold the mouse button down. The cursor will change to a crosshair with an ellipsis (...) in it. This indicates that a Bezier curve creation is in progress. This first point is the first vertex
4. Drag the mouse down along the curve you are trying to fit. A straight line will be created
5. Release the mouse button
6. At a point further down the curve, press and hold the mouse button down. This point will become the next vertex
7. Drag the mouse down further along the curve. Move the mouse up and down. A smooth curve will be displayed. Try to fit the curve as close to the edge that you are trying to trace
8. Continue this process until done
9. Double-click the mouse button to end the closed Bezier curve. The curve will be closed automatically by joining the beginning and end points. The cursor will change back to the Closed Bezier cursor

A closed Bezier curve will be created that has a curve smoothed around the specified vertices. As with other objects, the closed Bezier curve object's graphical attributes may be altered by configuring the structure associated with it. In this case, only the line width, color, and pattern may be changed.

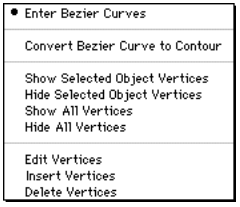
The area and length of the last closed Bezier curve in real world coordinates (microns) will be displayed in the Mapping Window Info window.

Area and length of the last Closed Bezier Curve object entered



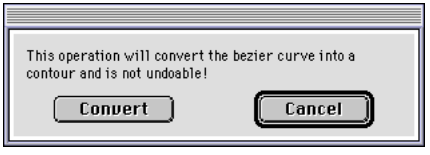
Popup Menu

There is also a popup menu associated with the tool.



1. **Enter Bezier Curves** - This is the default mode of the tool. New closed Bezier curves can be entered with this mode. A bullet character appears before this mode in the menu if selected.

2. **Convert Bezier Curve to Contour** - One unlocked, selected closed Bezier curve object must be selected before this menu item can be used. If not, an error window opens. This converts the closed Bezier curve object to an closed contour object. A confirmation opens because this action is not undoable.



Press **Cancel** if you do not want to convert the Bezier curve. Press **Change** to convert the Bezier curve to a contour. As many vertices are created in the contour object to maintain smoothness at the current scale of the window. Note that while the data object type is changed to closed contour, the selected tool remains as the **Closed Bezier** tool.

3. **Show Selected Object Vertices** - Selecting this forces the *selected* closed Bezier curves to *show* their vertices.

4. **Hide Selected Object Vertices** - Selecting this forces the *selected* closed Bezier curves to *hide* their vertices.

5. **Show All Vertices** - Selecting this forces *all* closed Bezier curves to *show* their vertices.

6. **Hide All Vertices** - Selecting this forces *all* closed Bezier curves to *hide* their vertices.

7. **Edit Vertices** - One unlocked, selected closed Bezier curve object must be selected before this menu item can be used. When selected, the vertices of the closed Bezier curve object may be moved to new locations. A bullet character also appears before the selected mode in the menu. The cursor changes to a **Vertex Edit** cursor when inside a vertex of the closed Bezier curve. Each vertex of an closed Bezier curve can be moved independently of others. In addition, the handle for the bezier vertex displays. Moving the cursor into the endpoints of the handle allows the shape of the vertex segments to be altered.

8. **Insert Vertices** - One unlocked, selected closed Bezier curve object must be selected before this menu item can be used. When selected, new vertices of the closed Bezier curve object may be added. A bullet character also appears before the selected mode in the menu. Move the cursor into a Bezier vertex and the cursor changes to a **Vertex Add** cursor.

Clicking the mouse button adds the new vertex at a point on the segment halfway between the vertex clicked on and the next vertex.

9. **Delete Vertices** - One unlocked, selected closed Bezier curve object must be selected before this menu item can be used. When selected, vertices of the closed Bezier curve object may be deleted. A bullet character also appears before the selected mode in the menu. The cursor changes to a **Vertex Delete** cursor when on a vertex that can be deleted. The selected vertex is deleted. You cannot delete the vertex if only two are remaining in the closed Bezier curve.

Arbor Tool

Arbor Tool

Arbor Cursor

In Progress Cursor

The **Arbor** tool is used to enter in 3D arbor data, associated with the current structure of the mapping window. The cursor will change to an *Arbor* cursor. There is a little number 1 in the cursor indicating that it is waiting for a first point. An arbor is a tree like structure that starts with a primary or first branch, and branches outward from the primary branch. It can be used to represent dendritic arborization.

To create an arbor object:

1. Select the Arbor Tool
2. Position the cursor on the first point of the primary branch. This might be right on the cell body if tracing out a cell
3. Press the mouse button once and release it. The cursor will change to a crosshair with an ellipsis (...) in it. This indicates that an arbor creation is in progress
4. Without having to hold the mouse button down, move the mouse to the next vertex of the current branch. A line will be drawn from the last vertex to the current mouse location
5. Click the mouse button once to store the current mouse location as a vertex
6. Double-click the mouse button to end the current branch and to enter the last vertex. The cursor will change back to the Arbor cursor
7. Position the cursor at a bifurcation point on any of the branches. The cursor will change to a X, indicating that a cross branching is to occur at that point
8. Repeat steps 4 - 7 until all branches are traced

- To add to a *sub-branch* to the end of an *existing* branch (that is, it blends perfectly with the sub-branch with no bifurcation), press the **Option** key and move the cursor onto the endpoint. The cursor will change to and X. Repeat steps 4 - 7 until the current branch is done.

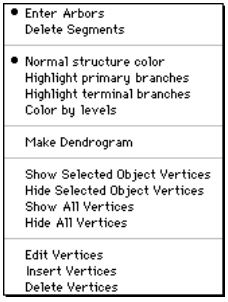
An arbor will be created that has all branches drawn around the specified vertices. The hierarchy of the branching is preserved.

The length of the last branch in real world coordinates (microns) will be displayed in the Mapping Window Info window.

Length of the last branch object entered

Popup Menu

There is also a popup menu associated with the tool.



1. **Enter Arbors** - This is the default mode of the tool. New arbors can be entered with this mode. A bullet character appears before this mode in the menu if selected.

- **Extending an arbor** - This mode is also used to extend an existing arbor. Move the cursor onto an endpoint of any branch of an arbor. The cursor will change to the **In Progress** cursor. *Press and release* the mouse button to connect to the endpoint. Move the cursor to the desired location of the next vertex and press the mouse button. A new segment will be made.
- **Adding a sub-branch to the end of the arbor** - To add to a *sub-branch* to the end of an *existing* branch (that is, it blends perfectly with the sub-branch with no bifurcation), press the **Option** key and move the cursor onto the endpoint. The cursor will change to and X. Move the cursor to the desired location of the next vertex and press the mouse button. A new segment will be made. Continue until done.

2. **Delete Segments** - Selecting this allows deletion of segments of an arbor. A bullet character appears before this mode in the menu if selected. The cursor changes to a *black* colored **Segment Eraser** cursor.

Moving the cursor onto a segment of an arbor that can be deleted changes the color of the cursor to *red*. Click the mouse button while it is red will delete the segment under the cursor.

- If this is the last segment of an arbor, the arbor object will be completely removed.
- If this is a segment in the middle of an arbor branch, the original arbor branch will decompose into two separate arbors. The orphaned branch will become a primary branch.

  An arbor does not have to be selected in order to use this tool mode. Any unlocked arbor can be acted on.

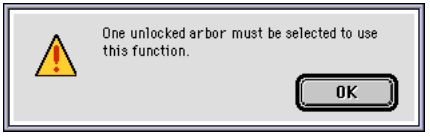
3. **Normal structure color** - The assigned color of the structure associated with the arbor is used for the graphical display.

4. **Highlight primary branch** - The primary branches of all arbors are displayed in the highlighted color selected for the computer.

5. **Highlight terminal branch** - The terminal branches of all arbors are displayed in the highlighted color selected for the computer.

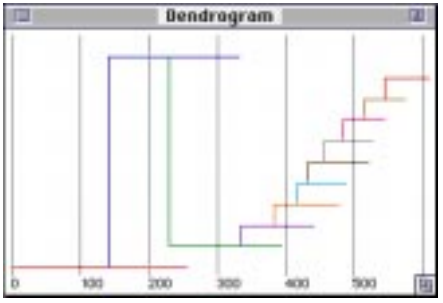
6. **Color by levels** - Each branch from primary to terminal is color coded with a different color

7. **Make dendrogram** - One unlocked, selected arbor object must be selected before this menu item can be used. If not, an error window opens.



When selected, a dendrogram is made from the branch descriptions of the arbor. The individual lengths of the branches are schematically displayed.

Dendrogram



8. **Show Selected Object Vertices** - Selecting this forces the *selected* arbors to *show* their vertices.

9. **Hide Selected Object Vertices** - Selecting this forces the *selected* arbors to *hide* their vertices.

10. **Show All Vertices** - Selecting this forces *all* arbors to *show* their vertices.

11. **Hide All Vertices** - Selecting this forces *all* arbors to *hide* their vertices.

12. **Edit Vertices** - One unlocked, selected arbor object must be selected before this menu item can be used. When selected, the vertices of the arbor object may be moved to new locations. A bullet character also appears before the

selected mode in the menu. The cursor changes to a **Vertex Edit** cursor when inside a vertex of the arbor. Each vertex of an arbor can be moved independently of others.

13. **Insert Vertices** - One unlocked, selected arbor object must be selected before this menu item can be used. When selected, new vertices of the arbor object may be added. A bullet character also appears before the selected mode in the menu. The cursor changes to a **Vertex Add** cursor when on a segment that allows the addition of vertices. The vertex is added at the cursor location.

14. **Delete Vertices** - One unlocked, selected arbor object must be selected before this menu item can be used. When selected, vertices of the arbor object may be deleted. A bullet character also appears before the selected mode in the menu. The cursor changes to a **Vertex Delete** cursor when on a vertex that can be deleted. The selected vertex is deleted. Note however that if you delete the vertex that serves as the bifurcation point of a branch, the orphaned branch will separate into a separate segment and become a primary branch.

Arbor with Diameter Tool

Arbor with Diameter Tool

Arbor with Diameter Cursor

In Progress Cursor

The **Arbor with Diameter** tool is used to enter in 3D arbor with diameter data, associated with the current structure of the mapping window. The cursor will change to an *Arbor with Diameter* cursor. There is a little number 1 in the cursor indicating that it is waiting for a first point. An arbor is a tree like structure that starts with a primary or first branch, and branches outward from the primary branch. It can be used to represent dendritic arborization. This arbor has diameters that can be assigned to each of the branches to give it more detail.

To create an arbor with diameter object:

1. Select the Arbor with Diameter Tool
2. Position the cursor on the first point of the primary branch. This might be right on the cell body if tracing out a cell
3. Press the mouse button once and release it. The cursor will change to a crosshair with an ellipsis (...) in it. This indicates that an arbor creation is in progress
4. Without having to hold the mouse button down, move the mouse to the next vertex of the current branch. A line will be drawn from the last vertex to the current mouse location
5. Click the mouse button once to store the current mouse location as a vertex
6. To change the diameter, do not release the mouse button. While pressing the mouse button, move the mouse from left to right. The thickness of the current branch as seen on the monitor will change. Release the mouse button to accept the displayed thickness
7. Double-click the mouse button to end the current branch and to enter the last vertex. The cursor will change back to the Arbor cursor
8. Position the cursor at a bifurcation point on any of the branches. The cursor will change to a X, indicating that a cross branching is to occur at that point
9. Repeat steps 4 - 8 until all branches are traced

- To add to a *sub-branch* to the end of an *existing* branch (that is, it blends perfectly with the sub-branch with no bifurcation), press the **Option** key and move the cursor onto the endpoint. The cursor will change to and X. Repeat steps 4 - 8 until the current branch is done.

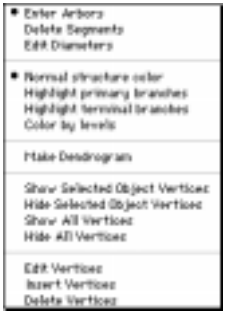
An arbor with diameter will be created that has all branches drawn with a specified diameter around the specified vertices. The hierarchy of the branching is preserved.

e length of the last branch in real world coordinates (microns) will be displayed in the Mapping Window Info window.

Length of the last branch object entered

Popup Menu

There is also a popup menu associated with the tool.



1. **Enter Arbors** - This is the default mode of the tool. New arbors can be entered with this mode. A bullet character appears before this mode in the menu if selected.

- **Extending an arbor** - This mode is also used to extend an existing arbor. Move the cursor onto an endpoint of any branch of an arbor. The cursor will change to the **In Progress** cursor. *Press and release* the mouse button to connect to the endpoint. Move the cursor to the desired location of the next vertex and press the mouse button. A new segment will be made.

- **Adding a sub-branch to the end of the arbor** - To add to a *sub-branch* to the end of an *existing* branch (that is, it blends perfectly with the sub-branch with no bifurcation), press the **Option** key and move the cursor onto the endpoint. The cursor will change to and X. Move the cursor to the desired location of the next vertex and press the mouse button. A new segment will be made. Continue until done.

2. **Delete Segments** - Selecting this allows deletion of segments of an arbor. A bullet character appears before this mode in the menu if selected. The cursor changes to a *black* colored **Segment Eraser** cursor.

Moving the cursor onto a segment of an arbor that can be deleted changes the color of the cursor to *red*. Click the mouse button while it is red will delete the segment under the cursor.

- If this is the last segment of an arbor, the arbor object will be completely removed.
- If this is a segment in the middle of an arbor branch, the original arbor branch will decompose into two separate arbors. The orphaned branch will become a primary branch.

  An arbor does not have to be selected in order to use this tool mode. Any unlocked arbor can be acted on.

3. **Edit Diameters** - Selecting this allows changing of diameter of segments of an arbor. A bullet character appears before this mode in the menu if selected. The cursor changes to a *black* colored **Diameter** cursor.

Moving the cursor onto a segment of an arbor that can be changed changes the color of the cursor to *red*. Click the mouse button while it is red and moving the cursor up and down will alter the current diameter of the segment under the cursor.

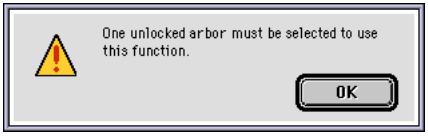
4. **Normal structure color** - The assigned color of the structure associated with the arbor is used for the graphical display.

5. **Highlight primary branch** - The primary branches of all arbors are displayed in the highlighted color selected for the computer.

6. **Highlight terminal branch** - The terminal branches of all arbors are displayed in the highlighted color selected for the computer.

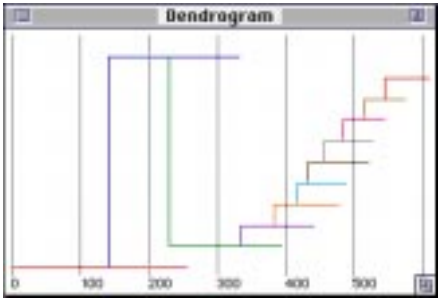
7. **Color by levels** - Each branch from primary to terminal is color coded with a different color

8. **Make dendrogram** - One unlocked, selected arbor object must be selected before this menu item can be used. If not, an error window opens.



When selected, a dendrogram is made from the branch descriptions of the arbor. The individual lengths of the branches are schematically displayed.

Dendrogram



9. **Show Selected Object Vertices** - Selecting this forces the *selected* arbors to *show* their vertices.

10. **Hide Selected Object Vertices** - Selecting this forces the *selected* arbors to *hide* their vertices.

11. **Show All Vertices** - Selecting this forces *all* arbors to *show* their vertices.

12. **Hide All Vertices** - Selecting this forces *all* arbors to *hide* their vertices.

13. **Edit Vertices** - One unlocked, selected arbor object must be selected before this menu item can be used. When selected, the vertices of the arbor object may be moved to new locations. A bullet character also appears before the selected mode in the menu. The cursor changes to a **Vertex Edit** cursor when inside a vertex of the arbor. Each vertex of an arbor can be moved independently of others.

14. **Insert Vertices** - One unlocked, selected arbor object must be selected before this menu item can be used. When selected, new vertices of the arbor object may be added. A bullet character also appears before the selected mode in the menu. The cursor changes to a **Vertex Add** cursor when on a segment that allows the addition of vertices. The vertex is added at the cursor location.

15. **Delete Vertices** - One unlocked, selected arbor object must be selected before this menu item can be used. When selected, vertices of the arbor object may be deleted. A bullet character also appears before the selected mode in the menu. The cursor changes to a **Vertex Delete** cursor when on a vertex that can be deleted. The selected vertex is deleted. Note however that if you delete the vertex that serves as the bifurcation point of a branch, the orphaned branch will separate into a separate segment and become a primary branch.

Cavalieri Tool

The **Cavalieri** tool is a stereology tool used to estimate volume of objects. This tool cannot be used normally in the mapping window until a stereology protocol has been established. See the reference chapters on ***Stereology*** or ***Stereology Windows*** for more information on how to use this tool.

Cycloid Tool

The **Cycloid** tool is a stereology tool used to estimate surface area of objects. This tool cannot be used normally in the mapping window until a stereology protocol has been established. See reference chapters on ***Stereology*** or ***Stereology Windows*** for more information on how to use this tool.

Nucleator Tool

The **Nucleator** tool is a stereology tool used to estimate mean volume of objects. This tool cannot be used normally in the mapping window until a stereology protocol has been established. See reference chapters on ***Stereology*** or ***Stereology Windows*** for more information on how to use this tool.

Rotator Tool

The **Rotator** tool is a stereology tool used to estimate mean volume of objects. This tool cannot be used normally in the mapping window until a stereology protocol has been established. See reference chapters on ***Stereology*** or ***Stereology Windows*** for more information on how to use this tool.

View Window

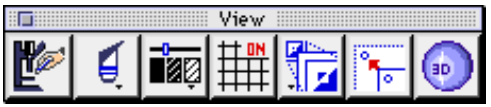
The **View** window contains functions that pertains to the Views.

Opened by:

- View Window in Map menu
- Command - 2
- Pressing Window Toggler in the Mapping window 
- Opening a new Mapping window if the preference is set to automatically open the View Window

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.

A *view* is a combination of a microscope, a camera, and the lens objective. Each view is scaled within NeuroZoom so that the precise ratio of real world units (microns) is know for every device unit (pixels). The presentation of these views, and other related functions are controlled from the **View** window. Views are configured using the **Configuration** window.



Alert: The control buttons on this window act as push buttons. Click once to activate a function. The button image may change to reflect a change in state.

The details of each button follows.

Microscope Setup Window

Press this button to open the **Microscope Setup** window showing the selections available for choosing devices for the current mapping window.

Opened by:

- Press Microscope Setup button in View window
- Microscope Setup... in Map menu
- Opening a new Mapping window if the preference is set to automatically open the Microscope Setup Window.

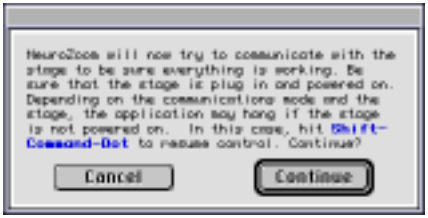
From this window the microscope, the camera, the lens objective, and the stage are chosen from the configured devices. The microscope, the camera, and the lens objective make up the View that is being used by the mapping window. The microscope and the camera generally will not change during the analysis of the microscope slide section. However, the lens objectives will change. All of the lens objectives that you intend to use for this slide session should already have been scaled into a view device using the **Configuration** window. See the chapter on ***Configuring NeuroZoom Devices*** to do this.

If there are no objectives scaled for the particular microscope and camera selected, the **Lens Objective** popup menu will be grayed out. If the microscope and camera selections are correct, you will have to use the **Configuration** window to scale in new *View* devices using those lens objectives.

Likewise, if the microscope and camera selected does have views with configured lens objectives, only those lens objectives will appear in the popup menu. For example, the following figure shows the Zeiss Axiophot selected as the microscope, and a Dage CCD70 with QuickTime digitizer selected as the camera. Of this pairing, the 5x Plan-apochromat, the 10x Plan-apochromat, the 20x Plan-apochromat, and the 40x Plan-apochromat are selectable because there is four view devices configured with this microscope and camera. Think of this as a box full of objectives. They are useless unless attached to a microscope with a camera. The microscope, camera, and lens objective is what makes the working view.

When a stage is selected, NeuroZoom may ask for you to verify that the stage controller is connected, powered on, and ready to use.

NeuroZoom will then attempt to communicate to the stage and prepare it for use. If there is any error, NeuroZoom will alert you.



You cannot proceed to use NeuroZoom with a motorized stage until you successfully pass this point. If there is difficulty in communicating with a stage controller, use the **Configuration** window to configure the stage controller. Be sure to pay attention to the communications mode (i.e., GPIB, Communications Toolbox Serial Tool, etc.).

To summarize the steps in using the Microscope Setup Window:

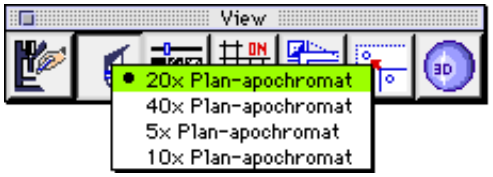
1. Select the proper microscope from the microscope popup menu
2. Select the proper camera from the camera popup menu
3. Select the proper lens objective from the lens objective menu that you are starting with. This will list all the objectives that are available based on the selected microscope and camera
4. Select the proper stage controller from the stage controller popup menu. If the stage has an electronic controller, NeuroZoom will try to communicate with it to initialize it to a known state

Lens Objective Popup Menu

Press this button to get a popup menu showing all of the lens objectives available with the microscope and camera combination selected with the **Microscope Setup** window.

Activated by:

- Press Lens Objective button in View window
- Select Microscope Objective... in Map menu



As stated in the section on **Microscope Setup**, only those lens objectives that are in view devices that use the selected microscope and camera will appear in the popup menu. If a particular lens objective is not listed, you will have to create a new *View* device using the **Configuration** window for this combination of microscope, camera, and lens objective.

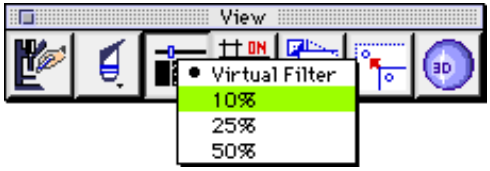
Alert: The lens objective itself must still be manually switched into the focal path. Using the popup menu above only alerts NeuroZoom that you changed the lens.

Filter Popup Menu

Press this button to get a popup menu showing all of the filters available. These filters are made with the **Configuration** window.

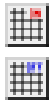
Activated by:

- Press lens Filter button in View window
- Select Filter Objective... in Map menu



Currently, these filters have no affect in NeuroZoom. Selecting them does enter their values into reports that are generating by NeuroZoom.

Grid Toggle



Press this button to toggle the appearance of a grid on the mapping window. The grid displays the stage location in the mapping coordinate system in real time. The button will show the word ON if the grid is on, and the word OFF if the grid is off.

Activated by:

- Press Grid button in View window
- Grid in Map menu

Grid Setup Window - Pressing the button with the **Option** key down will open the **Grid Setup** window where you can adjust the size and appearance of the grid. This window can also be opened by selecting **Grid Setup...** in the **Map** menu.

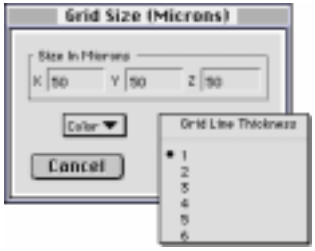
Opened by:

- Option-Press Grid Setup button in View window
- Grid Setup... in Map menu

Enter in the value for the distance between tics for the X and Y axis. The Z axis value has no effect at this time.

The color of the grid can be selected with the **Color Popup** menu.

The thickness of the grid lines can be selected with the **Thickness Popup** menu.



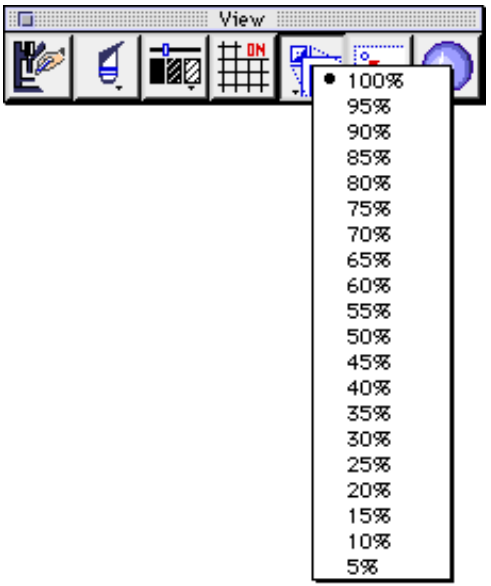
Press **Accept** to accept the changes and to redraw the Grid, or press **Cancel** to cancel all changes made in this window.

Image Size Popup Menu

Press this button to get a popup menu showing all of the sizes available for this mapping window.

Activated by:

- Press Image Size button in View window
- Image Size Popup Menu in Imaging menu
- Mapping Window Image Size Field in Mapping window



This is the same menu that is popped up from the **Mapping Window Image Size** field at the bottom left of the mapping window, or by selecting **Image Size** in the **Imaging** menu. The mapping area will change to the selected percentage of the camera format. For example, if 50% is chosen, a 640 by 480 format camera will

display in a 320 by 240 window. The video will not be cropped, but instead, the video will be scaled down 50%. Likewise, all document data that is visible in the mapping area will also be scaled down 50%.

Alignment Correction

Press this button to correct slight alignment errors. If the preferences are set to display informational windows, a help window will appear to guide you.

Activated by:

- Press Alignment Correction button in View window
- Alignment Correct in Imaging menu
- Realign Data to Image... in Imaging menu

The error vector is measured by indicating the graphics data point and its corresponding data point in the image. Use this function to make corrections that may appear for a number of reasons (spherical aberrations, camera axis misalignment, non-square pixels, etc.). The correction that is entered remains in effect while the document is opened, and is applied to all new data entered. The following dialog appears when you enter in the correction with the mouse.

Open 3D Window

Press this button to open a window that displays the data in 3D. The **3D** button must be enabled. If it is not, either QuickDraw™ 3D is not installed, there is insufficient RAM to load QuickDraw™ 3D, or NeuroZoom is not running on a Power Macintosh. When you press the **3D** button, NeuroZoom will initialize QuickDraw™ 3D for the first time, and check its version. Any outdated version will display an error dialog, and you will not be able to continue with 3D visualization.

Activated by:

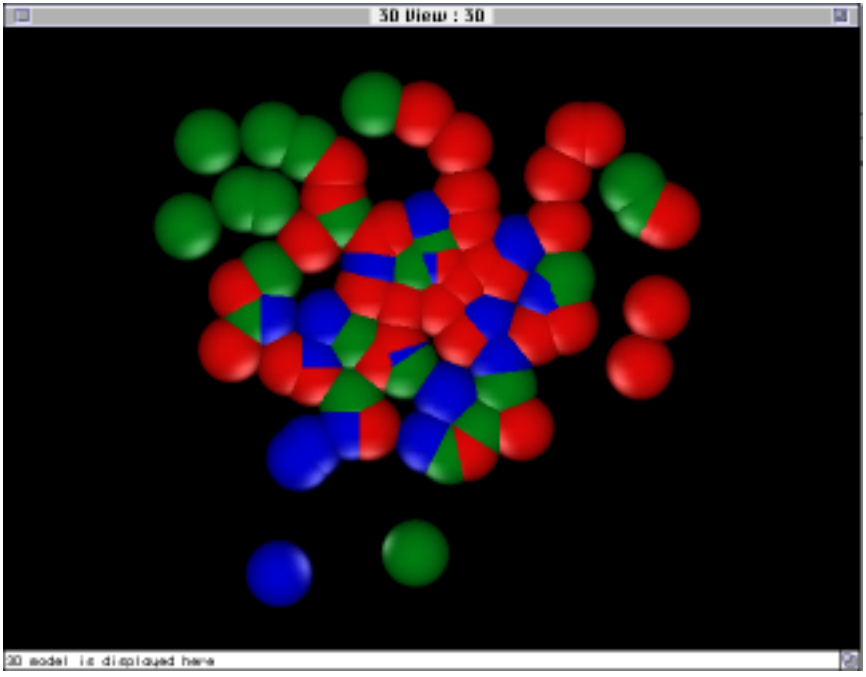
- Press Open 3D Window button in View window
- 3D View in 3D submenu of Modules menu

A 3D model is created from the current data in the frontmost mapping window.

- If there are data selected, those selected data are used to create the model.
- If there are no data selected, all the data in the mapping window are used to create the model.

If there is no data in the mapping window, a alert will open.

A progress window is displayed that shows the progress of creating the model from the data. A new window is then opened displaying the 3D model. The name of the window is the same name of the mapping window, with 3*D View:* prepended to it.



Refer to the chapters on 3D ***Visualization*** to use this 3D window and its windows.

Imaging Window

The **Imaging** window contains functions that pertains to Imaging.

Opened by:

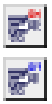
- Imaging Window in Map menu
- Command - 3
- Pressing Window Toggler in the Mapping window 
- Opening a new Mapping window if the preference is set to automatically open the Imaging Window

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.

Alert: The control buttons on this window act as push buttons. Click once to activate a function. The button image may change to reflect a change in state.

This window contains functions that deal with video, imaging, or the use of files that contains images. The details of each button follows.

Live Video Toggle



Pressing this button toggles the live video display on the mapping window. The button shows the word ON if video display is on, and the word OFF if video display is off.

Activated by:

- Press Live Video button in Imaging window
- Select Turn Video On/Off in Imaging menu
- Various functions throughout NeuroZoom that require a live image

Live video is defined as the display of continuous video frames in the mapping area of the mapping window. It may be 30 frames per second as in the case with Macintosh models that have color key digitizers, or less than 30 frames if it uses offscreen digitizing, such as with mask type digitizers.

In all cases except when expressively brought to your attention by NeuroZoom, the data that is in the mapping window is displayed as *graphic overlays* on top of the live video. This allows you to see what data has already been mapped from the live video images.

Grab Image

Press this button to grab one frame from the live video input of the camera. If the video is not on, it is turned on automatically, the image is grabbed, and the video then turned off. The image is then stored in NeuroZoom as a background image.

Activated by:

- Press Grab Image button in Imaging window
- Select Grab Image in Imaging menu
- Various functions throughout NeuroZoom that require a digitized image
- Press Grab button in Background Images window

Background images appear just like live video. They are in the background, meaning that graphical data appear overlaid on top of the image.

The image that was just grabbed will be saved to the document when the document is saved. The currently displayed image can also be saved to a file as a PICT or TIFF image. The currently grabbed image is also known as the current image.

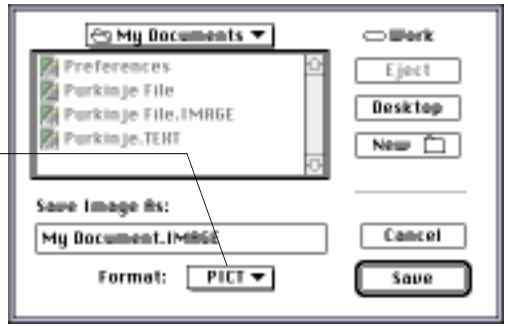
By The Way: Images scale and translate and remained fixed to the mapping coordinate system at their original location.

Save Image to File

Press this button to open a file dialog to save the current background image to file.

Activated by:

- Press Save Image to File button in Imaging window
- Select Save Image to File... in Imaging menu
- Press Save button in Background Images window



Press and hold on the **Format** button to get a popup for the two choices of file format for the image as PICT or TIFF.

If no background image is available (you can use the **Background Images** window to view all background images) and if live video is on, one image is grabbed. Live video is then turned off and the grabbed image becomes the first background image. This image is then saved to disk as a file.

Open Image from File

Press this button to open a file dialog to select and open a disk file containing a PICT or TIFF image as the current background image.

Activated by:

- Press Open Image from File button in Imaging window
- Select Open Image from File... in Imaging menu
- Press Open button in Background Images window

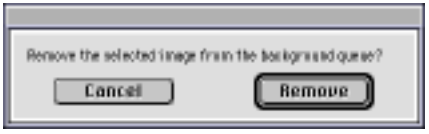
The size of the mapping area of the mapping window will adjust to the size of the image in the file.

Remove Current Background Image

Press this button to remove the currently displayed background image. You will be given a warning that you need to confirm.

Activated by:

- Press Delete Current Background Image in Imaging window
- Select Remove Image from List... in Imaging menu
- Press Remove button in Background Images window



The next background image in the document, if any, will then become the new current background image. If there are no background images in the document, an alert will appear.

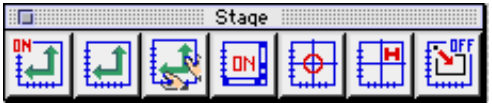
Stage Window

The **Stage** window contains functions that pertains to the stage.

Opened by:

- Stage Window in Map menu
- Command - 4
- Pressing Window Toggler in the Mapping window 
- Opening a new Mapping window if the preference is set to automatically open the Stage Window

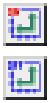
The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.



Alert: The control buttons on this window act as push buttons. Click once to activate a function. The button image may change to reflect a change in state.

This window contains functions that deal with aspects of the stage. The details of each button follows.

Stage Toggle



Pressing this button toggles the stage controller on the microscope. The button shows the word ON if the stage controller is on, and the word OFF if the stage controller is off.

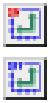
Activated by:

- Press Stage Toggle button in Stage window
- Select Turn Stage On/Off in Stage menu
- Various functions throughout NeuroZoom that require a the stage to be on or off

Many other NeuroZoom functions will turn off the stage. For example, zooming and grabbing the mapping window will turn this off. Use this button if you manually want to turn it off. Turning it off only affects what NeuroZoom can and cannot do programmatically with the stage controller. If it is off, NeuroZoom will not attempt to communicate with the stage. However, the stage is still physically powered on. You could still move it with the trackballs or joysticks attached to the stage controller.

When the stage controller is off, the other buttons in the Stage Window will be grayed out, since they function only on a stage controller that is on.

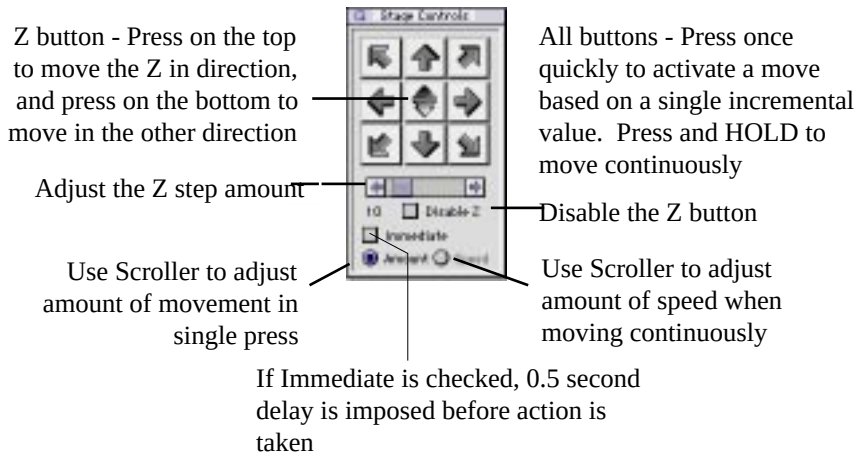
Stage Controls Toggle



Press this button to toggle the stage controller window on and off.

Activated by:

- Press Stage Controls Toggle button in Stage window
- Select Stage Controls window in Stage menu
- Pressing Window Toggler in the Mapping Window 



See section on **Stage Controls** window for more information.

Manual Move of Stage To New Location

Press this button when you want to move the stage to a new location, or when you actually have moved the stage to a new location.

Activated by:

- Press Manual Move of Stage To New Location button in Stage window
- Select Move Stage Manually... in Stage menu
- Command - M

This function is needed when the Automatic Behavior preference for automatically updating the stage preference during mapping is off. If it is on, the stage position is updated when data are entered. This function would only be needed if an explicit update from the stage controller is required.

Mapping Window XYZ Scrollers Toggle

Press this button to toggle the scrollbars in the mapping window for moving the X, Y, or Z axes. The button will show the word ON if the scrollbars are on, and the word OFF if the scrollbars are off.

Activated by:

- Press Mapping Window XYZ Scrollers Toggle button in Stage window
- Select Turn Map Scrollers On/Off in Stage menu

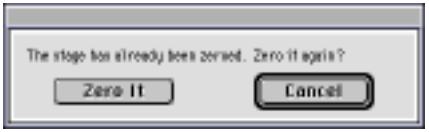
The scrollers will activate the computerized stage and move the stage in a particular axis. They are used to move the stage, not the image per se.

Zero the Stage

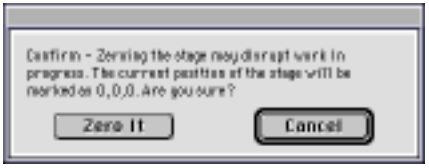
Pressing this button zeroes the stage controller to the current location of the microscope stage. If the stage controller has already been zeroed, a warning confirmation dialog appears.

Activated by:

- Press Zero the Stage button in Stage window
- Select Zero the Stage... in Stage menu



The final confirmation dialog then appears before zeroing the stage controller.



A zeroed stage controller establishes an origin in the mapping coordinate system so that all subsequent data entry are relative to this origin. A stage controller is zeroed usually for new documents. A fiducial that can be located with ease is typically positioned over the origin. This becomes the origin of the mapping coordinate system when the stage is zeroed, and the grid if displayed will show the origin as two solid X and Y lines in the center of the mapping window. Once the stage controller is zeroed, all data are mapped relative to this origin. If there is data already mapped to another fiducial, the data will appear misaligned.

The indication of the zero point can be altered by holding down the **Option** key. In this case, you move the mouse to the desired zero location in the mapping window and click the mouse button on that location to be the origin. The origin of the grid is then moved to that location, rather than defaulting to the center of the mapping window.

Tip: Use this method when the structure you want to be the zero fiducial point is on the screen already. It really doesn't matter where on the mapping window the zero fiducial is, as long as the origin of the mapping system falls on top of it.

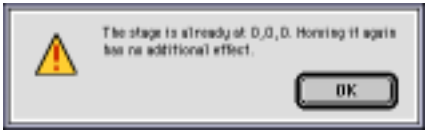
Home the Stage

Pressing this button homes the stage controller back to 0,0,0. A confirmation dialog displays.

Activated by:

- Press Home the Stage button in Stage window
- Select Home the Stage... in Stage menu

If the stage has already been homed, a warning dialog appears saying that this will have no effect.



Homing the stage does not destroy data. It only moves the stage back to the last known origin.

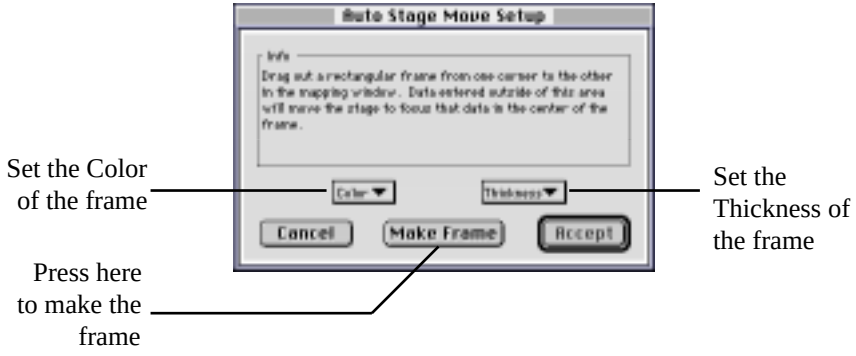
Auto Stage Move

Pressing this button toggles whether NeuroZoom moves the stage automatically when you position the mouse outside of a rectangular region. This rectangular region is the auto stage move frame. The button shows the word ON if the auto stage move is on, and the word OFF if the auto move is off.

Activated by:

- Press Auto Stage Move button in Stage window
- Select Auto Stage Move in Stage menu

This auto stage move frame must be initially configured. If this is the first time, the **Auto Stage Move Setup** window opens where you can create or adjust the auto stage move frame.



Subsequent presses of this button will toggle the frame on and off. Pressing the button with the **Option** key down will open the **Auto Stage Move Setup** window again.

Mapping Window Info Window

The **Mapping Window Info** window displays real time information pertaining to the configuration of NeuroZoom, the mapping window, tools, or data.

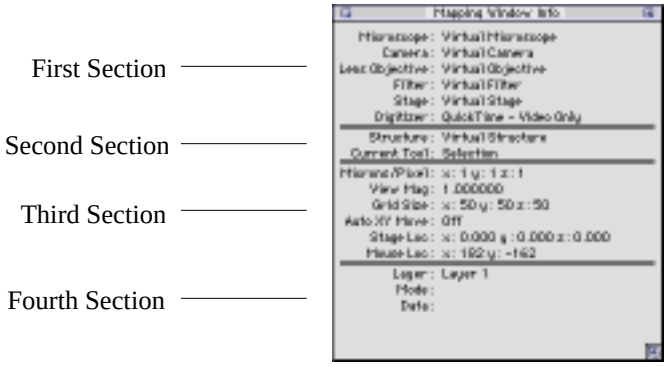
Opened by:

- Info Window in Map menu
- Command - 5
- Pressing Window Toggler in the Mapping window 
- Opening a new Mapping window if the preference is set to automatically open the Info Window

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.

The window is divided into 4 major sections.

- The first section shows the devices that are currently selected for use by the mapping window - the microscope, the camera, the lens objective, the filter, the stage, and the digitizer.
- The second section shows the currently selected structure that is in use by the mapping window, and the currently selected tool in operation.
- The third section shows some of the parameters associated with the devices - the scale ratios in microns/pixel for each of the three axes, the current magnification of the view selected, the size of the overlay grid in microns, whether Auto Stage Move is on or off, the current location of the stage in microns from the established home position, and the current mouse location in microns from the 0,0,0 origin.
- The fourth section shows the current layer selected for the mapping window, the mode of entry (ex., standard vs. stereology), and data pertaining to the last operation performed (ex., the area and length of a contour that was just closed).



Drag and Drop

The text in the window is draggable to the desktop. Click and hold the mouse down anywhere in the content area of the **Mapping Window Info** window and drag it to the Finder. A text clipping is deposited at the mouse location where the mouse button is released. The clipping is a text output of the information in the window.

Data Type Window

The **Data Type** window displays all of the structures and their data type currently mapped in the current mapping window. The layer for each structure object is also displayed. This window is useful to control the visibility of specific kinds of structures.

Opened by:

- Data Type Window in Map menu
- Command - 6
- Pressing Window Toggler in the Mapping window 
- Opening a new Mapping window if the preference is set to automatically open the Data Type Window

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.

Visibility of Data Type

The columns are:

V - Visibility of the data type. If checked, this structure of this data type is visible in the mapping window. To toggle the visibility, put the mouse cursor on the line and in the visibility column and press the mouse button once to toggle the visibility state. The effect is immediate.

Name - Name of the structure.

Type - The data type of the structure. Refer to the section in this chapter on Mapping Tools window for the different kinds of data types in NeuroZoom.

Layer - The data layer of the structure. Refer to the section in this chapter on the Layer Window. If the data belongs to a stereology layer, then Stereology is displayed.

Various buttons at the bottom of this window control different actions.

Sort - Toggles whether the sorting is by Type or by Name.

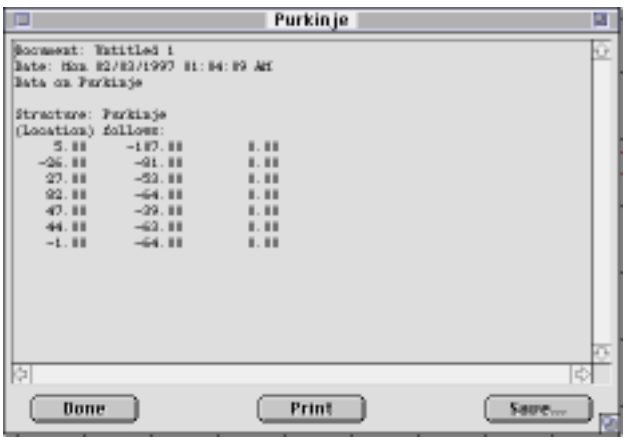
Show All - All data types are made visible.

Hide All - All data types are made invisible.

Print - All selected data types are printed as a report. This button is enabled only if there are selected items in the list.

Copy - All selected data types are copied to the clipboard as a report. This button is enabled only if there are selected items in the list.

Rprt - All selected data types are opened into a text window as a report. This button is enabled only if there are selected items in the list.



Drag and Drop

Dragging selected items to the Finder will also produce a text clipping of each of the selected items.

Alert: Press and hold the Option key when generating a report, or when dragging to the desktop to create a more simplified report of some stereology data suitable for copy and paste into spreadsheets. Also be aware of the preferences for Copy/Paste that can affect the output as well.

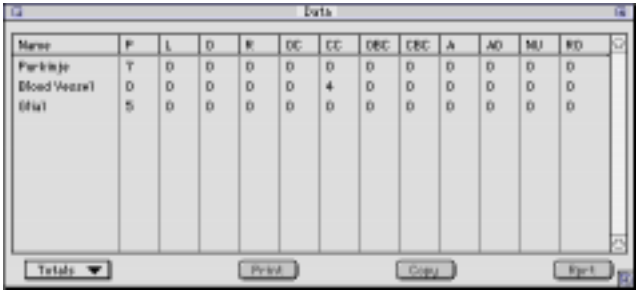
Data Window

The **Data** window displays all structures currently mapped in the current mapping window, and lists their total count per type of data.

Opened by:

- Data Window in Map menu
- Command - 7
- Pressing Window Toggler in the Mapping window 
- Opening a new Mapping window if the preference is set to automatically open the Data Window

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.



Each of the data types corresponds to one column. Refer to the section in this chapter on **Mapping Tools** window for the different kinds of data types in NeuroZoom. The column abbreviations are:

P. . . . . Point
L . . . . . Line
O . . . . . Oval
R . . . . . Rectangle

OC . . . . . Open Contour
CC . . . . . Closed Contour
OBC . . . . . Open Bezier Curve
CBC . . . . . Closed Bezier Curve
A. . . . . Arbor
AD . . . . . Arbor with Diameter
NU . . . . . Nucleator
RO . . . . . Rotator

Various buttons at the bottom of this window control different actions.

Totals - This is a popup menu that selects the mode with which totals are displayed:

Normal Mapping Only - Only those that are entered with normal mapping (i.e., not stereological data) are displayed as totals.

Stereology Mapping Only - Only the data collected with normal mapping tools during a stereological session are displayed. Note that these are not the data collected by the stereological probes. Those are generated only by reports by the stereological protocols.

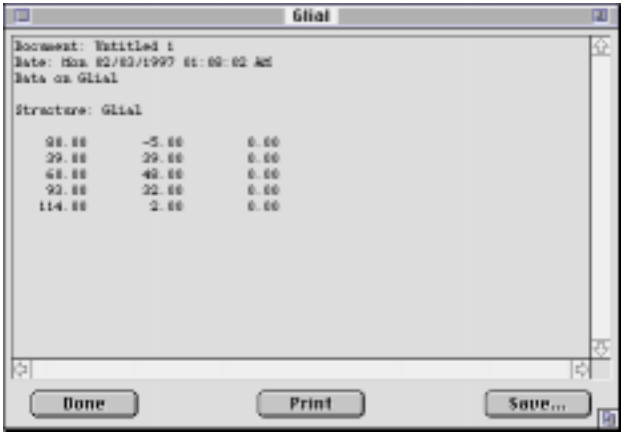
Total Mapping - The sum total of both the normal and stereological totals are displayed.

Print - All selected data are printed as a report. This button is enabled only if there are selected items in the list.

Copy - All selected data are copied to the clipboard as a report. This button is enabled only if there are selected items in the list.

Rprt - All selected data are opened into a text window as a report. This button is enabled only if there are selected items in the list.

Alert: Press and hold the Option key when generating a report, or when dragging to the desktop to create a more simplified report of some stereology data suitable for copy and paste into spreadsheets. Also be aware of the preferences for Copy/Paste that can affect the output as well.



Drag and Drop

Dragging selected items to the Finder will also produce a text clipping of each of the selected items.

Alert: Press and hold the Option key when generating a report, or when dragging to the desktop to create a more simplified report of some stereology data suitable for copy and paste into spreadsheets. Also be aware of the preferences for Copy/Paste that can affect the output as well.

Background Images Window

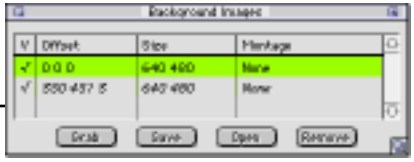
The **Background Images** window displays all background images opened in the current document.

Opened by:

- Background Images... in Imaging menu
- Pressing Window Toggler in the Mapping window 
- Opening a new Mapping window if the preference is set to automatically open the Background Images window

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.

Visibility of background image



These background images display behind the data, acting as a kind of template on which data are mapped. In this figure, the first image is positioned exactly on the origin. The second image is 350 mm to the right in the X axis, 451 mm down in the Y axis, and 3 mm up in the Z axis.

The current image is displayed in italics. In this case, the second image is the current image. The current image is the image that is operated on by the Image **Alignment Tool**, and is also the one that is displayed when **Spatial Image Mapping** is off. *Double-click* on an image to make it the current image.

The columns are:

V - Visibility of the background image. If checked, this image is visible in the mapping window. To toggle the visibility, put the mouse cursor on the line and in the visibility column and press the mouse button once to toggle the visibility state. The effect are immediate. Only that image of all images is affected.

Offset - This is the amount of offset in microns that the center of the image is offset from the mapping coordinate system origin.

Size - This is the amount of image in pixels as width and height.

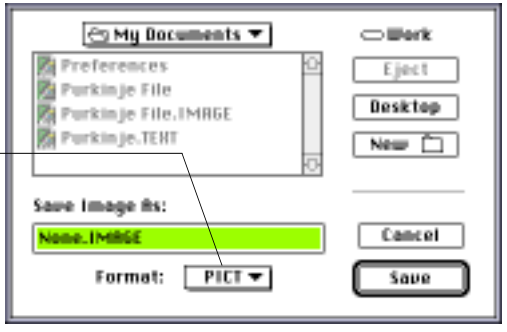
Montage - The name of the montage with which this image is associated is displayed here. If the image was grabbed separately, or opened from a file, the word None will be displayed here instead.

Various buttons at the bottom of this window control different actions.

Grab - This will grab one image from the video input, if the video input digitizer supports image digitization. The image grabbed is stored in the list of background images, and becomes the current background image.

Save - This will display a file dialog from which the selected images may be saved to the disk as a PICT or TIFF file. This button is enabled only if there are selected items in the list.

Press here for a popup menu with Image File Format choices



Open - This will display a file dialog from which a PICT or TIFF file can be selected and opened. The image opened is stored in the list of background images, and becomes the current background image.

Remove - This will remove the selected image from the list of background images. This button is enabled only if there are selected items in the list.

Drag and Drop

Dragging selected items to the Finder will also a picture clipping of each of the selected items.

Stage Controls Window

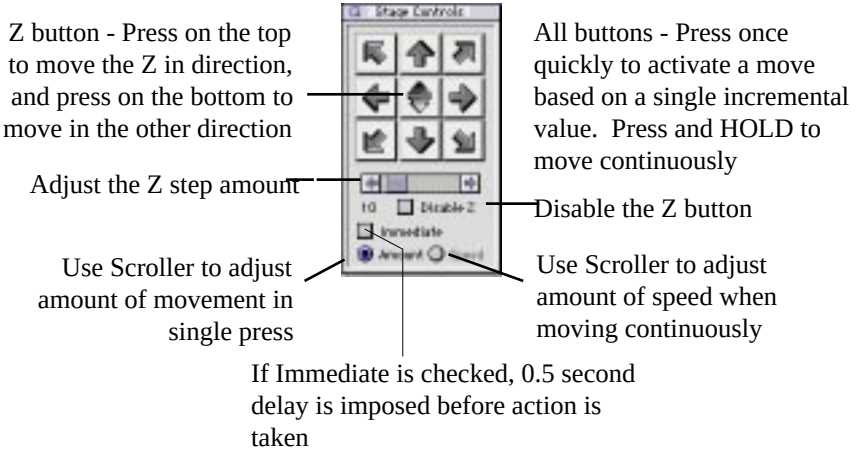
The **Stage Controls** window displays a software based controller for the stage controller that will work with any of the stages selected for the mapping window.

Opened by:

- Stage Controls Window in Stage menu
- Pressing Window Toggler in the Mapping window 
- Opening a new Mapping window if the preference is set to automatically open the Stage Controls Window

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.

This software joystick should not be used as a replacement for any hardware joystick or trackball supplied with your stage controller. Those will be much easier to use since this software joystick will have a certain amount of inherent non-responsiveness due to the nature of how the computer communicates with the stage controllers.



This controller moves the stage in any of the three axes - X, Y, or Z. The buttons are activated by one of two methods:

1. Press quickly to activate a single movement in the direction specified
2. Press and hold to move the stage continuously in the direction specified

Not all stage controllers support continuous movement. The virtual and manual keyboard do not support continuous movement.

The two radio buttons labelled **Amount** and **Speed** are used to determine how the scroller should operate. When Amount is on, the scroller will adjust the amount of movement that stage controller will move with one single press. When Speed is on, the scroller will adjust the speed of movement in continuous motion. 10% of the Amount is used for Z axis.

The **Speed** button will not be enabled if the controller has not been previously configured for a suitable amount of movement, or if the controller is not capable of moving continuously.

Alert: The target for the continuous motion is not really infinity. It is a value computed with regard to the Amount specified. Therefore, if the Amount is high, the target for continuous movement will be a long distance from the starting point. If the Amount if small, the continuous movement may only move a short distance. All of this is to provide a measure of safety and prevent the stage controllers from moving passed practical and safe limits, perhaps damaging the microscope slide, an objective, or the microscope itself.

Click on the **Disable Z** checkbox when you want to prevent the Z button from being active. This will prevent unintentional focus changes.

If the **Immediate** checkbox is selected, the movement, either single or continuous, is immediate. Otherwise, there is a 1/2 second delay before any action is taken.

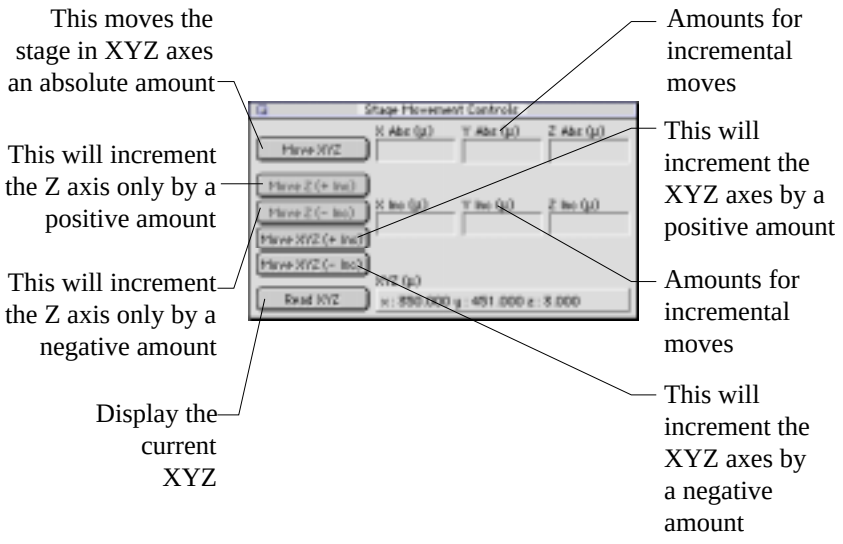
Stage Movement Controls Window

The **Stage Movement Controls** window can be used to move the stage in certain prescribed amounts, for example, to perform systematic stepwise movements though the tissue section.

Opened by:

- Stage Movement Controls... in Stage menu
- Opening a new Mapping window if the preference is set to automatically open the Stage Controls window

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.



Moving Absolute

To move the XYZ axes to an absolute location, enter in the target values for each axis in the fields labelled **X Abs (μ)**, **Y Abs (μ)**, and **Z Abs (μ)**. Only a floating point number can be entered into the fields. Then press the button labelled **Move XYZ**. The stage will immediately move this location. The field at the bottom of the window labelled **XYZ (μ)** will be updated with this new position. The **Mapping Window Info** window will also show this new position.

Moving Relative

To move relative to the current position, enter in the values into the fields labelled **X Inc (μ)**, **Y Inc (μ)**, and **Z Inc (μ)**. If you do not want a particular axis to move, enter 0 or leave the field empty. To move to the new position relative to these values, press the **Move XYZ (+ Inc)** button to move to the current position + the values, or press **Move XYZ (- Inc)** to move to the current position - the values. To move only the Z values regardless of the values in **X Inc (μ)** and **Y Inc (μ)**, press the button **Move Z (+ Inc)** or **Move Z (- Inc)**. The current position will be updated in all cases.

Stage Location Window

The **Stage Locations** window displays all stored stage locations for this document.

Opened by:

- Stage Locations... in Stage menu
- Opening a new Mapping window if the preference is set to automatically open the Stage Controls window

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.

Stage locations are wherever the stage happens to be when the position is recorded. By using this window, those locations can be returned to immediately.

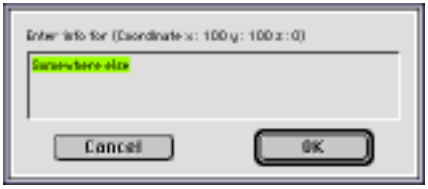
The columns are:

Info - Information that you entered when storing this location is displayed here.

Location - This is the location in microns of the stage when the location was stored.

Various buttons at the bottom of this window control different actions.

Store - The current stage location is stored when you press on this button. A dialog window opens asking for the information that you an optionally stored with this location.



Go To - The stage is moved to the selected stage location when this button is pressed. This button is enabled only if there are selected items in the list.

Info - The information for the selected stage location may be changed when this button is pressed. This button is enabled only if there are selected items in the list.

Remove - The selected stage location may be removed when this button is pressed. A confirmation will appear. This button is enabled only if there are selected items in the list.

Set Stage Limits Window

The **Set Stage Limits** window can be used to configure in software based limits on the XYZ movement.

Opened by:

- Set the Stage Limits... in Stage menu

This is useful if the stage controller does not have physical limit switches on the stage, or if you want to program in additional safety protecting the microscope equipment and the microscope slides.

To set the limits, move to the limits in each axis, left and right for the X, top and bottom for the Y, and front and back for the Z, and press the appropriate button to record the limit. The stage should already have been zeroed properly before using this feature. If there is any error, for example, the left side is greater than the right side, NeuroZoom will display the error so that you may correct it by moving the stage to the proper location.

If you make an error, press the **Restore Last Limits** button to put the last known values in place.

Once all of the limits are set, the **Store** button is enabled. Pressing on this button will store the limits in memory. You then need to explicitly turn on the feature to use the stored limits by selecting **Ignore Stage Limits** from the **Stage** menu so

that it is *NOT* checked. Check this item if a limit is reached and you decide that you really need to bypass the stored limit values.

When the limits are reached in any condition, for example, you attempt to move the stage using the software joystick or some other stage movement command, NeuroZoom will alert you that you have exceeded a limit on an axis.

Note that the virtual stage presets the limits at 8000 and -8000 microns in all directions. You can override this, but since the virtual stage does not actually move a physical stage, there is never any danger of any damage.

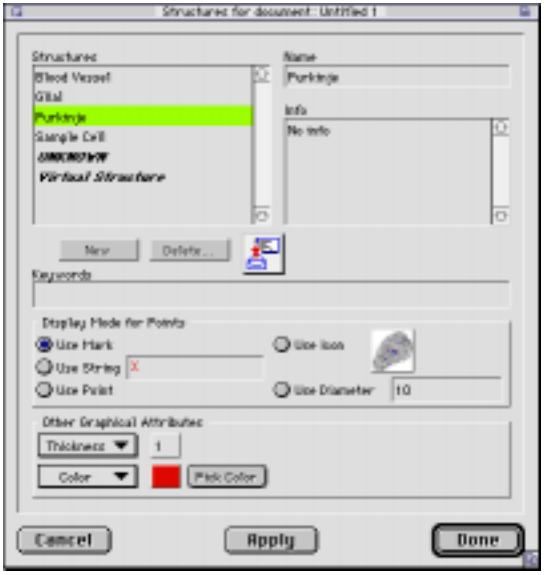
Structure Configuration Window

The **Structure Configuration** window contains all of the structures that can be used from the current document.

Opened by:

- Configure Structures... in Objects menu
- Pressing Window Toggler in the Mapping window 
- Pressing the Current Structure Field Popup menu in the Mapping window and selecting Configure all structures..., Configure current structure..., or Configure new structure...

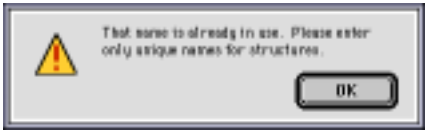
The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.



Editing Structures

The special structures named *UNKNOWN* and *Virtual Structure* cannot be edited.

- To edit a specific structure, select it from the scrolling field. The name and the information will be displayed in the **Name** and **Information** field.
- To change the name of a structure, select any part or all of its name from the **Name** field. Enter in the new text. The structure name in the scrolling field will be updated with each character entered. Only names that are unique are acceptable. When you click outside of the **Name** field, or select another function from this window, the uniqueness of the name will be verified. If it is not unique, an alert will be displayed asking you to reenter a unique name.



- To change the information associated with a structure, select any or all part of its information from the **Information** field. Enter in the new text.

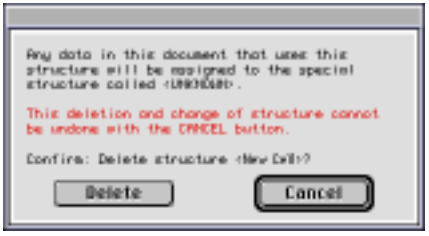
Making a New Structure

To create a new structure, press **New**. A new structure beginning with the name *Untitled* will be added to the scrolling list and automatically selected. This name will be unique. You can edit the name of the structure at this point.

Deleting a Structure

To delete an existing structure, select the structure from the scrolling list. The **Delete** button will be enabled. Press the button. You will be asked to confirm the deletion. Press **Delete**.

A second confirmation dialog appears warning that any data associated with the structure to be deleted will be relinked to the structure named *Unknown*. Press **Delete** to really delete the structure, or press **Cancel** to abort the deletion.



Alert: Note that data are never deleted. Only the structure definition is deleted.

Exporting Structures

To **export** selected structures to a file that may be stored for later use in another mapping document, select the structures (shift clicking to select multiple structures), then drag the selection to the desktop. A file named *Structure n* will be created. The number n will be some number chosen to keep the file name unique in case there are other structure files on the desktop.

Alert: System 7 and come copy utilities combine to produce a strange delayed effect on the Macintosh when dragging and dropping to create files. It is possible that the structure file will not appear immediately on the desktop. At some later point when the Finder decides to update the contents viewable on the Desktop will the file icon then be seen. One workaround is to use the Finder Find command and actually search for the file based on its root name of Structure. When the Find command finds the file, select it and open its

enclosing folder (which is the Desktop). This will force the Finder to immediately display the file.

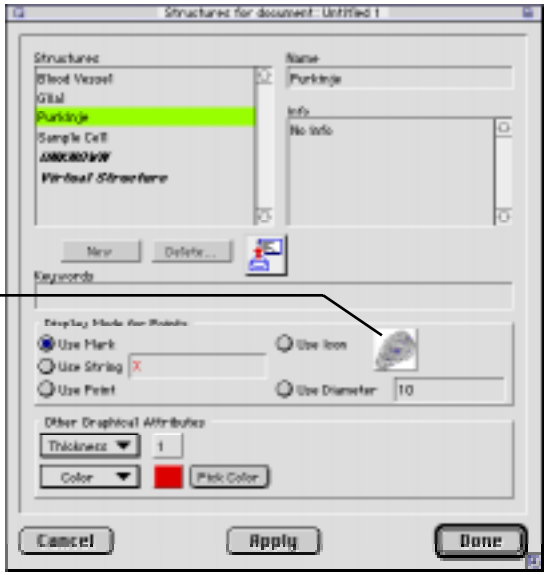
Importing Structures

To **import** a structures file, drag the file from the Finder and drop onto the import icon. Only unique structures will be imported.

Editing Graphical Attributes

To edit the attributes of an existing structure, select the structure from the scrolling list. The controls under the **Keyword** field will be enabled. All of the options in the **Display Mode for Points** box have to do only with point objects. Every point is displayed at its location in the mapping window as one of the following:

- **Mark** - a 3 by 3 square pixel box is drawn
- **String** - a string of any styled text is drawn. Enter in the text by clicking the mouse in the field next to the Use String radio button, and typing any characters
- **Point** - a single pixel is drawn
- **Icon** - an ICON is displayed. One default icon is used for all new structures. To enter in one of your own, copy into the clipboard any PICT object from any other application. Then press on the ICON button



Press here to import a PICT as an ICON for the selected structure

A window will appear asking if you want to import the PICT object as the ICON. Press **Import**. Another way to import a PICT is to drag and drop a Finder graphics clip file to the ICON button.

Alert: Please note that not all PICT objects will behave properly. NeuroZoom will attempt to scale down the image to fit within a 32 by 32 pixel boundary for the ICON. If the ICON is too large, that particular PICT image is not a suitable import source.

- **Diameter** - a round, filled circle is drawn whose diameter is the size specified in microns. Enter in the size by clicking the mouse in the field next to the **Use Diameter** radio button, and typing any floating point value.

In the Other Graphical Attributes box, other attributes can be configured:

- **Thickness** - the thickness of the line drawn for lines, ovals, rectangles, contours, and bezier curves can be selected from this popup menu as a value 1 to 6. The selected value is shown in the field to the right of this menu button.

- **Color** - the color of any graphical object can be selected from this popup menu of popularly used colors. If you want a specific color, press on **Pick Color**, and the Apple color wheel window will open. The selected color is shown in the field to the right of this menu button.

Storing Changes

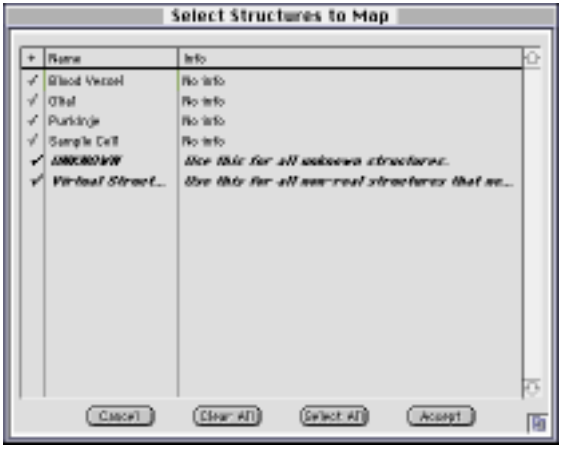
The changes may be viewed by pressing on the **Apply** button. All data affected by structure changes will be redrawn in the mapping window for these structures. Pressing the **Done** button will store these changes in the document. Pressing the **Cancel** button will abort all changes and redraw the mapping window to the state before the current changes, and not store any changes to the document.

Select Structures to Map Window

The **Select Structures to Map** window lists all of the structures from the current document that can be used in the mapping window for mapping.

Opened by:

- Select Structures to Map... in Objects menu
- Pressing the Current Structure Field Popup menu in the Mapping window and selecting Add/Delete more structures to this map window list...



Many structures may be defined for a document, but only a small subset may actually be used in one experiment of the document. Press in the first column labelled + to select a particular structure to be available in the list of structures for a mapping window. Press again to clear the column to remove that structure from eligibility as a mapping structure. Note that this does not delete data. It only removes the structure from being selectable as a current mapping structure.

Press the **Clear All** button to clear all objects from being selectable. The exception is *UNKNOWN* and *Virtual Structure*. These are always available in the mapping window as a selectable structure. Press the **Select All** button to select all

of the structures. Press the **Accept** button to accept the current list as the selectable structures. Press the **Cancel** button to abort any changes.

Layers Window

The **Layers** window displays all layers of the document. Whether a layer is active or visible can be controlled from this window.

Opened by:

- Configure Layers... in Objects menu
- Pressing Window Toggler in the Mapping window 

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.

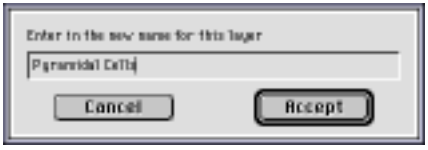
Layers are used to organize the data into manageable parts. Data can go into containers known as layers. Each layer can be shown, hidden, activated for data entry, or inactivated to protect it against data entry (and thus change).

Layers can be used to hold different parts of an entity that you might be mapping. For example, boundaries of the cortical surface can go into a layer called pial surface. Different regions can go into a layer called regions. Cortical layers can

go into a layer called cortical layers. Cell can go into a layer called cells, and fiber can go into a layer called fibers.

Making a New Layer

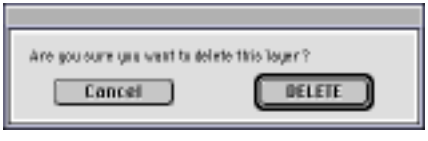
Press the **New** button to create a new layer. A unique name will be created for this new layer. If you want to rename it, select the layer and press on the **Rename** button and enter in new name. Its uniqueness will be verified.



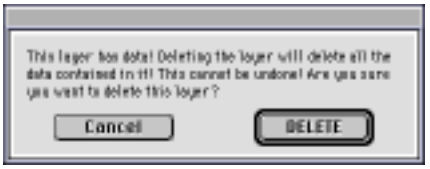
Likewise, select the layer and press the **Info** button to enter in information for this layer.

Deleting a Layer

To delete a layer, select the layer and press the **Delete** button. Only one layer may be selected at a time in this window. A confirmation window displaying information is displayed if this layer has no data. Press the **Delete** button will immediately delete the layer.



If there is data in this layer, another confirmation will be displayed warning that there is data, and that deletion of the layer cannot be undone.



Pressing the **Delete** button will delete the layer immediately. Note that the last layer in the document cannot be deleted.

Accepting Changes

The **Accept** button will make any changes immediate and permanent to the document. This includes any deletion of layers. Therefore, if you make a mistake in deleting a layer, do not press the **Accept** button. Instead, press the **Cancel** button to abort all changes. Note that once the **Accept** button is pressed, the **Cancel** button is disabled.

Controlling Layers

- To show all layers, press the **Show All** button. All layers will be checked as visible. Press the **Hide All** button to uncheck visibility of all layers.
- To activate all layers, press the **Activate All** button. All layers will be checked as activated. Press the **Inactivate All** button to uncheck activation of all layers. An activated layer is eligible to receive new data. Otherwise, it is considered locked and protected against changes.
- To check specific layers for visibility or activation, press the mouse in the column labelled **A** for activation, or **V** for visibility. The checkmark will toggle on and off indicating the state of the layer for that action.

  The data column will show a *Yes* if there is data in a layer, or *No* if there is none.

Alert: There is always one current layer. This current later is drawn in italics style. *Data can still go into an inactive layer if that layer is selected as the*

current layer. Inactive layers are best used to prevent accidental editing of existing data.

Create Montage Window

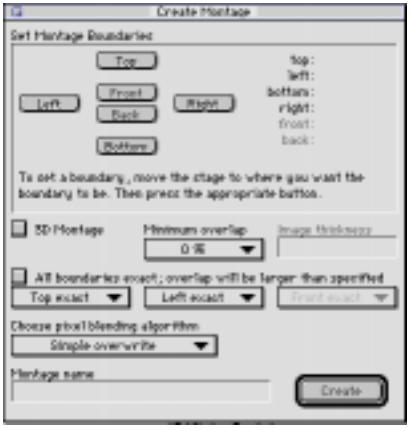
The **Create Montage** window contains all of the functions needed to create a montage across the microscope slide section.

Opened by:

- Create Montage... in Stage menu

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.

If a motorized stage is configured, the entire montage can be captured automatically. Because images act precisely the same way as live video, this montage can be treated as a “mounted” tissue section, and analyzed off-line from the microscope.



Move the stage to the top boundary and press the **Top** button. This will be the top of the montage being assembled. Move the stage to the bottom boundary and press the **Bottom** button. This will be the bottom of the montage being assembled. Repeat for the left and right sides. These four dimensions are sufficient to begin a 2D montage. The **Create** button will now be highlighted.

Select the amount of minimum overlap desired by pressing on the **Minimum Overlap** button for a popup menu. This is a value from 5 to 40% and represents the amount that the an image will overlap an adjacent image. This could be useful if there is some geometric aberrations from the lens objectives that are preventing a planar image from being captured.

Choose from the popup menu labelled **Top Exact** to choose whether then top edge or the bottom edge will be exact (*Top Exact* or *Bottom Exact*); i.e., the edge remains as it was imaged, and the overlap is on the opposite side. Therefore, depending on the amount overlapped, the opposite side will be truncated. For example, if *Top Exact* is chosen, the top will be as imaged, but the bottom will be truncated by the amount of overlap specified. Make the same selection for *Left Exact* of *Right Exact*.

If all boundaries are to be exact, check the checkbox labelled **All boundaries exact, overlap will be larger than specified**. In this case, the boundaries will be as imaged by adjusting the internal boundaries will be adjusted. The amount of internal overlap will thus be a little larger than specified, depending on the size of the montage.

Select the method of blending the pixels in the overlap area. The default is **Simple overwrite**, meaning that the overlapping boundary pixels simply replace the underlying ones. There are no other algorithms in place yet.

Enter in the **Montage Name** for this montage for identification purposes. The name is associated with each image to help distinguish it from other images captured for this document.

When ready to create the montage, press the **Create** button. A window will open displaying how many images will be collected. All images are cached on disk. They are not stored in memory. Therefore, you can collect a fairly large montage, limited only by disk space. However, the montage does have to be rendered, and depending on the final magnification of the mapping window, the rendering of the images can be time-consuming.

If video is available, live video is turned on automatically. The stage, if controlled by NeuroZoom, will move to the first calculated field of view for the montage.

A progress dialog is displayed showing which image of the set is being captured. Press on the dialog's **Cancel** button to stop a montage collection.

When completed, the montage is rendered in the mapping window. The current magnification is used, and since this is generally the field of view captured for the montage, not much will be seen at this point. Use the **Zoom** tool to zoom the montage in and out.

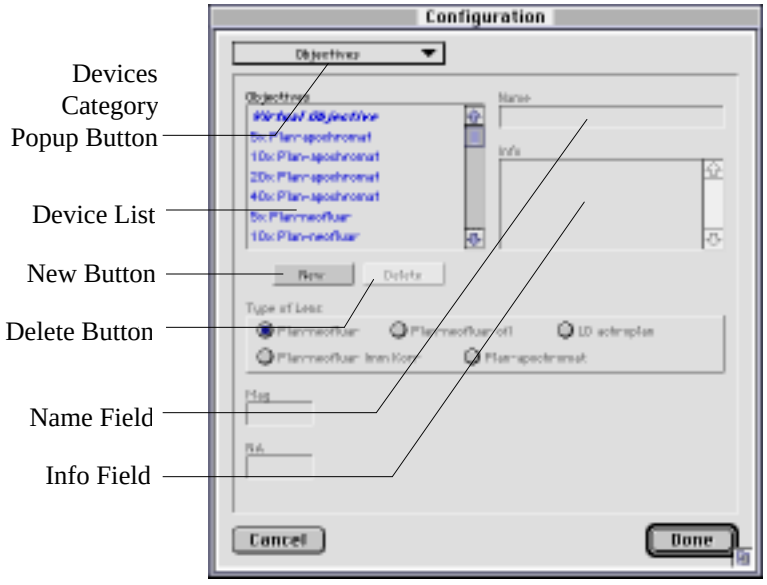
Tip: Some montages can be large, and more images slow down the final rendering of the map image. The time to render depends on how many images are cached total, how many of these intersect the viewable region of the mapping window, and the amount of data to be drawn on top of the images. If you are experimenting with different zoom levels, try using the menu selection **Enter Zoom...** from the **Objects** menu. The current scale is shown as a default, and from this you can estimate what scale you prefer, enter it in directly as a floating point value, and NeuroZoom will scale directly to that value.

Configuration Window

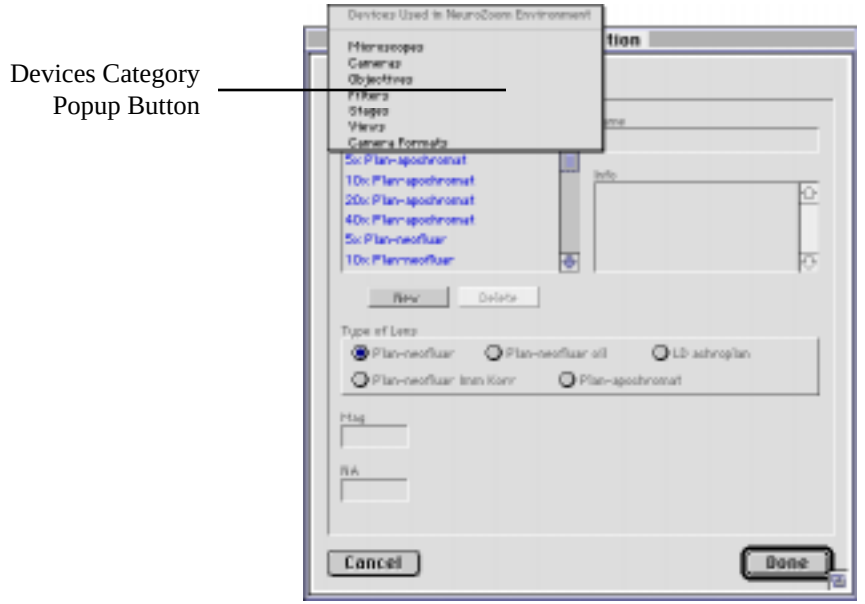
The **Configuration** window contains all of the functions needed to configure the hardware used in NeuroZoom.

Opened by:

- Configure Devices... in File menu



The window opens with Objectives displayed in the popup menu button at the top left of the window. This is the **Devices Category Popup** button. Click and hold on this button to see the different categories of devices.



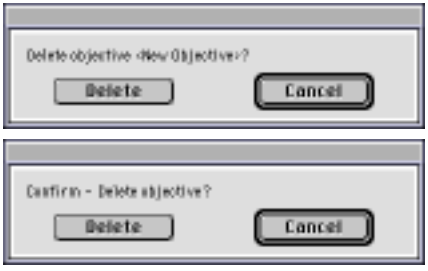
Each of the devices displays different information. The upper half of the window remains the same where the list of each device in the current device category is displayed in the scrolling field, along with its name and any information associated with it, and two buttons named **New** and **Delete**. The bottom half of the window changes depending on the device category.

General Information

Any devices beginning with the word Virtual cannot be edited.

- To edit a specific device, select it from the scrolling field. The name and the information is displayed in the **Name** and **Information** field.
- To change the name of a device, select any part or all of its name from the **Name** field. Enter in the new text. The device name in the scrolling field will be updated with each character entered. Only names that are unique within the current device category are acceptable. When you click outside of the **Name** field, or select another function from this window, the uniqueness of the name will be verified. If it is not unique, an alert will be displayed asking you to reenter a unique name.

- To change the information associated with a device, select any or all part of its information from the **Information** field. Enter in the new text.
- To change the keywords associated with a device, select any or all part of its keywords from the **Keywords** field. Enter in the new text. These keywords are a special field that can be used to locate specific structures.
- To create a new device in the current device category, press **New**. A new device beginning with the name *Untitled* will be added to the scrolling list and automatically selected. This name will be unique for this category. You can edit the name of the device at this point.
- To delete an existing device, select the device from the scrolling list. The **Delete** button will be enabled. Press the button. You will be asked to confirm the deletion twice.

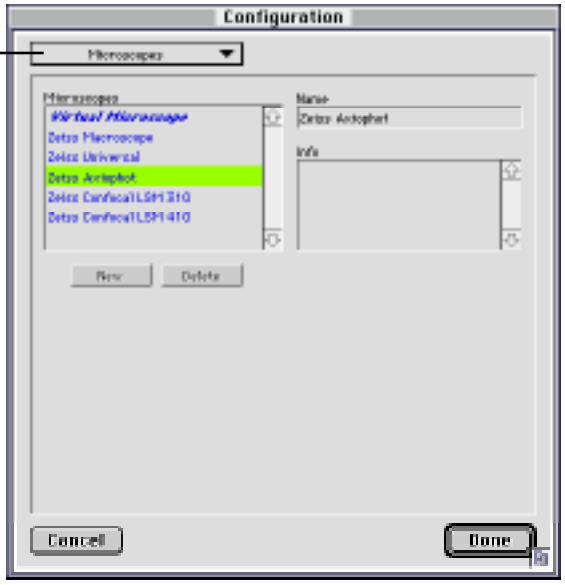


Alert: The devices that are configured or made here apply to ALL documents opened and used by NeuroZoom on this particular Macintosh. The configurations are stored in the System Preference folder, and not in the document. In fact, a document does not have to be opened in order to configure the devices.

Microscopes

All microscopes to be used by NeuroZoom on a given Macintosh need to be configured. To do this, select **Microscopes** from the **Devices Category Popup** button to get to the **Microscopes Devices** window.

Select Microscopes from this popup menu



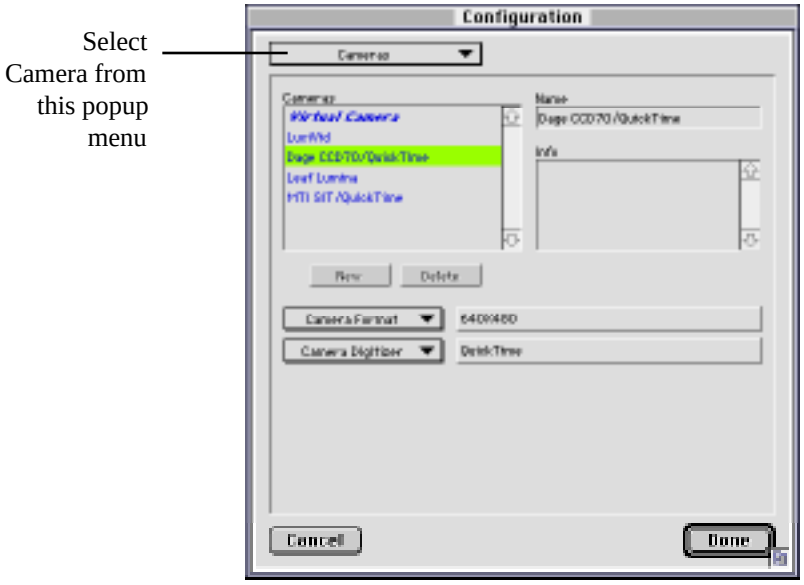
Follow these steps to create or edit a microscope device:

1. Press **New** to create a new microscope, or single click on a stored microscope to select it for editing.
2. If this is a new microscope, enter in a name and any information you want to associate with it.

Microscopes will be used to create **View** devices, described later.

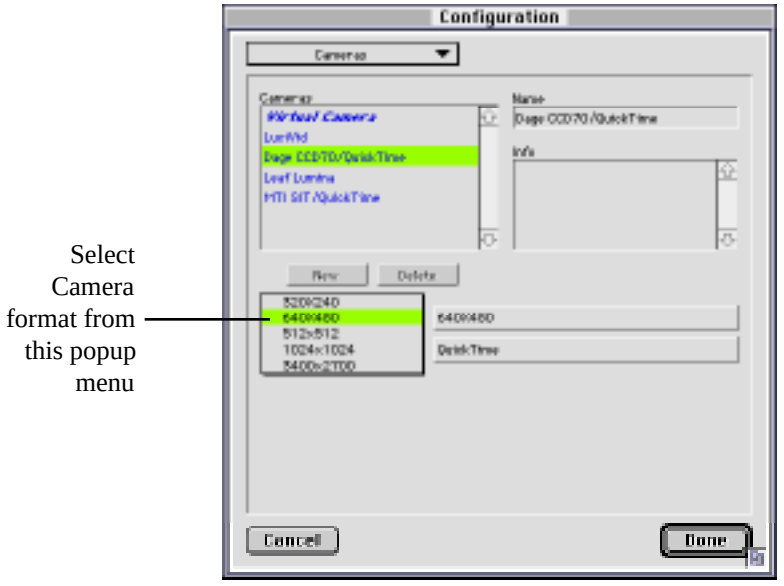
Cameras

All cameras to be used by NeuroZoom on a given Macintosh need to be configured. To do this, select **Cameras** from the **Devices Category Popup** button to get to the **Cameras Devices** window.



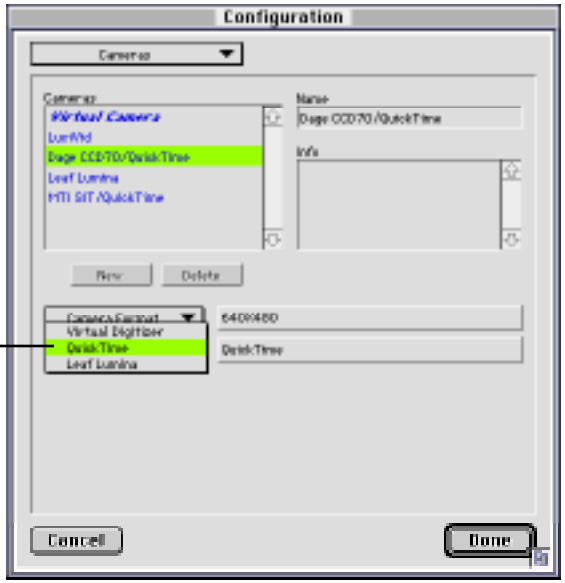
Follow these steps to create or edit a camera:

1. Press **New** to create a new camera, or single click on a stored camera to select it for editing.
2. If this is a new camera, enter in a name and any information you want to associate with it.
3. Press and hold on the **Camera Format** popup menu button to select the format for this camera. The format is the width and height of the video image. These are the actual available configurations from the **Camera Format** category.



Select Camera format from this popup menu

4. Press and hold on the **Camera Digitizer** popup menu button to select the digitizer for this camera. These are the actual available configurations from the **Camera Digitizer** category.



Select Camera Digitizer from this popup menu

Choose the proper camera format that matches the camera. Most NTSC and RS170 cameras in the US are preset at 640 by 480 pixels of resolution when digitized by a Macintosh QuickTime digitizer set for NTSC scan rates.

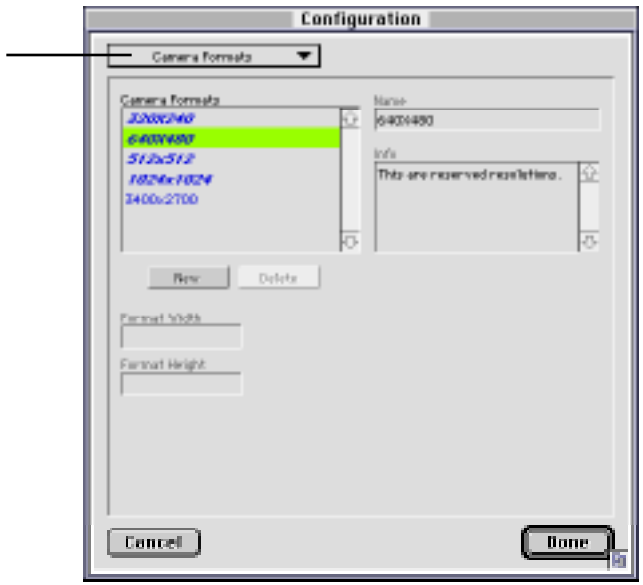
AV capable Macintosh models come with onboard electronics that can digitize a video signal and display it in a Macintosh window as live color video. In this case, the camera digitizer will be QuickTime. The Virtual Digitizer is used when there is no available electronics digitizer, such as a PowerBook. The Leaf Lumina digitizer is selected when Leaf Systems Lumina digital camera is connected to the SCSI port of the Macintosh for high-resolution digital images.

Alert: The Leaf Lumina driver is currently unsupported.

Cameras will be used to create View devices, described later.

Camera Formats

All camera formats are configured with this device category. To do this, select **Camera Formats** from the **Devices Category Popup** button to get to the **Camera Formats Devices** window.

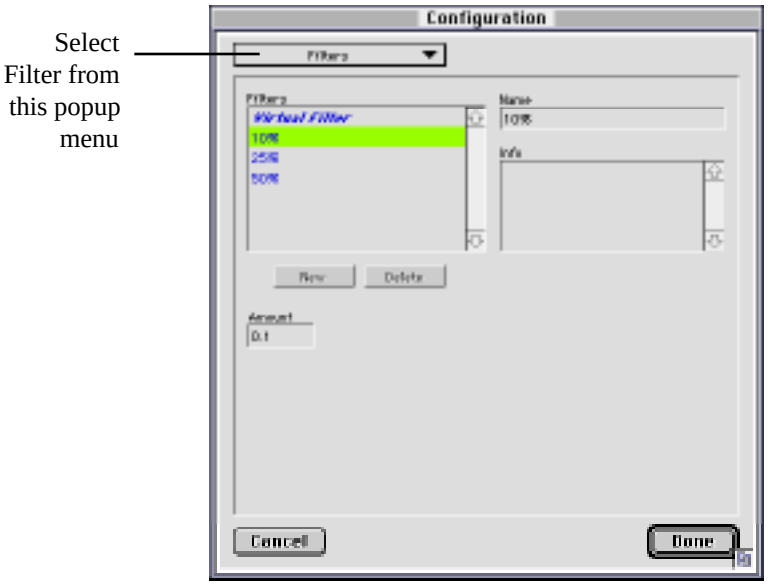


Follow these steps to create or edit a camera format device:

1. Press **New** to create a new camera format, or single click on a stored camera format to select it for editing.
2. If this is a new camera format, enter in a name and any information you want to associate with it.
3. Enter in the **format width** and the **format height** in pixels in the text fields. This information will be used to resize the mapping window when a camera with this format is chosen for use in the mapping window. Typical formats are 640 x 480 for NTSC cameras, and 512 x 512 for square pixel cameras.

Filters

All filters are configured with this device category. To do this, select **Filters** from the **Devices Category Popup** button to get to the **Filters Devices** window.



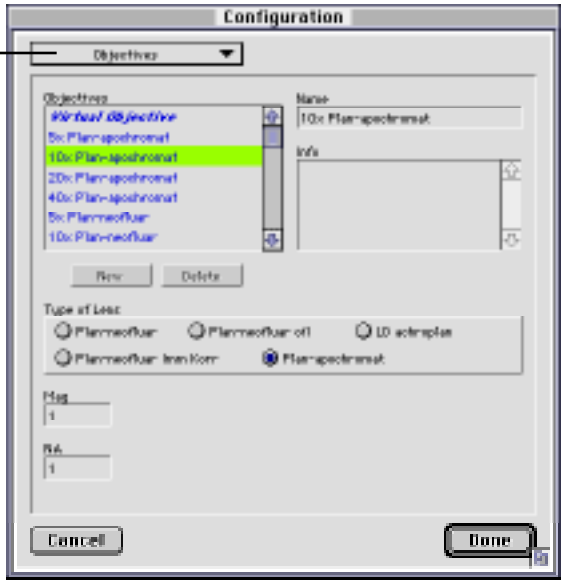
Follow these steps to create or edit a filter device:

1. Press **New** to create a new filter, or single click on a stored filter to select it for editing.
2. If this is a new filter, enter in a name and any information you want to associate with it.
3. Enter in the amount of filter that is expect from this filter into the field named **Amount**. This value should vary from 0 to 1.

Objectives

All lens objectives are configured with this device category. To do this, select **Objectives** from the **Devices Category Popup** button to get to the **Objectives Devices** window.

Select Objectives from this popup menu



Follow these steps to create or edit a lens objective device:

1. Press **New** to create a new lens objective, or single click on a stored lens objective to select it for editing.
2. If this is a new lens objective, enter in a name and any information you want to associate with it.
3. Enter in the type of lens - Plan-neofluar, Plan-neofluar Oil, Plan-neofluar 1mm Korr, LD acroplan, Plan-apochromat.
4. Enter in the **Magnification** and the **Numerical Aperture** (NA) into the fields.

Lens Objectives will be used to create **View** devices, described later. All physical lens objectives that you will use on the microscope with NeuroZoom should be listed here.

Stages

NeuroZoom can control a motorized stage on the microscope to drive the field of view in the X and Y direction, and in the Z axis for focusing. The motorized stage generally has its own controller interface. In the case of the Ludl, the Zeiss, the Prior, and the ASI stage systems, a separate chassis connects to the stepper motors of the stage, and a cable interfaces between the chassis and the computer. The type of the cable and the connection to the Macintosh will vary.

Stage Controllers

Either GPIB (IEEE) or RS232/422 serial communications may be used with the Zeiss or the Ludl. The Prior and ASI only supports RS232 serial communications. Please refer to appendix on ***Stage Controllers*** if one is not supplied by the stage manufacturer.

- **GPIB** - the only supported GPIB board is from National Instruments. Either the NuBUS or the PCI bus board may be installed in the Macintosh. Please refer to the instructions supplied with the board to install both the board and software drivers. You will also need to know the address and board number when configuring the board within NeuroZoom.
- **Serial** - if you are using Localtalk, the Printer port will be occupied. You must then use the Modem port to connect the microscope stage controller to the Macintosh. BUS based serial boards may be used if the manufacture provides the proper software drivers to make the board appear as a communications toolbox serial port, that is controllable by the Communications Toolbox Manager.

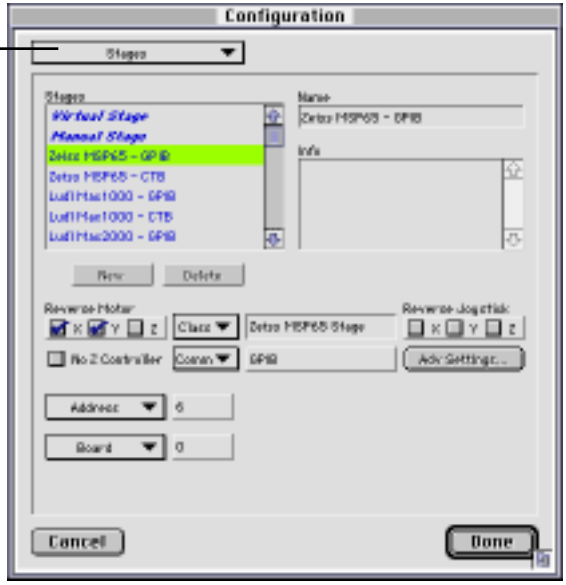
In addition to the motorized stages, there are two other special stage drivers:

- Virtual Stage
- Keyboard Stage

These will be explained later.

Once the stage controller is connected properly to the Macintosh computer, some configuration of parameters may be necessary. To do this, select **Stages** from the **Devices Category Popup** button to get to the **Stages Devices** window.

Select Stages from this popup menu



Virtual Stage and *Keyboard Stage* are special devices. These cannot be altered.

- **Virtual Stage** - this is a virtual device as described in the section on ***Devices*** in the previous chapter on ***The Basics***. If no physical stage is available, choose this one so that most of the NeuroZoom functions may operate properly. The coordinate system in the mapping window of NeuroZoom will act as if a stage controller were really moving a motorized stage. The coordinates of data will be offset properly when the scrollbars or the stage controller are used. The virtual stage should also be used when multiple images are being imported and montaged manually with the **Image Alignment** tool. This will allow you to traverse the entire montage as if you were using a real, physical stage.
- **Keyboard Stage** - this is a stage controller device that intercepts all requests to and from a stage controller, and brings up a dialog box asking for stage coordinates for the three axes, or to display the coordinates that you need to enter in separately, or to record on paper. Use this when you might have a keyboard readout stage controller system on the microscope, but it is not currently connected to the Macintosh. When NeuroZoom prompts you to move the stage, use your stage controller keyboard to enter in the coordinates displayed by NeuroZoom. When NeuroZoom wants a coordinate readout,

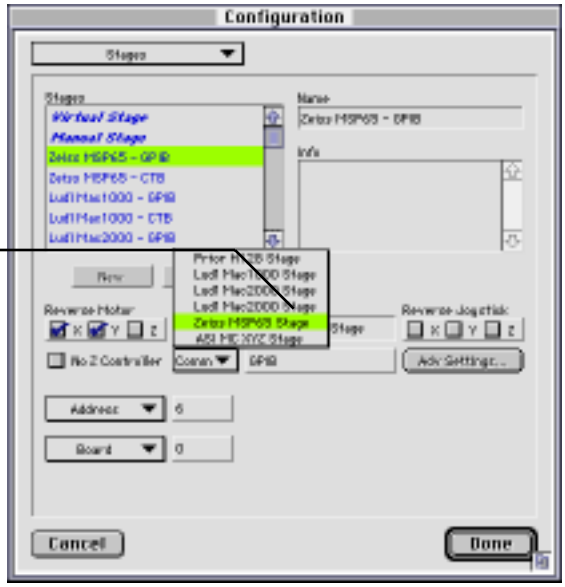
enter in the coordinates as displayed by your stage controller system into the NeuroZoom dialog window. In essence, you are acting as the stage controller driver, interpreting requests, translating them, and providing the communications that is missing physically between NeuroZoom and the stage controller.

Follow these steps to create or edit a stage device other than virtual or keyboard.

1. Press **New** to create a new stage, or single click on a stored stage to select it for editing.
2. If this is a new stage, enter in a name and any information you want to associate with it.

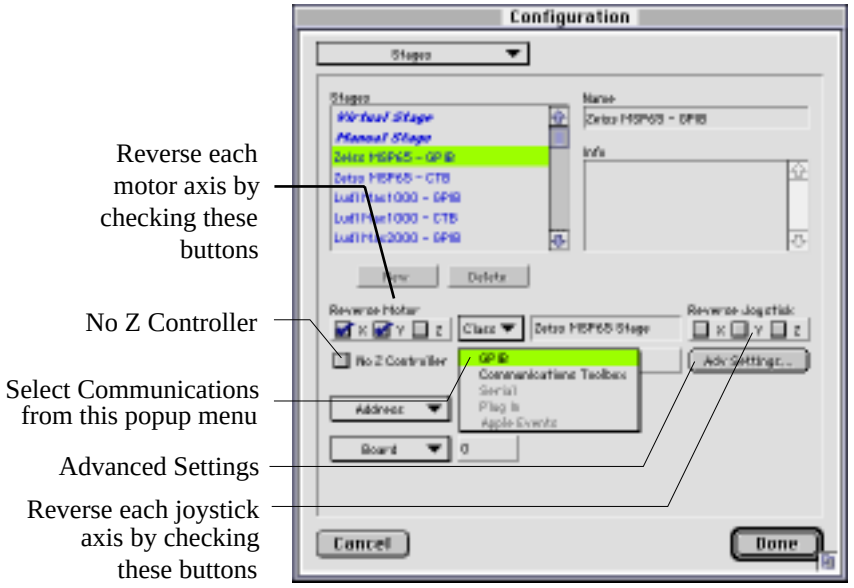
Once a new stage device is created, or an existing one is being configured, there are several options available.

Select Class of stage from this popup menu



Stage Class

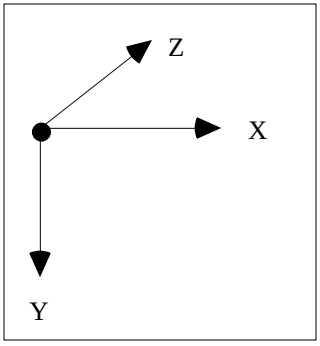
Choose the correct class of the stage connected to the Macintosh. The classes are preconfigured with a driver written in software code for supported controllers. New controllers from other manufacturers cannot be added without additional software code. Press and hold on the button named **Class** to get a popup menu.



Reverse Axis

If an axis is to be reversed because moving the stage controller in NeuroZoom produces the opposite affect, click on the appropriate axis. Most of the pre-configured stage devices have the proper axis direction configured.

In particular, you should be sure that the Z is properly configured such that focusing through the tissue section from top to bottom produces an *increase* in the Z stage position (by noting the readout from NeuroZoom in the Mapping Window Info window). The mapping system is configured so that Z increases in value numerically as you focus through the section.



Noting the position on the microscope focus knob is not sufficient to determine whether the motor for Z should be reversed. This needs to be determine in concert with the stage controller for your microscope. The stage controllers supported directly by NeuroZoom should be preset properly for most microscopes, but if you need to confirm this, follow these steps:

1. Be sure that the stage controller communicates with the computer via NeuroZoom. Configure the communications settings using the information in this chapter section, and connect the proper cables. Then opening a mapping window and use the Microscope Setup Window to select your stage controller
2. Open the Stage Movement Controls Window. In this window, set up a Z movement amount that is discernible by visually reading the focus knob on the microscope. For example, 10 microns. Make sure that the lens objectives are position away from the stage!
3. Note the position as indicated by the *focus knob* on the microscope.
4. Press the Move Z (+ Inc) button. This will move the stage to a new position.
5. Note the new position as indicated by the *focus knob* on the microscope.
6. If the second reading is *lower* than the first reading, the checkbox for the Reverse Motor for Z should *not* be checked. This is the normal default setting.
7. If the second reading is *higher* than the first reading, the checkbox for the Reverse Motor for Z *should* be checked. This combination of stage controller and microscope are reversing the focus readout positions that NeuroZoom wants for its mapping system. Reversing the Z axis will correct for this.

Reverse Joystick

If an axis is to be reversed on the joystick because it feels incorrect (i.e., you would like to push the joystick to the right to move the view relative to the camera, rather than relative to the field), click on the appropriate axis.

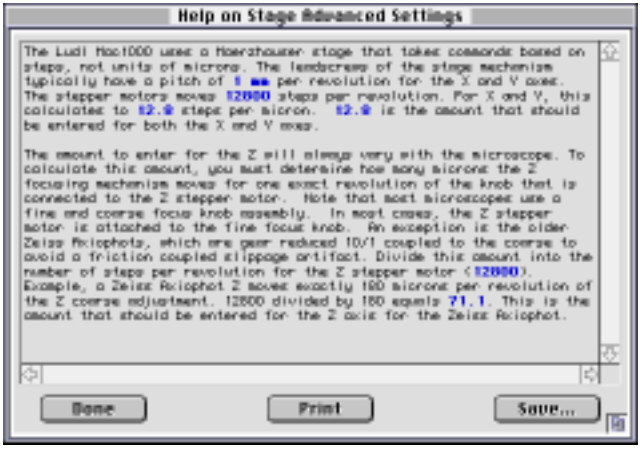
No Z Controller

If no Z controller is available, or if you want to disable it, check this box. Virtual and Manual stage always have this Z controller enabled.

Advanced Settings

Press this button to get additional advanced settings. What displays depends on the stage controller that is selected. The following figures apply to the Ludl Mac1000 controller.

This asks for the steps per micron for each axis. The Ludl moves the stepper motors by sending pulses as steps. The number of steps per micron must be calculated and entered. The default values for the X, Y, and Z axes are shown. Press **Help** for more information.



Both Ludl Mac1000 and Mac2000 have an additional setup window for the motor addresses.

Enter the proper address for each motor axis. The defaults are displayed for each controller. Press Help for information on motor addresses for this controller.

See the appendix on ***Stage Controllers*** for more information on understanding this information and what values to enter for advanced settings.

Communications

Press and hold on the **Communications** button to get a popup menu. Choose from the following selections.

- GPIB
- Communications Toolbox
- Serial
- Plug In
- Apple Events

Any grayed out item indicates that it is not available for use. Once again, pre-configured stage controllers already have the proper communications mode selected.

GPIB communications requires an installed NuBUS or PCI GPIB (IEEE) board, with its software drivers loaded. Only those boards from National Instruments are supported by NeuroZoom.

Communications Toolbox uses the *Serial Tool* extension. This provides for RS232 and RS422 serial mode communications with the stage controller.

Serial communications uses the serial ports as above, but does not require the Communications Toolbox Manager.

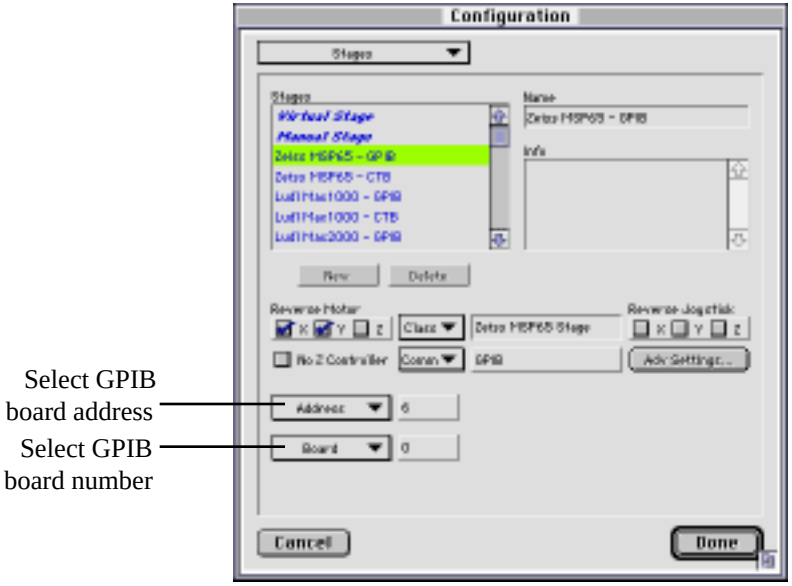
Plug In communications uses a plug-in that is specifically designed for the microscope stage controller.

Apple Events communications require a separate application communicating with the stage controller. That application becomes a proxy for NeuroZoom. NeuroZoom communicates with the proxy application via Apple Events. This allows users to program their own applications to control a non-supported stage controller.

Alert: Some options are not yet available and will be disabled.

GPIB Settings

If GPIB is chosen as the communications mode, additional options appear to configure the GPIB parameters.



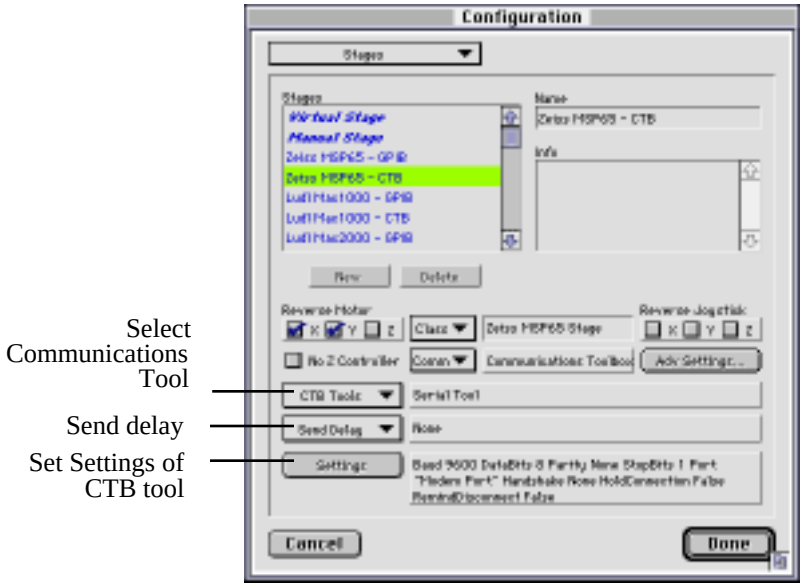
Select GPIB board address

Select GPIB board number

The **address** and the **board number** must be entered. Both depend on how the GPIB board is configured. If there is only one board, the board is number 0. The address, however, depends on the address that the stage controller is set for. Refer to the manual of your stage controller to set and determine this value.

Communications Toolbox Settings

If the Communications Toolbox is chosen as the communications mode, additional options appear to configure those parameters.



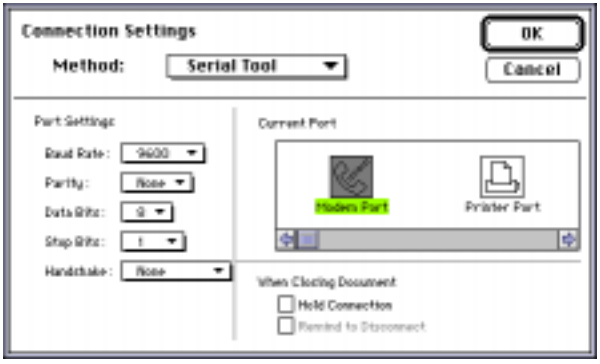
Select Communications Tool

Send delay

Set Settings of CTB tool

The **CTB Tool** must be selected. The **CTB Tools** button pops up a menu displaying all available tools located in your Macintosh. The Serial Tool should be selected for most stage controllers communicating via serial mode. Once selected, click on the **Settings** button to further configure the tool via the Communications Toolbox Manager.

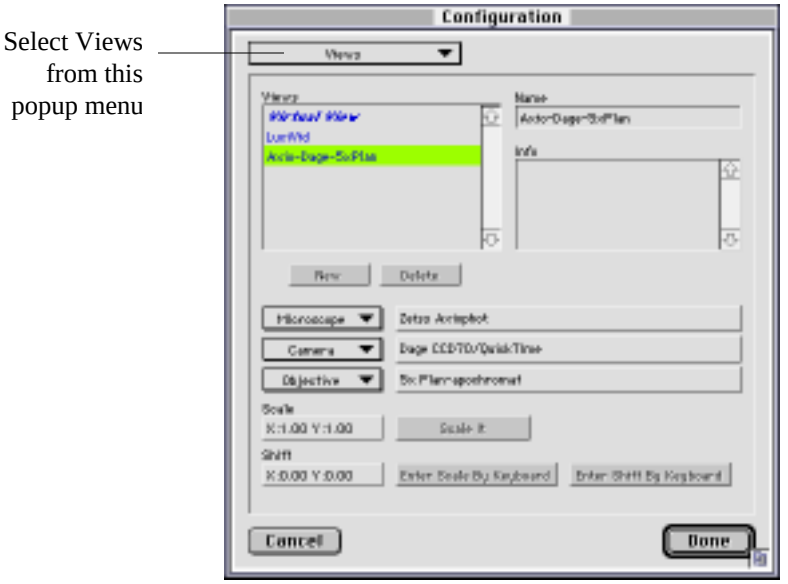
The **Send Delay** is the amount of delay introduced for each character that is sent via a serial device. Some controllers for microscope stages do not use handshaking, and the computer sometimes sends the data too quickly. Choose from the delay options in this menu to slow down the character send.



Views

A *view* is a combination of a microscope, a camera, and the lens objective. Each view is scaled within NeuroZoom so that the precise ratio of real world units (microns) is know for every device unit (pixels).

When mapping, a view is selected from the combination of devices available. In general, the microscope and the camera does not change during an analysis of the tissue section. The only variable to change is the lens objective. Therefore, you should configure in all of the possible lens combinations using the same microscope and camera. To do this, select the **Views** from the **Devices Category Popup** button to get to the **Views Devices** window.



Follow these steps to create or edit a view device.

1. Press **New** to create a new view, or single click on a stored view to select it for editing.
2. If this is a new view, enter in a name and any information you want to associate with it. The name should reflect the view combination to make it easier to recognize it from a list of view names.
3. Use the popup menu button **Microscope** to select the correct microscope. All of the microscopes previously configured appear in this popup menu.
4. Use the popup menu button **Camera** to select the correct camera connected to this microscope. All of the cameras previously configured appear in this popup menu.
5. Use the popup menu button **Objective** to select the correct lens objective that you want to use with this microscope/camera combination. All of the lens objectives previously configured appear in this popup menu.
6. The view must now be scaled. Press **Scale It** if you want to use the video input from the camera attached to the microscope with a stage controller to produce the scale factors. Or if the scale is known, press **Enter Scale By Keyboard**.

Before scaling, please refer to the appendix on ***The Microscope and Camera Adjustments*** to align the camera.

Scaling the View

If you press **Scale It**, a dialog appears offering three choices by which to scale the view.

Select one of these methods to scale a view

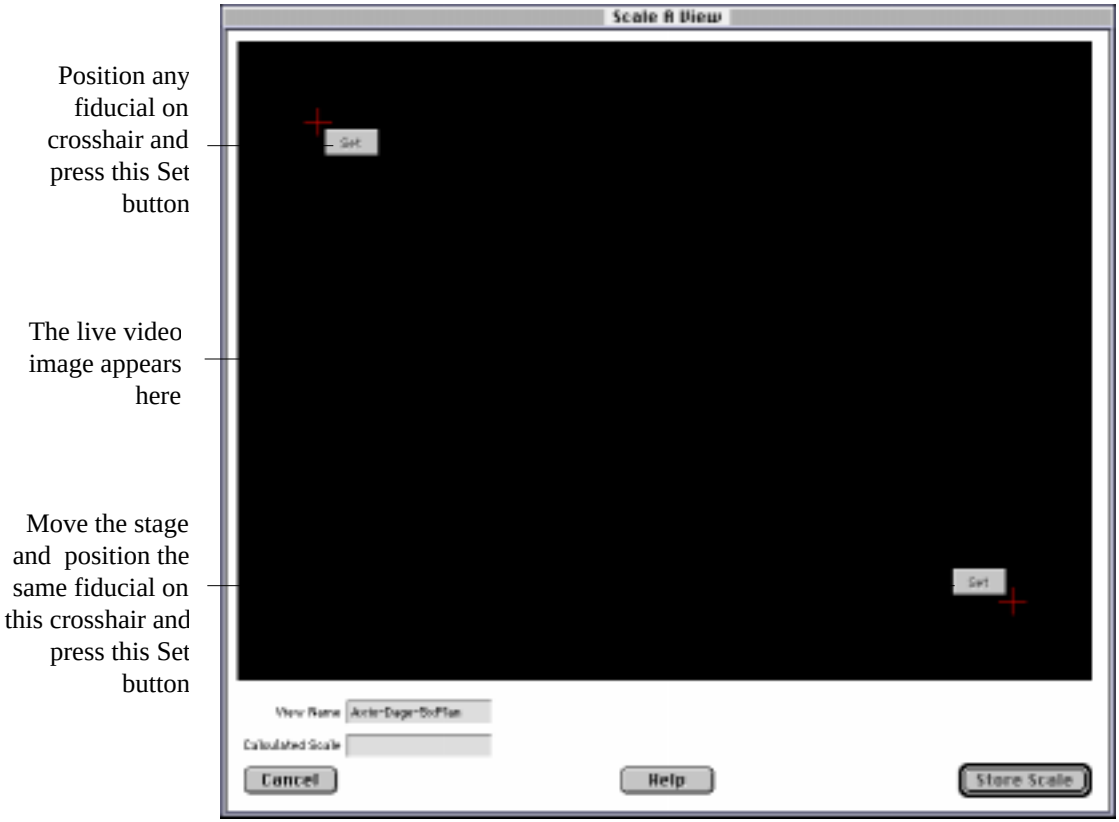
- **Use live video on motorized stage** - this will be the most common method of scaling a view. The motorized stage will be used to move a fiducial from one corner of the screen to the other. Both the amount of stage movement in microns, and the amount displayed on the Macintosh monitor will be measured. A ratio of microns to pixels is produce for the X and Y axes and stored for this view. This is currently the only method that supports correction for parcentration.
- **Use a live video image of a calibrated grid** - this can be used when a motorized stage is not available. A grid of known dimensions is placed on the microscope and imaged with the video camera and lens objective. The ratios in the X and Y axes are computed from information that you enter in via the keyboard and stored for this view. This method does not support correction for parcentration.
- **Use a digitized video image of a calibrated grid** - this is similar to the live image of a calibrated grid, except that live video may not be possible. In this case, a stored image captured separately using the microscope, camera, and lens objective is displayed instead of the live image. The ratios in the X and Y axes are computed from information that you enter in via the keyboard and stored for this view.

Alert: Use a digitized video image of a calibrated grid is currently not implemented.

If you choose **Use live video on motorized stage**, the following window opens asking you to choose the stage connected to the Macintosh. Obviously, the stage needs to be configured and working before you can proceed with this step. This is necessary because NeuroZoom will be communicating with the stage controller during this kind of scaling method

Select the stage is is currently attached and configured to the computer

After a stage is chosen, a window displaying live video is opened.



Scaling - There are two crosshairs and buttons at the top left and the bottom right corners of the window. A fiducial is positioned directly over each of the crosshairs. The fiducial can be anything that you can focus on sharply and move from corner to corner. The fiducial can be placed anywhere on the crosshair that is convenient, as long as the placement is consistent for both crosshairs. The XY displacement in microns as measured by how much the stage moves is what is important here.

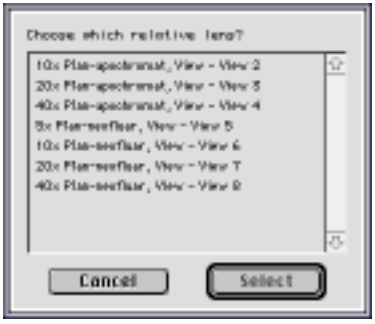
1. Position a fiducial on the top left crosshair. Use the hardware stage locator that came with the stage controller (for example, trackball or joystick).
2. Press on the **Set** button in the top right corner. The name of the button will change to **Reset**.

3. Position the same fiducial on the bottom right crosshair. Again, use the hardware stage locator to move the stage.
4. Press on the **Set** button in the top bottom right. The name of the button will change to **Reset**.

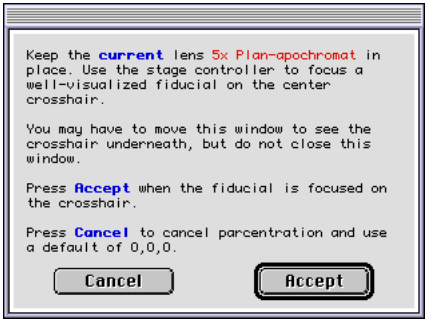
If this is the first lens objective that you are scaling with this particular combination of microscope and camera, scaling is complete. If you make a mistake, you may start over at any time with either of the two crosshairs.

If there are other lens objectives with this particular combination of microscope and camera, scaling is complete, and **parcentration** can be measured. Parcentration is the measure of the amount of shift in the center of the field when lens objectives are switched into place on the microscope. A new dialog window opens showing more information.

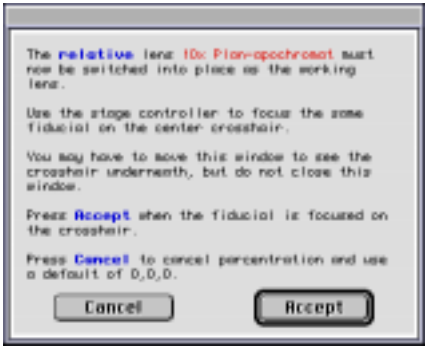
Another lens is required to be mounted and switched into place to measure the parcentration. You select that lens in the following window which opens if you press **Measure**. Or press **Cancel** to dismiss this window, cancel parcentration measure, and enter 0,0,0 as the default correction factor.



This window lists all of the lens objectives using this particular microscope and camera. Select any lens that is currently mounted in the microscope. This becomes the relative lens from which to measure parcentration. Press **Cancel** to dismiss this window, cancel parcentration measure, and enter 0,0,0 as the default correction factor. Press **Select** when a lens has been highlighted. Another window opens explaining the next step.



In this example, 5x Plan-apochromat is selected as the current lens objective. Keep this lens in place as the working lens. In the middle of the live video window, a red crosshair appears. Position any fiducial on the crosshair. When positioned and focused, press **Accept** to continue and to open another window. Press **Cancel** to dismiss this window, cancel parcentration measure, and enter 0,0,0 as the default correction factor. You will also be reminded to switch the lens back to the currently selected lens.



In this example, 10x Plan-apochromat is selected as the relative lens objective. This lens must be switched into place as the working lens and focused on the same fiducial. When positioned and focused, press **Accept** to continue and to open another window. Press **Cancel** to dismiss this window, cancel parcentration measure, and enter 0,0,0 as the default correction factor. You will also be reminded to switch the lens back to the currently selected lens. This completes the measure of parcentration.

Alert: Remember to switch back to the original current lens if you had this originally selected in a mapping window.

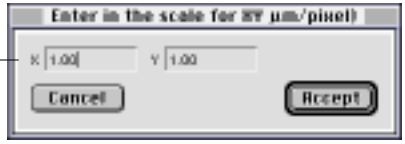
See the section on ***Parcentration*** in appendix on ***The Microscope and Camera Adjustments*** for more information.

The **Store Scale** button will highlight when scaling has been completed and parcentration has been measured if this is not the first lens. Press on this button and the scale and parcentration will be stored for this view. The window will then close and you will be returned to the **Configuration** window.

Enter Scale By Keyboard

If you choose this option, a window appears asking for the scale for the X and Y axes. The scale is microns/pixel. Press **Accept** to store these scales for this view. Press **Cancel** to dismiss the window with no changes stored.

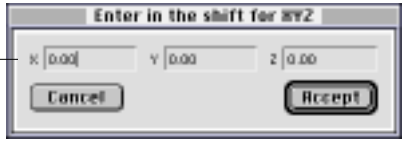
The known X and Y scale can be entered directly in for a view



Enter Shift By Keyboard

If you choose this option, a window appears asking for the parcentration shift for X, Y, and Z. The shift is the measure of displacement of a fiducial when switching from one lens objective to another. These displacements are relative to the other lens. Press **Accept** to store these shift for this view. Press **Cancel** to dismiss the window with no changes stored.

Enter the shift of XYZ from the indicated relative objective



Preference Window

Opened by:

- Preferences... in File menu

All preferences for NeuroZoom are configured from one window named **Preferences**.

The preferences are grouped by similarity. Each group is selected by pressing on the popup menu at the top of the window. New options are displayed for each group. As you pass the mouse cursor over each option, a description displays in the field at the bottom of the window.

Automatic Behavior

These items control automatic behavior of some features in NeuroZoom.

Automatically save every ... minutes - If this is selected, every unsaved document is automatically saved to disk for the time interval specified. If the document currently exists, the same name will be used. If the document is new, a file dialog opens asking for the name to use for the file. If a document has not been altered, no action will be taken.

Automatically save a backup of the document - If this is selected, a copy of the older document is made prior to saving the new one to disk. The older document will have the same name with the character * appended to it (Ex. filename*). This method is useful when there is sufficient capacity on the hard drive, and for critical data. Although NeuroZoom never deletes any file before a new one is successfully created, this ensures that previous data are automatically maintained. This could be considered a one version control system where only the last version is maintained.

Automatically hide 2D map window floating palettes - If this is selected, all of the floating palettes associated with the **Mapping** window (2D) are hidden when the **Mapping** window goes into the background. For example, when a text window is opened. This is useful for small monitors that tend to get cluttered with all of the opened windows.

Automatically hide 3D map window floating palettes - If this is selected, all of the floating palettes associated with the **3D Mapping** window are hidden when the **3D Mapping** window goes into the background. For example, when a text window is opened or when the **2D Mapping** window is brought to the foreground. This is useful for small monitors that tend to get cluttered with all of the opened windows.

Open microscope setup dialog when opening a new map window - If this is selected, the **Microscope Setup** window will open automatically whenever a Mapping Window opens. This includes both new and existing documents. If two documents are opened simultaneously, such as selecting two or more NeuroZoom documents from the Finder and selecting **Open** from the **File** menu of the Finder, the **Microscope Setup** window will only apply to the last window opened. This option is useful to help you remember to set the microscope equipment up properly when starting a new document.

Always update stage position during mapping - If this is selected, the microscope stage controller is always queried for a current position before a data point is entered in the map window. In other words, as soon as you indicate a point, a vertex, or other similar point during mapping, the XYZ location of the stage is read automatically. Normally, you can leave this preference off to speed up data entry, as most data are “collapsed” to the current plane of focus, which doesn’t change, and the location of the current field of view, which also doesn’t change. However, in some conditions, such as tracing out arbors that course in and out of the tissue section, this preference is useful when focusing on different points of the fiber for vertex entry using the arbor tool. Note that a there would be a slight delay during entry as the stage controller is queried. Also, this option does not work for stereology, as the plane of focus should not be altered manually.

Copy/Paste

These items control the behavior of copying and pasting in NeuroZoom.

Copy and drag objects in verbose mode - If this is selected, objects are copied to the clipboard or dragged to files with more information added to the data. If not selected, only the data are copied or dragged in a tab-delimited format more suitable for pasting into spreadsheets. Turn verbose more on for generating reports.

Expand out multilines into vertices when dragging or copying - If this is selected, multiline objects (contours, curves, etc.) are copied to the clipboard or dragged to files with expanded data on the individual vertices. When used in combination with non-verbose mode, this can be useful for recreating the lines in other application.

Copy PICT object of map window when dragging or copying - If this is selected, copying to the clipboard or dragging to files will also export a PICT object of the mapping window (either the whole mapping window or selected objects). This can slow down the copy or the drag. Use this only if you really need a PICT object.

File I/O

These items control aspects of file I/O.

Export PICT as Multiple Objects - If this is selected, a map is exported to PICT as a collection of objects. If not selected, only a single PICT object is exported. A collection is useful if you need every QuickDraw element separately created in the exported file. Note that this can be unwieldy, especially if objects like the Grid is on. Every line of the grid is an object. A single object is useful when you have the precise mapping window presentation, and you need to export it a one PICT object that can be scaled together as a group in another graphics application.

> **By The Way:** Graphics applications vary with how they handle PICT files. Deneba Canvas will properly open the PICT files created by NeuroZoom, whether as a single or a collection of PICT objects. Adobe Illustrator opens the individual objects, but hides them behind a frame.

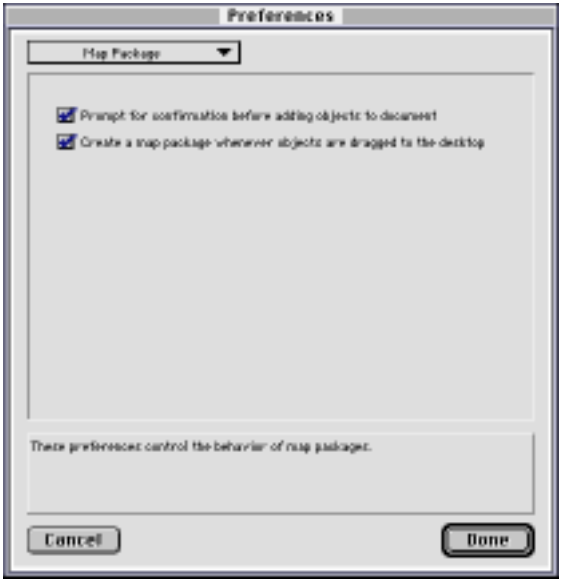
Imaging

When grabbing one image - There are two choices:

1. **Do NOT delete the previously grabbed image** - Choose this if grabbing a new image adds to the list of captured images for the current document. A montage will eventually be created if the menu item **Spatially Map All Images** is on in the **Imaging** menu.
2. **DELETE the previously grabbed image** - Choose this if grabbing a new image should delete the last grabbed image. This is useful if you want to capture a lot of images, perhaps when mapping quenching material, but don't want to store them persistently in the document.

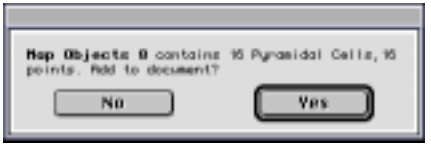
Map Package

These items control the use of map packages.



Map Packages are files that are created when dragging data from the **Mapping** window to the Finder. The file is self-contained and can be sent directly to another use using the **Networking Module**. A map package can also be dragged and dropped directly into an opened **Mapping** window. The contents of the package are then merged into the document and displayed. The name of the Map Package file is *Map Objects* followed by a number that makes the name unique.

Prompt for confirmation before adding objects to document - If this is selected, a confirmation will be displayed before adding any objects from the dropped map package into the document.



A window then opens displaying the action and number of new objects added to the document.

Create a map package whenever objects are dragged to the desktop - If this is selected, a map package is always created for every drag and drop to the Finder desktop. A window opens to ask for an optional description and an optional password.

The password is requested when the Map Package is reopened by NeuroZoom.

By The Way: Due to limitations in how the drag and drop mechanism works, you can drag to any folder on any drive, but the Map Package file will always be created on the Desktop. Furthermore, System 7 may delay the display of the file until the file is *accessed*. This makes the file difficult to locate at times. At some point after creation, the file will be displayed. If you need to get the file more quickly, Use the Finder File command to *locate* the file. Opening its enclosure will force the Finder to immediately draw it on the Desktop.

Mapping 3D Window

These items control the initial state of the **Mapping 3D** window when opened from a **Mapping** window.

Surface Smoothing - This is a popup menu to select the method for smoothing surfaces. The choices are:

- None
- Smooth
- Very Smooth

Polygons Backfacing - This is a popup menu to select the method for drawing polygons that are facing away from the user. The choices are:

- Visible
- Removed
- Flipped

Polygon Drawing - This is a popup menu to select the method for drawing polygons. The choices are:

- Solid
- Only Edges
- Only Points

Shading - This is a popup menu to select the shading method. The Lambert method is used when the option selected is **Some Shading**. The Phong method is used when the option selected is **Better Shading**. The choices are:

- None
- Some Shading
- Better Shading

Camera - This is a popup menu to select the type of camera or projection. The choices are:

- Orthographic
- Perspective

Image - This is a popup menu to select the rendering type to use to create the 3D image. The choices are:

- Wireframe
- Realistic

Axis of Rotation - This is a popup menu to select the default axis of rotation when the **Rotate** tool is the active tool. The choices are:

- About X
- About Y
- About Z
- About XYZ

Rotate Relative To - This is a popup menu to select the center of rotation. If you select **Camera**, then the center of the camera is used. If you select **Model**, then the center of rotation will be the model. The choices are:

- Camera
- Model

Subdivision Method - This is a popup menu to select the method for curve and surface subdivision. **Constant** indicates a fixed number of line segments. **Microns** indicates a length in microns of the line segments. **Pixel** indicates a length in pixels of the line segments. The choices are:

- Constant
- Microns
- Pixel

Background Color - This button opens a color picker to choose the starting color for the background of the **3D Mapping** window.

Show Bounding Box - If this is selected, the bounding box of the data is displayed.

Double Buffer - If this is selected, double buffering is used when rendering the model. The result is smoother animation.

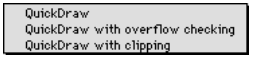
Show Axis - If this is selected, the axes of the model are displayed.

Auto Rotate - If this is selected, the model will auto-rotate when the **Rotate** tool is selected, and the model is moved in any of the three axes.

Mapping Window

Most of these items control whether a window is opened automatically when a mapping window opens.

2D Graphics Rendering - Press this for a popup menu to choose the method of rendering 2D graphics in the mapping window.



- **QuickDraw** does not check for 16 bit overflow when scaling data objects up. This produces the faster rendering, but artifacts due to the overflow may result at higher magnifications. An alert is displayed in the Mapping window message box field if any data object overflows.
- **QuickDraw with overflow checking** does check for 16 bit overflow when scaling data objects up. If any data object overflows, it simply will not be rendered. This produces fast rendering as well and the artifacts are suppressed. An alert is displayed in the Mapping window message box field if any data object overflows.
- **QuickDraw with clipping** does not rely on QuickDraw for 16 bit overflow checking when scaling data objects up. All objects are computed and clipped in floating point math. This produces the slowest rendering, but all objects are displayed faithfully. Searching may be fastest with this method because all

vertices of objects are precomputed. As with the other two options, an alert is displayed in the Mapping window message box field if any data object could overflow. This alerts you to use the **QuickDraw** only method for faster rendering.

Open Mapping Tools Window - If this is selected, the Mapping Tools Window is opened when a new mapping window is opened.

Open View Tools Window - If this is selected, the View Tools Window is opened when a new mapping window is opened.

Open Imaging Tools Window - If this is selected, the Imaging Tools Window is opened when a new mapping window is opened.

Open Stage Tools Window - If this is selected, the Stage Tools Window is opened when a new mapping window is opened.

Open Info Window - If this is selected, the Mapping Window Info Window is opened when a new mapping window is opened.

Open Background Images Window - If this is selected, the Background Images Window is opened when a new mapping window is opened.

Open Stage Controller Window - If this is selected, the Stage Controller Window is opened when a new mapping window is opened.

Open Stage Location Window - If this is selected, the Stage Locations Window is opened when a new mapping window is opened.

Open Stage Movement Controls Window - If this is selected, the Stage Movement Controls Window is opened when a new mapping window is opened.

Open Data Type Window - If this is selected, the Data Type Window is opened when a new mapping window is opened.

Open Data Window - If this is selected, the Data Window is opened when a new mapping window is opened.

Open Montage Window - If this is selected, the Create Montage Window is opened when a new mapping window is opened.

Stage Scrollers Initially Displayed - If this is selected, the stage scrollers are displayed when a new mapping window is opened.

Mapping Window - Grid

These items control aspect of the grid on the mapping windows.

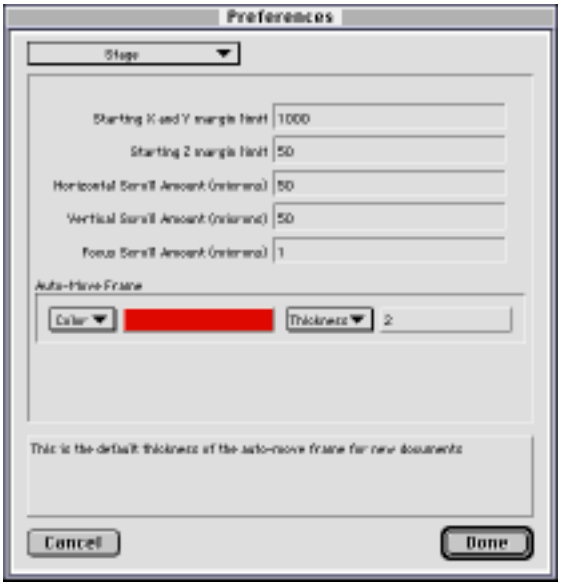
Grid Initially Displayed - If this is selected, the grid is displayed when a new mapping window is opened.

Grid Size Width and Grid Size Height - These values in µ indicate the default width and height of the grid for all new documents.

Grid Color and Thickness - Color and Thickness are popup menus from which the default color and thickness for the grid may be selected for new documents.

Stage

These items control aspects of the stage.



Starting X and Y margin limit - Enter in the default starting values to be used when limiting the movement of the X and Y axis when the option for Stage Limits is on.

Starting Z margin limit - Enter in the default starting values to be used when limiting the movement of the Z axis when the option for Stage Limits is on.

Horizontal Scroll Amount (microns) - Enter in the default starting values in microns that determines the displacement in the X axis when the scrollbar for the stage controller in the Mapping Window is pressed.

Vertical Scroll Amount (microns) - Enter in the default starting values in microns that determines the displacement in the Y axis when the scrollbar for the stage controller in the Mapping Window is pressed.

Focus Scroll Amount (microns) - Enter in the default starting values in microns that determines the displacement in the Z axis when the scrollbar for the stage controller in the Mapping Window is pressed.

Auto-Move Frame Color and Thickness - Color and Thickness are popup menus from which the default color and thickness for the auto-move frame may be selected for new documents.

Stereology

These items control general aspects of Stereology.

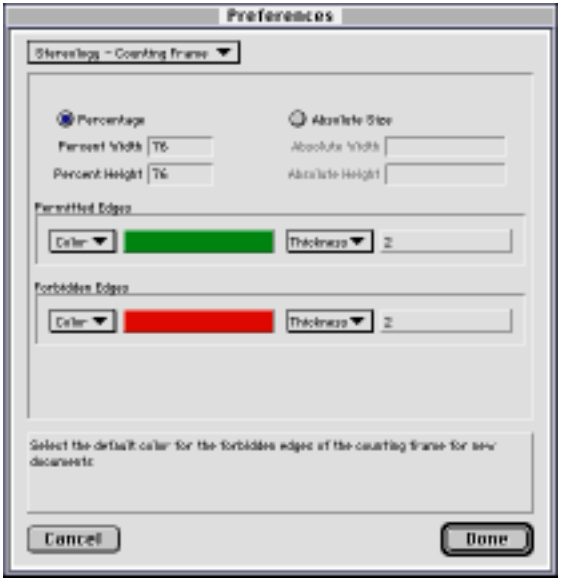
Display multisector name in map window - If this is selected, name of the multisector is displayed in the multisector itself.

Units - This is a popup menu used to select the units used in most of the stereology reports. Choose from microns, millimeters, centimeters, decimeters, and meters.

Default Fraction for Fractionator - This is the default fraction to be used for all Fractionator based protocols in new documents. Enter a percent floating point value.

Stereology - Counting Frame

These items control aspects of the counting frame in Stereology.



Percentage - If this is selected as one of the two options, the default size of the counting frame will be calculated as a percentage of the **Mapping** window dimensions. Enter the **Percent Width** and **Percent Height**.

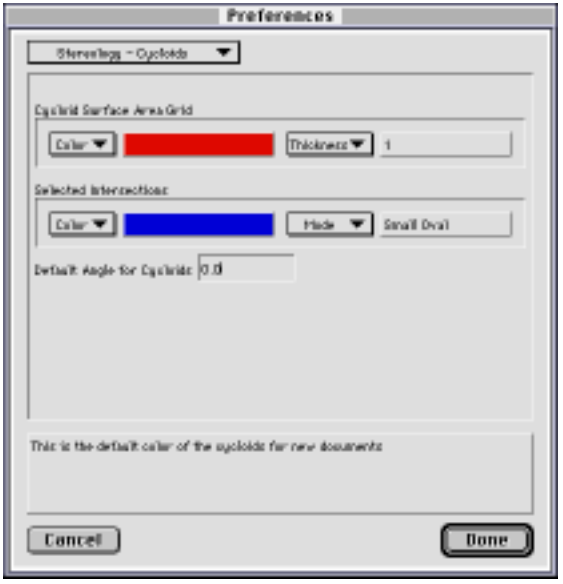
Absolute Size - If this is selected as one of the two options, the default size of the counting frame will be calculated as an absolute size in microns, regardless of the magnification or scale of the **Mapping** window. This also means that the counting frame may not be valid at some magnifications. Enter the **Absolute Width** and **Absolute Height**.

Permitted Edges Color and Thickness - Color and Thickness are popup menus from which the default color and thickness for the permitted edges of the counting frame may be selected for new documents.

Forbidden Edges Color and Thickness - Color and Thickness are popup menus from which the default color and thickness for the forbidden edges of the counting frame may be selected for new documents.

Stereology - Cycloids

These items control aspects of the cycloids in Stereology.



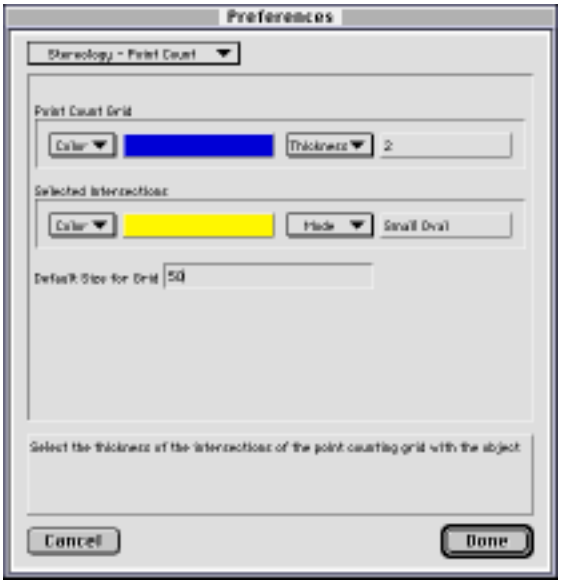
Cycloid Surface Area Grid Color and Thickness - Color and Thickness are popup menus from which the default color and thickness for the cycloid surface area grid may be selected for new documents.

Selected Intersections Color and Thickness - Color and Thickness are popup menus from which the default color and thickness for the selected intersections between the cycloids and the objects may be selected for new documents.

Default Angle for Cycloids - This is the default angle to be used for the orientation of the cycloid grid for new documents.

Stereology - Point Count

These items control aspects of the point counting grid in Stereology



Point Count Grid Color and Thickness - Color and Thickness are popup menus from which the default color and thickness for the point count grid may be selected for new documents.

Selected Intersections Color and Thickness - Color and Thickness are popup menus from which the default color and thickness for the selected intersections between the point count grid and the objects may be selected for new documents.

Default Size for Grid - This is the default size to be used for the width and height of the cycloid grid for new documents.

Warnings

These items control warnings used throughout NeuroZoom.

Confirmation dialog for manual stage move - If this is selected, a confirmation dialog will always open when selecting the **Move Stage** command from the **Stage** menu or the **Stage Tools** window.

Show popup information windows on certain functions - If this is selected, popup help windows will appear for certain tasks throughout NeuroZoom to assist you in the execution of that tasks.

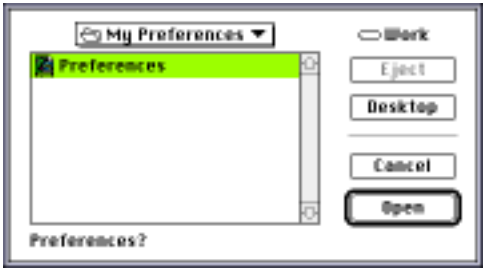
Saving and Reading Preferences

Preferences may also be saved to and restored from disk files. To save the preferences to a file, select **Save to File...** from the **Preferences** submenu.

A file dialog opens asking for the name of the file in which to store the preferences.

To restore the preferences from a file, select Restore from File... from the Preferences submenu.

A file dialog opens asking for the file from which to restore the preferences.



Once the preferences are read in, they remain in effect as the default preferences for NeuroZoom.

CHAPTER 4 *Stereology Windows*

All the windows in NeuroZoom are opened by activating a menu command. In this chapter, all of the 3D Mapping windows in NeuroZoom will be detailed.

- See the chapter on ***Mapping Windows*** for specific information on the 2D windows.
- See the chapter on ***3D Mapping Windows*** for specific information on the 3D windows.

Please be sure to read the previous chapter on **Menus** to understand fully how these windows are opened.

Introduction to Stereology Windows

The stereology windows in NeuroZoom are all opened by commands in the **Stereology** menu. Stereology is task driven in NeuroZoom. A stereology protocol is selected for a specific experiment, and NeuroZoom presents windows and options pertaining to that protocol. Because of this, this chapter will present windows not separately as in the previous chapter, but rather as sequential actions taken when conducting a session with the stereology probes.

Stereology Probes

The stereology probes, or tools used to acquire the data, are grouped around 4 categories: estimating number, estimating length, estimating surface area, and estimating volume. Be sure to read the chapters on ***Stereology*** in the ***User Guide Manual*** to fully understand which probe to use for your experiments.

The protocols for the experiments are set up through the menu selections in NeuroZoom. The protocols are stored in the document, and may be opened at any time for subsequent data acquisition, retrieval, or analysis.

Each of the probes open a dialog window initially to present four options:

3. Make a new protocol
4. Open an existing protocol
5. Delete an existing protocol
6. Get info on an existing protocol

Doing this in a dialog window reduces the menu clutter from too many options, and also lets NeuroZoom present helpful information on each step involving stereology.

This chapter will discuss each window from the point of view of the probe. We will begin with the point estimators, then the surface area estimators, and then the volume estimators.

Alert: Currently, there are no length estimators in NeuroZoom.

The following chapters do not try to present the fundamentals of stereology. They do not go into depth on the reasons why certain parameters are used over others.

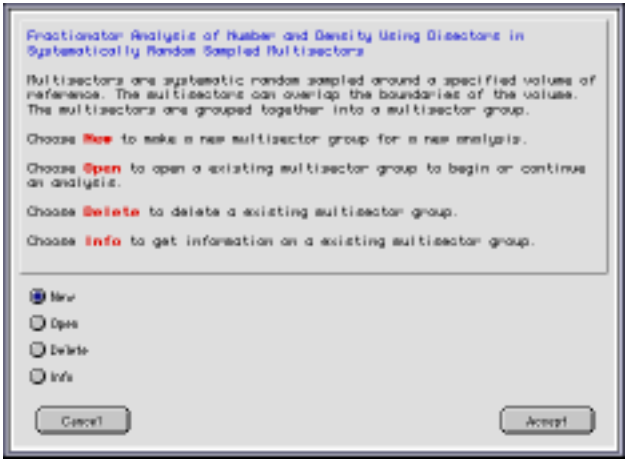
They only show the windows, how to get to the windows, and the meaning of the components on the windows. For an understanding of the stereology reasoning, please refer to the chapters on ***Stereology*** in the ***User Guide Manual*** .

Number by Fractionator Systematic Sampling

Opened by:

- Estimate Number By Fractionator Systematic Sampling... in Stereology menu

The **Number By Fractionator Systematic Sampling** window, for lack of a better name, is a choice window. From this you are to select one of four options to proceed with estimating numbers.



Information on what this probe accomplishes and the four options are presented.

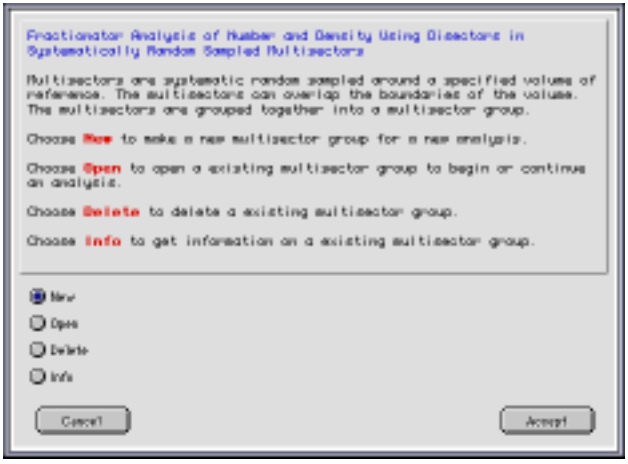
- **New** - make a new multisector group to begin a new analysis on estimating the number of some structure
- **Open** - open an existing multisector group for additional data entry or analysis
- **Delete** - delete an existing multisector group from the document
- **Info** - get information on an existing multisector group

Choose one of the options my pressing the mouse button the radio button. The radio button will highlight indicating that it is selected. Press **Accept** to continue

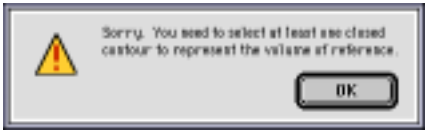
with the process, or press **Cancel** to dismiss this window with no options or changes made to the document.

New Multisector Group

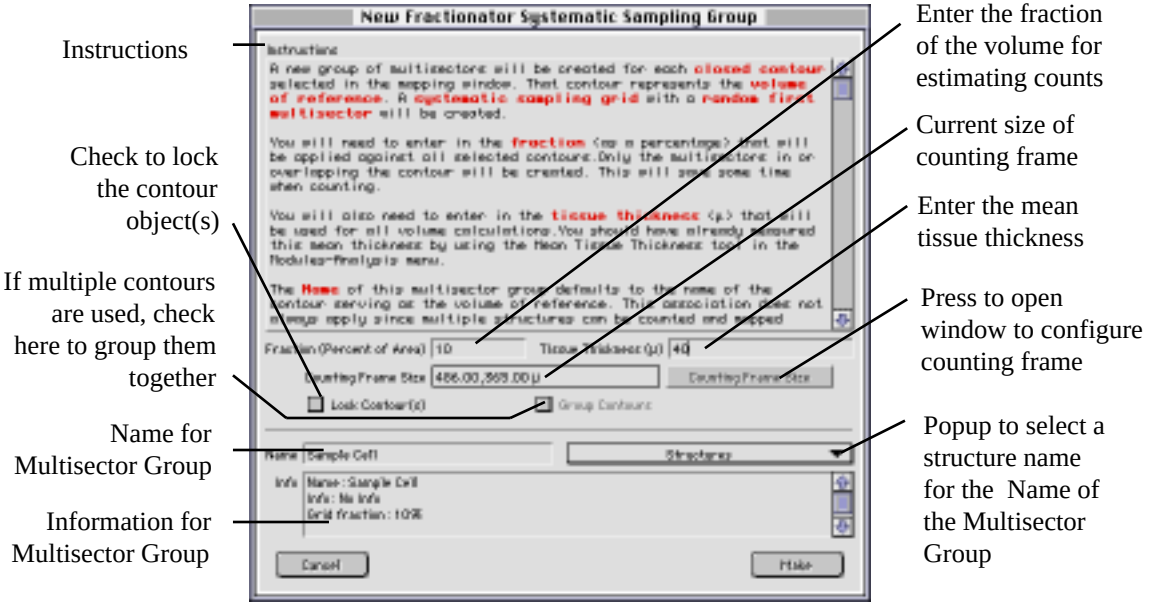
Make a new multisector group for estimating numbers using a Fractionator protocol.



If you choose this option, and if you do not have at least one contour created and selected in the mapping window, an error window opens indicating that at least one selected contour is needed to represent the *volume of reference*.



Go back and use the mapping tools to create a closed contour around the volume of reference. When you have done so, select it, choose **Estimate Number By Fractionator Systematic Sampling...** in the **Stereology** menu again, and select **New** and press **Accept**. A new window opens.



This window is the **New Fractionator Systematic Sampling Group** window. All of the parameters to create a multisector group for a Fractionator analysis are entered here. The multisectors are calculated on a systematic random sample through the selected contour. Only those multisectors that intersect the contour will be presented. Disectors are created in those multisectors in which the estimates are places for cell counts.

Fraction (Percent of Area)

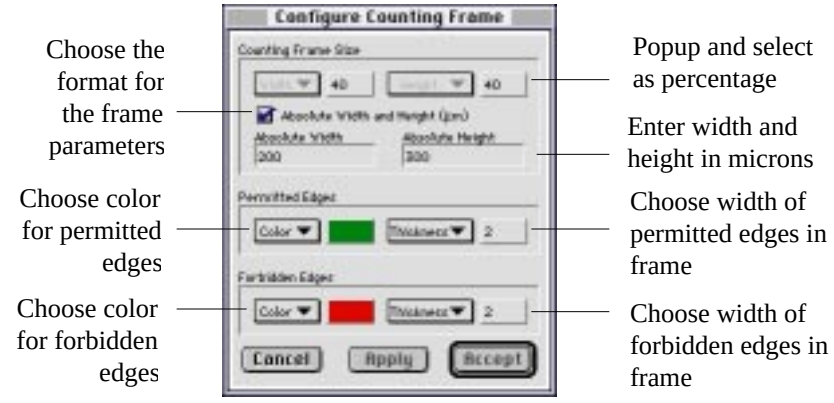
Enter the fraction that is desired for the Fractionator analysis. For example, if you want to estimate counts of cells in 10% of the contour volume, enter 10. The default is 10%. Please refer to the chapters on ***Stereology*** in the ***User Guide Manual*** for more specific information on this value.

Tissue Thickness (μ)

Enter the mean tissue thickness in microns for this section. This can be measured with the **Measure Mean Tissue Thickness** tool located in the **Analysis** submenu of the **Modules** menu.

Counting Frame Size

This is a button that opens a window from which to adjust the size of the counting frame. The current frame size is displayed in the field to its left.

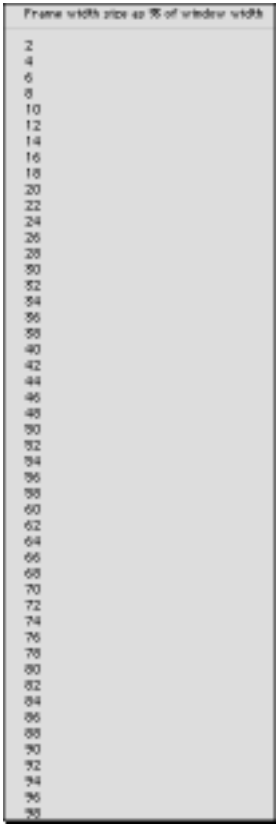


The size of the counting frame may be entered as a *percentage* of the screen size, or as an absolute width and height in microns. The major difference between the two techniques is that a percentage will always ensure that the counting frame fits in the mapping window regardless of the *lens objective* used. However, you cannot switch lens objectives in the middle of a stereology experiment using the counting frame because the absolute size of the frame would change.

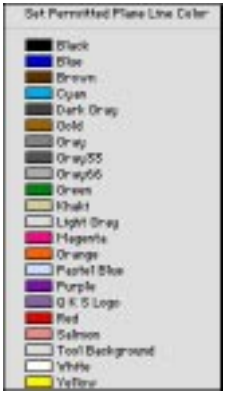
When you enter the size in *absolute microns*, the displayed size of the counting frame in the mapping window will change as you change the lens objective. The frame will always adjust to the absolute width and height entered. However, the problem here is that the size of the counting frame may exceed the size of the mapping window with lower power objectives. If so, NeuroZoom will alert you.

In all cases of stereology, you cannot change the lens objectives once the experiment is underway. However, one method of entering in the size may be more appropriate than the other, depending on how you go about the business of setting up the stereology experiment. You choose the method which best suits your needs. Please also refer to the chapters on ***Stereology*** in the ***User Guide Manual*** and the ***Reference Manual*** more information.

When choosing the width and height as a percentage, press and hold on the width or height button. A menu pops up for selectons from 2 to 98%.



Press and hold on the **Color** button for the *permitted and forbidden colors*. A popup menu shows the color choices.



Press and hold on the **Thickness** button for the *permitted and forbidden thicknesses*. A popup menu shows the choices from 1 to 6. 6 is thickest.

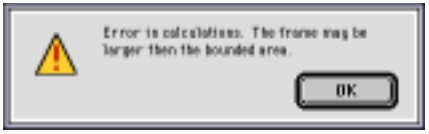
Press on the **Apply** button to see the changes. Press on **Cancel** to dismiss the window and cancel any changes you made with this window. Press **Accept** to dismiss the window and to store the changes made with this window.

Name and Info

Enter the name and information for this multisector group. The default name is the structure name of the contour that is selected when this window was opened. Press and hold on the **Structure Popup** menu button to get a list of all the structures for this mapping window and select one for the name. You can also enter in any other name you desire.

When all parameters are entered, the **Make** button is enabled. Press **Cancel** to dismiss the window and not make the multisector group. Press **Make** to make the multisector group.

If the counting frame is too large to accurately place a group of multisectors over the selected contour representing the volume of reference, a window opens displaying this error.



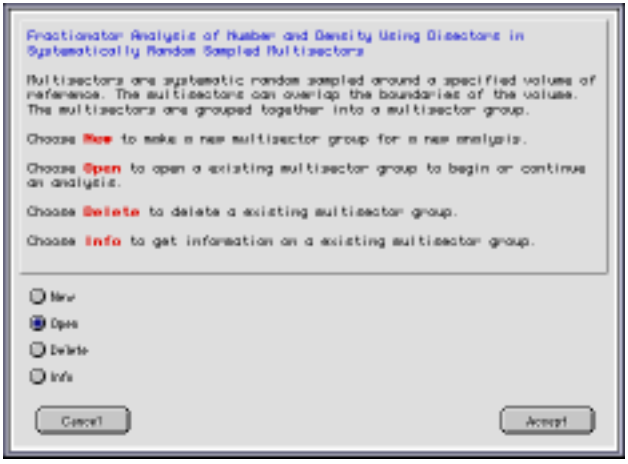
Once the multisector group has been made successfully, a window opens presenting the option of starting the stereology session immediately on this new group.

Press **No** to close window and return to the Mapping window. Press **Yes** to continue immediately to the Fractionator analysis on this multisector group.

Answering **Yes** is the same as opening an existing multisector group for analysis. This is presented in the next session.

Open Existing Multisector Group

Open an existing multisector group for estimating numbers using a Fractionator protocol.



If you choose this option, and if there are no multisector groups for this fractionator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing fractionator protocols in the document.

Tip: Holding down the OPTION when choosing Estimate Number By Fractionator Systematic Sampling in the Stereology Menu will automatically preselect this OPEN button.

Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

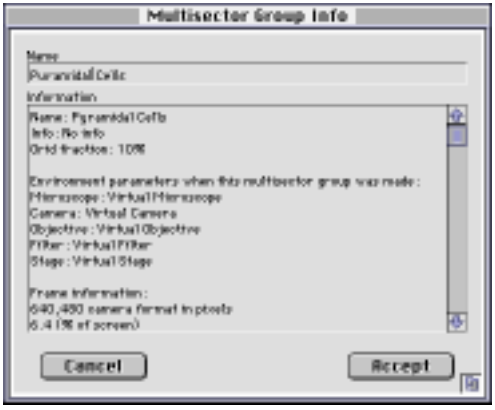
T - This first column indicates the Type of Multisector Group. It should show F for Fractionator.

Name - The second column is the name assigned to it when the multisector group was made.

#MS - The third column indicates the number multisectors in the group.

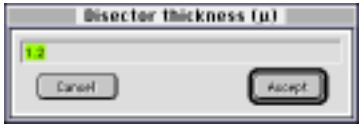
Info - The fourth column is the information assigned to it when the multisector group was made.

Select the multisector group to reopen and press the **Count** button to continue counting with that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.

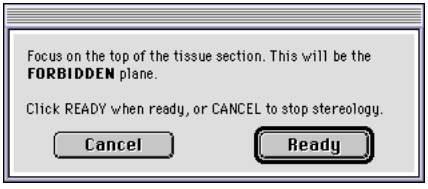


Fractionator Control Window

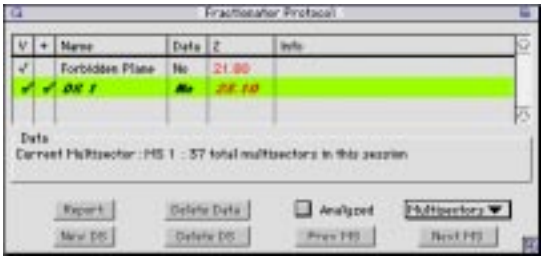
If a multisector group is selected and opened for counting, live video is turned on. If this is the first time the multisector group is being opened, the thickness of the disector must be entered in the following window.



Once the thickness is specified, another window prompts for you to focus on the top of the tissue section.



After the top is specified, the following window opens.



This is the **Fractionator Protocol Control** window from which the probe is controlled. There are various components to this window.

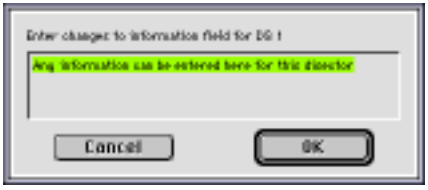
1. **Disectors** - The disectors for the current multisector that the microscope stage are located in the only scrolling field on this window.

Of these disectors, the disector in Italics is the current disector. The current disector is the one that the microscope is focused on. Of the two planes of a disector, the microscope focuses on the reference plane. This is indicated by the column labelled Z. The selected disector is the disector which is highlighted in the highlight color. The selected disector and the current disector are not necessarily the same, although in this example, they are.

Double clicking on a disector makes the selected disector the current disector. The microscope stage will move to that new focus point, and the disector will be displayed in Italics.

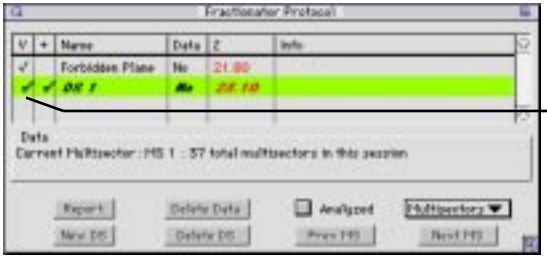
The **Name** for each disector is auto-generated when the multisector is made. The first disector is named *Forbidden Plane* because the microscope focuses on the first plane in the first disector, which by NeuroZoom convention, is the forbidden plane. No data is usually counted in this plane. The subsequent disectors are then named DS 1, DS 2, and so on.

The **Info** field is blank initially for each disector. However, you can Option-*Double Click* on a disector and a dialog window opens for you to enter in new information.



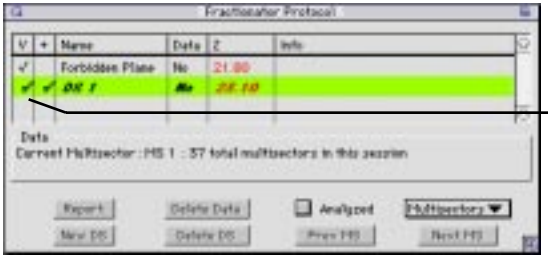
Tip: Option Double Click on a disector to change its information.

Visibility of data can be controlled by clicking on the leftmost column labelled **V**. A single click will toggle the visibility of all of the data for this disector. This is particularly useful if there is a large concentration of data in the multisectors, and they are visually collapsing on top of each other.



Single click in the V column to toggle visibility of the data in the selected disector

Inclusion of data can be controlled by clicking on the column labelled **+**. A single click will toggle the inclusion of all of the data for this disector. Use this to remove a disector completely from reports that are generated, including any statistics generated for the multisector or the multisector group.



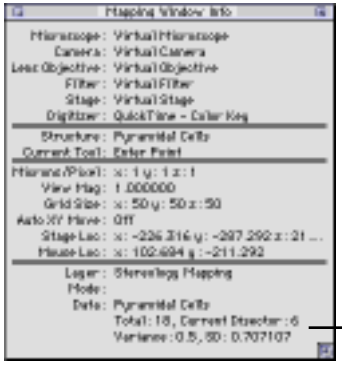
Single click in the + column to toggle inclusion of the data in the selected disector

The column labelled **Data** shows *No* if there is no data in the disector, or *Yes* if there is data.

2. **Data Field -** This shows the current multisector that the microscope stage is located on, and whether it has been “analyzed” or not. When a multisector is “analyzed”, NeuroZoom knows how to treat its data for generating reports and statistics. For example, an unanalyzed multisector is not used in generating reports before the experiment is over, because there may be more data to enter. The total number of multisectors in the group is also shown. The current structure selected in the mapping window is then displayed, along with the number tallied for this structure for both the current multisector and the current disector.

   Multiple structures may be estimated simultaneously with the Fractionator probe. However, only the current structure’s totals are shown in the data field.

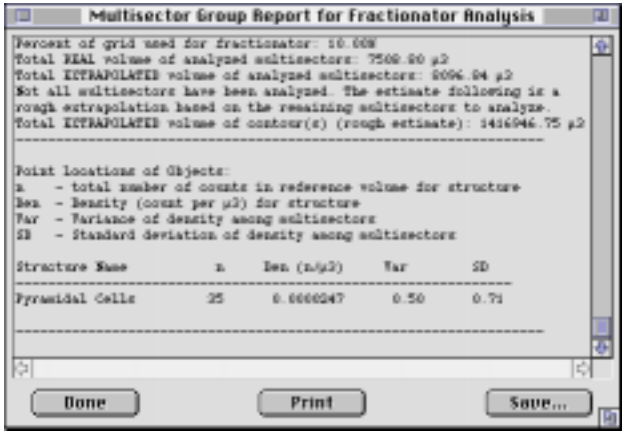
   All data are estimated for number by using the **Point** tool (see the chapters on ***Stereology*** in the ***User Guide Manual*** to understand how to estimate structure number using this tool. Also see the section in this manual on the **Point** tool). The **Mapping Window Info** window will show information on the current structure that is being estimated. If there are two or more analyzed multisectors in the group, the *Variance* and the *Standard Deviation* for the number estimates among the multisectors are expressed in the **Mapping Window Info** window. The multisector total and the disector total are also displayed for the current structure.



Variance and SD are displayed only if there are 2 or more analyzed multisectors

Several control buttons in the **Control** window perform actions on the multisectors.

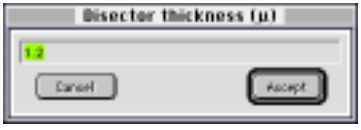
3. **Report** - pressing this button generates a report and displays it in a separate window.



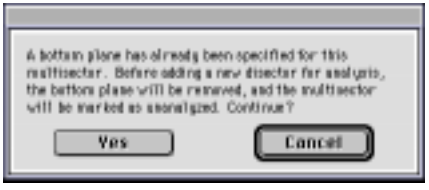
The report can be closed by pressing **Done**, printed to the selected printer by pressing **Print**, or saved to a Text file by pressing **Save....** In this window, the end of the report shows each structure estimated for number in this

experiment and lists the total count (n), the density of the structure expressed as n per μ^3 and the variance and standard of the structure among the multisectors. This is zero unless at least two multisectors have been marked as “analyzed”.

4. **New Disector** - pressing the **New DS** button creates a new disector for the current multisector. The position of the disector is immediately adjacent to the current disector. The thickness of the disector is the same as all other disectors, and is the number first specified when this multisector group was made. However, if the option is held down when **New DS** is pressed, a window opens for you to enter in a new disector thickness value in microns.



If **New DS** is pressed and the current multisector has already been marked as analyzed, a window opens to alert you to this fact.

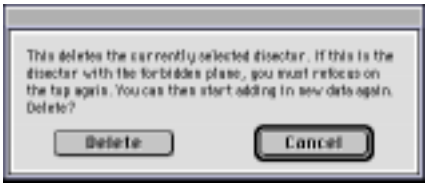


Press **Cancel** to dismiss the window and to not create a new disector. Press **Yes** to indicate that the new disector will be added to the bottom of the multisector, and will push the multisector stack downward to incorporate the new thickness of this new disector. The multisector will then be marked as “unanalyzed”.

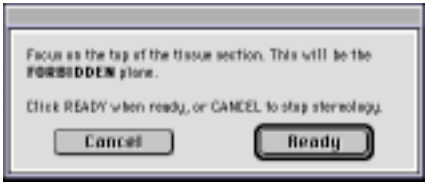
5. **Delete Disector** - The **Delete DS** button is enabled only if the last disector in the multisector is the current disector. Pressing this button deletes the current disector completely from the multisector.

Alert: The CURRENT disector is deleted, not the SELECTED disector. The current disector is always displayed in Italics. Please note this difference between the two.

A window opens alerting you to certain conditions if this is the last disector.



If the current disector contains the forbidden disector (meaning also it is the last disector in the multisector), you need to refocus on the top of the multisector again to begin estimating again for this multisector.



If you press on **Cancel**, the last disector without its data is reattached to the multisector. This is not a serious problem, because generally you do not estimate data when on the forbidden plane.

If you press **Ready**, the current position of the microscope stage is read from the controller, and two disectors are made. The first disector contains the forbidden plane at the current focus you indicated. The second disector contains the first permitted plane. NeuroZoom automatically moves to the first permitted plane of the second disector, and makes that one the current disector.

If the current disector being deleted is not the last disector, the next to last disector then becomes the current disector after deletion.

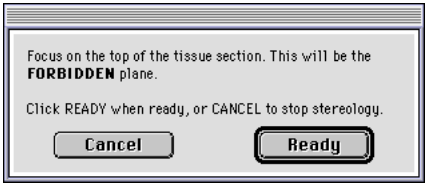
6. **Delete Data** - pressing the **Delete Data** button will delete all data from the current disector. Similar to the previous command, this acts on the current, not the selected disector. If there is no data in the current disector, an error window opens.

If it does contain data, a warning window opens.

Only the data are deleted. The planes and their location are not affected. You can continue estimating data directly into this disector.

7. **Previous Multisector** - pressing the **Prev MS** button moves the microscope stage to the previous multisector for this group. This movement wraps around, so if you are currently on the first multisector, the last multisector is selected.

If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.

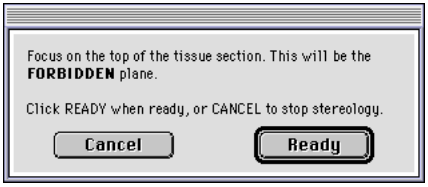


Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

The **Data** field updates to show the current multisector. The Disector list also updates to show the disectors in this multisector. The last disector in this multisector becomes the current disector. The microscope focuses on the permitted plane of this disector.

8. **Next Multisector** - pressing the **Next MS** button moves the microscope stage to the next multisector for this group. This movement wraps around, so if you are currently on the last multisector, the first multisector is selected.

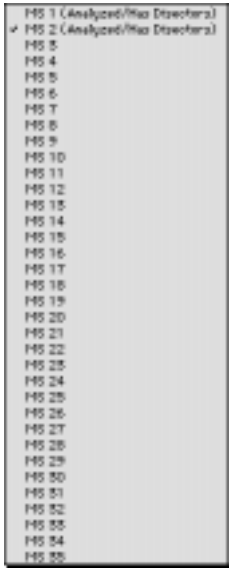
If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.



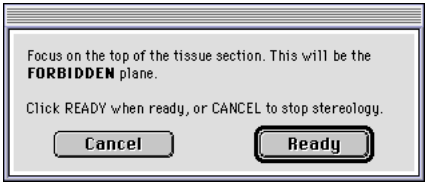
Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

The **Data** field updates to show the current multisector. The Disector list also updates to show the disectors in this multisector. The last disector in this multisector becomes the current disector. The microscope focuses on the permitted plane of this disector.

9. **Multisector** - this is a popup menu showing all the multisectors in this group. You can select any of them and NeuroZoom automatically goes to that multisector. The menu indicates whether a multisector has been analyzed or not. The current multisector is preceded with a checkmark.



If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.

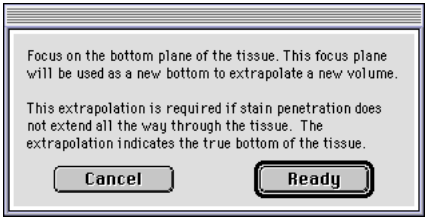


Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

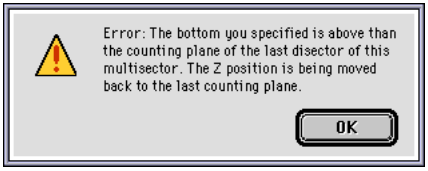
10. **Analyzed -** checking this button marks the current multisector as”analyzed”. This makes the multisector eligible for calculations involving global volumes and counts, such as when generating reports.

When a multisector has been marked as “analyzed”, and you uncheck the box to mark it as “unanalyzed” again, a warning window opens.

The counting plane of the last disector in this multisector is used as the bottom of the multisector. If the tissue has *incomplete staining*, this counting plane may not be the true bottom of the multisector. In this case, *Option-Click* on the **Analyzed** checkbox. A window opens asking you to focus on the true bottom of the tissue section. This plane will then be used as the bottom and all volumes will be extrapolated to this plane. Note that the density of the structures if it is being calculated, remains the same regardless of extrapolation.



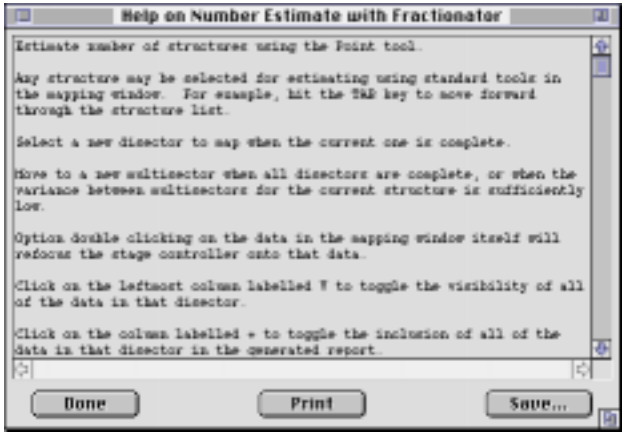
If the plane you specify as the bottom is above the counting plane of the last disector of this multisector, an error window opens, and the stage moves back to the last known position.



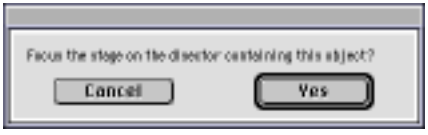
The bottom cannot be above the last counting plane, otherwise data will be excluded from the calculations. If a disector needs to be deleted, use the **Delete DS** button to delete the disector completely.

11.

12. **Help** - If the Help button on the keyboard is pressed, a **Help** window opens displaying some helpful comments for this protocol.



13. **Moving to the Data's Disector** - In the **Mapping** window, *control-double-clicking* on object that belongs to the current multisector focuses the microscope stage to the data's disector. A warning window opens asking for confirmation.



Press **Yes** to move the stage to the disector holding that data. That disector then becomes the current disector. Press **Cancel** to dismiss the window without moving the stage or changing disectors.

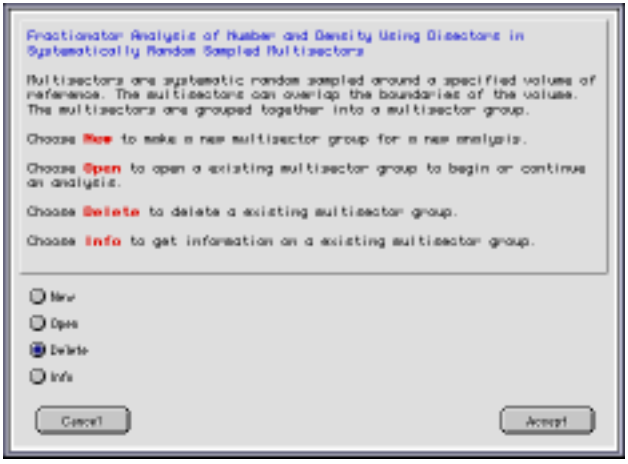
If you control-double-click on an object that is in the current disector, a warning window opens.

Alert: Be sure to have the **Selection** wool selected from the **Mapping Tools** window in order to control-double-click on it. Otherwise, you will only put a new data point on top of what you are clicking on.

14.

Delete Existing Multisector Group

Delete an existing multisector group for estimating numbers using a Fractionator protocol.



If you choose this option, and if there are no multisector groups for this Fractionator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Fractionator protocols in the document.

Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

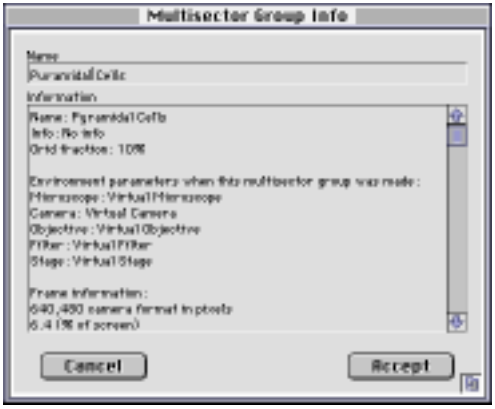
T - This first column indicates the Type of Multisector Group. It should show F for Fractionator.

Name - The second column is the name assigned to it when the multisector group was made.

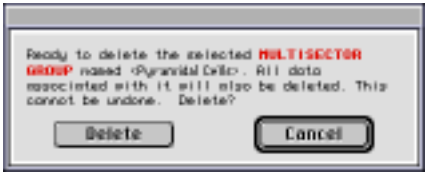
#MS - The third column indicates the number multisectors in the group.

Info - The fourth column is the information assigned to it when the multisector group was made.

Select the multisector group to delete and press the **Delete** button to delete that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.



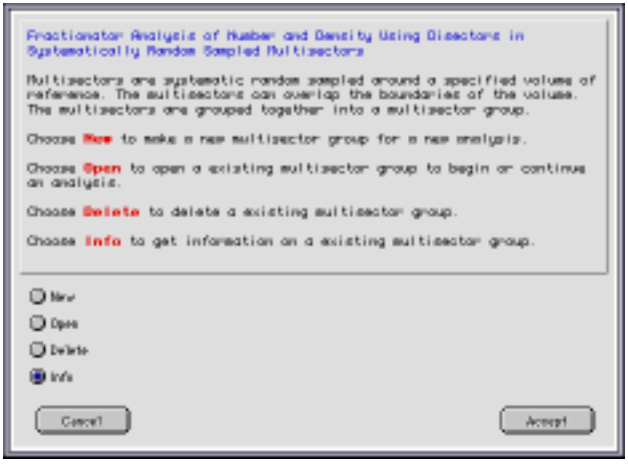
When deleting a group, a confirmation window opens warning that the deletion is permanent and cannot be undone.



Press **Delete** to delete the group, or press **Cancel** to dismiss the window without any changes.

Info on Existing Multisector Group

Get information by reports on existing multisector groups for estimating numbers using a Fractionator protocol.



If you choose this option, and if there are no multisector groups for this Fractionator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Fractionator protocols in the document.

Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

+ - This first column may be toggled on and off by pressing the mouse in this column. Toggling on selects the group for a report. Multiple selections may be made in this manner to report on more than one multisector group at one time.

T - This second column indicates the Type of Multisector Group. It should show F for Fractionator.

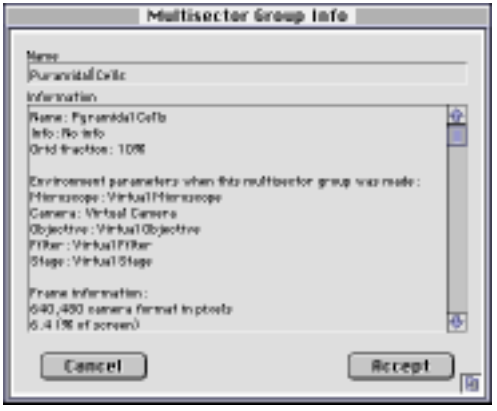
Name - The third column is the name assigned to it when the multisector group was made.

#MS - The fourth column indicates the number multisectors in the group.

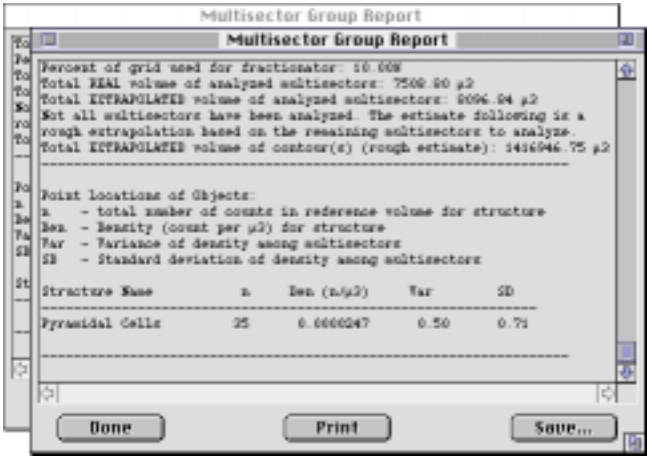
Info - The fifth column is the information assigned to it when the multisector group was made.

Select the multisector groups to report on and press the **Report** button to report on that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and

Information can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.



Multiple group reporting opens multiple report windows. A report window shows the same information as generating a report when conducting the experiment.



Surface Area by Cycloids/Point Counting in Vertical Sections

Opened by:

- Estimate Surface Area by Cycloids/Point Counting in Vertical Sections... in Stereology menu

The **Surface Area by Cycloids/Point Counting in Vertical Sections** window, for lack of a better name, is a choice window. From this you are to select one of four options to proceed with estimating volumes. A Cavalieri point counting grid is displayed along with the cycloids. Both surface area and volume can be estimated simultaneously.

Information on what this probe accomplishes and the four options are presented.

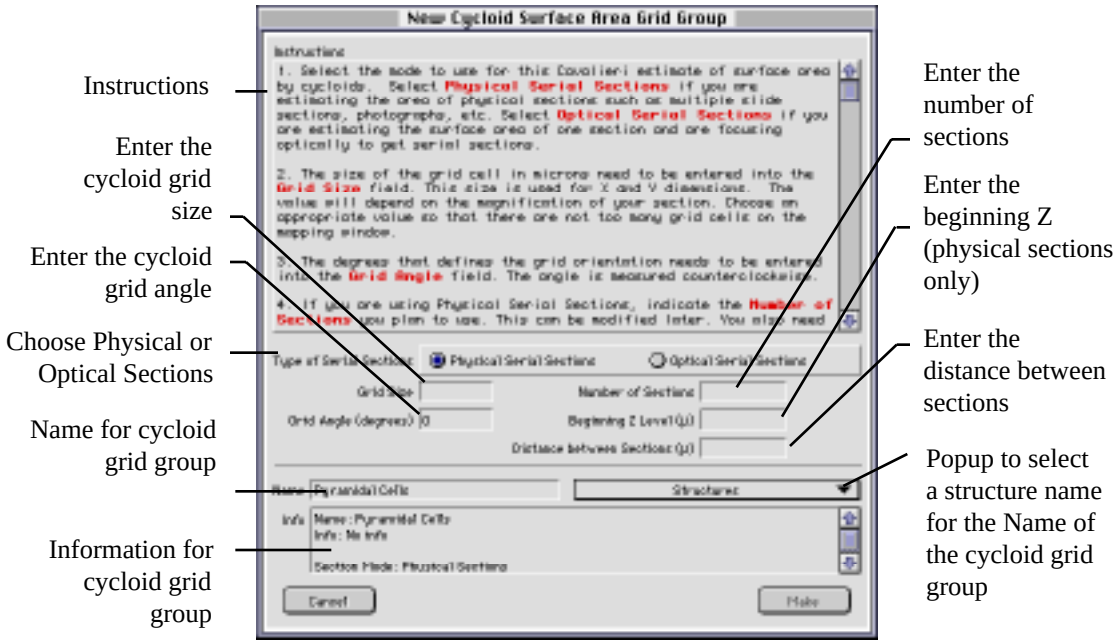
- **New** - make a new cycloid grid group to begin a new analysis on estimating the surface area of some structure
- **Open** - open an existing cycloid grid group for additional data entry or analysis
- **Delete** - delete an existing cycloid grid group from the document
- **Info** - get information on an existing cycloid grid group

Choose one of the options my pressing the mouse button the radio button. The radio button will highlight indicating that it is selected. Press **Accept** to continue with the process, or press **Cancel** to dismiss this window with no options or changes made to the document.

New Cycloid Grid Group

Make a new cycloid grid group for estimating surface area using a Cycloid protocol.

Select **New** and press **Accept**. A new window opens.



This window is the **New Cycloid Surface Area Grid Group** window. All of the parameters to create a cycloid grid group for a Cycloid analysis are entered here. A Cavalieri point counting grid is also made along with the cycloids. Both surface area and volume can be estimated simultaneously.

Physical or Optical Serial Sections

Either physical serial sections (photographs) or optical serial sections (from microscope) can be specified. Please refer to the chapters on ***Stereology*** in the ***User Guide Manual*** for more specific information on how to use these two different methods.

The previous figure shows Physical serial sections selected. Certain fields on the window will be visible.

Grid Size

This is the size of the point counting grid, measured in microns. A full cycloid is placed at every two point counting intersections.

Grid Angle

The slope of the grid can be altered by entering a value here from.

Number of Sections

The number of sections used as physical sections is entered here. Typically this is the number of images you would be imported.

Beginning Z Level

Physical sections are not read from the microscope. Therefore, the beginning Z level is needed to place the entire set of images properly into 3 space.

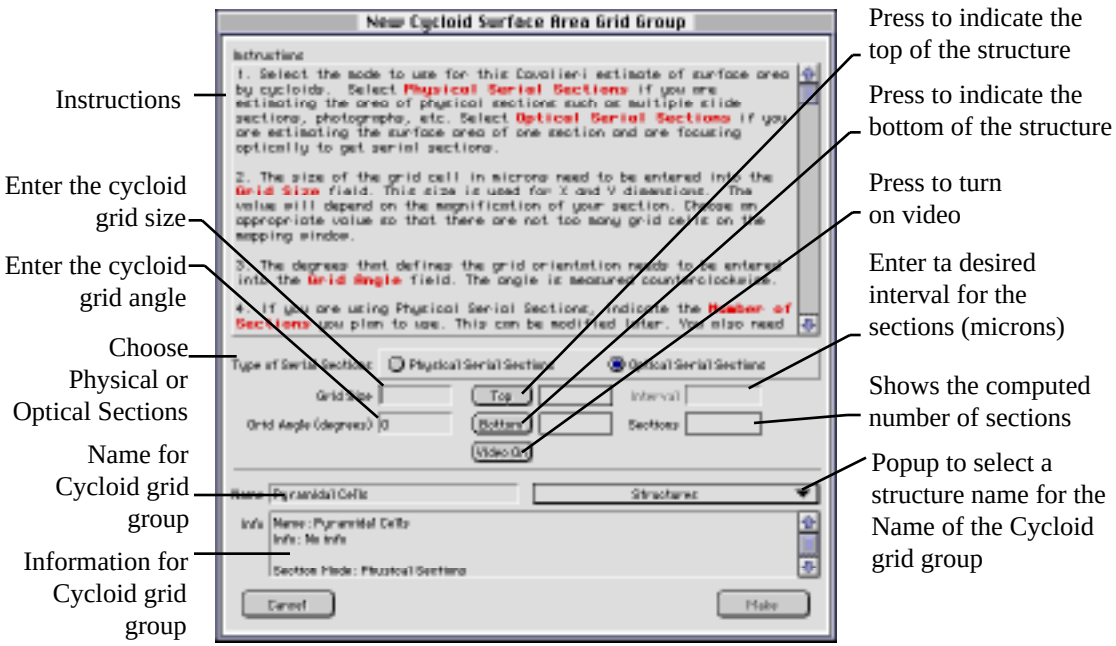
Distance Between Sections

Physical sections are not read from the microscope. Therefore, the distance between the sections is needed to place the entire set of images properly into 3 space.

Name and Info

Enter the name and information for this cycloid grid group. The default name is the current structure of the mapping window. Press and hold on the Structure Popup menu button to get a list of all the structures for this mapping window and select one for the name. You can also enter in any other name you desire.

If the **Optical Serial Sections** button is pressed, a new set of fields are displayed.



Optical serial sections are acquired from the microscope. NeuroZoom controls the microscope by moving the stage. Therefore, the structure whose surface and volume is being estimated needs to focused on, so that the top and bottom of the structure are known, and the number of optical sections can be computed. The parameters for optical sections are listed below.

Video Press the **Video On** button to turn on video. This button is provided here because this window is modal. No other window is active. This is the only window with which you can interact.

Top Focus on the top of the structure and press the **Top** button. The current position from the microscope will be read.

Bottom Focus on the bottom of the structure and press the **Bottom** button. The current position from the microscope will be read.

Interval Enter the desired interval based on 1 interval = 1 micron. The number of serial sections is then computed and displayed in the field named **Sections**. The first serial section is randomly placed to ensure a systematic random sample. The random position generated is shown in parentheses. You can adjust the **Interval** number until a comfortable number of sections is displayed.

When all parameters are entered, the **Make** button is enabled. Press **Cancel** to dismiss the window and not make the cycloid grid group. Press **Make** to make the cycloid grid group.

Once the cycloid grid group has been made successfully, a window opens presenting the option of starting the stereology session immediately on this new group.

Press **No** to close window and return to the **Mapping** window. Press **Yes** to continue immediately to the Cycloid analysis on this cycloid grid group.

Answering **Yes** is the same as opening an existing cycloid grid group for analysis. This is presented in the next session.

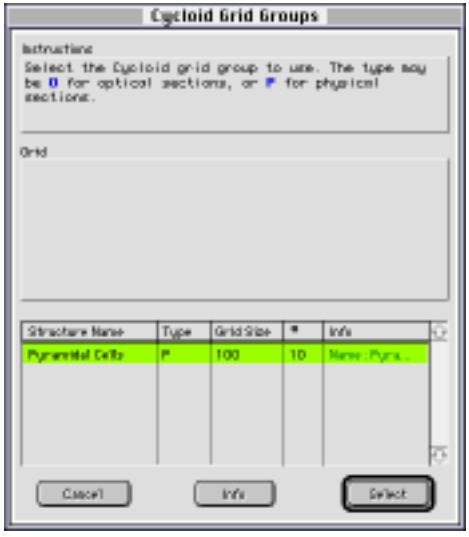
Open Existing Cycloid Grid Group

Open an existing cycloid grid group for estimating surface area using a Cycloid protocol.

If you choose this option, and if there are no cycloid grid groups for this Cycloid protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Cycloid protocols in the document.

Tip: Holding down the OPTION when choosing Estimate Surface Area By Cycloid and Point Counting in Vertical Sections... in the Stereology Menu will automatically preselect this OPEN button.



Structure Name - The first column is the name assigned to it when the cycloid grid group was made.

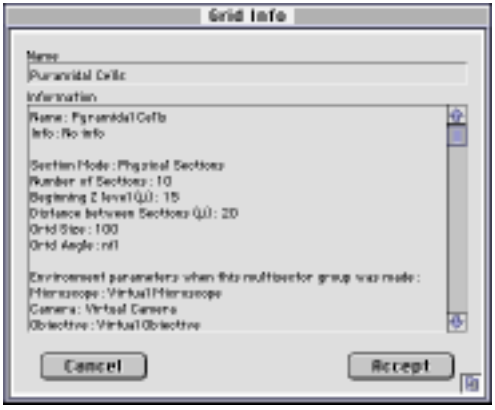
Type - This second column indicates the type of serial section. It should show P for Physical or S for Serial.

Grid Size- The third column indicates the size of the point counting grid.

- The fourth column is the number of sections.

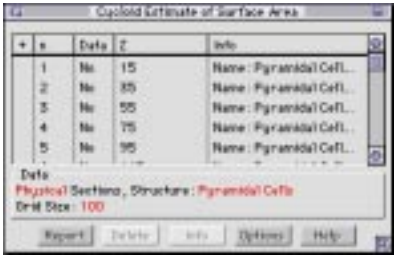
Info - The fifth column is the information assigned to it when the cycloid grid group was made.

Select the cycloid grid group to reopen and press the **Select** button to continue counting with that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.



Cycloid Control Window for Physical Sections

If a cycloid grid group for physical sections is selected and opened for additional estimating, the following window opens.



This is the **Cycloid Estimate of Surface Area Control** window from which the probe is controlled. There are various components to this window.

1. **Grids** - The grids for the current group that the microscope stage are located in the only scrolling field on this window.

Of these grids, only one may be displayed at any one time. These are overlaid on top of the image. To select a grid for display, press in the leftmost column (the column labelled +). This will toggle the visibility of the grid on and off.

The **selected** grid is the grid which is operated on by certain buttons located at the bottom of the window. The selected grid and the current grid are not necessarily the same, although in this example, they are.

The column labelled **n** indicates the number of the grid from the top section downward to the bottom.

The column labelled **Data** indicates if there is any data in this grid. The column shows *No* if there is no data in the disector, or *Yes* if there is data. Data are positive for any intersected point on any cycloid in the grid, or any selected intersection on the point counting grid.

The column labelled **Z** indicates the Z level of the grid. Z levels start at a random point between the top and the first section, and then are placed systematically thereafter based on the specified distance between sections.

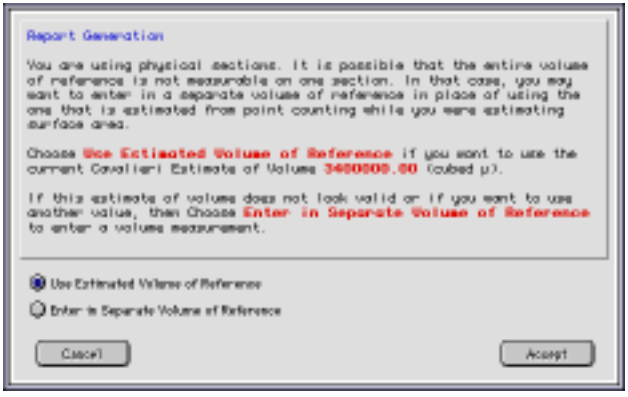
The **Info** for each disector is auto-generated when the cycloid grid is made. This can be changed with the button labelled **Info**.

2. **Data Field** - This shows whether physical or optical serial sections are being used, the name of the group, which is probably also the name of the structure whose surface area you are estimating, the grid size, and the currently calculated surface area as a real time measurement.

   All data are estimated for surface area by using the **Cycloid** tool (see the chapters on ***Stereology*** in the ***User Guide Manual*** to understand how to estimate structure surface area using this tool. Also see the section in this manual on the **Cycloid** tool).

Several control buttons in the **Control** window perform actions on the grids.

3. **Report** - pressing this button generates a report. There are two options for reports when physical serial sections are used. A window opens from which to select one option.

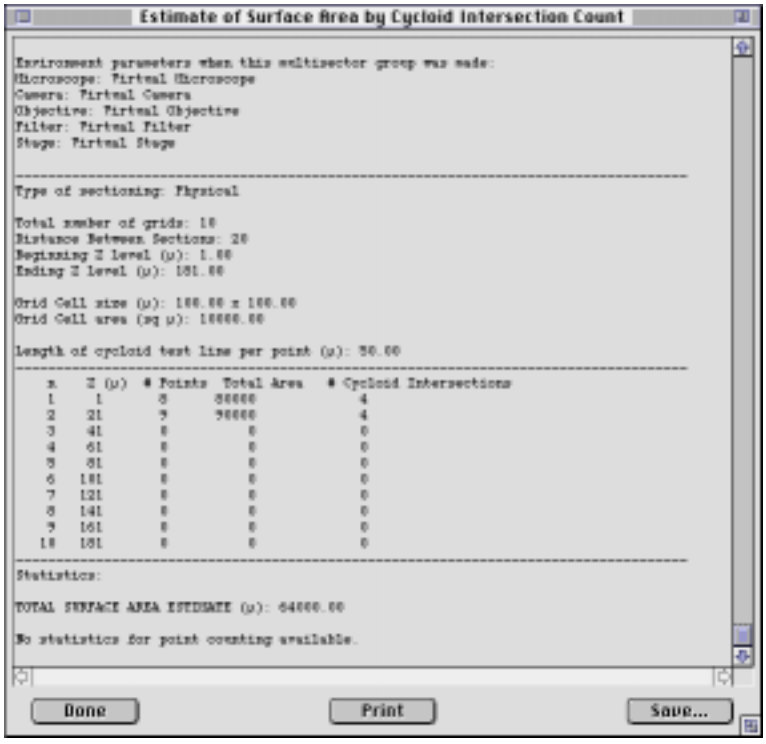


When physical sections are used, the volume of reference may or may not be known. For example, it may be too large to estimate with the point counting (Cavalieri) grid. You can choose to enter in the volume from the keyboard.

- Choose **Use Estimated Volume of Reference** to use the volume computed from the point counting (Cavalieri) grid.

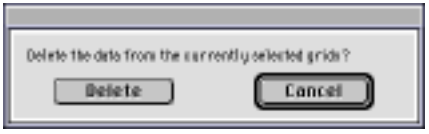
- Choose **Enter in Separate Volume of Reference** if entering from the keyboard.

The report generated displays in a separate window.

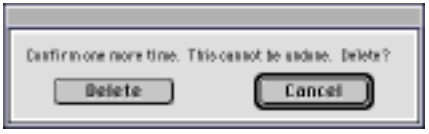


The report can be closed by pressing **Done**, printed to the selected printer by pressing **Print**, or saved to a Text file by pressing **Save...**. In this window, the end of the report shows each grid and its estimates for surface area. The total surface area is the totalled and displayed.

4. **Delete** - pressing the **Delete** button deletes all of the current data in the current grid. The data deleted are the cycloid and point counting grid intersections. A confirmation window opens for confirmation.

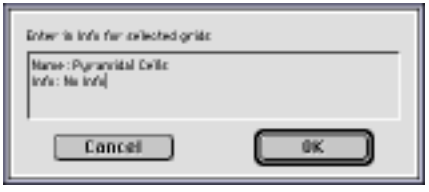


A second window opens again for confirmation. This data deletion cannot be undone.



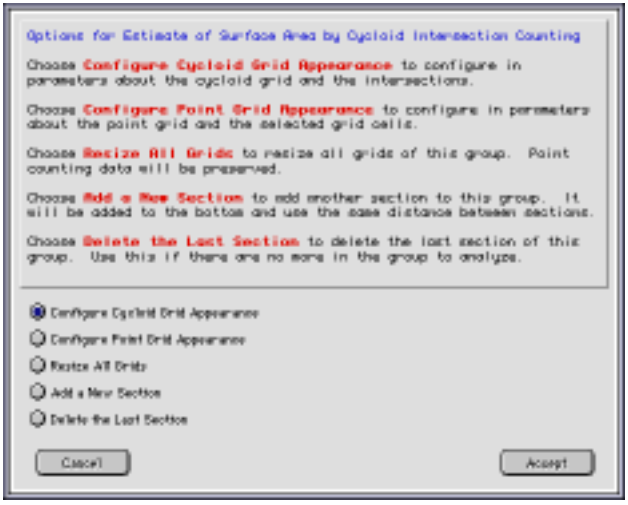
Multiple grids may be selected by *Shift-Clicking* on each grid. Pressing **Delete** will delete the data from all the grids simultaneously.

5. **Info** - pressing the **Info** button opens a window in which to enter in new information for the selected grid. Note that this is for the selected grid, and does not have to be the current grid.

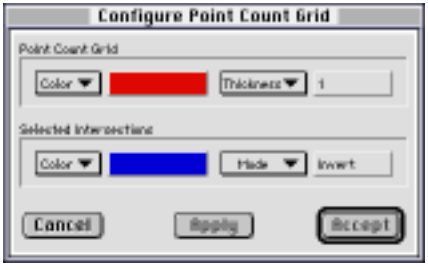


Multiple grids may be selected by *Shift-Clicking* on each grid. Pressing **Info** will change the information for all the grids to the new value simultaneously.

6. **Options** - pressing the **Options** button opens a window with options to change certain parameters for the grids.



Configure Point Grid Appearance - opens a window to adjust the appearance of the point grid.



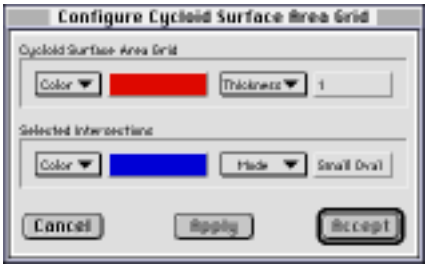
Press and hold on the **Color** button for the color of the grid itself, and of intersections points with the structure. A popup menu shows the color choices.

Press and hold on the **Thickness** button for the thickness of the grid. A popup menu shows the choices from 1 to 6. 6 is thickest.

Press and hold on the **Mode** button for the symbol to use on the *intersections*. A popup menu shows the choices from.

Press **Cancel** to dismiss the window and cancel any changes. Press **Apply** to see the effects. Press **Accept** to accept the changes and stored them with the grids.

Configure Cycloid Grid Appearance - opens a window to adjust the appearance of the cycloid grid.



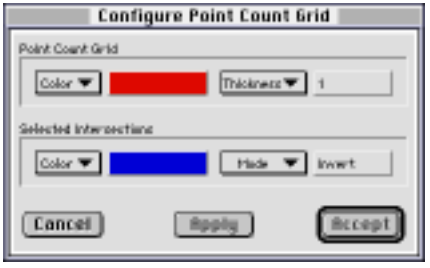
Press and hold on the **Color** button for the *color of the grid* itself, and of intersections points with the structure. A popup menu shows the color choices.

Press and hold on the **Thickness** button for the *thickness of the grid*. A popup menu shows the choices from 1 to 6. 6 is thickest.

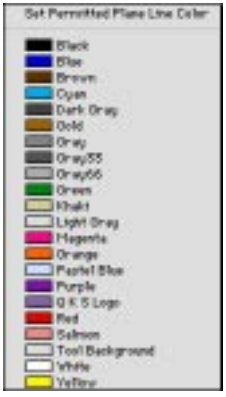
Press and hold on the **Mode** button for the symbol to use on the *intersections*. A popup menu shows the choices from.

Press **Cancel** to dismiss the window and cancel any changes. Press **Apply** to see the effects. Press Accept to accept the changes and stored them with the grids.

Configure Point Grid Appearance - opens a window to adjust the appearance of the point grid.



Press and hold on the **Color** button for the color of the grid itself, and of intersections points with the structure. A popup menu shows the color choices.

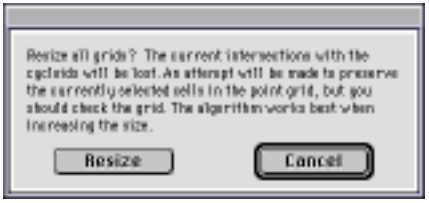


Press and hold on the **Thickness** button for the thickness of the grid. A popup menu shows the choices from 1 to 6. 6 is thickest.

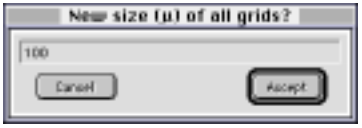
Press and hold on the **Mode** button for the symbol to use on the *intersections*. A popup menu shows the choices from.

Press **Cancel** to dismiss the window and cancel any changes. Press **Apply** to see the effects. Press **Accept** to accept the changes and stored them with the grids.

Resize all Grids - this option allows all the grids to be resize. The cycloid intersection data on the surface area will be lost. An attempt will be made to preserve the data for the point grid

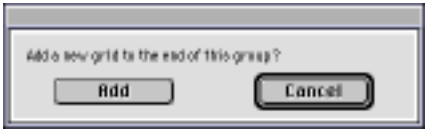


Press **Cancel** to dismiss the window with resizing. Press **Resize** to continue. A window opens in which to enter the new grid size.



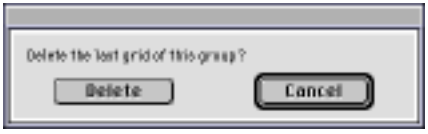
Enter the new value for the size of the grid in microns. The current size is the default size shown. Press **Cancel** to dismiss the window with resizing. Press **Accept** to accept the new size. The grids resize and redisplay.

Add a New Section - this option adds a new section (grid) to the end of the current list.



Press **Cancel** to dismiss the window without any changes. Press **Add** to add the new section.

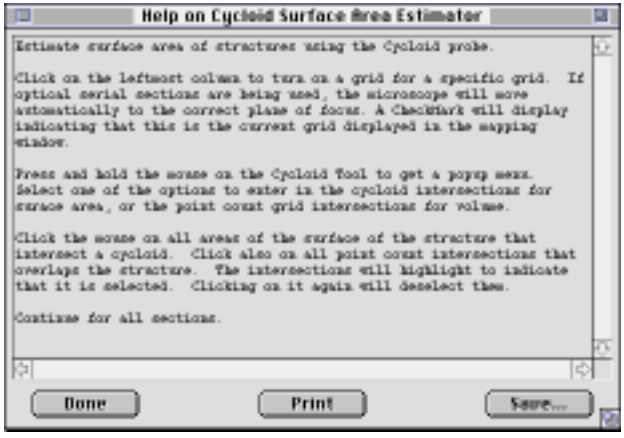
Delete the Last Section - this option deletes the last section (grid).



Press **Cancel** to dismiss the window without any changes. Press **Delete** to continue with another confirmation window.

Press **Cancel** to dismiss the window without any changes. Press **Delete** to delete the last section.

7. **Help -** If the Help button on the keyboard is pressed, a **Help** window opens displaying some helpful comments for this protocol.

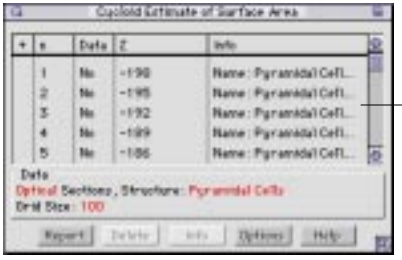


Cycloid Control Window for Optical Sections

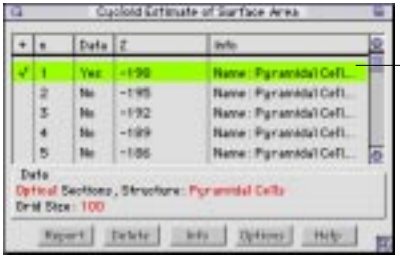
If a cycloid grid group for optical sections is selected and opened for additional estimating, the following window opens.

This is the **Cycloid Estimate of Surface Area Control** window from which the probe is controlled. There are various components to this window.

1. **Grids** - The grids for the current group that the microscope stage are located in the only scrolling field on this window.



Of these grids, only one may be displayed at any one time. These are overlaid on top of the image. To select a grid for display, press in the leftmost column (the column labelled +). This will toggle the visibility of the grid on and off.



Currently displayed grid

The *selected* grid is the grid which is operated on by certain buttons located at the bottom of the window. The selected grid and the current grid are not necessarily the same, although in this example, they are.

The column labelled **n** indicates the number of the grid from the top section downward to the bottom.

The column labelled **Data** indicates if there is any data in this grid. The column shows *No* if there is no data in the disector, or *Yes* if there is data. Data are positive for any intersected point on any cycloid in the grid, or any selected intersection on the point counting grid.

The column labelled **Z** indicates the Z level of the grid. Z levels start at a random point between the top and the first section, and then are placed systematically thereafter based on the specified distance between sections.

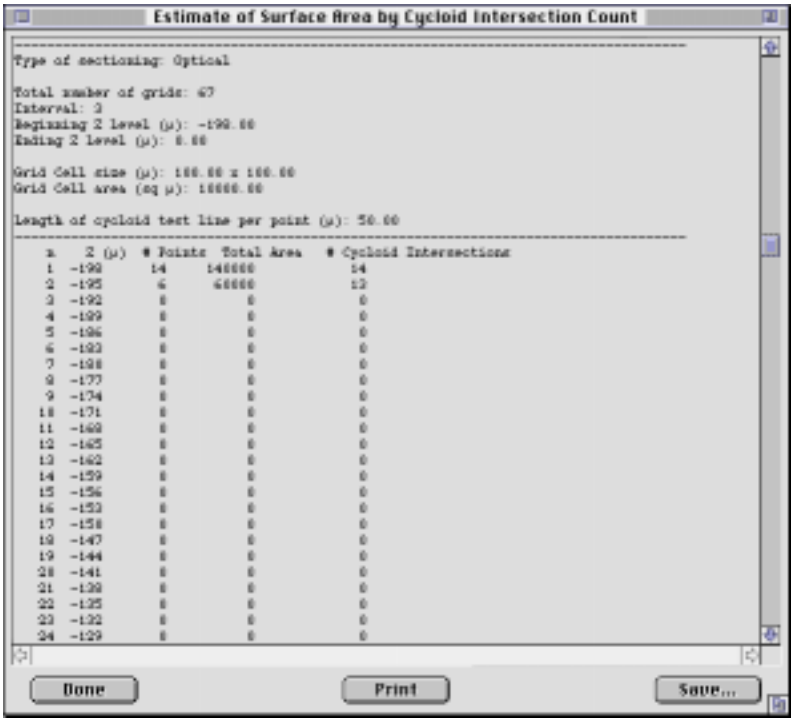
The **Info** for each disector is auto-generated when the cycloid grid is made. This can be changed with the button labelled **Info**.

2. **Data Field** - This shows whether physical or optical serial sections are being used, the name of the group, which is probably also the name of the structure whose surface area you are estimating, the grid size, and the currently calculated surface area as a real time measurement.

   All data are estimated for surface area by using the **Cycloid** tool (see the chapters on ***Stereology*** in the ***User Guide Manual*** to understand how to estimate structure surface area using this tool. Also see the section in this manual on the **Cycloid** tool).

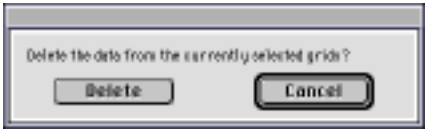
Several control buttons in the **Control** window perform actions on the grids.

3. **Report** - pressing this button generates a report. For optical serial sections, no options appear because the volume of reference can always be calculated from the existing point counting grid. A report window opens immediately.

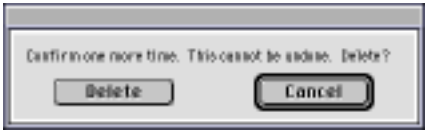


The report can be closed by pressing **Done**, printed to the selected printer by pressing **Print**, or saved to a Text file by pressing **Save...**. In this window, the end of the report shows each grid and its estimates for surface area.

4. **Delete** - pressing the **Delete** button deletes all of the current data in the current grid. The data deleted are the cycloid and point counting grid intersections. A confirmation window opens for confirmation.

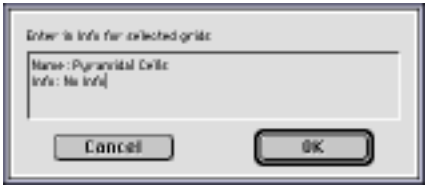


A second window opens again for confirmation. This data deletion cannot be undone.



Multiple grids may be selected by *Shift-Clicking* on each grid. Pressing **Delete** will delete the data from all the grids simultaneously.

5. **Info** - pressing the **Info** button opens a window in which to enter in new information for the selected grid. Note that this is for the selected grid, and does not have to be the current grid.

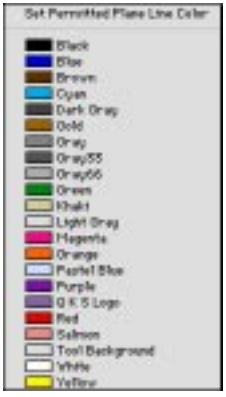


Multiple grids may be selected by *Shift-Clicking* on each grid. Pressing **Info** will change the information for all the grids to the new value simultaneously.

6. **Options** - pressing the **Options** button opens a window with options to change certain parameters for the grids.

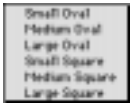
Configure Cycloid Grid Appearance - opens a window to adjust the appearance of the cycloid grid.

Press and hold on the **Color** button for the *color of the grid* itself, and of intersections points with the structure. A popup menu shows the color choices.



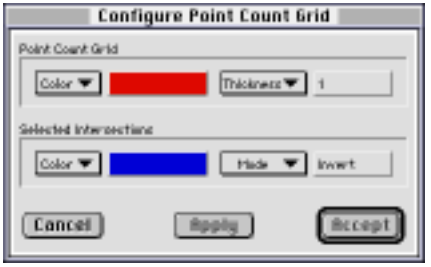
Press and hold on the **Thickness** button for the *thickness of the grid*. A popup menu shows the choices from 1 to 6. 6 is thickest.

Press and hold on the **Mode** button for the symbol to use on the *intersections*. A popup menu shows the choices from.



Press **Cancel** to dismiss the window and cancel any changes. Press **Apply** to see the effects. Press Accept to accept the changes and stored them with the grids.

Configure Point Grid Appearance - opens a window to adjust the appearance of the point grid.



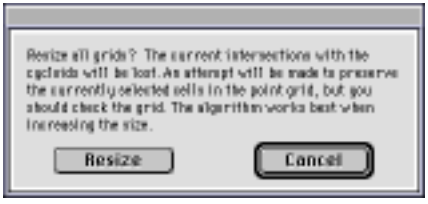
Press and hold on the **Color** button for the color of the grid itself, and of intersections points with the structure. A popup menu shows the color choices.

Press and hold on the **Thickness** button for the thickness of the grid. A popup menu shows the choices from 1 to 6. 6 is thickest.

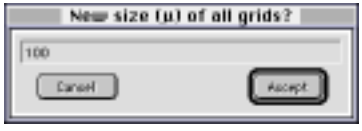
Press and hold on the **Mode** button for the symbol to use on the *intersections*. A popup menu shows the choices from.

Press **Cancel** to dismiss the window and cancel any changes. Press **Apply** to see the effects. Press **Accept** to accept the changes and stored them with the grids.

Resize all Grids - this option allows all the grids to be resize. The cycloid intersection data on the surface area will be lost. An attempt will be made to preserve the data for the point grid

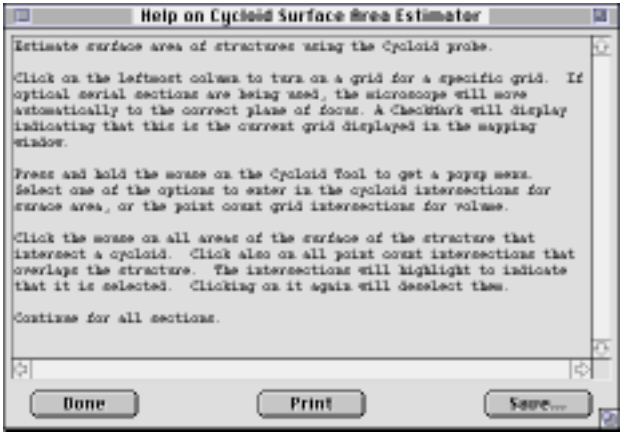


Press **Cancel** to dismiss the window with resizing. Press **Resize** to continue. A window opens in which to enter the new grid size.



Enter the new value for the size of the grid in microns. The current size is the default size shown. Press **Cancel** to dismiss the window with resizing. Press **Accept** to accept the new size. The grids resize and redisplay.

7. **Help** - If the Help button on the keyboard is pressed, a **Help** window opens displaying some helpful comments for this protocol.

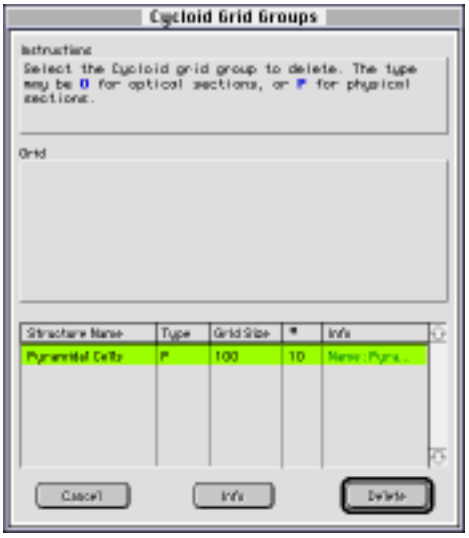


Delete Existing Cycloid Grid Group

Delete an existing cycloid grid group for estimating surface area using a Cycloid protocol.

If you choose this option, and if there are no cycloid grid groups for this Cycloid protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Cycloid protocols in the document.



Structure Name - The first column is the name assigned to it when the cycloid grid group was made.

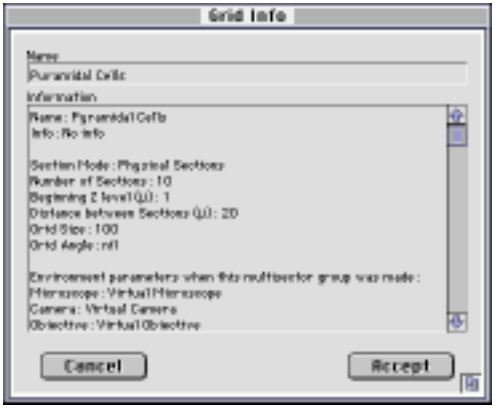
Type - This second column indicates the type of serial section. It should show P for Physical or S for Serial.

Grid Size- The third column indicates the size of the point counting grid.

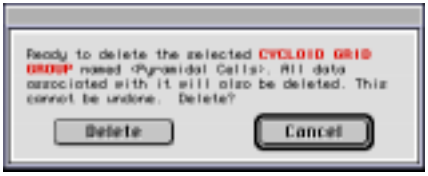
- The fourth column is the number of sections.

Info - The fifth column is the information assigned to it when the cycloid grid group was made.

Select the cycloid grid group to delete and press the **Delete** button to delete that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.



When deleting a group, a confirmation window opens warning that the deletion is permanent and cannot be undone.



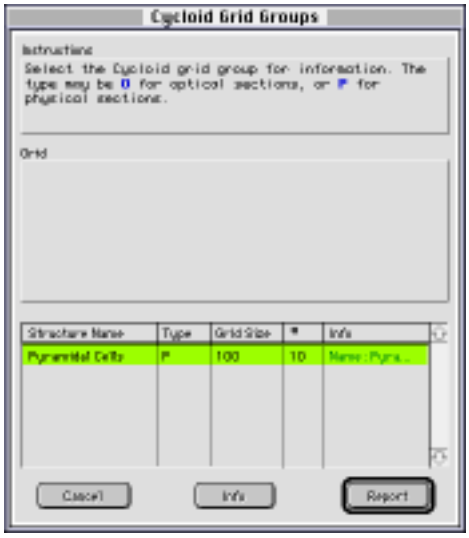
Press **Delete** to delete the group, or press **Cancel** to dismiss the window without any changes.

Info on Existing Cycloid Grid Group

Get information by reports on existing cycloid grid groups for estimating surface area using a Cycloid protocol.

If you choose this option, and if there are no cycloid grid groups for this Cycloid protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Cycloid protocols in the document.



Structure Name - The first column is the name assigned to it when the cycloid grid group was made.

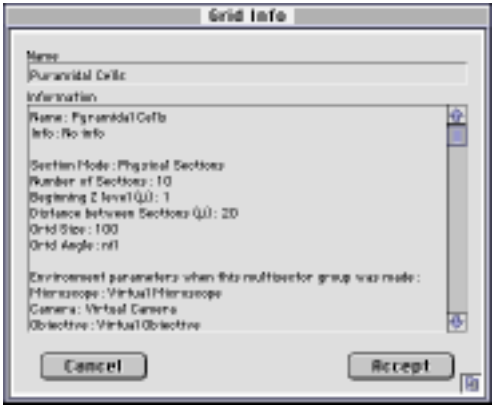
Type - This second column indicates the type of serial section. It should show P for Physical or S for Serial.

Grid Size- The third column indicates the size of the point counting grid.

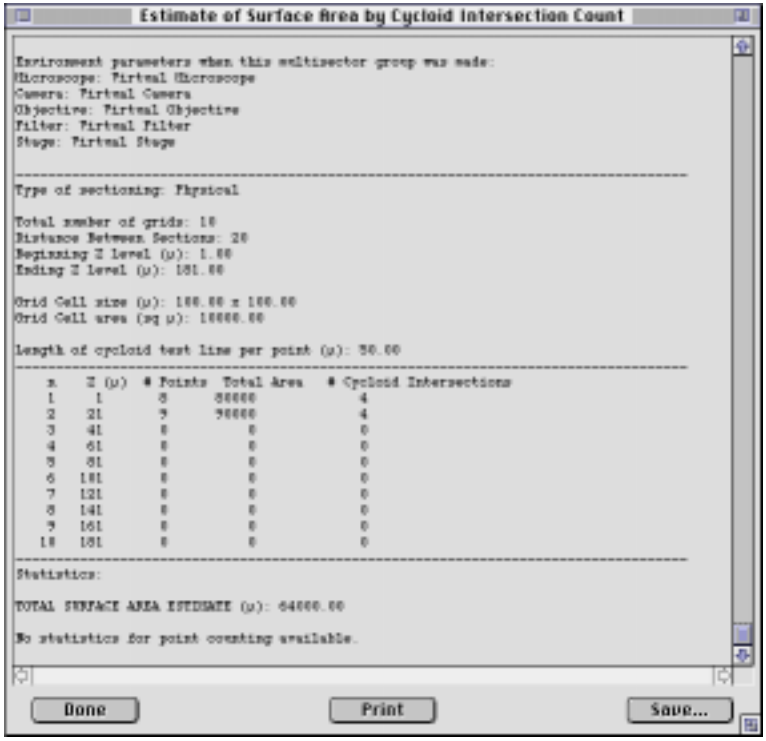
- The fourth column is the number of sections.

Info - The fifth column is the information assigned to it when the cycloid grid group was made.

Select the cycloid grid groups to report on and press the **Report** button to report on that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.



Multiple group reporting opens multiple report windows. A report window shows the same information as generating a report when conducting the experiment.


Estimate of Surface Area by Cycloid Intersection Count
Environment parameters when this multisector group was made:
Microscope: Virtual Microscope
Camera: Virtual Camera
Objective: Virtual Objective
Filter: Virtual Filter
Stage: Virtual Stage
Type of sectioning: Physical
Total number of grids: 10
Distance Between Sections: 20
Beginning Z level (µ): 1.00
Ending Z level (µ): 181.00
Grid Cell size (µ): 100.00 x 100.00
Grid Cell area (sq µ): 10000.00
Length of cycloid test line per point (µ): 50.00
n Z (µ) # Points Total Area # Cycloid Intersections
1 1 8 80000 4
2 21 9 90000 4
3 41 0 0 0
4 61 0 0 0
5 81 0 0 0
6 101 0 0 0
7 121 0 0 0
8 141 0 0 0
9 161 0 0 0
10 181 0 0 0
Statistics:
TOTAL SURFACE AREA ESTIMATE (µ): 64000.00
No statistics for point counting available.
Done
Print
Save...

Volume by Point Counting (Cavalieri)

Opened by:

- Estimate Volume by Point Counting (Cavalieri)... in Stereology menu

The **Volume by Point Counting (Cavalieri)** window, for lack of a better name, is a choice window. From this you are to select one of four options to proceed with estimating volumes.

Information on what this probe accomplishes and the four options are presented.

- **New** - make a new Cavalieri grid group to begin a new analysis on estimating the volume of some structure

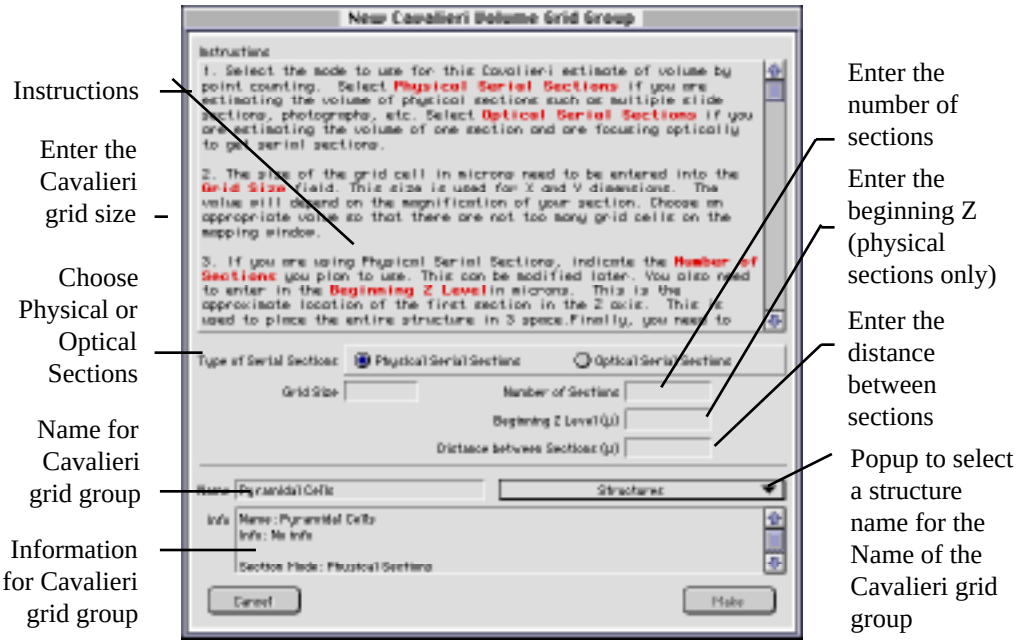
- **Open** - open an existing Cavalieri grid group for additional data entry or analysis
- **Delete** - delete an existing Cavalieri grid group from the document
- **Info** - get information on an existing Cavalieri grid group

Choose one of the options my pressing the mouse button the radio button. The radio button will highlight indicating that it is selected. Press **Accept** to continue with the process, or press **Cancel** to dismiss this window with no options or changes made to the document.

New Cavalieri Grid Group

Make a new Cavalieri grid group for estimating volume using a Cavalieri protocol.

Select **New** and press **Accept**. A new window opens.



This window is the **New Cavalieri Volume Grid Group** window. All of the parameters to create a Cavalieri grid group for a Cavalieri analysis are entered here.

Physical or Optical Serial Sections

Either physical serial sections (photographs) or optical serial sections (from microscope) can be specified. Please refer to the chapters on ***Stereology*** in the ***User Guide Manual*** for more specific information on how to use these two different methods.

The previous figure shows Physical serial sections selected. Certain fields on the window will be visible.

Grid Size

This is the size of the point counting grid, measured in microns.

Number of Sections

The number of sections used as physical sections is entered here. Typically this is the number of images you would be imported.

Beginning Z Level

Physical sections are not read from the microscope. Therefore, the beginning Z level is needed to place the entire set of images properly into 3 space.

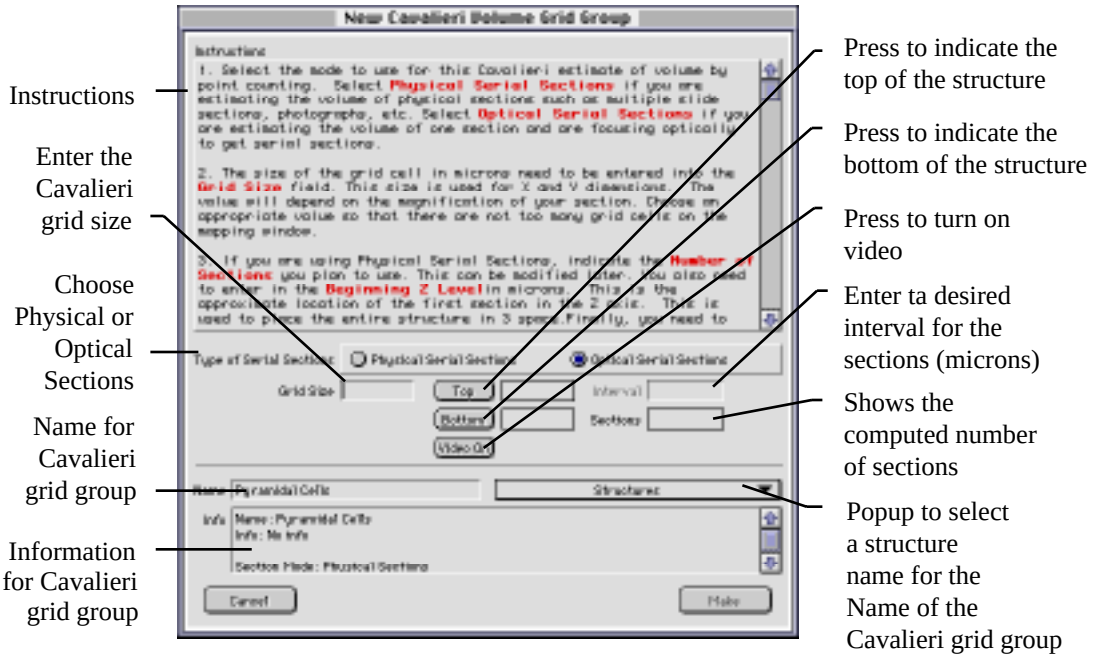
Distance Between Sections

Physical sections are not read from the microscope. Therefore, the distance between the sections is needed to place the entire set of images properly into 3 space.

Name and Info

Enter the name and information for this Cavalieri grid group. The default name is the current structure of the mapping window. Press and hold on the Structure Popup menu button to get a list of all the structures for this mapping window and select one for the name. You can also enter in any other name you desire.

If the **Optical Serial Sections** button is pressed, a new set of fields are displayed.



Optical serial sections are acquired from the microscope. NeuroZoom controls the microscope by moving the stage. Therefore, the structure whose volume is being estimated needs to focused on, so that the top and bottom of the structure are known, and the number of optical sections can be computed. The parameters for optical sections are listed below.

Video

Press the **Video On** button to turn on video. This button is provided here because this window is modal. No other window is active. This is the only window with which you can interact.

Top

Focus on the top of the structure and press the **Top** button. The current position from the microscope will be read.

Bottom

Focus on the bottom of the structure and press the **Bottom** button. The current position from the microscope will be read.

Interval

Enter the desired interval based on 1 interval = 1 micron. The number of serial sections is then computed and displayed in the field named **Sections**. The first serial section is randomly placed to ensure a systematic random sample. The random position generated is shown in parentheses. You can adjust the **Interval** number until a comfortable number of sections is displayed.

When all parameters are entered, the **Make** button is enabled. Press **Cancel** to dismiss the window and not make the Cavalieri grid group. Press **Make** to make the Cavalieri grid group.

Once the Cavalieri grid group has been made successfully, a window opens presenting the option of starting the stereology session immediately on this new group.

Press **No** to close window and return to the **Mapping** window. Press **Yes** to continue immediately to the Cavalieri analysis on this Cavalieri grid group.

Answering **Yes** is the same as opening an existing Cavalieri grid group for analysis. This is presented in the next session.

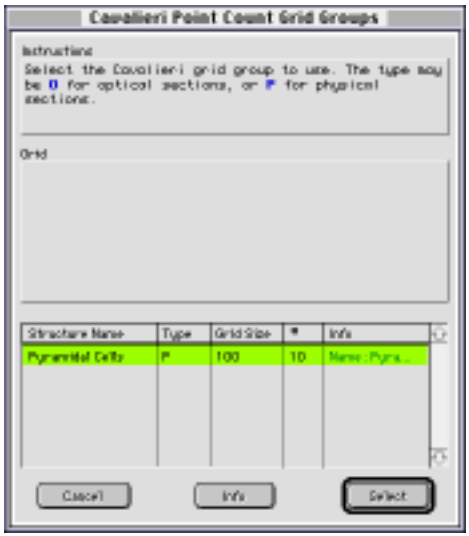
Open Existing Cavalieri Grid Group

Open an existing Cavalieri grid group for estimating volume using a Cavalieri protocol.

If you choose this option, and if there are no Cavalieri grid groups for this Cavalieri protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Cavalieri protocols in the document.

Tip: Holding down the OPTION when choosing Estimate Volume By Point Counting (Cavalieri)... in the Stereology Menu will automatically preselect this OPEN button.



Structure Name - The first column is the name assigned to it when the Cavalieri grid group was made.

Type - This second column indicates the type of serial section. It should show P for Physical or S for Serial.

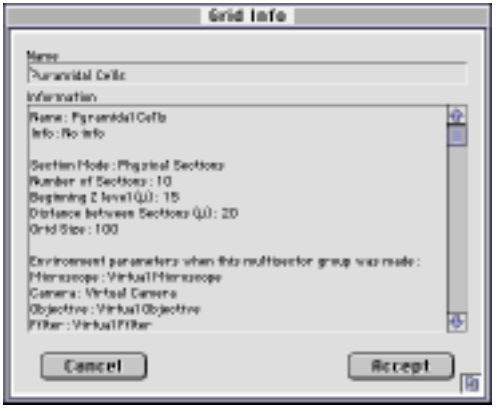
Grid Size- The third column indicates the size of the point counting grid.

- The fourth column is the number of sections.

Info - The fifth column is the information assigned to it when the Cavalieri grid group was made.

Select the Cavalieri grid group to reopen and press the **Select** button to continue counting with that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the

Name and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.



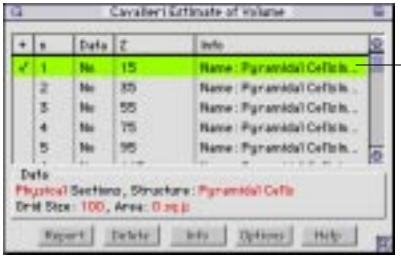
Cavalieri Control Window for Physical Sections

If a Cavalieri grid group for physical sections is selected and opened for additional estimating, the following window opens.

This is the **Cavalieri Estimate of Volume Control** window from which the probe is controlled. There are various components to this window.

1. **Grids** - The grids for the current group that the microscope stage are located in the only scrolling field on this window.

Of these grids, only one may be displayed at any one time. These are overlaid on top of the image. To select a grid for display, press in the leftmost column (the column labelled +). This will toggle the visibility of the grid on and off.



The **selected** grid is the grid which is operated on by certain buttons located at the bottom of the window. The selected grid and the current grid are not necessarily the same, although in this example, they are.

The column labelled **n** indicates the number of the grid from the top section downward to the bottom.

The column labelled **Data** indicates if there is any data in this grid. The column shows *No* if there is no data in the disector, or *Yes* if there is data. Data are positive for any selected intersection on the point counting grid.

The column labelled **Z** indicates the Z level of the grid. Z levels start at a random point between the top and the first section, and then are placed systematically thereafter based on the specified distance between sections.

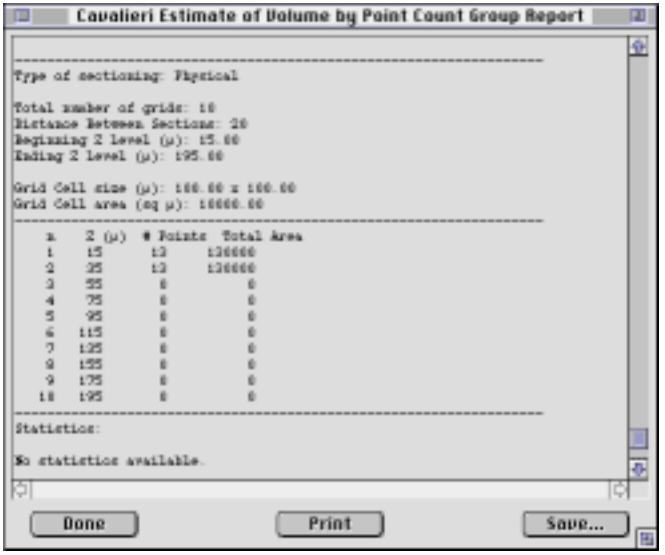
The **Info** for each disector is auto-generated when the Cavalieri grid is made. This can be changed with the button labelled **Info**.

2. **Data Field** - This shows whether physical or optical serial sections are being used, the name of the group, which is probably also the name of the structure whose volume you are estimating, the grid size, and the currently calculated volume as a real time measurement.

   All data are estimated for volume by using the **Cavalieri** tool (see the chapters on ***Stereology*** in the ***User Guide Manual*** to understand how to estimate structure volume using this tool. Also see the section in this manual on the **Cavalieri** tool).

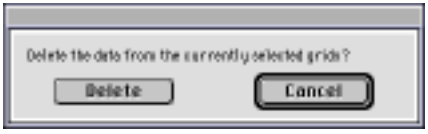
Several control buttons in the **Control** window perform actions on the grids.

3. **Report** - pressing this button generates a report.

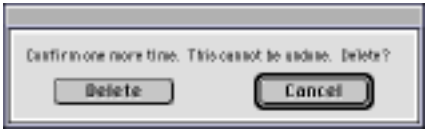


The report can be closed by pressing **Done**, printed to the selected printer by pressing **Print**, or saved to a Text file by pressing **Save...**. In this window, the end of the report shows each grid and its estimates for volume.

4. **Delete** - pressing the **Delete** button deletes all of the current data in the current grid. The data deleted are the point counting grid intersections. A confirmation window opens for confirmation.

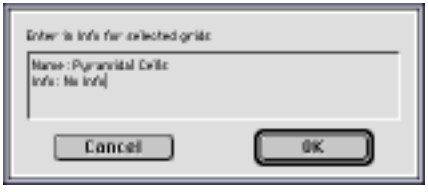


A second window opens again for confirmation. This data deletion cannot be undone.



Multiple grids may be selected by *Shift-Clicking* on each grid. Pressing **Delete** will delete the data from all the grids simultaneously.

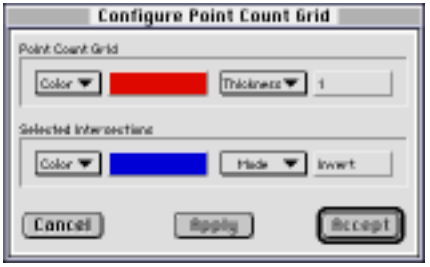
5. **Info** - pressing the **Info** button opens a window in which to enter in new information for the selected grid. Note that this is for the selected grid, and does not have to be the current grid.



Multiple grids may be selected by *Shift-Clicking* on each grid. Pressing **Info** will change the information for all the grids to the new value simultaneously.

6. **Options** - pressing the **Options** button opens a window with options to change certain parameters for the grids.

Configure Point Grid Appearance - opens a window to adjust the appearance of the point grid.



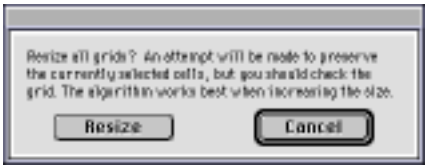
Press and hold on the **Color** button for the color of the grid itself, and of intersections points with the structure. A popup menu shows the color choices.

Press and hold on the **Thickness** button for the thickness of the grid. A popup menu shows the choices from 1 to 6. 6 is thickest.

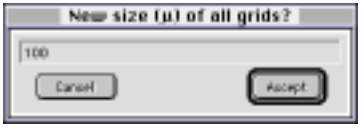
Press and hold on the **Mode** button for the symbol to use on the *intersections*. A popup menu shows the choices from.

Press **Cancel** to dismiss the window and cancel any changes. Press **Apply** to see the effects. Press **Accept** to accept the changes and stored them with the grids.

Resize all Grids - this option allows all the grids to be resize. An attempt will be made to preserve the data for the point grid

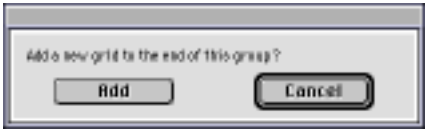


Press **Cancel** to dismiss the window with resizing. Press **Resize** to continue. A window opens in which to enter the new grid size.



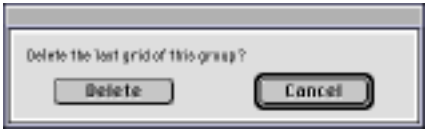
Enter the new value for the size of the grid in microns. The current size is the default size shown. Press **Cancel** to dismiss the window with resizing. Press **Accept** to accept the new size. The grids resize and redisplay.

Add a New Section - this option adds a new section (grid) to the end of the current list.



Press **Cancel** to dismiss the window without any changes. Press **Add** to add the new section.

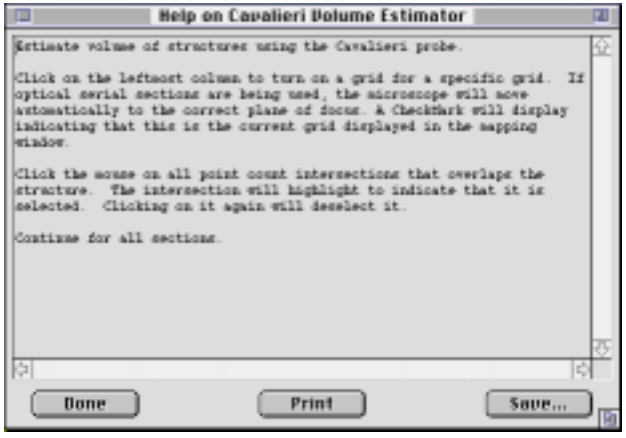
Delete the Last Section - this option deletes the last section (grid).



Press **Cancel** to dismiss the window without any changes. Press **Delete** to continue with another confirmation window.

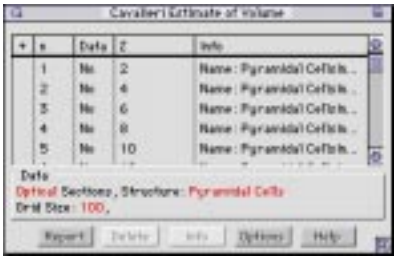
Press **Cancel** to dismiss the window without any changes. Press **Delete** to delete the last section.

7. **Help -** If the Help button on the keyboard is pressed, a **Help** window opens displaying some helpful comments for this protocol.



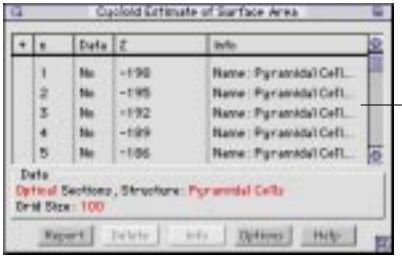
Cavalieri Control Window for Optical Sections

If a Cavalieri grid group for optical sections is selected and opened for additional estimating, the following window opens.

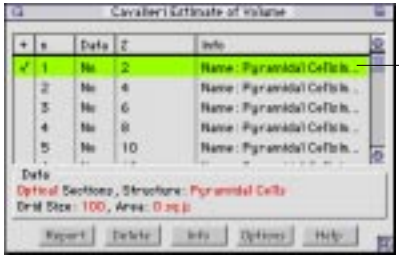


This is the **Cavalieri Estimate of Volume Control** window from which the probe is controlled. There are various components to this window.

1. **Grids** - The grids for the current group that the microscope stage are located in the only scrolling field on this window.



Of these grids, only one may be displayed at any one time. These are overlaid on top of the image. To select a grid for display, press in the leftmost column (the column labelled +). This will toggle the visibility of the grid on and off.



The *selected* grid is the grid which is operated on by certain buttons located at the bottom of the window. The selected grid and the current grid are not necessarily the same, although in this example, they are.

The column labelled **n** indicates the number of the grid from the top section downward to the bottom.

The column labelled **Data** indicates if there is any data in this grid. The column shows *No* if there is no data in the disector, or *Yes* if there is data. Data are positive for any selected intersection on the point counting grid.

The column labelled **Z** indicates the Z level of the grid. Z levels start at a random point between the top and the first section, and then are placed systematically thereafter based on the specified distance between sections.

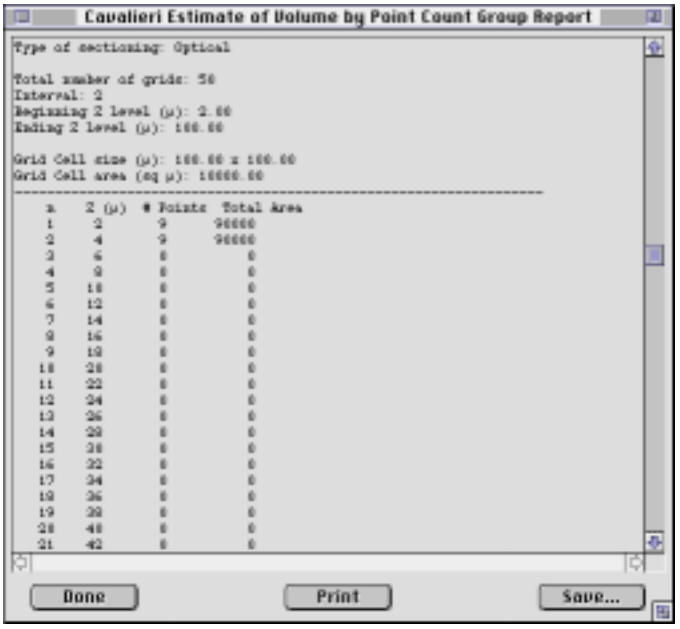
The **Info** for each disector is auto-generated when the Cavalieri grid is made. This can be changed with the button labelled **Info**.

2. **Data Field** - This shows whether physical or optical serial sections are being used, the name of the group, which is probably also the name of the structure whose volume you are estimating, the grid size, and the currently calculated volume as a real time measurement.

   All data are estimated for volume by using the **Cavalieri** tool (see the chapters on ***Stereology*** in the ***User Guide Manual*** to understand how to estimate structure volume using this tool. Also see the section in this manual on the **Cavalieri** tool).

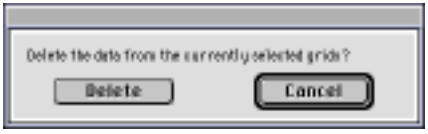
Several control buttons in the **Control** window perform actions on the grids.

3. **Report** - pressing this button generates a report. For optical serial sections, no options appear because the volume of reference can always be calculated from the existing point counting grid. A report window opens immediately.

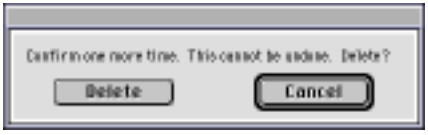


The report can be closed by pressing **Done**, printed to the selected printer by pressing **Print**, or saved to a Text file by pressing **Save...**. In this window, the end of the report shows each grid and its estimates for volume.

4. **Delete** - pressing the **Delete** button deletes all of the current data in the current grid. The data deleted are the point counting grid intersections. A confirmation window opens for confirmation.

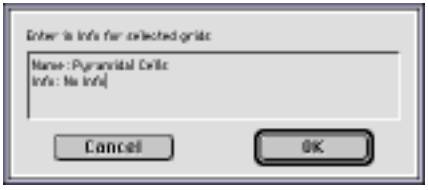


A second window opens again for confirmation. This data deletion cannot be undone.



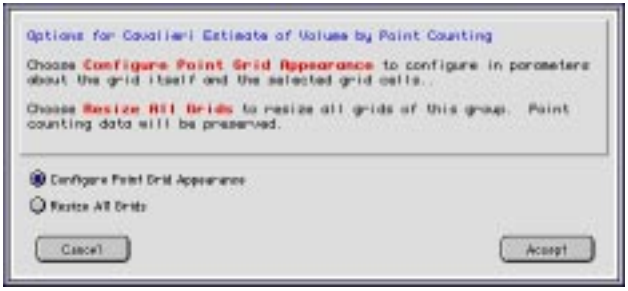
Multiple grids may be selected by *Shift-Clicking* on each grid. Pressing **Delete** will delete the data from all the grids simultaneously.

5. **Info** - pressing the **Info** button opens a window in which to enter in new information for the selected grid. Note that this is for the selected grid, and does not have to be the current grid.

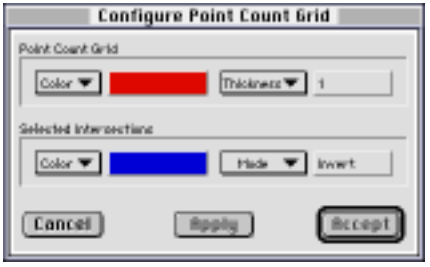


Multiple grids may be selected by *Shift-Clicking* on each grid. Pressing **Info** will change the information for all the grids to the new value simultaneously.

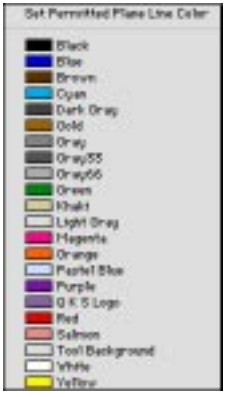
6. **Options** - pressing the **Options** button opens a window with options to change certain parameters for the grids.



Configure Point Grid Appearance - opens a window to adjust the appearance of the point grid.



Press and hold on the **Color** button for the color of the grid itself, and of intersections points with the structure. A popup menu shows the color choices.

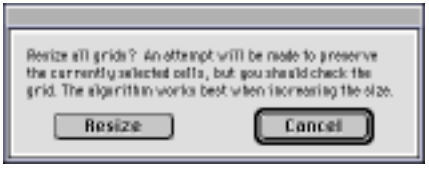


Press and hold on the **Thickness** button for the thickness of the grid. A popup menu shows the choices from 1 to 6. 6 is thickest.

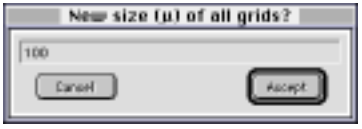
Press and hold on the **Mode** button for the symbol to use on the *intersections*. A popup menu shows the choices from.

Press **Cancel** to dismiss the window and cancel any changes. Press **Apply** to see the effects. Press **Accept** to accept the changes and stored them with the grids.

Resize all Grids - this option allows all the grids to be resize. An attempt will be made to preserve the data for the point grid

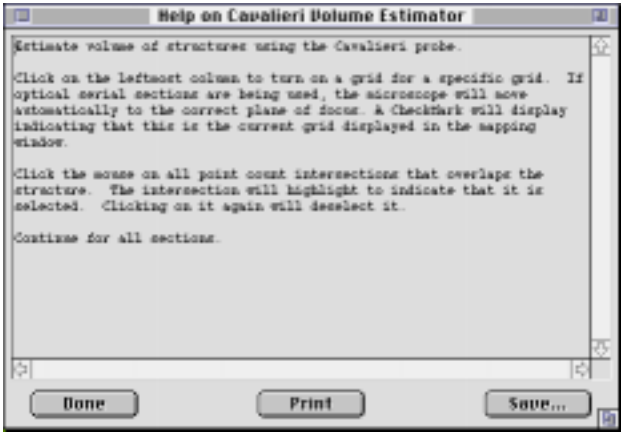


Press **Cancel** to dismiss the window with resizing. Press **Resize** to continue. A window opens in which to enter the new grid size.



Enter the new value for the size of the grid in microns. The current size is the default size shown. Press **Cancel** to dismiss the window with resizing. Press **Accept** to accept the new size. The grids resize and redisplay.

7. **Help** - If the Help button on the keyboard is pressed, a **Help** window opens displaying some helpful comments for this protocol.

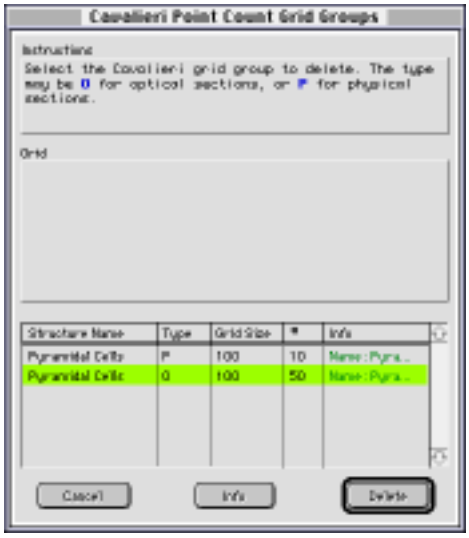


Delete Existing Cavalieri Grid Group

Delete an existing Cavalieri grid group for estimating volume using a Cavalieri protocol.

If you choose this option, and if there are no Cavalieri grid groups for this Cavalieri protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Cavalieri protocols in the document.



Structure Name - The first column is the name assigned to it when the Cavalieri grid group was made.

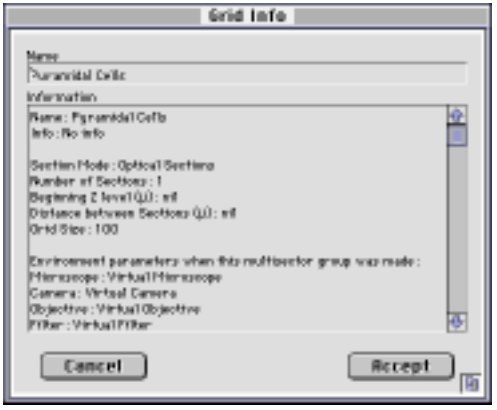
Type - This second column indicates the type of serial section. It should show P for Physical or S for Serial.

Grid Size- The third column indicates the size of the point counting grid.

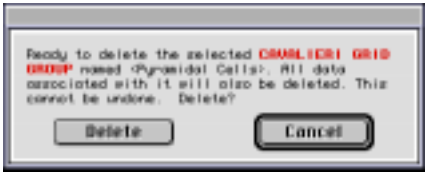
- The fourth column is the number of sections.

Info - The fifth column is the information assigned to it when the Cavalieri grid group was made.

Select the Cavalieri grid group to delete and press the **Delete** button to delete that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.



When deleting a group, a confirmation window opens warning that the deletion is permanent and cannot be undone.



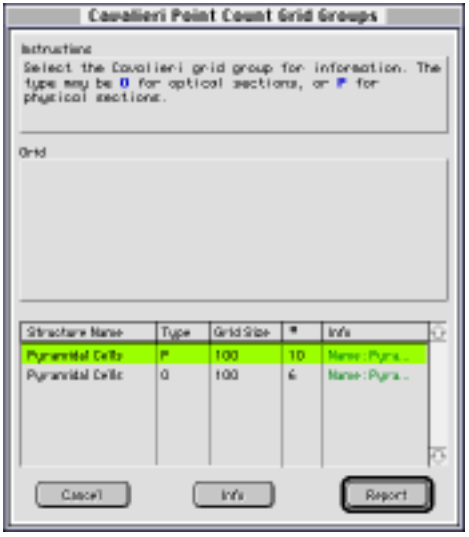
Press **Delete** to delete the group, or press **Cancel** to dismiss the window without any changes.

Info on Existing Cavalieri Grid Group

Get information by reports on existing Cavalieri grid groups for estimating volume using a Cavalieri protocol.

If you choose this option, and if there are no Cavalieri grid groups for this Cavalieri protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Cavalieri protocols in the document.



Structure Name - The first column is the name assigned to it when the Cavalieri grid group was made.

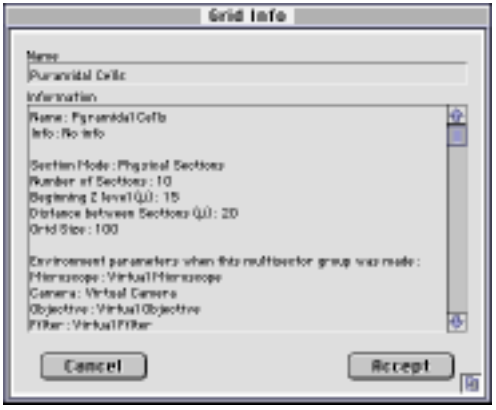
Type - This second column indicates the type of serial section. It should show P for Physical or S for Serial.

Grid Size- The third column indicates the size of the point counting grid.

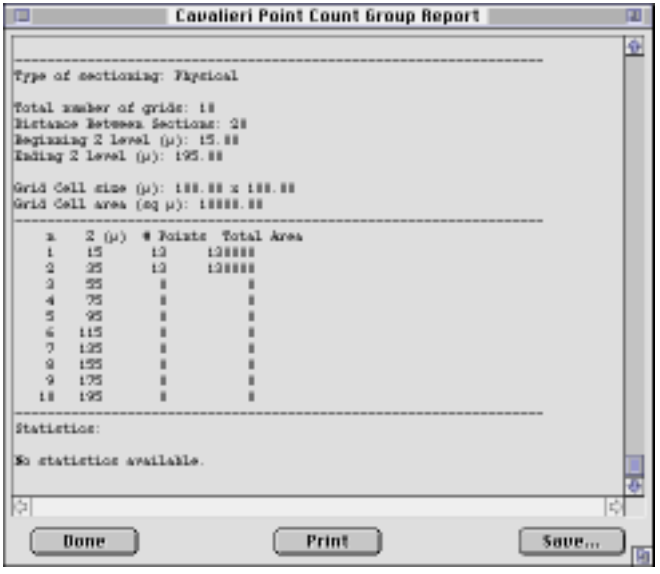
- The fourth column is the number of sections.

Info - The fifth column is the information assigned to it when the Cavalieri grid group was made.

Select the Cavalieri grid groups to report on and press the **Report** button to report on that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.



Multiple group reporting opens multiple report windows. A report window shows the same information as generating a report when conducting the experiment.

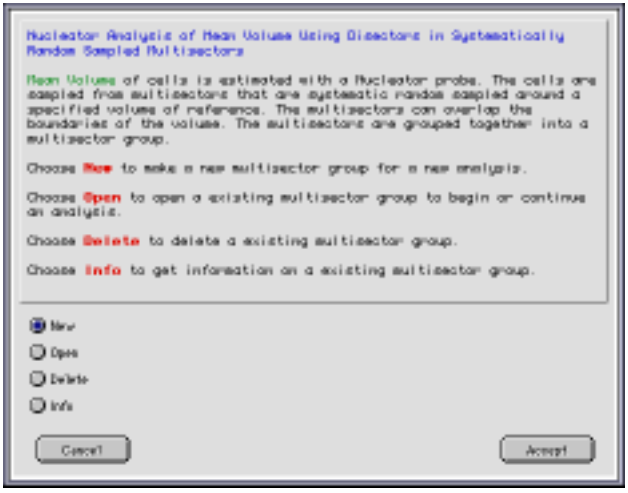


Volume by Nucleator in Vertical Sections

Opened by:

- Estimate Volume By Nucleator in Vertical Sections... in Stereology menu

The **Volume By Nucleator in Vertical Sections** window, for lack of a better name, is a choice window. From this you are to select one of four options to proceed with estimating volumes.



Information on what this probe accomplishes and the four options are presented.

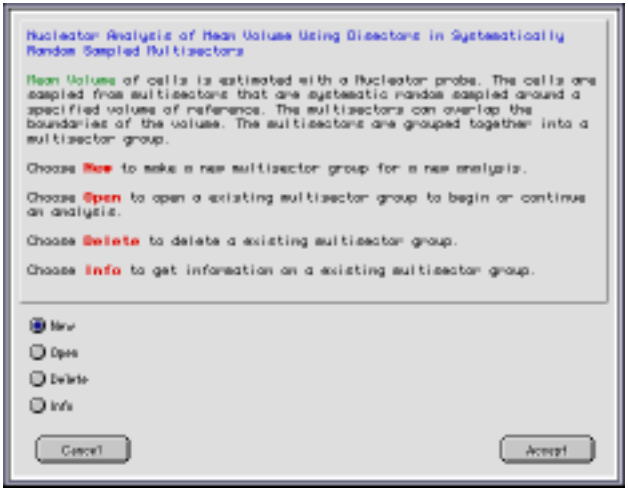
- **New** - make a new multisector group to begin a new analysis on estimating the mean volume of some structure
- **Open** - open an existing multisector group for additional data entry or analysis
- **Delete** - delete an existing multisector group from the document
- **Info** - get information on an existing multisector group

Choose one of the options my pressing the mouse button the radio button. The radio button will highlight indicating that it is selected. Press **Accept** to continue

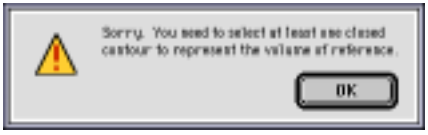
with the process, or press **Cancel** to dismiss this window with no options or changes made to the document.

New Multisector Group

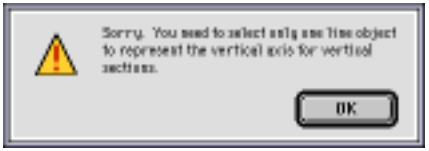
Make a new multisector group for estimating mean volume using a Nucleator protocol.



If you choose this option, and if you do not have at least one contour created and selected in the mapping window, an error window opens indicating that at least one selected contour is needed to represent the *volume of reference*.

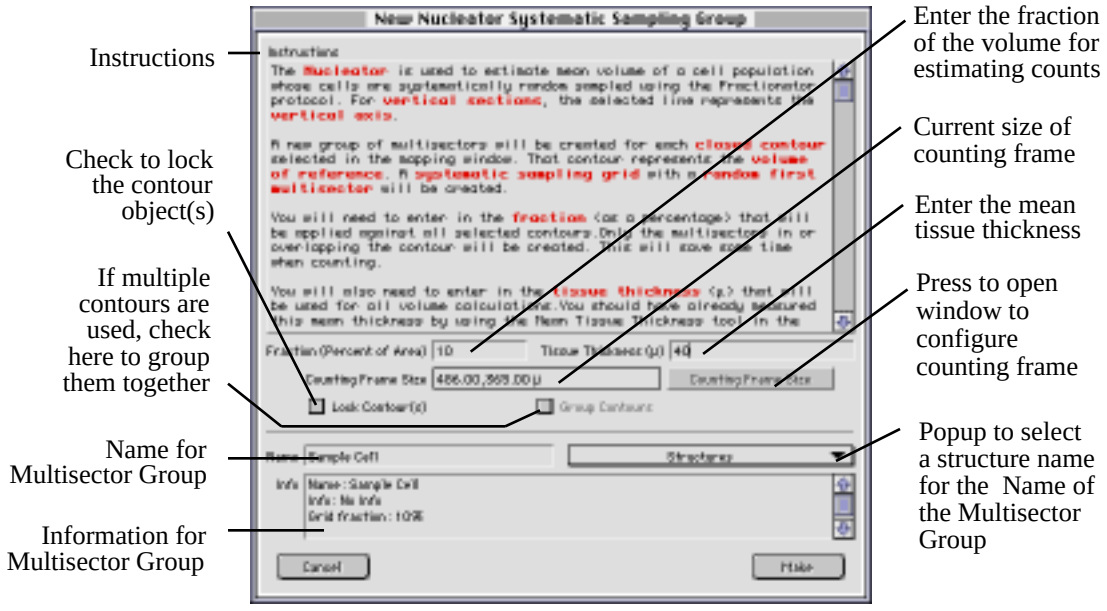


You also need one line object, drawn parallel to the *vertical axis* of the tissue section. This line object needs to be selected or else an error window opens. The same error window will open if more than one line object is selected.



Go back and use the mapping tools to create a closed contour around the volume of reference, and a line drawn parallel to the vertical axis. When you have done so, select them both, choose **Estimate Volume By Nucleator in Vertical Sections...** in the **Stereology** menu again, and select **New** and press **Accept**. A new window opens.

This window is the **New Nucleator Systematic Sampling Group** window. All of the parameters to create a multisector group for a Nucleator analysis are entered here. The multisectors are calculated on a systematic random sample through the selected contour. Only those multisectors that intersect the contour will be



presented. Disectors are created in those multisectors in which the estimates are places for cell volume estimates.

Fraction (Percent of Area)

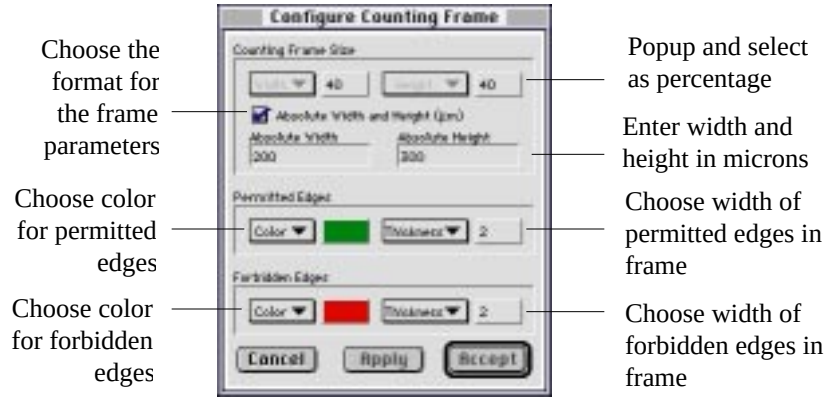
Enter the fraction that is desred for the Nucleator analysis. For example, if you want to estimate counts of cells in 10% of the contour volume, enter 10. The default is 10%. Please refer to the chapters on ***Stereology*** in the ***User Guide Manual*** for more specific information on this value.

Tissue Thickness (µ)

Enter the mean tissue thickness in microns for this section. This can be measured with the **Measure Mean Tissue Thickness** tool located in the **Analysis** submenu of the **Modules** menu.

Counting Frame Size

This is a button that opens a window from which to adjust the size of the counting frame. The current frame size is displayed in the field to its left.

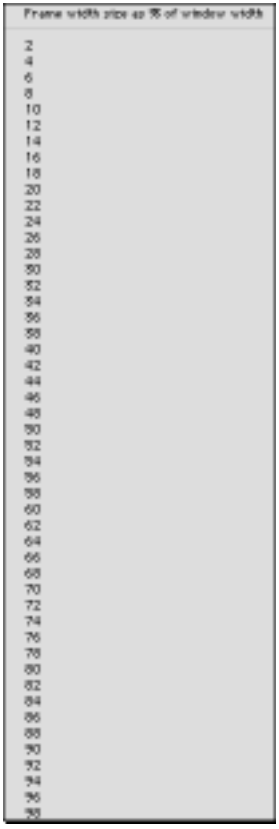


The size of the counting frame may be entered as a *percentage* of the screen size, or as an absolute width and height in microns. The major difference between the two techniques is that a percentage will always ensure that the counting frame fits in the mapping window regardless of the *lens objective* used. However, you cannot switch lens objectives in the middle of a stereology experiment using the counting frame because the absolute size of the frame would change.

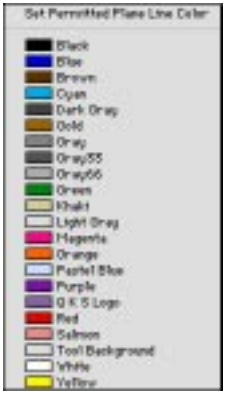
When you enter the size in *absolute microns*, the displayed size of the counting frame in the mapping window will change as you change the lens objective. The frame will always adjust to the absolute width and height entered. However, the problem here is that the size of the counting frame may exceed the size of the mapping window with lower power objectives. If so, NeuroZoom will alert you.

In all cases of stereology, you cannot change the lens objectives once the experiment is underway. However, one method of entering in the size may be more appropriate than the other, depending on how you go about the business of setting up the stereology experiment. You choose the method which best suits your needs. Please also refer to the chapters on ***Stereology*** in the ***User Guide Manual*** and the ***Reference Manual*** more information.

When choosing the width and height as a percentage, press and hold on the width or height button. A menu pops up for selectons from 2 to 98%.



Press and hold on the **Color** button for the *permitted and forbidden colors*. A popup menu shows the color choices.



Press and hold on the **Thickness** button for the *permitted and forbidden thicknesses*. A popup menu shows the choices from 1 to 6. 6 is thickest.

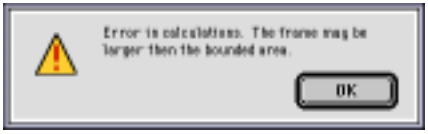
Press on the **Apply** button to see the changes. Press on **Cancel** to dismiss the window and cancel any changes you made with this window. Press **Accept** to dismiss the window and to store the changes made with this window.

Name and Info

Enter the name and information for this multisector group. The default name is the structure name of the contour that is selected when this window was opened. Press and hold on the **Structure Popup** menu button to get a list of all the structures for this mapping window and select one for the name. You can also enter in any other name you desire.

When all parameters are entered, the **Make** button is enabled. Press **Cancel** to dismiss the window and not make the multisector group. Press **Make** to make the multisector group.

If the counting frame is too large to accurately place a group of multisectors over the selected contour representing the volume of reference, a window opens displaying this error.



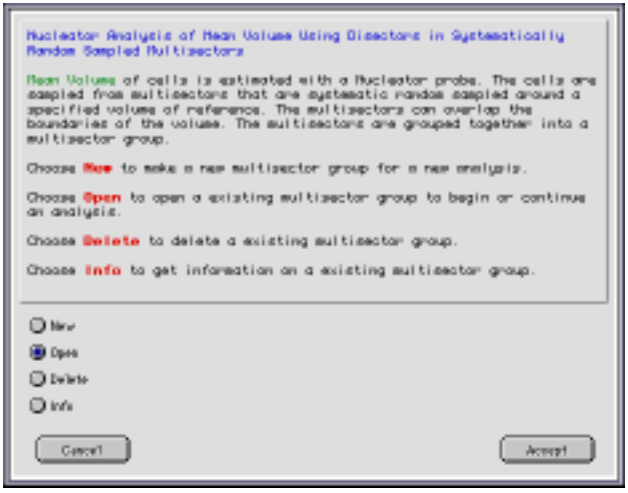
Once the multisector group has been made successfully, a window opens presenting the option of starting the stereology session immediately on this new group.

Press **No** to close window and return to the Mapping window. Press **Yes** to continue immediately to the Nucleator analysis on this multisector group.

Answering **Yes** is the same as opening an existing multisector group for analysis. This is presented in the next session.

Open Existing Multisector Group

Open an existing multisector group for estimating numbers using a Nucleator protocol.



If you choose this option, and if there are no multisector groups for this Nucleator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Nucleator protocols in the document.

Tip: Holding down the OPTION when choosing Estimate Volume By Nucleator in Vertical Sections... in the Stereology Menu will automatically preselect this OPEN button.

Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

T - This first column (T) indicates the Type of Multisector Group. It should show N for Nucleator.

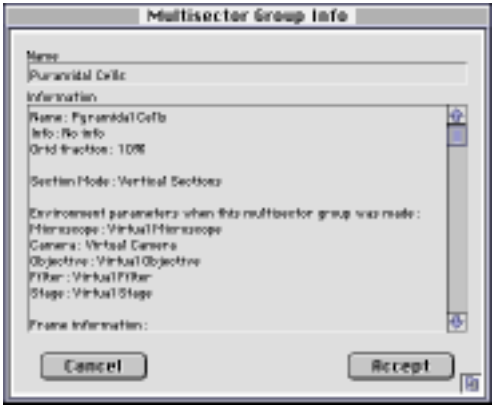
Name - The second column is the name assigned to it when the multisector group was made.

#MS - The third column indicates the number multisectors in the group.

Info - The fourth column is the information assigned to it when the multisector group was made.

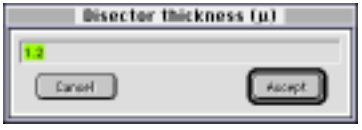
Select the multisector group to reopen and press the **Count** button to continue counting with that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both

the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.

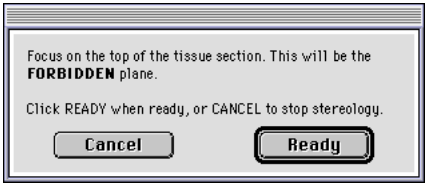


Nucleator (Vertical Sections) Control Window

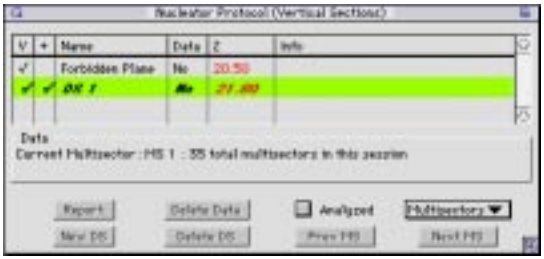
If a multisector group is selected and opened for counting, live video is turned on. If this is the first time the multisector group is being opened, the thickness of the disector must be entered in the following window.



Once the thickness is specified, another window prompts for you to focus on the top of the tissue section.

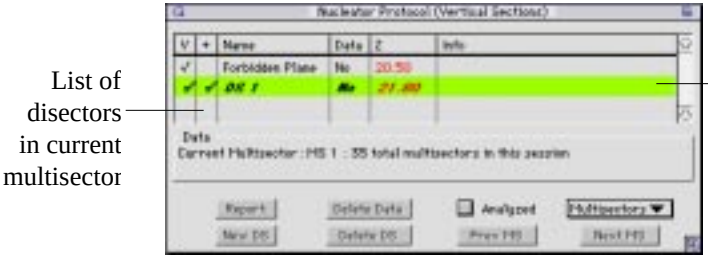


After the top is specified, the following window opens.



This is the **Nucleator Protocol (Vertical Sections) Control** window from which the probe is controlled. There are various components to this window.

1. **Disectors** - The disectors for the current multisector that the microscope stage are located in the only scrolling field on this window.

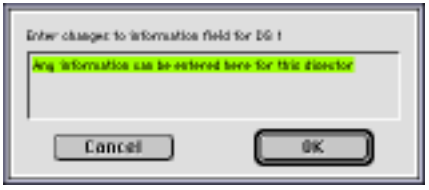


Of these disectors, the disector in *Italics* is the current disector. The current disector is the one that the microscope is focused on. Of the two planes of a disector, the microscope focuses on the reference plane. This is indicated in the column labelled Z. The selected disector is the disector which is highlighted in the highlight color. The selected disector and the current disector are not necessarily the same, although in this example, they are.

Double clicking on a disector makes the selected disector the current disector. The microscope stage will move to that new focus point, and the disector will be displayed in *Italics*.

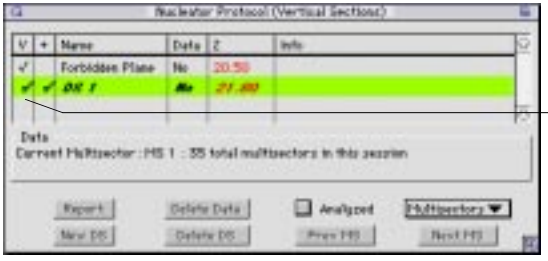
The **Name** for each disector is auto-generated when the multisector is made. The first disector is named *Forbidden Plane* because the microscope focuses on the first plane in the first disector, which by NeuroZoom convention, is the forbidden plane. No data is usually counted in this plane. The subsequent disectors are then named DS 1, DS 2, and so on.

The **Info** field is blank initially for each disector. However, you can Option-*Double Click* on a disector and a dialog window opens for you to enter in new information.



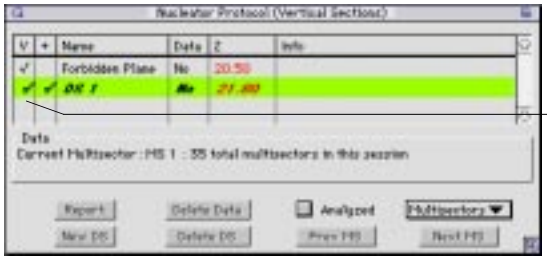
Tip: Option Double Click on a disector to change its information.

Visibility of data can be controlled by clicking on the leftmost column labelled **V.** A single click will toggle the visibility of all of the data for this disector. This is particularly useful if there is a large concentration of data in the multisectors, and they are visually collapsing on top of each other.



Single click in the V column to toggle visibility of the data in the selected disector

Inclusion of data can be controlled by clicking on the column labelled **+**. A single click will toggle the inclusion of all of the data for this disector. Use this to remove a disector completely from reports that are generated, including any statistics generated for the multisector or the multisector group.



Single click in the + column to toggle inclusion of the data in the selected disector

The column labelled **Data** shows *No* if there is no data in the disector, or *Yes* if there is data.

2. **Data Field** - This shows the current multisector that the microscope stage is located on, and whether it has been “analyzed” or not. When a multisector is “analyzed”, NeuroZoom knows how to treat its data for generating reports and statistics. For example, an unanalyzed multisector is not used in generating reports before the experiment is over, because there may be more data to enter. The total number of multisectors in the group is also shown. The current structure selected in the mapping window is then displayed, along with the number tallied for this structure for both the current multisector and the current disector.

   Multiple structures may be estimated simultaneously with the Nucleator probe. However, only the current structure’s totals are shown in the data field.

   All data are estimated for mean volume by using the **Nucleator** tool (see the chapters on ***Stereology*** in the ***User Guide Manual*** to understand how to estimate structure mean volume using this tool. Also see the section in this manual on the **Nucleator** tool). The **Mapping Window Info** window will show information on the current structure that is being estimated. If there are two or more analyzed multisectors in the group, the *Variance* and the *Standard Deviation* for the number estimates among the multisectors are expressed in the **Mapping Window Info** window. The multisector total and the disector total are also displayed for the current structure.

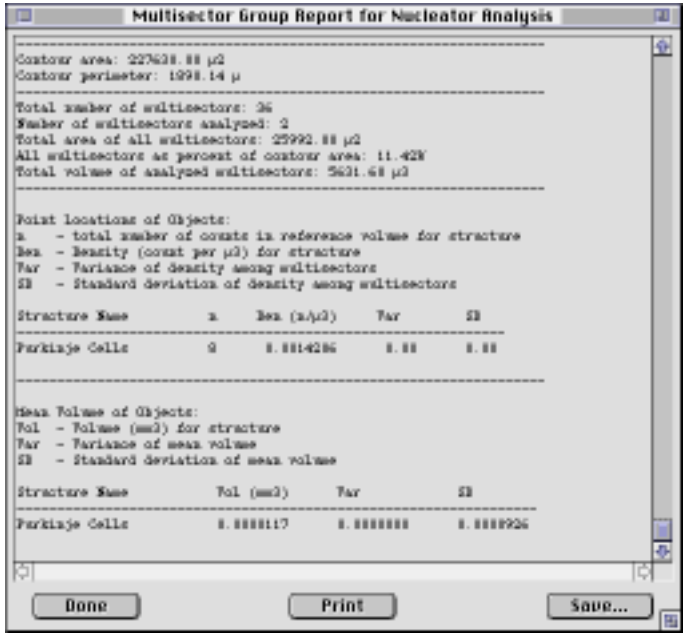
Variance and SD are displayed only if there are 2 or more analyzed multisectors

When the tool closes the current data object and the volume is calculated for that data structure, the volume is displayed in the **Mapping Window Info** window.

Volume of cell just estimated shows as cubed mm

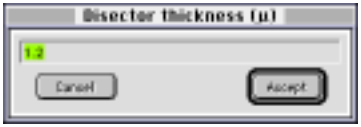
Several control buttons in the **Control** window perform actions on the multisectors.

3. **Report** - pressing this button generates a report and displays it in a separate window.

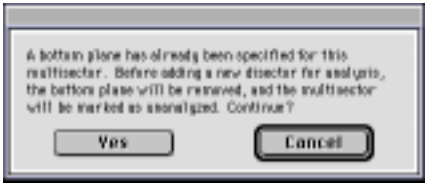


The report can be closed by pressing **Done**, printed to the selected printer by pressing **Print**, or saved to a Text file by pressing **Save....** In this window, the end of the report shows each structure estimated for number in this experiment and lists the total count (n), the density of the structure expressed as n per μ^3 and the variance and standard of the structure among the multisectors. This is zero unless at least two multisectors have been marked as “analyzed”. Following this are the mean volume estimates, the variance, and the standard deviation for each structure.

4. **New Disector** - pressing the **New DS** button creates a new disector for the current multisector. The position of the disector is immediately adjacent to the current disector. The thickness of the disector is the same as all other disectors, and is the number first specified when this multisector group was made. However, if the option is held down when **New DS** is pressed, a window opens for you to enter in a new disector thickness value in microns.



If **New DS** is pressed and the current multisector has already been marked as analyzed, a window opens to alert you to this fact.

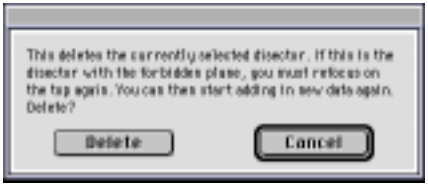


Press **Cancel** to dismiss the window and to not create a new disector. Press **Yes** to indicate that the new disector will be added to the bottom of the multisector, and will push the multisector stack downward to incorporate the new thickness of this new disector. The multisector will then be marked as "unanalyzed".

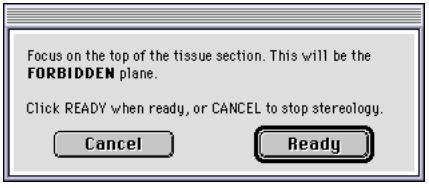
5. **Delete Disector** - The **Delete DS** button is enabled only if the last disector in the multisector is the current disector. Pressing this button deletes the current disector completely from the multisector.

Alert: The CURRENT disector is deleted, not the SELECTED disector. The current disector is always displayed in Italics. Please note this difference between the two.

A window opens alerting you to certain conditions if this is the last disector.



If the current disector contains the forbidden disector (meaning also it is the last disector in the multisector), you need to refocus on the top of the multisector again to begin estimating again for this multisector.



If you press on **Cancel**, the last disector without its data is reattached to the multisector. This is not a serious problem, because generally you do not estimate data when on the forbidden plane.

If you press **Ready**, the current position of the microscope stage is read from the controller, and two disectors are made. The first disector contains the forbidden plane at the current focus you indicated. The second disector contains the first permitted plane. NeuroZoom automatically moves to the first permitted plane of the second disector, and makes that one the current disector.

If the current disector being deleted is not the last disector, the next to last disector then becomes the current disector after deletion.

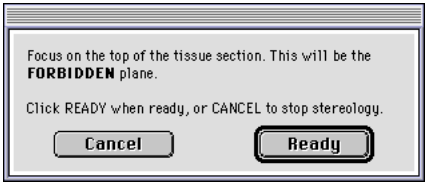
6. **Delete Data** - pressing the **Delete Data** button will delete all data from the current disector. Similar to the previous command, this acts on the current, not the selected disector. If there is no data in the current disector, an error window opens.

If it does contain data, a warning window opens.

Only the data are deleted. The planes and their location are not affected. You can continue estimating data directly into this disector.

7. **Previous Multisector** - pressing the **Prev MS** button moves the microscope stage to the previous multisector for this group. This movement wraps around, so if you are currently on the first multisector, the last multisector is selected.

If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.

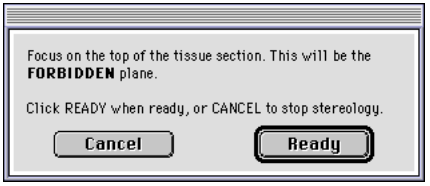


Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

The **Data** field updates to show the current multisector. The Disector list also updates to show the disectors in this multisector. The last disector in this multisector becomes the current disector. The microscope focuses on the permitted plane of this disector.

8. **Next Multisector** - pressing the **Next MS** button moves the microscope stage to the next multisector for this group. This movement wraps around, so if you are currently on the last multisector, the first multisector is selected.

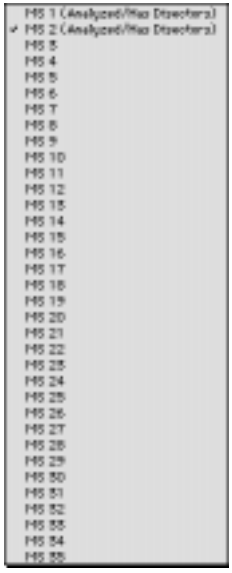
If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.



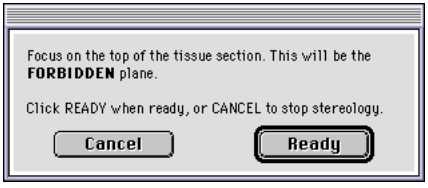
Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

The **Data** field updates to show the current multisector. The Disector list also updates to show the disectors in this multisector. The last disector in this multisector becomes the current disector. The microscope focuses on the permitted plane of this disector.

9. **Multisector** - this is a popup menu showing all the multisectors in this group. You can select any of them and NeuroZoom automatically goes to that multisector. The menu indicates whether a multisector has been analyzed or not. The current multisector is preceded with a checkmark.



If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.

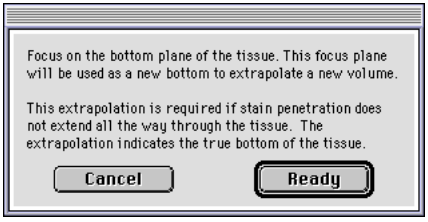


Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

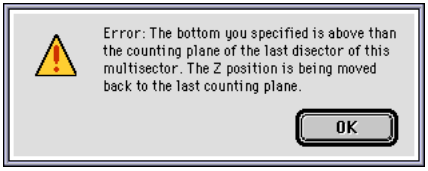
10. **Analyzed -** checking this button marks the current multisector as”analyzed”. This makes the multisector eligible for calculations involving global volumes and counts, such as when generating reports.

When a multisector has been marked as “analyzed”, and you uncheck the box to mark it as “unanalyzed” again, a warning window opens.

The counting plane of the last disector in this multisector is used as the bottom of the multisector. If the tissue has *incomplete staining*, this counting plane may not be the true bottom of the multisector. In this case, *Option-Click* on the **Analyzed** checkbox. A window opens asking you to focus on the true bottom of the tissue section. This plane will then be used as the bottom and all volumes will be extrapolated to this plane. Note that the density of the structures if it is being calculated, remains the same regardless of extrapolation.



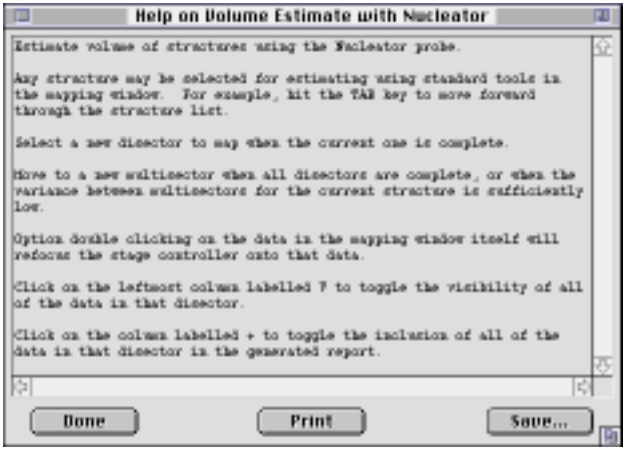
If the plane you specify as the bottom is above the counting plane of the last disector of this multisector, an error window opens, and the stage moves back to the last known position.



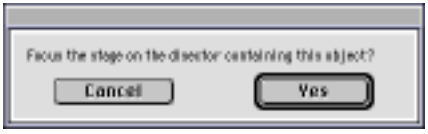
The bottom cannot be above the last counting plane, otherwise data will be excluded from the calculations. If a disector needs to be deleted, use the **Delete DS** button to delete the disector completely.

11.

12. **Help** - If the Help button on the keyboard is pressed, a **Help** window opens displaying some helpful comments for this protocol.



13. **Moving to the Data's Disector** - In the **Mapping** window, *control-double-clicking* on object that belongs to the current multisector focuses the microscope stage to the data's disector. A warning window opens asking for confirmation.



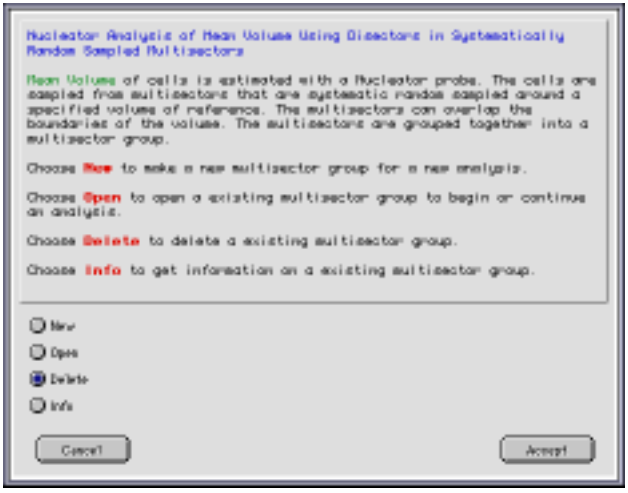
Press **Yes** to move the stage to the disector holding that data. That disector then becomes the current disector. Press **Cancel** to dismiss the window without moving the stage or changing disectors.

If you control-double-click on an object that is in the current disector, a warning window opens.

Alert: Be sure to have the **Selection** wool selected from the **Mapping Tools** window in order to control-double-click on it. Otherwise, you will only put a new data point on top of what you are clicking on.

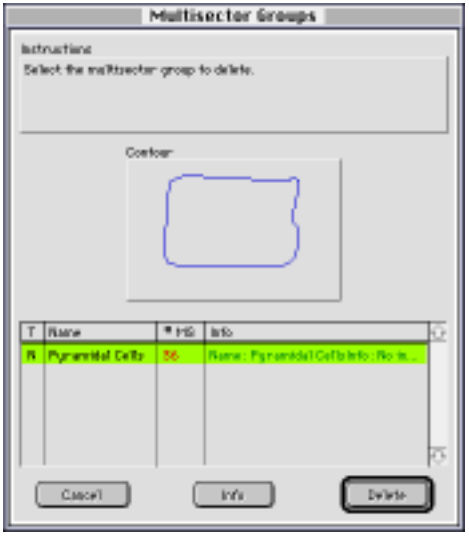
Delete Existing Multisector Group

Delete an existing multisector group for estimating numbers using a Nucleator protocol.



If you choose this option, and if there are no multisector groups for this Nucleator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Nucleator protocols in the document.



Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

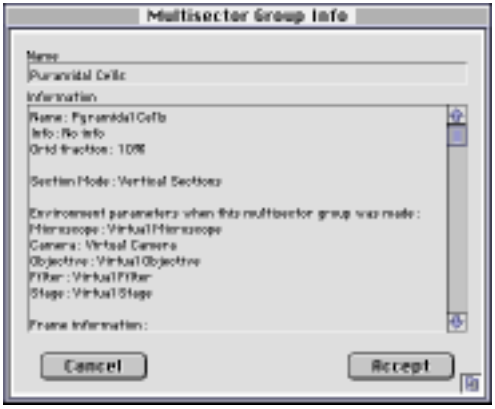
T - This first column indicates the Type of Multisector Group. It should show N for Nucleator.

Name - The second column is the name assigned to it when the multisector group was made.

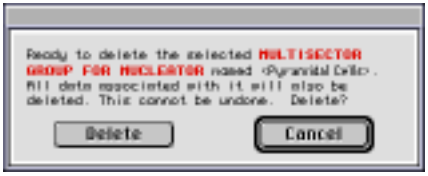
#MS - The third column indicates the number multisectors in the group.

Info - The fourth column is the information assigned to it when the multisector group was made.

Select the multisector group to delete and press the **Delete** button to delete that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.



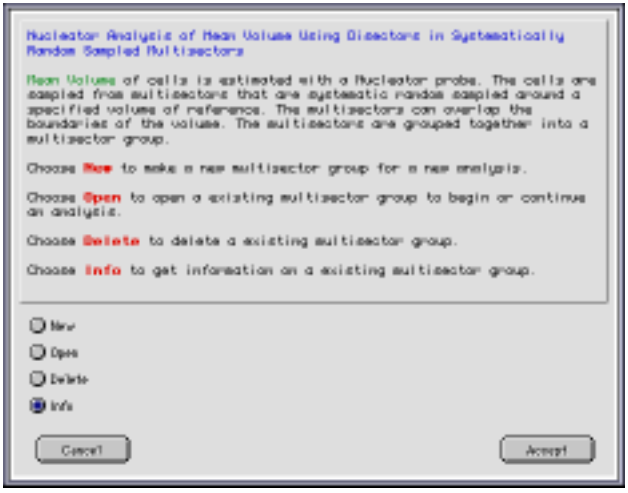
When deleting a group, a confirmation window opens warning that the deletion is permanent and cannot be undone.



Press **Delete** to delete the group, or press **Cancel** to dismiss the window without any changes.

Info on Existing Multisector Group

Get information by reports on existing multisector groups for estimating numbers using a Nucleator protocol.



If you choose this option, and if there are no multisector groups for this Nucleator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Nucleator protocols in the document.

Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

+ - This first column may be toggled on and off by pressing the mouse in this column. Toggling on selects the group for a report. Multiple selections may be made in this manner to report on more than one multisector group at one time.

T - This second column indicates the Type of Multisector Group. It should show N for Nucleator.

Name - The third column is the name assigned to it when the multisector group was made.

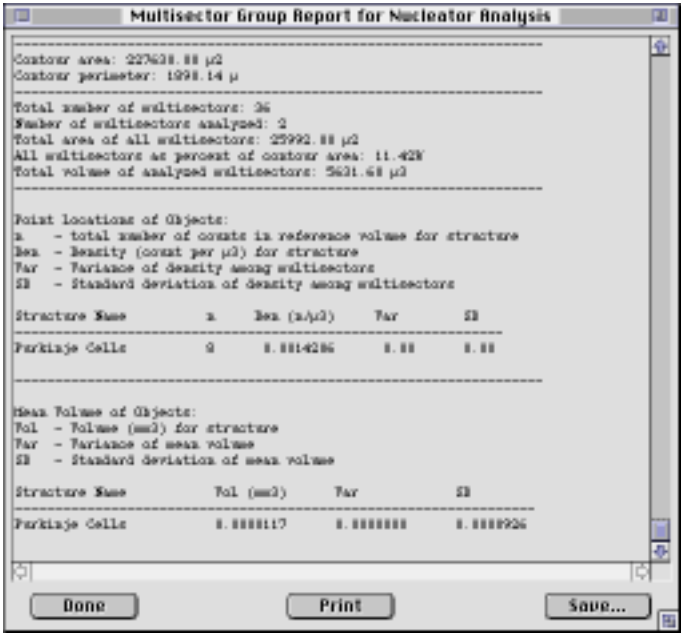
#MS - The fourth column indicates the number multisectors in the group.

Info - The fifth column is the information assigned to it when the multisector group was made.

Select the multisector groups to report on and press the **Report** button to report on that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and

Information can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.

Multiple group reporting opens multiple report windows. A report window shows the same information as generating a report when conducting the experiment.

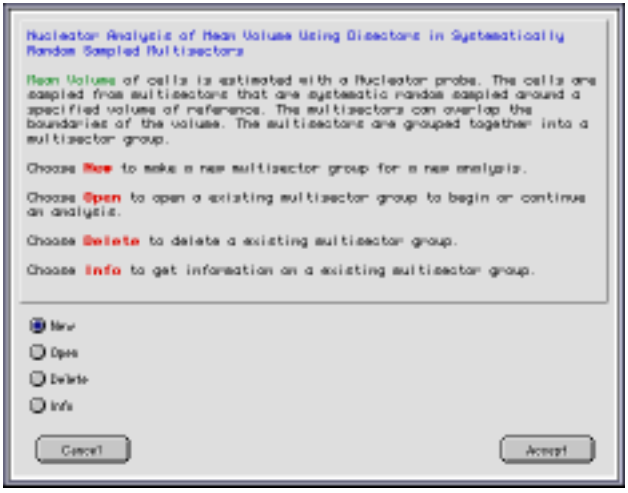

Multisector Group Report for Nucleator Analysis
Contour area: 227638.88 µ2
Contour perimeter: 1998.14 µ
Total number of multisectors: 36
Number of multisectors analyzed: 2
Total area of all multisectors: 25992.88 µ2
All multisectors as percent of contour area: 11.42%
Total volume of analyzed multisectors: 5621.68 µ3
Point locations of Objects:
n - total number of counts in reference volume for structure
Den - Density (count per µ3) for structure
Var - Variance of density among multisectors
SD - Standard deviation of density among multisectors
Structure Name n Den (n/µ3) Var SD
Purkinje Cells 9 0.0014286 0.00 0.00
Mean Volume of Objects:
Vol - Volume (mm3) for structure
Var - Variance of mean volume
SD - Standard deviation of mean volume
Structure Name Vol (mm3) Var SD
Purkinje Cells 0.0000117 0.0000000 0.0000926
Done
Print
Save...

Volume by Nucleator in Isotropic, Random Sections

Opened by:

- Estimate Volume By Nucleator in Isotropic, Random Sections... in Stereology menu

The **Volume By Nucleator in Isotropic, Random Sections** window, for lack of a better name, is a choice window. From this you are to select one of four options to proceed with estimating volumes.



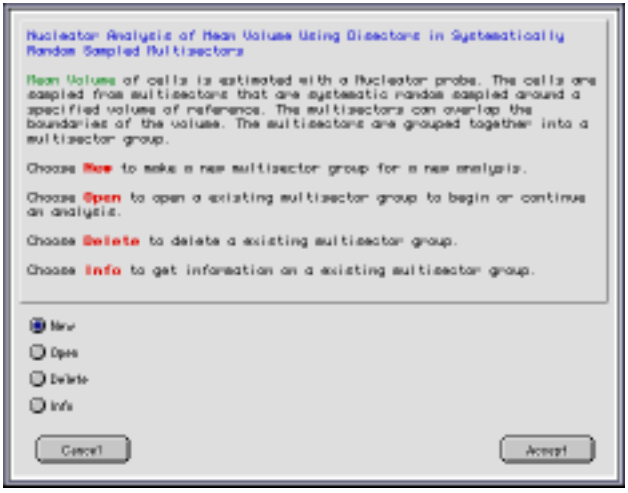
Information on what this probe accomplishes and the four options are presented.

- **New** - make a new multisector group to begin a new analysis on estimating the mean volume of some structure
- **Open** - open an existing multisector group for additional data entry or analysis
- **Delete** - delete an existing multisector group from the document
- **Info** - get information on an existing multisector group

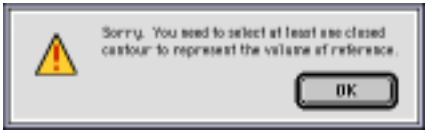
Choose one of the options my pressing the mouse button the radio button. The radio button will highlight indicating that it is selected. Press **Accept** to continue with the process, or press **Cancel** to dismiss this window with no options or changes made to the document.

New Multisector Group

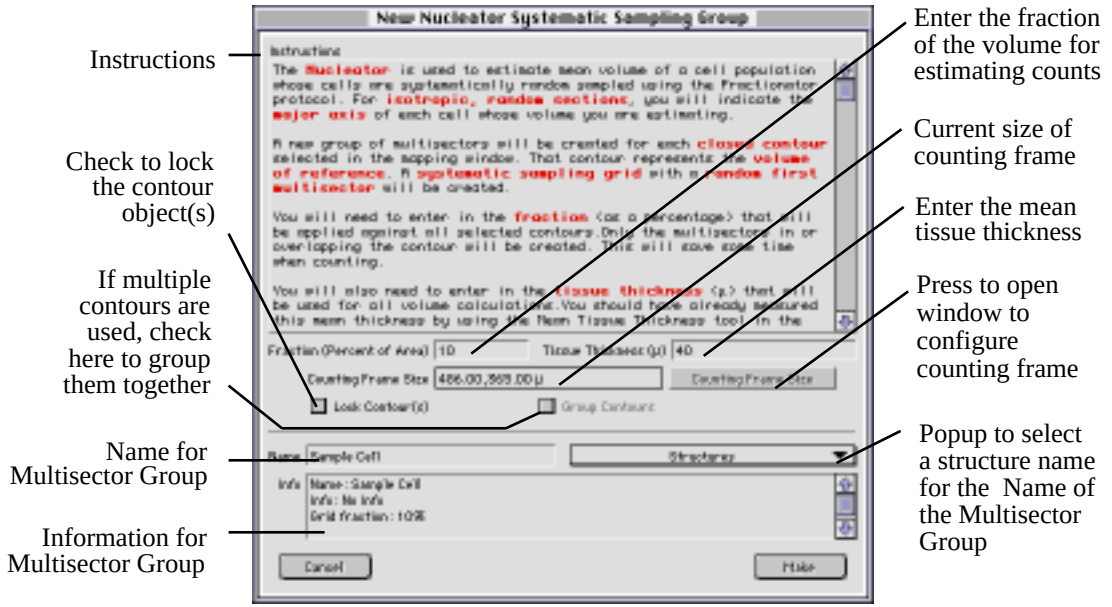
Make a new multisector group for estimating mean volume using a Nucleator protocol.



If you choose this option, and if you do not have at least one contour created and selected in the mapping window, an error window opens indicating that at least one selected contour is needed to represent the *volume of reference*.



Go back and use the mapping tools to create a closed contour around the volume of reference. When you have done so, select them both, choose **Estimate Volume By Nucleator in Isotropic, Random Sections...** in the **Stereology** menu again, and select **New** and press **Accept**. A new window opens.



This window is the **New Nucleator Systematic Sampling Group** window. All of the parameters to create a multisector group for a Nucleator analysis are entered here. The multisectors are calculated on a systematic random sample through the selected contour. Only those multisectors that intersect the contour will be presented. Disectors are created in those multisectors in which the estimates are places for cell volume estimates.

Fraction (Percent of Area)

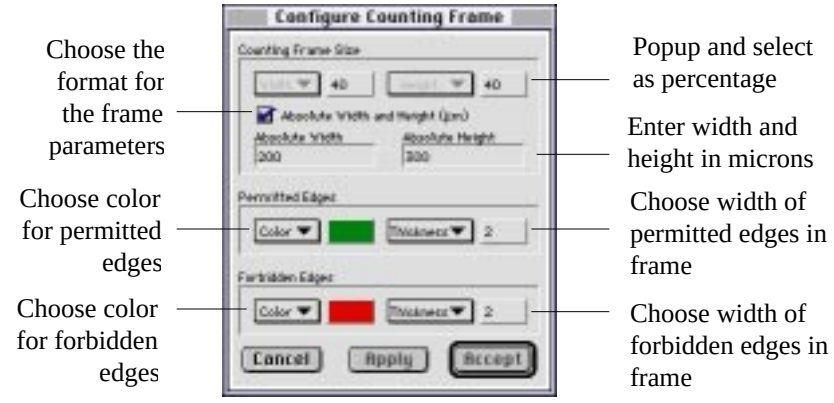
Enter the fraction that is desred for the Nucleator analysis. For example, if you want to estimate counts of cells in 10% of the contour volume, enter 10. The default is 10%. Please refer to the chapters on ***Stereology*** in the ***User Guide Manual*** for more specific information on this value.

Tissue Thickness (μ)

Enter the mean tissue thickness in microns for this section. This can be measured with the **Measure Mean Tissue Thickness** tool located in the **Analysis** submenu of the **Modules** menu.

Counting Frame Size

This is a button that opens a window from which to adjust the size of the counting frame. The current frame size is displayed in the field to its left.

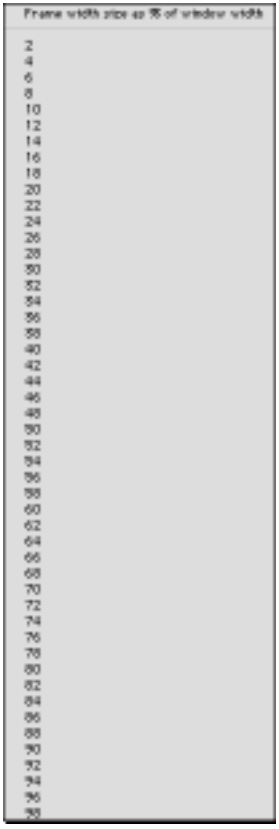


The size of the counting frame may be entered as a *percentage* of the screen size, or as an absolute width and height in microns. The major difference between the two techniques is that a percentage will always ensure that the counting frame fits in the mapping window regardless of the *lens objective* used. However, you cannot switch lens objectives in the middle of a stereology experiment using the counting frame because the absolute size of the frame would change.

When you enter the size in *absolute microns*, the displayed size of the counting frame in the mapping window will change as you change the lens objective. The frame will always adjust to the absolute width and height entered. However, the problem here is that the size of the counting frame may exceed the size of the mapping window with lower power objectives. If so, NeuroZoom will alert you.

In all cases of stereology, you cannot change the lens objectives once the experiment is underway. However, one method of entering in the size may be more appropriate than the other, depending on how you go about the business of setting up the stereology experiment. You choose the method which best suits your needs. Please also refer to the chapters on ***Stereology*** in the ***User Guide Manual*** and the ***Reference Manual*** more information.

When choosing the width and height as a percentage, press and hold on the width or height button. A menu pops up for selectons from 2 to 98%.



Press and hold on the **Color** button for the *permitted and forbidden colors*. A popup menu shows the color choices.

Press and hold on the **Thickness** button for the *permitted and forbidden thicknesses*. A popup menu shows the choices from 1 to 6. 6 is thickest.

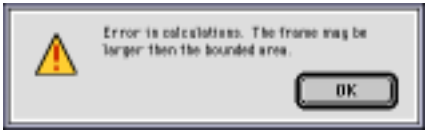
Press on the **Apply** button to see the changes. Press on **Cancel** to dismiss the window and cancel any changes you made with this window. Press **Accept** to dismiss the window and to store the changes made with this window.

Name and Info

Enter the name and information for this multisector group. The default name is the structure name of the contour that is selected when this window was opened. Press and hold on the **Structure Popup** menu button to get a list of all the structures for this mapping window and select one for the name. You can also enter in any other name you desire.

When all parameters are entered, the **Make** button is enabled. Press **Cancel** to dismiss the window and not make the multisector group. Press **Make** to make the multisector group.

If the counting frame is too large to accurately place a group of multisectors over the selected contour representing the volume of reference, a window opens displaying this error.



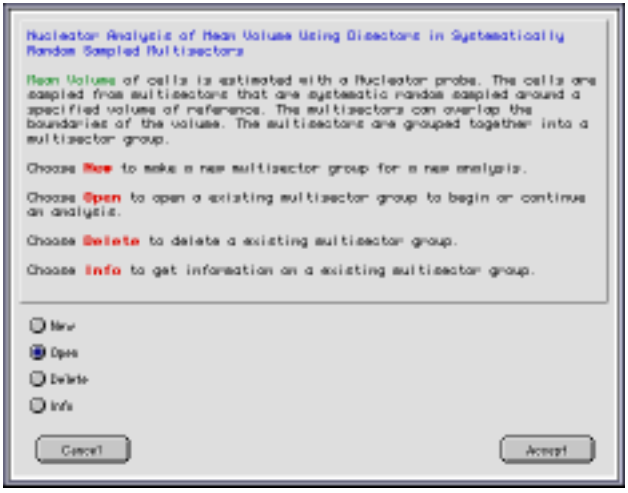
Once the multisector group has been made successfully, a window opens presenting the option of starting the stereology session immediately on this new group.

Press **No** to close window and return to the Mapping window. Press **Yes** to continue immediately to the Nucleator analysis on this multisector group.

Answering **Yes** is the same as opening an existing multisector group for analysis. This is presented in the next session.

Open Existing Multisector Group

Open an existing multisector group for estimating numbers using a Nucleator protocol.



If you choose this option, and if there are no multisector groups for this Nucleator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Nucleator protocols in the document.

Tip: Holding down the OPTION when choosing Estimate Volume By Nucleator in Isotropic, Random Sections... in the Stereology Menu will automatically preselect this OPEN button.

Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

T - This first column (T) indicates the Type of Multisector Group. It should show N for Nucleator.

Name - The second column is the name assigned to it when the multisector group was made.

#MS - The third column indicates the number multisectors in the group.

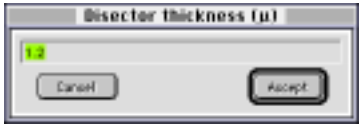
Info - The fourth column is the information assigned to it when the multisector group was made.

Select the multisector group to reopen and press the **Count** button to continue counting with that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both

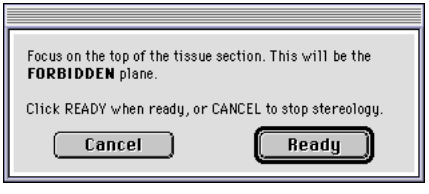
the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.

Nucleator Control Window

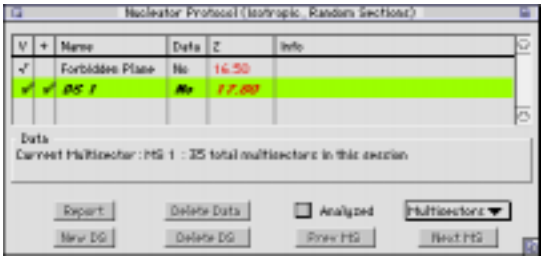
If a multisector group is selected and opened for counting, live video is turned on. If this is the first time the multisector group is being opened, the thickness of the disector must be entered in the following window.



Once the thickness is specified, another window prompts for you to focus on the top of the tissue section.



After the top is specified, the following window opens.



This is the **Nucleator Protocol (Isotropic, Random Sections) Control** window from which the probe is controlled. There are various components to this window.

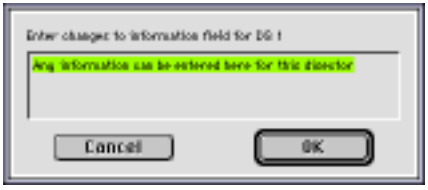
1. **Disectors -** The disectors for the current multisector that the microscope stage are located in the only scrolling field on this window.

Of these disectors, the disector in *Italics* is the current disector. The current disector is the one that the microscope is focused on. Of the two planes of a disector, the microscope focuses on the reference plane. This is indicated in the column labelled Z. The selected disector is the disector which is highlighted in the highlight color. The selected disector and the current disector are not necessarily the same, although in this example, they are.

Double clicking on a disector makes the selected disector the current disector. The microscope stage will move to that new focus point, and the disector will be displayed in *Italics*.

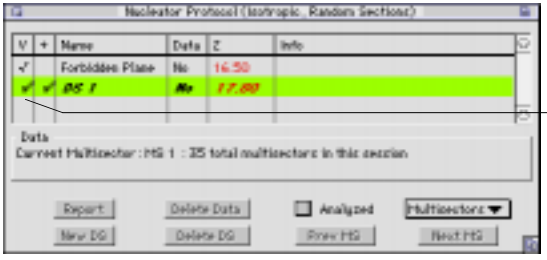
The **Name** for each disector is auto-generated when the multisector is made. The first disector is named *Forbidden Plane* because the microscope focuses on the first plane in the first disector, which by NeuroZoom convention, is the forbidden plane. No data is usually counted in this plane. The subsequent disectors are then named DS 1, DS 2, and so on.

The **Info** field is blank initially for each disector. However, you can Option-*Double Click* on a disector and a dialog window opens for you to enter in new information.



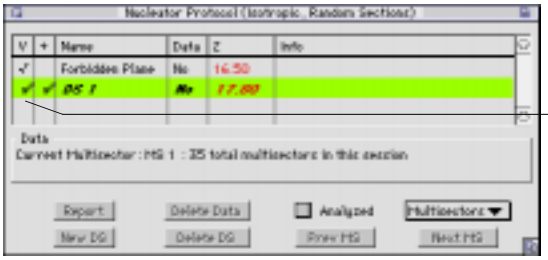
Tip: Option Double Click on a disector to change its information.

Visibility of data can be controlled by clicking on the leftmost column labelled **V**. A single click will toggle the visibility of all of the data for this disector. This is particularly useful if there is a large concentration of data in the multisectors, and they are visually collapsing on top of each other.



Single click in the V column to toggle visibility of the data in the selected disector

Inclusion of data can be controlled by clicking on the column labelled **+**. A single click will toggle the inclusion of all of the data for this disector. Use this to remove a disector completely from reports that are generated, including any statistics generated for the multisector or the multisector group.



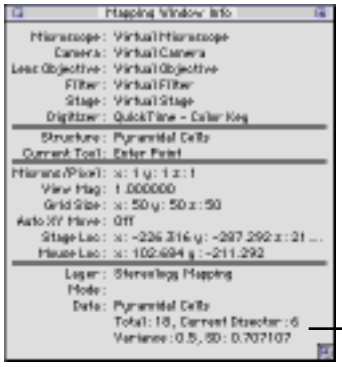
Single click in the + column to toggle inclusion of the data in the selected disector

The column labelled **Data** shows *No* if there is no data in the disector, or *Yes* if there is data.

2. **Data Field** - This shows the current multisector that the microscope stage is located on, and whether it has been “analyzed” or not. When a multisector is “analyzed”, NeuroZoom knows how to treat its data for generating reports and statistics. For example, an unanalyzed multisector is not used in generating reports before the experiment is over, because there may be more data to enter. The total number of multisectors in the group is also shown. The current structure selected in the mapping window is then displayed, along with the number tallied for this structure for both the current multisector and the current disector.

   Multiple structures may be estimated simultaneously with the Nucleator probe. However, only the current structure’s totals are shown in the data field.

   All data are estimated for mean volume by using the **Nucleator** tool (see the chapters on ***Stereology*** in the ***User Guide Manual*** to understand how to estimate structure mean volume using this tool. Also see the section in this manual on the **Nucleator** tool). The **Mapping Window Info** window will show information on the current structure that is being estimated. If there are two or more analyzed multisectors in the group, the *Variance* and the *Standard Deviation* for the number estimates among the multisectors are expressed in the **Mapping Window Info** window. The multisector total and the disector total are also displayed for the current structure.



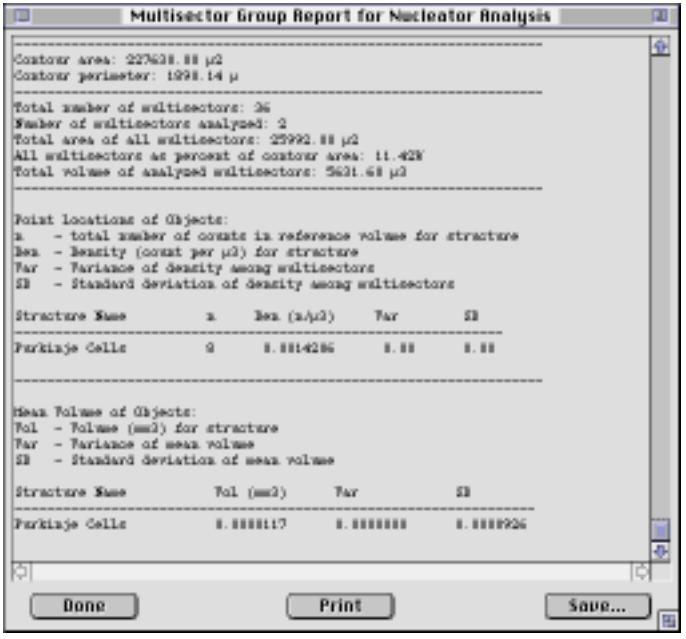
Variance and SD are displayed only if there are 2 or more analyzed multisectors

When the tool closes the current data object and the volume is calculated for that data structure, the volume is displayed in the **Mapping Window Info** window.

Volume of cell just estimated shows as cubed mm

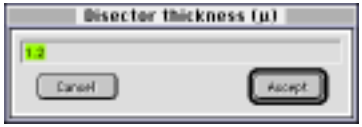
Several control buttons in the **Control** window perform actions on the multisectors.

3. **Report** - pressing this button generates a report and displays it in a separate window.

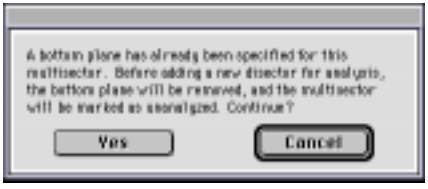


The report can be closed by pressing **Done**, printed to the selected printer by pressing **Print**, or saved to a Text file by pressing **Save....** In this window, the end of the report shows each structure estimated for number in this experiment and lists the total count (n), the density of the structure expressed as n per μ^3 and the variance and standard of the structure among the multisectors. This is zero unless at least two multisectors have been marked as "analyzed". Following this are the mean volume estimates, the variance, and the standard deviation for each structure.

4. **New Disector** - pressing the **New DS** button creates a new disector for the current multisector. The position of the disector is immediately adjacent to the current disector. The thickness of the disector is the same as all other disectors, and is the number first specified when this multisector group was made. However, if the option is held down when **New DS** is pressed, a window opens for you to enter in a new disector thickness value in microns.



If **New DS** is pressed and the current multisector has already been marked as analyzed, a window opens to alert you to this fact.



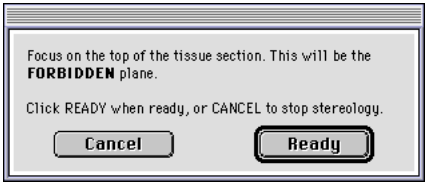
Press **Cancel** to dismiss the window and to not create a new disector. Press **Yes** to indicate that the new disector will be added to the bottom of the multisector, and will push the multisector stack downward to incorporate the new thickness of this new disector. The multisector will then be marked as “unanalyzed”.

5. **Delete Disector** - The **Delete DS** button is enabled only if the last disector in the multisector is the current disector. Pressing this button deletes the current disector completely from the multisector.

Alert: The CURRENT disector is deleted, not the SELECTED disector. The current disector is always displayed in Italics. Please note this difference between the two.

A window opens alerting you to certain conditions if this is the last disector.

If the current disector contains the forbidden disector (meaning also it is the last disector in the multisector), you need to refocus on the top of the multisector again to begin estimating again for this multisector.



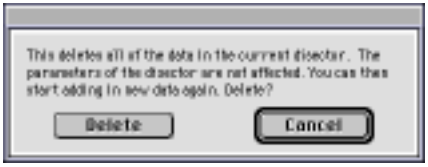
If you press on **Cancel**, the last disector without its data is reattached to the multisector. This is not a serious problem, because generally you do not estimate data when on the forbidden plane.

If you press **Ready**, the current position of the microscope stage is read from the controller, and two disectors are made. The first disector contains the forbidden plane at the current focus you indicated. The second disector contains the first permitted plane. NeuroZoom automatically moves to the first permitted plane of the second disector, and makes that one the current disector.

If the current disector being deleted is not the last disector, the next to last disector then becomes the current disector after deletion.

6. **Delete Data** - pressing the **Delete Data** button will delete all data from the current disector. Similar to the previous command, this acts on the current, not the selected disector. If there is no data in the current disector, an error window opens.

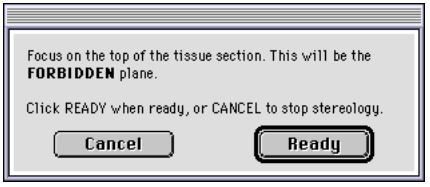
If it does contain data, a warning window opens.



Only the data are deleted. The planes and their location are not affected. You can continue estimating data directly into this disector.

7. **Previous Multisector** - pressing the **Prev MS** button moves the microscope stage to the previous multisector for this group. This movement wraps around, so if you are currently on the first multisector, the last multisector is selected.

If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.

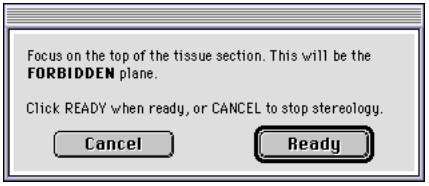


Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

The **Data** field updates to show the current multisector. The Disector list also updates to show the disectors in this multisector. The last disector in this multisector becomes the current disector. The microscope focuses on the permitted plane of this disector.

8. **Next Multisector** - pressing the **Next MS** button moves the microscope stage to the next multisector for this group. This movement wraps around, so if you are currently on the last multisector, the first multisector is selected.

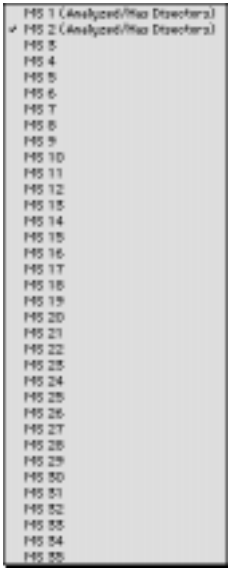
If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.



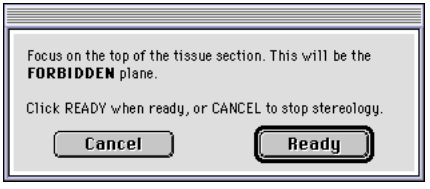
Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

The **Data** field updates to show the current multisector. The Disector list also updates to show the disectors in this multisector. The last disector in this multisector becomes the current disector. The microscope focuses on the permitted plane of this disector.

9. **Multisector** - this is a popup menu showing all the multisectors in this group. You can select any of them and NeuroZoom automatically goes to that multisector. The menu indicates whether a multisector has been analyzed or not. The current multisector is preceded with a checkmark.



If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.

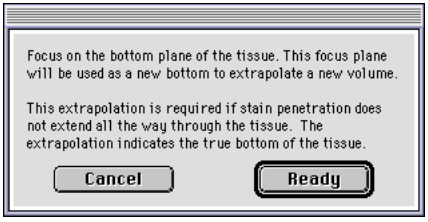


Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

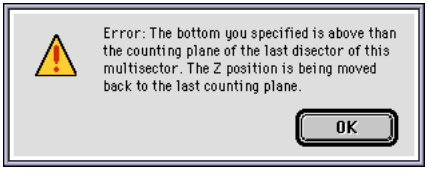
10. **Analyzed** - checking this button marks the current multisector as”analyzed”. This makes the multisector eligible for calculations involving global volumes and counts, such as when generating reports.

When a multisector has been marked as “analyzed”, and you uncheck the box to mark it as “unanalyzed” again, a warning window opens.

The counting plane of the last disector in this multisector is used as the bottom of the multisector. If the tissue has *incomplete staining*, this counting plane may not be the true bottom of the multisector. In this case, *Option-Click* on the **Analyzed** checkbox. A window opens asking you to focus on the true bottom of the tissue section. This plane will then be used as the bottom and all volumes will be extrapolated to this plane. Note that the density of the structures if it is being calculated, remains the same regardless of extrapolation.



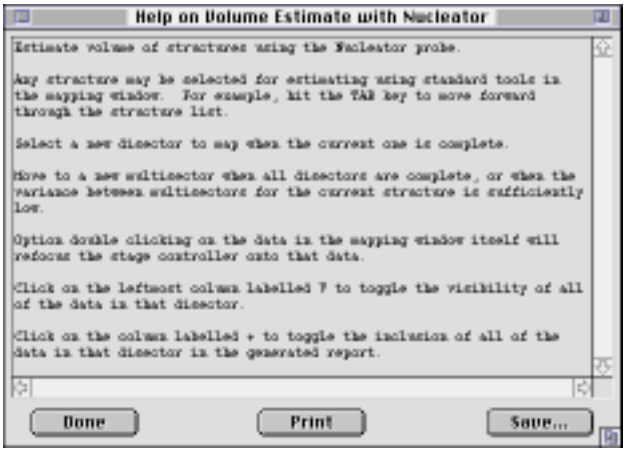
If the plane you specify as the bottom is above the counting plane of the last disector of this multisector, an error window opens, and the stage moves back to the last known position.



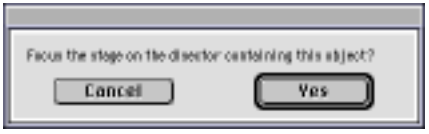
The bottom cannot be above the last counting plane, otherwise data will be excluded from the calculations. If a disector needs to be deleted, use the **Delete DS** button to delete the disector completely.

11.

12. **Help** - If the Help button on the keyboard is pressed, a **Help** window opens displaying some helpful comments for this protocol.



13. **Moving to the Data's Disector** - In the **Mapping** window, *control-double-clicking* on object that belongs to the current multisector focuses the microscope stage to the data's disector. A warning window opens asking for confirmation.



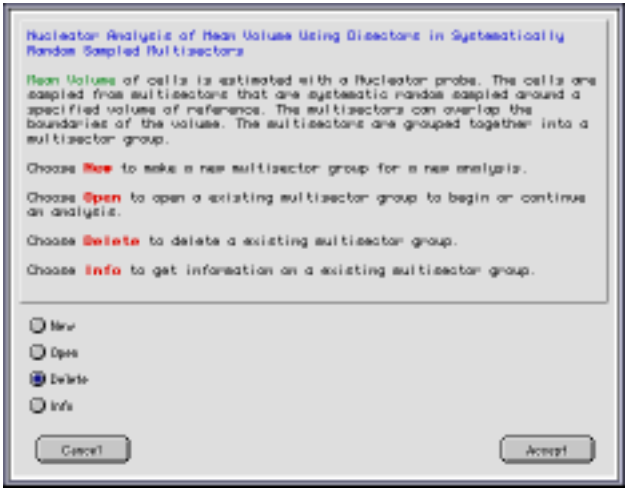
Press **Yes** to move the stage to the disector holding that data. That disector then becomes the current disector. Press **Cancel** to dismiss the window without moving the stage or changing disectors.

If you control-double-click on an object that is in the current disector, a warning window opens.

Alert: Be sure to have the **Selection** wool selected from the **Mapping Tools** window in order to control-double-click on it. Otherwise, you will only put a new data point on top of what you are clicking on.

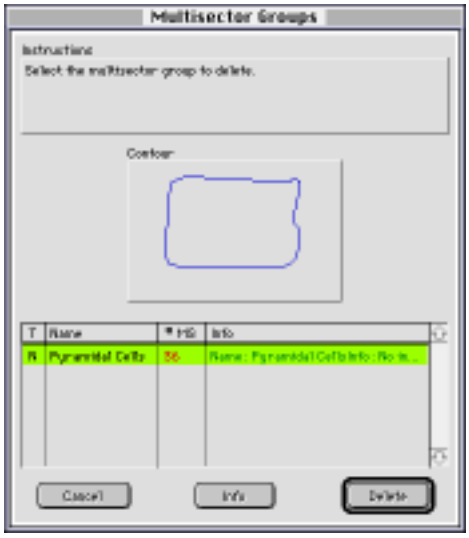
Delete Existing Multisector Group

Delete an existing multisector group for estimating numbers using a Nucleator protocol.



If you choose this option, and if there are no multisector groups for this Nucleator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Nucleator protocols in the document.



Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

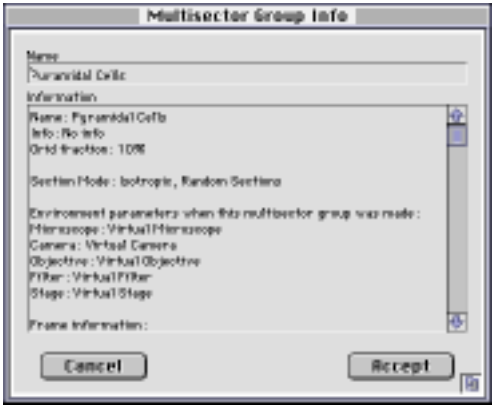
T - This first column indicates the Type of Multisector Group. It should show N for Nucleator.

Name - The second column is the name assigned to it when the multisector group was made.

#MS - The third column indicates the number multisectors in the group.

Info - The fourth column is the information assigned to it when the multisector group was made.

Select the multisector group to delete and press the **Delete** button to delete that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.

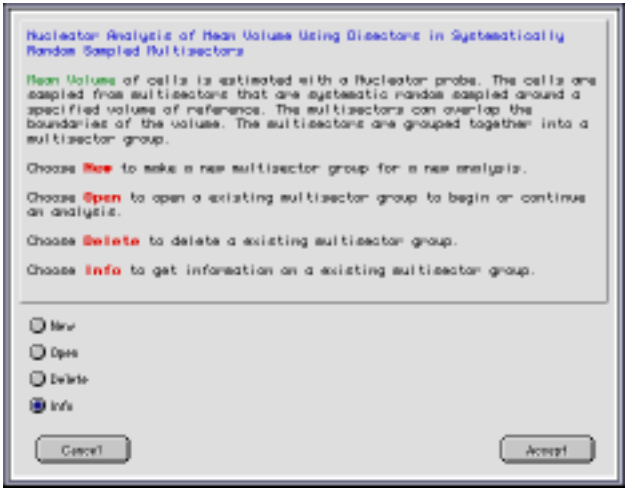


When deleting a group, a confirmation window opens warning that the deletion is permanent and cannot be undone.

Press **Delete** to delete the group, or press **Cancel** to dismiss the window without any changes.

Info on Existing Multisector Group

Get information by reports on existing multisector groups for estimating numbers using a Nucleator protocol.



If you choose this option, and if there are no multisector groups for this Nucleator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Nucleator protocols in the document.

Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

+ - This first column may be toggled on and off by pressing the mouse in this column. Toggling on selects the group for a report. Multiple selections may be made in this manner to report on more than one multisector group at one time.

T - This second column indicates the Type of Multisector Group. It should show N for Nucleator.

Name - The third column is the name assigned to it when the multisector group was made.

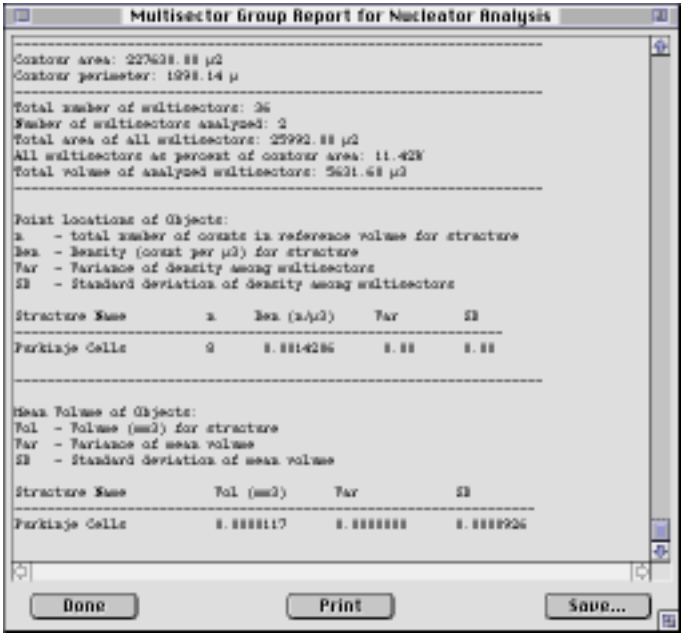
#MS - The fourth column indicates the number multisectors in the group.

Info - The fifth column is the information assigned to it when the multisector group was made.

Select the multisector groups to report on and press the **Report** button to report on that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and

Information can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.

Multiple group reporting opens multiple report windows. A report window shows the same information as generating a report when conducting the experiment.

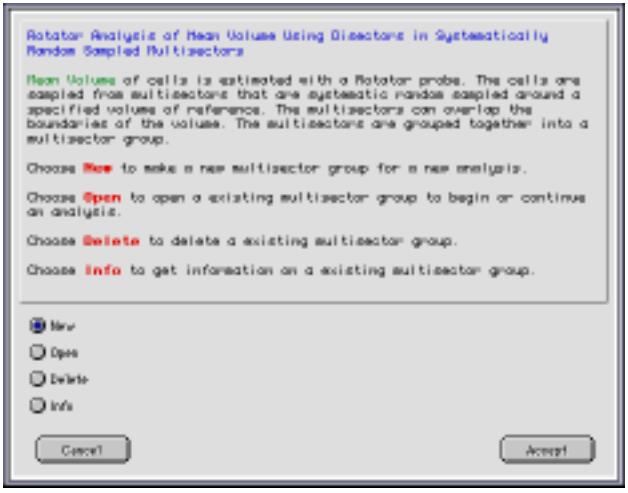

Multisector Group Report for Nucleator Analysis
Contour area: 227628.88 µ2
Contour perimeter: 1998.14 µ
Total number of multisectors: 36
Number of multisectors analyzed: 2
Total area of all multisectors: 25992.88 µ2
All multisectors as percent of contour area: 11.42%
Total volume of analyzed multisectors: 5631.68 µ3
Point locations of Objects:
n - total number of counts in reference volume for structure
Den - Density (count per µ3) for structure
Var - Variance of density among multisectors
SD - Standard deviation of density among multisectors
Structure Name n Den (n/µ3) Var SD
Purkinje Cells 9 0.0014286 0.00 0.00
Mean Volume of Objects:
Vol - Volume (mm3) for structure
Var - Variance of mean volume
SD - Standard deviation of mean volume
Structure Name Vol (mm3) Var SD
Purkinje Cells 0.0000117 0.0000000 0.0000926
Done
Print
Save...

Volume by Rotator in Vertical Sections

Opened by:

- Estimate Volume By Rotator in Vertical Sections... in Stereology menu

The **Volume By Rotator in Vertical Sections** window, for lack of a better name, is a choice window. From this you are to select one of four options to proceed with estimating volumes.



Information on what this probe accomplishes and the four options are presented.

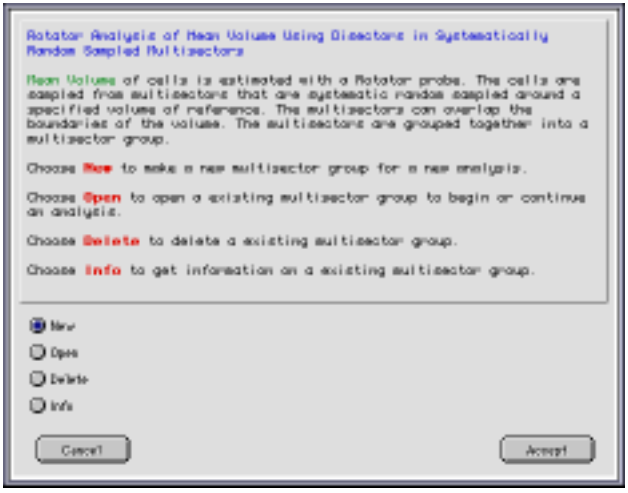
- **New** - make a new multisector group to begin a new analysis on estimating the mean volume of some structure
- **Open** - open an existing multisector group for additional data entry or analysis
- **Delete** - delete an existing multisector group from the document
- **Info** - get information on an existing multisector group

Choose one of the options my pressing the mouse button the radio button. The radio button will highlight indicating that it is selected. Press **Accept** to continue

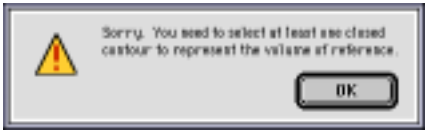
with the process, or press **Cancel** to dismiss this window with no options or changes made to the document.

New Multisector Group

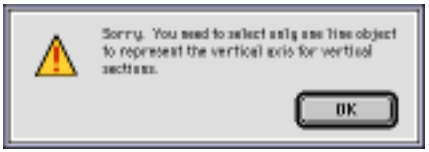
Make a new multisector group for estimating mean volume using a Rotator protocol.



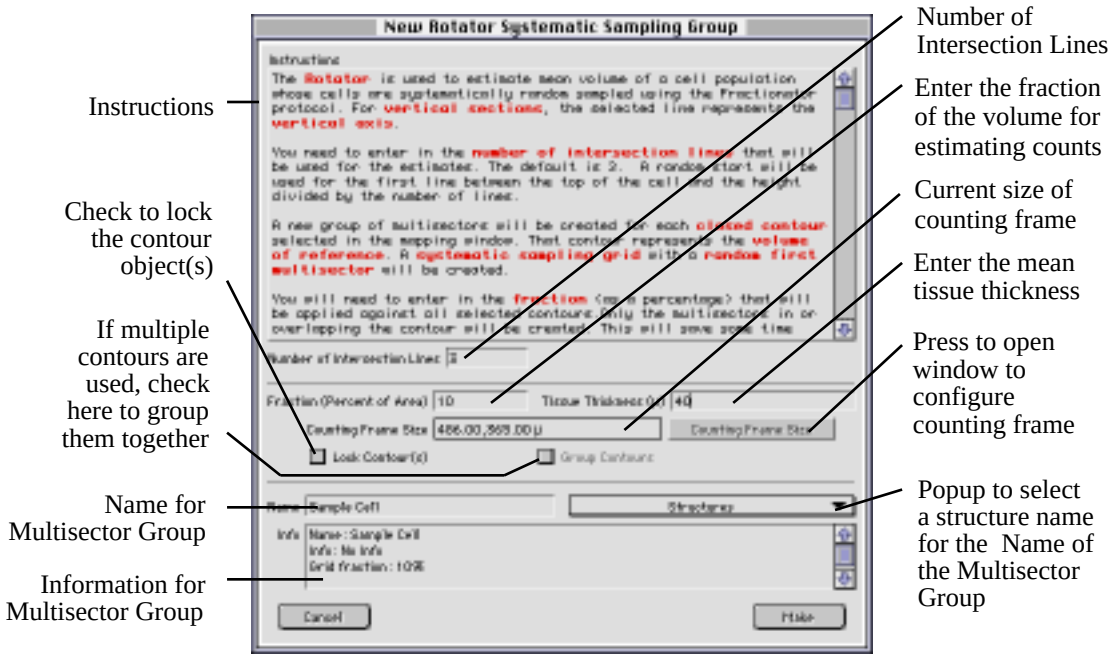
If you choose this option, and if you do not have at least one contour created and selected in the mapping window, an error window opens indicating that at least one selected contour is needed to represent the *volume of reference*.



You also need one line object, drawn parallel to the *vertical axis* of the tissue section. This line object needs to be selected or else an error window opens. The same error window will open if more than one line object is selected.



Go back and use the mapping tools to create a closed contour around the volume of reference, and a line drawn parallel to the vertical axis. When you have done so, select them both, choose **Estimate Volume By Rotator in Vertical Sections...** in the **Stereology** menu again, and select **New** and press **Accept**. A new window opens.



This window is the **New Rotator Systematic Sampling Group** window. All of the parameters to create a multisector group for a Rotator analysis are entered

here. The multisectors are calculated on a systematic random sample through the selected contour. Only those multisectors that intersect the contour will be presented. Disectors are created in those multisectors in which the estimates are places for cell volume estimates.

Fraction (Percent of Area)

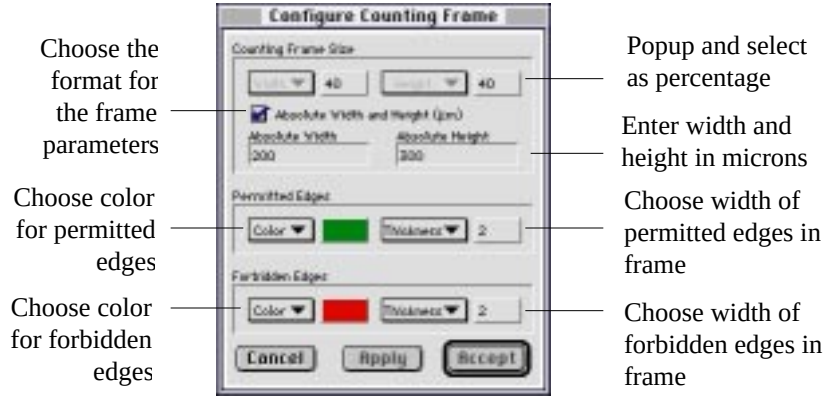
Enter the fraction that is desred for the Rotator analysis. For example, if you want to estimate counts of cells in 10% of the contour volume, enter 10. The default is 10%. Please refer to the manual ***NeuroZoom Stereology*** for more specific information on this value.

Tissue Thickness (μ)

Enter the mean tissue thickness in microns for this section. This can be measured with the **Measure Mean Tissue Thickness** tool located in the **Analysis** submenu of the **Modules** menu.

Counting Frame Size

This is a button that opens a window from which to adjust the size of the counting frame. The current frame size is displayed in the field to its left.

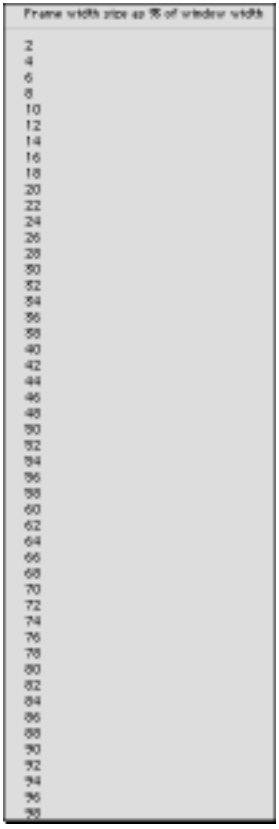


The size of the counting frame may be entered as a *percentage* of the screen size, or as an absolute width and height in microns. The major difference between the two techniques is that a percentage will always ensure that the counting frame fits in the mapping window regardless of the *lens objective* used. However, you cannot switch lens objectives in the middle of a stereology experiment using the counting frame because the absolute size of the frame would change.

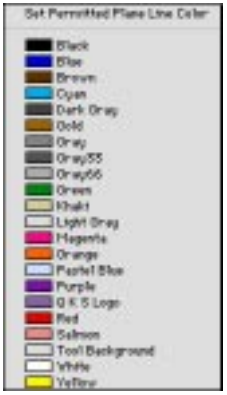
When you enter the size in *absolute microns*, the displayed size of the counting frame in the mapping window will change as you change the lens objective. The frame will always adjust to the absolute width and height entered. However, the problem here is that the size of the counting frame may exceed the size of the mapping window with lower power objectives. If so, NeuroZoom will alert you.

In all cases of stereology, you cannot change the lens objectives once the experiment is underway. However, one method of entering in the size may be more appropriate than the other, depending on how you go about the business of setting up the stereology experiment. You choose the method which best suits your needs. Please also refer to the chapters on ***Stereology*** in the ***User Guide Manual*** and the ***Reference Manual*** more information.

When choosing the width and height as a percentage, press and hold on the width or height button. A menu pops up for selectons from 2 to 98%.



Press and hold on the **Color** button for the *permitted and forbidden colors*. A popup menu shows the color choices.



Press and hold on the **Thickness** button for the *permitted and forbidden thicknesses*. A popup menu shows the choices from 1 to 6. 6 is thickest.

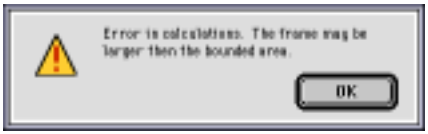
Press on the **Apply** button to see the changes. Press on **Cancel** to dismiss the window and cancel any changes you made with this window. Press **Accept** to dismiss the window and to store the changes made with this window.

Name and Info

Enter the name and information for this multisector group. The default name is the structure name of the contour that is selected when this window was opened. Press and hold on the **Structure Popup** menu button to get a list of all the structures for this mapping window and select one for the name. You can also enter in any other name you desire.

When all parameters are entered, the **Make** button is enabled. Press **Cancel** to dismiss the window and not make the multisector group. Press **Make** to make the multisector group.

If the counting frame is too large to accurately place a group of multisectors over the selected contour representing the volume of reference, a window opens displaying this error.



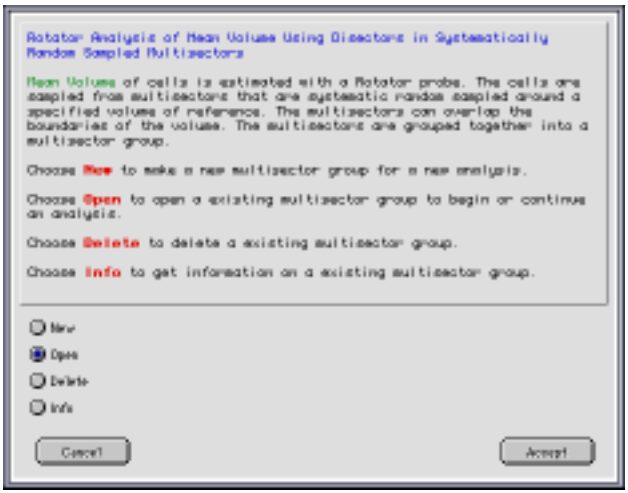
Once the multisector group has been made successfully, a window opens presenting the option of starting the stereology session immediately on this new group.

Press **No** to close window and return to the Mapping window. Press **Yes** to continue immediately to the Nucleator analysis on this multisector group.

Answering **Yes** is the same as opening an existing multisector group for analysis. This is presented in the next session.

Open Existing Multisector Group

Open an existing multisector group for estimating numbers using a Rotator protocol.



If you choose this option, and if there are no multisector groups for this Rotator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Rotator protocols in the document.

Tip: Holding down the OPTION when choosing Estimate Rotator By Nucleator in Vertical Sections... in the Stereology Menu will automatically preselect this OPEN button.

Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

T - This first column (T) indicates the Type of Multisector Group. It should show R for Rotator.

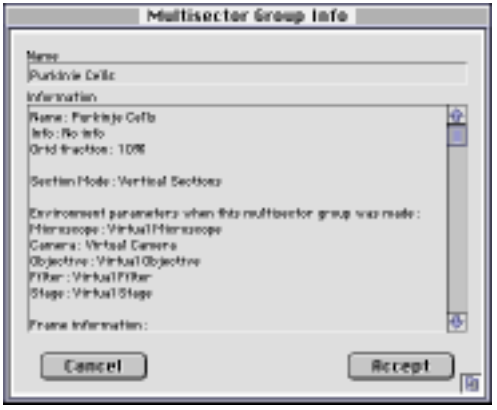
Name - The second column is the name assigned to it when the multisector group was made.

#MS - The third column indicates the number multisectors in the group.

Info - The fourth column is the information assigned to it when the multisector group was made.

Select the multisector group to reopen and press the **Count** button to continue counting with that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both

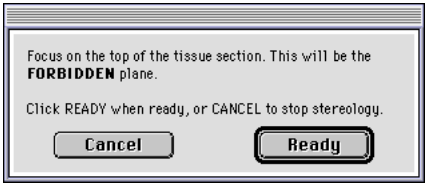
the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.



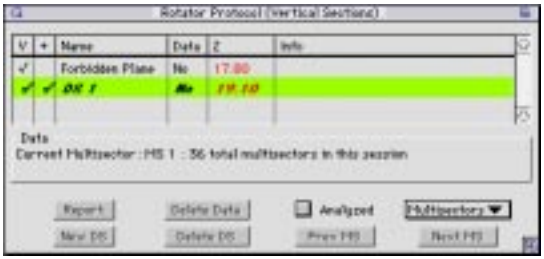
Rotator (Isotropic, Random Sections) Control Window

If a multisector group is selected and opened for counting, live video is turned on. If this is the first time the multisector group is being opened, the thickness of the disector must be entered in the following window.

Once the thickness is specified, another window prompts for you to focus on the top of the tissue section.

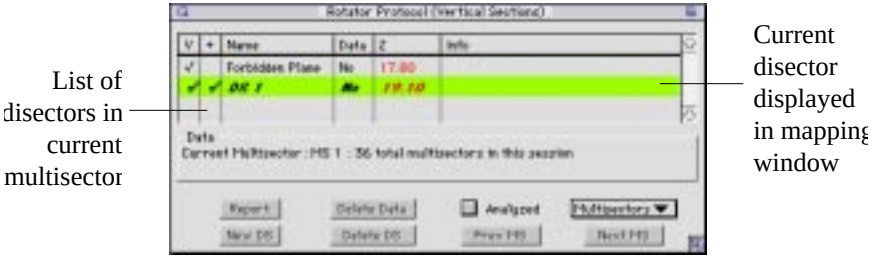


After the top is specified, the following window opens.



This is the **Rotator Protocol (Vertical Sections) Control** window from which the probe is controlled. There are various components to this window.

1. **Disectors** - The disectors for the current multisector that the microscope stage are located in the only scrolling field on this window.

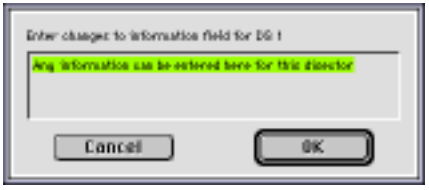


Of these disectors, the disector in *Italics* is the current disector. The current disector is the one that the microscope is focused on. Of the two planes of a disector, the microscope focuses on the reference plane. This is indicated in the column labelled Z. The selected disector is the disector which is highlighted in the highlight color. The selected disector and the current disector are not necessarily the same, although in this example, they are.

Double clicking on a disector makes the selected disector the current disector. The microscope stage will move to that new focus point, and the disector will be displayed in *Italics*.

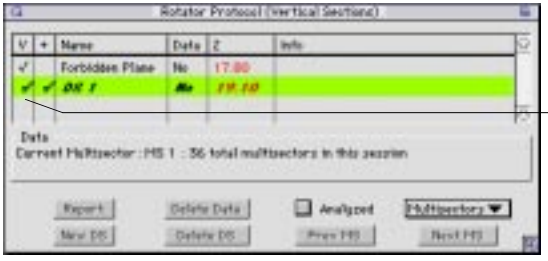
The **Name** for each disector is auto-generated when the multisector is made. The first disector is named *Forbidden Plane* because the microscope focuses on the first plane in the first disector, which by NeuroZoom convention, is the forbidden plane. No data is usually counted in this plane. The subsequent disectors are then named DS 1, DS 2, and so on.

The **Info** field is blank initially for each disector. However, you can Option-*Double Click* on a disector and a dialog window opens for you to enter in new information.



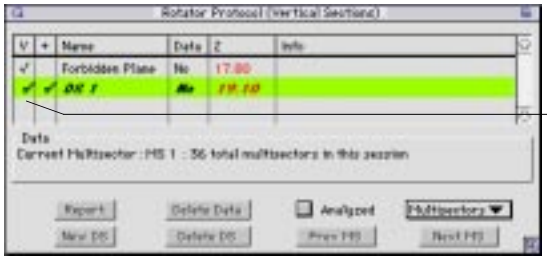
Tip: Option Double Click on a disector to change its information.

Visibility of data can be controlled by clicking on the leftmost column labelled **V.** A single click will toggle the visibility of all of the data for this disector. This is particularly useful if there is a large concentration of data in the multisectors, and they are visually collapsing on top of each other.



Single click in the V column to toggle visibility of the data in the selected disector

Inclusion of data can be controlled by clicking on the column labelled **+**. A single click will toggle the inclusion of all of the data for this disector. Use this to remove a disector completely from reports that are generated, including any statistics generated for the multisector or the multisector group.



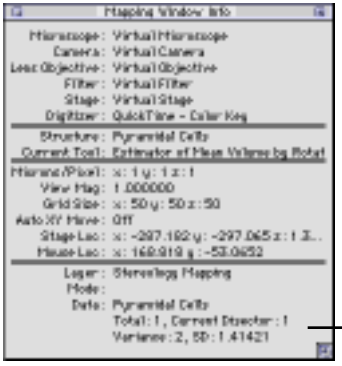
Single click in the + column to toggle inclusion of the data in the selected disector

The column labelled **Data** shows *No* if there is no data in the disector, or *Yes* if there is data.

2. **Data Field** - This shows the current multisector that the microscope stage is located on, and whether it has been “analyzed” or not. When a multisector is “analyzed”, NeuroZoom knows how to treat its data for generating reports and statistics. For example, an unanalyzed multisector is not used in generating reports before the experiment is over, because there may be more data to enter. The total number of multisectors in the group is also shown. The current structure selected in the mapping window is then displayed, along with the number tallied for this structure for both the current multisector and the current disector.

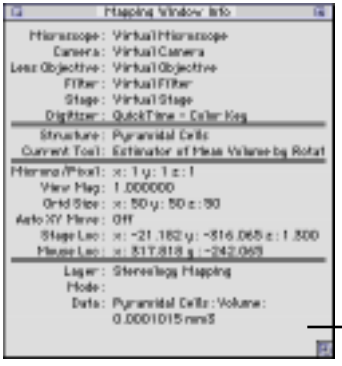
   Multiple structures may be estimated simultaneously with the Rotator probe. However, only the current structure’s totals are shown in the data field.

   All data are estimated for mean volume by using the **Rotator** tool (see the chapters on ***Stereology*** in the ***User Guide Manual*** to understand how to estimate structure mean volume using this tool. Also see the section in this manual on the **Rotator** tool). The **Mapping Window Info** window will show information on the current structure that is being estimated. If there are two or more analyzed multisectors in the group, the *Variance* and the *Standard Deviation* for the number estimates among the multisectors are expressed in the **Mapping Window Info** window. The multisector total and the disector total are also displayed for the current structure.



Variance and SD are displayed only if there are 2 or more analyzed multisectors

When the tool closes the current data object and the volume is calculated for that data structure, the volume is displayed in the **Mapping Window Info** window.

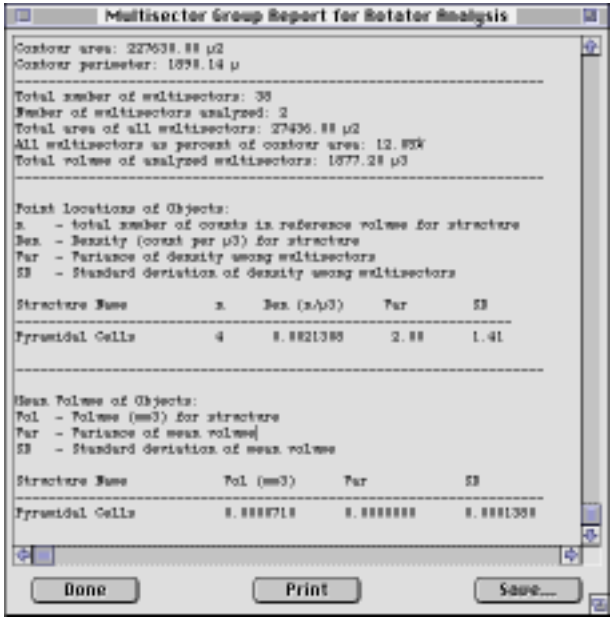


Volume of cell just estimated shows as cubed mm

3.

Several control buttons in the **Control** window perform actions on the multisectors.

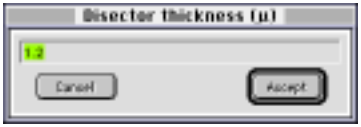
4. **Report** - pressing this button generates a report and displays it in a separate window.



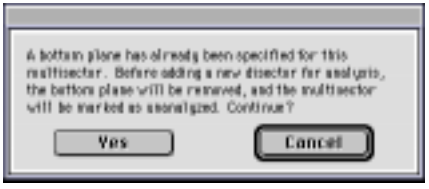
The report can be closed by pressing **Done**, printed to the selected printer by pressing **Print**, or saved to a Text file by pressing **Save....** In this window, the end of the report shows each structure estimated for number in this experiment and lists the total count (n), the density of the structure expressed as n per μ^3 and the variance and standard of the structure among the multisectors. This is zero unless at least two multisectors have been marked as “analyzed”. Following this are the mean volume estimates, the variance, and the standard deviation for each structure.

5.

6. **New Disector** - pressing the **New DS** button creates a new disector for the current multisector. The position of the disector is immediately adjacent to the current disector. The thickness of the disector is the same as all other disectors, and is the number first specified when this multisector group was made. However, if the option is held down when **New DS** is pressed, a window opens for you to enter in a new disector thickness value in microns.



If **New DS** is pressed and the current multisector has already been marked as analyzed, a window opens to alert you to this fact.

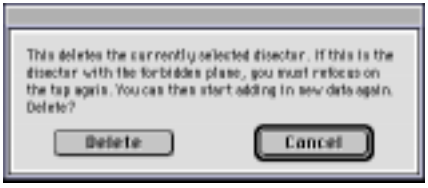


Press **Cancel** to dismiss the window and to not create a new disector. Press **Yes** to indicate that the new disector will be added to the bottom of the multisector, and will push the multisector stack downward to incorporate the new thickness of this new disector. The multisector will then be marked as "unanalyzed".

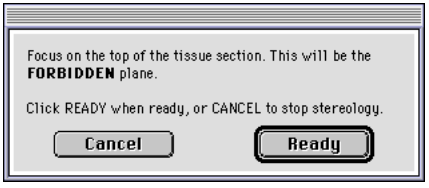
7. **Delete Disector** - The **Delete DS** button is enabled only if the last disector in the multisector is the current disector. Pressing this button deletes the current disector completely from the multisector.

Alert: The CURRENT disector is deleted, not the SELECTED disector. The current disector is always displayed in Italics. Please note this difference between the two.

A window opens alerting you to certain conditions if this is the last disector.



If the current disector contains the forbidden disector (meaning also it is the last disector in the multisector), you need to refocus on the top of the multisector again to begin estimating again for this multisector.



If you press on **Cancel**, the last disector without its data is reattached to the multisector. This is not a serious problem, because generally you do not estimate data when on the forbidden plane.

If you press **Ready**, the current position of the microscope stage is read from the controller, and two disectors are made. The first disector contains the forbidden plane at the current focus you indicated. The second disector contains the first permitted plane. NeuroZoom automatically moves to the first permitted plane of the second disector, and makes that one the current disector.

If the current disector being deleted is not the last disector, the next to last disector then becomes the current disector after deletion.

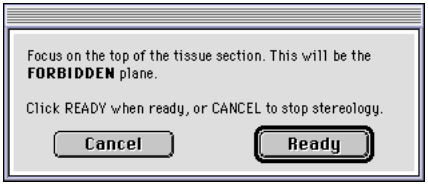
8. **Delete Data** - pressing the **Delete Data** button will delete all data from the current disector. Similar to the previous command, this acts on the current, not the selected disector. If there is no data in the current disector, an error window opens.

If it does contain data, a warning window opens.

Only the data are deleted. The planes and their location are not affected. You can continue estimating data directly into this disector.

9. **Previous Multisector** - pressing the **Prev MS** button moves the microscope stage to the previous multisector for this group. This movement wraps around, so if you are currently on the first multisector, the last multisector is selected.

If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.

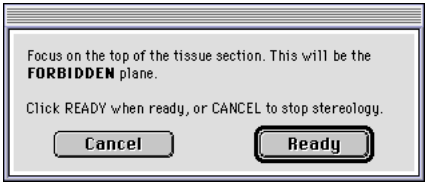


Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

The **Data** field updates to show the current multisector. The Disector list also updates to show the disectors in this multisector. The last disector in this multisector becomes the current disector. The microscope focuses on the permitted plane of this disector.

10. **Next Multisector** - pressing the **Next MS** button moves the microscope stage to the next multisector for this group. This movement wraps around, so if you are currently on the last multisector, the first multisector is selected.

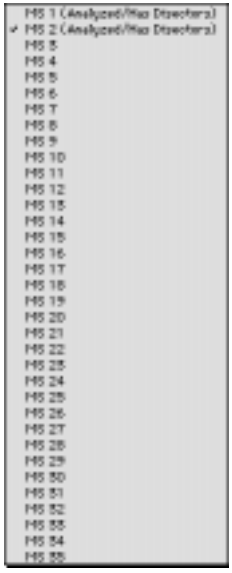
If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.



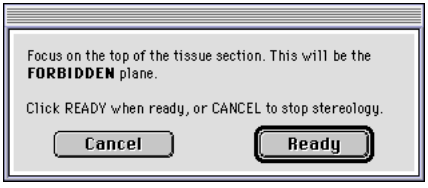
Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

The **Data** field updates to show the current multisector. The Disector list also updates to show the disectors in this multisector. The last disector in this multisector becomes the current disector. The microscope focuses on the permitted plane of this disector.

11. **Multisector** - this is a popup menu showing all the multisectors in this group. You can select any of them and NeuroZoom automatically goes to that multisector. The menu indicates whether a multisector has been analyzed or not. The current multisector is preceded with a checkmark.



If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.

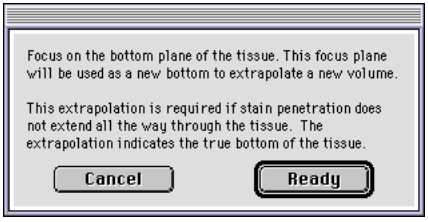


Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

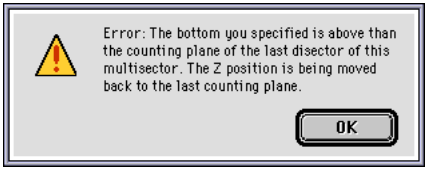
12. **Analyzed -** checking this button marks the current multisector as”analyzed”. This makes the multisector eligible for calculations involving global volumes and counts, such as when generating reports.

When a multisector has been marked as “analyzed”, and you uncheck the box to mark it as “unanalyzed” again, a warning window opens.

The counting plane of the last disector in this multisector is used as the bottom of the multisector. If the tissue has *incomplete staining*, this counting plane may not be the true bottom of the multisector. In this case, *Option-Click* on the **Analyzed** checkbox. A window opens asking you to focus on the true bottom of the tissue section. This plane will then be used as the bottom and all volumes will be extrapolated to this plane. Note that the density of the structures if it is being calculated, remains the same regardless of extrapolation.



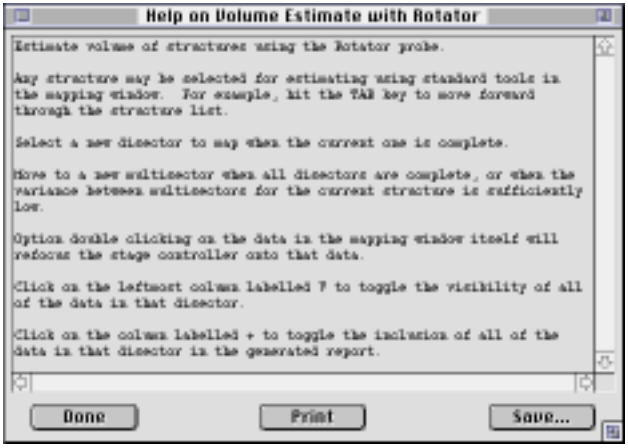
If the plane you specify as the bottom is above the counting plane of the last disector of this multisector, an error window opens, and the stage moves back to the last known position.



The bottom cannot be above the last counting plane, otherwise data will be excluded from the calculations. If a disector needs to be deleted, use the **Delete DS** button to delete the disector completely.

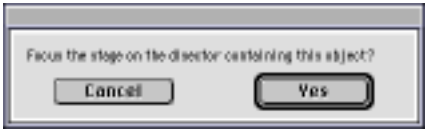
13.

14. **Help** - If the Help button on the keyboard is pressed, a **Help** window opens displaying some helpful comments for this protocol.



15.

16. **Moving to the Data's Disector** - In the **Mapping** window, *control-double-clicking* on object that belongs to the current multisector focuses the microscope stage to the data's disector. A warning window opens asking for confirmation.



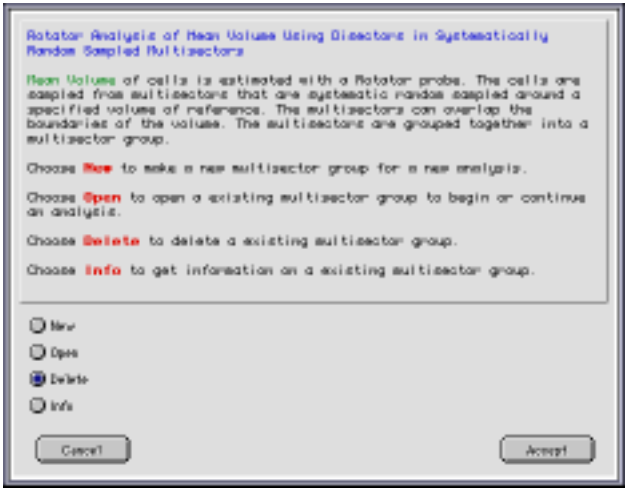
Press **Yes** to move the stage to the disector holding that data. That disector then becomes the current disector. Press **Cancel** to dismiss the window without moving the stage or changing disectors.

If you control-double-click on an object that is in the current disector, a warning window opens.

Alert: Be sure to have the **Selection** wool selected from the **Mapping Tools** window in order to control-double-click on it. Otherwise, you will only put a new data point on top of what you are clicking on.

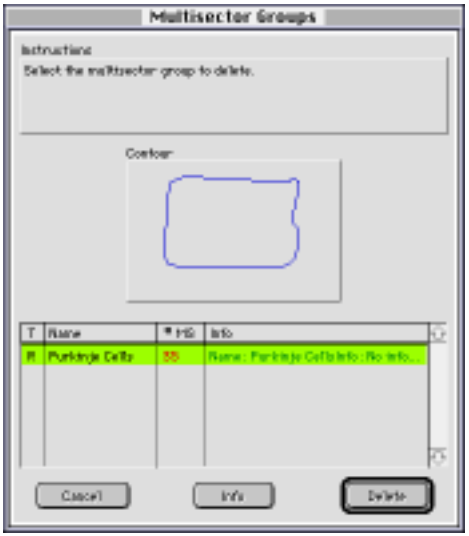
Delete Existing Multisector Group

Delete an existing multisector group for estimating numbers using a Nucleator protocol.



If you choose this option, and if there are no multisector groups for this Rotator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Rotator protocols in the document.



Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

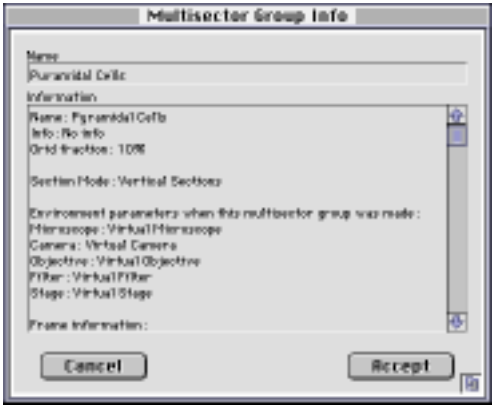
T - This first column indicates the Type of Multisector Group. It should show R for Rotator.

Name - The second column is the name assigned to it when the multisector group was made.

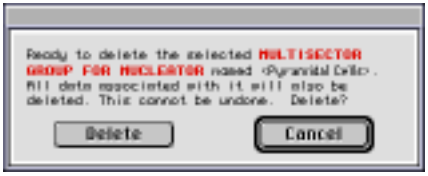
#MS - The third column indicates the number multisectors in the group.

Info - The fourth column is the information assigned to it when the multisector group was made.

Select the multisector group to delete and press the **Delete** button to delete that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.



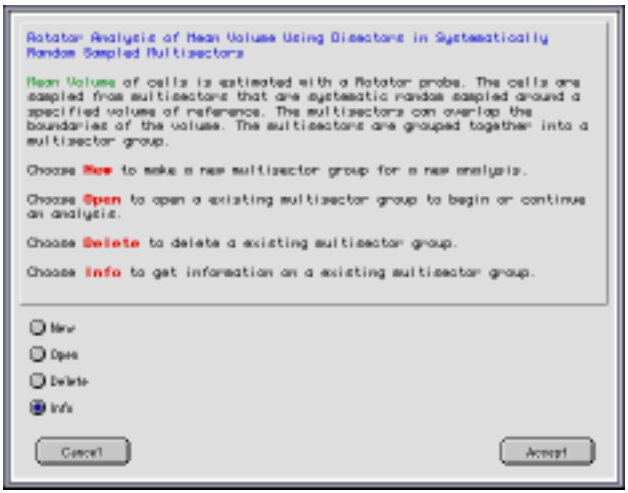
When deleting a group, a confirmation window opens warning that the deletion is permanent and cannot be undone.



Press **Delete** to delete the group, or press **Cancel** to dismiss the window without any changes.

Info on Existing Multisector Group

Get information by reports on existing multisector groups for estimating numbers using a Rotator protocol.



If you choose this option, and if there are no multisector groups for this Rotator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Rotator protocols in the document.

Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

+ - This first column may be toggled on and off by pressing the mouse in this column. Toggling on selects the group for a report. Multiple selections may be made in this manner to report on more than one multisector group at one time.

T - This second column indicates the Type of Multisector Group. It should show R for Rotator.

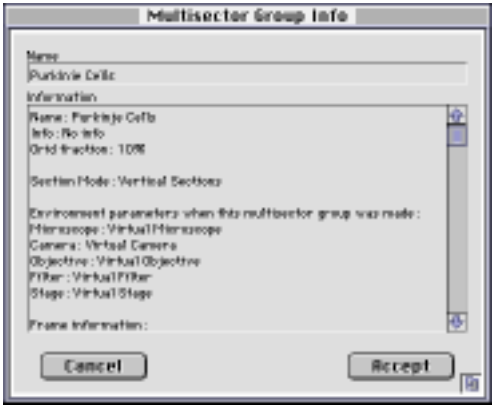
Name - The third column is the name assigned to it when the multisector group was made.

#MS - The fourth column indicates the number multisectors in the group.

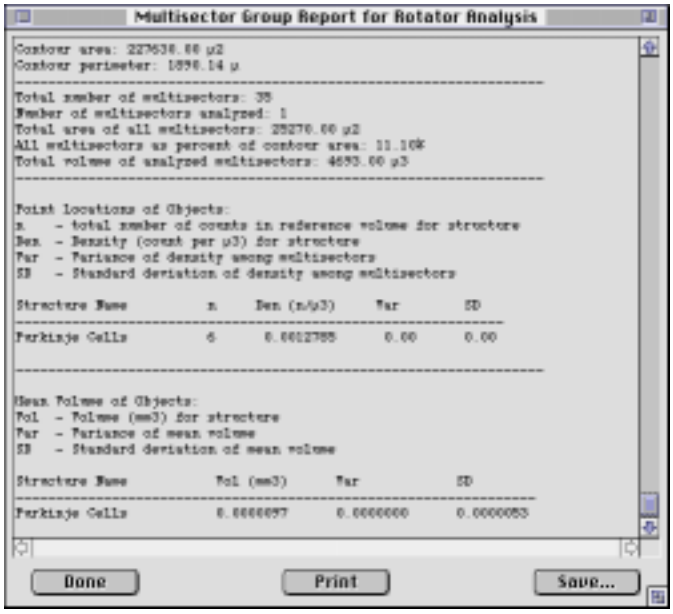
Info - The fifth column is the information assigned to it when the multisector group was made.

Select the multisector groups to report on and press the **Report** button to report on that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and

Information can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.



Multiple group reporting opens multiple report windows. A report window shows the same information as generating a report when conducting the experiment.

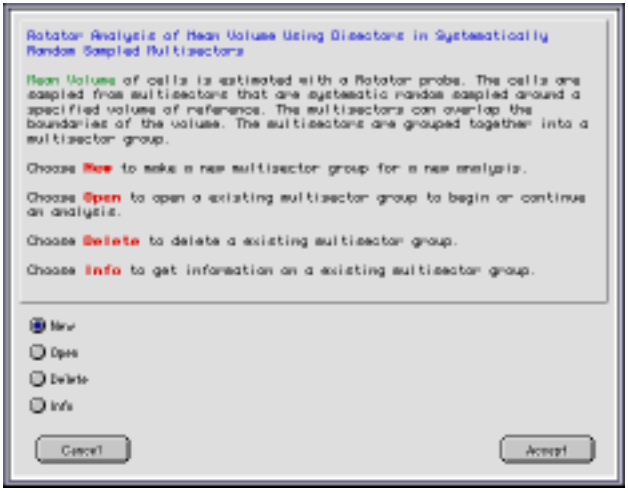

Multisector Group Report for Rotator Analysis
Contour area: 227630.00 µ2
Contour perimeter: 1890.14 µ
Total number of multisectors: 35
Number of multisectors analyzed: 1
Total area of all multisectors: 25270.00 µ2
All multisectors as percent of contour area: 11.10%
Total volume of analyzed multisectors: 4693.00 µ3
Point Locations of Objects:
n - total number of counts in reference volume for structure
Den - Density (count per µ3) for structure
Var - Variance of density among multisectors
SD - Standard deviation of density among multisectors
Structure Name n Den (n/µ3) Var SD
Purkinje Cells 6 0.0012785 0.00 0.00
Mean Volume of Objects:
Vol - Volume (mm3) for structure
Var - Variance of mean volume
SD - Standard deviation of mean volume
Structure Name Vol (mm3) Var SD
Purkinje Cells 0.0000097 0.0000000 0.0000053
Done
Print
Save...

Volume by Rotator in Isotropic, Random Sections

Opened by:

- Estimate Volume By Rotator in Isotropic, Random Sections... in Stereology menu

The **Volume By Rotator in Isotropic, Random Sections** window, for lack of a better name, is a choice window. From this you are to select one of four options to proceed with estimating volumes.



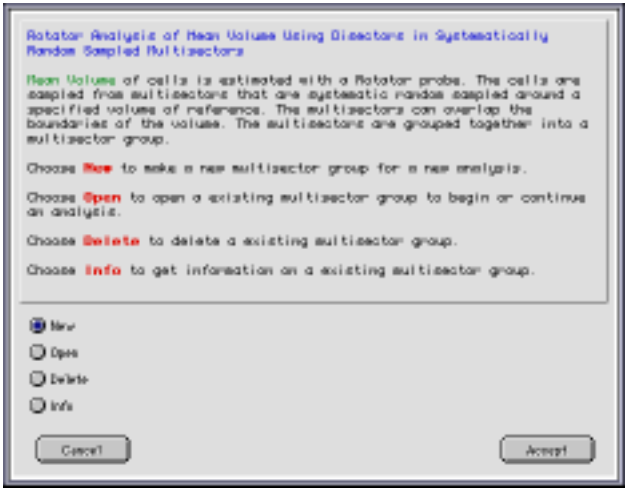
Information on what this probe accomplishes and the four options are presented.

- **New** - make a new multisector group to begin a new analysis on estimating the mean volume of some structure
- **Open** - open an existing multisector group for additional data entry or analysis
- **Delete** - delete an existing multisector group from the document
- **Info** - get information on an existing multisector group

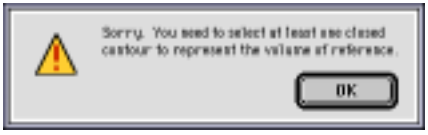
Choose one of the options my pressing the mouse button the radio button. The radio button will highlight indicating that it is selected. Press **Accept** to continue with the process, or press **Cancel** to dismiss this window with no options or changes made to the document.

New Multisector Group

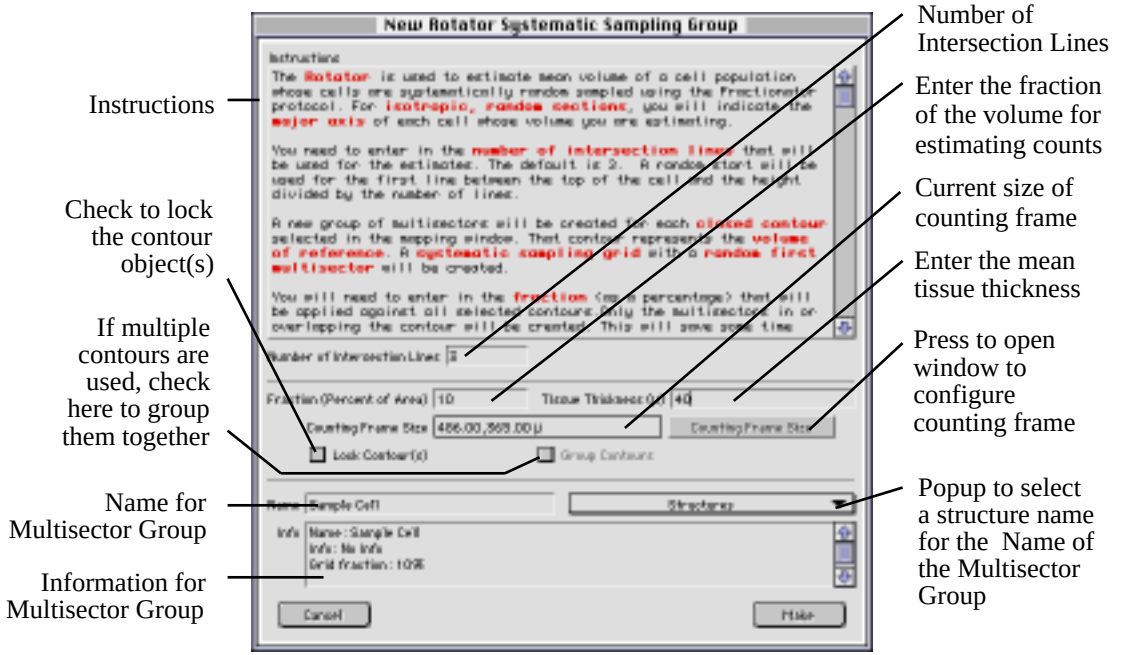
Make a new multisector group for estimating mean volume using a Rotator protocol.



If you choose this option, and if you do not have at least one contour created and selected in the mapping window, an error window opens indicating that at least one selected contour is needed to represent the *volume of reference*.



Go back and use the mapping tools to create a closed contour around the volume of reference. When you have done so, select them both, choose **Estimate Volume By Rotator in Isotropic, Random Sections...** in the **Stereology** menu again, and select **New** and press **Accept**. A new window opens.



This window is the **New Rotator Systematic Sampling Group** window. All of the parameters to create a multisector group for a Rotator analysis are entered here. The multisectors are calculated on a systematic random sample through the selected contour. Only those multisectors that intersect the contour will be presented. Disectors are created in those multisectors in which the estimates are places for cell volume estimates.

Fraction (Percent of Area)

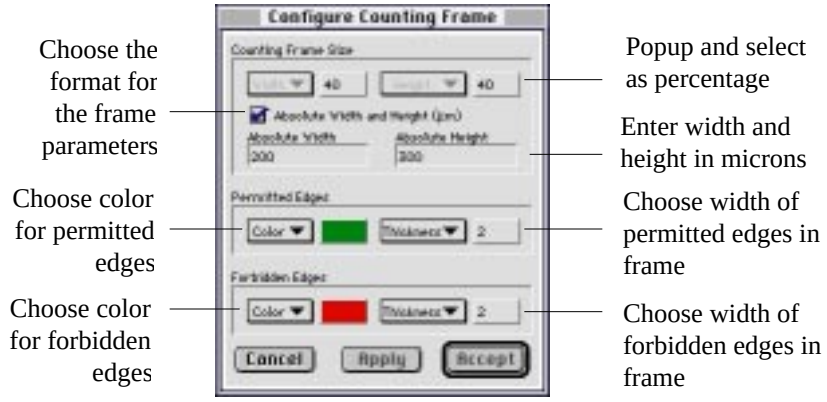
Enter the fraction that is desred for the Rotator analysis. For example, if you want to estimate counts of cells in 10% of the contour volume, enter 10. The default is 10%. Please refer to the manual ***NeuroZoom Stereology*** for more specific information on this value.

Tissue Thickness (µ)

Enter the mean tissue thickness in microns for this section. This can be measured with the **Measure Mean Tissue Thickness** tool located in the **Analysis** submenu of the **Modules** menu.

Counting Frame Size

This is a button that opens a window from which to adjust the size of the counting frame. The current frame size is displayed in the field to its left.

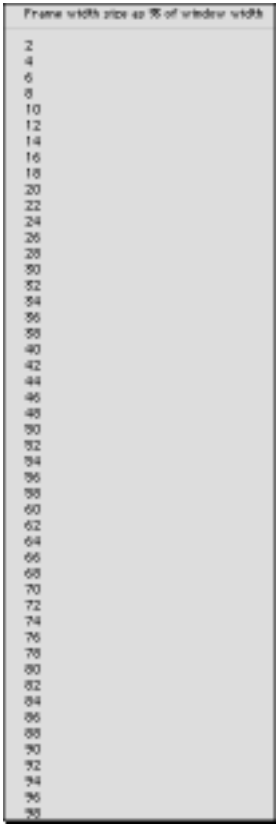


The size of the counting frame may be entered as a *percentage* of the screen size, or as an absolute width and height in microns. The major difference between the two techniques is that a percentage will always ensure that the counting frame fits in the mapping window regardless of the *lens objective* used. However, you cannot switch lens objectives in the middle of a stereology experiment using the counting frame because the absolute size of the frame would change.

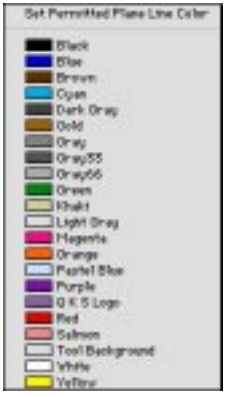
When you enter the size in *absolute microns*, the displayed size of the counting frame in the mapping window will change as you change the lens objective. The frame will always adjust to the absolute width and height entered. However, the problem here is that the size of the counting frame may exceed the size of the mapping window with lower power objectives. If so, NeuroZoom will alert you.

In all cases of stereology, you cannot change the lens objectives once the experiment is underway. However, one method of entering in the size may be more appropriate than the other, depending on how you go about the business of setting up the stereology experiment. You choose the method which best suits your needs. Please also refer to the chapters on ***Stereology*** in the ***User Guide Manual*** and the ***Reference Manual*** more information.

When choosing the width and height as a percentage, press and hold on the width or height button. A menu pops up for selectons from 2 to 98%.



Press and hold on the **Color** button for the *permitted and forbidden colors*. A popup menu shows the color choices.



Press and hold on the **Thickness** button for the *permitted and forbidden thicknesses*. A popup menu shows the choices from 1 to 6. 6 is thickest.

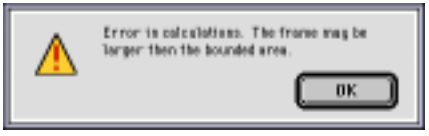
Press on the **Apply** button to see the changes. Press on **Cancel** to dismiss the window and cancel any changes you made with this window. Press **Accept** to dismiss the window and to store the changes made with this window.

Name and Info

Enter the name and information for this multisector group. The default name is the structure name of the contour that is selected when this window was opened. Press and hold on the **Structure Popup** menu button to get a list of all the structures for this mapping window and select one for the name. You can also enter in any other name you desire.

When all parameters are entered, the **Make** button is enabled. Press **Cancel** to dismiss the window and not make the multisector group. Press **Make** to make the multisector group.

If the counting frame is too large to accurately place a group of multisectors over the selected contour representing the volume of reference, a window opens displaying this error.



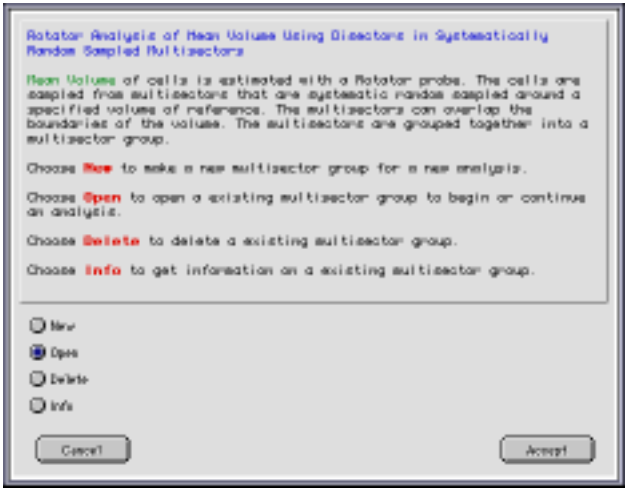
Once the multisector group has been made successfully, a window opens presenting the option of starting the stereology session immediately on this new group.

Press **No** to close window and return to the Mapping window. Press **Yes** to continue immediately to the Nucleator analysis on this multisector group.

Answering **Yes** is the same as opening an existing multisector group for analysis. This is presented in the next session.

Open Existing Multisector Group

Open an existing multisector group for estimating numbers using a Rotator protocol.



If you choose this option, and if there are no multisector groups for this Rotator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Rotator protocols in the document.

Tip: Holding down the OPTION when choosing Estimate Volume By Rotator in Isotropic, Random Sections... in the Stereology Menu will automatically preselect this OPEN button.

Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

T - This first column (T) indicates the Type of Multisector Group. It should show R for Rotator.

Name - The second column is the name assigned to it when the multisector group was made.

#MS - The third column indicates the number multisectors in the group.

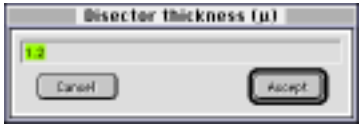
Info - The fourth column is the information assigned to it when the multisector group was made.

Select the multisector group to reopen and press the **Count** button to continue counting with that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both

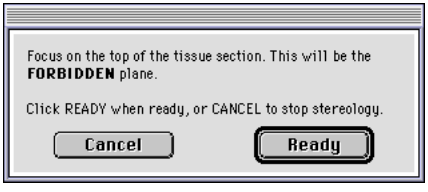
the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.

Rotator (Isotropic, Random Sections) Control Window

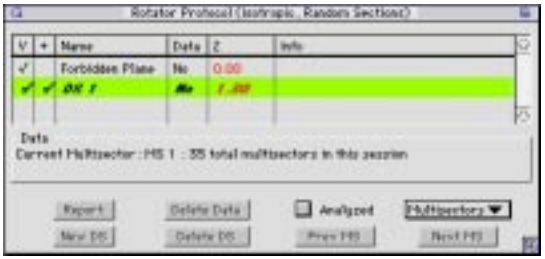
If a multisector group is selected and opened for counting, live video is turned on. If this is the first time the multisector group is being opened, the thickness of the disector must be entered in the following window.



Once the thickness is specified, another window prompts for you to focus on the top of the tissue section.

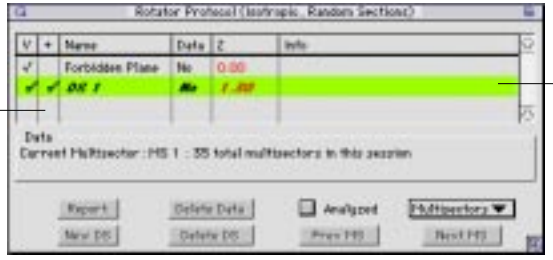


After the top is specified, the following window opens.



This is the **Rotator Protocol (Isotropic, Random Sections) Control** window from which the probe is controlled. There are various components to this window.

1. **Disectors -** The disectors for the current multisector that the microscope stage are located in the only scrolling field on this window.



List of disectors in current multisector

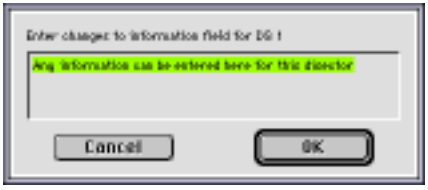
Current disector displayed in mapping window

Of these disectors, the disector in *Italics* is the current disector. The current disector is the one that the microscope is focused on. Of the two planes of a disector, the microscope focuses on the reference plane. This is indicated in the column labelled Z. The selected disector is the disector which is highlighted in the highlight color. The selected disector and the current disector are not necessarily the same, although in this example, they are.

Double clicking on a disector makes the selected disector the current disector. The microscope stage will move to that new focus point, and the disector will be displayed in *Italics*.

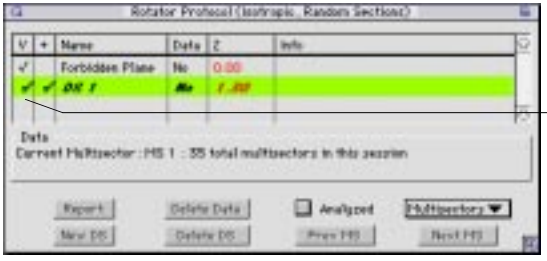
The **Name** for each disector is auto-generated when the multisector is made. The first disector is named *Forbidden Plane* because the microscope focuses on the first plane in the first disector, which by NeuroZoom convention, is the forbidden plane. No data is usually counted in this plane. The subsequent disectors are then named DS 1, DS 2, and so on.

The **Info** field is blank initially for each disector. However, you can Option-*Double Click* on a disector and a dialog window opens for you to enter in new information.



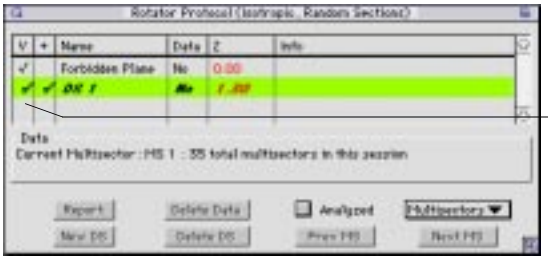
Tip: Option Double Click on a disector to change its information.

Visibility of data can be controlled by clicking on the leftmost column labelled **V**. A single click will toggle the visibility of all of the data for this disector. This is particularly useful if there is a large concentration of data in the multisectors, and they are visually collapsing on top of each other.



Single click in the V column to toggle visibility of the data in the selected disector

Inclusion of data can be controlled by clicking on the column labelled **+**. A single click will toggle the inclusion of all of the data for this disector. Use this to remove a disector completely from reports that are generated, including any statistics generated for the multisector or the multisector group.



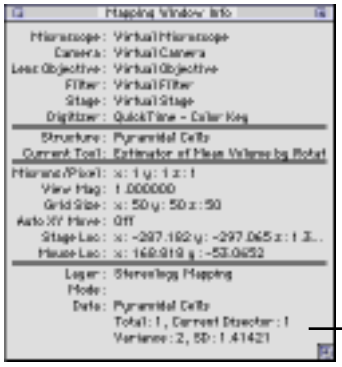
Single click in the + column to toggle inclusion of the data in the selected disector

The column labelled **Data** shows *No* if there is no data in the disector, or *Yes* if there is data.

2. **Data Field** - This shows the current multisector that the microscope stage is located on, and whether it has been “analyzed” or not. When a multisector is “analyzed”, NeuroZoom knows how to treat its data for generating reports and statistics. For example, an unanalyzed multisector is not used in generating reports before the experiment is over, because there may be more data to enter. The total number of multisectors in the group is also shown. The current structure selected in the mapping window is then displayed, along with the number tallied for this structure for both the current multisector and the current disector.

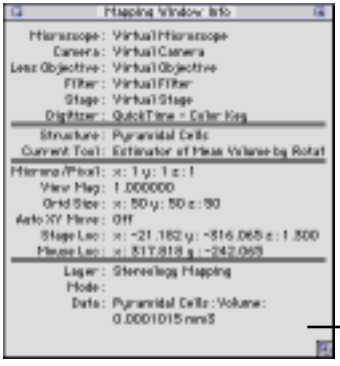
   Multiple structures may be estimated simultaneously with the Rotator probe. However, only the current structure’s totals are shown in the data field.

   All data are estimated for mean volume by using the **Rotator** tool (see the chapters on ***Stereology*** in the ***User Guide Manual*** to understand how to estimate structure mean volume using this tool. Also see the section in this manual on the **Rotator** tool). The **Mapping Window Info** window will show information on the current structure that is being estimated. If there are two or more analyzed multisectors in the group, the *Variance* and the *Standard Deviation* for the number estimates among the multisectors are expressed in the **Mapping Window Info** window. The multisector total and the disector total are also displayed for the current structure.



Variance and SD are displayed only if there are 2 or more analyzed multisectors

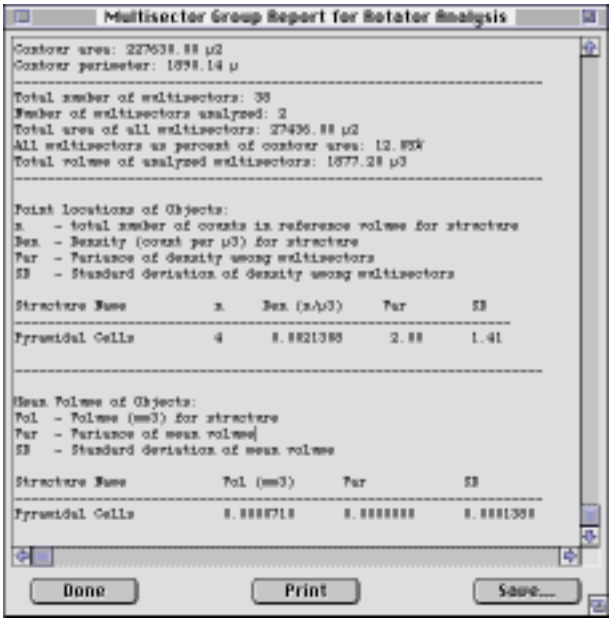
When the tool closes the current data object and the volume is calculated for that data structure, the volume is displayed in the **Mapping Window Info** window.



Volume of cell just estimated shows as cubed mm

Several control buttons in the **Control** window perform actions on the multisectors.

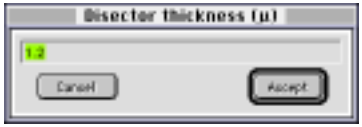
3. **Report** - pressing this button generates a report and displays it in a separate window.



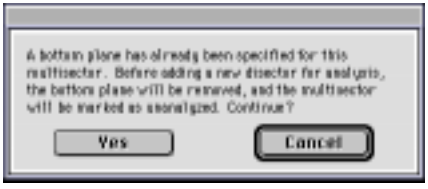
The report can be closed by pressing **Done**, printed to the selected printer by pressing **Print**, or saved to a Text file by pressing **Save....** In this window, the end of the report shows each structure estimated for number in this experiment and lists the total count (n), the density of the structure expressed as n per μ^3 and the variance and standard of the structure among the multisectors. This is zero unless at least two multisectors have been marked as “analyzed”. Following this are the mean volume estimates, the variance, and the standard deviation for each structure.

4.

5. **New Disector** - pressing the **New DS** button creates a new disector for the current multisector. The position of the disector is immediately adjacent to the current disector. The thickness of the disector is the same as all other disectors, and is the number first specified when this multisector group was made. However, if the option is held down when **New DS** is pressed, a window opens for you to enter in a new disector thickness value in microns.



If **New DS** is pressed and the current multisector has already been marked as analyzed, a window opens to alert you to this fact.



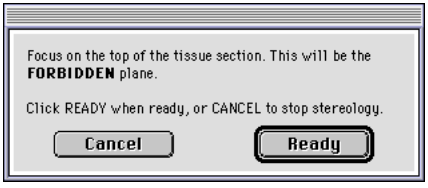
Press **Cancel** to dismiss the window and to not create a new disector. Press **Yes** to indicate that the new disector will be added to the bottom of the multisector, and will push the multisector stack downward to incorporate the new thickness of this new disector. The multisector will then be marked as “unanalyzed”.

6. **Delete Disector** - The **Delete DS** button is enabled only if the last disector in the multisector is the current disector. Pressing this button deletes the current disector completely from the multisector.

Alert: The CURRENT disector is deleted, not the SELECTED disector. The current disector is always displayed in Italics. Please note this difference between the two.

A window opens alerting you to certain conditions if this is the last disector.

If the current disector contains the forbidden disector (meaning also it is the last disector in the multisector), you need to refocus on the top of the multisector again to begin estimating again for this multisector.



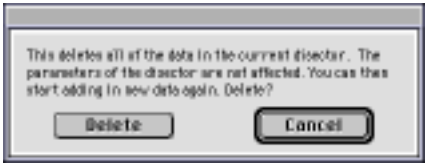
If you press on **Cancel**, the last disector without its data is reattached to the multisector. This is not a serious problem, because generally you do not estimate data when on the forbidden plane.

If you press **Ready**, the current position of the microscope stage is read from the controller, and two disectors are made. The first disector contains the forbidden plane at the current focus you indicated. The second disector contains the first permitted plane. NeuroZoom automatically moves to the first permitted plane of the second disector, and makes that one the current disector.

If the current disector being deleted is not the last disector, the next to last disector then becomes the current disector after deletion.

7. **Delete Data** - pressing the **Delete Data** button will delete all data from the current disector. Similar to the previous command, this acts on the current, not the selected disector. If there is no data in the current disector, an error window opens.

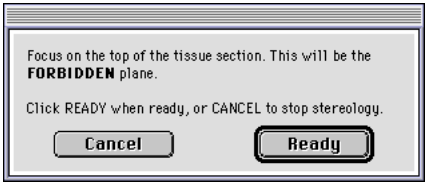
If it does contain data, a warning window opens.



Only the data are deleted. The planes and their location are not affected. You can continue estimating data directly into this disector.

8. **Previous Multisector** - pressing the **Prev MS** button moves the microscope stage to the previous multisector for this group. This movement wraps around, so if you are currently on the first multisector, the last multisector is selected.

If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.

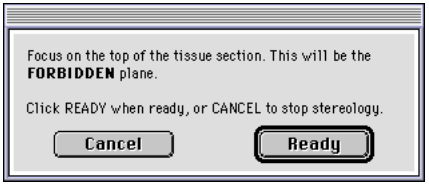


Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

The **Data** field updates to show the current multisector. The Disector list also updates to show the disectors in this multisector. The last disector in this multisector becomes the current disector. The microscope focuses on the permitted plane of this disector.

9. **Next Multisector** - pressing the **Next MS** button moves the microscope stage to the next multisector for this group. This movement wraps around, so if you are currently on the last multisector, the first multisector is selected.

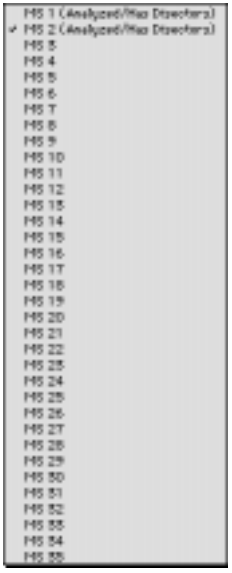
If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.



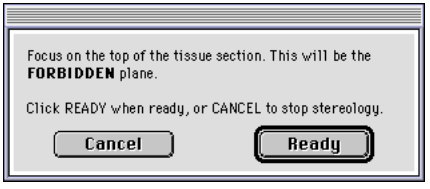
Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

The **Data** field updates to show the current multisector. The Disector list also updates to show the disectors in this multisector. The last disector in this multisector becomes the current disector. The microscope focuses on the permitted plane of this disector.

10. **Multisector** - this is a popup menu showing all the multisectors in this group. You can select any of them and NeuroZoom automatically goes to that multisector. The menu indicates whether a multisector has been analyzed or not. The current multisector is preceded with a checkmark.



If this is a new multisector being visited, and a first plane has not been specified, NeuroZoom will ask you to focus on the first plane of the first disector.

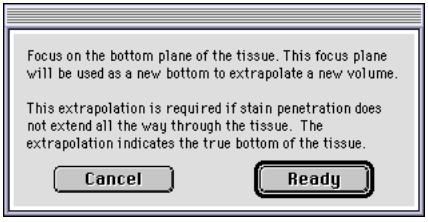


Press **Ready** and the current microscope stage location will be read as the first plane of the first disector. Press **Cancel** to dismiss the window, and cancel the multisector move command. The last used multisector will be moved back into location.

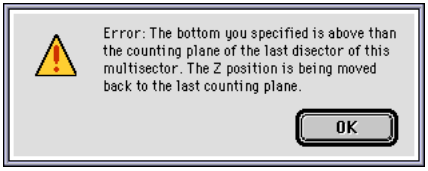
11. **Analyzed** - checking this button marks the current multisector as”analyzed”. This makes the multisector eligible for calculations involving global volumes and counts, such as when generating reports.

When a multisector has been marked as “analyzed”, and you uncheck the box to mark it as “unanalyzed” again, a warning window opens.

The counting plane of the last disector in this multisector is used as the bottom of the multisector. If the tissue has *incomplete staining*, this counting plane may not be the true bottom of the multisector. In this case, *Option-Click* on the **Analyzed** checkbox. A window opens asking you to focus on the true bottom of the tissue section. This plane will then be used as the bottom and all volumes will be extrapolated to this plane. Note that the density of the structures if it is being calculated, remains the same regardless of extrapolation.



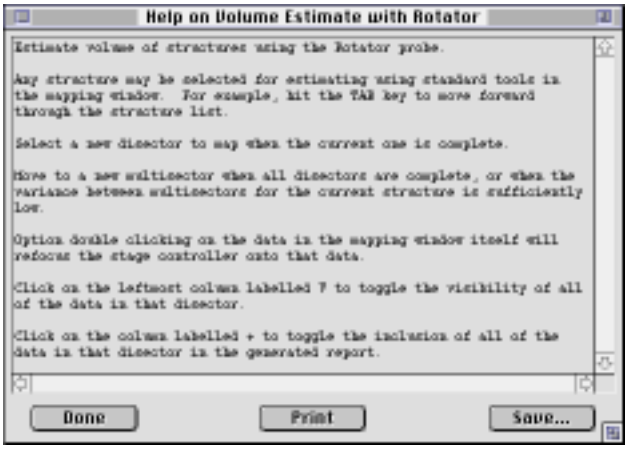
If the plane you specify as the bottom is above the counting plane of the last disector of this multisector, an error window opens, and the stage moves back to the last known position.



The bottom cannot be above the last counting plane, otherwise data will be excluded from the calculations. If a disector needs to be deleted, use the **Delete DS** button to delete the disector completely.

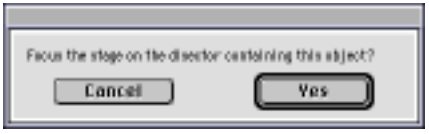
12.

13. **Help** - If the Help button on the keyboard is pressed, a **Help** window opens displaying some helpful comments for this protocol.



14.

15. **Moving to the Data's Disector** - In the **Mapping** window, *control-double-clicking* on object that belongs to the current multisector focuses the microscope stage to the data's disector. A warning window opens asking for confirmation.



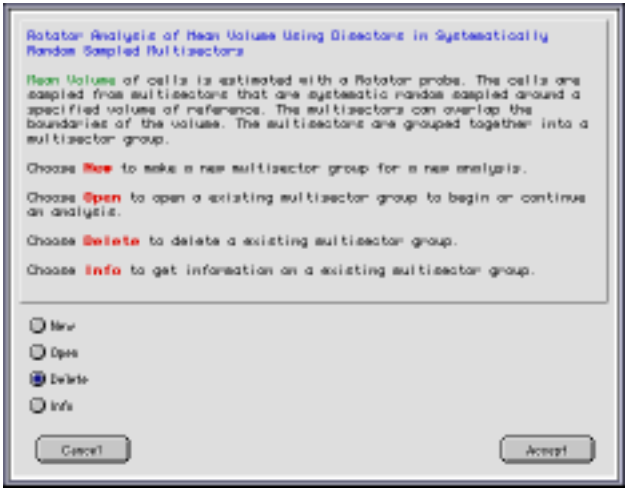
Press **Yes** to move the stage to the disector holding that data. That disector then becomes the current disector. Press **Cancel** to dismiss the window without moving the stage or changing disectors.

If you control-double-click on an object that is in the current disector, a warning window opens.

Alert: Be sure to have the **Selection** wool selected from the **Mapping Tools** window in order to control-double-click on it. Otherwise, you will only put a new data point on top of what you are clicking on.

Delete Existing Multisector Group

Delete an existing multisector group for estimating numbers using a Nucleator protocol.



If you choose this option, and if there are no multisector groups for this Rotator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Rotator protocols in the document.

Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

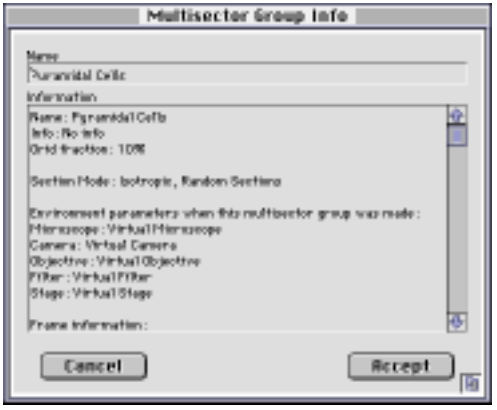
T - This first column indicates the Type of Multisector Group. It should show R for Rotator.

Name - The second column is the name assigned to it when the multisector group was made.

#MS - The third column indicates the number multisectors in the group.

Info - The fourth column is the information assigned to it when the multisector group was made.

Select the multisector group to delete and press the **Delete** button to delete that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and **Information** can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.

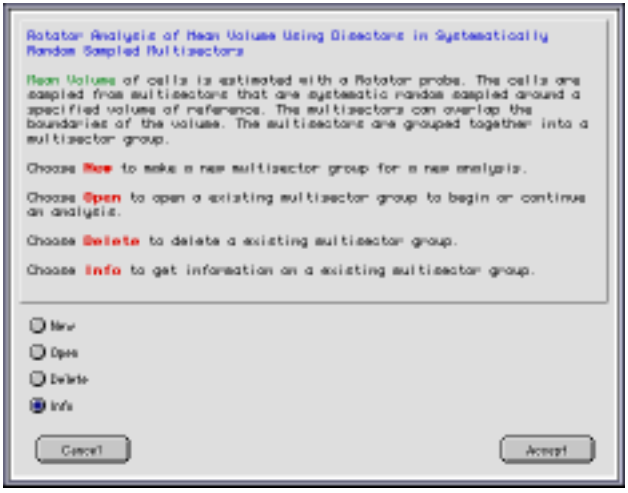


When deleting a group, a confirmation window opens warning that the deletion is permanent and cannot be undone.

Press **Delete** to delete the group, or press **Cancel** to dismiss the window without any changes.

Info on Existing Multisector Group

Get information by reports on existing multisector groups for estimating numbers using a Rotator protocol.



If you choose this option, and if there are no multisector groups for this Rotator protocol stored in the document, an error window opens to alert you.

Otherwise, a window opens that allows you to select from any existing Rotator protocols in the document.

Notice the contour drawn to remind you of the volume of reference. This is handy if there are several protocols stored in the document.

+ - This first column may be toggled on and off by pressing the mouse in this column. Toggling on selects the group for a report. Multiple selections may be made in this manner to report on more than one multisector group at one time.

T - This second column indicates the Type of Multisector Group. It should show R for Rotator.

Name - The third column is the name assigned to it when the multisector group was made.

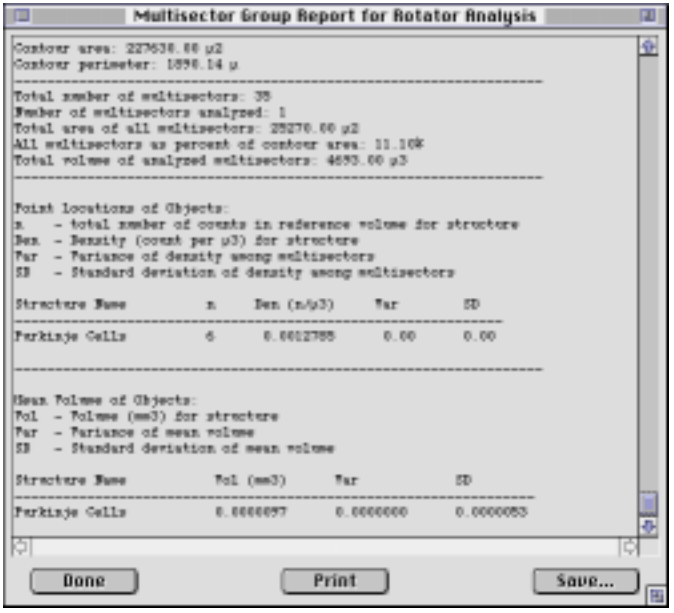
#MS - The fourth column indicates the number multisectors in the group.

Info - The fifth column is the information assigned to it when the multisector group was made.

Select the multisector groups to report on and press the **Report** button to report on that group. Press the **Cancel** button to dismiss the window and cancel any selection. Or press the **Info** button to see more information. Both the **Name** and

Information can be edited. Pressing **Accept** will store the changes. Pressing **Cancel** will dismiss the window without storing any changes.

Multiple group reporting opens multiple report windows. A report window shows the same information as generating a report when conducting the experiment.


Multisector Group Report for Rotator Analysis
Contour area: 227630.00 µ2
Contour perimeter: 1898.14 µ
Total number of multisectors: 35
Number of multisectors analyzed: 1
Total area of all multisectors: 25270.00 µ2
All multisectors as percent of contour area: 11.10%
Total volume of analyzed multisectors: 4853.00 µ3
Point Locations of Objects:
n - total number of counts in reference volume for structure
Den - Density (count per µ3) for structure
Var - Variance of density among multisectors
SD - Standard deviation of density among multisectors
Structure Name n Den (n/µ3) Var SD
Purkinje Cells 6 0.0012355 0.00 0.00
Mean Volume of Objects:
Vol - Volume (mm3) for structure
Var - Variance of mean volume
SD - Standard deviation of mean volume
Structure Name Vol (mm3) Var SD
Purkinje Cells 0.0000097 0.0000000 0.0000053
Done
Print
Save...

CHAPTER 5 *3D Mapping Windows*

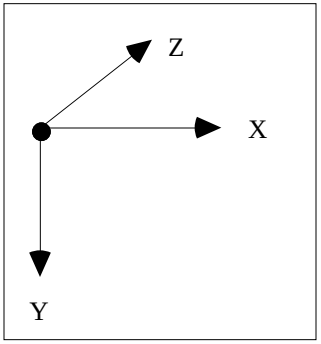
All the windows in NeuroZoom are opened by activating a menu command. In this chapter, all of the 3D Mapping windows in NeuroZoom will be detailed.

- See the chapters on ***Stereology Windows*** for specific information on the stereology windows.
- See the chapters on ***Mapping Windows*** for specific information on the 2D windows.
- See the chapters on ***3D Visualization*** for basic information on using 3D visualization windows.

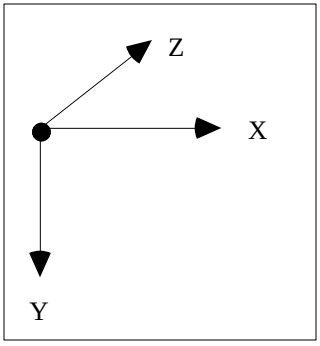
Please be sure to read the previous chapter on **Menus** to understand fully how these windows are opened.

3D Window

The *initial view of the 3D model* is centered around the data bounds or data bounding box and scaled appropriately to show all the data without distortion. The coordinate axes are oriented in the same way as in the mapping windows: positive X to the right, positive Y to the bottom and positive Z away from the user. The origin of this coordinate system is equal to the center of the data bounds.



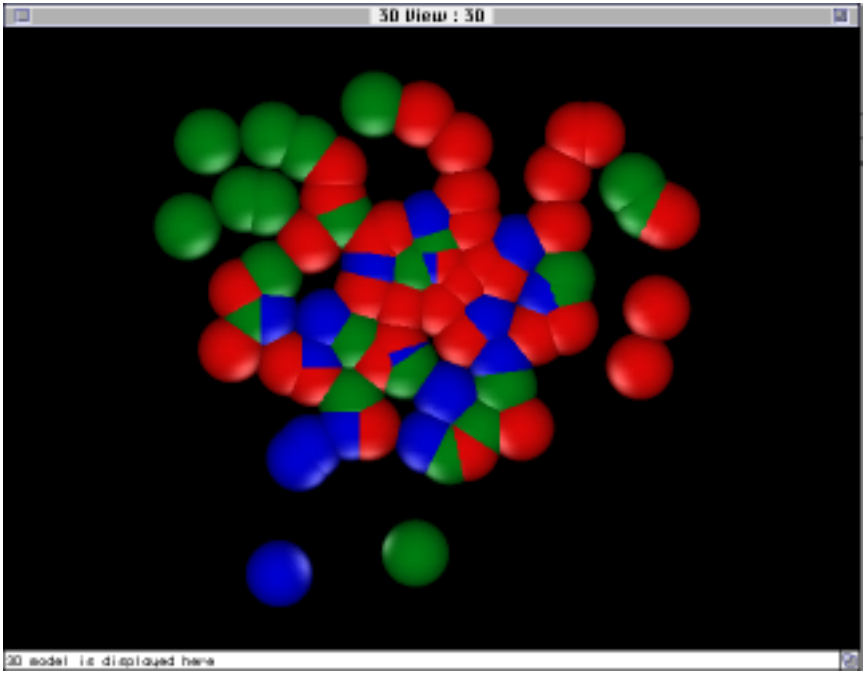
The *initial view of the 3D model* is centered around the data bounds or data bounding box and scaled appropriately to show all the data without distortion. The coordinate axes are oriented in the same way as in the mapping windows: positive X to the right, positive Y to the bottom and positive Z away from the user. The origin of this coordinate system is equal to the center of the data bounds.



The 3D mapping window is where a 3D model generated from the data in a 2D mapping window is displayed.

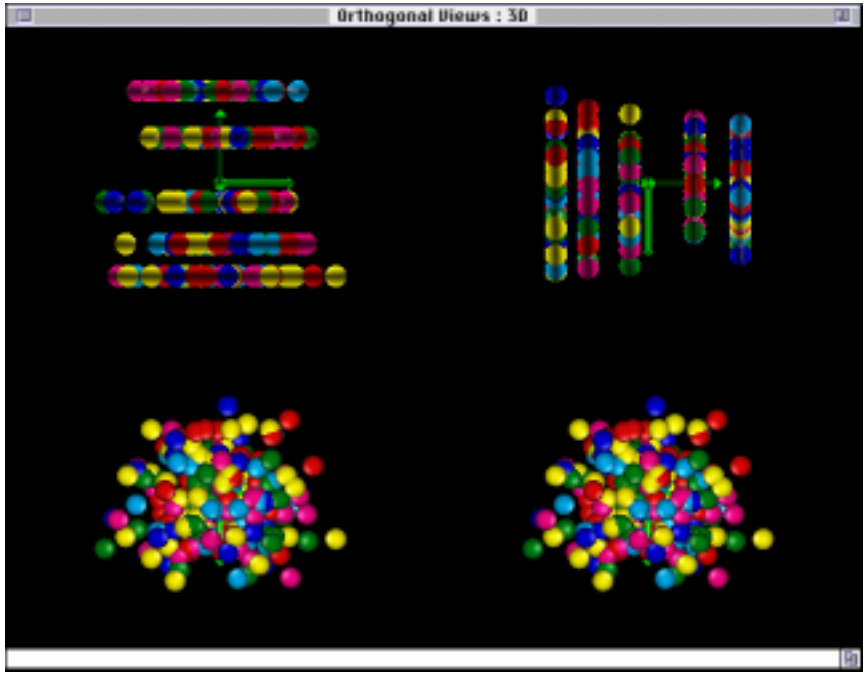
Opened by:

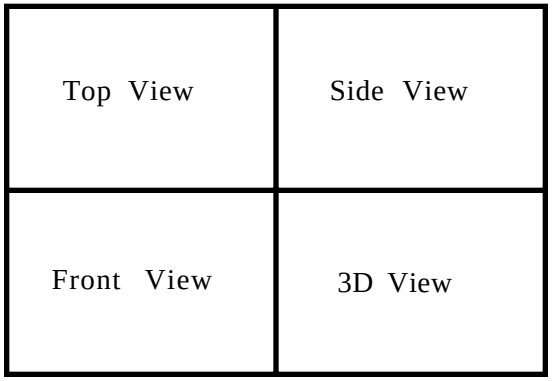
- 3D button in the View palette
- 3D View of the 3D submenu of the Modules menu
- 3D View of the 3D menu



The display of the model is controlled by various 3D tools. See the section on ***3D Tools Window*** for more information.

Orthogonal Window





The **Top**, **Side** and **Front** Views show the orthographic projection of the 3D model into the plane perpendicular to the direction of the y, x and z axis respectively. The **3D View** shows either a one point perspective projection or an orthographic parallel projection of the 3D model onto the front plane (parallel to the z direction) that upon initialization will look exactly like the Front View if the type of camera selected is Orthographic. Note that the 3D View will change as the 3D Tools are used to move through the 3D model.

The 3D mapping window is where a 3D model generated from the data in a 2D mapping window is displayed.

Opened by:

- Orthogonal Views of the 3D submenu of the Modules menu
- Orthogonal Views of the 3D menu

The display of the model is controlled by various 3D tools. See the section on ***3D Tools Window*** for more information.

3D Tools Window

The **3D Tools** window is used to change the way a model displays.

Opened by:

- 3D Tools Window in Views menu

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.

The control buttons on this palette act as a push buttons. The currently selected tool is the one with its button shown as pressed. The **Picking Tool** is currently selected in the following figure. To select another tool, click once on that tool to make it active

Picking Tool

Zoom Tool

Rotate Tool

Translate Tool

Target Tool

The details of each tool follows.

Picking Tool

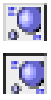
Press this button to toggle the **Picking** tool. This tool is used to select 3D objects in the 3D window. When the **Picking** tool is the current tool, the cursor is a blue 3D arrow

.

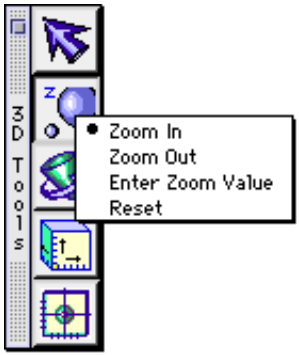
Moving the cursor over objects changes the color of the cursor from blue to red when directly over an object. The **3D Window Info** window shows information about the object in the **Current 3D Location**, **Data** and **Structure** fields. The **Current 3D Location** shows the real coordinates of the cursor in microns. The contents of this field are only valid when the cursor is on an object.

Pressing the mouse button when the cursor is on an object selects the object. The object will change to the highlighted color. Information about the selected object will be displayed on a text window when the option **Object Info** is selected from the **3D** menu.

Zoom Tool



Press this button to toggle the **Zoom** tool. This tool is used to zoom the 3D model in and out. This tool has a popup menu associated with it. Holding down the mouse button on the tool button displays the menu.



When the **Zoom** tool is the current tool, the cursor is the 3D magnifying glass with a plus or minus sign on it, depending on the current zoom mode.

Clicking the cursor anywhere in the 3D window zooms in or zooms out the entire model by 100%, with the location of the cursor as the anchor point. The **3D Window Info** window updates to show the current zoom factor in the Magnification field. A zoom factor can also be entered manually by selecting the menu item **Enter Zoom Value** from the tool popup menu.

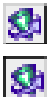
Option clicking the mouse performs the opposite effect. If the current mode is Zoom In, option clicking will zoom out. If the current mode if Zoom Out, option clicking will zoom in.

The current zoom mode can be changed by selecting the mode **Zoom In** or **Zoom Out** from the tool popup menu. The cursor always shows the current zoom mode.

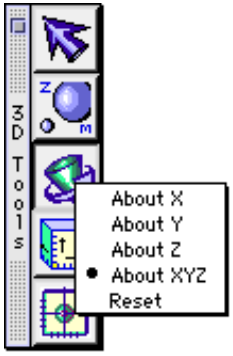
Selecting the menu item **Reset** from the tool popup menu sets the zoom factor back to 1.

By The Way: Zooming doesn't change the orthographic views of the model. This views remain unchanged because they serve as a reference when navigating through the model.

Rotation Tool



Press this button to toggle the **Rotation** tool. This tool is used to rotate both the 3D model and the point of view around the XYZ axes. This tool has a popup menu associated with it. Holding down the mouse button on the tool button displays the menu.



When the **Rotation** tool is the current tool, the cursor is a 3D blue curved arrow.

To use this tool, hold down the mouse button while dragging in the 3D window. The model rotates with the cursor. The value of the current axis of rotation is displayed in the Axis of Rotation field of the **3D Window Info** window. If the axes are visible, the current axis of rotation is displayed in the highlighted color. The specific rotation axis is selected from the tool popup menu. Modifier keys change the current selection of the axis of rotation:

<control key> - rotates about the X axis

<option key> - rotates about the Y axis

<command key> - rotates about the Z axis

The rotation angle about the Y and Z axis is proportional to the mouse movement in the horizontal direction. The rotation angle about the X axis is proportional to the mouse movement in the vertical direction.

Selecting the menu item **Reset** from the tool popup menu resets all the angles back to their initial values such that the model appears unrotated. It also resets the center of rotation back to the center of the model. Changing the center of rotation is discussed in the **Anchor** tool.

There are two modes when rotating a model. Both are set from the **Views** menu.

16. Rotate Relative to Model
17. Rotate Relative to Camera

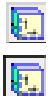
If the menu item **Rotate Relative to Model** is checked, rotating the model is equivalent to rotating an imaginary camera about the model. The scene represents the image that is viewed through that camera. This imaginary camera is also equivalent to an observer looking at the model from a certain distance. The axes and the model appears rotated.

If the menu item **Rotate Relative to Camera** is checked, rotating the model is equivalent to spinning the model about the axes in the camera coordinate system. The camera remains fixed while the model is rotated.

If the menu item **Auto Rotate** is checked, the model rotates as long as the mouse button is depressed. The speed and direction of the rotation is proportional to the mouse distance and direction from the location where the button was initial depressed.

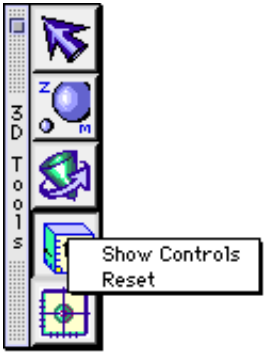
By The Way: The Rotation tool and other navigational tools affect only the way the 3D model is displayed on the screen. It doesn't affect the real coordinates of the data.

Translation Tool



Press this button to toggle the **Translation** tool. This tool is used to translate the model in the horizontal and vertical directions.

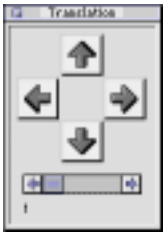
This tool has a popup menu associated with it. Holding down the mouse button on the tool button displays the menu.



When the **Translation** tool is the current tool, the cursor is a hand (grab) cursor.

To use this tool, press and hold the mouse button in the 3D window. A small square will appear in the location where the button was depressed. While the mouse button is still down, move the mouse around in the horizontal and vertical directions. A rubber band line from the initial location to the current location is drawn as a feedback. Release the mouse button to translate the model to the new location.

Select menu item **Show Controls** from the **Translation** tool popup menu to open the **Translation** window. This window controls the model movements via directional buttons.

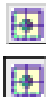


See ***NeuroZoom Reference*** on **Translation** window for more information on how to use this window.

To translate the model to its original position, select the menu item **Reset** from the tool popup menu. This resets the model back to the center of the 3D window. The magnification factor is reset back to 1 to ensure the visibility of the model. Rotation angles are not changed.

> **By The Way:** The Translation tool and other navigational tools, only affects the way the 3D model is displayed on the screen. It doesn't affect the real coordinates of the data.

Anchor Tool



Press this button to toggle the **Anchor** tool. This tool is used to change the center of rotation. The center of rotation acts as an anchor for the Rotation tool. Only a specific object in the model can be selected as a new anchor. When the **Anchor** tool is the current tool, the cursor is a green 3D crosshair cursor.

Moving the cursor over objects changes the color of the cursor from green to red when directly over an object. The **3D Window Info** window shows the object in the **Current 3D Location**, **Data**, and **Structure** fields. The **Current 3D Location** shows the real coordinates of the cursor in microns. The contents of this field are only valid when the cursor is on an object.

Pressing the mouse button while on an object changes the center of rotation to the location on the object, and sets the new anchor. The real coordinates of the new center are shown in the **Center of Rotation** field of the **3D Window Info** window. Note that the anchor may be fine-tuned to any position on the object. Zoom up to higher magnification if needed.

If the axes are visible, they are drawn at the current anchor. The model will rotate around the new anchor. The anchor doesn't affect the origin of the coordinate system.

By The Way: The anchor tool is particularly helpful when viewing the model at a high magnification. In this case, if the **Rotation** tool is used and the center of rotation is out of sight, perhaps far away from the current location, the model will probably disappear at the slightest cursor movement. To avoid this, change the center of rotation to any object that is visible at the current magnification before rotating.

View Locations Window

Different views of the model are obtained after rotating, translating, or zooming the model. The parameters that define a view (rotation angle, magnification, translation, center of rotation, etc.) can be stored at any time. Restoring the parameters recreates the view.

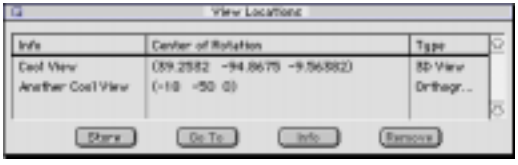
The **View Locations** window is used to store locations of the 3D model.

Opened by:

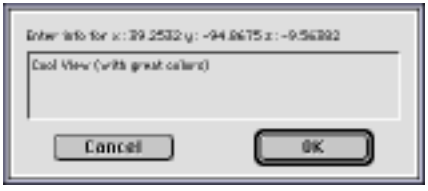
- View Locations Window in Views menu

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.

Select **View Locations** from the **Views** menu to open this window.



Pressing the **Store** button stores a view. A dialog opens asking for information about the view.



This information helps identify the view. The current value of the center of rotation is shown as a reference. Press the **OK** button to accept the view. The newly stored view is added to the scrollable list of the **View Locations** window. The column named **Type** indicates if the view was saved from a standard 3D view or an Orthogonal view.

To restore a view, select the view from the list and press the **Go To** button. The model will be rendered with the restored view parameters.

To remove a view, select the view from the list and press the **Remove** button. The view will be removed from the list. This has no effect on the currently displayed model.

To change the description of a view, select the view from the list and press the **Info** button. The same information window will open. Enter the new information and press **OK** to accept the changes, or **Cancel** to dismiss the window without changing the information.

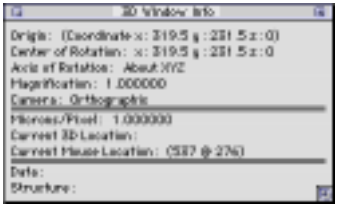
3D Window Info Window

The **3D Window Info** window shows the origin of the 3D coordinate system which is equal to the center of the data bounds, the current center of rotation which initially is equal to the origin, the current axis of rotation, the view magnification or zoom factor, the camera type, the current scale in use as microns/pixel, the current 3D location of the cursor in microns, the current mouse location in pixels and information of the current selected data and its structure.

Opened by:

- 3D Info Window in Views menu

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.



Lights Window

Toggles the **Lights** window. This window can be used to edit some of the attributes of the light sources in the light group that provide illumination for the objects in the 3D model. For 3D windows with four views (Orthogonal View), the light group associated with each view is the same. Changing the light sources with orthogonal views affects all the views displayed in that window.

Opened by:

- Lights Window in Views menu

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.

There are currently three predefined light sources in the light group associated with the current 3D window:

1. ambient light
2. point light
3. directional light

To edit these lights, use the **Lights** popup menu and select the light type that you want to edit. The attributes that can be edited for the selected light are displayed in the light pane area of the window. The definition of the lights is currently very limited. New lights can not be added to the light group and the location and direction of the predefined lights can not be changed either. This is not a QuickDraw™ 3D limitation.

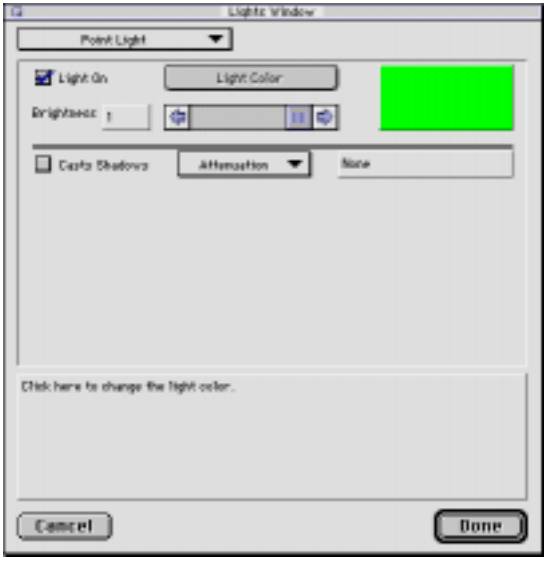
The **Lights** window has also a message area where information about the window fields is displayed. Moving the cursor across the fields of the window displays information about the field where the cursor is on in this message area.

Press the **Done** button to dismiss this window. Changes are applied in real time. The current 3D window is updated as changes are made. Press the **Cancel** button to undo the changes made to the last light object selected.

1. **Ambient Light -** An ambient light is a light that is applied equally everywhere in the scene, providing a flat, uniform light. All attributes of the predefined ambient light source can be edited and are shown in the light pane.

- **Light On** - Use this check button to toggle the ambient light on and off.
- **Light Color** - Click on this button to select the ambient light color. The current ambient light color is shown in the field located to the right.
- **Brightness** - This field displays the current value of the ambient light intensity. Use the scroll bar right next to it to change the light intensity.

2. **Point Light** - There is only one predefined point light source in the light group associated with the current 3D window. A point light is a light source that emits rays of light in all directions from a specific location. The point light is placed in the center of the 3D model and away from it in the z direction. The location of the point light can not be currently edited and new point light sources can not be added to light group.



The illumination that a point light contributes to a surface depends on the light intensity and color together with the orientation of the surfaces and its distance from the light source. A point light can suffer attenuation, in which objects closer to the light source receive more illumination than objects farther away.

All the attributes of the predefined point light source that can be edited are shown in the light pane.

- Light On - Use this check button to toggle the point light on and off.
- Light Color - Click on this button to select the point light color. The current point light color is shown in the field located to the right.
- Brightness - This field displays the current value of the ambient light intensity. Use the scroll bar right next to it to change the light intensity.
- Cast Shadows - Use this check button to toggle the cast shadows attribute of the light on and off.

- Attenuation - Use this popup menu to select the attenuation type for the point light. The current value of the atenuation type is displayed on the field right next to the popup menu. The attenuation type determines how quickly the intensity of a light changes as a function of the distance of the illuminated object from the light source.
- None - The intensity of the light is not affected by the distance from the illuminated object.
- Inverse Distance - The intensity of the light is inversely proportional to the distance from the illuminated object.
- Inverse Distance Squared - The intensity of the light is inversely proportional to the square of the distance from the illuminated object.

3. **Directional Light** - There is only one predefined directional light source in the light group associated with the current 3D window. A directional light is a light source that emits infinite parallel rays of light in a specific direction. The vector that specifies the direction of the predefined directional light can not be currently edited and new directional light sources can not be added to light group. The direction of the predefined directional light is equal to the z direction.

All the attributes of the predefined directional light source that can be edited are shown in the light pane.

- Light On - Use this check button to toggle the directional light on and off.
- Light Color - Click on this button to select the directional light color. The current directional light color is shown in the field located to the right.
- Brightness - This field displays the current value of the directional light intensity. Use the scroll bar right next to it to change the light intensity.
- The Cast Shadows check button is available for this light object.

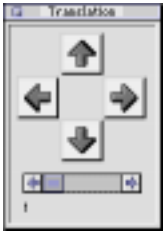
Translation Window

Select menu item **Show Controls** from the **Translation** tool popup menu to open the **Translation** window. This window controls the model movements via directional buttons.

Opened by:

- Show Controls from the Translation tool popup menu in the 3D Tools menu

The position of this window remains fixed to its last location for all subsequent openings until NeuroZoom exits. This makes it easy to place the window to a new location.



Press once or press and hold on one of the arrow keys to move the model in the direction of the arrow. Use the scrollbar to increase or decrease the amount of movement per button press. This amount is in pixels.

Note that the controls in the **Translation** window can be used at anytime even when the Translation tool is not the current tool.

Index