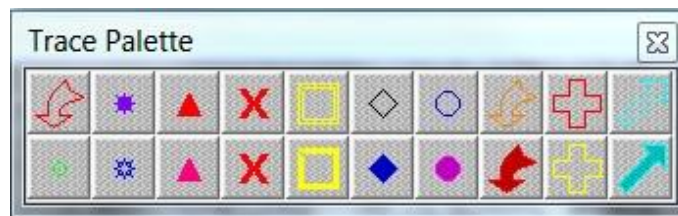


# Reconstruct Basics

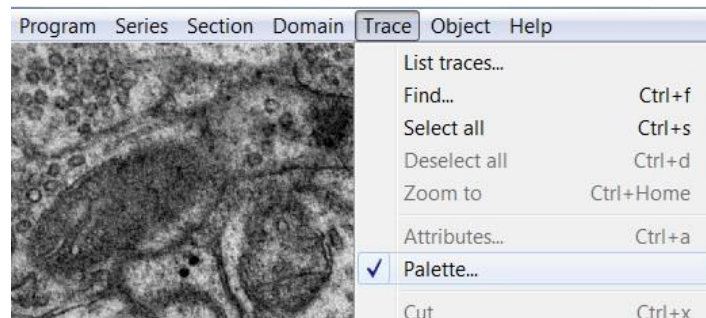
Patrick Parker  
Aug. 2017

## Trace Palette

From the trace palette, you can select the color, name, and shape of the trace you want to make. Here's the Trace Palette:



If the Trace Palette is not already open, in the top left drop-down menu, select **Trace > Palette...** and make sure it's checked:

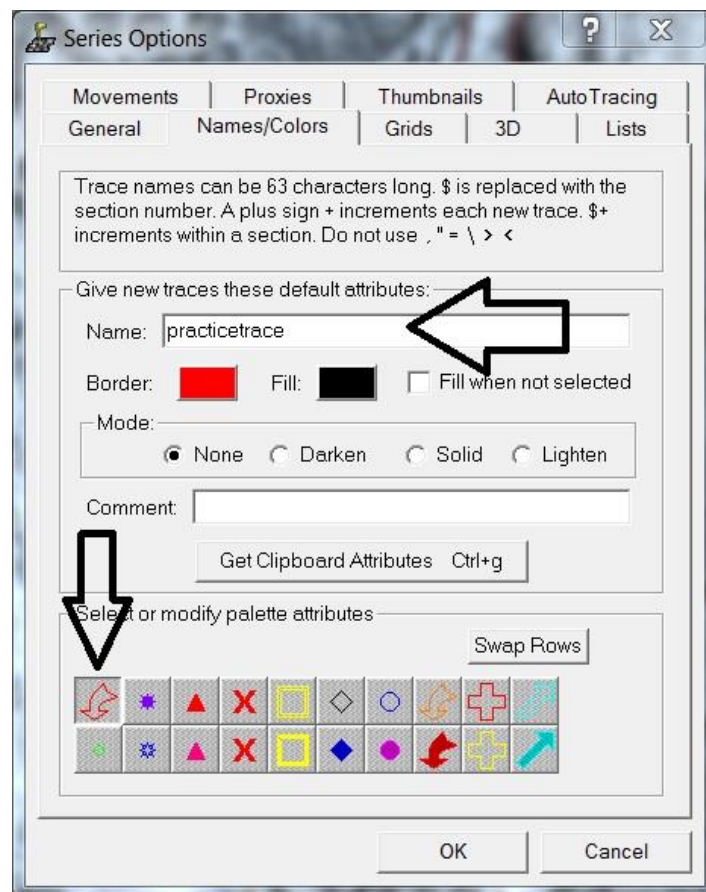


For each one of these squares, you can change the trace name, color, and shape. So you can set up your palette in advance and click on what palette square you need for each type of trace you are making.

### Choose the name and color for your palette square:

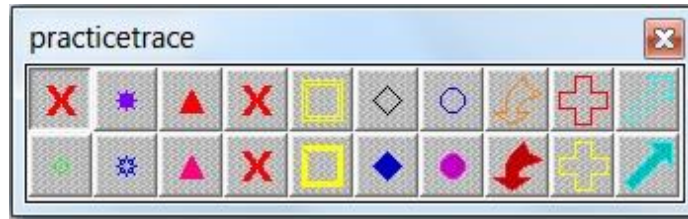
- In the top left drop-down menu, open the Series Option window: go to Series > Options... (or just hit Ctrl-o).
- In the Series Option window, go to the Names/Colors tab.
- Under "Select or modify palette attributes" at the bottom, click on a palette square. Selecting that palette square will allow you to name it or change its attributes. (Note: If you don't click on a specific square first, your changes will not be saved.)
- Choose a name: Under "Give new traces these default attributes", next to "Name", type in a name for your trace.

- Choose a color: Click the colored square next to “Border” (i.e. the border outline color of your trace) > pick your favorite color > OK. Mode should be “None” if you don’t want your trace filled with color.
  - Follow this step only if you want your trace to be filled with color:
    - a) Click the colored square next to “Fill” (i.e. the fill color of your trace) > pick your favorite color > OK.
    - b) Under “Mode”, select “Darken,” “Solid,” or “Lighten” depending on how opaque you want your color fill.
    - c) Check “Fill when not selected” if you want the trace filled with color when it is not selected. If left unchecked, the default will be that the trace is filled when it is selected. (Select traces with the Select Traces cursor tool. See: Tools Window.)



### Choose shapes in your palette:

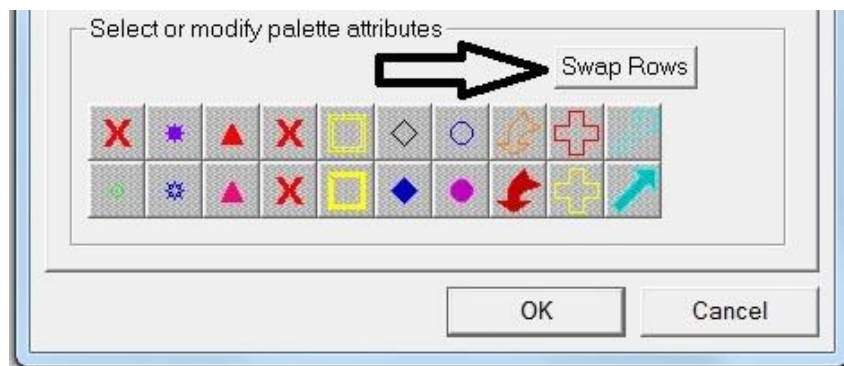
- Let’s say you want to change your first palette square from an arrow to an X.
- Exit out of Series Options. Click on the red X (or whatever shape you want) in the Trace Palette. Click on the Stamp Shape tool in the Tools Window.
- Stamp the red X onto your image. Now hit Control-0 (zero) on your keyboard.
- Your first palette square will now be a red X:



- There are 10 squares per row in the Trace Palette. The squares in the top row are indicated numerically in order from left to right by 0, 1, 2, 3, 4, 5, 6, 7, 8, 9.
- Placing the original X stamp indicated what new shape you wanted for your palette square. Hitting Control-0 (zero) indicated that you wanted to change the palette square in position zero – that is, the first palette square. Or you could hit Control-1 to change the second square, Control-2 to change the third square, etc.

Recap: Just make a stamp of the new shape you want and hit Control-# to indicate which palette square you want to change. (Note: Make sure you keep at least one of each shape that you need. If you run out of a shape, you can import a Trace Palette from another series.)

If you want to change palette squares on the bottom row: Go to Series Options (control-o) > Swap Rows. Then you can change the shapes of the top row as described above.












## Tools Window

In the Tools Window, you can select a variety of tools for tracing, viewing, and measuring. Here's the Tools Window:



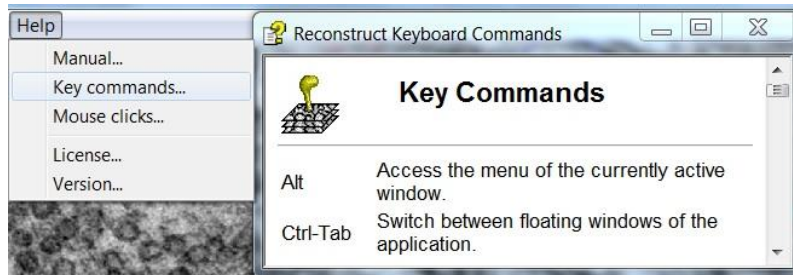
When opening Reconstruct, the Tools Window should open automatically. (If you can't see your Tools Window, go to the top left menu option Program > Windows > check Tools Window.) Here are the tools a beginner should be familiar with:

Icon	Name	What it does and how to use it
	Select Traces	This cursor tool allows you to select traces. Select: left-click on or nearby the trace. De-select: right-click on or nearby the trace. Ctrl-S: Select all traces. Ctrl-D: De-select all traces.
	Pan and Zoom Section	This allows you to move the image and zoom. Hold down the left button and move the mouse around to move the image left, right, up, and down. Hold down the right button and move the mouse toward or away from you to zoom the image in and out.
	Draw Point by Point	Tracing tool. Left-click to begin the trace, left-click to add each additional point, and right-click to end the trace. This tool will not automatically connect the ends of the trace – this tool makes an open trace.
	Draw Freehand	Tracing tool. Hold down the left mouse-button and carefully move your mouse around to trace the object. Release the left mouse-button when you are done with the trace. This tool will automatically connect the ends of the trace – this tool makes a closed trace.
	Draw Closed Point by Point	Tracing tool. Left-click to begin the trace, left-click to add each additional point, and right-click to end the trace. This tool will automatically connect the ends of the trace – this tool makes a closed trace.
	Stamp Shape	This will stamp a shape trace. Select the desired shape on the Trace Palette and left-click to place a stamp.
	Draw Line	This will draw a straight line. Left-click to begin the line and left-click to end it. It can also be used to measure length – left-click to draw a line without ending it, and the length is listed in the bottom left of the main screen in the Status Bar, next to “Length”.
	Clip, Backspace and Retrace	Scissor tool. This will allow you to correct tracing mistakes. Left-click on the desired spot on the erroneous trace; hit backspace repeatedly to un-do the trace (counter-clockwise). After deleting the erroneous area, the tool becomes the Draw Point by Point tool, so you can add more to correct the trace. Right-click to end the trace.
	Slice Trace into Pieces	Scalpel. This allows you to cut a trace into pieces. This tool is temperamental and difficult to use accurately. For best results, cut at a ninety-degree angle, perpendicular to the trace you are cutting. Use at your own risk.

Other tools will be covered as needed later in this manual.

# How to use the Keyboard

The keyboard commands and hotkeys are all listed in Reconstruct. In the top menu bar, go to Help > Key commands:



## Some of the most important key commands:

**Delete:** Delete selected traces or selected domain.

**Page Up:** Make next higher section in series the displayed section.

**Page Down:** Make preceding lower section the displayed section.

**Spacebar:** Blend the current section with the last section viewed. Useful when comparing two sections.

**/ :** Switch back & forth between the current section and last section you were looking at. Hold to flicker. Useful when comparing two sections or trying to think of the sections in 3D.

**Ctrl-A:** Open the trace attributes dialog if a trace is selected.

**Ctrl-B:** Paste attributes of clipboard traces onto selected traces (i.e., select a trace, hit Ctrl-C to copy its attributes, de-select, then select a second trace, hit Ctrl-B to paste the attributes of the first trace onto the second. That will change its name, color, etc).

**Ctrl-C:** Copy the selected traces.

**Ctrl-D:** Deselect all traces on section.

**Ctrl-F:** Find trace in series. (Or, go to Object List > select desired trace > Find First.)

**Ctrl-H:** Hide the selected traces. (Use Trace List to Unhide them.)

**Ctrl-M:** Merge the selected traces. (This will combine two traces into one, as long as they have some overlap.)

**Ctrl-O:** Open the Series Options dialog.

**Ctrl-S:** Select all traces on section.

**Ctrl-V:** Paste traces into section.

**Ctrl-X:** Cut selected traces from section for later pasting.

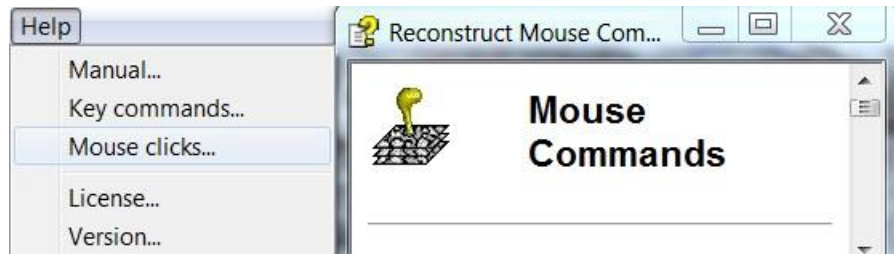
**Ctrl-Z:** Undo.

**Backspace:** Delete last trace point entered when drawing.

**Ctrl-0,...,Ctrl-9:** Set current default attributes (and stamp profile) onto key. (See: Trace Palette - Choose shapes in your palette.)

## How to use the Mouse

The mouse commands and hotkeys are all listed in Reconstruct. In the top menu bar, go to Help > Mouse clicks:

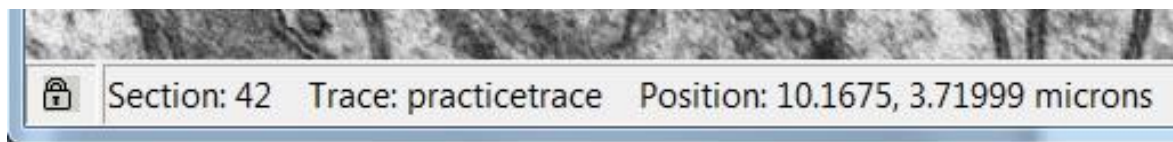


For the most part, these are intuitive – left-click to use tool, right-click to stop using it – or they are covered in the Tools Window chart above.

In the Tools Window, make sure you are familiar with Pan and Zoom Section, which allows you to move the image and zoom. After selecting the Pan and Zoom tool, hold down the left button and move the mouse around to move the image left, right, up, and down. Hold down the right button and move the mouse toward or away from you to zoom the image in and out.

## Status Bar

The bottom left of the main screen is known as the Status Bar:



The Status Bar indicates, from left to right:

- Lock icon: whether a section is locked or unlocked
- Section: current section number, i.e. the section you are looking at
- Trace: name of the trace closest to the cursor
- Position: the x-y coordinates position of the cursor
- Length: when using the Draw Line tool, the length of the line will be listed (see: Tools Window, above).

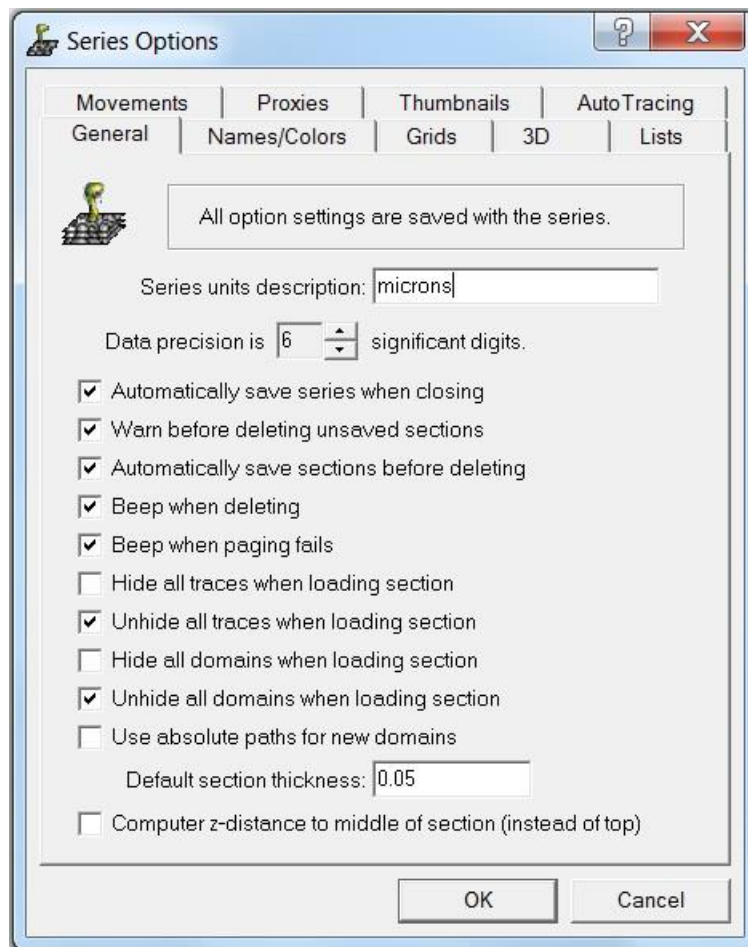
## Series Options

We've already looked at Series Options > Names/Colors, but here we'll go into more detail on other useful tabs.

You can open it by going to Series > Options..., but the quickest way is the hotkey **Control-o**.

### Series Options > General

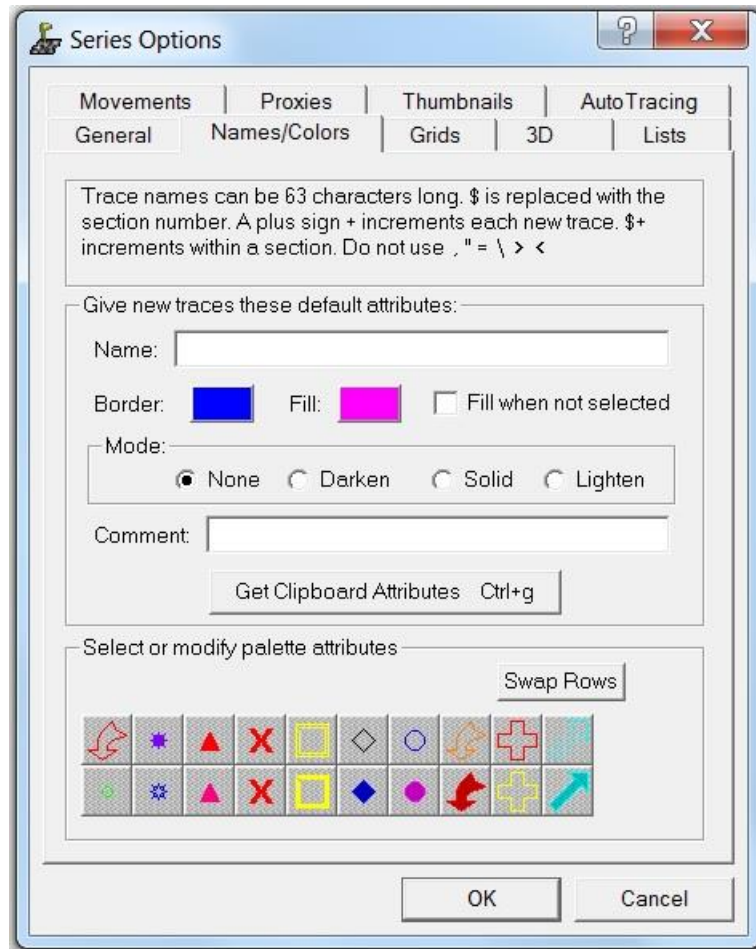
Hit Control-o then choose the tab General:



There are a lot of general options here. Read through all of them so you can see what options you have. Generally, you will want the options checked above.

## Series Options > Names/Colors

Hit Control-o then choose the tab Names/Colors:



Here you can change the names and colors of your Trace Palette squares (see: Trace Palette, above).

Under “Select or modify palette attributes” at the bottom, click on a palette square that you want to change. Selecting that palette square will allow you to name it or change its attributes. (Note: If you don’t click on a specific square first, your changes will not be saved.)

By “Name” you can change the name. By clicking on the color square next to “Border” and “Fill”, you can select the border color and fill color for your trace or stamp.

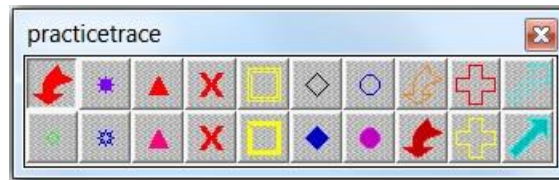
If the trace or stamp has a color fill, the default is that it will be filled only when you select it, but you can check “Fill when not selected” if you want it filled when not selected.



By “Mode” you can choose whether you want your trace or stamp filled with color:

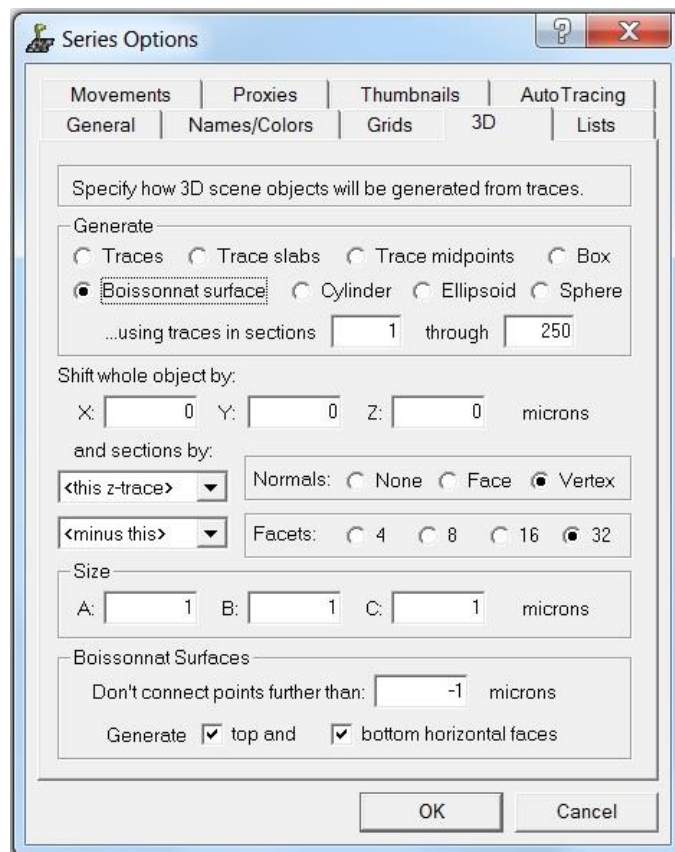
- None: no color fill
- Darken: a dark color fill, but not opaque
- Solid: a solid color fill, completely opaque
- Lighten: a light color fill, partially transparent

The icon for that trace in the Trace Palette will indicate whether it is filled or not and what name and color you have chosen. Here we see that “practicetrace” (the first palette square) has a red border, red fill, with the Solid fill chosen:



## Series Options > 3D

Hit Control-o then choose the tab 3D:



This will allow you to choose among a variety of options regarding how your image is displayed in 3D. To make a 3D reconstruction, go to the Object > List Objects and double-click on your trace name. (See: Reconstruct: Intro for Beginners - How to Make a 3D Reconstruction.)

For most 3D reconstructions, you will want the options as they are pictured above:

- **Generate:** Boissonnat – this will create a smooth surface over your image. (Named after French computer scientist Jean-Daniel Boissonnat.)
- **Normals:** Vertex.
- **Don't connect points further than:** -1. When the value is negative or zero no limits are applied. This is usually recommended, unless there are traces being erroneously connected, in which case you could choose a small positive value.
- **Generate top and bottom horizontal faces:** Check both.

The [Reconstruct User Manual](#) shows examples of 3D representations of one object with different options selected:

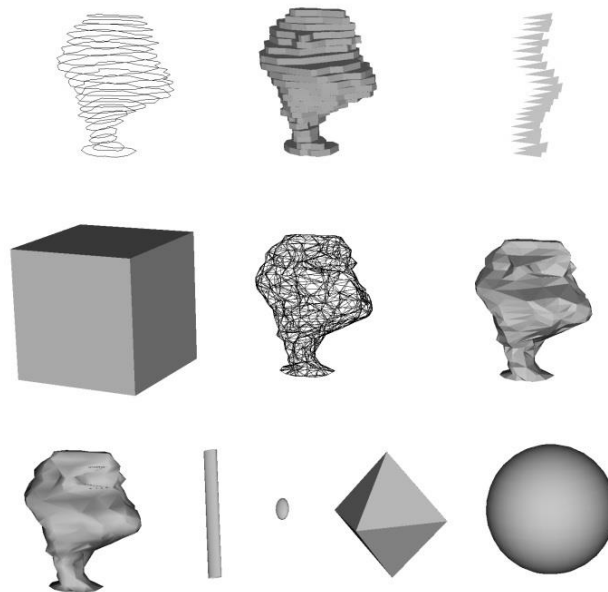
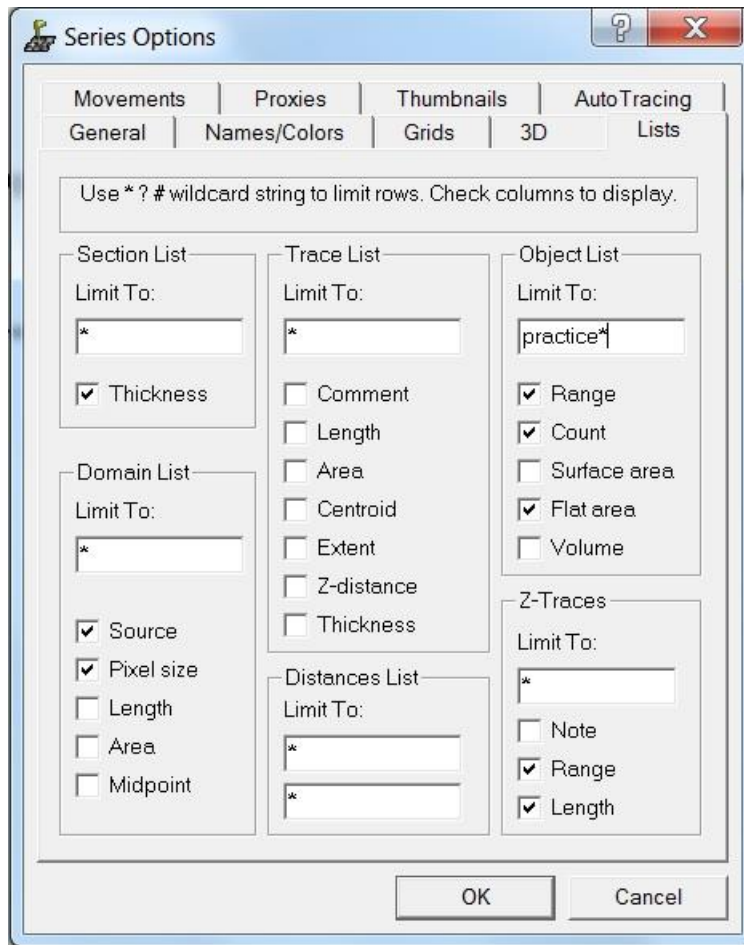


Figure 11.3 Examples of 3D representations of one object. The object is a dendritic spine about 0.5 microns tall, reconstructed from serial section electron microscopy. All images were saved from the 3D Scene at the same magnification and viewing angle. A. Traces. B. Trace Slabs. C. Trace Midpoints with a Size A and B set to 0.25 microns. D. Box (Size: A=1,B=1,C=1). E. Boissonnat surface drawn with front and back faces in wireframe mode instead of filled. F. Boissonnat surface with face normals. G. Boissonnat surface with vertex normals. H. Cylinder with the length (Size A) set to 0.5 microns. I. Ellipsoid (Size: A=-1, B=-1, C=-1). J. Sphere with a faceting density of 4 and face normals. K. Sphere with a faceting density of 32 and vertex normals.

The smooth reconstruction of the bottom left image in Fig 11.3 above – Boissonnat surface with vertex normals – is what you will want in most cases. (However, when reconstructing vesicles, you will want to select Generate: Sphere.)

## Series Options > Lists

Hit Control-o then choose the tab Lists:



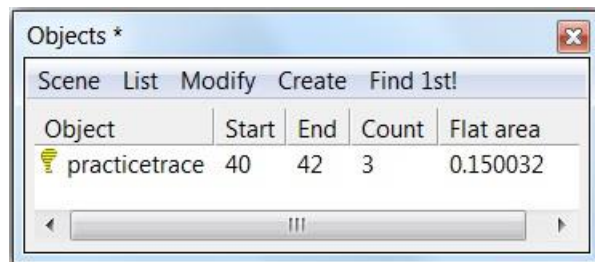
Here you can select which traces and columns are displayed in all the various List windows. You will most commonly use the Object List (Object > List objects...).

Under “Limit To”, you can limit which objects are listed. Here I have limited it to “practice\*”. The asterisk (\*) is a wildcard, so that all traces that begin with “practice” will be listed.

There are various columns of data that can be listed in the Objects List:

- Range: This lists the first section (Start) and the last (End) section on which a trace is found.
- Count: This lists the number of traces made with that name.
- Surface Area: This is the area of a surface, e.g. if you want to know the area of the membrane surface of a spine.
- Flat Area: This is the area calculation you will use to determine the area of a synapse. (Do not confuse this with Surface Area! In Reconstruct, the Surface Area of a synapse is meaningless.)
- Volume: This is the volume of the object.

Here is the Object List with columns for Range, Count, and Flat Area:



Object	Start	End	Count	Flat area
practicetrace	40	42	3	0.150032

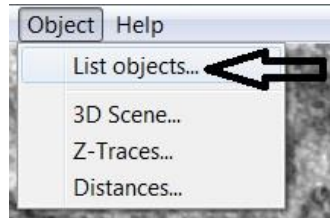
As mentioned above, the asterisk (\*) is a wildcard. For example, if you want to see all the traces in the Objects List that are associated with dendrite “d01”, list “d01\*” under “Limit To” – that will list all of the objects that begin with the prefix “d01” (e.g. d01ax01, d01ax02, d01p01, d01p02, etc.).

Other wildcards are the question mark (?) and pound (#). The ? takes the place of any individual character, letter, or number, and the # takes the place of any individual number.

For example, if you want to see all of the d traces, list “d##” under “Limit To” – that will list all of the objects that begin with “d” followed by two numbers: d01, d02, d03, etc. Or if you list “?01” under “Limit To,” that will list all of the objects that start with any character, letter, or number followed by 01: a01, b01, c01, etc.

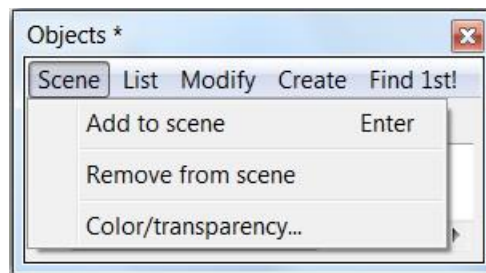
## Objects List

Now that we've seen how to limit the traces and columns in the Series Options, let's look at the Objects List. To open the Objects List, go to Object > List objects...



Here are the various dropdown menus in the Objects List and what you can do in each one:

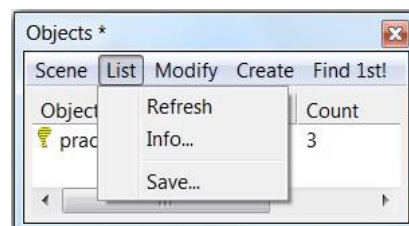
### Objects List > Scene



This menu affects the 3D scene, i.e. the 3D reconstruction. First, select an object from the Objects List.

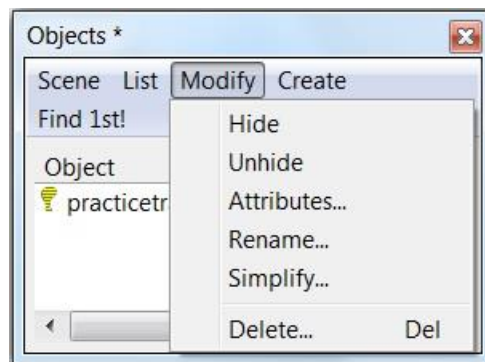
- Add to scene: creates a 3D reconstruction of the object.
- Remove from scene: removes the 3D reconstruction of an object that was already reconstructed in the scene.
- Color/transparency: allows you to change the color and transparency of the object in the 3D scene.

### Objects List > List



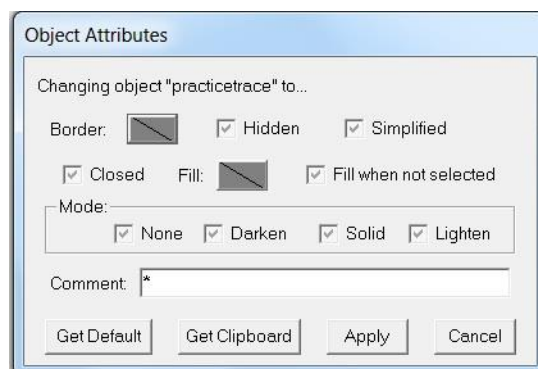
- Refresh: refreshes the Objects List, if you have made any changes to it (e.g. changing which traces and columns are listed in the Series Options > Lists, above).
- Info: lists number of items and which are highlighted. Not too useful in most cases.
- Save: allows you to save a .csv file of the Objects List, which can be opened in Excel. This is how you export data into Excel.

## Objects List > Modify



First, select an object from the Objects List to modify.

- Hide: hides the trace from the main window.
- Unhide: unhides the trace from the main window.
- Attributes: allows you to change the color, name, and other attributes of a trace. You can also select the trace in the main window and hit Control-A to bring up the attributes.



- Rename: allows you to rename the object.
- Simplify: not necessary for beginners. See more in the Reconstruct User Manual.
- Delete: deletes the object. You can also highlight the object in the Objects List and hit the Delete key.

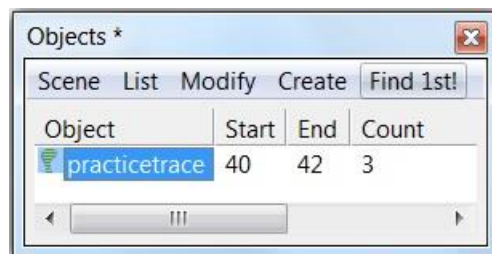
## Objects List > Create

You will pretty much just use this to create a copy of a trace. The other options are not commonly used.

## Objects List > Find 1st!

Find 1st! helps you find a particular trace in the main window.

Say you're a hundred sections away and you want to quickly find "practicetrace". Just select "practicetrace" in the Objects List and hit "Find 1st!", and it will show you "practicetrace" on the first section it appears.



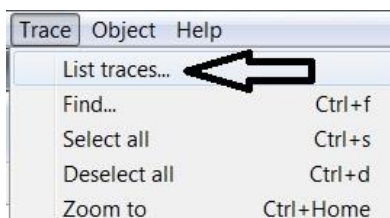
## Traces, Z-Traces, and Sections List

You can open several other lists and select which traces or columns to view in Series Options > Lists.

The two other lists you will probably use the most are the Traces List and the Z-traces List.

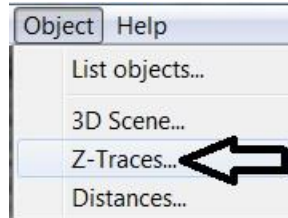
### Traces List

Go to Trace > List traces. This list allows you to view all of the traces on the current section you're on. If you scroll to another section, the list will repopulate with the traces for the current section.



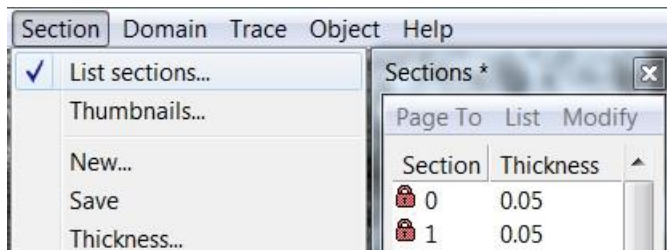
## Z-Traces List

Go to Object > Z-Traces. This list allows you to view all of the z-traces. Z-traces are traces through the z-axis, across numerous sections, generally made to determine the length of an object that spans across many sections. (See more on how to make Z-traces later in this manual.)



## Sections List

Go to Section > List sections... to open the Sections List.



In the Sections List either double-click on a section or highlight the section and hit “Page To” in order to view that section. This is a whole lot faster than scrolling through 200 sections.

The Sections List also lists the section thickness for each section.



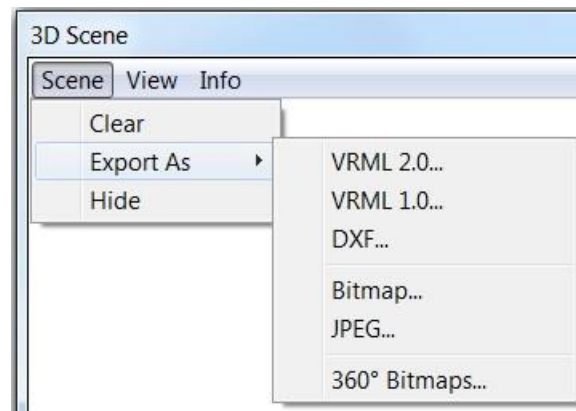
## 3D Scene

In Objects List > Scene (described above), you can add or remove objects from the 3D Scene and change their color and transparency. You can also add an object to the 3D Scene by double-clicking on the object's name in the Objects List.

Zoom in and out by holding the right mouse button and moving the mouse toward or away from you. You can move or spin objects by holding the left button and moving the mouse. (Note: Center point of rotation will always be the first object generated in the scene, so plan ahead.)

Here are the drop-down menu options:

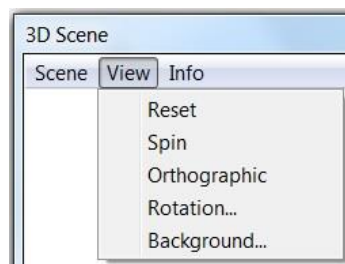
### 3D Scene > Scene



There are three dropdown options:

- Clear: clears all objects from the 3D Scene. Start over from scratch.
- Export As: allows you to export the 3D Scene as a variety of file types.
- Hide: removes the 3D Scene window.

### 3D Scene > View



- Reset: resets all of the below options to the default.
- Spin: the object will slowly spin.
- Orthographic: the scene can only be magnified by enlarging the window, not by zooming.
- Rotation: changes the degree of Spin.
- Background: allows you to choose the background color.

### 3D Scene > Info

This menu provides info about the number of objects, etc.

Here's a 3D reconstruction of a rat brain cell (dendrite in yellow, synapses in red):

