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This chapter gives the steps for reconstructing serial sections. You will learn the basics of using Reconstruct to import a series of images, view and align the sections, trace profiles, and generate a 3D view of the traces.

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### *Getting Started*

The only requirements for getting started are that the Reconstruct application is installed and that a set of image files is available for input. To install the software download the latest version from <http://synapses.bu.edu/> or from the Users Group [http://groups.yahoo.com/group/reconstruct\\_users/files/](http://groups.yahoo.com/group/reconstruct_users/files/). Run the downloaded file and a dialog should appear asking you whether you want to install Reconstruct. Select Yes and proceed through the installation dialogs until the process is complete.

To test your installation, start Reconstruct and select *Open...* from the Series menu. If this is the first time you've installed and used Reconstruct, a series file called *example.ser* should be visible in the Open dialog. Open the example series. When

the series opens you should see some text on the screen. Follow these text instructions to get familiar with the basic operations of Reconstruct.

This chapter only covers a small fraction of Reconstruct functionality. To get a better idea of the full functionality, review the Key and Mouse commands listings available through the Help menu. Then open the *Options...* dialog from the Series menu and review the contents of all the Options tabs. This will give you an idea of the features of the program that can be reconfigured to suit your purpose. Details about these features can be found in subsequent chapters.

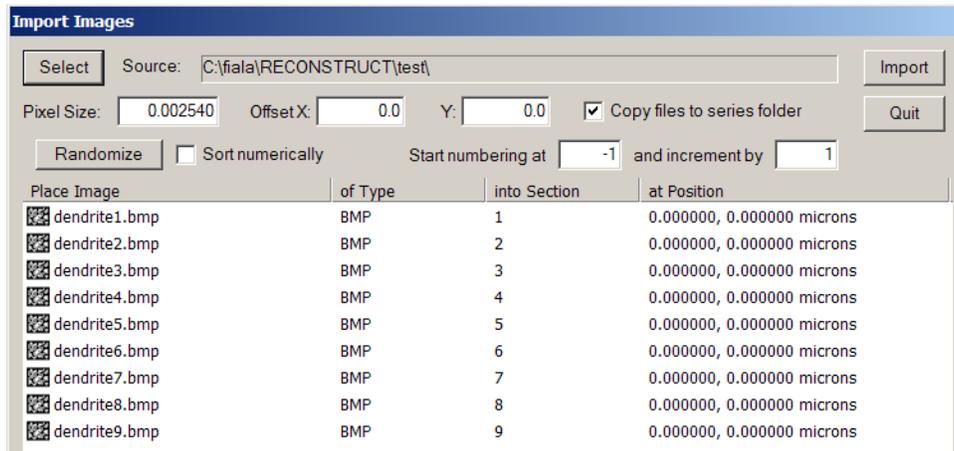
### CREATE A SERIES

Start the Reconstruct program and from the Series menu select the *New...* menu item. A file open dialog will appear. Navigate to a folder on the hard drive where the new series files can be written. If desired you may create a new folder from this dialog. The word “newSeries” should appear in the Filename box near the bottom of the dialog. This will be the name of the series files created to store the series data. Select the Save button in the New Series dialog to create the series.

### IMPORT SECTION IMAGES

Import section images into the series using the *Images...* item from the Import sub-menu of the Series menu. This will open a dialog for creating the section files from a set of image files (Fig. 1.1). Image files can be in a variety of formats including Windows Bitmaps, GIF, TIFF, and/or JPEG. Each image file should contain a single image (not an image stack) representing one section in a series, and should use a representation of 8 or 24 bits per pixel.

Choose image files using the Select button. This button will open a file selection dialog. In this dialog navigate to the folder containing the image files and highlight the image files using the mouse. Multiple files can be highlighted using the Ctrl or Shift keys. The filenames will appear in quotes in the Filename box when multiple files are selected. When all desired images have been selected, use the Open button to transport the filenames into the Import Images list.



**Figure 1.1.** The Import Images dialog box after images have been selected using the Select button. Since the file names contain the section index, automatic numbering is realized by having a -1 specified in the First Section box.

Figure 1.1 shows the Import Images dialog after some images have been selected. The source directory is given at the top of the dialog. The filenames of recognized image files and their formats are listed in the left-most columns of the list. The third column gives the section number to which each image will be imported. Set the first section number of the sequence by typing the number, typically 1, in the First Section box. If the sections are not numbered correctly and the filenames contain numbers indicating the correct order, you can use the Sort Numerically checkbox to reorder the files in the list. For finer control of section numbering you may have to import images in stages.

Create the section files by selecting the Import button in the dialog. As the image files are added to the series they will be deleted from the list. After the images have been imported you can repeat the process to add more images to your series. When all images have been added, use the Quit button to close the Import Images dialog.

To view the sections, open the *List sections...* from the Section Menu and double-click on one of the listed sections. This will move the display to that section in the series. Press the Home key. After a brief delay, the image of the first section will be centered in the main window. Use the Page Up and Page Down keys to advance



**Figure 1.2.** The Tools window. This window should be visible at the top of the Reconstruct main window. Use the left mouse button to select a tool. The Line Tool used for calibration lines is the 8th button. The Pencil Tool is the 10th button. The Wildfire tool button is depressed and so a description of the tool appears in the title bar.

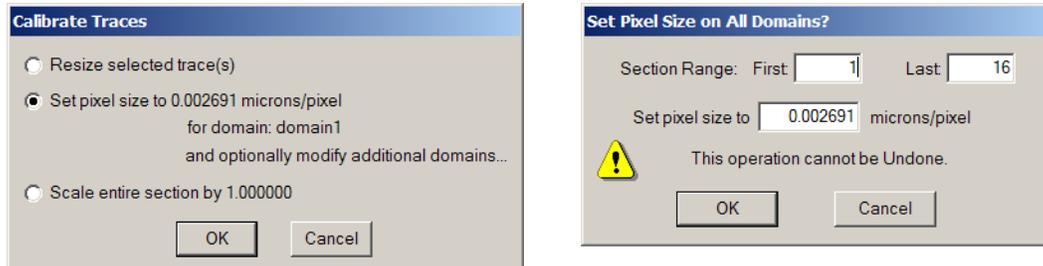
section by section through the series. Verify that the images were imported as expected with the correct section numbers. The number of each section appears in the title bar of the main window.

### CALIBRATE PIXEL SIZE

An important parameter during image import is the pixel size, which we ignored in the previous section. This parameter specifies the size of image picture elements (pixels) in terms of the units of measure of the series (Chapter 9). If the pixel size in the Import Images dialog was accurate, no further calibration is required. However in most cases the pixel size should be adjusted using the image of a calibration grid or scale that was photographed with the series. This is best done before any alignments or tracing, since changing the pixel size later can invalidate these adjustments.

The process of calibration in Reconstruct involves drawing traces of known length and then entering that length value into the program. Select the Line Tool (Fig. 1.2) from the Tools window and draw a line trace of known length on a section containing a calibration grid image. (For more details on calibration grids and lines see Chapter 9.)

After the calibration trace is drawn, select the *Calibrate...* item from the Trace menu. A dialog box will appear for each calibration trace displaying the current length of the trace. Enter the actual length of the trace in place of the current length.



**Figure 1.3.** The Calibrate dialogs as filled out for a global calibration of the pixel size of images. *Left:* The first dialog requests that a pixel size calibration be done for the image on which the calibration lines were traced. *Right:* The next dialog allows this same pixel size to be applied to every image in every section of the series.

When the lengths for all selected traces have been entered, a Calibrate Traces dialog will appear (Fig. 1.3) offering three options. Select the second option to set the correct pixel size for the current section. When the “Set Pixel Size on All Domains?” dialog appears, set the First Section to the first section of the series and the Last Section to the last section. Verify the value in the “Set pixel size to...” box and click the OK button. This will set the pixel size of all images in the series to the value determined from the calibration image. The pixel size for any image can be verified by using the Domain List while viewing the section in the main window.

Note that after calibration of pixel size, the calibration lines are no longer accurately matched to the calibration image on which they were drawn. This is to be expected. Since these calibration lines are no longer useful they can be deleted from the calibration section. An additional check on the calibration would be to redraw one or two calibration lines and verify that their length is now correct.

### ALIGN SECTIONS

Tissue that has been physically sectioned and then imaged must be realigned for accurate 3D measurements and visualizations. Section images can be manually moved into relative alignment using keyboard commands (Chapter 10), but it is usually much easier to align sections by specifying correspondence points and then using one of the Align commands from the Trace menu. This option is outlined in

the following steps. It will only work if appropriate landmarks are visible in adjacent sections. For best alignment results, read Chapter 10.

*Step 1.* Go to a section which will serve as the stationary reference. Then page to the adjacent section that will be aligned to the stationary one. Press the ‘/’ key to switch between the two sections.

*Step 2.* In the Trace menu, select *Palette...* to open the Trace Palette window. Click on the first palette button and pick up the Stamp Tool from the Tools window. (It is assumed that this is the default palette for a new series such that the first palette button is an orange circle with the default name “a\$+”.)

*Step 3.* Use the Stamp Tool to place traces at points to be aligned. Use the ‘/’ key to alternate between sections as points are entered. Enter the landmarks in the same order on both sections so that traces of corresponding points will be numbered the same. To delete a point if you make a mistake, use Ctrl-D to deselect all the points, pick up the Arrow Tool (1st tool button) and select the point to be deleted, then use the Delete key to delete it. Use Ctrl-S to reselect all the points to be aligned.

*Step 4.* After entering 3 or 4 correspondence points, switch to the section that will be moved. Verify in the title bar that the stationary section is not displayed.

*Step 5.* Choose *Linear* from the “Align section” submenu of the Trace menu. When the section is not unlocked this submenu will be titled “Align traces” instead of “Align section”. Enter Ctrl-L to unlock section movement. The Status bar will display an open lock image. If traces are accidentally aligned instead of the images, use Ctrl-Z to undo the trace movement. If the message “Alignment needs more traces” is displayed, make sure you have entered at least 3 points on each section and that all points are selected (Ctrl-S) on both sections. If this message still appears make sure the corresponding traces in each section have the same name. In other words, “a1” in section 1 must be matched to a trace “a1” in section 2, “a2” must match “a2”, and so on.

*Step 6.* After *Linear* has moved the section, use ‘/’ or blend (spacebar) to confirm that all parts of the section are aligned (Chapter 10). If the alignment is acceptable, lock (Ctrl-L) the section to prevent further movements.

*Step 7.* If the alignment is not acceptable, use Ctrl-Z to undo the movement. Check the names and positions of the traces entered and modify or add traces as needed. Go to Step 5. As a last resort, delete all traces by entering Ctrl-S followed by the Delete key. Do this for each of the two sections and then start over with Step 3.

*Step 8.* Once the alignment is acceptable you can delete the alignment traces from both sections. If you decide to keep the alignment traces for later editing, you will need to switch the color and name of future correspondence points to avoid confusion with the existing ones. Switching to a new trace name ensures that traces with the same numbering will be created on both sections. To switch correspondence point names, simply select the second button on the Trace Palette. Later, when you repeat this step after aligning the next section, go back to the first palette entry, alternating between trace names after each alignment.

*Step 9.* Page Up (or Down) to the next section to be aligned and go to Step 3.

### TRACE PROFILES

Information about sectioned objects is created by tracing the profiles of the objects on the sections. To do this, return to the first section in the series by using Page Down or by selecting the first section in the list generated from the *List sections...* item of the Section menu.

From the Series menu, select *Options....* This opens a dialog that will allow you to configure the default parameters for tracing. Select the Names/Colors options tab at the top the dialog. Put the cursor in the Name edit box and type a name for the object to be traced, such as “myObject”. This will be the name given to the profiles you draw. All profiles with the same name are considered part of the same object. Hit Enter or select the OK button to close the Options dialog.

The Tools window (Figure 1.2) should be visible at the top of the main window. Use the cursor to select the tool button that looks like a pencil. The cursor will change to a pencil when the cursor is moved back into the main window.



**Figure 1.4.** Drawing a trace with the freehand drawing tool. (*left*) Start of the tracing. The pencil is positioned at the profile to be traced and the left mouse button pressed and held down. (*middle*) Just before release of the mouse button. The pencil left a trail where it was moved with the mouse from the start position. (*right*) Right after release of the mouse button. The trace is closed and filled with color.

Place the tip of the pencil on the edge of the profile to be traced as shown in Figure 1.4). Press and hold down the left mouse button. Drag the tip of the pencil around the profile and release the mouse button when the tip gets near the start of the trace. The trace will be completed (closed) and filled with a color.

Press the Page Up key to advance to the next section. The trace on the previous section will be saved automatically as long as the default save options were not changed in the Series Options dialog. Repeat the process of tracing the profile with the mouse. Continue paging and tracing until several sections have been traced. Use the Page Down button to go back and verify that the data was saved correctly.

#### VIEW THREE-DIMENSIONAL OBJECT

Select *List objects...* from the Object menu to see the list of objects defined by traces in the series. Use the left mouse button to double-click the object name in the list (or use a single mouse click and select *Add to scene* from the Scene menu at the top of the list window.)

A three-dimensional view of the object traces will appear in a 3D Scene window after a brief delay. You can rotate the object representation by placing the mouse in the 3D Scene window and pressing and dragging with the left mouse button. You

can zoom in or stop the object's spin by pressing the right mouse button. Pressing both mouse buttons together allows you to pan the scene.

To surface the object, open the Series Options dialog and select the 3D tab. In the 3D options, change the Generate option from Traces to Boissonnat surface and click OK. Go back to the Object List window and again double-click on the object name. The object will be surfaced and replace the trace representation in the scene.

Note that traces must conform to certain criteria for Boissonnat surfacing to work correctly. For example, traces of the same object within a section cannot overlap. For a detailed discussion of tracing rules related to Boissonnat surfacing see Chapter 11.

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### *About the Rest of the Book*

This concludes the Quick Start tutorial. To take full advantage of the software, be sure to read the remaining chapters as well.

***Note: Spending an hour skimming through all the chapters could save you many hours of frustration.***

Chapter 3 gives an overview of how data is organized and stored, and includes technical details on the system requirements and file formats. This chapter introduces the basic data elements: series, sections, domains, traces, and objects. Chapters 5-8 describe how these data elements are created, edited, saved and deleted.

Chapter 4 describes the user interface and includes important information on what particular keystrokes and mouse buttons do in the various program windows. This information is also available from the Key and Mouse Commands help menu items.

Chapter 9 explains in detail how to set and calibrate the units of measurement used in Reconstruct. This chapter explains the meaning of the image pixel size param-

ter and how its value can be accurately determined. Chapter 9 also explains how to get quantitative measurements of the traces created on serial sections.

Chapter 10 covers movements of section data, including all the operations that aid in aligning sections. Chapters 4-9 are required reading to understand how to use the full capabilities of the software. Chapter 10 is necessary only if sections or traces will be moved or aligned.

Chapters 11-12 cover 3D object visualization. In Chapter 11 the representation and editing of objects is discussed, as well as the methods for generating 3D shapes. The 3D Scene window and the rendering of reconstructed objects is covered in Chapter 12.