

Reconstruct Alignment Protocol

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Ch 1 & Ch 10 of the [Reconstruct User Manual](#) discuss manual alignment.

(Note that automatic elastic alignment in TrakEM2 might be more accurate and less time-consuming than alignment in Reconstruct.)

From the Reconstruct User Manual Ch 1:

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.

Read the entirety of Reconstruct User Manual Ch 10 for a full explanation of alignment.



Further Details on the Alignment Process

1. Alignment works by aligning *identically named* stamps on adjacent sections *when they are selected*. Stamps must be *selected* in order to be aligned.
2. In order to be aligned, the sections must first be unlocked. Section > select the sections > Modify > Unlock. To lock a section after aligning, hit Ctrl-L.
3. Choose a “golden section.” Start around the central section of the series (i.e. if the series has 200 sections, then start looking for a good section around section 100). When choosing a “golden section” to base all the alignments on, choose a central section with a zone (of several sections) that is free of flaws. Lock the golden section (Ctrl-L) to ensure it is not moved.
4. Name the stamps **Align_ \$+**. Use a square or cross stamp—more correspondence points. Use a different name/color for the adjacent sections to avoid confusion.
5. Cross-sectioned mitochondria work best. Try to put the stamp in the same place on both sections, at the same zoom level. Zoom in close enough that the stamp fills the mitochondrion.
6. Don't pick the far edges—they're not going to align anyways. Choose center and corners (but not far corners; don't go too close to the edge).
7. Never use the same structure for more than 2 sections, or you could falsely straighten out the field. For example, don't follow the same mitochondria throughout the whole series. Stamp it on two adjacent sections and then switch to a different one.
8. If you move away from the section, you can't un-do it. You can only do space bar to blend. If you scroll any more, you can't un-do.
9. After making the alignment stamps, select the stamps: **Trace > Align Section > Linear**. Best to do Linear alignment because the others will distort the image. (Deformal is useful if something is tilted. Quadratic is only if a section has a lot of distortion; this will force your alignment markers to align perfectly.)
10. Traces must be selected in order to align. If they're all de-selected, you can't choose Align (it will be grey and you can't click on it).
11. “Section > Movement > Propagate” can be used to move multiple sections simultaneously. Example: if sections 3-200 are uniformly misaligned, perform conventional alignment on sections 1-2, and propagate movement to sections 3-200
12. Check how good the alignment looks each pair at a time by blending with the space bar. (The edges will typically not align well.)
13. Don't begin to trace dendrites, synapses, axons, etc until you are happy with the alignment.

Example alignment process:

1. Starting on golden section 103, stamp on 103 then up to 104 (can only lay stamps in 1 direction) for 5-15 points (depends on size of EM).
2. Click Trace > Align Section > Linear to pull 103 into alignment with 104.
3. Then move to align 104 to 105, and so on until you reach the end of the series.
4. At the very end of the alignment, page through the entire series on blend (hit Space Bar) to see if anything seems out of alignment. Spot repair the misalignment and propagate thru the rest of the sections as needed. Example, sections 1-22 aligned beautifully and 23-61 were also good, but something weird happened between 22 and 23. Fix the misalignment and propagate back from 22 to 1.
5. Try to align around folds, tears, and stretches. Use Quadratic as need to force the mid section into alignment. As in, align section 103 to 105 because 104 has the tear. Then use quadratic to force 104 to align to 105.