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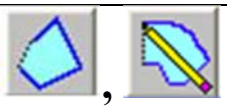
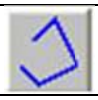
Tracing Synapses

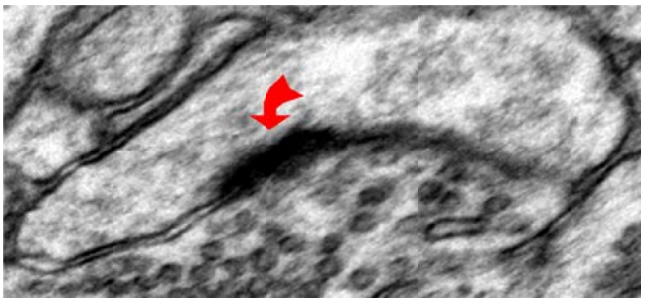
Two Types of Tracing Tools

The two basic types of tracing tools in Reconstruct are closed and open. Both "Draw Closed Point by Point" and "Draw Freehand" (pencil) create closed traces; you can choose which of these you prefer. Open and closed traces will both be needed to trace synapses.

Tracing tool(s)	Purpose	Flat area calculation	Tools Icon
Closed ("Draw Closed Point by Point" and "Draw Freehand")	Closed traces (like circles)	Area of the closed trace	
Open ("Draw Point by Point")	Open traces (like lines)	Length times section thickness	

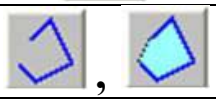
The Postsynaptic Density (PSD)

Synapses can be identified by the darkly staining postsynaptic density (PSD) in the postsynaptic dendrite and vesicles in the presynaptic axon. A trace of the PSD area supplies the area of the synapse. In the electron microscope image below, the arrow is pointing at the PSD in a dendritic spine head; vesicles are visible in the adjacent presynaptic axon.



Two Types of PSD Traces

The two types of PSD trace are contact and contact flat area. ("Contact" refers to synaptic contact.) The contact trace creates an image of the synapse in a 3D reconstruction, and the contact flat area trace measures the synaptic area. In Reconstruct, the contact trace is named d##c##, and the contact flat area trace is named d##cfa##. d## is the dendrite number, while c## and cfa## correspond to the protrusion number—for example, spine 05 in dendrite 01 would have synapse traces named d01c05 and d01cfa05. Contact and contact flat area traces are often referred to as c-traces and cfa-traces. Make the c-trace and cfa-trace different colors to avoid confusion.

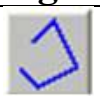
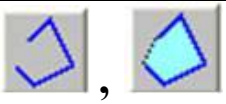
Trace name	Purpose	Color	Tools Icon
d##c##	3D visualization in Reconstruct	blue	
d##cfa##	Synapse area calculation	green	

Three Types of PSD Orientation

1. Cross Section – Cross section PSDs occur when the section is cut perpendicular to the synapse. In a distinct cross section synapse, you can see a presynaptic membrane, a postsynaptic membrane, and a synaptic cleft (the space between the pre- and postsynaptic membrane). If the synaptic cleft and synaptic membranes are not visible, it might still be cross-sectioned if nearby membranes are visible and the PSD width is less than two times the width of a normal cross-sectioned PSD.

2. Oblique – Oblique PSDs occur when the section is cut at an angle to the synapse such that the PSD "slopes" across more than one section. It is likely oblique if the PSD is greater than or equal to two times the normal width of a cross-sectioned PSD (look at other nearby synapses for examples of PSD width). If the adjacent membrane (on either side of the PSD) is visible, it might not be oblique.

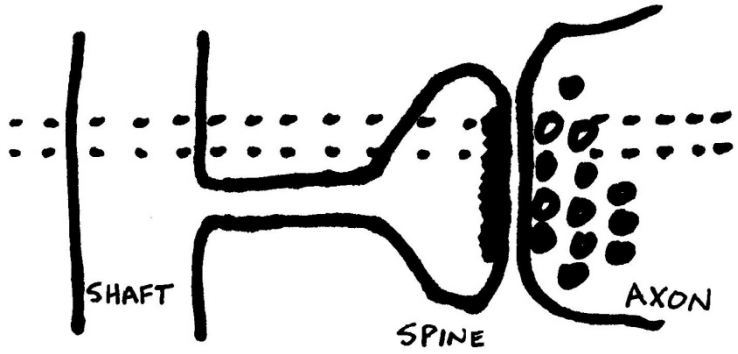
3. En Face – *En face* is a French phrase meaning "opposite" or "across the street". En face PSDs occur when the section is cut parallel to the synapse and the PSD is contained in the width of one section, so that the entire "face" of the synapse is visible, "opposite" from the presynaptic axon.

Orientation	Criteria	cfa tracing tool
Cross Section	Two visible membranes and a synaptic cleft.	
Oblique	PSD ≥ 2 times the width of a cross-sectioned PSD, no visible membranes.	
En Face	"Opposite". PSD fully visible in one section.	

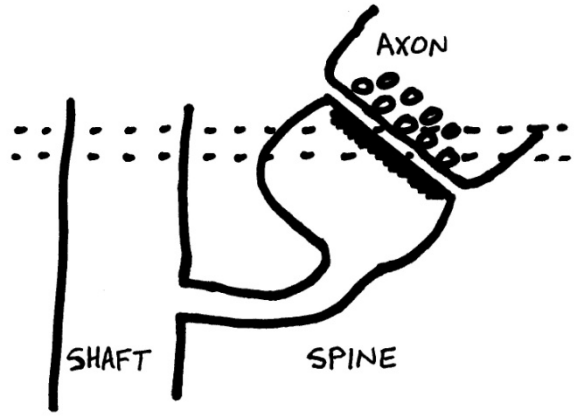
Cartoons of the Three Types of PSD Orientation

In the following shoddy cartoons, the dashed lines indicate a single section (sections are typically around 55nm; these drawings are not to scale). In an ideal dendrite, the section (the dashed lines) will be perpendicular to the shaft, so that the shaft is nicely cross sectioned. The cartoons indicate how, depending on the orientation of the spine with respect to the shaft, a synapse can be sectioned in different orientations.

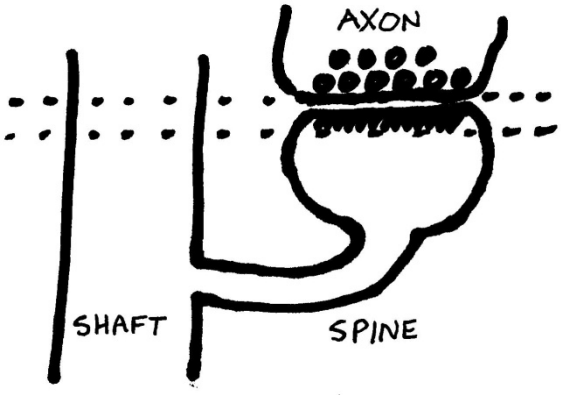
1. Cross Section – Cross section PSDs occur when the section is cut perpendicular to the synapse.



2. Oblique – Oblique PSDs occur when the section is cut at an angle to the synapse such that the PSD "slopes" across more than one section.



3. En Face – En face PSDs occur when the section is cut parallel to the synapse, so that the entire "face" of the synapse is visible in one section. Note that the entire PSD is contained in the section.

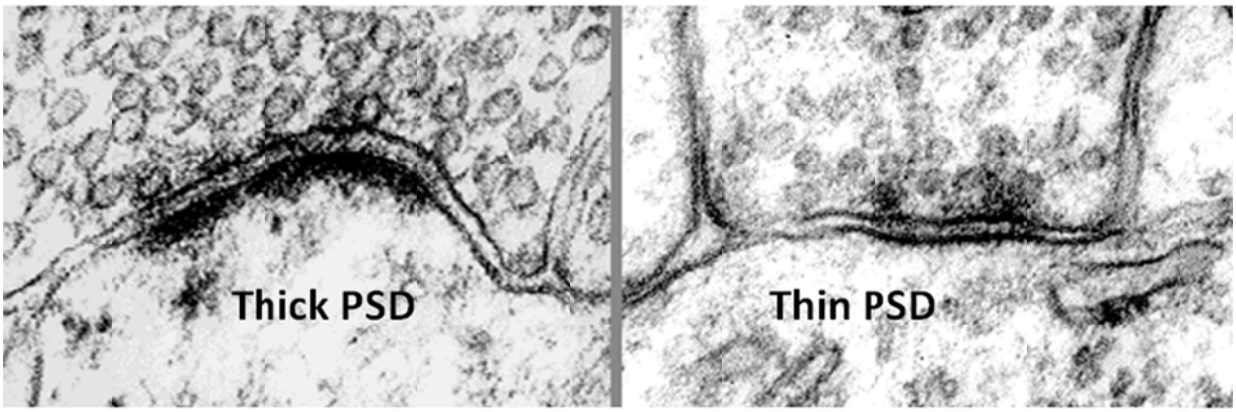


Two Types of Synapses

Inhibitory
Location: usually located on the dendritic shaft
Vesicles: "pleiomorphic," multiple shapes (flattened, oblong, etc.)
PSD width: thin PSD
Axon: axon synapses with other shafts / inhibitory synapses
A.K.A.: symmetric

Excitatory
Location: usually located on spines
Vesicles: round vesicles
PSD width: thick PSD
Axon: axon synapses with other spines / excitatory synapses
A.K.A.: asymmetric

	Inhibitory	**Excitatory**
Location	Usually on the shaft	Usually on a spine head
Vesicles	Pleiomorphic	Round
PSD width	Thin	Thick
Axon	Synapses with other shafts / inhibitory synapses	Synapses with other spines / excitatory synapses
A.K.A.	Symmetric	Asymmetric



Atlas of Ultrastructural Neurocytology, http://synapseweb.clm.utexas.edu/16-chemical-synapses-11

Tracing PSDs

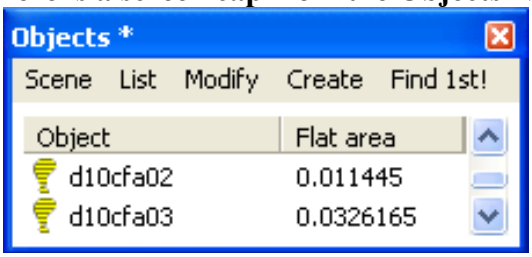
1. Cross Section:
a. Trace an open cfa-trace along the edge of the PSD on each section.
b. Circle the cfa-trace with a c-trace for visualization. (Extend the c-trace outside of the membrane trace to make it visible.)

2. En Face:
a. Trace the outline of the PSD with a closed cfa-trace.
b. Circle the cfa-trace with a c-trace for visualization. (For an en face trace, the c-trace does not necessarily need to be extended outside of the membrane trace to be visible. Just do what makes sense aesthetically.)
c. Copy the c-trace to the next section to create volume for visualization. (If a c-trace is only on one section, it will not be visible in the reconstruction.) Make sure that the copied trace is on the adjacent section where the presynaptic axon appears.

3. Oblique:
a. Some sections of an oblique synapse might be cross-sectioned; sections with cross-sectioned PSD are traced as described previously, with an open trace drawn along the width of the PSD.
b. An oblique PSD will be thicker than a cross-sectioned PSD and will overlap with an axon on the adjacent section. PSD significantly overlapping an adjacent axon is likely to be oblique, whereas PSD overlapping adjacent PSD is cross-sectioned.
c. Determine what part of the PSD overlaps the axon on the adjacent section, and measure its width; if the width is more than or equal to twice the width of a typical PSD for that region, then the PSD on that section safely can be considered oblique.
d. Draw a closed trace only around the section of the PSD that overlaps an axon. For adjacent closed traces of oblique PSD, draw an open trace connector along the edge of the abutment in order to include the section thickness in the area.

Calculating Flat Area of Synapses

1. Go to Object → List objects → you should see the flat areas listed for your synapses. (The unit of measurement is square microns.) Be sure you're looking at the *flat* area, *not* the surface area. (If you don't see Flat Area listed, go to Series → Options → Lists → under Object list, make sure flat area is selected → Click OK.) Here is a screen cap from the Objects list:



2. Go to Object → List objects → List → save → It will save it as a Comma separated file (.csv) that you can open with Excel. Open the .csv objects file and copy and paste the values listed for d##cfa## into the appropriate column of your spreadsheet.

3. Flat area = $\Sigma_{\text{all sections}}$ [$\Sigma_{\text{open traces}}$ length x thickness + $\Sigma_{\text{closed traces}}$ area]
(See the Reconstruct manual chapter on "Calibration and Measurement".)

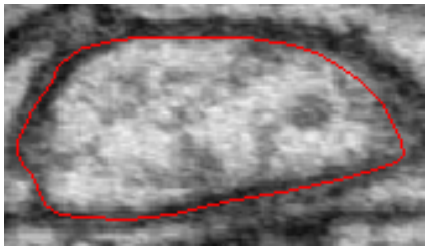
TRACING PSDs – CROSS-SECTIONED

(CLZBJ d04p03)

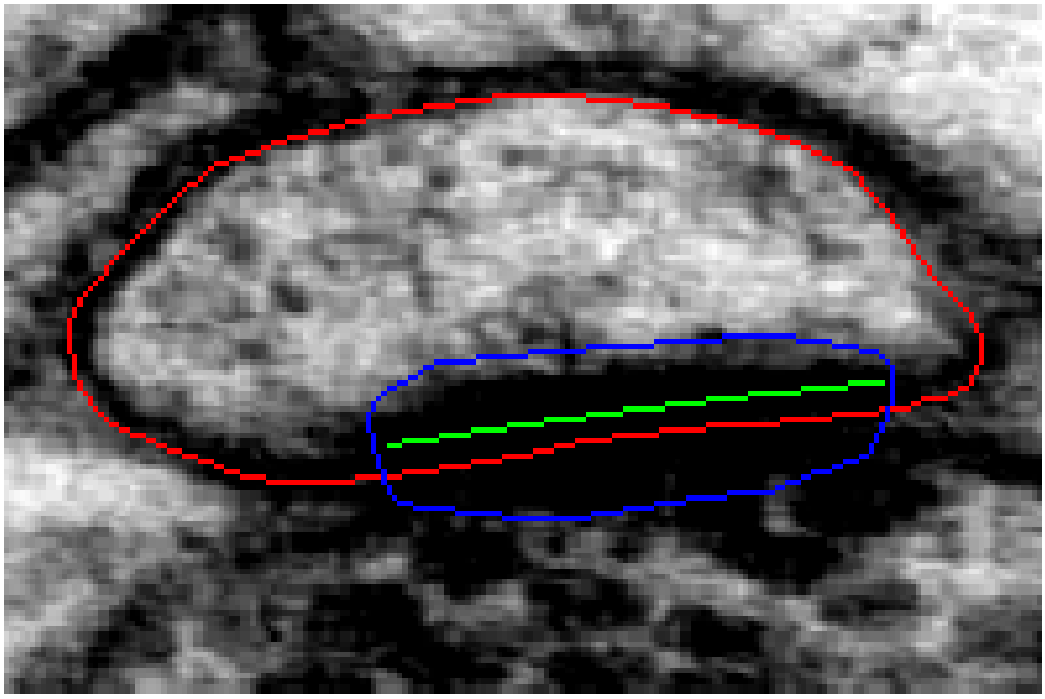
Legend:

Red = spine trace
Blue = 3D c-trace
Green = flat area cfa-trace

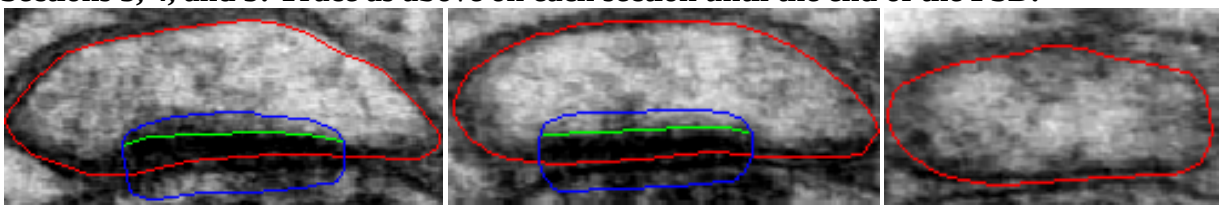
Section 1: No PSD.



Section 2: The PSD is traced with an open cfa-trace line (green) along length of PSD. The cfa-trace is then circled by a closed c-trace (blue) which extends outside of the spine membrane trace.

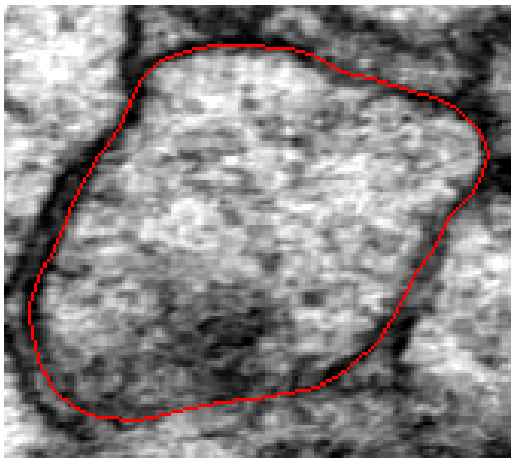


Sections 3, 4, and 5: Trace as above on each section until the end of the PSD.



Tracing PSDs – En Face

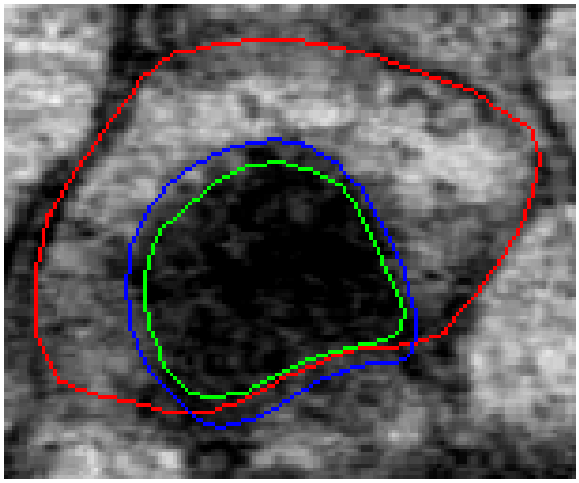
(CLZBJ d18p34)



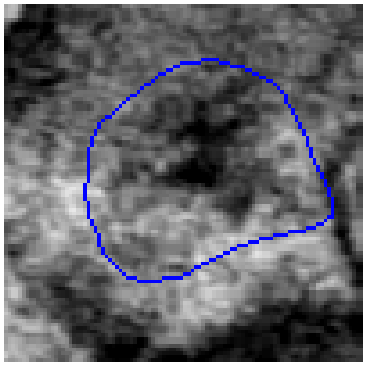
Legend:

Red = spine trace
Blue = 3D c-trace
Green = flat area cfa-trace

Section 1 – No PSD (faint/questionable).



Section 2 – En face PSD, traced with closed cfa-trace (green), circled by closed c-trace (blue).



Section 3 – Additional c-trace to give volume to the 3D reconstruction.

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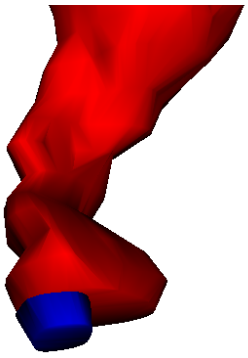
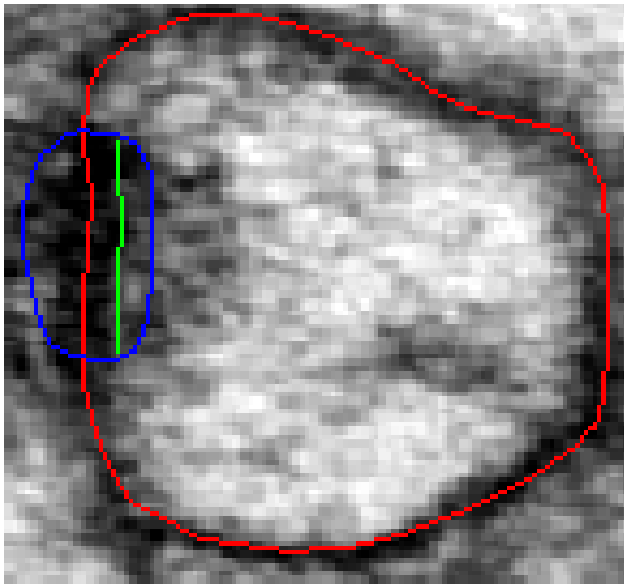
Reconstruction:



Tracing PSDs – Oblique, example 1

(CLZBJ d04c51)

Section 1:



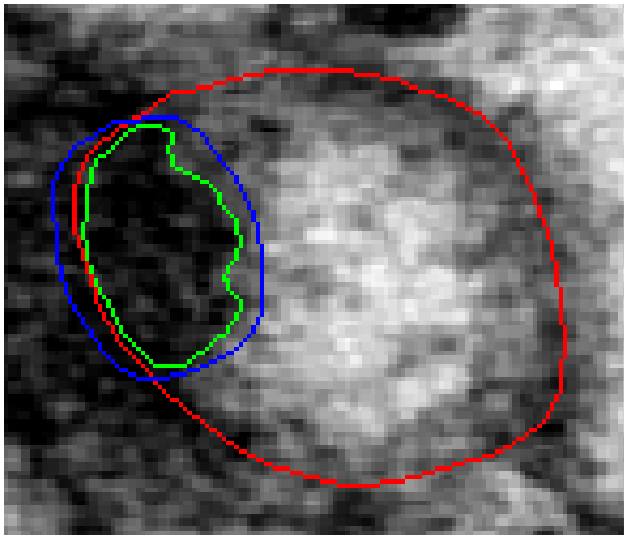
Legend:

Red = spine trace
Blue = 3D c-trace
Green = flat area cfa-trace

Section 1: Cross-section, traced with open cfa-trace (green) and circled by a closed c-trace (blue).
(There is no PSD in section "0".)

Section 2: This section is oblique. The PSD is traced with a closed cfa-trace (green) which is circled by a c-trace (blue).

Section 2:



Section 3:
An additional c-trace (blue) is drawn to give volume to the c-trace on section 2. (Copy the cfa-trace from section 2 and change its attributes with Control-B.)

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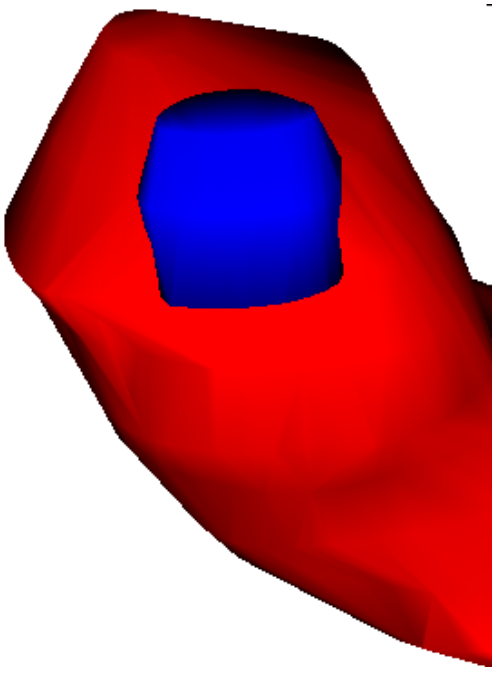
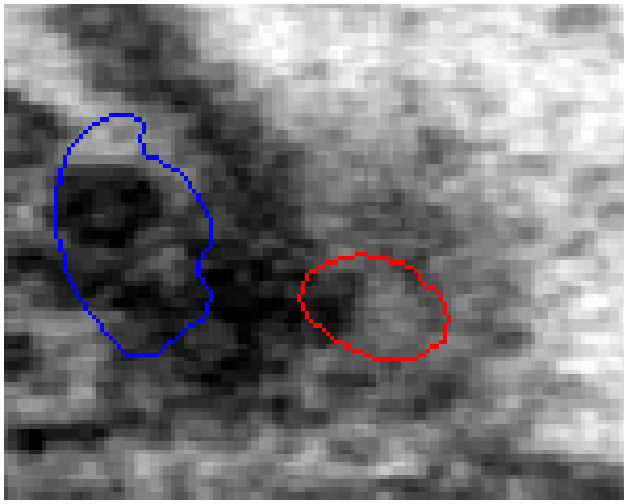
3D Reconstruction:



Section 3:



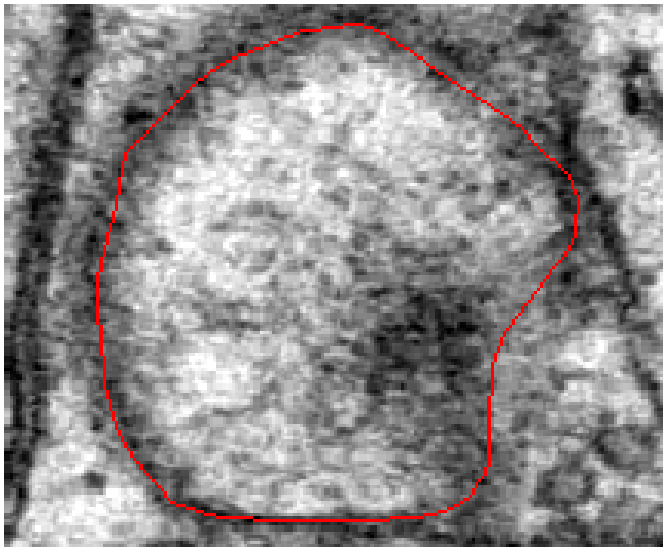
Tracing PSDs – Oblique, example 2

(RJZQR d01p31a)

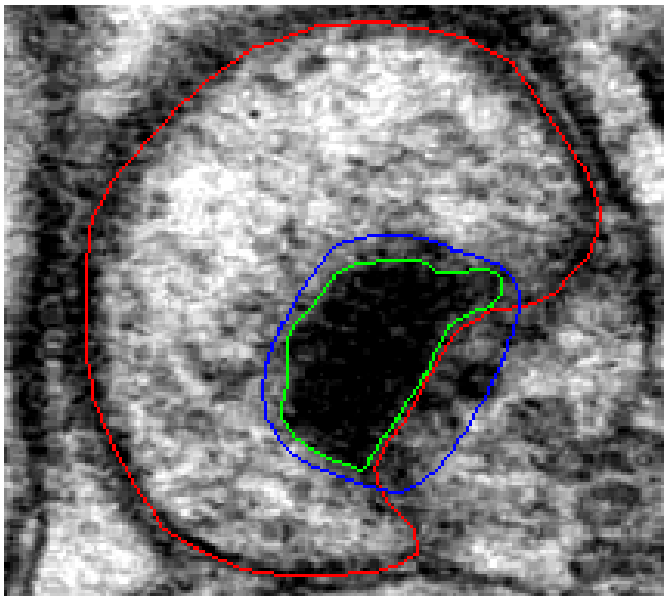
Legend:

Red = spine trace
Blue = 3D c-trace
Green = flat area cfa-trace

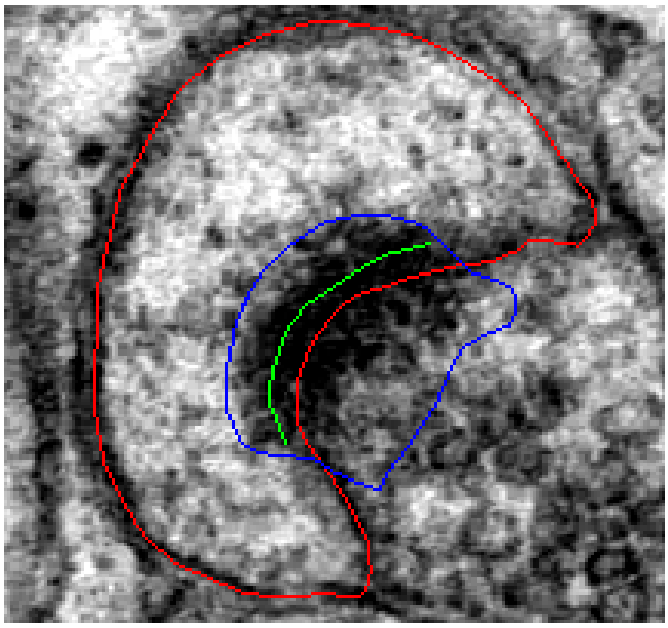
Section 1: No PSD (very faint, overlapping PSD on next section).



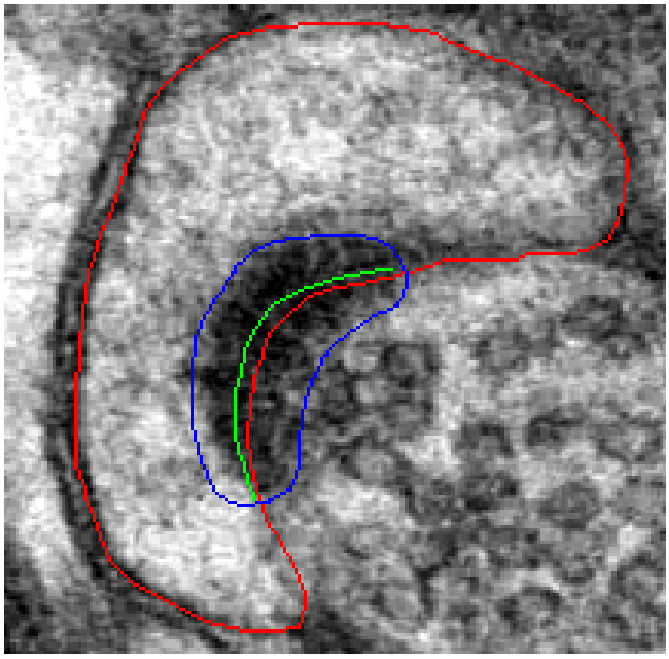
Section 2: This section is oblique. The PSD is traced with a closed cfa-trace (green) which is circled by a c-trace (blue).



Section 3: Cross-section, traced with open cfa-trace (green). The cfa-trace is copied from Section 2 and pasted in this section with its attributes changed to c-trace using Control-B, in order to give volume for the 3D visualization. A c-trace is then made around the cfa-trace, and is then connected and merged with the pasted c-trace from Section 2, to create a single c-trace.



Section 4: Typical cross section PSD; last section with PSD.



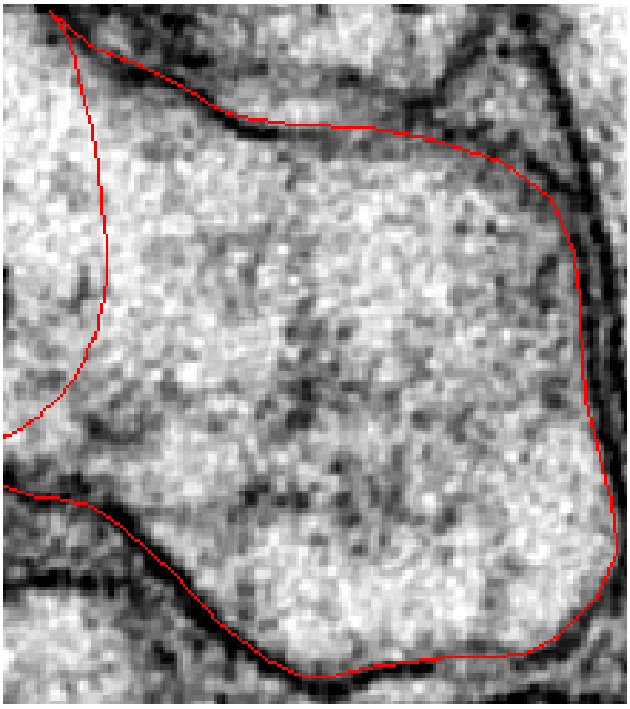
Tracing PSDs – Oblique, example 3

(RJZQR d10sp18)

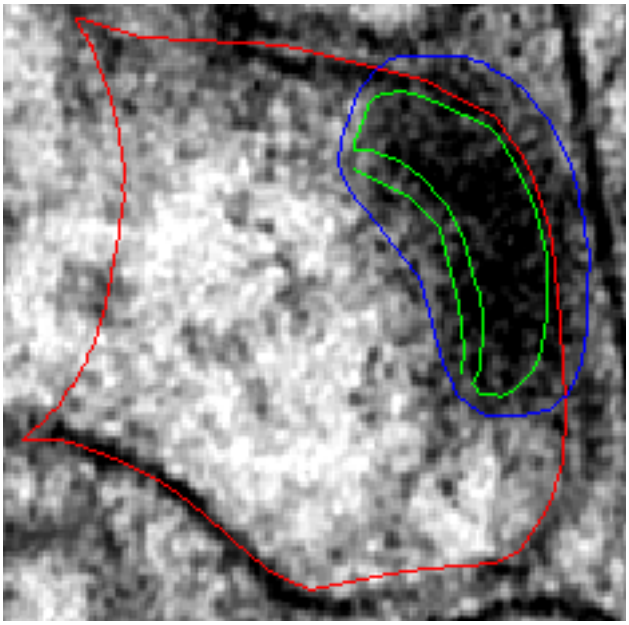
Legend:

Red = spine trace
Blue = 3D c-trace
Green = flat area cfa-trace

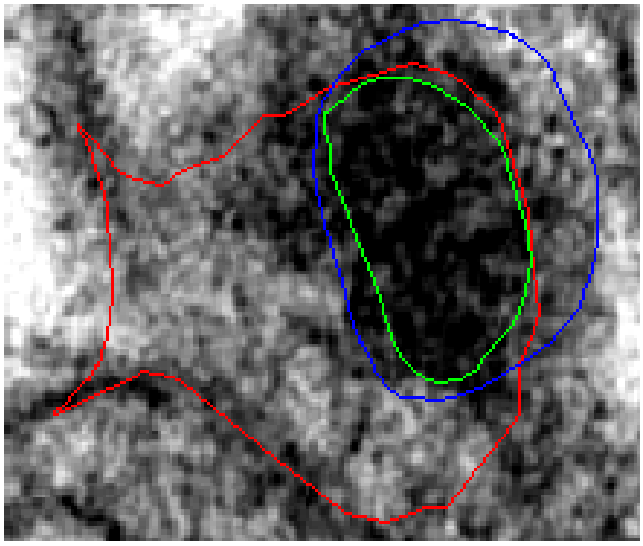
Section "0": No PSD.



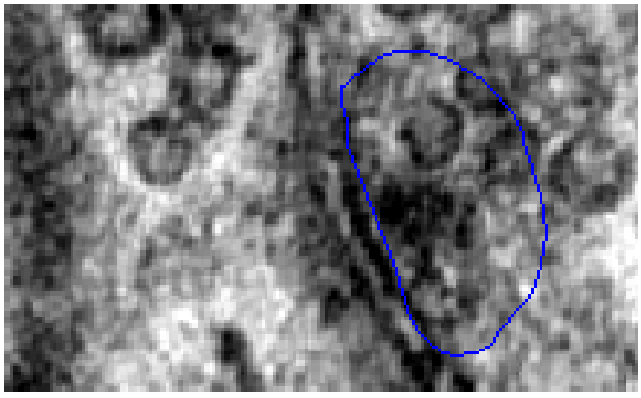
Section 1: Oblique PSD traced with closed c-trace and cfa-trace, with open cfa-trace to connect between the sections.



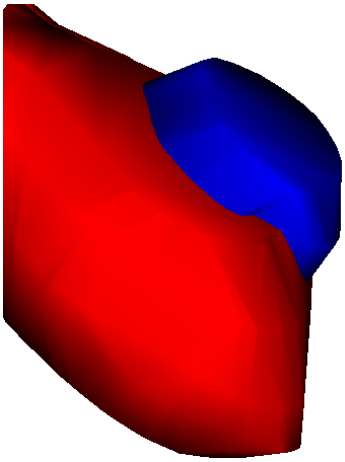
Section 2: Oblique PSD.



Section 3: cfa-trace copied from Section 2 (its attributes changed to c-trace) to give volume to the 3D visualization.



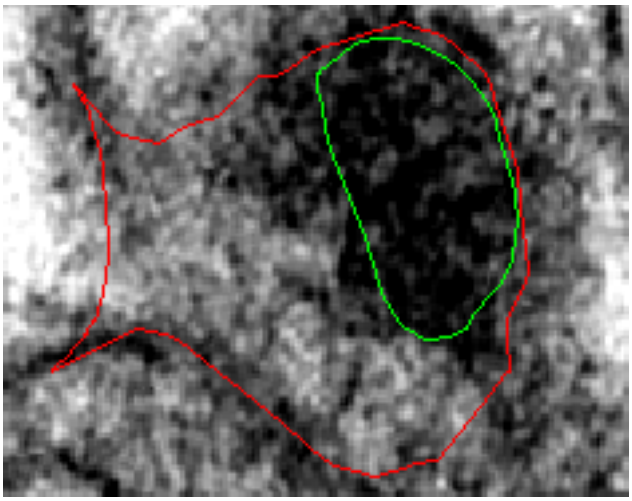
3D Reconstruction:



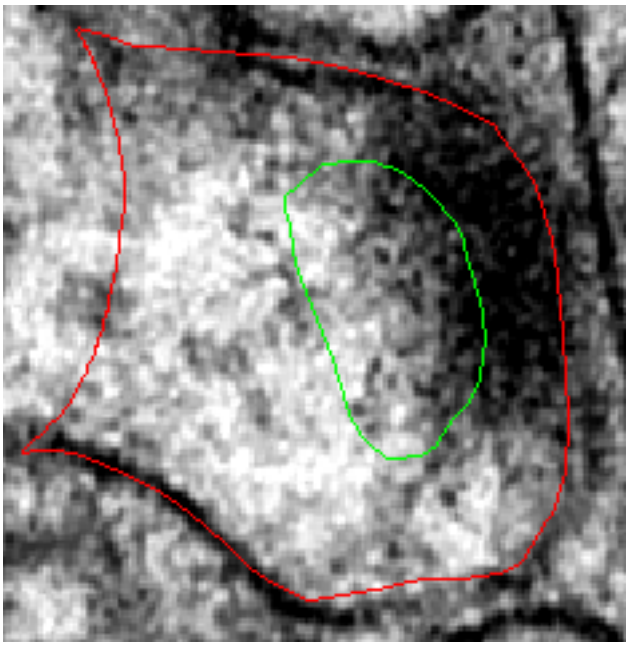
Step by Step Tracing Instructions:

1. First, trace the cfa-traces.

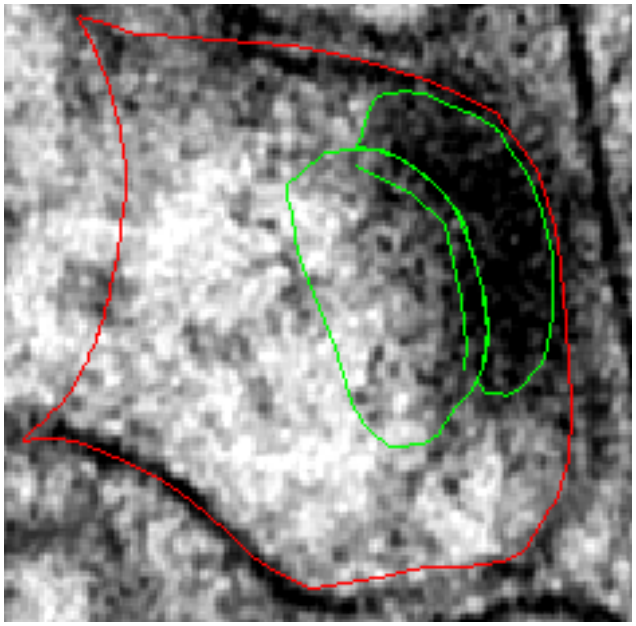
Section 2: Start with the section that has all of its PSD adjacent to the axon on the next section (section 3).



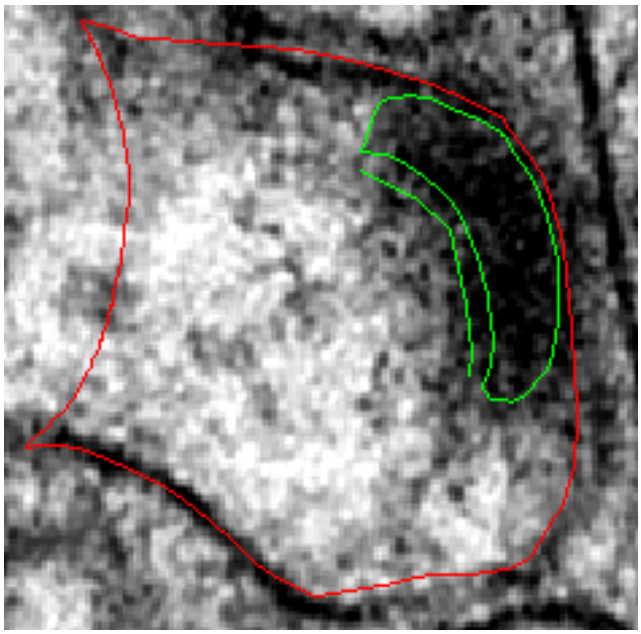
Section 1: Copy the cfa-trace from section 2 to section 1. Adjust for any possible misalignment.



Section 1 cont'd: Use the copied cfa-trace to avoid tracing the PSD which overlaps the PSD in section 2. Draw the open trace connector where the PSDs from section 1 and 2 abut.



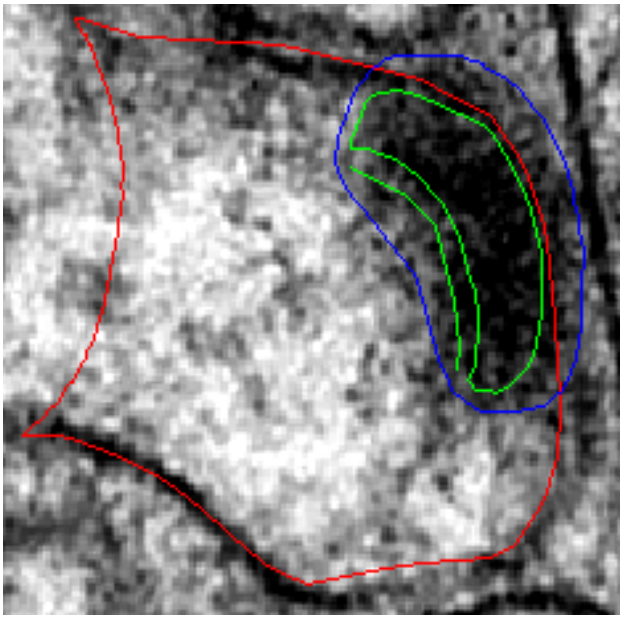
Section 1 cont'd: Delete the cfa-trace that was copied from section 1.



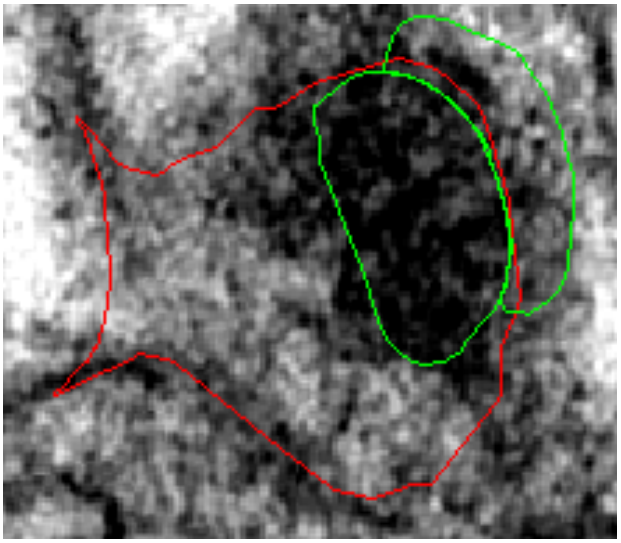
The cfa-traces are now complete.

2. Second, trace the c-traces.

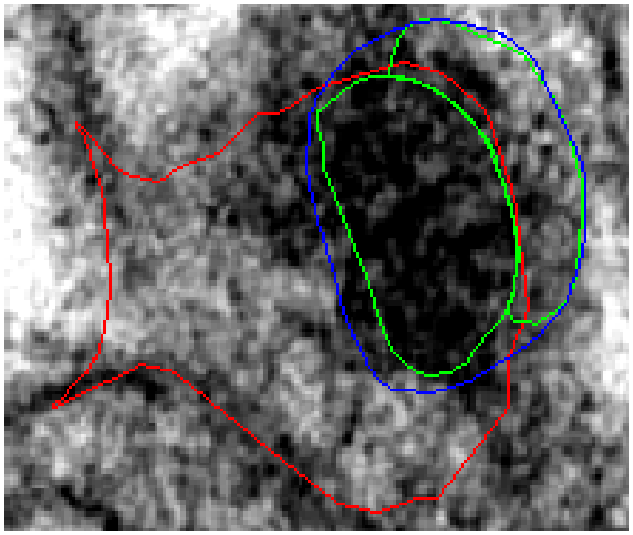
Section 1: Trace a c-trace around the cfa-trace. Extend it outside of the spine trace.



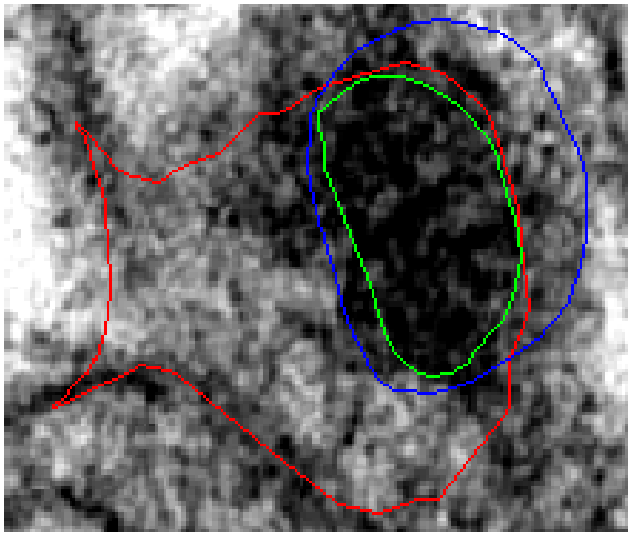
Section 2: Copy & paste the cfa-trace from Section 1 onto Section 2. (Alternately, when you make the c-trace on this section, you can just extend it farther past the spine trace. That gives the PSD from section 1 more volume for visualization.)



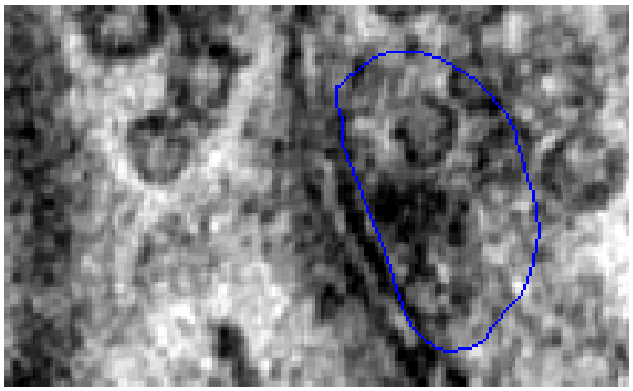
Section 2, cont'd: Trace a c-trace around both of the cfa-traces.



Section 2, cont'd: Delete the cfa-trace you copied from section 1.



Section 3: Copy the cfa-trace from Section 2, paste it in Section 3, and change its attributes to be a c-trace (using Control-B).



That's it!

Further Tips & Principles for Tracing Synapses

1) Finding the membrane. Due to dark smudges, it can sometimes be difficult to identify and trace the spine membrane which is adjacent to the PSD. For help, look at the nearby membrane (of both the spine and the axon). Follow what membrane you can see clearly in order to make an educated guess.

2) The PSD is inside the spine. It is a common mistake to trace the PSD as though it is outside of the spine membrane. However, the cfa trace is *always* inside the membrane trace. PSDs are completely bound within the spine membrane (Fig. 1).

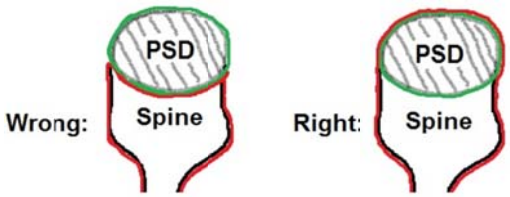


Figure 1. The PSD trace (green) is inside the spine membrane trace (red).

3) Closed cfa traces must overlap axon. En face closed traces must be overlapping an axon on the adjacent section – measure 40nm *that overlaps axon*. (Blend the two sections to see if they are overlapping.) Understand the principle that when you circle a cfa trace, that means the whole traced area is a synapse – the whole thing is forming a synapse with an axon; thus it must be overlapping an axon.

4) Closed cfa traces must not overlap each other. Make sure closed cfa traces are not overlapping each other – otherwise you're double tracing the PSD. A closed cfa trace should overlap axon, and nothing else.

5) Closed cfa trace next to membrane trace. Make the closed cfa trace *next to the membrane trace*. If, in order not to double-trace/overlap the closed cfa traces, you move the closed cfa trace farther away from the membrane – you're doing it wrong. Start from the other direction (up or down) in the series (Fig. 2).

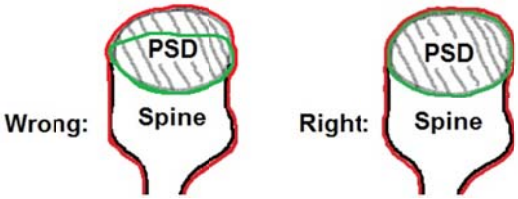


Figure 2. Place closed cfa trace (green) next to the membrane trace (red).

5) Connector trace placement. Make the connector trace *on the other side from the membrane trace*. If 4 trace lines (connector line, cfa trace, spine membrane trace, and axon membrane trace) are all jumbled next to each other, it will be visually confusing (Fig. 3).

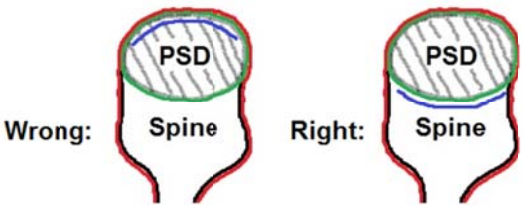


Figure 3. Place the connector line (blue) on the opposite side from the membrane trace (red). (The connector trace is a cfa trace and thus will be the same color as the cfa trace; in this figure, we made it a different color in order to identify it.)