nTracer 1.0

An interactive tool for reconstructing and analyzing complex neural networks from multi-color fluorescent images

Instruction Manual with

Step-by-Step Tutorial

Written and published by
The Cai Lab

Contents

I. Introduction

1. What is nTracer?

nTracer was developed as a solution to the challenge of analyzing images produced from Brainbow labeled brain tissues. The software utilizes Fiji and operates as a plug-in within the program. nTracer allows users to accurately trace individual neurons as well as identify connections between neurons in densely labeled, three-dimensional, multicolored environments to explore their synaptic connectivity. The user-generated tracing results are assigned as numerical data for further analysis and interpolation.

2. Application Examples

Single Cell Analysis

sample image

Connectomics
(Divergence)

sample image

Dense Reconstruction

sample image

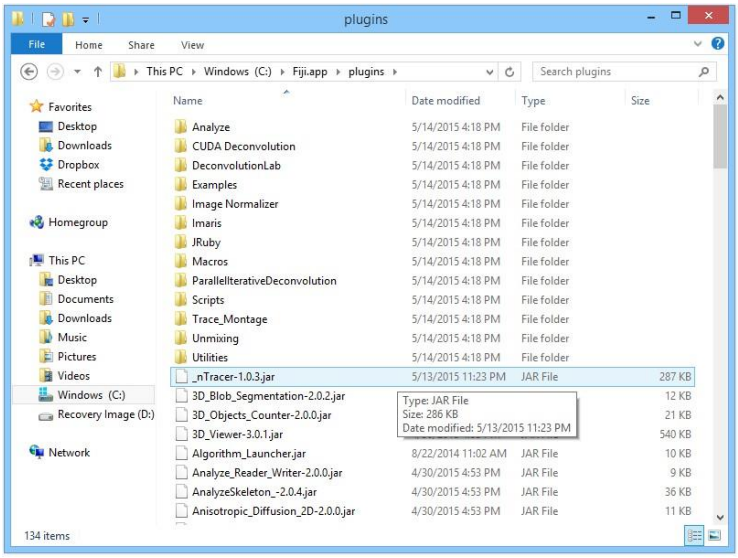
Connectomics
(Convergence)

sample image

II. Starting nTracer

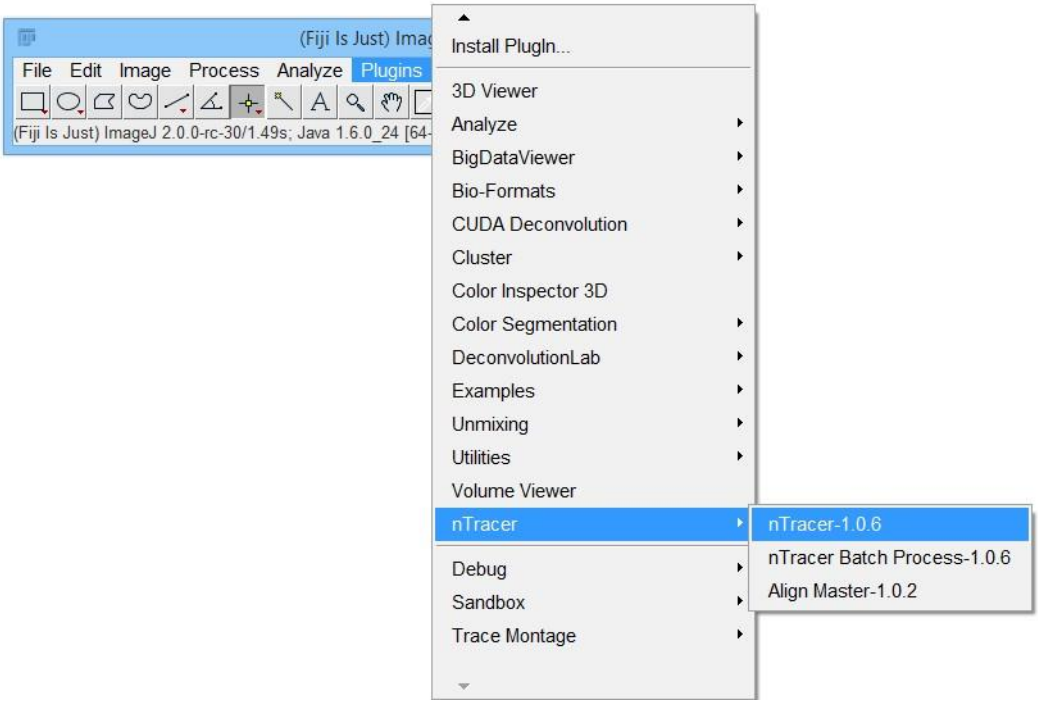
1. Install and run nTracer

Download <nTracer.jar> from https://www.cai-lab.org/ntracer-tutorial and place it into ImageJ/Fiji application's plugin folder.



You have to restart ImageJ/Fiji if you have it opened.

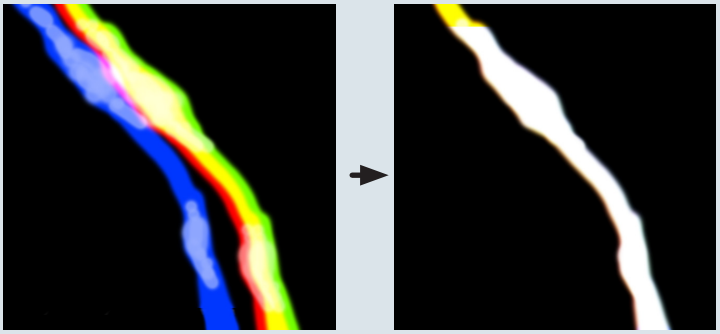
After opening ImageJ/Fiji, you can open nTracer from the <Plugins> pull-down menu.



Align Master

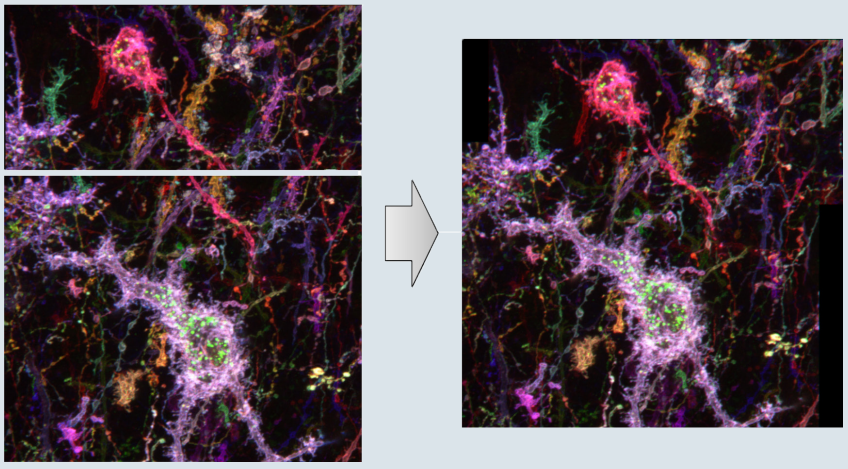
In most cases, you will need to carry out some post-processing for the images acquired from the microscope before loading them in nTracer to do the tracing. nTracer simplifies this process with Align Master, which is included in the package.

Using Intensity/Color Normalization tool, histogram of each color channel across z-stack will be matched to that of a reference image. (Left: Reference image chosen from the stack with good contrast and color intensity. Middle: Image from a different z-position before normalization. Right: Image after normazliation).



With Channel Registration, images from different channels are automatically calculated and shifted in x,y,z directions for perfect alignment.

There is also the option of 3D Stitching in case your image covers a large area, and consists of multiple image stacks. Using 3D Stitching, multiple images can be arranged to automatically be stitched together to create a seamless scene of a larger area of analysis.



As nTracer opens, you will be prompted to open an image file.

*__Note:__ nTracer opens images that are saved as "Composite" or RGB format.

a) Select and open the desired hyperstack image file by double clicking or by clicking "open." *It might take a while to load the images, depending on the size of your file.*

b) When prompted, set the x,y,x resolution.

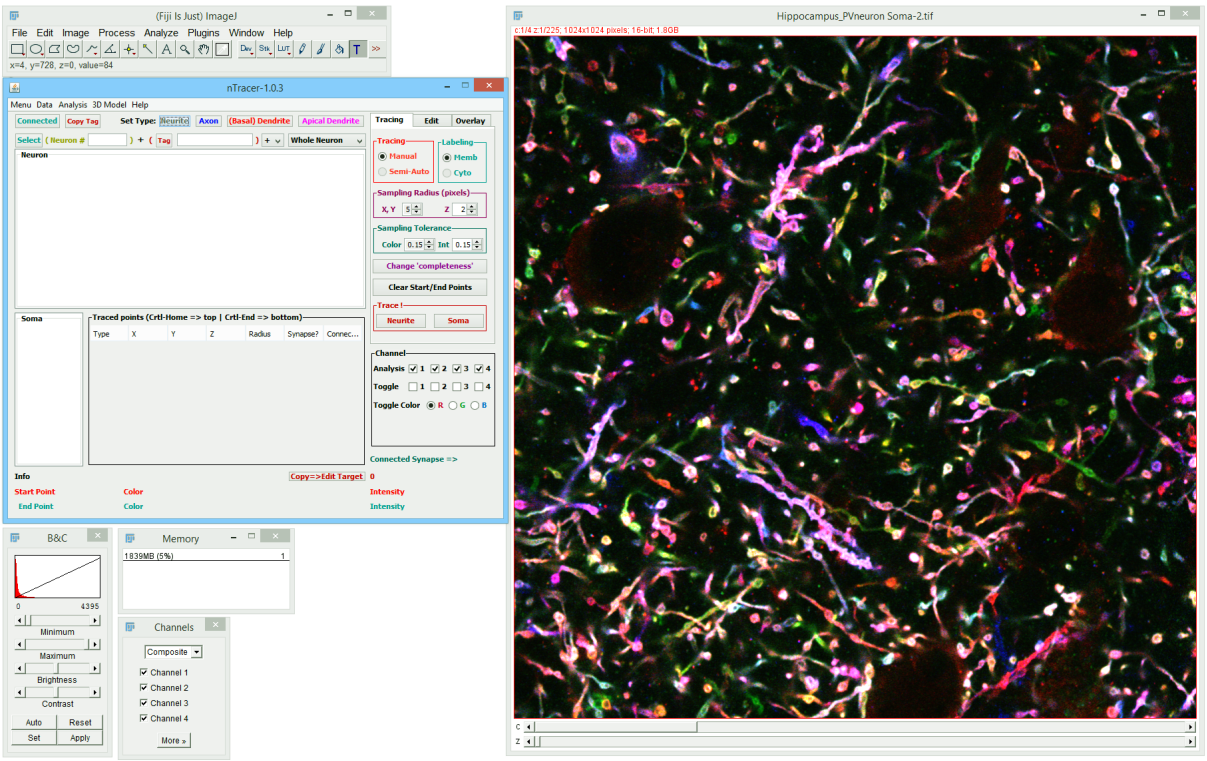
The dialog default values are taken from the image if meta data header is saved from the microscope during image acquisition. If no resolution information is on file, it will show 0 in all fields. Having 0 as the resolution value will show distance unit in Pixel.

Next, you will be prompted to load a tracing data file.

a) If you have previously saved tracing data, select the desired data file (.zip).
This will load the saved tracing data and display parameters with the image.
Any tracing data from the previous session will be saved as a <.zip> file with the same file name as the image file by default.

b) If you do not have saved data, or wish to create new tracing from scratch, click " "cancel."

<nTracer> will launch two windows: the control panel and the image viewer.



The control panel (left) displays an overview of the architecture of your tracing in addition to showing different command options and settings for creating and editing the tracing data.

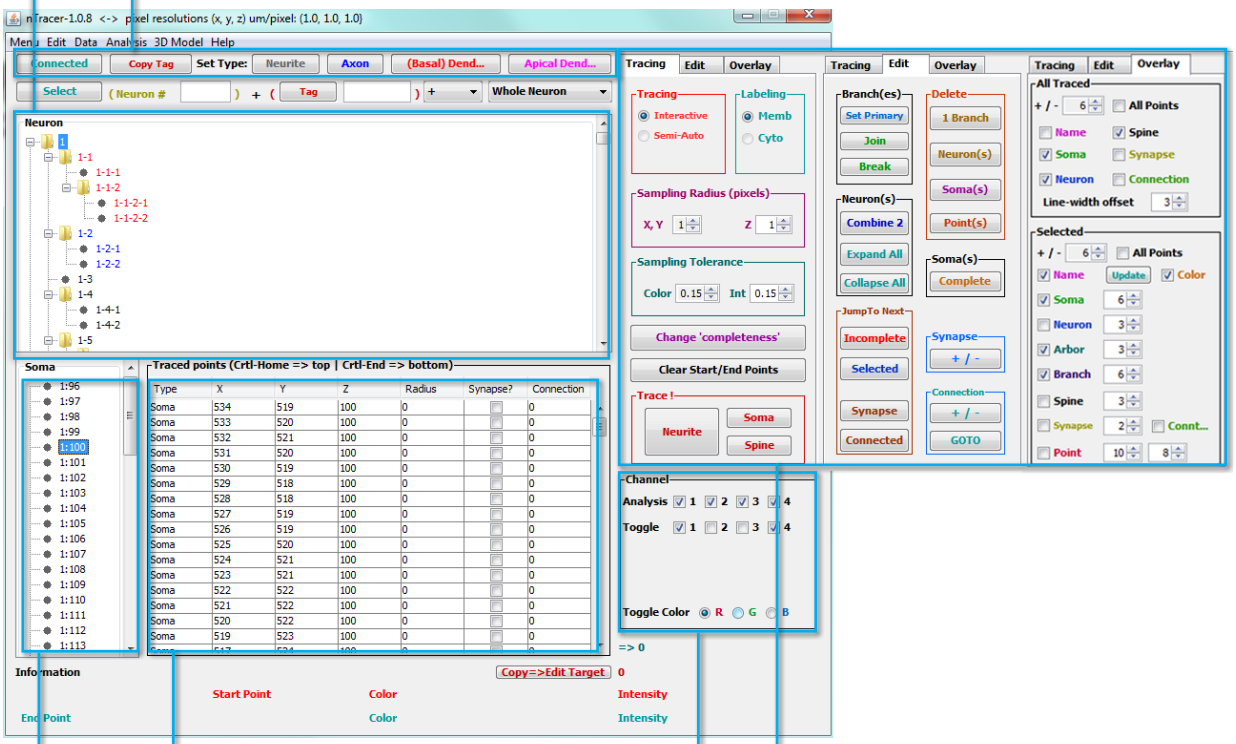
The image viewer (right) is where you perform the actual tracing. Scroll up and down to move through the hyperstack. You can also zoom in and out centered on the cursor by pressing <-> and <+> on your keyboard.

2. Control panel overview

Familiarize yourself with different menu options and command buttons in the control panel. Most of the menus will display a short description as a rollover text. Some of the basic menus are briefly described below.

Trace diagram
The overall architecture of the tracing data is displayed as a tree diagram

Set type
Assign different identities to the individual traces



Channel
Select color channels and specify color toggle option
*'Toggle': refer to page 11

Toolbox (Tracing/Edit/Overlay)
Change the settings in each window as you perform your tracings and data analysis
We will go over each menu as part of the following tutorials:
- Tracing (chapter 3)
- Editing and overlay (chapter 4)

Traced points databox
This window displays the vector information for the selected trace as well as the synapse and connectivity data corresponding to each point

Soma databox
Tracings of soma is organized and displayed by z-stack
(1:100 means soma tracing of neuron #1 in image slice 100)

III. Tracing tutorial

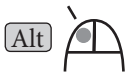
1. Trace soma

Once the image file is loaded, you can begin tracing. You may start by tracing any neuron of your choosing. Here, we will start by tracing a clearly identifiable soma.

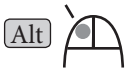
There are two ways to create a trace for a soma:

- Interactive: the computer automatically determines the "best-fit" path between two points based on color and intensity information
- Freehand: directly draw the trace with your mouse by using the freehand tool

a) Interactive Tracing

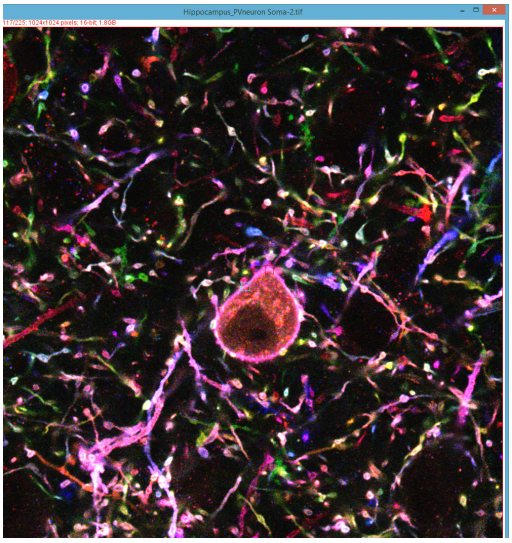


i. <Alt-left click> anywhere on the soma outline. A red box will appear marking this point as the starting point.

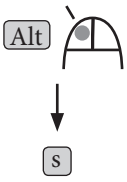


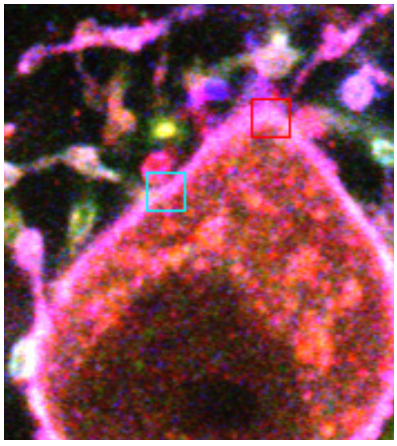
ii. <Alt-left click> once more somewhere on the somatic boundary to mark the end point. A blue box will appear.

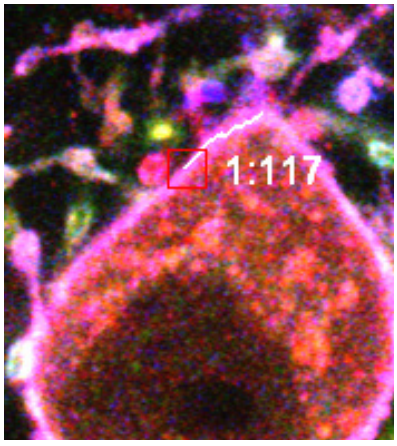
iii. Press hotkey <s> or click on "Soma" under "Trace!" in the Tracing toolbox to assign this trace as a soma. The program will connect the two points with the best-fit path found according to the user-set Sampling Radius and Sampling Tolerance values.



iv. The path will be shown as a white line, and the end point automatically becomes the new starting point (red box).
Continue tracing by clicking along the somatic boundary in the same manner.



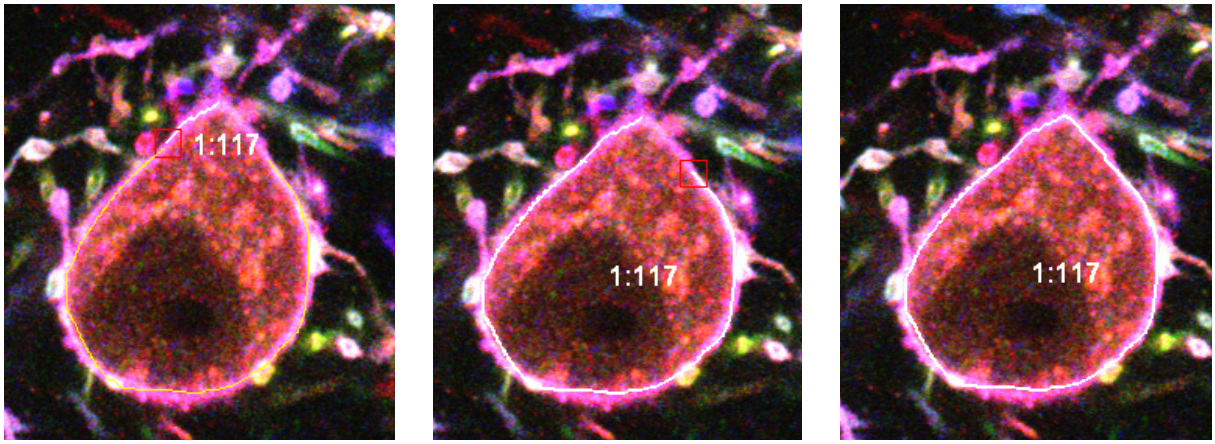




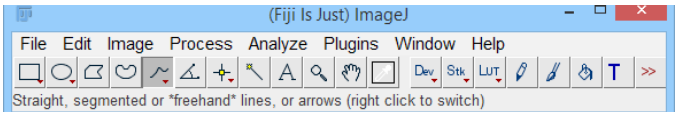
o

v. To finish, press hotkey <o> to complete the current tracing.
The program will automatically connect the most recent endpoint to the first starting point.

vi. Before starting to trace the soma at a new z position, <Double right click> to deselect the last tracing you just made while keeping the root neuron selected. The red box from the previous slice will disappear and allow you to assign a new starting point on the new z position.



b) Freehand Tracing



i. Select the "Freehand Line" tool.

ii. <Left click-down> and trace around the edge of the soma. The path is visible as a thin yellow line (you do not have to go all the way around the soma in one stroke).

s

iii. Release the mouse and press hotkey <s> to assign the trace to the soma.

iv. To continue tracing, simply <left click-down> near the new starting point and trace while holding down the mouse.

o

v. When you are ready to close off the trace, press hotkey <o>.

vi. <Double right click> to deselect the current slice.

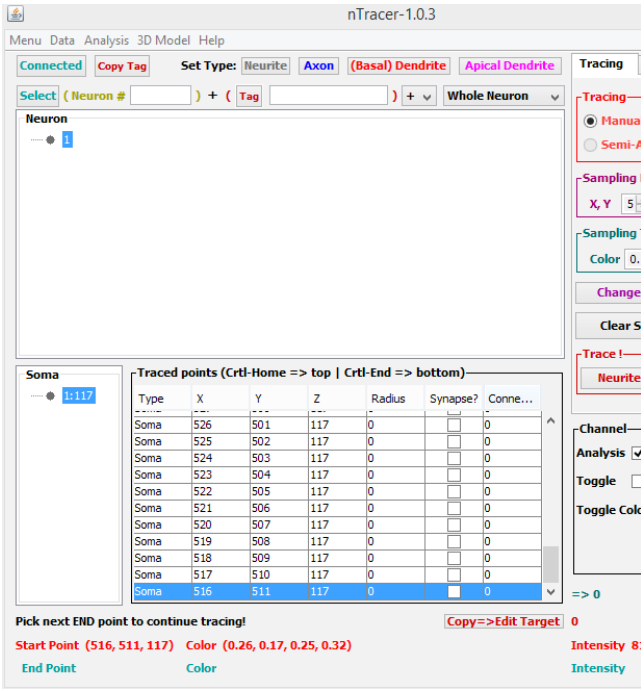
vii. Scroll up/down to the next z-position and repeat.

You can use the two methods interchangeably.

Ctrl z

You can un-do tracing by pressing <Ctrl-z>.

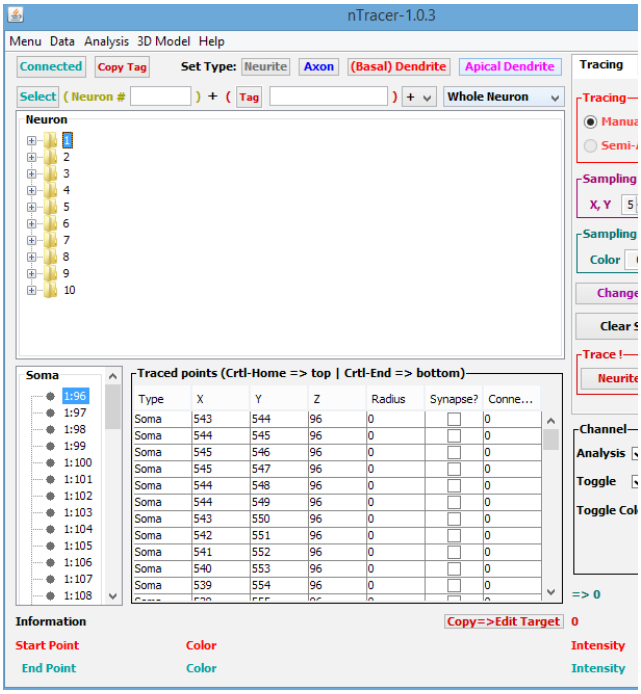
Upon creating a trace, you will see your first soma trace liste=d in the control panel.



In this example, the trace was done for a soma in image slice #117. This new entry created a new neuron <1> in the Tracing Diagram. Additional traces of this neuron will be listed under <1> as they are generated, and each entry will be accessible through the Soma and Tracing Points windows.

For soma trace, entries appear as numbers in the form A:B. 'A' represents the neuron identity (1 as this trace is part of the first neuron to be traced) and 'B' represents the z- coordinate.

Using the Interactive and/or the Freehand tracing method, trace through the entire stack to outline the soma. Once you finish tracing the soma of neuron <1>, make sure you don't miss any layers by examining the soma data box in the control panel.

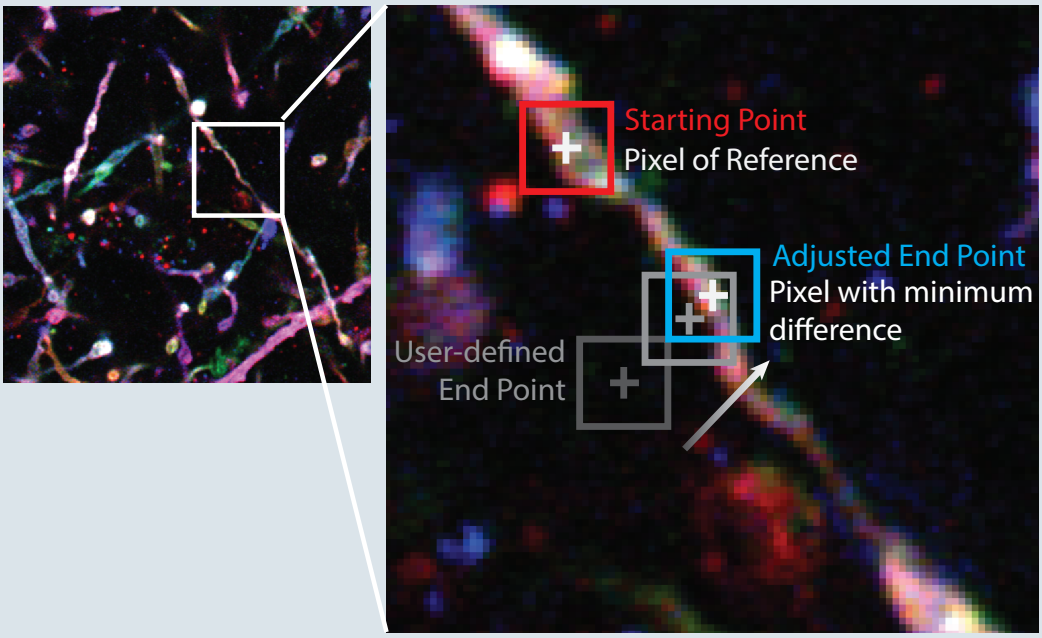


Here, the entire stack has been traced and entered for the soma of neuron <1>. All traces from different slices are listed as entry 1:96, 1:97, 1:98,..., etc in the soma databox.

Take a look at the A:B values.
If the A value is different in some entries, that means the program doesn't recognize those traces as being part of the same neuron. Delete that entry and re-trace, making sure that the right neuron is selected in the Tracing Diagram.
If the B value is not consecutive, you must have skipped a slice. Scroll to that position and trace to fill in the missing trace entry.

To trace the next neuron and its soma, <Triple right click> on the image or <Double left click> at the empty space in the Trace diagram to deselect the previous neuron and to allow creating a new neuron entry (in the above example, you can see traces have been made for ten different neurons, and a folder is created for each).

Tracing Algorithm



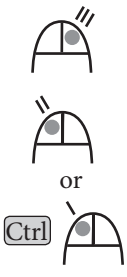
nTracer finds the tracing start point by moving a mouse click input to a local point with high intensity based on a mean-shift algorithm. The end point is determined by moving a second mouse click input to a local maxima that is similar to the start point in color and light intensity. An algorithm that determines series of pixels with the minimum total color and intensity difference relative to the two selected points calculates a path between the two points based on "Sampling Radius" and "Sampling Tolerance" in the control panel.

2. Trace Neurite

While soma can only be traced within each z slice, you can move freely through different z slices when tracing neurites. <nTracer> determines the path in-between the starting point and the end point in 3D.

Here, we will show you how to a) trace a neurite, and b) assign it to a neuron as its dendrite.

a-1) Trace Neurite



i. <Triple right click> to deselect all previous entries.

ii. Select a starting point.

*__Note:__ If tracing a projection from a soma, start from the soma by <double-clicking> on the soma trace. If not, <Ctrl-click> where the neurite first appears with to define the starting point (red box).

iii. Follow the path of the neurite, scrolling through the z-stack if necessary, and <Ctrl-click> once more to set the end point (blue box).

Ctrl or Alt

*__Note:__ You can use <Alt-click> instead of <Ctrl-click> to override the color restriction set by "Sampling Tolerance" and constrain the point selection to the current z slice.

Point Selection

There are three different methods of defining an end point:

Ctrl — Use the mean-shift algorithm with variables defined in "Sampling Radius" and "Sampling Tolerance" to find the point that is most similar to the starting point

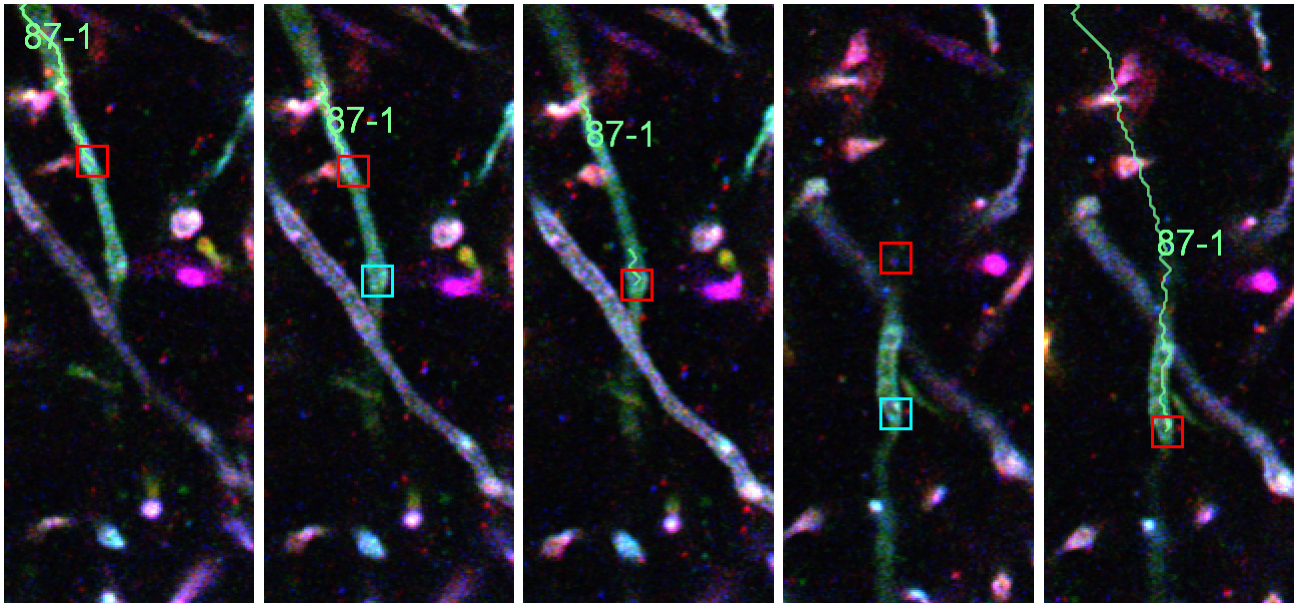
Alt — Use the mean-shift algorithm to find the end point, but using only X,Y and Intensity Tolerance as variables (End point is on the same image slice as the starting point)

Ctrl Alt — Overrides the mean-shift selection algorithm to manually select a point

a

iv. Press hotkey <a> to connect the points and create an entry as a neurite.

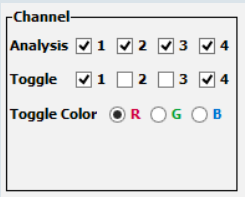
v. Continue tracing by repeating steps iii-iv.



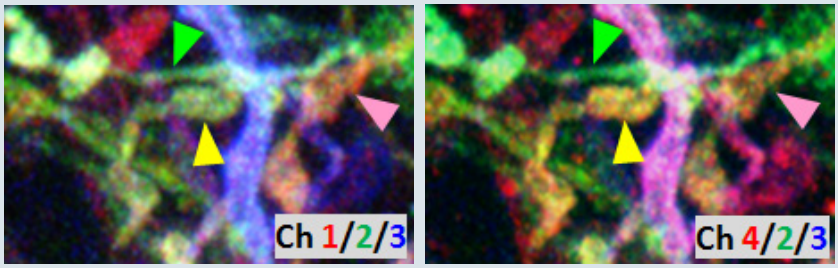
Toggle Color

If two neurons are very similar in color, and you are unable to identify which is which, you can toggle between different combinations of color channels. For instance, if you acquired the image through four different channels, you can assign channel 1 and 4 to the same color (in this case, red) and toggle between color combination 1,2,3 and 2,3,4 using the hotkey <1> or <4>.

1 4



Red color is set to toggle between channel 1 and 4

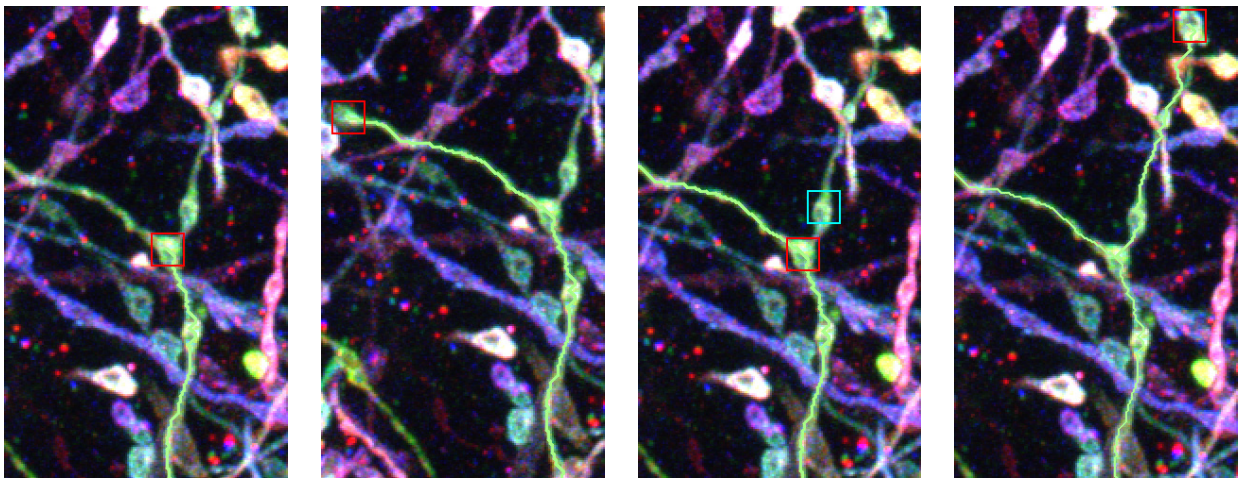


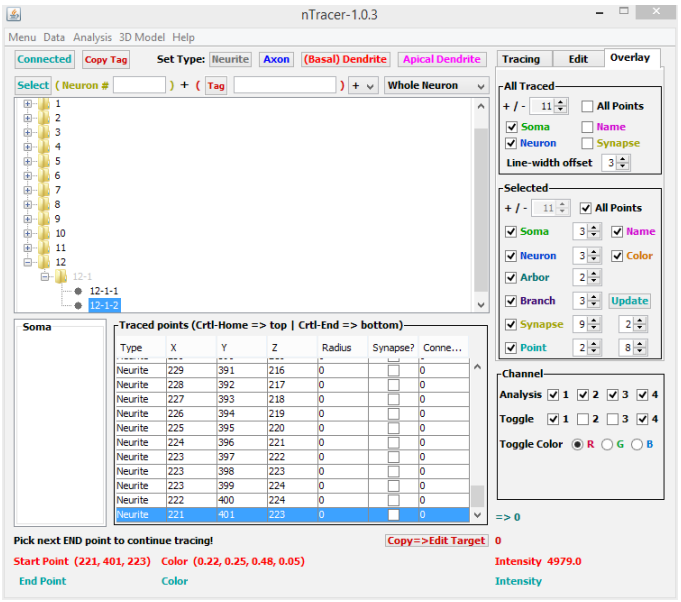
Channel 1 as red color (left) and channel 4 as red color (right). Compare how the three neurites (arrowheads) can be distinguished from each other by toggling the 4th channel.

a-2) Branching

While tracing a neurite, you may come to a fork, where the neurite splits into two or more branches.

i. Continue tracing neurite along one of the branches.
ii. Return to the fork and <double click> to select the branching point on the trace and start tracing along the second branch.
iii. You will be asked if you want to 'add a new branch.' Click "Yes".
iv. The new branch will be assigned a new name.





Creating a branch will automatically be recorded in the Trace Diagram, and each branch will be listed under the primary branch.

In this example, secondary branches 12-1-1 and 12-1-2 is created under primary branch, 12-1.

***Note:** As you encounter multiple branches, it may be hard to keep track of all the branches that are left unfinished and require you to go back to continue tracing. One method to make this easier is to use the <Change 'completeness'> option in the Tracing toolbox. When you encounter a 'fork in the road' and switch to start tracing a new branch, clicking on 'completeness' or pressing hotkey <x> will mark the current trace as 'incomplete' and its name on the Trace Diagram will be faded grey. Each time you encounter a split in the neurite, you can mark one branch as 'incomplete' and continue tracing the other one. You can revisit all the points marked as 'incomplete' by clicking <Jump To Next 'Incomplete'> option in Edit toolbox or by pressing hotkey <i>. Once you finish tracing the segment, click on <Change 'completeness> or press <x> to remove the 'incomplete' label.

***Note:** <Triple left click> will automatically select the end point of the trace. This is a convenient way to continue where you left off.

b) Assigning Types and Tagging

In the control panel, you can assign identities, such as axon or dendrites, to neurites that you traced by clicking on one of the options listed under 'Set Type.' This assignment is reflected in the Trace Diagram as color labels.

You can also "Tag" a neuron with keywords separated by semicolon.

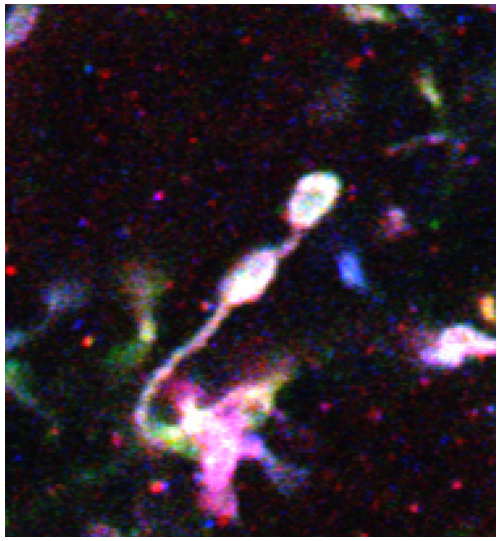
For more information about how to use the Tag option, refer to page 21.

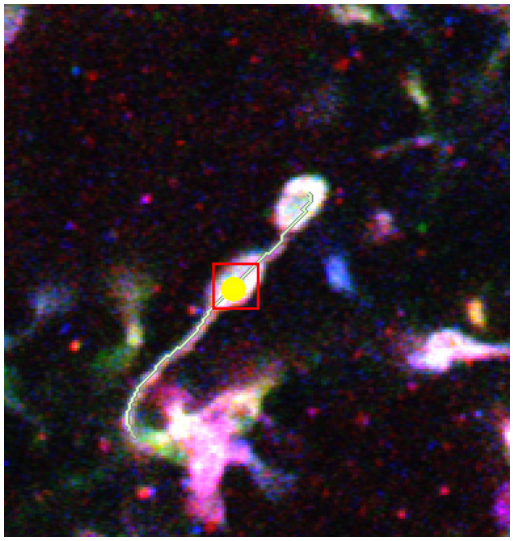
3. Identify Synapses and make Connections

While tracing the neurite, you might have noticed some places where the neurite bulges, or appears as a bulb, with increased color contrast and brightness. This is a synapse. On your traces, you can mark the synapse locations and specify its connection.

i. <Double left click> on the trace where you see a synapse to select the point.

ii. Press hotkey <e>. A yellow circle will appear to indicate the synapse, and a check mark will appear next to the corresponding point in the Traced Points data box of the control panel.





*__Note:__ To speed up the tracing process, you may want to mark the synapses as you trace the neurite. Select the synapse as the end point and press <a> followed by <e> for each tracing segment.

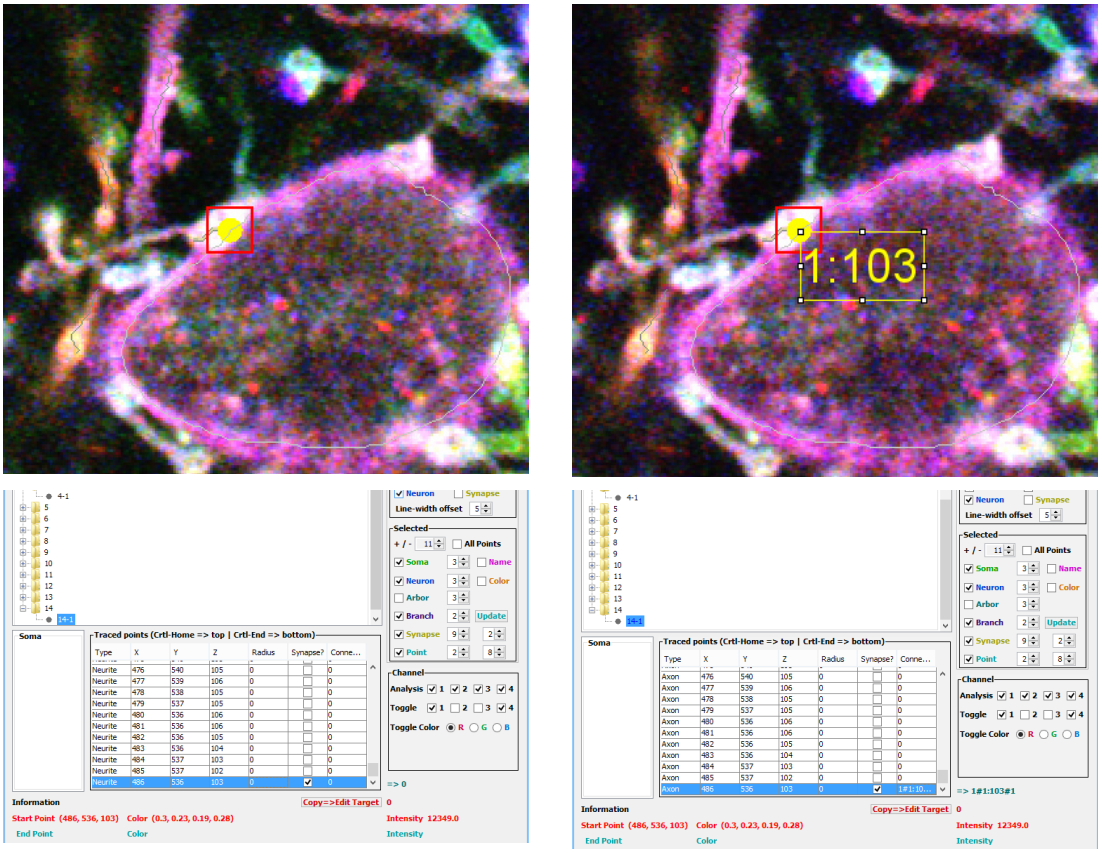
Occasionally, a synapse will form between two neurons. You can make note of this by marking the connection.

i. Before making a connection, a synapse should have been created and both elements assigned its identity (soma and axon, in the example below).

ii. <Double left click> on the image to select the point of synapse, or directly from the Traced Points data box (the point with the 'Synapse?' box checked).

iii. <Shift-left click> the second element, in this case a point on neuron #1's soma. A yellow box with coordinates of the selected point will appear. Make sure you have selected the correct neuron.

iv. With both parties selected, press hotkey <n> to form a connection.

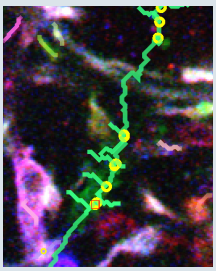


Upon making a connection, a green circle is created on top of the corresponding synapse. In addition, an identical green circle will appear on the point of the soma (or dendrite) closest to the synapse indicating the pre and post-synaptic sites. The data box will also reflect the connection next to the corresponding point.

Dendritic Spines

Depending on your research interest, you might want to indicate the spines on dendrites. You can do so by tracing the spines as you would a neurite branch, and pressing <d> to assign the trace as a spine. This will not create a new dataset in the control panel, but instead will assign the spine trace information to that point on the neurite, similar to how synapses are assigned.

d



Traced points (Crtl-Home => top | Crtl-End => bottom)

Type	X	Y	Z	Radius	Synapse?	Connection
Dendrite	646	752	96	0	☐	0
Dendrite	647	751	95	0	☐	0
Dendrite	648	750	95	0	☐	0
Spine#30	649	749	94	0	☑	0
Spine#31	650	748	94	0	☑	0
Dendrite	651	747	94	0	☐	0
Dendrite	652	746	94	0	☐	0
Dendrite	652	745	93	0	☐	0
Dendrite	652	744	93	0	☐	0
Dendrite	651	743	94	0	☐	0
Dendrite	652	742	93	0	☐	0
Dendrite	653	741	92	0	☐	0

Information for all the spines on a neuron, along with synapse information, can be exported as an Excel file by doing Data>Export Synapse.

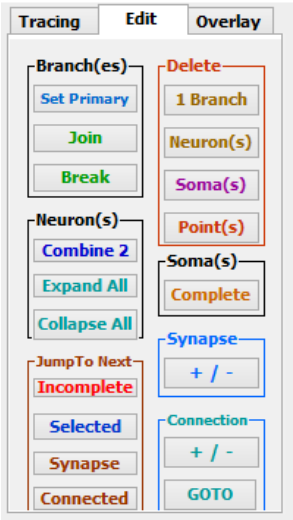
e

To remove a spine, select the point of interest and press <e> or use the “Toggle Synapse” button.

IV. Editing and Overlay tutorial

Once you have traced all the components of your interest from the virtual image stack, you have the option to edit the tracing results and adjust the visual output (overlay) to better suit your needs. The menus are self-explanatory and a short description appears upon rolling your mouse over each menu option. Here, we go through some examples of using the 'Edit' and 'Overlay' toolbox, but it is recommended that you do hands-on exploration to get a better sense of when and how to use each function.

1. Editing



The Edit toolbox gives you the option to manually edit your tracing data and image during or after the tracing process.

A few of the panels, such as the "Soma(s)", "Synapse" and "Connection" panels, are used in the tracing tutorial. While the tracing tutorial suggests the use of hotkeys for these editing functions, you may use the Edit toolbox as well.

The rest of the panels will generally not be used during tracing, but are instead available in case a mistake in tracing is discovered later.

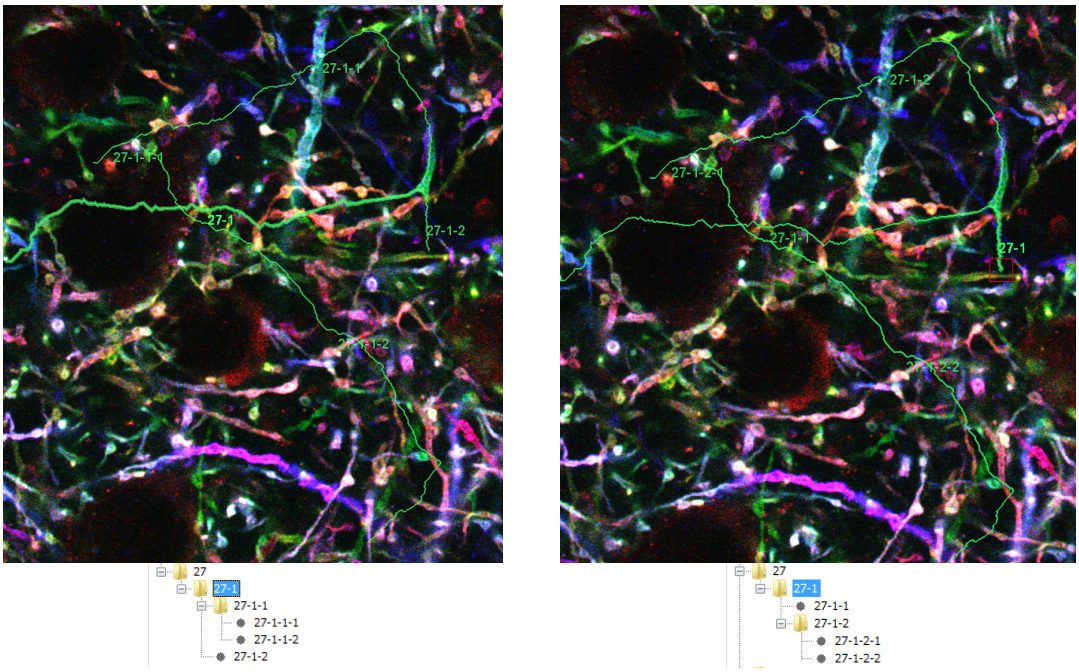
a) Branch(es)

Adjust how different branches of neurite are recognized by the program.

i. "Set Primary"

Change which branch of a neuron is recognized as its 'primary' branch.
The default in nTracer is to assign the first neurite to be traced as the primary branch. However, there are times when the branch order needs to be edited post-tracing. In such case, simply select the branch that needs to be assigned as the primary and click <Set Primary>. The change will take place and branch order will be reorganized accordingly.

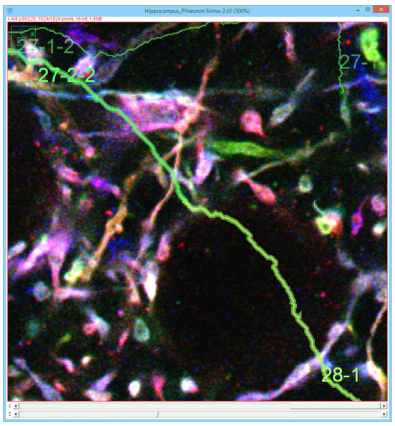
Example of using “Set Primary” to switch from left to right.



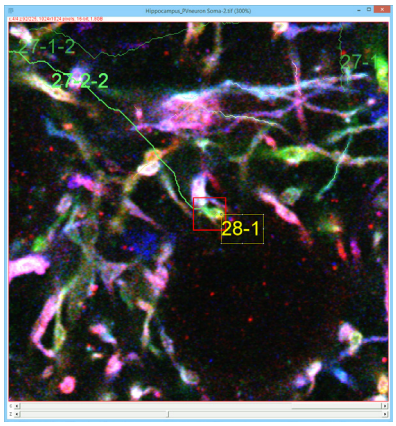
ii. “Join” & “Break”

Join two separate branches from the same neuron and/or break a branch into two.

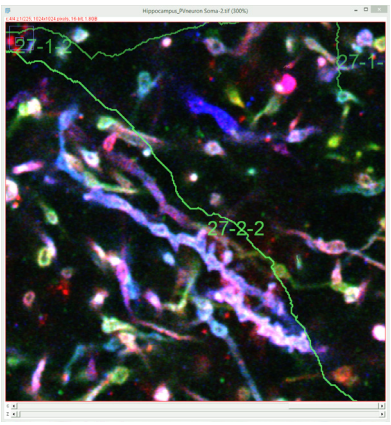
To join two neurites, select one branch from the Trace Diagram and <shift-left click> on the second branch from the image. Press “Join” to join the two branches into one continuous branch.



Two separate branches, 27-2-2 and 28-1, to be joined



Branch 27-2-2 is selected from the Trace Diagram and 28-1 is co-selected from the image.



Two are joined, and become a continuous branch, 27-2-2.

Use the “Break” option to break a trace into two.

Select the point at which you wish to split the trace into two and press <Break>.

Doing so will create a new neuron and list the broken-off trace as a separate neurite.

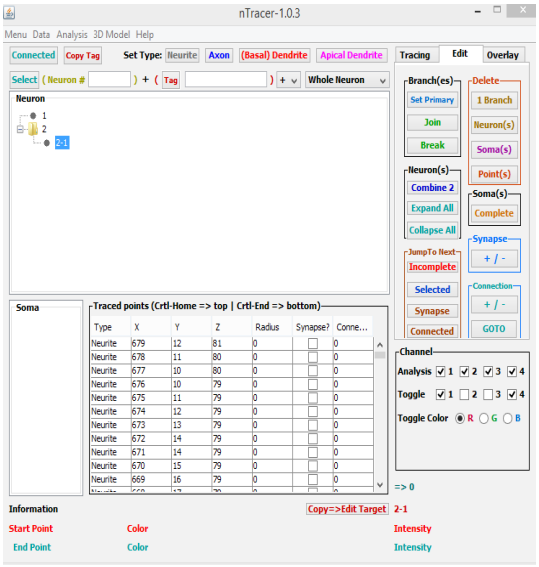
b) Neuron(s)

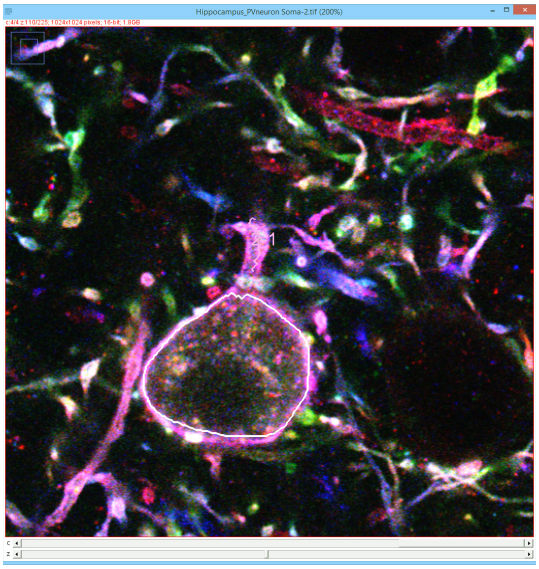
This panel allows you to combine separate neurons and navigate the Trace Diagram.

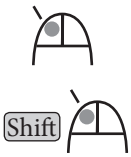
i. “Combine 2”

You may find that a neurite you traced as a separate neuron is actually part of another neuron. In this case, you would need to ‘Combine’ the two neurons together.

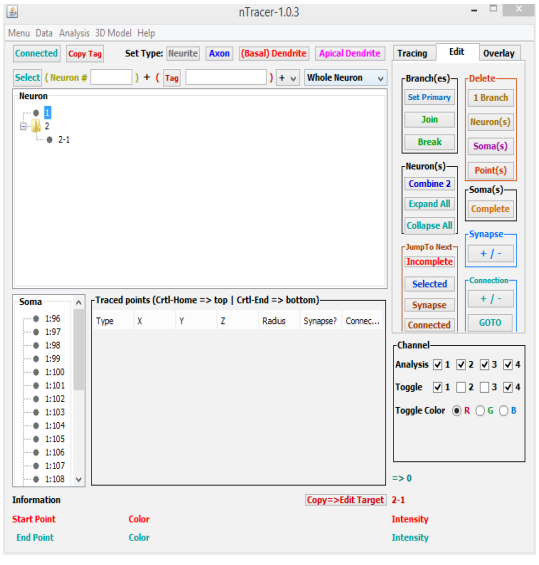
In the following example, a new entry <2> is created for a neurite. However, it actually is a dendrite of neuron <1>. So we want to combine <2> with <1> and assign the neurite as a dendrite.

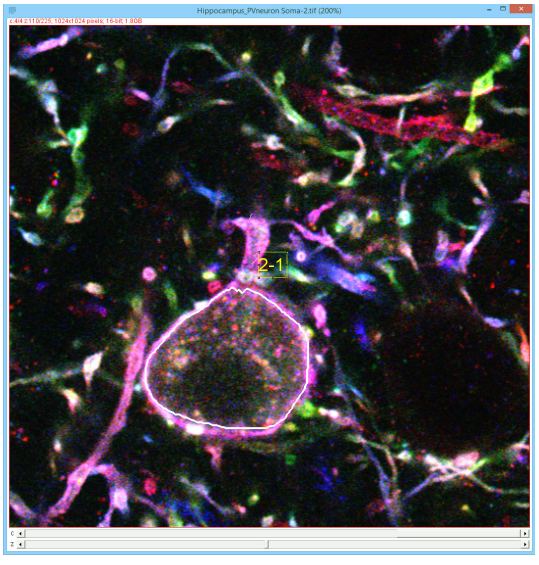






a. Select neuron <1> from the Trace Diagram in the control panel
b. Select (<Shift-click>) the neurite <2-1> from the screen. A yellow text will appear to indicate the selection.



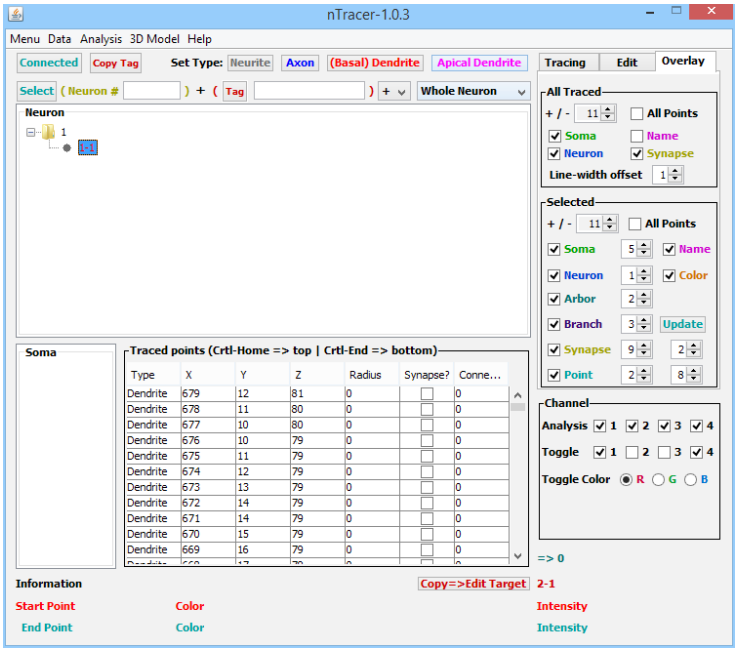


c. Click <Neuron(s) 'Combine2'> in the Edit Toolbox.

This will merge neuron <2> with neuron <1>, and thus change its name from <2-1> to <1-1>. Now the neurite belongs to neuron <1>.

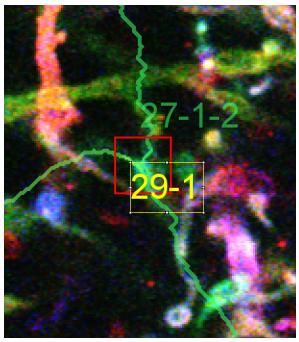
d. Select <1-1> and click 'Dendrite' under 'Set Type' menu to assign its identity.

Now <1-1> appears red in the Trace Diagram and its new identity is reflected in the Traced points window as below.

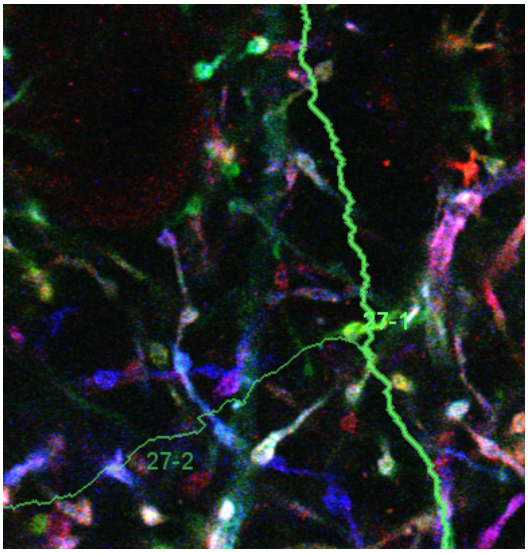


***Note:** "Combine 2" can also be used to join two neurites:

After tracing two neurites as separate neurons, you might realize they are really a part of the same neuron. This gives you the option to combine components from different neurons and merge them into a single neuron.



Select one of the neurons to be combined from the Trace Diagram, and <shift-left click> to select the other one from the image. Press "Combine 2" to merge them into a single neuron.



ii. "Expand All" & "Collapse All"

Expand or collapse the neuron folders in the Trace Diagram.
These options help you navigate the diagram with ease.

c) Jump to Next

i. "Incomplete"

Pressing "Incomplete" will select traces that are marked as being "incomplete" (refer to the note under Ch.1-a2).

ii. "Selected"

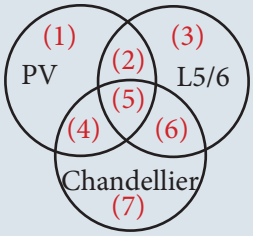
Navigate through neurons selected by its name and/or under a common tag as specified in "Select" panel.

Tag

You can attach a 'tag' to a neuron or group of neurons according to your research and analysis needs. For instance, let's say you have identified some of the neurons you have traced to be Parvalbumin-positive GABAergic neurons, which some but not all are Chandellier cells, and you have sorted them out by cortical layer location. You can tag each neuron with tags such as 'PV' 'L5/6' 'Chandellier' separated with a semicolon (;) to indicate this in you trace, and make selections based on your tags.

***Syntax:**

=	exact match	=PV;L5/6	cells that are tagged with "PV;L5/6" and nothing else (2)
&	both	PV&L5/6	all PV+ cells that are in layer 5/6 (2) (5)
|	or	PV|L5/6	cells that are either PV+ or in layer 5/6 (1) (2) (3) (4) (5) (6)
!	not	PV!L5/6	PV+ cells that are not in layer 5/6 (1) (4)



iii. "Synapse" & "Connected"

Jump through the points of a selected neuron marked as a 'synapse' or as being 'connected.'

d) Delete

Ctrl 

Delete a set of tracing data by selecting the data from the Control Panel (select multiple neurons or points by <Ctrl-left click>) and pressing the corresponding option under the "Delete" panel.

e) Soma - Synapse - Connection

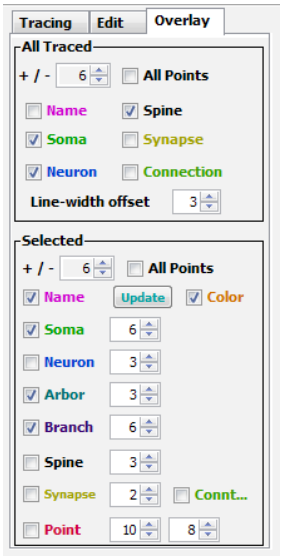
Most of the functions carried out in these panels have been described in Ch.III as hotkeys, since they are extensively used during tracing.

The “GOTO” option under “Connection” panel can be used to cycle through connected points. If a point on a neurite is involved in a connection with a soma, select the point on the neurite, then click “GOTO.” The corresponding connected point on the soma will then be selected.

2. Working with Overlay

The Overlay toolbox gives you the option to change the visual output of the traces. You can adjust the overlay to help make the tracing process easier, create a convincing 3D representation of your data, highlight and/or hide different elements of trace, etc.

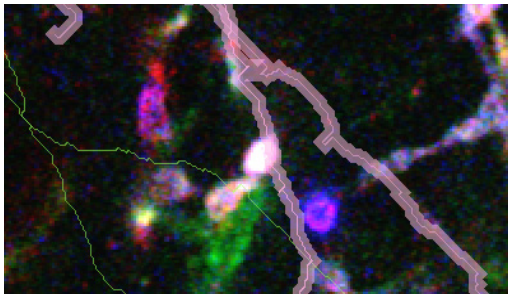
a) Basic Principles



The <All Traced> panel gives you the option of adjusting the overlay settings for your entire tracing. Select different components to have them in the image viewer. You can adjust the z-range from which you want to see the traces by setting the '+/-' option. Selecting 'All Points' will show tracings from the entire stack on a single plane. 'Line-width offset' adjusts the thickness of the traces in relation to those that are selected (see below).

The <Selected> panel will adjust the overlay for a (or group of) selection. Changing the numbers next to each component will change the line thickness. Also you will notice that line thickness of non-selected traces gets changed accordingly, based on the 'Line-width offset' value. For instance, the pink neuron is selected below and is set to have a thickness of 11. With an offset value of 10, the non-selected green neuron is shown with a thickness of 1.

Different settings can be applied to different parts of the same neuron to differentiate, for example, the primary branch from the secondary branch, or the axon from dendrites. You can adjust the setting to hide what you do not need to be visualized as well.

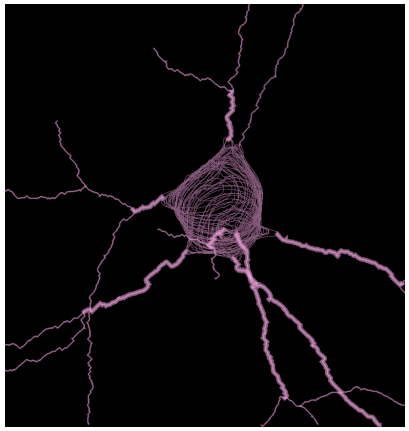


- Name: Lists names of individual trace on the image. *It may be helpful to make this invisible during tracing.*
- Soma: Soma trace by layer. "All points" option does not apply.
- Neuron: Neurite trace of selected neuron, or the entire trace if selected under "All Traced."
- Arbor: Shows the entire arbor tree of the selected neurite, starting from the primary branch.
- Branch: Shows the selected branch (ie, each segment as listed in the trace diagram).
- Spine: Shows the spines traced on the selected neuron, or all the trace if selected under "All Traced."
- Synapse: Controls the visibility and size of the synapse marker.
- Connection: Shows the connections made by the selected neuron as a pair of green circles.
- Point: Controls the size and thickness of the selection box.
- Color: The default color of traces resembles that of the neuron as imaged. Turning this off will display the neuron according to its assigned type.

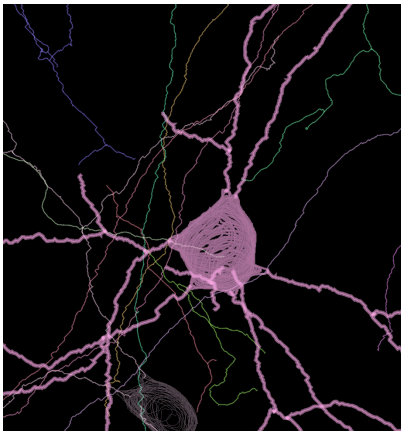
b) Rendering Examples

Following images show examples of image rendering that you can achieve by adjusting the overlay.

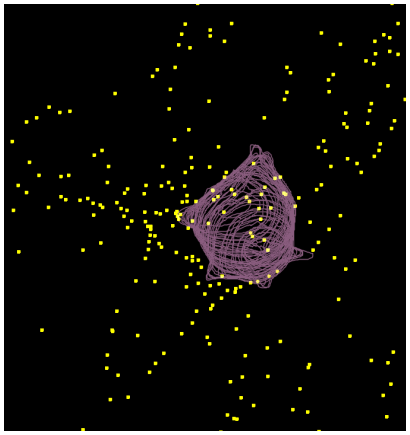
Images were created using <3D Model -> Skeleton> option from the nTracer menu.



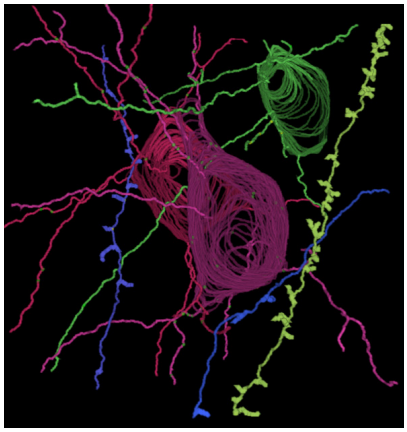
A single neuron rendered with its primary branches highlighted



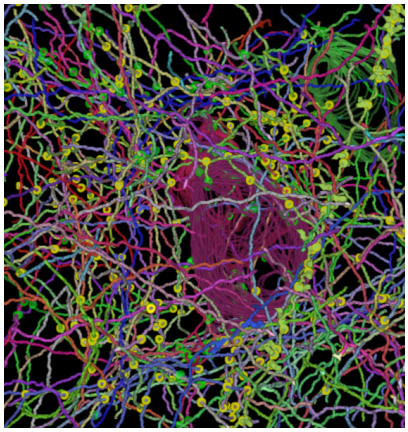
A select neuron highlighted against other neurons



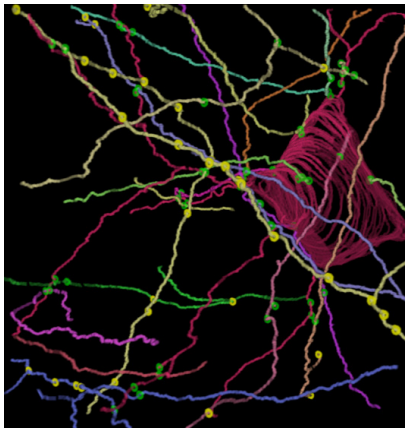
A soma and location of synapses of surrounding neurites



Rendering of spines along with group of other neurons



Reconstruction of a sample with dense neural projections



Neurons that make connections to the red neuron

In combination with the image processing tools of ImageJ, nTracer Overlay provides effective visualization option for complex microscopy data. Experiment with different Overlay settings and rendering option according to your purpose of producing the visual output.

V. Export and Analysis

1. Export Data

nTracer allows several different options to export the tracing data. Here, we will briefly go over each of the options to prepare the data for further analysis and/or visualization.

a) SWC

The raw tracing data can be exported as a SWC file. A SWC file is a text file with a header beginning with a # character, and a series of numbers referring to each trace points.

```
# CREATION_DATE 2015-05-14
# Hippocampus_PVneuron Soma-2_Neuron-3_stdSWC.swc
# CREATED BY nTracer1.0
# Dawen Cai, University of Michigan (dwcai@umich.edu)
#
# standard SWC format (n T x y z R P)
# n = point indentifier
# T = type indentifier:
#  0 = undefined; 1 = soma; 2 = axon; 3 = (basal) dendrite;
#  4 = apical dendrite; 5 = fork point; 6 = end point; 7 = custom
# x, y, z = cartesian coordinates (pixel)
# R = radius at the point (pixel)
# P = parent point; P = -1 indicates the origin point

1 0 290 979 1 0 -1
2 0 291 978 2 0 1
3 0 292 977 2 0 2
4 0 291 976 2 0 3
5 0 290 975 3 0 4
6 0 291 974 4 0 5
7 0 290 973 5 0 6
8 0 289 972 6 0 7
9 0 288 971 7 0 8
10 0 287 970 7 0 9
11 0 288 969 7 0 10
12 0 287 968 8 0 11
13 0 287 967 8 0 12
14 0 287 966 8 0 13
15 0 286 965 9 0 14
16 0 285 964 9 0 15
17 0 285 963 9 0 16
18 0 285 962 9 0 17
19 0 285 961 10 0 18
20 0 285 960 10 0 19
21 0 286 959 11 0 20
22 0 285 958 12 0 21
23 0 284 957 12 0 22
24 0 283 956 12 0 23
25 0 282 955 12 0 24
```

To export a SWC file, select the neuron(s) of your interest from the image or the trace diagram and go to <Data -> Export SWC> from the nTracer menu. Select the destination for the SWC file, and correct the xyz resolution according to the condition of your image acquisition.
An example of a SWC file is shown on the left.
The generated SWC file can then be imported into other applications, such as Microsoft Excel or MATLAB, to carry out further trace analysis.

***Note:** You can also export synapse information of the selected neuron(s) in <Data -> Export Synapse>. This option will generate an excel file that contains the xyz coordinates for all synapse points.

b) 3D Model

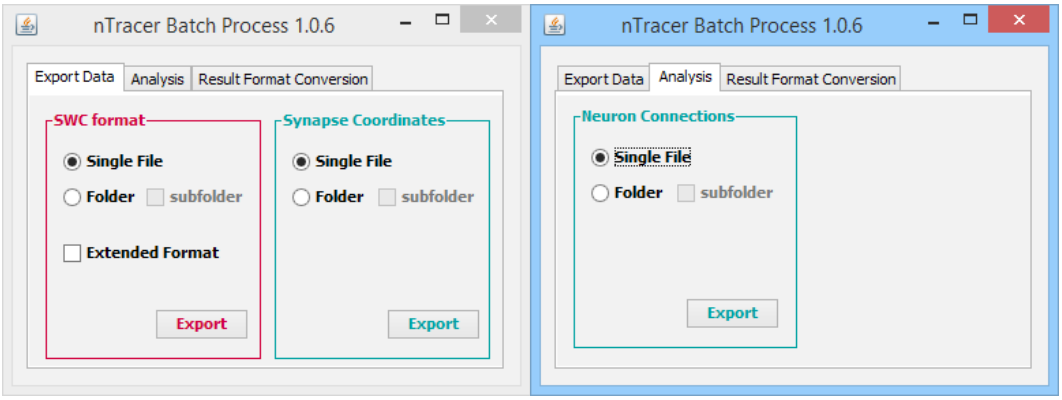
<3D Model -> Skeleton> function gives you a simple way to visualize your tracings by creating a hyperstack of the overlay.
Adjust the overlay options to reflect the visual output you want.
At least one neuron has to be selected in order to process this function.
Go to <3D Model -> Skeleton>. The program will give you the option to create one image stack or multiple image stacks, each containing one neuron. The “Crop frame” option will crop the

image frame to fit the selected neuron(s). Leave this option unchecked if you wish to image the entire field. Press "OK" to start creating the image stack.
It may take a while for the image stack to be generated. You can indirectly monitor the process by looking at memory usage.

The new image stack of your trace will open up as a new window in Fiji. From here, you can handle the trace hyperstack as you would any other image stack using the 3D viewer and/or different image processing tools available in Fiji.

2. Batch Process

As part of the latest nTracer package, there is a separate program that allows you to export and analyze multiple datasets without opening nTracer. This is called "Batch Process" and can be located in the nTracer plugin folder.



There are two sub-menus that you can use in Batch Process: Export Data and Analysis.

a) Export Data: Export SWC files or as Synapse Coordinates from one or more tracing data

b) Analysis: Export Connections data from one or more tracing data