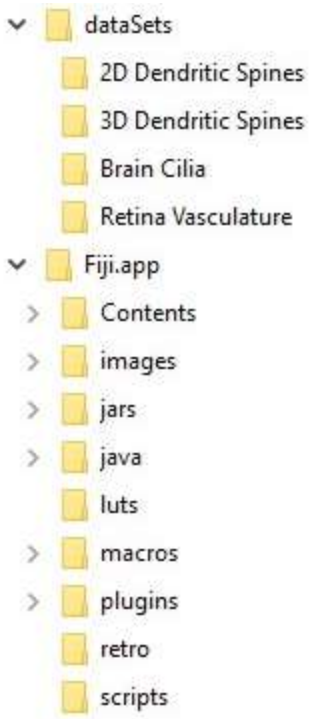
The Fiji.app contained within this zip folder is a version of the Fiji IJ distribution lacking some of the plugins. We have used Fiji with an additional plugin we developed and called Morphometry for our analysis.

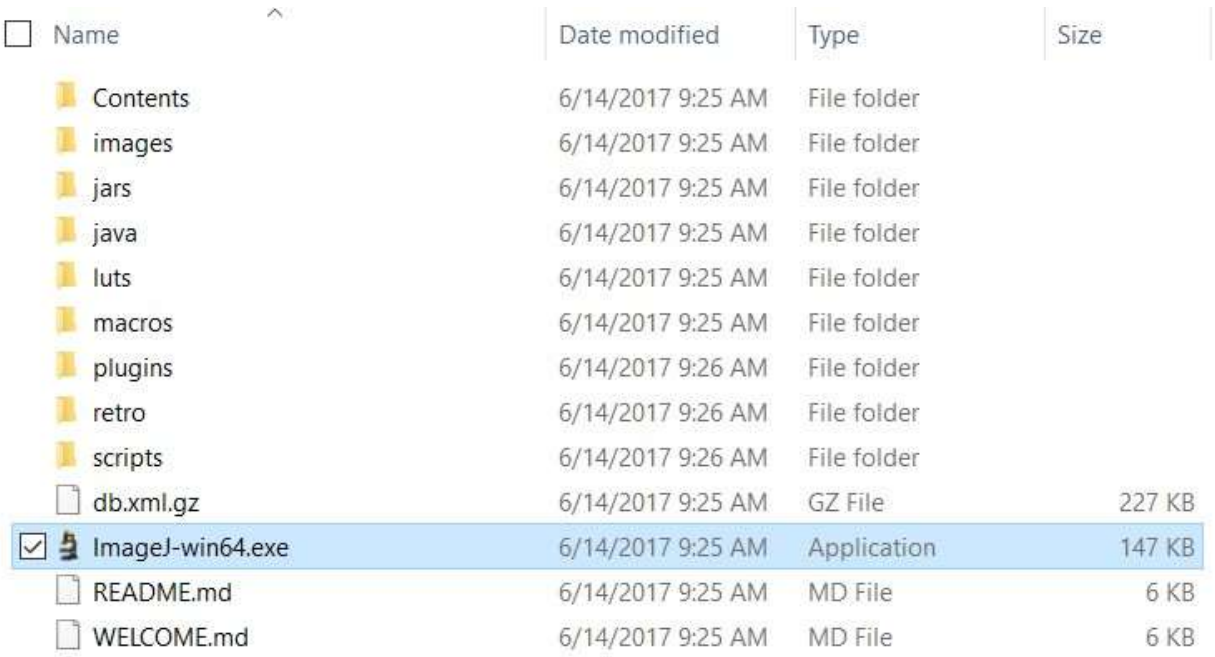
The folder contains a runtime distribution of Java8 which is necessary to run Fiji with our algorithm. The plugins folder of Fiji has also been trimmed down from the standard distribution to include our plugin BranchAnalysis 2D/3D and a few others necessary for image processing.

This set contains files in the structure as shown in the following image:



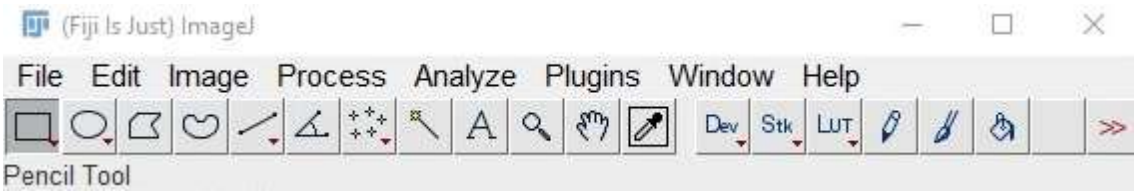
Drag the whole Fiji.app folder from the CD to the desktop, and follow the following steps.

To open Fiji ImageJ click on the highlighted application as shown below:

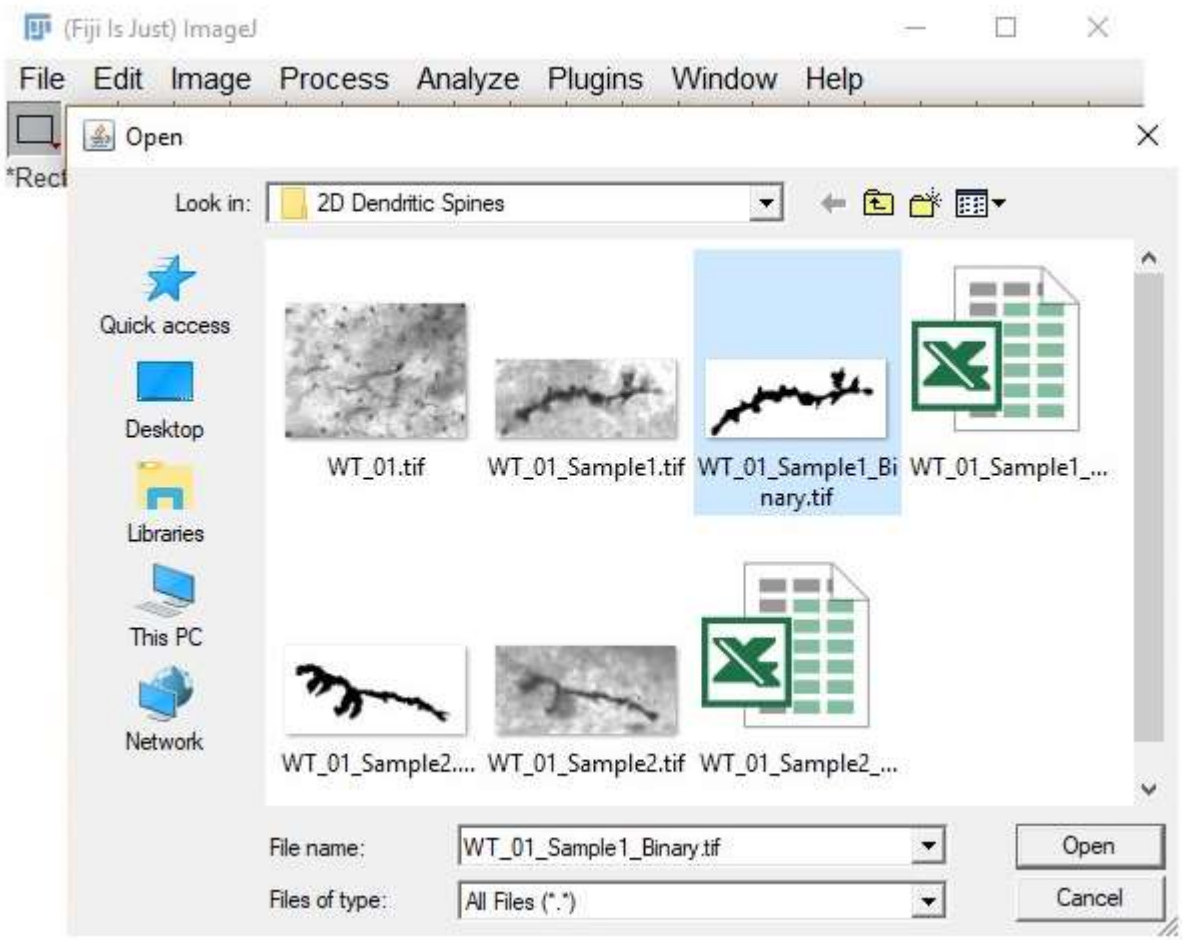


Name	Date modified	Type	Size
Contents	6/14/2017 9:25 AM	File folder	
images	6/14/2017 9:25 AM	File folder	
jars	6/14/2017 9:25 AM	File folder	
java	6/14/2017 9:25 AM	File folder	
luts	6/14/2017 9:25 AM	File folder	
macros	6/14/2017 9:25 AM	File folder	
plugins	6/14/2017 9:26 AM	File folder	
retro	6/14/2017 9:26 AM	File folder	
scripts	6/14/2017 9:26 AM	File folder	
db.xml.gz	6/14/2017 9:25 AM	GZ File	227 KB
ImageJ-win64.exe	6/14/2017 9:25 AM	Application	147 KB
README.md	6/14/2017 9:25 AM	MD File	6 KB
WELCOME.md	6/14/2017 9:25 AM	MD File	6 KB

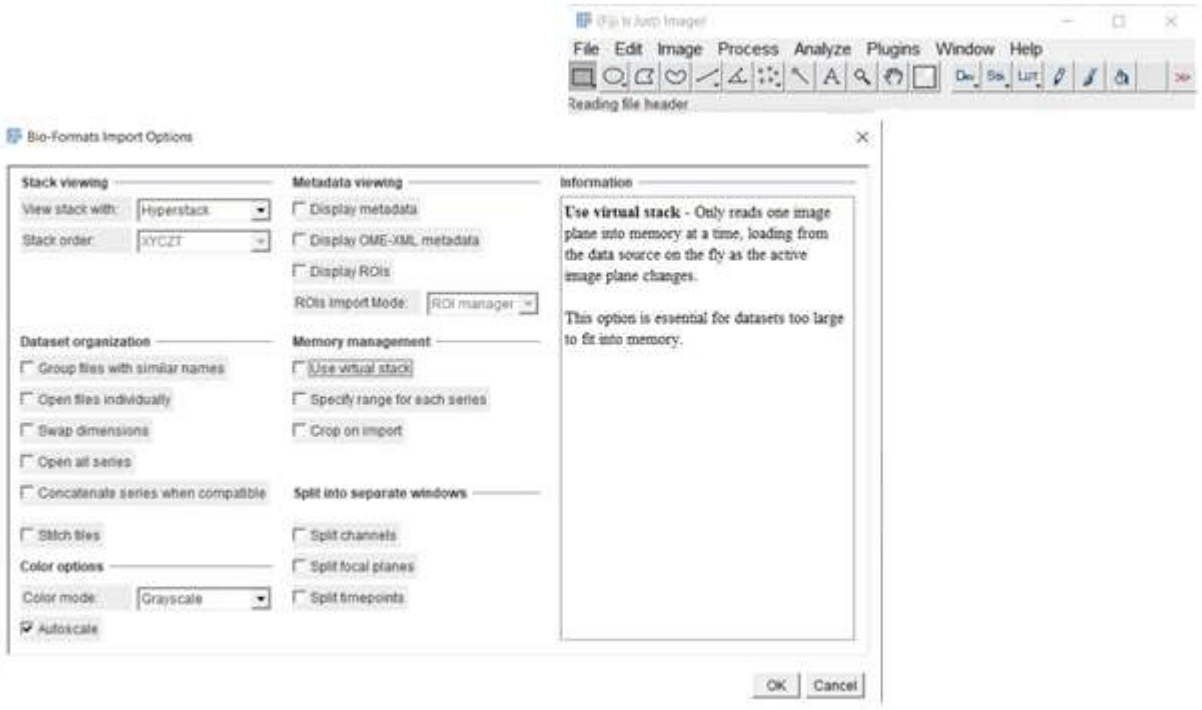
Clicking on ImageJ-win64.exe in the folder Fiji.app brings up the following dialog:



Through this dialog, one can open the image/stacks of interest provided in the sample dataSets folder (File->Open) as is shown here:


(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
*Rect
Open
Look in: 2D Dendritic Spines
Quick access
Desktop
Libraries
This PC
Network
WT_01.tif
WT_01_Sample1.tif
WT_01_Sample1_Binary.tif
WT_01_Sample1_...
WT_01_Sample2....
WT_01_Sample2.tif
WT_01_Sample2_...
File name: WT_01_Sample1_Binary.tif
Files of type: All Files (*.*)
Open
Cancel

For stacks, the following dialog will appear. Ensure the parameters follow those in the image, and accept.



Once opened, the images/stacks may require cropping for regions of interest. The datasets included here include a few sample cropped and segmented images to delineate the analysis performed through the plugin.

The pre-processing which we have most often used is for single channel images (2D spine data, 3D spine data, retina vasculature):

Process → Noise → Despeckle
Process → Noise → Remove outliers (Accept Default)
Process → Enhance Contrast (Accept Default) Image → Type → 8bit
Process → Binary → Make Binary (Accept Default)
Process → Binary → Fill Holes (Do not perform this step for stacks)

For primary cilia images, there is an additional step since the image has two color channels – nuclei in blue, primary cilia in green.

The pre-processing is, for primary cilia:

Image → Color → Split Channels

Using the red channel only:

Process → Noise → Despeckle Process → Noise → Remove outliers Process → Enhance Contrast Process → Binary → Make Binary

Note that adjusting these settings can lead to alterations in the binary image obtained, and the binary images that are generated may not resemble the binary images we present. Any extraneous pixel noise can be removed manually using the paintbrush tool in ImageJ, but proper pre-processing should make this step unnecessary. A more complete guide to image pre-processing can be found with the Fiji IJ documentation wiki (http://imagej.net/Segmentation).

If one is opening a file labeled _Binary only the following preprocessing will be required before proceeding to the analysis

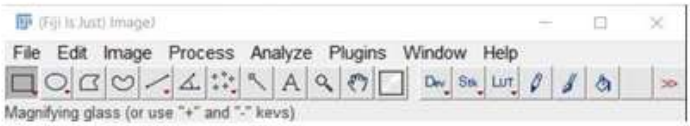
Process → Binary → Make Binary

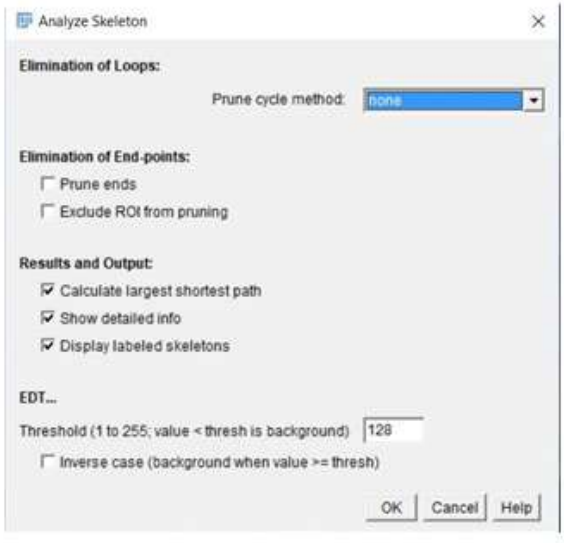
Once a satisfactory preprocessing has been done one can proceed to the analysis by clicking

Analyze → Morphometry → Branch Analysis (2D/3D)

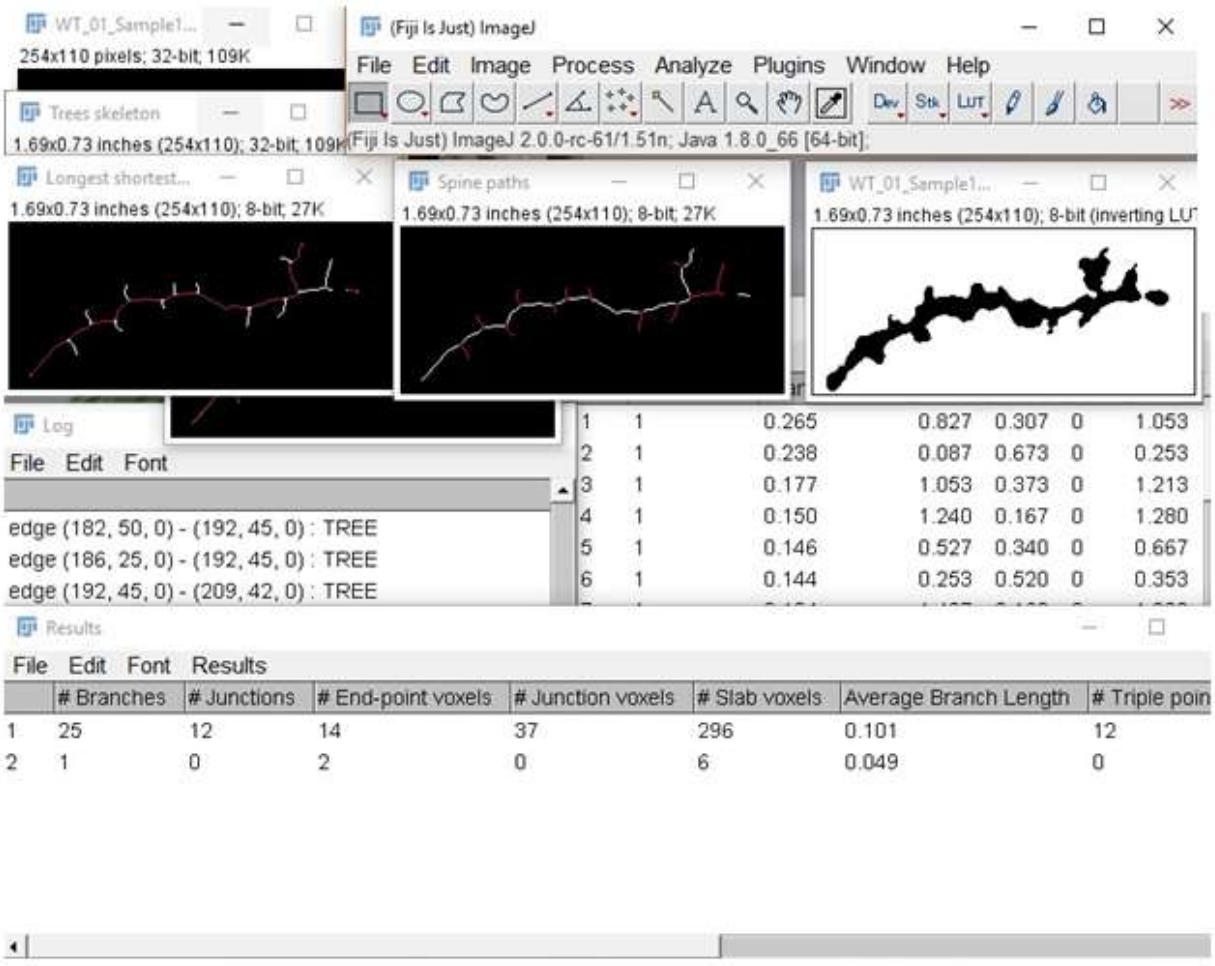
Branch Analysis (2D/3D) is part of the Morphometry submenu which we also developed.

The options available during analysis are shown in the screen below. For our analysis, we used the window presented below:





A capture of the mutiple windows that open during the analysis are shown in the following illustration which include the original images/stacks, the trees skeleton, a depiction of the longest shortest path, the spine paths, and the branch information which can then be saved.



This set contains data and samples from the following (each set includes the original, samples of the cropped and segmented sections of the original, a thresholded version of the samples (8 bit) and the resultant branch information) 1. 2D dendritic spines

2. Brain Cilia
3. Retina Vasculature
4. 3D dendritic spines (as Z-stacks)

It is important to note that input binary images must also be binarized via (**Process → Binary → Make Binary**) prior to analysis.