1. Download Latest version of FIJI: <https://imagej.net/Fiji/Downloads>

2. Unzip files and open FIJI

3. Install the following plugins:

Mexican Hat Filter

Vessel Analysis

Particle remover

4. Open FIJI, go to Edit -> Options -> Colors, make sure foreground color is black and background color is white

5. Open vein image

6. go to Edit -> Invert to invert color of image

7. go to Plugins -> vessel analysis -> vessel analysis(complete)

8. stop the process when the binary graph is generated

9. for the binary graph go to Plugins -> particle remover to remove all particles

10. After remove particles, use the image for diameter measurement and vascular density

I simplified the above steps by modifying the vessel analysis plugin. By replacing the file Vessel\_Analysis\_(complete).ijm in plugin folder, you should be able to skip steps from 8 to 10.

A clear image of vein can be obtained from the above procedure

The following steps incorporate the image of vein and the output from the pipeline. They are performed in a free software called QGIS. There is a python package for it. But I used its graphical interface. <https://qgis.org/en/site/>

1. import the vein image into QGIS

2. vectorize the image using the function in ‘Vector’

3. There are two python scripts: overlay.py and transform.py

4. First, run overlay.py in QGIS python console. Manually find two pairs of reference points, 1 from spot 1 from vein. The python script will calculate, scale, and rotate the vein geojson file.

5. Then, run transform.py, find 2 reference points, 1 from spot 1 from vein. The script will transform the vein geojson file to the appropriate coordinate system.