Vessel Analysis User Manual

The following manual offers instructions on using the *Vessel Analysis* plugin with *Fiji* (version 1.47g or higher required).

① Download & Save *Vessel Analysis*

Download the *Vessel Analysis.zip* package and save an unzipped copy in Fiji's *Plugins* folder. Open a new session in Fiji and you should now see *Vessel Analysis* listed near the bottom of the *Plugins* drop down menu.

Before using the plugin, make sure your version of Fiji comes pre-downloaded with the *Auto Threshold* and *Geometry to Distance* plugins. They should be listed under:

- o Image > Adjust > Auto Threshold
- o Analyze > Local Thickness > Geometry to Distance Map

② Using the Plugin: Pre-processing

Open a multi-channel image in Fiji and execute the *Vessel Analysis* plugin via *Plugins > Vessel Analysis > Vessel Analysis (complete)*. The "complete" program will guide you through pre-processing steps to prepare the image for vascular density and diameter measurements.

You may skip directly to *Vessel Analysis > Vascular Density* or *Vessel Analysis > Diameter Measurements* if you already have the appropriate pre-processed image.

As the image is processed, new images will be generated and automatically saved in the source directory of the original image. You may re-use these images for future processing.

Vessel Analysis (complete)

Using the rectangular selection tool, you will be asked to select the image area you would like to analyze. Once complete, click *OK*. A cropped image will be generated, followed by its binary version. The following measurements will be collected using this binary image.

Choose a metric...

You will now be prompted to perform either vascular density or diameter measurements. At the end of each individual program, you can choose to analyze the other metric as well.

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3 Using the Plugin: Collecting Measurements

Vascular Density

Provides measurements of vascular density & vascular length density (expressed as *%Area*).

   o *Vascular Density*: ratio of vasculature area to total selection area
   o *Vascular Length Density*: ratio of skeletonized vasculature area to total area

Using the polygon selection tool, you will be prompted to select a region of interest. Once complete, click *OK*.

Measurements will be recorded as percentages in 2 windows: *Vascular Density Measurements* and *Vascular Length Density Measurements*. Note that one or both windows may initially be minimized.

You will be then asked to analyze another region of interest. If so, the above steps will be repeated. Subsequent measurements will appear in the corresponding windows, in a new row below the last measurement.

Once you have collected all of your measurements, the vascular density program will terminate and you will be prompted to perform diameter measurements. If you choose to do so, you will be guided through the steps below. If not, the entire program will terminate; however, you will still be able to browse through the measurement windows.

Retaining the image: A copy of the image with the most recent ROI selection will be saved under a file name prefixed with "VD-". The *Vessel Analysis > Vascular Density* program can run directly on this image to generate further measurements.

Retaining the measurements: The measurement windows are not automatically saved - they must be manually saved (*File > Save As*) if you would like to re-access the data.

Diameter Measurements

Provides measurements of the average vessel diameter in the selected region (in mm).

Using the polygon selection tool, you will be asked to select a region of interest. Once complete, click *OK* and a coloured, skeletonized image will be generated.

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A visual representation: Each pixel's colour represents the relative thickness of the vessel in that area. Thicker vessels range from white to orange in colour, whereas thinner vessels vary from red to purple. See the following scale for reference: *Thin* ▬▬▬▬▬▬ *Thick*

You will then input measurements for scaling: enter a known distance in *pixels* and in *mm*. Generally, this will be the mm measurement of the image's full width. The corresponding pixel width of the image will be calculated by Fiji and noted in the image's title bar. This data will be used to formulate the diameter measurements.

Now, using the rectangle selection tool, select a region of interest and click *OK*. An overlay will be created to mark the ROI and the average diameter of the vasculature in that area will be superimposed as a label. These overlays and labels are only visible in Fiji. They do not alter the image and can be removed manually via *Image > Overlay > Remove Overlay*.

Retaining the image: A copy of the image with all overlays and labels will be saved under a file name prefixed with "EDM-". The *Vessel Analysis > Diameter Measurements* program can run directly on this image to generate further measurements.

The program will offer to measure multiple ROIs and will repeat the above steps if prompted. Once you have collected all of your diameter measurements, you can save a flattened copy of the image, which will merge all of the image's layers to allow the overlays and labels to be visible in external image viewers.

The diameter measurements program will terminate and you will have the option to analyze vascular density next. If you choose to do so, you will be guided through the steps above. If not, the entire program will terminate.

4 Further Reference

Sample images are provided in the *Vessel Measurements* package to showcase the various operations available in the *Vessel Analysis* plugin.

For more information on Fiji:

 o *Downloading Fiji*

 o *Installing a plugin*

To download the supplementary plugins:

 o *Auto Threshold*

 o *Local Thickness: Geometry to Distance Map*