

Retinal area analysis using ImageJ

ImageJ

<http://rsbweb.nih.gov/ij/>

Fiji (enhanced ImageJ, comes pre-loaded with useful plugins)

<http://fiji.sc/Fiji>

Retinal area analysis

1. Open image in ImageJ by either clicking File -> Open or by dragging the image into ImageJ
2. (Optional) If the image is too dim, enhance contrast by Process -> Enhance Contrast and select % value

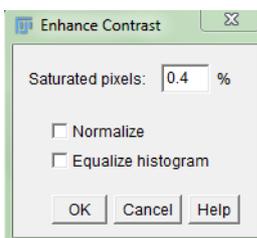


Figure 1. Enhance contrast window prompting user to select percentage value

3. Use either 'polygon selection' or 'freehand selection' tool to trace total retinal area

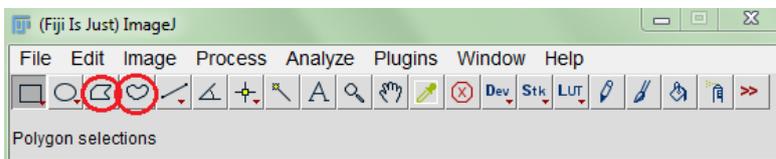


Figure 2. 'Polygon selection' tool is on the left (red circle) and 'freehand selection' tool is on the right (red circle).

Autofluorescence arising from retina is used to trace total retinal area. Notice the heavy contrast enhancement which allows visualisation between retina and background, and between avascular area and capillary network in the image below.

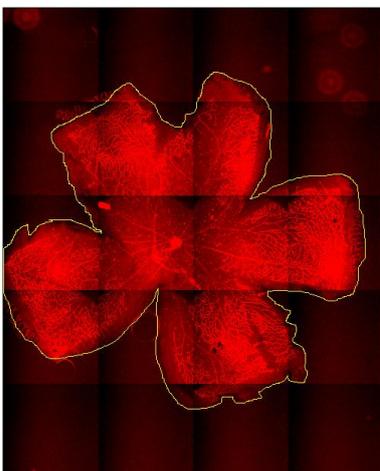


Figure 3. Trace of total retinal area.

4. The area is measured using Analyze -> Measure. Keep the table with area value opened
5. Trace avascular area and measure it by Analyze ->Measure

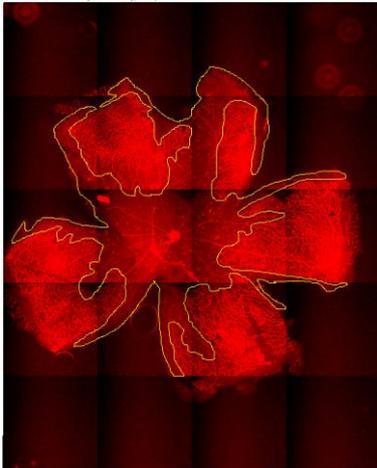
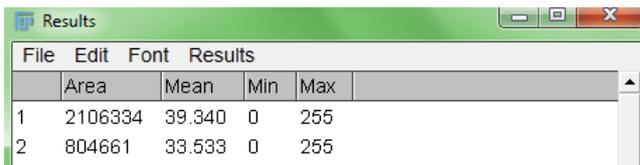


Figure 4. Trace of avascular area. Note that several more measurements of avascular areas will have to be traced for this image.

6. Copy two rows of values into Excel Spreadsheet. Keep only the 'Area' value



	Area	Mean	Min	Max
1	2106334	39.340	0	255
2	804661	33.533	0	255

Figure 5. Results table. The top row represents total retinal area and the bottom row represents avascular area. If more than one avascular areas are measured, add the values in Excel.

7. The values for the two areas pasted into Excel spreadsheet will most likely be in pixels (Figure 6). We need a meaningful value of distance (meters, μm normally). To convert the area measured in pixels to actual distance multiply the area measured in pixels by pixel size²



Figure 6. This information appears on top of the image when opened in ImageJ. Ignore 'cm' as it does not represent centimetres. First two numbers are x and y dimensions of digital image given in pixels.

In Figure 5, total retinal area is 2106334. This value is a product of multiplication of x and y image dimensions in pixels. Pixel size in actual distance units (normally μm) can usually be found in metadata .txt file (Figure 7). Multiply 2106334 by 2.564^2 to obtain area in μm .

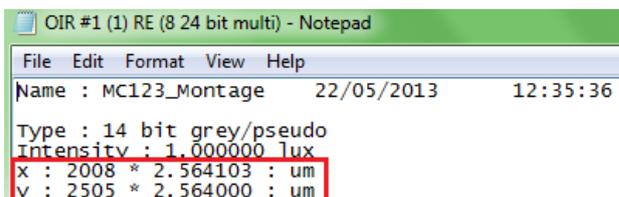


Figure 7. The first number in every row in red box signifies number of pixels that make up the digital image. The second number is the size of individual pixel. Using 10x magnification in this case produced a pixel with dimensions of 2.564 μm in both x and y.

8. Calculate percentage of avascular area: $(\text{avascular area}/\text{total retinal area}) \times 100\%$

The final table in Excel spreadsheet should look like this:

	Sample 1	Sample 2	Sample 3
Total Retinal Area (μm^2)			
Avascular Area (μm^2)			
% Avascular Area			