Supplementary material:

A tutorial guide to ImageJ and Vessel_width-plug-in

Original article: Indirect measurement of the vascular endothelial glycocalyx layer thickness in human submucosal capillaries with a plug-in for ImageJ

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1. Dowload ImageJ

Go to http://rsbweb.nih.gov/ij/download.html and dowload the version of ImageJ that matches the configuration of your hardware and operating system.

Save the ImageJ setup file on your computer and run the installation program. If necessary, use the ImageJ instructions on the download page (Instructions) to solve any platform specific problems encountered during the installation process.

2. Download the Vessel_width.jar

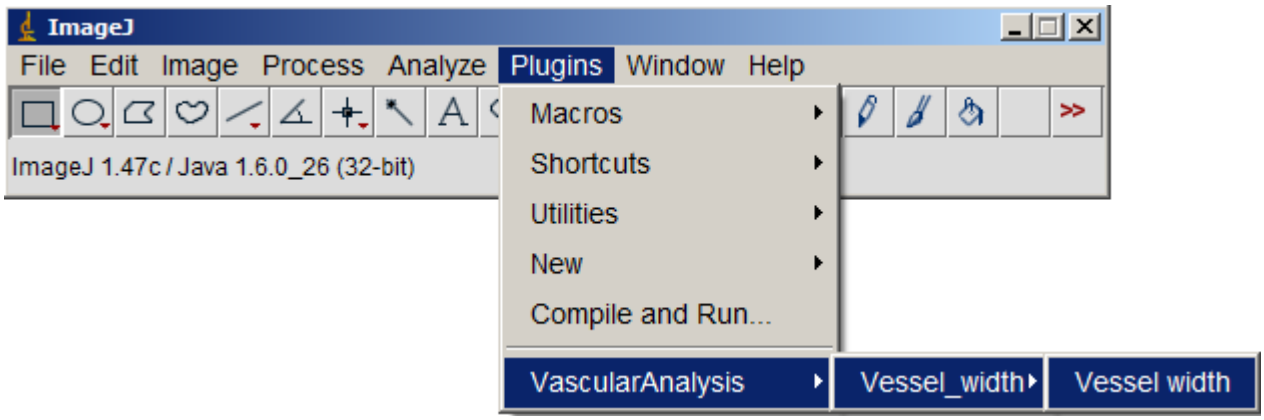
Go to http://www.bioinformatics.org/microcirc/ and download the “Vessel_width.jar” -file.

Place the “Vessel_width.jar” file at the Plugins folder of the ImageJ directory.

3. Run ImageJ.

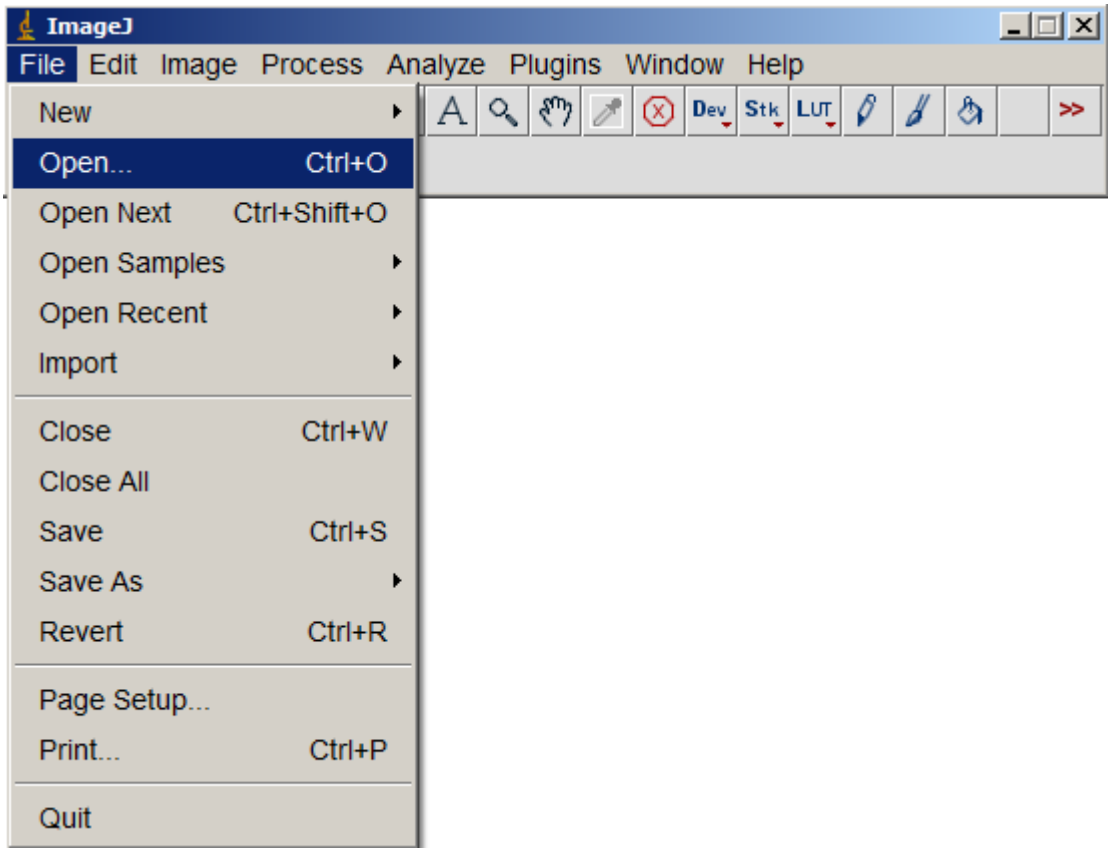
4. Locate the plugins icon and the Vessel_width plug-in

The Vessel_width plug-in should appear listed in the available plugins list under “Vascular analysis”. If not, make sure you have placed the Vessel_width.jar in the correct folder of the ImageJ directory.

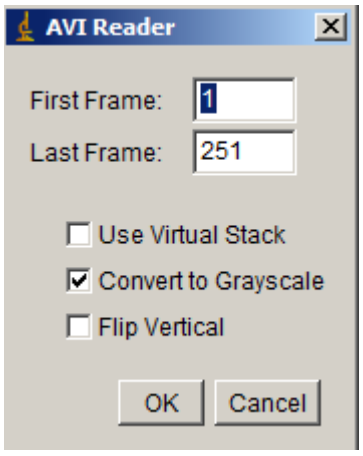


5. Open a microcirculatory AVI file with ImageJ

Open the AVI file by using the File -> Open command.

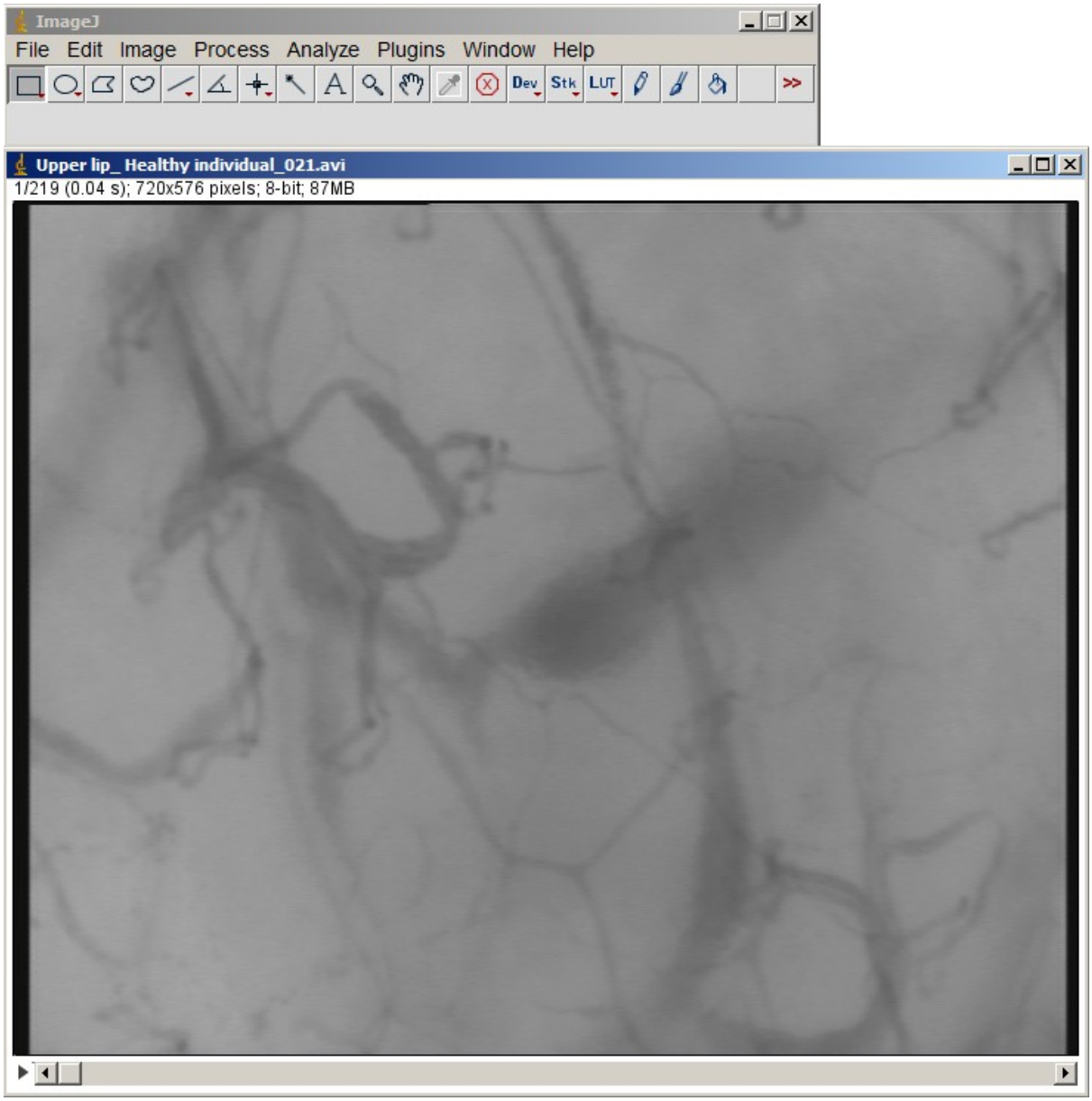


The ImageJ AVI reader box will appear. Choose the AVI frame numbers you want to open (default: First and Last frames of the file).



Optionally you may choose to convert the AVI file to 8-bit grayscale at this step. However, this is not necessary, as the Vessel_width plugin will temporally convert the opened AVI file to 8-bit grayscale to save memory and to make the measurement process faster.

An opened AVI file and the ImageJ command icons will appear like these:

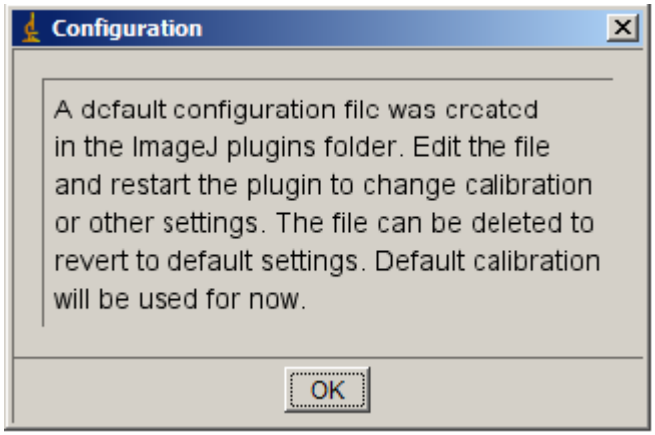


6. Activate the Vessel_width.jar on top of the opened AVI file.

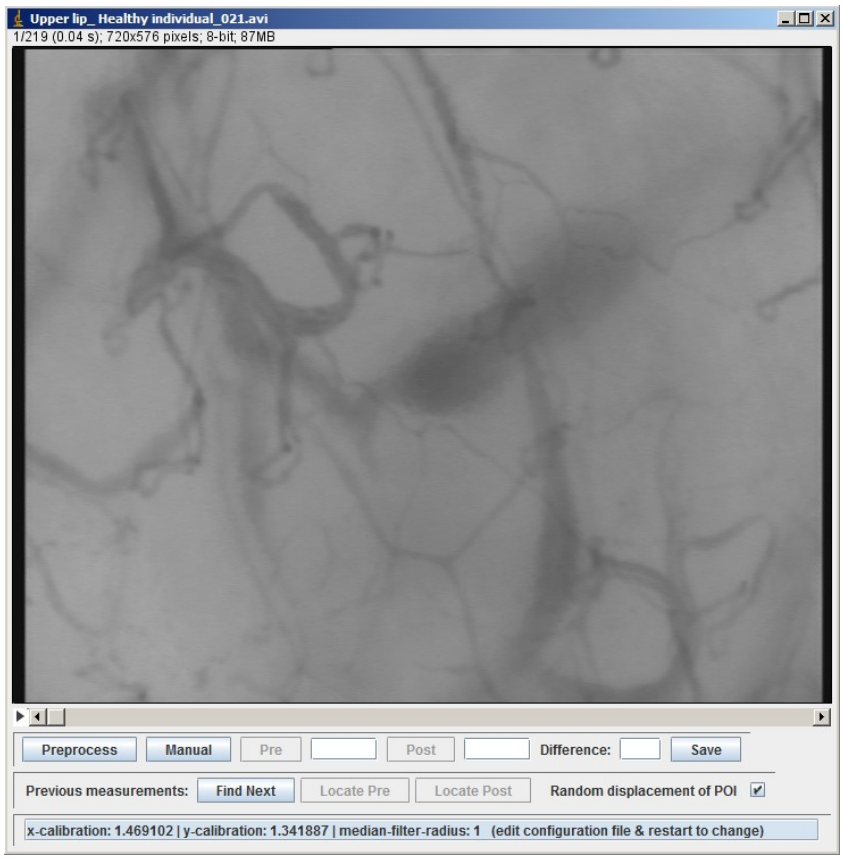
Follow the path: Plugins -> VascularAnalysis -> Vessel_width ->Vessel_width.

A default configuration file, (Vessel_width-config.txt) will be automatically created in the plugins folder, when the Vessel_width plug-in is used for the first time. The configuration (X- and Y-calibration, and the applied median filter radius) can be edited in a text editor, and saved to the Vessel_width-config.txt -file in the plugins folder.

Press "OK". (Exit ImageJ at this step to edit the configuration prior to any measurements, and restart).



The AVI-file will open with the Vessel_width plug-in in a new window.



7. Locate a spontaneous white blood cell passage in a point-of-interest (POI) of a capillary.

Verify, that the shadow of the white blood cell also persists in the post-capillary venule (Figures A-D), before you start measuring the glycocalyx thickness.

Figure A. Point-of-interest in a capillary (beneath the asterix). The AVI frame preprocessed for demonstrative purposes (see the text of the original article for clarification of "preprocess").

Figure B. A White blood cell (black-arrow) about to enter the capillary of interest. The AVI frame preprocessed for demonstrative purposes.

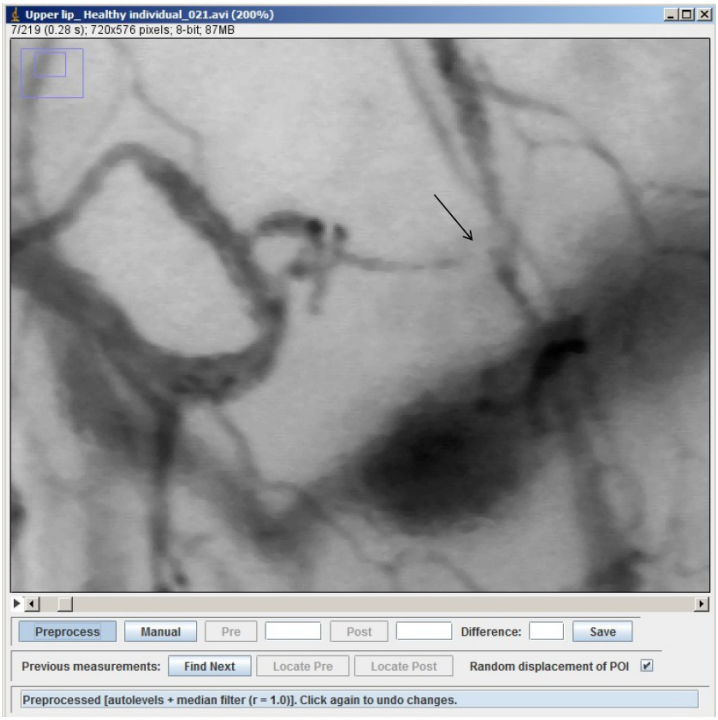
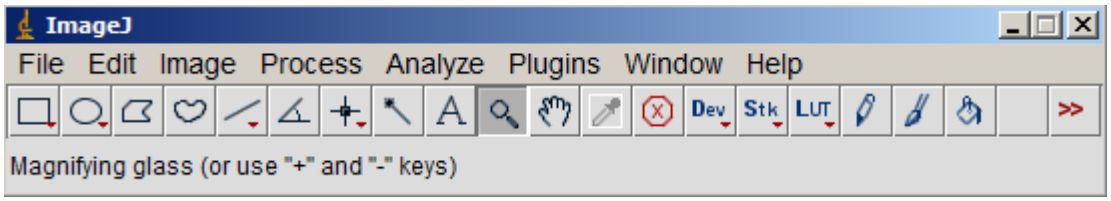


Figure C. A white blood cell passing the point-of-interest (black arrow). The AVI frame preprocessed for demonstrative purposes.

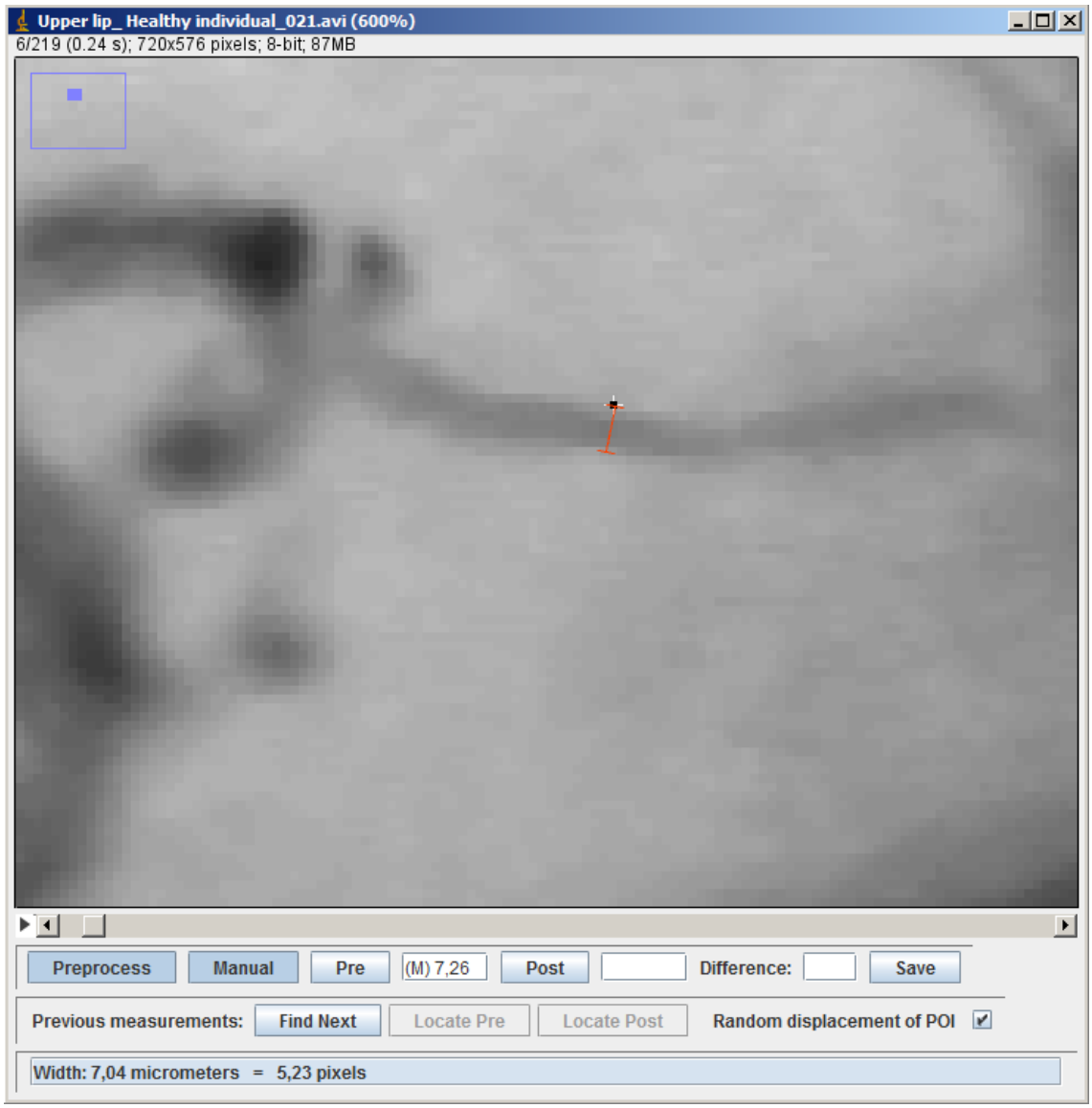
Figure D. The shadow of a passed white blood cell followed into a post-capillary venule (black arrow). The AVI frame preprocessed for demonstrative purposes.

8. Manual measurements (Pre and Post).

Use the 600% magnification by activating the ImageJ magnifying glass tool on the image.



PRE: Activate the “Manual” icon of the Vessel_width -plugin. Manual measurement tool (a free angle digital micrometer, red line on in the images below) will appear on the screen as you select a point on the image. A transection of the vessel (an ‘eye-ball’ estimate of diameter) can be drawn by drawing with a left mouse click. Visually approximate the diameter (edges of the red blood cell column) at the POI, where the WBC is about pass in the capillary. Then activate the “Pre”-icon, to measure the manually (M) drawn diameter.



POST: Measure the post diameter immediately after the passage of the WBC (need to scroll frames). Then activate the “Post”-icon, to measure the manually (M) drawn diameter.

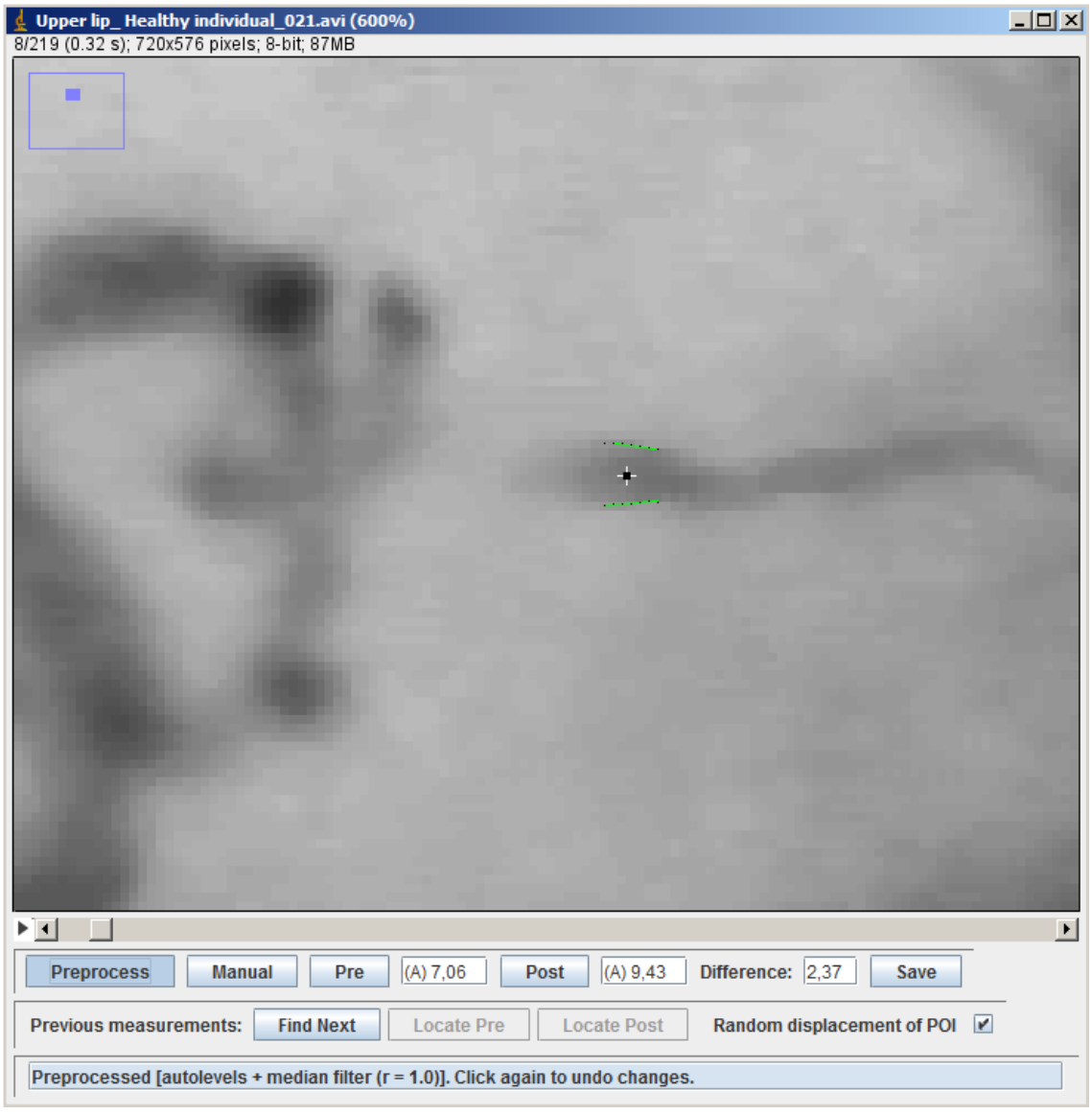
Finally, press the “Save” icon to save the measurements in the log.

9. Semi-automatic measurements (Pre and Post)

Deactivate the “Manual” icon of the Vessel_width -plugin (by clicking it), if it is active.

PRE: Place the point selection in the middle of the capillary at the POI, where the white blood cell is about to pass. Press the “Pre” icon, and the software will automatically detect the capillary thickness. Visually reassure the position and angle of detected red blood cell column edges (green lines). If the measurement is acceptable, proceed to post measurement.

POST: Place the point selection in the middle of the capillary at the POI, where the white blood cell has just passed. Press the “Post” icon, and the software will automatically derive the capillary thickness. Visually reassure the position and angle of detected red blood cell column edges (green lines).



Finally, press the “Save” icon to save the measurements in the log.

10. Troubleshooting:

"No image - There are no images open"

You attempt to run Vessel_width plug-in before you have opened the AVI file. Press "OK" and proceed to open a microcirculatory AVI file first (See section 5).

Can't locate the Vessel_width plug-in in the plugins tray

Reposition the Vessel_width.jar into the correct folder of the ImageJ directory.

You can even download the Vessel_width.jar again, and place the reloaded file in the correct directory. Malpositioned .jar -files will not harm the software functions.