

Segmentation of fibers

How to run the Fiji pipeline

1. Input directory

Figure 1 shows an example of an input directory containing the images to be analyzed. Images should be grouped by pairs of BF (brightfield) and PL (polarized light) images.

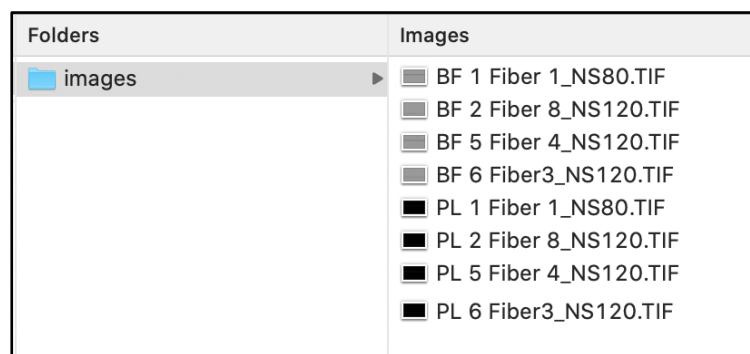


Fig. 1. Visualization of the input directory.

2. Requirements

- a) Fiji: <https://fiji.sc>

3. How to run the pipeline

Open Fiji and go to “Plugins” – “Macros” – “Edit...” and browse the directory in which the file “pipeline.ijm” is located. The file extension must be “ijm” (ImageJ macro). Next, the interface showed in Fig. 2 will appear. Between lines 1 and 29, different parameters can be updated, which are described next:

- a) **minDist**: the minimum distance between any two pixels of the fiber (minimum diameter)
- b) **maxDist**: the minimum distance between any two pixels of the fiber (maximum diameter)
- c) **pixPerMic**: number of pixels per micron
- d) **scale**: true or false, whether to use or not the given scale.
- e) **thresholdMethod1**: automatic threshold method for the segmentation of the BF image
- f) **thresholdManual1**: user-defined threshold value for the segmentation of the BF image

- g) **useThresholdManual1**: true or false - whether or not to use the manual threshold for the BF image, if false then the corresponding automatic threshold is used.
- h) **thresholdMethod2**: automatic threshold method for the segmentation of the PL image
- i) **thresholdManual2**: user-defined threshold value for the segmentation of the PL image
- j) **useThresholdManual2**: true or false - whether or not to use the manual threshold for the PL image, if false then the corresponding automatic threshold is used.
- k) **diagonal_fiber**: true or false - whether set of fibers are diagonal or not
- l) **nPixelsCorner**: how many pixels should be eliminated from the fiber if endings are in the corners (check Fig. 3.)
- m) **nPixelsSide**: how many pixels should be eliminated from the fiber if endings are not in the corners (check Fig. 3.)
- n) **Lines 22 to 28**: parameters for the histogram of area and average intensity of the particles segmented in the PL - ending points and bin size.

After choosing the values of each parameter, press the “Run” button (highlighted in Fig. 2). Wait for the execution of the pipeline. The execution time will depend on the number of images in the input directory. During the execution, the “Run” button will be disabled.

4. Pipeline output

After the execution of the pipeline, a folder named *segmentation* will be created containing the following output files (Fig. 4):

- **Image files**: For each BR/PL pair of images in the input directory, three image files are created: segmentation overlay of the BF image; segmentation overlay of the PL image and the binary mask of the fiber.
- **summaryBF.csv**: A summary file containing the measurements of the BF image
- **summaryPL.csv**: A summary file containing the measurements of the PL image (diameter is measured based on BF segmentation)
- **histDiameter.csv**: A file containing the histograms of the diameters of the fibers
- **histBF.csv**: A file containing the histograms of the pixel intensities of the segmented fiber for each BF image
- **histPL.csv**: A file containing the histograms of the pixel intensities of the segmented fiber for each PL image
- **areaIntensityPerFiberPL.csv**: A file containing the average area and average intensity of all the segmented objects inside the fiber region

- **areaPerFiberPL_perObj.csv**: A file containing the area values of each segmented object inside the fiber region
- **intensityPerFiberPL_perObj.csv**: A file containing the average intensity of each segmented object inside the fiber region
- **histogramAreaPerFiberPL.csv**: area histogram of the segmented object inside the fiber region
- **histogramIntensityPerFiberPL.csv**: intensity histogram of the segmented object inside the fiber region

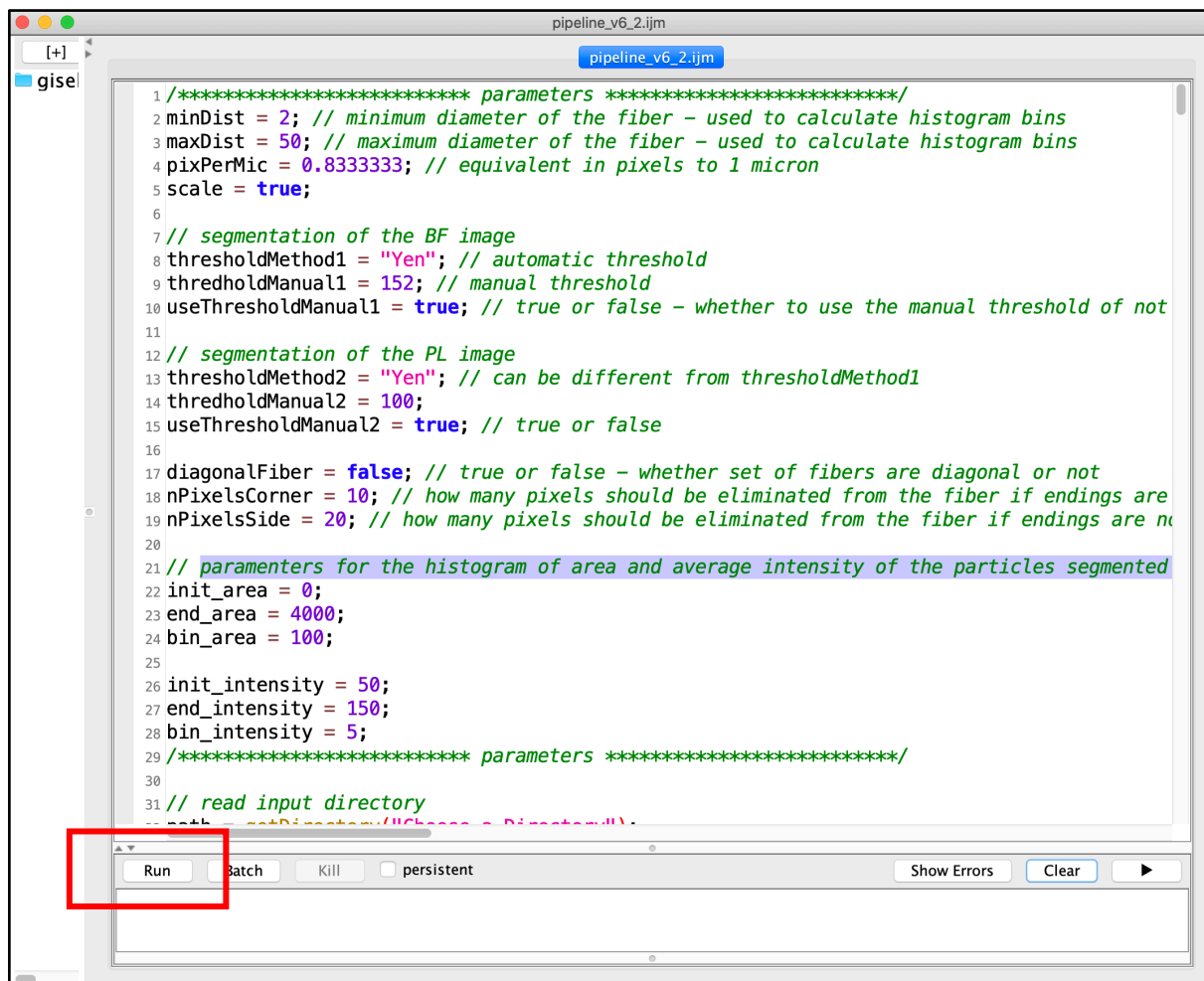


Fig. 2. Interface showing the implemented pipeline (macro).

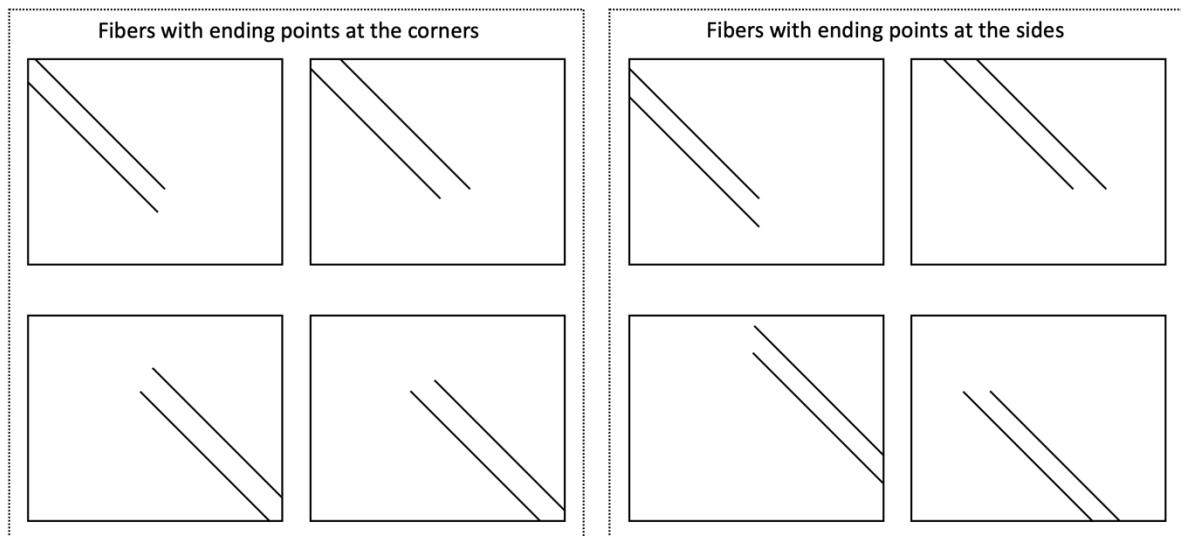


Fig. 3. Different ending points of the fibers.

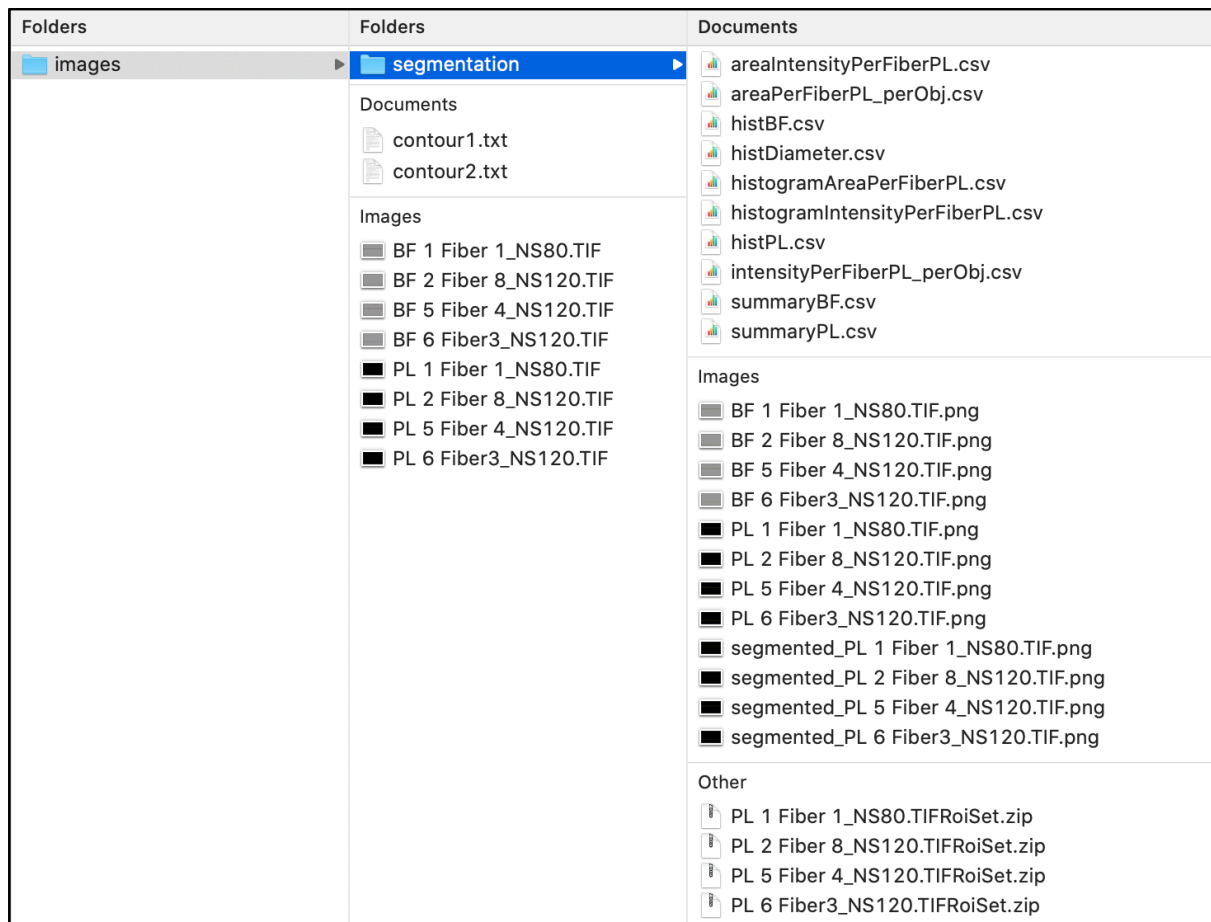


Fig. 4. Segmentation folder containing the processed files.