MyoAnalyst User Manual

Overview of MyoAnalyst

MyoAnalyst is an ImageJ/Fiji plugin designed for fully automatic analysis of both immunofluorescence (IF)- and H&E-stained skeletal muscle cross sections. This plugin features a user-friendly interface, thus will be as an efficient, versatile tool for myofiber quantification, tailored to meet the needs of both researchers and clinicians.

Installation of MyoAnalyst

As an ImageJ/Fiji plugin, MyoAnalyst is easy to install. You can just need two steps (Figure 1):

1) Download the "MyoAnalyst_v3.jar" from the github (https://github.com/ZhangHongbo-Lab/MyoAnalyst/tree/master/jar).

2) Copy the "MyoAnalyst_v3.jar" file into the ImageJ/Fiji plugins folder and restart the ImageJ/Fiji. You will find the plugin in the menu bar of "plugins" in ImageJ/Fiji.

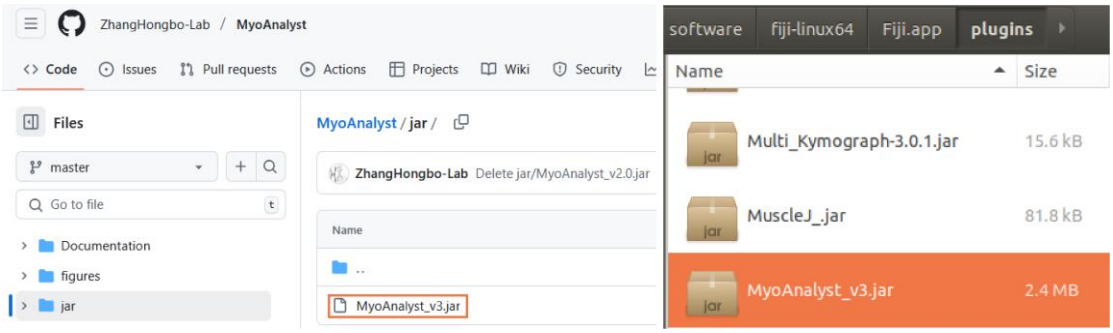

Figure 1. steps to install MyoAnalyst

Usage of MyoAnalyst

MyoAnalyst can analyze multiple images consecutively just by running one time, as long as these images are under the same folder and are stained by same staining technology. MyoAnalyst also supports various image files formats, including .TIFF, .PNG, .JPEG and .BMP .

1. segmentation and measure skeletal muscle cross-section stained by immunofluorescence (IF)

For skeletal muscle cross-section stained by IF, single channel images of myofiber boundary staining are only required. If you want to analyze the IF-stained skeletal muscle slides, please place these IF-stained skeletal muscle images in a folder and fellow these steps (Figure 2) :

1) Select the folder that contained the IF-stained skeletal muscle images (Step 1);

2) Choose the Sample Type as "IF" through the drop-down menu (Step 2);

3) Select the folder in which to save the output data following image analysis (Step 3);

4) Select the mode of parameter setting : "Automation parameters" or "Manual parameters" (Step 4 and 6).

4.1) If you choose "Automation parameters" (Step 4), then you just click the "OK" button, MyoAnalyst automatically analyzes all the images in the input folder and save the corresponding result files to the designated output folder (Step 5).

4.2) If you choose "Manual parameters" (Step 6), then you should set three parameters by manually.

4.2.1 set three parameters by manually (Step 7). A detailed description of the parameters is given below:

- the number of Dilation: This parameter specifies the number of dilation operations applied to skeletal muscle fiber boundaries. A larger value results in inward contraction of the boundaries, while a smaller value leads to outward expansion of the skeletal muscle fiber boundaries. The default value is 6, and it is recommended that this value be set within the range of 1 to 10.
- the Size of Filtering Area (pixel^2): This parameter specifies the minimum value of the cross-sectional area of skeletal muscle fibers (pixel^2). Skeletal muscle fibers with an area smaller than this value will be filtered out. The default value is 250 and it is recommended that this value be set within the range of 250 to 600.
- the Circularity (0.0 to 1.0): This parameter specifies the circularity of the closed curve formed by the boundaries of skeletal muscle fibers. Skeletal muscle fibers with a value less than this will be filtered out. The default value is 0.25 ant the value must be set within the ranges from 0.0 to 1.0.

4.2.2) After the parameter settings are complete, click the "OK" button (Step 7);

4.2.3) Then a dialog box will pop up with the parameters you have set. Please click the “OK” button to confirm (Step 8).

4.2.4) Finally, click the “OK” button, MyoAnalyst automatically analyzes all the images in the input folder and save the corresponding result files to the designated output folder (Step 9).

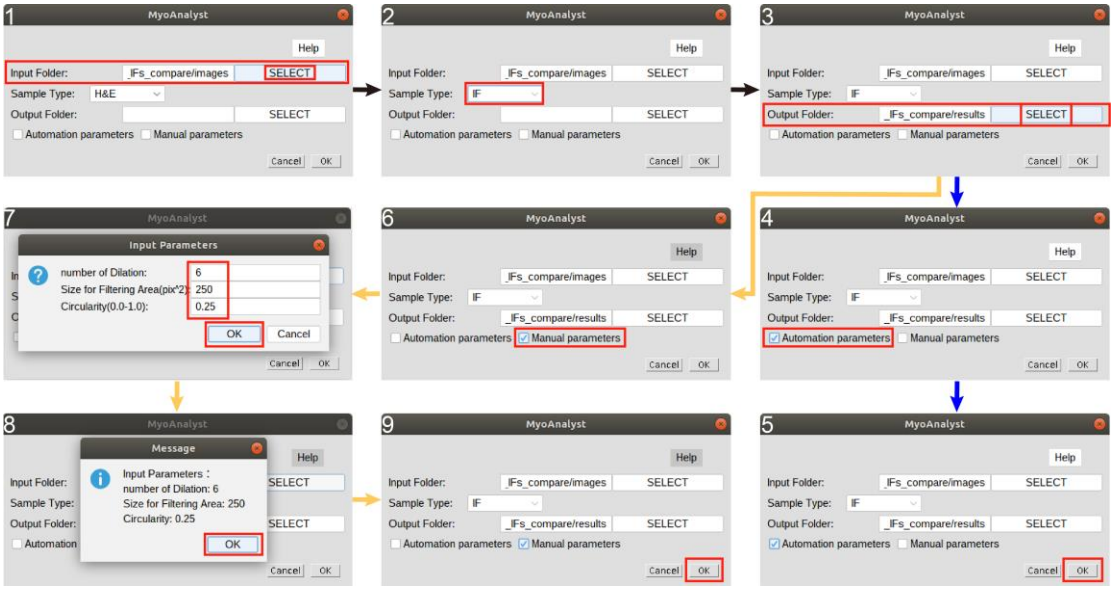

Figure 2. steps to segment and measure skeletal muscle cross-section stained by IF.

It is worth noting that we recommend first selecting the automated parameter mode to run our program, and then deciding whether to use the manual parameter setting mode based on whether the output results meet the user's requirements. For example, if the boundaries of skeletal muscle fibers obtained from the automated parameter mode are smaller than the actual boundaries, the value for the number of dilations can be set smaller. If the results from the automated parameter mode include many false positives located in the extracellular matrix, the value for the cross-sectional area can be set larger to filter these false positives results.

1. segmentation and measure skeletal muscle cross-section stained by Hematoxylin and eosin (H&E)

The workflow for segmentating and measuring skeletal muscle cross-section stained by Hematoxylin and eosin (H&E) is very similar with the above workflow for

segmentating and measuring skeletal muscle cross-section stained by IF. If you want to analyze the H&E-stained skeletal muscle slides, please place these H&E -stained skeletal muscle images in a folder and fellow these steps (Figure 3):

1) Select the folder that contained the IF-stained skeletal muscle images (Step 1);

2) Choose the Sample Type as "H&E" through the drop-down menu (Step 2);

3) Select the folder in which to save the output data following image analysis (Step 3);

4) Select the mode of parameter setting : "Automation parameters" or "Manual parameters" (Step 4 and 5).

4.1) If you choose "Automation parameters" (Step 4), then you just click the "OK" button, MyoAnalyst automatically analyzes all the images in the input folder and save the corresponding result files to the designated output folder (Step 5).

4.2) If you choose "Manual parameters" (Step 6), then you should set three parameters by manually.

4.2.1 set three parameters by manually (Step 7). A detailed description of the parameters is given below:

- the number of Dilation: This parameter specifies the number of dilation operations applied to skeletal muscle fiber boundaries. A larger value results in inward contraction of the boundaries, while a smaller value leads to outward expansion of the skeletal muscle fiber boundaries. The default value is 6, and it is recommended that this value be set within the range of 1 to 10.
- the Size of Filtering Area (pixel^2): This parameter specifies the minimum value of the cross-sectional area of skeletal muscle fibers (pixel^2). Skeletal muscle fibers with an area smaller than this value will be filtered out. The default value is 250 and it is recommended that this value be set within the range of 250 to 600.
- the Circularity (0.0 to 1.0): This parameter specifies the circularity of the closed curve formed by the boundaries of skeletal muscle fibers. Skeletal muscle fibers with a value less than this will be filtered out. The default value is 0.25 ant the value must be set within the ranges from 0.0 to 1.0.

4.2.2) After the parameter settings are complete, click the "OK" button (Step 7);

4.2.3) Then a dialog box will pop up with the parameters you have set. Please click the “OK” button to confirm (Step 8).

4.2.4) Finally, click the “OK” button, MyoAnalyst automatically analyzes all the images in the input folder and save the corresponding result files to the designated output folder (Step 9).

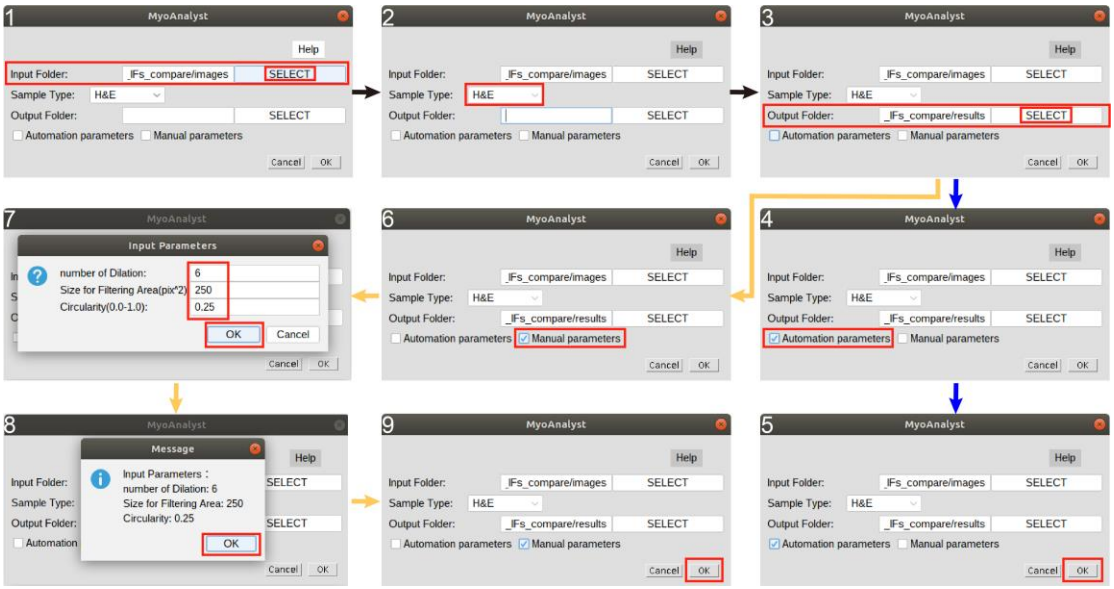

Figure 3. steps to segment and measure skeletal muscle cross-section stained by H&E.

Output of MyoAnalyst

Upon completion of MyoAnalyst execution, two files are generated in the user-specified output directory: a *.results_ROISet.zip and a *.results.csv file

The *.results_ROISet.zip file archives myofiber segmentation metadata and can be opened by ImageJ/Fiji directly (*File▷Open…*) and further processed through the ROI Manager toolset. For example, users may execute operations such as removal of erroneously segmented ROIs or addition of new ROIs to compensate for omitted myofibers. Comprehensive processing methodologies are detailed in the official ROI Manager documentation(ImageJ User Guide - IJ 1.46r | Analyze Menu). Additionally, user can also visualize the segmentation of myofibers by importing the corresponding *.results_ROISet.zip file (Figure 4).

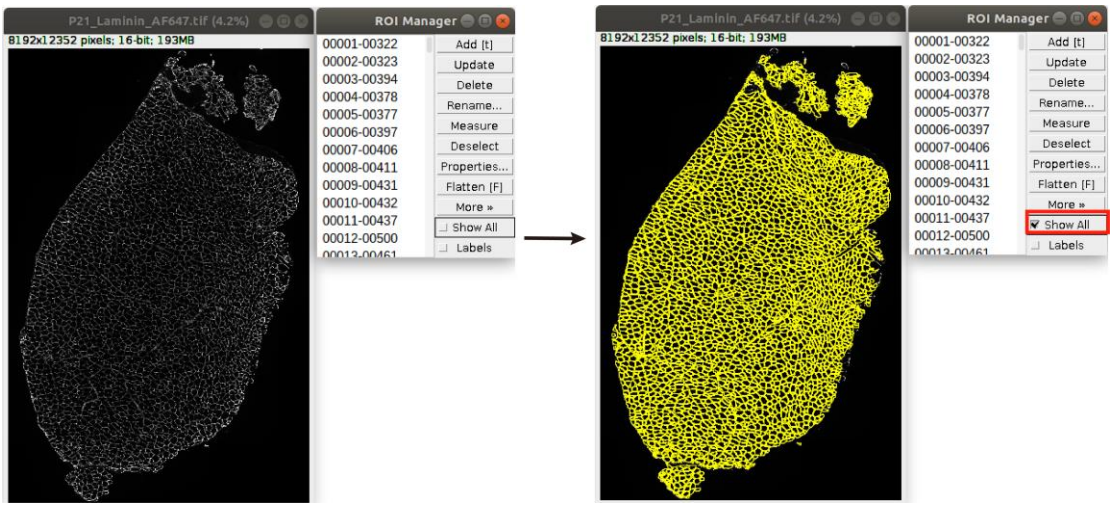

Figure 4. visualization of the segmentation results.

The *.results.csv file is a results table of statistical measurements, including Area and Perimeter, Feret's Diameter, Shape descriptors, Centroid and Circularity. Additionally, if users want to perform other measurement, the measurement parameters can be reconfigured through choosing "*Analyze* > *Set Measurements....*" in ImageJ/Fiji and then users can perform the measurement again based on the ROIs file output by MyoAnalyst (Figure 5).

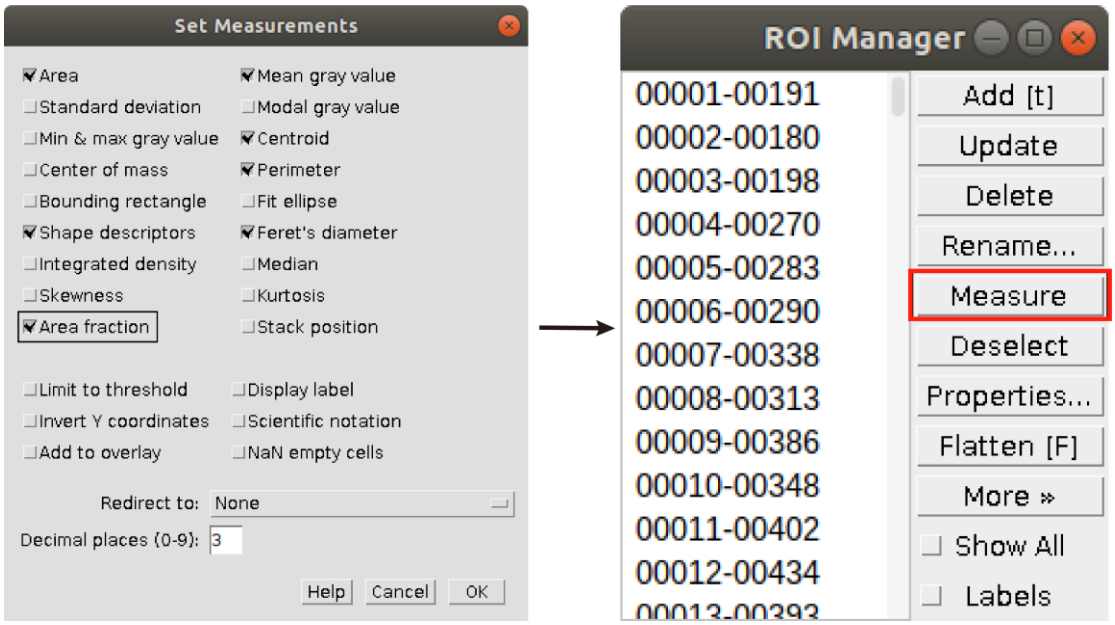

Figure 5. steps to set measurements and re-measure.

Notably, the numerical values of these measurement results are pixel-scale metrics. For comparative analysis of statistical results across multiple images, users should first use the "*Set Scale*..." dialog in ImageJ/Fiji to define the spatial scale of the corresponding

image and then re-measure via the "*Measure*" in the ROI Manager toolset utilizing the *.results_ROISet.zip file output by MyoAnalyst (Figure 6). So, measurement results can be presented in calibrated units, such as mm or μm.

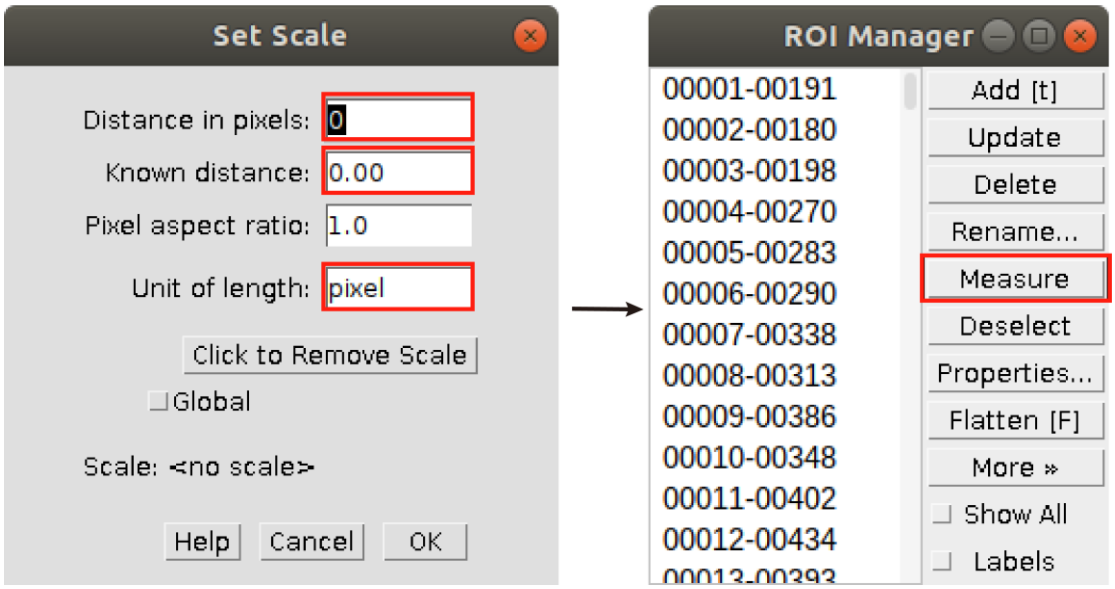

Figure 6. steps to define the spatial scale and re-measure

Further help

In case of difficulties using MyoAnalyst, you can create an issue in the following link so we or someone from the community can help you: https://github.com/ZhangHongbo-Lab/MyoAnalyst/issues. Or you can contact us: zhangbao5@mail.sysu.edu.cn