

# 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\_Vx.x.jar” from the github (<https://github.com/ZhangHongbo-Lab/MyoAnalyst>).
- 2) Copy the “MyoAnalyst\_Vx.x.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.

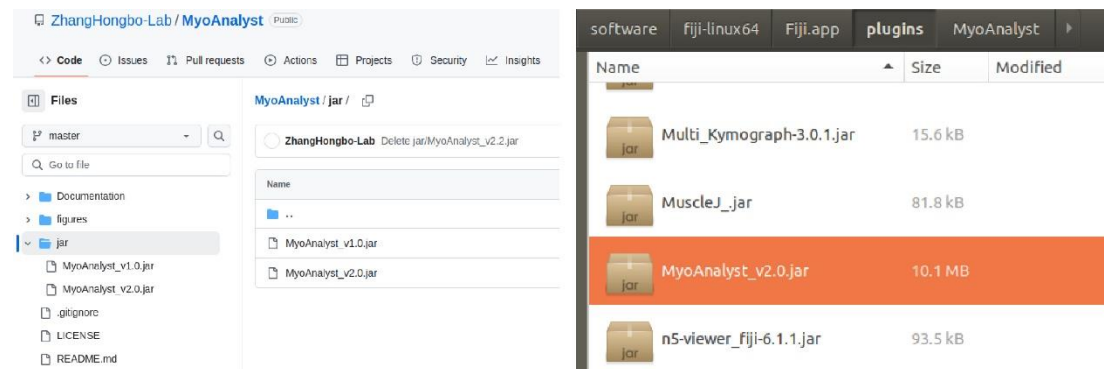


Figure 1. steps to install MyoAnalyst

## Version of MyoAnalyst

In GitHub, we provided two version of MyoAnalyst. One is the [MyoAnalyst\\_v1.0.jar](#) and the other is [MyoAnalyst\\_v2.0.jar](#). The difference between the two versions is that in [MyoAnalyst\\_v1.0.jar](#), users cannot customize the parameters of image segmentation, including the number of the dilation, the area size and circularity for filtering the myofiber, while they can when using [MyoAnalyst\\_v2.0.jar](#) (Figure 2).

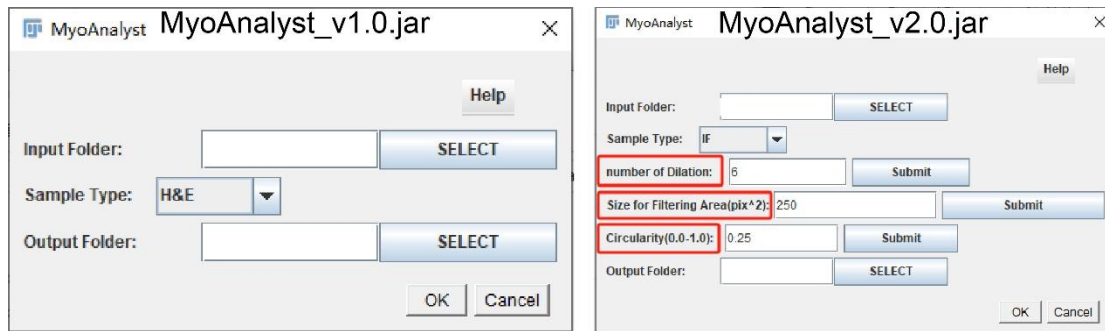


Figure 2. The difference between the two versions of MyoAnalyst

## 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. Here, we take the [MyoAnalyst\\_v2.0.jar](#) as an example to introduce the use of MyoAnalyst.

### 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 follow these steps (Figure 3): 1) selected the folder that contained the IF-stained skeletal muscle images; 2) choose the Sample Type as IF through the drop-down menu; 3) set the number of Dilation (the default value is 6, The value ranges from 1 to 10) and click the “Submit”; 4) set the Size of Filtering Area ( $\text{pix}^2$ ) (the default value is 250) and click the “Submit”; 4) set the Circularity (the default value is 0.25, The value ranges from 0.0 to 1.0) and click the “Submit”; 5) selected the folder in which to save the output data following image analysis; 6) After that, clicked the “OK” button, MyoAnalyst automatically analyzes all the images in the input folder and save the corresponding result files to the designated output folder.

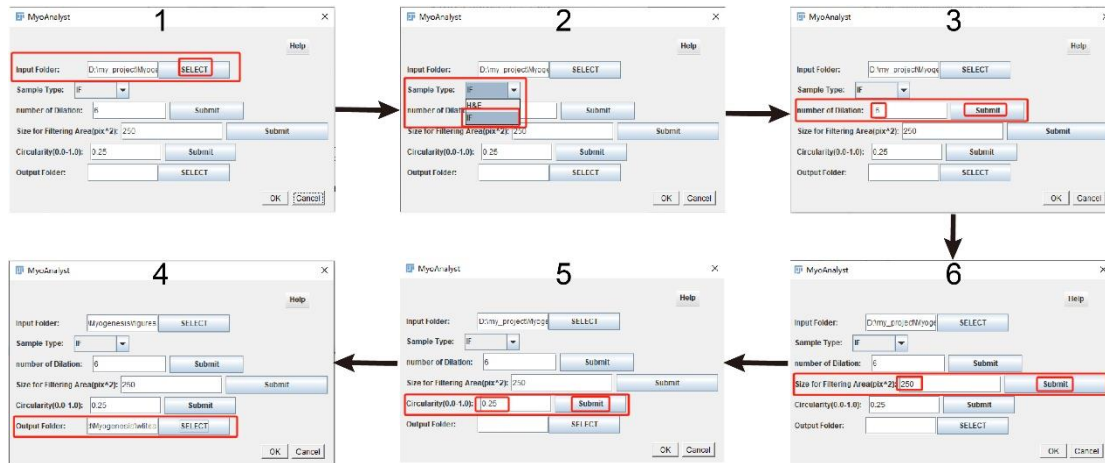


Figure 3. steps to segment and measure skeletal muscle cross-section stained by IF.

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

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 follow these steps (Figure 4): 1) selected the folder that contained the H&E-stained skeletal muscle images; 2) choose the Sample Type as H&E through the drop-down menu; 3) set the number of Dilation (the default value is 6, The value ranges from 1 to 10) and click the “Submit”; 4) set the Size of Filtering Area ( $\text{pix}^2$ ) (the default value is 250) and click the “Submit”; 4) set the Circularity (the default value is 0.25, The value ranges from 0.0 to 1.0) and click the “Submit”; 5) selected the folder in which to save the output data following image analysis; 6) After that, clicked the “OK” button, MyoAnalyst automatically analyzes all the images in the input folder and save the corresponding result files to the designated output folder.

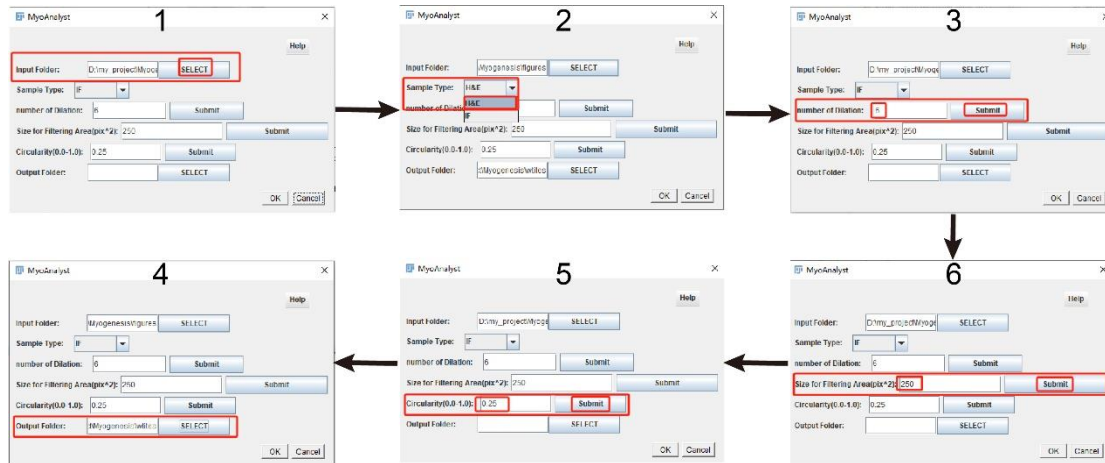


Figure 3. steps to segment and measure skeletal muscle cross-section stained by H&E.

## Help

In case of difficulties using MyoAnalyst, create an issue in the following link so we or someone from the community can help you: <https://github.com/ZhangHongbo-Lab/MyoAnalyst/issues>.