MuscleJ Tutorial

MuscleJ: A high content analysis method to study skeletal muscles.

The MuscleJ macro is a compilation of tools allowing for the analysis of fiber phenotypes.

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Features

Fiber morphology – Centro Nuclei Fiber detection (CNF) – Vessel detection – Satellite Cell detection (Sat) – Fiber typing

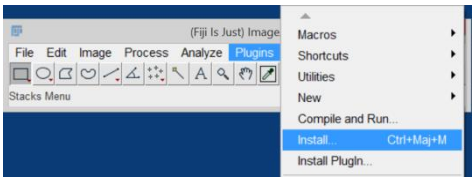
Contents

I. Installation of MuscleJ in the FiJi environment

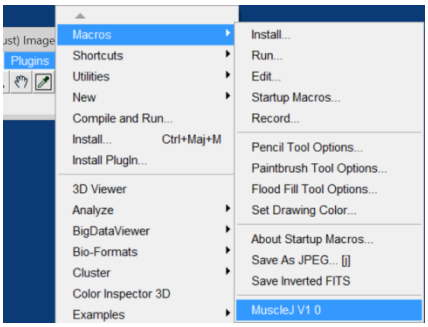
The automated image analysis workflow was implemented in the FiJi (NIH, Bethesda, MD, USA) environment as a macro and will be upgrade to a plugin with further functionalities.

Installation

- Download FiJi from *https://imagej.net/Fiji/Downloads* and following the installation instructions. Or update your FiJi version to meet the minimum version requirements.
- The minimum version requirements are:
  Fiji version from 1.51e, tested on 1.52a
  Java version: Java 1.8.0-66 (64 bits)
  Used Plugins: Bio-Formats Plugins for Fiji (from release 5.5.3, tested on 5.8.2)



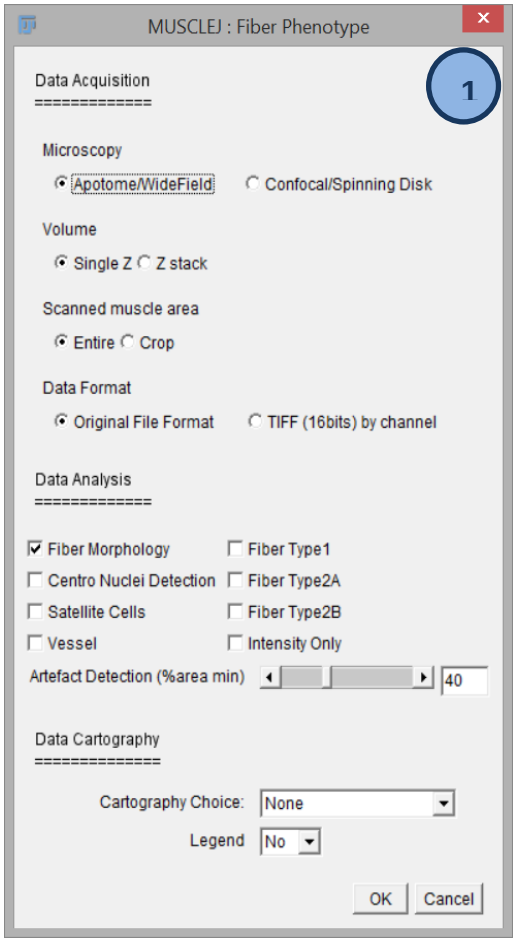
- Installation of Muscle J
  - Download the MuscleJ_V1_00.ijm file onto your computer.
  - From *Plugins➔Install…* menu, open the MuscleJ macro and save it into the FiJi 'Plugins/Macros' folder.
  - Restart FiJi to complete the macro installation.
  - MuscleJ will appear in the *Plugins➔Macros* menu.



I. Description: MUSCLEJ Fiber Phenotype Dialog Boxes

The first step : The user provides the requested information

Data Acquisition



- *Microscopy* selection
  Select the type of microscopy used when generating the data. Based on the selection a specific pretreatment will be performed.
- *Volume* choice
  The *Z stack* option will apply a maximum projection along z axis.
- *Scanned muscle area* choice
  Entire: the whole section or a large part of the section (min. 50%) will be been scanned.
  Crop: if only a small area is to be scanned the analysis will be less efficient despite a series of additional filters that track the maximum intensity of the fiber contours in Laminin channel.
- *Data Format*
  The *Original File Format* is for files readable by the *Bioformat Importer* Plugin such as czi, lsm, lif, etc. formats. Additionally, tiff files containing all channels (a stack file from FiJi or ImageJ) are readable.
  The *TIFF (16bits) by channel* format is for cases when your images have been exported from your acquisition system by channel.

Data Analysis

- *Analysis* check boxes: multiple options – you can select the series of analysis to be performed. However, the *Fiber Morphology* analysis is mandatory whenever you start a new batch run.
- *Artefact Detection* option: sets the minimum threshold for the fiber area detection. This is used for the initial quality check of the fiber shape signal (Laminin).
  Example: %area min=xx, if less than xx% of the total area of the section does not contain segmentable fibers, the section will be automatically placed into the "Artefact" directory and the analysis will not be performed for this section.

Data Cartography

- *Cartography*
  Automatically backs up the *in situ* cartography according to the analysis performed (single choice): "*Fiber Area Classes*", "*Centro Nuclei Classes*", "*Sat Cell*", "*Fiber Types*" or "*Vessels*". Note: If "*Fiber Morphology*" analysis has been performed during a previous run, the corresponding cartography ("Fiber Area Classes") can be chosen independently from the current analysis.
- *Legend*
  If chosen, for the Centro Nuclei Fibers, Sat Cells, Types, and Vessels a legend will be automatically drawn onto the cartography *in situ* on the bottom left. For the distribution by fiber surface the legend is put in a column at the top left of the cartography with the range of surface distribution displayed in μm^2.

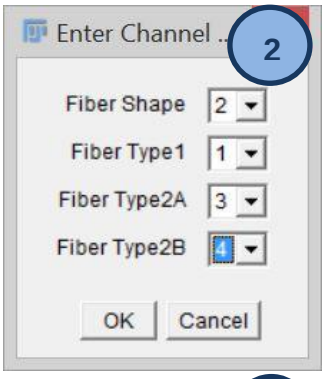
Click on the OK button to continue.

Channel Information

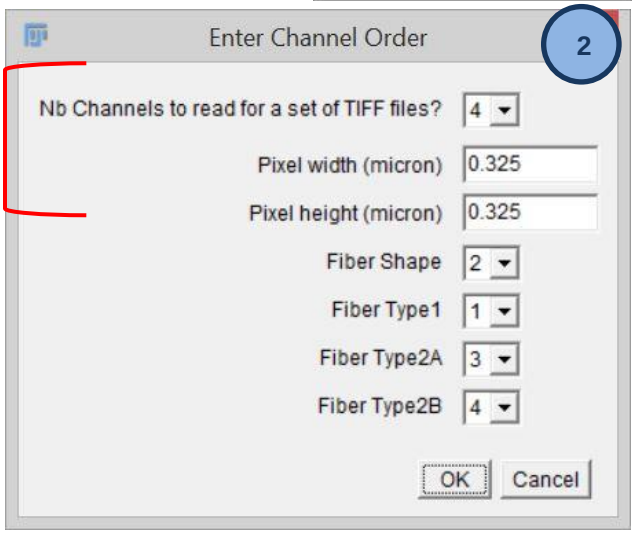
To identify the channel order, please open the first image from the input folder with the *Bioformat Importer*.

Depending on the analysis requested by the user, the order of channels will be need to be set.

In the right dialog box, the analysis of fiber typing has been demanded. You must enter the corresponding fiber channel number to the track of the fiber shape, before the analysis of intensity by fiber typing channel can be performed.



For the same analysis request, if the *tiff format by channel* option has been checked the information about the number of channels, the x pixel size in microns and the y pixel size in microns must be entered before the analysis can be performed.

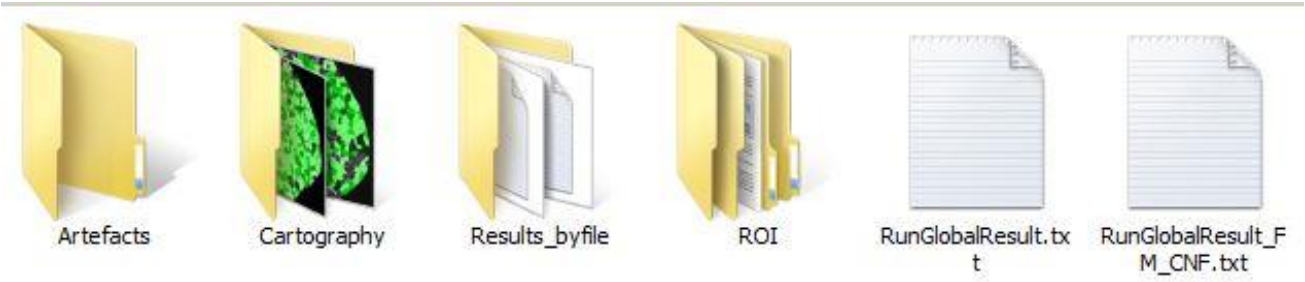


Click on the OK button to continue.

Note: all fields in the two previous dialog boxes are mandatory

The second step: Setting the directory path for the Input and the Output data

- Select the Image File Folder by Batch run
- Select an empty folder to save the ROIs, the Results by file and the Cartographies During the first run, a series of directories will be automatically created in the root of the selected folder:



II. Possible analysis combinations

One channel is dedicated to the detection of the shape of the fiber (Laminin).

+ 1 channel	+ 2 channels	+ 3 channels
• CNF (Dapi) • Vessels (CD31) • Intrafiber staining (Fiber type or other intra-staining) • Intensity per channel	• Sat Cells (Dapi+Pax7) • CNF (Dapi) + Vessels (CD31) • CNF (Dapi) + Sat Cells (Pax7) • CNF (Dapi) + one intrafiber staining • Two intrafiber staining	• CNF (Dapi) + Sat Cells (Pax7)+ Vessels (CD31) • Three intrafiber staining (3 Fiber types or other intra-staining) • CNF (Dapi) + Vessels (CD31) + One intrafiber staining • CNF (Dapi) + Sat Cells (Pax7)+ one intrafiber staining • CNF (Dapi) + two intrafiber staining.

III. Recommendations / Limitations

Images obtained from any microscope magnification (10x, 20x, 25x, 40x, 63x) can be used. The limitations are relative to the quality of image staining and a minimum of 1.5x1.5mm scanned surface area.

For an optimal utilization of MUSCLEJ we recommend using the tool under the following conditions:

When gather the data use an Apotome / Widefield microscope when imagining a single Z slice or use a confocal/Spinning Disk microscope when imaging a Z-stack. When analyzing a whole section (set the minimum detection threshold of 40 % to reject section artefacts).

Use the Original File Format to keep the metadata associated.

Images folder: all images of the batch folder should have the same parameters (number of channels, channel orders, stainings and formats). The input and output folders have to be named without using spaces and symbols.

File format that are not supported are: Jpeg, Png, Tiff-8bits, a time series.

For the option « *Tiff 16 bits by channel»:* Each file must contain one channel and have the following nomenclature **FileName1_C#** where # is the number of channel (1,2,3 or 4). Do not use spaces and symbols when naming the files.

Note: All the files have to be gather into the image folder

IV. Examples

Data set description

Input Folder Name	Channel Order	Acquisition	Raw Data	Output Folder
• CNF • FiberMorphology	Dapi(1) Laminin(2)	Widefield 20X	Yes	• ResultsCNF • ResultsFMorpho
Fiber Type	Laminin(1) MyhI(2) IIB(3) IIA(4)	Widefield 20X	Yes	ResultsTypes
SatCells	Dapi(1) Laminin(2) Pax7(3)	Widefield 20X	No	ResultsSatCells
Vessels	Dapi(1) CD31(2) Laminin(3)	Widefield 20X	Yes	ResultsVessels

All the data corresponding to the described analysis can be downloaded via a request to the corresponding authors.

Single analysis

Analysis	Options to check	Results by batch run	Results by fiber
Fiber Morphology	• Apotome/Widefield • Single Z • Whole section • Original file format • Quality Check: 40% • Cartography • No legend	***GlobalResults_FM.txt*** • Whole section area (µm^2) • Numbers of segmented fibers • Fiber Area mean (µm^2) • Distribution of the fiber areas.	• Area • Minimum and maximum Ferret • 2D Localisation (GC)
+CNF	• Apotome/Widefield • Single Z • Crop • Original file format • Quality Check: 40% • No Cartography	***GlobalResults_FM_CNF.txt*** • Total Centronucleated fibers	• Area • Minimum and maximum Ferret • Numbers of centronuclei • Numbers of perinuclei
+Sat Cells	• Apotome/Widefield • Single Z • Crop • Original file format • Quality Check: 40% • Cartography • No Legend	***GlobalResults_FM_SC.txt*** • Numbers of satellite cells (Pax7$^+$ cells) • Total of satellite cells associated fibers	• Area • Minimum and maximum Ferret • Numbers of satellite cells associated to the fiber
+Vessels	• Apotome/Widefield • Single Z • Crop • Original file format • Quality Check: 40% • Cartography • No Legend	***GlobalResults_FM_V.txt*** • Total of vessels • Total of vessels associated fibers • Vascularisation Surface(%) • Number of vessels by mm^2	• Area • Minimum and maximum Ferret • Numbers of vessels associated to the fiber

Multiple analysis

Analysis	Option check	Results by batch run	Results by fiber
FM+ 3 Fiber Types	• Apotome/Widefield • Single Z • Crop • Original file format • Quality Check: 40% • Cartography • Legend	***GlobalResults_FM_I_IIA_IIB.txt***	• Area • Minimum and maximum Ferret • 2D Localisation (GC) • Fiber typing

FM+CNF+Vessels	• Apotome/Widefield • Single Z • Crop • Original file format • Quality Check: 40% • Cartographies • Legend	***GlobalResults_FM_CNF_V.txt*** • Tot Centro Nuclei • Tot Vessels • Fibers with Vessel • Vascularisation surface(%) • Vessels by mm^2	• Area • Minimum and maximum Ferret • 2D Localisation (GC) • Numbers of centronuclei • Numbers of perinuclei • Numbers of vessels associated to the fiber

Example of cartographies corresponding to multiple analysis

FM+CNF+Vessels