

Automatic Micrograph Analyser

User Manual

Version 1.1

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Author: Pawel Cislo

Contact: pawel.cisloo@gmail.com

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Note: this manual was generated for the use of a script designed for a master's project and included on the attached CD with the essential files

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1. Overview

Micrograph Analyser is a script written for ImageJ computer application to help the user analyse micrograph more effectively with a great time save. The user specifies the outputs so that the system can be applied to various images. The optional outputs are defined in the 11th point of the list in [chapter 1.1 \(Performed Operations\)](#).

The following script's user access mode is performed entirely through a graphical user interface (GUI) for the ease of use.

1.1 Performed Operations

The script execution is performed with the following workflow:

- 1) Ask the user for input image or directory of images
- 2) Check if the specified input has a supported format and is not empty
- 3) Request to determine the output directory
- 4) Using a GUI, ask the user for:
 - a) desired output (specified in point 11)
 - b) preference of analysing interparticle range
 - c) minimum and maximum particle size taken into analysis
 - d) minimum and maximum particle circularity taken into analysis
 - e) analysis preferences (specified in point 9)
 - f) preference of setting the scale (specified in point 7)
 - g) preference of removing the label (specified in point 8)
- 5) Ask to specify output images (optionally)
- 6) Ask to specify area distribution plot analysis (optionally)
- 7) Ask to set accurate scale (optionally)
 - a) manually (by drawing a line over scale)
 - b) typing in the known distances
 - c) using predefined scale
 - d) skipping the part (by default 1 pixel = 1 known distance)
- 8) Crop the image to remove the bottom label (optionally)
 - a) provide predefined option to remove the label as for micrographs taken at Coventry University
 - b) provide an option to crop the image manually
 - c) leave the image without cropping
- 9) Perform segmentation
 - a) run machine learning segmentation instead of automatic thresholding (optionally)
 - b) transform input into 8-bit image
 - c) apply automatic threshold
 - d) convert input into binary values (optionally)
 - e) remove small particles from the image (outliers) (optionally)
 - f) fill holes in particles on the image (optionally)

- g) apply watershed algorithm on the image (optionally)
 - h) exclude particles on edges during the analysis (optionally)
 - i) include holes of particles in analysis (optionally)
- 10) Ask the user if he is satisfied with the particles taken into analysis (optionally)
- a) if not, specify particle size and circularity again, till the user is satisfied
 - b) if yes, continue to run the script
- 11) Ask to specify interparticle range analysis (optionally)
- 12) Analyze particles, close windows, optionally save:
- a) overall results (including nearest neighbor distances from centroids) (saved in "RESULTS..." file)
 - b) summary (saved in "SUMMARY..." file)
 - c) interparticle distances (saved in the "INTERPARTICLE_DISTANCES" folder)
 - d) area distribution plot (saved in the "PLOTS" folder)
 - e) cluster indication (saved in the "CLUSTERS" folder)
 - f) images
 - g) specifications used in analysis (saved in "ANALYSIS_INFO(...)" file)
 - h) time of running manual and automatic operations of the script (saved in "ANALYSIS_INFO..." file)
 - i) machine learning results (saved in the main folder)

2. Requirements

2.1 System Requirements

ImageJ/Fiji is a cross-platform computer application which runs on the following systems:

- Windows XP, Vista, 7, 8 and 10
- Mac OS X 10.8 "Mountain Lion" or later
- Linux on amd64 and x86 architectures

2.2 Software Requirements

- 1) ImageJ/Fiji distribution
 - a) Fiji is recommended: <https://imagej.net/Fiji/Downloads>
- 2) NND plugin:
https://icme.hpc.msstate.edu/mediawiki/index.php/Nearest_Neighbor_Distances_Calculation_with_ImageJ
NOTE: if the website is not working, please find the required file in the "required plugins" folder of this CD
- 3) Graph plugin:
<https://imagej.nih.gov/ij/plugins/graph/index.html>
NOTE: if the website is not working, please find the required file in the "required plugins" folder of this CD
- 4) Activation of BioVoxel Toolbox:
https://imagej.net/BioVoxel_Toolbox
- 5) Activation of BAR Toolbox:
<http://imagej.net/BAR>
- 6) 3 files in the "macros" folder of ImageJ
 - a) "Micrograph_Analysis.ijm"
 - b) "Micrograph_Analysis_Single.ijm"
 - c) "Micrograph_Analysis_Multiple.ijm"
- 7) The input files must have one of the extensions specified below. The inclusion of different formats requires code modification
 - a) TIFF (.tiff, .tif)
 - b) PNG (.png)
 - c) JPEG (.jpeg, .jpg)
 - d) BMP (.bmp)

3. Installation

- 1) Install ImageJ/Fiji distribution from the official website
 - a) Fiji is recommended
<https://imagej.net/Fiji/Downloads>
- 2) Install all the requirements mentioned in points 2-5 of the previous [subchapter 2.2 \(Software Requirements\)](#).
The up to date installation instructions are placed on the websites mentioned in the points.
- 3) Find Fiji installation directory and place “Micrograph_Analysis” folder from this CD in the “Fiji/Macros” folder.
- 4) In the Fiji toolbar menu, choose “Plugins > Install...”
 - a) From the file selection menu, locate and choose “Micrograph_Analysis.ijm” file
 - b) Locate Fiji installation directory and save the file in “Fiji/plugins/Macros” folder

4. Using the System

After successful installation, please find the “Micrograph Analysis” entry in the “Plugins” menu of the ImageJ/Fiji toolbar:

“Plugins > Macros > Micrograph Analysis”.

After selecting “Micrograph Analysis” entry, please follow the on-screen instructions.

4.1 Using own functions

To use own functions on the processed image, it is recommended to open the “PROCESSED_(...)” file after performing the analysis. This image is already segmented, so the program knows that white color represents the particles and black color identifies the background.

4.2 Adding Features/Suggestions

In case of adding the features and knowing the work of ImageJ Macro, you can try adding the features by yourself as the code is commented in detail. It is recommended to open the files in an advanced text editor, like [Sublime Text](#) or [Atom](#) for a better view. Moreover, please select “Java” syntax to get an accurate variable highlighting of the code.

Otherwise, please find the contact to the developer in [chapter 5.2 \(Support\)](#).

4.3 Cautions & Warnings

For the best experience, please read all instructions that appear in the dialogue boxes.

If there is an unfamiliar term during the script execution, please contact the developer or try to research it on ImageJ user guide.

<https://imagej.nih.gov/ij/docs/index.html>

In case of any other errors, please refer to [chapter 5.2 \(Support\)](#) of this user manual.

5. Troubleshooting & Support

5.1 Error Messages

In case of any Error Messages, please follow the on-screen instructions directing to the solution. If the solution is not satisfying, please make sure that the script was properly installed using the provided installation guide in [chapter 3](#). If the error messages still occur, please find help [in chapter 5.2 \(Support\)](#).

5.2 Support

In case of any additional support required, please do not hesitate to contact the developer using the details provided below.

Table 1 - Support Points of Contact

Contact	Email	Website
<i>Pawel Cislo</i>	pawel.cisloo@gmail.com	pawelcislo.com

In case of not getting a response or any other issues, please:

- Search through ImageJ resources
<http://search.imagej.net/>
- Browse ImageJ user manual
<https://imagej.nih.gov/ij/docs/index.html>
- Write a post on ImageJ official forum to get extensive help from the fantastic community
<http://forum.imagej.net/>