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Cell Image Analysis User Manual – V0.5

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Executive Summary

This manual provides a step-by-step guide to run the developed programs. It also illustrates the possible output and how to use them to analyze the results.

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Installation Requirements

- A 64bits PC – 8GB (or more) of memory recommended
- Microsoft Windows
- Java Runtime Environment version 1.7 or higher
- Matlab 2011 or higher, for 32bits and 64 bits

A configuration file named “config.cfg” should be edited to specify the path/location of the Matlab(s) installation. The configuration file is located in the same path/directory of the program to run. An example of the configuration file content is the following:

M32=c:\Program Files (x86)\MATLAB\R2011b\bin\matlab.exe

M64=c:\Matlab\R2011b\bin\matlab.exe

Actin, nuclei & micropattern analysis program

Installation Requirements

- A PC (64bits) – at least 2GB of memory recommended
- Microsoft Windows (preferable Win7 or higher)
- Java Runtime Environment version 1.7 or higher
- Matlab 2011 or higher, for 32bits and for 64bits (two installations in the same PC)

Folders structure of the input data

Folders Structure

The data folders should include one folder for each condition to be process as illustrate in Figure 1. Within each condition folder you should create three folders containing the different data channel: the actin channel, the nucleus channel and the pattern channel, respectively.

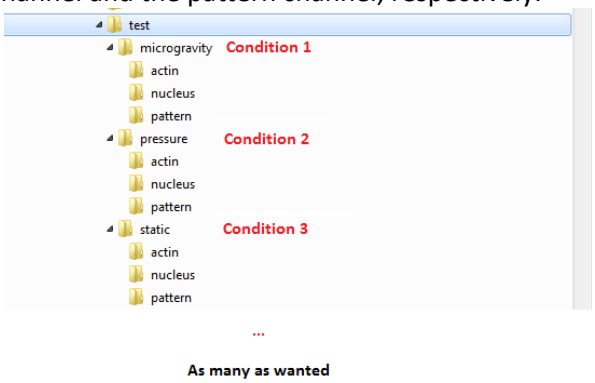


Fig 1. The data to be analyzed shall be structured in folders.

Expected data

Each folder should contain a list of .tif files, depending on how many images where taken in such condition.

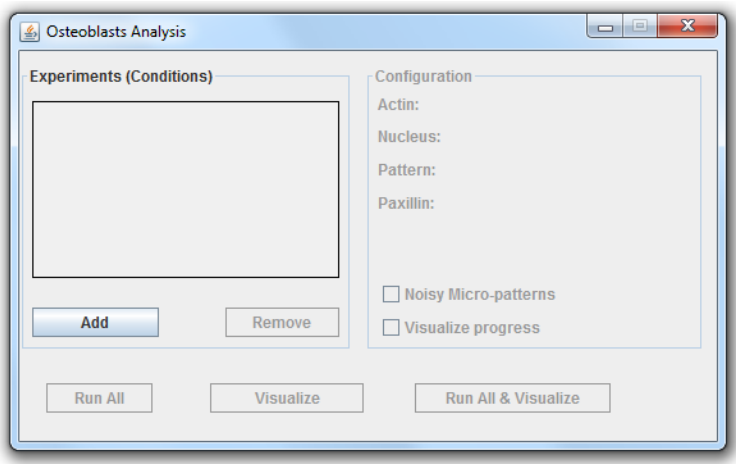
- In the case of actin and nucleus folders, the .tif image should be a multipage tif, each one representing a z-stack taken at different focal planes (z-axis).
- The pattern image is expected to be a single-page tif.

Running the program:

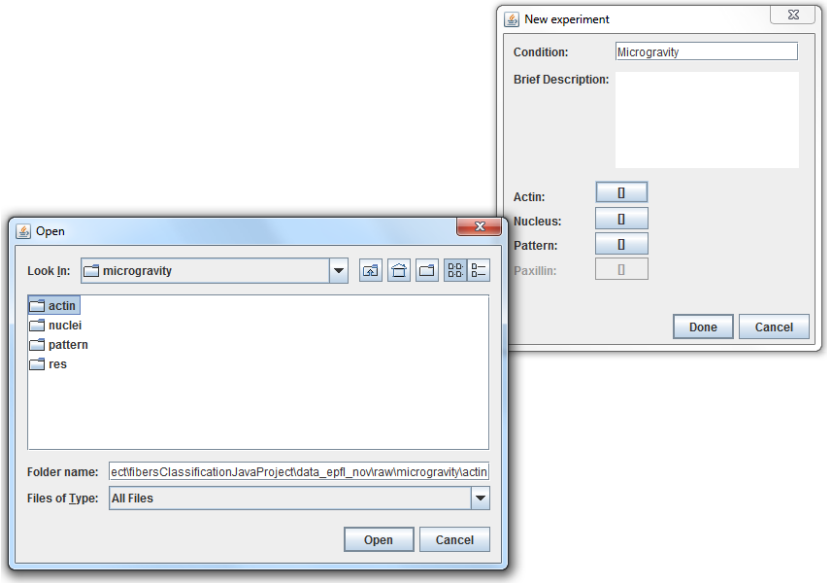
To run the program, you should double click the file "OsteoblastsAnalysis_vXXX.jar" and the program will be automatically executed if the Java was properly installed. A window will appear to add the different conditions and set the parameters.

Adding conditions:

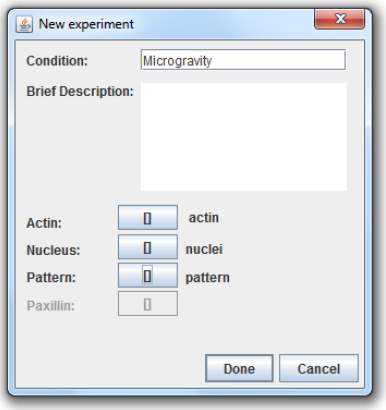
Once the Actin/Nuclei/Pattern program runs, the first step is to add each of the "Experiments (conditions)" to be analyzed.



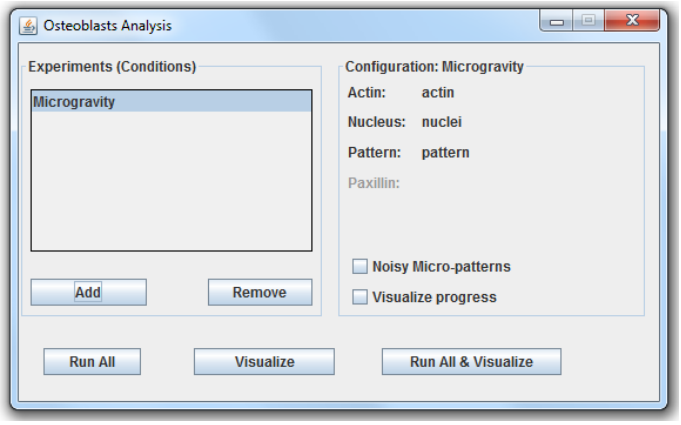
To add a new condition or experiment, one should specify a name for it and (optionally) a brief description. Then click in each and every button to specify the corresponding folder for the actin, nuclei and micropattern images.



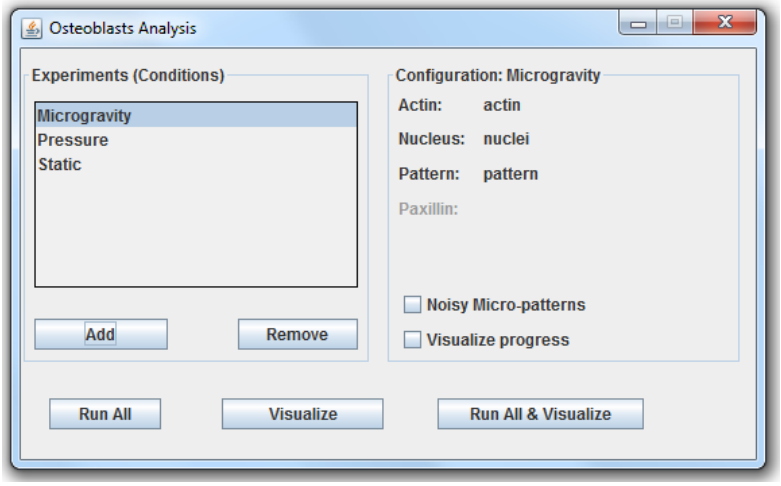
This should be repeated for all the three channels of information. After specifing the three channels you should end up with something like this:



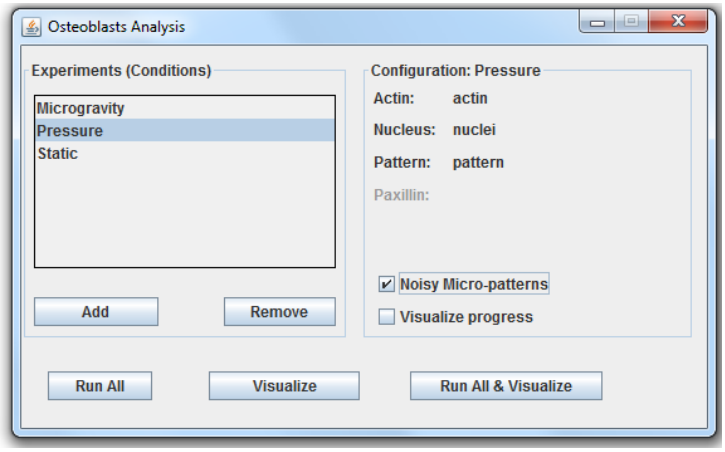
Finally, one should click in “Done” to return to the previous windows, and the newly added condition should appear in the Conditions list.



The same process should be repeated for the other conditions until all the conditions to be analyses are introduced in the “Experiments (Conditions)” list.



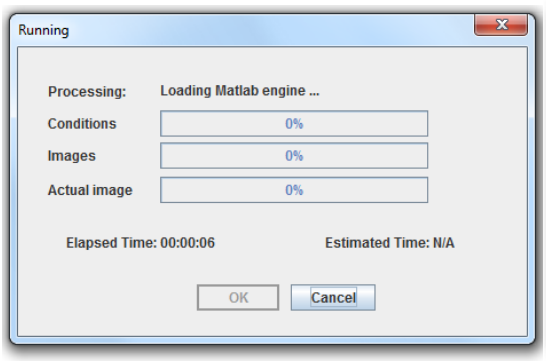
The configuration/options are related to each individual condition. For instance, in the pressure images the pattern image is very noisy and you can barely see the pattern. Thus by clicking the Pressure condition and checking the Noisy Micro-Patterns will signal the program to use another method for the processing micro-pattern of the micro-pattern images. We recommend to first run without checking this option, and if the results are not good then re-run the experiments setting the Noisy Micro-Pattern option.

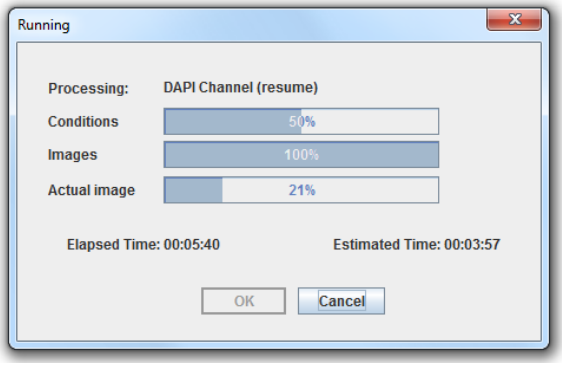


Once the experiments have been added and configured, the next step is to click “Run All” or “Run All & Visualize” to run the experiments or run and visualize the results, respectively. The “Visualize” option should be active whenever some results are available for some of the Conditions in the list.

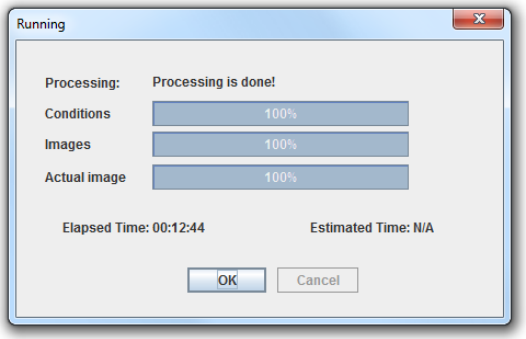
Running:

After clicking on Run (or Run & Visualize), the processing will start and a windows will show the progress achieved in the processing. It will first start the Matlab engine, which usually takes less than 4mins.





Then it will start processing all the channels for each condition, and will show the elapsed and estimated execution time (for the actual channel and actual condition only). The estimated time will be provided after a few minutes of execution. Once the results are ready, each of the progress bars should be in 100%.



Results:

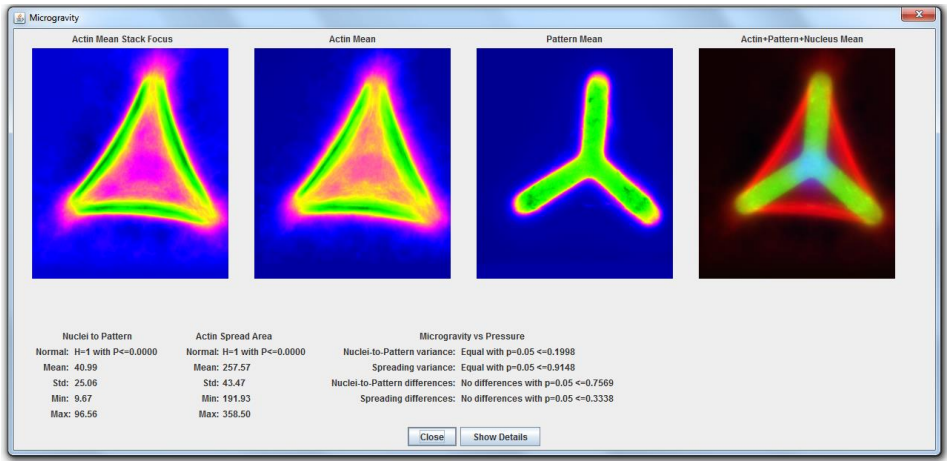
Once the processing is complete, one can click on “Visualize” to visualize the results. Then one window per condition will popup, where the overall results are visualized.

Visualization

Average images, computed after aligning all the images according to the micro-pattern displacements, are then visualized.

The first three images are shown using colormaps in order to highlight differences on the mean values, and are related to actin and the micropattern channels;

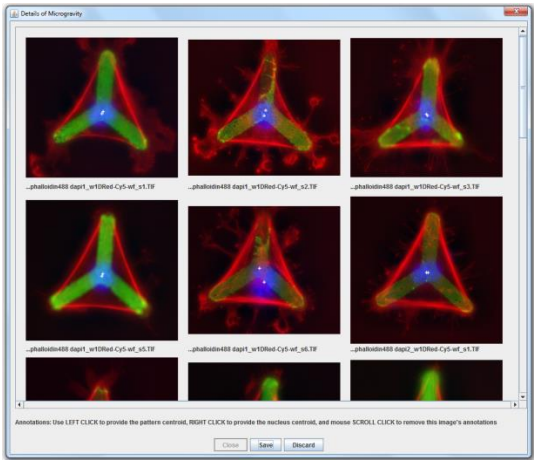
The forth image is an RGB combining the average image for the three channels.



In the case of the actin channels, two images are provided: “Actin Mean Stack Focus” and “Actin Mean”. The “Actin Mean Stack Focus” combines all the z-stack images into one using a stack focus technique (Remember that in the case of the actin (and nuclei) channels, a z-stack of images is expected in each tif image). Then the average of all stack focus images is provided using colormap.

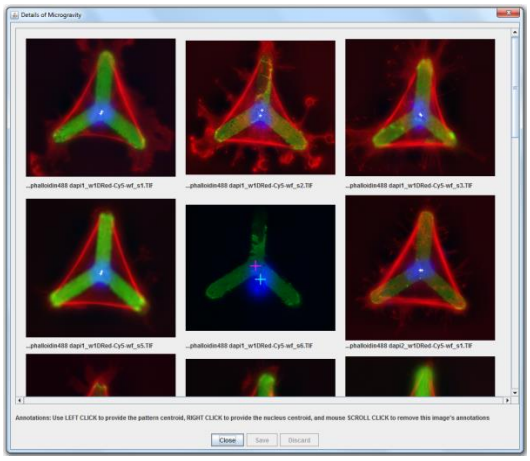
The “Actin Mean” select the z-stack that exhibit the best focus, and we provide the average of all the best focused images, visualized using colormap.

The average images can be always visually inspected by clicking in “Show Details”, where each individual image will be shown, along with the detected nucleus and micropattern center.



Quality control & Annotations:

A quality control step can be performed by simply hovering any of images and correct the estimated centroids. Left clicking within the image will provide a correction of the estimated micro pattern's centroid while right clicking will provide the same for the nuclei's centroid. Scroll clicking (or middle button click) will erase the provided corrections, keeping only the centroids estimated by the programs.



Once the desired corrections has been provided, clicking the "Save" button will allow saving the changes permanently, while the "Discard" button allow to erase all the corrections. The provided annotations (centroids) will serve as correction for the estimated values, and also as a mean to assess the programs performance.

Stored Results

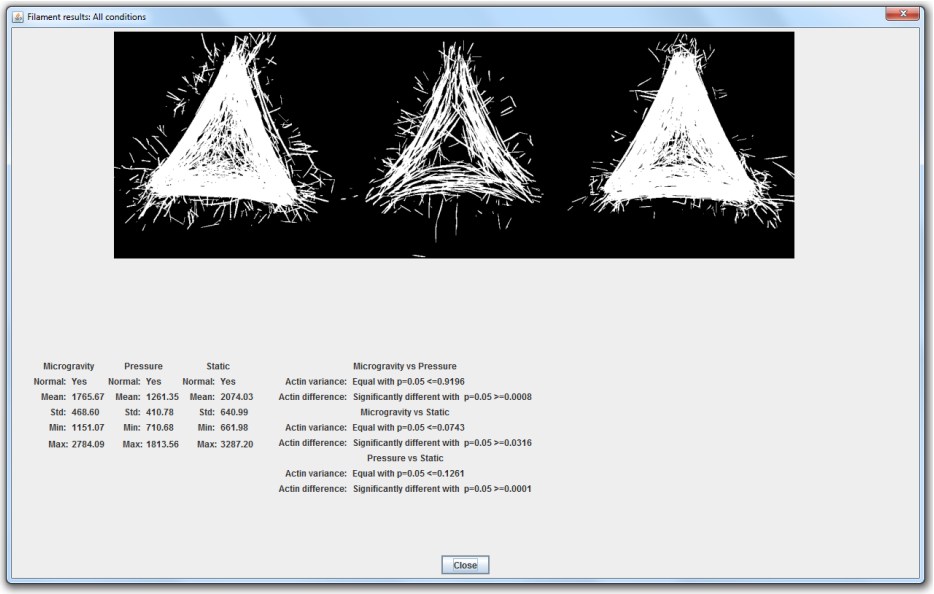
The overall processing results along with statistics are also stored in the respective folder, within a folder named "res".

Statistics related to sampled population measurements, in particular the nuclei center to pattern center distance, as well as the spreading degree quantification. For the two measurements, normality is tested using Kolmogorov-Smirnov and their mean, standard deviation, minimum and maximum values are provided.

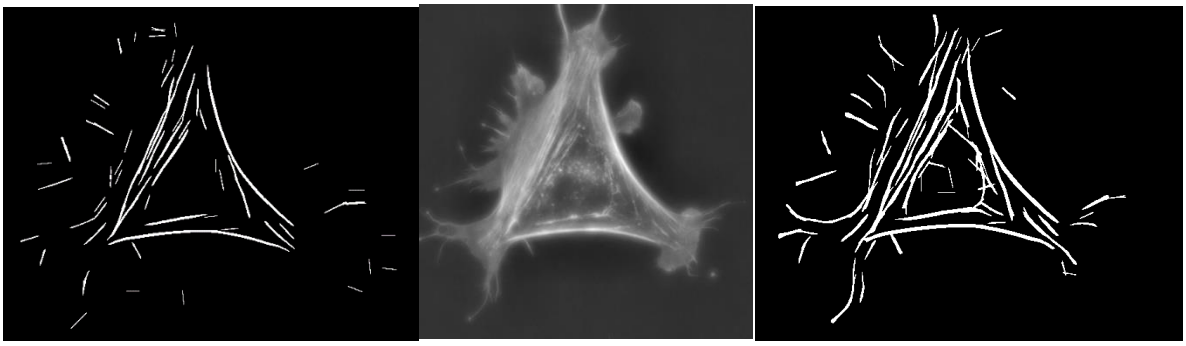
Then pairwise combination of conditions follows after these two initial statistics. Equality of variances (homoscedastic) is tested first using Levene's test, and if both variance are similar then a Student t-test is performed; otherwise a Welch test is performed instead. All the tests are performed for the nucleus-to-pattern center distances, and for the spreading degree.

Results for micropatterns analysis:

The result provides one composite filaments for each condition, and statistics related to actin filaments in the different conditions. First, the individual statistics for each condition is shown, followed by statistics related to pairwise combinations of different conditions. The computed statistics follows the same strategy and methods than in the previous program. The order of the images corresponds to the order of the statistics shown for each individual condition.



Although the default parameters pre-set in the program should be good enough to run most of the experiments, in some cases it is necessary to tune the parameters. Wrong parameters tuning might lead to wrong filaments extraction results, as shown in the following examples for one of the images (good parameters in the left picture, actin stack focus in the center, suboptimal parameters in the right picture).



Actin filament analysis program

Installation Requirements

- A PC (can be 32bits or 64bits) – at least 2GB of memory recommended
- Microsoft Windows (preferable Win7 or higher)
- Java Runtime Environment version 1.7 or higher
- Matlab 2011 or higher, for 32bits

The same configuration file of the previous program is required.

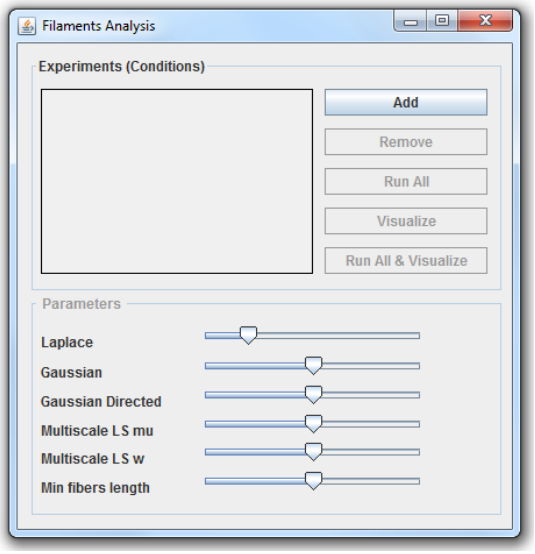
Folders structure of the input data

A similar structure as in the case of the Actin/Nuclei Micropattern Program are required as input data is for the filaments analysis program, the only difference is that only the actin filaments channel is used/required.

Ideally (but not a requirement), the previous program should be run first, then the filaments analysis will benefit of some of the output data an pre-processing already performed, thus saving computational costs.

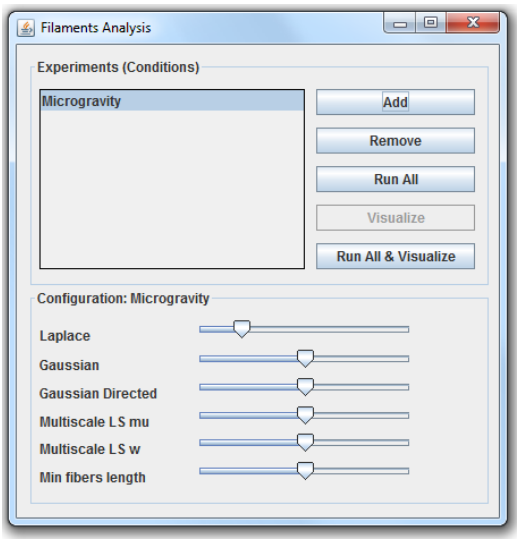
Running the program:

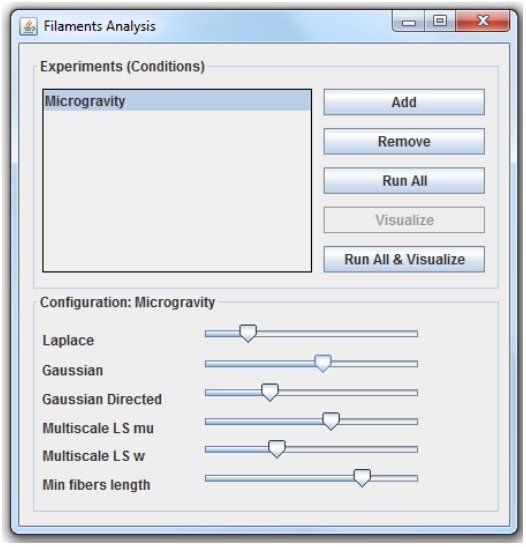
To run the program, you should double click the file "FilamentsAnalysis_vXXX.jar" and the program will be automatically executed. A window will appear to add the different conditions and set the parameters. Once clicked Run All (or Run All & Visualize), a small window showing the running progress will appear, just like in the previous program.



Adding conditions:

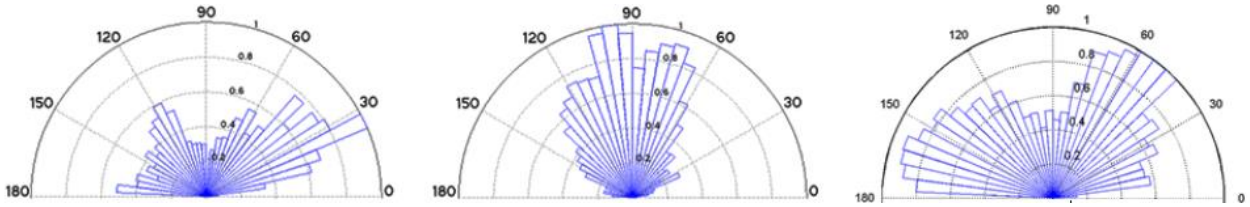
The conditions are added in similar way as in the Actin/Nuclei Micropattern Program, only the parameters are different. Likewise in the previous program, the default parameters should work most of the time. Otherwise, they should be tune up using just a few images and checking until satisfactory results are obtained. The same set of parameters is used for all the conditions.





Angular distribution results:

The result is a plot with the angular distribution per each condition, covering of all filaments of all cells in the related experiment, as illustrated next.



The plot is provided as a PNG file located in the "res" folder within the folder of each condition under assessment. The files name are:

- angulardist-Filaments.mat.png: corresponding to the angular distribution of all filaments
- angulardist-Filaments.mat_nuclei.png: corresponding to the angular distribution of those filaments located in the peri-nuclear area of the cells

A file in Matlab format is also provided containing the detailed list of all filaments of all the cells associated that particular condition, including: segments position within the image, length, width, ….