MitosisAnalyser GUI - User Guide

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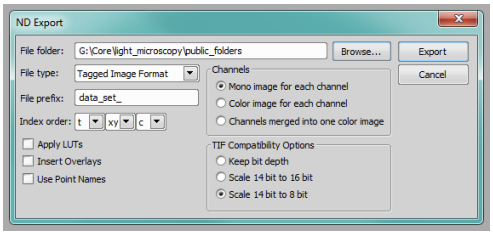
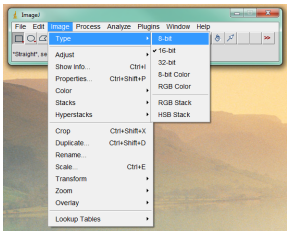
1 Some preliminaries





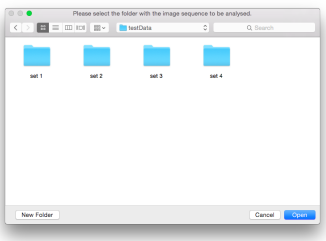
In the latest version, *MitosisAnalyser* only reads 8-bit tif or tiff images. If you have your underlying sequence saved as an nd2 file and need to scale your images and convert them from 16-bit to 8-bit, you can easily do so by using the NIS-Elements or Fiji/ImageJ software.

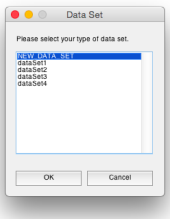
2 Running *MitosisAnalyser*

After having started MATLAB® make sure that your Current Folder is **MitosisAnalyser**. It should always contain the subfolders **presetDataSets** and **results**. Please also make sure that you never manually delete the "NEW_DATA_SET" file in the **presetDataSets** folder. The folder with the data set to be analysed does not necessarily need to be saved in the current directory.

In order to run the GUI, simply type `MiA` in the Command Window and press the return/enter key.

3 Choosing the data set to be analysed



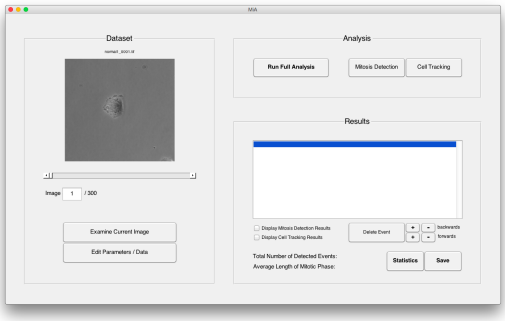


Next, a new window opens asking you to select the folder with the image sequence to be analysed. The images should be 8-bit grey-scale or RGB tif/tiff images[1]. However, colour images are converted into grey-scale images automatically.

[1] Loading nd2 files and other formats might be possible soon by using BioFormats.

Another window opens asking you to select your type of data set. You can either select “NEW_DATA_SET”, which will load default preset parameters, or choose a specific cell line or type of data, for which a set of variables already exists.

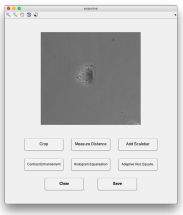
4 Main window



4.1 Dataset

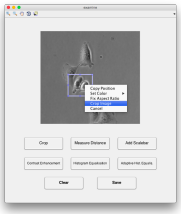
On the top left, you can click through all frames of your image sequence by using the slider or entering a specific frame number.

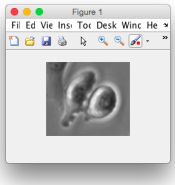
Furthermore, pressing the “Examine current image” pushbutton opens a new window:



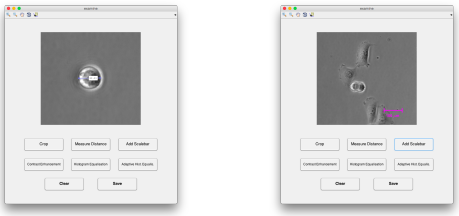
You can use the toolbar in order to zoom in, zoom out, pan, rotate and use the data cursor. Moreover, you can examine your image by using the pushbuttons:

- **Crop:** Select a rectangular part of the image you want to crop, right-click on it and choose “Crop Image”. A new window opens where you are able to save your result.



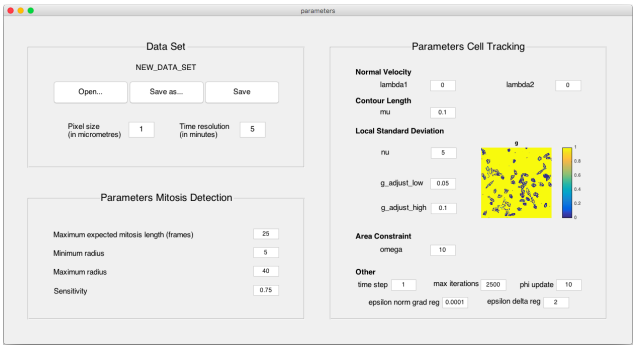


- **Measure distance:** With this tool, you can measure the distance (in pixels) between two positions inside of the image. You can add multiple distance lines by pressing the pushbutton several times. There are more options after right-clicking on the lines.
- **Add scalebar:** Displays a scalebar on the right bottom of the image. The width is 100 pixels multiplied with the pixel size in µm specified in the data settings.



- **Enhance contrast:** A built-in contrast and brightness adjustment toolbox opens in a new window where you are able to change histogram settings.
- **Equalise histogram:** Performs histogram equalisation on the current image (tries to use the full range of the grey values).
- **Adaptive histogram equalisation:** Performs adaptive histogram equalisation on the current image rather locally than on the whole image in contrast to the above method. Usually works better on phase contrast images than the plain histogram equalisation.
- **Clear:** Deletes changes on the current image and redisplays the original one.
- **Save:** Saves the current image as a png file in the current directory. A new window opens where you can enter the file name.

If you want to change your data settings and edit parameters, press the "Edit Parameters / Data" button:



On the top left, the name of your parameter / data set is displayed and you can quickly save changes by pressing "Save". "Save as..." enables you to save your settings in a new file. Change the current parameter set by pressing the "Open..." button and selecting a file. You can also manually edit the pixel size and the time resolution.

On the bottom left you can change the parameters in connection to the Mitosis Detection:

1. **Maximum expected mitosis length** (in frames)
2. Radius interval [**minimum radius**, **maximum radius**]: You can check whether these values seem reasonable by using the "Measure distance" tool
3. **Sensitivity**: Value between 0 and 1; the higher the sensitivity, the more circular objects are detected, but the more likely are false positives at the same time

On the right you can edit several parameters associated with the Cell Tracking:

1. Normal Velocity: **lambda1** and **lambda2** are weighting the normal velocity term inside and outside of the cell contour, respectively
2. Contour Length: the higher **mu**, the smoother is the contour
3. Local Standard Deviation: **nu** weights the local standard deviation term, which propagates the contour towards regions of high local standard deviation (inside of the cell) and stops

its evolution at the cell boundary; the edge detection map g is adjusted by stretching values between **g_adjust_low** and **g_adjust_high** (values between 0 and 1, usually roughly inside the interval [0.05 0.25]; immediate changes to g can be seen on the right)

4. Area Constraint: **omega** weights the term, which prevents the cell area from becoming too small
5. Other: Five additional parameters ensuring that the algorithm converges and works properly

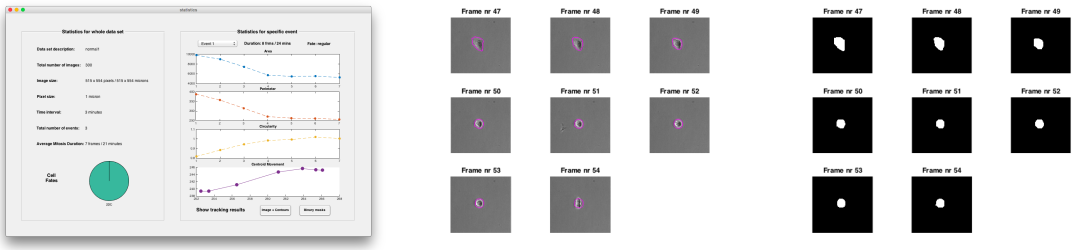
4.2 Analysis

You can either run the "Mitosis Detection" and "Cell Tracking" separately or directly in one go by pressing "Run Full Analysis". Note that cell tracking cannot be performed without previous mitosis detection. A waitbar will very roughly show you the progress of the analysis. Moreover, some comments are displayed in the Command Window.

4.3 Results

After Mitosis Detection, the total number of events is shown at the bottom and results are displayed in the listbox as follows: "Event nr | Frame nr". Once you click on a specific event and the checkbox "Display Mitosis Detection Results" is ticked, the corresponding image with the encircled mitotic cell is display on the top left in the Dataset panel. You can delete events by clicking on the corresponding row in the listbox and pressing "Delete Event" and you can save your data as mat files (they are automatically saved in the "results" folder) by pressing "Save" and entering a file name.

After having run the Cell Tracking, your analysis is complete and the listbox displays the results in the following order: "Event Nr. | Frames | Duration (frames) | Duration (minutes) | Cell Fate". In addition to the total number of events you can now see the average of the mitotic phase displayed at the bottom. Again, you can enable display of the tracking contours by ticking the checkbox "Display Cell Tracking Results". Moreover, you can not only delete entire events by pressing "Delete Event", but also add and delete single frames to the beginning or end of the mitosis phase by pressing the "+" and "-" buttons, respectively. Please note that you should only use this feature after having looked at statistics / tracking results. By pressing the "Save" button all your results are saved in the "results" folder.



Pressing the "Statistics" button opens a new window where you can see a summary of the mitosis analysis. On the left statistics for the whole data set are displayed and at the bottom you can see a pie chart immediately visualising the proportion of the different cell fates. On the right you can switch between all events and display specific statistics for single events including plots that visualise changes of area, perimeter and circularity as well as centroid movement. You can see tracking results at a glance and have them displayed in a new window showing the original images with the corresponding cell contours and binary segmentation masks by pressing "Image + Contours" and "Binary masks", respectively.