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Live or Dead on DIC

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Overview

"Live or Dead on DIC" is an analysis module that tracks and classifies living cells acquired under DIC illumination. The calls are classified based on the shape either as "live" or "dead".

The input of the analysis is a single channel time-lapse movie.

The module

1. detects the cells,
2. tracks them and
3. measures relevant dynamic and morphometric features.

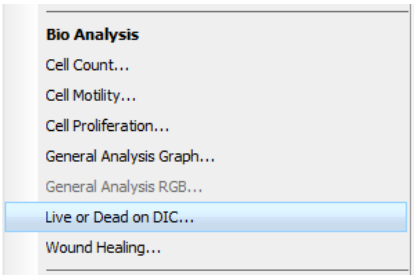
Based on the cell circularity the module assigns each cell into appropriate class.

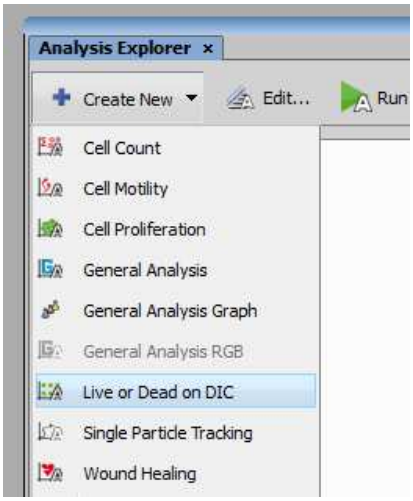
Interactive Operation

This section describes how to setup the module parameters for a given image. The image to be analyzed must be opened fits.

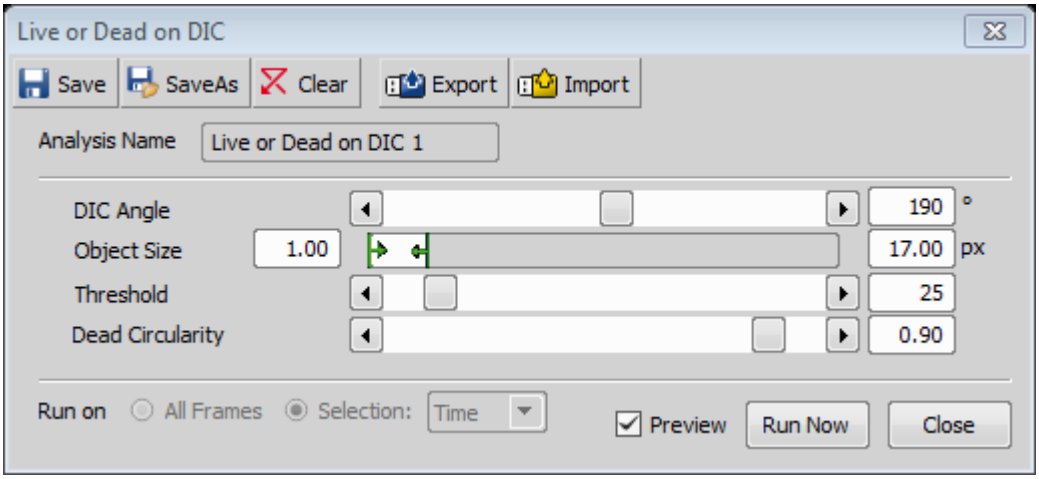
There are two ways to invoke the module window.

- From main menu in the "Applications" popup, find the "Bio Analysis" section and select the "Live or Dead on DIC…"
- In the "Analysis Explorer" floating element select "Live or Dead on DIC" from the picker.





The parameters window is displayed…



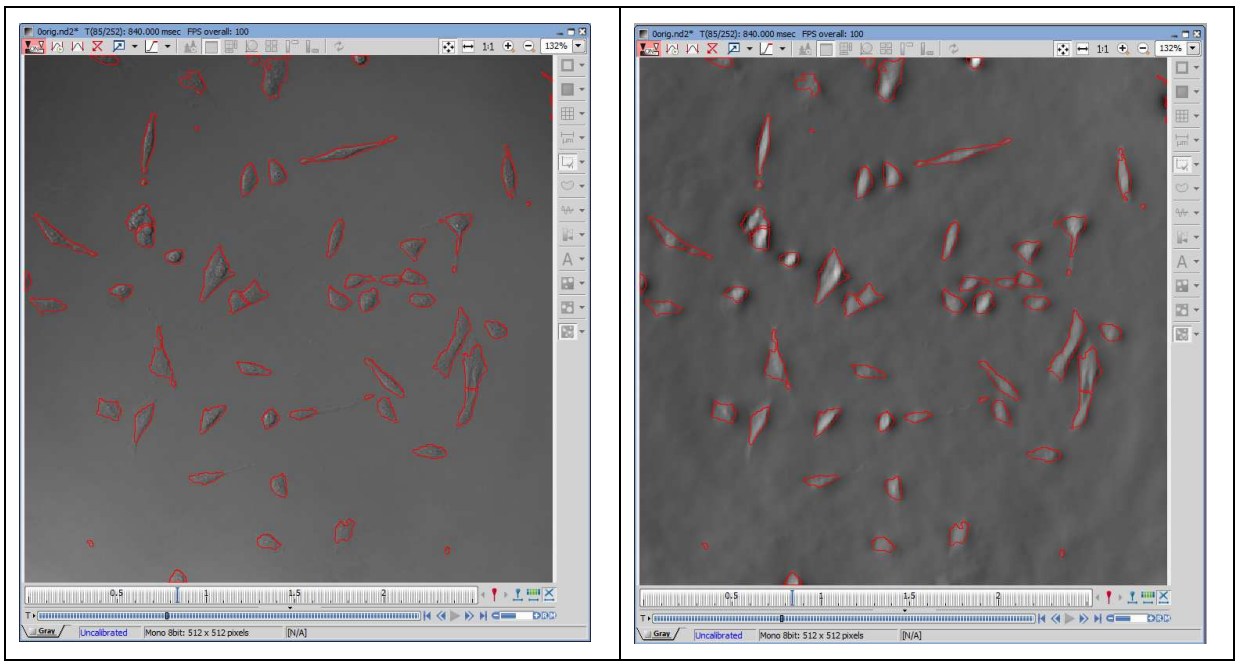
Detection

Detecting structures on DIC is difficult because of low contrast of such images. It is said that the image is reconstructed from the differential image. There are three parameters affecting the reconstruction:

- DIC Angle of illumination
- Minimum and Maximum Object Size (round objects are assumed)

The preview of segmented cells can be visualized as overlay on the DIC original image. It changes as the parameters are being changed.

However it is beneficial to see the reconstructed image while changing the reconstruction parameters. The reconstructed image is shown while manipulating (dragging the sliders of the three relevant parameters).



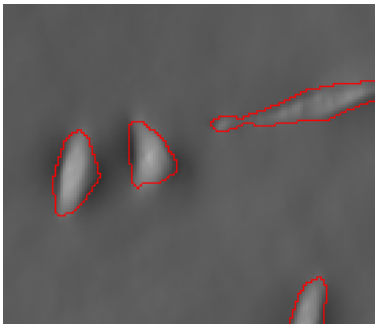
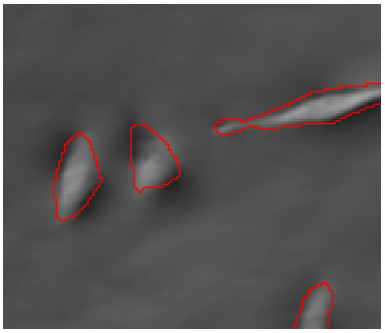
The goal in setting these three parameters is to make the cells bright compared to their background on the reconstructed image.

The reconstructed image is used to detect regional maxima and mark the cells. It affects the final number cells.

Angle

This is the most critical parameter of the reconstruction. It must be set correctly.

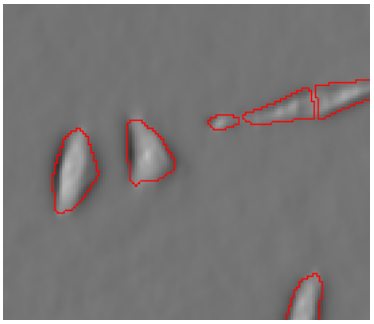
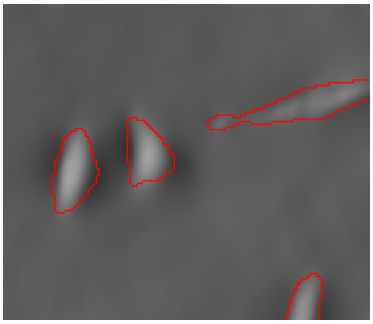
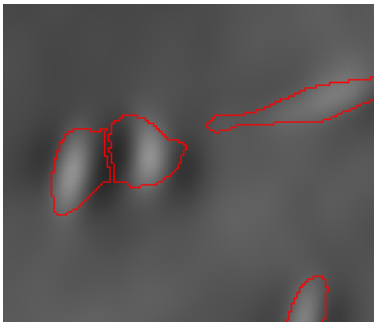
The following strip shows the effect of angle on the reconstructed image.

Angle: 130 (bad)	Angle: 190 (best)	Angle: 250 (good)
		

Object Size

The reconstruction takes into account expected size of objects being reconstructed. As the real cells tend not to be circular some compromises must be made. The minimum must be small enough to accommodate elongated structures but not so much as to separate objects. More images from different portion of the movie should be checked.

The strip of images shows the effect of size on the reconstructed image.

Size: 5-10 (too small)	Size: 10-30 (best)	Size: 30-50 (too big)
		

Threshold and Circularity

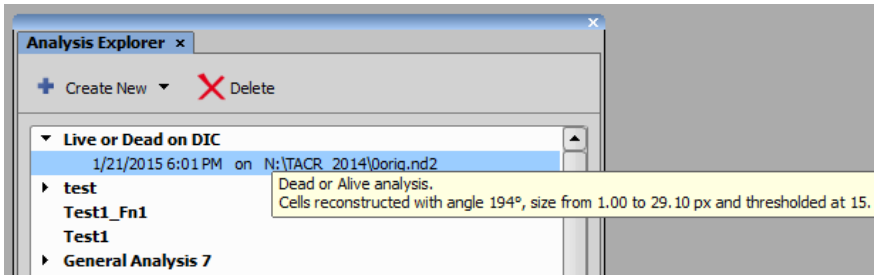
This parameter affects the shape of segmented cells. The lower the parameter the farther the cell will extend from its center.

The “Dead Circularity” parameter defines the threshold where a cell is considered live or dead. Specifically if the circularity is higher than the value (1 [one] is a perfect circle) specified it is dead. Otherwise it is considered alive.

Saving the analysis definition

The analysis definition can be saved for later use or for batch analysis. It can be also exported and transferred to different computer.

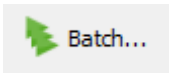
Saved analysis under a default name “Live or Dead on DIC” inside Analysis Explorer. The tooltip shows the description of definition and tree one run analysis from January 21 2015.



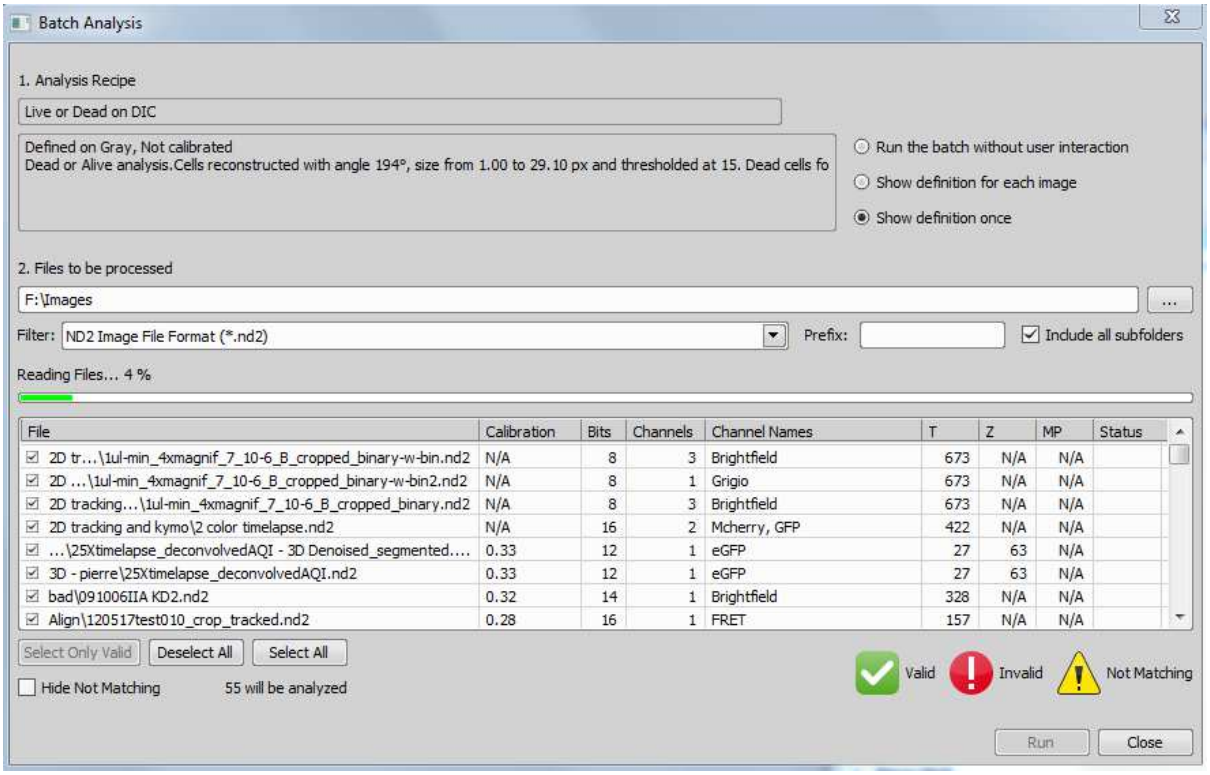
Running the analysis

By running the analysis on current image segmented binary objects are created and then tracked. Several features are measured. In case the result is not as expected the results can be undone and the analysis can be run with different parameters.

Batch analyzing multiple files



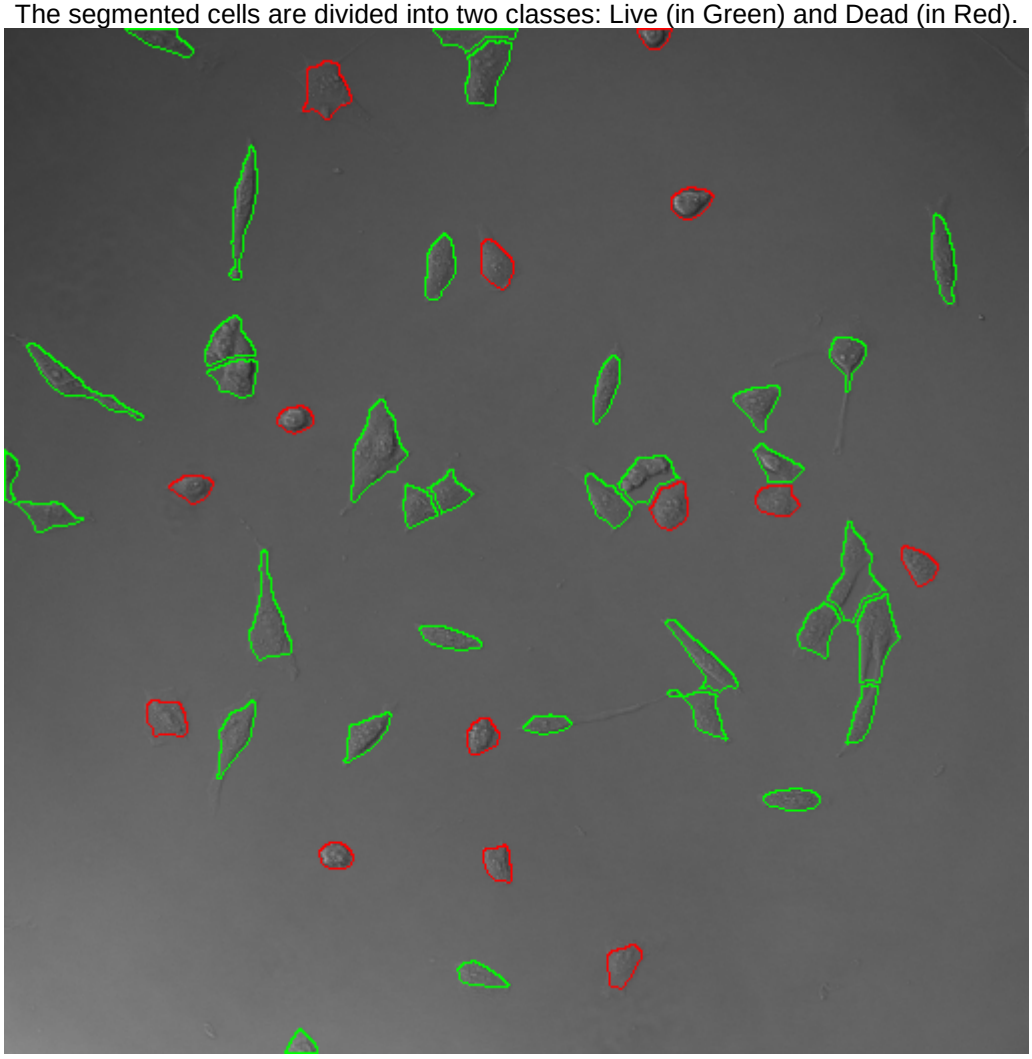
Each saved analysis can be run on several files from Analysis Explorer.



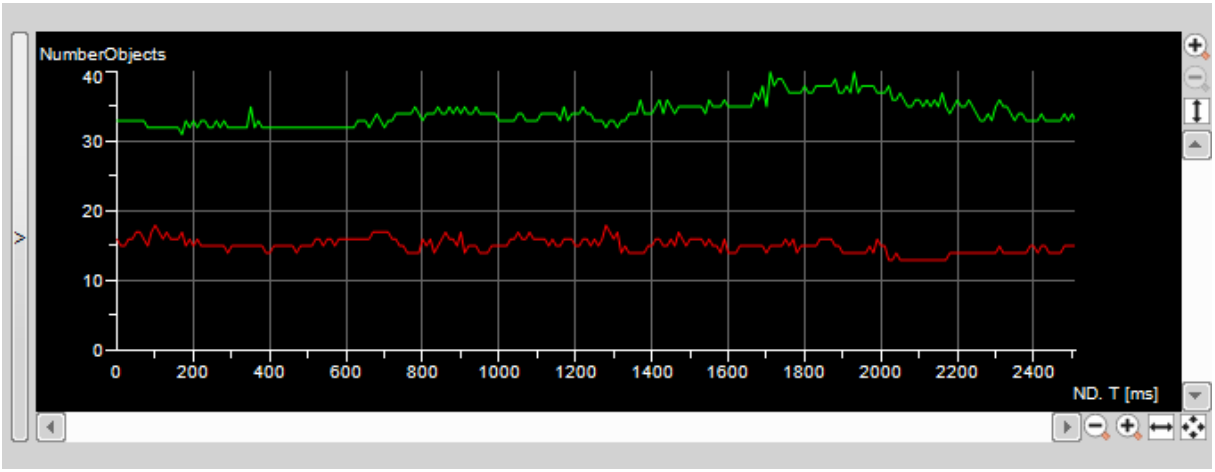
After batch analysis is finished, tracked cells are stored in each processed file.

Results

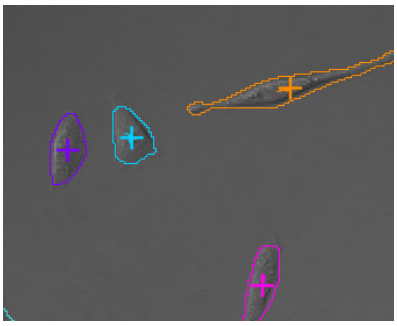
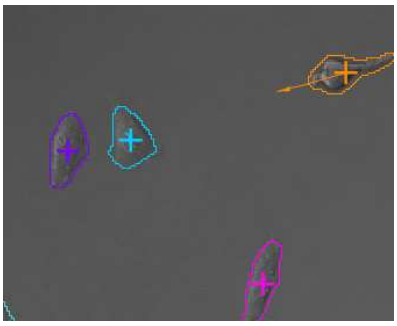
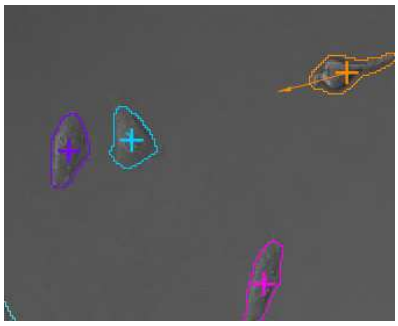
The segmented cells are divided into two classes: Live (in Green) and Dead (in Red).



The number of Live/Dead cells can be visualized in a table or graph in relation with time.



Tracking

The tracked cells can be inspected and their measured properties viewed in graph or table. Here, a cell is shown at three different time points. In the middle (in frame 101) it contracts abruptly. This rapid change can be seen on the graph of circularity (yellow line – right axis) and area (orange – left axis).

T: 83/252	T: 101/252	T: 119/252