Transmitted light timelapse images correction
ImageJ macro
User guide
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ImageJ macro "DivideStackByRunningAverage_29.ijm"

About the macro name: should be changed because current version is not limited to a division of input frames by running sub-stack Average T-projections.

Designed to correct transmitted-light time-lapse images in cases such as:

- Dirt on the camera or optical surfaces;
- Illumination unevenness due to:
  1. imperfections in illumination system;
  2. distortion of light beam by sample (e. g. imaging near border of wells).

Two working modes:

- Active image processing (**single image processing**);
- Processing of a time-lapse images folder (**batch processing**).

Accepted image dimensions: XYT, XYZT, XYCZT.
Processed dimensions: XYT.
Multi-channel images are reduced to their transmitted-light channel.
Z-stacks are reduced to their Average Z-projection.

If an acceptable image is selected, the macro displays a suite of user interfaces and processes it after eventual reduction to its XYT dimensions.
If no images are open, the macro switches to batch-processing mode and asks for input-output folders and for including and excluding file filters and processing parameters.

The macro was first designed to work without any empty-field correction-image to process images close to the border of well, in which case illumination and images are distorted by the surface of the culture medium.
If no such problems occurred during acquisition, the macro can be run using a correction-image consisting in an empty-field image (the same for all positions in case of a multi-position time-lapse).

Algorithms:

Algorithm 1a: division of frames by running sub-stack average-projection
For each frame of active transmitted-light time-lapse image:
- creates a running sub-stack of constant, adjustable size (timeAverageRange):
  first slice = current input image frame - timeAveragingRange/2
  last slice = current input image frame + timeAveragingRange/2-1
- does average T-projection of running sub-stack;
- divides current input slice by projection and multiplies the result
  by input stack mean value;
  in order to remove jumps of slice brightness.

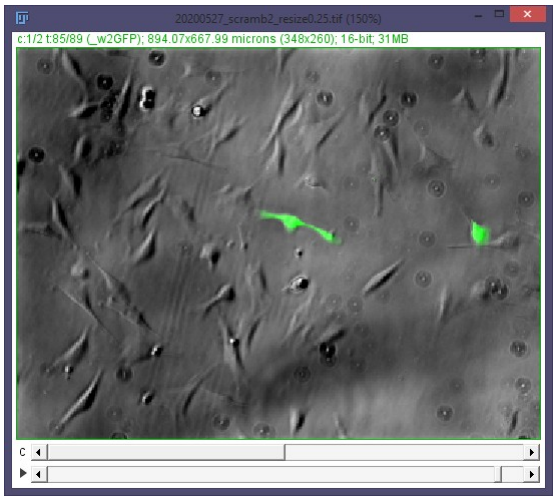
Algorithm 1b: division of each frame by empty-field image (correction-image).

Algorithm 2: division of each frame by an image obtained from the frame by:
- removal of brightest and darkest regions that are smaller than objects to be analyzed;
- gaussian blur with a sigma calculated from objects' size.

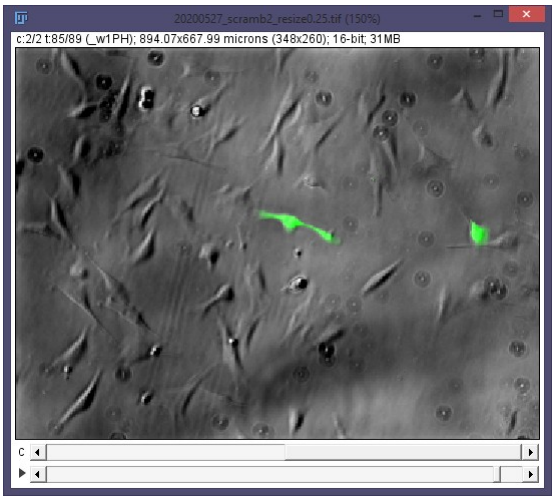
Algorithm 2 has similar effect as FFT-Bandpass but is faster for small objects and provides results of better quality. Improvements of the method are under devloppement.

Algorithms 1 and 2 are independent.
Process can be limited to 1a or 1b, exclusive of each other.
It can be followed by or limited to Algorithm 2.

Example of accepted input image:

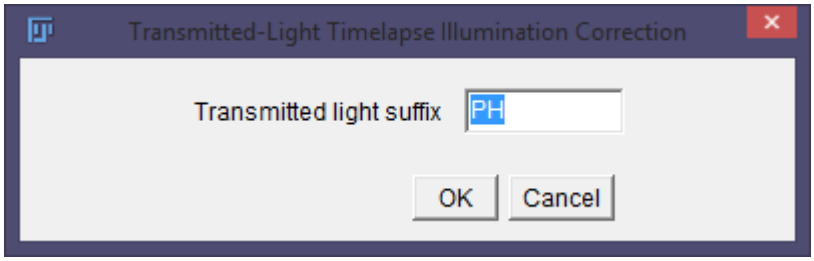


Channel slider on GFP (_w2GFP)

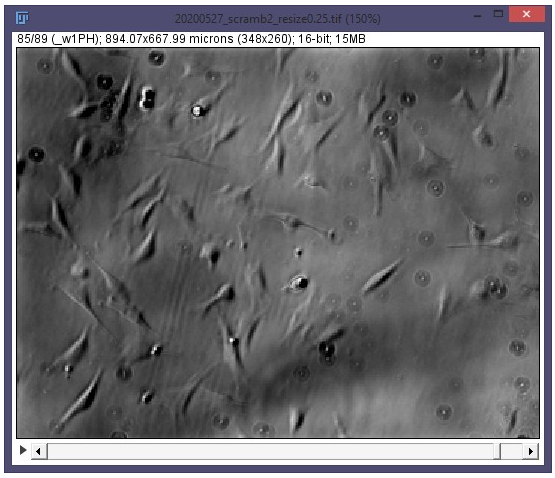


Channel slider on phase contrast (_w1PH)

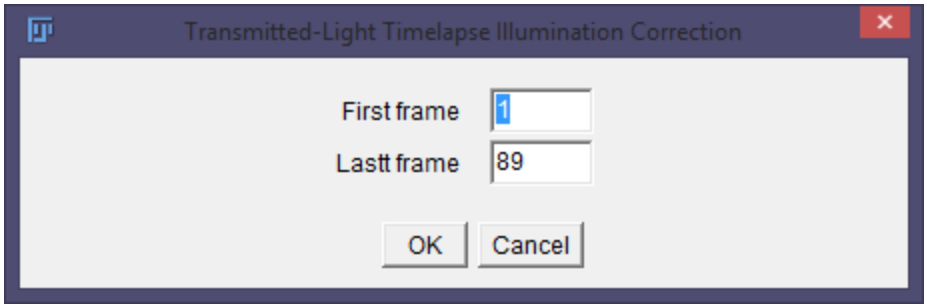
Enter a characteristic string from label of transmitted-light channel:



Pressing OK reduces input image to its phase contrast channel:



Enter time-range to be processed:

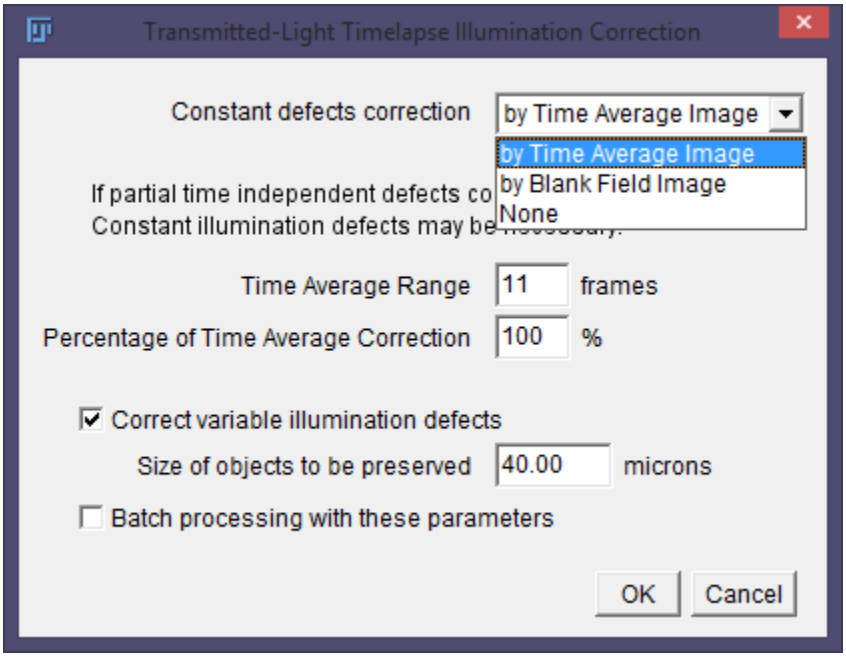


Defaults: first frame = 1, last frame = number of time-points.
Allows to limit process to a part of time-lapse to be analyzed.

Quality of result strongly depends on:

- nature and severity of defects;
- used algorithm(s);
- processing parameters.

Processing algorithms and parameters dialog:



1)

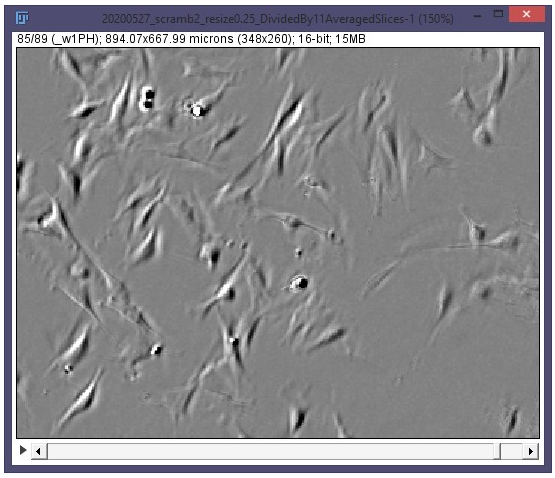
Constant defects correction:

- **by Time Average Projection Image**:
  Time Average Range is the width of the running time window around processed frame.
  Setting it to number of output frames should be OK if there are no immobile objects.
  In case of very bad illumination, low contrast or highly visible camera dirt, try 3, which is the lowest value.
  Percentage of Time Average Correction: result is a mix of processed and original frame. In case of a large Time Average Range and objects move slowly, processed frames may exhibit grabs tracing their movement. If the grabs are a problem for tracking the objects, try to do a partial correction, for instance 50%, 33% or 25%.
- **by Blank Field Image**:
  This option accepts no parameters. It's the best if the camera-optical-system dirt image is the same with and without the sample (is not disturbed by the sample, the medium or the holder).
- **None**:
  Do no **Constant defects correction**

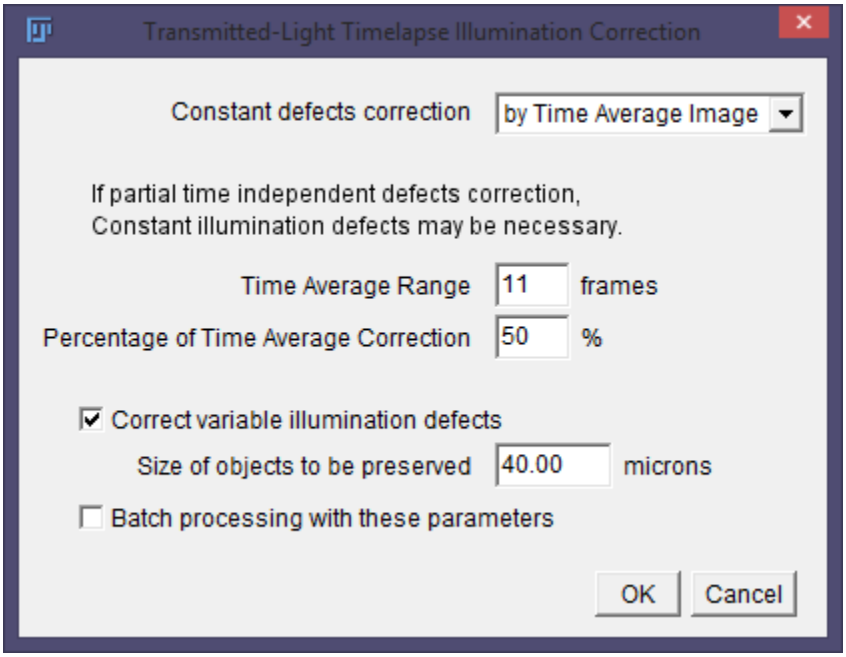
Correct variable illumination defects:
Variable defects may occur when imaging objects close to the border of a well. In such regions, the surface of the culture medium is not flat and induces a distortion of the illumination beam, resulting in poor phase contrast and defects depending on position in case of a multi-position time-lapse.

Size of objects to be preserved:
Expressed in geometrical units if images are spatially calibrated, in pixels otherwise. Advantage of calibrated images: the number to be entered here is independent of image scale. It will be the same for original image and for down-sampled copies, whatever the scale factor.

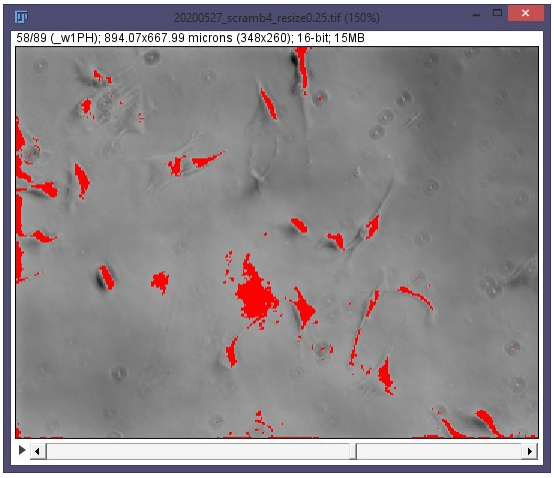
Result obtained with parameters from the example:



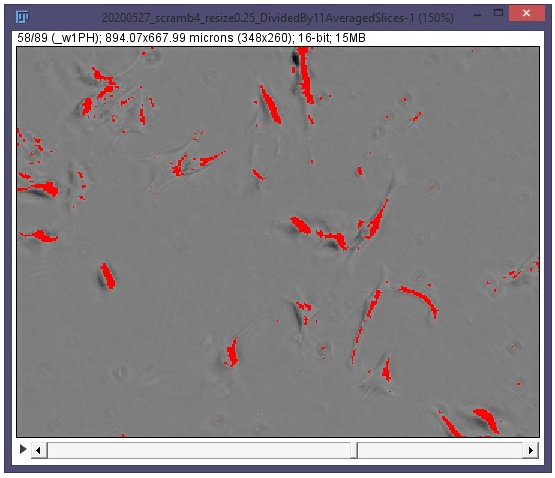
Other example:
Processing parameters:



Thresholded images:



Unprocessed



Processed

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