GUI - Image Treatment
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Version 1.2

Custom Matlab-based software to process timelapse stacks and scan stacks.

Active:
- FOV alignment
- Drift correction
- Wiener filter
- Background subtraction

Inactive:
- Wallis filter
- Photobleaching correction
- Parallel computing (available with Matlab script)
- Default calibration (available with Matlab script)

Version log

1.1
- Added support to load irregular-sized stacks for background subtraction

1.2
- Drift correction works for more than 2 wavelengths
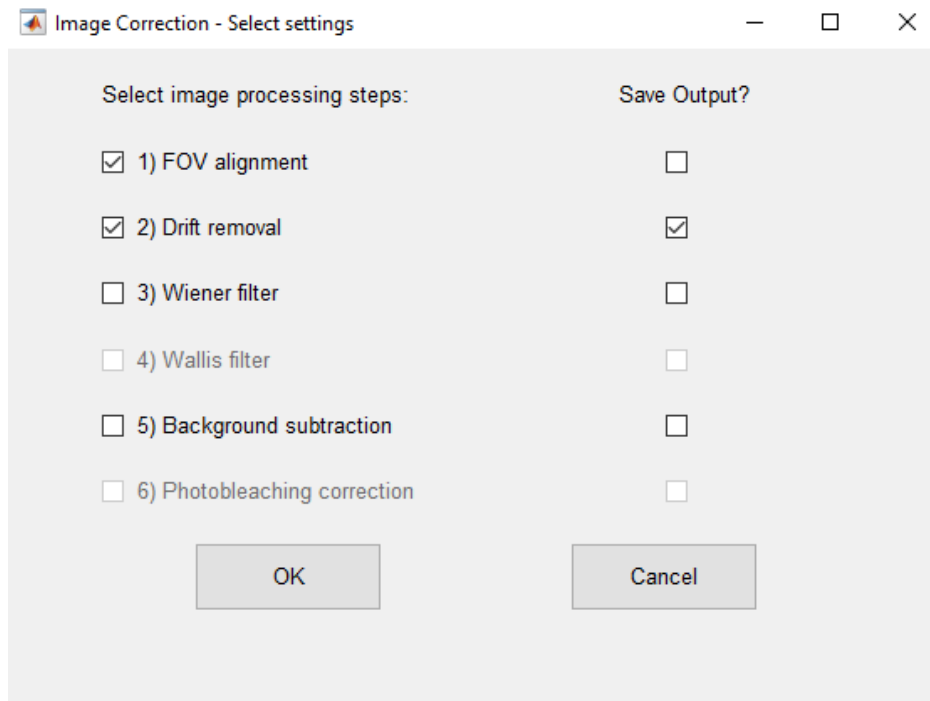- Naming output files fixed

1) Installation

Follow the on-screen instructions.

First-time users require an internet connection to download the Matlab Compiler Runtime (about 500 Mb)

2) Settings selection

Dialog window to select the processing steps (see section 7 for details). The user can choose which output(s) to save. Be aware that the processing are sequential and cumulative. For example, selecting the options as in the screenshot below, saves the stacks after FOV alignment and drift correction.

3.1) Options

If FOV alignment is selected, please select the channel identifier from the list [w1, w2, 405, 488, 561, 640]. This identifier needs to be present in the file names that require the FOV alignment.
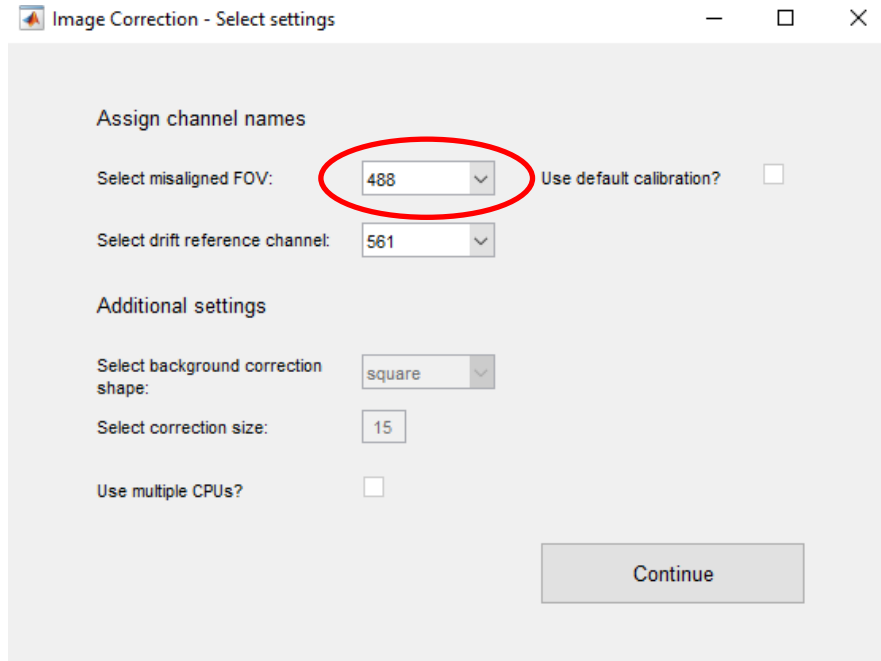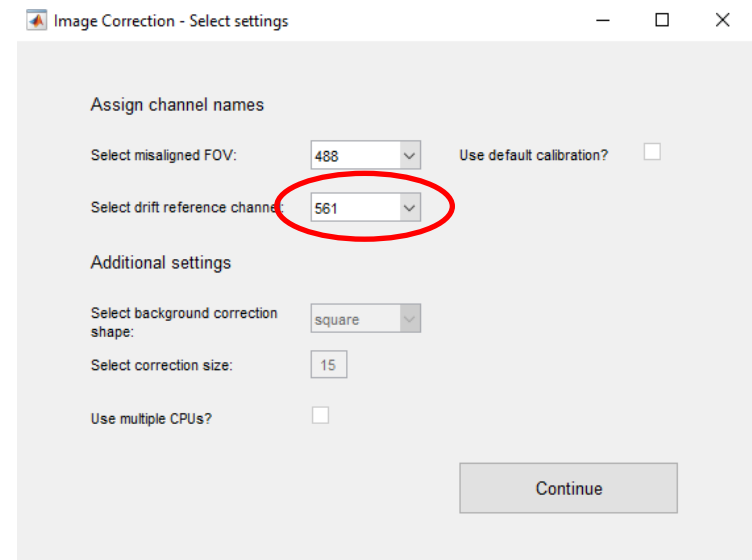
3.2) Options

If Drift correction is selected, please provide the channel identifier for the reference channel (see section 7.2) from the list [all,w1, w2, 405, 488, 561, 640]. This identifier needs to be present in the file name, except for "all". If two channels require the same drift correction, when for example using two camera's during simultaneous acquisition, the names need to be identical apart from the identifier. The identifier in the second (non-reference) channel needs to correspond with one from the identifier list.

"All" is used when no identifier is present in the file names and all files require a separate drift correction.

Example
The files "TIRF_488_1" and "TIRF_561_1" are identical, apart from the wavelength identifier. The default identifier for MetaMorph is "w1"and "w2".

3.3) Options

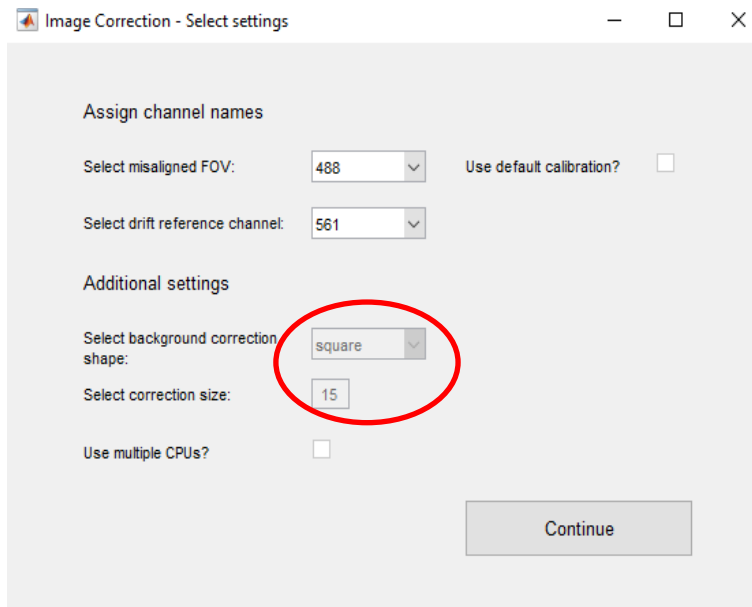If background subtraction is selected, the size (in pixels) and shape of the subtraction kernel can be selected.
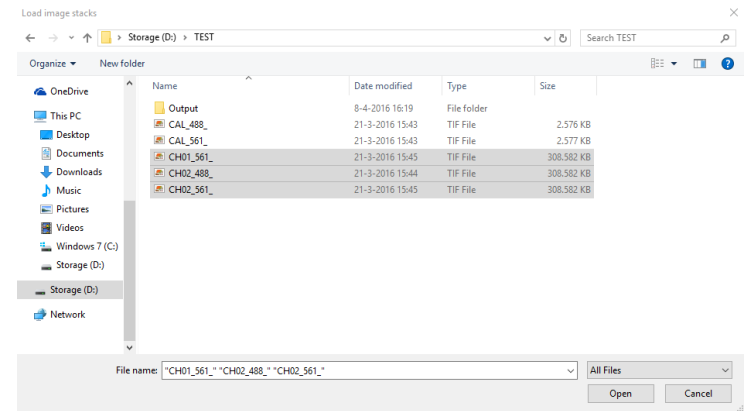
4.1) Select image files

The following (multiple) inputs are permitted:
- TIF stacks
- STK stacks
- .ND files (MetaMorph)
- .SCAN files (MetaMorph)

- Files containing two FOV's, i.e. images with size 512x1024, are recognized and separated (MetaMorph)

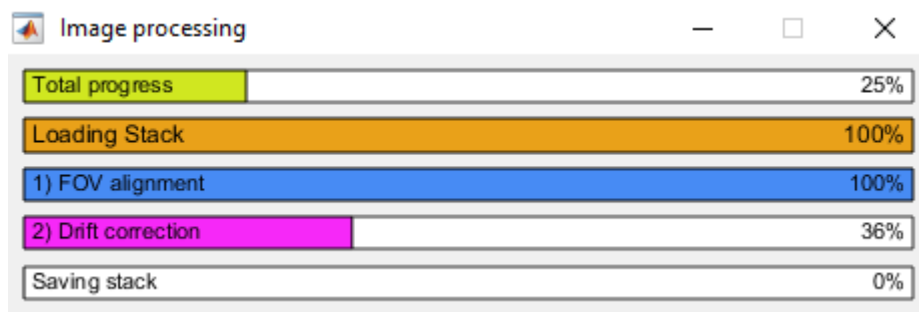## 4.2) Select FOV calibration files

Select the calibration stacks. These require a wavelength identifier, one of which needs to correspond to the chosen misaligned FOV wavelength (section 3.1).
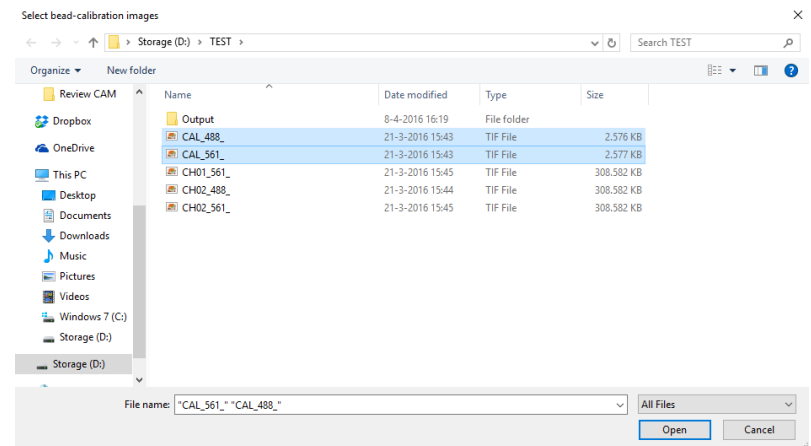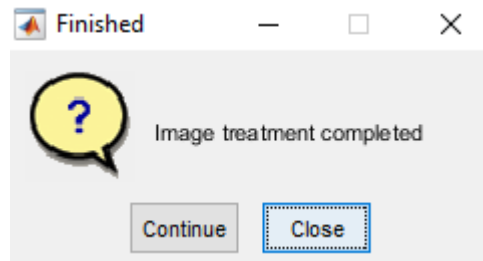
5) Progress

The progress bar is visible during the process.
Note, no abort function is currently present.


After completion, the user can choose to process more stacks or close the program.

6) Output

The output files can be found in the folder "Output", which is located in the folder containing the stacks.

The numbers preceding the original filename indicate the order of the processing steps. This can be useful in keeping track of the Output files after multiple processing rounds.
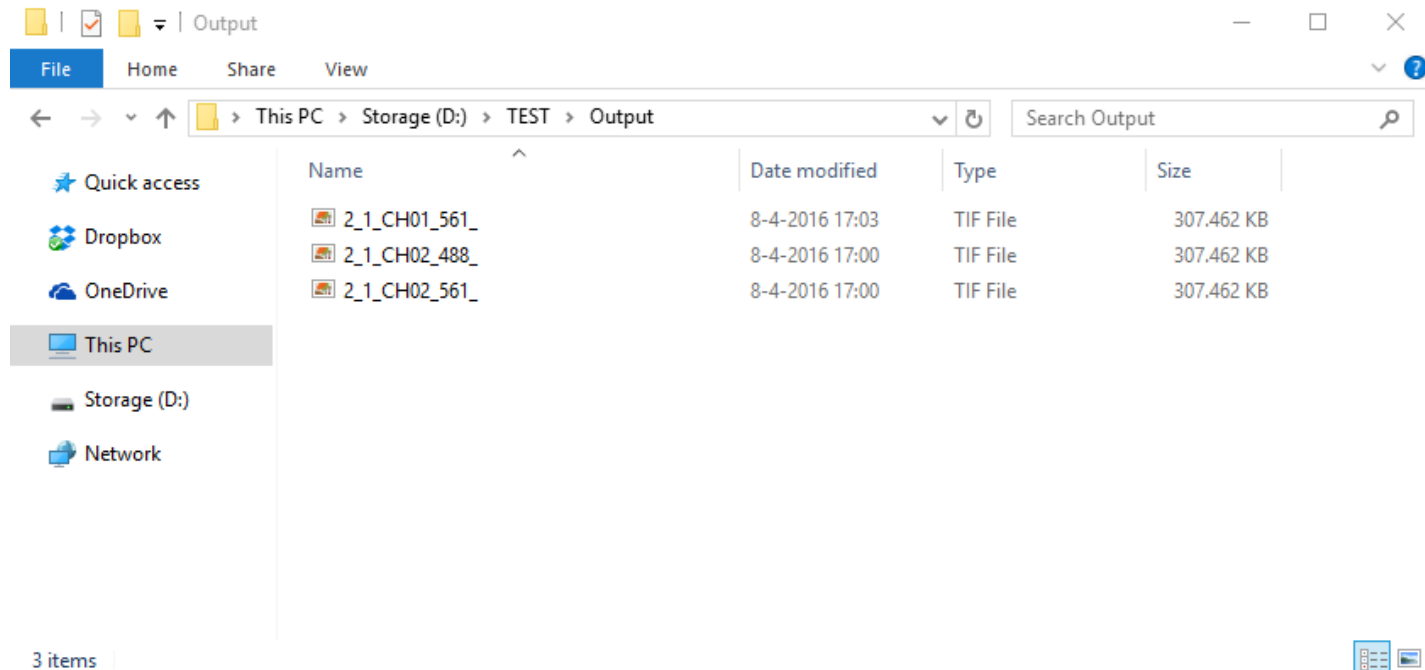
7.1) FOV alignment
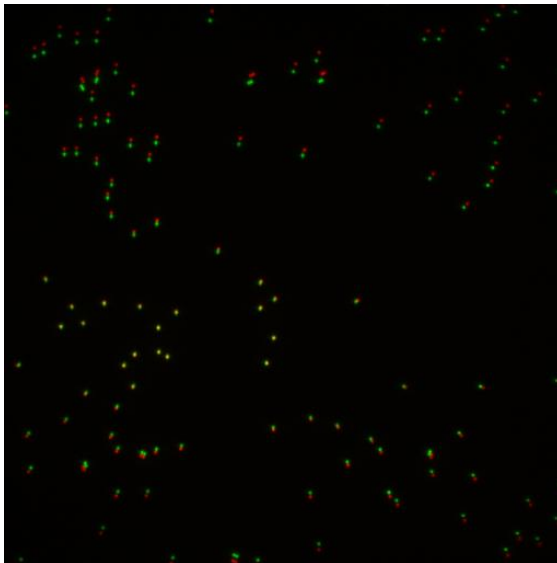
This processing step corrects misalingment and (non-linear) distortion between the two Field-of-Views of two camera's. It requires an image of beads from both camera's.

The program automatically finds the center of the beads and matches these positions between both FOV's. It then applies the correction to the distorted stacks.

Note, the edges of the FOV can be difficult to correct for.
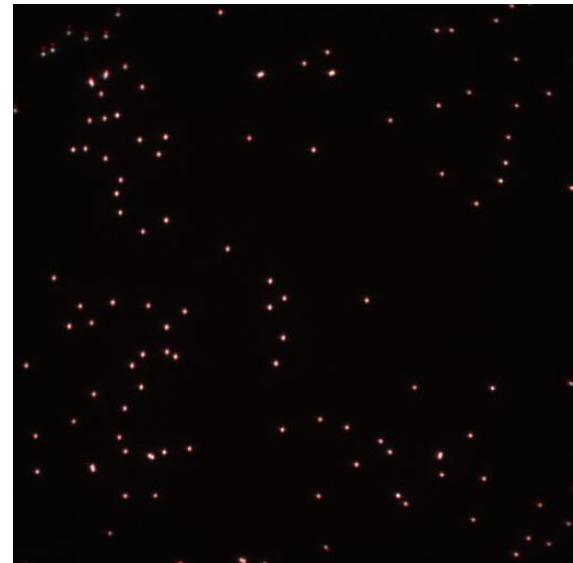
7.2) Drift correction

This processing step corrects for drift during time-lapse acquisition. It requires features that are constant and fixed (such as beads or labelled microtubule seeds) in a reference channel. Through a cross-correlation algorithm between consecutive frames in this reference channel, it finds the drift between each frame.

The drift correction is then applied to the reference channel and, if you're using two camera's, to the second channel as well.
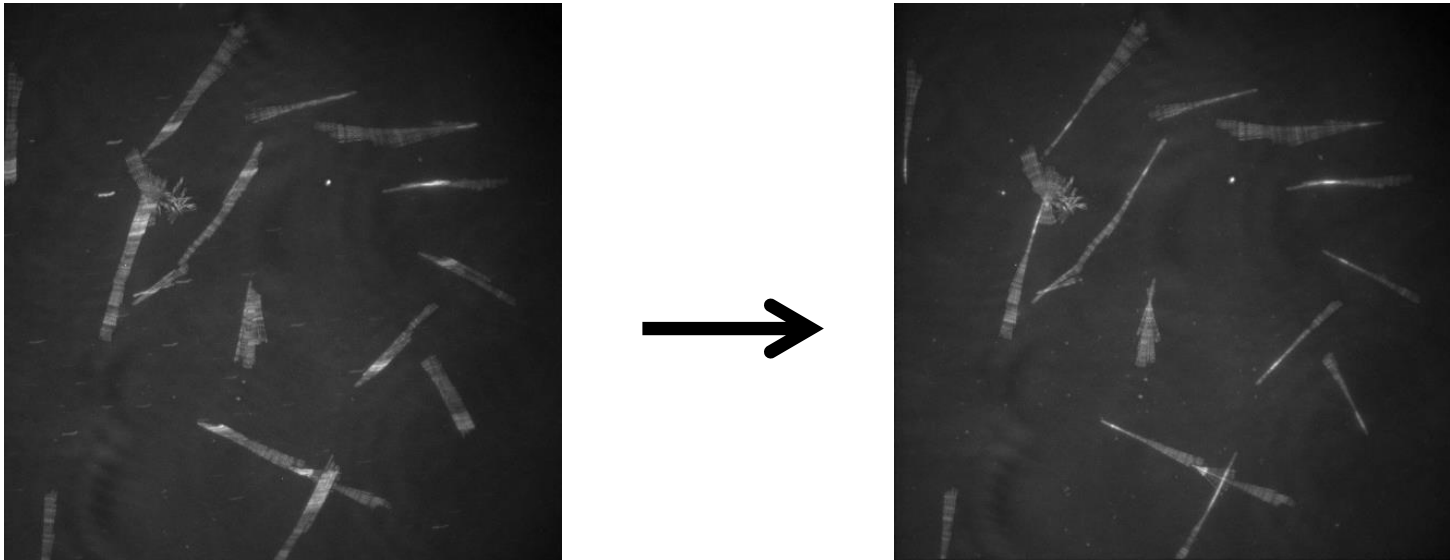
Max projection of 10 min timelapse of dynamic microtubules

7.3) Wiener filter

The Wiener filter is used to remove motion blur in each frame and effectively sharpens the image through a deconvolution step. The deconvolution kernel is a Gaussian.

7.4) Wallis filter (inactive)

The Wallis filter performs a local contrast stretch to correct for inhomogenous illumination of the sample.

7.5) Background subtraction

This feature calculates the mean intensity value in each frame through a kernel. The size and shape of the kernel can be changes by the user in the Options dialog. Subsequently, the mean background value over all frames is subtracted from each frame.

7.6) Photobleaching correction (inactive)

This processing step corrects for photobleaching.