Tutorial for using the image shifting macro, for diSPIM data acquired in stage-scanning mode

The image shifting macro preprocesses diSPIM data acquired in stage-scanning mode before further analysis (e.g. deconvolution, segmentation etc.). The macro offers background subtraction, image shifting and automated region of interest detection in a graphical user interface for easy user interaction and review.

The macro enables users to process both single-view (iSPIM) images and dual-view (diSPIM) images. Its main task is to transform the stage-scanning coordinate system into the more conventional coordinate system (stage stationary, light-sheet and detection plane swept through sample). In stage-scanning mode, the diSPIM moves the stage (and sample) at constant speed, but keeps the light sheet and detection objective stationary. The relative movement between the sample and detection lens leads to the following relationship between the two coordinate systems:

$$\begin{cases} x' = x + z \\ y' = y \\ z' = \sqrt{2}z \end{cases}$$

where x, y, z are the coordinates in conventional mode and x', y, z' the corresponding coordinates in stage-scanning mode. To perform the inverse transformation, the macro shifts the acquired image stacks in the x direction slice by slice, and sets the slice thickness to $\frac{\sqrt{2}}{2}$ of the slice step in stage-scanning mode (i.e. a 45 degree relation between light-sheet and stage is assumed).

Since background subtracted images sometimes show better contrast after joint deconvolution, the macro also provides multiple ways to subtract image background based on either background images acquired during an experiment or background information manually or automatically derived from the raw images.

To save storage as well as speed deconvolution after image-shifting, the macro allows the user to crop the shifted stack and save the cropped result, thus reducing the final image size as much as possible. The macro first tests one time point to determine the cropping volume. During processing of this test time point, the macro detects a region of interest in the XY maximum intensity projection, and semi-automatically selects the slice range automatically. Based on this slice range, a cropped volume is generated and used as a benchmark for cropping volumes corresponding to all time points.

The macro should work within Fiji that has ImageJ version 1.48c or later on a PC with the Windows 7 Professional operation system. We have also used it with good results within Fiji: Life-Line version, 2013 July 15, Windows 7 (64-bit).

Running the ImShifting macro:

1. Run ImShifting.ijm within Fiji

Download Fiji at http://fiji.sc/Downloads#Fiji

Open the macro with in Fiji and run it.

2. Choose the view and color option when the pop-up dialog shows up (Fig. 1).

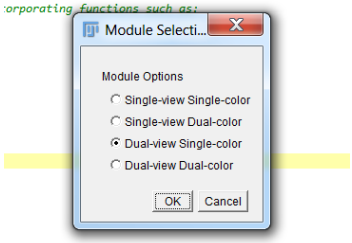

Fig. 1

3, Following the pop-up dialogs, sequentially select folders:

For two color: SPIMA folder→SPIMB folder→ Output folder;

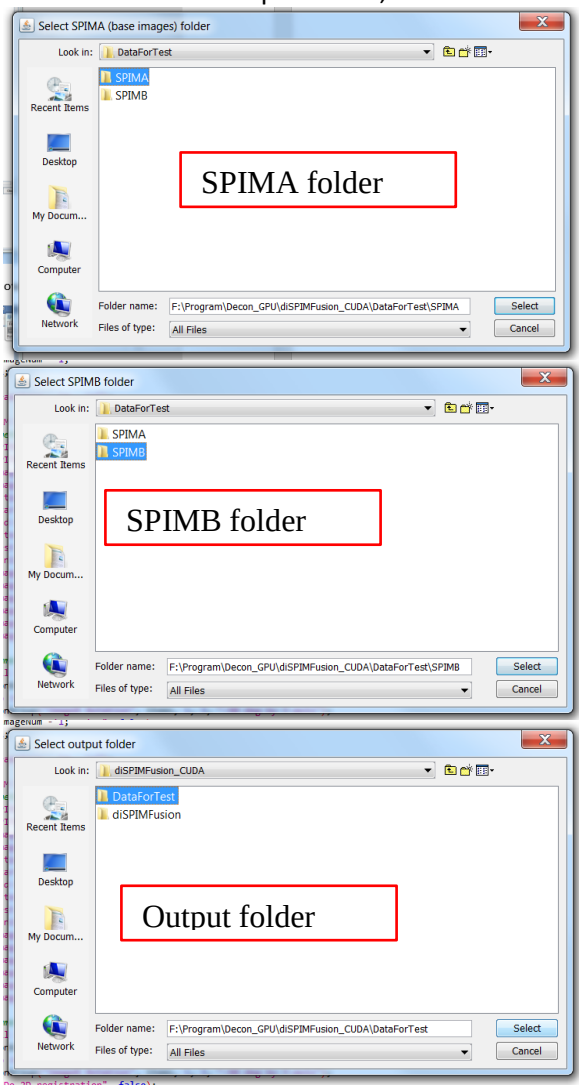

Fig. 2

4. Set parameters

Users need to specify all the parameters shown in the dialog window, the default parameters are for Shroff Lab's diSPIM configuration, but users can make their own default settings by modifying the parameters in the macro code.

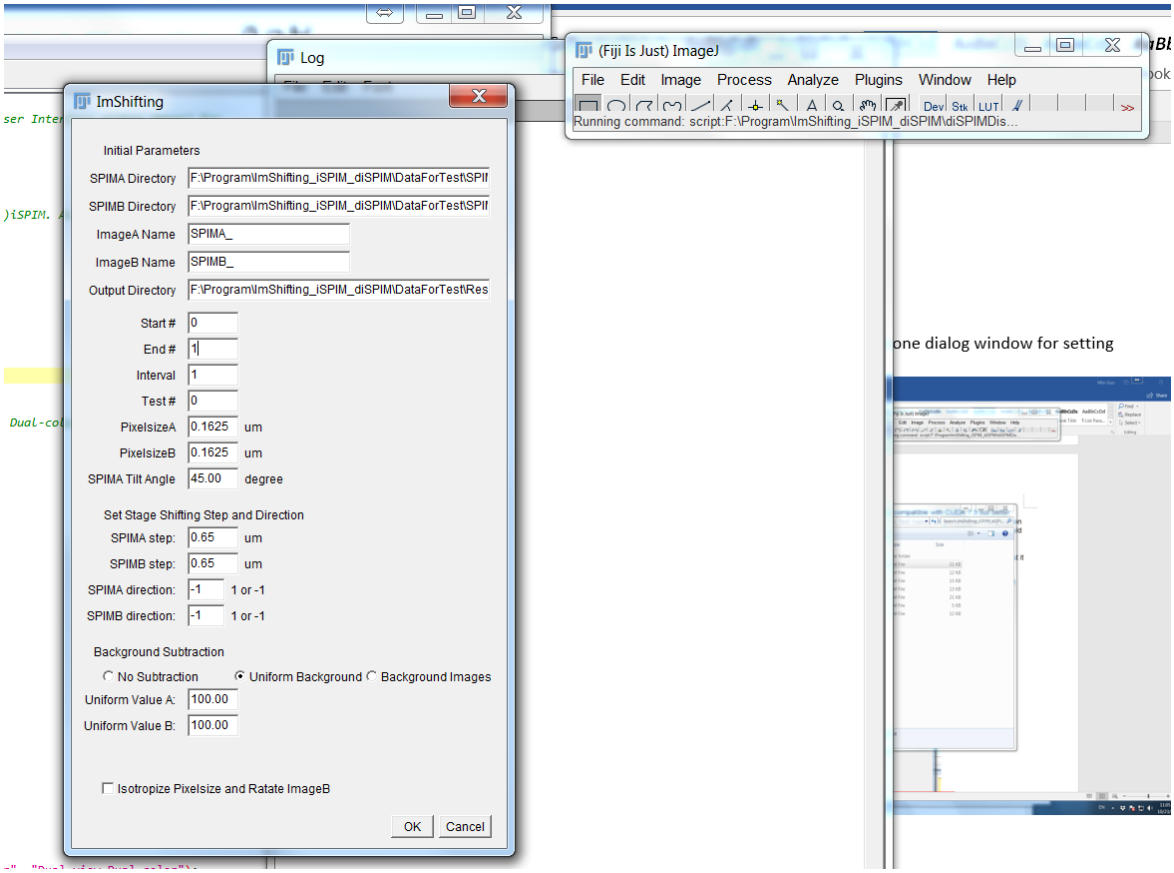

Fig. 3

Folder directories: the directories are just the SPIMA, SPIMB and Output folder set in last step, but users can modify them here. The program uses a folder convention as in Fig 4

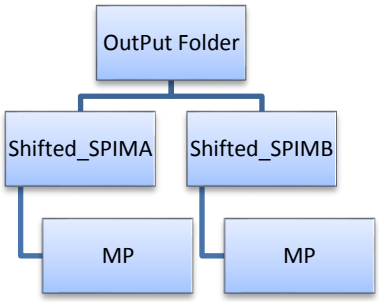

Fig. 4 Default file and folder naming convention.

Images Name: provide images name prefix.

the image stack name prefix should be set. For example, if the stacks' names are SPIMA_1.tif, SPIMA_2.tif, SPIMA_3.tif…, then "SPIMA_" should be set as the name prefix;

Set Time Points: set the time range of the image volumes and the test image for selecting Region of Interest (ROI).

If Test Time < Start Time or Test Time > End Time, the Start Time point is implicitly set as the Test Time point.

- The #s will be appended to the name prefix to generate a total name of the image at each time point

Pixel Size: size of the image pixels

SPIMA Tilt Angle: The horizontal angle for SPIMA arm. For regular diSPIM (symmetric structure), this angle should be 45 degrees.

Stage shifting step: The moving steps of the stage

Set Shifting Direction: the shifting direction depends on the particular diSPIM's stage setup and will affect the rotation direction for subsequent dual-view registration. The default shifting direction is specified for our diSPIM configuration, and may need to be modified by the user. If the input shifting direction is wrong, the maximum projection of the shifted image stacks is obviously blurred and distorted (Fig. 5). Once the correct shifting direction has been determined, the result should work for any imaging experiment unless the users change the diSPIM stage's direction of movement.

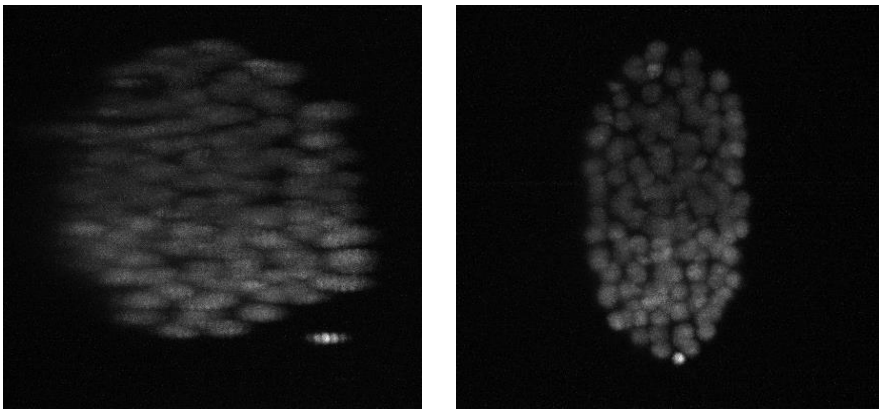

Fig. 5 Projections of images with wrong (left) and correct (right) shifting direction.

Background subtraction:
Three options are provided for background subtraction:
a) no subtraction: no background subtraction will be used, i.e. users don't need to take any action.
b) background mean value: users need to specify the estimated average values of the background (typically the camera offset):
c) background images: the program will subtract the background, based on background images provided by users. Users will then be guided to

- Open a 2D background image for A View;
- Open a 2D background image for B View;

Isotropize Pixel size and Rotate SPIMB: do interpolation to make the output images' pixels isotropic and rotate/flip the SPIMB image appropriately to match its orientation with SPIMA image. The default setup is for Shroff Lab diSPIM, users may need to modify the function within the macro to customize for their own system.

5. Select Region of Interest (ROI)
The program pops up four image windows, and the selections are based on the two projection images.

- 2 ROIs will be automatically generated
- The ROIs will be cropped and saved as outputs
- If users want to modify the selections, please keep them same size for both views (important for subsequent deconvolution)
- When modifying, use "ctrl+shift+E" to copy one selection to other view so that you can keep them the same size. (click window frame of the selection to copy from→ click window frame of the selection to copy to→"ctrl+shift+E"→move the selection to cover the ROI)

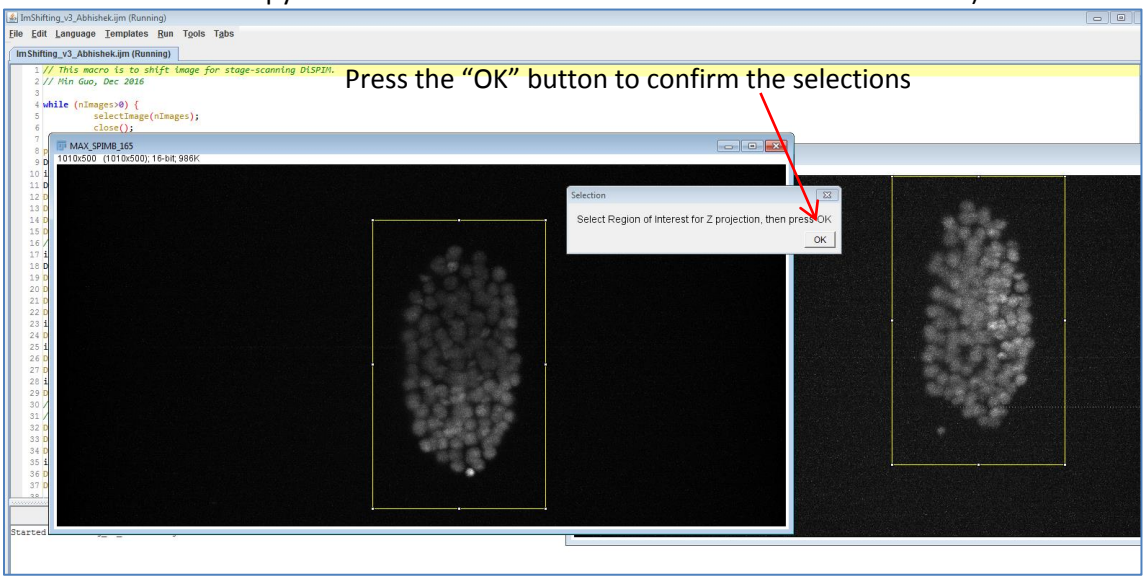

Fig. 6 Selecting ROIs in the z projection

6. Select slice range

We don't input the slice range directly, but determine the slice range by detecting the ROI of the XZ maximum projection. Users need to select the ROI as in step 4.

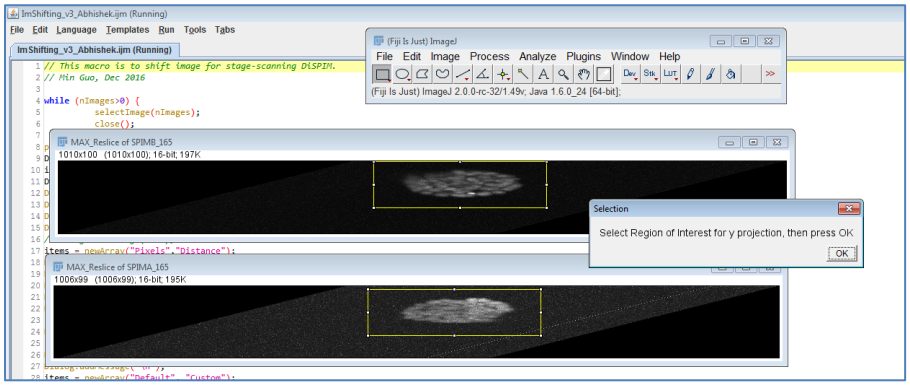

Fig. 7 Selecting ROIs in XZ projection

7. Batch Processing

After selecting the slice range, background subtraction and shifting are batched, as shown in the log window (Fig. 8).

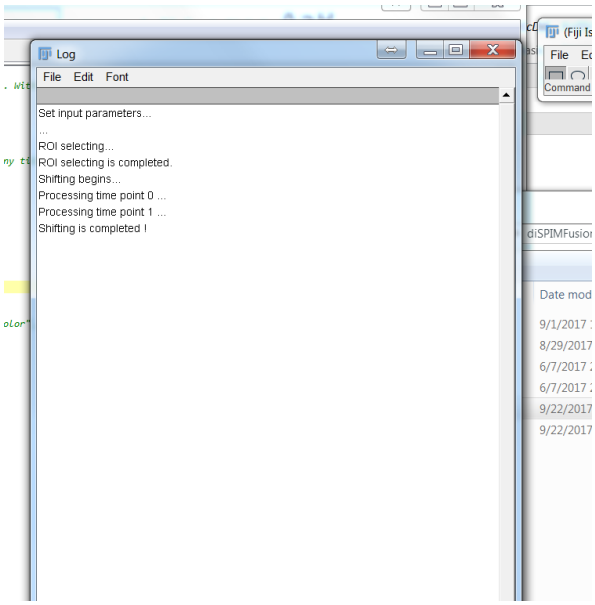

Fig. 8 Log window