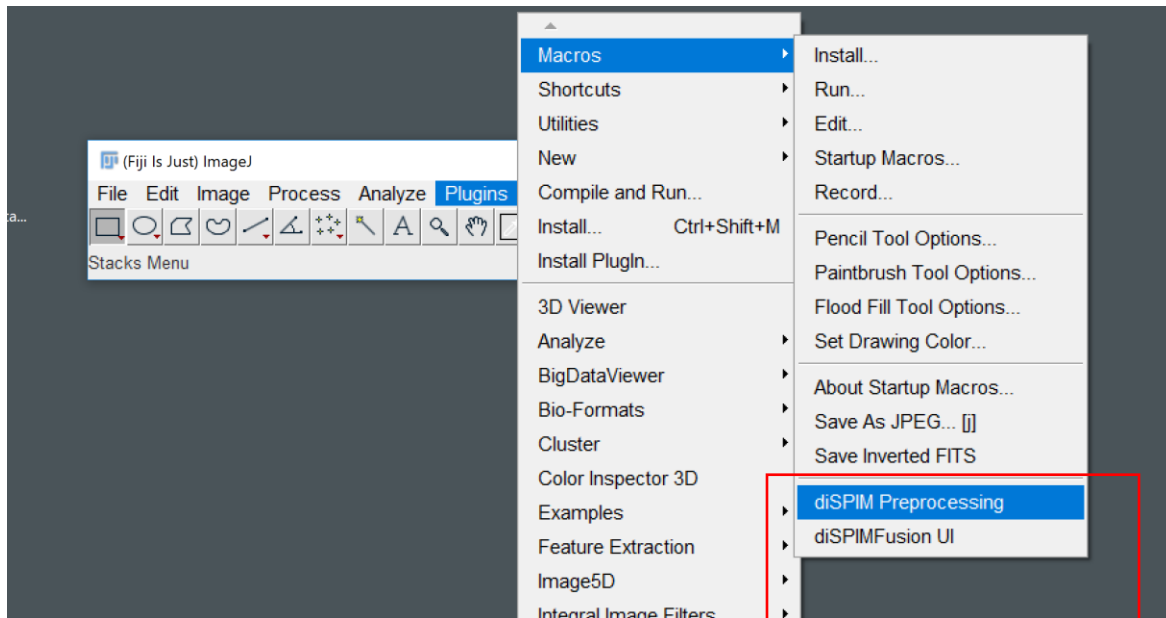


Data processing for diSPIM images: an overview

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Aiming to get a final image with 3D isotropic resolution, registration and deconvolution are usually applied to the two view images of diSPIM. But before the registration, data preprocessing is also need to handle the raw dada, such as background subtraction, region of interest (ROI) cropping and 3D orientation arranging. Additionally, the stage-scanning acquisition introduces distortion to the 3D raw images with regards to the regular light-sheet scanning acquisition. We'll need to correct this distortion before we can further fuse these the views and get a final deconvolved image (please see our paper on this topic <https://www.ncbi.nlm.nih.gov/pubmed/27638693>). So, the processing for diSPIM images should mainly include two parts: preprocessing and fusion. Accordingly, we have written appropriate programs: an image preprocessing program and a GPU-based CUDA/C++ program to fuse the two views (including image registration and joint deconvolution). The software have been onto the MBL workstation and there is detailed description and user manual for each program, but I'd like to specify some the configurations and parameters for MBL diSPIM.



1) The image preprocessing macro.

“diSPIM_Preprocessing.ijm” is an imageJ macro which aims to correct the images distortion. It is supposed to work for both iSPIM (single-view imaging) and diSPIM (dual-view imaging). But somehow, we have not added the function for iSPIM yet. But it should be all right for single color or dual color diSPIM processing. The macro, the test data and the user manual should be contained within the diSPIM Data Processing Package. Specifically, datasets in the folder “TestDataForPreprocessing” were acquired during the MBL diSPIM imaging workshop, they should be good examples for the macro. Users can find a log file in the benchmark result folder (“Result_backup”), which contains the processing parameters of each dataset.

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

The screenshot shows the 'diSPIM Preprocessing' dialog box. It is divided into two main sections, each highlighted with a red border. The first section, 'Directories and Files', contains fields for 'SPIMA Directory', 'SPIMB Directory', 'ImageA Name', 'ImageB Name', and 'Output Directory', all pointing to a directory on the D: drive. Below these are 'Image Number/Range' settings: 'Start #' (0), 'End #' (2), 'Interval' (1), and 'Test #' (0). A red box with the text 'need to specify for each new data' points to these settings. The second section, 'Stage Scanning configurations', includes 'SPIMA Tilt Angle' (45.00 degree), 'Set Stage Shifting Step and Direction' (SPIMA direction: -1, SPIMB direction: 1), and 'Background Subtraction' (Uniform Background selected). A red box with the text 'Good parameters for MBL diSPIM' points to the direction settings. At the bottom, there is a checkbox for 'Isotropize Pixelsize and Ratate ImageB' and a note: 'Note: this function works only during the ROI selecting.' The 'OK' and 'Cancel' buttons are at the bottom right.

diSPIM Preprocessing

Directories and Files

SPIMA Directory D:\Workshop_diSPIMdataprocessingpackage\DataForPreprocessir

SPIMB Directory D:\Workshop_diSPIMdataprocessingpackage\DataForPreprocessir

ImageA Name SPIMA-

ImageB Name SPIMB-

Output Directory D:\Workshop_diSPIMdataprocessingpackage\DataForPreprocessir

Image Number/Range

Start # 0

End # 2

Interval 1

Test # 0

PixelsizeA 0.1625 um

sliceThicknessA 1.0000 um

PixelsizeB 0.1625 um

sliceThicknessB 1.0000 um

need to specify for each new data

Stage Scanning configurations

SPIMA Tilt Angle 45.00 degree

Set Stage Shifting Step and Direction

SPIMA direction: -1 1 or -1

SPIMB direction: 1 1 or -1

Good parameters for MBL diSPIM

Background Subtraction

☐ No Subtraction ☒ Uniform Background ☐ Background Images

Uniform Value A: 100.00

Uniform Value B: 100.00

☐ Isotropize Pixelsize and Ratate ImageB

Note: this function works only during the ROI selecting.

OK Cancel

2) diSPIM fusion software.

To register the two view images and do the joint deconvolution, we provide another imageJ macro “diSPIMFusion_UI.ijm” that can also create a user interface. The macro calls some executable apps written with CUDA/C++. To run the software, the PC needs to have a) windows 7 or 10 operation system, b) either imageJ or Fiji and c) a graphics card supported by CUDA 7.5 and with appropriate drivers (Most nowadays graphics card in Nvidia should be compatible with CUDA 7.5 but better check here <https://developer.nvidia.com/cuda-gpus>). This software runs fast based on parallelized computation by GPU card, but the images size is limited by the GPU memory. For regular running, the GPU memory should be > 24 times of the image size (16-bit, after interpolation), with memory saved running, the GPU memory should be >12 times of the image size (16-bit, after interpolation). We also want to note that there is another diSPIM fusion software that does not need a GPU card, so image size is only limited by the CPU memory, but it runs much slower (MIPAV, GenerateFusion: http://dispim.org/software/mipav_generatefusion).

The screenshot shows the diSPIM Fusion software interface. It has a title bar "diSPIM Fusion" and a close button. The main window is divided into several sections:

- Directories and Files:** Contains text boxes for "SPIMA Directory", "SPIMB Directory", "ImageA Name", "ImageB Name", and "Output Directory". All are set to "D:\Workshop_diSPIMdataprocessingpackage\DataForPreprocessir".
- Image Number/Range:** Contains text boxes for "Start #", "End #", "Interval", and "Test #". Values are 0, 2, 1, and 0 respectively. A red box highlights this section with the text "need to specify for each new data".
- Initial Pixel Size:** Contains text boxes for "ImageA x, y, z" and "ImageB x, y, z". Each has three sub-boxes for x, y, and z values. For ImageA, values are 0.1625, 0.1625, 1.0000. For ImageB, values are 0.1625, 0.1625, 1.0000. Units are "um".
- Set Registration Options:** Contains radio buttons for "All images dependently" (selected), "All images independently", "One image only", and "No registration". Below are "ImageB Rotation" options: "No rotation", "90 deg by Y-axis", and "-90 deg by Y-axis" (selected). There are checkboxes for "Do 2D registration", "Customize initial transformation matrix", and "Save Registered Images" (checked). A "Convergence Threshold" text box has the value "0.000100".
- Set Deconvolution Options:** Contains text boxes for "Iterations" (10) and "Output Image Bit" (16). A red box highlights this section with the text "Good parameters".
- Set GPU Options:** Contains a checked checkbox for "Show GUP Device Information" and a "GPU Device #" text box with the value "0".
- GPU Memory Save Mode:** An unchecked checkbox at the bottom.

At the bottom right are "OK" and "Cancel" buttons.

