User manual for diSPIMFusion

The diSPIMFusion is a software package that performs registration and joint deconvolution on the two view images acquired by diSPIM microscope. The software package contains an ImageJ macro "diSPIMFusion_UI.ijm", several CUDA/C++ executable applications, and PSFs for the diSPIM microscope. Basically, the ImageJ macro creates a user interface for parameter configuration and calls the executable applications which complete all the processing based on GPU device. To run the software, the PC needs to have

a) Windows 7 or 10 operation system,

b) either ImageJ or Fiji

c) a graphics card supported by CUDA 9. Most nowadays graphics card from Nvidia should be compatible with CUDA 9, but better check here https://developer.nvidia.com/cuda-gpus.

d) an appropriate driver for the graphics card. A default driver, especially an old version, may be enough for the GPU card working for monitor display, but it is possible that the GPU card can't be recognized by the CUDA program. Usually, a later driver from Nvidia should support the GPU cards to do parallelized computation http://www.nvidia.com/Download/index.aspx .

This software runs fast based on parallelized computation by GPU card, but the images size is limited by the GPU memory. To run the software, the GPU memory should be > 8.5 times of the image size (16-bit, after interpolation). Specifically:

1) if the GPU memory > 24 times of image size, the processing runs in a most efficient way; Otherwise,

2) if the GPU memory > 8.5 times of image size, the processing runs slower as a tradeoff of efficiency and memory usage;

How to install the program

1, copy the folder "diSPIMFusion" to ImageJ or Fiji's main folder (e.g, C:\Fiji.app\).

2, install the UI macro:

ImageJ (or Fiji) Plugins--> Macros-->Install..., Choose the macro file "diSPIMFusion_UI.ijm" (within the "diSPIMFusion" folder). Then there will be a dispimfusion option listed on the Plugins--> Macros menu.

How to use the program

1. run the program:

ImageJ (or Fiji) Plugins--> Macros--> dispimfusion.

or

Directly open the "diSPIMFusion_UI.ijm" within imageJ (or Fiji) and run it;

2. Choose “Single color” or “Multiple colors” option when the pop-up dialog shows up;

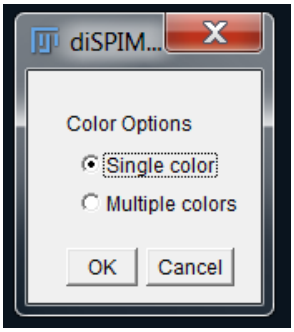

Fig. 1, pop-up window for color option.

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

1) For single color: SPIMA folder→SPIMB folder→ Output folder;

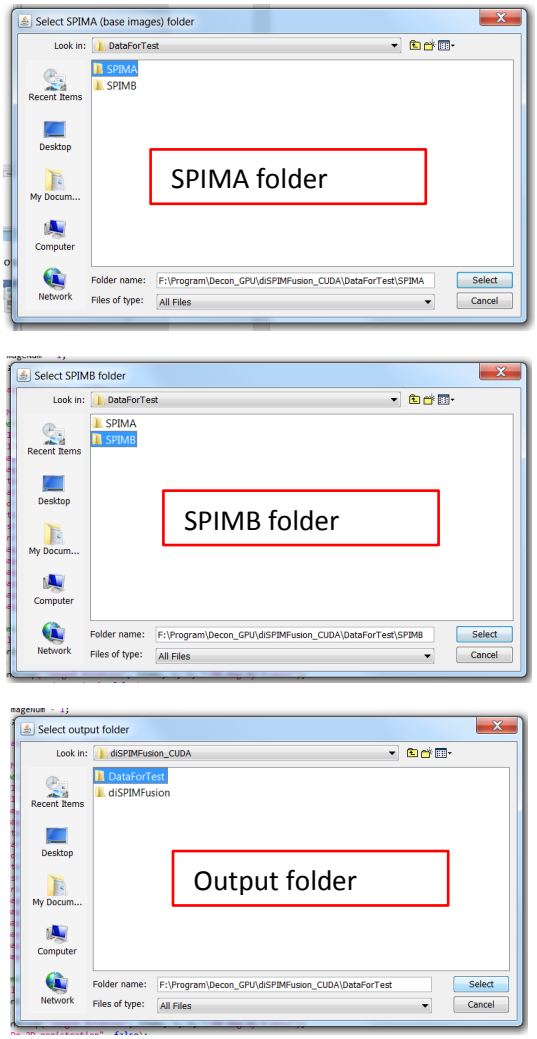

Fig. 2, pop-up dialog windows for selecting folder in single color case.

2) For multiple colors: Main folder → Output folder.

Users need to organize the input data as shown in Fig. 3. The program automatically takes each subfolder within the main folder as the input of each color.

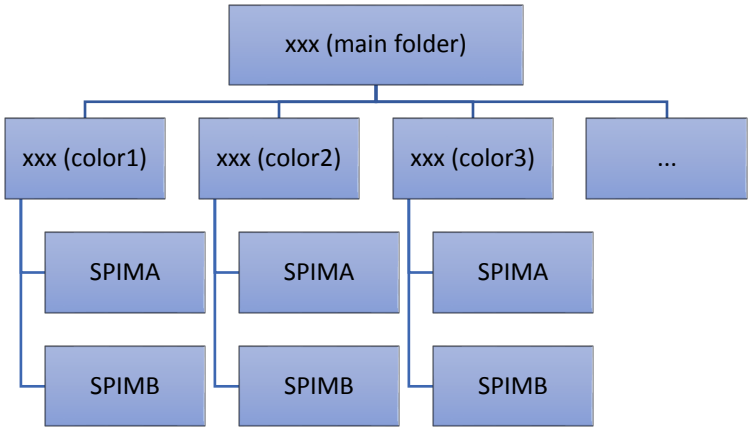

Fig. 3, Folder convention of multiple colors data. The "xxx ( )" indicates the name for the folders.

4. Confirm and modify the parameters in the next pop-up panel.

The panel contains all parameters that are needed for the executable apps. Users are allowed to modify any of the parameters at this step. The panel along with the explanations for the parameters are shown in Fig. 4.

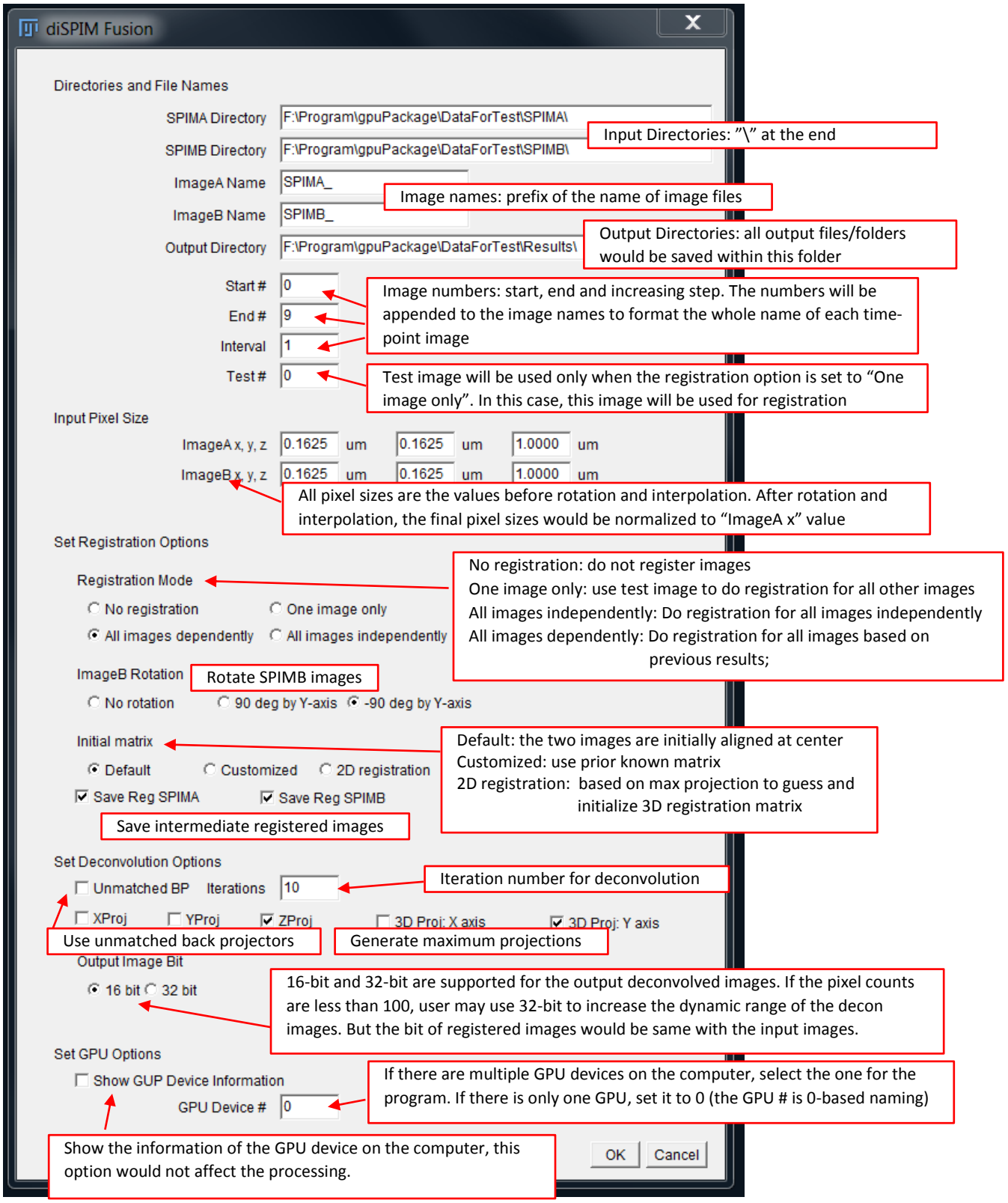

Fig. 4, the diSPIM Fusion panel

5. Specify input matrix file (optional).

If “Customized” is selected as initial matrix, users will be guided to choose a matrix file. (A text file is provided within the test data)

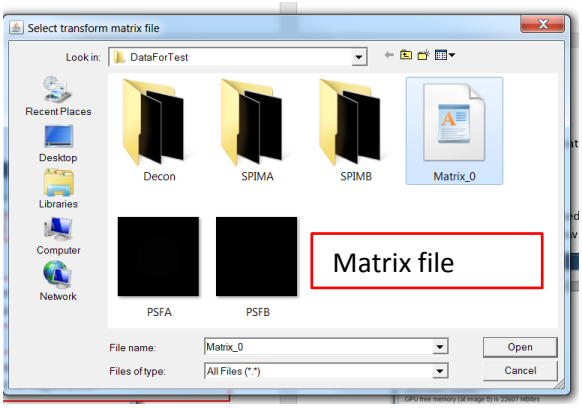

Fig. 5, dialog for choosing matrix file.

6. GUP-based apps running

Then the macro calls executable applications and starts the processing. Once the processing is completed, all messages will show up in the ImageJ log window.

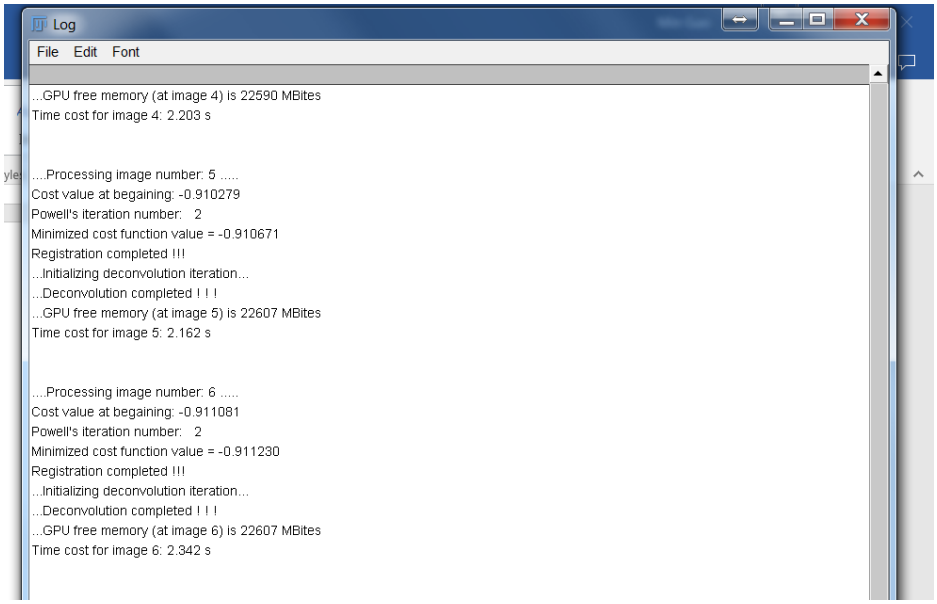

Fig. 6, an example of the ImageJ log window.

7. Output files and folders

Decon: deonvolved imges and corresponding 2D/3D maximum intensity projections.

RegA/RegB (Only if the “Save Reg SPIMA” and/or “Save Reg SPIMB” option is checked): registered images.

TMX: transformation matrixes of each registration.

ProcessingLog.txt: log information including basic configurations, memory usage, cost function values and time cost. The log file also provides an opportunity to check the status during the running of the CUDA app.

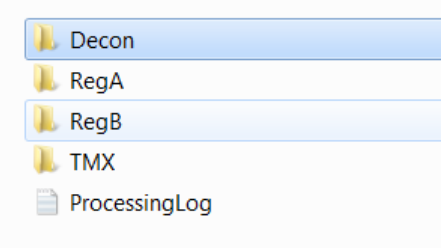

Fig. 7, output files and folders.

Other notes

(1) If it's the first time for the GPU device to run on the computer, it may take some time (possibly up to minutes, but usually less than 5 minutes) to initialize the device. Once the device is initialized, it won't need initialization next time, even after the computer is restarted.

(2) Once the CUDA app has been launched the by the ImageJ macro, its running is independent on the ImageJ or Fiji anymore. But its running status will be shown as "spimfusion.exe" in system Task Manager, you can kill it there if needed.

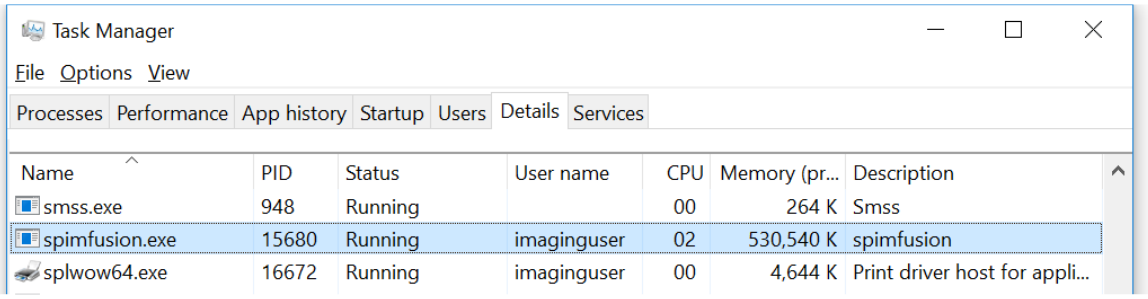

Fig. 8, CUDA app status in Task Manager.

(3) Users could also customize the default parameters by modifying the codes in the ImageJ macro as shown in Fig. 9.

(4) It is also recommended to do cert

```
//====================================================================
//=========You can customize your default parameters here!!!=======
//====================================================================
//*** Default setup****
// Default folders/files for CUDA app
appPath = ".\\diSPIMFusion\\cudaApp\\";
filePSFA = ".\\diSPIMFusion\\PSFA.tif";
filePSFB = ".\\diSPIMFusion\\PSFB.tif";
filePSFA_bp = ".\\diSPIMFusion\\PSFA_BP.tif";
filePSFB_bp = ".\\diSPIMFusion\\PSFB_BP.tif";

// Default parameters
colorChoice="Single color";

nameA = "SPIMA_";
nameB = "SPIMB_";
pixelSizeAx = 0.1625;
pixelSizeAy = 0.1625;
pixelSizeAz = 1;
pixelSizeBx = 0.1625;
pixelSizeBy = 0.1625;
pixelSizeBz = 1;

regChoice = "All images dependently"; // registration mode
rotChoice = "-90 deg by Y-axis"; // rotation angle for SPIMB
tmxChoice= "Default"; //flagInitialTmx = 0;
FTOL = 0.0001;
itLimit= 3000;
saveRegA = true; // save registerred image A or not
saveRegB = true; // save registerred image B or not

iteration = 10; //iteration number for deconvolution
flagUnmatch = false; // use customized unmatched backprojector or not
saveXProj = false; //
saveYProj = false; //
saveZProj = true; //
saveXaxisProj = false; //
saveYaxisProj = false; //

outputBit = "16 bit"; // output data bit: 16 or 32
dQuery = false; // show GPU information or not
deviceNum = 0; // GPU device #
//====================================================================
//==================Customization End!!!!=========================
//====================================================================
```

In case ImageJ/Fiji fails to find the default directory, user will need to replace these paths with full directories.

filePSFA_bp and filePSFB_bp are used only if "unmatched BP" is set for deconvolution

File name prefixe of each view

Pixel sizes

Set convergence threshold and maximum iteration number

Save maximum projections or not

Fig. 9, Parameters customization in ImageJ macro.