User guide (Windows)

1. Install anaconda from https://www.anaconda.com/download/success

2. Download the script file (2024-08-19_eSPIM_reconstruction_multiprocessing_singlechannel.py) and the pyimagej.yml file to your PC. Save them to a folder (e.g., D:\Example). Avoid space(s) in the path (e.g., D:\Example data is not recommended).

3. Download and install Fiji from https://imagej.net/software/fiji/downloads

Save the Fiji folder to D:\Example\Fiji\Fiji.app

4. Run 'Anaconda prompt' from the start menu.

5. Change the working directory to the folder that contains the pyimagej.yml file.

For example, input 'cd /d D:\Example', where D:\Example is the path of the folder that contains the .yml file. Press enter to execute the command.

6. Input 'conda env create -f pyimagej.yml' in the Anaconda prompt and press enter. The environment will be set up automatically.

7. Input 'conda activate pyimagej' in the Anaconda prompt and press enter. The environment will be activated. Keep this window open.

8. Open the script file with Notepad or any Python IDE and edit the path to your image folders. The folder structure MUST follow the following format:

Parent folder - D:\Example\cell_image

Position subfolders - D:\Example\cell_image\pos1; D:\Example\cell_image\pos2; …

Image subfolders - D:\Example\cell_image\pos1\638_1; D:\Example\cell_image\pos1\638_2; …

Each image subfolder contains one .tif file, which is a raw stacked image generated by MicroManager.

Change the following line in the Python script to point to the parent folder:

path = r'D:\Example\cell_image'

Change the following line in the Python script to point to the folder that contains Fiji:

path_fiji = r'D:\Example\Fiji\Fiji.app'

Save the changed script.

9. Open the image stack file using Fiji and run 'Record'. Select the rectangular area that contains the illuminated cell(s). A line containing the coordinates of the boundary of the rectangular ROI will show in the Fiji recorder window (looks similar to the following line):

makeRectangle(226, 503, 622, 129);

10. Change the following line in the Python script to indicate the boundary of the ROI (ensure the numbers are the same as shown in Fiji):

roi = "226, 503, 622, 129"

Save the Python script.

11. In the Anaconda prompt window, input:

python 2024-08-19_eSPIM_reconstruction_multiprocessing_singlechannel.py

Press enter to run the saved Python script. A new folder should be created parallel to the parent folder named ' r'D:\Example\cell_image_Analysis'. This folder will contain the same number of position subfolders, each containing four folders: Sub-bg (raw images with subtracted background); Processed (deskewed images); Projection (projected deskewed images rotating around the y-axis); and Resliced (resliced deskewed image stacks viewed from a different axis).