Yangyang Bai yangyanb@usc.edu

FLFM Reconstruction Manual

I. Data Preparation (using 10x as an example)

Before starting the processing, you would need to be already familiar with FIJI or at least know how to look for answers. The documents are very well documented online and should not be hard to find certain functions just by Googling. Apart from that, you would need an extra plugin installed called: Mask from ROI. The plugin can be downloaded and installed from the website: *Mask(s) from ROI(s) (imagej.net)* . After successful installation, it should show up in FIJI in Edit > Selection > Mask from Rois. The details are all explained on the website.

We need to manually generate the following files in FIJI before running the code:

1) Aperture region of interest (Ap_Mask.roi)
2) Cropped PSF stack with background value removed (folder: Raw_Cropped_Bg-190)
3) Cropped FLFM movie with background value removed (folder: Raw_Cropped_Bg400)
4) MLA mask (MLA_Mask.tif)
5) First Lens-let's mask (Lenslet_Mask.tif)
6) Initial PSF mask (*Inipsf_Mask.tif*)
7) Initial lens-let's PSF mask (*Inipsflet_Mask.tif*)

A. Procedures to generate 1), 2), 3):

a) Open your raw PSF stack folder (Raw) in FIJI. Circle out a region where the lens-lets that are well illuminated (Figure 1.). Then save the roi file (Ap_Mask.roi).
b) Cropped the psf stack using the roi and you will have a cropped PSF stack (Figure 2). Save it for future reference (folder: Raw_Cropped).
c) Subtract a background value from the PSF stack. This value can be estimated using the average intensity of the blank region in one or multiple lens-lets. At this end, you will have a cropped PSF stack with background value removed (Figure 3). Save the stack in the folder for PSF extraction in MATLAB (folder: Raw_Cropped_Bg-190).

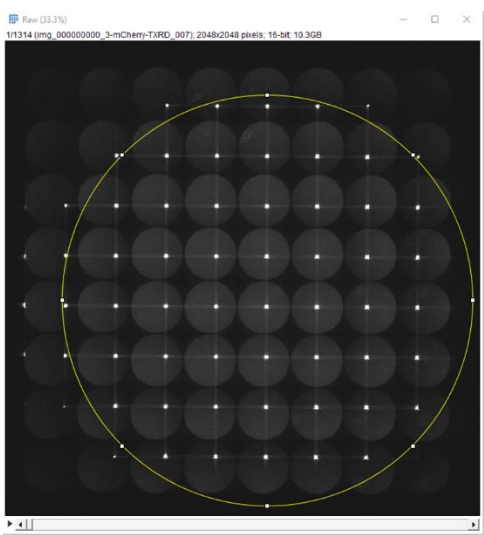

Figure 1. Example of an aperture region of interest (Yellow) . Here, the diameter includes 7 lens-lets.

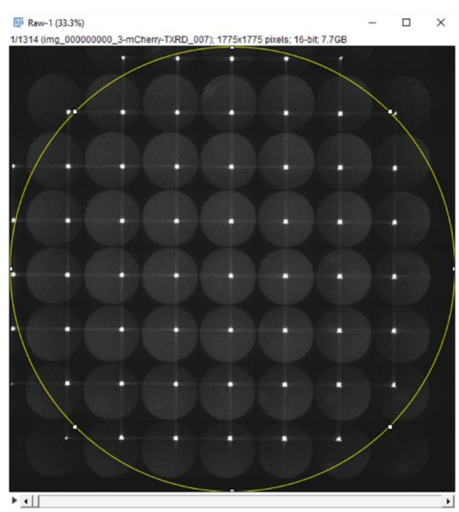

Figure 2. Cropped PSF stack from Figure 1. The image size is reduced and this will increase the processing and reconstruction speed, especially when the aperture is much smaller than the camera chip size.

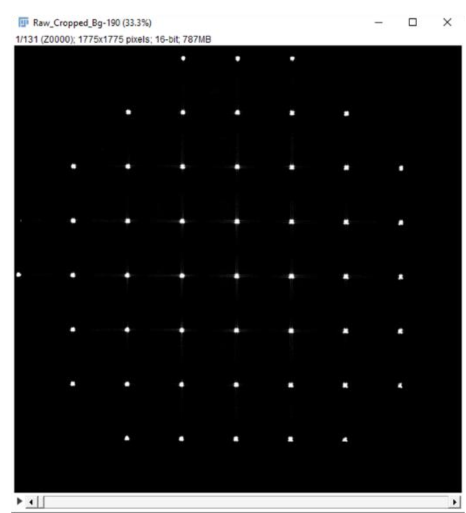

Figure 3. Cropped PSF stack with background value removed. Here, for this data I used 190 as an average estimate of a constant background value from the PSF

We can do the same processing now to generate 7). Open your raw FLFM movie in FIJI (folder: Raw). Then, repeat the above procedure b) and c) for the raw FLFM movie stack. The movie can also just have one frame which represents one FLFM image. Save the cropped and background removed FLFM movie stack in folder (folder: Raw_Cropped_Bg400).

B. Procedures to generate 4), 5), 6), 7):

The masks are all generated by the *Mask from Rois* function we pre-installed.

a) Open a separate window for the first slice of the Cropped PSF in Figure 2. Circle out the MLA pattern and save the Rois in the ROI Manager (MLA_Mask.zip). An example is shown in Figure 4. Note here that we only consider the lens-lets that are illuminated with in the aperture. Half illuminated lens-lets will be discarded. Using *Mask from Rois* function, we can generate Figure 5 which is the MLA mask. Save the MLA mask (MLA_Mask.tif).

b) Select the ROI of the first lens-let from the ROI manager and create the first lens-let's mask (Lenslet_Mask.tif) as shown in Figure 6.

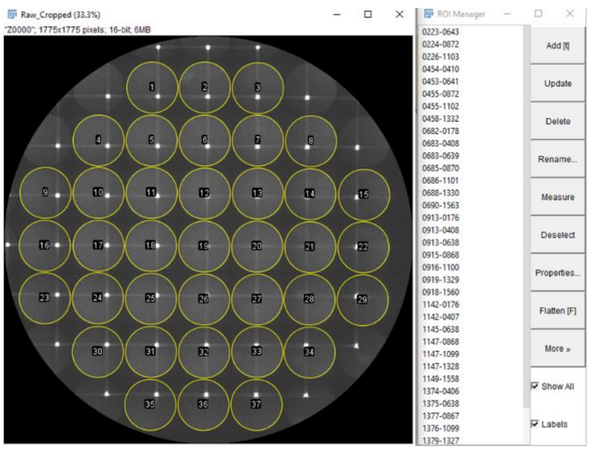

Figure 4. Example of the MLA Rois. At the aperture cut-off, the lens-lets' data are discarded by simple keeping them out of the binary mask in Figure 5.

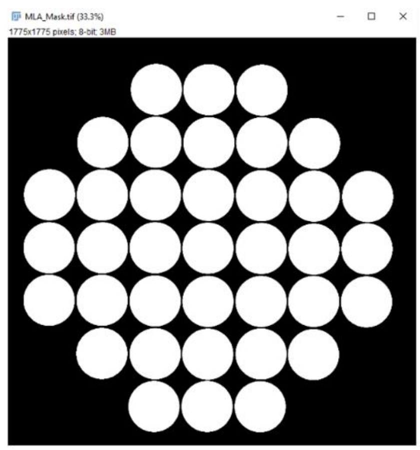

Figure 5. The MLA mask created from Figure 4. The mask is binary and includes the lens-lets' views that are used for 3D reconstruction.

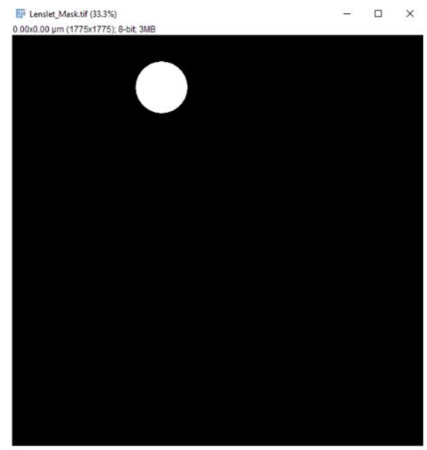

Figure 6. The first lens-let mask. It is used for certain calibration functions in the reconstruction which could be skipped in the future but for now we'll keep it.

c) Now close the ROI Manager and open the first slice of 2). Then circle out the PSF profiles in all the selected lens-lets to create the initial PSF mask Rois (Figure 7) as described for the MLA Mask Rois. Saved the ROIs in ROI manager (Inipsf_Mask.tif.zip) and create the initial PSF mask (*Inipsf_Mask.tif*) using Mask from Rois function (Figure 8).

d) Select the ROI of the first lens-let's PSF from the ROI manager and create the first lens-let's PSF mask (Inipsflet_Mask.tif) as shown in Figure 9.

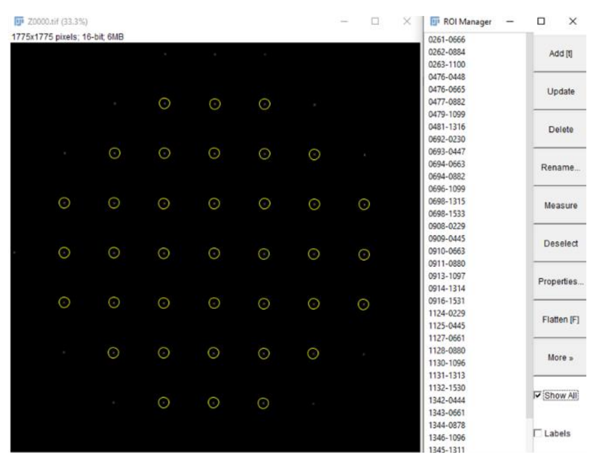

Figure 7. Initial PSF mask Rois. The cirle diameter should includes the complete PSF profile and should be an odd number. Here, I chose 51.

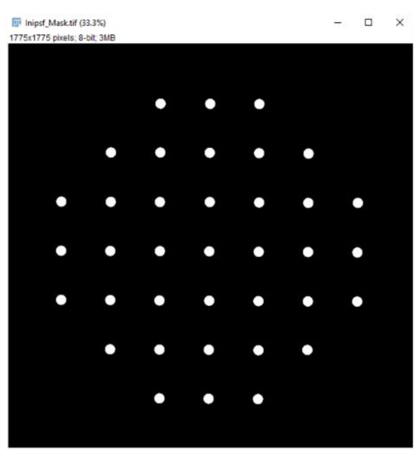

Figure 8. Initial PSF mask. Block out all the other beads or irrelevant objects outside to create a single bead image within each lens-let.

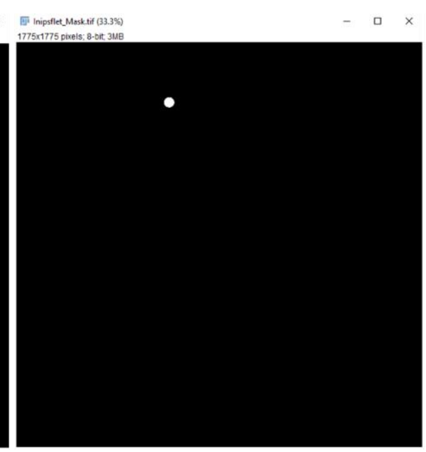

Figure 9. The first lens-let's PSF mask . It is used for tracing the shifting PSF profile over entire depth.

Now, we have generated all the files we need for PSF extraction and 3D reconstruction. This is a tedious procedure but only need to performed once as long as your MLA pattern remains the same between each data acquisition. You can check the pattern before or after the experiments with the bead image you took last time. To check it, just save a bead image or an MLA pattern image on the workstation from last time and overlay it with the current MLA pattern.

II. PSF Extraction

All the MATLAB code to be used are in *FLFM_Reconstruction \Code \internal.* Since it is in Matlab, basic Matlab programming is required to understand and execute the procedures properly. The code is not complicated and changes of your own should not be hard.

A. Procedures to run PSF extraction:

a) Open MATLAB file: Psf_exp_extraction_tracing_Trim.m

b) Plug in the directory for file 6) and 7) in Section 1

```
%% Section 1: Read in psf Mask
datasetdir='E:\yangyang\FLFM_reconstruction_My\Data_Seisure\202230516_4umBeads10X0.4NA\Center_3C\Mask\';
fileName='Inipsf_Mask.tif';
InipsfMask=makeOddSize(double(imread([datasetdir,fileName])));
```

c) Plug in the directory for file 2) and set PSF profile diameter in Section 2

```
%% Section 2:Read in raw psf image stack
psf_datasetdir='E:\yangyang\FLFM_reconstruction_My\Data_Seisure\202230516_4umBeads10X0.4NA\Center_3C\TXRD\';
folderName='Raw_Cropped_Bg-190\';
imglist=dir([psf_datasetdir,folderName]);
```

d) Plug in the directory for configuration file where you set the basic parameters for the system in Section 3.

```
%% Section 3:Read configuration file
% Get the centers of each mask and the mask diameter
conf_datasetdir='E:\yangyang\FLFM_reconstruction_My\Data_Seisure\202230516_4umBeads10X0.4NA\Center_3C\TXRD';
fileName='configFile.xlsx';
```

e) Set saving directory in Section 6.

```
%% Section 6:Set saving directory
psf_savedir='E:\yangyang\FLFM_reconstruction_My\Data_Seisure\202230516_4umBeads10X0.4NA\Center_3C\TXRD\';
savefolderName=['Extracted_All_profiled-',num2str(psfprofile_d),'\'];
```

f) Run the code.

The result will be two folders containing extracted PSF stack (Extracted_All_profiled-51\H) and its transpose (Extracted_All_profiled-51\Ht). Both of them are required to perform the reconstruction.

III. 3D Reconstruction

There are two files for the reconstruction. The first one is an initiation file (*mainFLFM_wExppsf_loop_ini.m*) for setting up the reconstruction. The second one is the execution file (*mainFLFM_wExppsf_loop.m*) for reconstructing the data. The results are saved in mat for in-MATLAB processing.

Please note here that when the depth of view is much larger than the sample (Ex: 10x for zebrafish brain), reconstruction should be performed twice illustrated in the following steps. The reason is to perform reconstruction more efficiently. Take the seizure data for example, it takes ~140 seconds per image to reconstruct the entire depth of 131 Z slices.

- **First, perform the reconstruction using all the PSF slices over the entire depth of view for one or two images in FLFM movie. Therefore, in the code, be sure to change the for loop to t=1:2 which represents the first two frames of the movie.**

- **Second, pick a proper sub depth range that covers the fish brain. This step is checked in FIJI after converting the mat files to tiff files.**

```
%% Section 2: Richardson Lucy deconvolution
iter =10; % number of iterations for each image

for t=1:2
% for t=1:length(imglist_FLFMimg)
```

- **Third, create a sub stack folder (Substack (47-97) and copy the corresponding H and Ht slices from the entire the H and Ht.**
- **Lastly, repeat the reconstruction for the whole movie using the sub stack H and Ht and remember to change the for loop back to the whole movie range.**

A. Procedures to run reconstruction:

a) Open MATLAB file: *mainFLFM_wExppsf_loop_ini.m*

b) Plug in the directory for file 3) in Section 1

```
%% Section 1: Set FLFM Images directory
FLFMimg_datasetdir='E:\yangyang\FLFM_reconstruction_My\Data_Seisure\2023_Sebfish_seisure\2023_05_26_Sample1_15s_Activity\';
FLFMimg_folder='Raw_Cropped_Bg400\';
imglist_FLFMimg=dir([FLFMimg_datasetdir,FLFMimg_folder]);
```

c) Plug in the directory for configuration file in Section 2

```
%% Section 2: read in Configuration file
conf_datasetdir='E:\yangyang\FLFM_reconstruction_My\Data_Seisure\202230516_4umBeads10X0.4NA\Center_3C\TXRD\';
configfileName='configFile.xlsx';
[MLANump_d,LensletCenters]=FLFM_Getcenters([conf_datasetdir,configfileName],MLAMask,Lenslet_Mask);
```

d) Plug in the directory for H and Ht in Section 3

```
%% Section 3: read in PSF data
psf_datasetdir='E:\yangyang\FLFM_reconstruction_My\Data_Seisure\202230516_4umBeads10X0.4NA\Center_3C\TXRD\Extracted_profiled-51\';
psfHt_folderName='All\Ht\';
imglist_Ht=dir([psf_datasetdir,psfHt_folderName]);
imglist_Ht = extractfield(imglist_Ht,'name');
imglist_Ht=imglist_Ht(3:end);

psfH_folderName='All\H\';
imglist_H=dir([psf_datasetdir,psfH_folderName]);
imglist_H = extractfield(imglist_H,'name');
imglist_H=imglist_H(3:end);
```

e) Set saving directory in Section 5

```
%% Section 5: Set output directory
Rc_datasetdir='E:\yangyang\FLFM_reconstruction_My\Data_Seisure\2023_Sebfish_seisure\Reconstruction\2023_05_26_Sample1_15s_Activity\matfile\';
if ~exist(Rc_datasetdir,'dir')
    mkdir(Rc_datasetdir);
end
```

f) Run the code.

V. Normalization and saving as tiff file

For FLFM movie, especially for event related recordings, there is another mat file (Mat2Tiff.m) to be configurated and run for the data to be saved as tiff files properly. This file would only run after reconstruction file *mainFLFM_wExppsf_loop_ini.m*.

A. Procedures to run mat-to-tiff:

a) Open MATLAB file: Mat2Tiff.m or you can comment off the last section of *mainFLFM_wExppsf_loop_ini.m* for automatic conversion. However, to do this you would need to configure the Mat2Tiff.m file first.

```
%% Section 3: for video normaliztion
% Mat2Tiff
```

b) Plug in the directory for reconstructed mat files in Section 1

```
%% Section 1: Read in Mat Files
Mat_datasetdir='E:\yangyang\FLFM_reconstruction_My\Data_Seisure\2023_Sebfish_seisure\Reconstruction\2023_05_26_Sample1_15s_Activity\';
img_folder='matfile\All\';
imglist=dir([Mat_datasetdir,img_folder]);
```

c) Set the normalization value for the entire recording in Section 2. This value should be an estimate of the maximum value of the event. Note here that ***MaxV_Frame*** is the **frame** that contains the maximum value. You should plug in the **frame number** that contains the maximum value. To estimate the frame, you could use the raw FLFM movie. Alternatively, you could always change the code and just directly set the value in this section.

```
%% Section 2: Setting Nomalization
MaxV_Frame=411; % 411
Numcolor = 65536;
```

d) Set tiff saving directory in Section 3

```
%% Section 3: Write out Tiff file
Tiff_datasetdir='E:\yangyang\FLFM_reconstruction_My\Data_Seisure\2023_Sebfish_seisure\Reconstruction\2023_05_26_Sample1_15s_Activity\Tiffile\All\';
if ~exist(Tiff_datasetdir,'dir')
    mkdir(Tiff_datasetdir);
end
```

e) Run the file

Okay! This is everything I cleaned out for reconstruction. If you have any questions, let me know and I'll be happy to arrange for meetings on zoom or in person. Good luck!