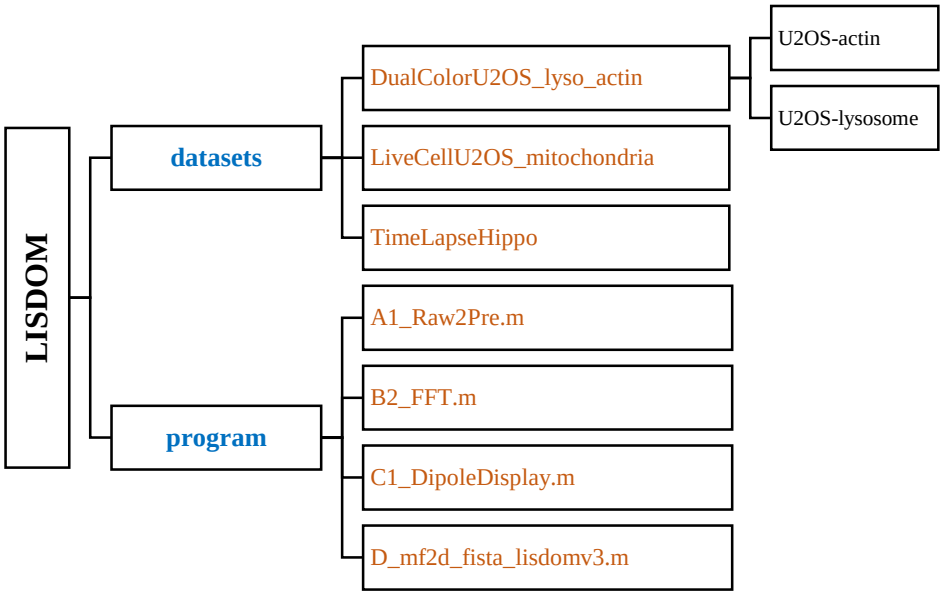
LiSDOM Program Description

This program was written by MATLAB 2016b and the post-show images are processed by Fiji, such as contrast adjustment, merge color channels and video generation.

1. Folder description:

This sample program consists of two parts: datasets and program.



datasets:

- DualColorU2OS_lyso_actin: Bicolor imaging of U2OS cells, in which two subfiles are fluorescent images of Lysosome and Actin at the same location, respectively.
- LiveCellU2OS_mitochondria: Mitochondrial imaging of U2OS living cells.
- TimeLapseHippo: In vivo LiSDOM imaging of dendritic spines stained with lipophilic tracer dil in live hippocampal neurons.

each set of data contains two subfolders:

- **0_System:**
  1_modulated.txt: It contains acquisition time, polarization modulation signal, and camera acquisition pulse. According to this file, read and sort out the acquisition signal and the modulation signal synchronization;
  Psf_xxx.tif: the PSF of the system under the current acquisition conditions.
- **1_Raw:** All images were captured from the camera.

The document structure in the **program** section is the same as the document structure in the **dataset** section. The internal procedures include:

A1_raw2pre.m: unifies the name of the image file based on the information in *1_Modulation.txt* file.

B2_fft.m: read the images calculated from *A1_raw2pre.m* file, separate the same information and background information as the modulation signal in the acquired image, and save it.

C1_dipoledisplay.m: calculates and displays the dipole orientation according to *D_mf2d_fista_lisdomv3.m* reconstruction information.

D_mf2d_fista_lisdomv3.m: LiSDOM reconstruction of the image.

2. Calculation processing

Run as A1_raw2pre.m →B2_fft.m→D_mf2d_fista_lisdomv3.m→C1_dipoledisplay.m.

Parameter description:

B2_fft. m: In the OLID process, select the image for the period of *li_periods* to participate in the calculation. Read idx1: idx2 files in the current file order, and modify the *time* variable according to the file name. In long-duration imaging, OLID for multiple points in time can be generated, followed by LISDOM reconstruction in *D_mf2d_fista_lisdomv3.m*.

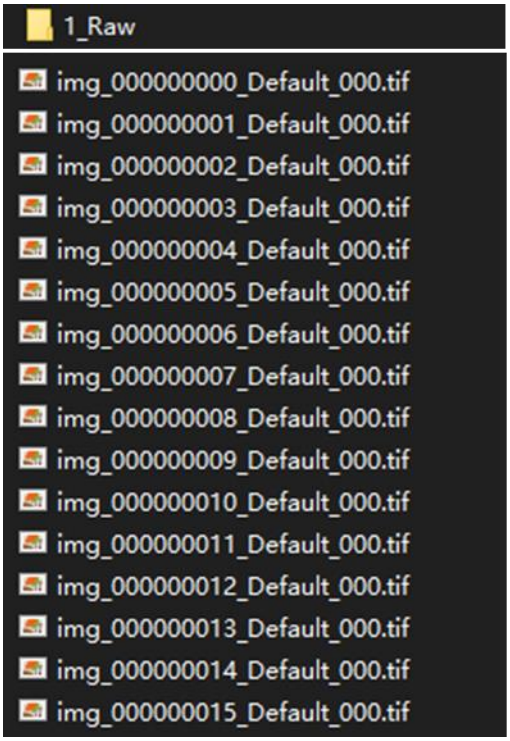
D_mf2d_fista_lisdomv3.m: LISDOM reconstruction. In the process of reconstruction, the file name should be regenerated into a file according to *B2.fft.m* and appropriately modified. The number of iterations *n_iter* and calculated parameters *lk* in FISTA can be adjusted, and the calculated images and *.mat* data files can be saved in the **4_Recon** file for reviewing and saving data.

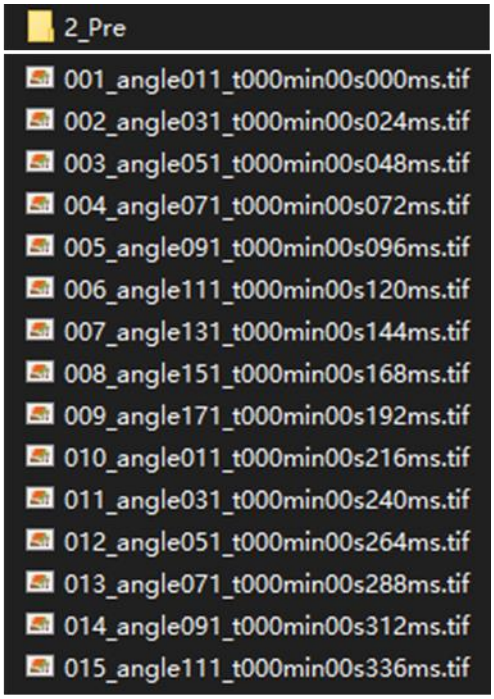
C1_dipoledison.m: This file displays the OLID image and the dipole orientation map. Adjust the *data_dir* according to the **4_Recon** floder. When *ang* is calculated, you need to add a constant correction, which is measured during the collection.

Then Fiji should be used for color Channel Merge, or video for growth time imaging.

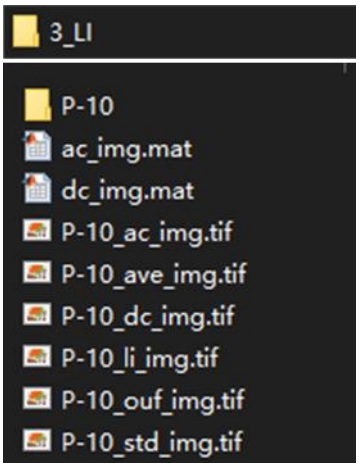
3. Take bicolor imaging as an example:

1. Run *A1_raw2pre.m* to generate the **2_Pre** folder, as follows:



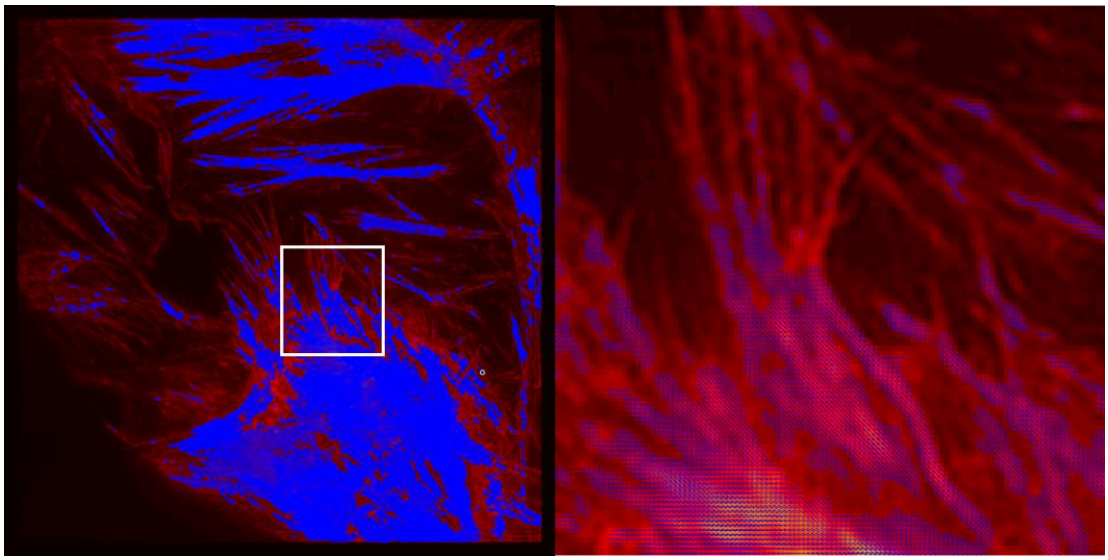


2. Run *B2_fft.m* to generate **3_LI** folder. Where **p-10** is a periodic modulated image generated after OLID processing, and '**P-10_AC_img. tif**', '**P-10_DC_img. tif**' and '**P-10_li_img.tif**' are respectively DC, AC and LISDOM images of OLID to be reconstructed. '**P-10_ave_img.tif** 'is the average image.



3. Run *D_mf2d_fista_lisdomv3.m* to generate **4_Recon** folder, including the reconstruction images after each iteration.

4. Run *C2_DipoleDisplay.m* to get the dipole orientation mapping. In addition, the threshold of present OUF can be adaptively modified.



Calculating the other folder which including lysosome imaging data of U2OS, and doing the step of Image/Color/Merge Channels… in **Fiji** can get the bicolor image of actin and lysosome.