PyLorentz GUI Manual

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Table of Contents

Getting Started

Purpose

Installation

This document assumes you have already installed the required packages and files by following the instructions ____here___. The only thing left required to use the full capabilities of the GUI is to store install the plugin responsible fore the bUnwarpJ registration procedure.

1. Open a file browser and navigate to:

                    `/PyLorentz/GUI`

2. Locate the file named: *bUnwarpJ_2_images.bsh*
3. Open another file browser and navigate to the Fiji installation you will use for your image registration.
4. Next you need to move the .bsh script into Fiji's Plugin folder
    a. Mac:
        i.   Right click on Fiji and choose *Show Package Contents*
        ii. Open the Plugins folder
        iii. Drag and drop the *bUnwarpJ_2_images.bsh* into the *Plugins* folder.
    b. Windows/Linux:
        i.   ...

File Setup

It is important to note that the there is a particular file setup necessary for image alignment and reconstruction, which can be found ___here____ and reemphasized below.

For image registration or reconstructing a stack utilizing a double-sided through focal series of images, the image working directory must look like the following:

```
        datafolder/    flip/      -im1.dm3
                                  -im2.dm3
                                    ...
                                  +im1.dm3
                                  +im2.dm3
                                    ...
                                   0im.dm3
                            [flip_fls.fls (opt.)]
                        unflip/    -im1.dm3
                                  -im2.dm3

                                     .
                                     .
                                  +im1.dm3
                                  +im2.dm3

                                     .
                                     .
                                   0im.dm3
                          [unflip_fls.fls (opt.)]
                      [fls_file.fls]
                            or
                      [unflip_fls.fls,
                       flip_fls.fls]
                       full_align.tif
```

Figure 1. An example image working directory and its contents for a two-sided through focal series reconstruction.

The files in brackets are the *.fls files that contain information about the number of images, the change in defocus length between images, and the order in which the images should be aligned. The order for the files within the *.fls should be as follows

- largest underfocus
- …
- smallest underfocus
- focused image
- smallest overfocus
- …
- largest overfocus.

This should be the case for both orientations, *flip* and *unflip*, and all images listed in the *.fls should exist within their respective folders. For a two-sided through focal series there should be an unflip.fls file and a flip.fls file. These can be stored inside the *unflip* and *flip* folders or in the image working directory itself, referenced as *datafolder* above. If the images share the same name, only one *.fls (represented by fls_file.fls above) is needed and can be used for both orientations. Here is an example for a shared *.fls file for a two-sided through focal series. Take note that alignment and reconstruction will only work for an odd number of images, symmetric on either side of the infocus image.

```
// fls_file.fls
7                <- Number of files in (un)flip folder.
10000            *
20000            * The changing defocus lengths.
30000            *
-60000.dm3       #
-30000.dm3       #
-10000.dm3       # The file names, following the order
      0.dm3      # described above.
+10000.dm3       #
+30000.dm3       #
+60000.dm3       #
```

Table 1. An example *.fls file for a two-sided through focal series.

For a single-sided through focal series, the image working directory should look like the following:

```
datafolder/    tfs/     -im1.dm3
                        -im2.dm3
                            ...
                        +im1.dm3
                        +im2.dm3
                            ...
                         0im.dm3
                     [fls_file.fls (opt.)]
                    fls_file.fls
                    full_align.tif
```

Figure 2. The directory for a single-sided through focal-series

The requirements for the *.fls file are the same as that for a two-sided through focal series. However the name for the folder storing the images should be *tfs*, otherwise the GUI will not catch which folder to check.

If that setup is complete, and the installation of PyLorentz and its dependencies are complete, you can proceed to starting the GUI.

Start Up

1. Open a command line or terminal.
2. Activate the conda environment that has the dependencies for PyLorentz.
3. Navigate to the PyLorentz folder. (cd ../PyLorentz)
4. Initiate the GUI with the command

$ python PyLorentz_GUI.py

5. The GUI takes a few moments to start up
   - If on Mac, there may be a few system authorizations necessary for subprocess to allow Fiji to run in the background when called.

- Additionally on Macs, Finder may jump to the front of the screen if it is open. This is ok, it is a process that enables the menu options of PyLorentz specifically for Mac that are inherently capable on Windows.
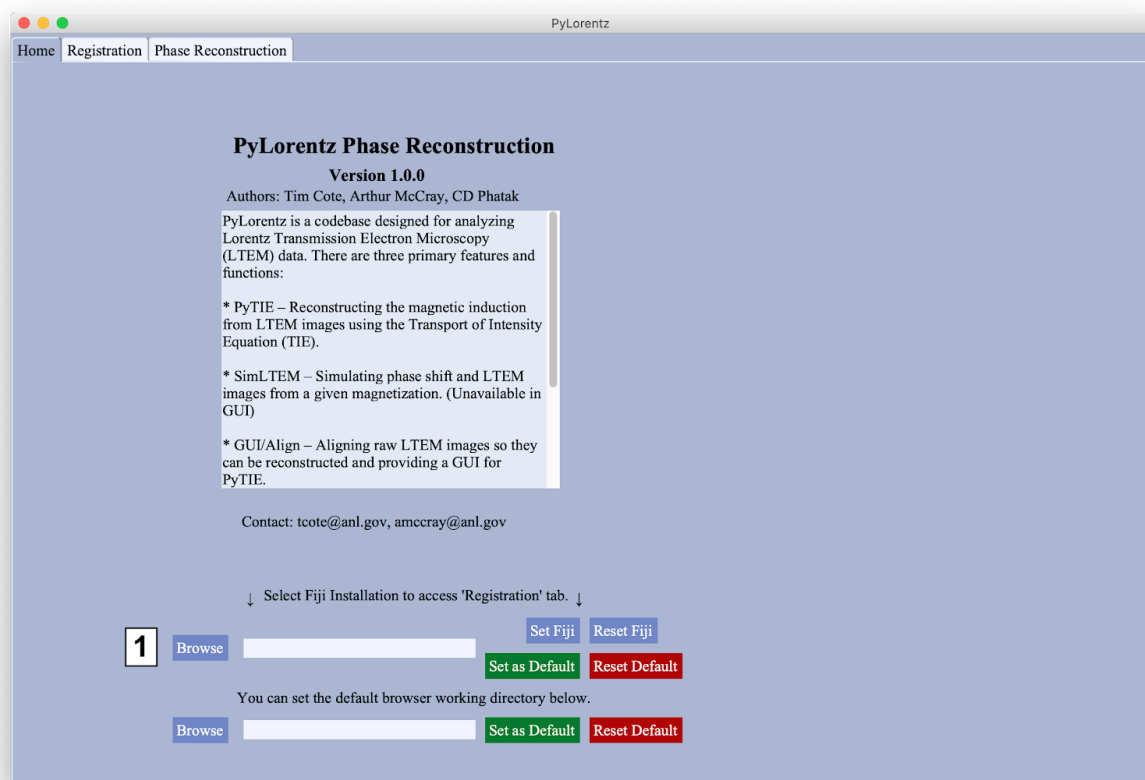6. Start up complete, move on to using the GUI

The GUI

Home

Figure 1. The *Home* tab of the GUI. For performing any of the image registration routines, it is required that you set the path to your local installation of Fiji.

Setting Fiji

To perform any image registration procedures, the first thing you need to do is confirm a path to a local Fiji installation. The *Registration* tab can't be accessed otherwise.

1. Navigate to your Fiji installation using *Browse (1)*. Once you have confirmed a selection, the input field will fill with the Fiji path. You can manually enter the Fiji path by hand if you choose.
2. Click *Set Fiji*. Once Fiji is set, you will be able to access the *Registration* tab. If an incorrect Fiji installation is chosen, no change will occur and an error message will be sent to the *Log*.
3. If for whatever reason you need to reset the Fiji path, just click the *Reset Fiji*.

Setting Defaults

It is likely that you will use the same Fiji installation for each registration. This may be true for the working directory where your images are stored as well. The GUI allows you to select default options for both FIJI and the working directory.
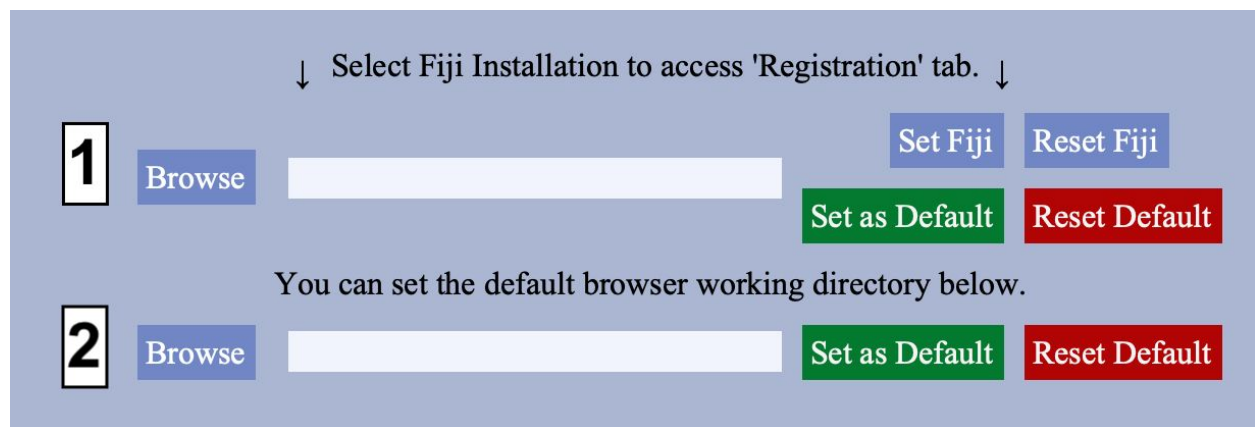

Figure 2. The default setting/resetting buttons on the *Home* tab.

1. **Fiji**
   ○ *Set as Default (1)* Saves a selected Fiji path in the 'defaults.txt' file. On subsequent uses of the GUI, this Fiji installation will already be populated in the Fiji input field.
   ○ *Reset Default (1)* Clears any Fiji path from the 'defaults.txt' file.
2. **Working Directory**
   ○ *Browse (2)* Navigate to and select an image working directory.
   ○ *Set as Default (2)* Saves the working directory path in the ''defaults.txt' file. On subsequent uses of the GUI, the default working directory input field will be filled as well as the working directory input fields for the *Registration* and *Reconstruction* tabs.
   ○ *Reset Default (2)* Clears the working directory from the 'defaults.txt' file.
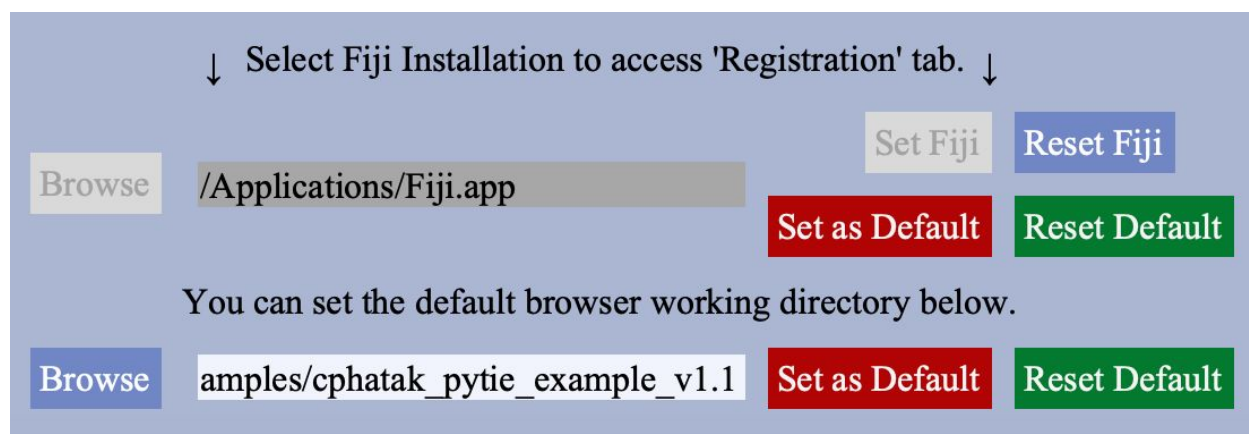
Figure 3. An example where the Fiji path is set. The Fiji path and working directory defaults are also set.

Registration

The GUI offers two different image alignment procedures (image registration) via subprocess routines that call two different plugins of Fiji. **Linear Stack Alignment with SIFT** is a registration technique that aligns images utilizing scale invariant feature extraction (SIFT) based off a paper written by David Lowe. For more information on the parameters used for registering images, visit either of the following urls.

- [Linear Stack Alignment with SIFT Plugin](#)
- [Feature Extraction](#)

The second alignment procedure, **bUnwarpJ**, was written by Ignacio Arganda-Carreras, Jan Kybic, and Albert Cardona and registers images through elastic deformations modeled as B-splines. The accuracy of registration is determined by minimize a weighted 'energy' function between different images. The version of bUnwarpJ used by the GUI is slightly modified from the plugin distributed by Fiji, as it is written to bypass the internal bUnwarpJ I/O. More about the parameters of bUnwarpJ can be found here.

- [bUnwarpJ](#)

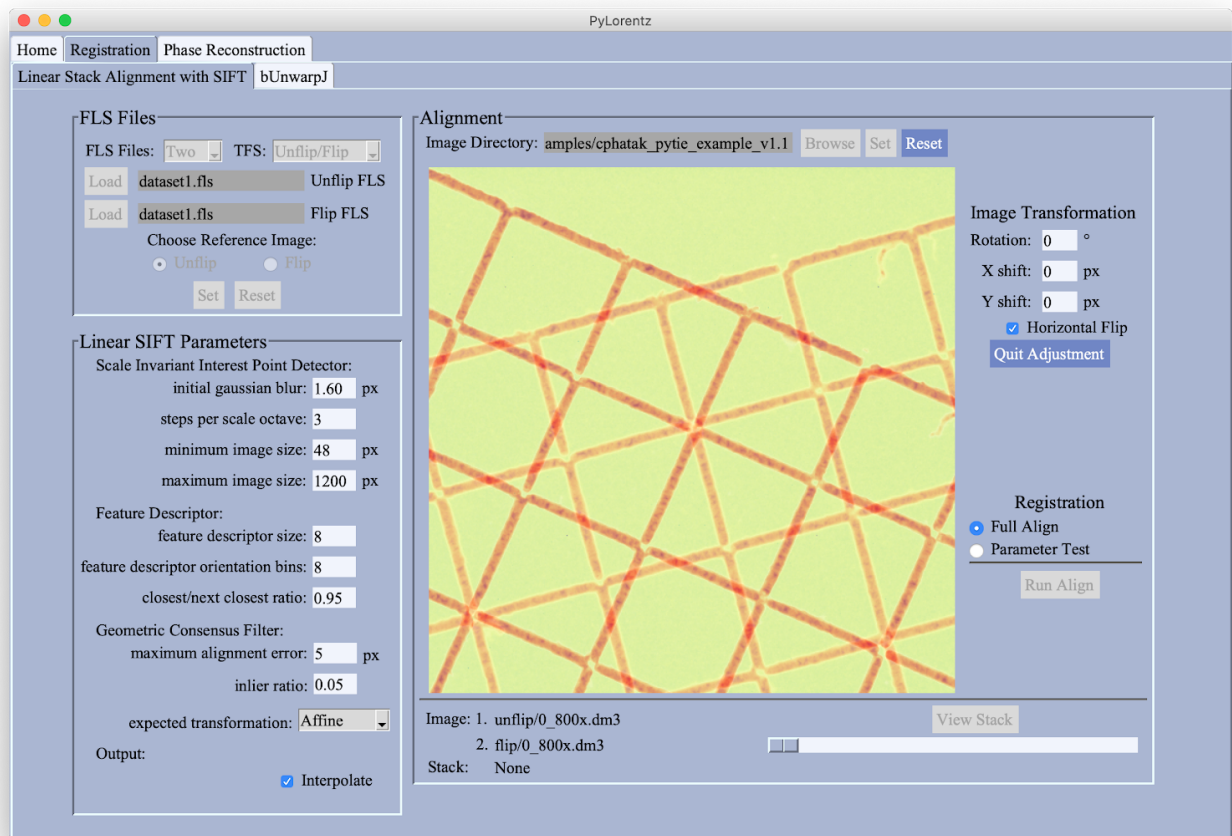# Linear Stack Alignment with SIFT (*LS Tab*)

Figure 4. An in progress alignment for the Linear Stack Alignment with SIFT tab.

Reconstruction