Grr User Guide

Getting Started

Once you have the program open, the first step is to load data. For this, you have a few different options. If your data is a tiff stack or a ser file, you can directly import these files. If your data is in another format, the best course of action is to use another program to extract the files to a folder of tif images or a tiff stack.

Once you have one of these file types ready, you can now proceed. If you have ser file, then go to File>Import .ser files. This will open a file explorer. Navigate to a .ser file containing image data and select 'Open'

If you have a tif stack, go to File>Import .tif stack, which will open a file explorer. Navigate to a .tif file containing a stack of images and select 'Open'.

If you have extracted tif files, they should all be in one folder with no other files in said folder. Go to File>Import Extract .tif Files and navigate to the folder with the .tif files. Go into this folder and select any one of the files within this folder and select 'Open'.

The program will now create an image stack, look at the shifts between the images, calculate the outliers of the shifts between the images, disregard those shifts, use the remaining shifts to calculate an averaged image, and display this image, along with its Fourier transformation. You will also be able to see the shifts between each image in the x and y direction and see which data points were deemed outliers and not used in the generation of the averaged image.

In the display, you see, the screen is divided into 6 sections. In the top left is the final output: the average image and the Fourier transformation. Directly under this plot is a toolbar that allows users to zoom in on either one of the images displayed and move around it to explore. You may also use the Home button to revert to the original review. In the top left, the Fourier transformation mask is featured. On the left side of this image, the Fourier transformation from one slice from the stack is shown with the mask placed on top. On the right side of this image, the Fourier transformation after the mask has been applied is shown. Like the averaged image, there is a toolbar under this plot that allows users to zoom in on either one of the images displayed.

On the middle row, we have the graphs of the shifts in the X and Y direction. These are the shifts between each image that occur due to the stage moving. On the left is the shifts without a mask, and on the right is the shifts with the mask covering the outlying points. The image on the right may look different since it removes the extreme values from the data, so the colors may appear brighter in this image. To help compare the original and masked data set, you can hold down the mouse button on the left image without a mask and it will cause the mask to overlay on this image. If you hold down the mouse button on the right image that has a mask, you can see the version of the image without the extreme values removed.

On the bottom row, you can look at each slice in the stack. On the left, the unregistered images are shown so you may use the arrow buttons on either side of the figure to move through the stack and see the shifts between them. On the right are the registered images so you may go through the stack and see (hopefully) little shifts between the images, which is a good way of checking to see if the correlation method worked on the data.

Editing Output

If you are not satisfied with the output, there are some things you can control to fix it, most having to do with how the image shifts are determined and how the outliers from these are determined.

The first option is to change the range of images used in the stack of images. To change this, go to Edit > Change nz range. A popup will come up, allowing you to change the nz min value and max value. These values refer to the stack of images produced. If some of the images at the beginning or at the end of the stack are not good to include for whatever reason, they can be removed from the stack temporarily and the average image recalculated without using these images. Underneath the min and max value, in the 'Bad Images' text box, users may input the index number or numbers of bad images that are in the middle of the data. If inputting more than one number, give each index separated by a comma.

Another option is to change how the outliers are calculated. To access these controls, go to Edit > Change Outlier Method. There are two methods you can choose from. The first method the outlier's method uses is a polynomial fit. In this method, a multidimensional polynomial is fit to the outlier shifts since in most cases there appears to be a pattern in these shifts due to the moving stage. After this is found, the program then finds the z-value for each point in the data set compared to the expected value found from the polynomial. If the z-score is too high, the point is considered an outlier and rejected. If you wish to use this method, select the Use Polynomial Fit radio button at the top of the popup. This will then allow you to edit the order of the polynomial used to fit the data and the z-score that each data point must fall under. Note: It is not recommended to use large order polynomials (greater than 4) since it will take a large amount of time to calculate and may not be accurate. The other method goes through each point and compares it to its direct neighbors. If the difference in the value between the given point and its neighbors, then the program considers it to be a jump. If there are multiple jumps between one point and its neighbors, it is considered an outlier and removed. If you wish to use this method, you may select the radio button 'Use Nearest Neighbors' at the top of the popup. This will then allow you to change the max jump – the difference between the point examined and its neighbor before it is considered an outlier.

Lastly for changing the outliers, you can also create a custom mask. This can be found by going to Edit>Custom Mask. A popup will appear showing the shifts in the X and Y direction with the mask overlaid. You can click on any point in either of the two plots to toggle the mask at that point on or off. Note that since the mask on both plots is the same and is diagonally symmetrical, up to 4 points may change with one single click.

Another option to edit how everything is calculated is changing the Fourier mask. As mentioned earlier, we can see the mask on top of the Fourier transformation and the result of this in the top right pane. If this mask does not encompass the correct information, this can be changed by going to Edit>Fourier Transformation Mask. The first option you are presented with is the n value, which defines the upper frequency that is allowed. Features that are smaller than n pixels will be smoothed. The second option you are presented with is the type of mask used. There are three options for this: None, Low pass, or Bandpass.

Lastly, some edits to the method of the correlation can be made. To change these settings, go to Edit>Change Image Shift Method. There are two major things you can change: the correlation method and the method used to find the maxima. There are three different correlation method options: cross correlation, mutual correlation, and phase correlation. For the finding maxima, there are also three options: pixel, Gaussian fit, and center of mass. Pixel identifies the maximum pixel; Gaussian fit creates a fit around *n* maximum pixels; Center of mass takes the center of mass of the whole image, then the center of mass in a smaller section around the center of whole image. For the Gaussian fit and center of mass options there are more options that can be changed. When selecting one of these two options, the respective options will become active allowing you to edit these. For the Gaussian fit, you may change the number of peaks used, the sigma value, and the window radius. For the center of mass, you may change the number of iterations, and the fraction of the window used in each iteration.

After doing any of these edits, make sure to hit the save button in the popup menu. After selecting the Save button, the popup menu will automatically close and the program will recalculate and display the data with the new parameters.

<u>Saving Output</u>

After going through all the calculations for, you naturally will want to export your results to another format to be saved. You can save your averaged image as an image in .tif or another format, a PDF report of your results, or you can get the data from the shifts in a .txt file.

There are a couple different ways to save the averaged image. You can go to File > Save Menu and a file selector will come up for you to select the location of the file and name it. Another way of doing this is selecting the save icon at the very right side of the toolbar. Using this method will allow you to save as an image with a variety of file types.

If you wish to get a PDF report of your results, which includes a graph of the shifts, both with and without the mask and shows the averaged image along with the Fourier transformation. To do this, go to File > Generate Report. This will open a file selector for you to select the destination of the PDF to be saved.

If you wish to export the shifts between the images, you can go to File > Export Shift Data and select the location and file name for the shift data to be saved to. This will create a text file that could be imported into another data editing program, using spaces as the delimitating character.