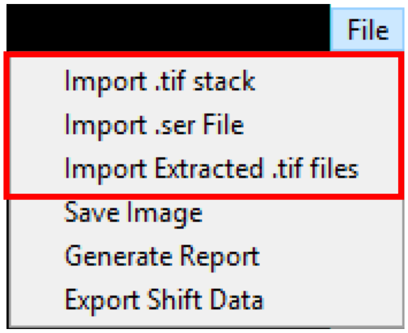
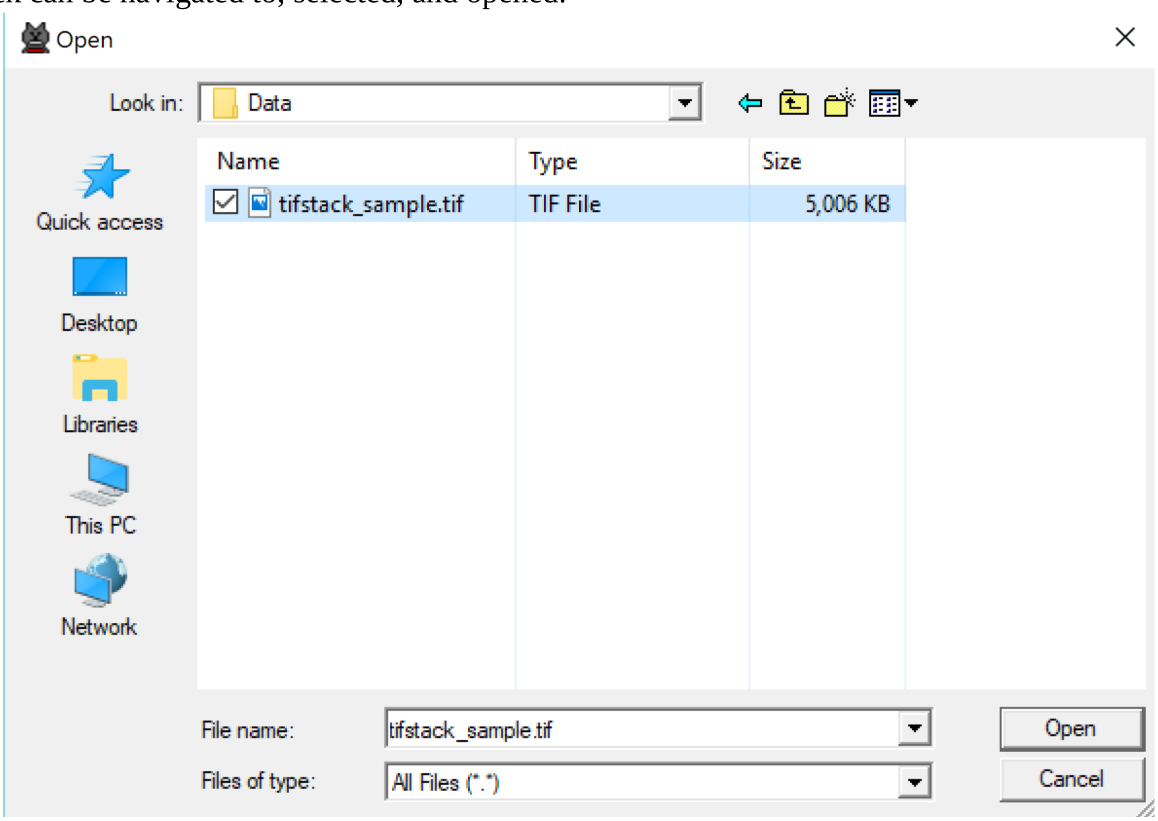
Grr User Guide

Import Data

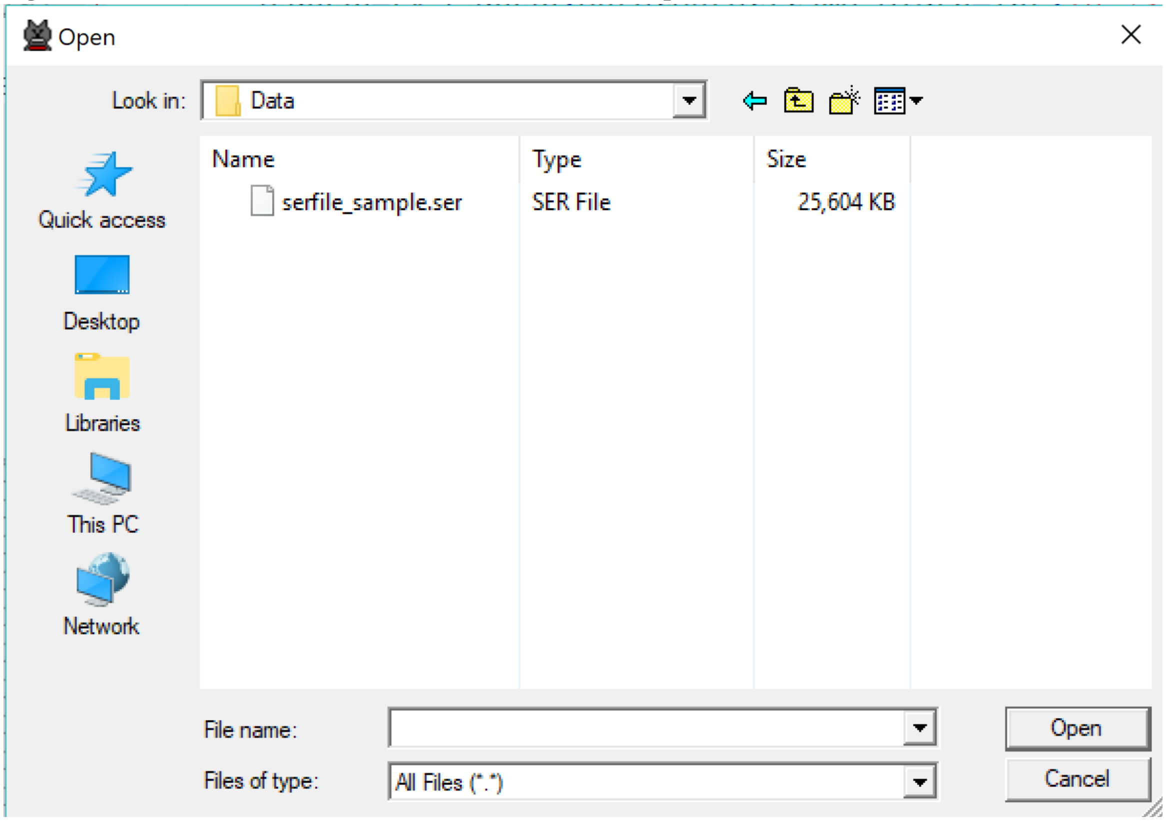
After launching Grr, the first step is to load the data you wish to analyze. There are three different options for loading in data, all of which are found under the File menu.



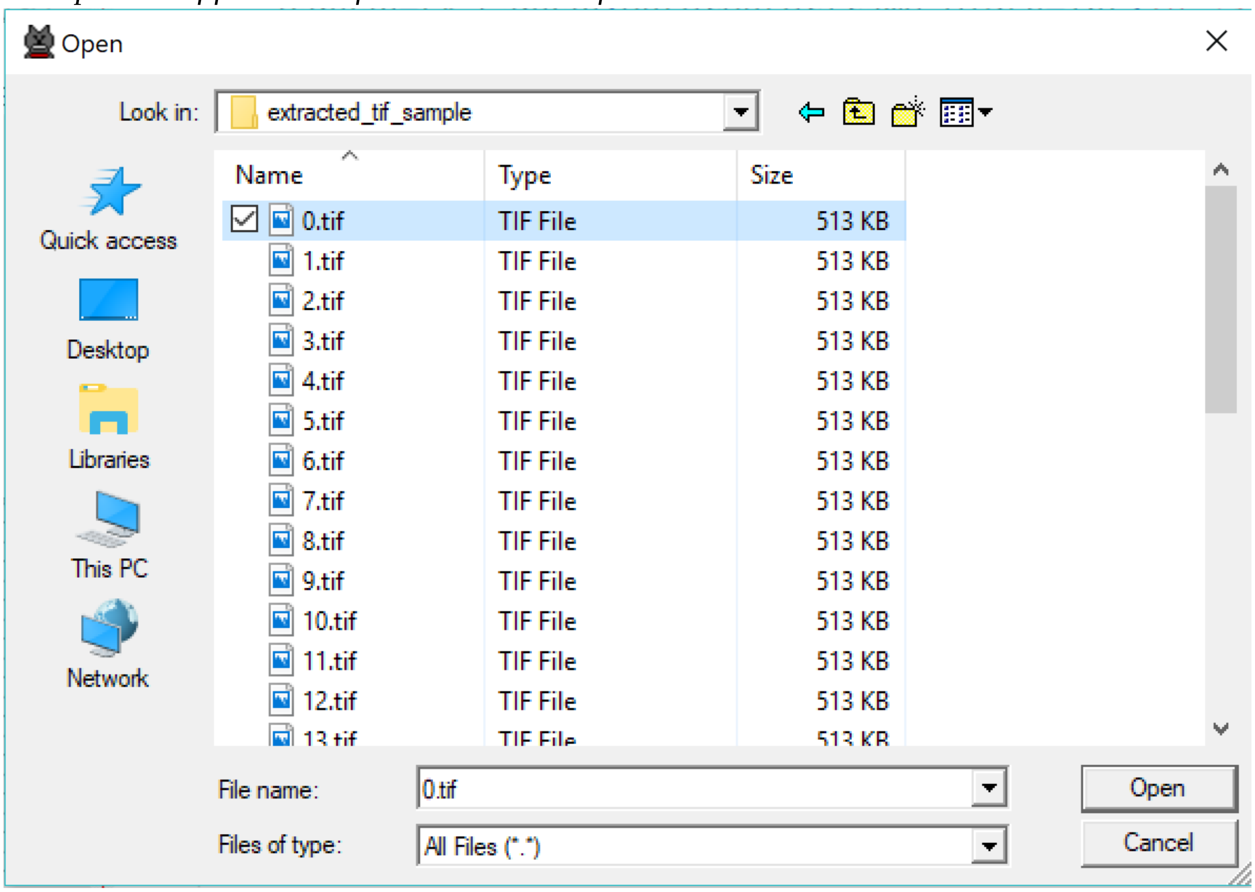
The first option, Import .tif stack, allows a stack of data that is in a single .tif file to be used. To do this, select this option from the File menu. This will open a file selection dialog in which .tif stack can be navigated to, selected, and opened.



The second option is Import .ser File. In this option, a. ser file that contains a stack of data may be imported. To do this, select this option from the File menu. This will open a file selection dialog in which a. ser file can be found, selected, and opened.



The last option is to Import Extracted .tif files. In this option, a folder with a .tif file of each slice in a stack of data should already exist. This option is best for when the original data is not in a .tif stack or a. ser file and must first do some amount of processing to make the data readable to Grr. To use this option, select Import Extracted .tif files from the File dropdown menu and use the file selection popup to navigate to a folder that contains the .tif files. Enter the folder containing all the .tif files and select any .tif file within the folder, and click open. *The program will open all .tif files in the folder the selected file is in.*

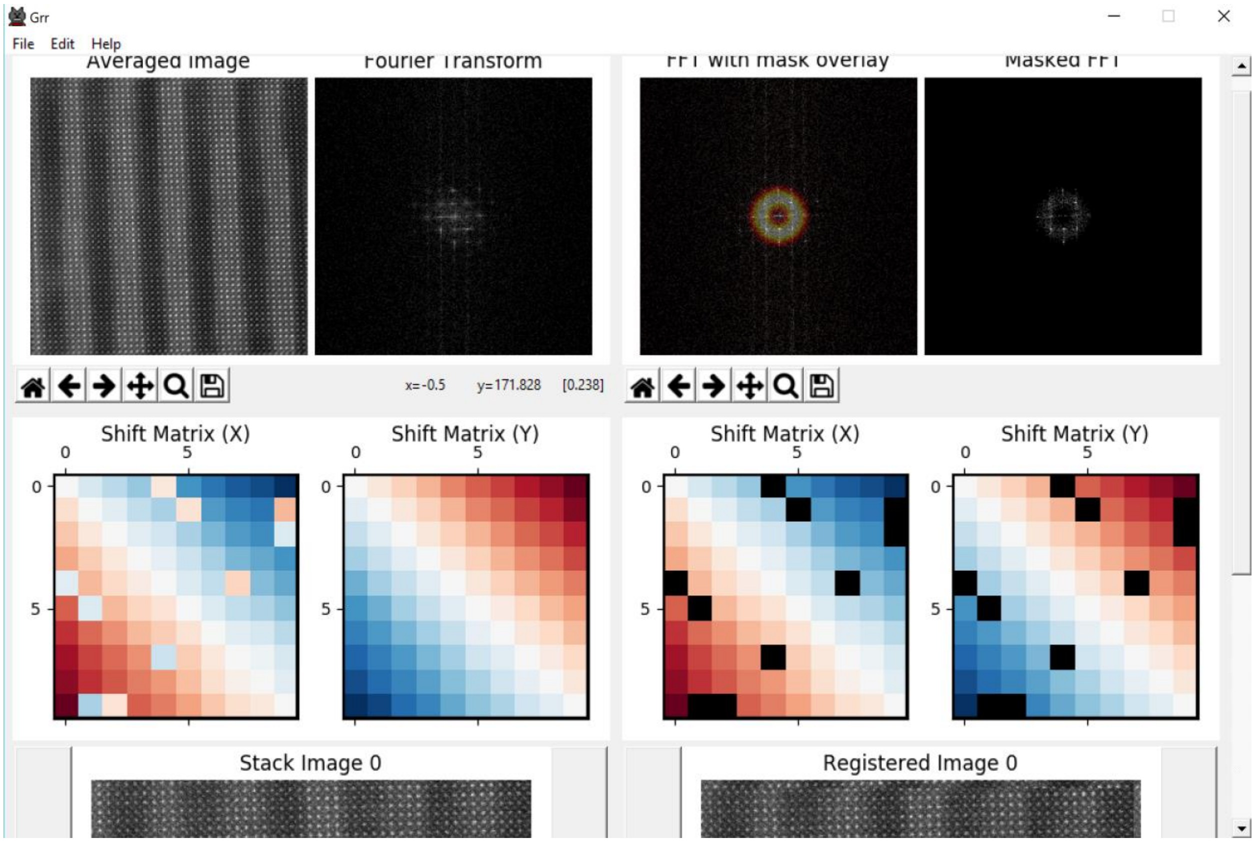


Note: The file selection popup appears different on different operating systems; regardless of the appearance of the file selection popup, the necessary steps are identical.

The program will now use the 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.

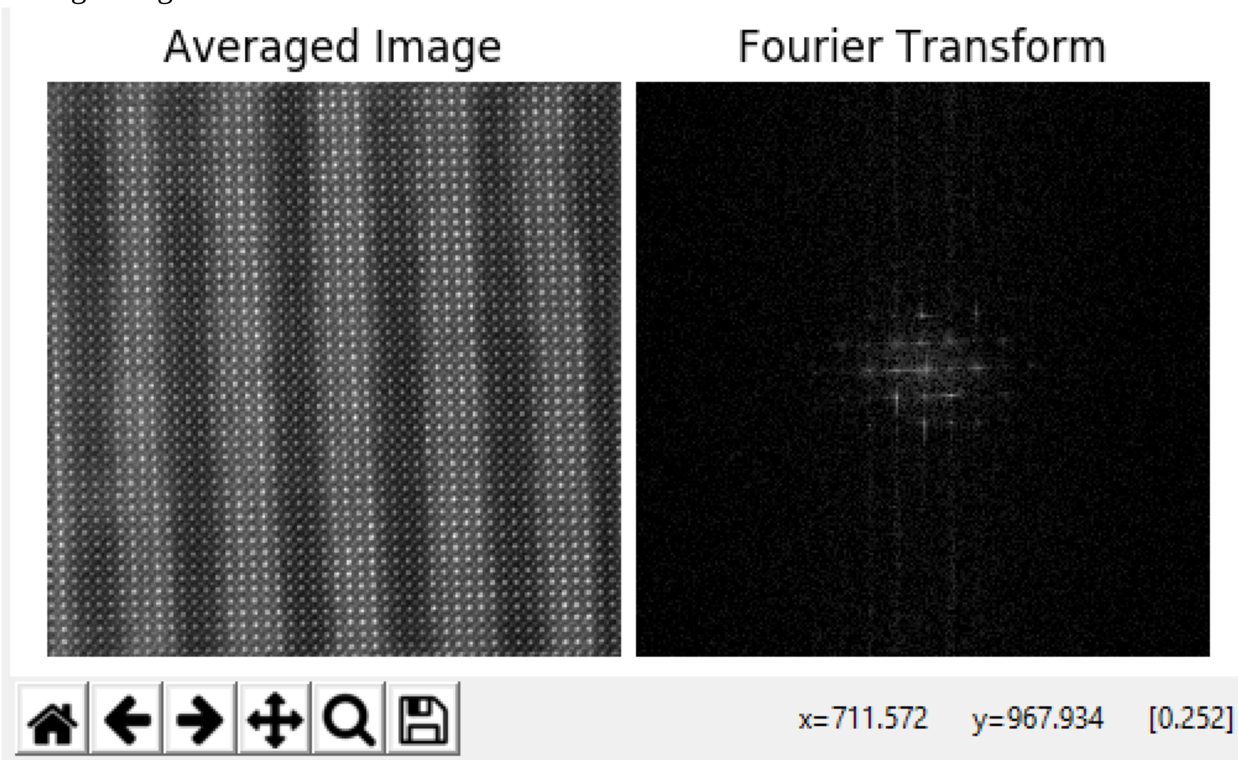
The Main Display

After all calculations have been completed, the window will display 6 different panes, in a two by three grid. *Note: It can take seconds to minutes to complete all calculations. During such time, the window may not respond and will appear blank. Do not stop the process, rather let it finish.*



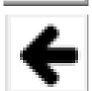
Note that all panes are not currently all the way in the field of view. The scrollbar can be used to move the window up and down to see all panes.

The first pane in the upper left corner shows the final results of the registration. Here the averaged image from the data is given on the left and the Fourier transformation of the averaged image on the right. Underneath this pane is a toolbar that can be used to change the view of the average image and Fourier transform.

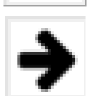


On the right, underneath the plot, the x and y coordinates of the mouse pointer are displayed. On the left is the 6-button toolbar. The buttons are described below:

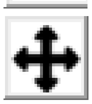
The “Reset to original view” or home button restores the view to the originally displayed view, which shows the entire image and Fourier transform.



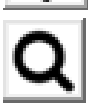
The “Back to previous view” button returns to the most recently used view of the image.



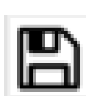
The “Forward to next view” button returns user to a more recent view after the user has used the back button.



The Pan button allows for moving around the image by simply clicking and holding on the image. The image must first be zoomed in.

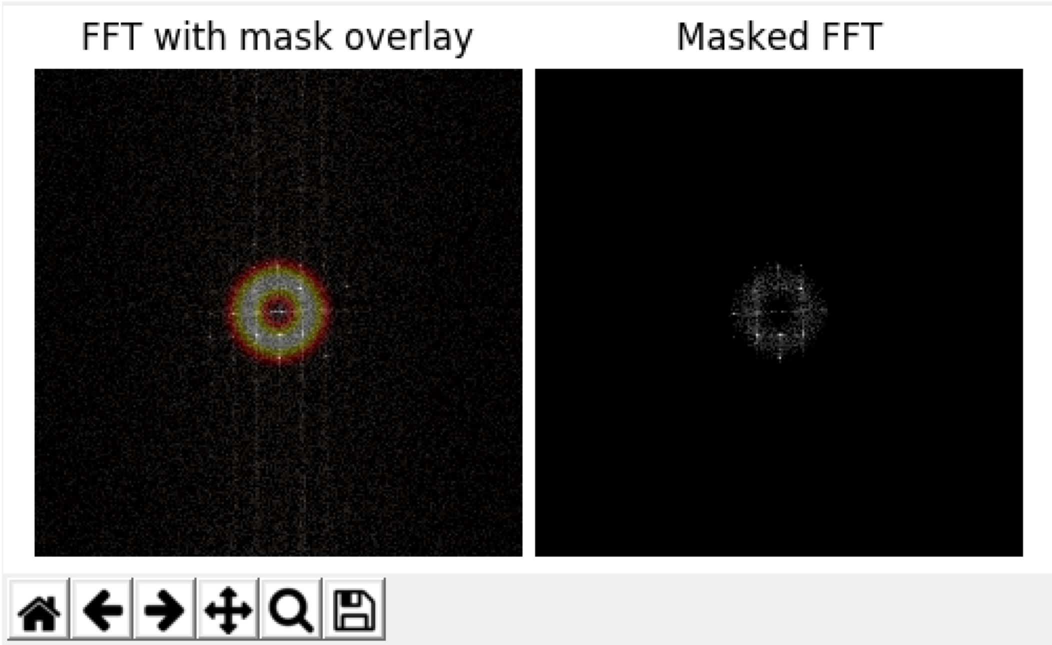


The zoom button allows a specific part of the image to be viewed. Select the zoom button and click and hold the cursor on the image. Drag the cursor to create a rectangle and release the mouse. The selected rectangle will now be the entire field of view.

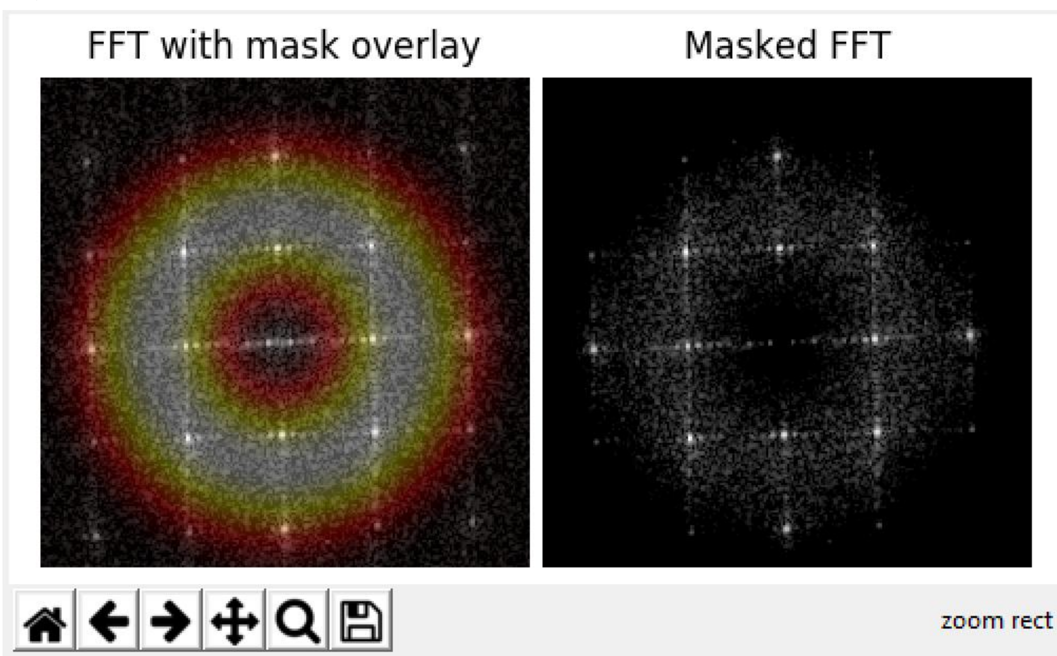


The save button saves this image to a file destination of their choosing. This will save the average image alongside its Fourier transform as well as the white space and titles.

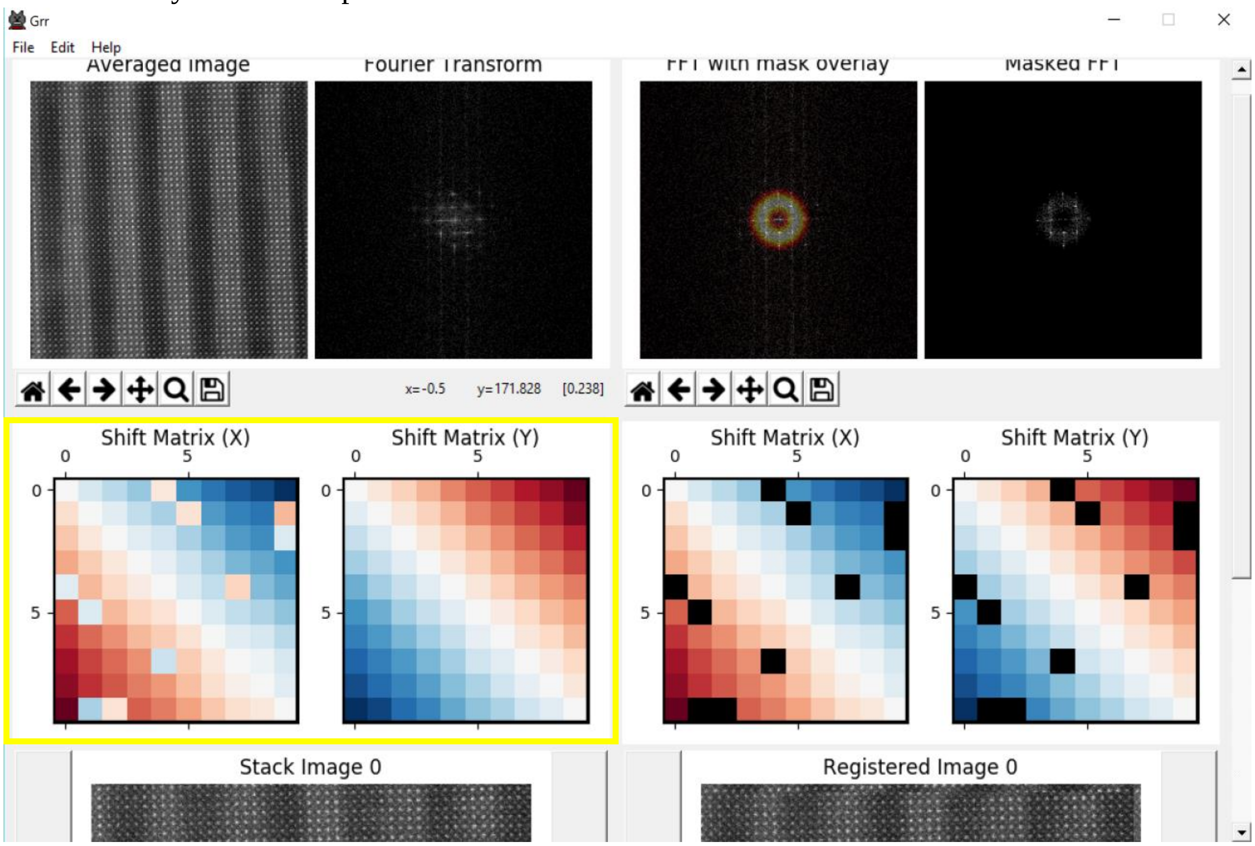
To the right of the first pane is the Fourier transform pane. One of the most crucial steps in registering images is creating a mask in Fourier space to crop the frequencies used when getting the cross-correlations between two images.



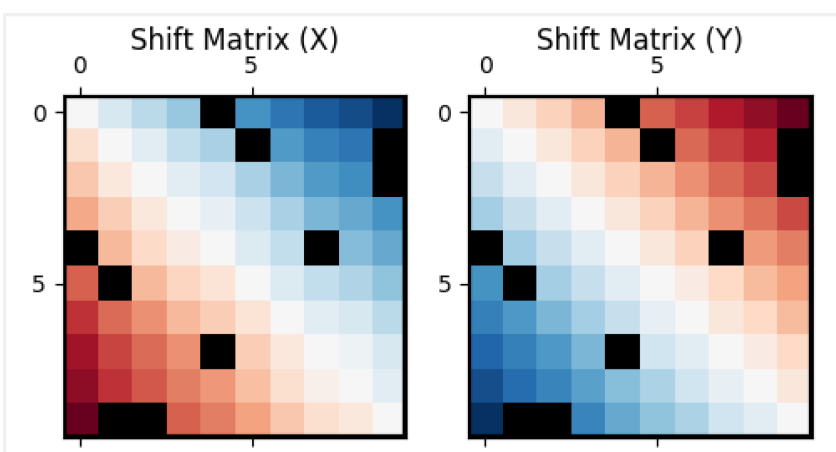
Like in the average image pane, there is a toolbar in the bottom left corner that can control the view of the transformations. The only difference between this pane and the first in terms of functionality is that when one of the Fourier transforms is zoomed in on, the other will zoom to the same amount as well, making it easy to compare the before and after masked Fourier transform.



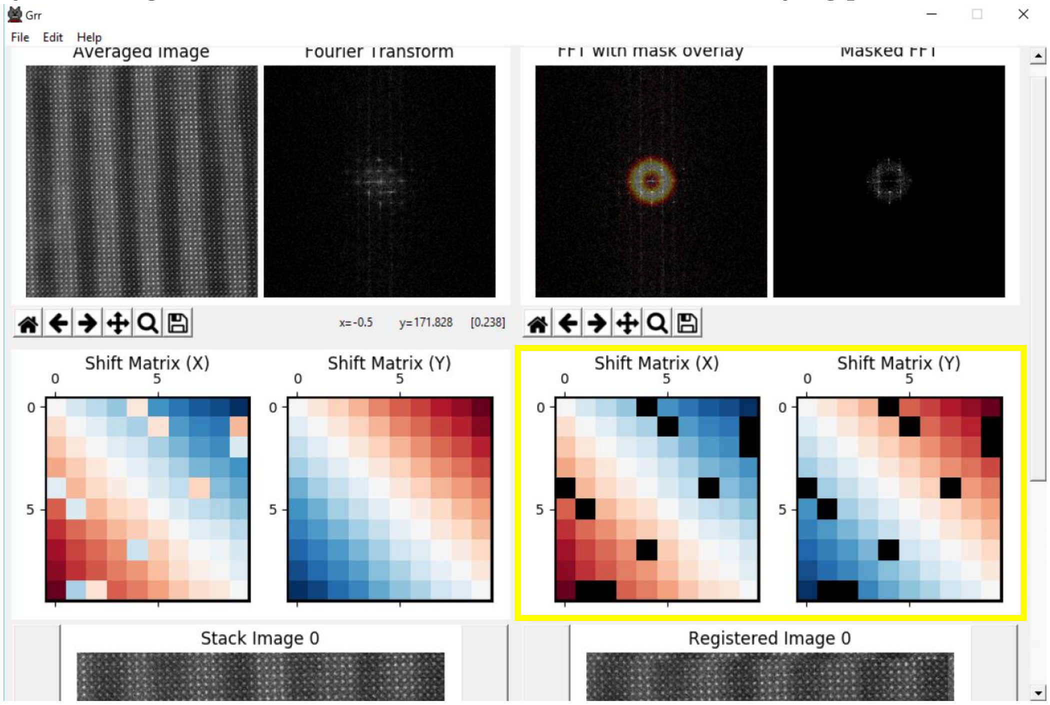
The third panel, the second one in the first column, shows the shift matrix without a mask on it. The shift matrix shows how much of a shift there are between each of the images in the stack based off the cross correlations. This occurs in both the X and Y directions, and the results are shown side by side in this panel.



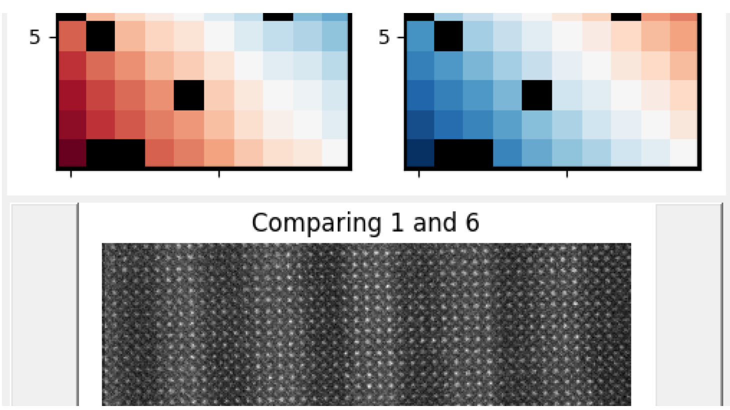
One interesting feature of this panel is that the mask can be overlaid on top by clicking and holding anywhere on this pane. This will put the mask, which shows where all the outliers are in this matrix and removes them, over the original matrixes. This provides an easy way to check the mask and make sure that all outliers are being removed without any good data being removed as well.



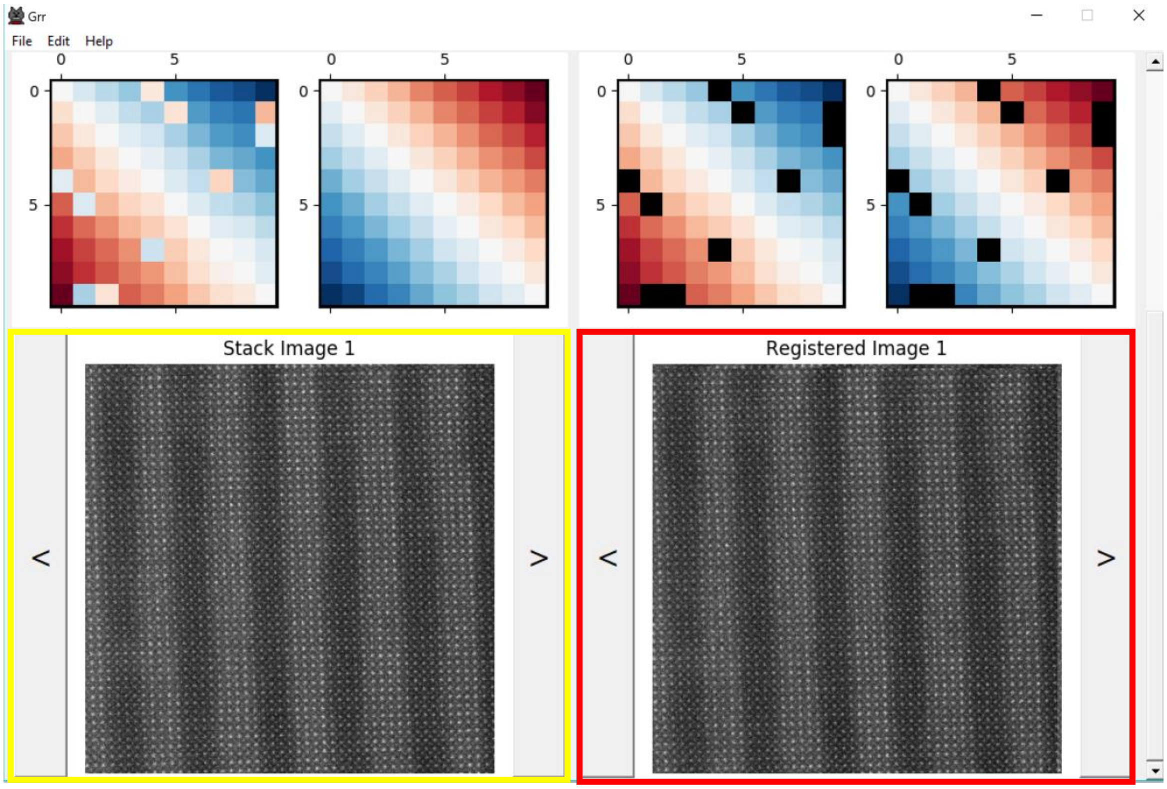
To the right of the shift matrices panel is a very similar looking panel: this shows the shift matrices with the mask on it. This one is also normalized such that if the largest values from the original are outliers, the next largest value that is not covered by the mask will become the darkest color. If there are a small number of very large outliers, the underlying pattern may be hidden by these large outliers. So once these are removed, the underlying pattern is more visible.



One useful feature of this panel is the ability to click on any point and see two images represented by the point clicked will be displayed in the individual slice viewer underneath this panel (see next page for individual slice viewer information). To select two images, click any point on either matrix to select those two images. To get the stack viewer underneath to return to scrolling through the entire stack, click this shift matrix panel anywhere in the white space around the plots.



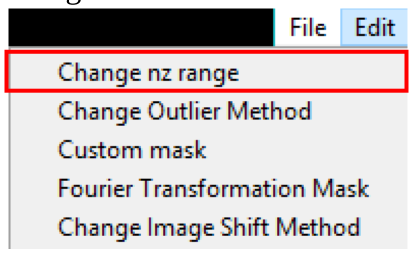
The last two panes also look very similar. On the left (highlighted in yellow below) is the stack viewer. Using the left and right arrow buttons on either side of this pane, the original stack of data can be viewed. The shifts between the slices from a stack can be observed using this tool.



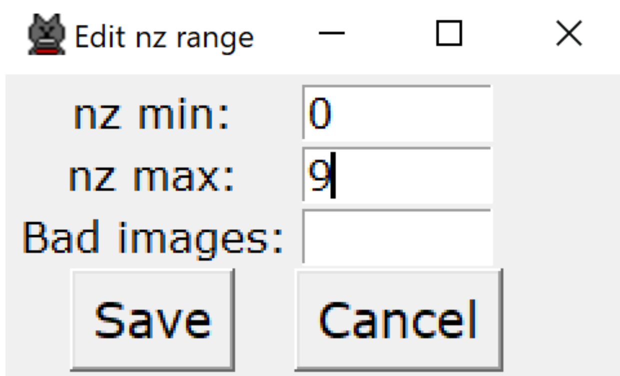
On the right, highlighted in red here, is the registered stack viewer. Using the left and right arrow buttons on either side of this pane, the registered images can be viewed. In theory, there should not be shifts between any of these images since they are already registered. This pane is ideal for checking the registration and making sure all registered images align properly. This registered viewer can also be used to compare two images that were selected in the masked shift matrix panel (for more information, see previous page).

Editing the Averaged Image

If in the shift matrix, there is one row and column that look entirely like they do not fit with the rest of the shift matrix, the best way to handle it may just be to remove the image causing this from the stack and make the average image with just the remaining images. To remove images from stack, go to Edit>Change nz range.



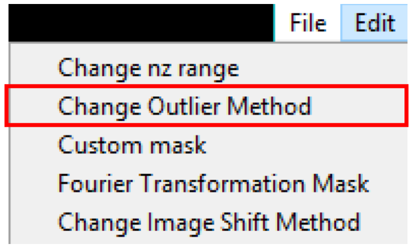
This will bring up a popup with three text boxes.



Nz min and max represent the edges of the stack. If the user wishes to remove images from the edges of the stack, the best way to do this would be to simply change the bounds of the images within the stack used. Note that the stack is 0-indexed, so the first image is 0 and the last image is the size of the stack minus one. Below is the result of decreasing the nz max by one.

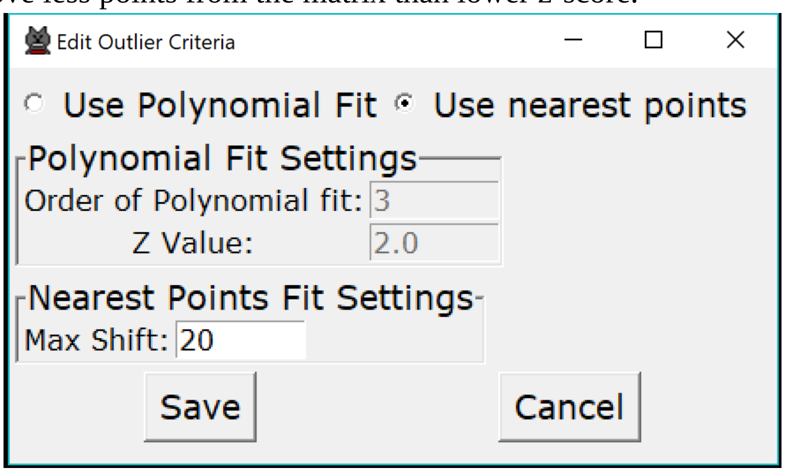
If an image in the middle of the stack should be removed, the bad images text box can be used. Enter the images to be removed with commas separating each image number. When this is finished, select save. At any point, cancel may be selected to close the popup without changing anything in the average image calculations.

If the outlier mask does not correctly capture the outliers while retaining good points in the shift matrix, there are multiple ways of calculating the outliers and one or more parameters that go with each method. To change how the outliers of the shift matrix are calculated, go to Edit>Change Outlier Method



This will bring up the popup shown below. The two radio buttons at the top "Use Polynomial Fit" and "Use nearest points" select which of the two outlier methods is used. Underneath are the parameters for each method. When a method is selected, the text boxes to edit the parameters for that method become active and the text boxes for the other become inactive.

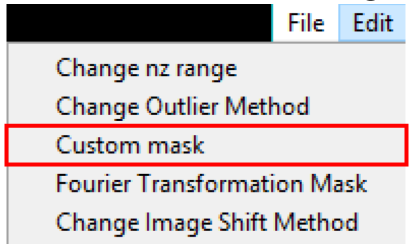
"Use Polynomial Fit" creates a two-dimensional polynomial to model the shift matrix. The highest order of this polynomial is user defined by editing the value of the text box labeled "Order of Polynomial fit". *Note: Large orders are not recommended*. If a point has a large difference between the shift found and the expected value relative to the standard deviation, the point is deemed an outlier and the mask is applied at this point; this is what the z-score is. Higher z-scores will remove less points from the matrix than lower z-score.



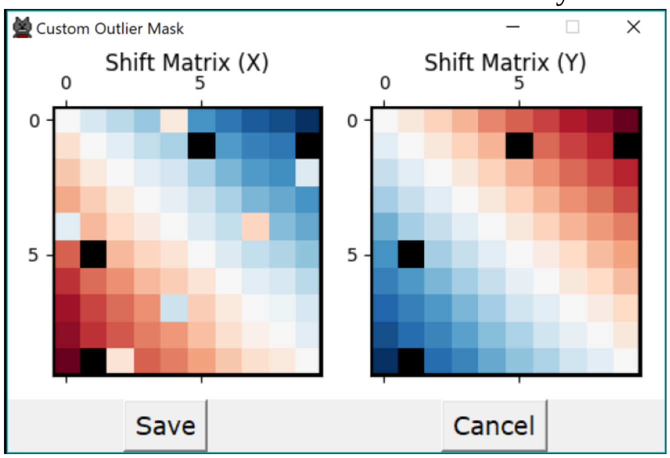
The other method, "Use nearest points" examines each point and compares the point to all its neighbors. If there is a large jump between this point and multiple neighbors, the point does not fit in the matrix (in theory the matrix should have a smooth pattern) and this point is masked since it is considered an outlier. The limit on the size of the jump between two points allowed can be defined by the user, by editing the "Max Shift" value.

After making all edits to the outlier method and parameters, click Save, which will close the dialog and rerun the necessary calculations using the outlier method selected. At any point, click cancel to close the window without recalculating anything.

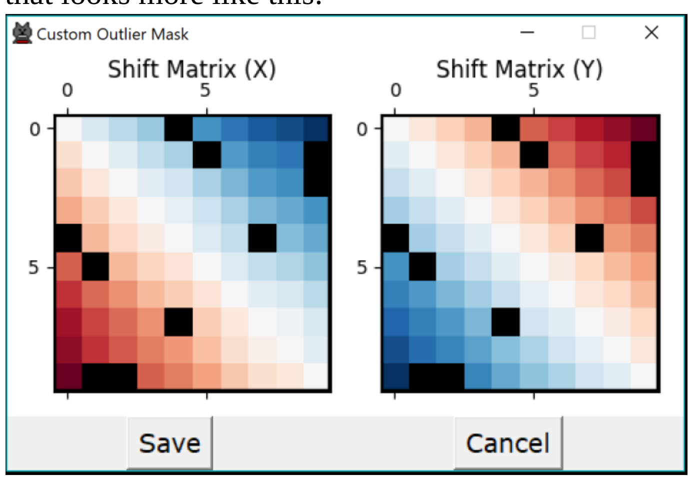
If changing the method used for detecting the outliers in the shift matrix does not give the best mask for the data, a custom mask can be created. To do this, go to Edit > Custom Mask.



This will open a new window showing the shift matrix with the mask overlaying it. Any point may be clicked on either the X or Y shift matrix to toggle the mask on and off at that point. Note that the mask is mirrored across the diagonal and the mask on the X and Y shift matrix are the same so clicking one point could toggle the mask in up to 4 points on the two figures. This tool is useful, for example, if a mask like the one below was calculated by the software.

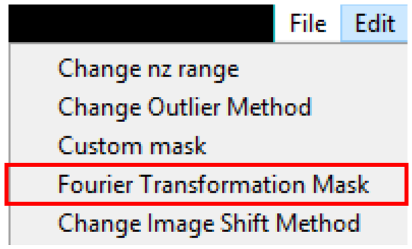


Using the custom mask tool, the points that are obviously outliers can be quickly selected to create a custom mask that looks more like this:

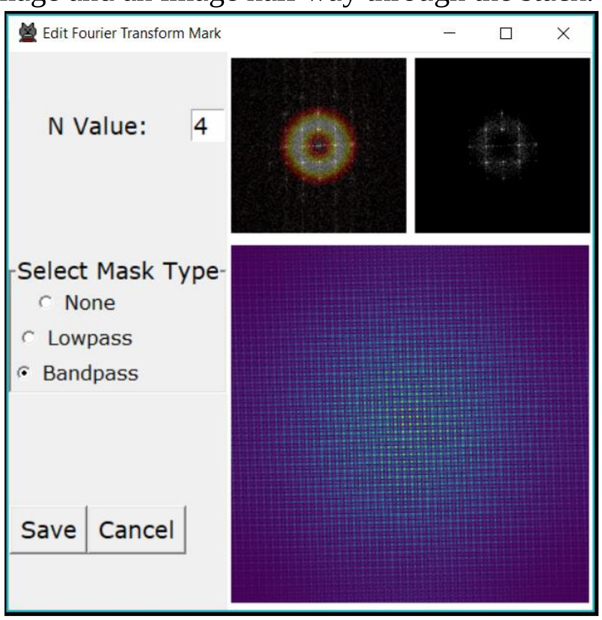


Once satisfied with the mask, click Save and the average imaged will be recalculated based on the outliers selected. At any time, the Cancel button may be selected to exit the window without recalculating the averaged image.

One of the most crucial stages of the registration is applying a mask to the Fourier transform of each image in the stack. Low frequency and high frequency information must be correctly balanced to give good cross correlations. If one is favored to high over the other, the cross correlation may look like streaks rather than dots. To edit the Fourier space Mask, go to Edit>Fourier Transform Mask.



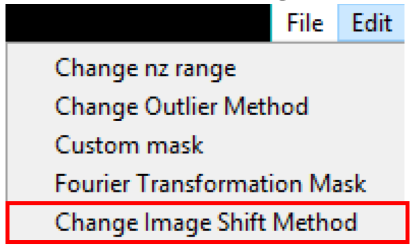
The popup shown below will appear. On the left are the main parameters of the mask that can be changed and on the right, is a zoomed in Fourier transform of one image from the stack and a cross correlation. The left Fourier transform does not have the mask applied, but has it overlaid. The transform on the right is the result of the mask being applied. The cross correlation below this is between the first image and an image half way through the stack.



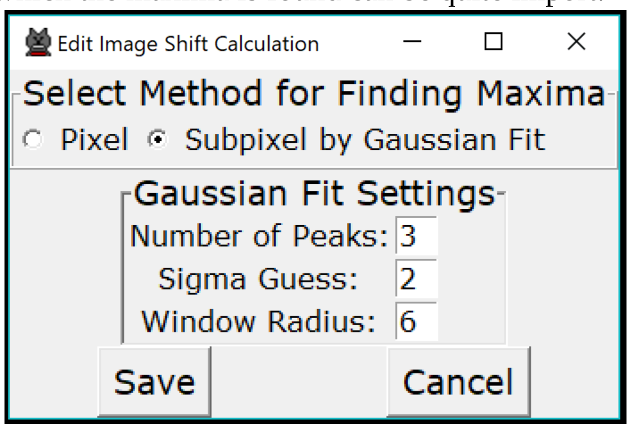
The Mask type has three options. The first "None" removes any mask from the data. This may be good for data that isn't atomic lattices. The second option is "Lowpass", which keeps data at the center and throws out data towards the edges. Finally, the last option "Bandpass" throws out data at the very center and data towards the edges. The N value gives the limit on the size of features that get smoothed. For example, a value of 4 will smooth features that are 4 or less pixels big. When changing this value, hit enter and the cross correlation and Fourier transform will update.

Once the cross-correlation demonstrates a correct balance between the high and low frequency information, select Save and this mask will be applied and all subsequent calculations performed, with the result being displaying on the main display. Cancel may be selected at any time to quit the popup without recalculating anything.

There are also a couple options in how the shifts between each image is calculated. This will change the values in the shift matrix itself, and by changing how the shifts are calculated, the average image could be improved as well. To do this, go to Edit>Change Image Shift Method.



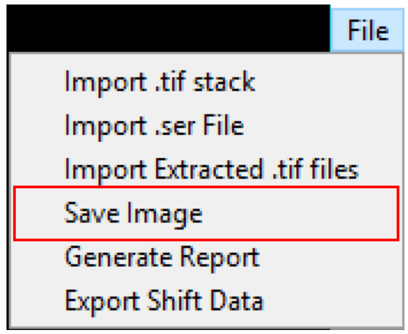
This will bring up a popup with two sections. The first section gives a prompt to select a method for finding maxima and gives the options of “Pixel” and “Subpixel by Gaussian Fit”. When finding the shift, the program finds the maximum overlap in the cross correlation between two images. The method in which the maxima is found can be quite import.



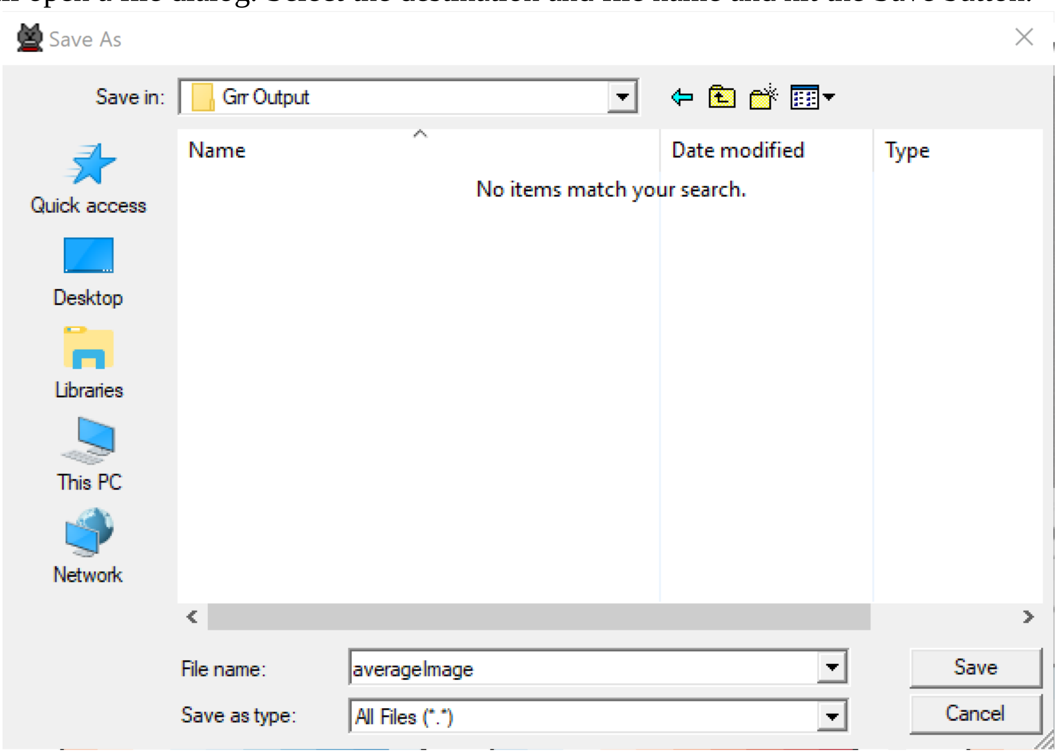
The pixel method simply finds the maximum pixel, hence the lack of parameters for this method to edit. The subpixel by Gaussian Fit fits a Gaussian curve about maximal pixels. This has multiple parameters to edit, which can be edited using the text boxes in the popup. These text boxes are only active if the subpixel by Gaussian fit radio button is selected. The number of peaks gives the number of pixels that should have a Gaussian fit around them. The sigma guess is the standard deviation used in calculating the Gaussian fit and the window radius determines how large the distribution should be. The Gaussian fit is done around the *n* largest pixels and determines the maximum from the fit rather than the pixel itself.

When finished editing these settings, select Save; the popup will close and image shift and subsequent calculations will be redone and then outputted to the main display. At any point, select Cancel to close the popup without recalculating anything.

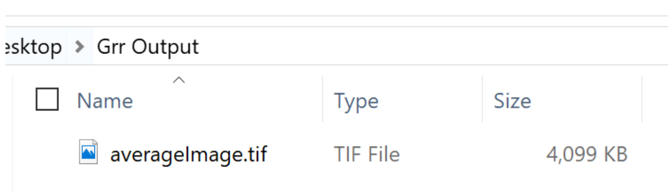
<u>Saving the Results</u>
After getting an output that correctly registers the images and creates a good average image, the next step is to export the average image so it may be used elsewhere. There are three different save types. The first is to save the average image itself as a .tif image. To do this, go to File > Save Image.



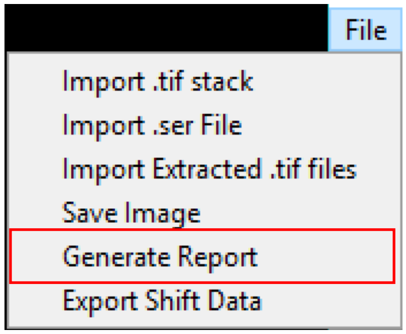
This will open a file dialog. Select the destination and file name and hit the Save button.



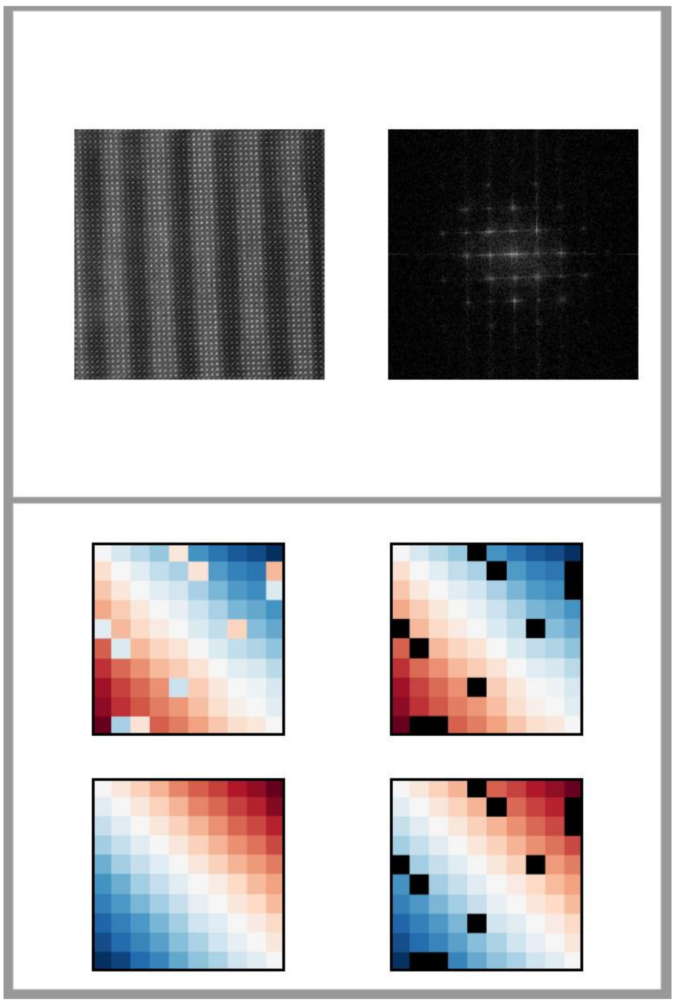
This will create a .tif image of the averaged image with metadata giving the parameters used to create the average image.



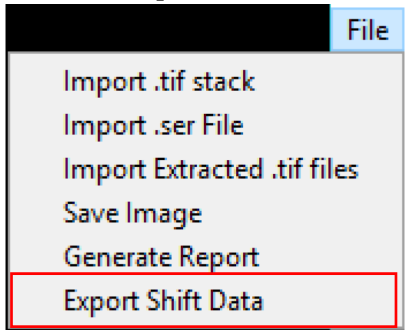
The next save option is to save a pdf report of the registration, which includes the shift matrix with and without the mask, as well as the averaged image and its Fourier transform. To do this, go to File > Generate Report.



This will open a file dialog. Like in saving the image, select the destination and file name and select Save. This will put a 2-page pdf in this location. The resulting pdf looks similar to the one shown below.



The third and final way to save some of the information from the rigid registration is to export the shift matrices. To do this, go to File > Export Shift Data.



This will open a file dialog. From here, select the destination and file name and select Save. This will save a .txt file that contains both shift matrices, the values being delimited by spaces and line breaks. Upon opening in a text file editor, the output will look similar to this.

shiftMatrices.txt - Notepad

File Edit Format View Help

```
X shifts
0.00    6.00    10.00   14.00   -4.00   22.00   27.00   31.00   33.00   37.00
-6.00   0.00    4.00    9.00    12.00   -5.00   21.00   25.00   27.00   -12.00
-10.00  -4.00   0.00    4.00    7.00    12.00   17.00   21.00   23.00   5.00
-14.00  -9.00   -4.00   0.00    3.00    8.00    12.00   17.00   19.00   22.00
4.00    -12.00  -7.00   -3.00   0.00    5.00    9.00    -8.00   16.00   19.00
-22.00  5.00    -12.00  -8.00   -5.00   0.00    5.00    9.00    11.00   15.00
-27.00  -21.00  -17.00  -12.00  -9.00   -5.00   0.00    4.00    6.00    10.00
-31.00  -25.00  -21.00  -17.00  8.00    -9.00   -4.00   0.00    2.00    6.00
-33.00  -27.00  -23.00  -19.00  -16.00  -11.00  -6.00   -2.00   0.00    4.00
-37.00  12.00   -5.00   -22.00  -19.00  -15.00  -10.00  -6.00   -4.00   0.00

Y Shifts
0.00    -4.00   -8.00   -12.00  -17.00  -21.00  -24.00  -28.00  -31.00  -35.00
4.00    0.00    -4.00   -8.00   -12.00  -17.00  -20.00  -24.00  -27.00  -32.00
8.00    4.00    0.00    -4.00   -8.00   -12.00  -16.00  -20.00  -23.00  -27.00
12.00   8.00    4.00    0.00    -4.00   -8.00   -12.00  -16.00  -19.00  -23.00
17.00   12.00   8.00    4.00    0.00    -4.00   -8.00   -12.00  -15.00  -18.00
21.00   17.00   12.00   8.00    4.00    0.00    -3.00   -7.00   -11.00  -14.00
24.00   20.00   16.00   12.00   8.00    3.00    0.00    -4.00   -7.00   -11.00
28.00   24.00   20.00   16.00   12.00   7.00    4.00    0.00    -3.00   -7.00
31.00   27.00   23.00   19.00   15.00   11.00   7.00    3.00    0.00    -4.00
35.00   32.00   27.00   23.00   18.00   14.00   11.00   7.00    4.00    0.00
```