This tutorial contains navigation buttons that enable you to move through the tutorial. Please use the navigation buttons and not the page up/page down or arrow keys to navigate through the tutorials.

- This is the 'Next' button. It takes you to the next frame or step.
- This is the 'Previous' button. It takes you to the previous frame or step.
- This is the 'Go to frame' button. It takes you to a specified frame.
- This is the 'Go to URL' button. It takes you to a website link.

Press the 'Next' button below to start this tutorial.
This tutorial will introduce the functions on the main window of the ZcorrectorGui.

Before using the ZcorrectorGui you must create a .bit (V4) or .bifs (V6) file for each slice of your depth profile. Each .bit file must be created using the same peak list.

All of the .bit files should be named sequentially (i.e. name01.bit, name02.bit, name03.bit) and placed in the same directory.

No other files can be located in the directory where the .bit files are located.
To start the GUI type 'zcorrectorgui' at the MATLAB prompt.

NOTE: You must restart the GUI each time you want to work with a new data set.
This is the main window of the ZcorrectorGui.
To load some data press the 'Import' button.
Once you have selected the folder where the files are located, press the 'OK' button.
The files will be loaded into Matlab and the ZcorrectorGui.
The number of layers loaded will be shown here.
If you have a large number of layers (>100) or a large number of peaks in each layer, you should downbin the data to avoid 'Out of Memory' errors with Matlab.

Downbinning reduces the dimensions of the image by a factor of 2 on each side, so a 256x256 pixel image is reduced to 128x128 pixels.

If you start with 128x128 pixel images, you should be fine and probably do not need to downbin.
The ZcorrectorGui uses a thresholded total counts image to find the 'true' z=0 line. Noise in the data can cause artifacts in the corrected data.

To adjust the threshold used in this calculation press the 'Adjust Total Counts Threshold Value' button.
This brings up this view. There are two sliders.
This slider allows you to change the Threshold value. It is currently set at 9 so anything below 9 counts is set to black and everything above 9 is set to white.

When you load your data the threshold value is set to the mean of the intensities for the entire data stack. This is typically a good starting point.
Now we have changed the threshold value to 11. As you can see the noise in the image (individual white pixels) has been removed.

It is recommended to set the threshold value at the lowest value that gives good contrast to avoid changing the data too much.
After setting a threshold value, it is a good idea to check other slices to see if that threshold is good across the whole data stack, since the same threshold is used for all data slices.
Down binning the data cannot be undone. You must reload the original data in order to restore the original matrix.

Adjust Total Counts Threshold Value

☐ Work with Uncorrected Data

Peak List

Total Counts

<table>
<thead>
<tr>
<th>Total Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>58,10555</td>
</tr>
<tr>
<td>70,13471</td>
</tr>
<tr>
<td>86,19242</td>
</tr>
<tr>
<td>104,2413</td>
</tr>
<tr>
<td>125,1904</td>
</tr>
<tr>
<td>166,2641</td>
</tr>
<tr>
<td>184,3039</td>
</tr>
<tr>
<td>224,3978</td>
</tr>
</tbody>
</table>

Threshold value: 11

Slice #: 131

If you want to save the movie, frames check this box before creating the movie.

Create XY Corr Slice Movie
Create XZ Corr Slice Movie
Create YZ Corr Slice Movie

Once you have set the threshold, press the 'Close' button. The threshold is automatically saved whenever the Threshold slider is adjusted.
Before viewing any corrected data, we need to initialize the corrected data matrix. This calculates the corrected data for all peak images across all slices. It takes a bit of time, but makes further processing faster since the software doesn't have to recalculate the correction later.

To start the process, press the 'Initialize Corrected Data' button.
A progress bar will be shown as each peak image is corrected. Depending on the speed of your computer, this could take a while. So sit back and relax.
Once all of the data has been corrected we can select a peak and look at the corrected data.

Click on a peak from the list...
The data being displayed is shown here.
Down binning the data cannot be undone. You must reload the original data in order to restore the original matrix.

This slider allows you to browse the corrected X-Y data slices.
Adjust the sliders for X-Z and Y-Z to see the corrected data and the calculated data used for the correction.
The green line shows the location along the X-Z axis of the slice shown in the X-Z plots.

The red line shows the location along the Y-Z axis of the slice shown in the Y-Z plots.
You must click on the sliders to update the displays to show the newly chosen data.
These plots show the original data along the X-Z and Y-Z axes.
These plots show the thresholded total counts image for the X-Z and Y-Z axes.
Down binning the data cannot be undone. You must reload the original data in order to restore the original matrix.

Data Being Displayed
Multiple

Layer number: 29

Peak List

<table>
<thead>
<tr>
<th>Total Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>58.10555</td>
</tr>
<tr>
<td>70.13471</td>
</tr>
<tr>
<td>86.19242</td>
</tr>
<tr>
<td>104.2413</td>
</tr>
<tr>
<td>125.1904</td>
</tr>
<tr>
<td>166.2641</td>
</tr>
<tr>
<td>104.3038</td>
</tr>
<tr>
<td>224.3978</td>
</tr>
</tbody>
</table>

If you want to save the movie, check this box before creating the movie.

These plots show the corrected data for the chosen X-Z or Y-Z slice.
Down binning the data cannot be undone. You must reload the original data in order to restore the original matrix.

Adjust Total Counts Threshold Value

Work with Uncorrected Data

Overlay Tools

3D Tools

Initialize Corrected Data

Layer number: 27

Layer number: 29

Peak List

Total Counts
58.10955
70.13471
86.19242
104.2413
125.1904
166.2841
104.3038
224.3978

The corrected X-Z or Y-Z images can be saved by pressing the 'Save Corr XZ' or 'Save Corr YZ' buttons.
A copy of the image is saved.
A movie viewing sequential slices through X-Y, X-Z or Y-Z can be made by using these buttons.

NOTE:
Matlab's movie making function can be problematic. Once you start making a movie, you must let it finish and you must save the file. If you do not Matlab will probably give an error and you will not be able to create another movie until you restart Matlab.

I am exploring how to avoid this, but haven't figured it out yet.
An avi movie file is automatically generated. If you also want to save the individual images for each slice, check this box.

NOTE: You do not have to save the individual frames. You only need to do this if you want to import the frame images into another program.
To create the movie, press the corresponding button for the desired view.
Here we choose 'Create XY Corr Slice Movie'.

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If you want to save the movie, check this box before creating the movie.
And will show the movie being created.
When it is done, choose where you want to save the file, give it a name and press the 'Save' button.
It may take a while to save the movie because they are created as uncompressed avi files. Once it is done saving you can close the popup window.
That's it for the main page of the ZcorrectorGui.

Continue on to another tutorial to learn how to use the Overlay Tools or 3D Tools.

Press the green button on the left to go back to the previous step. Press the button on the right to go back to the beginning of the tutorial.