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

- **Next button**: Takes you to the next frame or stop point.
- **Previous button**: Takes you to the previous frame or stop point.
- **Go to frame button**: Takes you to a specified frame.
- **Go to URL button**: Takes you to a website link.

Press the 'Next' button below to start this tutorial.
This tutorial will introduce the functions on the Overlay Tools panel of the ZcorrectorGui.

Please see the tutorial about the Main page of the ZcorrectorGui before reading through this tutorial.

The Overlay Tools panel allows you to create RGB overlays of the data in a 3d depth profile. You can visualize the data in X-Y, X-Z, Y-Z and in 3D.

You can export snapshots of any view and movies of both 2D slices and 3D data.
Though it is also shown in the tutorial about the main page of the ZcorrectorGUI, we will show how to import data and set it up in this tutorial as well.
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.
To load some data press the 'import' button.
Once you have selected the folder where the files are located, press the 'OK' button.
Notice that the 'Overlay Tools' button is not active even after loading the data. That is because you must either choose to work with uncorrected data, or initialize the corrected data first.

If you want to save the movie frames check this box before creating the movie.
If you want to work with uncorrected data you can check this box.

Using uncorrected data is advisable when working with data sets collected from samples that have minimal topography and therefore would need minimal if any correction.
In this case we want to work with corrected data since we are using a cell depth profile. First we will adjust the total counts threshold value as explained in the tutorial about the main ZcorrectorGui window.
For details on how this works, see the tutorial about the main ZcorrectorGui window.

Here we have chosen a threshold value and now can press 'Close' to continue.
Since we want to work with corrected data, we must first initialize the corrected data matrix. This corrects the data for each peak for all data in the 3D stack.

Press the 'Initialize Corrected Data' button to start the process. Depending on the speed of your computer this may take awhile.
Once the data correction has been calculated these buttons become active.

Press the 'Overlay Tools' button to open the 'Overlay Tools' panel.

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

Layer number: 1

Thresholded total counts XZ
Z line XZ
Z corrected image XZ

Layer number: 1

Thresholded total counts YZ
Z line YZ
Z corrected image YZ

Layer number: 1

Import Data From Directory
Import

Loaded Data
Number of image layers: 55

Down binning the data cannot be undone. You must reload the original data in order to restore the original matrix.

Downbin Data

Adjust Total Counts Threshold Value
This is the Overlay Tools Panel.
These selection boxes allow you to choose which peaks you want included in each of the data channels (Red, Green and Blue).

You can select multiple peaks from each list. You must select something from each list. If you do not want any peaks displayed in a given channel, select 'None' from the list.
We will select 2 peaks in the Red channel. These peaks are from the lipids in the cell.
We will select 1 peak in the Green channel. This peak is from protein in the cell.
We will not select any peaks in the Blue channel, so we choose 'None'.

Threshold for overlay = 0

Create Composite Image
If desired you can choose a threshold value. This can be used to filter out noise in the images. Any pixels with intensity below the threshold are set to zero.

Typically the default value of 0 works well. This means the data is not modified.
Press the 'Create Composite Image' button to create the RGB overlay.
A progress bar appears and shows the time remaining. The process can take some time as it has to calculate the RGB overlay for all peaks selected and all slices in the data set.
###瘤细胞的检测

####瘤细胞的检测

- **Red Peak(s):**
  - None
  - Total Counts: 56.2
  - Non-Overlaid Counts: 0

- **Green Peak(s):**
  - None
  - Total Counts: 56.2
  - Non-Overlaid Counts: 0

- **Blue Peak(s):**
  - None
  - Total Counts: 56.2
  - Non-Overlaid Counts: 0

####参数设置

- **Alpha value for 3D overlay:** 1
- **View in 3D:**
- **Choose Background Color:**
- **Current background color:**
- **Number of degrees for rotation:** 360
- **Check which axes you want to rotate around:**
  - X
  - Y
  - Z

####图像操作

- **Create Composite Image:**
- **Create 3D Snap Shot:**
- **Create 3D Movie:**

####图示说明

- **After the RGB data is calculated, new windows appear including the XY, XZ, and YZ displays and 3D plotting options.**

####坐标轴

- **Layer number:** 1
- **Row #:** 1
- **Column #:** 1
Moving the sliders will update the view. Here we adjust the slider for the XY slice RGB plot.
To save a snapshot of any of the three 2D views press the appropriate button. The file is automatically saved to the active Matlab directory.

Here we will save the current XY slice.
And contains the respective image.
You can also save a movie showing sequential slices in XY, XZ or YZ by pressing the appropriate button.

Here we will make an XY slice movie by pressing the 'Create XY Movie' button.
A window will pop up and play the movie as it is created.
Figure 4
Select File to Write

After it is done a dialog will open to allow you to save the files. Choose a location, give the file a name and press the 'Save' button. Saving can take awhile because the movies are saved as uncompressed .avi files.
You can close the popup window AFTER the file is saved.
This area contains the controls for creating a 3D RGB overlay plot.

**Red/Blue Channel**
- None
- Total
- 58.105 70.134 86.192
- 104.241 125.194 166.264
- 184.303 184.303 184.303

**Green/Red Channel**
- None
- Total
- 58.105 70.134 86.192
- 104.241 125.194 166.264
- 184.303 184.303 184.303

Threshold for overlay = 0

**Alpha value for 3D overlay**
- 1

**View in 3D**

**Choose Background Color**
- Current background color

**Number of degrees for rotation**
- 360

**Check which axes you want to rotate around**
- X
- Y
- Z

**Create 3D Snap Shot**

**Create XY Movie**

**Create XY Snap shot**

**Create XZ Movie**

**Create XZ Snap shot**

**Create YZ Movie**

**Create YZ Snap Shot**

**Close Panel**
Press the "View in 3D" button to create a 3D plot.
The 3D volume will be rendered. This can take a bit of time.
You can rotate the image by clicking and dragging on the plot in any direction.
You can change the background color by pressing the 'Choose Background Color' button.

Threshold for overlay = 0

Create Composite Image

Layer number: 26

Create XY Movie
Create XY Snap shot
Create XZ Movie
Create XZ Snap shot
Create YZ Movie
Create YZ Snap shot

Close Panel
Choose the color you want from the popup window...
And then press the 'OK' button.
You must press the 'View in 3D' button again to update the plot.

Alpha value for 3D overlay = 1

Check which axes you want to rotate around

Number of degrees for rotation = 360

Create 3D Snap Shot

Create 3D Movie

Create XY Movie

Create XY Snap shot

Create XZ Movie

Create XZ Snap shot

Create YZ Movie

Create YZ Snap Shot

Create Composite Image
You can add transparency to the image by changing the transparency value here. Lower values make the image more transparent. Here we will lower it to 0.1.
Press the 'View in 3D' button to update the image.

Threshold for overlay = 0

Layer number: 26

Create Composite Image

Number of degrees for rotation: 360

Create 3D Snap Shot

Create XY Movie
Create XY Snap Shot
Create XZ Movie
Create XZ Snap Shot
Create YZ Movie
Create YZ Snap Shot

Close Panel
The data is displayed with transparency.
It is a bit hard to see the transparency against a black background. So let's change it to white.
And update the plot by pressing 'View in 3D'.
That is a bit easier to see.
Click and drag to rotate the image and the transparency becomes more apparent.
The z scale in the Matlab 3D plots is somewhat arbitrary. The height is determined by the number of slices in the profile and by the aspect ratio defined when the plot is made.
The zscale can be adjusted using this slider. Here we change the z scale factor to 3.

You can see that the cell is flattened out. This is more 'realistic' based on the real cell shape, but this is purely for viewing purposes since the Z axis scale is arbitrary.
With these controls you can create a 3D movie rotating the image in any combination of axes you want.

Check the box for the axes you want to rotate the image around.

Z scale factor: 3

Number of degrees for rotation: 360

Check which axes you want to rotate around:
- X
- Y
- Z

Create Composite Image
Create XZ Movie
Create XZ Snap Shot
Create YZ Movie
Create YZ Snap Shot
Create XY Movie
Create XY Snap Shot

Layer number: 26

Row #: 56
Here we will use the X and Y axes.

Check which axes you want to rotate around:
- X
- Y
- Z

Number of degrees for rotation: 360°

Threshold for overlay = 0

Z scale factor = 3

Create Composite Image

Create XY Movie
Create XY Snapshot
Create XZ Movie
Create XZ Snapshot
Create YZ Movie
Create YZ Snapshot

Close Panel
You can enter the number of degrees you want the volume rotated during the movie. The default is 360.
To preview what the movie will look like, press the Preview button.
The preview will happen in the 3D viewing area.
The preview will happen in the 3D viewing area.
Once you are happy with the way it looks, press the 'Create 3D Movie' button.
The movie will be rendered in a pop up window.

Do not close the window until the movie is done and you have saved it.
When the movie is done rendering a save dialog will appear. Choose where to save the file and press the "Save" button.
You can then close the popup window.
That's it for the Overlay Tools tutorial.

Continue on to the third tutorial to learn how to use 3D Tools.

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