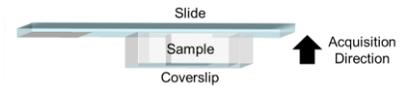# Quick guide to z-stacks (with correction) in Zen by Emily Wyatt, Grad Student CCE

1) Focus to approximate starting position:

In the **Left Tool Area** click on the **Locate** tab. Click on **Brightfield**.

Focus to starting position using the eyepiece (adjacent to coverslip).

Click on **Brightfield** again to turn off.

2) Activate z-stack acquisition:

In the **Left Tool Area** click on the **Acquisition** tab.

Select **Z-Stack** just below the primary imaging buttons to activate.

Z-stack controls will now be available in the **Multidimensional Acquisition** panel.

Make sure **Show All** is activated.

3) Select a mode of operation:

There are two modes: **First/Last** and **Center**. The first option allows the user to set the start and stop Z-positions for the volume being recorded. The second sets the current Z-position as the center position for the stack and allows the user to set a number of slices above and below this center point. The **First/Last** option is described here.

Click on **First/Last**.
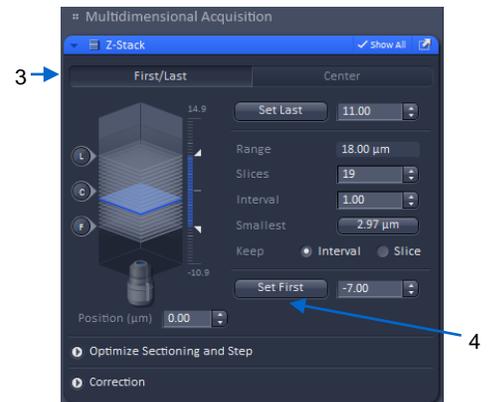
4) Define the range of the z-stack:

Highlight channel that best characterizes your range (e.g. nuclear stain). With the camera running live adjust the fine focus using the **TFT Remote** to the starting position.

Note: The current position is displayed in **Position (um)** field.

Click **Set First** to set as the first position in the stack.

Adjust to anticipated last position in z-stack (thumb forwards moves deeper in sample). With the camera running live, adjust the fine focus using the **TFT Remote** to the final position.

Caution: Check working distance of objective – do not plow into coverslip!

Click **Set Last**. The new z-stack **Range** will appear.

5) Select step size:

Click on **Optimize Sectioning and Step**. Several options are available.

For good Z resolution, click **Optimal**. Optimal step size is calculated by software based on focal depth of your objective (property determined by the magnification and NA of the objective). However, in many cases you may want to choose a larger step size.

The number of slices in z-stack will be automatically calculated to match your selection.

6) Apply correction (optional):

Click on **Correction**. Several corrections are available.

Enter RI of mounting medium in the **Refractive Index Correction** field.

Select **Use Correction** under **Auto Z Brightness Correction**. **Enable Test** function is now available. When possible, solely apply laser power correction to retain image quality, but **Master Gain** may be used for further correction if needed.

Click the **F** button next to the z-stack graphic in the main z-stack window. This will move to the first position and **Snap**.

Adjust laser power settings for channels individually under **Channels**. Make sure all channels are activated, then click **Add** in the correction wizard.

Click the **L** button. This will move to the last position and **Snap**.

Again, adjust laser power settings, then click **Add**.

Confirm that **Spline Interpolation** is selected. This will linearly interpolate between added corrections.

Note: Multiple additional corrections may be added, but it is best practice to set two corrections (at start and end) as to not introduce bias.
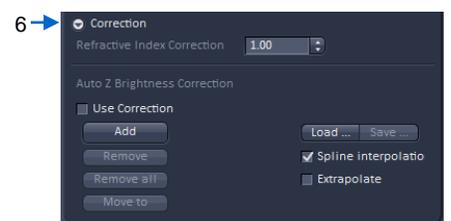
7) Test Correction (optional):

Click **Enable Test**. This function allows you to **Snap** at any position in z-stack to test correction. Test correction by either (1) clicking on the **F**, **C**, or **L** buttons to test at first, center, or last positions, or (2) manually adjusting the fine focus position to move to any position in the stack, then clicking **Snap**.

Leave **Enable Test** selected to be able to watch the laser power change as the stack is collected.

8) Collect z-stack:

Begin acquisition by clicking **Start Experiment** with channels you have activated under **Channels**. Progress in position of z-stack can be tracked with the graphic in the z-stack window. The blue slice shows the active plane of acquisition relative to the whole z-stack. Save the data!