Z-Stack Application

Zeiss 880
Zen 2.3
Acquisition Tab

• Select the **Acquisition** tab and then select **Z-Stack**.

• Scroll down the panel to locate the **Z-Stack** window.
Z-Stack via First/Last

There are a few different ways to acquire a Z-Stack, this explanation will involve acquiring a stack from a defined First and Last position.

Select the First/Last tab at the top of the window.
Be sure that throughout your volume, your gain and threshold settings are optimal and not saturating your image, gain too high, or masking information, threshold too low. Undesirable settings will greatly distort your 3-D reconstructed image and is not a scientifically sound representation of your data.
First and Last Positions

You may want to acquire the whole volume of your sample or you may want a certain region within your volume; the choice depends on your sample size and what you wish to view.

It does not matter where you mark the first and last positions, however please note that the system will start closest to the coverslip and finish the volume farthest from the coverslip.

When you reach the **First** position, press the **Set First** button to accept the position. Focus through your image. When you reach the **Last** position, press the **Set Last** button to accept the position.
Interval and Optimal

The selections you make for the options, Slices, Interval, Optimal and Keep will depend on your sample thickness, however, the following explanation describes the acquisition of a Z-Stack based on Optimal settings. This is the most accurate means for acquiring a Z-Stack and translating your data from an analog to digital representation.

Select Keep Interval, and press the Optimal button. The Slices and Interval fields will automatically update based on these selections.
The optimal step size is automatically calculated as 1/2 of the z resolution, according to the Nyquist Theorem.
Nyquist Theorem

The Nyquist Theorem states that in order to accurately reproduce a signal, it should be sampled at two times the size of the smallest structure. Therefore, the step size should be 1/2 the size of the z resolution.

Nyquist Sampling Rate

Under Sampled

Missed Features
Multi-Channel Z-Stack

The Optimal Step, 1, is calculated the same as with the single channel configuration, however, note that the z resolution must be the same between the multiple channels, 2 and 3. This is adjusted by the Pinhole slider and should have been adjusted as one of the initial scanning parameters, prior to the Z-Stack acquisition.
Once your parameters have been established, press the **Start Experiment** button to initiate the Z-Stack acquisition.

**SAVE YOUR FILE**
Z-Stacks generate large files, therefore be sure to save your image file first and then proceed to view it in order to avoid data loss.
Viewing your Z-Stack

There are multiple ways to view your Z-Stack images: 2-D, 3-D, Cut View, and Ortho View. You can find detailed descriptions for each of these views in their respective sections, which is not included in this explanation.