

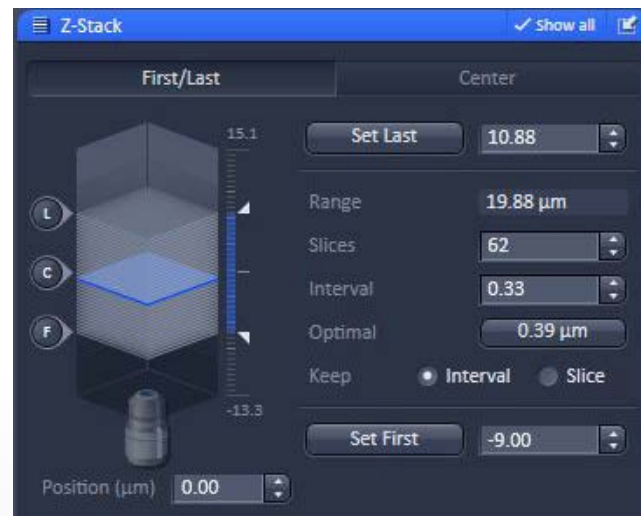
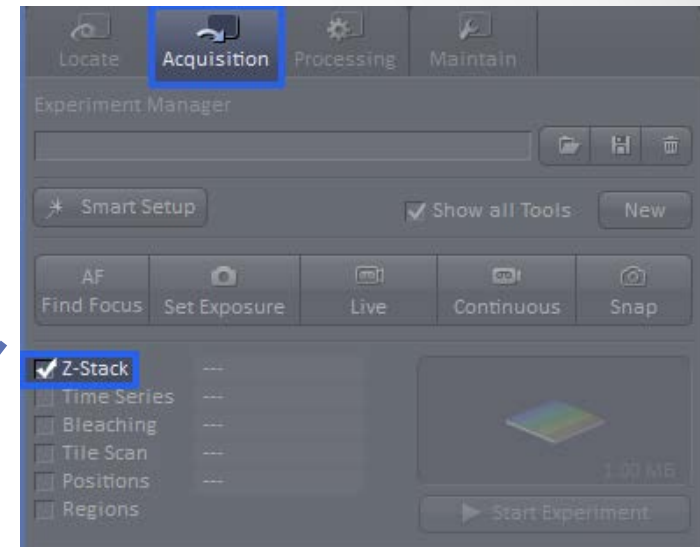
# Z-Stack Application

Zeiss 880

Zen 2.3

# Acquisition Tab

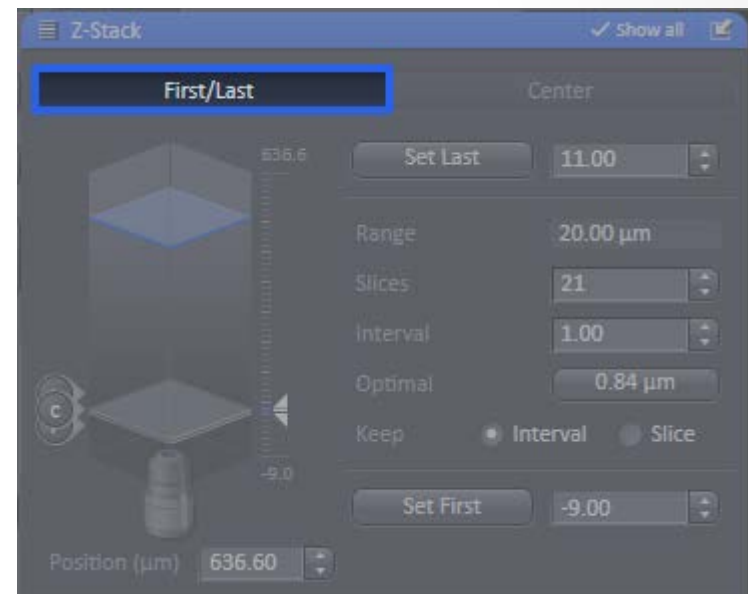
- Select the **Acquisition** tab and then select **Z-Stack**.
- Scroll down the panel to locate the **Z-Stack** window.



# 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.

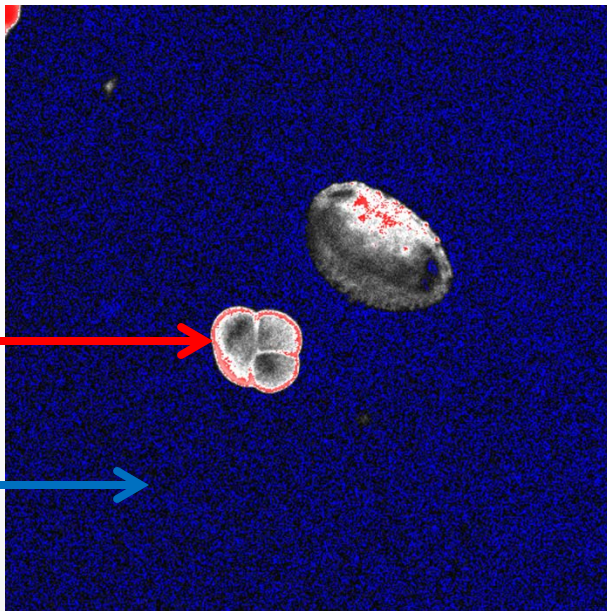
Select the **First/Last** tab at the top of the window.



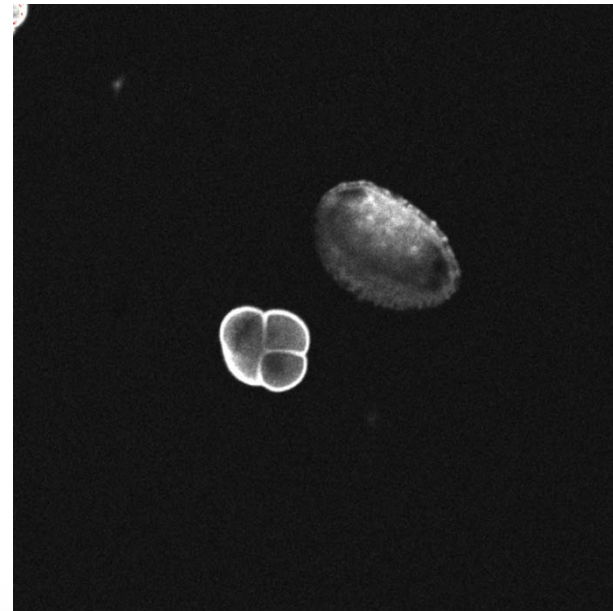
# Note Your Settings

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.

Undesirable Settings



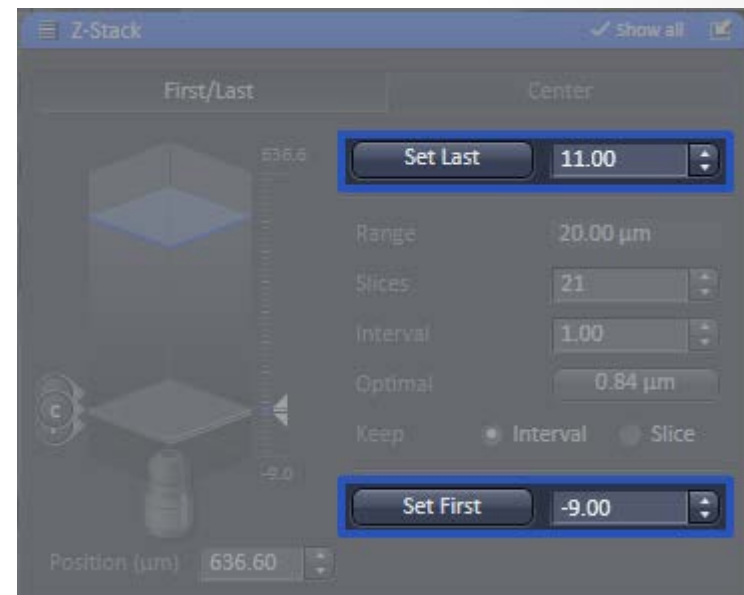
Optimal Settings



# 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.

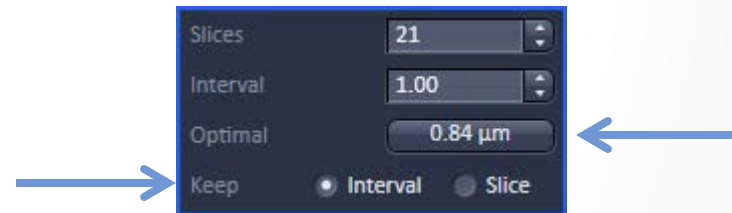


When you reach the **First** position, press the **Set First** button to accept the position. Focus through your image. When you reach the **Last** position, press the **Set Last** button to accept the position.

# Interval and Optimal

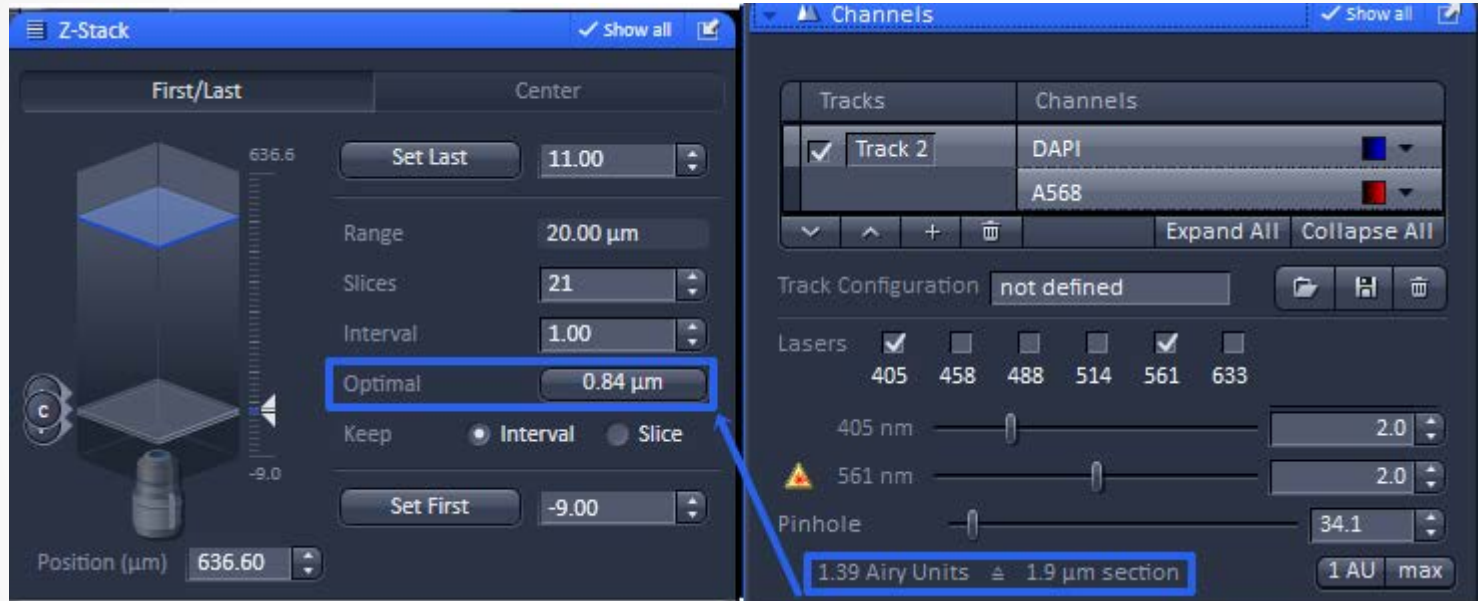
The selections you make for the options, **Slices**, **Interval**, **Optimal** and **Keep** will depend on your sample thickness, however, the following explanation describes the acquisition of a Z-Stack based on Optimal settings. This is the most accurate means for acquiring a Z-Stack and translating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





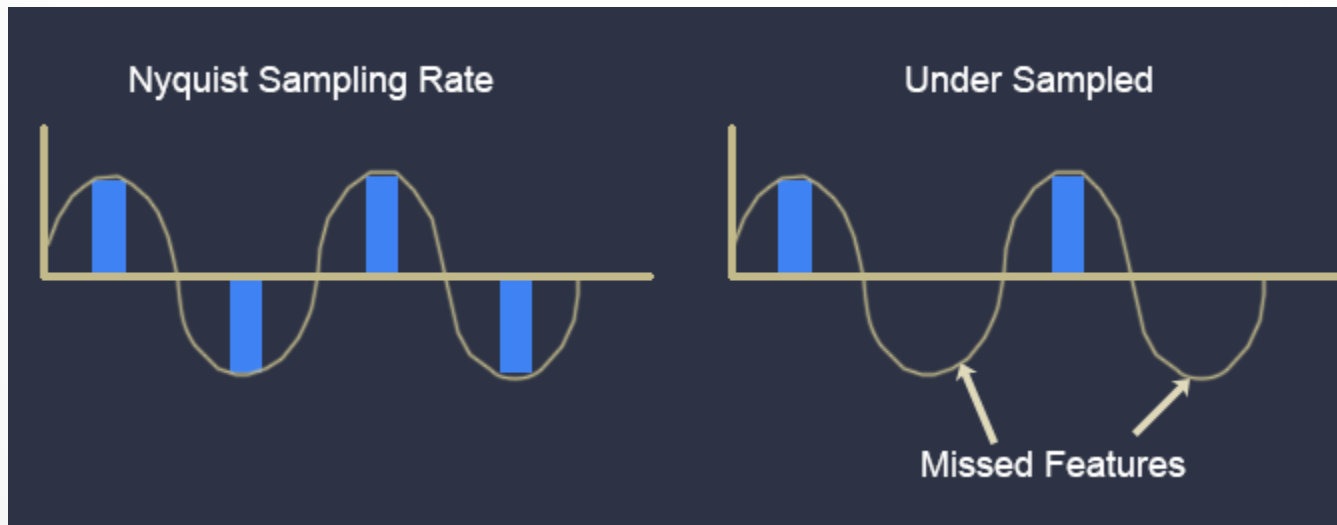
# Single Channel Z-Stack



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 smallest structure. Therefore, the step size should be  $1/2$  the size of the  $z$  resolution.





# 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.

The image displays two panels from a microscope software interface. The left panel is the 'Z-Stack' window, and the right panel is the 'Channels' window. Both panels have blue circular callouts with numbers 1, 2, and 3 pointing to specific settings.

**Z-Stack Panel:**

- 1:** Points to the 'Optimal' step size, which is set to 0.84  $\mu\text{m}$ .
- 2:** Points to the 'Pinhole' setting, which is set to 1.39 Airy Units (1.9  $\mu\text{m}$  section).
- 3:** Points to the 'Pinhole' setting in the Track 4 - LSM section, which is set to 1.23 Airy Units (0.8  $\mu\text{m}$  section).

**Channels Panel:**

Tracks	Channels
<input checked="" type="checkbox"/> Track 1	A647
<input checked="" type="checkbox"/> Track 2	A568
<input checked="" type="checkbox"/> Track 3	A488
<input checked="" type="checkbox"/> Track 4	DAPI

**Track 2 - LSM:**

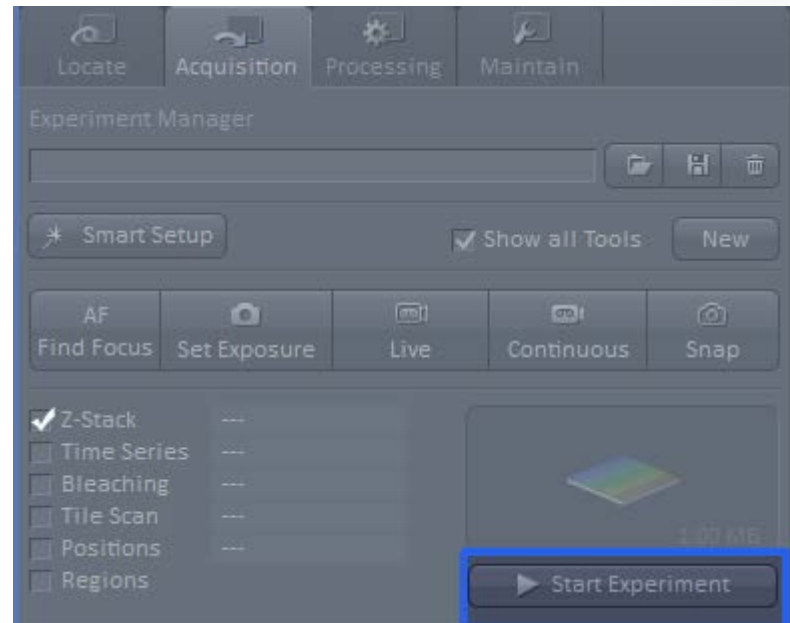
- Lasers: 405, 458, 488, 514, 561, 633
- Pinhole: 1.39 Airy Units (1.9  $\mu\text{m}$  section)
- A568 Gain (Master): 188
- Digital Offset: 0
- Digital Gain: 1.0

**Track 4 - LSM:**

- Lasers: 405, 458, 488, 514, 561, 633
- Pinhole: 1.23 Airy Units (0.8  $\mu\text{m}$  section)
- DAPI Gain (Master): 188
- Digital Offset: 0
- Digital Gain: 1.0

# Start Experiment

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.

