Acquiring a Z-Stack Using the ZEISS LSM 510 META

Method 1 – Mark First/Last

1. **Scan Control** - choose from the acquire submenu.

2. Click on **Z Stack** to open the Z Settings window.

3. Select **Mark First/Last**

4. Click **Fast XY** from the right side of the Scan Control Window and adjust the focus to find your approximate center slice and perform color adjustment. Continue focusing to where you want the first slice of the z-stack – click **Mark First**. Then, continue to adjust the focus to where you want the last slice of the z-stack – click **Mark Last**.

6. Click on **Z Slice** and **Optimal Interval (Optional).**

7. If you are using an oil objective with embedding media of 1.33 refractive index, type .875 for **Reff. Corr.**

8. Return to the Mode screen to make sure the scan settings are set for the quality of image that you need.

9. Click **Start** to begin acquiring the z-stack.
Method 2 – Z Sectioning

1. **Scan Control** - choose from the acquire submenu.

2. Click on Z Stack to open the Z Settings window.

3. Select **Z Sectioning**

4. Click on Z Slice and **Optimal Interval (Optional)**.

5. Enter enough Num Slices to exceed the width of your specimen.

6. **Fast XY** - focus to find your approximate center slice and perform color adjustment - crop as desired.

7. **Line Sel** - the middle XZ cut line should be placed through any area of interest that is fluorescent – preferably close to the center of the screen. If this line needs to be moved, **first** click on the arrow button, then click and hold on the line to move it to another position.

8. **Range** - click and hold to move the red lines where you want your top and bottom slice. The green line represents your current plane of focus and is usually not moved.
9. If you are using an oil objective with embedding media of 1.33 refractive index, type \(0.875\) for Reff. Corr.

10. Return to the Mode screen to make sure the scan settings are set for the quality of image that you need.

11. Click Start to begin acquiring the z-stack.