Nikon SDC 100x z-stack PSF and SNR Protocol (Slide 2)

1) Select the 488nm Laser on the LUNV laser box. Deselect all other illuminated laser indicators.

2) Select the **100x 1.45 NA** objective (<u>Position 5</u>). Put immersion media on the lens.

3) From the **OC** panel, select the **GFP** Optical Configuration, allowing you to image Green fluorescent microspheres: 488nm ex, 514nm em.

4) Within the **Camera Settings** panel, select the following imaging conditions:

   a. No Bin (Live and Capture)
   b. 300ms Exposure Time
   c. Readout Mode = EM Gain 10MHz
   d. EM Gain = 300
   e. Conversion Gain = 2
   f. Frame Average = 4x

5) For the 488nm laser, set the illumination power to **20%**.

6) Start a live scan and find a viable imaging region. An ideal region will have many beads in the field of view, but separate enough to generate distinct beads. Bring the beads into focus.

7) Select the **Pixel Saturation Indication** icon and check for saturated pixels.

8) Adjust your **laser power** and your **Exposure Time** to avoid saturation while providing a 10:1 signal to noise ration (use a Line Profile). Check your settings by scrolling through multiple z planes.

9) Set up the acquisition of Z stacks within the **ND Acquisition** window. Lower your objective to a focal plane just below the initial layer of microspheres. Choose the **Asymmetric** option within the **Z stack** tab within the **ND Acquisition** Window. Set the current focal plane to home by selecting the **Home** icon. Set **Below** as 0 and **Above** as +80 µm. Set the step size to **0.2 µm**. There should be 401 z steps.

10) Set the **Z Device** to Nidaq Piezo Z.

11) Within the **ND Acquisition** window, select **Save to File** and set the Path and Filename.

12) Press the **Run now** button to perform the acquisition.