

Nikon SDC 100x Step Size PSF and SNR Protocol (Slide 1)

- 1) Select the 488nm Laser on the LUNV laser box. Deselect all other illuminated laser indicators.
- 2) Select the **100x 1.45 NA** objective (Position 5). 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 (For Live and Capture)
 - b. Exposure Time = 300ms
 - c. Readout Mode: EM Gain 10MHz
 - d. EM Gain = 300
 - e. Conversion Gain = 2
 - f. Frame Average = 4x
- 5) Set the 488nm laser power to **20%** within the LUNV panel.
- 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) Within the **ND Acquisition** window, select **Save to File** and set the Path and Filename.
- 10) Press the **Run now** button to perform the acquisition.
- 11) Repeat steps 9 – 11 using the following **Bin sizes**: 2, 4, 8. Please note, you may need to increase your Illumination Power and/or Exposure Time settings to see the 100nm beads at larger bin sizes.