

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

Part 1

- 1) Start Nikon Elements by selecting the **Single Andor** startup icon.
- 2) Select the **100x 1.49 NA** objective (Position 5). Put immersion media on the lens.
- 3) From the **OC** panel, select the **GFP** Optical Configuration within the **Widefield Andor** subsection, allowing you to image Green fluorescent microspheres: 488nm ex, 514nm em.
- 4) Within the **Camera Control** 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 illumination power to **20%** using the LED control wheel.
- 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 **illumination 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, fill in the autosave fields (folder location and file name).
- 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.

Part 2

- 1) Close Nikon Elements.
- 2) Start Nikon Elements by selecting the **Single Hamamatsu** startup icon.
- 3) Select the **100x 1.49 NA** objective (Position 5). Put immersion media on the lens.
- 4) From the **OC** panel, select the **GFP** Optical Configuration within the **Widefield Hamamatsu** subsection, allowing you to image Green fluorescent microspheres: 488nm ex, 514nm em.
- 5) Within the **Camera Control** panel, select the following imaging conditions:
 - a. No Bin (For Live and Capture)
 - b. Exposure Time = 300ms
 - c. Scan Mode = Slow
- 6) Set the illumination power to **20%** using the LED control wheel.
- 7) 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.
- 8) Select the **Pixel Saturation Indication** icon and check for saturated pixels.
- 9) Adjust your **illumination 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.

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

- 10) Within the **ND Acquisition** window, fill in the autosave fields (folder location and file name).
- 11) Press the **Run now** button to perform the acquisition.
- 12) 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.