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.
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.