

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

### 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 Settings** panel, select the following imaging conditions:
  - a. No Bin (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 **LED 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  $\mu\text{m}$ . Set the step size to **0.2  $\mu\text{m}$** . There should be 401 z steps.
- 10) Set the **Z Device** to MCL NanoDrive PiezoZ Drive.
- 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.

### 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 Settings** panel, select the following imaging conditions:
  - a. No Bin (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) Set the camera ROI to **1024 x 1024** pixels by clicking on the down arrow immediately to the right of the **Camera ROI** icon located at the top of the active window.

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- 11) 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 under 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  $\mu\text{m}$ . Set the step size to **0.2  $\mu\text{m}$** . There should be 401 z steps.
- 12) Set the **Z Device** to MCL NanoDrive PiezoZ Drive.
- 13) Within the **ND Acquisition** window, select **Save to File** and set the Path and Filename.
- 14) Press the **Run now** button to perform the acquisition.