

Nikon A1R 100x z-stack PSF and SNR Protocol (Slide 2)

- 1) Select the **100x 1.45 NA** objective (Position 5). Put immersion media on the lens.
- 2) From the **OC** panel, select the **4Ch + DIC** Optical Configuration, allowing you to image Green fluorescent microspheres: 488nm ex, 514nm em.
- 3) Deselect (uncheck) the 405, 561, 640nm lasers, and the TD within the **A1plus Compact GUI**.
- 4) Within the **A1plus Compact GUI** panel, select the following imaging conditions:
 - a. Galvano
 - b. Unidirectional scan
 - c. Pixel Dwell = 2.2 μ s
 - d. Size = 512
 - e. Pinhole = 1.2 AU (calculated for 488nm)
 - f. Line Average = 4x
- 5) For the 488nm laser, set the illumination power to **1%**, and the **HV gain (HV(G))** for the PMT to **30** units. Finally, set the **Offset** to **0**.
- 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 **HV(G)** to avoid saturation while generating a peak pixel intensity value of approximately **3500 counts**. Check your settings by scrolling through multiple z planes.
- 9) In the **A1plus Scan Area** tab, select a square scan area (first **icon** on the top left, the “frame scan” mode). Choose a **Pixel size** of **0.07 μ m** per pixel.
- 10) 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 μ m. Set the step size to **0.2 μ m**. There should be 401 z steps.
- 11) Set the **Z Device** to Step By Step Nikon Piezo Z.
- 12) Within the **ND Acquisition** window, select **Save to File** and set the Path and Filename.
- 13) Press the **Run now** button to perform the acquisition.