Nikon A1R 100x Step Size PSF and SNR Protocol (Slide 1)

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 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 µs
    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 **0.05 µm** per pixel.
10) Within the **ND Acquisition** window, select **Save to File** and set the Path and Filename.
11) Press the **Run now** button to perform the acquisition.
12) Repeat steps 9 – 11 using the following **Pixel sizes**: 0.07, 0.11 (Nyquist), 0.25, 0.50, 1.00. Please note, you may need to increase your laser power or HV(G) settings to see the 100nm beads at larger step sizes.