ABRF-LMRG - 3D Microsphere Standard Sample Study #3: SNR

Imaging Protocol: Nikon A1R – 3D Signal-to-Noise (Slide 2)

This protocol is for the SNR acquisition only

1) Turn on and let your lasers warm up for at least 1 hour.
2) Put a **0.5 NA** or higher objective lens in place. If necessary, put immersion media on the lens.
3) From the **OC** panel, select the appropriate Optical Configuration(s) that will allow you to image:
   a. Green Fluorescent Microspheres: 488nm ex, 505nm em
   b. Red Fluorescent Microspheres: 555nm ex, 575nm em
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
   g. Channel Series
5) For the each 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**.

   Note: If you do not have GaAsP detectors, you may need to increase your **HV(G)** to 90. If you are unsure what detectors you do have, start at 30 and work your way up during a live scan.
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. Set the Pixel Saturation Indication color to one that can be clearly observed on both laser channels (Complimentary Color tends to work well).
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 as the beads vary in intensity.
9) In the **A1plus Scan Area** tab, select a square scan area (first **icon** on the top left, in frame scan mode). Choose a **Pixel size** to **0.2 µm** per pixel (or as close as possible).
10) Set up the acquisition of Z stacks within the **ND Acquisition** window (or press Ctrl+Alt+shift+Z). 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 +100 µm. Set the step size to **1.0 µm**. There should be 101 z steps.
11) Press the **Run Now** button to perform the acquisition.
12) Please follow this link to submit a sample information form for each dataset you submit: https://tinyurl.com/LMRG-vial1
13) Save the files as **.nd2** and also as **.tif**. Name the files as follows:

    "Lastname_Firstname_Microscope_Platform_MagnificationX_NAY_ImmersionMedia".
    For example: **Brown_Claire_NikonA1R_100x_NA1.45_Oil**