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

Imaging Protocol: Nikon A1R – 3D PSF (Slide 1)

This protocol is for the PSF acquisition only

1) Turn on and let your lasers warm up for at least 1 hour.
2) Put a **1.3 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 that will allow you to image Green fluorescent microspheres: 488nm ex, 514nm 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
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**.

   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.
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. The large beads will be much more intense than the small beads.
9) In the **A1plus Scan Area** tab, select a square scan area (first **icon** on the top left, a frame scan mode). Choose a **Pixel size** of **0.07 µm** per pixel.
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 **0.2 µm**. There should be 501 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 **8-bit .tif**. The image bit depth can be selected under File – Save As – .tif. At the bottom of the Save As window, select **More Options**. Choose “Scale 12 bit to 8 bit” from under the **Bit Depth** dropdown menu. Name the files as follows:

    “Lastname_Firstname_Microscope_Platform_MagnificationX_NAY_ImmersionMedia”
    For example: **Brown_Claire_NikonA1R_100x_NA1.45_Oil**