

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

## Imaging Protocol: Nikon C2 – 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 **Dye and Spectral Settings** 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:
  - b. Unidirectional scan
  - c. Pixel Dwell = 1.9  $\mu$ 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** for the PMT to **90** 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** 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 **C2plus 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  $\mu$ 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 +100  $\mu$ m. Set the step size to **0.2  $\mu$ m**. 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**