Light Microscopy Research Group – Study #3

Sample Preparation Protocol

Test Kit Includes:

1. **Tube 1 (5 µl) – PSF Analysis**
   0.1 µm FluoroSpheres microspheres 505/515 (ThermoFisher, Cat# F-8803)
   1.0 µm FluoroSpheres microspheres 540/560 (ThermoFisher, Cat# F-8820)
2. **Tube 2 (5 µl) – Intensity/SNR Analysis (3% and 30% intensity)**
   2.5 µm InSpeck microspheres 505/515 (ThermoFisher, Cat# I-7219)
   2.5 µm InSpeck microspheres 580/605 (ThermoFisher, Cat# I-7224)
3. 500 µl of CyGel (BioStatus, Cat# Cy10500)
4. Three (3) microscope slides (Fisherbrand, Cat# 12-522-3)
5. Three (3) double-sided adhesive spacers, 9 x 0.12 mm well
   (Electron Microscopy Sciences, Cat# 70327-8S)
6. Four (4) coverslips, 18 x 18 mm (Fisherbrand, Cat# 12541A)

Required Materials (Not Included):

1. Automatic Pipettes (2-20 µl, 20-200 µl)
2. Sonicator
3. Ice bath
4. Vortex
5. Fine forceps
6. Clear nail polish

Sample Storage:

- Store the whole sample kit between 2–8°C in a refrigerator,
- Do **NOT** freeze!

Helpful Tips & Information:

- If the microspheres in either Tube 1 or Tube 2 are desiccated, reconstitute the microspheres in 5 µl of PBS.
- If there is microsphere mixture in the cap of either tube, centrifuge the tubes.
- The CyGel-microsphere mixture requires a 20:1 dilution to set properly (minimum of 15:1).
- CyGel solidifies at room temperature and is liquid between 2–8°C. Keep the CyGel-microsphere mixture on ice at the bench so it does not solidify. Keep all other supplies (microscope slides, coverslips, pipette tips) at **room temperature**.
- Only two slides and two coverslips are required for the sample preparation. The extra materials are provided in the event of breakage or mistakes.
- For any additional help, **please refer to video posted in the LMRG Google Drive account.**

Directions:

1. Prepare a small ice bath. Keep the CyGel in the ice bath at all times.
2. Place Tube 1 and Tube 2 in an ice sonication bath for 15-20 minutes to break up any microsphere aggregates.
3. After the sonication is complete, remove 100 µl of CyGel and add to Tube 1. Mix gently to avoid bubbles by pipetting the mixture up and down **slowly**, and then return to the ice bath. Repeat for Tube 2.
4. Remove the plastic cover on one of the spacers located on the microscope slide.
5. Vortex Tube 1 for 5 seconds to ensure an even distribution of the microspheres in the CyGel. If bubbles in the tube have not dissipated, remove 7 µl of solution from the bottom of the tube, and pipette into the center well. Do not push the plunger on the pipette to its maximum as a measure to prevent the formation of bubbles.
6. Place one coverslip over the spacer and gently press down to seal it. To avoid desiccation, apply a double coat of clear nail polish around the coverslip.
   Note: A double or triple coat of nail polish is **critical**. If the CyGel dries, it will crack and all microspheres precipitate to the coverslip.
7. Invert the slide so the gel can set in the dark and at room temperature.
   Optional: Place slide in an incubation oven (37°C/98.6°F) until the gel has set.
8. Repeat steps 4-7 for Tube 2.