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Version 2

Tetraspeck Bead Imaging V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for imaging tetraspeck beads on glass coverslips.

Troubleshooting



Slide Preparation

- 1 Glass coverslips (Fisher Scientific, 12373128, #1 thickness 22 mm x 50 mm) were plasma cleaned for 30 min (Ar plasma cleaner, PDC-002, Harrick Plasma). 30m
- 2 Stick a frame-seal slide chamber (9 mm x 9 mm, SLF0201, Biorad) on the cover glass. Use some blunt tweezers to press down the sticker on the glass. 5m
- 3 Add 50 μ l of 0.01 % w/v poly-L-lysine (PLL, P4707, Sigma-Aldrich) to the well and wait for 10-20 min. 20m
- 4 Use a pipet to remove excess PLL. 2m
- 5 Wash with 50 μ l of filtered (0.02 μ m syringe filter, Whatman, 6809-1102) PBS. Pipet up and down in the corners of the well to wash. Repeat this step 3 times. 5m
- 6 Remove excess PBS and add 50 μ l of the diluted TetraSpeck (1:625 dilution, 0.1 μ m diameter TetraSpeck Microspheres, Thermo Fisher) beads to the well. Wait 2-3 minutes to let the beads settle and attach to the PLL-coated glass. 3m
- 7 Remove excess solution using a pipet. 2m
- 8 Wash with 50 μ l of filtered PBS. Pipet up and down in the corners of the well to wash. Repeat this step 3 times. 2m
- 9 Remove excess PBS and add 50 μ l filtered PBS to the well. The sample should not dry out! 1m

Imaging

- 10 Image the slide on a light microscope.