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Tetraspeck Bead Imaging

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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.

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	Slide Preparation
1	Glass coverslips (Fisher Scientific, 12373128, #1 thickness 22 mm x 50 mm) were plasma cleaned for min (Ar plasma cleaner, PDC-002, Harrick Plasma).
2	Stick a frame-seal slide chamber (9 mm x 9 mm, SLF0201, Biorad) on the cover glass. Use some blun tweezers to press down the sticker on the glass.
3	Add 50 μl of 0.01 % w/v poly-L-lysine (PLL, P4707, Sigma-Aldrich) to the well and wait for 10-20 min.
4	Use a pipet to remove excess PLL.
5	Wash with 50 μl of filtered (0.02 μm syringe filter, Whatman, 6809-1102) PBS. Pipet up and down in the corners of the well to wash. Repeat this step 3 times.
6	Remove excess PBS and add 50 µl of the diluted TetraSpeck (1:625 dilution, 0.1 µm diameter TetraSp 3m Microspheres, Thermo Fisher) beads to the well. Wait 2-3 minutes to let the beads settle and attach to the PLL-coated glass.

Remove excess solution using a pipet.

7

2m

- 8 Wash with 50 μl of filtered PBS. Pipet up and down in the corners of the well to wash. Repeat this step 2m times.
- 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.