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.

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Slide Preparation

1 Glass coverslips (Fisher Scientific, 12373128, #1 thickness 22 mm x 50 mm) were plasma cleaned fo 30m
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 blunt 5m
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. 20m

4 Use a pipet to remove excess PLL. 2m

5 Wash with 50 µl of filtered (0.02 µm syringe filter, Whatman, 6809-1102) PBS. Pipet up and down in th 5m
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.

7 Remove excess solution using a pipet. 2m

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

9 Remove excess PBS and add 50 µl filtered PBS to the well. The sample should not dry out! 1m

Imaging

10 Image the slide on a light microscope.