Sub-resolution and focal check beads solution for alignment and PSF measurements

Purpose

This protocol is used to prepare the mixed-beads control in well G9 of a pipeline 96-well plate

Reagents

1. Tetraspeck beads in suspension: 0.1 µm diameter, ~1.8×10^{11} particles/mL in deionized water containing 0.02% Tween®20 and 2 mM sodium azide, unit size of 0.5 mL. (https://www.thermofisher.com/order/catalog/product/T7284)
2. Focal check beads in suspension: 15 µm diameter, ~5×10^{5} beads/mL (0.1% solids) in deionized water containing 0.02% Tween®20 and 2 mM sodium azide, unit size of 0.5 mL. (https://www.thermofisher.com/order/catalog/product/F7239)
3. 96-well plate (cat P96-1.5H-N, Cellvis)
4. DPBS without Mg^{2+} or Ca^{2+} (Gibco #14190-144)

Requirements

1. The beads are to be added to well G9 of a 96-well plate.
2. The beads should be added to the wells at least 2 hrs before imaging.

Preparation

Mixed-bead solution preparation (this solution can be made ahead of time and kept either at RT or 4-8ºC for short term storage, which should not exceed -2 weeks):

1. Vortex bead stock solutions for 5 min at maximum speed to break aggregates of multiple beads and obtain a homogeneous solution. Do this for both stock solutions of Tetraspeck and Focal Check beads.

   Time sensitive

   Make sure this step occurs just before pipetting beads from the stock solution, as the beads will settle down and accumulate at the bottom of the vial within 15 min.

2. Add 4µL of vortexed Tetraspeck beads and 1µL of Focal Check beads stock solution to 1mL of PBS.

Adding mixed-bead solution to control wells

1. Vortex the final mixed-bead solution for 1 min at maximum speed. Pipetting up and down may not be sufficient especially if the solution was made ahead of time.

   Time sensitive

   Make sure this step occurs just before adding the beads to their respective wells, as beads will settle down and accumulate at the bottom of the vial within 15 min.

2. Add 100µL of mixed-bead solution to well G9.
3. Let sit for a minimum of 2 hrs before imaging.