Micro-oxen protocols:

Beads + cells

Binding differentiated neutrophil-like HL-60 cells to ConA-coated polystyrene beads, observation in 8-well chambered coverslips

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(Assuming ConA-bound beads from last step are at 1 mg/mL (0.1%))

Notes

Density of polystyrene particles:
1.05 g/cm3 = 1.05e6 g/m3 = 1.05e9 mg/m3

Density of polystyrene particles:
1.96 g/cm3 = 1.96e6 g/m3 = 1.96e9 mg/m3

To **convert mass % of bead solution to particles per volume**:
0.1% = 1 mg/mL
Vol of spherical particle = (4/3)*pi*r^3
For 1um diameter sphere, volume is: 5.236e-19 m^3

Particles per volume = (mass per vol) / [(density) * (vol of single particle)]
For **1um polystyrene beads, 0.1% (1 mg/mL)**:
(1 mg/mL) / [(1.05e9 mg/m3) * (5.236e-19 m3)] = 1818909382 = **1.82e9 particles / mL**

Reagents list

6-day differentiated HL-60 cells (1.3% DMSO)
ConA-coated polystyrene microspheres (coating method varies; optional: fluorescent)

Step-by-step

1) Spin down cells @ ~400g
2) Aspirate medium
3) Resuspend in warm (37C) mHBSS + 2% BSA @ 1.5e6/mL

4) Aspirate medium
5) Resuspend in warm (37C) mHBSS + 2% BSA @ 1.5e6/mL

Note: Do not wait longer than ~ 1 hour before observing cells under microscope (they get unhappy and will not move well or at all).

6) Prepare a separate working stock tube of ConA-coated polystyrene microspheres at ~1e8/mL.
For example, for a 0.1% solution of polystyrene microspheres, need 18.2x dilution (see Notes section). Pipet 10uL of beads (mix well, before pipetting!) into 172uL mHBSS.

7) Pipet 10uL bead suspension from step 6 into separate tube.
8) Pipet 1mL cell suspension from step 5 into tube with beads (from step 7).
9) Incubate 5 min @ 37 C (in incubator).

10) Pipet into wells of LabTek II 8-well Chambered Coverglass.
11) Let cells plate down 10 min @ 37 C.
12) Live cell microscopy - BF and/or fluorescence microscopy
(Note: Phase contrast / DIC methods have been problematic in the past due to the meniscus of the liquid in the wells of the chambered coverglass).