Live cell imaging of particle capturing during phagocytosis

Amir Horowitz

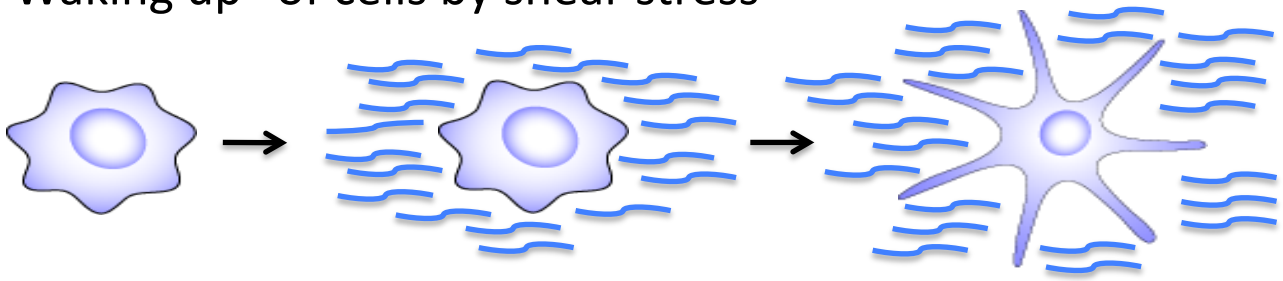
Robert Endres Group (Biochem building – 3rd Floor)

Center for Integrative Bioinformatics at Imperial College (CISBIC)

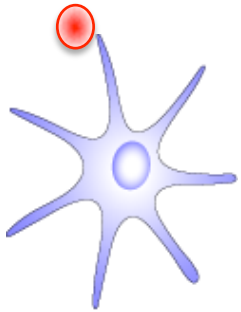
25.05.2011

Discussion points:

- "Waking up" of cells by shear stress



- Capture of bead particles by filopodia



- Visualization of FcγRIIA in live RAW cells

Particle capture: Active or Passive?

little is known about the very early events which lead to capture of target by phagocytes:

- Receptor dynamics – receptor diffusion along plasma membrane (Passive?)

- Role of filopodia - actin cortex and motor proteins (Active)

What do we know?

- Treatment of cells with jasplakinolide (actin deploymerization agent) restricted lateral diffusion of receptors and prevented particle binding (Mao et al. *J Cell Biol, 184: 281)* (suggests active process).

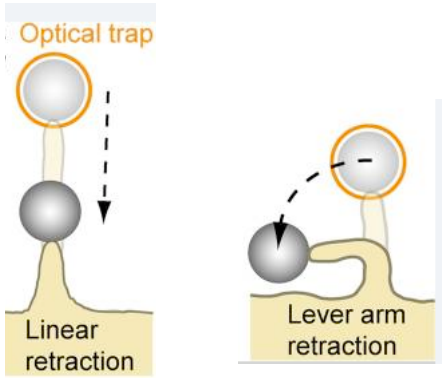
- Numerous studies found discrepancies with passive model of binding:
  - Herskowitz (1977) – cytochalasin impairs binding of IgG-treated RBCs
  - Sobota (2005) – tyrosine phosphorylation required for optimal binding
  - Dale (2009) – confirmed Sobota findings but also showed inhibition of actin polymerization inhibits binding

- Probably due to differences in experimental conditions:
  - Synchronization of cultures at 4°C
  - Lengthy incubations at 37°C
  - Density of opsinization by IgG
  - Probably many more

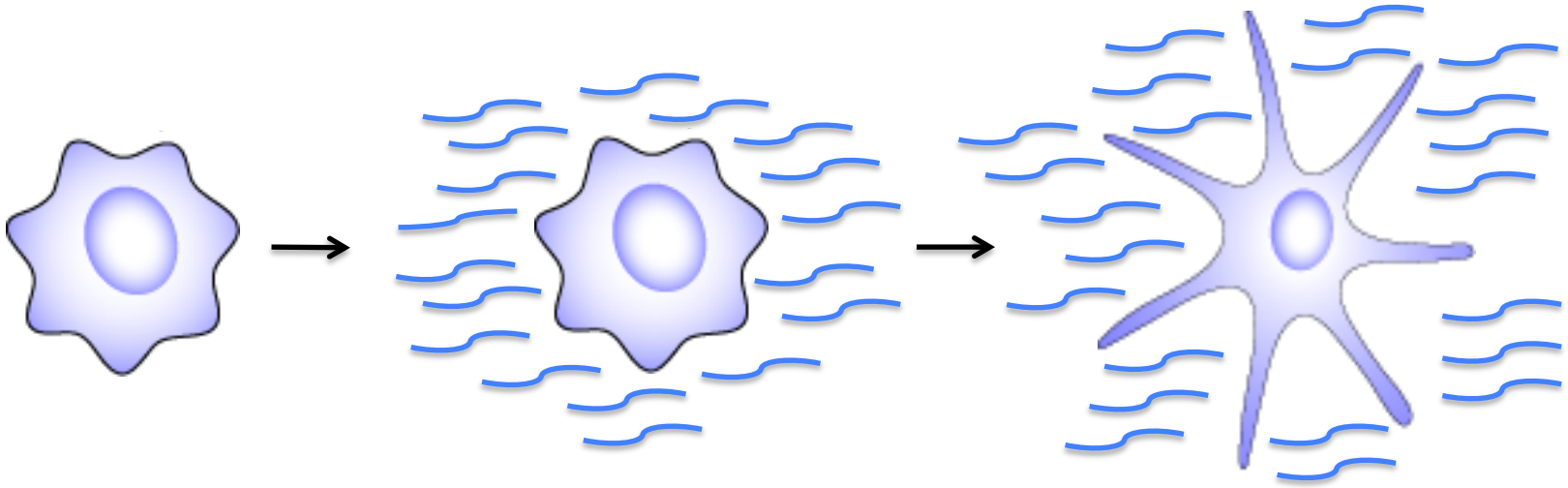
What we DON'T know:

- Do cells have ability of 'mechano-sensing' of environment as a mode of remaining alert for fast moving/circulating antigens?
- If so, then what are the cues?
- Is FcγRIIA actually expressed along the filopodia or just on surface of plasma membrane, whereby depends on either 'linear' or 'lever-arm' retraction of particle into plane of reach?

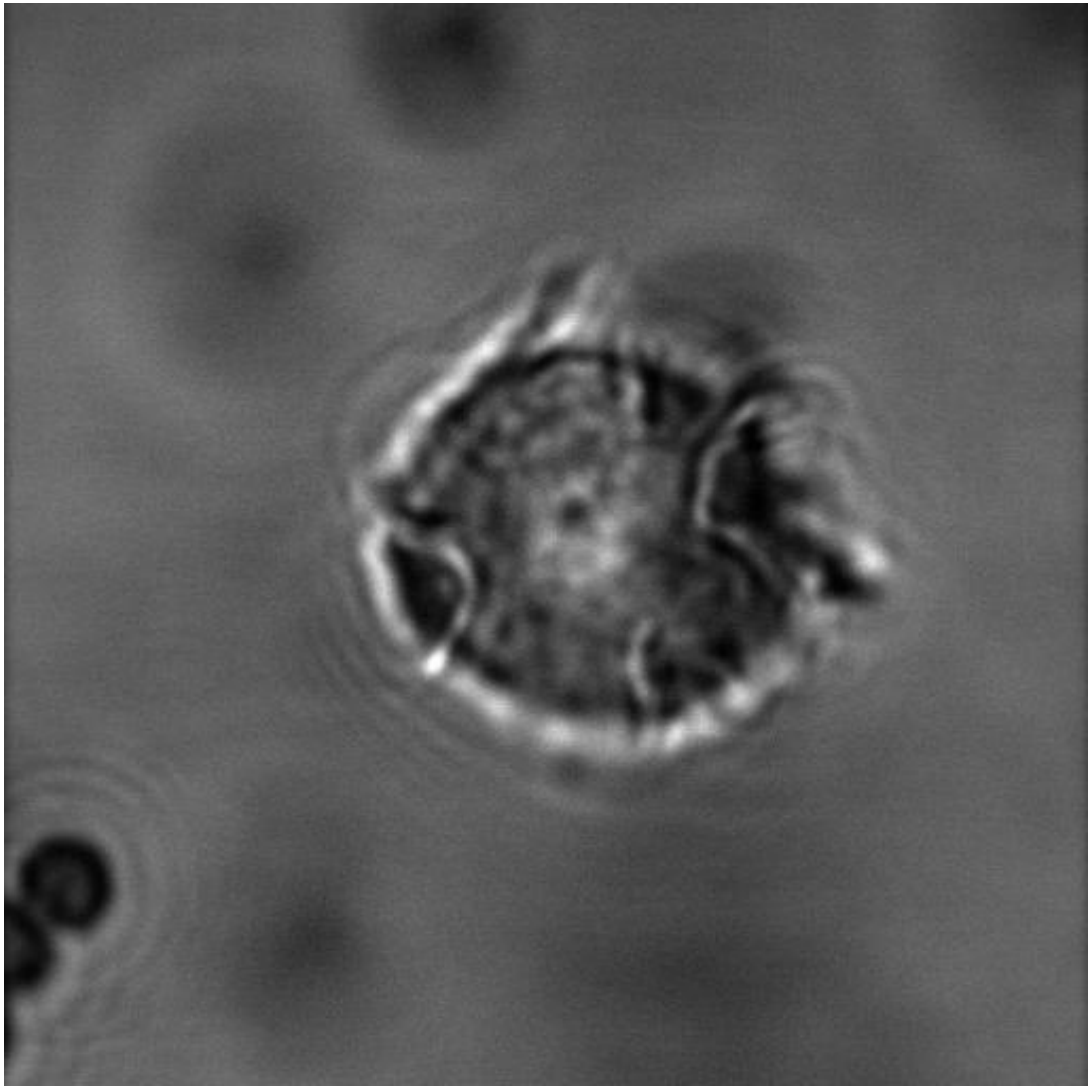
Kress et al., PNAS 104:11633



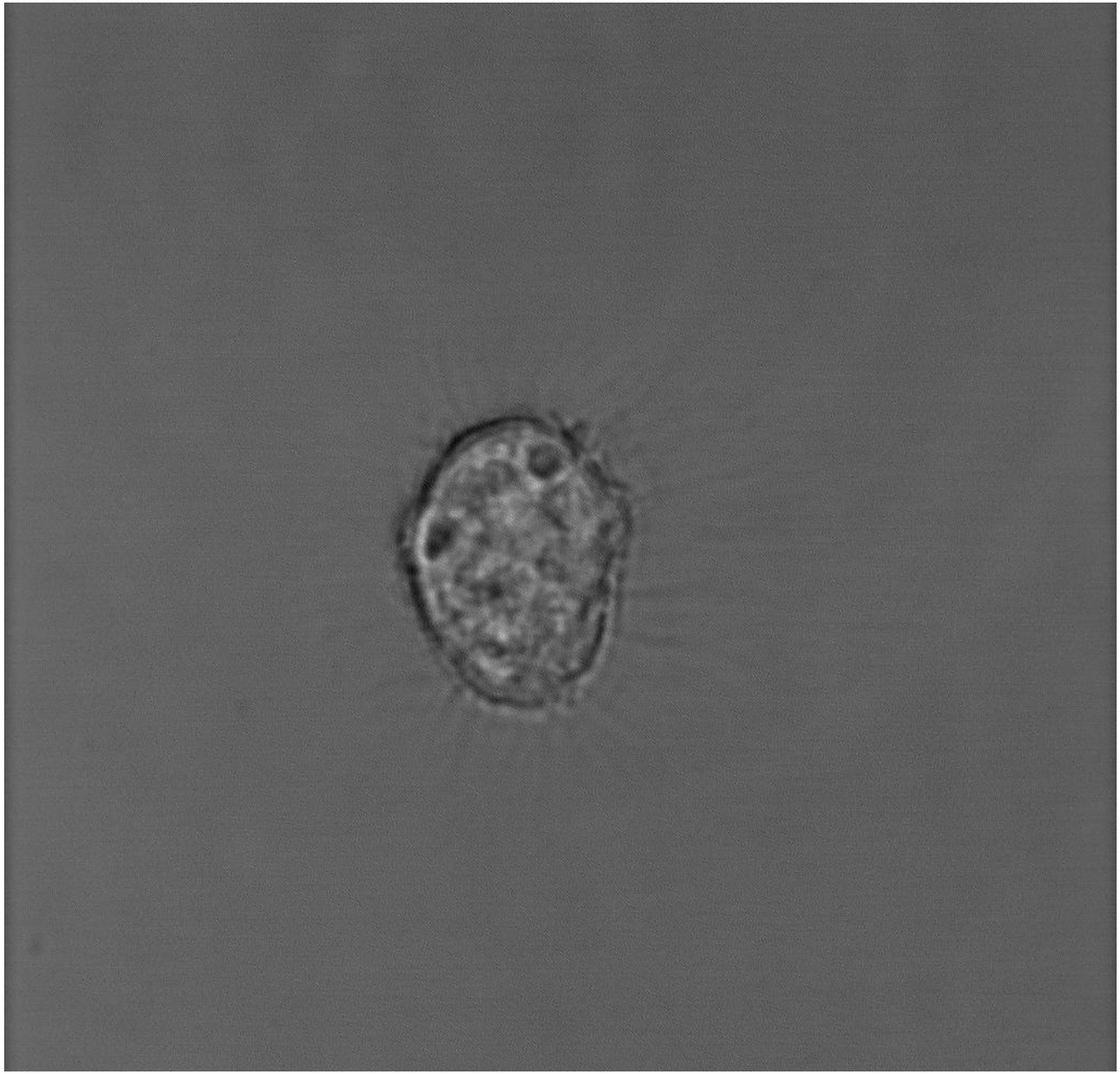
Waking up / mechano-sensing



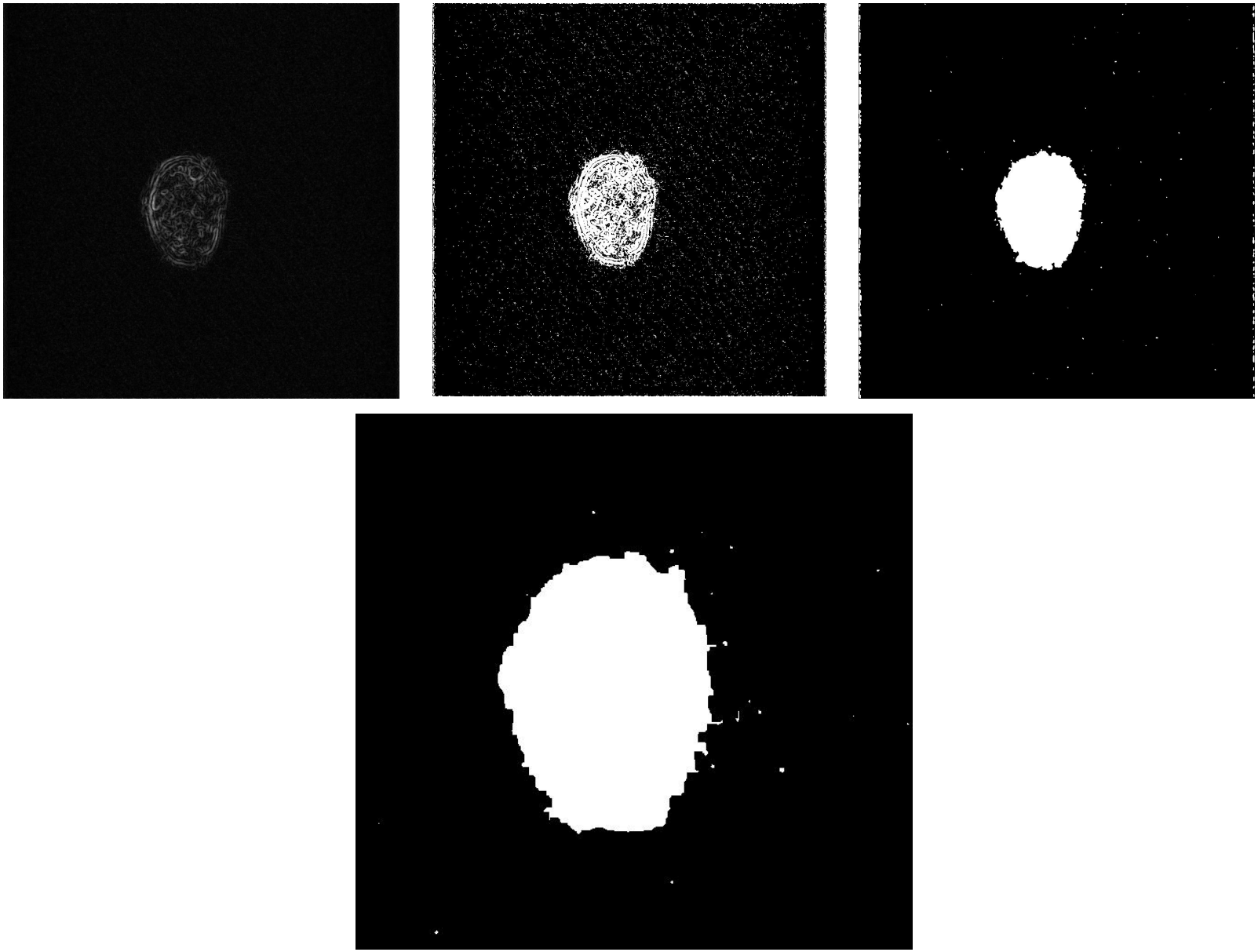
Observed that cells ‘woke up’ following addition of beads but well before beads were in range of contact

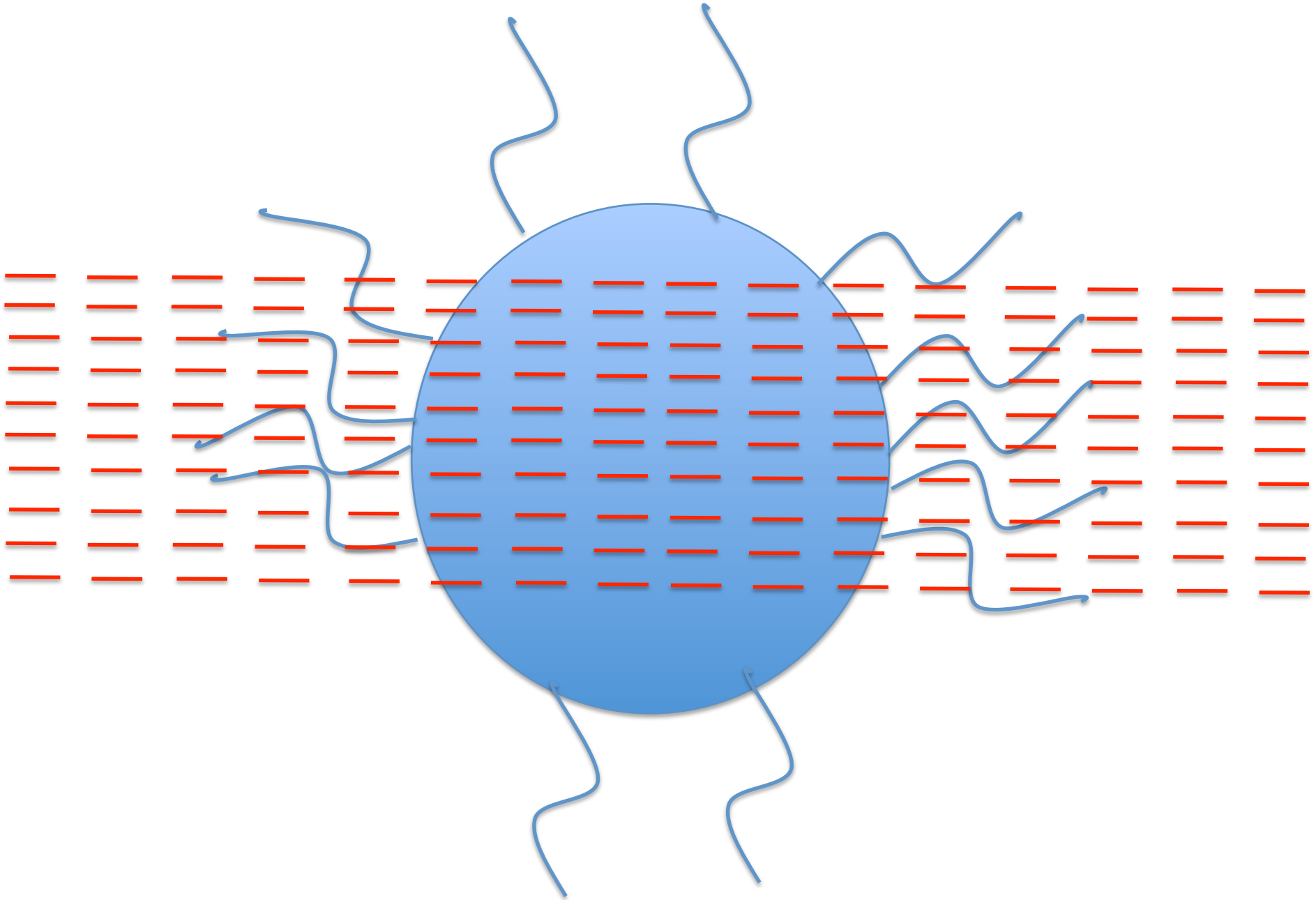


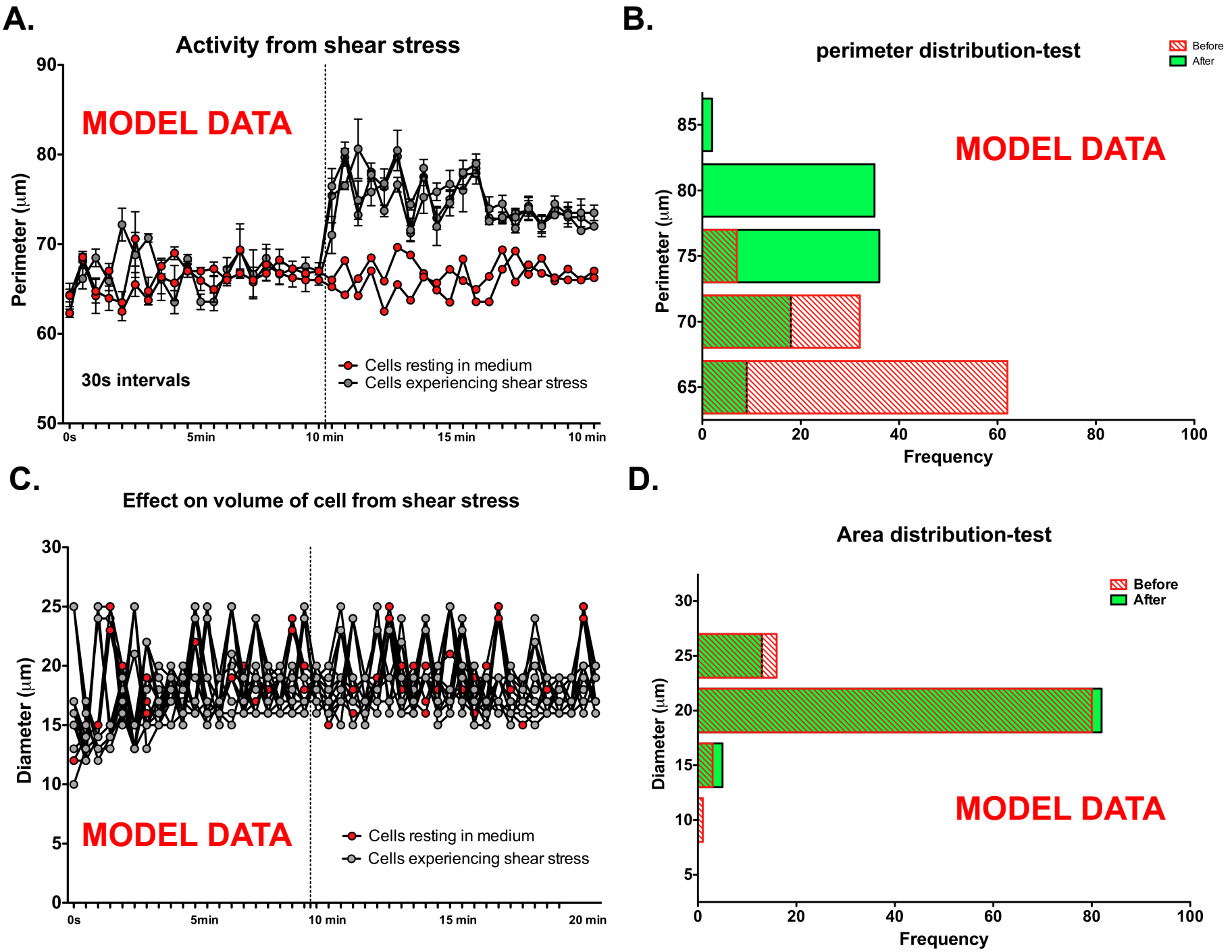
RAW 264.7 cells



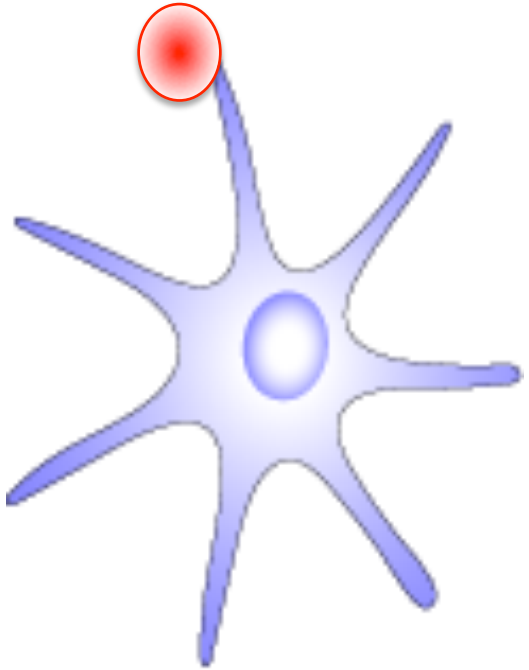
Using ImageJ software to calculate perimeter (μm) and surface area (μm^2)



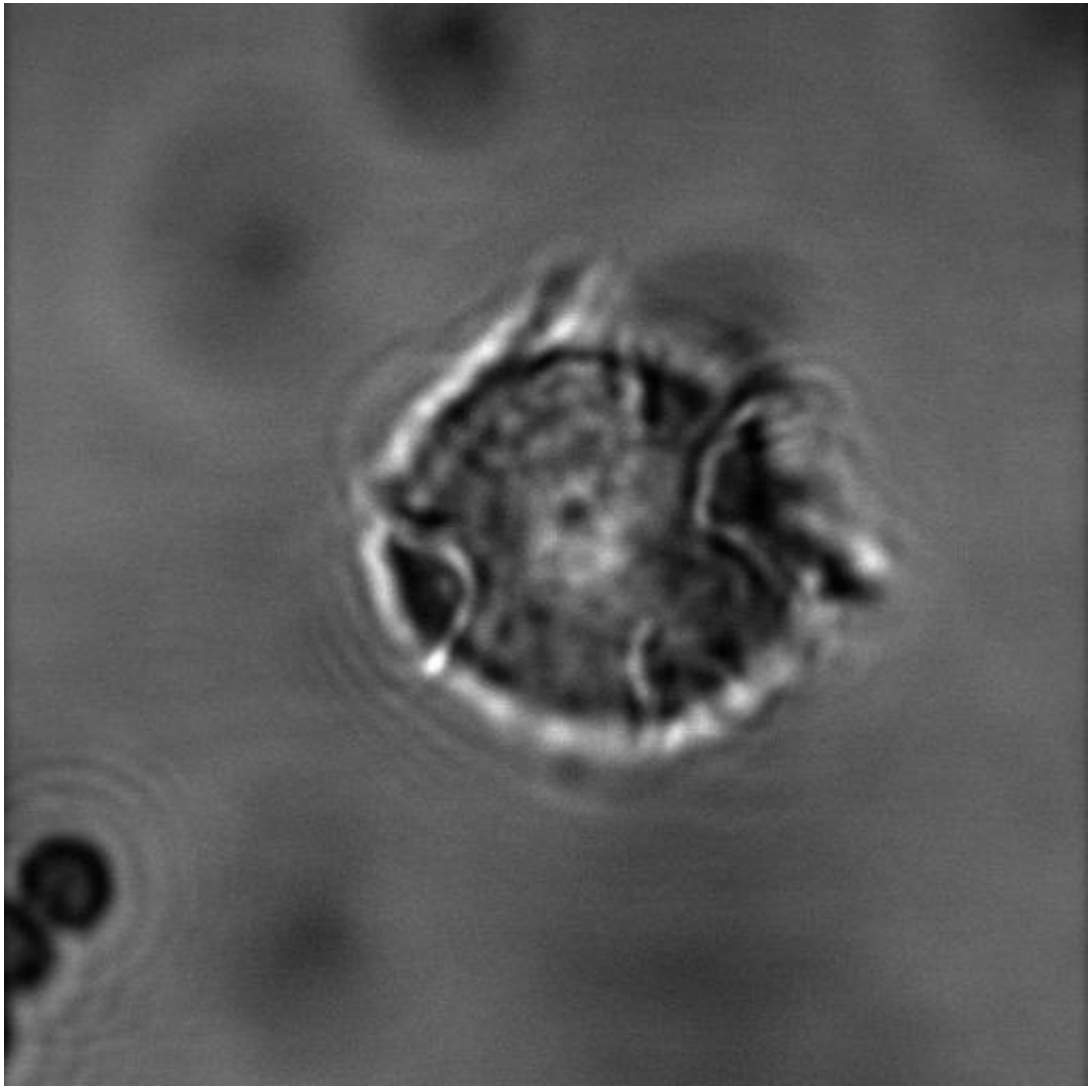



A.
Activity from shear stress
MODEL DATA
Perimeter (μm)
90
80
70
60
50
0s
5min
10 min
15 min
10 min
30s intervals
Cells resting in medium
Cells experiencing shear stress
B.
perimeter distribution-test
Before
After
MODEL DATA
Perimeter (μm)
85
80
75
70
65
0
20
40
60
80
100
Frequency
C.
Effect on volume of cell from shear stress
MODEL DATA
Diameter (μm)
30
25
20
15
10
5
0
0s
5min
10 min
15 min
20 min
Cells resting in medium
Cells experiencing shear stress
D.
Area distribution-test
Before
After
MODEL DATA
Diameter (μm)
30
25
20
15
10
5
0
20
40
60
80
100
Frequency

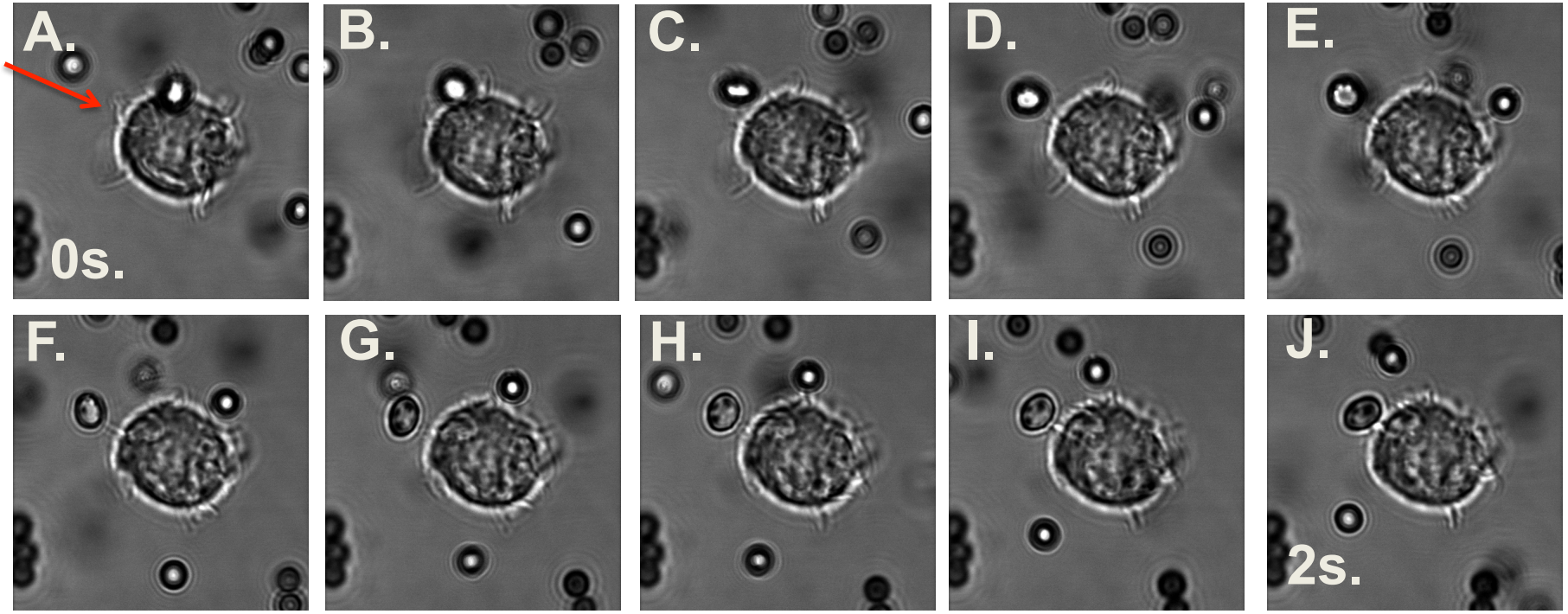
Capturing beads by filopodia



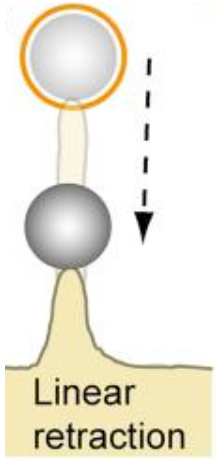
Bead Capture – Stochastic and Active



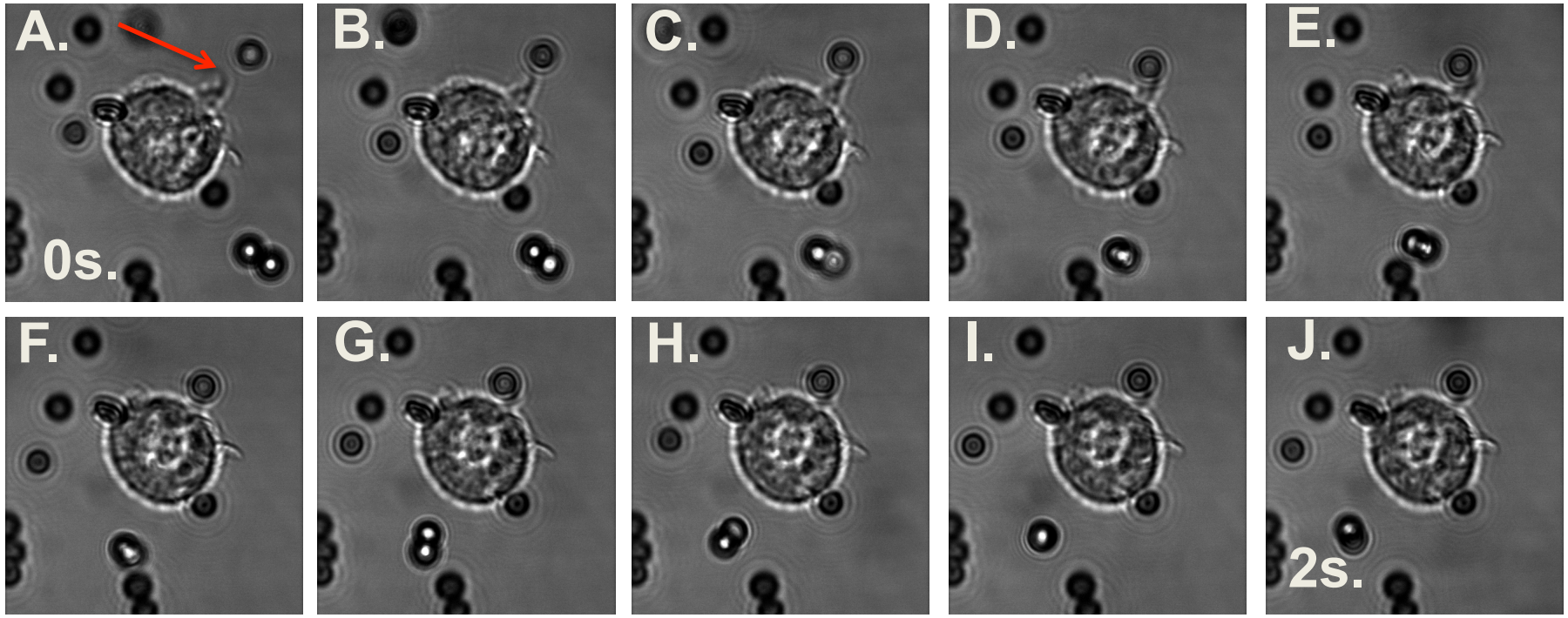
Example of linear capture

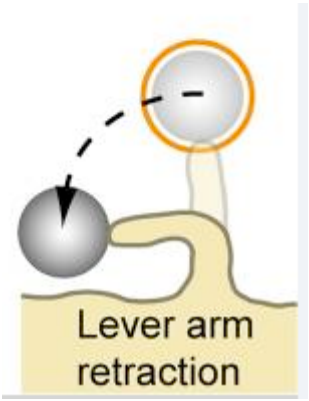


very rapid and appears completely stochastic
Although specific –
filopodia constantly surveying environment

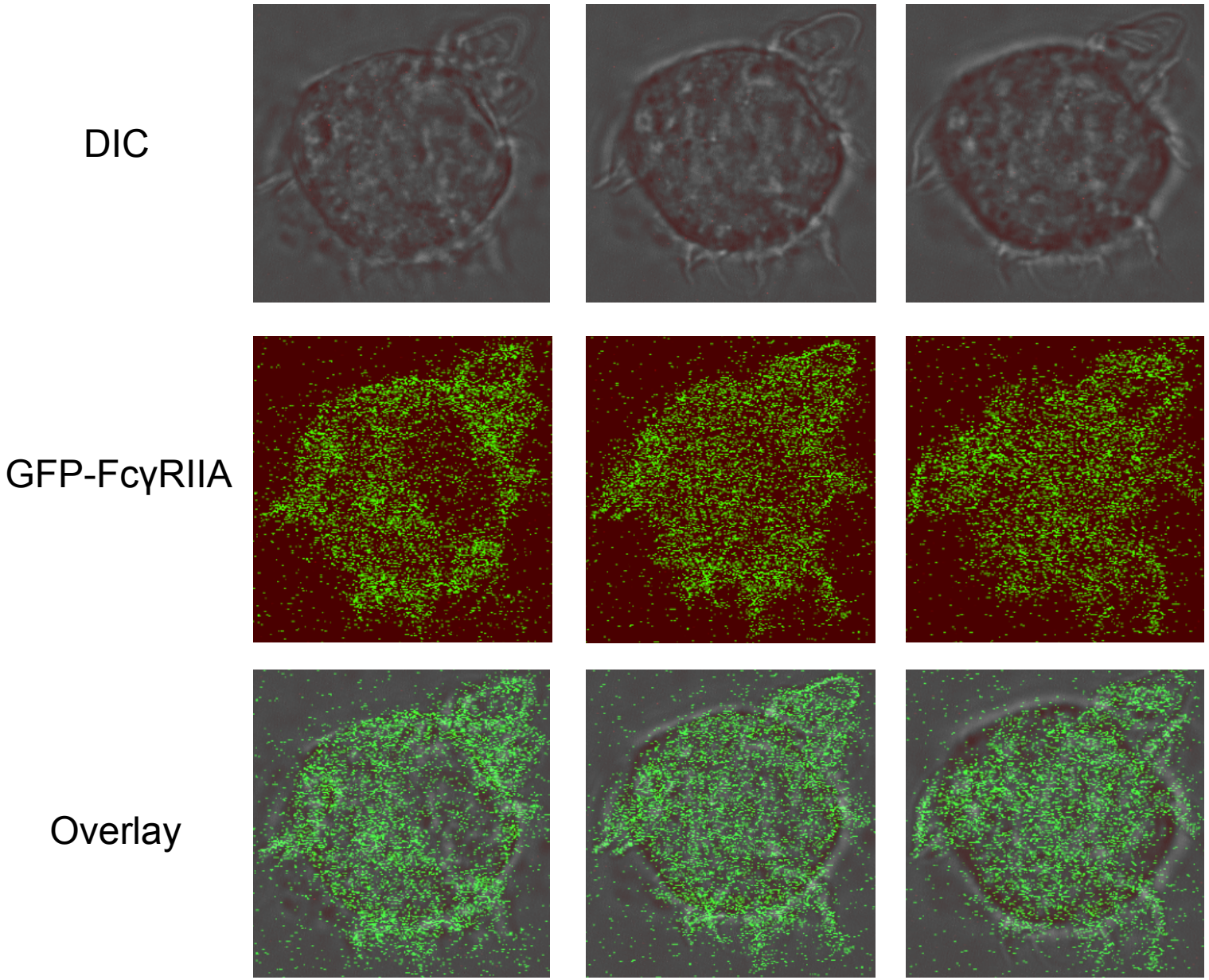


Example of lever-arm capture

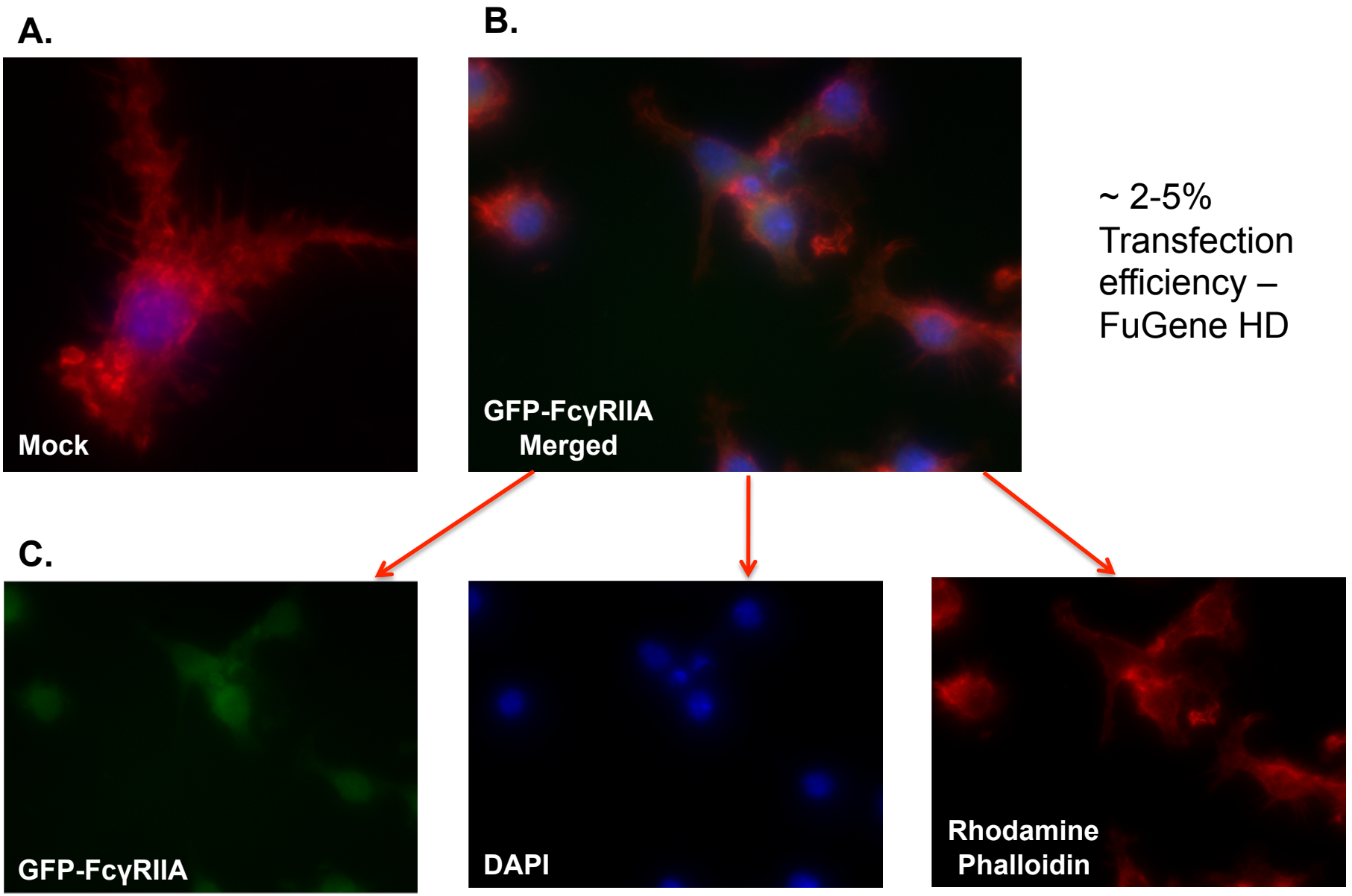




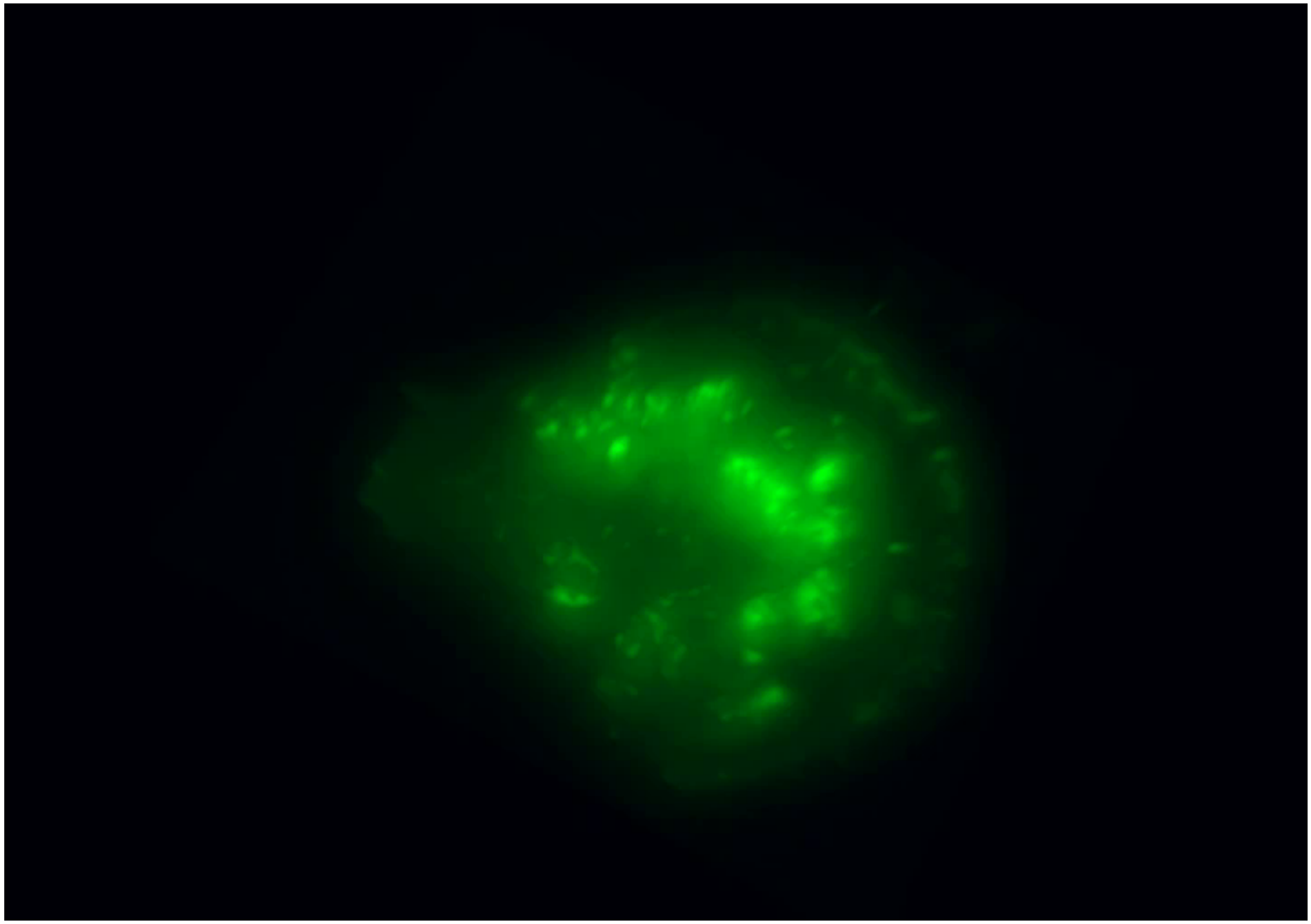
Do Filopodia express specific receptors?


DIC
GFP-FcγRIIA
Overlay

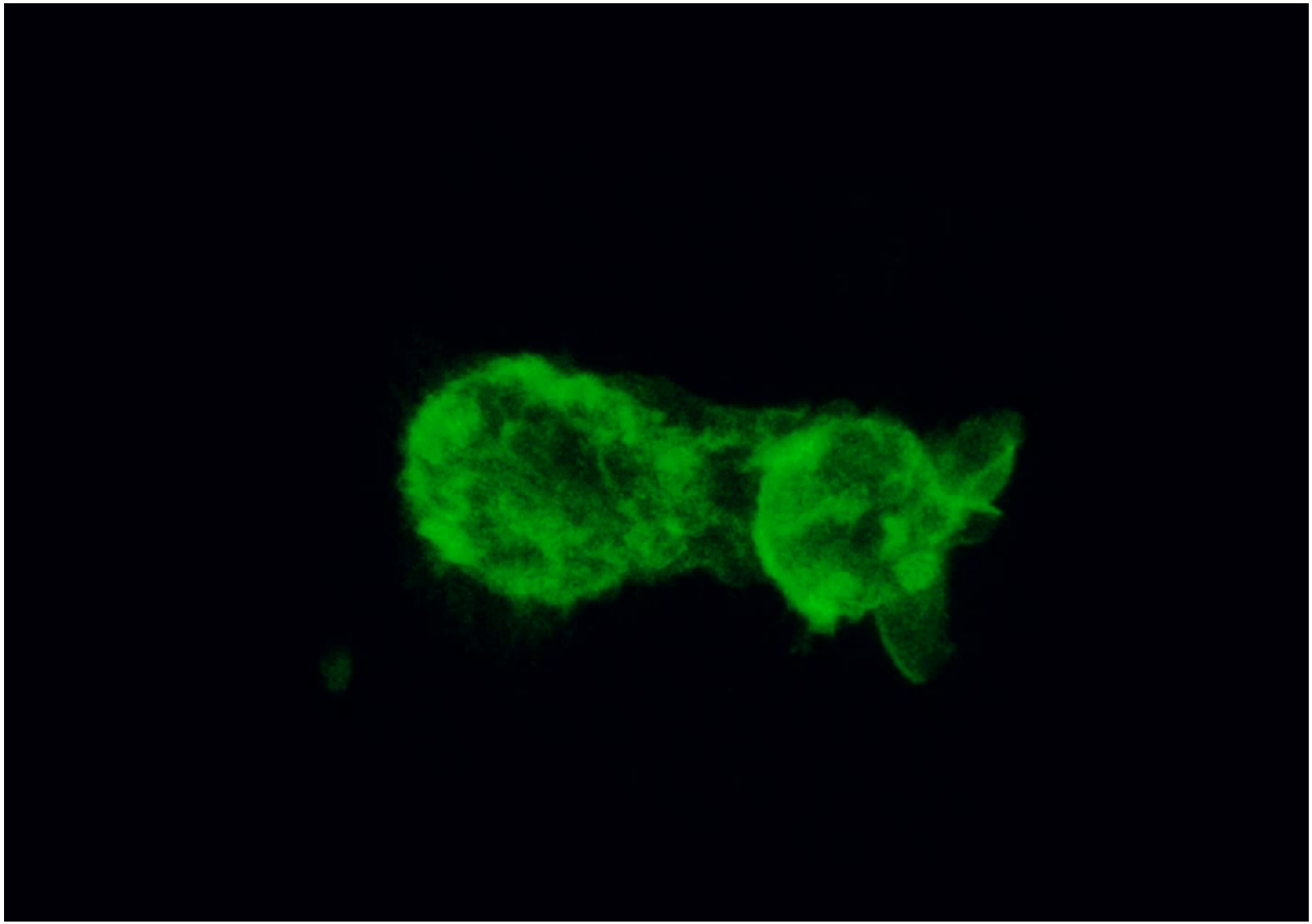
Transfection of RAW cells with GFP-FcγRIIA (**Endo-free)



3D reconstruction of GFP-FcγRIIA-transfected RAW cell



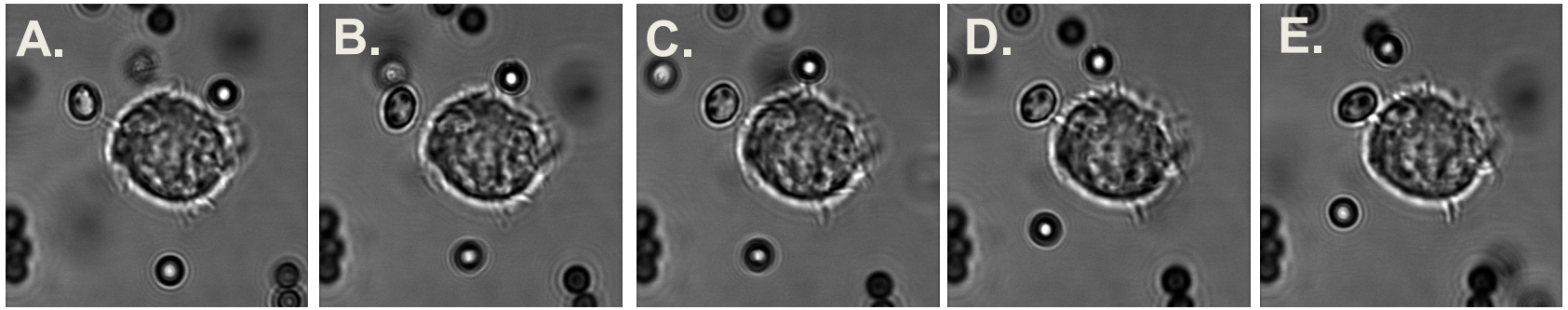
3D reconstruction of Live RAW cell



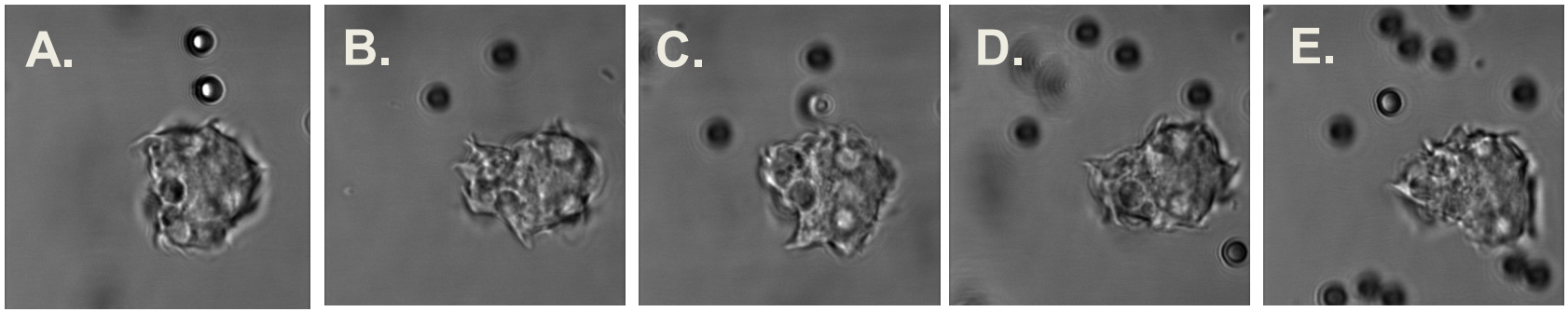
Does over-expression of FcγRIIA affect biological relevance?

- Interested in expression of FcγRIIA in filopodia – Need to compare to normal expression levels by using commercial α -CD32 Ab

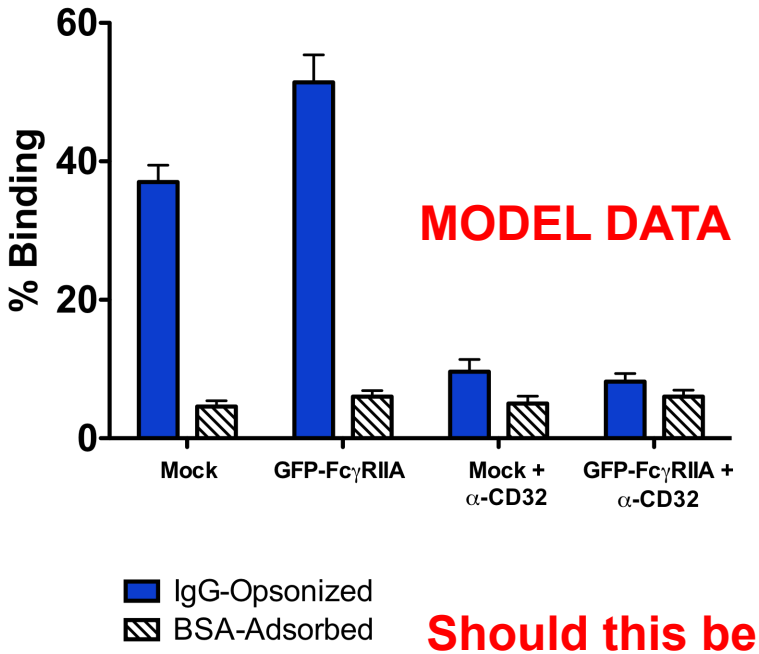
- Do we expect more binding because of over-expression of FcγRIIA?



IgG-Opsonized



BSA-Adsorbed



MODEL DATA

$$\% \text{ Binding} = \frac{(\Sigma \text{ cells with} \geq 1 \text{ bound bead})}{(\text{total cells})}$$

Should this be done based on IFA or from live cell imaging?

Conclusions

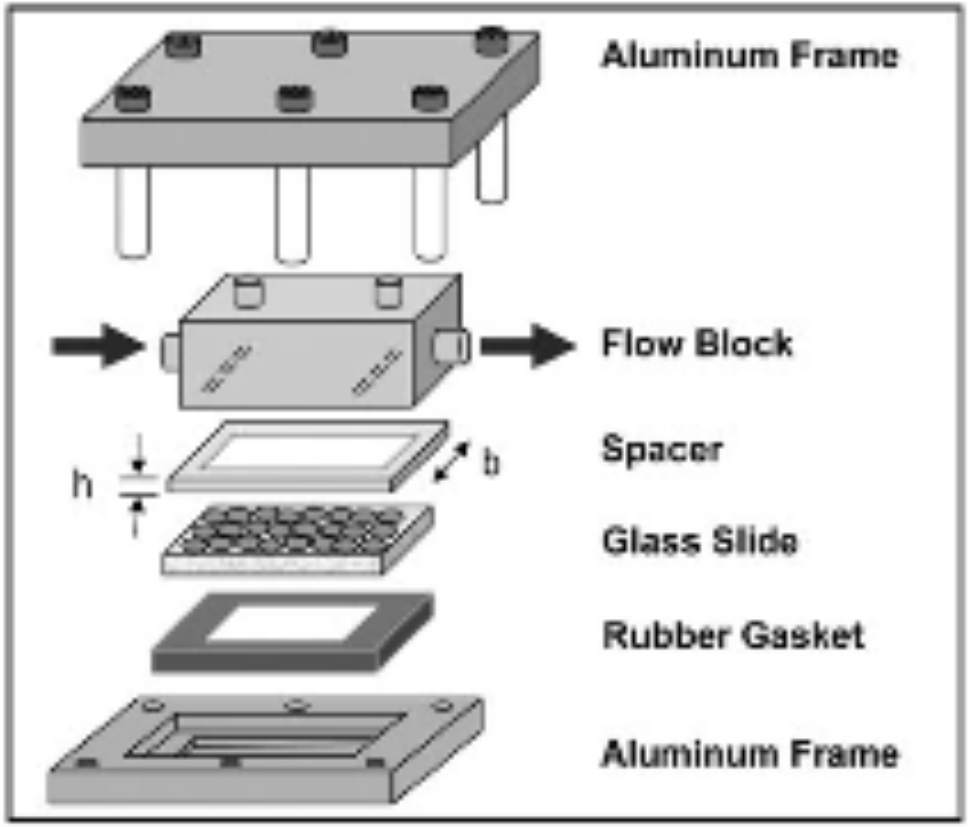
Want to design a closed system whereby we can quantify how much force is required to wake up cells as well as incorporate addition of beads

Micro-fluidics

- Established collaboration with Dr. Mark Steedman in Molly Stevens Lab, Bioengineering, Imperial College

- Already has experience designing and constructing fluidics chambers as well as experience with tissue culture (stem cell development)

Fluidics chamber



Acknowledgements

Robert Endres – Biochem / CISBIC
Luke Tweedy
Gerardo Aquino

Gad Frankel - Flowers
Joanna Young
Mike Bright
Leah Ensell

Dan Davis - SAF
Alice Brown
Anne Aucher
Sophie Pageon
Dominika Rudnika
Maryam Mehrabi

FILM Unit - SAF
Martin Spitaler
Mark Scott

Molly Stevens - Bioengineering
Mark Steedman
Benjamin White

Imperial College London