Biomedical Engineering Society
2014 Annual Meeting
San Antonio, Texas

10/23/2014
Cell Biomechanics I
9:15 AM, Room 006D

** Denotes movies played during talk

Dynamic Traction Forces of Spreading and Adherent Human Neutrophils

Steven J. Henry,
Christopher S. Chen, John C. Crocker, and Daniel A. Hammer

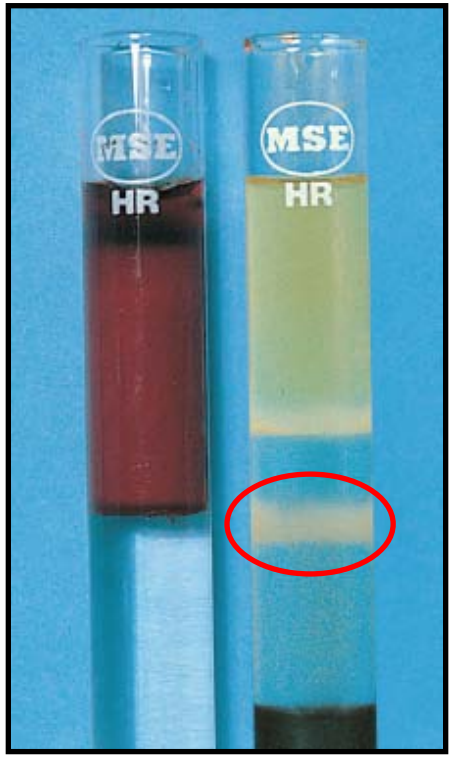
Funding:
NIH HL18208 to DAH
NSF GRFP to SJH

Penn
University of Pennsylvania

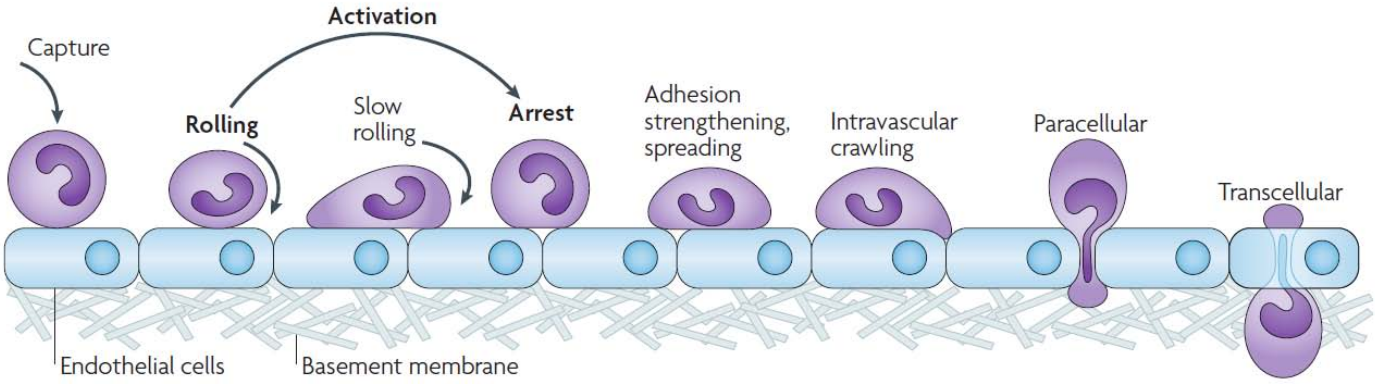
Neutrophils: first responders to trauma and infection

Whole Blood

After Density Centrifugation



Axis-Shield News Bulletin

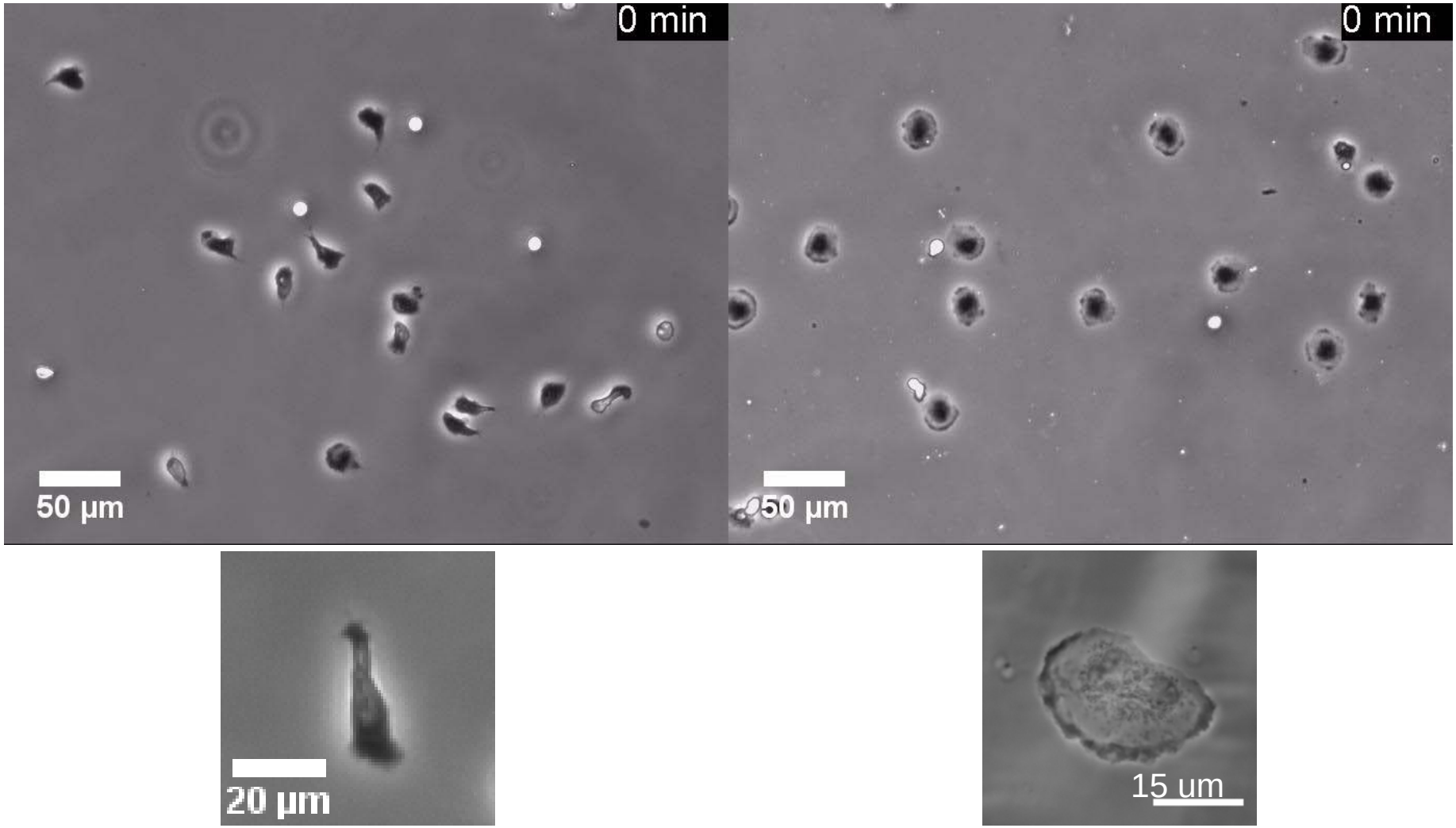


Ley et al. 2007. *Nat Rev Immunol.*

**Previously : fibronectin alone sufficiently stimulatory to induce neutrophil adhesion and motility

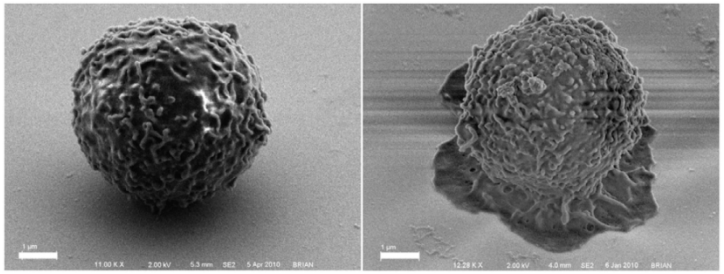
"Amoeboid"
High FN Density

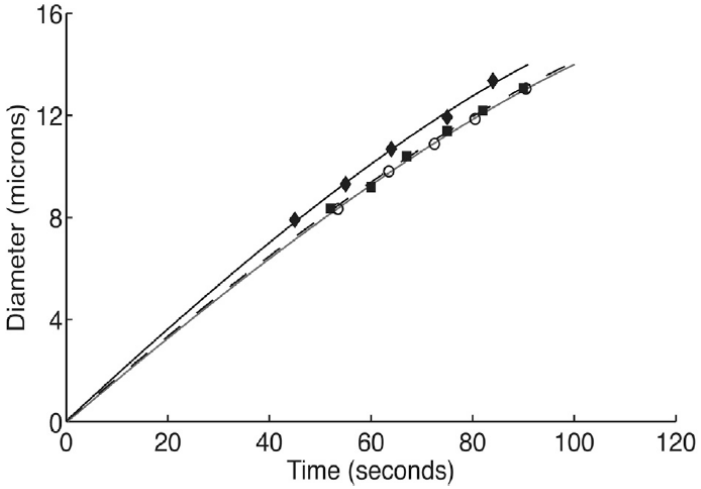
"Keratocyte-Like"
Intermediate FN Density



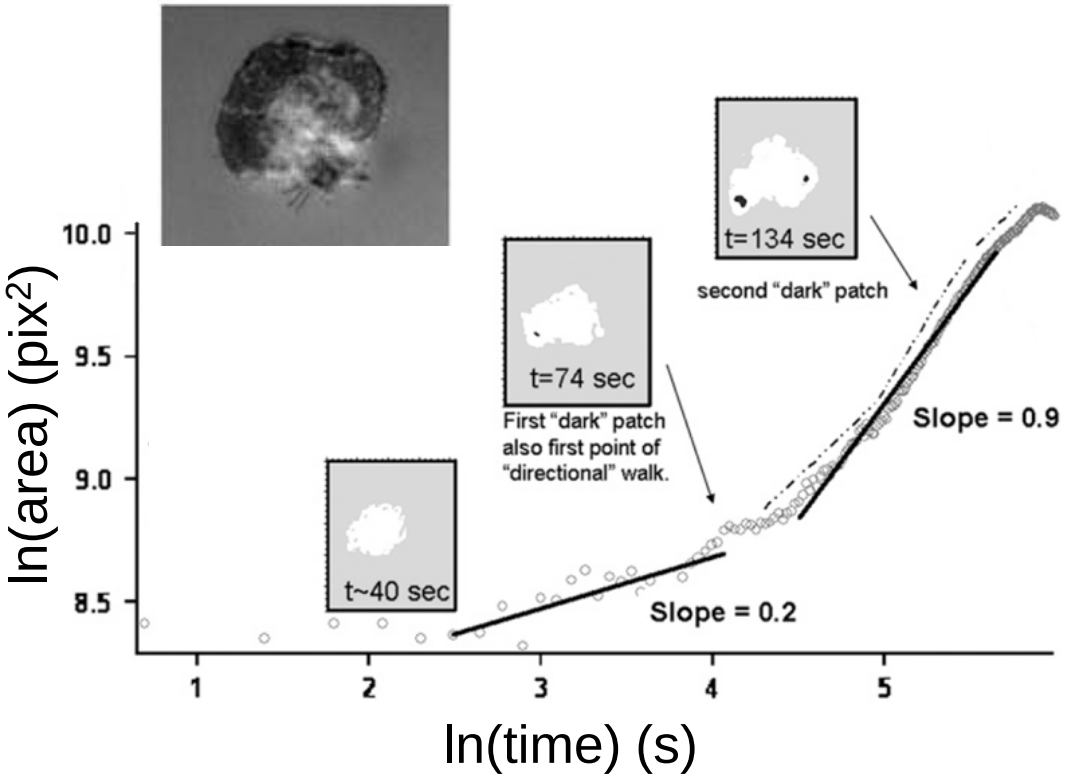
Henry et al. 2014. *Integrative Biol.*

Neutrophil spreading is fast.
Can we measure the associated forces?



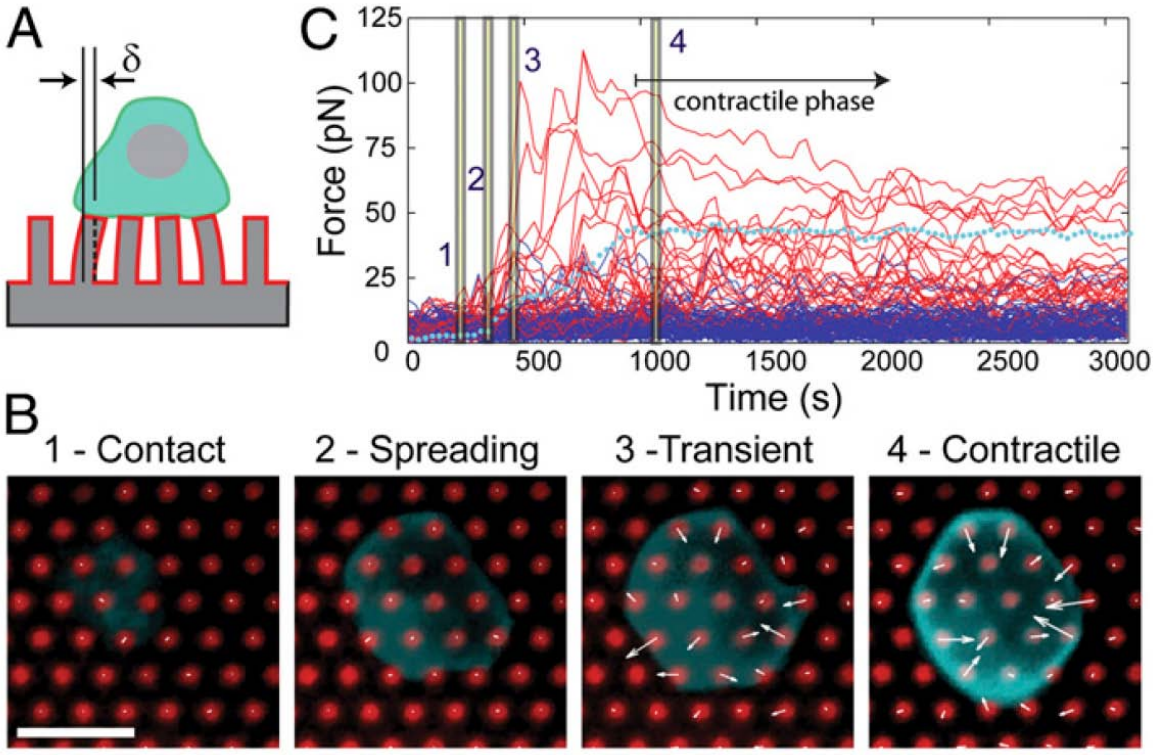


Lomakina et al. 2014. *Biophys J.*



Sengupta and Hammer et al. 2006. *Biophys J.*

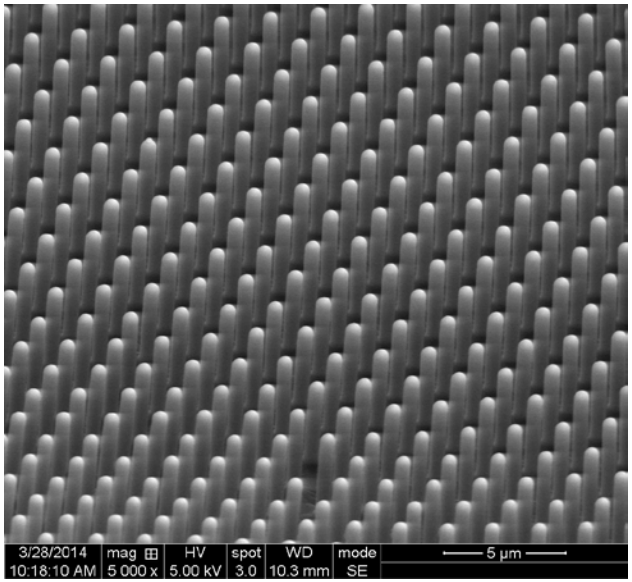
Precedent for immune cell spreading on mPADs: Traction forces of T-lymphocyte activation

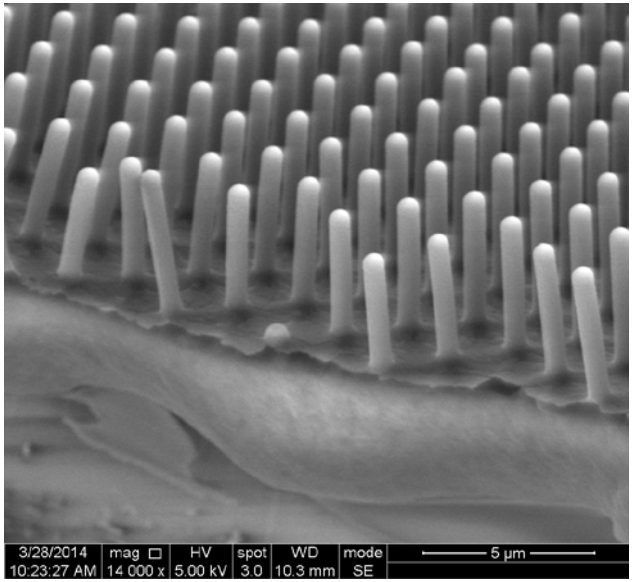


Bashour et al. 2014. *PNAS*

Dynamics of protrusive vs. contractile processes?
Role of the actin cytoskeleton?

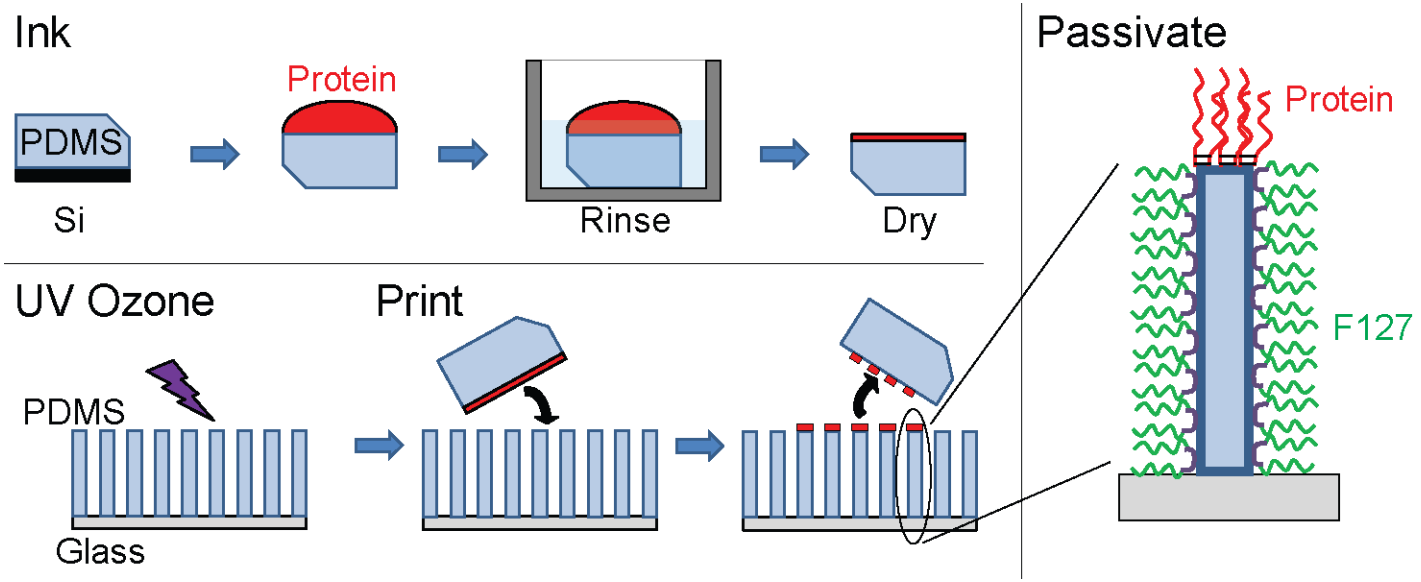
mPADs (microfabricated Post-Array-Detectors):



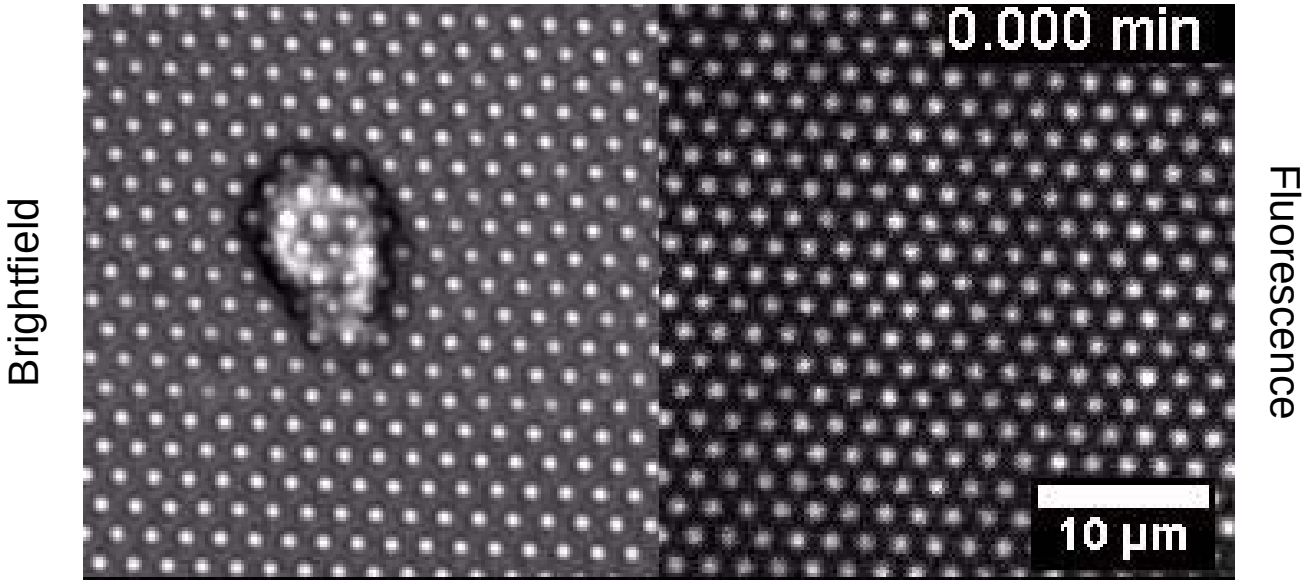


$k_{spring} = 0.28 \pm 0.07$ nN/um
G ~ 5 kPa

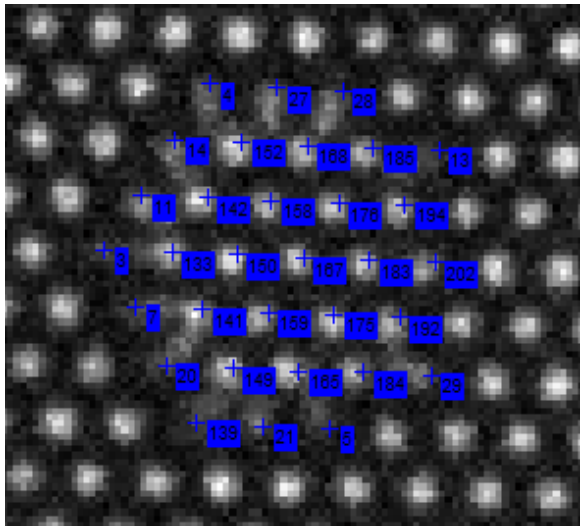
Schoen correction = 0.93
$k^*_{spring} = (0.93)(k_{spring})$
$k^*_{spring} = 0.26$ nN/um
Schoen et al. 2010. *NanoLett*.

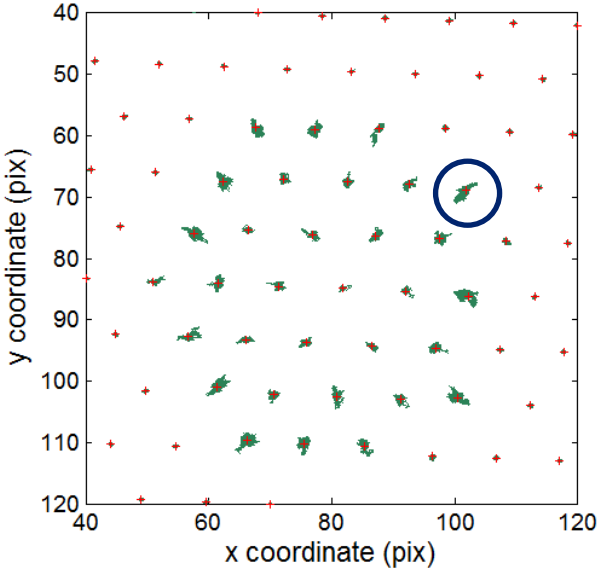


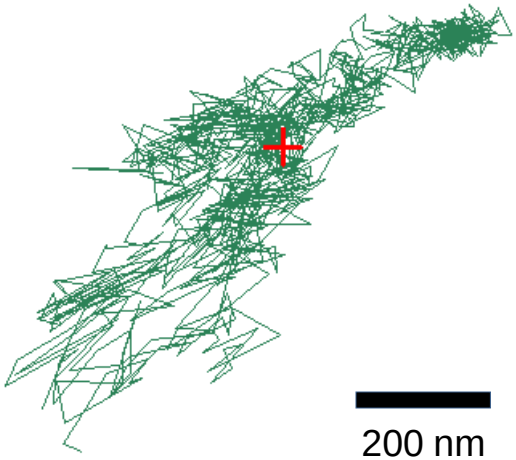
**hNeutrophil spreading on FN mPADs



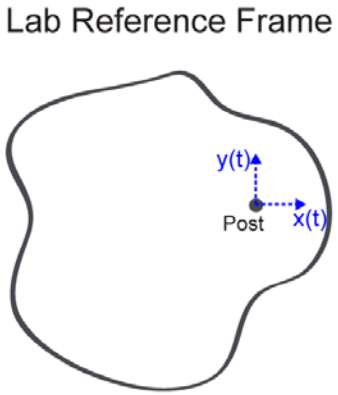
Cell-Engaged

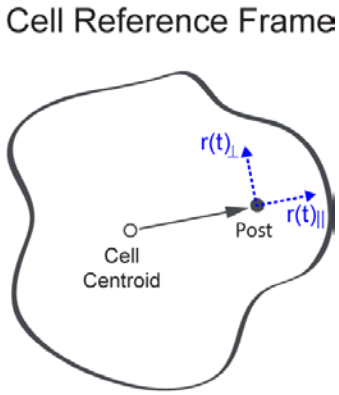


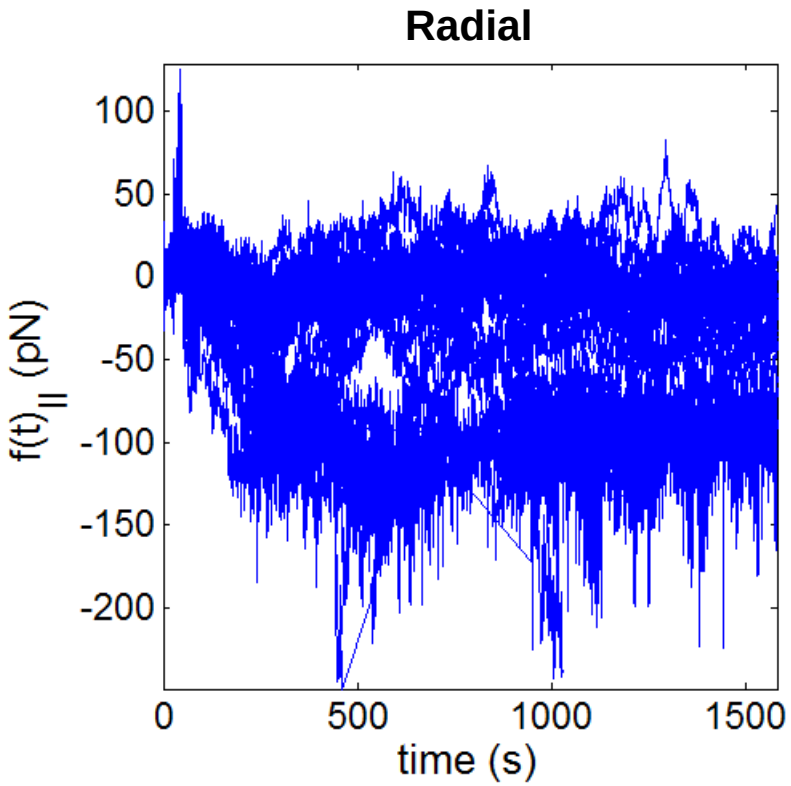


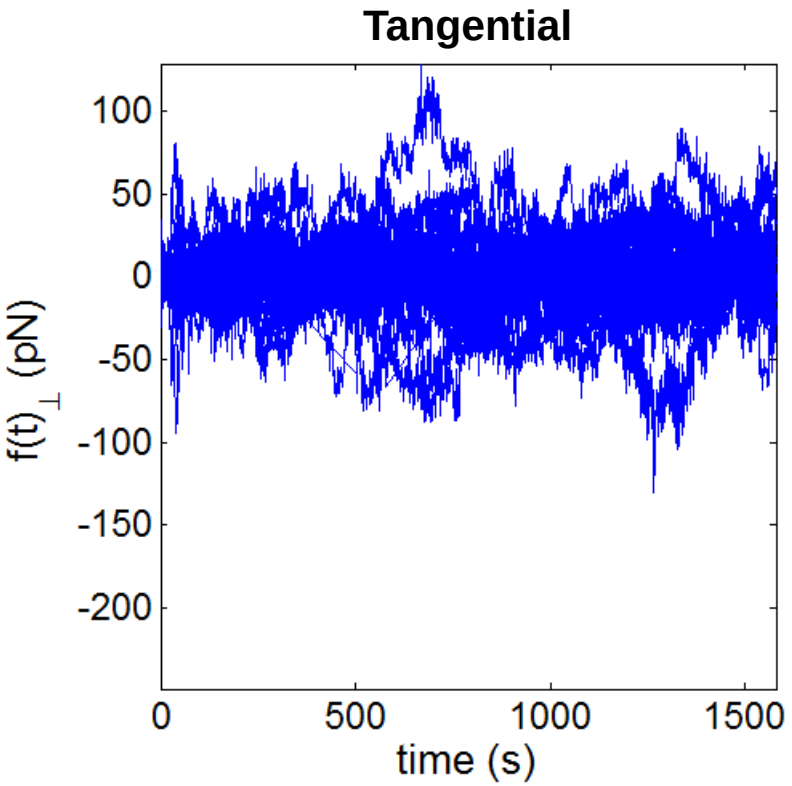


Plotting force trajectories in the cell reference frame

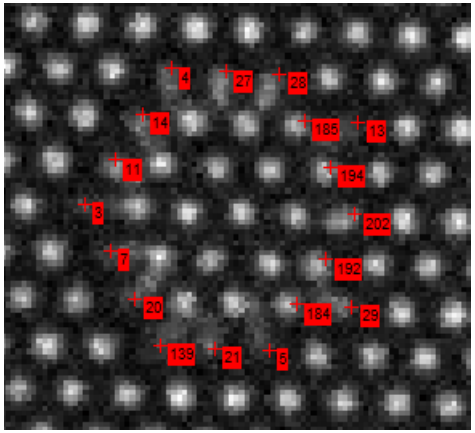


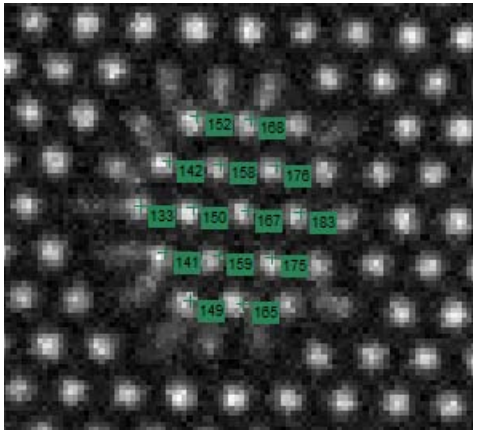






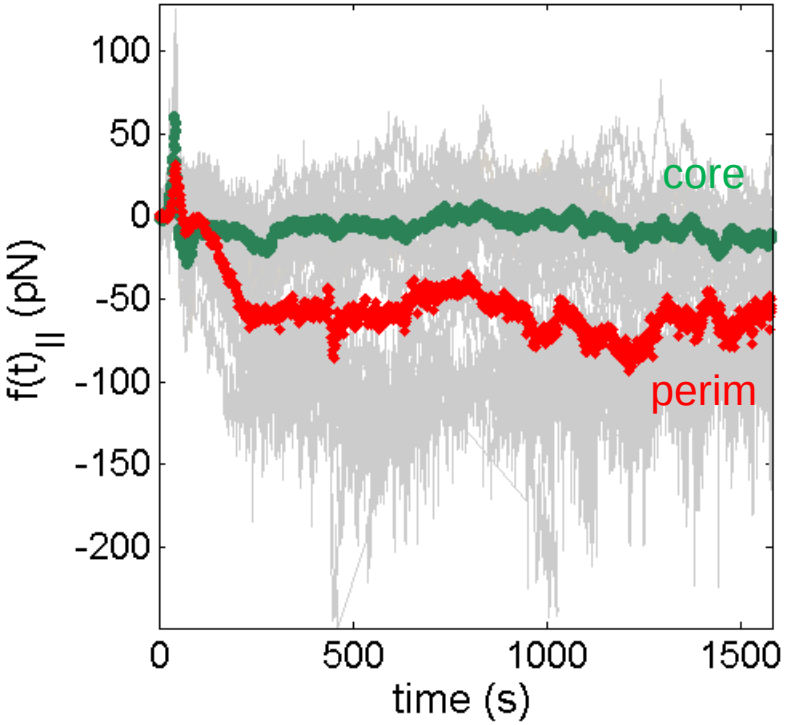
Dichotomizing data on geometric location

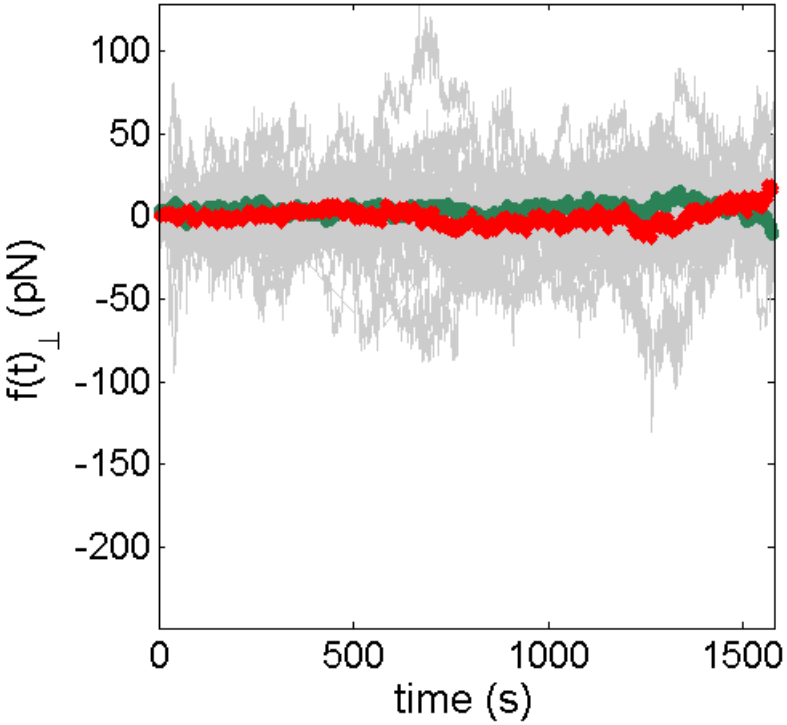




Radial

Tangential

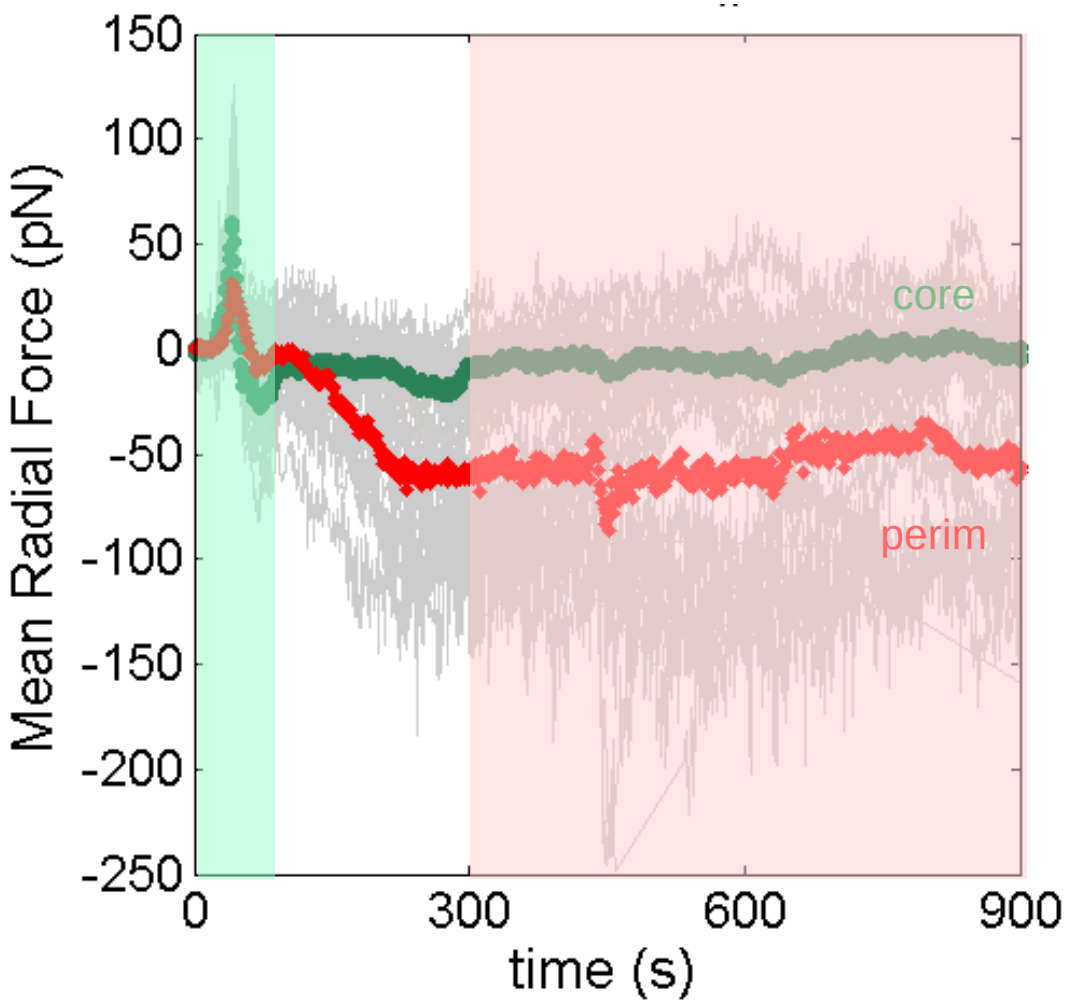




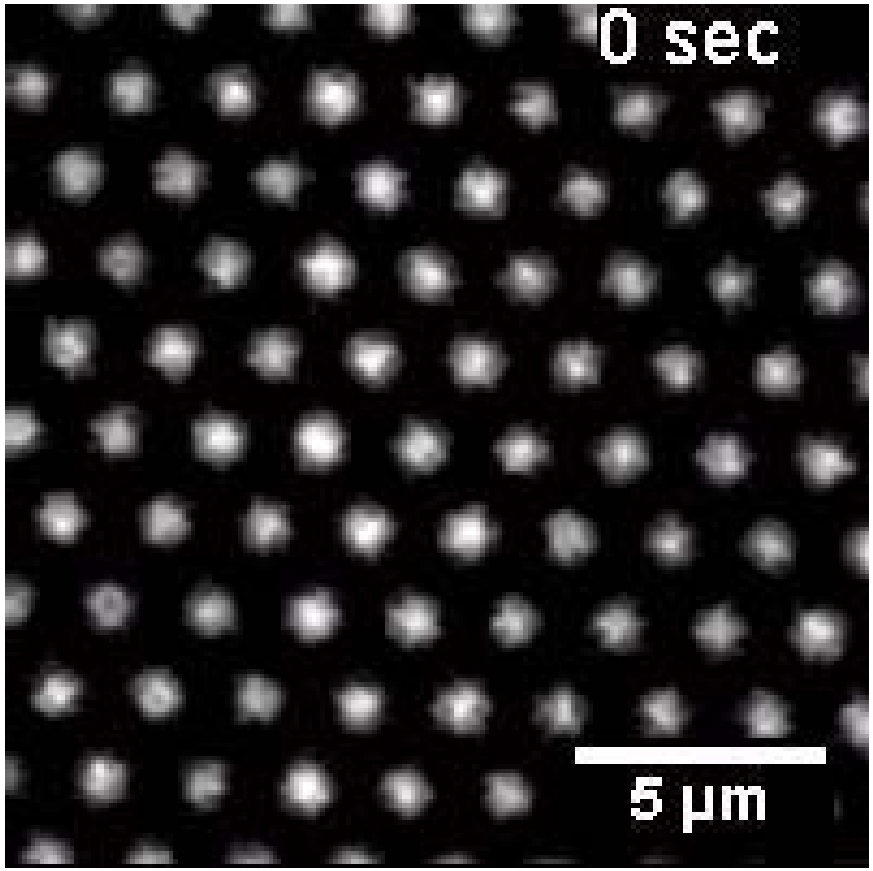
Distinct mechanical regimes are apparent

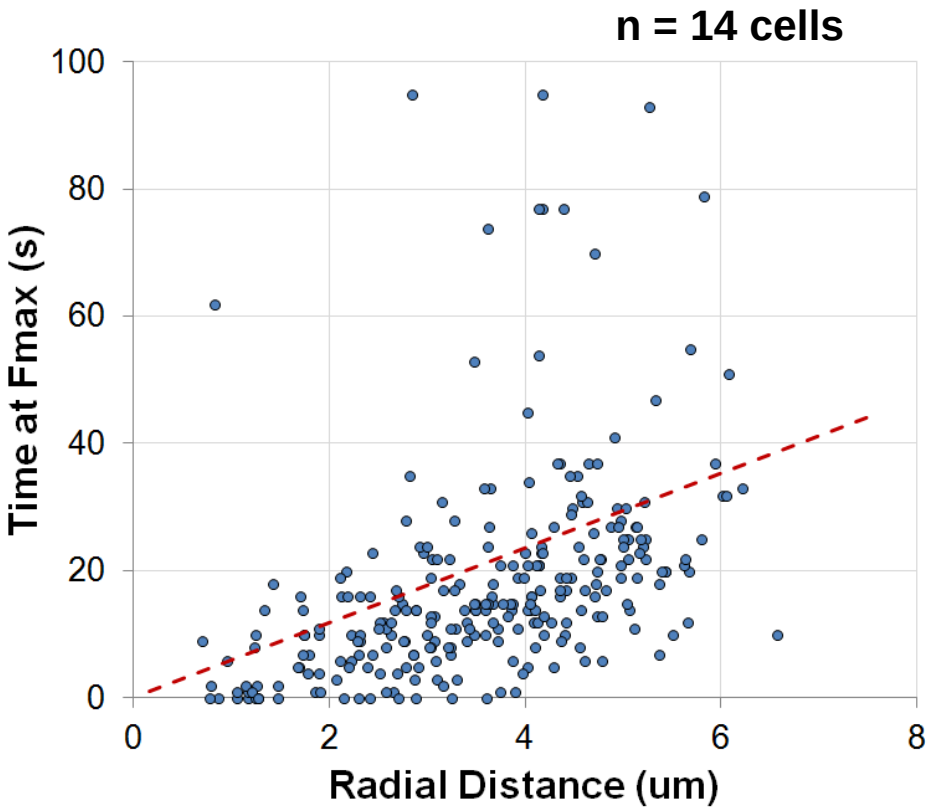
Transient Protrusion

Steady State Contraction



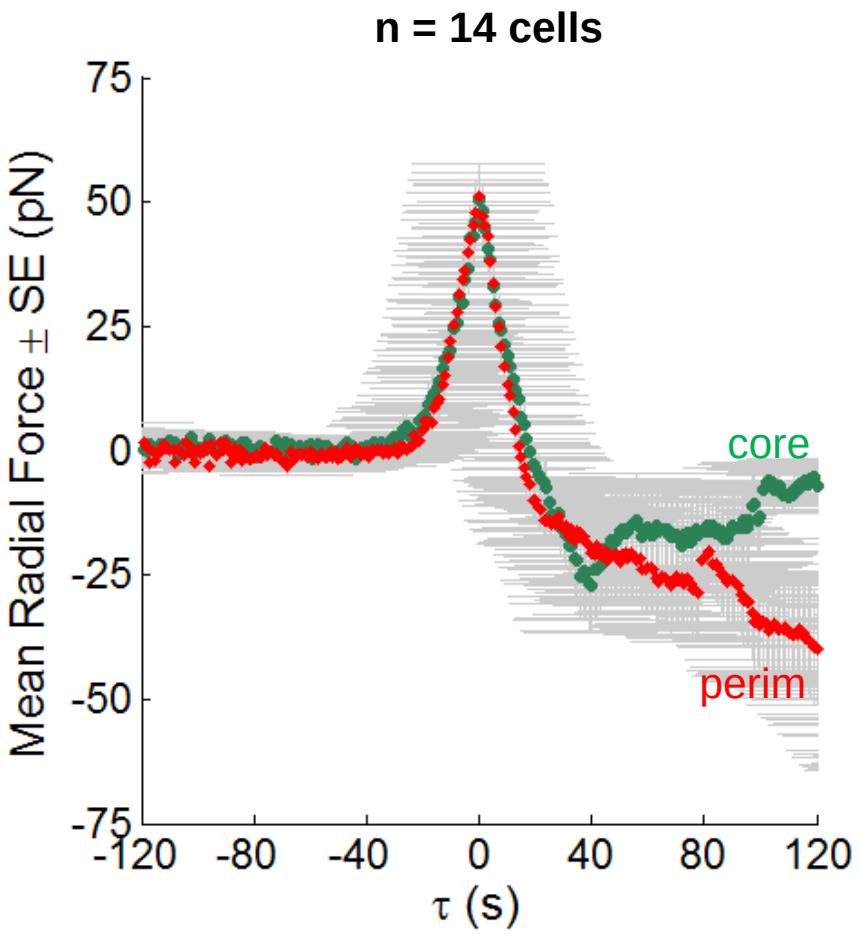
**Cell spreading as a protrusive wave

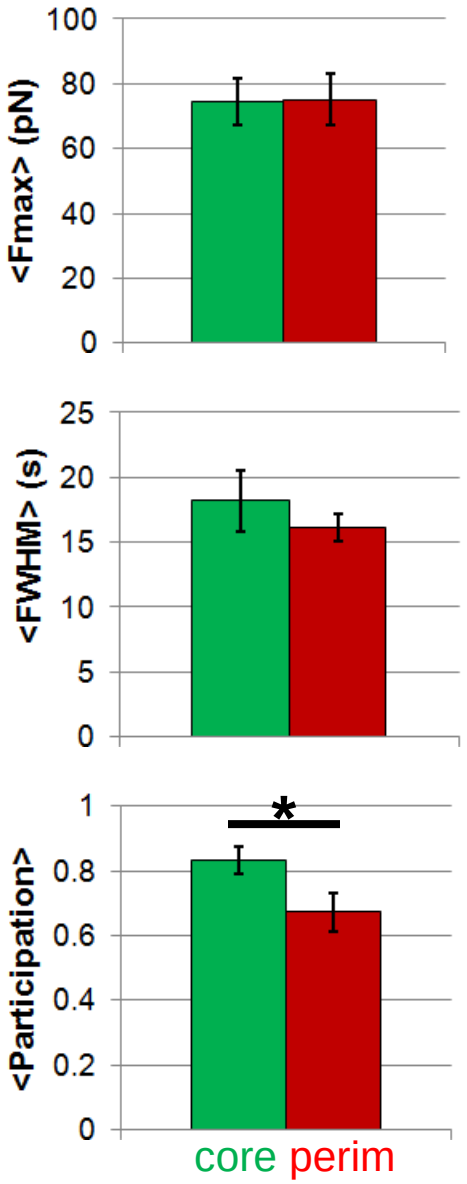




Spreading velocity = 170 nm / s

Characterizing the Protrusive Regime





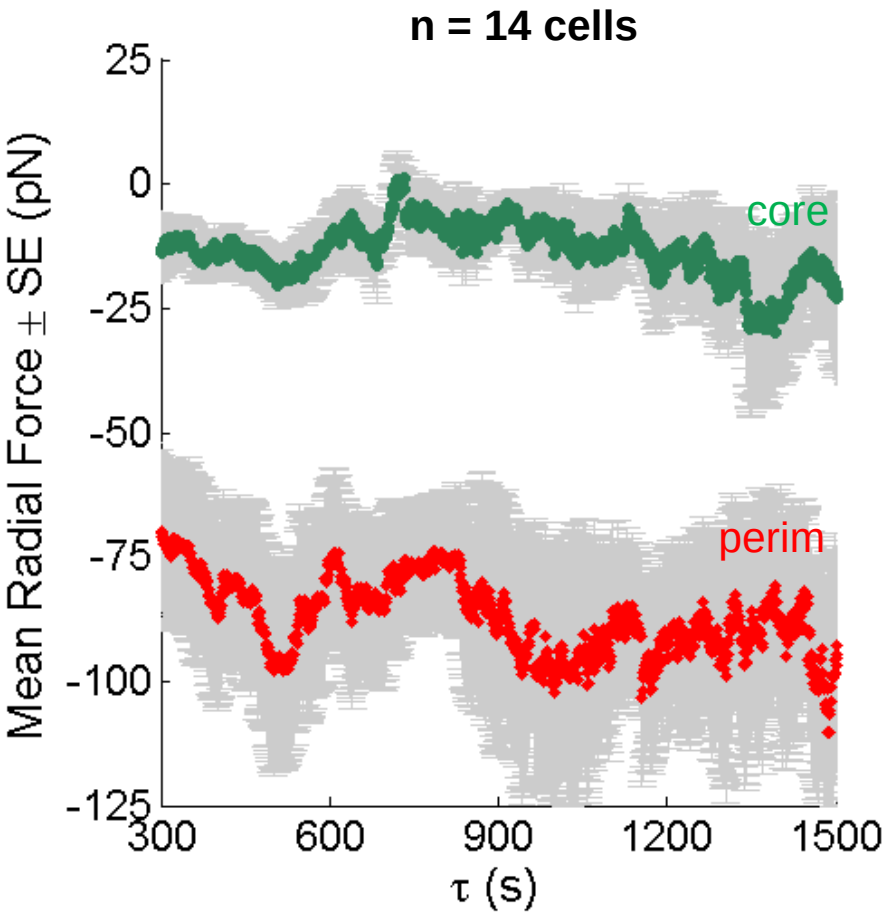
Protrusion force

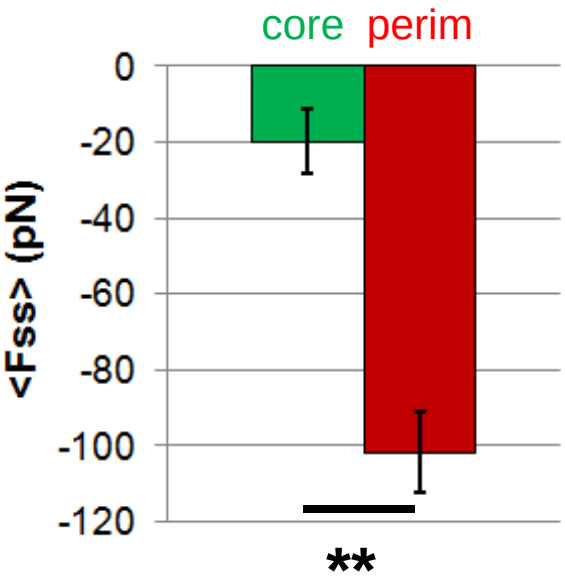
Protrusion duration

Posts with protrusive spike

* $p < 0.05$, unpaired t-test

Steady State Contractile Regime



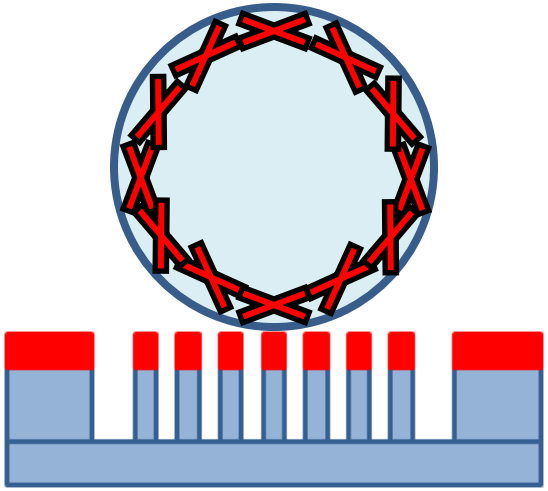


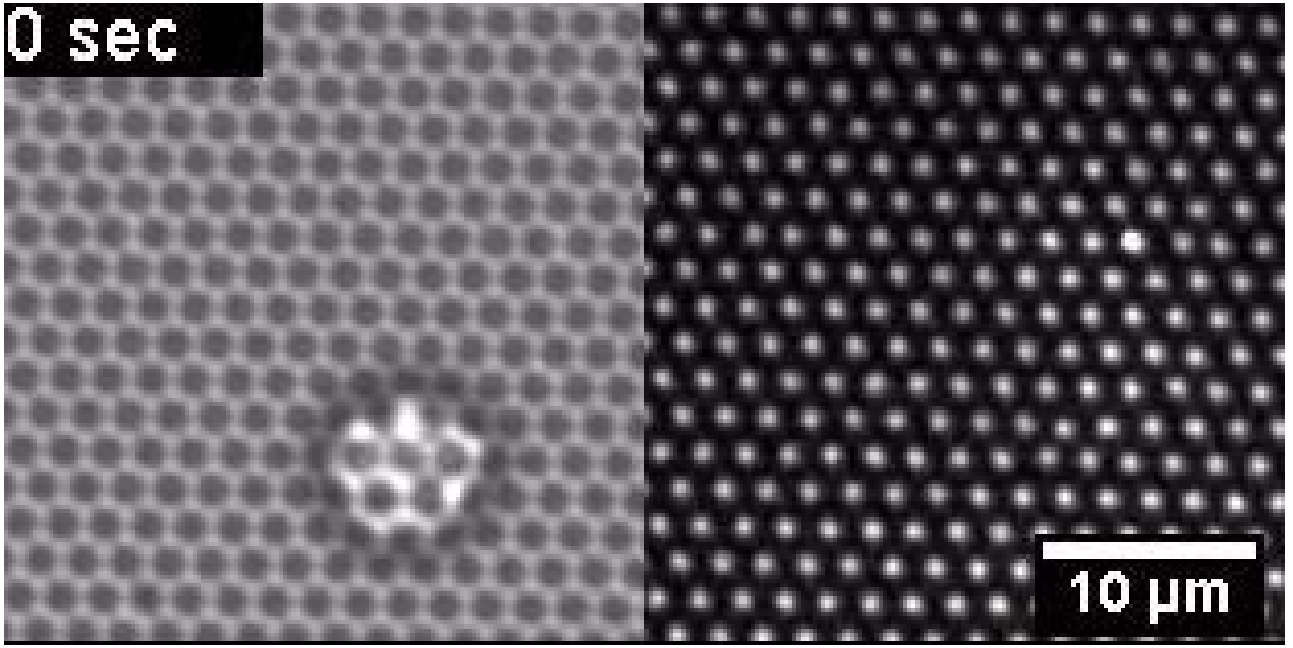
Steady State Contraction

** p < 0.01, unpaired t-test

**Actin rearrangement is essential, no spreading when depolymerization is inhibited

1 uM Jasplakinolide inhibits cortical actin depolymerization





Cross-linked filamenteous actin

Can we bias the protrusive and contractile regimes independently?

Hypothesis: Protrusion is lamellipodium formation

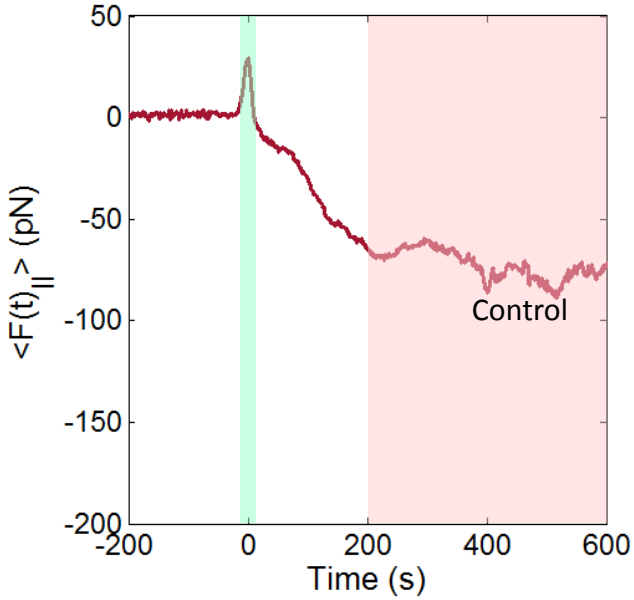
CK666 (1 uM) ⊣ Arp2/3

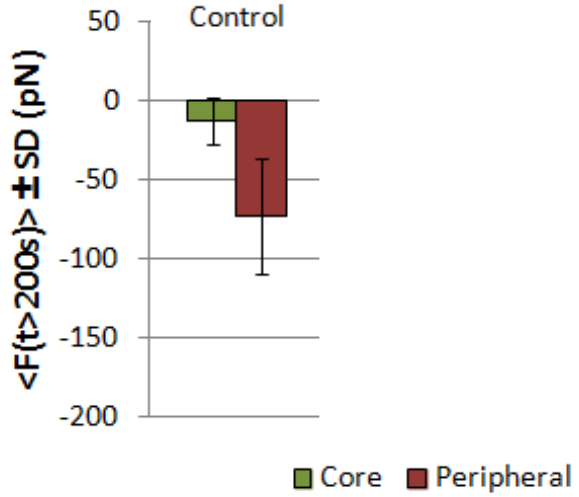
Hypothesis: Contraction is canonical RhoA/Rock and Myosin Mediated

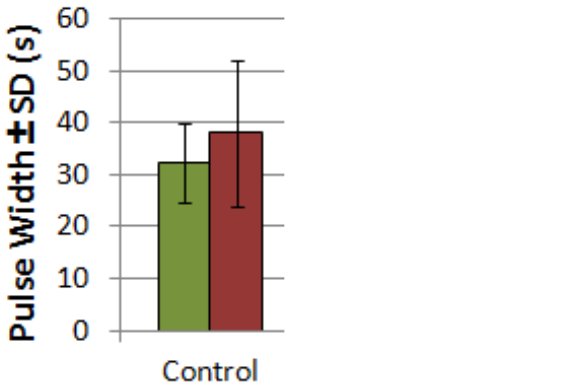
Y27632 (1 uM) ⊣ p160ROCK

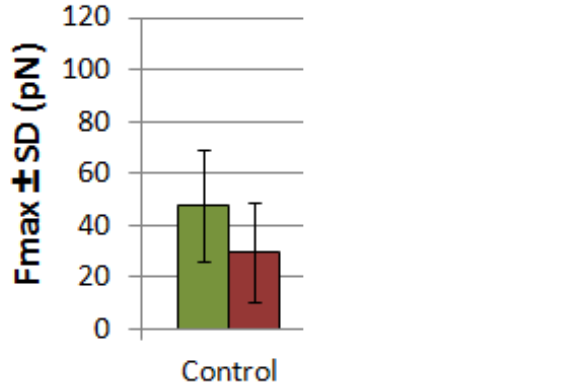
Blebbistatin (5 uM) ⊣ NM Myosin IIA

Looking for cytoskeletal inhibitor effects

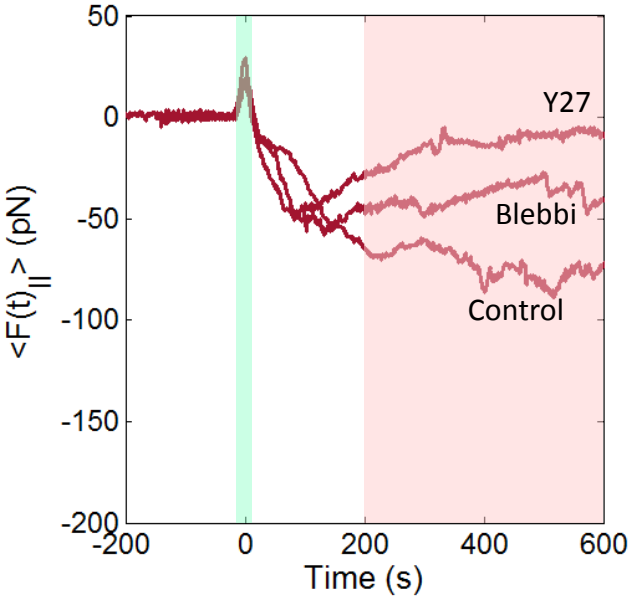


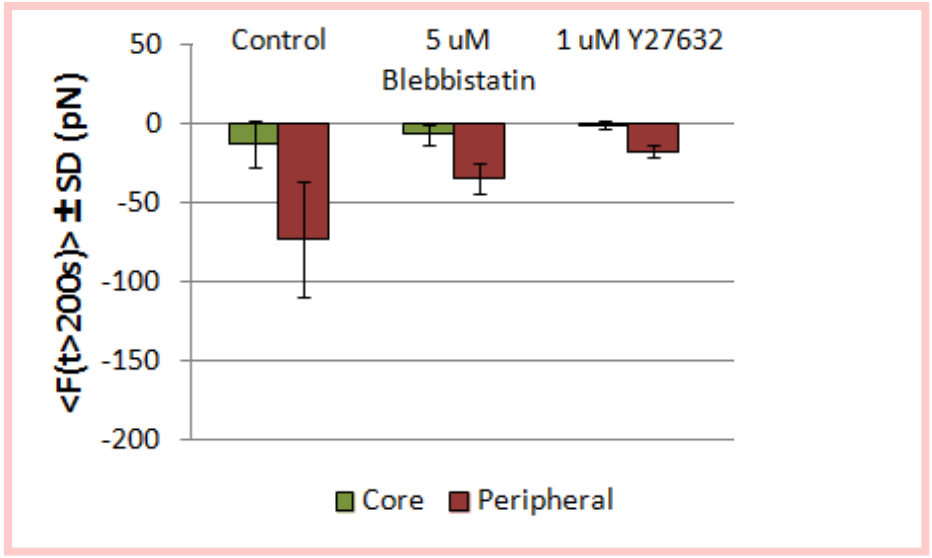


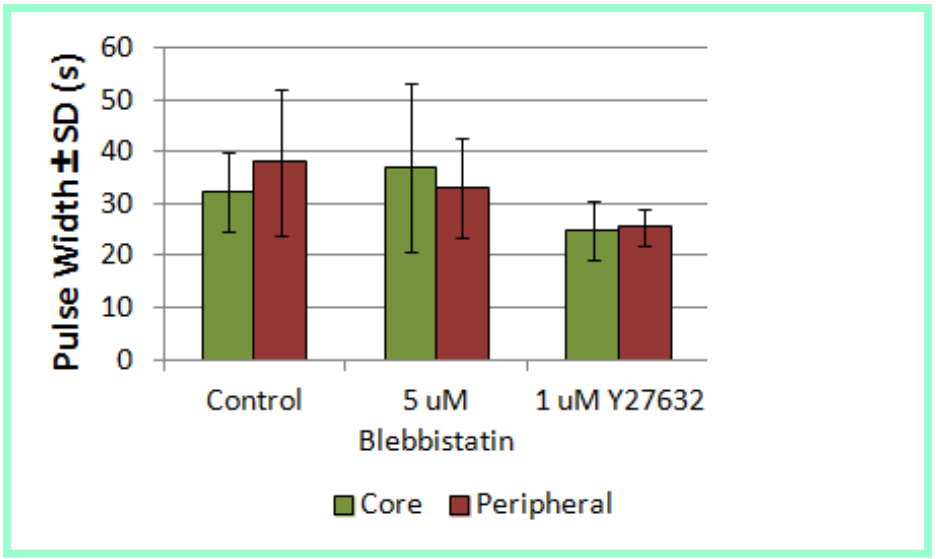


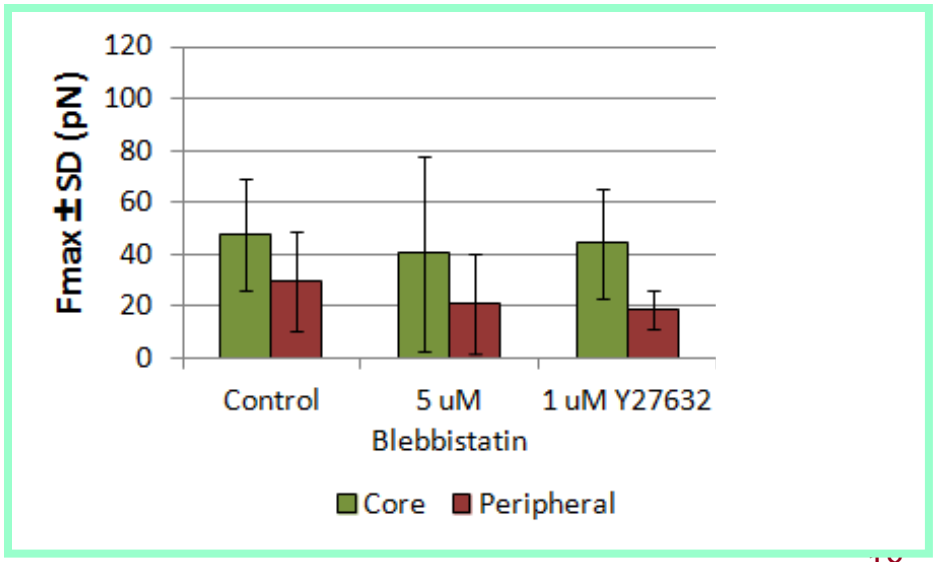


Y27632 & Blebbistatin interfere with contractility **maintenance**

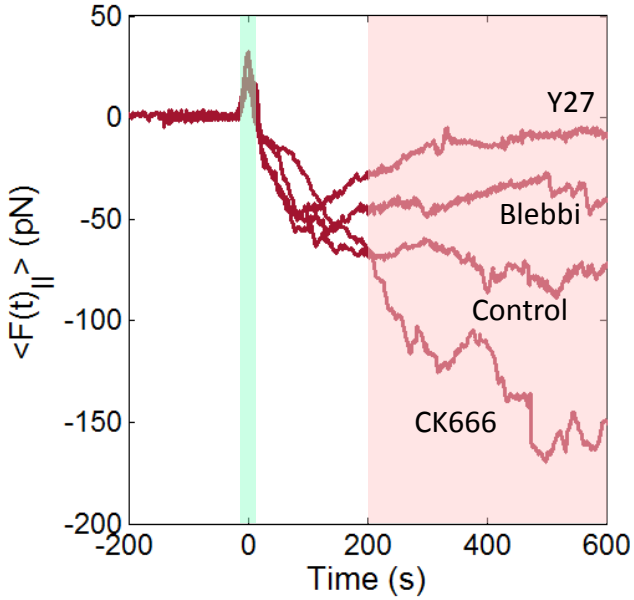


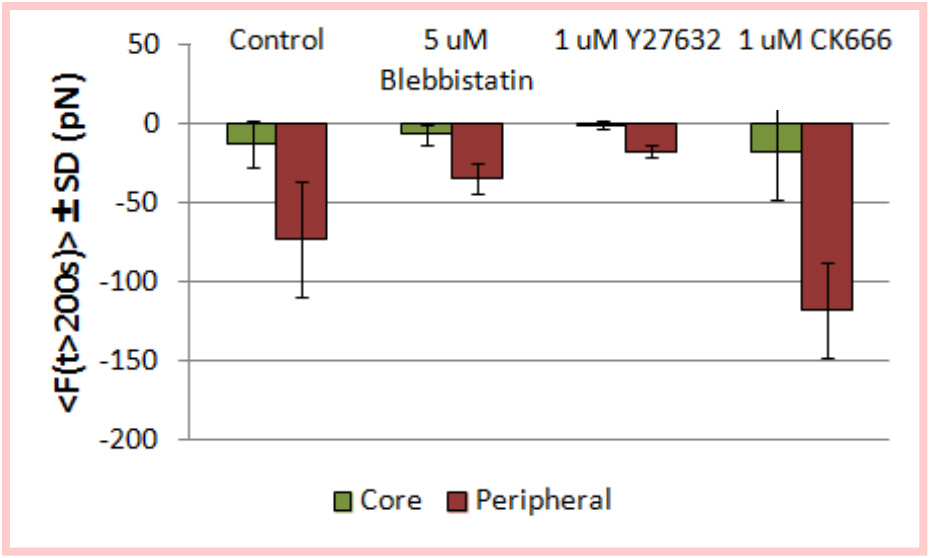


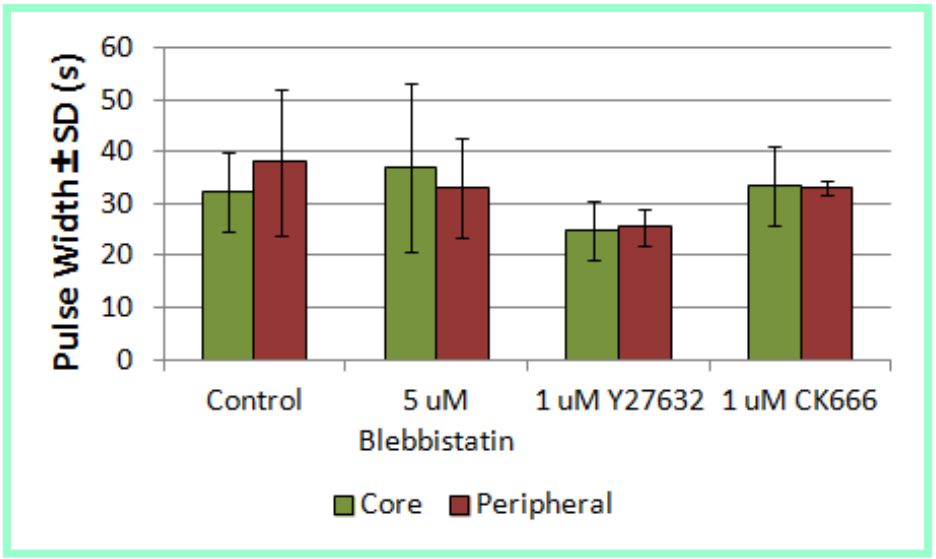


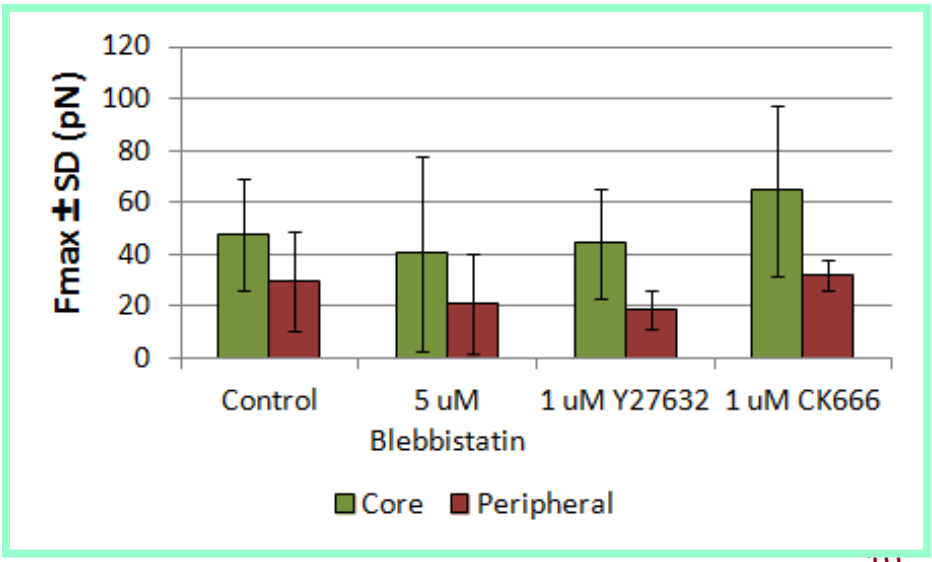


CK666 increases contractility, no effect on protrusion









Conclusions and Thank You!

- Post engagement highly dynamic
- Distinct behavior in periphery vs. core
- Two distinct mechanical regimes:
  - Short time: transient protrusion
  - Long time: steady state contraction
- Contractility maintenance:
  - Abrogated via Y27632 and Blebbistatin
  - Enhanced via CK666
- **Protrusion eliminated when actin can't depolymerize (Jasp) but not when Arp2/3 is inhibited (CK666)**