Biophysical Society
2015 Annual Meeting
Baltimore, MD

02/11/2015
Cell Mechanics, Mechanosensing, and Motility II
10:00 AM, Room 307/308

** Denotes movies played during talk

Dynamic Traction Forces of Human Neutrophil Adhesion

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

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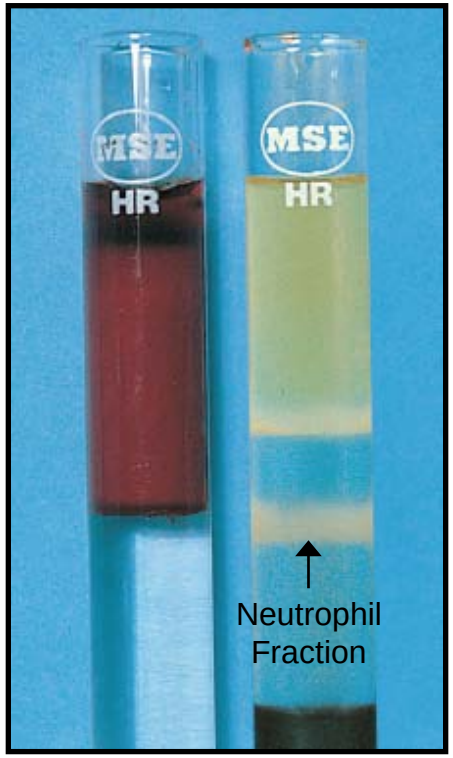
NIH HL18208 to DAH
NSF GRFP to SJH
Note:
Manuscript submitted to *Biophysical Journal* for review

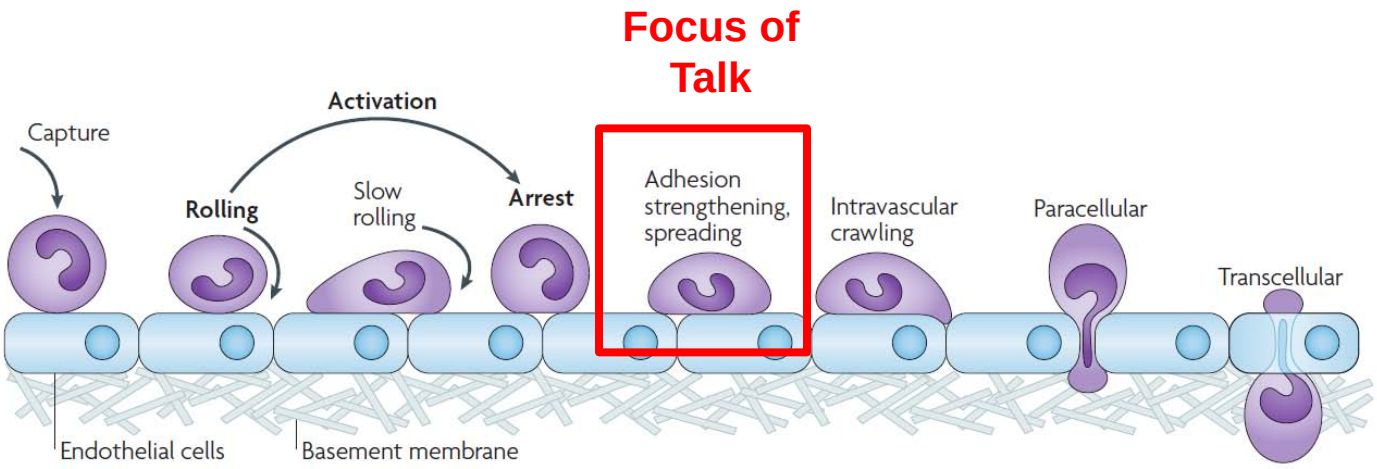
Penn
University of Pennsylvania

Neutrophils: first responders to trauma and infection

Whole Blood | After Density Centrifugation

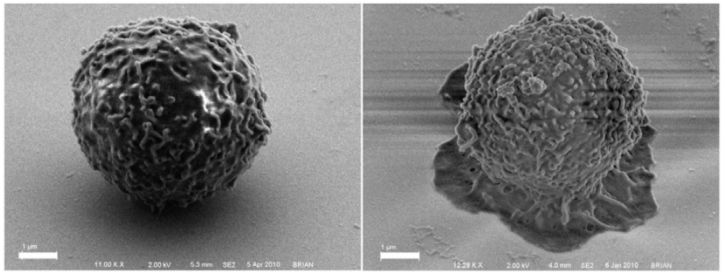


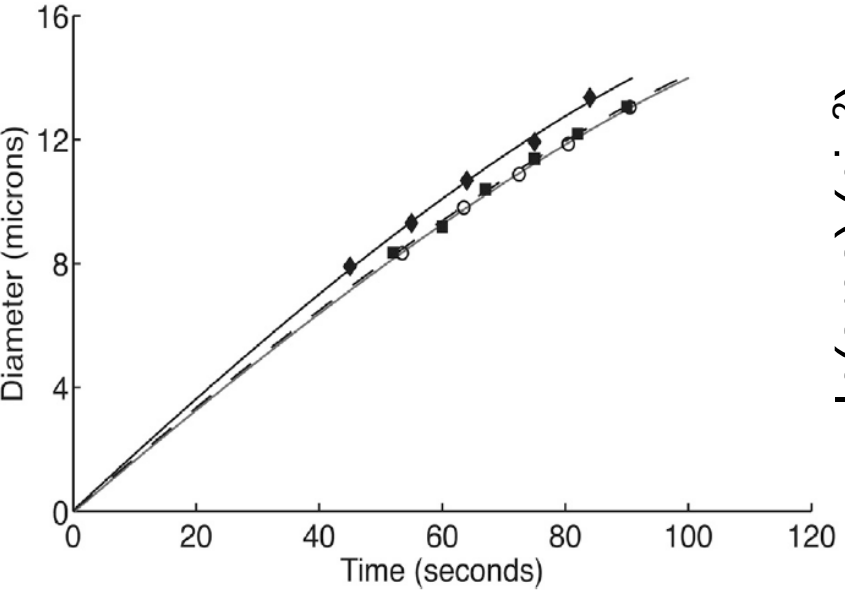
Axis-Shield News Bulletin



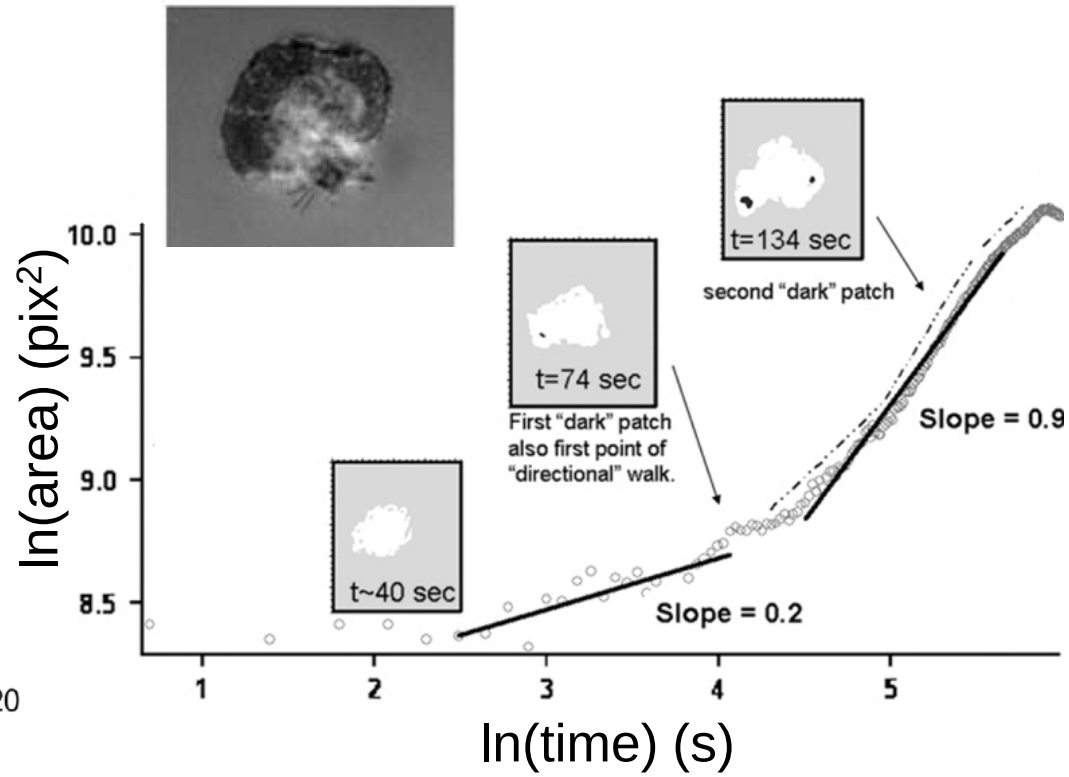
Ley et al. 2007. *Nat Rev Immunol.*

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



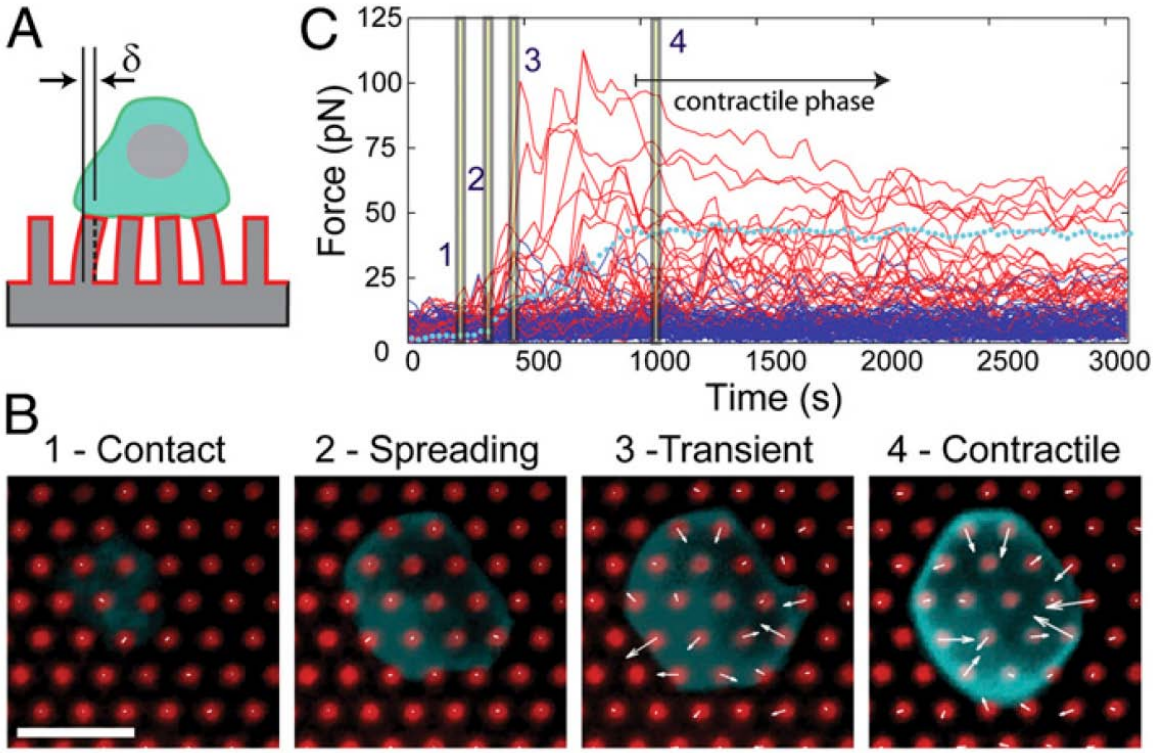


Lomakina et al. 2014. *Biophys J.*
(Waugh Lab)



Sengupta et al. 2006. *Biophys J.*
(Hammer Lab)

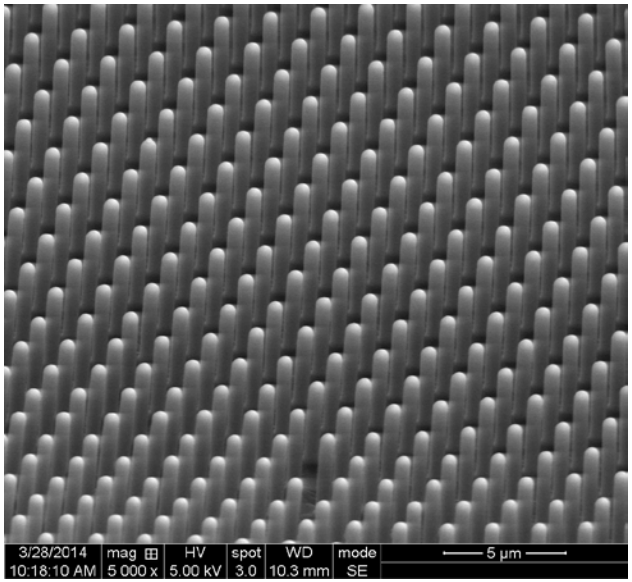
Traction forces of T-lymphocyte activation on microfabricated Post-Array-Detectors (mPADs)

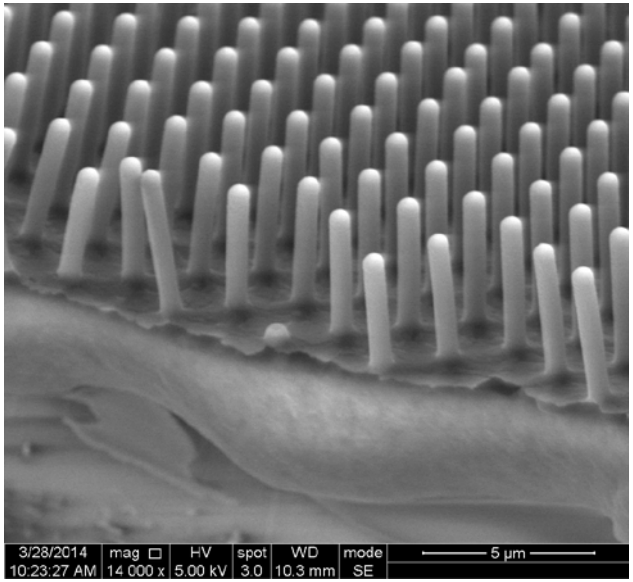


Bashour et al. 2014. *PNAS* (Kam Lab)

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

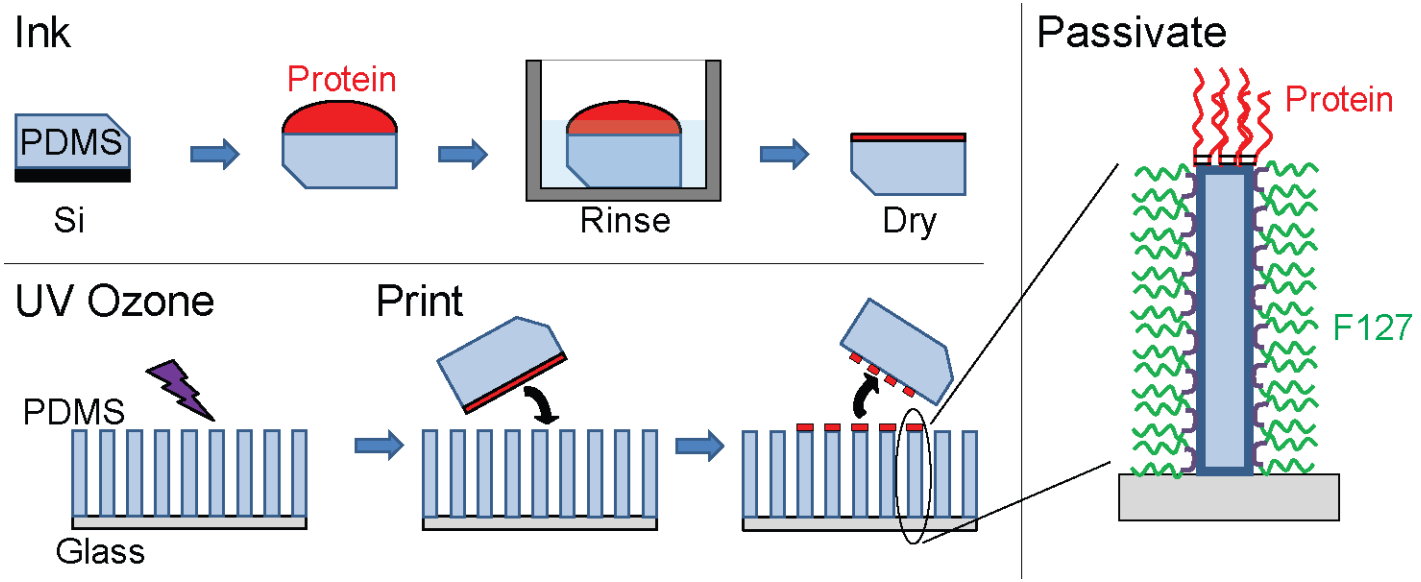
microfabricated Post-Array-Detectors (mPADs)



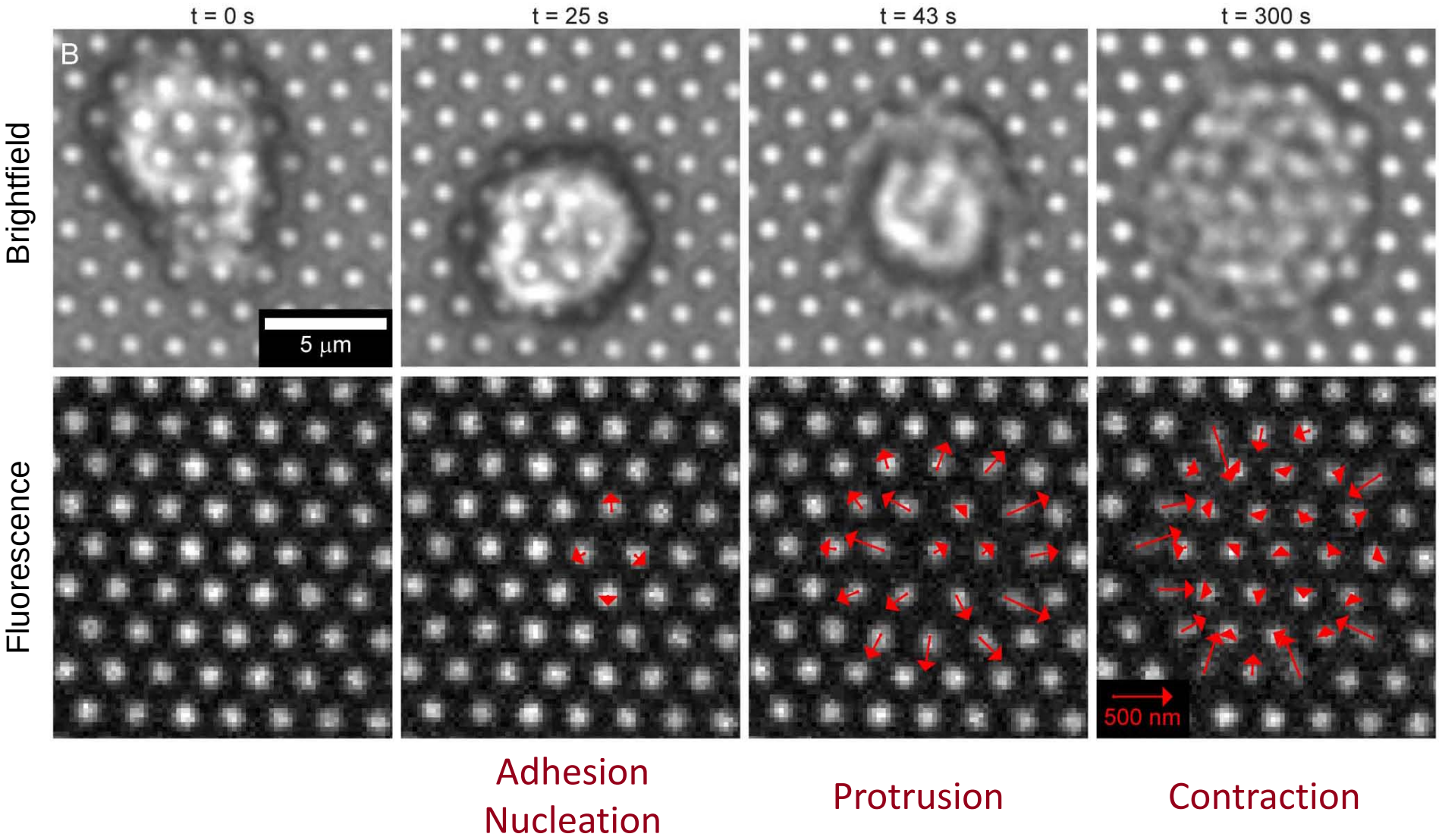


$k_{spring} = 0.28 \pm 0.07$ nN/um
G ~ 5 kPa

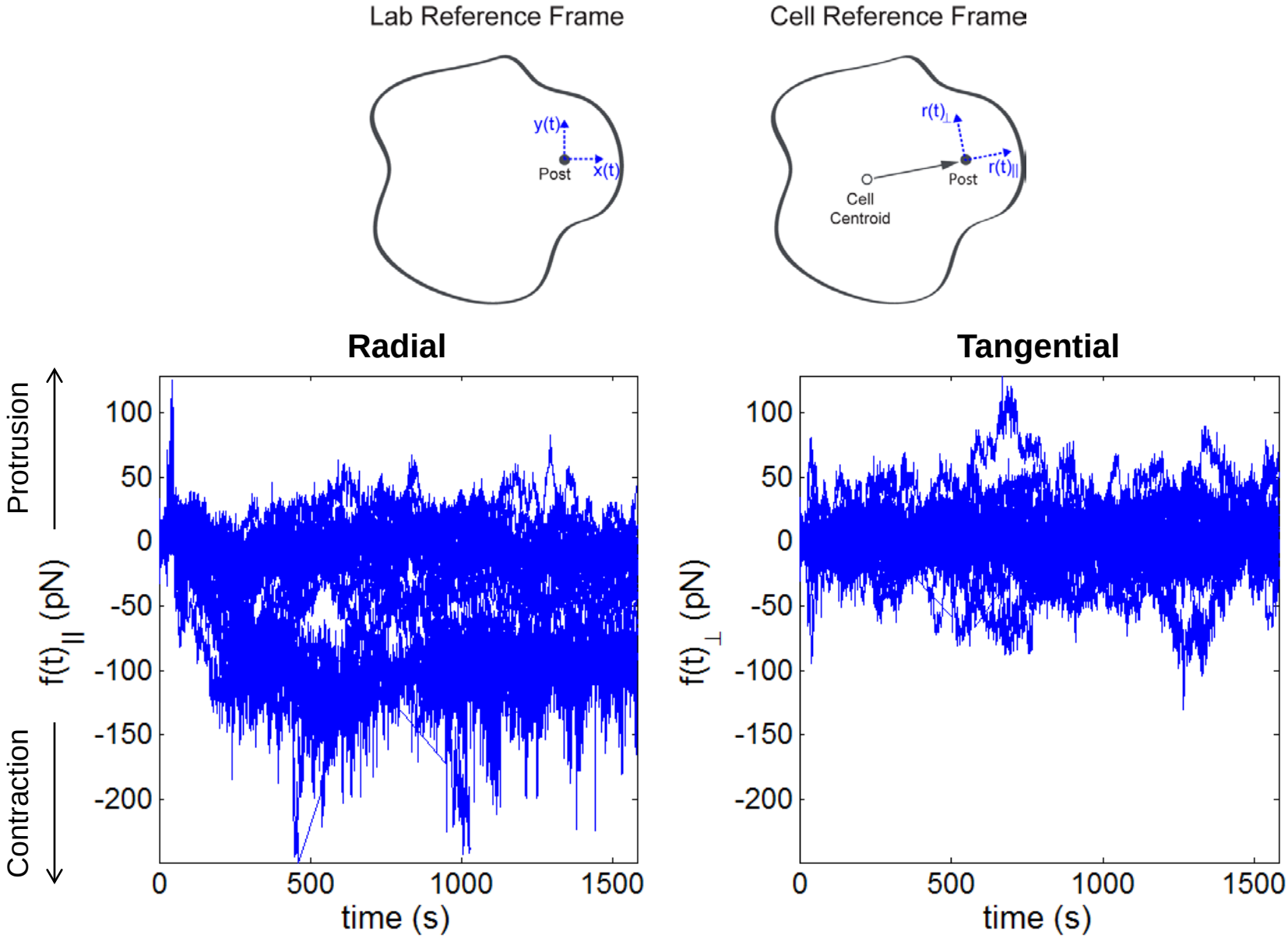
Schoen correction = 0.93
$k^*_{spring} = (0.93)(k_{spring})$
$k^*_{spring} = 0.26$ nN/um
Schoen et al. 2010. *NanoLett*.



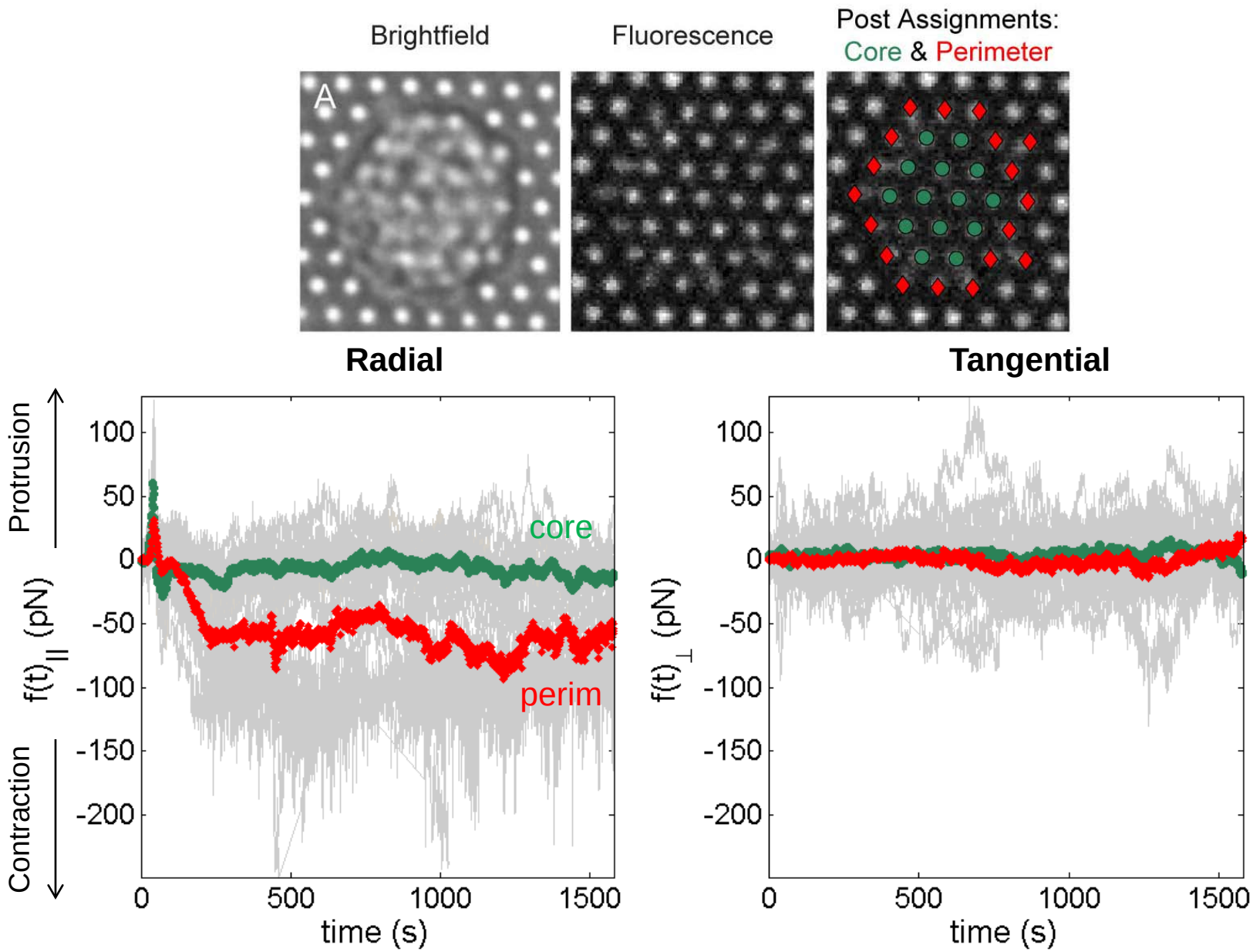
**Neutrophil spreading on mPADs



Plotting force trajectories in the cell reference frame



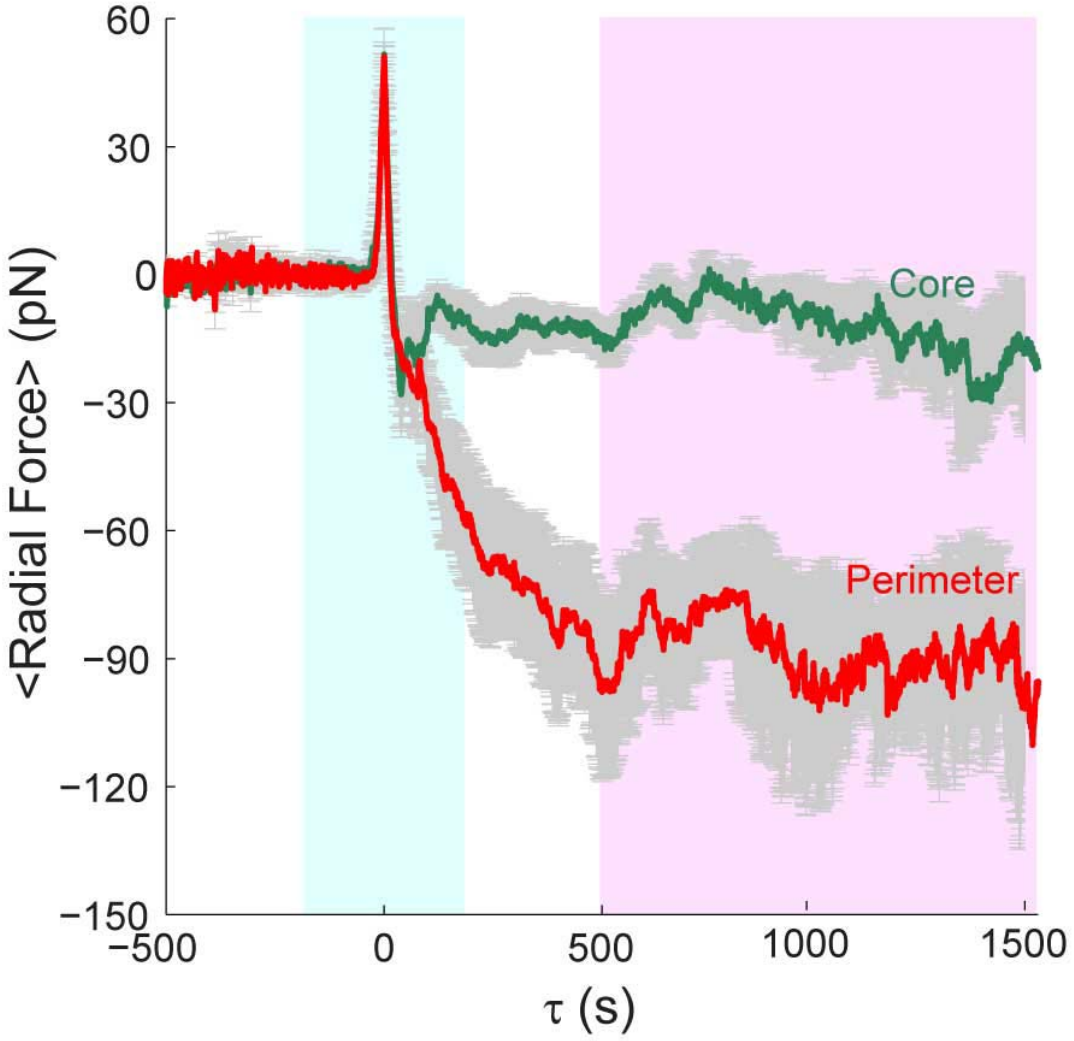
Dichotomizing data on geometric location



Distinct mechanical regimes are apparent

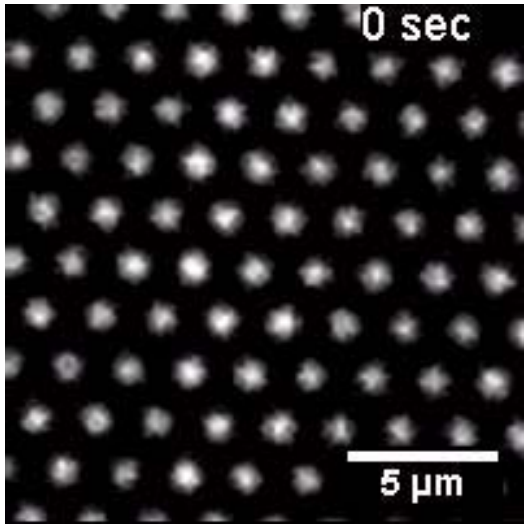
Transient Protrusion

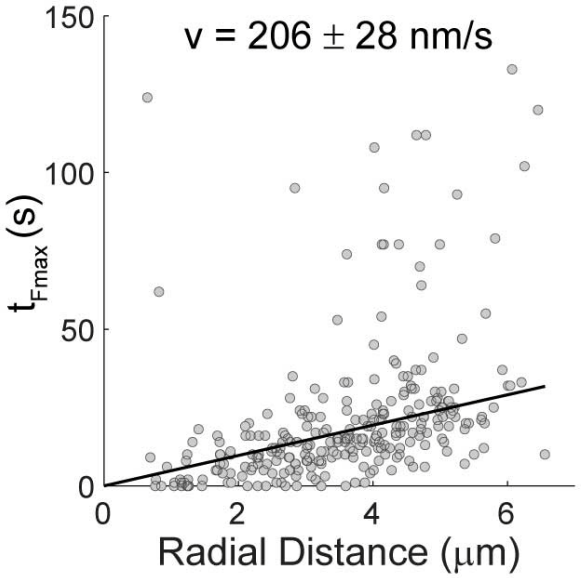
Steady State Contraction

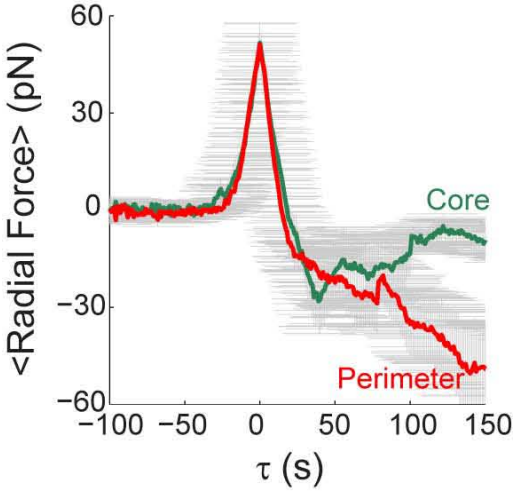


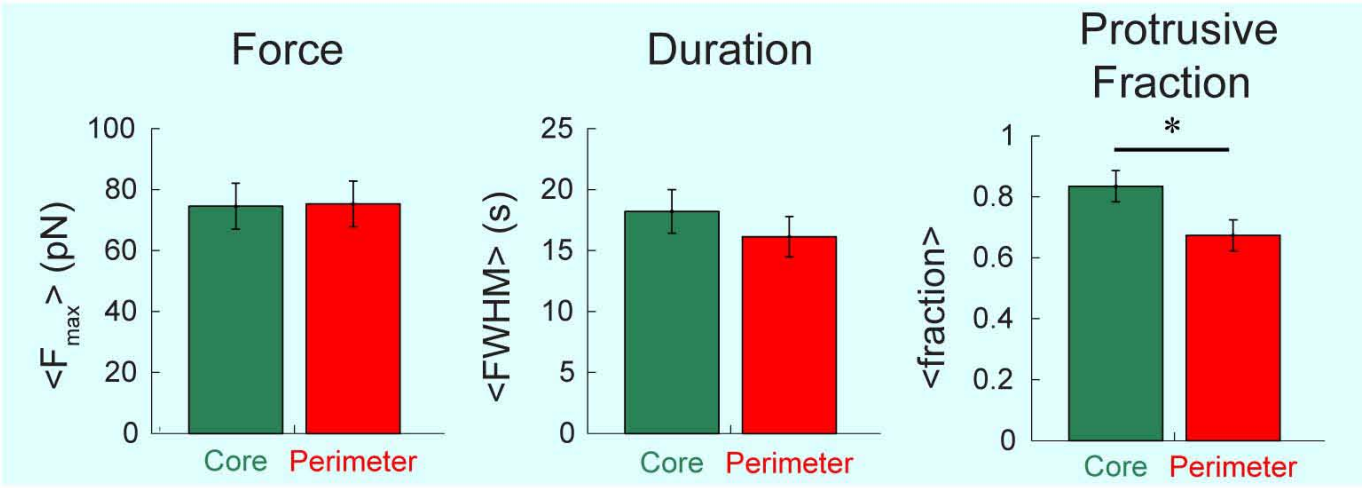
n = 14 cells

**Characterizing the protrusive wave



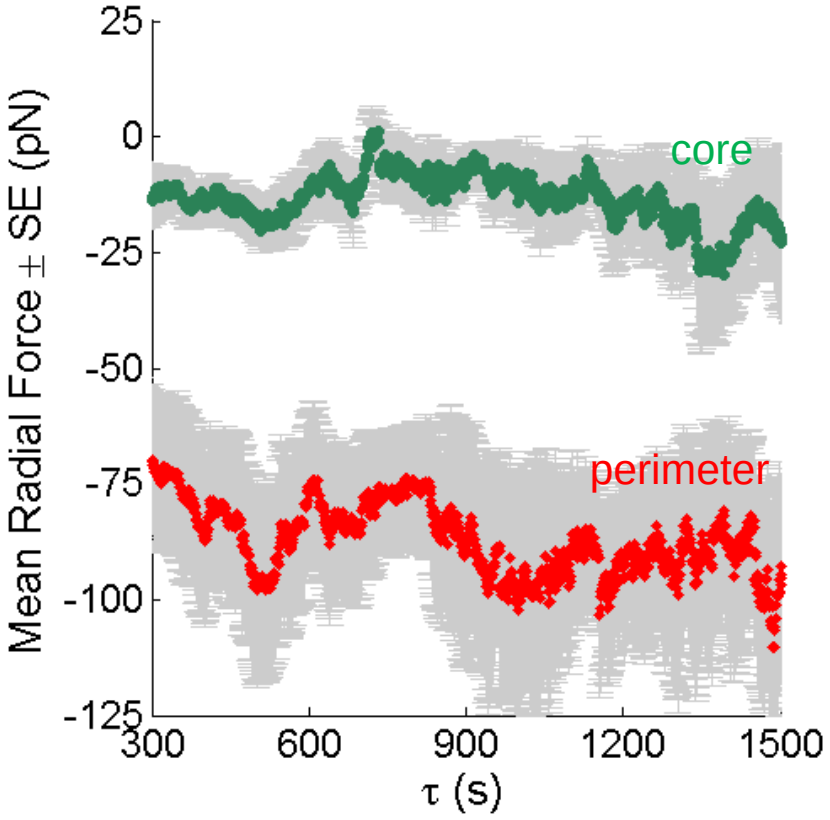


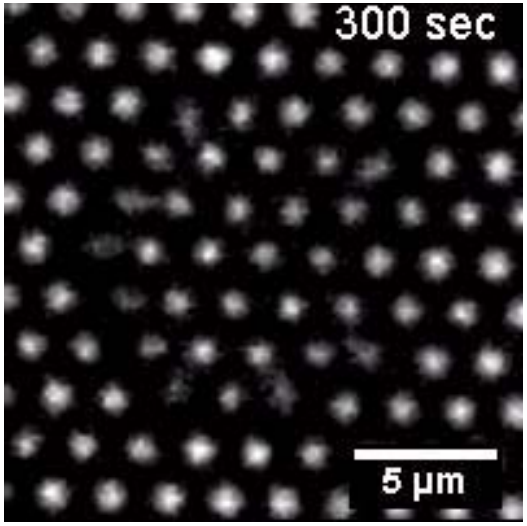


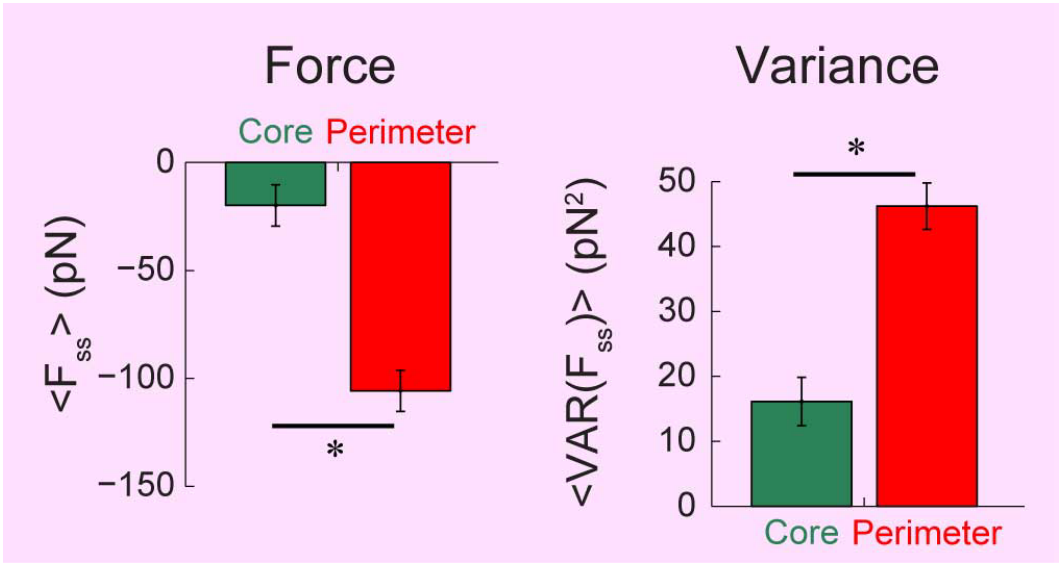


* p < 0.05, Tukey LSD method, n = 14 cells

**Characterizing the Steady State Contractile Regime







* p < 0.05, Tukey LSD method, n = 14 cells

Are protrusion and contraction biochemically distinct?

Hypothesis: Contraction is canonical RhoA/Rock and Myosin Mediated

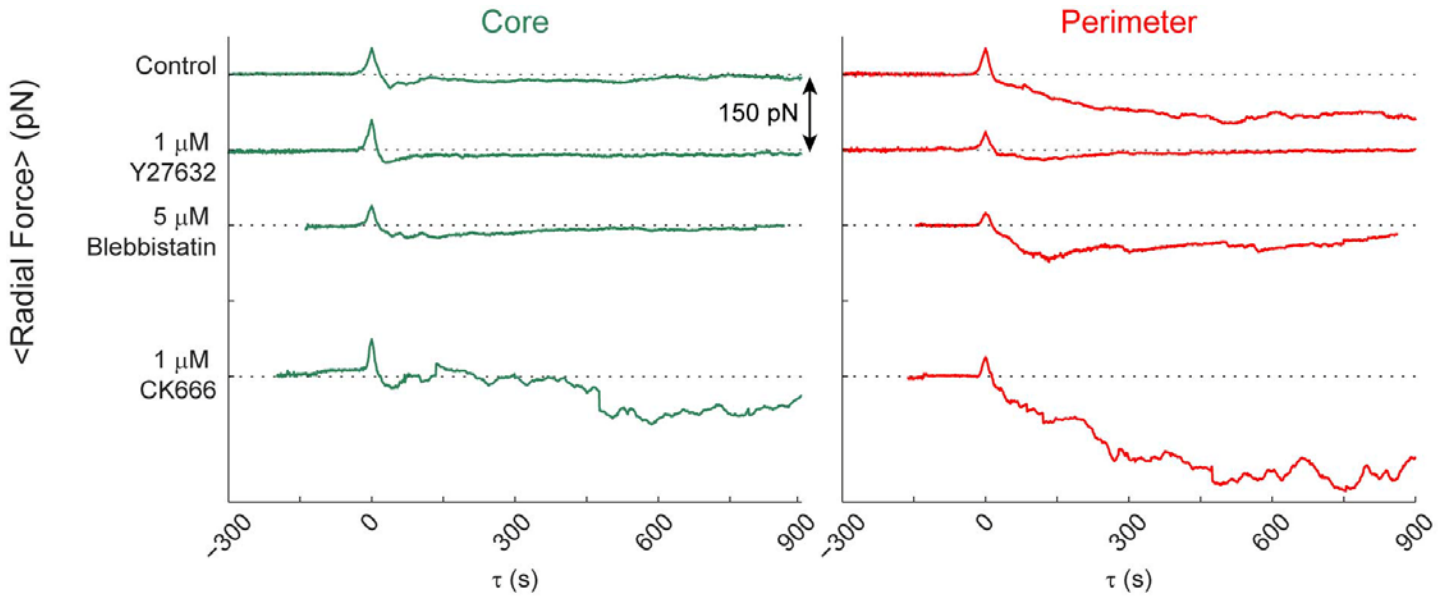
Y27632 (1 uM) ⊣ p160ROCK

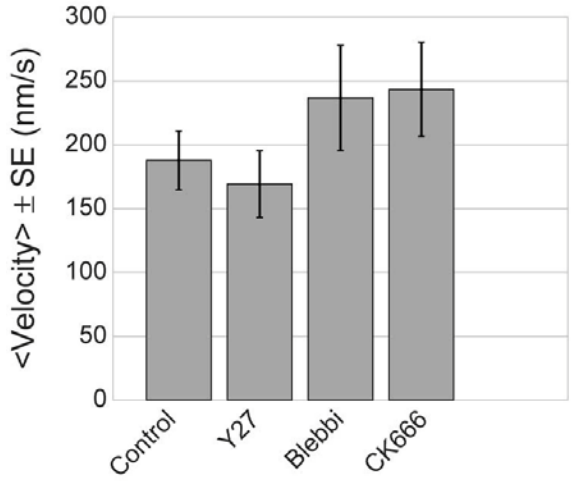
Blebbistatin (5 uM) ⊣ NM Myosin II

Hypothesis: Protrusion is lamellipodium formation

CK666 (1 uM) ⊣ Arp2/3

Sustained contractility ROCK and Myosin II mediated but spreading **not** analogous to lamellipodium formation



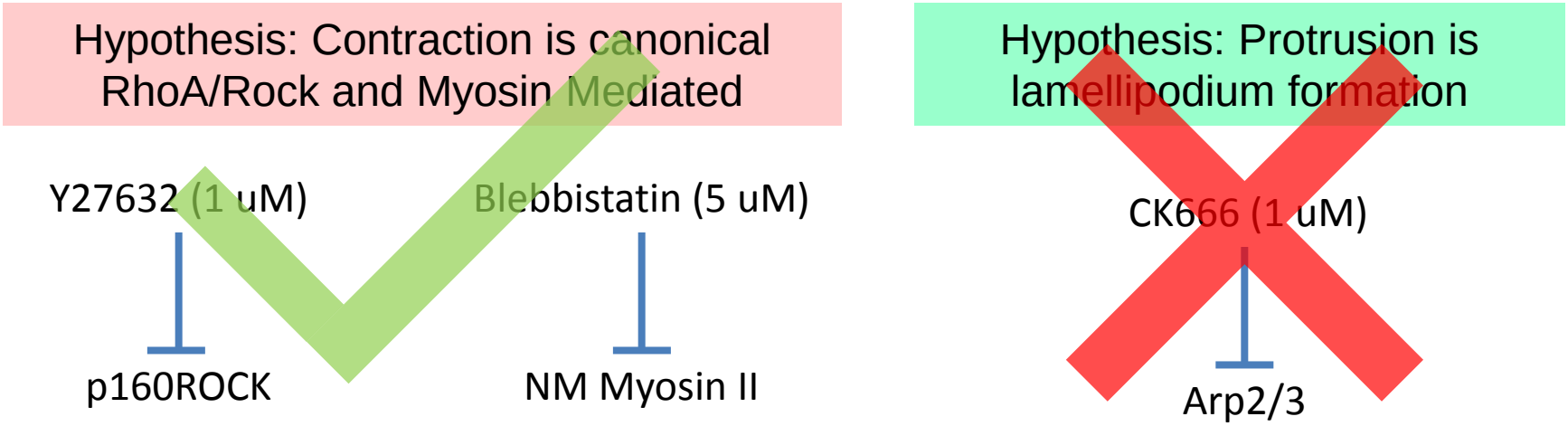


		Y27	Blebbi	CK666
n cells		10	4	5
n posts		325	117	161
Protrusion	<Fmax>	Core / Perimeter		
	<FWHM>	Core / Perimeter		
	<VAR(Fmax)>	Core / Perimeter		
	<Participation>	Core / Perimeter		
	<Velocity>	All Posts		
Contraction	<Fss>	Core / Perimeter	*↓	*↓
	<VAR(Fss)>	Core		*↑
		Perimeter	*↓	

= no sig. diff.

* $p < 0.05$, Tukey-Kramer multi. comp.

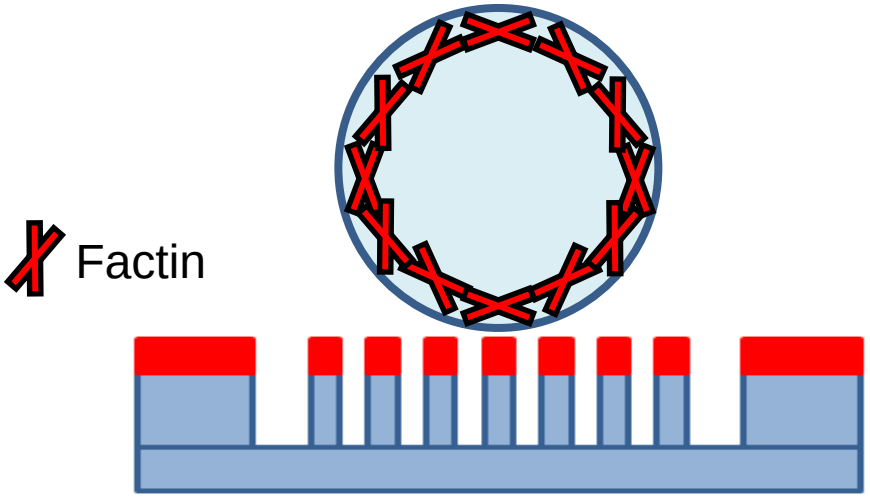
Spreading is not analogous to lamellipodium formation



Competition b/n adhesive energy and cortical stiffness?

Tension > Adhesive Energy

Tension < Adhesive Energy

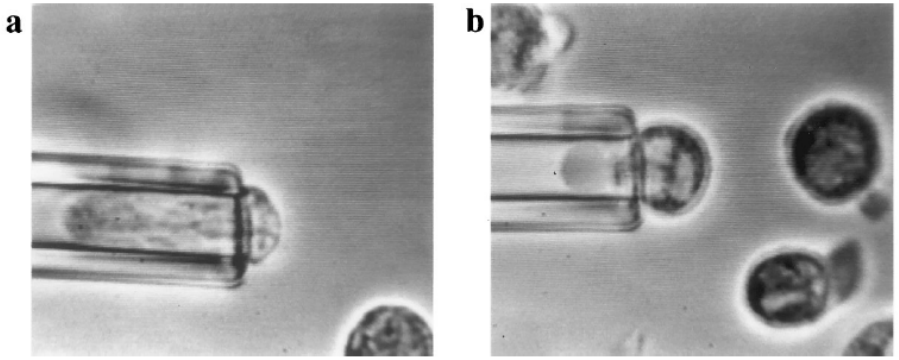


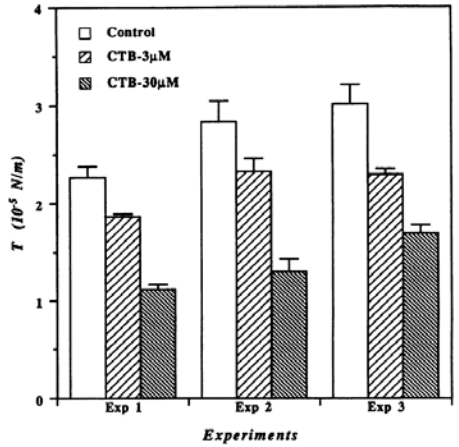
Jasplakinolide = stiffening

Cytochalasin B = softening

Control

10 uM

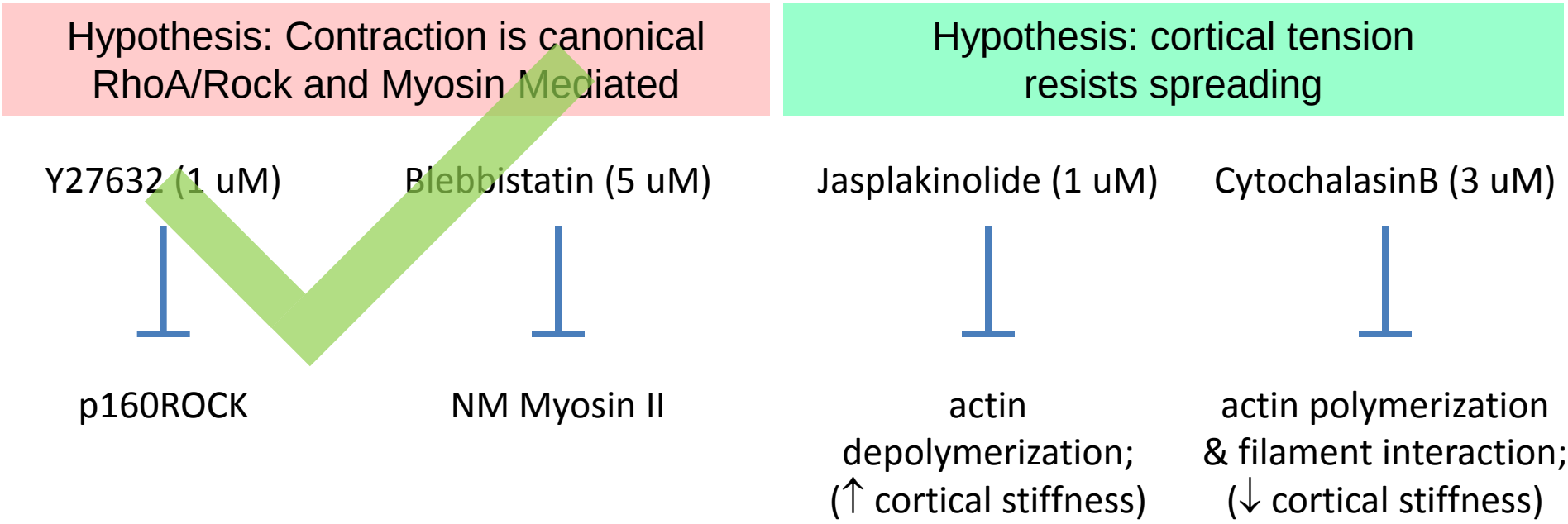




Sheikh et al. 1997. *BBRC.* (Nash Lab)

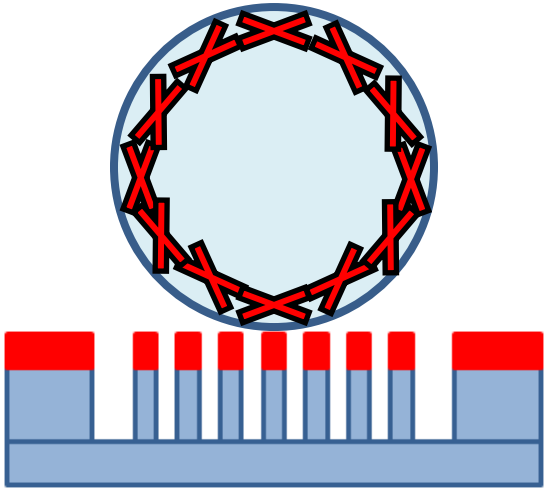
Tsai et al. 1994. *Biophys J.* (Waugh Lab)

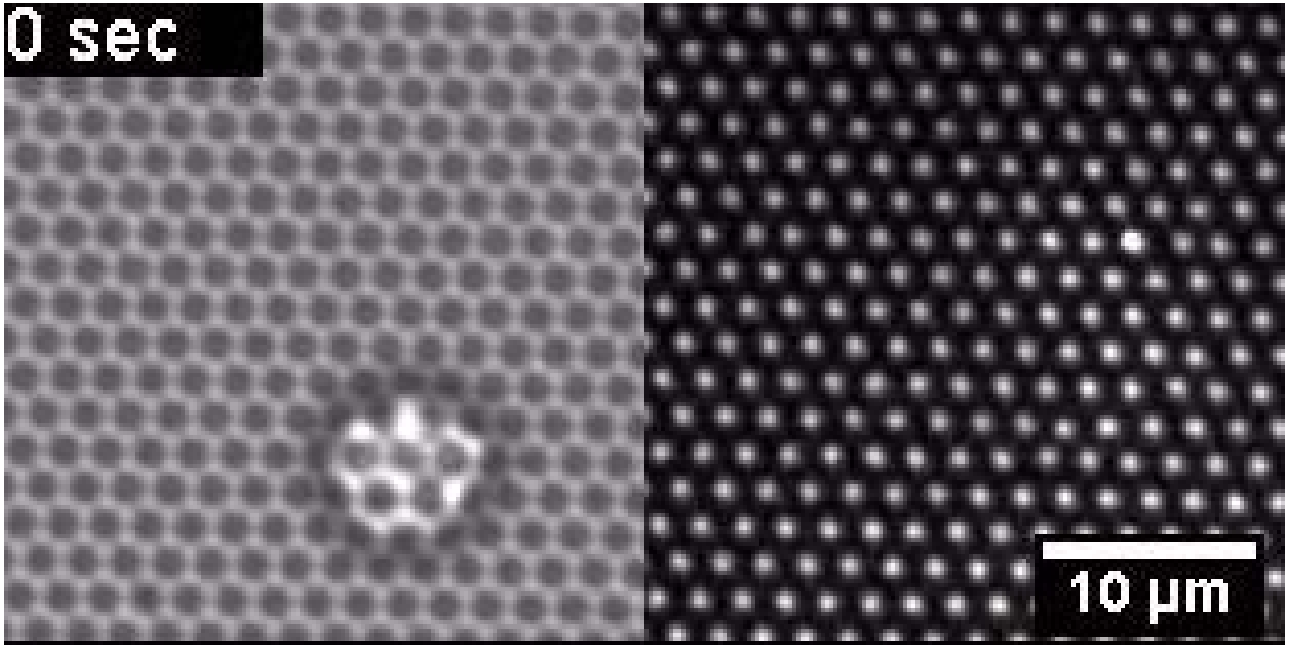
A revised hypothesis:



**Cortical stiffening via Jasplakinolide abrogates spreading

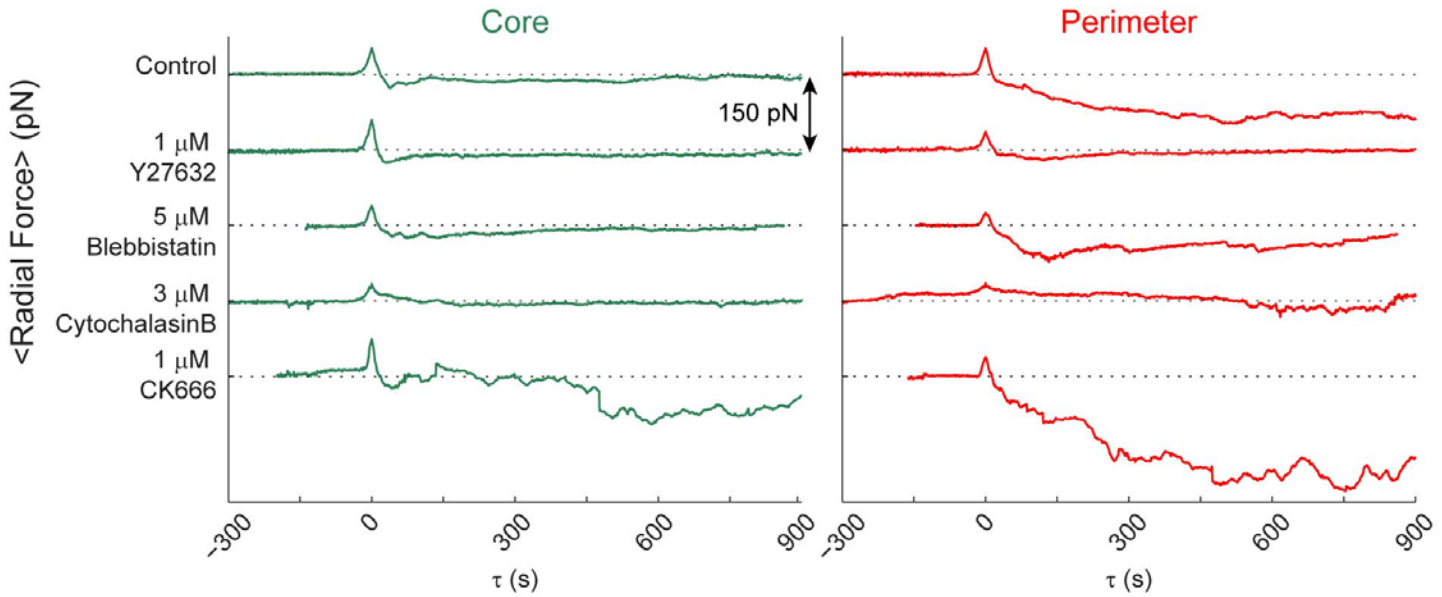
1 uM Jasplakinolide inhibits cortical actin depolymerization

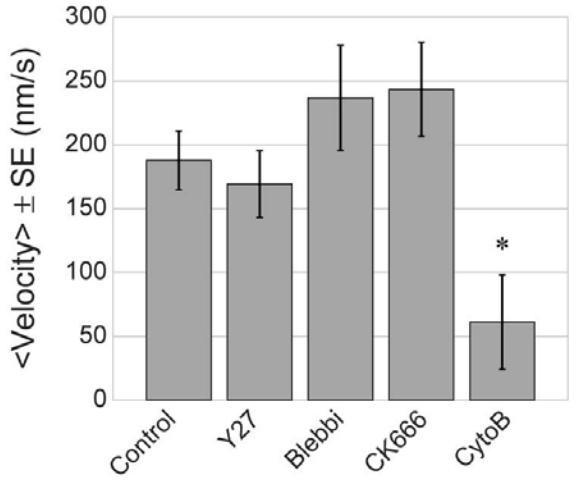




Cross-linked filamenteous actin

Cortical softening **slows** spreading
…implies release of pre-stress is important



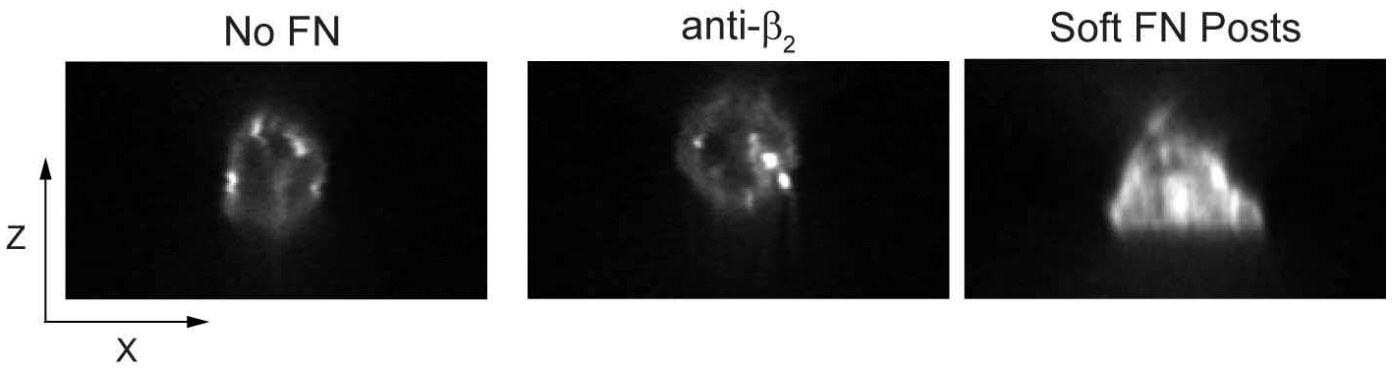


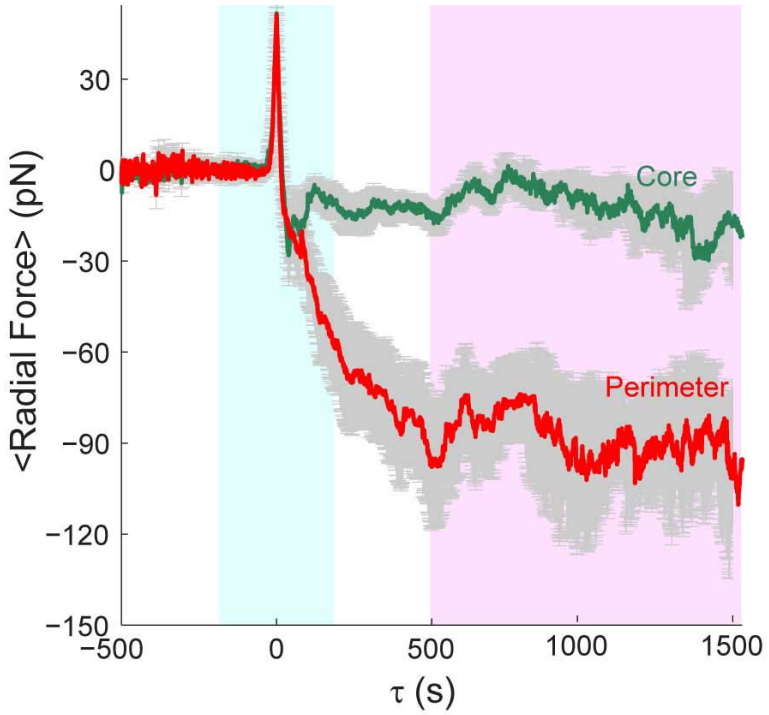
		Y27	Blebbi	CK666	Jasp	CytoB
	n cells	10	4	5	9	6
	n posts	325	117	161	n/a	177
Protrusion	<Fmax> Core				No Spreading	
	<Fmax> Perimeter					
	<FWHM> Core					*↑
	<FWHM> Perimeter					*↑
	<VAR(Fmax)> Core					
	<VAR(Fmax)> Perimeter					
	<Participation> Core					
	<Participation> Perimeter					
	<Velocity> All Posts					*↓
Contraction	<Fss> Core					
	<Fss> Perimeter	*↓	*↓			*↓
	<VAR(Fss)> Core			*↑		
	<VAR(Fss)> Perimeter	*↓				

= no sig. diff.

* $p < 0.05$, Tukey-Kramer multi. comp.

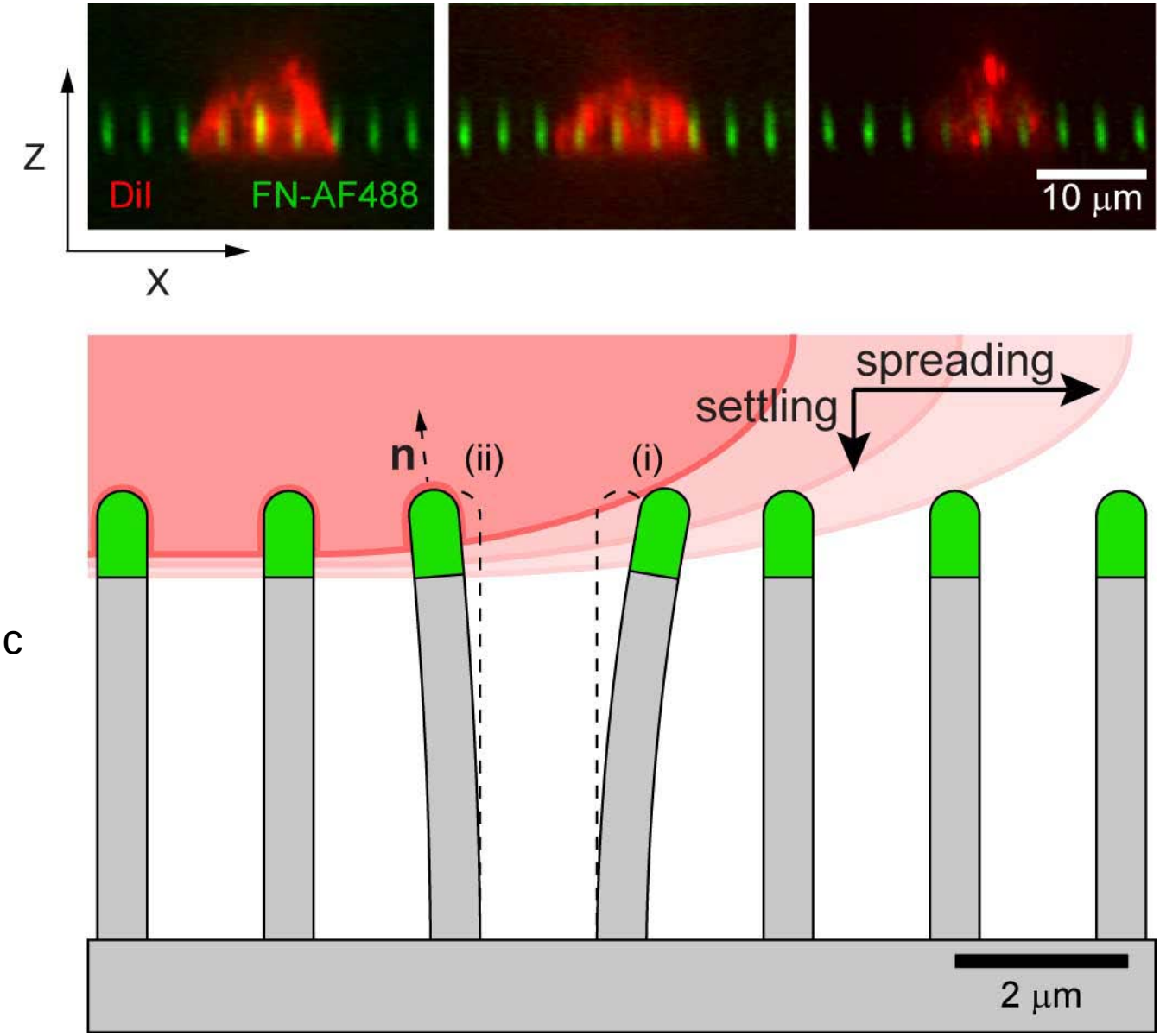
Spreading is integrin-mediated but connection to the actomyosin substructure takes minutes to develop





Why do we see protrusion at all?

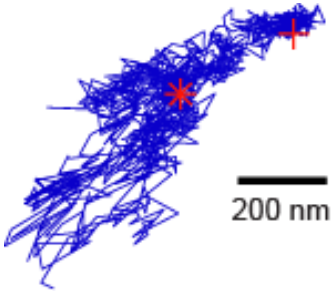
Invagination: a spreading neutrophil plows through posts

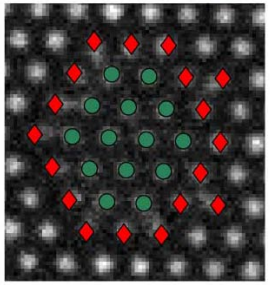


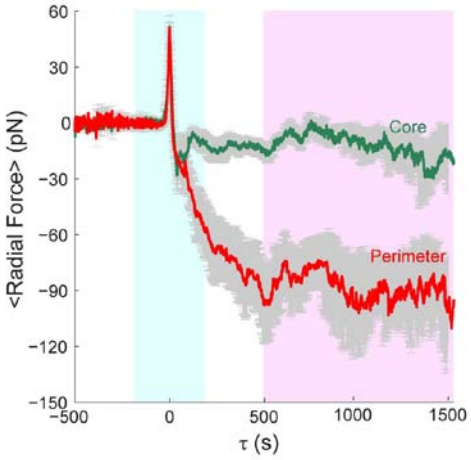
Schematic to scale

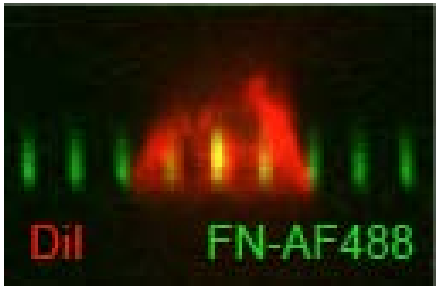
Conclusions and Thank You!

- Two mechanical regimes:
  - Short time: transient protrusion
  - Long time: steady state contraction
- Contractility maintenance via canonical ROCK/myosin II pathways
- Resting cortical tension and release are critical to spreading
- Invagination of post arrays reports force of shape change









Manuscript submitted to *Biophysical Journal* for review