Biophysics of Human Neutrophil Haptokinesis

Steven J. Henry

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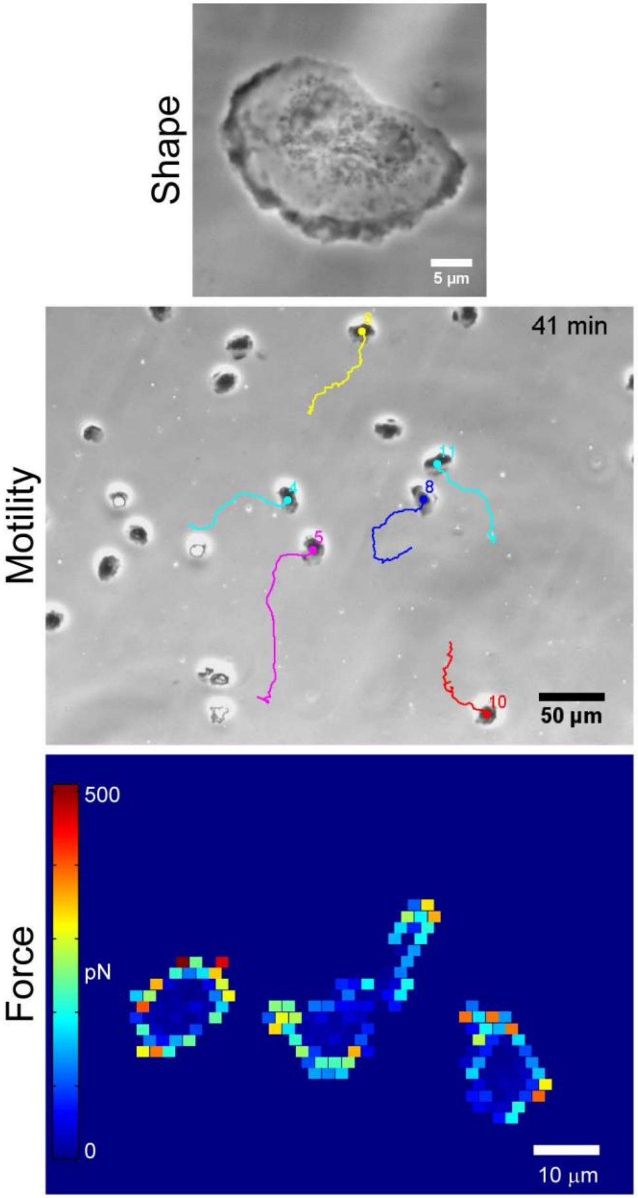
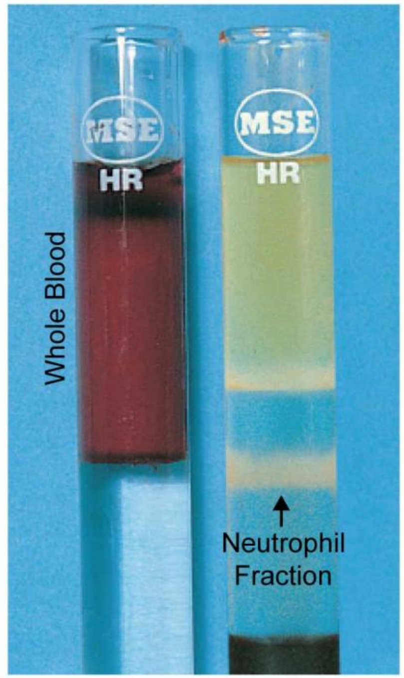
Committee:
Daniel A. Hammer, PhD (advisor)
Scott L. Diamond, PhD (chair)
John C. Crocker, PhD
Dongeun Huh, PhD

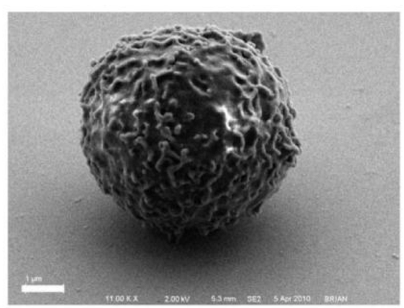
Penn
University of Pennsylvania

Biophysics of Human Neutrophil Haptokinesis

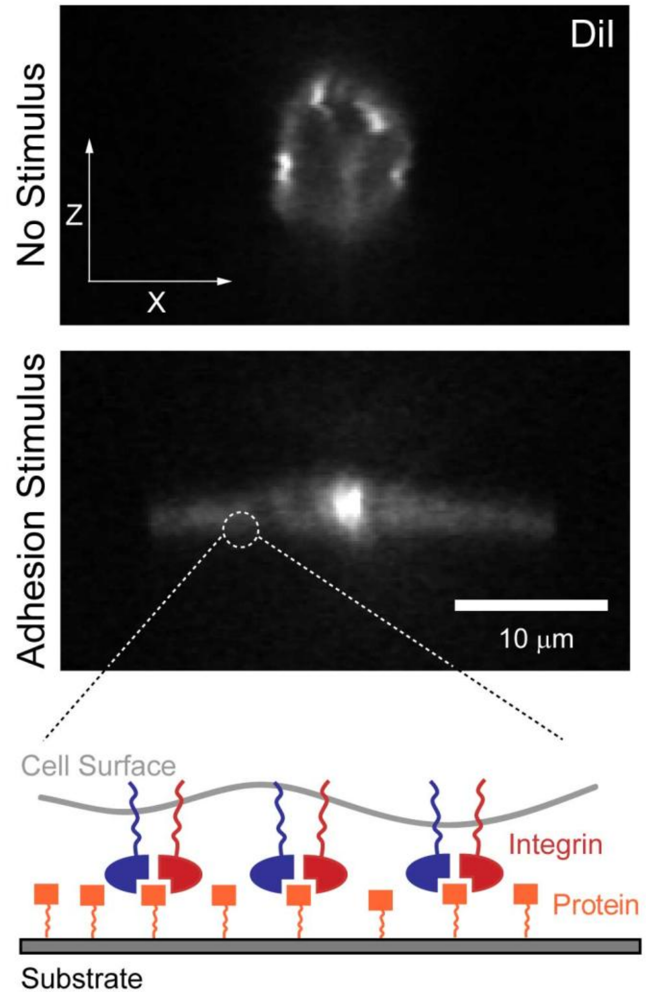




Axis-Shield

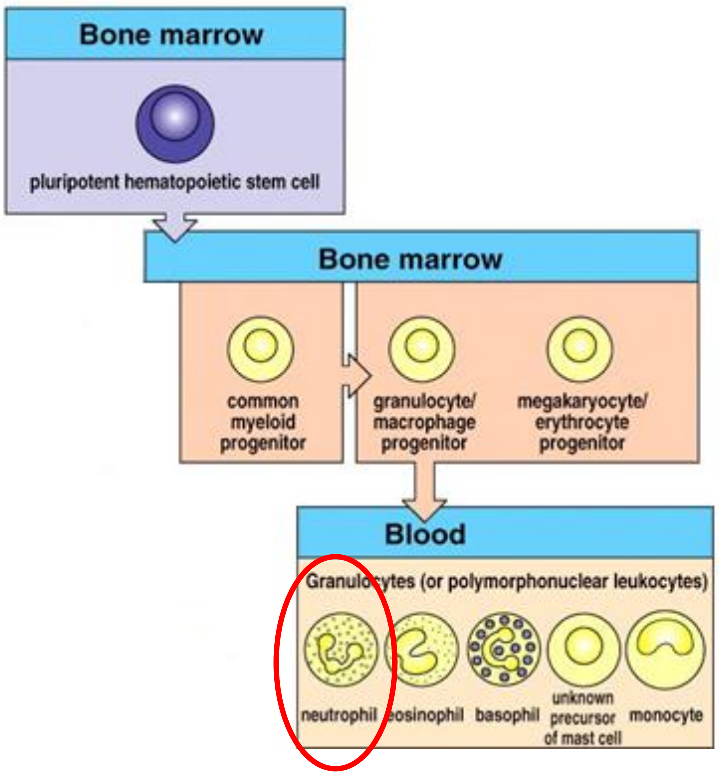


Lomakina et al. 2014. *Biophys J.*



Neutrophils: first responders to trauma and infection

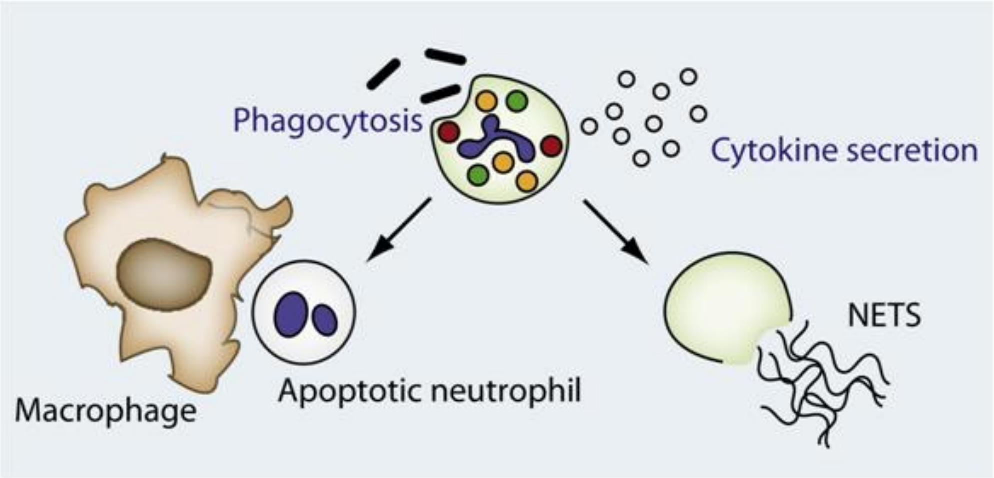
White Blood Cell



Janeway et al. Immunobiology. 6th Ed.

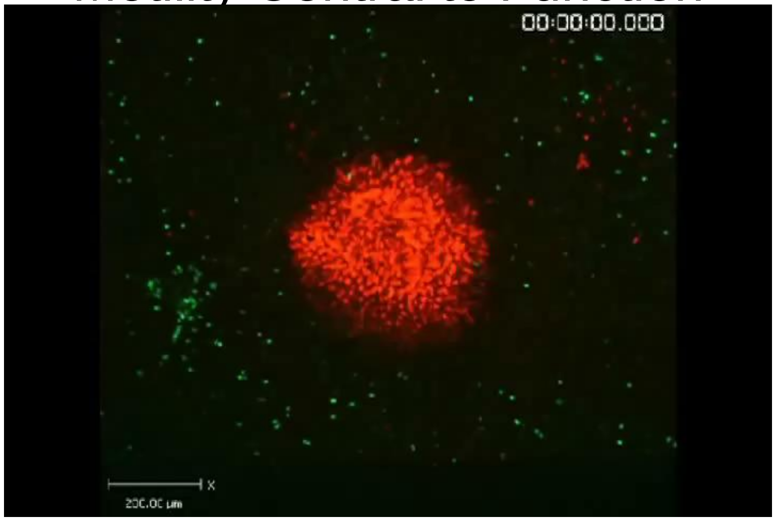
66% marrow production = neutrophils
10^{11} neutrophils/day

Fast (sec-min) Response Times



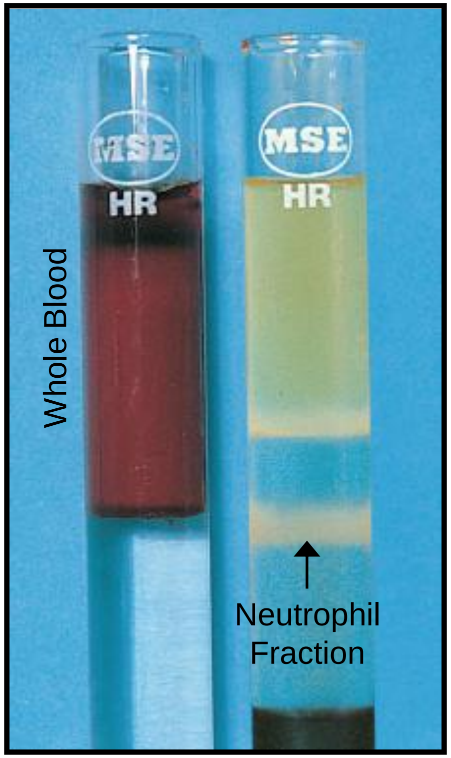
Borregaard. 2010. *Immunity.*

Motility Central to Function



McDonald et al. 2010. *Science.*

Neutrophils: a model cell type



Axis-Shield

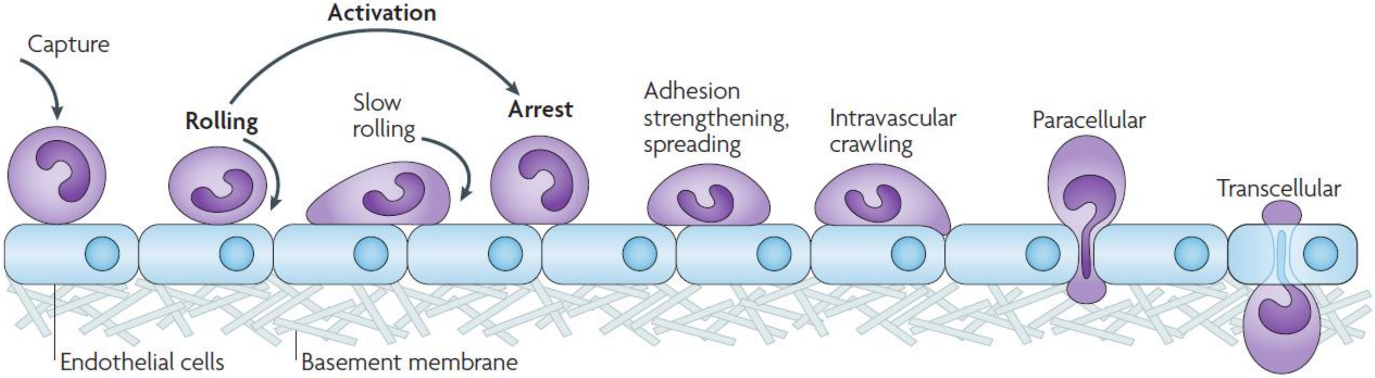
Minimally invasive: venipuncture

Ubiquitous: ~ 10^6 cells/mL whole blood

Fast-acting : sec-min

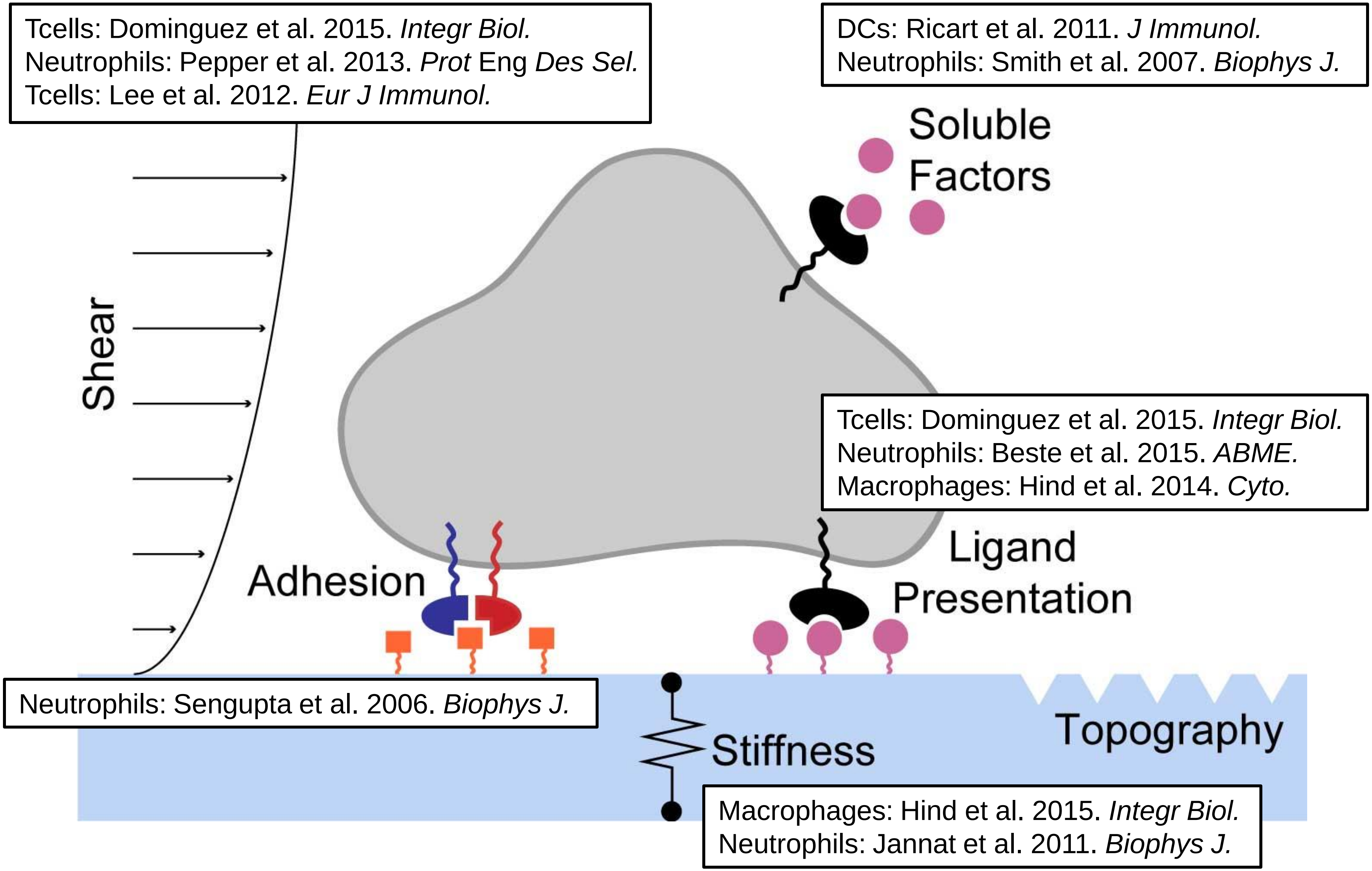
Highly motile: ~ 10 um/min

Leukocyte Adhesion Cascade

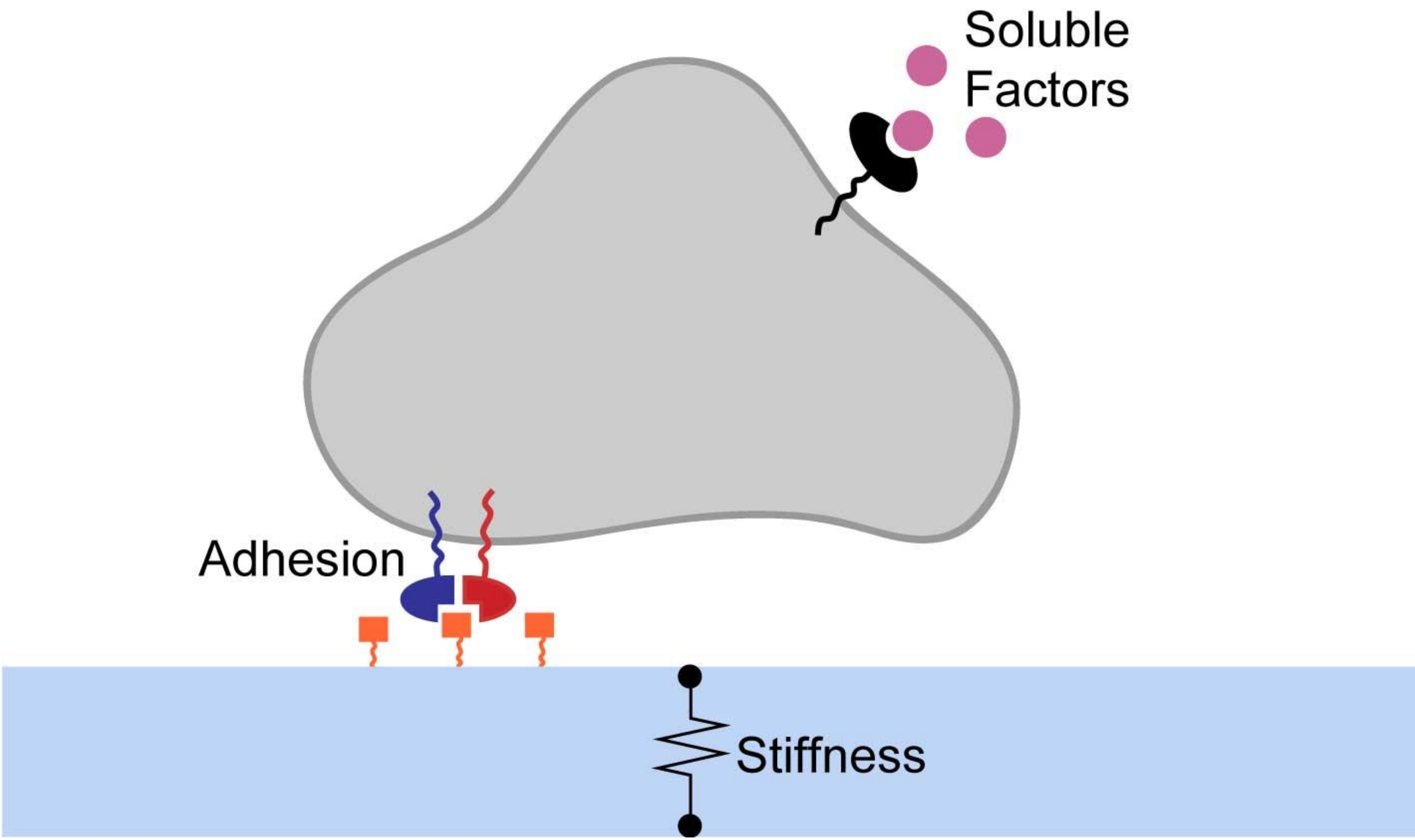


Ley. 2007. *Nat Rev Immunol.*

Cell environments are complex (multi-stimulatory)

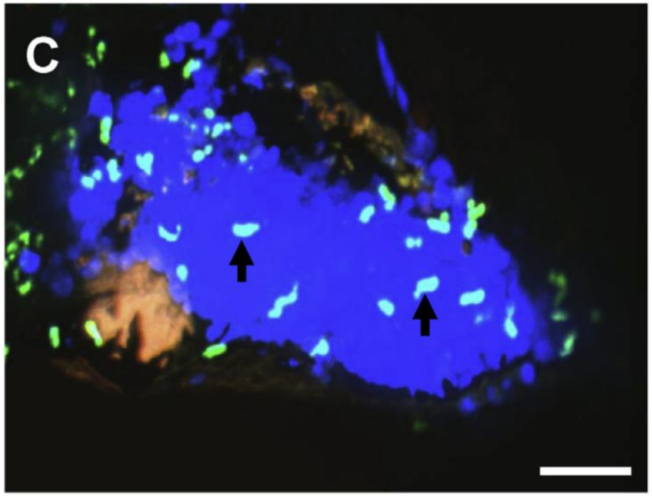


Today, neutrophil responses to:

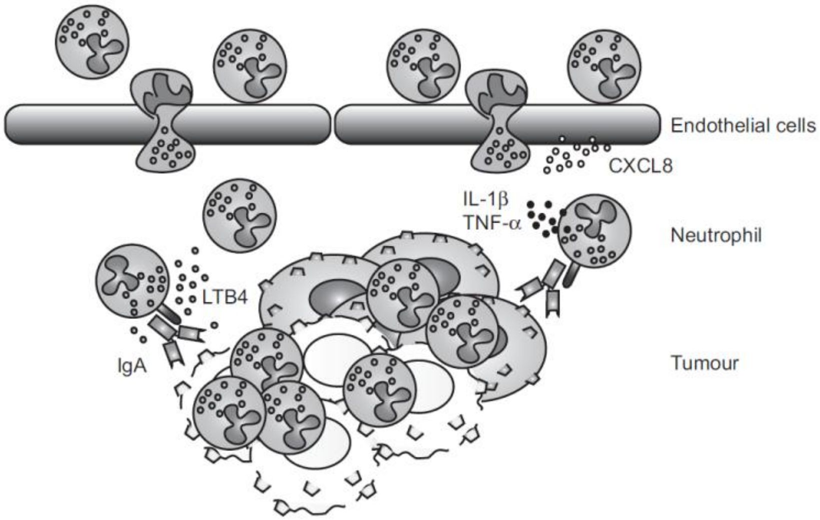


Why we should care … therapies of ~~tomorrow~~ Today!

Neutrophils Infiltrate **Tumors**



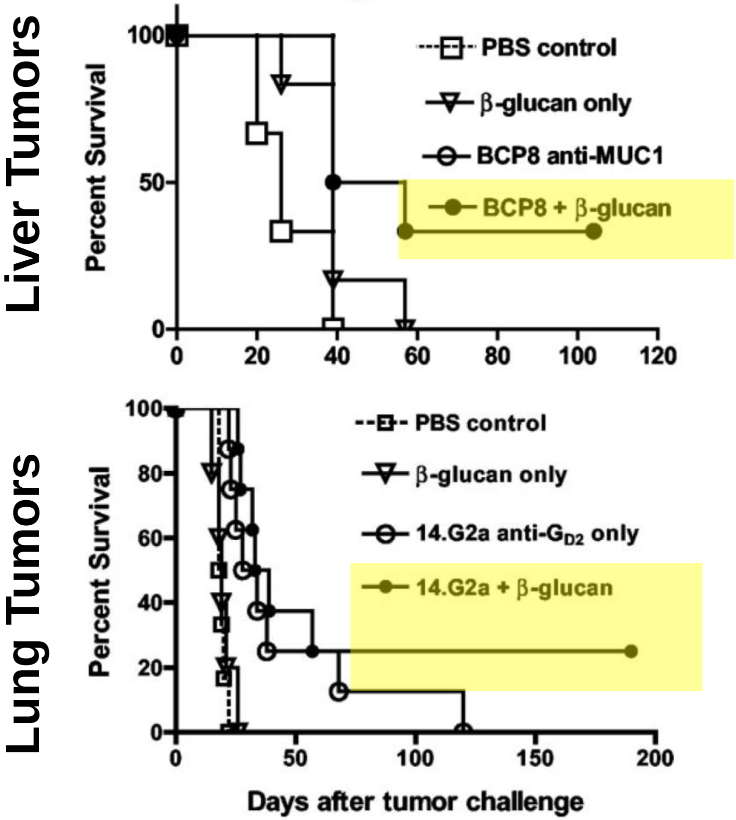
Tazzyman. 2013. *Sem Canc Bio*.



van Egmond et al. 2013. *Sem Canc Bio*.

Imprime PGG® (β-glucan as Leukocyte Adjuvant)

www.biothera.com



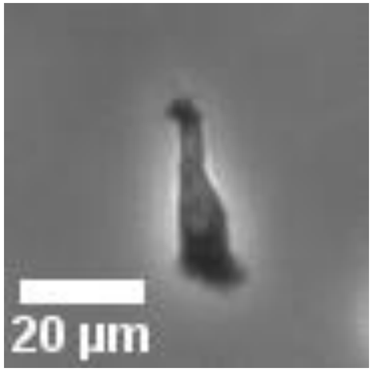
Hong et al. 2003. *Canc Res*.

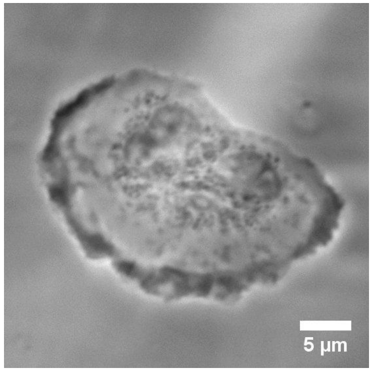
Outline

Shape and Motility

Ligand density elicits phenotypic switch in human neutrophils

Henry, Crocker, Hammer. 2014. *Integr Biol.*

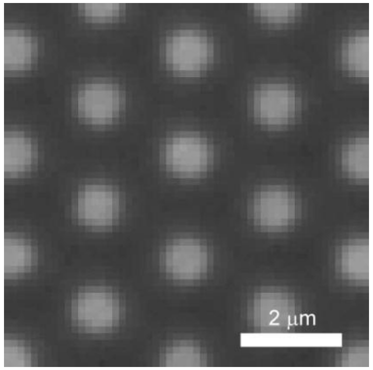


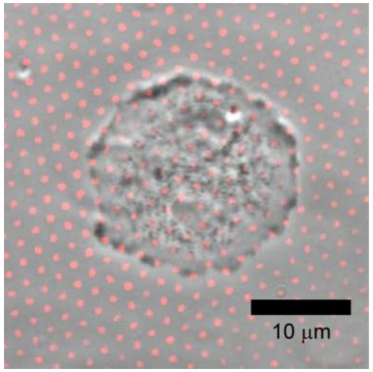


Density Sensing

Dynamic traction forces of spreading and adherent human neutrophils

Henry, Crocker, Hammer. 2015. *ABME* (In Prep)

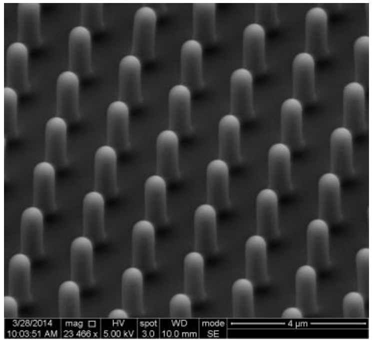


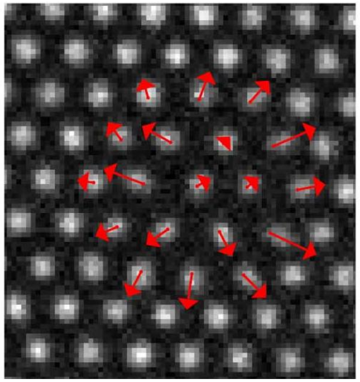


Spreading Mechanics

Dynamic traction forces of spreading and adherent human neutrophils

Henry, Chen, Crocker, Hammer. 2015. *Biophys J.* (Under Revision)





Shape and Motility

Ligand density elicits phenotypic switch in human neutrophils

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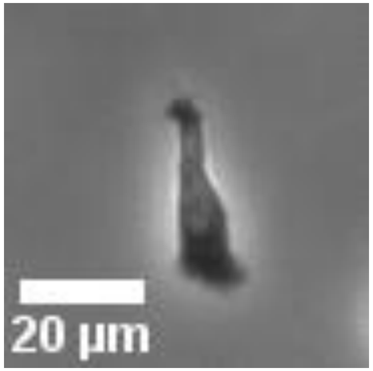
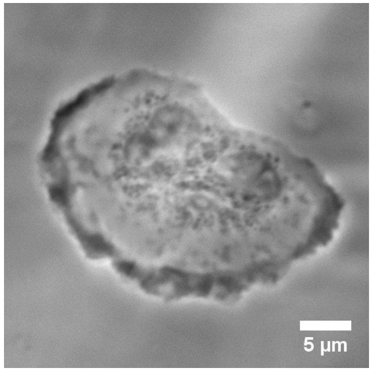




Aim:

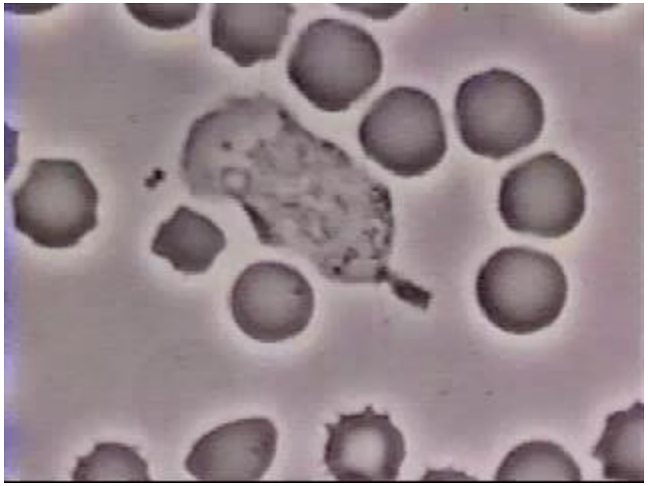
Quantify effect of adhesion density on neutrophil shape and motility

Hypotheses:

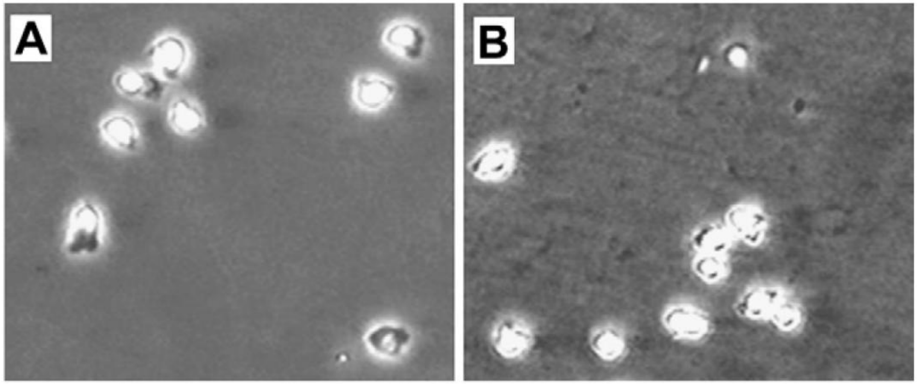
Neutrophil shape and motility are adhesion-sensitive

Integrin receptors will mediate this adhesion

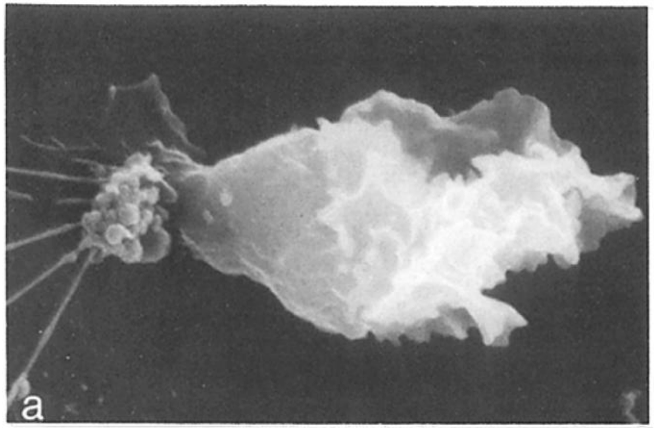
Canonical amoeboid phenotype of neutrophils



David Rogers, 1950s

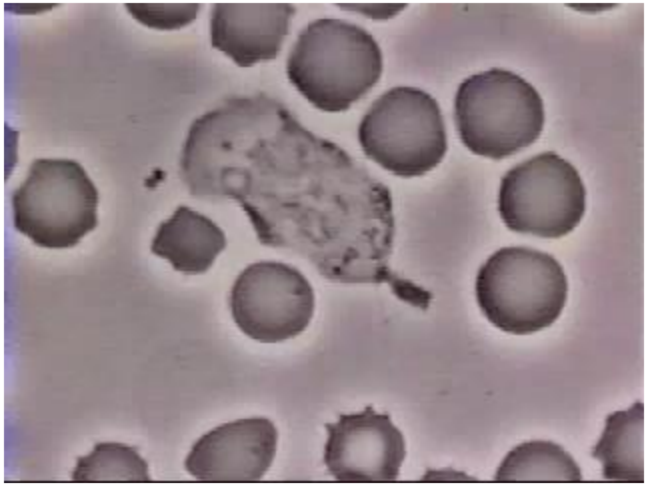


Butler et al. 2008. *Cell Immunol*.

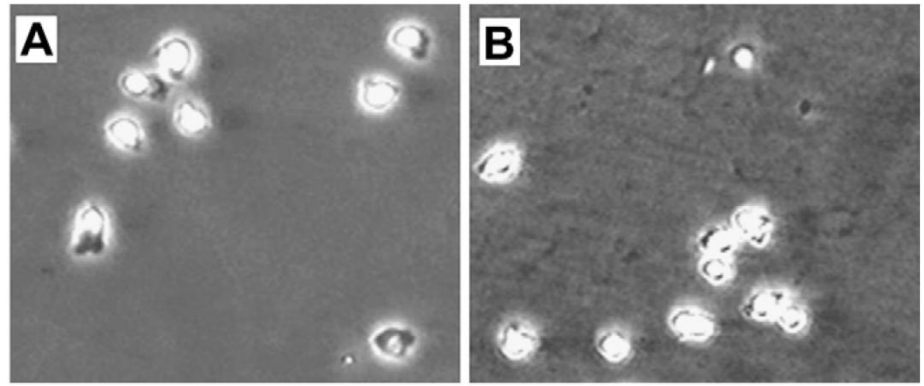


Cassimeris et al. 1990. *JCB*.

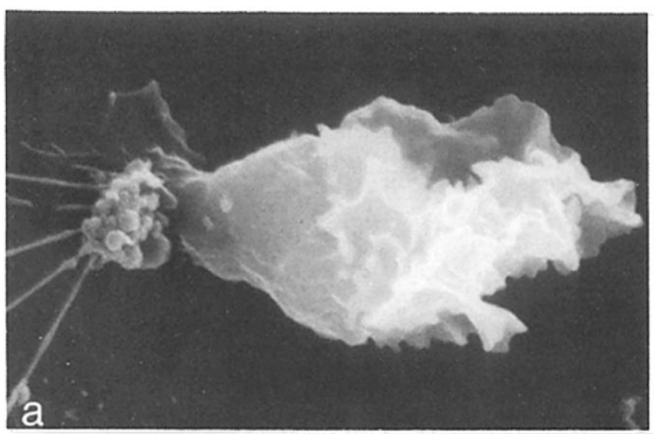
Can adhesivity reconcile these conflicting observations?



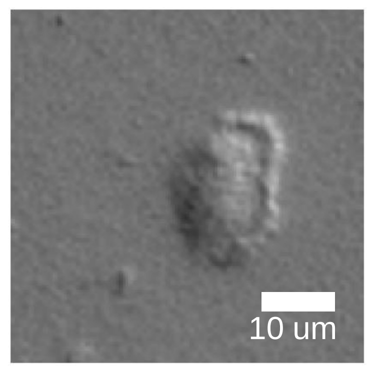
David Rogers, 1950s



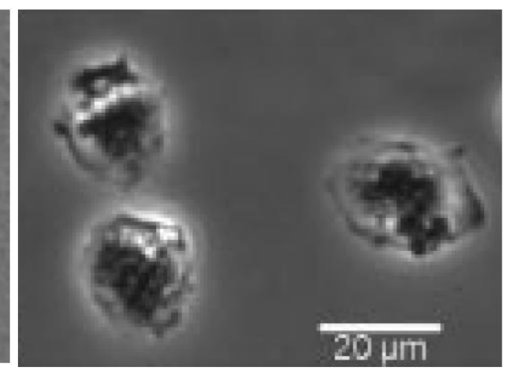
Butler et al. 2008. *Cell Immunol*.



Cassimeris et al. 1990. *JCB*.

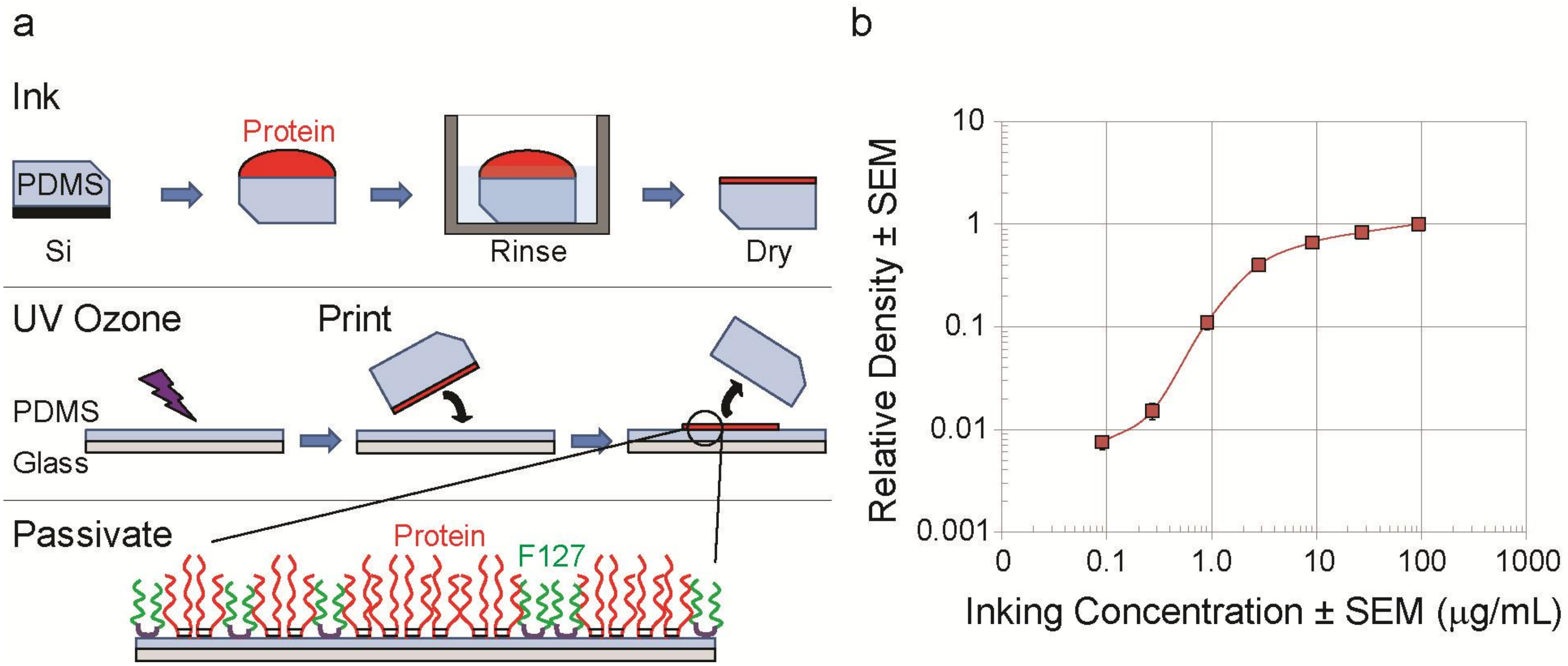


Oakes et al. 2009. *Blood*.



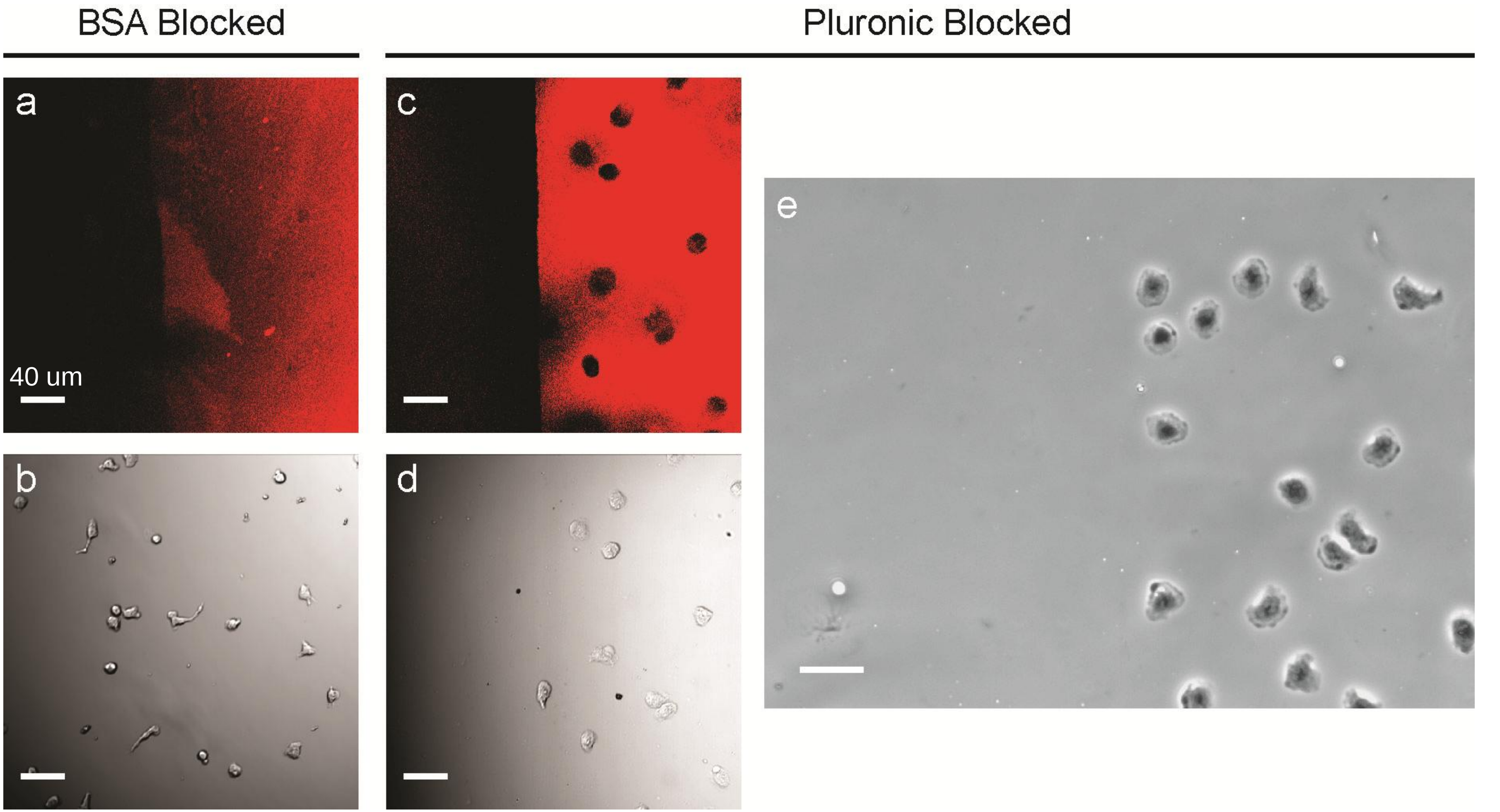
Stroka et al. 2009. *Cyto.*

Tuning Ahesivity via Microcontact Printing



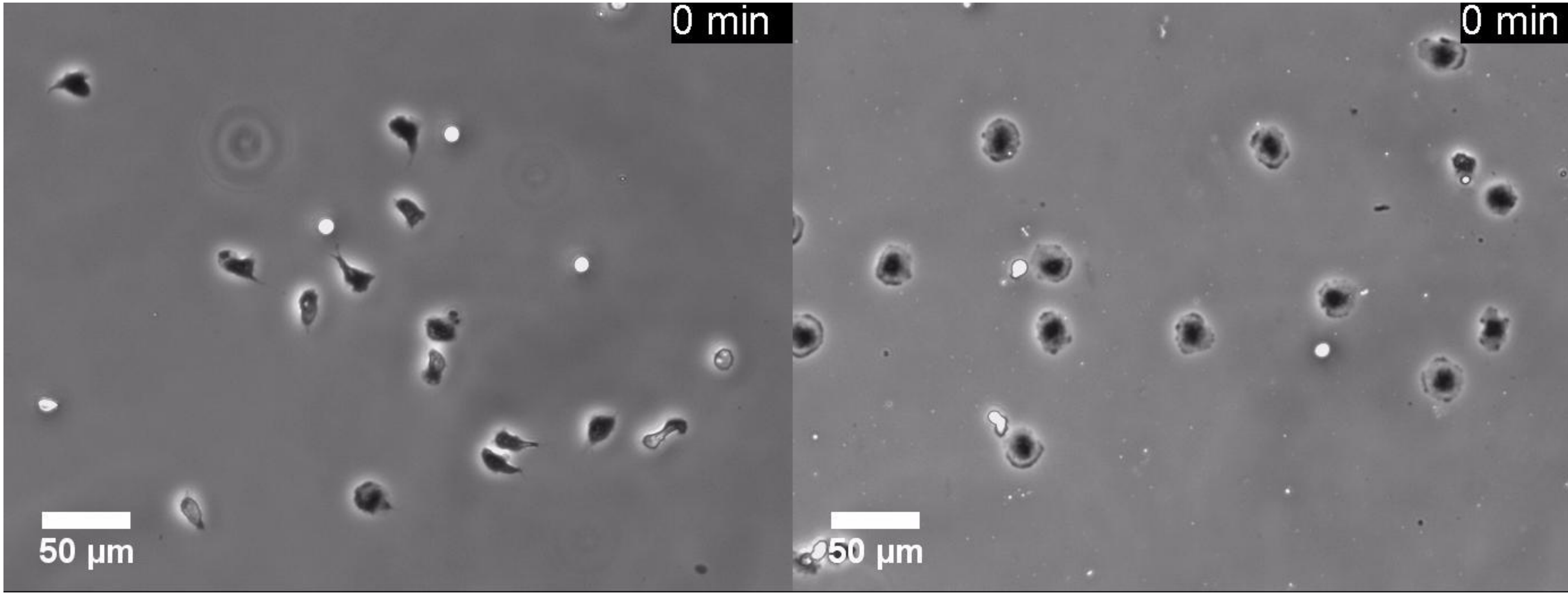
Henry et al. 2014. *Integr Biol.*

Exquisite cell-ligand specificity



Henry et al. 2014. *Integr Biol.*

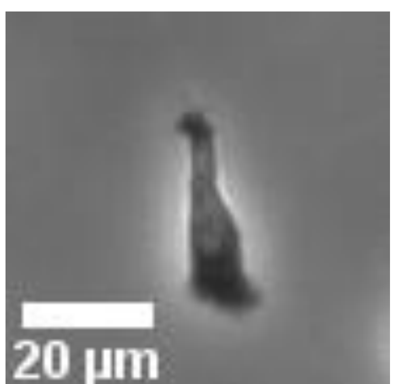
Two dramatically different modes of motility



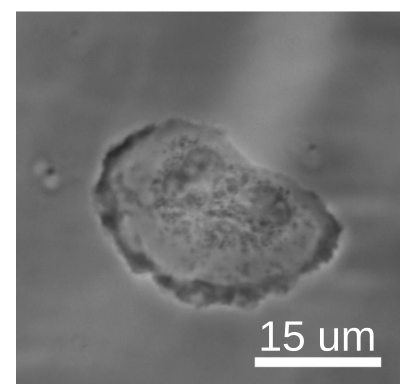
Amoeboid

“Keratocyte-Like”

Highly Adhesive Surface



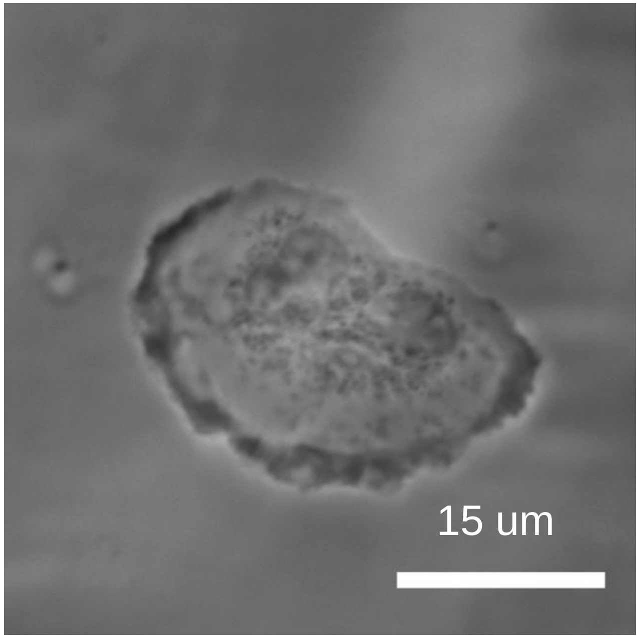
Moderately Adhesive Surface



Henry et al. 2014. *Integr Biol.*

"Keratocyte-like" morphology

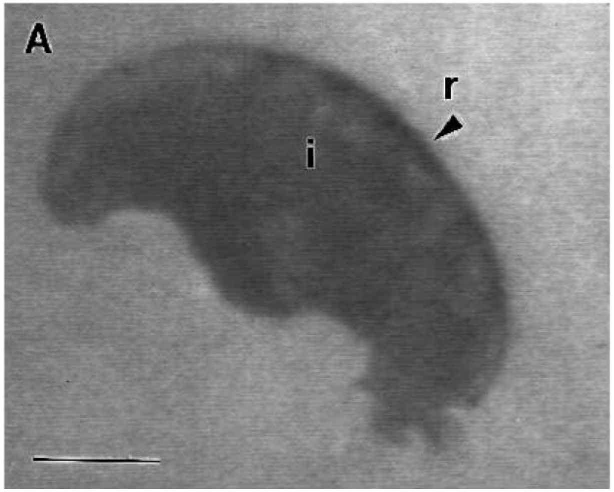
Neutrophils



Henry et al. 2014. *Integr Biol.*

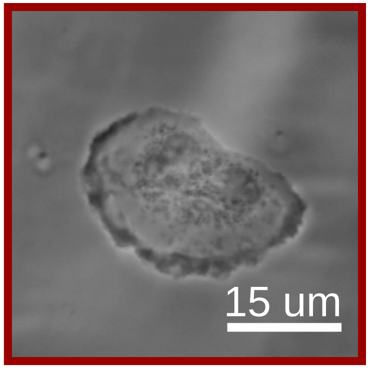
Epithelial Keratocytes

Keren et al. 2008. *Nature*.

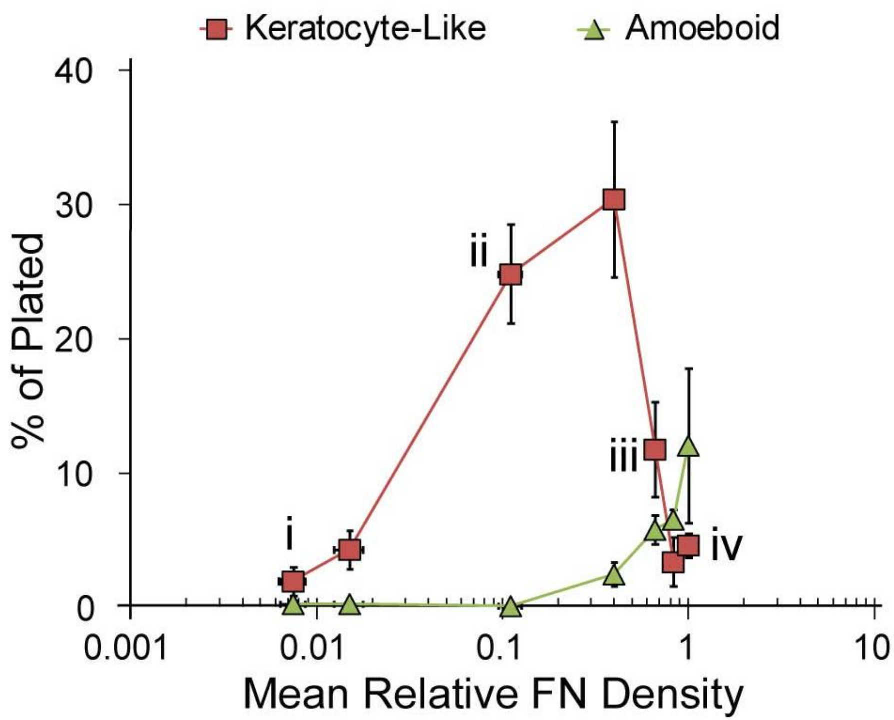


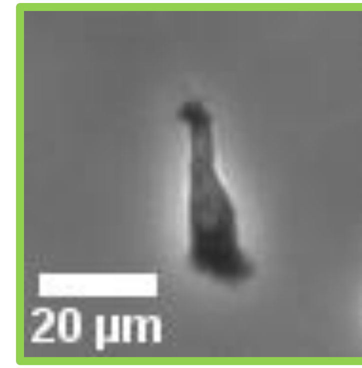
Lee et al. 1997. *JCS*.

Fibronectin density as controller of shape

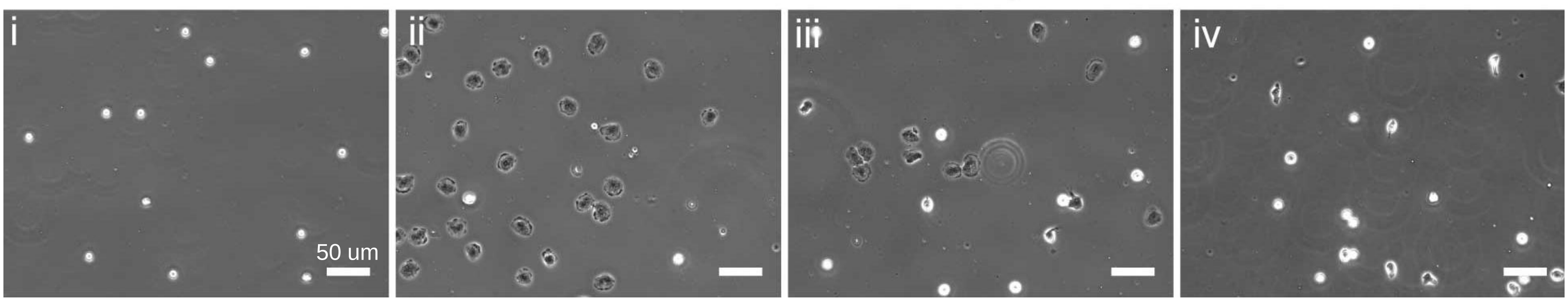


Keratocyte-Like





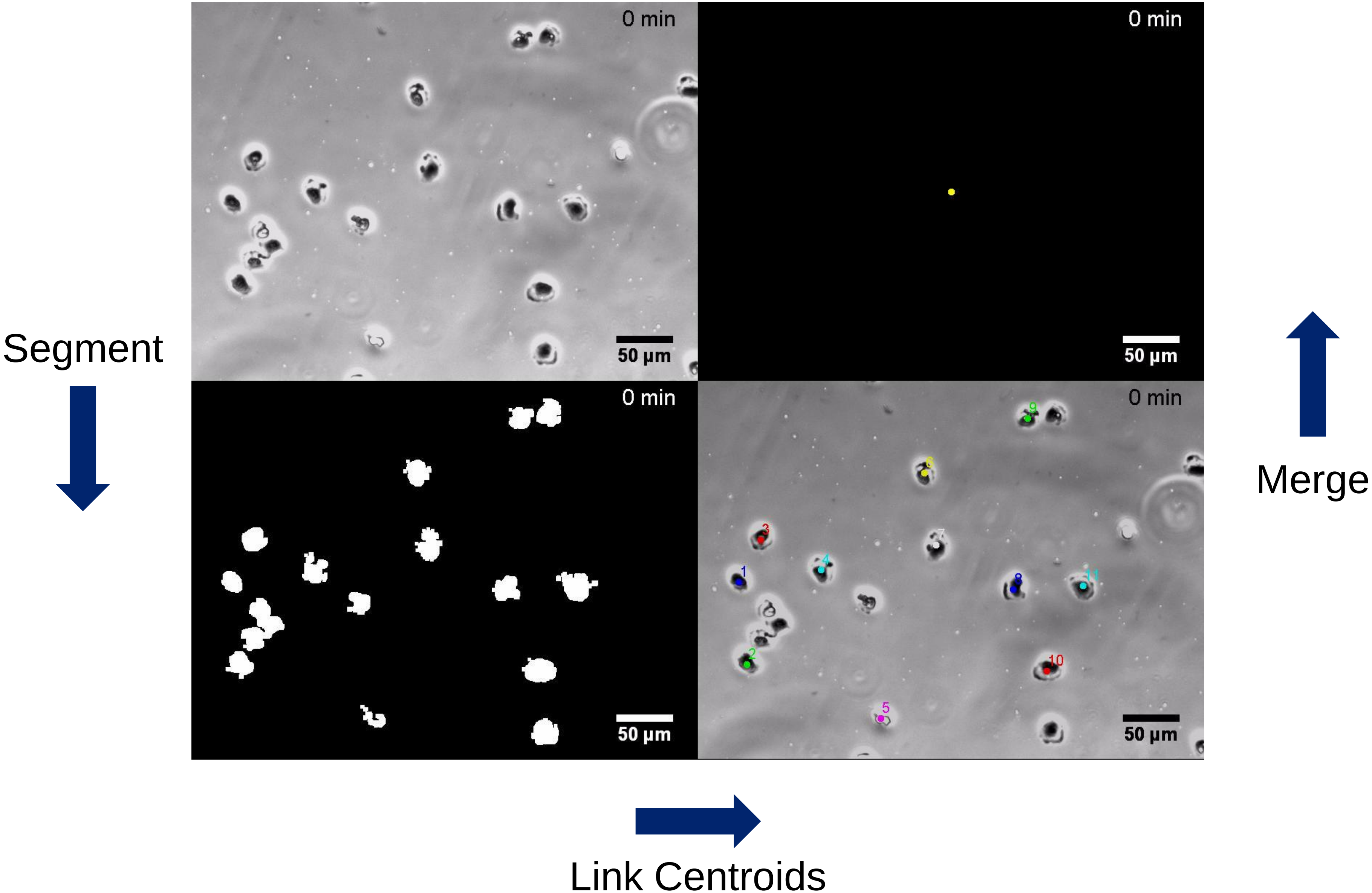
Amoeboid



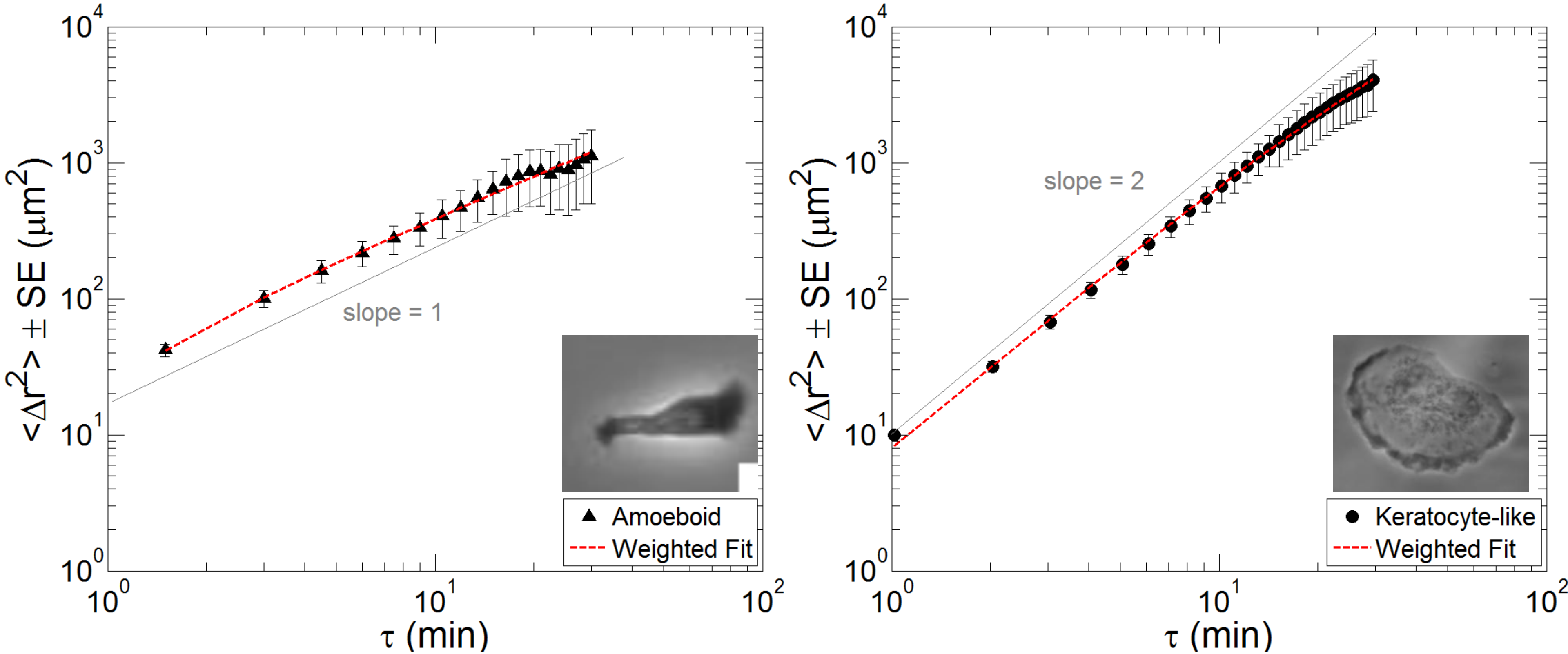
Increasing FN

Henry et al. 2014. *Integr Biol.*

Objective and reproducible cell tracking



Motility as a persistent random walk



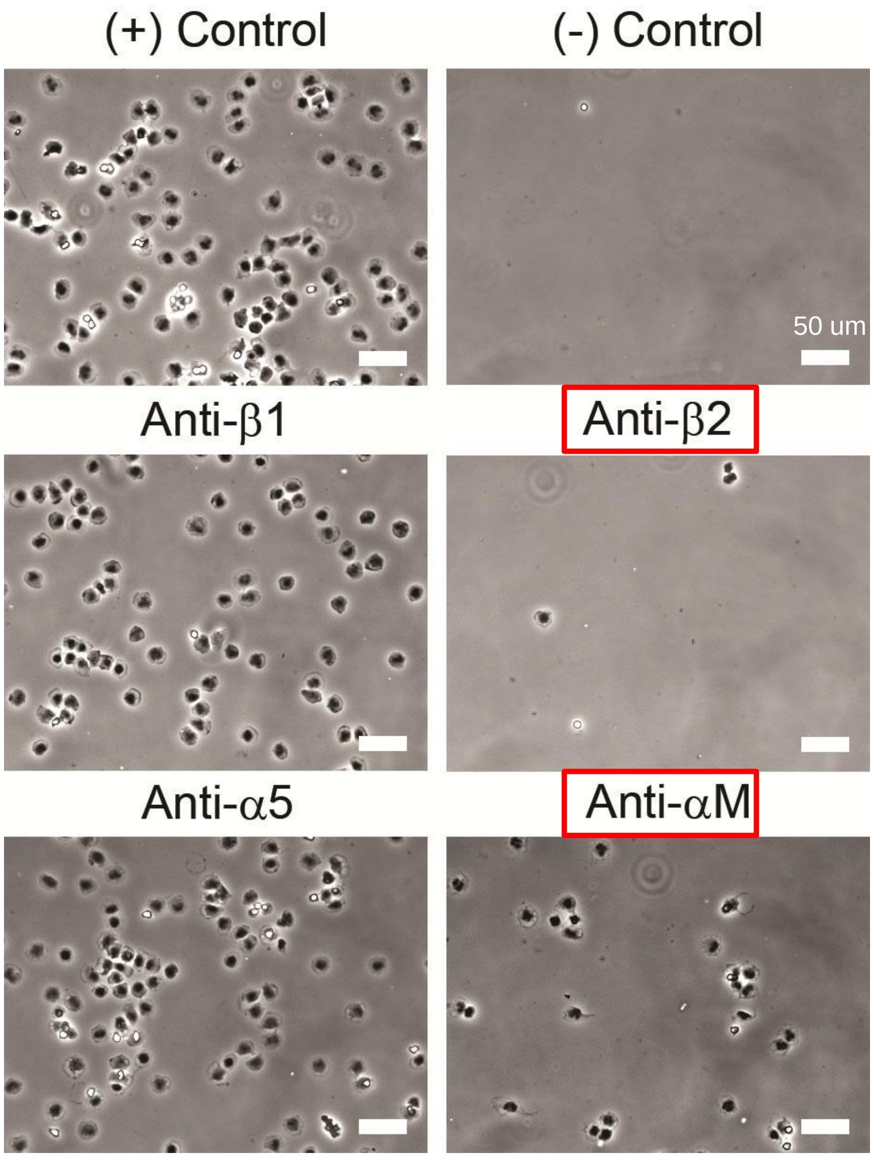
$$<\Delta r^2(\tau)> = 2S^2P[\tau - P(1-\exp(-\tau/P))]$$

S = 6 μm/min
P = 0.5min

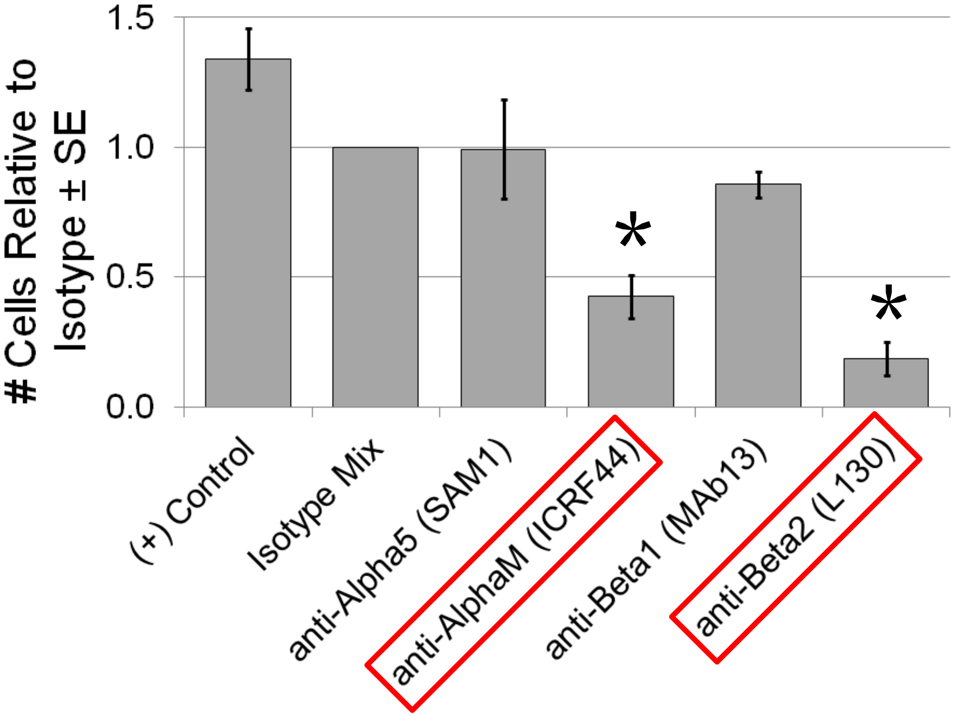
S = 3 μm/min
P = 15 min

Henry et al. 2014. *Integr Biol.*

Hyptothesis: integrins mediate adhesion



$\alpha_M\beta_2$ (Mac-1)

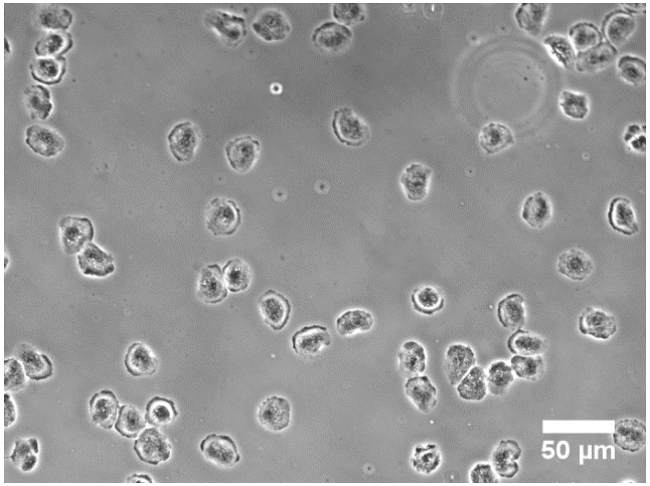


Henry et al. 2014. *Integr Biol.*

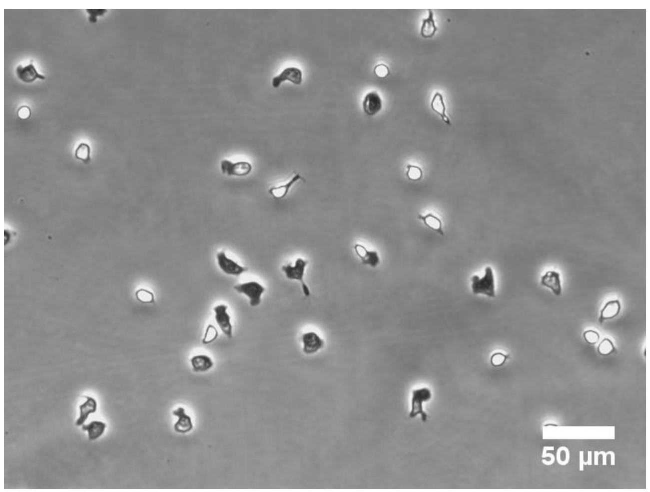
* $p < 0.05$, Dunnet's One Way ANOVA

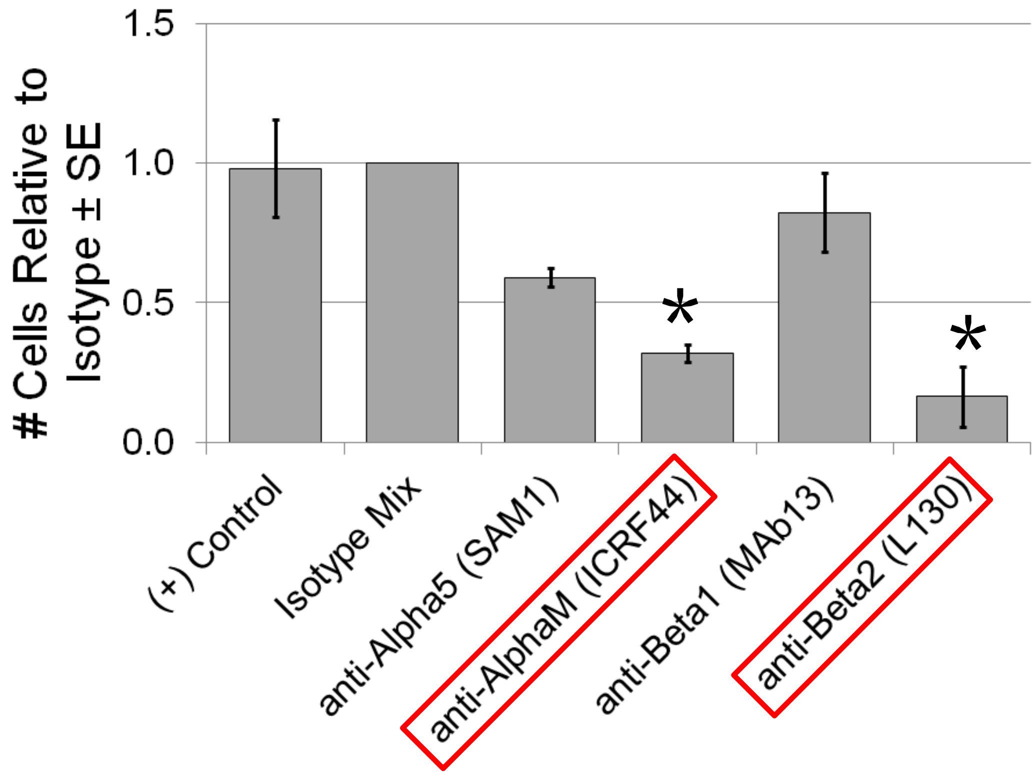
$\alpha_M\beta_2$ (Mac-1) is a promiscuous integrin
Hypothesis: density sensitivity is not FN specific

Intermediate density BSA ← Be careful about choice of "blocking" agent!



High density BSA



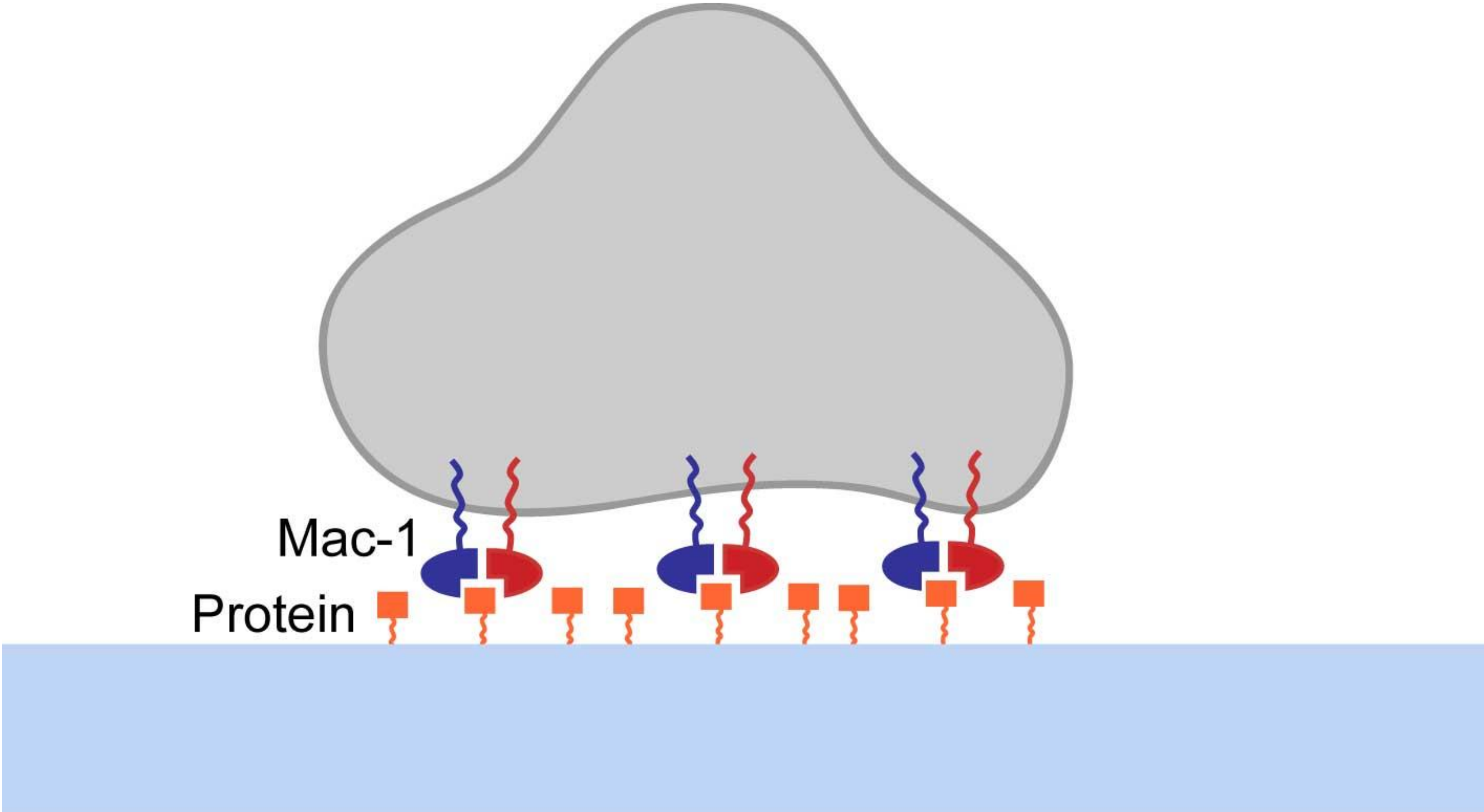


$\alpha_M\beta_2$ (Mac-1)

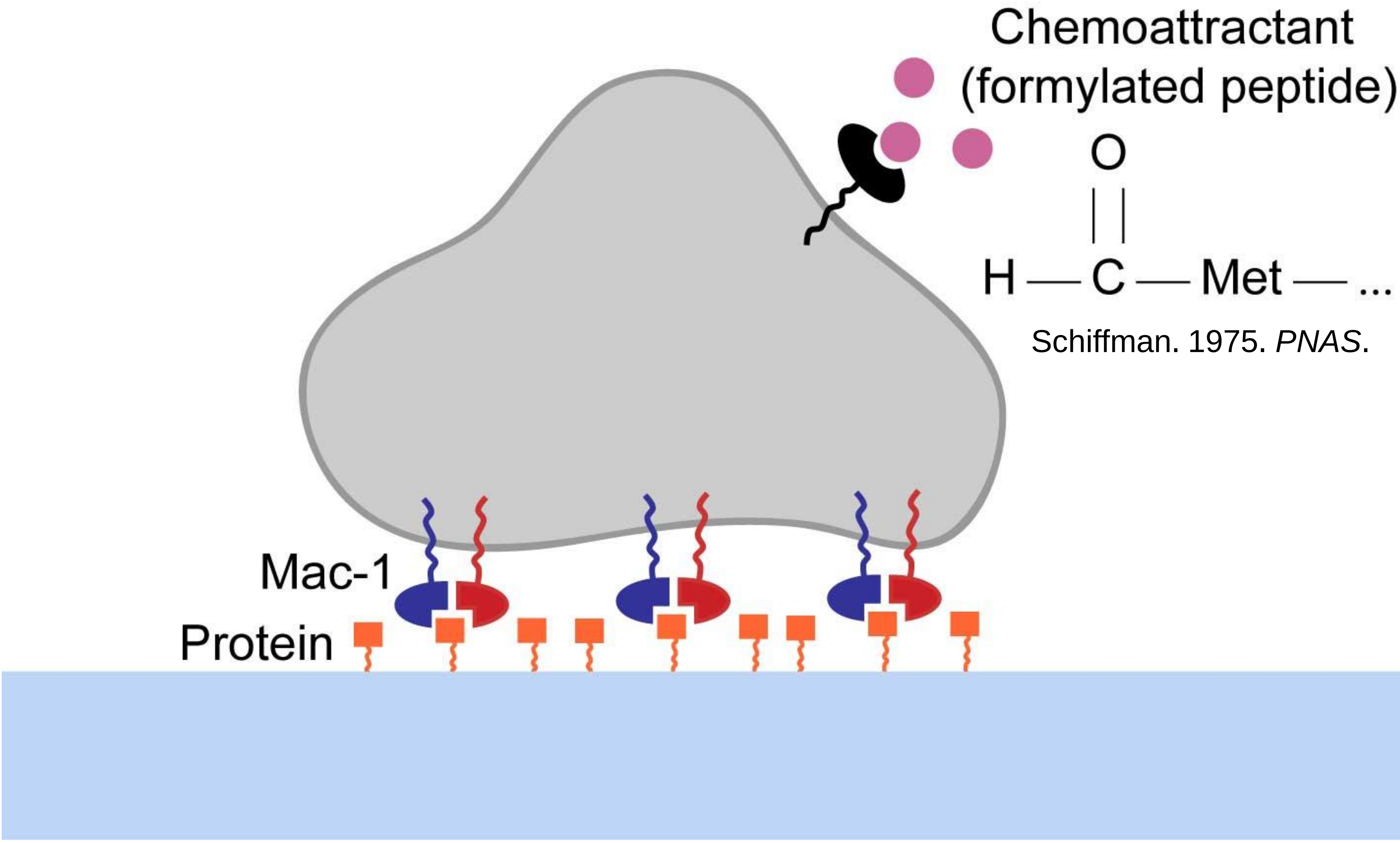
Henry et al. 2014. *Integr Biol.*

* $p < 0.05$, Dunnet's One Way ANOVA

So far, response to adhesive ligand alone (haptokinesis)

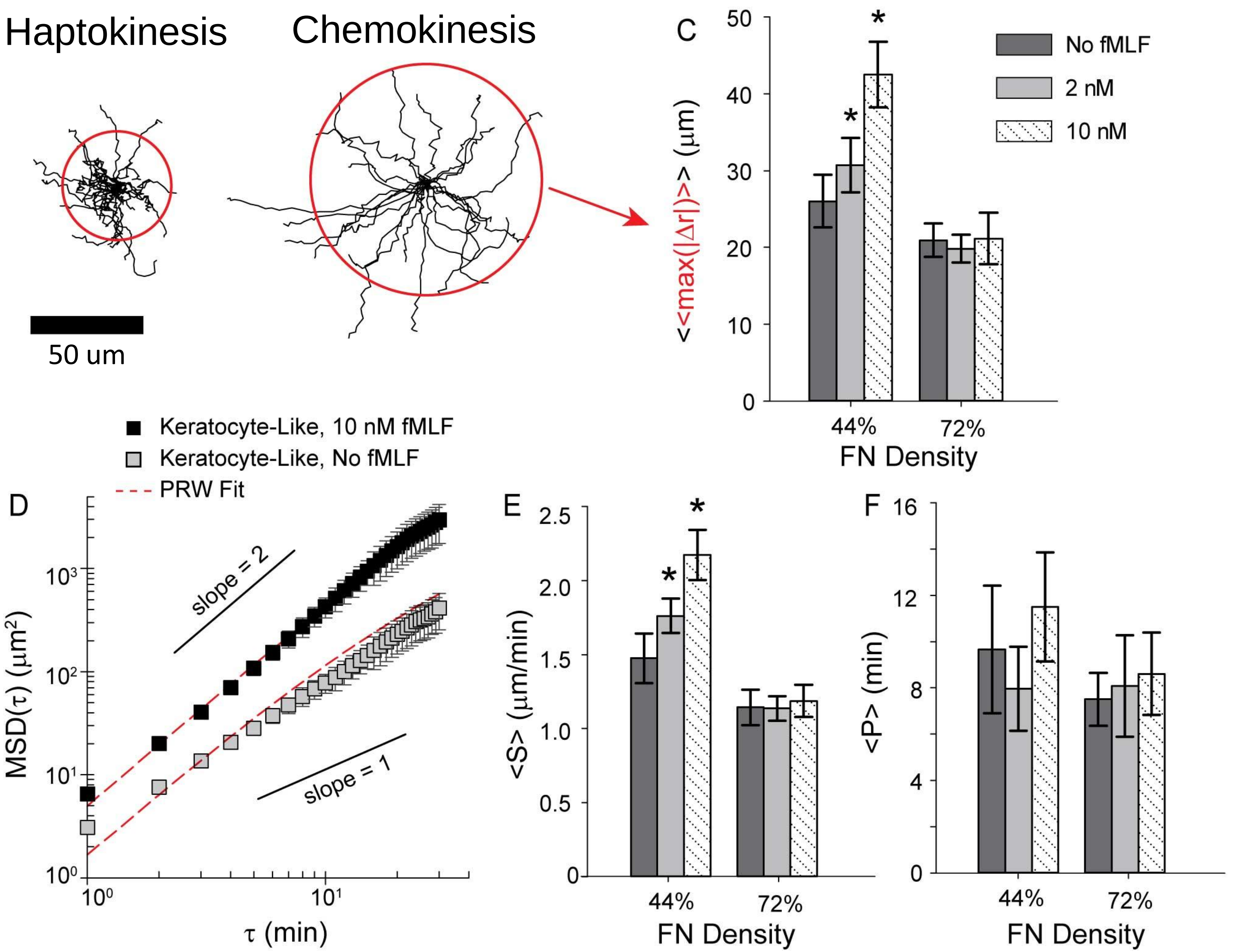


Response to adhesive ligand **and** chemoattractant?



Schiffman. 1975. *PNAS*.

Haptokinesis (surface stim.) → chemokinesis (soluble stim.) of keratocyte-like phenotype



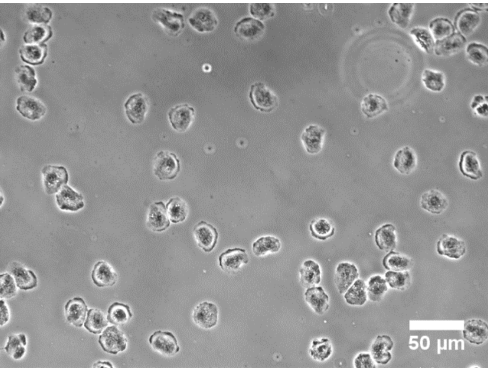
Henry et al. 2014. *Integr Biol.*

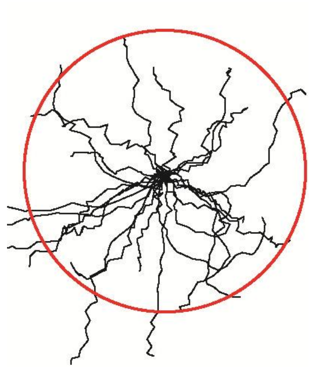
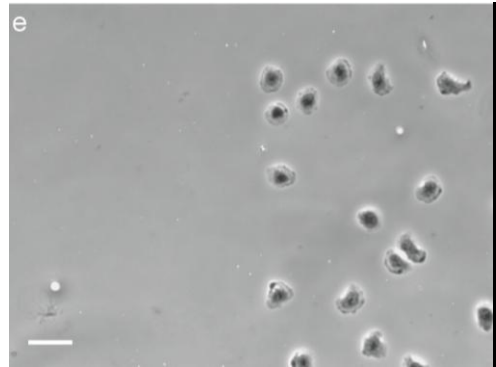
* $p < 0.05$, SNK multi. comp.

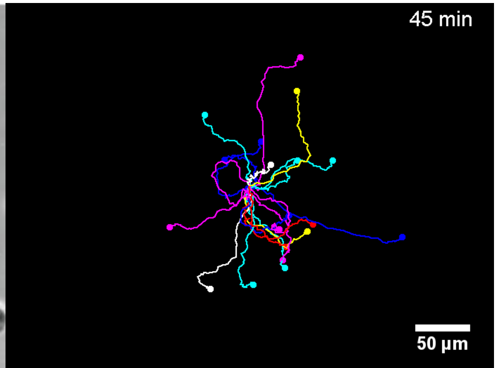
Part I Summary

Neutrophils are capable of an adhesion-driven phenotypic switch with respect to shape and motility.

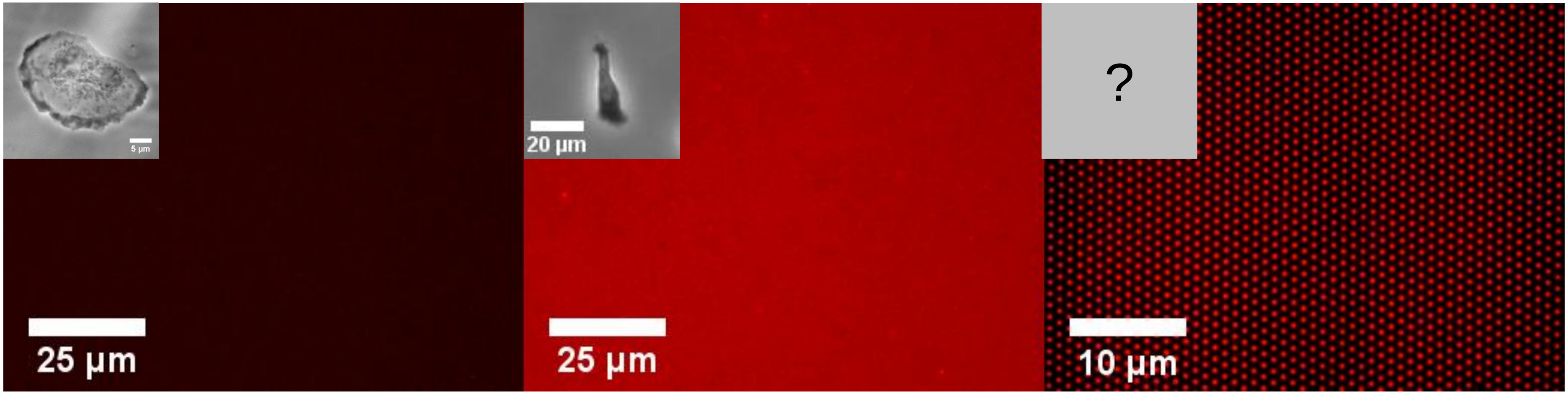
Promiscuous Mac-1 mediates this sensitivity.





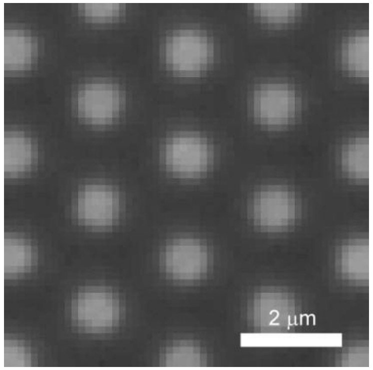
Length scale of density sensing?

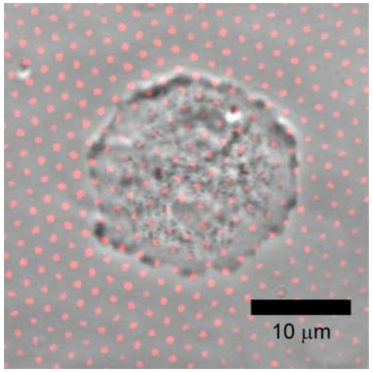


Density Sensing

Dynamic traction forces of spreading and adherent human neutrophils

Henry, Crocker, Hammer. 2015. *ABME* (In Prep)





Aim:

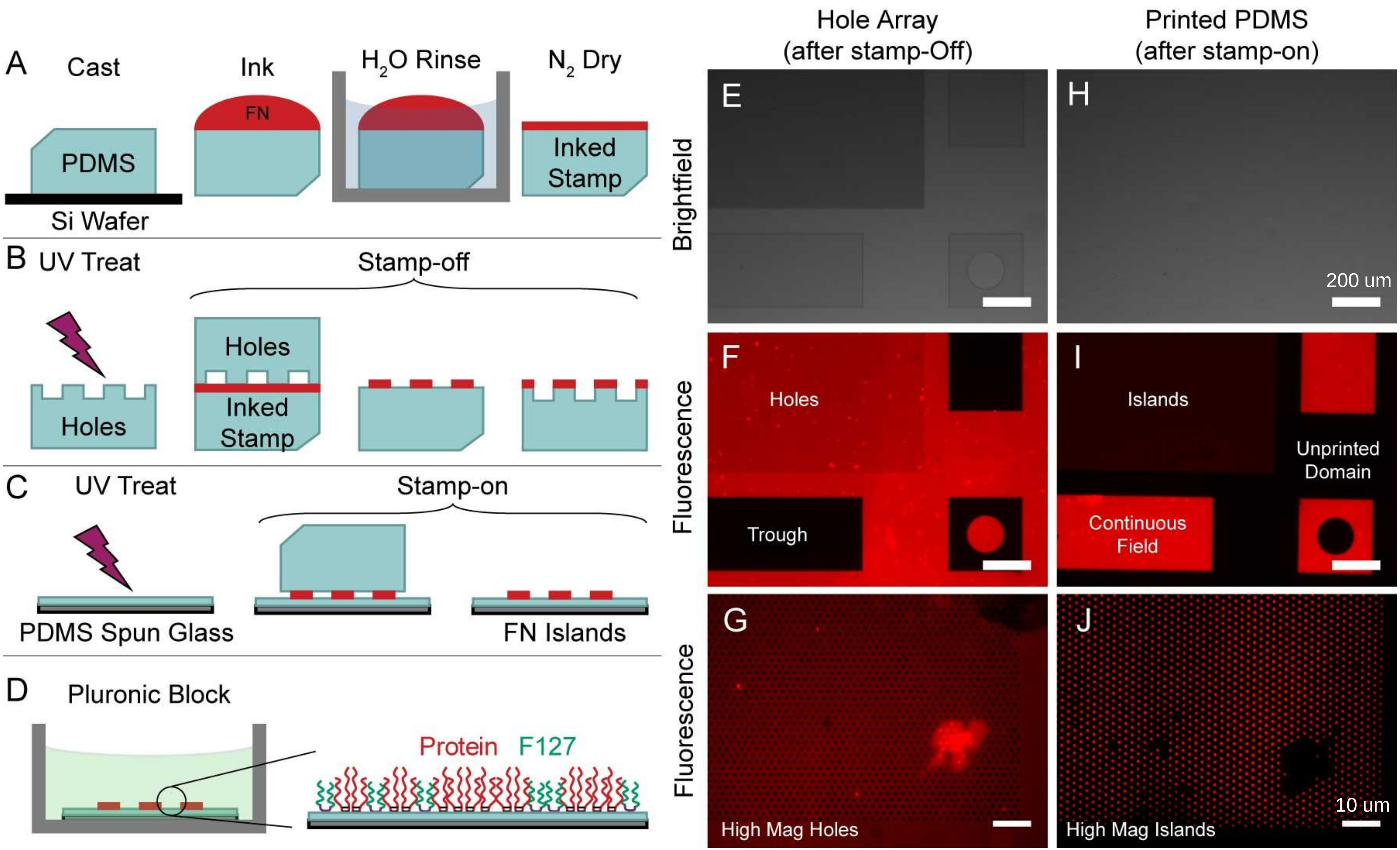
Elucidate length scale of density sensitivity

Hypotheses: (on dual adhesive environments)

Local (submicron) sensitivity → amoeboid

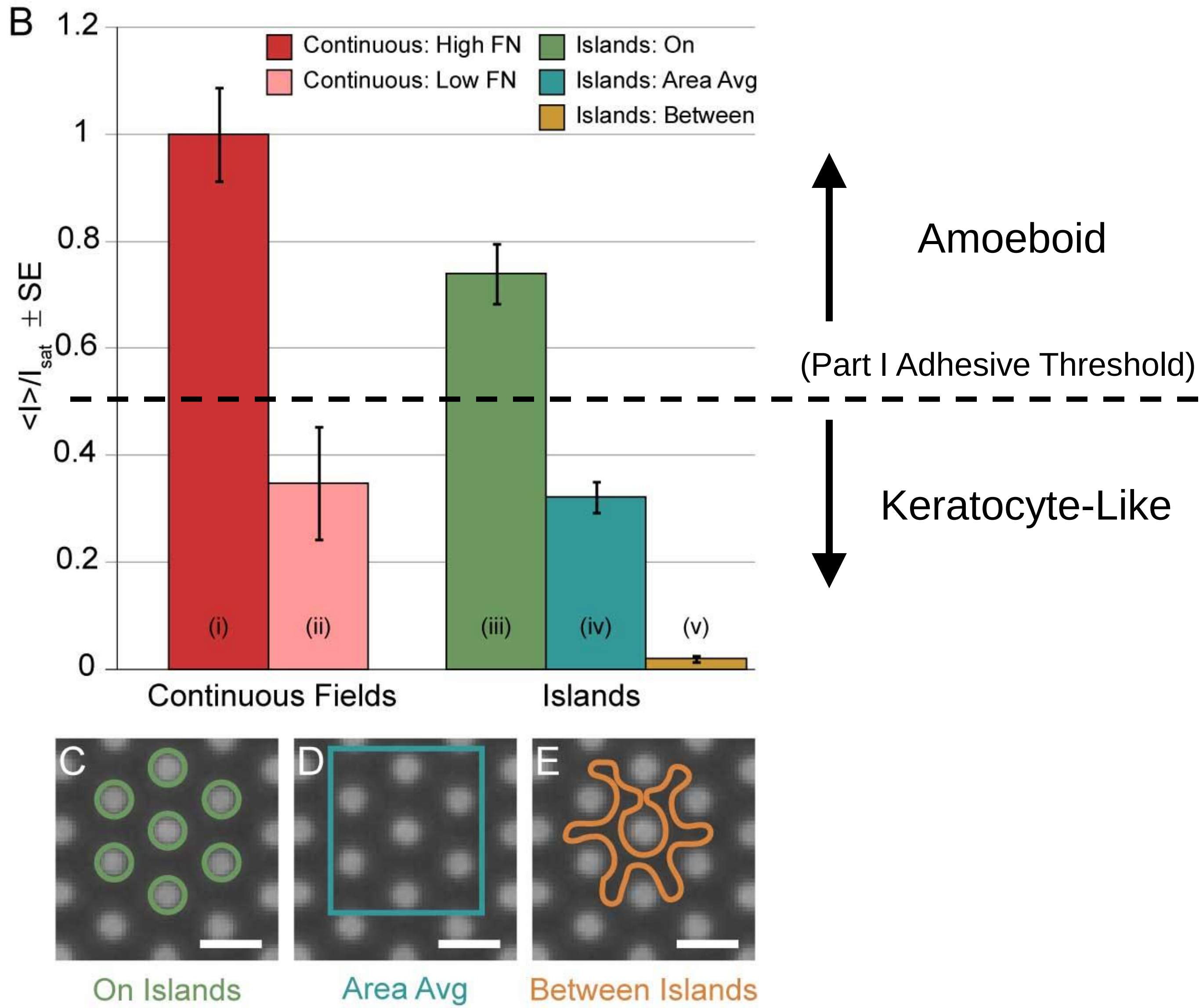
Global (whole cell) sensitivity → keratocyte-like

Arrays of discrete islands via “stamp-off”



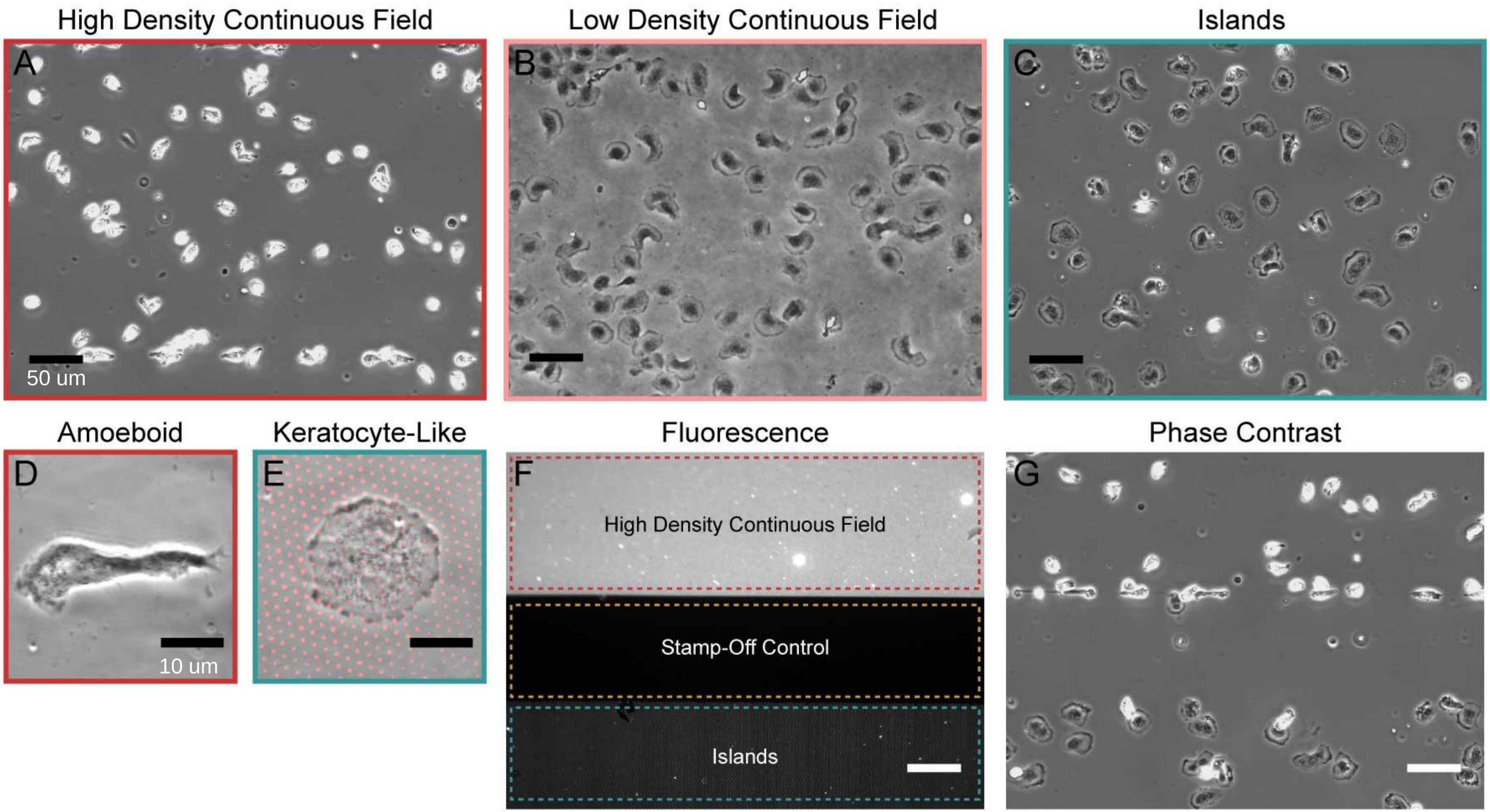
Henry et al. 2015. *ABME.* (In Prep)

Engineering dual adhesive length scales



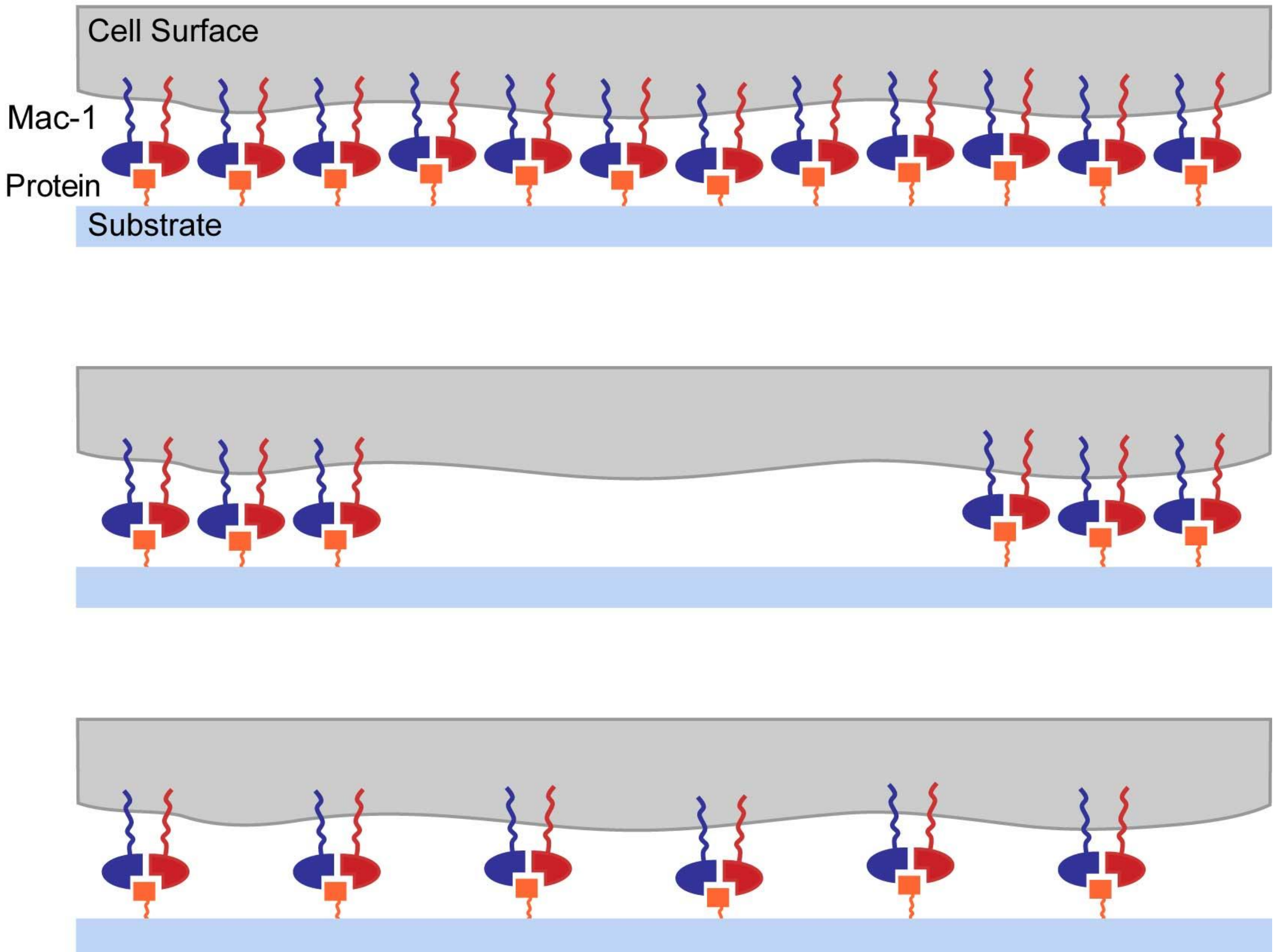
Henry et al. 2015. *ABME.* (In Prep)

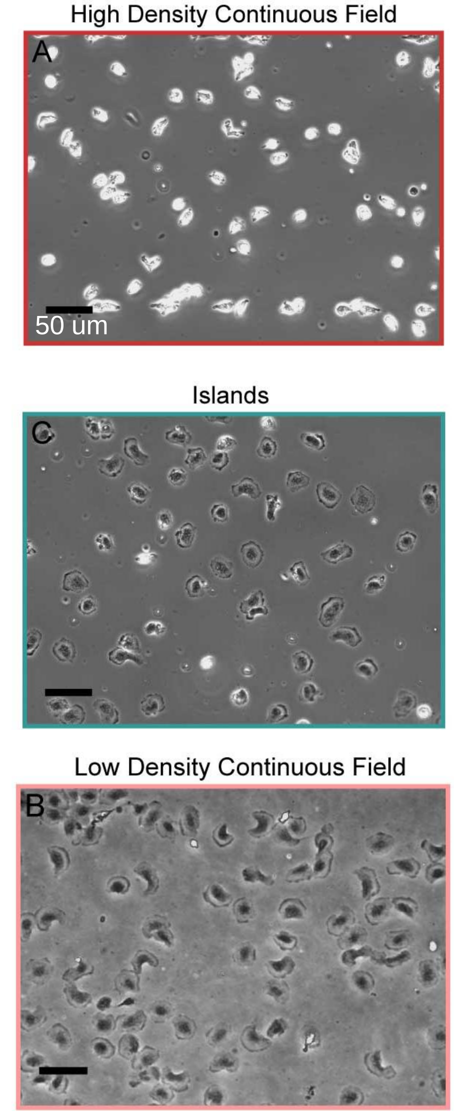
Neutrophil phenotype on islands? Keratocyte-Like!



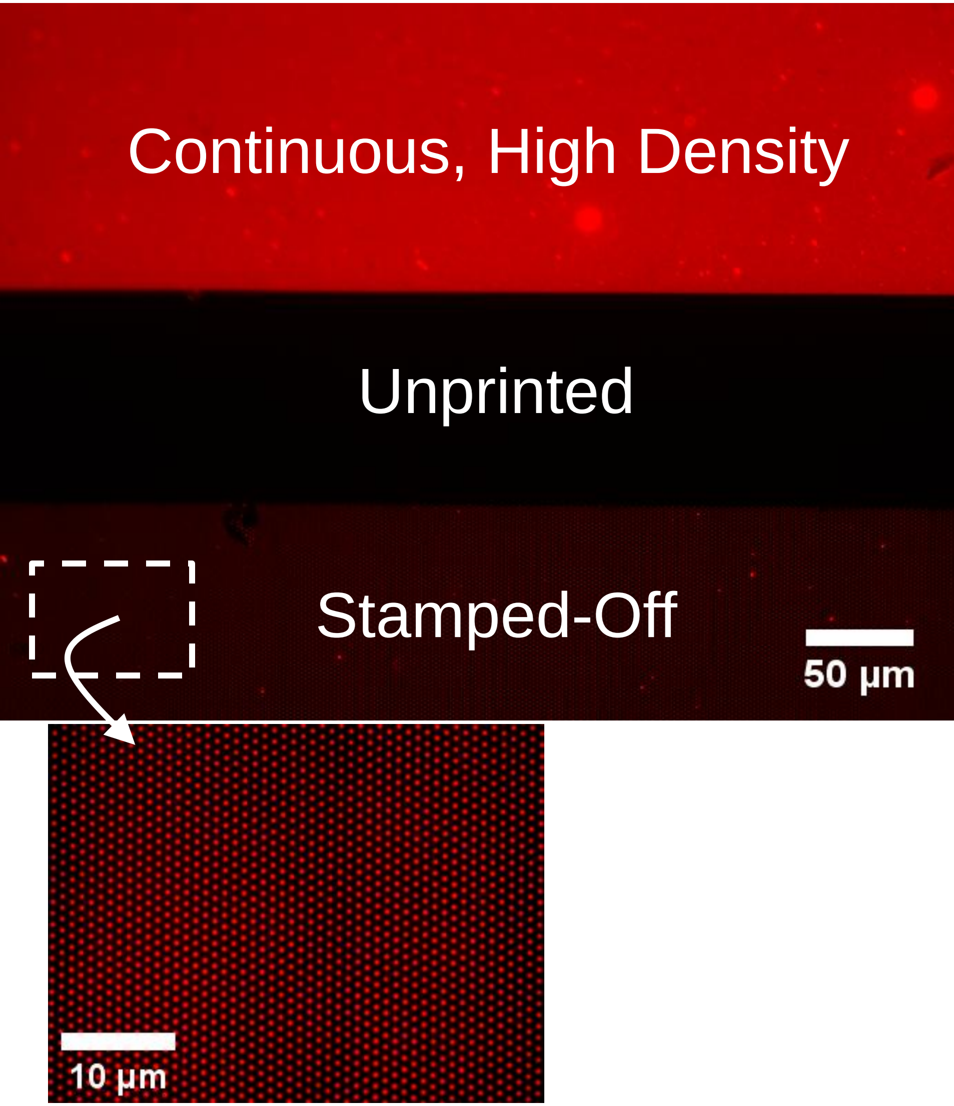
Henry et al. 2015. *ABME.* (In Prep)

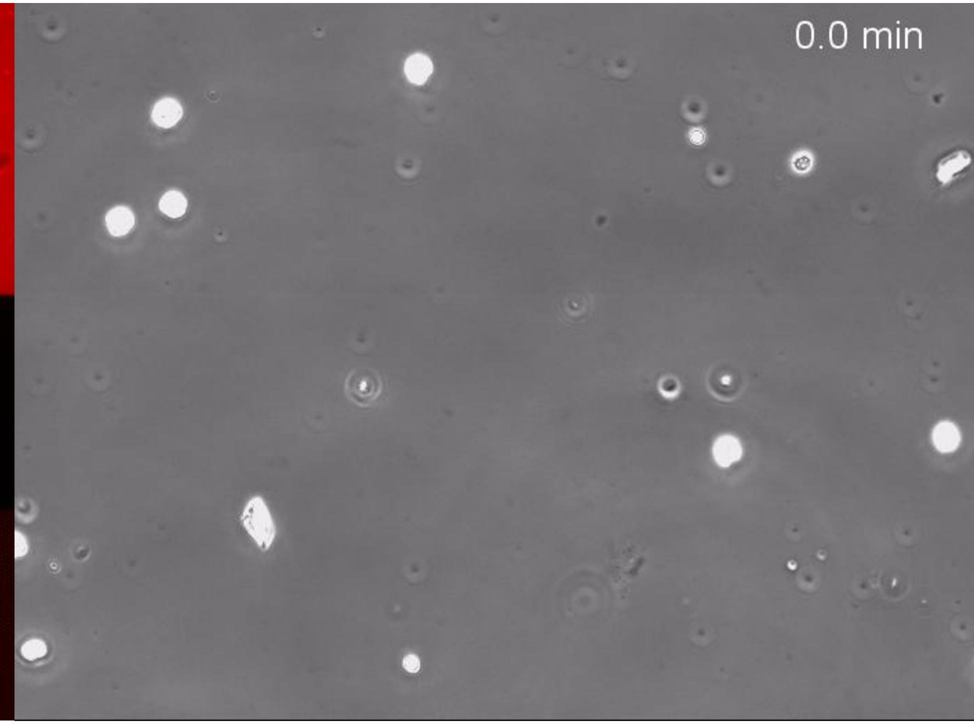
Neutrophils **integrate** adhesive stimulation





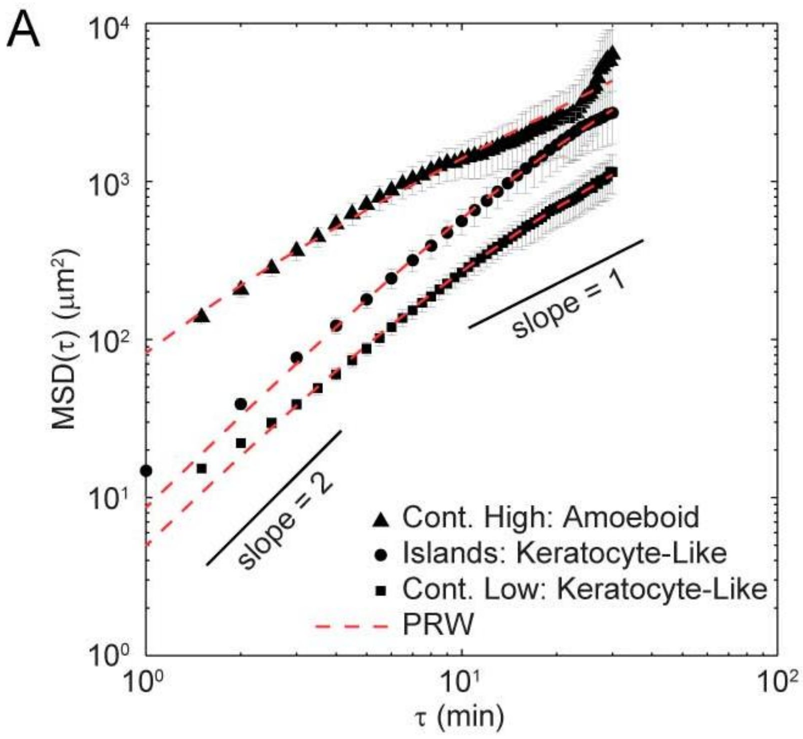
Neutrophils integrate adhesive stimulation
Rapid amoeboid → keratocyte-like transitions





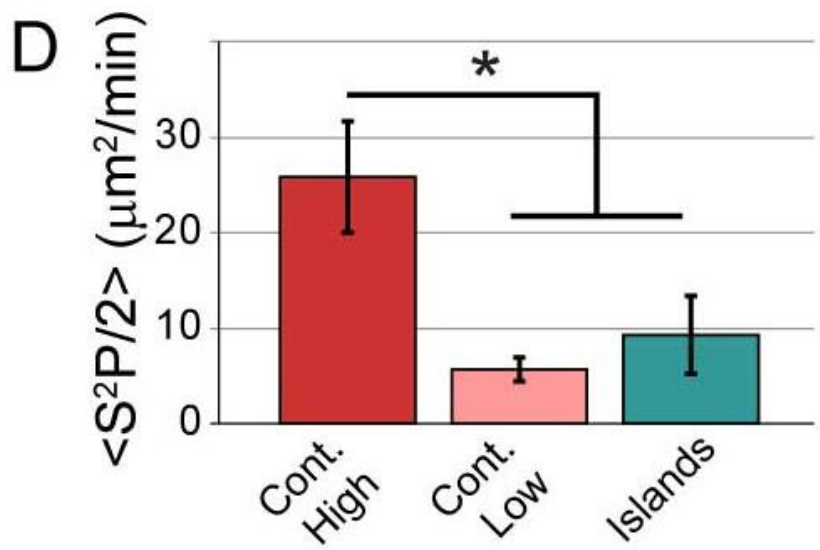
Henry et al. 2015. *ABME.* (In Prep)

Motility on islands ≈ moderate adhesivity continuous field



$$\langle \Delta r^2(\tau) \rangle = 2S^2P[\tau - P(1-\exp(-\tau/P))]$$

random motility coeff. = $S^2P/2$

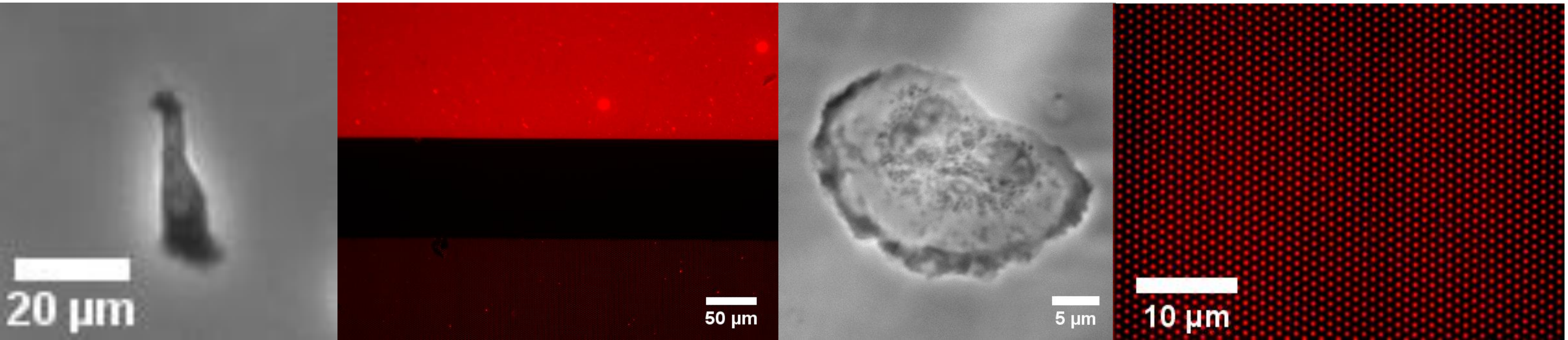


Henry et al. 2015. *ABME.* (In Prep)

* $p < 0.05$, post-hoc Dunn-Sidak multi. comp.

Part II Summary

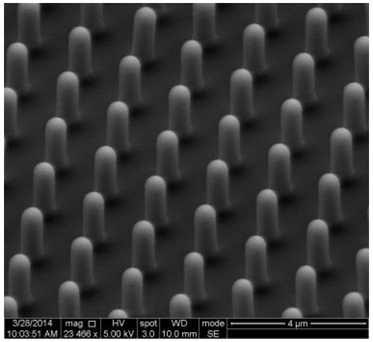
Neutrophils integrate local (submicron) adhesive stimuli and coordinate a global (whole cell) phenotypic response.

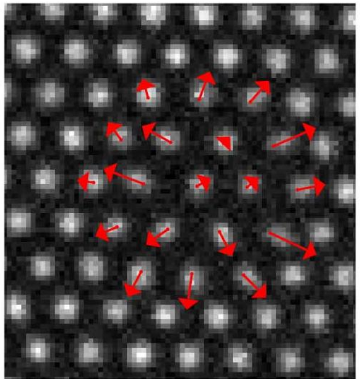


Spreading Mechanics

Dynamic traction forces of spreading and adherent human neutrophils

Henry, Chen, Crocker, Hammer. 2015. *Biophys J*. (Under Revision)





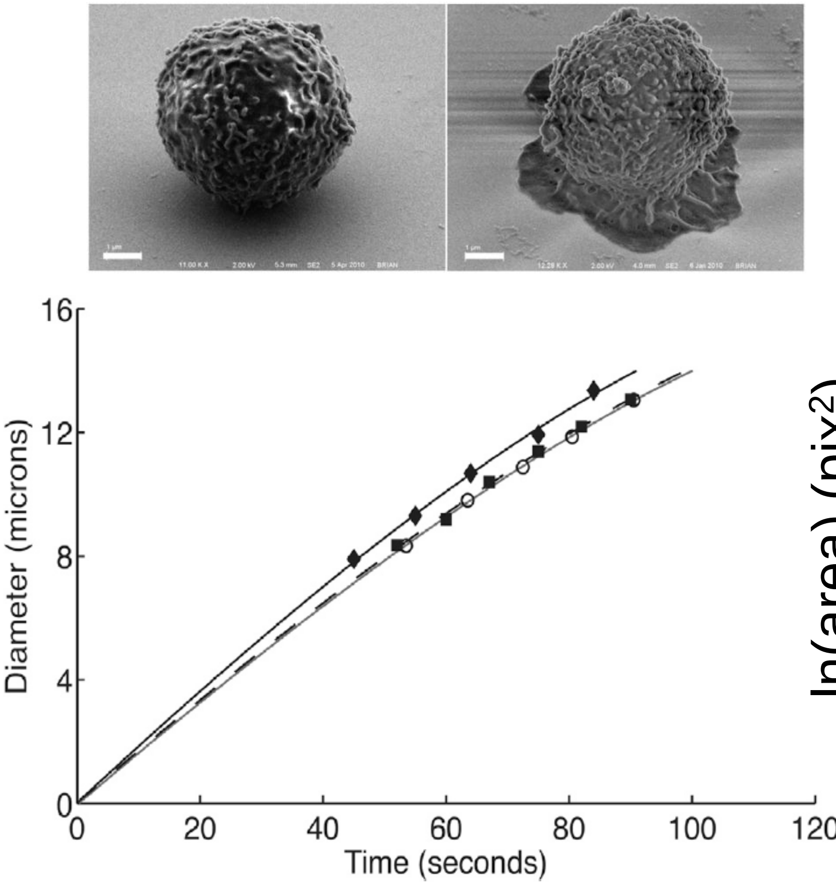
<u>Aim</u>:

Measure forces of adhesion-driven spreading

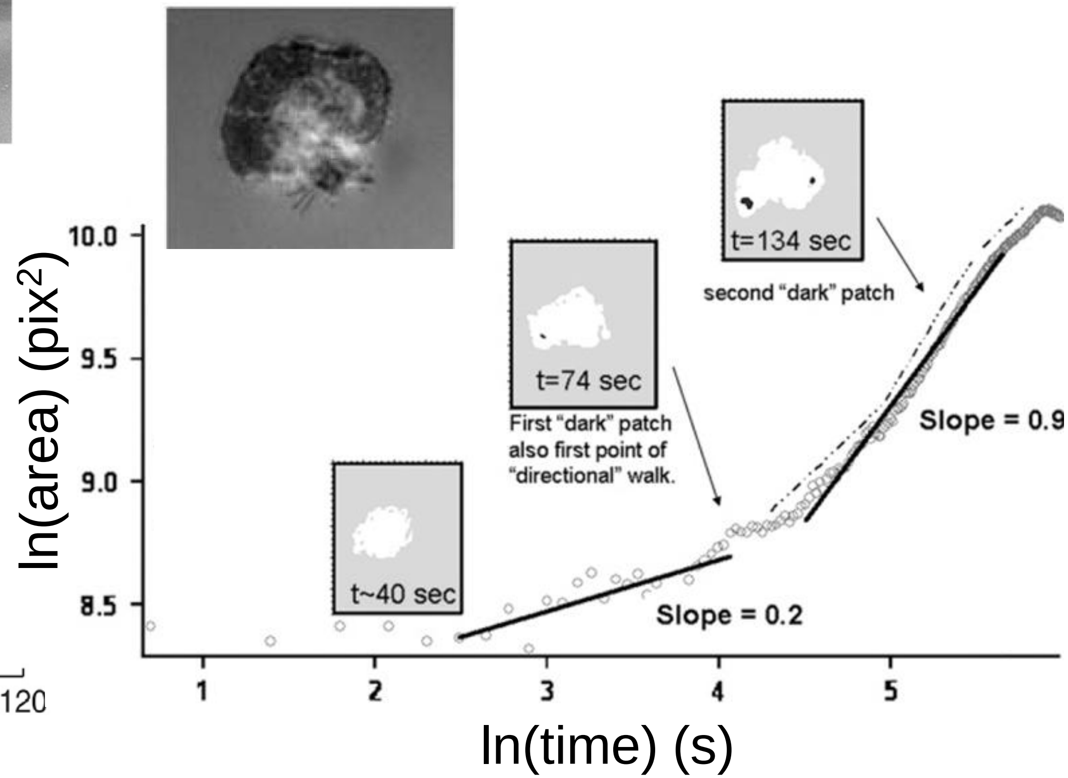
<u>Hypothesis</u>:

Spreading is an active process analogous to lamellipodium formation

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

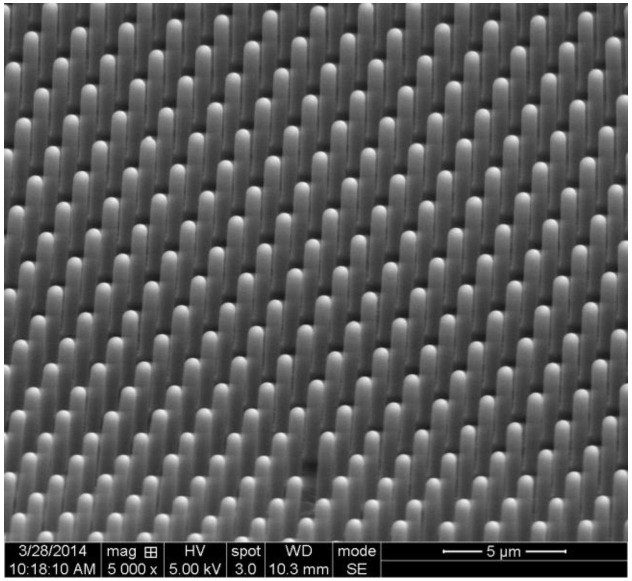


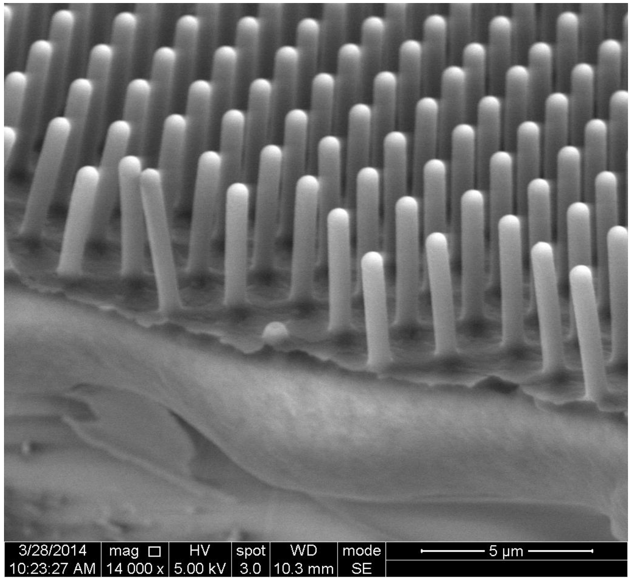
Lomakina et al. 2014. *Biophys J.*



Sengupta et al. 2006. *Biophys J.*

mPADs (microfabricated Post-Array-Detectors):





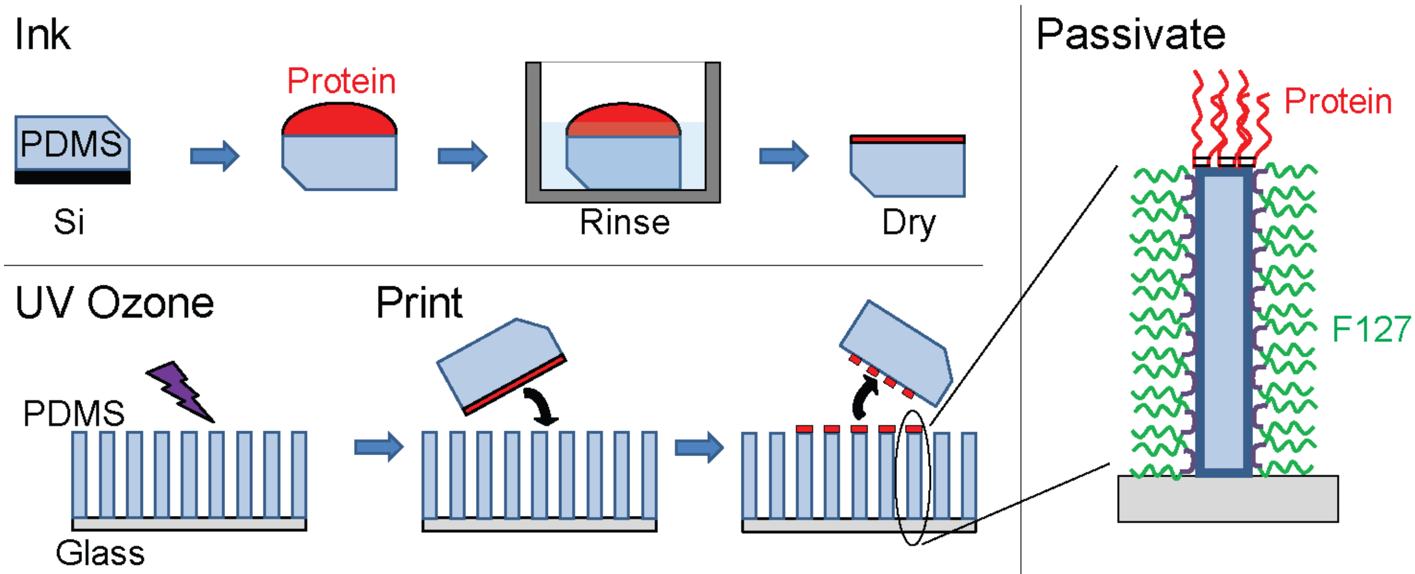
k_{spring} = 0.28 ± 0.07 nN/um
G ~ 5 kPa

Schoen correction = 0.93

k^*_{spring} = (0.93)(k_{spring})

k^*_{spring} = 0.26 nN/um

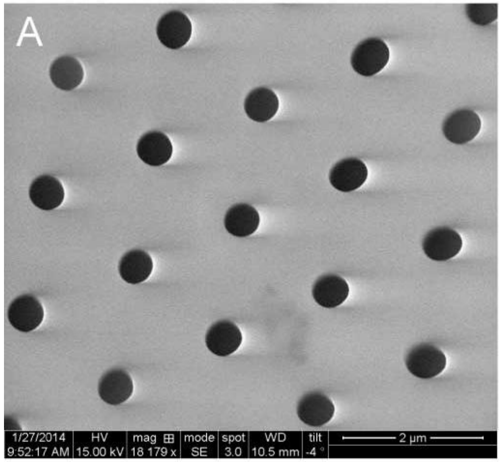
Schoen et al. 2010. *NanoLett*.



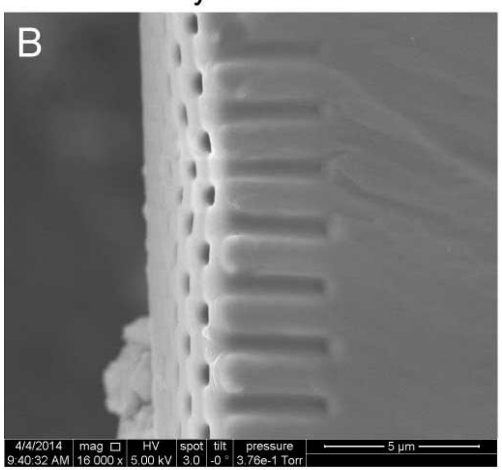
Henry et al. 2015. *Biophys J*. (Under Revision)

Array geometry preserved from Part II

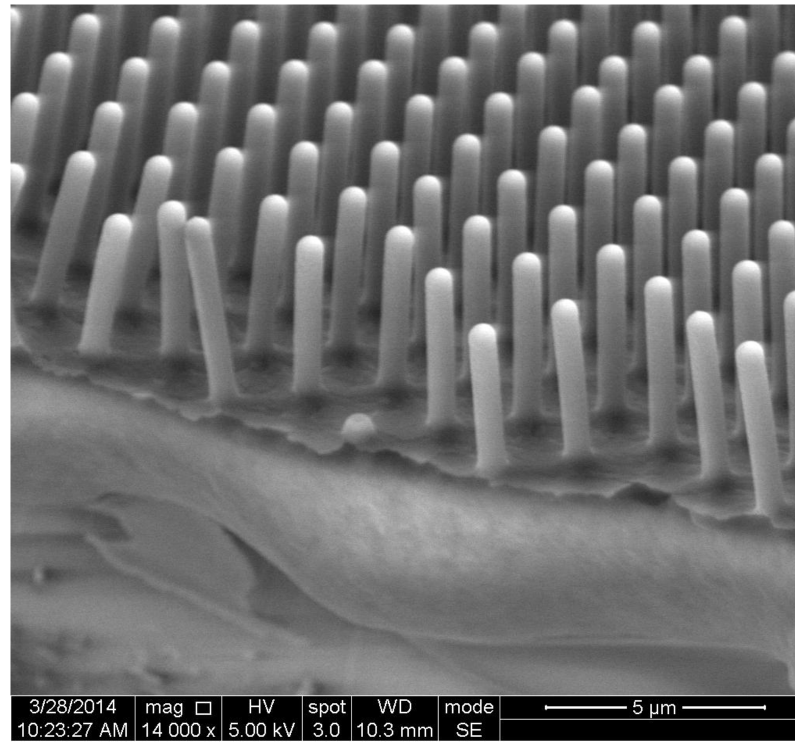
Hole Arrays: Plan View



Hole Arrays: Cross-Section



Henry et al. 2015. *ABME.* (In Prep)

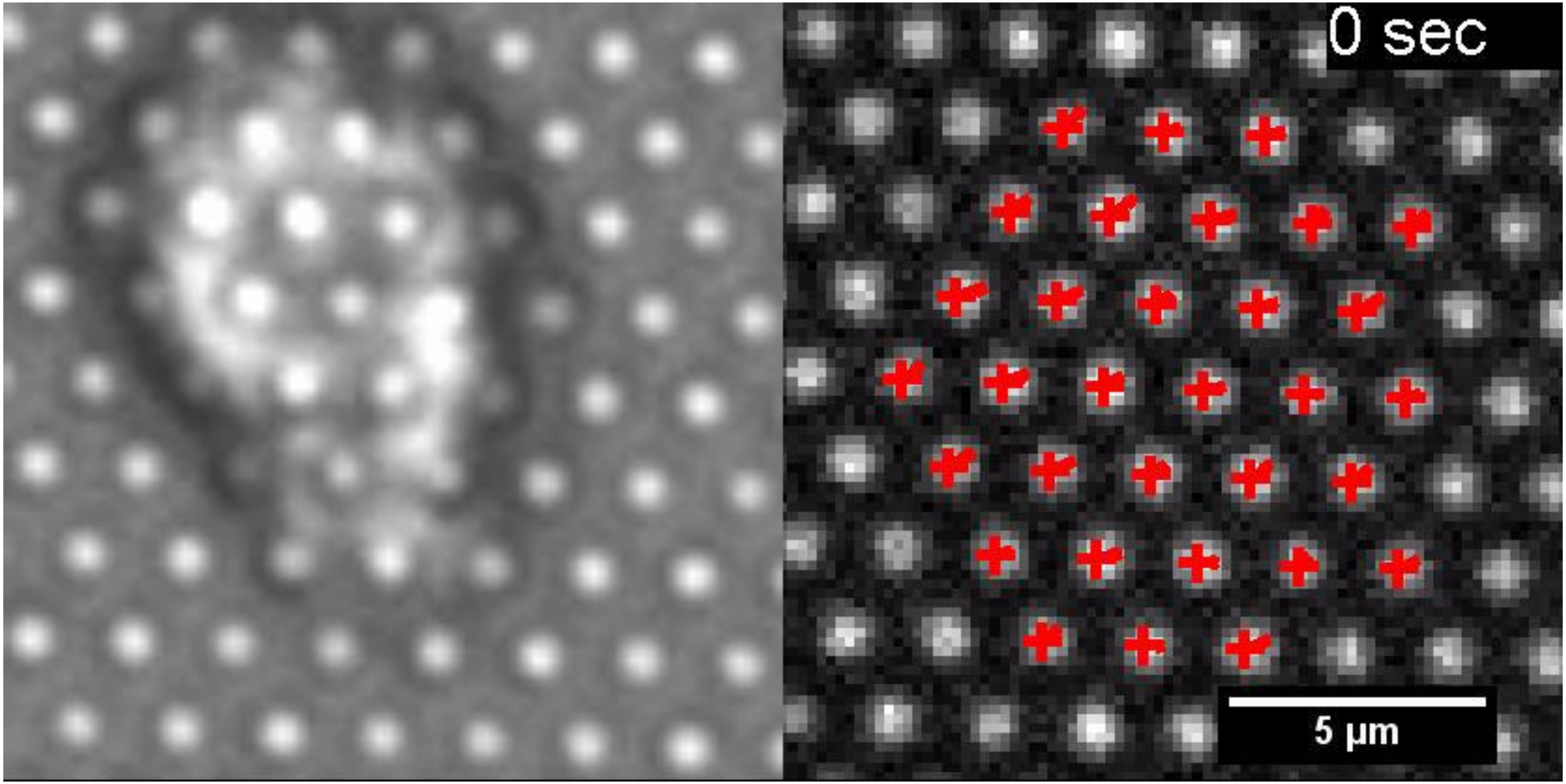


Henry et al. 2015. *Biophys J.* (Under Revision.)

Neutrophil spreading on mPADs: raw data

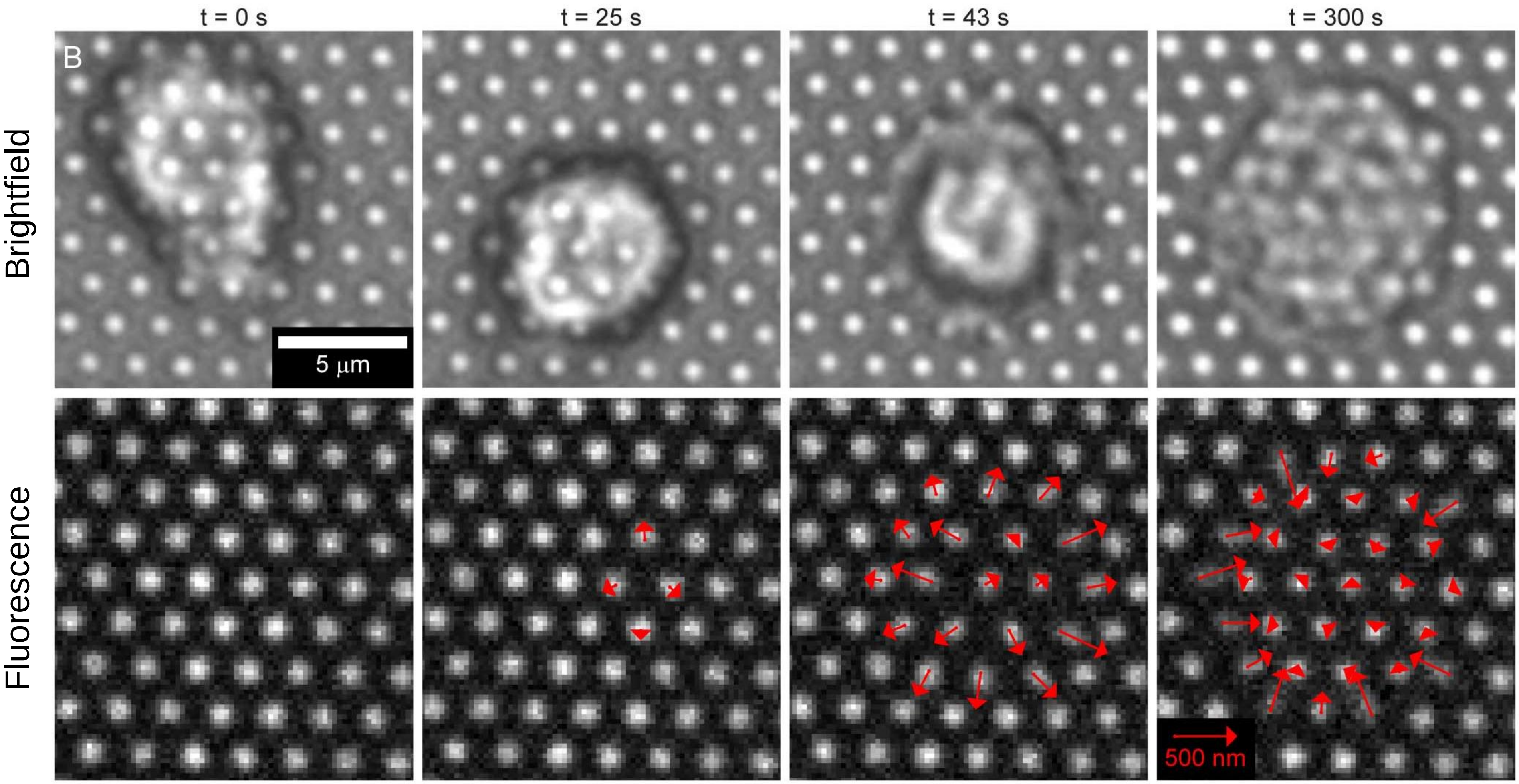
Henry et al. 2015. *Biophys J*. (Under Revision)

Neutrophil spreading on mPADs: force annotation



Henry et al. 2015. *Biophys J*. (Under Revision)

Neutrophil spreading on mPADs



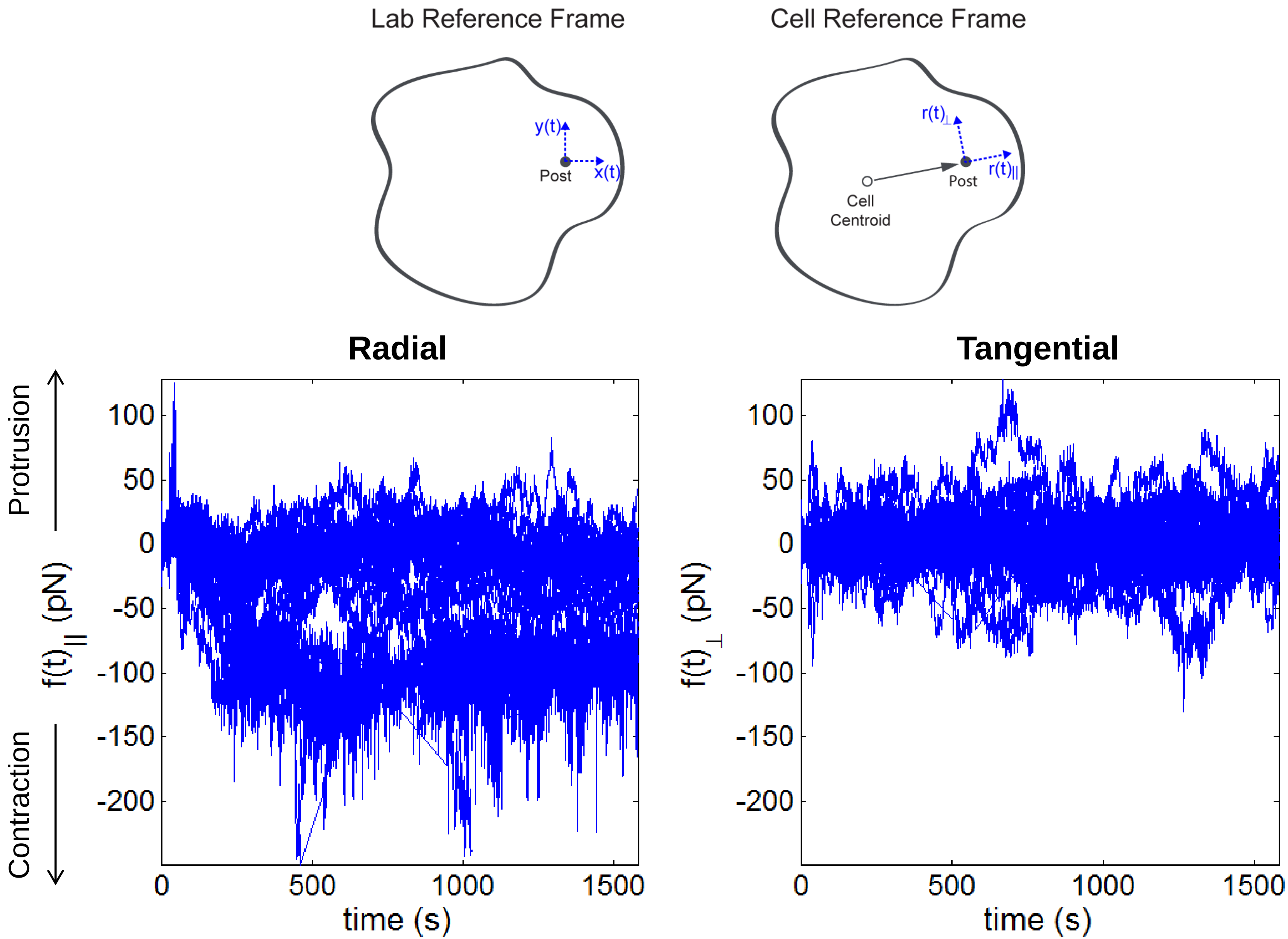
Adhesion Nucleation

Protrusion

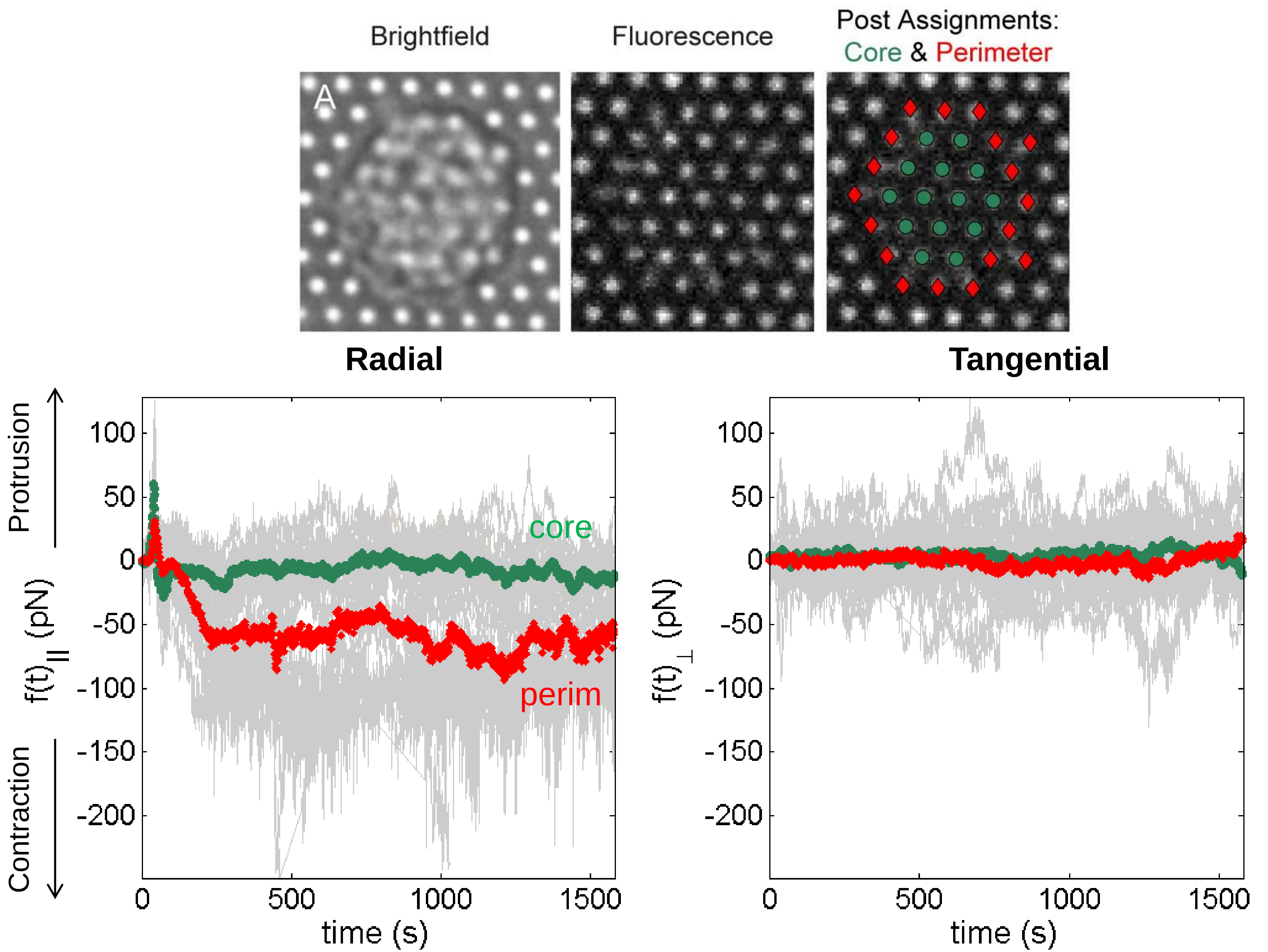
Contraction

Henry et al. 2015. *Biophys J*. (Under Revision)

Plotting force trajectories in the cell reference frame



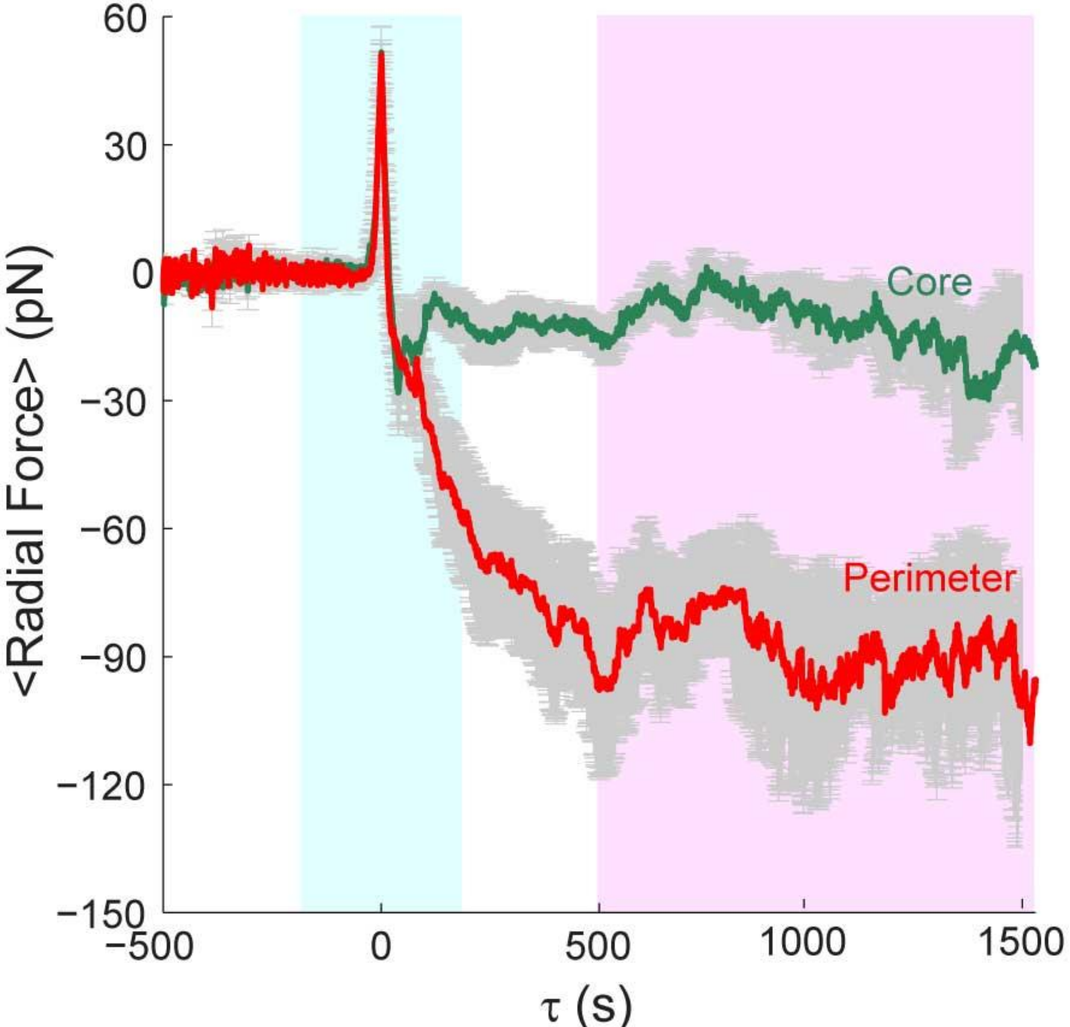
Dichotomizing data on geometric location



Ensemble avg makes mechanical regimes apparent

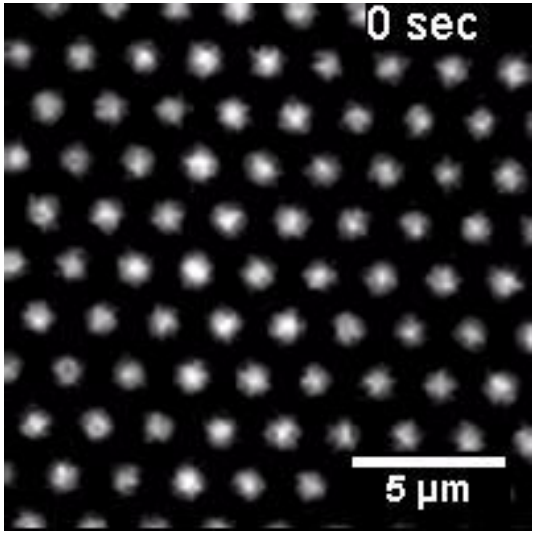
Transient Protrusion

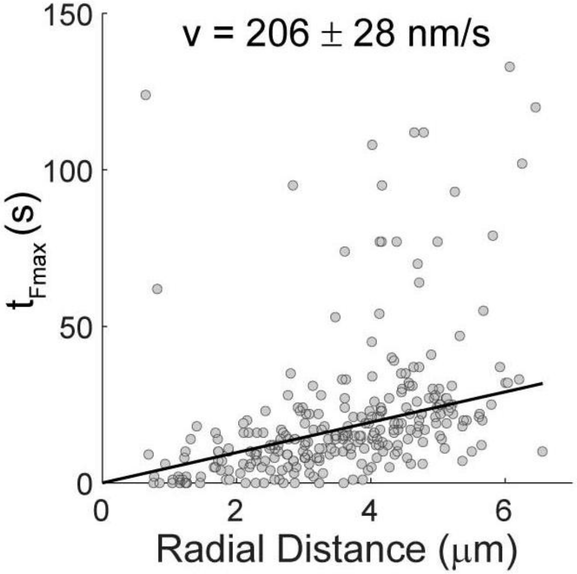
Steady State Contraction

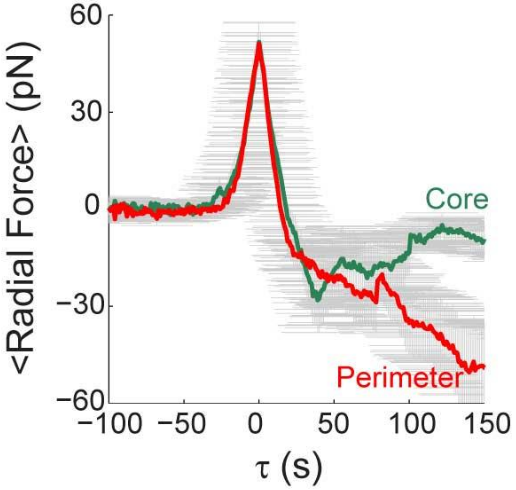


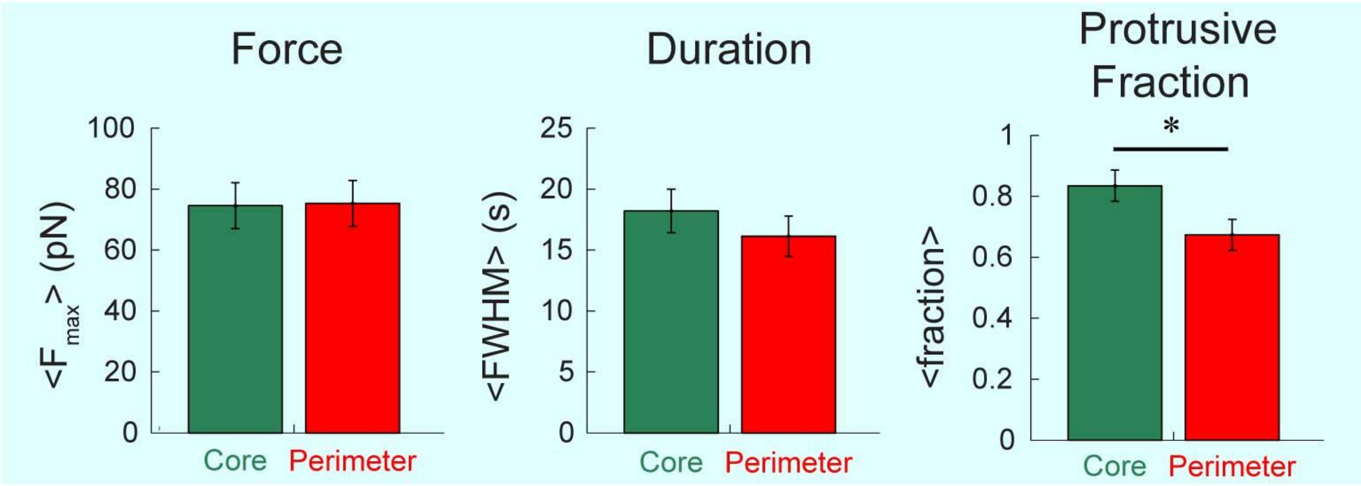
Henry et al. 2015. *Biophys J*. (Under Revision)

Characterizing the protrusive wave





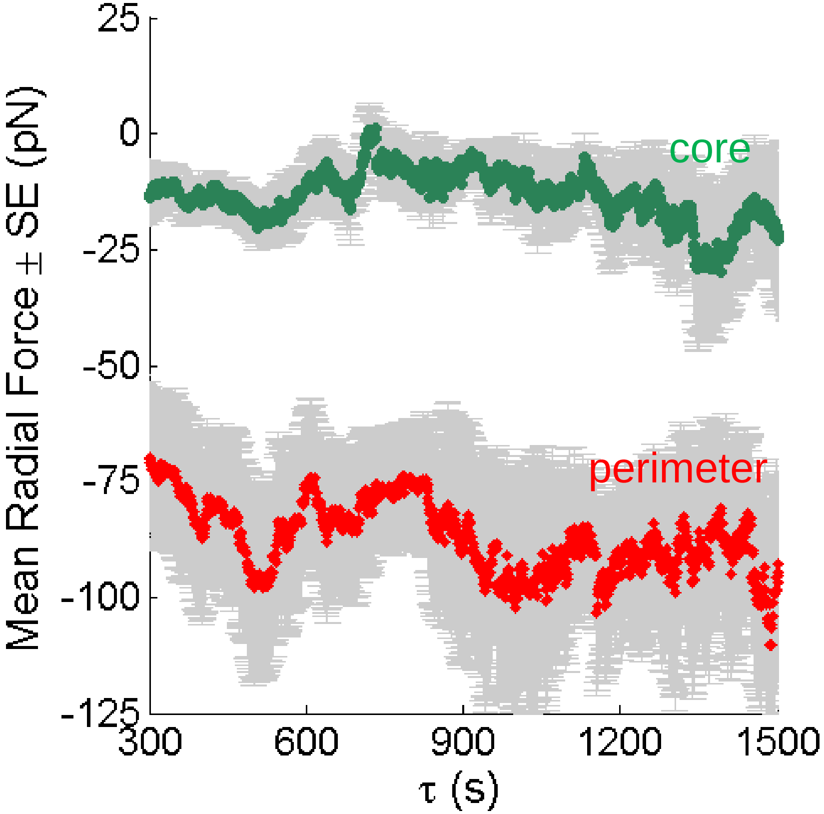


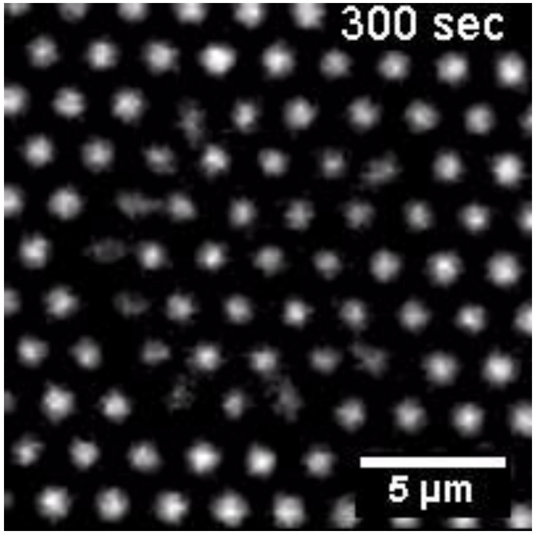


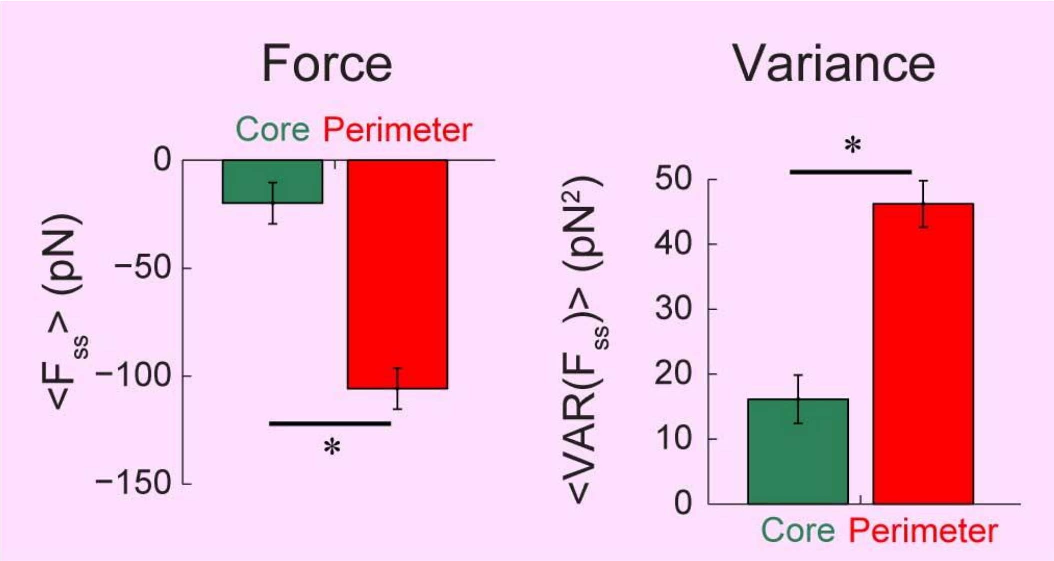
Henry et al. 2015. *Biophys J*. (Under Revision)

* $p < 0.05$, post-hoc Tukey LSD method

Characterizing the Steady State Contractile Regime







Henry et al. 2015. *Biophys J*. (Under Revision)

* $p < 0.05$, post-hoc Tukey LSD method

Are protrusion and contraction biochemically distinct?

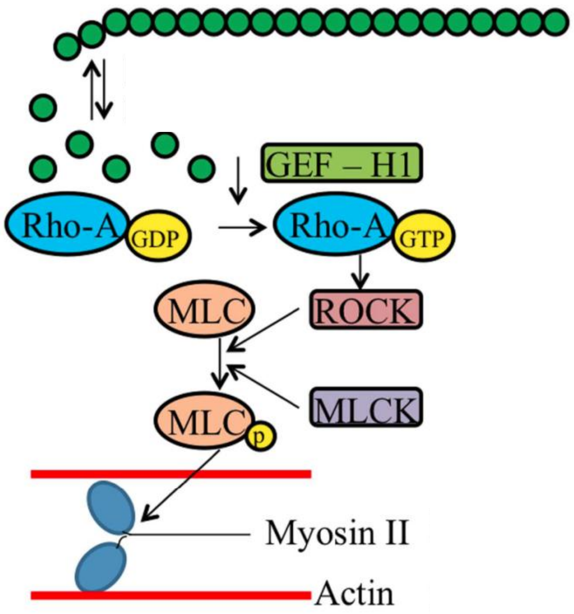
Hypothesis: Contraction is RhoA/Rock and Myosin Mediated

Y27632 (1 uM) ⊣ p160ROCK

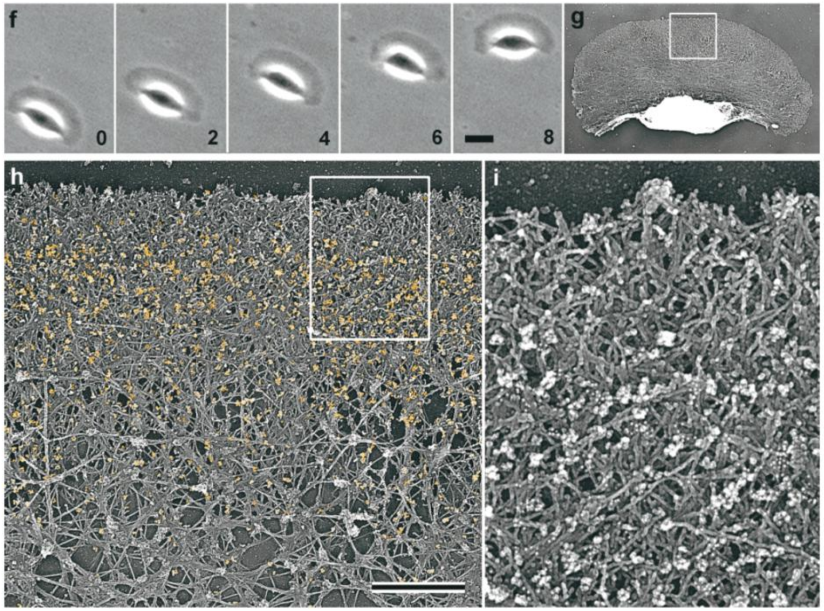
Blebbistatin (5 uM) ⊣ NM Myosin II

Hypothesis: Protrusion is lamellipodium formation

CK666 (1 uM) ⊣ Arp2/3

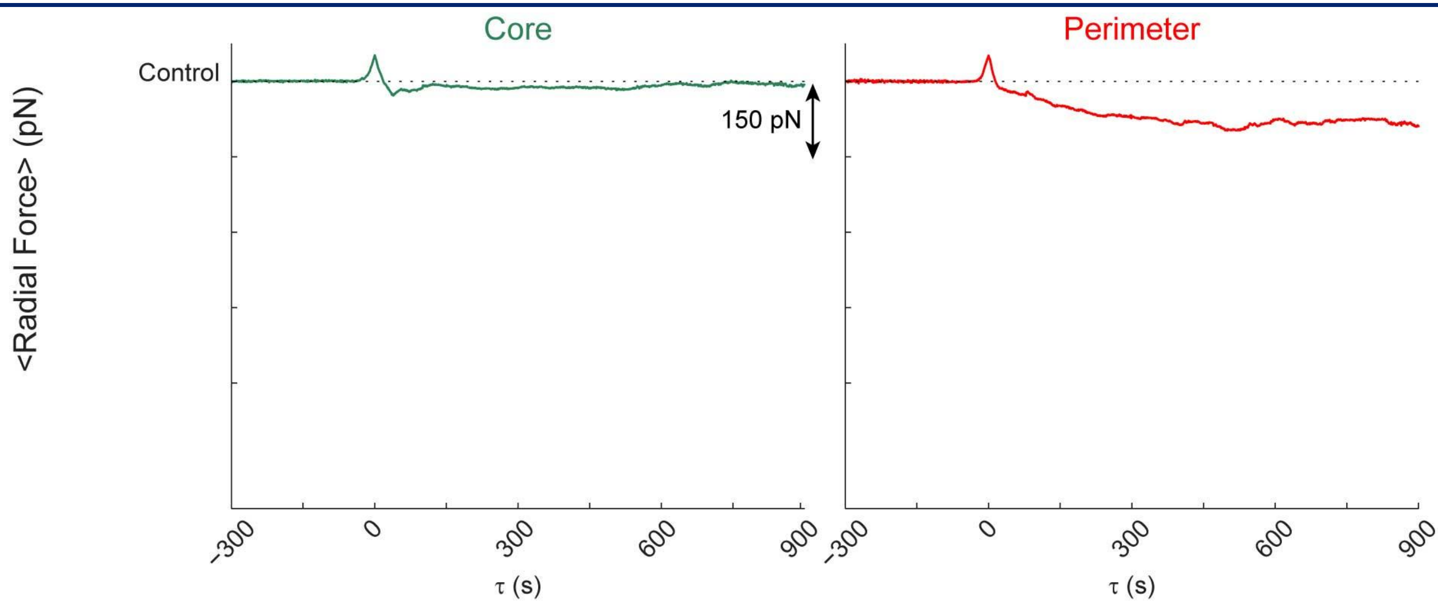


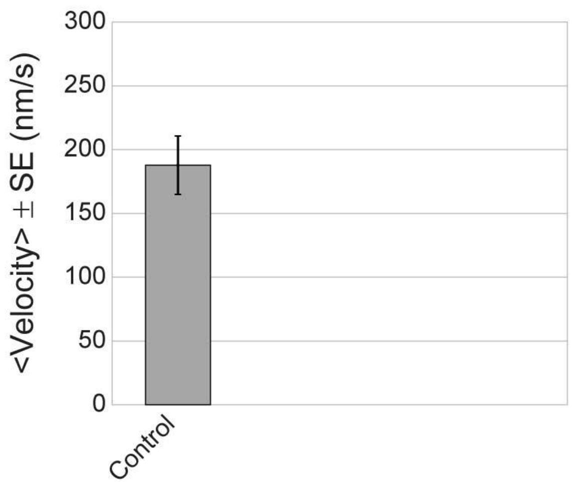
Modified from Stroka. 2013. *PLOS ONE*.



Svitkina. 1999. *JCB*.

Looking for inhibitor effects





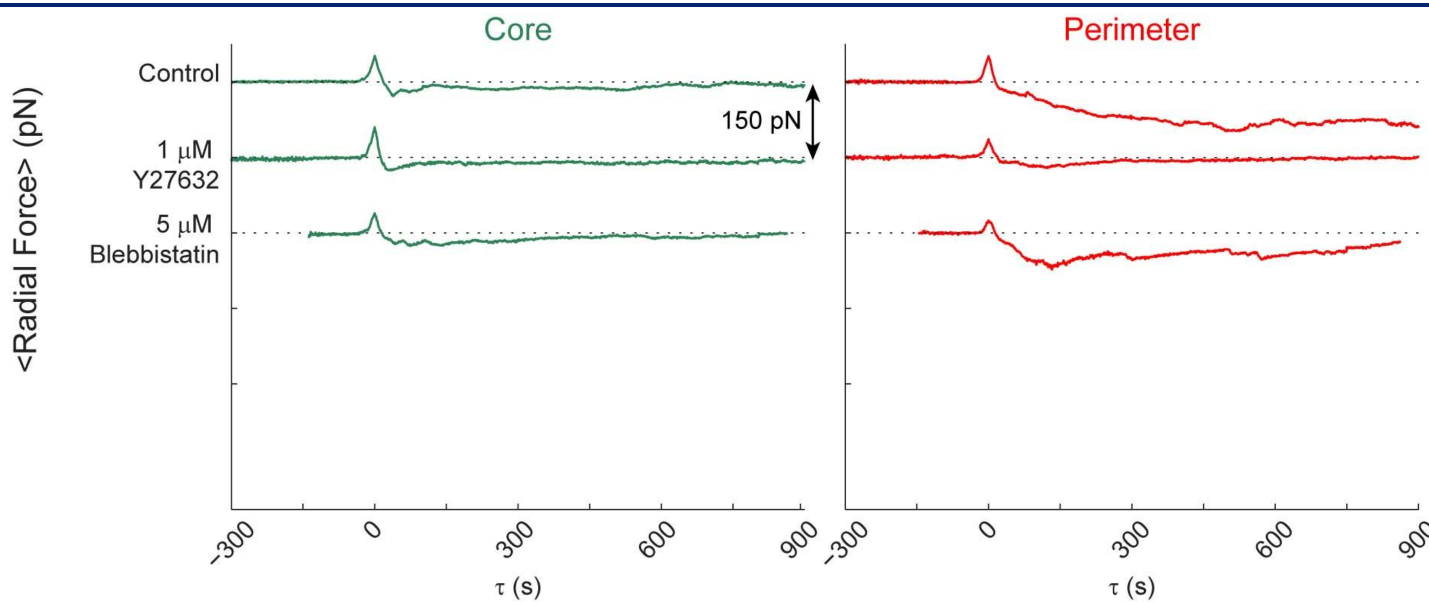
		n cells n posts					
Protrusion	Force	Core Perimeter					
Protrusion	Duration	Core Perimeter					
Protrusion	Variance	Core Perimeter					
Protrusion	Participation	Core Perimeter					
Protrusion	Velocity	All Posts					
Contraction	Force	Core Perimeter					
Contraction	Variance	Core Perimeter					

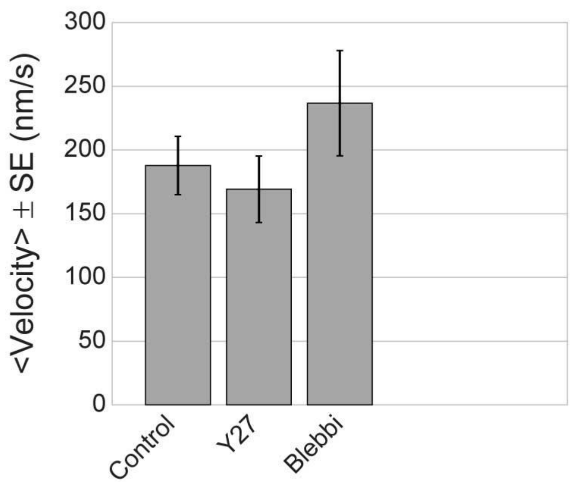
(grey box) = no sig. diff.

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

Henry et al. 2015. *Biophys J*. (Under Revision)

Sustained contractility is ROCK and Myosin II mediated





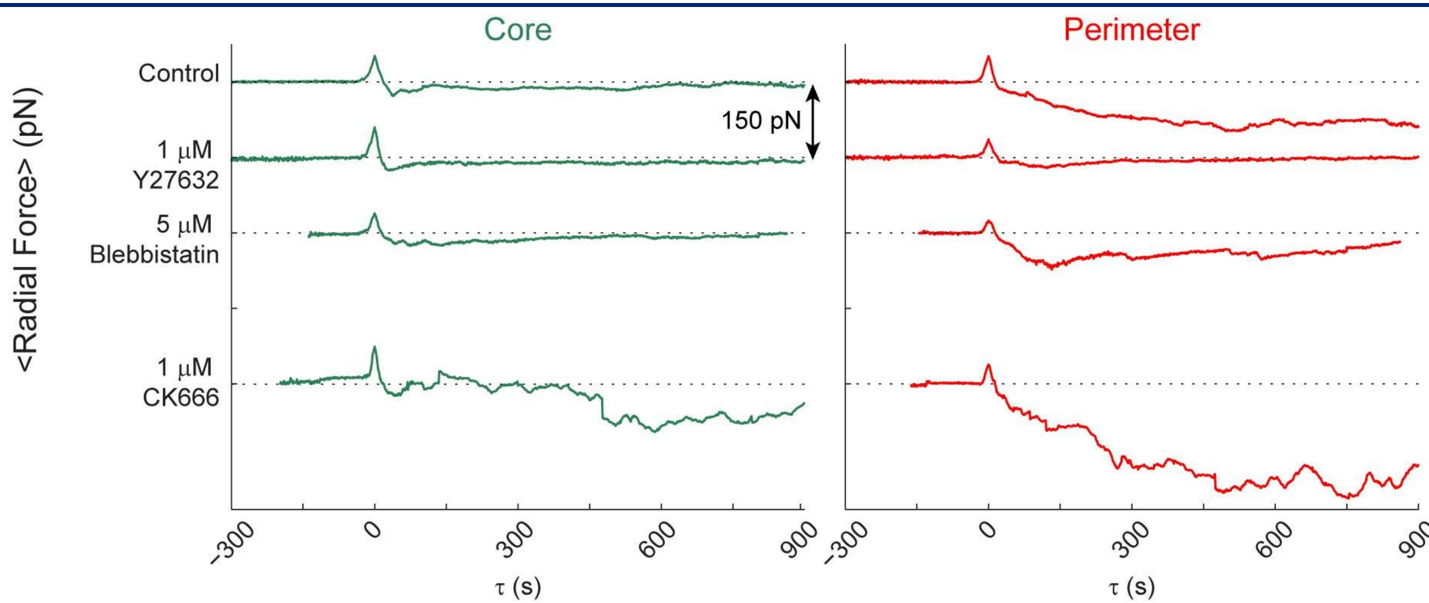
			Y27	Blebbi
		n cells	10	4
		n posts	325	117
Protrusion	Force	Core		
		Perimeter		
	Duration	Core		
		Perimeter		
	Variance	Core		
		Perimeter		
	Participation	Core		
		Perimeter		
	Velocity	All Posts		
Contraction	Force	Core		
		Perimeter	*↓	*↓
	Variance	Core		
		Perimeter	*↓	

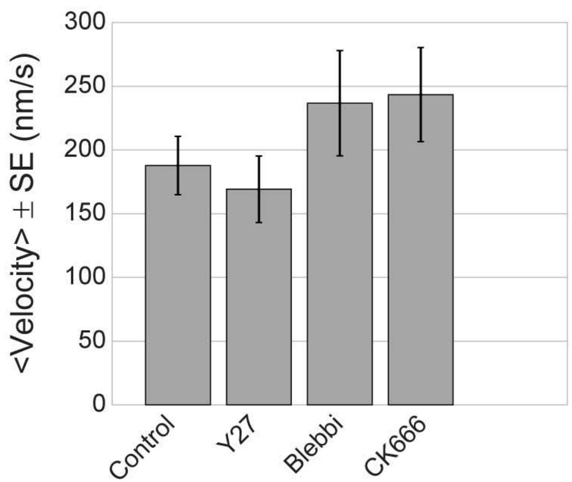
(grey box) = no sig. diff.

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

Henry et al. 2015. *Biophys J*. {Under Revision)

Spreading is **not** actin-branching liable





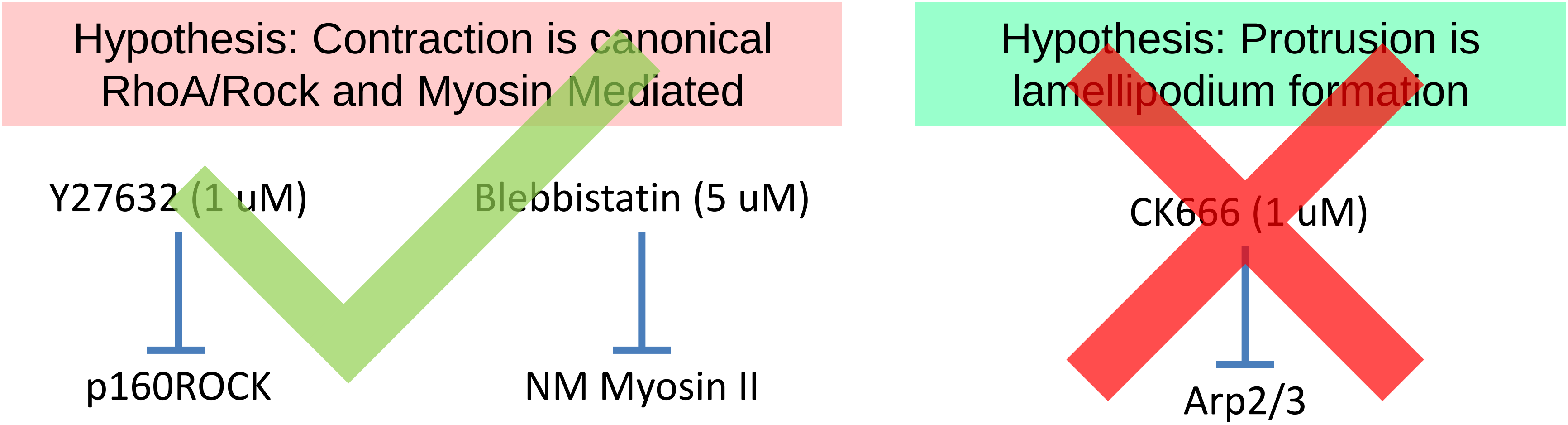
			Y27	Blebbi	CK666
		n cells	10	4	5
		n posts	325	117	161
Protrusion	Force	Core			
		Perimeter			
	Duration	Core			
		Perimeter			
	Variance	Core			
		Perimeter			
	Participation	Core			
		Perimeter			
	Velocity	All Posts			
Contraction	Force	Core			
		Perimeter	*↓	*↓	
	Variance	Core			*↑
		Perimeter	*↓		

(grey) = no sig. diff.

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

Henry et al. 2015. *Biophys J*. (Under Revision)

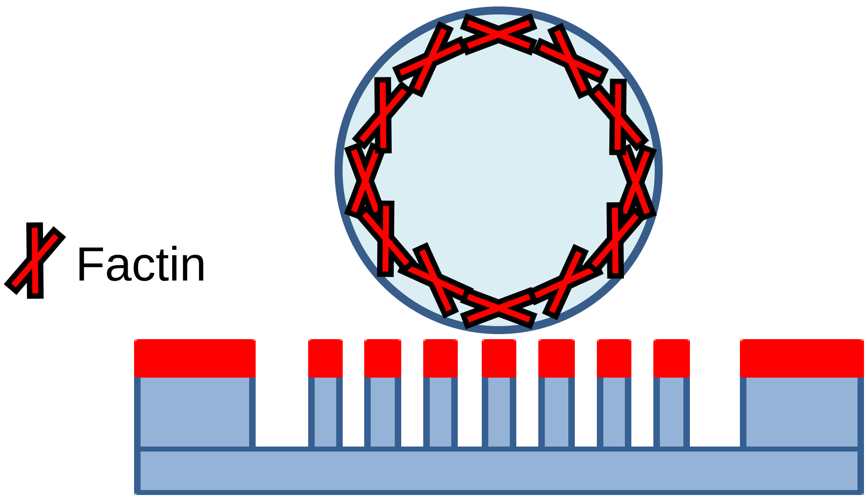
Spreading is not analogous to lamellipodium formation

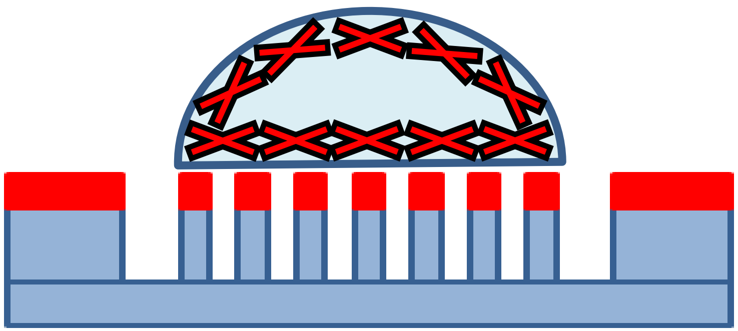


Competition b/n adhesive energy and cortical stiffness?

Tension > Adhesive Energy

Tension < Adhesive Energy

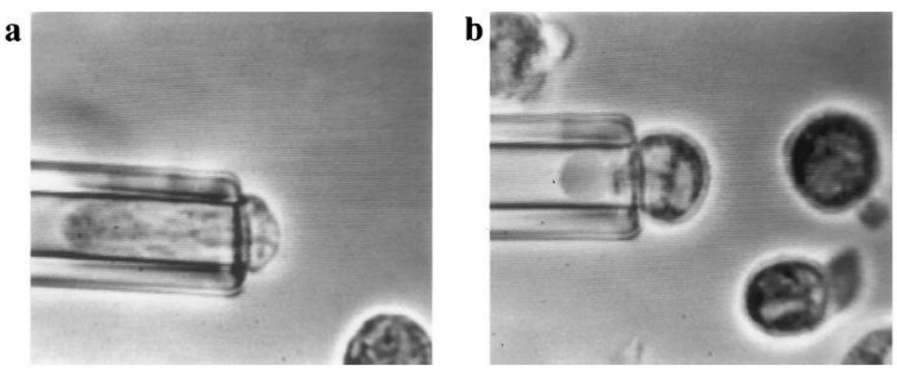




Jasplakinolide = stiffening

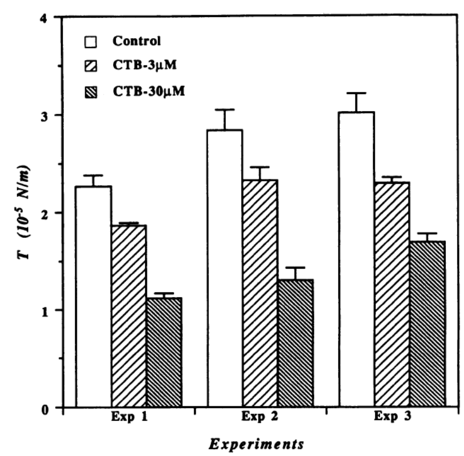
Control

10 uM



Sheikh et al. 1997. *BBRC.*

Cytochalasin B = softening



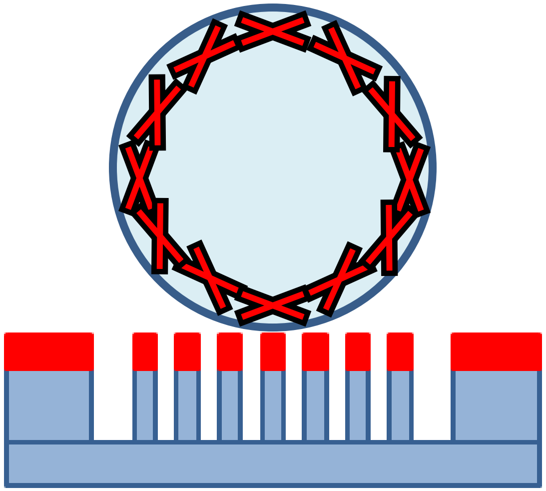
Tsai et al. 1994. *Biophys J.*

A revised hypothesis:

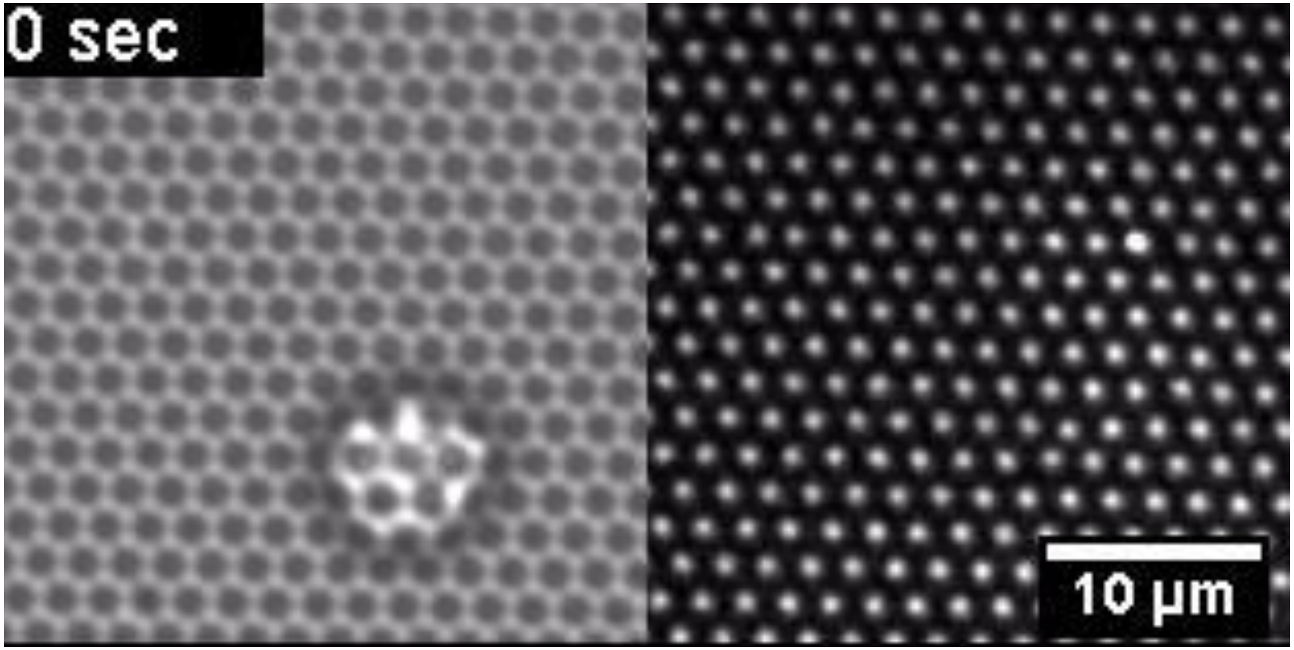
Hypothesis: Contraction is canonical RhoA/Rock and Myosin Mediated		Hypothesis: cortical tension resists spreading	
Y27632 (1 uM)	Blebbistatin (5 uM)	Jasplakinolide (1 uM)	CytochalasinB (3 uM)
⊥	⊥	⊥	⊥
p160ROCK	NM Myosin II	actin depolymerization; (↑ cortical stiffness)	actin polymerization & filament interaction; (↓ cortical stiffness)

Cortical stiffening via Jasplakinolide abrogates spreading

1 uM Jasplakinolide inhibits cortical actin depolymerization

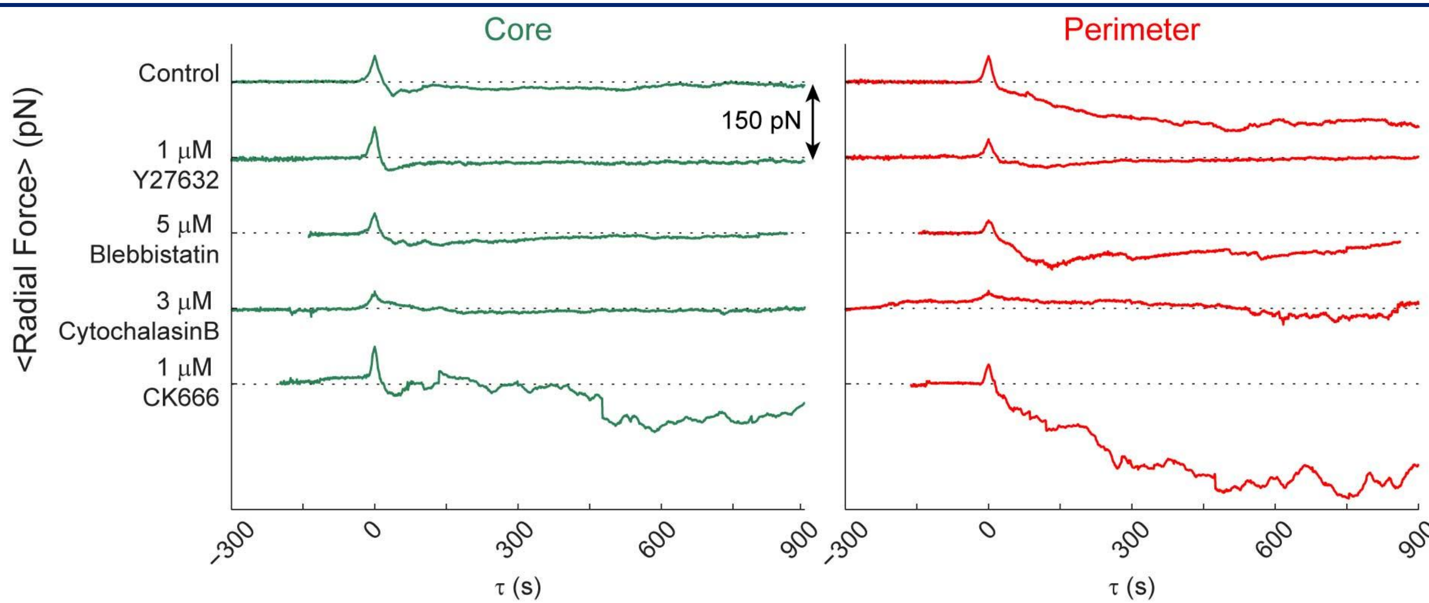


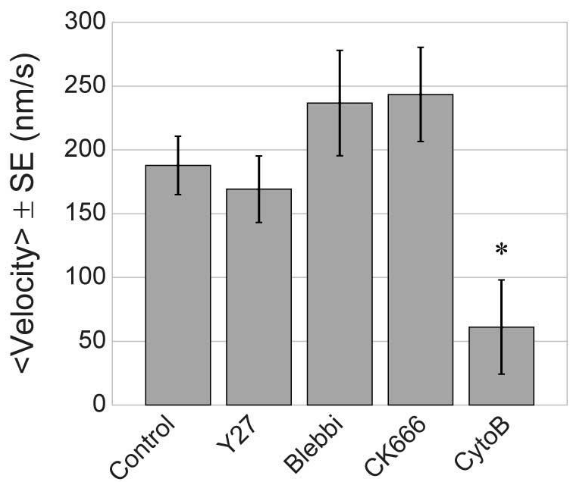
Cross-linked filamenteous actin



Henry et al. 2015. *Biophys J*. (Under Revision)

Cortical **stiffening eliminates** spreading
Cortical **softening slows** spreading





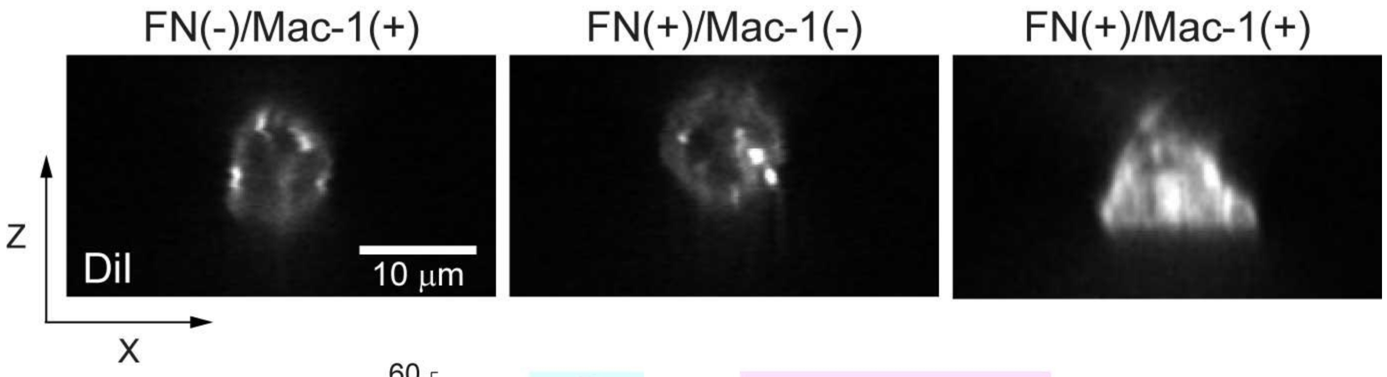
			Y27	Blebbi	CK666	Jasp	CytoB
		n cells	10	4	5	9	6
		n posts	325	117	161	n/a	177
Protrusion	Force	Core				No Spreading	
		Perimeter					
	Duration	Core					*↑
		Perimeter					*↑
	Variance	Core					
		Perimeter					
	Participation	Core					
		Perimeter					
	Velocity	All Posts					*↓
Contraction	Force	Core					
		Perimeter	*↓	*↓			*↓
	Variance	Core			*↑		
		Perimeter	*↓				

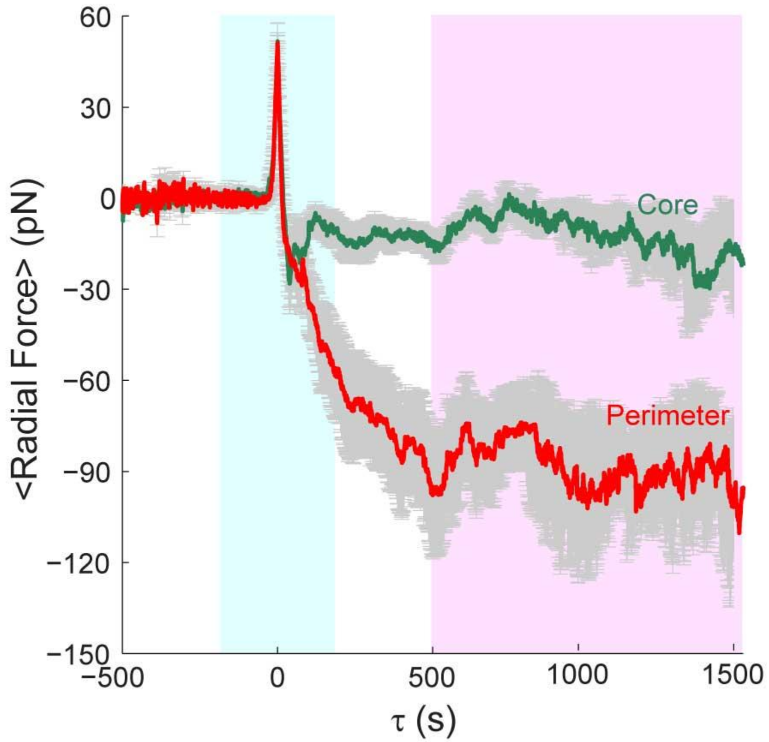
(grey box) = no sig. diff.

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

Henry et al. 2015. *Biophys J*. (Under Revision)

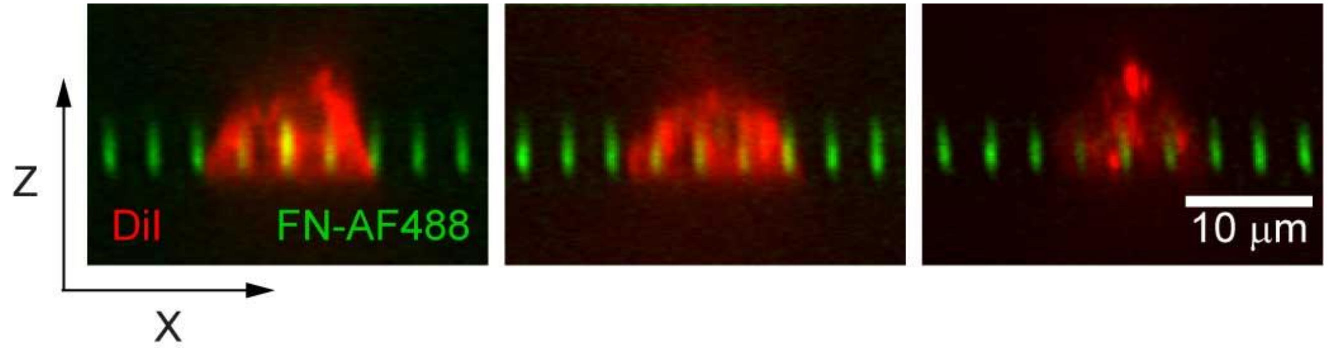
Spreading is integrin mediated **but** connection to the mature actomyosin substructure takes minutes to develop…

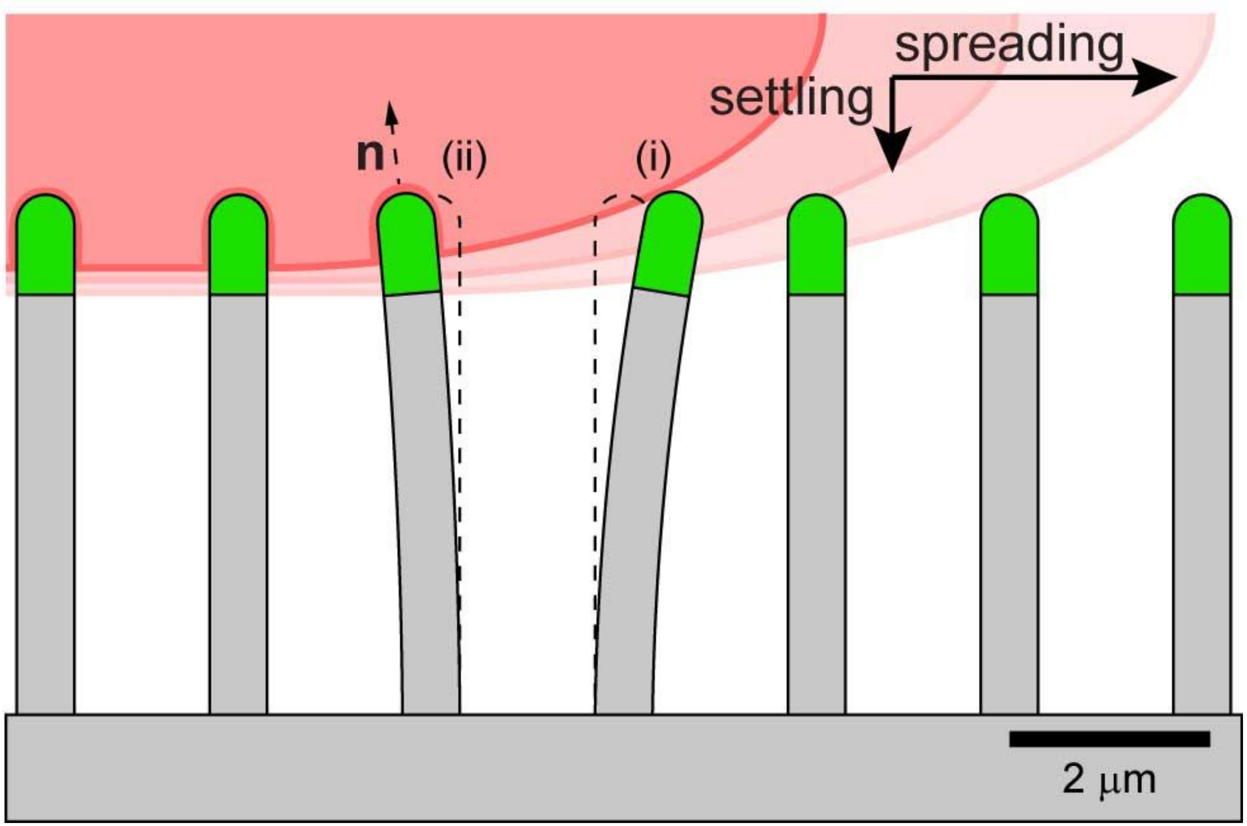




Why do we see protrusion at all?

Invagination: a spreading neutrophil pushing through post tips



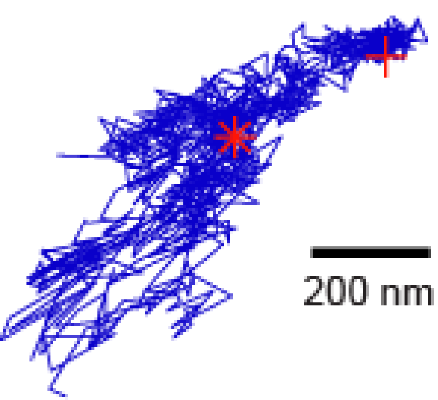


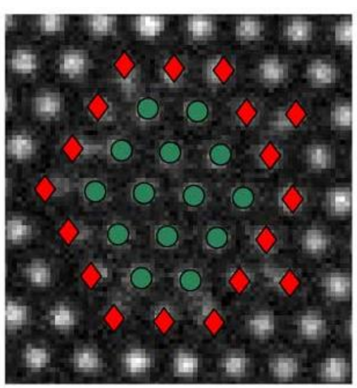
Calibrated invagination depth ~ 1 um

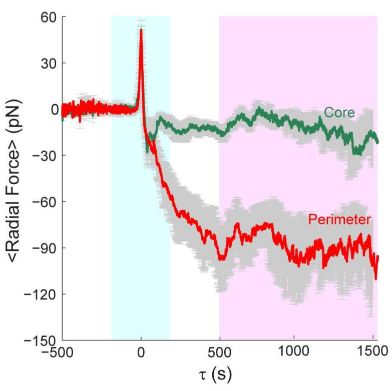
Schematic to scale

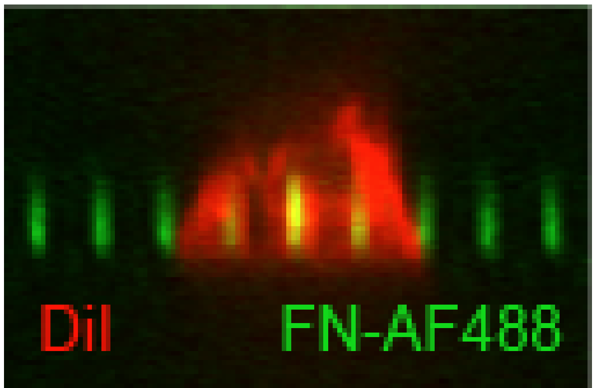
Part III Summary

Neutrophil adhesion-driven spreading is itself a phenotypic switch triggered by decrease in resting cortical tension.

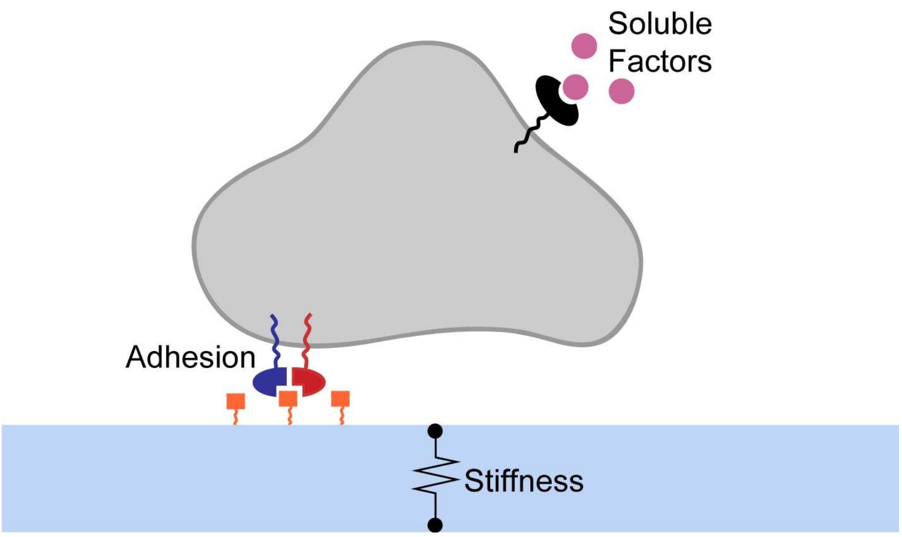


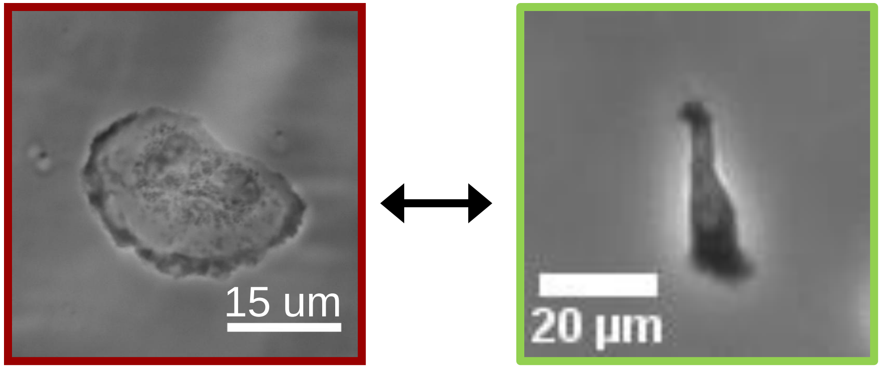


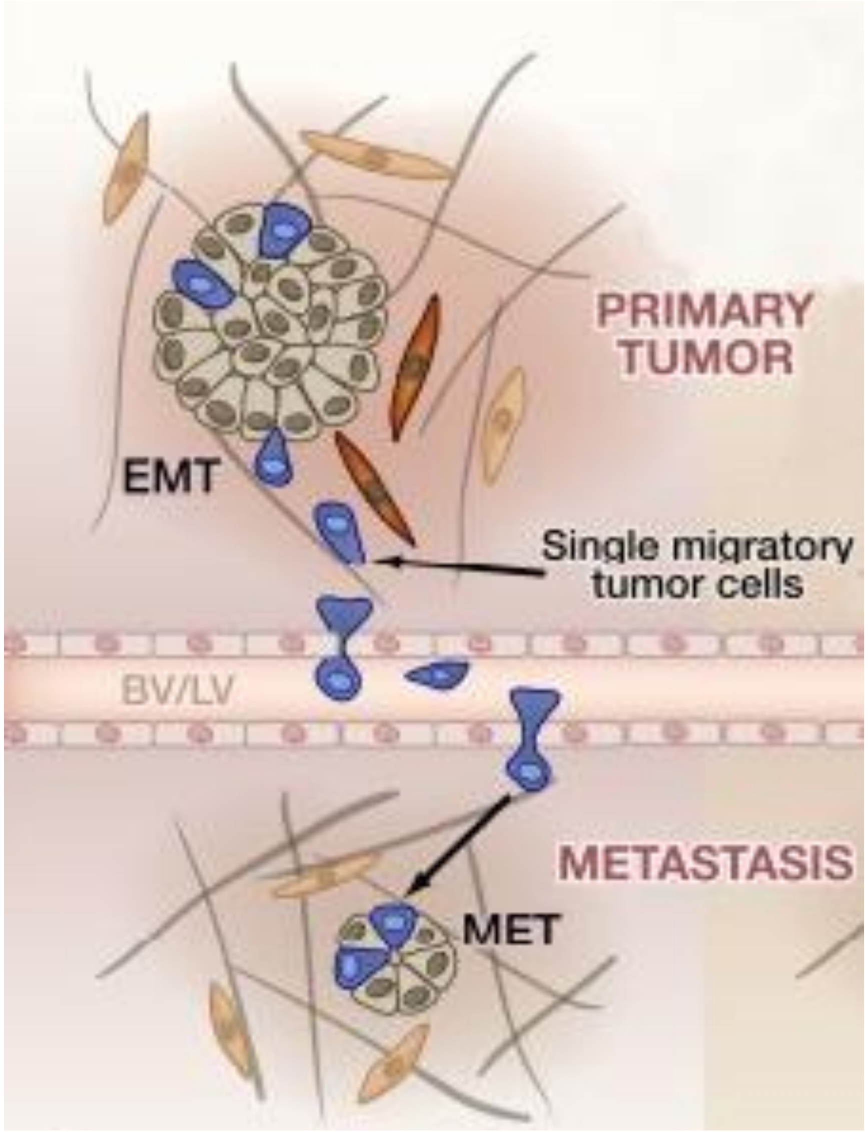




Role of adhesivity in cancer metastasis?







Modified from Thiery et al. 2009. *Cell*.

Thank you!

Advisor

Daniel A. Hammer, PhD

Committee

Scott L. Diamond, PhD (Chair)

John C. Crocker, PhD

Dongeun Huh, PhD

Scientific Collaborators

John C. Crocker, PhD

Christopher S. Chen, PhD

Neha P. Kamat, PhD

Daeyeon Lee, PhD

Hammer Lab

All members past and present

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National Institutes of Health

(HL18208 to DAH)

National Science Foundation

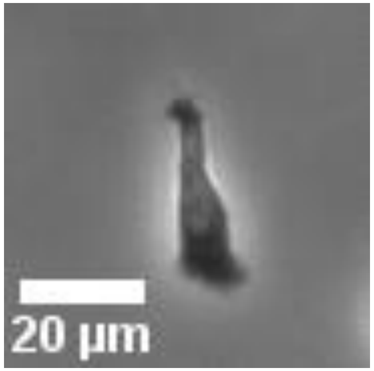
(GRFP to SJH)

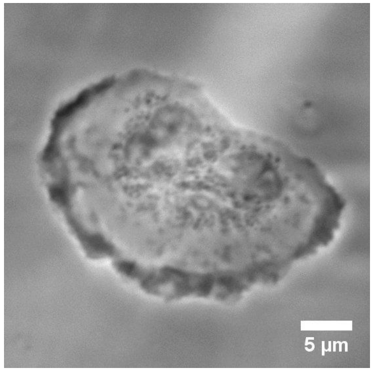
Questions?

Shape and Motility

Ligand density elicits phenotypic switch in human neutrophils

Henry, Crocker, Hammer. 2014. *Integr Biol.*

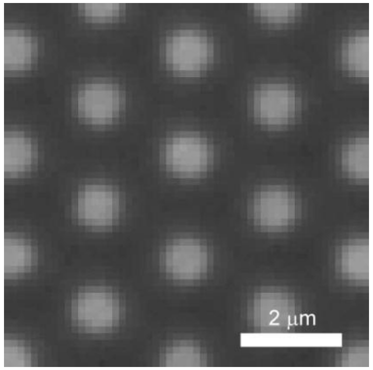


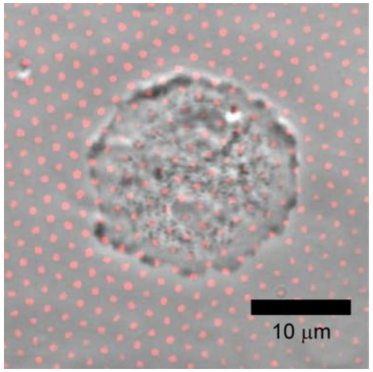


Density Sensing

Dynamic traction forces of spreading and adherent human neutrophils

Henry, Crocker, Hammer. 2015. *ABME* (In Prep)





Spreading Mechanics

Dynamic traction forces of spreading and adherent human neutrophils

Henry, Chen, Crocker, Hammer. 2015. *Biophys J.* (Under Revision)