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Motor Powered Alignment of Cytoskeletal Filaments

Shahid Khan

LUMS_SSE Lifesciences, Lahore. PAKISTAN

Molecular Biology Consortium, Chicago. USA

Team Members

LUMS_SSE

Lifescience

- *Tariq Butt*
- *Rehan Ali*
- *Nimra Asi*
- *Shahid Khan*

Computer Science

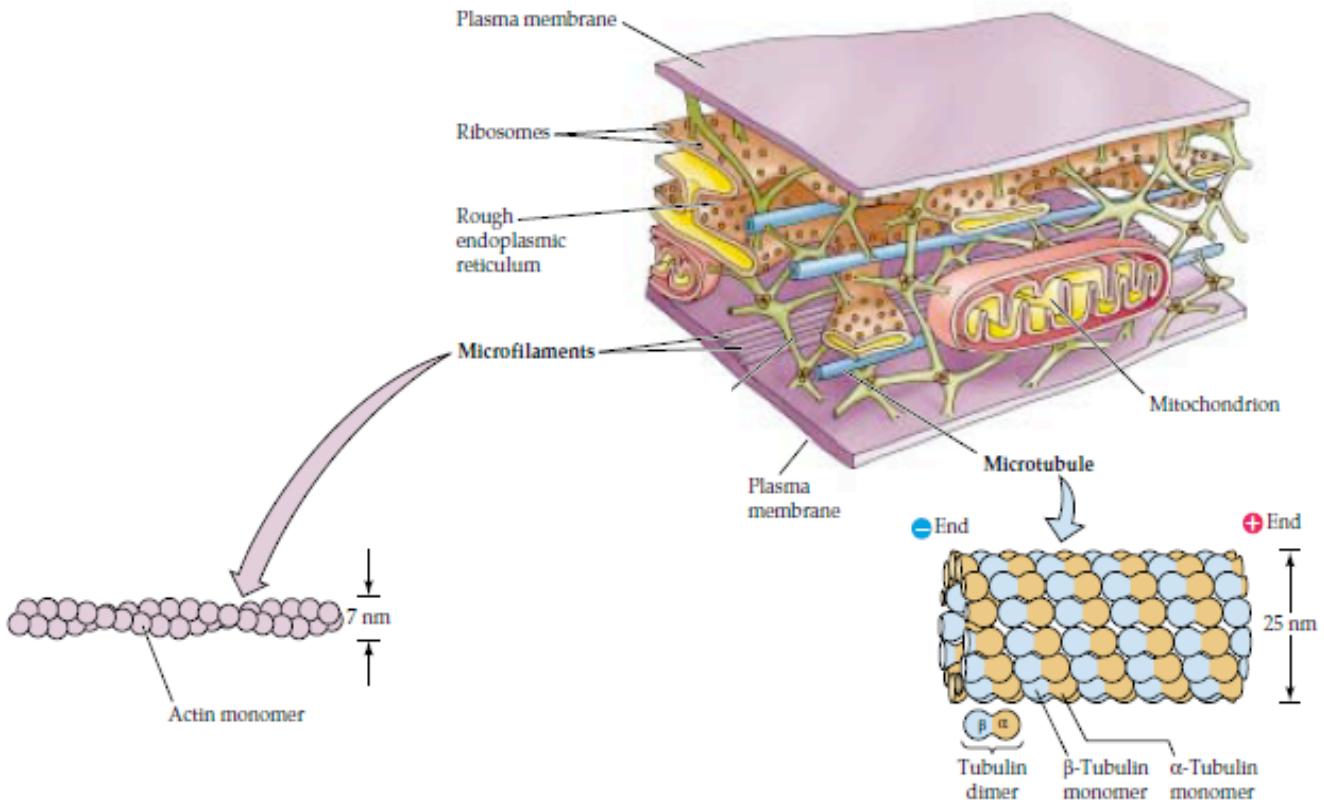
- *Tabish Mufti*
- *Ahmad Humayun*
- *Sohaib Khan*

NIMR

Physical Biochemistry

- *Steve Rosenthal*
- *Justin Molloy*

The Cell Cytoskekeleton



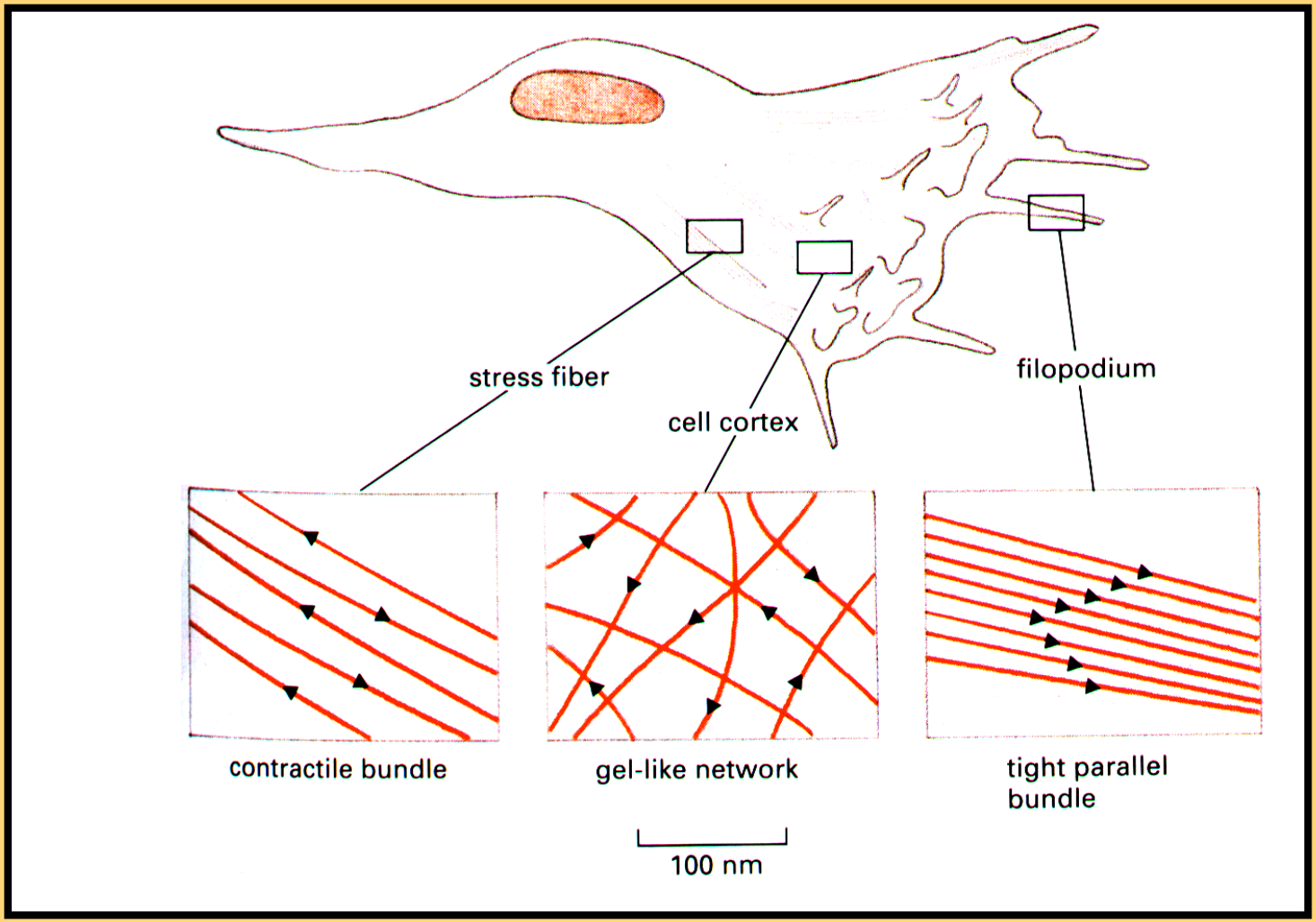
Functions

1.-Architecture
2.-Transport
3.-Cell \ Organelle Dynamics

Mechanical Modes

1.-Twisting
2.-Bending
3.-Breakage
4.-Alignment

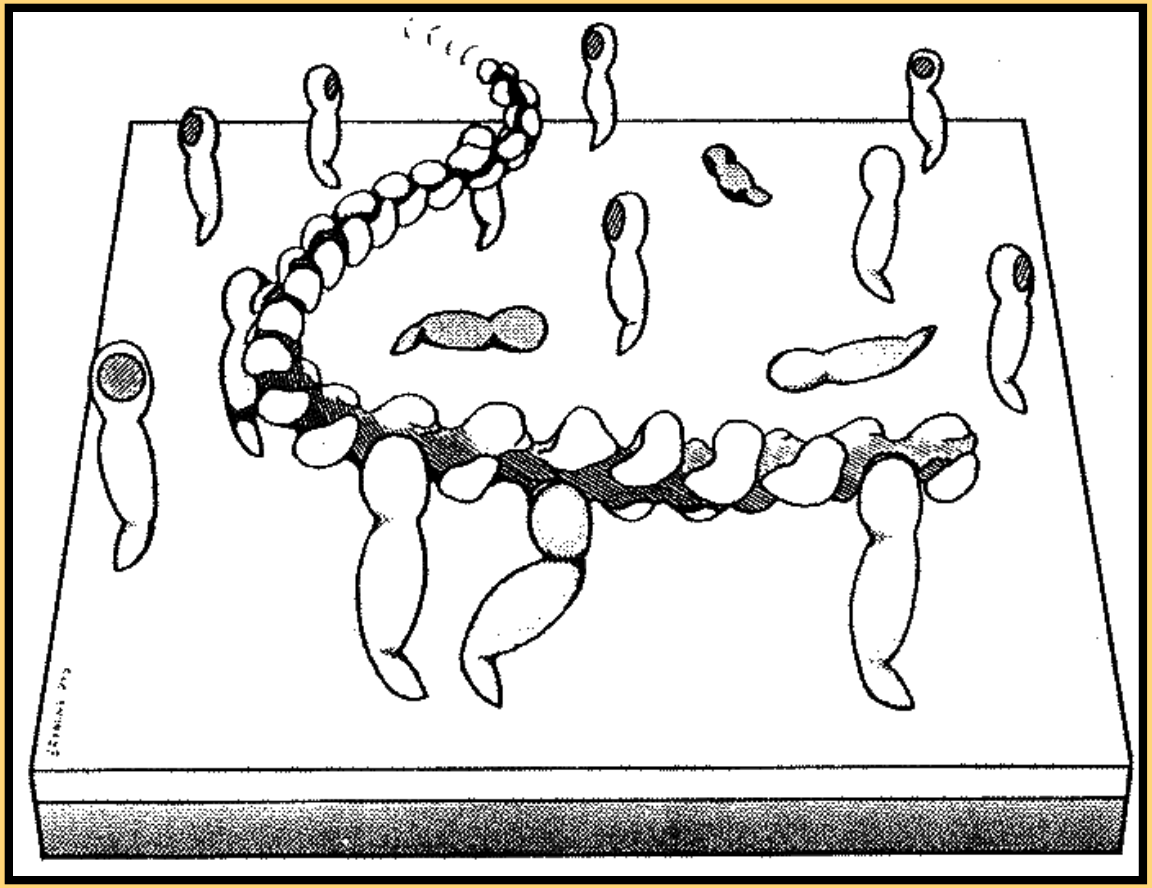
Actin Organization in Cells



[Actin] ~ 10 to 500μM (~1mM in muscle)

[Myosin] ~ 1 to 50μM (~0.2mM in muscle)

In Vitro Motility Assay



Ingredients

1.-Skeletal muscle meromyosin

2.-Rhodamine-phalloidin F-actin.

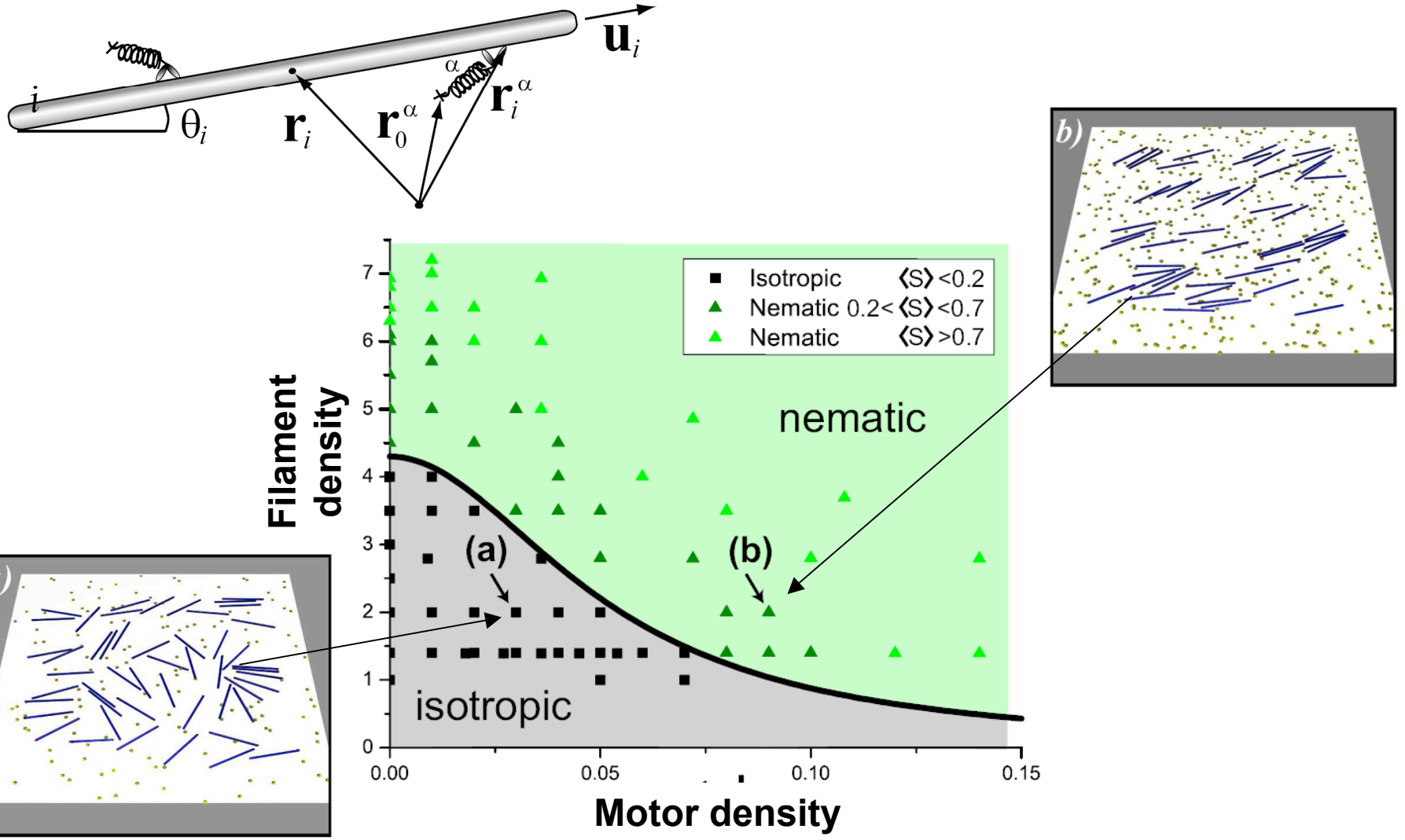
3.-2 mM ATP

4.-Nitrocellulose coated glass coverslips

5.-Non-fluorescent F-actin

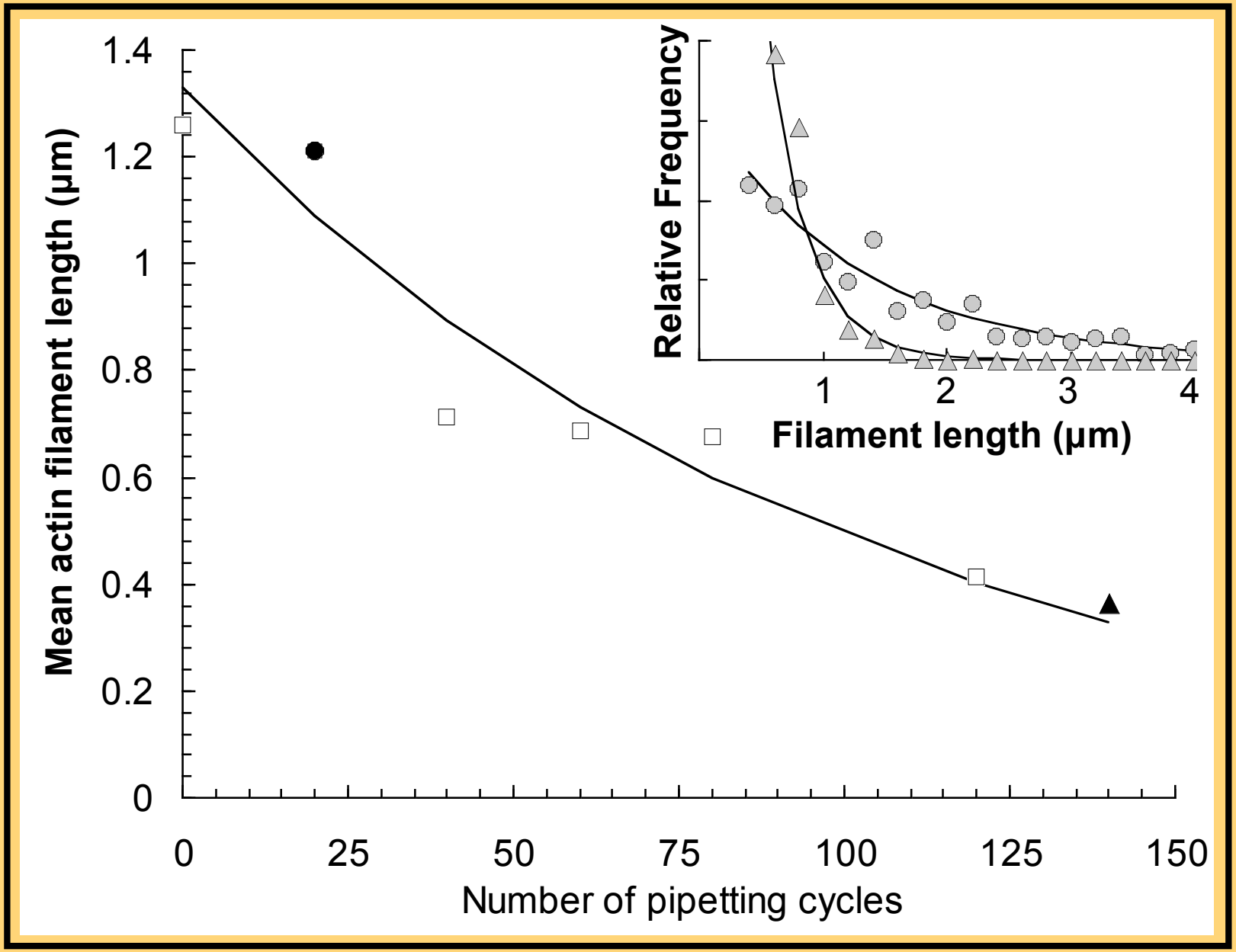
Butt, T., et al. (2010) J Biol Chem 285: 4964-4974

Alignment of Cytoskeletal Polymers - Theory

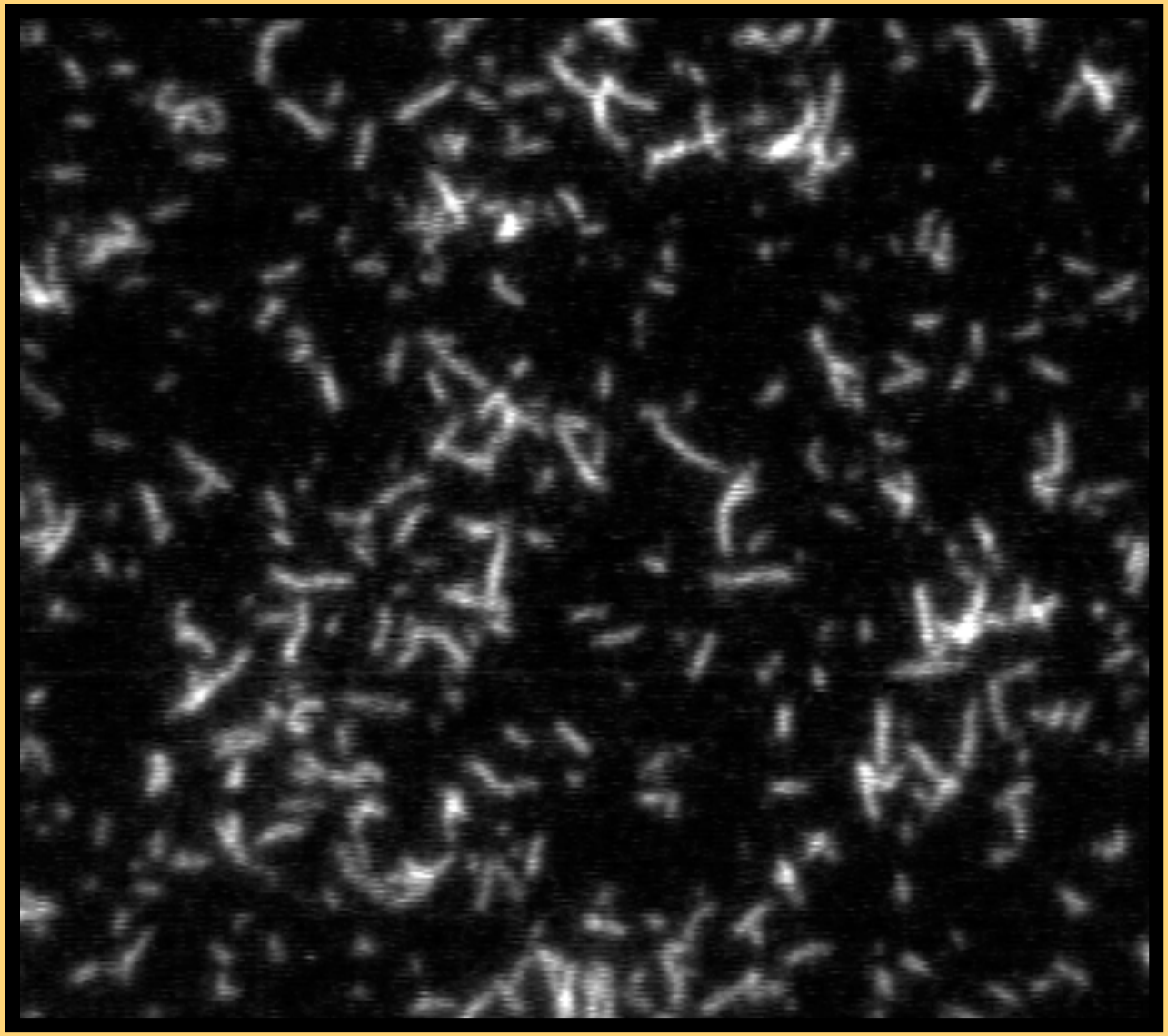


Kierfeld, Kraikivsky and Lipowsky. 2008. Biophys. Revs. Letts. 4:363-

Shearing - Control of Filament Length



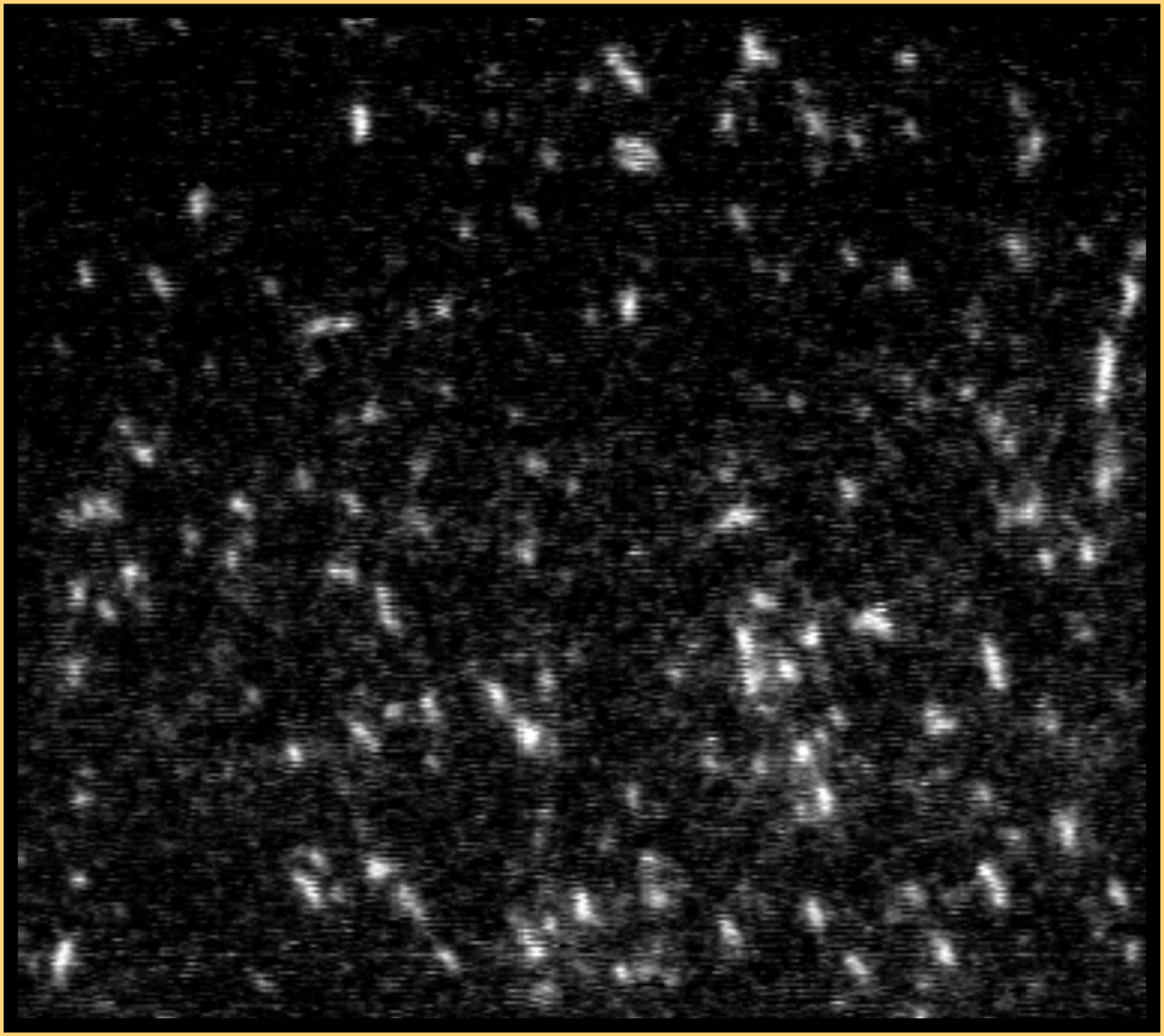
“Normal” *In vitro* Motility Assay (~ 2 pMolar F-actin)



10 μm

Real Time

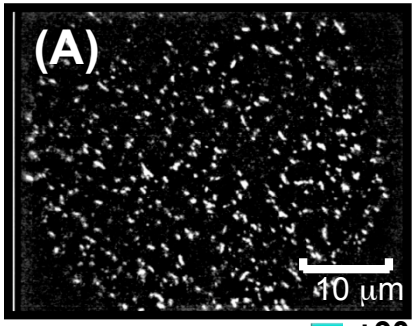
In vitro Motility with 20μM unlabelled F-actin (~10^7 x "Normal" amount)

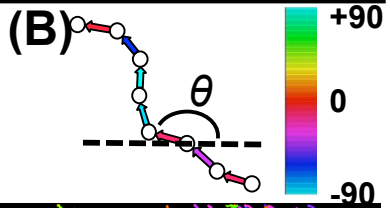


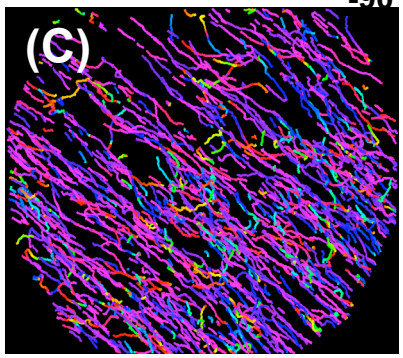
10 μm

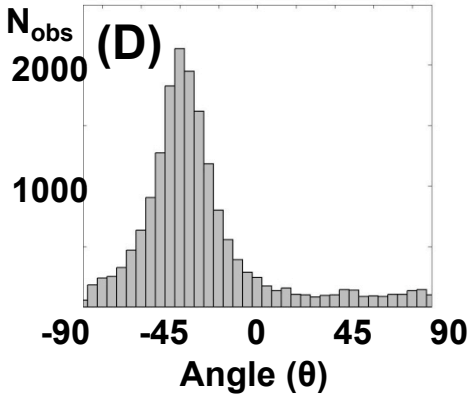
Real Time

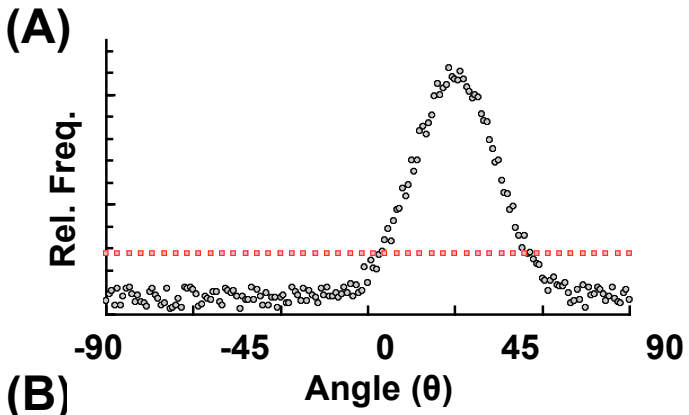
A Quantitative Measure of Alignment

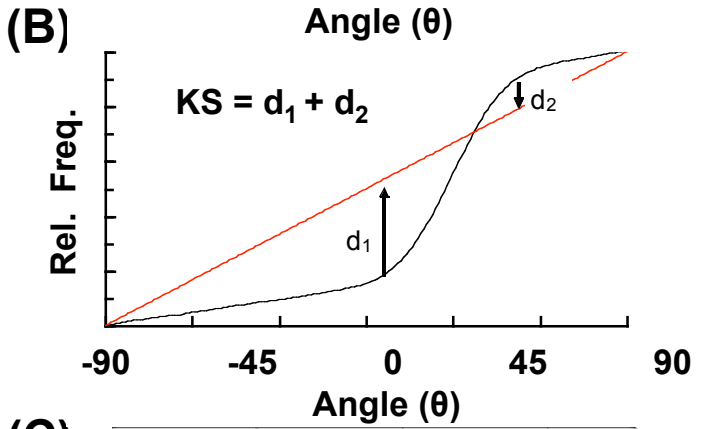


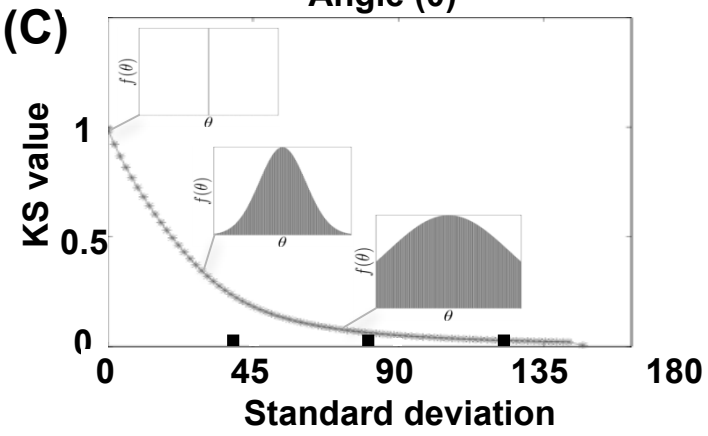




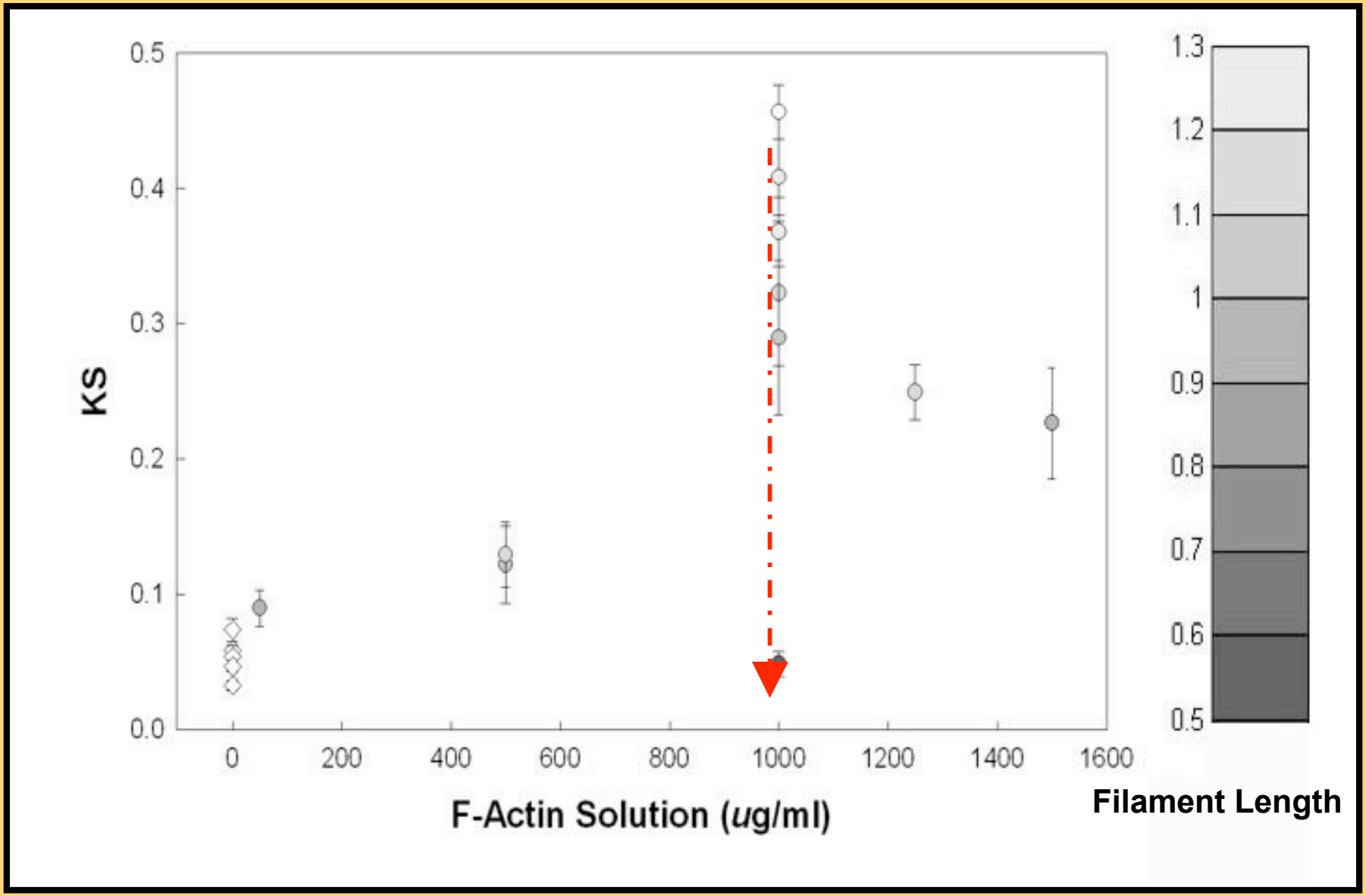




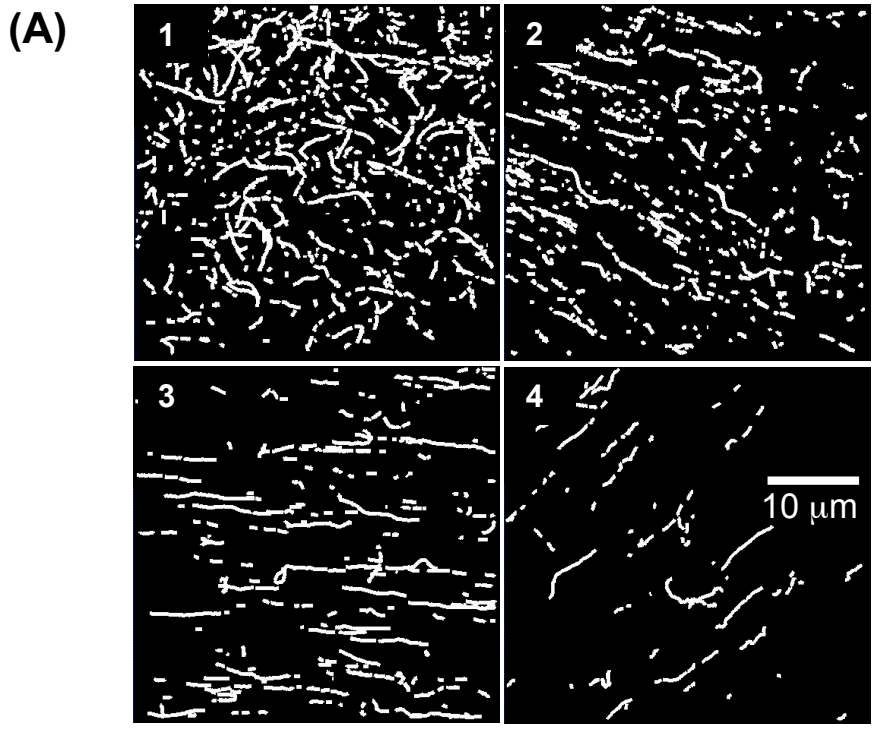


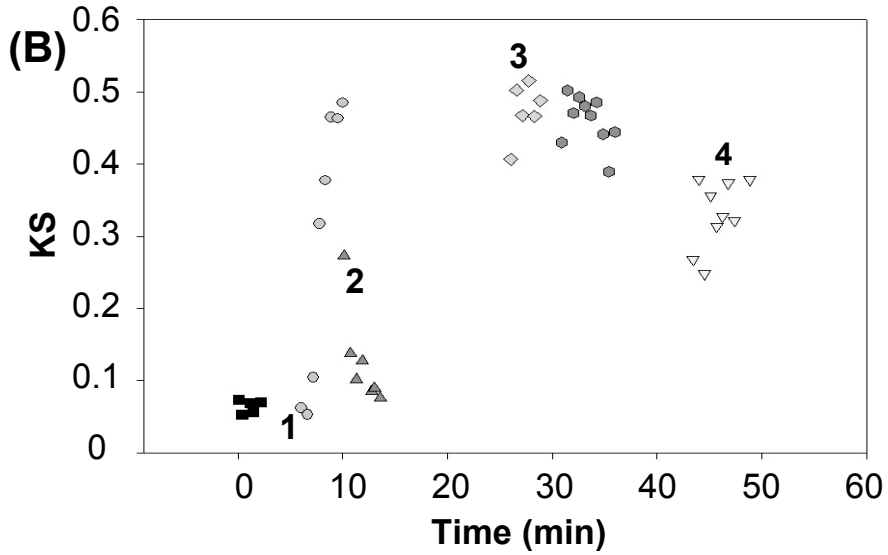


Alignment Strongest when Filament Length > 1μm



Formation of Aligned Domains - an Active Process

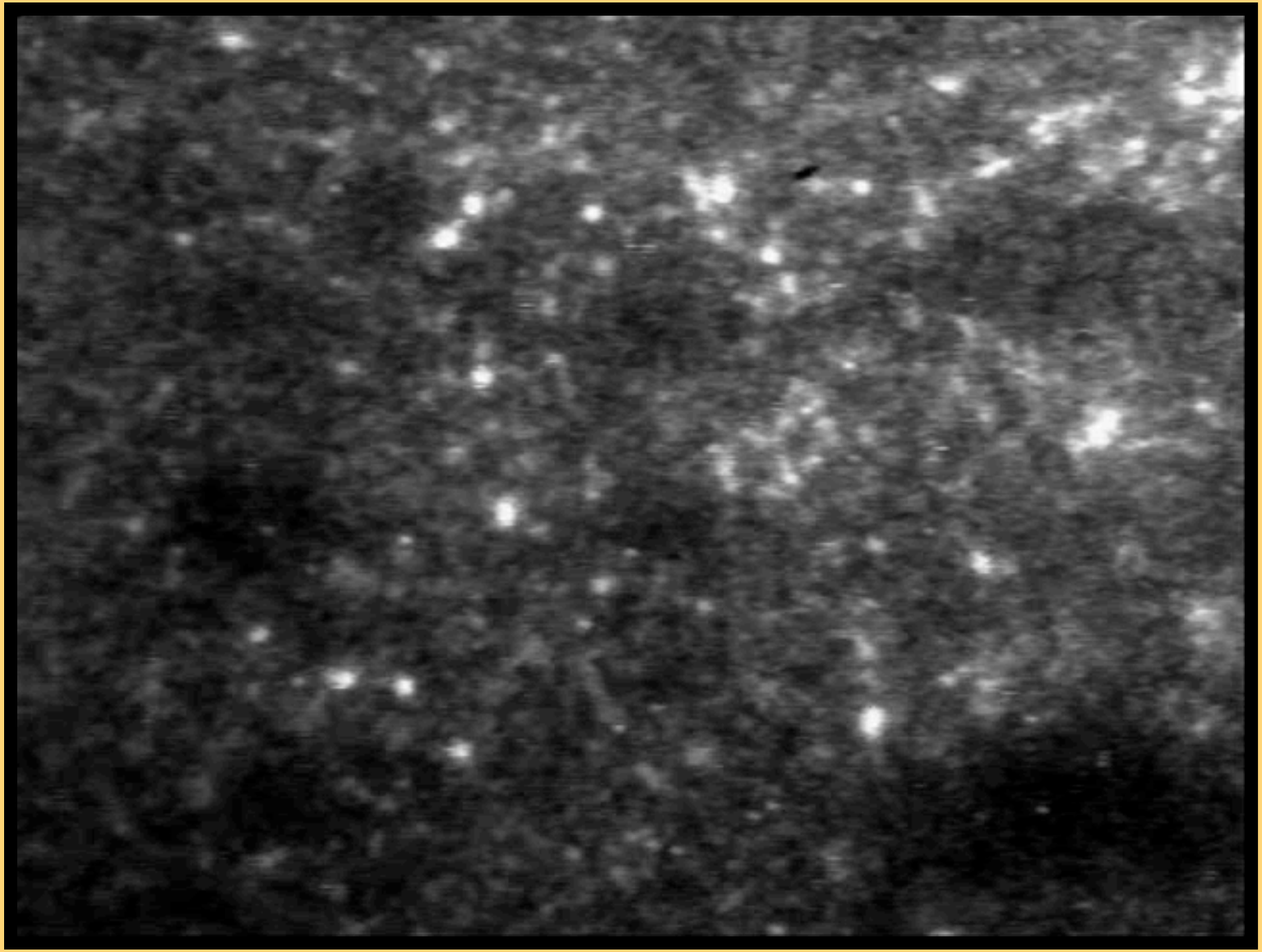




1. Alignment takes about 5-10 minutes to establish.

2. Rapid solution flow causes immediate alignment of filaments in bulk phase (in direction of flow).

Pattern Formation

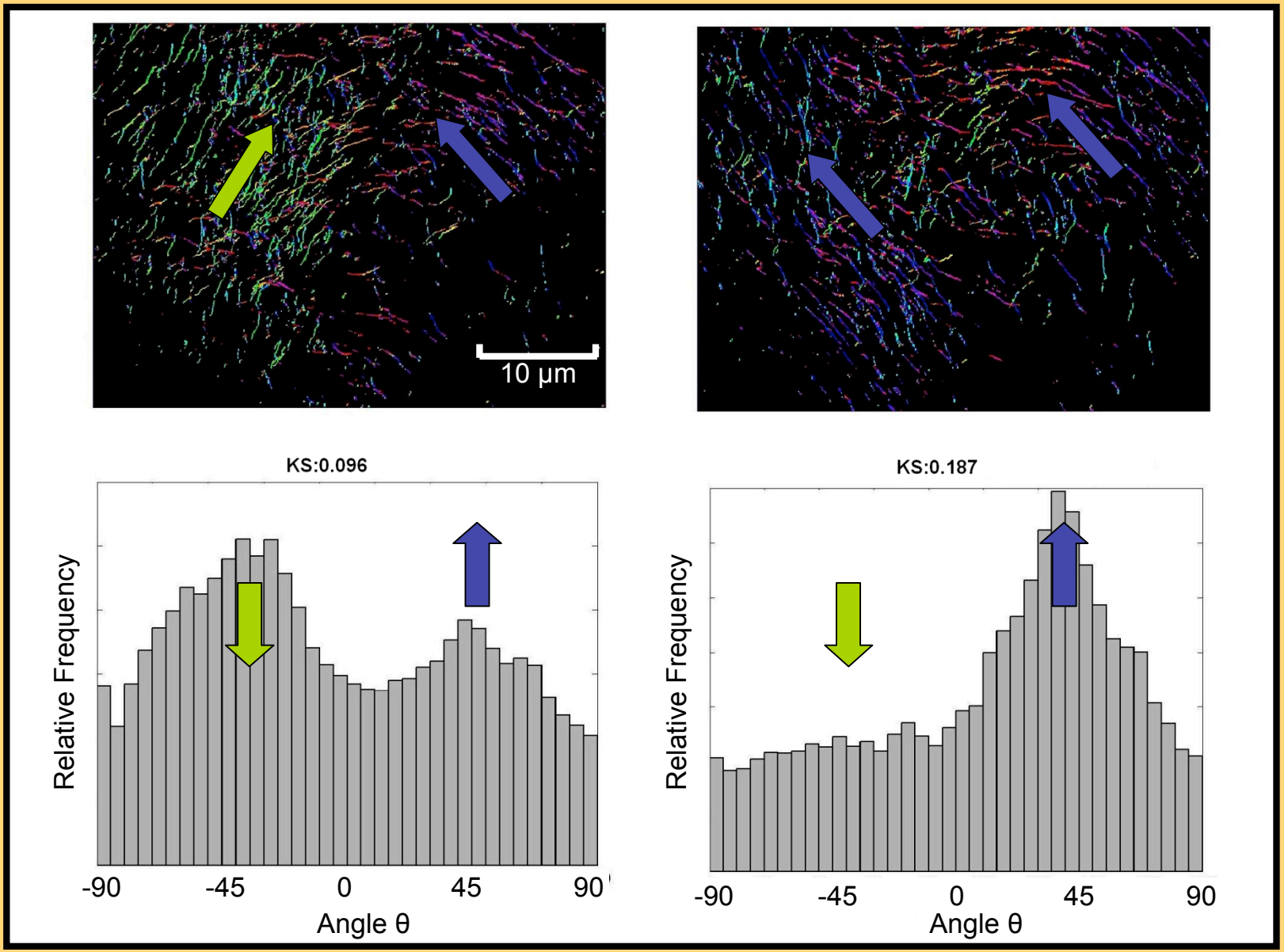


10μm

~*Real Time*

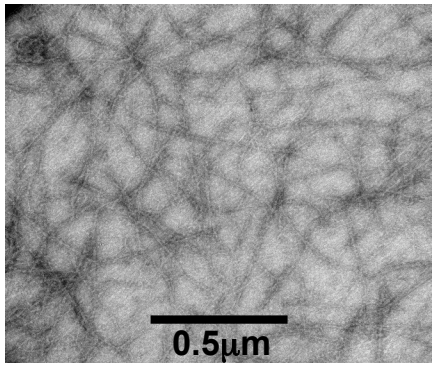
See also *Schaller, V et al. 2010. Nature. 467, 73.*

Microdomain Dynamics

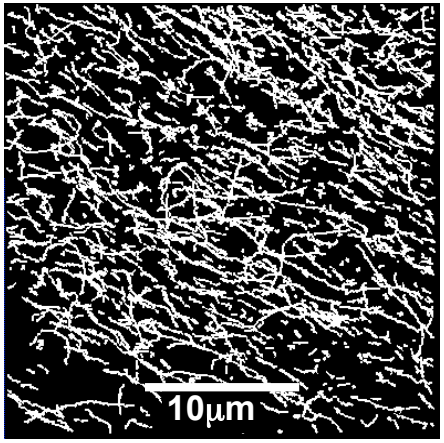


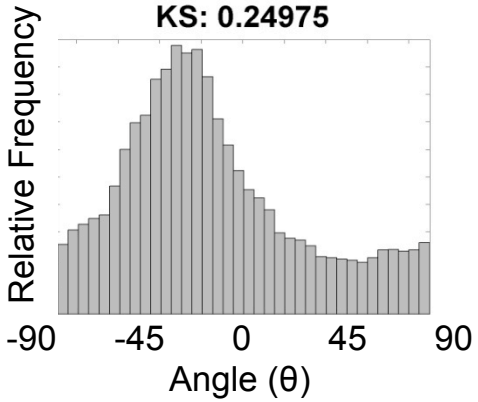
Myosin Filaments do not disrupt Oriented Actin Domains

MYOSIN E.M.

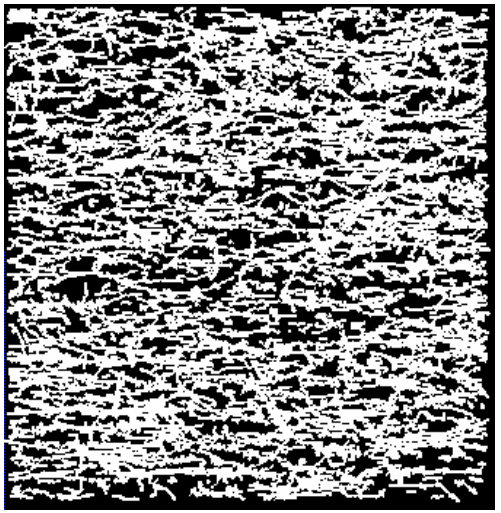


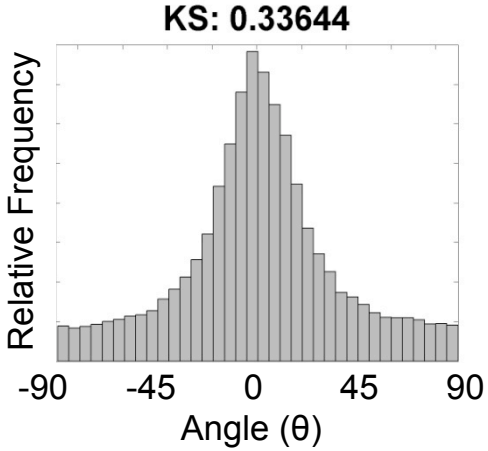
ACTIN Fluorescence



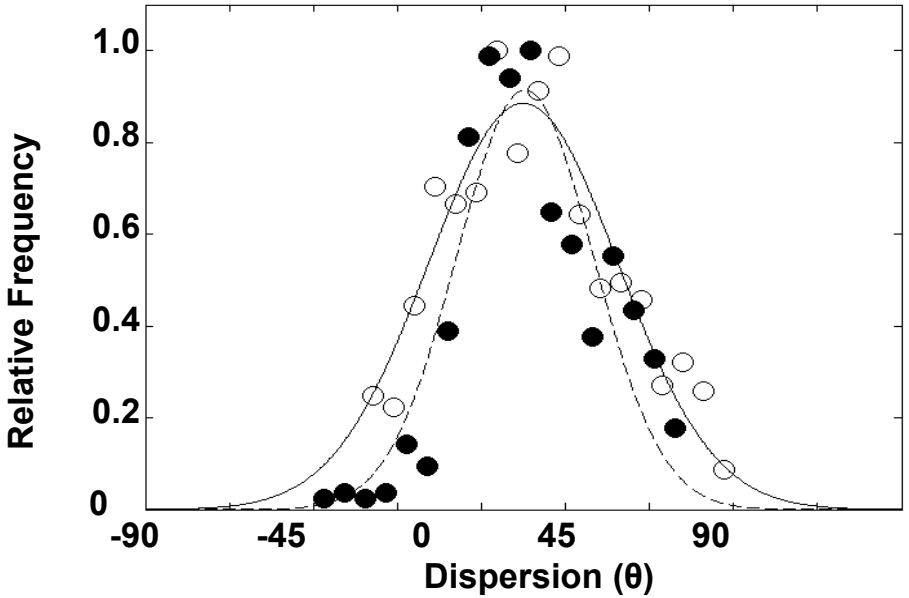


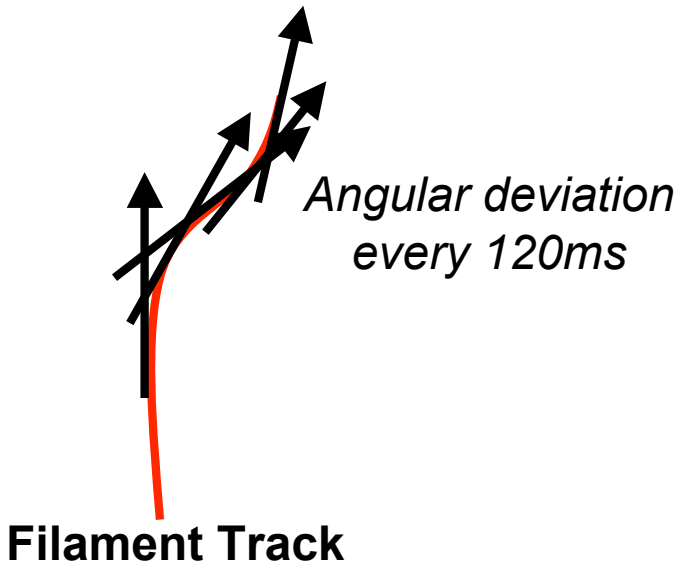
Fields of oriented actin also appear when synthetic filaments formed by native myosin are used





Persistence of motion is slightly greater at higher filament “crowding”

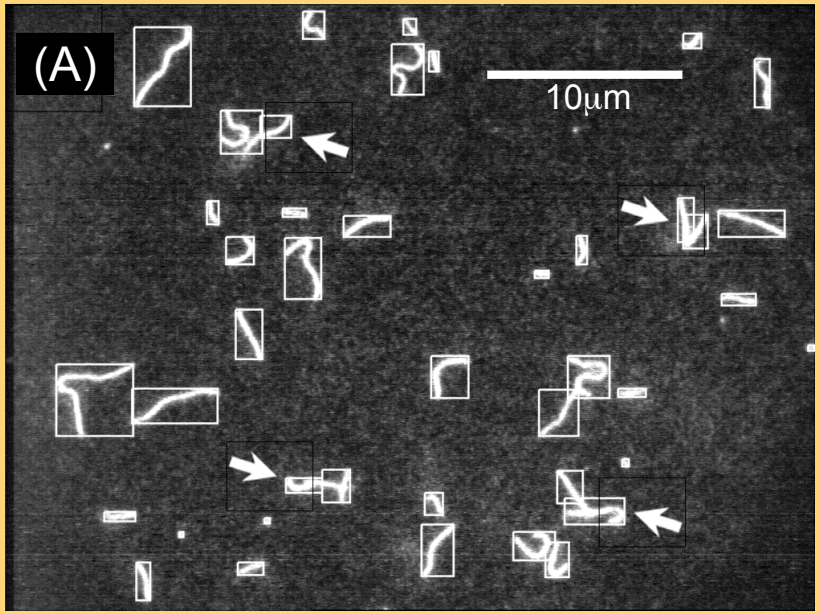




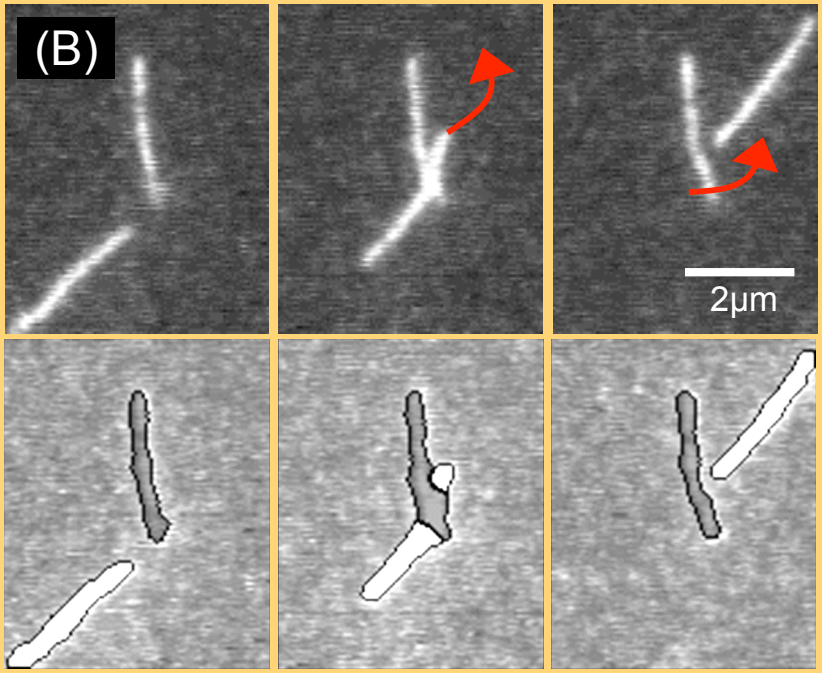
● +0.5mg/ml non-fluorescent actin

○ No added non-fluorescent actin

Filament Collisions



A. Filaments bend due to motor forces. Segmentation of a collision complex into individual filaments is a challenging computer vision problem

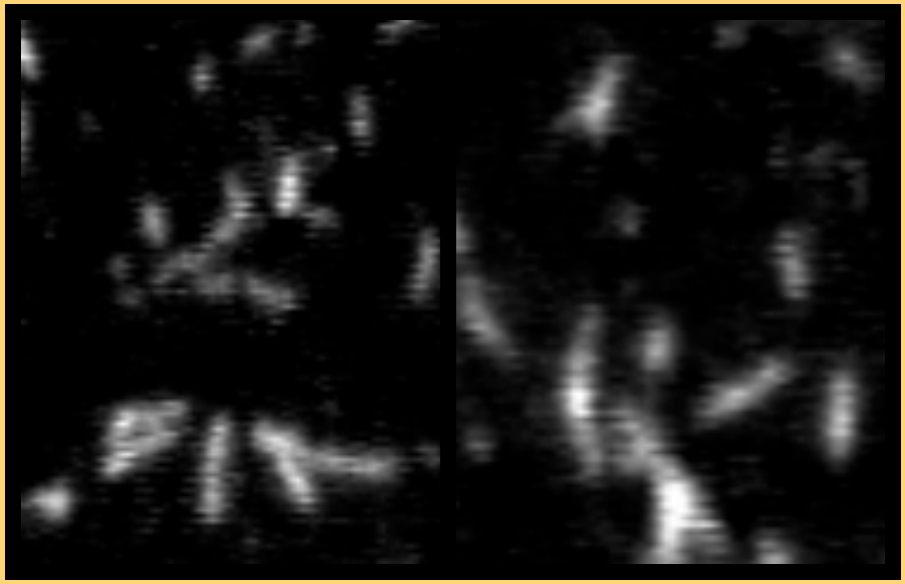


B. At low densities filaments transiently align by "kinking" upon collision

Observations of Single Collisions

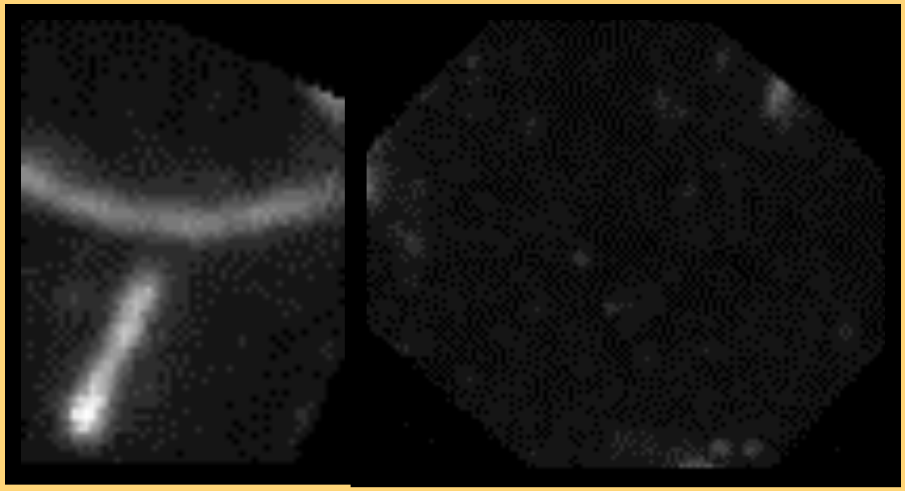
High Motor Density

Entrainment
Bending

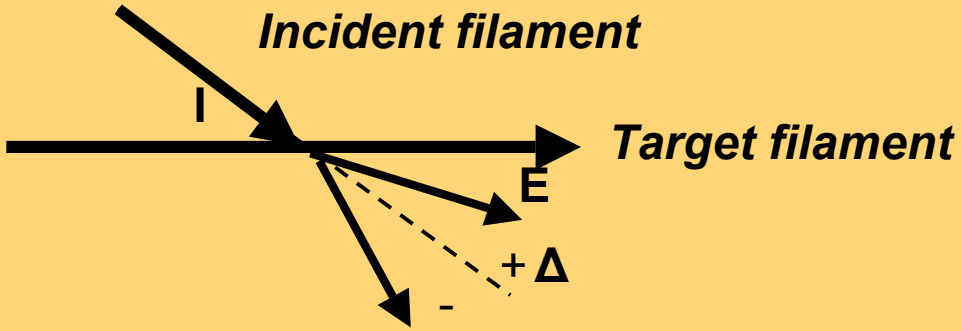


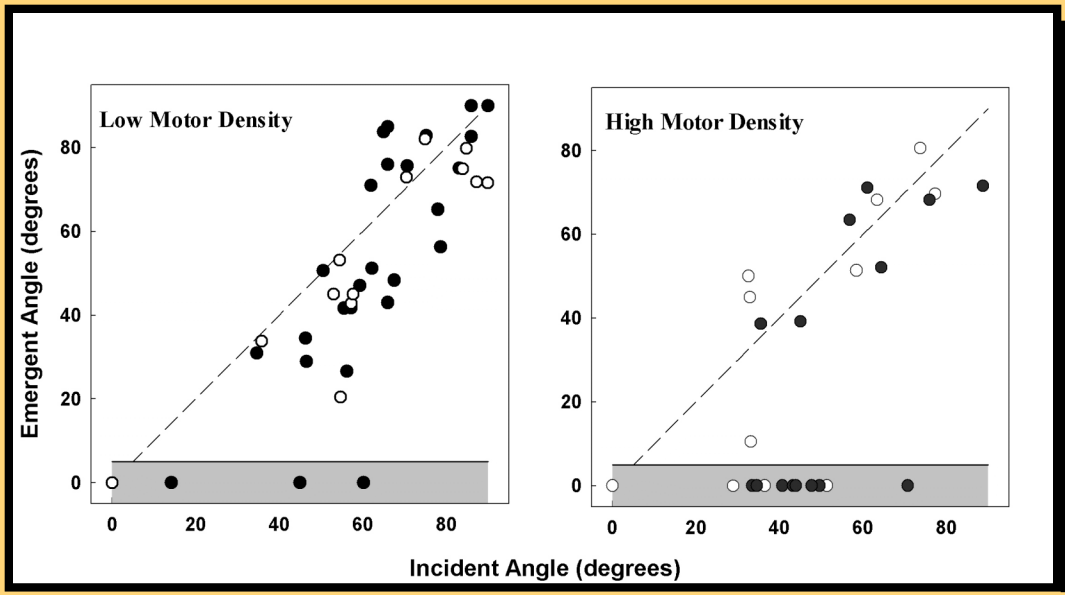
Low Motor Density

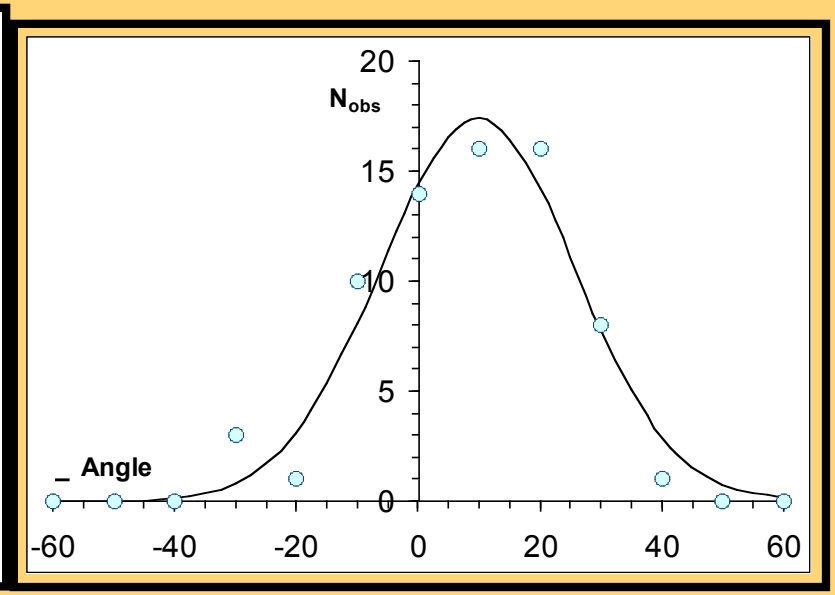
Crossover
Weak alignment



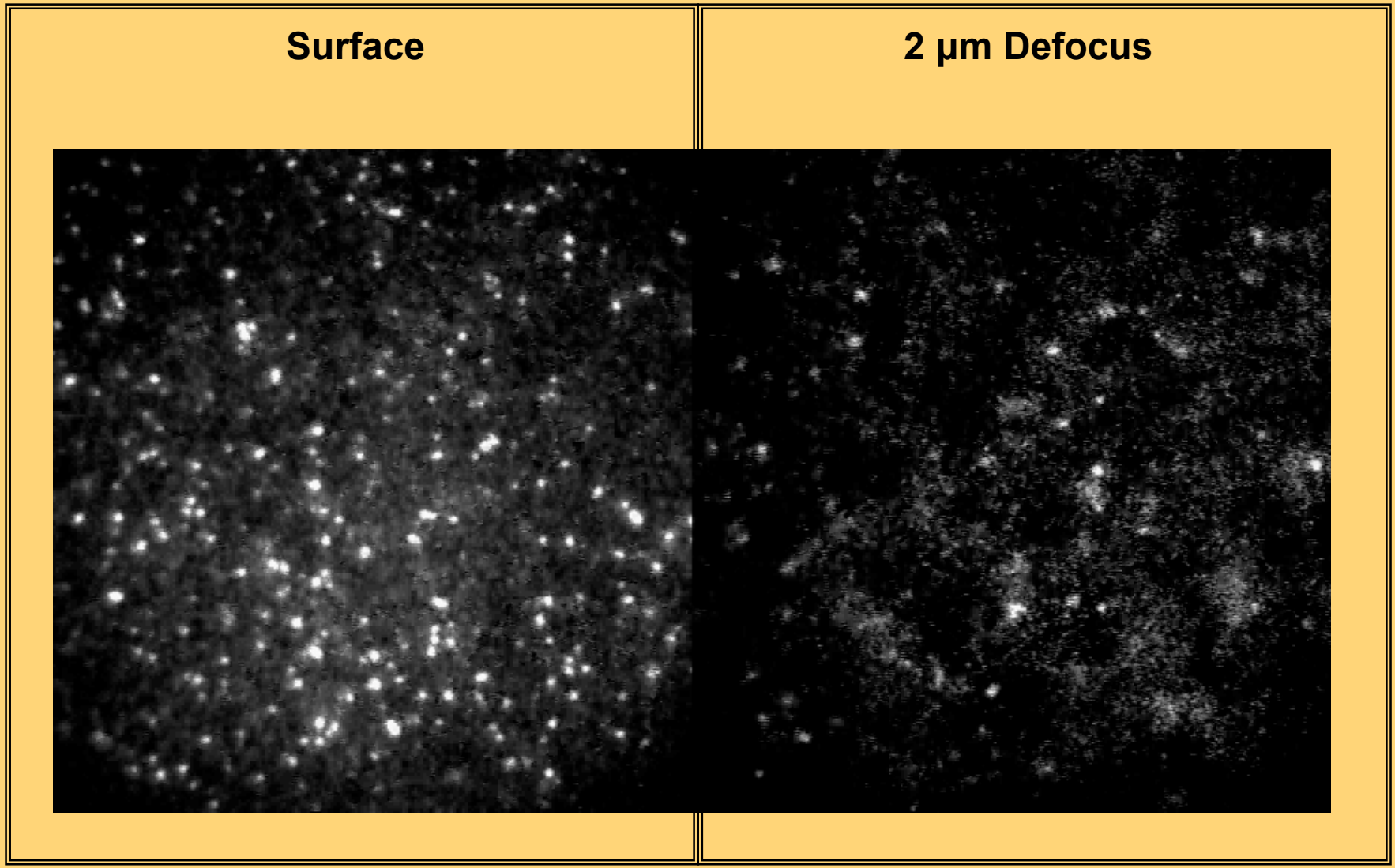
Alignment Probability depends on Motor Density







Does Surface Filament Sliding produce Convection Currents?



Liverpool & Marchetti. 2003. Phys. Rev. Lett. 90, 38012

CONCLUSIONS

1. Quantitative measure of population orientation.

2. Microdomain formation increases with filament crowding

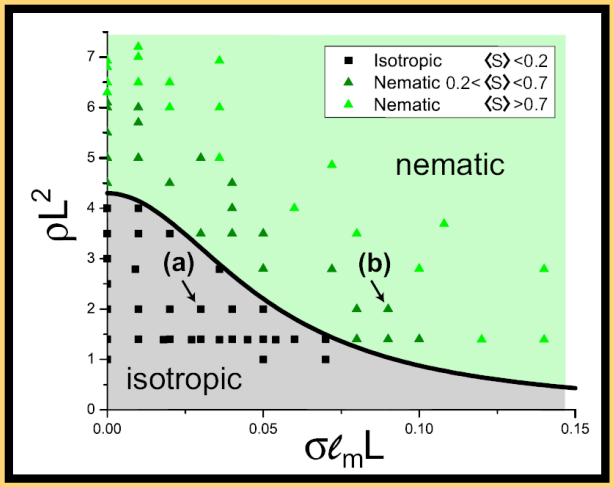
3. Microdomain formation is an active process.

4. Optimal filament length ca. 1 μm.

5. Actin microdomains reorganize on micrometer – second time scales.

6. Single collisions - Alignment probability depends on motor density.

PROSPECTS



Extension of theory and measurement in 2D and towards 3D

a- Finite crossover probability.

b- Relaxation of rigid rod approximation

c- Non-processive motors

d- Fluctuations in motor surface density

e-Weak forces