Physical properties of F-actin and filamentous phages: phase transitions, networks and motions

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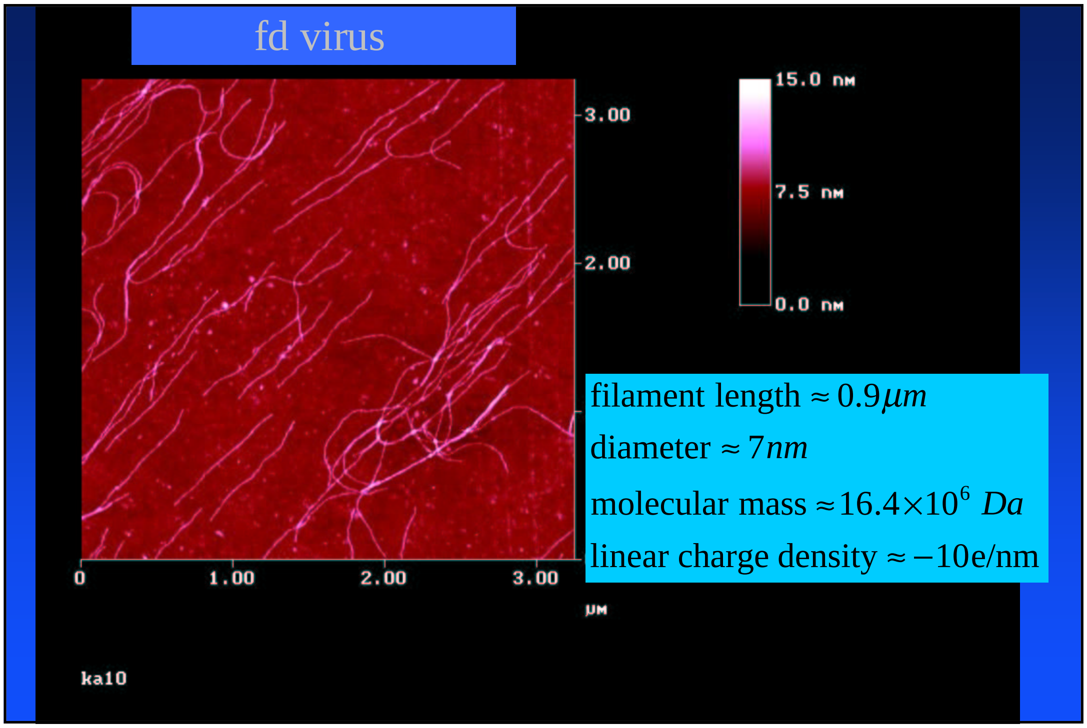
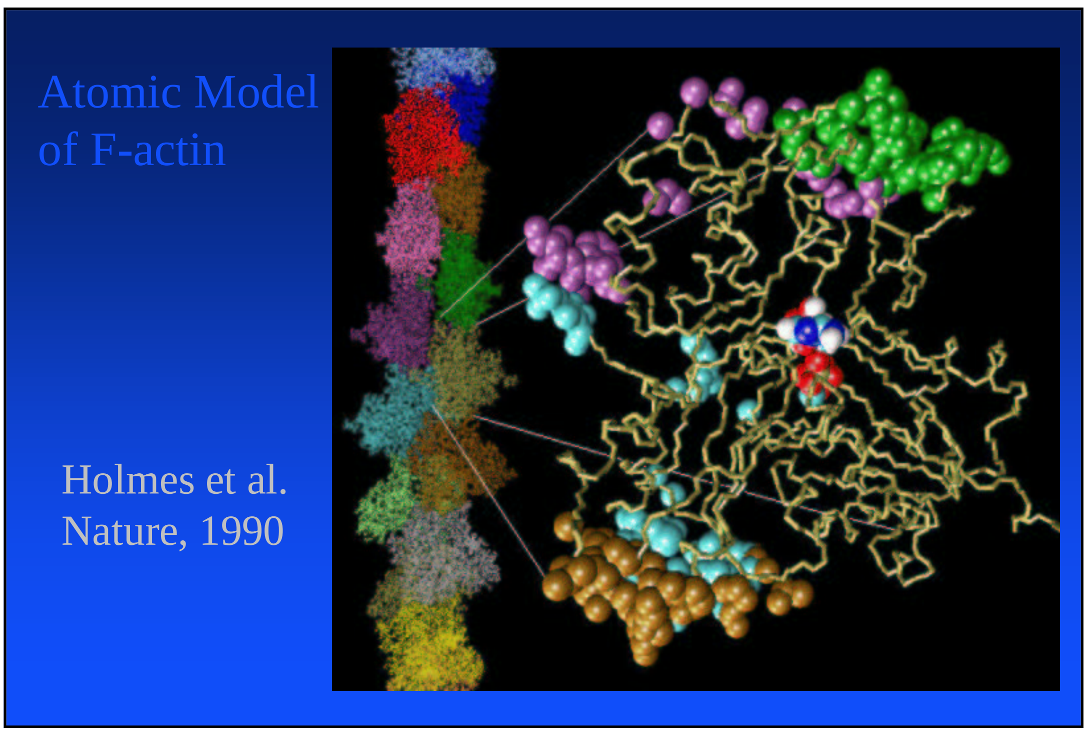
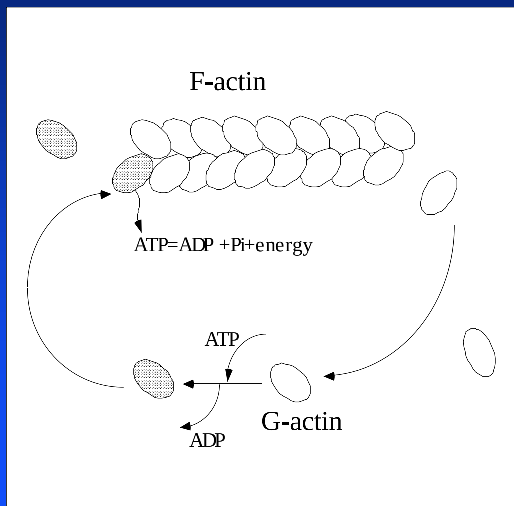
Outline

- Introduction: filamentous viruses and F-actin as model semiflexible polymers
  - Isotropic-nematic phase transition and filament dynamics
  - Rheology and mechanical manipulations
  - Cell motility: molecular mechanism of force generation and movement


fd virus
15.0 nm
7.5 nm
0.0 nm
3.00
2.00
0
1.00
2.00
3.00
μm
ka10
filament length ≈ 0.9μm
diameter ≈ 7nm
molecular mass ≈ 16.4×10^6 Da
linear charge density ≈ −10e/nm


Atomic Model of F-actin
Holmes et al. Nature, 1990

Polymerization of Actin



- MW=42,000 Dalton
- G-F transition
- Condensation model
  (F. Oosawa, 1960s)
- Binding energy ~14 kT
- Critical concentration
  <1μM, or 0.04 mg/ml
- ATP hydrolysis

Thermodynamics of Actin Polymerization

- **Exercise:**

a. The critical concentration of actin is 3 μM at 10°C, which decreases to 0.5 μM at 25°C. Calculate changes of both enthalpy & entropy?

b. Is the actin polymerization an endothermal or exothermal process?

F. Oosawa, 1975,
Thermodynamics of the Polymerization of Proteins

- **Solution:**

a. $K_d = c_c$ => $\ln c_c = G/RT$ =>

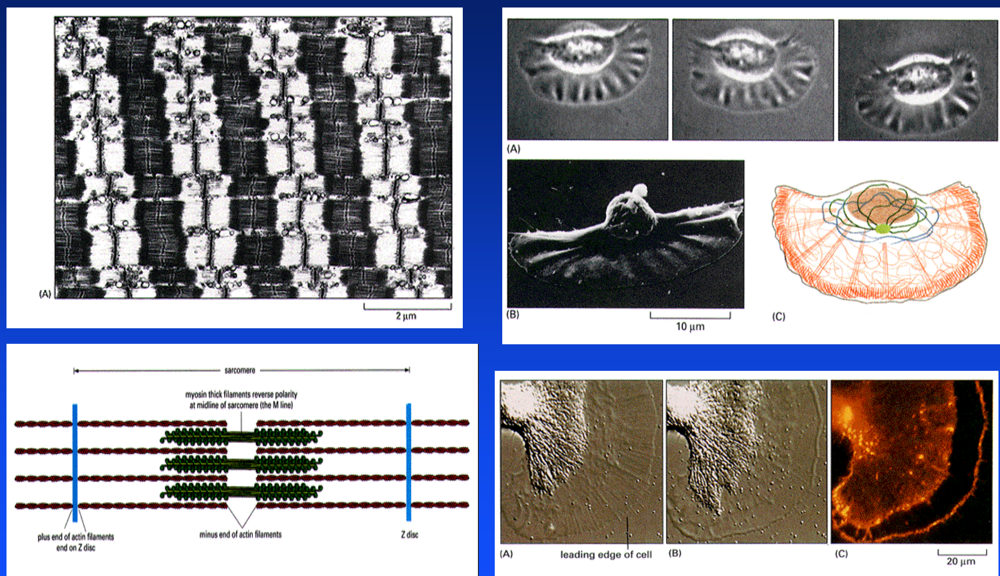
$\ln c_c = \Delta H/RT - \Delta S/R$ =>

ΔH=20 Kcal/mol,

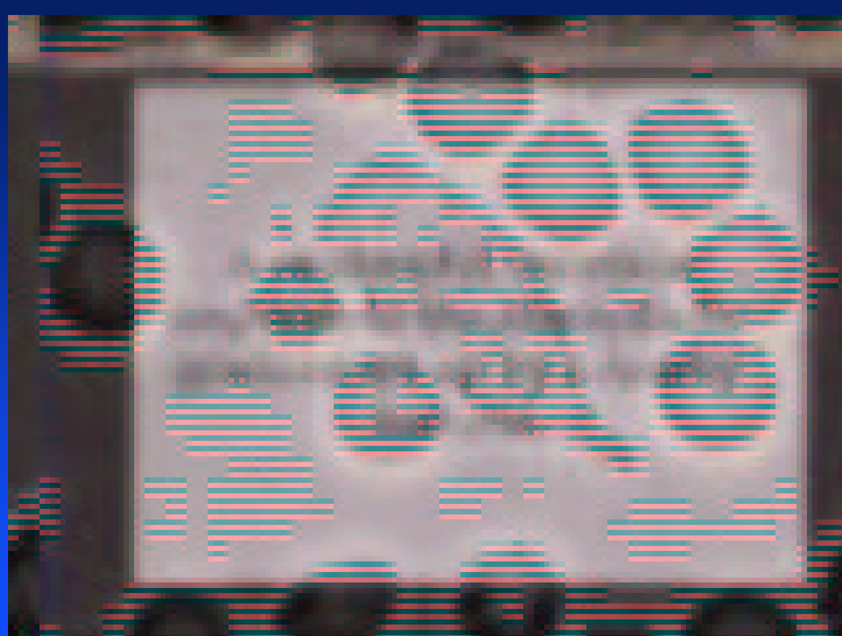
$T\Delta S$=28 Kcal/mol

b. Actin polymerization is an endothermal process. The process absorbs heat and gains entropy, via changes in interactions among protein, ions & water (hydration).

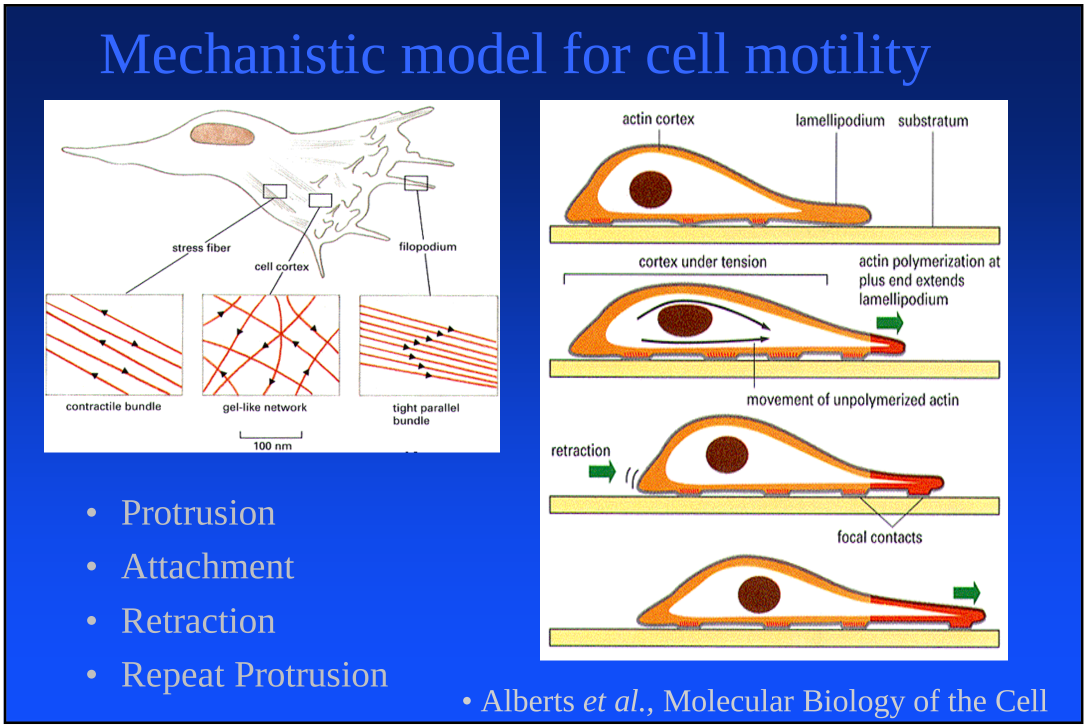
Actin in Cells

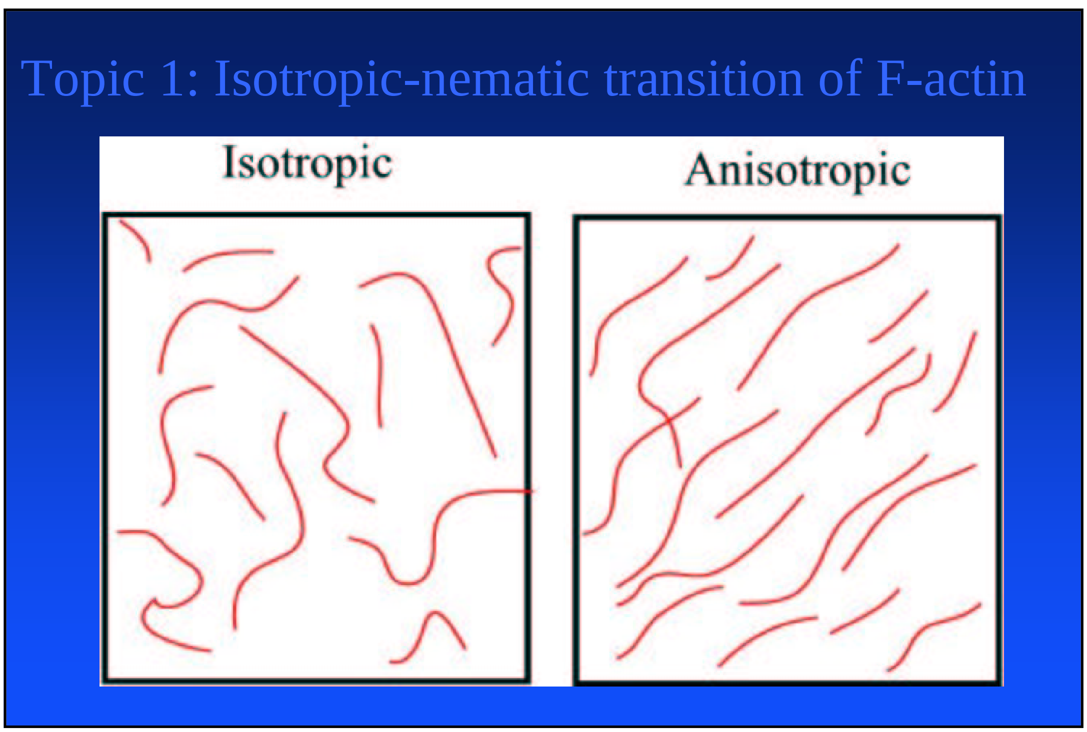


• Alberts *et al.*, Molecular Biology of the Cell

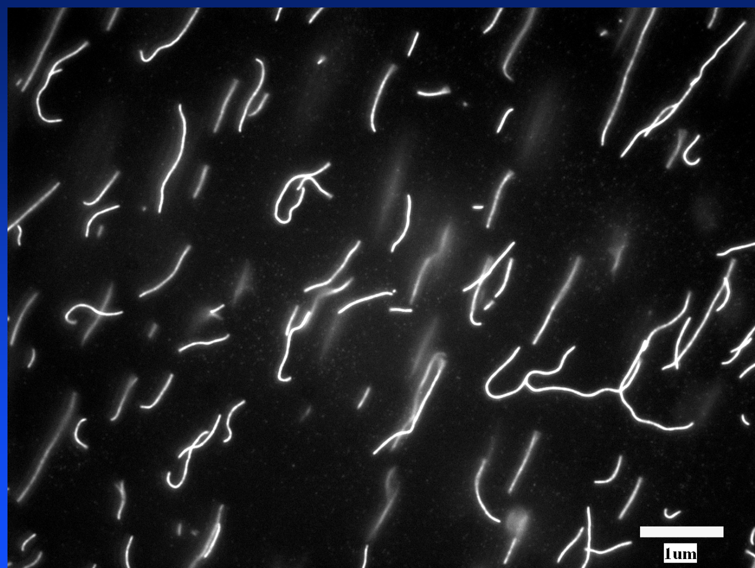


http://expmed.bwh.harvard.edu/projects/motility/neutrophil.html

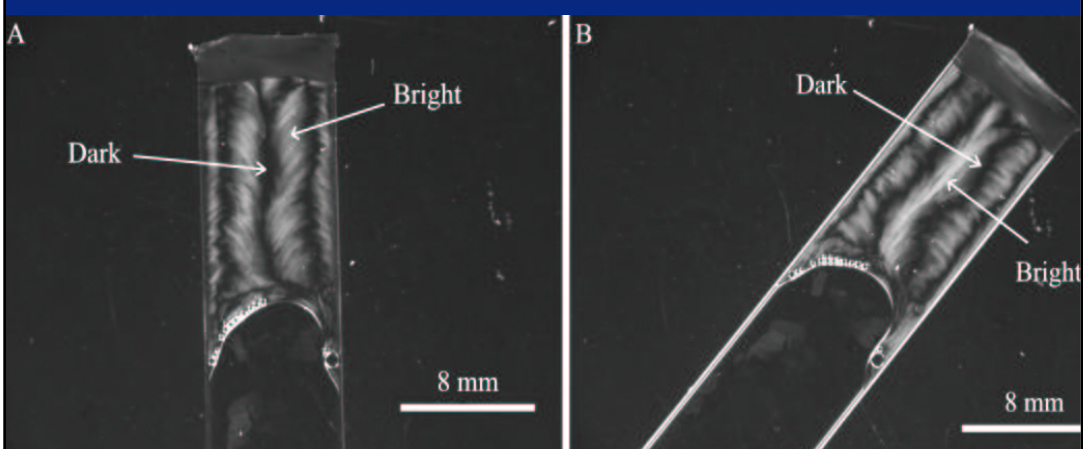

Mechanistic model for cell motility
stress fiber
cell cortex
filopodium
contractile bundle
gel-like network
tight parallel bundle
100 nm
• Protrusion
• Attachment
• Retraction
• Repeat Protrusion
actin cortex
lamellipodium
substratum
cortex under tension
actin polymerization at plus end extends lamellipodium
movement of unpolymerized actin
retraction
focal contacts
• Alberts et al., Molecular Biology of the Cell

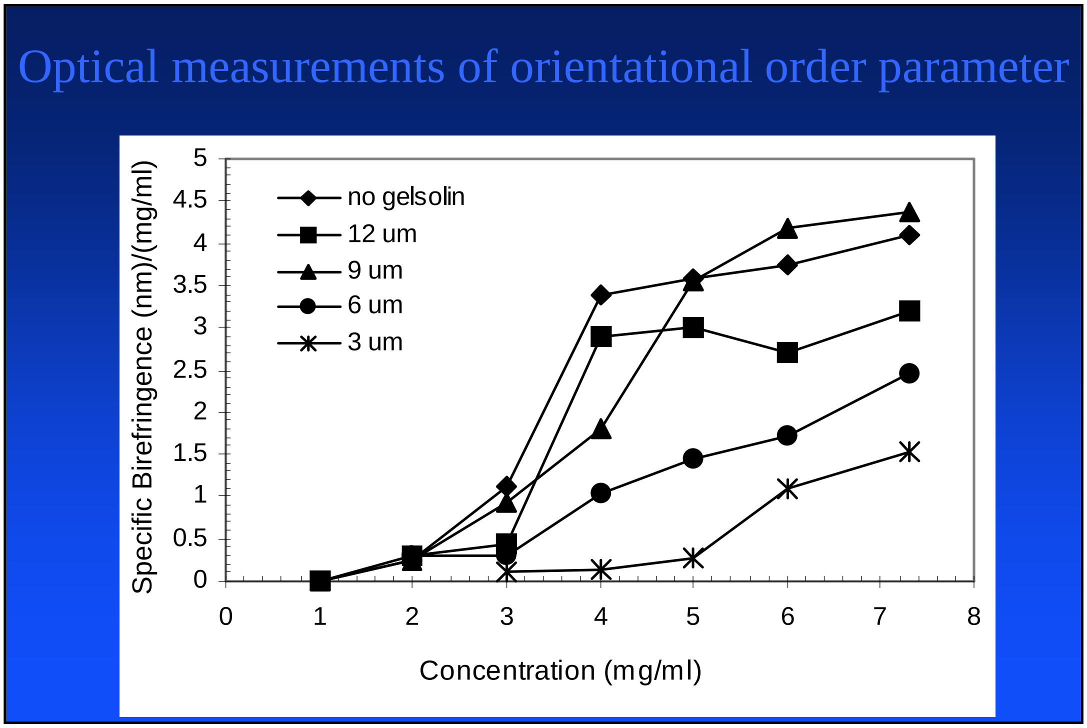

Topic 1: Isotropic-nematic transition of F-actin
Isotropic
Anisotropic

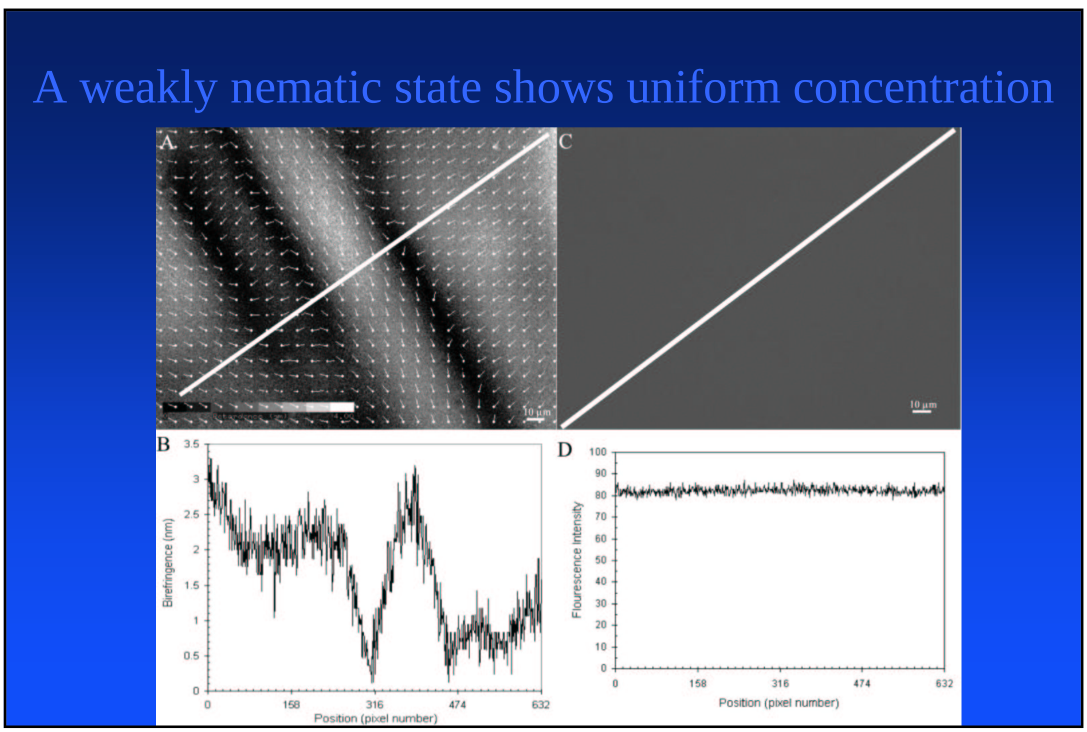
Labeled F-actin imbedded in a nematic network

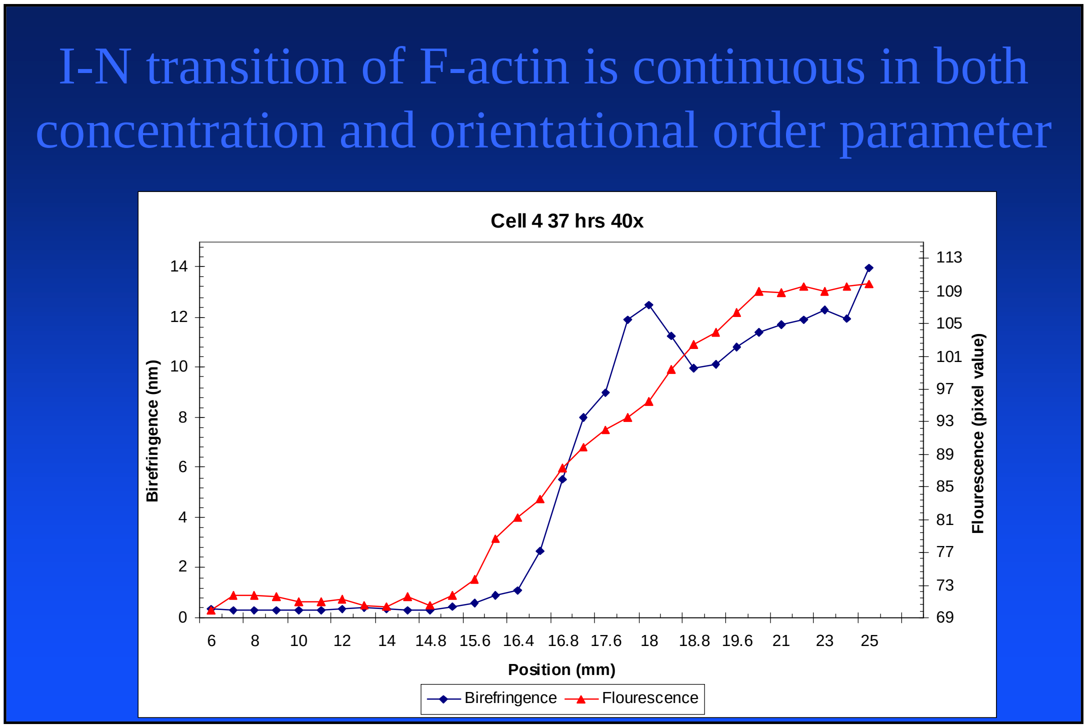


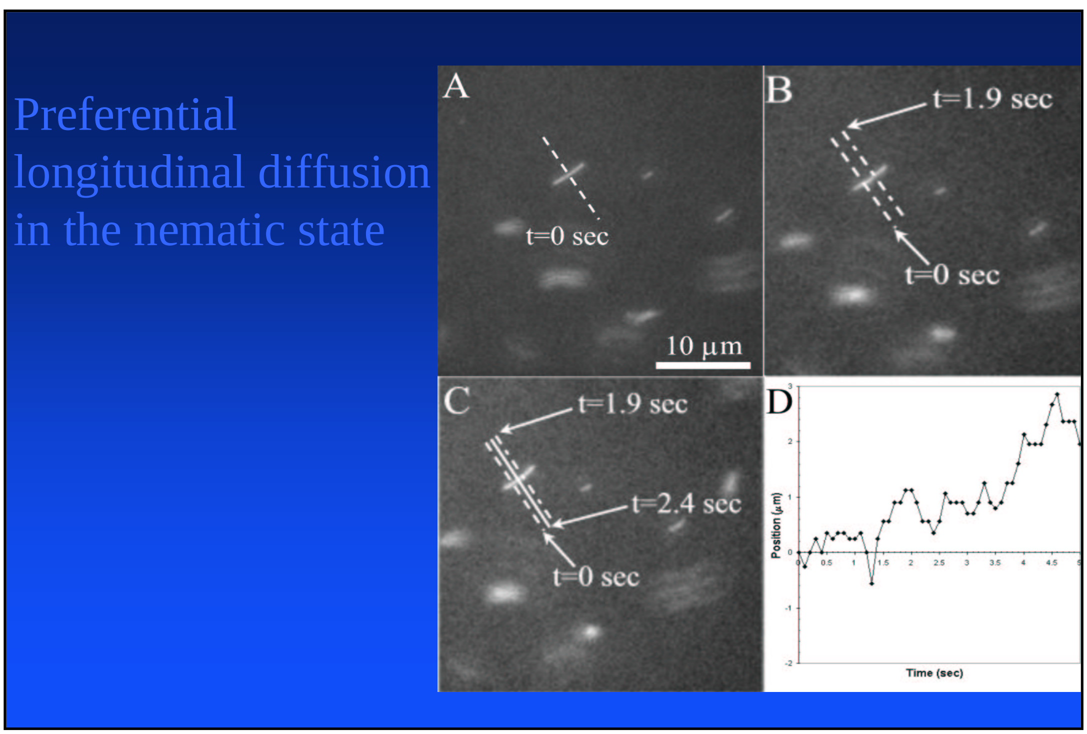
Domain alignment of nematic F-actin following shear




Optical measurements of orientational order parameter
Specific Birefringence (nm)/(mg/ml)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
no gelsolin
12 um
9 um
6 um
3 um
0
1
2
3
4
5
6
7
8
Concentration (mg/ml)


A weakly nematic state shows uniform concentration
A
C
10 µm
B
Birefringence (nm)
Position (pixel number)
D
Flourescence intensity
Position (pixel number)


I-N transition of F-actin is continuous in both concentration and orientational order parameter
Cell 4 37 hrs 40x
Birefringence (nm)
Flourescence (pixel value)
Position (mm)
Birefringence
Flourescence

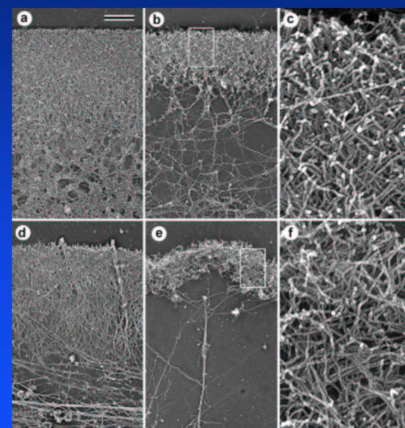

Preferential longitudinal diffusion in the nematic state
A
t=0 sec
10 µm
B
t=1.9 sec
t=0 sec
C
t=1.9 sec
t=2.4 sec
t=0 sec
D
Position (µm)
Time (sec)

Topic 2: Viscoelasticity of filamentous networks

- Biological motivation
- Semiflexible filaments in network
- Particle tracking microrheology
- Mechanical manipulation by AFM
- Nanoelectronics and mineralization

Biological Motivation

- Filamentous networks occur in many physiological settings
- Cell is not just a bag of enzymes, but instead has a filamentous skeleton
- Cytoskeletal structure is essential for scaffording, transport, signaling, force transmission, motility, etc.



Svitkina & Borisy, JCB, 1999

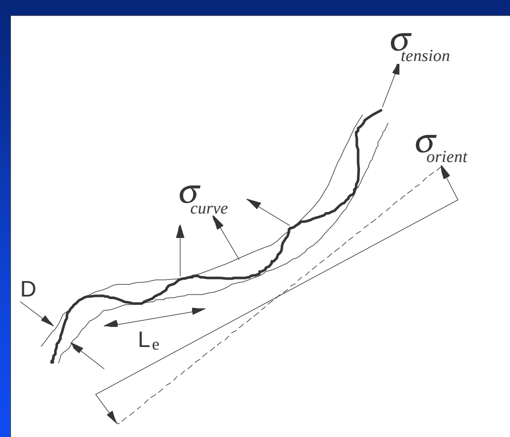
Viscoelasticity of semiflexible polymer network

Theory of polymer dynamics

de Gennes, 1970s
TUBE MODEL & REPTATION

Recent treatments for network of semiflexible polymers

A. Maggs, 1994, PRE
F. MacKintosh et al, 1995, PRL
R. Granek, 1997, J. Phys
E. Frey et al, PRE & PRL, 1998
D. Morse, Phys. Rev. E; Macro., 1998

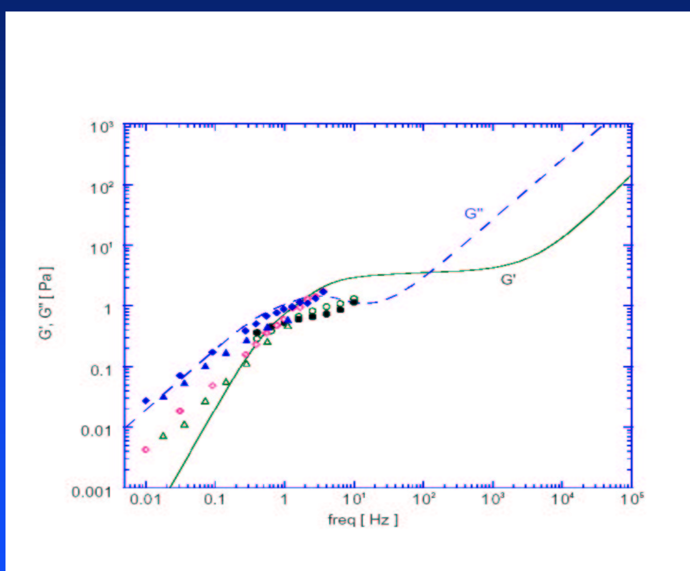


The stress tensor and response times

Measurement of Viscoelasticity

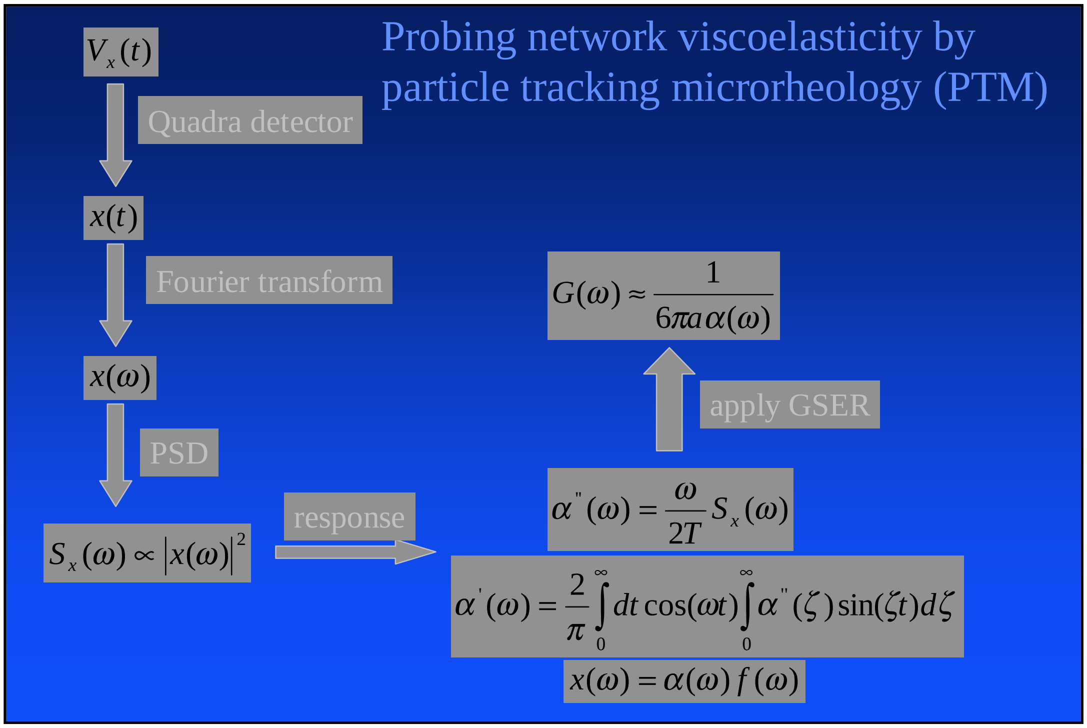
- Macrorheology
  - Parallel plates (cone-plate) mechanical rheometer
  - Magnet driven rotating disk rheometer (Sackmann)
- Microrheology (beads rheology)
  - Magnetic tweezers (Sackmann)
  - Diffusing wave spectroscopy (Weitz)
  - Particle tracking microrheology (PTM) (Schmidt, Kuo)
- Mechanical manipulation using AFM

Viscoelastic moduli of fd network



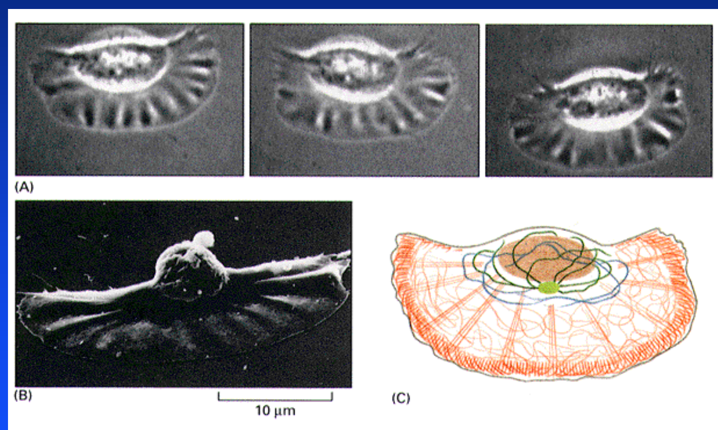
Theory Curves
D. Morse

Exp. Data
Schmidt, Hinner
Sackmann & Tang
PRE 2000


Probing network viscoelasticity by particle tracking microrheology (PTM)
$V_x(t)$
Quadra detector
$x(t)$
Fourier transform
$x(\omega)$
PSD
$S_x(\omega) \propto |x(\omega)|^2$
response
$x(\omega) = \alpha(\omega) f(\omega)$
$\alpha'(\omega) = \frac{2}{\pi}\int_0^\infty dt \cos(\omega t)\int_0^\infty \alpha''(\zeta)\sin(\zeta t)d\zeta$
$\alpha''(\omega) = \frac{\omega}{2T} S_x(\omega)$
apply GSER
$G(\omega) \approx \frac{1}{6\pi a \alpha(\omega)}$

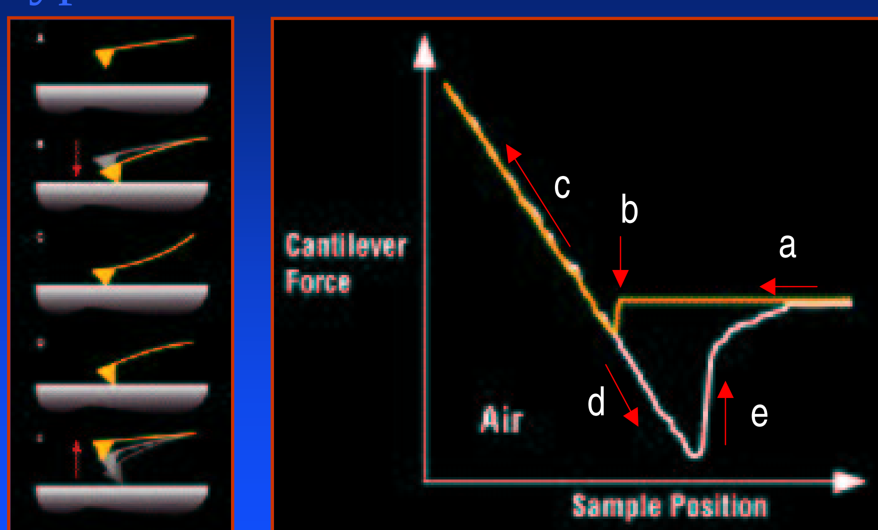

Promise of two-bead microrheology
Theory Levine & Lubensky, PRL 2000
• Separating local and bulk properties
• Measurement of compressibility
• Probing long range correlations of network dynamics
Experiments Crocker, et al., 1999
Addas, Levine, Schmidt & Tang, in progress

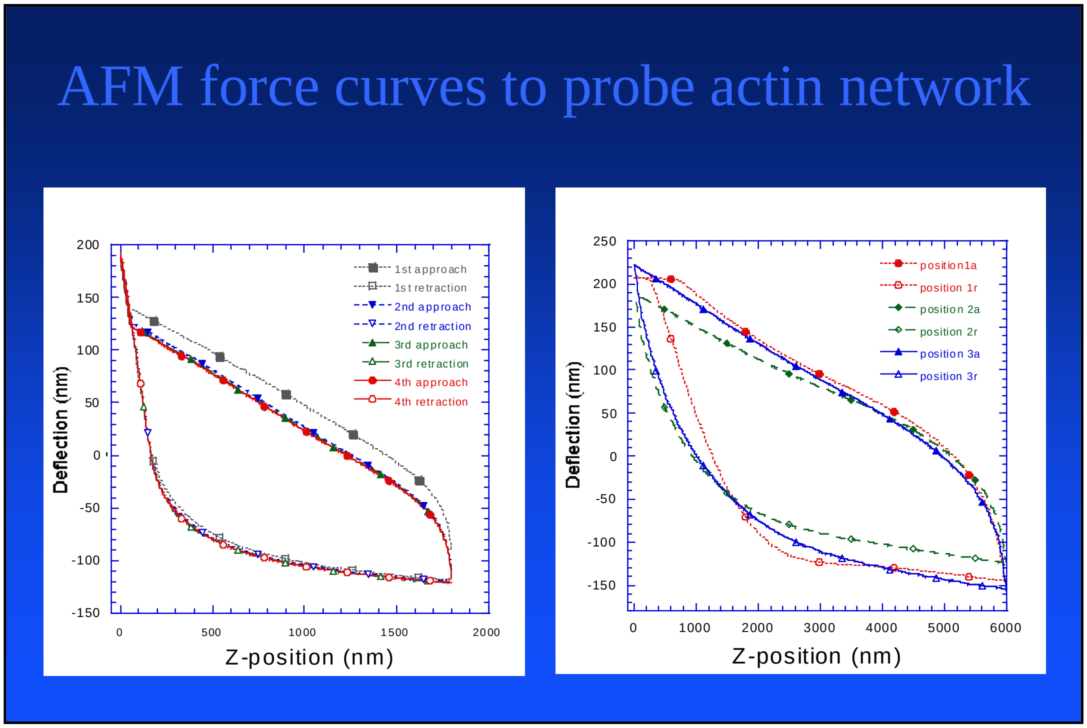
Force measurements and mechanical manipulation of actin network by AFM

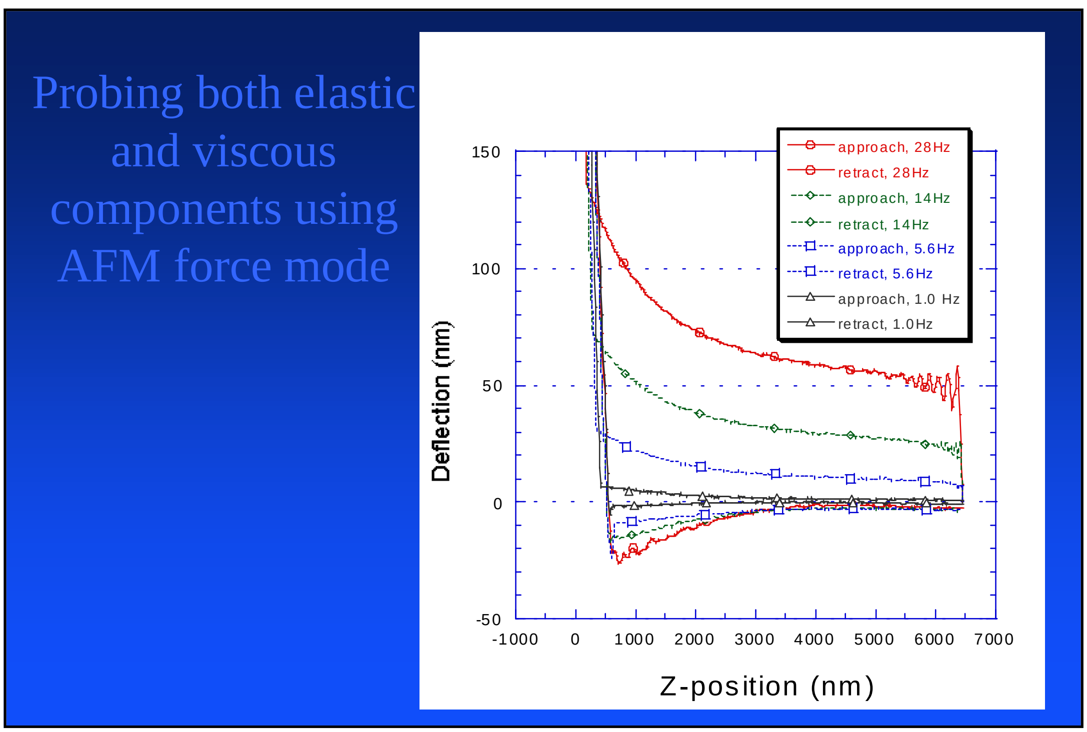


• Alberts *et al.*, Molecular Biology of the Cell

Typical force distance curves for AFM




AFM force curves to probe actin network
Deflection (nm)
Z-position (nm)
1st approach
1st retraction
2nd approach
2nd retraction
3rd approach
3rd retraction
4th approach
4th retraction
position1a
position 1r
position 2a
position 2r
position 3a
position 3r


Probing both elastic and viscous components using AFM force mode
Deflection (nm)
Z-position (nm)
approach, 28Hz
retract, 28Hz
approach, 14Hz
retract, 14Hz
approach, 5.6Hz
retract, 5.6Hz
approach, 1.0 Hz
retract, 1.0Hz

Other projects using solution AFM

- Single filament conductivity
- Nano-fabrication by mineralization

Is F-actin a conducting nanowire in solution?

- Interest and controversy of DNA conductivity (Fink, Nature, 1999; de Pablo, PRL, 2000)
- Importance of protein filaments in scafferding and transport in cells
- Ionic flux and physiological functions

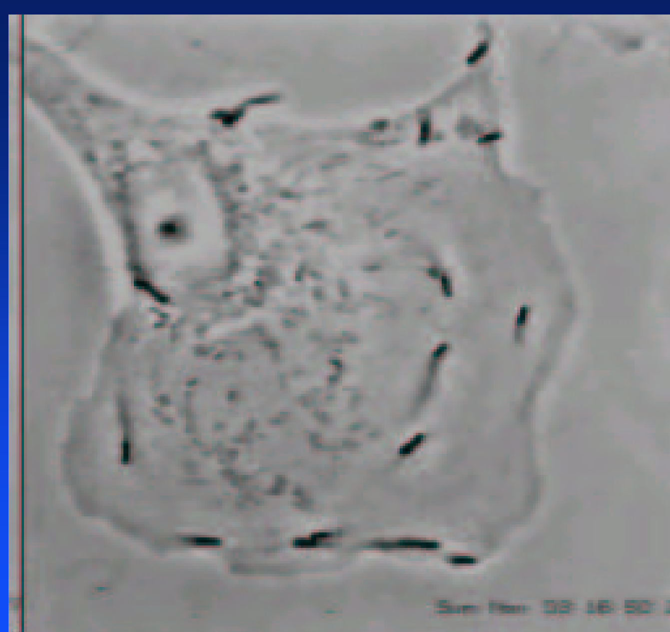
- Cable-like properties of F-actin in solution will be measured using conducting AFM tips in combination with the micro-patterning technique

Topic 3: Physics behind cell motility

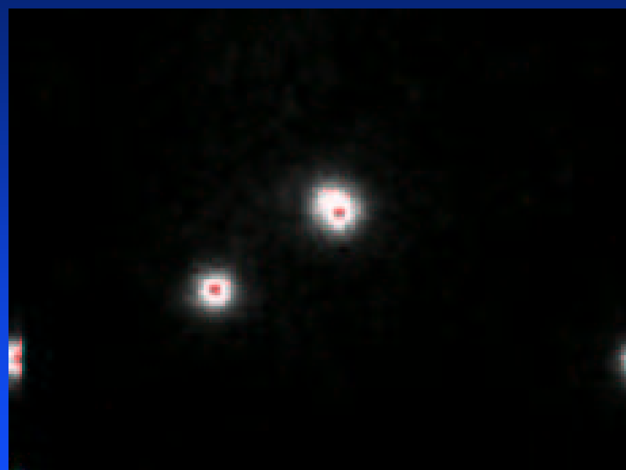
- Video segments on cell motility
- Understanding the listerial head rotation by extending a symmetry breaking concept
- Current effort towards understanding the helical tail growth

Listeria monocytogenes moving in PtK2 cells (150x)

http://cmgm.stanford.edu/theriot/movies.htm



Concept of spontaneous symmetry breaking to explain the initiation of directed motion



van Oudenaarden & Theriot, Nature Str. Bio., 1999

Rotatory motion of a bacterium while propelled by its own tail

http://cmgm.stanford.edu/theriot/movies.htm

Summary & Future Directions

- Features of I-N phase transitions for F-actin
- Polyelectrolyte properties of viruses and protein filaments
- Viscoelasticity of biopolymer network
- Actin network dynamics and force generation

- Self-assembly of bio-complexes (protein filaments, lipids, DNA, viruses, etc.)
- Rheological properties of semiflexible filament network
- Protein filament nano-electronics and transport properties
- Understanding physical mechanisms of cell motility
- Applying physics of protein-assembly and networks to cell biology and disease intervention

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