united nations
educational, scientific
and cultural
organization

international atomic
energy agency

the
abdus salam
international centre for theoretical physics

SMR 1495 - 6

WINTER COLLEGE ON BIOPHOTONICS:
Optical Imaging and Manipulation of Molecules and Cells
(10 - 21 February 2003)

"Elements of the cell cytoskeleton"

K. BERG-SORENSEN
Niels Bohr Institute, Copenhagen, Denmark

These are preliminary lecture notes, intended only for distribution to participants.

Elements of the cell cytoskeleton

K. Berg-Sørensen, Trieste, Feb. 2003

Overview of lectures

- Introduction: A little bit of biology
- Polymers of the cytoskeleton: Rods or ropes
- Networks of polymers
  - In 2D: Peptidoglycan layer, erythrocyte membrane
  - In 3D: "Full" cytoskeleton.
    - Percolation and connectivity
    - Viscoelasticity
- Force production by the cytoskeleton:
  - Polymerization forces

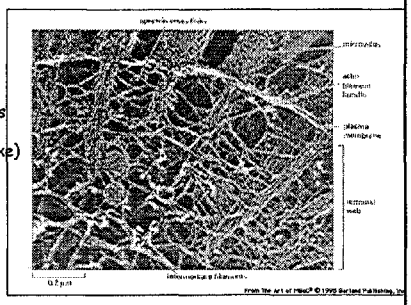
"Rods and ropes" of the cell and its skeleton

Filaments of the cell: Polymers, or collection of polymers:

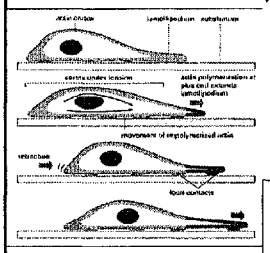
DNA (highly folded)

Cytoskeletal elements:
Actin
Intermediate filaments
Spectrin
Microtubules (string like)

Cellulose (plant cells)

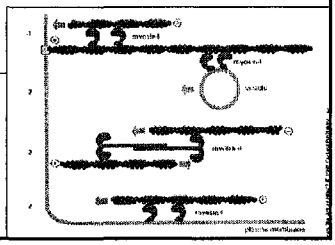


Actin:

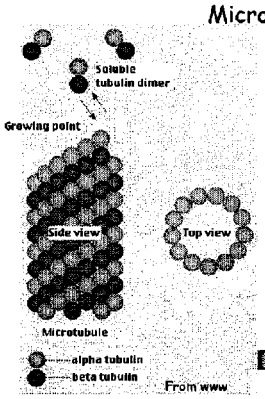


Thinnest filaments of cytoskeleton
Band beneath plasma membrane
"Linker" between proteins
Cellular locomotion
In muscle (actin - myosin)

Images from www

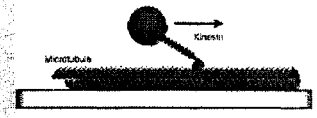


Microtubules



Stiff, rodlike structure.
Align chromosomes prior to cell division. "Active" in many steps of division.
Transport mediated by kinesin or dynein motion on microtubules

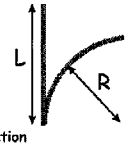
Animation of cell division in yeast



From S. M. Block, Nature, 1997

Elasticity of rods and ropes

Bending a stiff rod: $E_{arc} = \kappa_1 \frac{L}{2R^2}$



$\kappa_1 = Y \cdot I$ — Flexural rigidity

Youngs modulus — Moment of inertia of cross section

Cylinder: $I = \frac{\pi R^4}{4}$

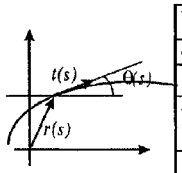
Thermal fluctuations introduces length scale:
Persistence length

$$\xi_p = \frac{\kappa_1}{k_B T}$$

$$L = \xi_p \Rightarrow E_{arc} = \frac{k_B T}{2} \frac{\xi_p^2}{R^2} \Rightarrow R \quad \frac{\xi_p}{\sqrt{2}} \text{ gives } E_{arc} = k_B T$$

Persistence length is the length over which the polymer appears straight (at temperature T).

Continous description of rods



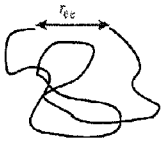
Tangent to curve	$\vec{t} = \partial \vec{r} / \partial s$
Contour length	L_c
Arc length (coordinate)	s
Curvature	C: $C \cdot \vec{n} = \frac{\partial \vec{t}}{\partial s} = \frac{\partial^2 \vec{r}}{\partial s^2}$
Local radius of curvature	R_c: $R_c = 1/C$
Normal vector to curve	$\vec{n} = \frac{\partial \vec{t}}{\partial \theta}$
Bending energy	$E_{bend} = \frac{\kappa_1}{2} \int_0^{L_c} \left(\frac{\partial \vec{t}}{\partial s} \right)^2 ds$

Interpretation of persistence length: $\langle \vec{t}(0) \cdot \vec{t}(s) \rangle = \exp\left(-\frac{s}{\xi_p}\right)$

Biopolymers: Rods or ropes?

$L_c \ll \xi_p \Rightarrow$ Rod

$L_c \gg \xi_p \Rightarrow$ Rope



Consider "long" polymer:
Calculate $\langle r_{ee}^2 \rangle$, r_{ee} : end-to-end distance

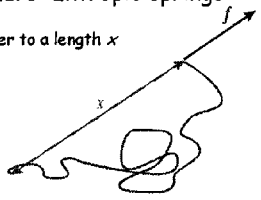
Characterizes erratic/stochastic nature of the convolutions of the filament. For a random chain (w.o. account for self-avoidance):

$$\langle r_{ee}^2 \rangle = 2\xi_p L_c - 2\xi_p^2 \left(1 - \exp\left(-\frac{L_c}{\xi_p}\right)\right) = \begin{cases} 2\xi_p L_c & \text{for } L_c \gg \xi_p \\ L_c^2 & \text{for } L_c \ll \xi_p \end{cases}$$

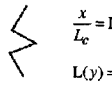
Scaling, random walk. Discrete description.

Energetics of biopolymers: Entropic springs

Apply a force f to stretch the polymer to a length x



- Freely-Jointed-Chain (FJC) model



$$\frac{x}{L_c} = L\left(\frac{2\xi_p}{k_B T} f\right)$$

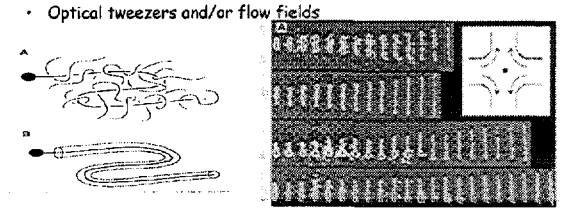
$$L(y) = \coth(y) - \frac{1}{y}$$

- Worm-Like-Chain (WLC) model
  Continous description, interpolation formula (Marko and Siggia)

$$\frac{\xi_p f}{k_B T} = \frac{1}{4}\left(1 - \frac{x}{L_c}\right)^2 - \frac{1}{4} + \frac{x}{L_c}$$

Measurements of ξ_p of biopolymers

- Dynamic light scattering
- Microscopy of fluorescently labelled filaments.
  - Thermal fluctuations
  - Driven oscillations
- Optical tweezers and/or flow fields

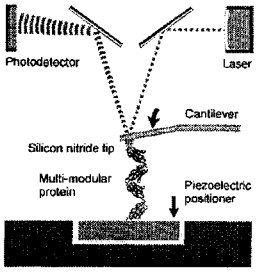


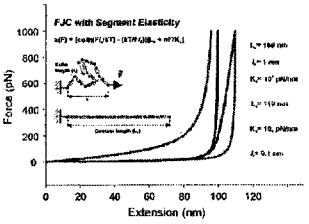
Figures from Perkins et al, Science (1994, 1997).

- From force-extension measurements

Amylose: Force-extension measurements

Experimental technique: AFM
Model: Extended FJC

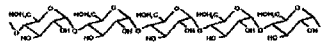




From Fisher et al, J. Physiol. (1999)

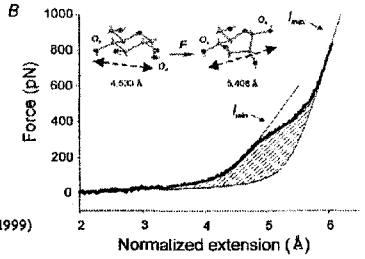
Amylose: Force-extension (contd.)

A



amylose

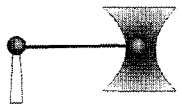
B



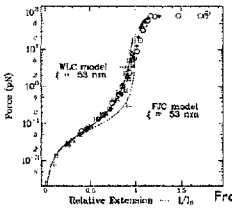
From Fisher et al, J. Physiol. (1999)

DNA: Force-extension curves

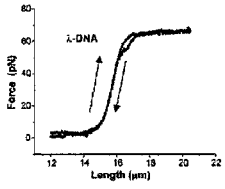
Typical experiment: Stretch DNA between optical tweezers and micropipette.



Measure force exerted versus length of the DNA. Fit to WLC model and extract the persistence length



From Strick et al, Ann. Rev. Biophys. Biomol. Struct., 2000

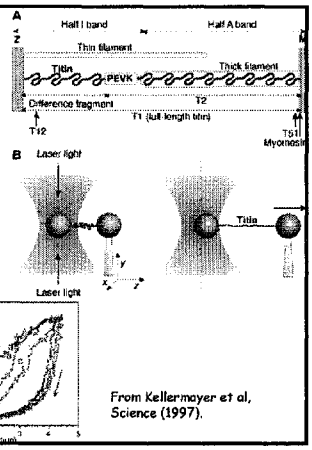


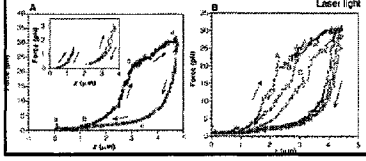
From Bennink et al, Nature Struct. Biol., 2002

Muscle protein titin

Stretching titin: WLC.

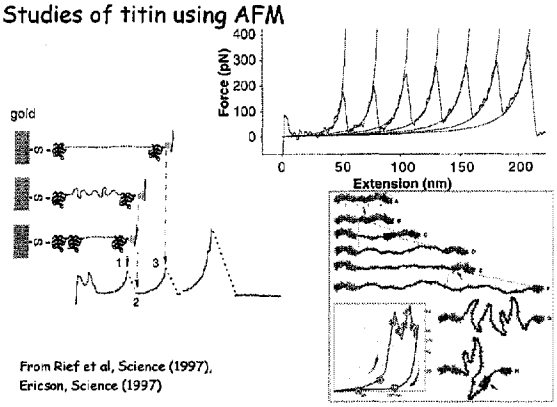
"Hysteresis", related to protein folding.





From Kellermayer et al, Science (1997).

Studies of titin using AFM



From Rief et al, Science (1997), Ericson, Science (1997)

Properties of biological rods and ropes

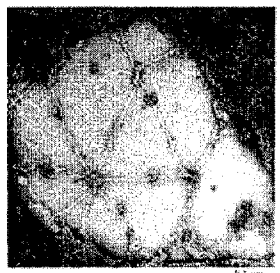
Actin	2 GPa	ξ_p=15 μm
Tubulin	2 Gpa	ξ_p =6 mm
Silk	~5 GPa	
Elastin	0.002 GPa	
Cellulose	80 GPa	Dry ramie fibers
Cellulose	20-40 Gpa	Wet fibers
DNA	~1 GPa	ξ_p =50 nm
Spektrin	0.002 GPa	ξ_p =10-20 nm

Source: table 2.2 in D. H. Boal: "Mechanics of the Cell", and table 8.4 in J. Howard: "Mechanics of Motor Proteins and the Cytoskeleton".

Rods and ropes: In 2D networks

Examples:
Membrane associated cytoskeleton,
Erythrocyte (red blood cell)

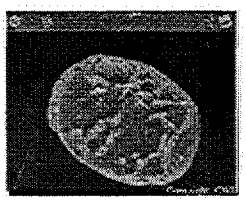
~6 fold symmetry in network

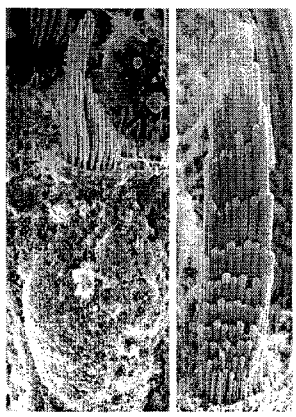


From www

Hair cell, lateral cortex of auditory outer hair cell.

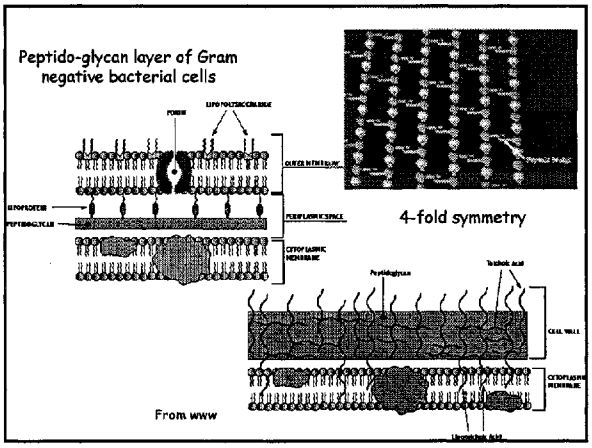
Nuclear lamina





From www

Peptido-glycan layer of Gram negative bacterial cells



From www

Elasticity of 2D networks

Symmetry important. Two relevant cases:
- Square symmetry. Four-fold connectivity
- Triagonal symmetry. Six-fold connectivity

General elasticity theory:

1D	2D
Displacement, x	Strain tensor u_{ij} (relative deformation)
Force, f	Stress tensor, σ_{ij} Stress is equivalent to pressure, force per unit area
$f=-k_{sp}x$ k_{sp}: spring constant	$\sigma_{ij} = \sum_{k,l} C_{ijkl} u_{kl}$; C_{ijkl} Elastic stiffness constants
$E = \frac{1}{2} k_{sp} x^2$	Density of free energy, ΔF: $\Delta F = \frac{1}{2} \sum_{ijkl} C_{ijkl} u_{ij} u_{kl}$

Elasticity in 2D, cont'd

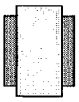
Free energy density, ΔF: Different modes of deformation

Area compression. K_A: Area compression modulus

Shear:

Simple shear. μ_s: Shear modulus (simple shear)

Pure shear. μ_p: Shear modulus (pure shear)



Simple networks of springs

Consider networks composed of short springs, each of spring constant k_{sp} :

Six-fold networks of springs (under tension, τ):

Area compression modulus, K_A : $\frac{\sqrt{3}}{2} k_{sp} \left(1 - \frac{\tau}{\sqrt{3} k_{sp}}\right)$

Shear modulus, μ : $\frac{\sqrt{3}}{4} k_{sp} \left(1 + \sqrt{3} \frac{\tau}{k_{sp}}\right)$

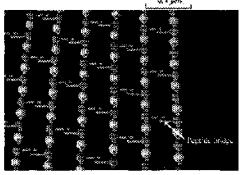
Four-fold networks of springs (under tension, τ, square symmetry):

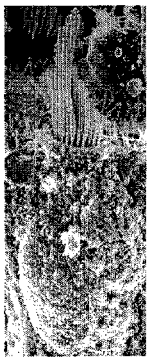
Area compression modulus, K_A $(k_{sp} - \tau)/2$

Shear modulus, pure shear, μ_p $(k_{sp} + \tau)/2$

Shear modulus, simple shear , μ_s τ

Biological reasons for the symmetry chosen?





Overview of lectures

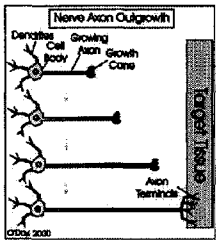
- Introduction: A little bit of biology
- Polymers of the cytoskeleton: Rods or ropes
- Networks of polymers
  - In 2D: Peptidoglycan layer, erythrocyte membrane
  - In 3D: "Full" cytoskeleton.
    - Connectivity
    - Viscoelasticity
- Force production by the cytoskeleton:
  - Polymerization forces

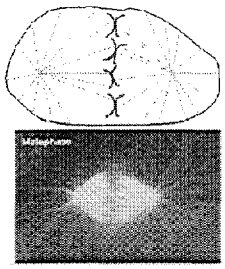
Rods and ropes: In 3D networks

Models for the networks of the cytoskeleton. Sometimes very different for the same polymer (e.g. Microtubules).

Composed of entropic vs. Hookean springs

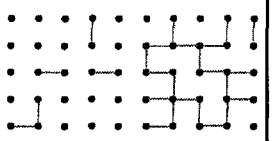
Density dependence (percolation theory). Viscoelasticity.





Connectivity of 3D networks

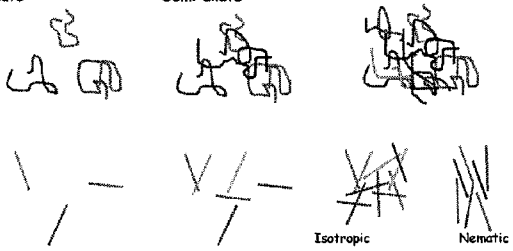
Density of polymers determine how "connected" a network is.



Dilute

Semi-dilute

Concentrated



Viscoelasticity

A semi-dilute polymer solution can behave like an elastic medium at short timescales, and like a viscous fluid at longer time scales. This behaviour is characterized by "the viscoelastic properties" of the medium.

Time-domain: relaxation modulus, $G(\tau)$

Frequency domain: shear moduli, $G'(\omega)$ and $G''(\omega)$

Apply oscillating strain $u_{xy}(t)$ at frequency ω, the resulting stress in the material is

$$\sigma_{xy} = G'(\omega) u_{xy}(t) + \frac{G''(\omega)}{\omega} \frac{du_{xy}}{dt}$$

$G'(\omega)$: role of a shear modulus⇒
$G'(\omega)$ termed "shear storage modulus"

$G''(\omega)/\omega$: role of a dynamic viscosity ⇒
$G''(\omega)$ termed "shear loss modulus"

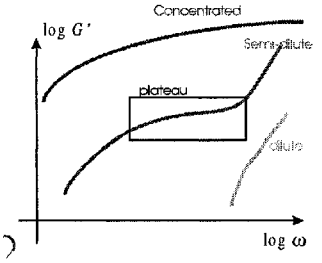
Rheology of biopolymer networks

In vitro investigations: The principal filaments of the cytoskeleton: *actin, intermediate filaments* and *microtubules.*

Semi-dilute polymer solutions:
$G'(\omega) \propto \omega^{\alpha}$ for high frequencies. Convenient to consider the power spectrum of thermal fluctuations:

$$\langle |\tilde{x}(\omega)|^2 \rangle \propto \frac{1}{\omega G'(\omega)}$$

$$\propto \omega^{-(1+\alpha)}$$



Interlude: power spectrum

Consider variable $x(t)$, describing (e.g.) the position of a random walker, moving in liquid with friction coefficient γ, at time t. The equation of motion for the random walker:

$$m\frac{d^2x}{dt^2} = -\gamma\frac{dx}{dt} + \tilde{F}(t)$$

Subdominant term (Low Reynolds number)

Stochastic, Langevin, force.

$$\langle F(t) \rangle = 0, \langle F(t) F(t') \rangle \propto \delta(t - t')$$

Equation for the Fourier transformed:

$$0 \simeq -i\omega\gamma\tilde{x}(\omega) + \tilde{F}(\omega)$$

Then, the power spectrum varies as

$$\langle |\tilde{x}(\omega)|^2 \rangle \propto \omega^{-2}$$

corresponds to α=1. Thus: Normal diffusion⇒α=1

Rheology of biopolymer networks

Actin filaments: d = 8 nm, ξ_p = 17 mm d = diameter of the filament

Microtubules: d = 25 nm, ξ_p = 6 mm

Filamentous networks with strong permanent cross-links:

G' is frequency -independent

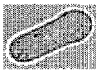
Corresponds to "elastic material" for all time-scales

Semidilute solutions of actin and microtubules individually show that at high frequencies:

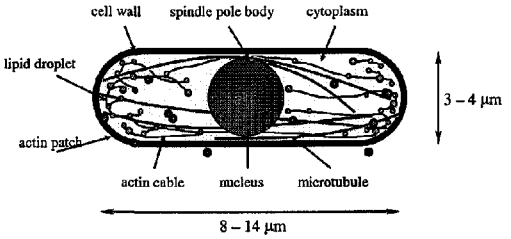
$G' \propto \omega^t \rightarrow \alpha = 0.75$

in agreement with theoretical predictions.

Rheology in living systems: Biological system



fission yeast cells
(Schizosaccharomyces pombe)



Work by E-L Munteanu, I M Tolic-Nørrelykke

Biological system

- Main components of the cytoskeleton

Actin network bundles

Microtubule

Robert J.P.Jr et al.
Nature Cell Biology 3(2001)

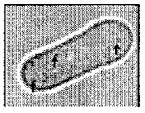
- Membranous structures

Vacuoles
Endoplasmatic reticulum
Golgi apparatus

Probes

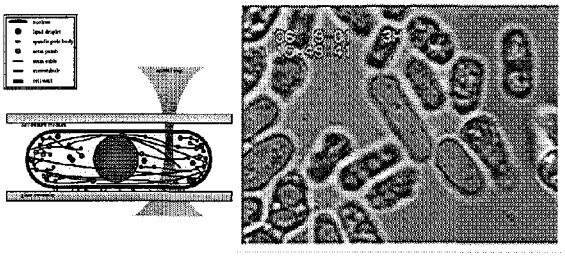
small round lipid droplets, naturally existing in the cell

size ≈ 100 - 200 nm



Viscoelasticity measurements in vivo

Optical tweezers trapping granuli in living yeast cells



The power spectrum

Sample the continuous signal $x(t)$ with frequency f_{sample} for a time T

$x_j \equiv x(t_j), \quad j = 1,\ldots,N$

$t_j = j\Delta t$

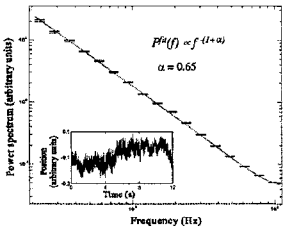
$T = N\Delta t$

- Fourier transformation

$x_j \rightarrow \tilde{x}_k \quad k = 0,\ldots,N/2$

- Power spectrum

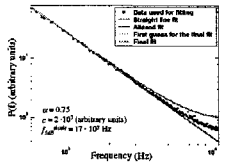
$P_k^{(ex)} \propto |\tilde{x}_k|^2$



Fitting the power spectrum with high precision

This fitting procedure of the power spectrum takes into account the effects of:

- *aliasing* due to the finite sampling frequency
- *electronic filters*
- power loss at high frequency due to the finite response time of *the photodiode*



Berg-Sørensen, K., and H. Flyvbjerg, submitted to *Phys. Rev. Lett.*
Berg-Sørensen, K., L. Oddershede, E.-L. Florin, and H. Flyvbjerg, *J. Appl. Phys.* (2003)

Procedure:

1) Fit the binned data to a power law expression

$$P^{theory}(f) = c\, f^{-(1+\alpha)}$$

2) Include aliasing

$$P^{aliased}(f) = \sum_n P^{theory}(f + 2nf_{Nyq})$$

3) Include electronic filters and the effect of the photodiode

$$P^{filtered}(f) = \frac{P^{theory}(f)}{\left(1+\left(\frac{f}{f_{3dB}^{e}}\right)^2\right)\left(1+\left(\frac{f}{f_{3dB}^{diode}}\right)^2\right)}$$

3 fitting parameters:

- power law exponent $-(1+\alpha)$
- constant c
- f_{3dB}^{diode}

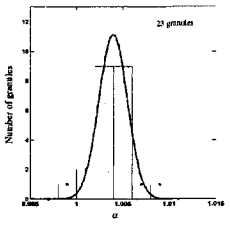
Results from optical tweezers experiments
Isolated granules in water

Protocol

- Cells were treated with a solution of lysing enzymes (1 mg/ml) in order to lyse the cell wall.
- The measurements were performed in water.

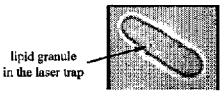
Result

- Isolated granules perform *normal diffusion* as expected for a pure viscous fluid like water



Black solid line is a Gaussian fit:
$\alpha = 1.004 \pm 0.002$

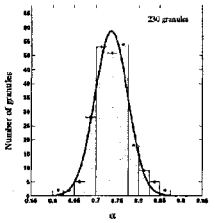
Results from optical tweezers experiments
Granules inside cells of fission yeast



- The cells of fission yeast are in interphase, in normal culture medium
- Time scale: $10^{-4} \div 10^{-2}$ sec

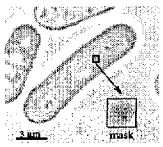
Results

- All the granules perform *subdiffusive motion*
- The histogram of the power law exponents is a broad one, with α ranging from 0.55 to 0.86



Black solid line is a Gaussian fit:
$\alpha = 0.736 \pm 0.038$

Methods
Single Particle Tracking



two main steps:

1) Choose a mask in the first frame that contains the object to be monitored
2) Apply the tracking algorithm: cross-correlation, on the series of images

- Spatial resolution ~ 5 nm = 0.1 pixel
- Sampling rate = 25 Hz

Cross-correlation algorithm:

- Calculate the cross-correlation matrix between *the mask K* and a *kernel* of a successive image *I*, which contains the tracked object. *K* and *I* have the same dimension *m* x *m*.

$$CC_{x,y} = \frac{\sum\sum [I_{x+i,y+j} - \bar{I}_{x,y}][K_{i,j} - \bar{K}]}{\sqrt{\sum\sum (I_{x+i,y+j} - \bar{I}_{x,y})^2} \sqrt{\sum\sum [K_{i,j}]^2}}$$

($CC_{x,y}$ element is in fact the normalized covariance)

- The parabolic fit of the nearest neighbors of the maximum value in the correlation matrix gives the subpixel value of the new position $(x,y)_{max}$

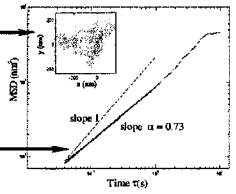
Mean Square Displacement

Series of images
(f_{sample} = 25Hz)

$\vec{r}(t) \equiv (x(t), y(t))$

$MSD \equiv \langle |\vec{r}(t) - \vec{r}(t+\tau)|^2 \rangle$

Example of a trajectory over 10 seconds and mean square displacement (MSD) measured on a lipid granule in a living cell. Solid black line is the power law fit.



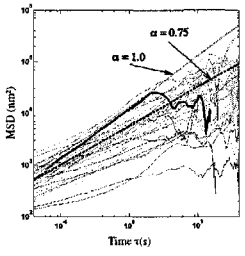
Results from single particle tracking experiments

- Mean square displacement as a function of time lag for 52 granules within living cells of fission yeast.
- Time scale: 0.04 – 40 sec

Important

At this time scale we can also see other types of motion like:
- Brownian motion
- directed and
- confined motion

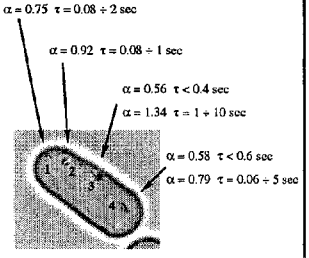
- The red solid line is an example of a granule that performs normal diffusion: $\alpha \cong 1.0$
- The green solid line has $\alpha \cong 0.75$ which is consistent with subdiffusive motion.



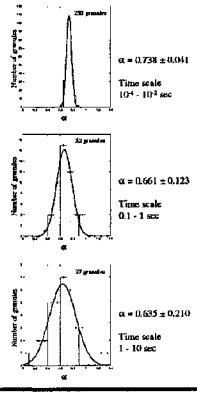
Results from single particle tracking experiments
Different types of motion within the same cell

- Subdiffusion can be seen for all time scales: from 0.1 up to 100 seconds (ex: granule 1 and 4)
- Normal diffusion or Brownian motion appears as well in all time scales that were explored in SPT measurements (ex: granule 2)
- Granule 3 shows an interesting behavior: at short time scale it "feels" the viscoelastic environment. But for longer time it shows directed motion.

the speed ~ 2 μm/min which is in the range of microtubule polymerization



Conclusions



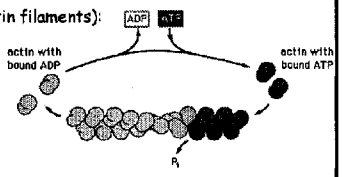
From both type of experiments, SPT and OT, we can see different scaling laws dependent on the time scale.

- At very short times (~ms) the granules perform subdifussive motion due to the viscoelastic surrounding. No other type of motion is observed.
- At intermediate times (~ 0.1 - 1 s) normal diffusion and confined motion is also observed. Some granules can diffuse freely or move in a confined space due to the geometrical arrangement of the cytoplasmatic structures.
- At long times (seconds) we also see directed motion that could be caused, for example, by the microtubule polymerization.
- At even larger times (up to 100 s) we see more examples of directed or confined motion. Subdiffusion is still present.

Polymers of cytoskeletal elements constitute a network, to "keep the cell in shape" – and for molecular motors to "do their thing". Can they do more?

Polymerization of cytoskeletal elements

- Mobility (caused by actin filaments):
  - Keratocyte cells
  - Listeria infection



- Nuclear positioning
  - Microtubule "pushing"

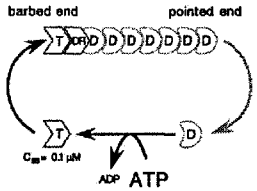
Simple polymerization dynamics

Intro to actin polymerization

Energy supply: ATP hydrolysis
Rate depend on "which end"

[*M*]: monomer concentration
n: number of monomers in a filament

Treadmilling:
$dn/dt=0$, i.e., $[M]=[M]_c=k_{off}/k_{on}$



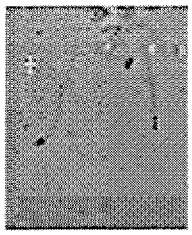
Microtubule polymerization
Position nucleus?

From Pantaloni et al, Science, 2001

Mobility, caused by actin polymerization

Keratocyte forward movement

Listeria propulsion

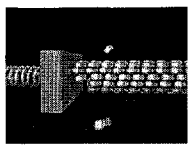


Images and movies from Pantaloni et al, Science, 2001

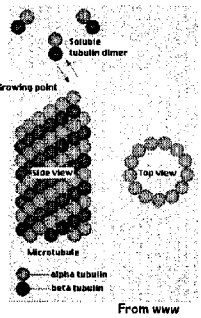
Microtubule pushing forces

Microtubules polymerize like actin, but also have a very intriguing dynamics – events of catastrophes, ...

Polymerization can induce forces:



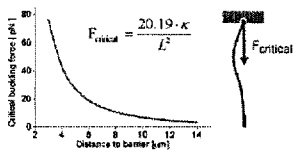
From webpage of M. Dogterom, AMOLF, NL



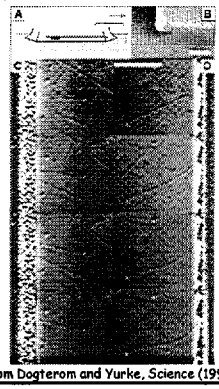
From www

Microtubules: Measure forces in vitro

Work in group of M. Dogterom, AMOLF, NL.



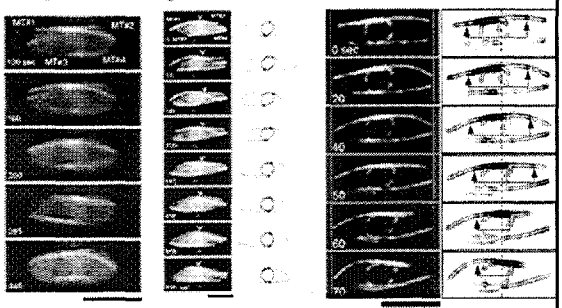
From webpage of M. Dogterom



From Dogterom and Yurke, Science (1997)

Microtubules: Position nucleus in yeast

Work in group of F. Chang, Columbia U., NY, USA



Opposing MT bundles

MT bundles pushes nuclear membrane

Fluorescent speckle microscopy

Images from Tran et al, J. Cell. Biol., 2001

Want to learn more?

Relevant books:

- "Mechanics of the Cell" by D. H. Boal
- "Mechanics of Motor Proteins and the Cytoskeleton" by J. Howard
- "Physics of Bio-Molecules and Cells", Les Houches lecture notes, session LXXV, ed. By H. Flyvbjerg, F. Jülicher, P. Ormos and F. David (e.g. Lecture by M. Dogterom)

(A very small selection of) papers:

- "Mechanism of Actin-Based Motility" by D. Pantaloni et al, Science **292**, 1502-1506 (2001).
- "A Mechanism for Nuclear Positioning in Fission Yeast Based on Microtubule Pushing", by P. T. Tran et al, J. Cell Biol. **153**, 397-411 (2001).