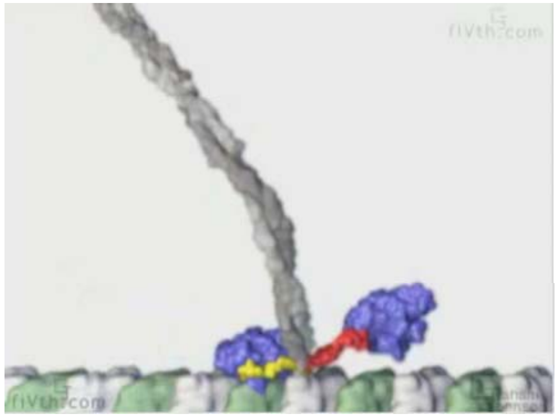
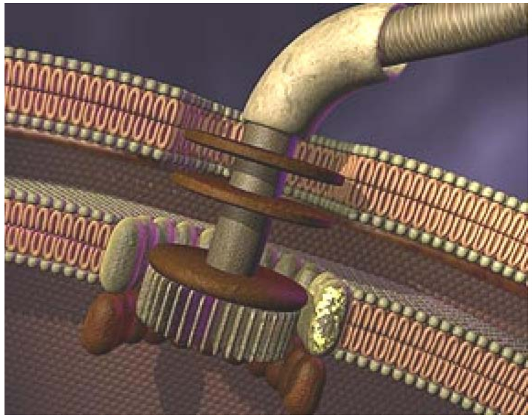
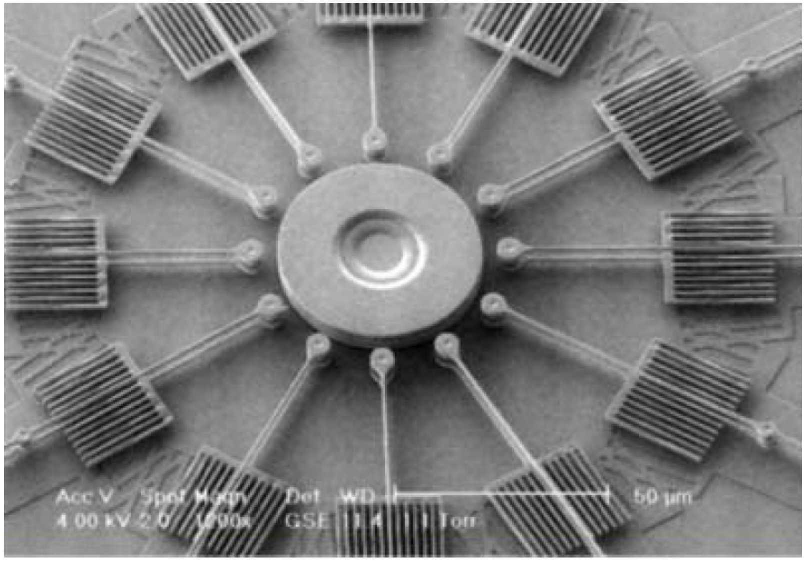
Biological Machines, Cell Mechanics and Nanotechnology

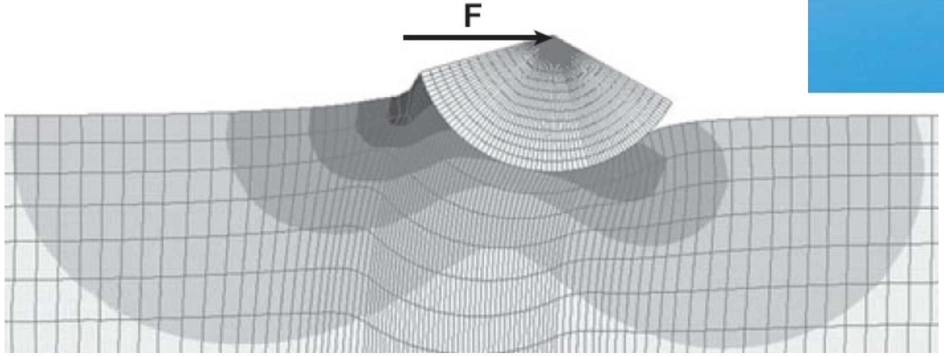


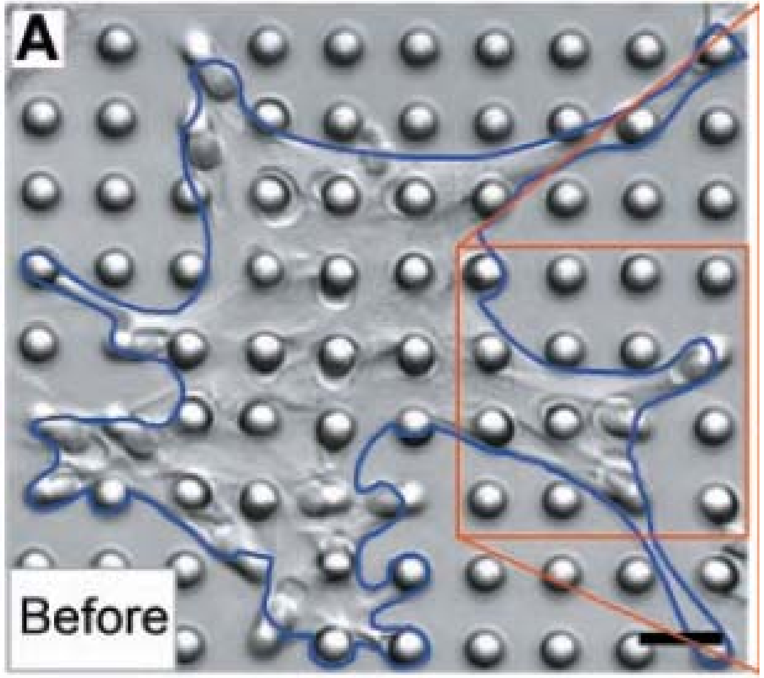




王歐力 副教授
Oliver I. Wagner, PhD
Associate Professor

National Tsing Hua University
Institute of Molecular & Cellular Biology
College of Life Science





Overlapping and merging subject matter, focus and expertise in biology

BIOLOGY: Zoology, Botany, Ecology, Microbiology…

Bio**chemistry**: Chemical reactions in the cell, structure of proteins…

Bio**physics**: Physical forces acting on organelles or macromolecules…

Biomedical **Engineering**: Tissue engineering, artificial implants…

Biomechanics: Physical properties of cells and tissues…

Bionanotechnology: Biochip design, lab-on-a chip…

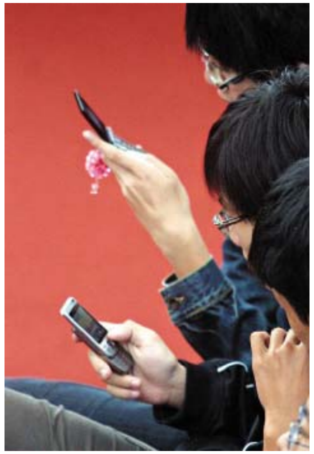
Bio**business** (Bioindustry)
·
·
·
… and more

People who should be interested in these <u>important fields</u> are:

- Engineers
- Material Scientist
- Chemists
- Physicist
- Involved in Biobusiness

Life Science Students
in Biology Class

Non-Life Science Students
in Biology Class



Teacher in class with
“awake” students

Awake students =
good and fun teaching

Teacher in class with
“absent-minded” students

Absent minded students =
bad and boring teaching

Regulation

-2 points deduction **for me** if:

I am teaching bad. My slides are bad. My lecture is boring.

-2 points deduction **for you** if:

- Sleeping
- Doing homework
- Playing with **cell phones** or laptop
- Talking to neighbor
- Do not come to class
- LEAVE THE CLASS AFTER THE FIRST HOUR

Which classes do we offer an which one have you chosen?

①

Wednesday class

10020LS 110301 Introduction to Life Science 生命科學導論

⇨ Textbook-based class! Requires ***little*** biology background

②

Thursday class

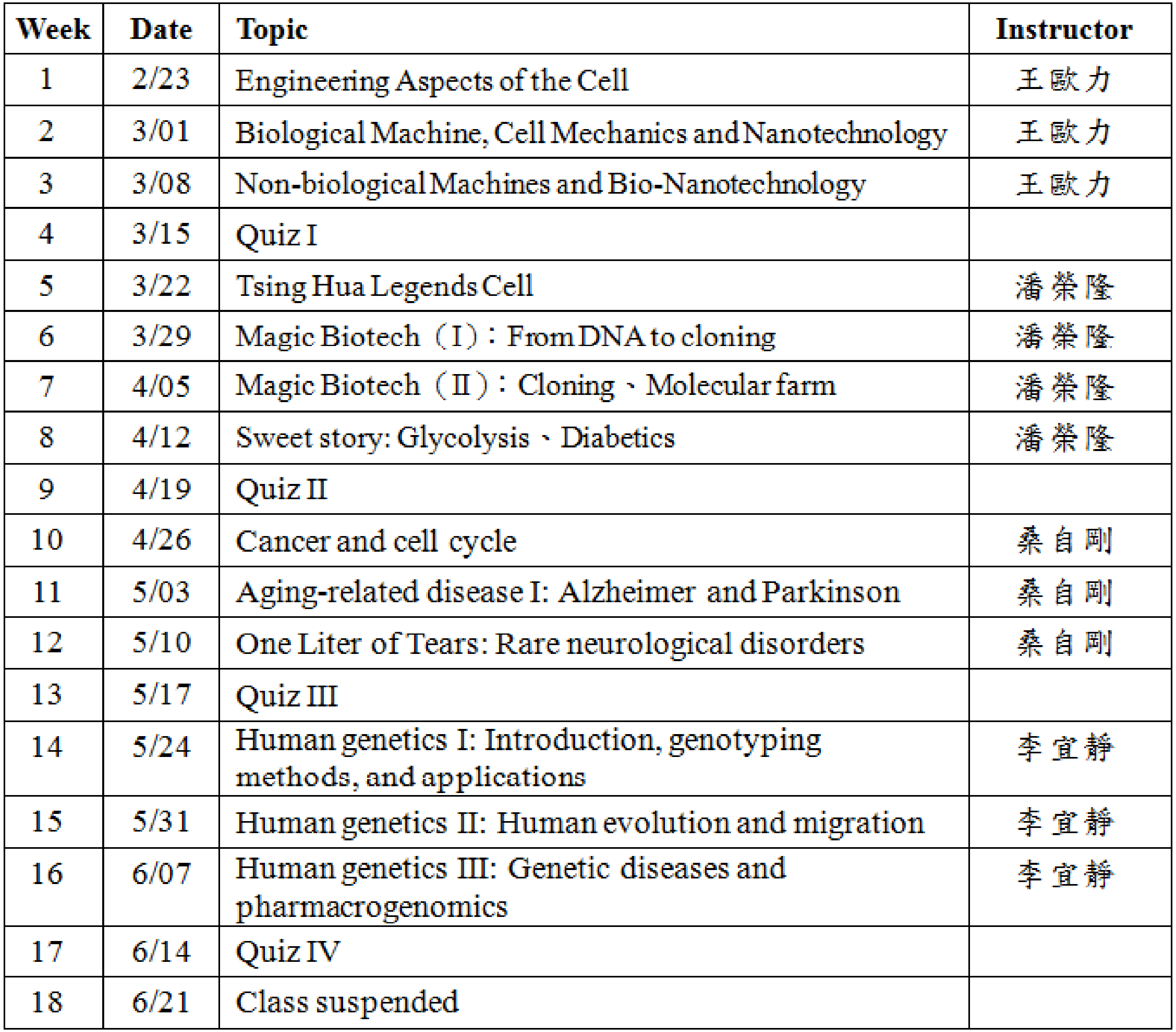
10020LS 110302 Introduction to Life Science 生命科學導論

⇨ Journal-based class! Requires ***more*** biology background
(for example, Biology classes at senior high school)

What does Journal-based mean?
- Discussing articles from “Scientific American Chinese Edition”
- Discussing articles from Scientific Journals as Nature, Science etc.

⇨ You have to download the reading material offered by the teachers from the e-learning system. And......... READ THEM!

Syllabus



Week	Date	Topic	Instructor
1	2/23	Engineering Aspects of the Cell	王歐力
2	3/01	Biological Machine, Cell Mechanics and Nanotechnology	王歐力
3	3/08	Non-biological Machines and Bio-Nanotechnology	王歐力
4	3/15	Quiz I	
5	3/22	Tsing Hua Legends Cell	潘榮隆
6	3/29	Magic Biotech（I）：From DNA to cloning	潘榮隆
7	4/05	Magic Biotech（Ⅱ）：Cloning、Molecular farm	潘榮隆
8	4/12	Sweet story: Glycolysis、Diabetics	潘榮隆
9	4/19	Quiz II	
10	4/26	Cancer and cell cycle	桑自剛
11	5/03	Aging-related disease I: Alzheimer and Parkinson	桑自剛
12	5/10	One Liter of Tears: Rare neurological disorders	桑自剛
13	5/17	Quiz III	
14	5/24	Human genetics I: Introduction, genotyping methods, and applications	李宜靜
15	5/31	Human genetics II: Human evolution and migration	李宜靜
16	6/07	Human genetics III: Genetic diseases and pharmacrogenomics	李宜靜
17	6/14	Quiz IV	
18	6/21	Class suspended	

English

30 min review in Chinese by TA

Chinese

Chinese

Chinese

The cell as a material

Karen E Kasza, Amy C Rowat, Jiayu Liu, Thomas E Angelini,
Clifford P Brangwynne, Gijsje H Koenderink and David A Weitz

To elucidate the dynamic and functional role of a cell within the tissue it belongs to, it is essential to understand its material properties. The cell is a viscoelastic material with highly unusual properties. Measurements of the mechanical behavior of cells are beginning to probe the contribution of constituent components to cell mechanics. Reconstituted cytoskeletal protein networks have been shown to mimic many aspects of the mechanical properties of cells, providing new insight into the origin of cellular behavior. These networks are highly nonlinear, with an elastic modulus that depends sensitively on applied stress. Theories can account for some of the measured properties, but a complete model remains elusive.

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Current Opinion in Cell Biology 2007, **19**:101–107

This review comes from a themed issue on
Cell structure and dynamics
Edited by Daniel P Kiehart and Kerry Bloom

Available online 15th December 2006

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2006.12.002

Introduction

Cells are highly dynamic: they crawl, change shape and divide. In many critical biological processes, cells both exert and respond to forces in their surroundings; the mechanical properties of the cell are intimately related to this behavior. Cells also continually remodel their internal structure and thereby change their mechanical properties. An integrated understanding of cell structure and mechanics is thus essential for elucidating many fundamental aspects of cell behavior, from motility to differentiation and development. Here we focus on the mechanical properties of cells and review recent developments in our understanding of the cell as a material.

A variety of experimental techniques show that cells have both elastic and viscous characteristics, and thus are viscoelastic materials: their stiffness is similar to Jello, but they continue to slowly deform under a steady stress (Figure 1a). Unlike most conventional materials, cells are highly nonlinear; their elastic modulus depends on the degree of applied or internal stress (Figure 2) [1••]. Moreover, their elastic behavior depends on the mechanical properties of their environment [2].

The mechanical properties of the cell are largely determined by the cytoskeleton, a biopolymer network consisting of three major components: filamentous actin (F-actin), intermediate filaments and microtubules (Figure 3a). In addition, a myriad of filament crosslinker, motor and regulatory proteins play a critical role in cytoskeletal structure and dynamics and hence in the mechanical properties of the cytoskeleton. The cytoskeleton is a complex, heterogeneous and dynamic structure, which makes the study of its properties extremely difficult. The two major approaches to this problem are *in vitro* studies of model networks designed to mimic the properties of individual components of the cytoskeleton, and studies of the mechanical properties of cells themselves.

Reconstituted cytoskeletal networks

A major advantage of reconstituted networks is that their viscoelastic properties can be probed by traditional engineering approaches [3•], as well as by more sophisticated optical methods; by measuring the time-dependent response to an imposed stress or strain, both the elastic and viscous properties can be determined. Networks of F-actin are among the most widely studied reconstituted systems. As with the other cytoskeletal filaments, F-actin is a semi-flexible polymer, neither completely flexible, like more traditional synthetic polymers, nor perfectly rigid. Instead, the filaments are soft enough to have some thermally induced shape fluctuations that play an important role in their elasticity. The effects of thermal fluctuations are particularly apparent in the network elasticity at the shortest timescales, leading to a characteristic time dependence [4]; the same behavior was also recently observed in cells [5••,6•]. Other recent measurements of F-actin networks demonstrate the important role of filament length [7] and additional relaxation mechanisms specific to semi-flexible filaments [8]. While earlier studies elucidate the behavior of solutions of entangled F-actin alone, current efforts focus primarily on the effects of crosslinking proteins and other actin-binding proteins (Figure 3b). The elasticity of the resultant crosslinked networks has a different physical origin, and can depend sensitively on both actin and crosslinker concentration [9–11,12••,13•]. Studies of crosslinked networks are likely to remain an area of active investigation.

The semi-flexible nature of the filaments constituting these networks is particularly important under increasing

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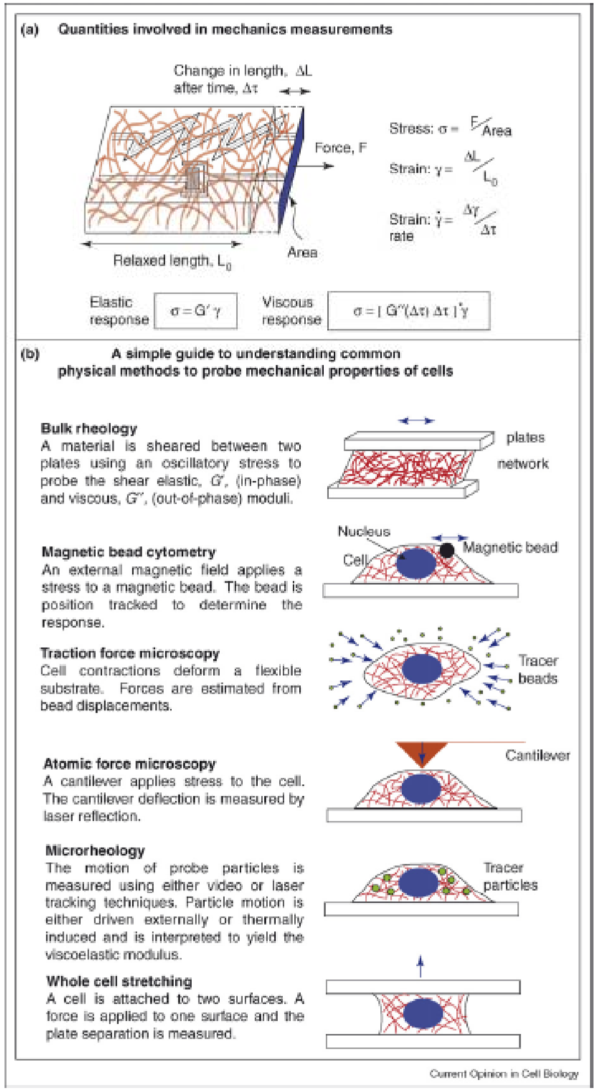
Figure 1



(a) Quantities involved in mechanics measurements

(b) A simple guide to understanding common physical methods to probe mechanical properties of cells

Bulk rheology
A material is sheared between two plates using an oscillatory stress to probe the shear elastic, *G*′, (in-phase) and viscous, *G*″, (out-of-phase) moduli.

Magnetic bead cytometry
An external magnetic field applies a stress to a magnetic bead. The bead is position tracked to determine the response.

Traction force microscopy
Cell contractions deform a flexible substrate. Forces are estimated from bead displacements.

Atomic force microscopy
A cantilever applies stress to the cell. The cantilever deflection is measured by laser reflection.

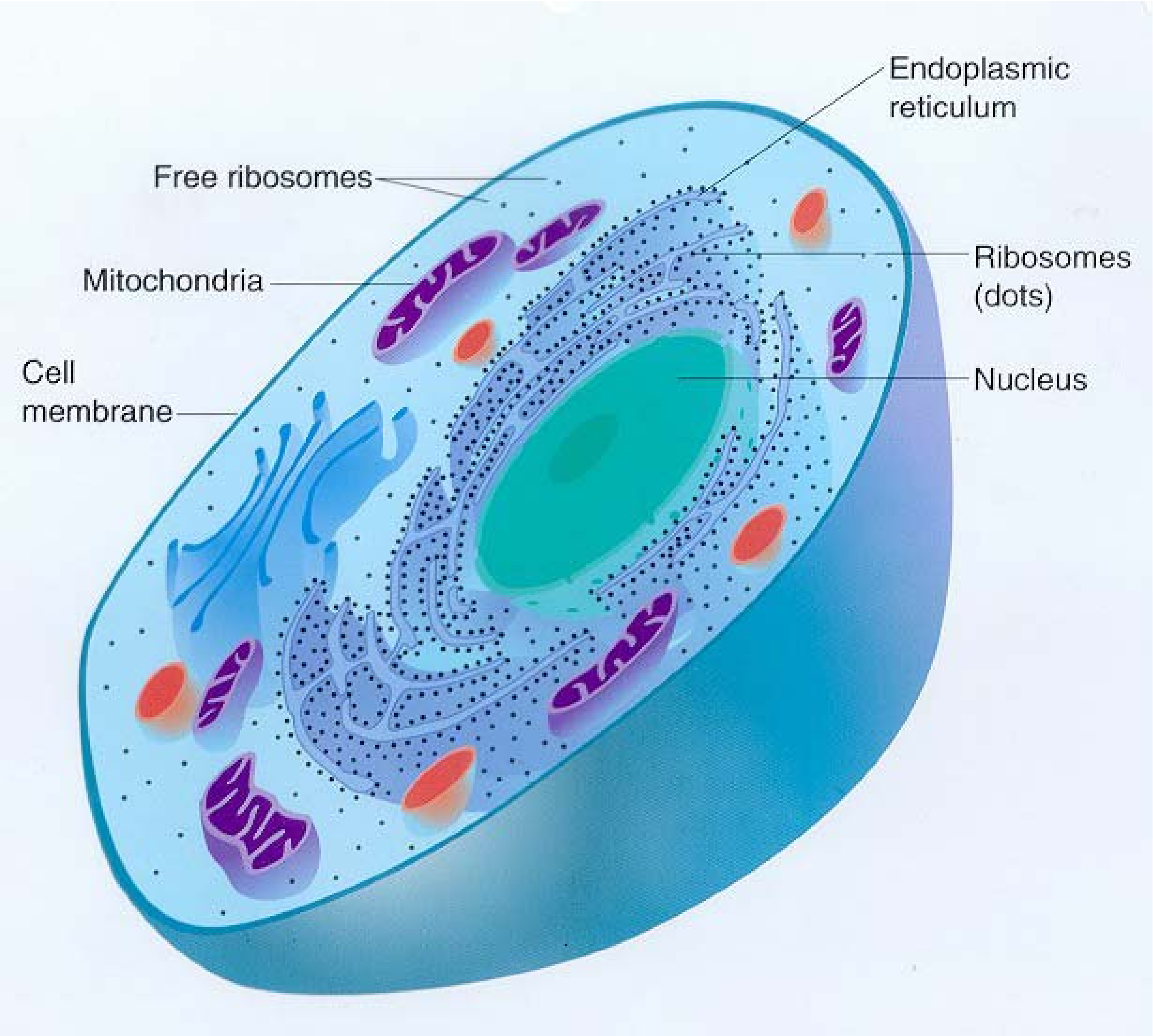
Microrheology
The motion of probe particles is measured using either video or laser tracking techniques. Particle motion is either driven externally or thermally induced and is interpreted to yield the viscoelastic modulus.

Whole cell stretching
A cell is attached to two surfaces. A force is applied to one surface and the plate separation is measured.

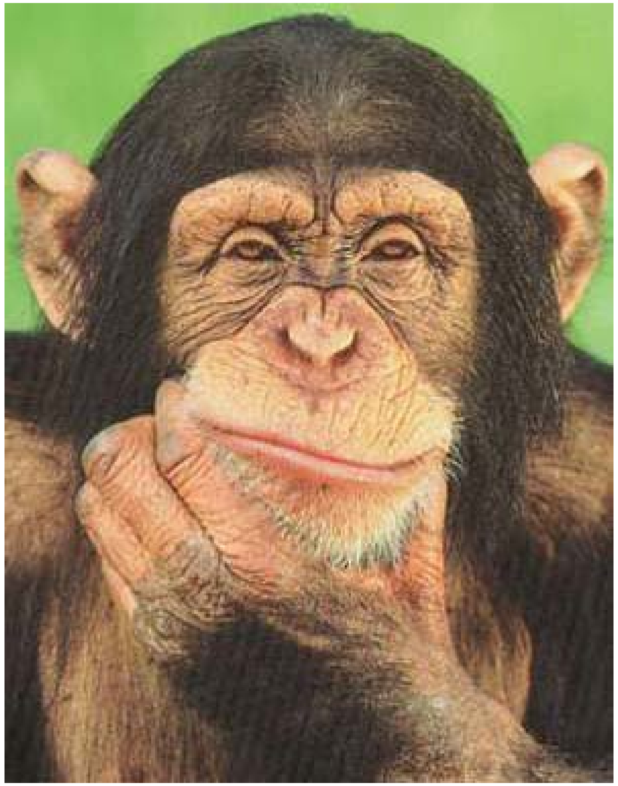
Current Opinion in Cell Biology

Mechanics of biopolymer networks and cells. **(a)** Quantities involved in mechanics measurements. Many materials have both elastic and viscous properties. The elasticity of biopolymer networks makes them resist deformation like a simple spring (grey, upper) for which the energy of deformation is stored in the material regardless of time; to quantify this we measure an elastic modulus, *G*′, which is analogous to a spring constant. The viscosity of

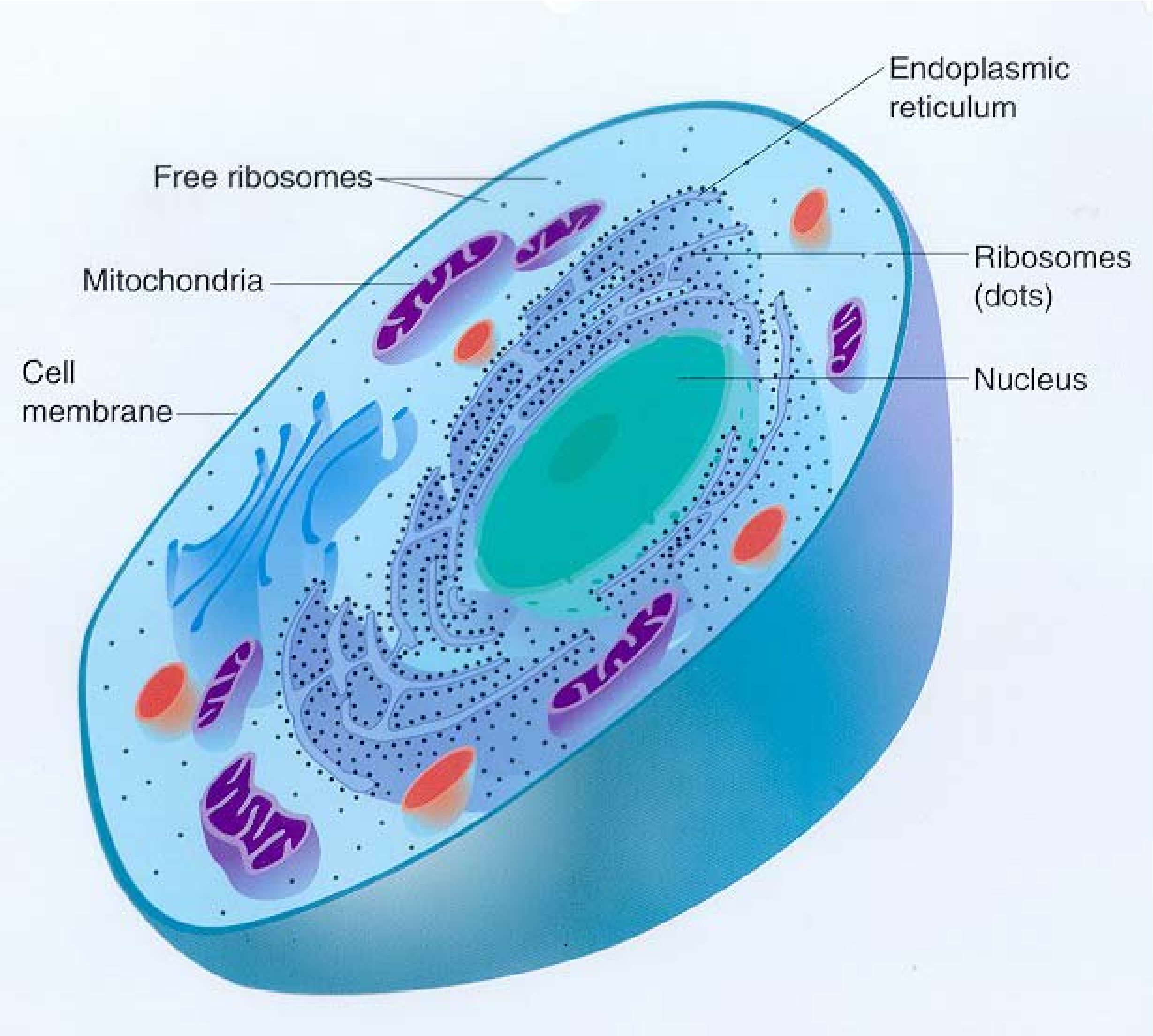
The eukaryotic cell



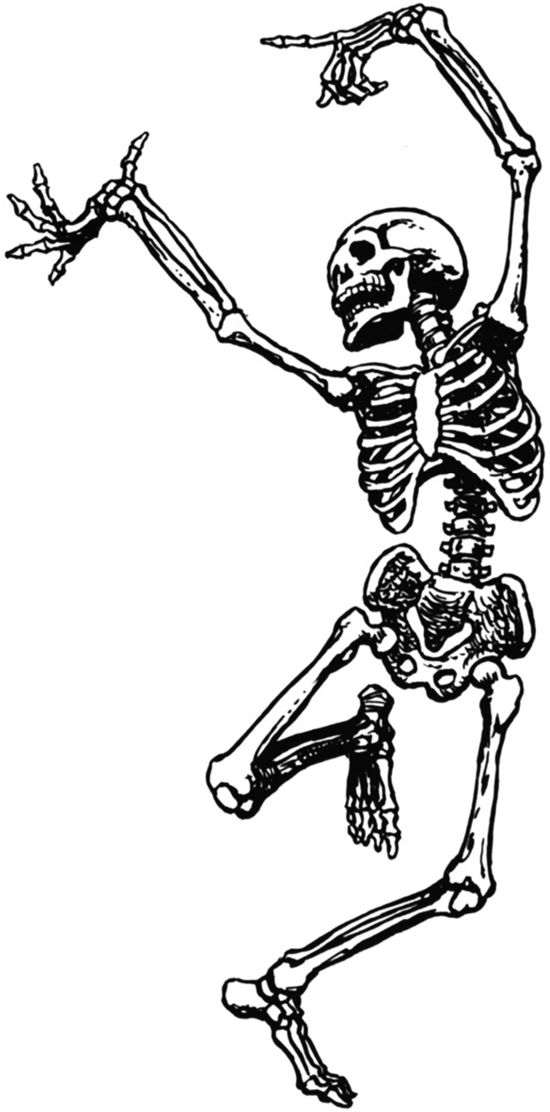
Um... something is missing?



The eukaryotic cell

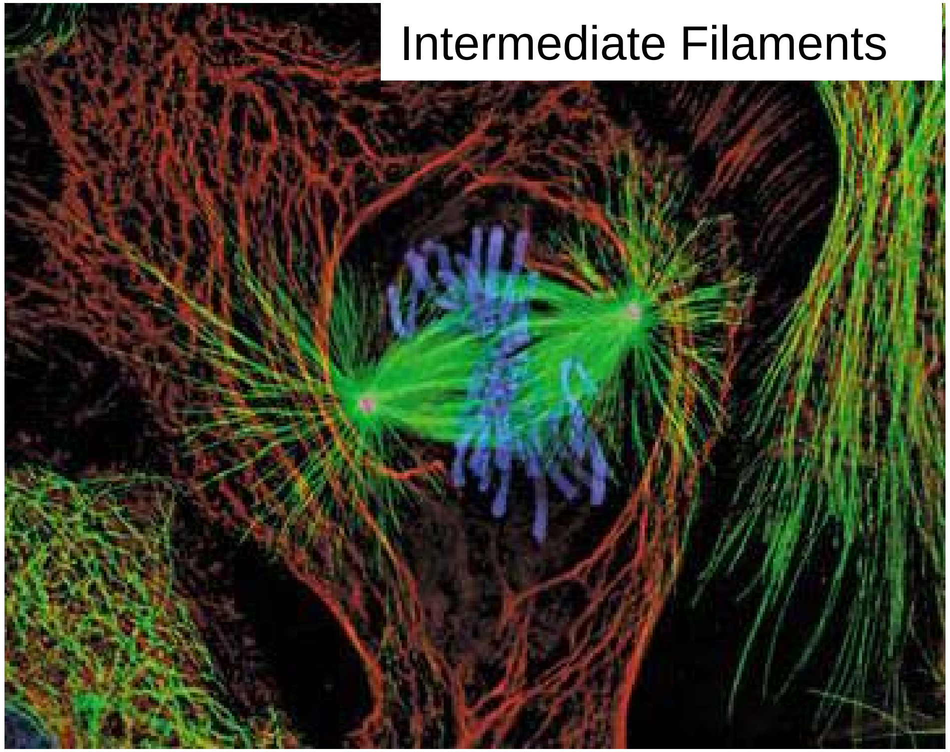


A skeleton!

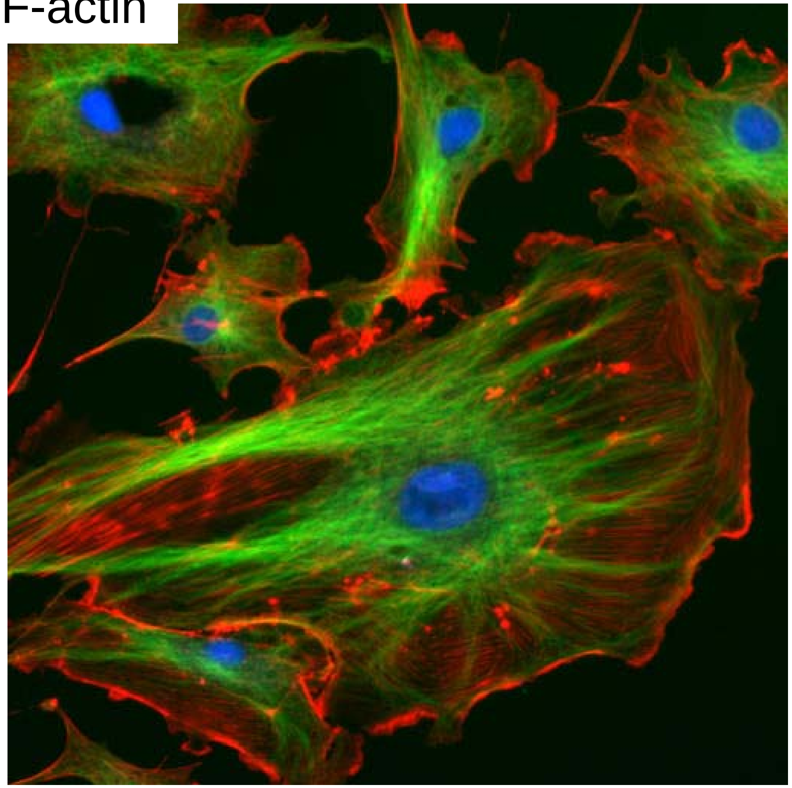


The **Cyto**skeleton!

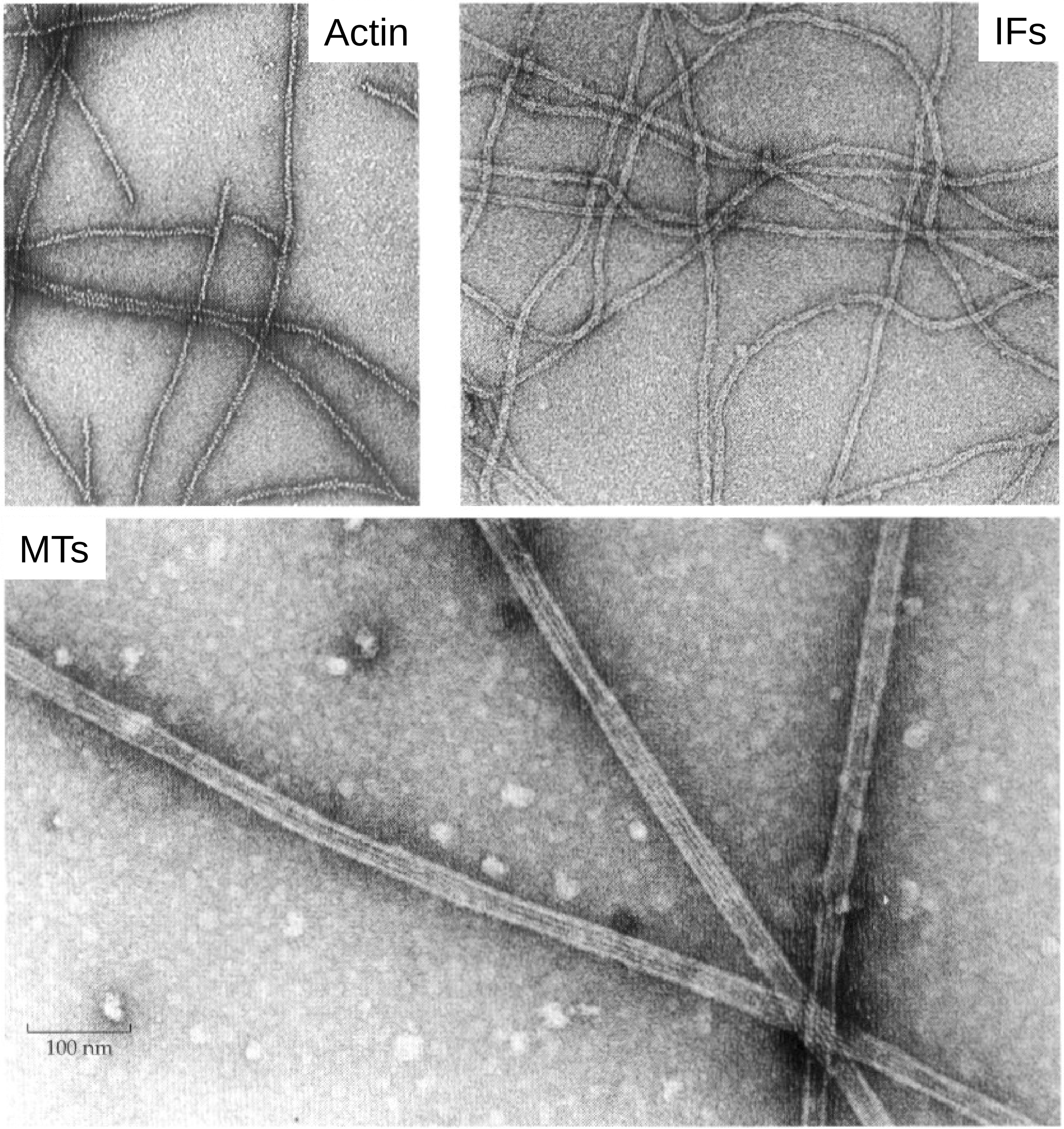
Intermediate Filaments



F-actin



Microtubules



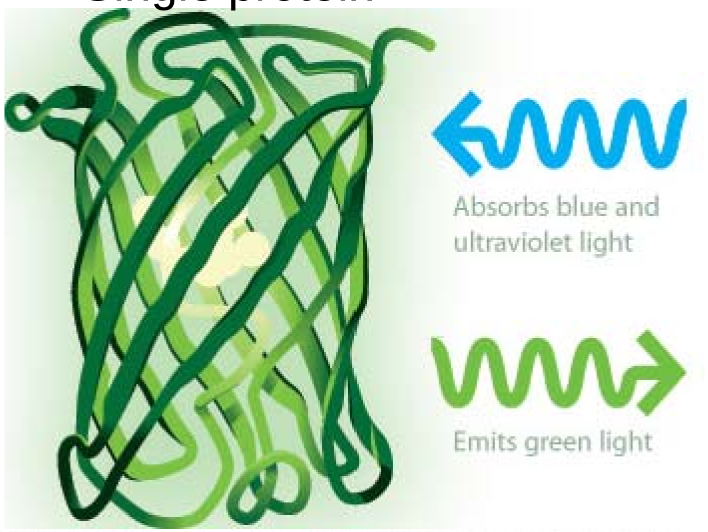
Cyto = “Cell”
Cytoskeleton =
“Skeleton of the cell”

Here:
Electron microscopy images of cytoskeletal elements (fibers/ filaments) drawn to scale

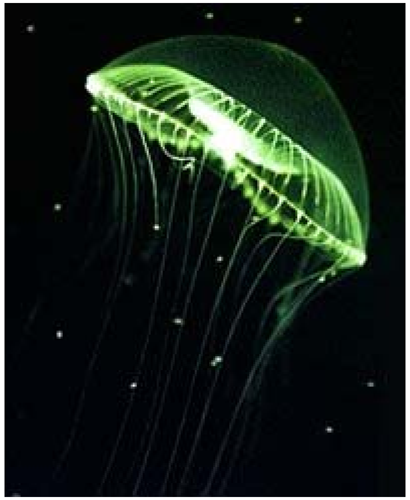
Howard, Mechanics of Motor Proteins, 1st Ed.

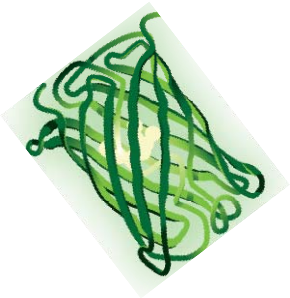
What is GFP?

Single protein



Green fluorescent protein
isolated from jellyfish
Aequorea victoria





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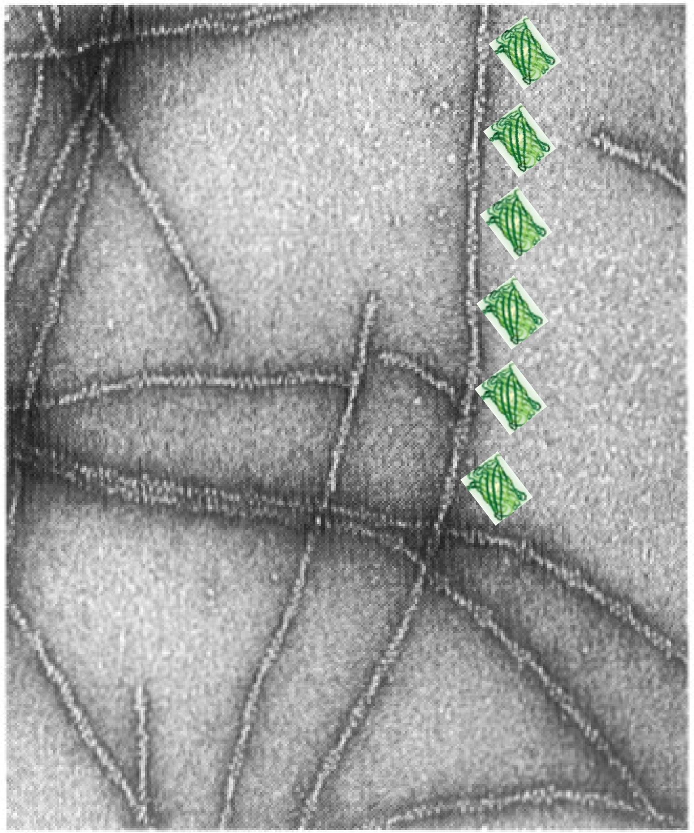

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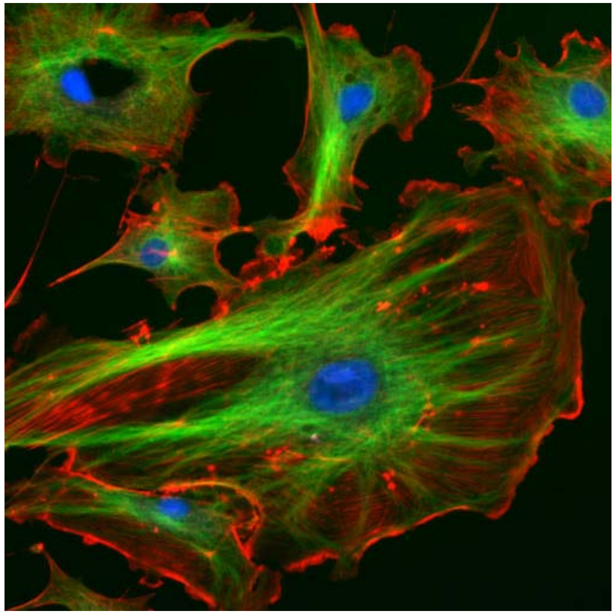
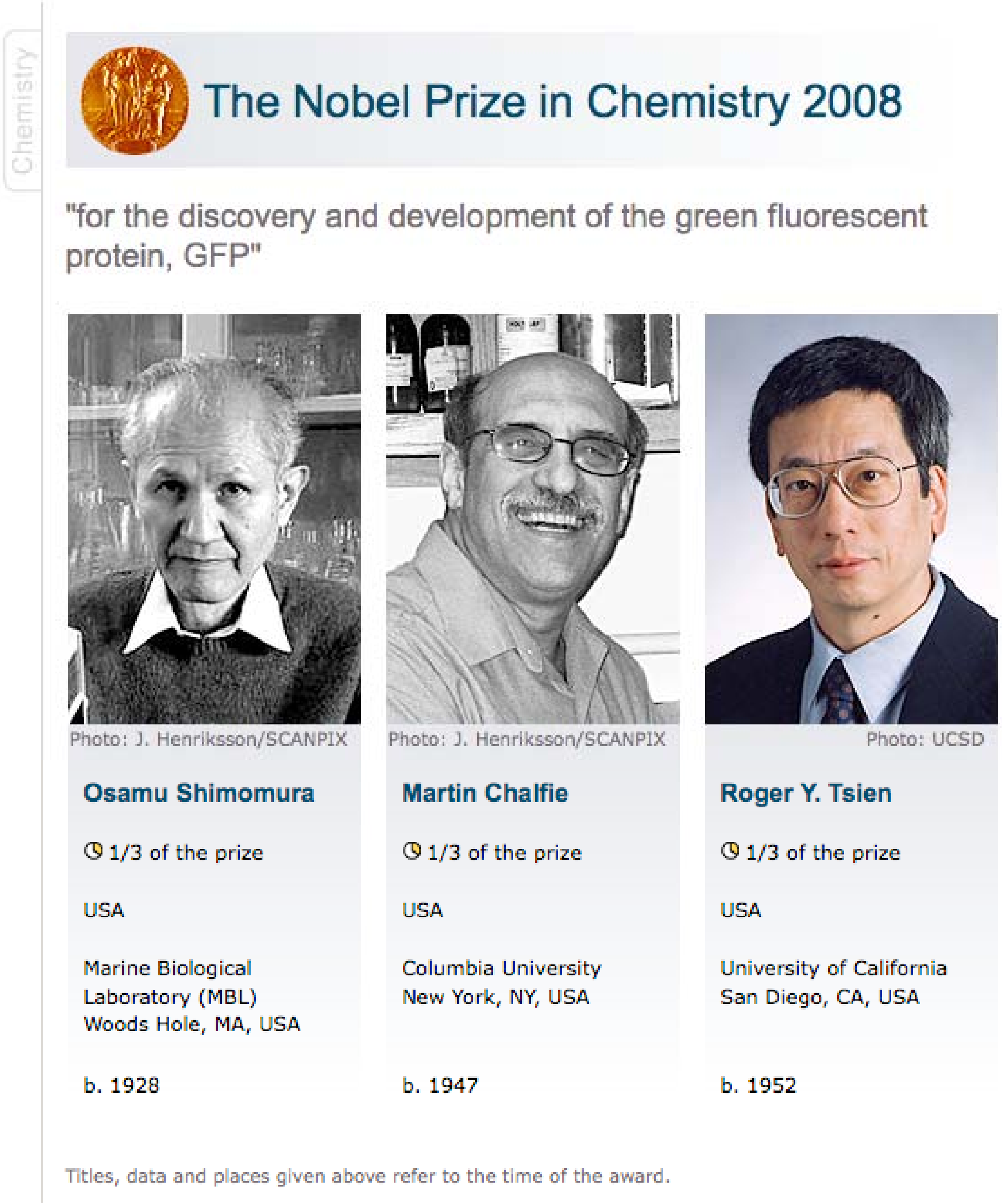




The Nobel Prize in Chemistry 2008

"for the discovery and development of the green fluorescent protein, GFP"

Photo: J. Henriksson/SCANPIX

Osamu Shimomura

◔ 1/3 of the prize

USA

Marine Biological
Laboratory (MBL)
Woods Hole, MA, USA

b. 1928

Photo: J. Henriksson/SCANPIX

Martin Chalfie

◔ 1/3 of the prize

USA

Columbia University
New York, NY, USA

b. 1947

Photo: UCSD

Roger Y. Tsien

◔ 1/3 of the prize

USA

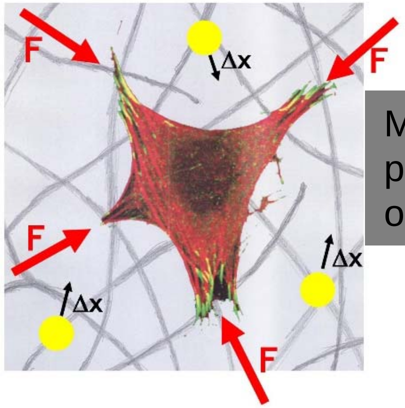
University of California
San Diego, CA, USA

b. 1952

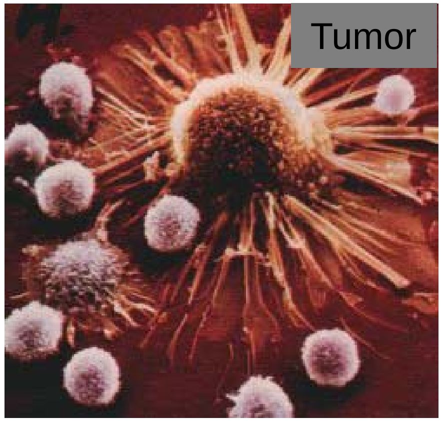
Titles, data and places given above refer to the time of the award.

Why do I think it is important to teach you about the cytoskeleton?

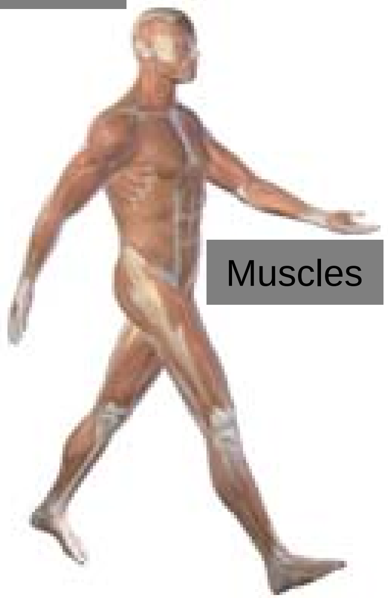
- It is related to **polymer science** => interesting to Chemists and Material Scientists
- It is related to **mechanics** => interesting to Physicists and Engineers
- The cytoskeleton **enables cells to move** => interesting to know why:
  - cancer cells move so fast (tumor migration/metastasis)
  - how sperm cells can move and find their target egg
  - how neurons grow so long and make networks in the brain (learning/memory)
- The cytoskeleton is important for **muscle contractions**: we can stand, walk and move things with our hands



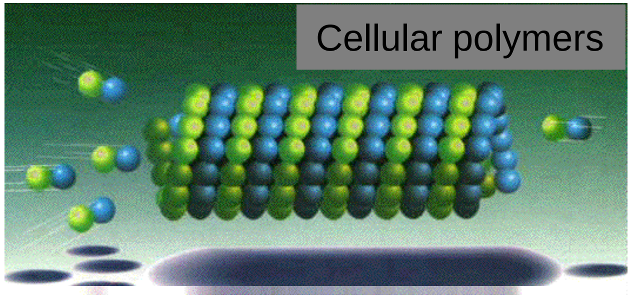
Mechanical properties of cells



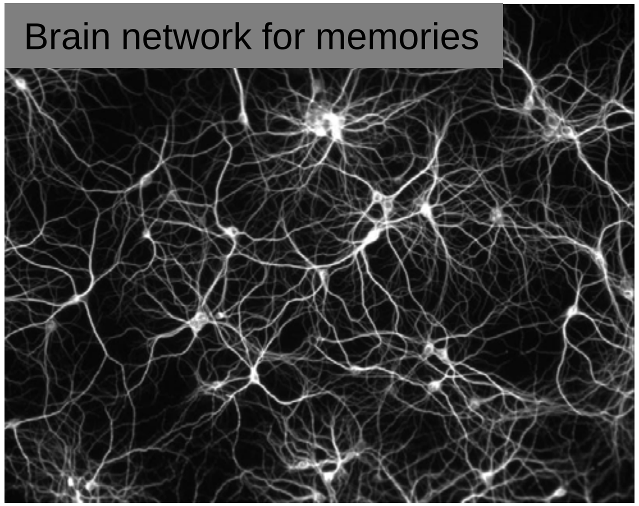
Tumor



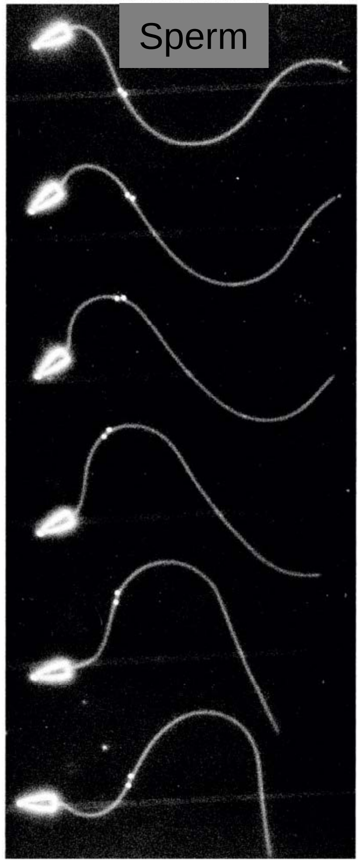
Muscles



Cellular polymers



Brain network for memories

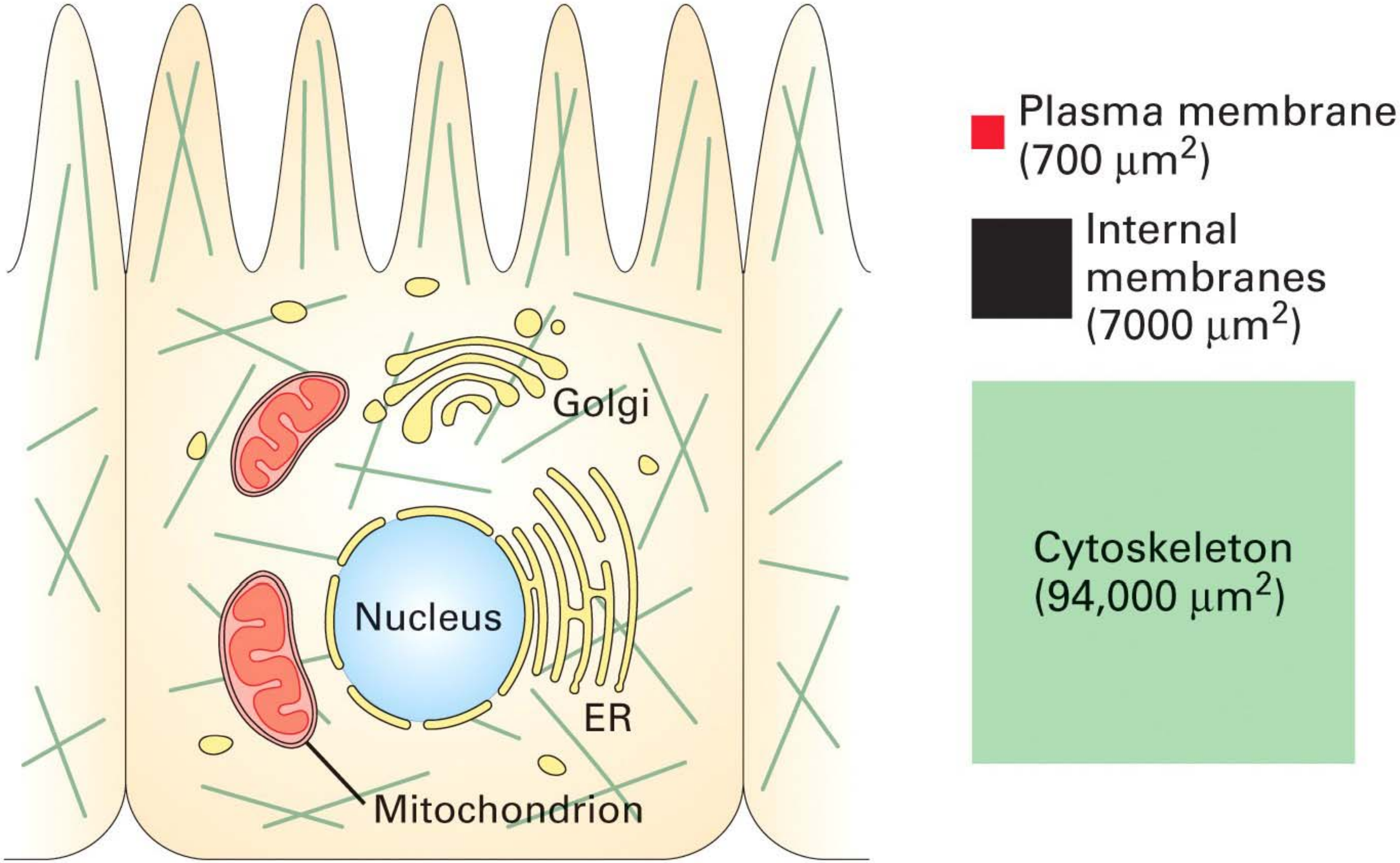


Sperm

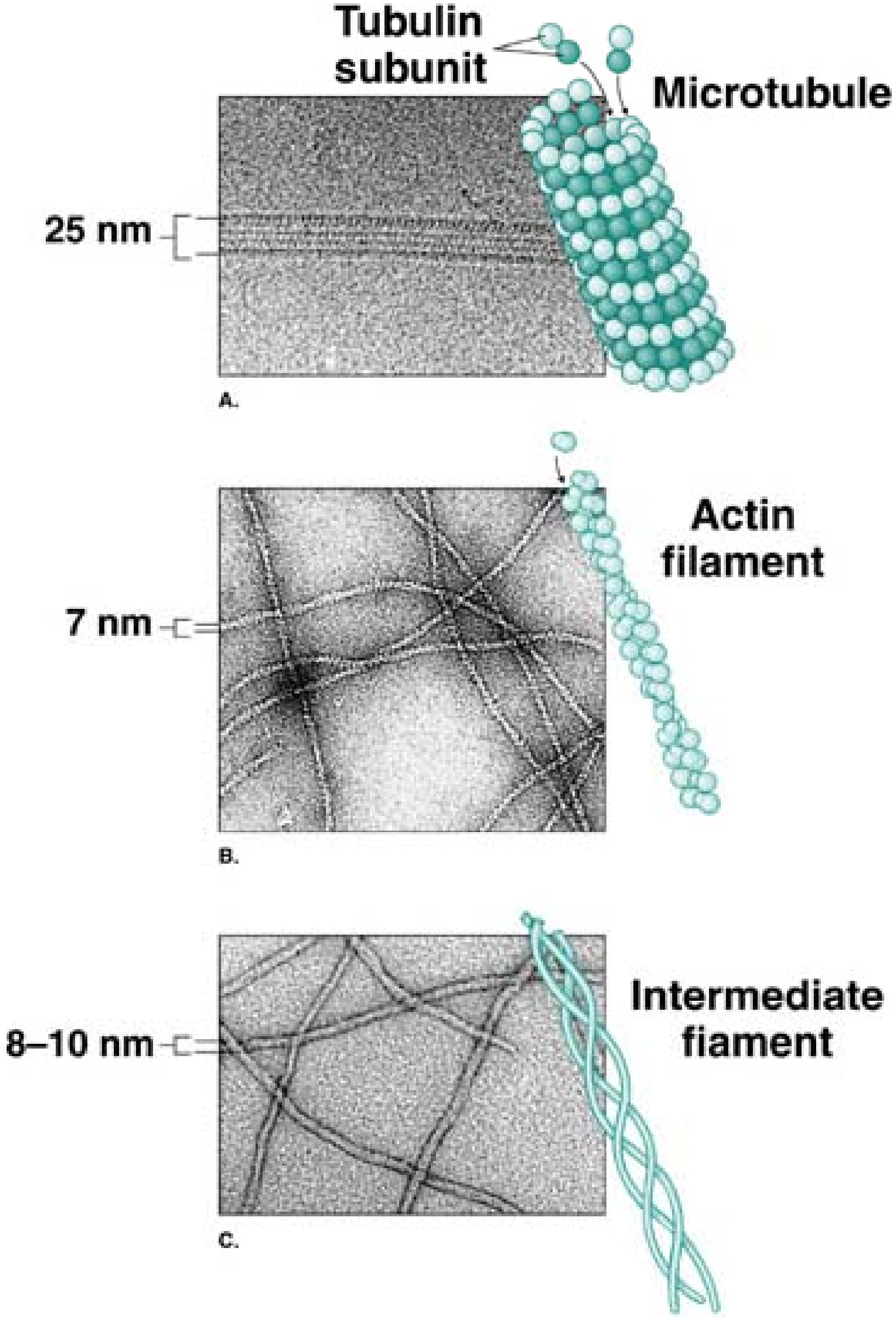
Why do we need the Cytoskeleton?

- Important for **cell shape** and **cell stiffness (cell mechanics)**
- Brings organelles into their correct positions
- **Highway** for molecular motors

⇒ **occupies lots of space**!



3 basic cytoskeletal elements

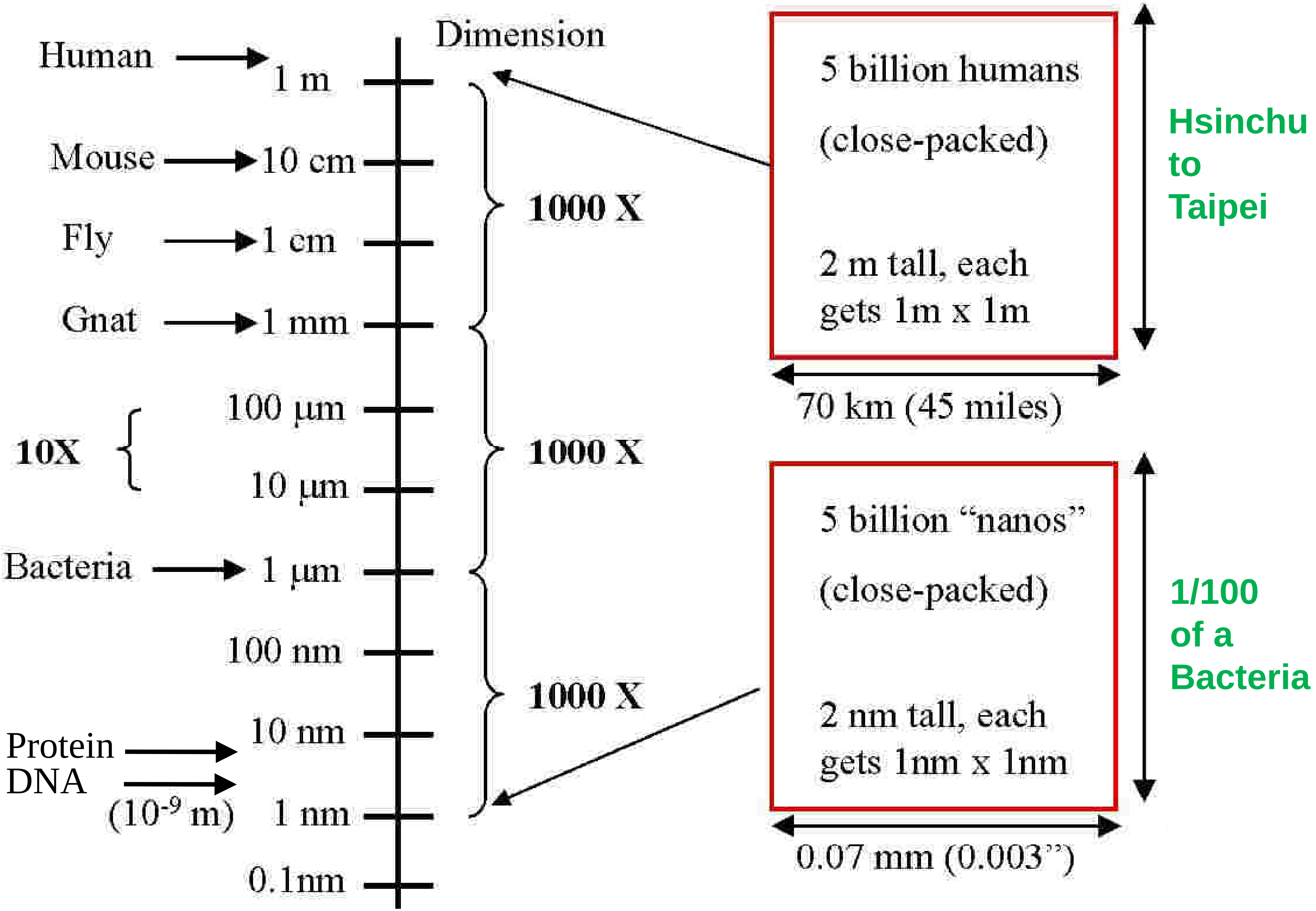


- Cytoskeleton is composed of **3 types of fibers** which are all polymers built from **globular protein subunits**
- The fibers can be **distinguished by** their **diameter**

Actin: twisted, two-stranded (pearl-string like) structure
⇒ **cell shape** and **highway** for molecular motors (cargo transport)
MT: hollow cylinder formed by proto-filaments made of tubulin-subunits
⇒ positioning of organelles; form **flagella**; chromosome separation; **highway**
IFs: rope-like structure
⇒ cell shape and **cell elasticity**

Movie
v20-01-microtubules.mov

What's a Nanometer?



Dimension

Human → 1 m

Mouse → 10 cm

Fly → 1 cm

Gnat → 1 mm

100 µm

10X

10 µm

Bacteria → 1 µm

100 nm

10 nm

Protein

DNA

$(10^{-9}$ m) 1 nm

0.1nm

1000 X

1000 X

1000 X

5 billion humans

(close-packed)

2 m tall, each
gets 1m x 1m

70 km (45 miles)

Hsinchu
to
Taipei

5 billion "nanos"

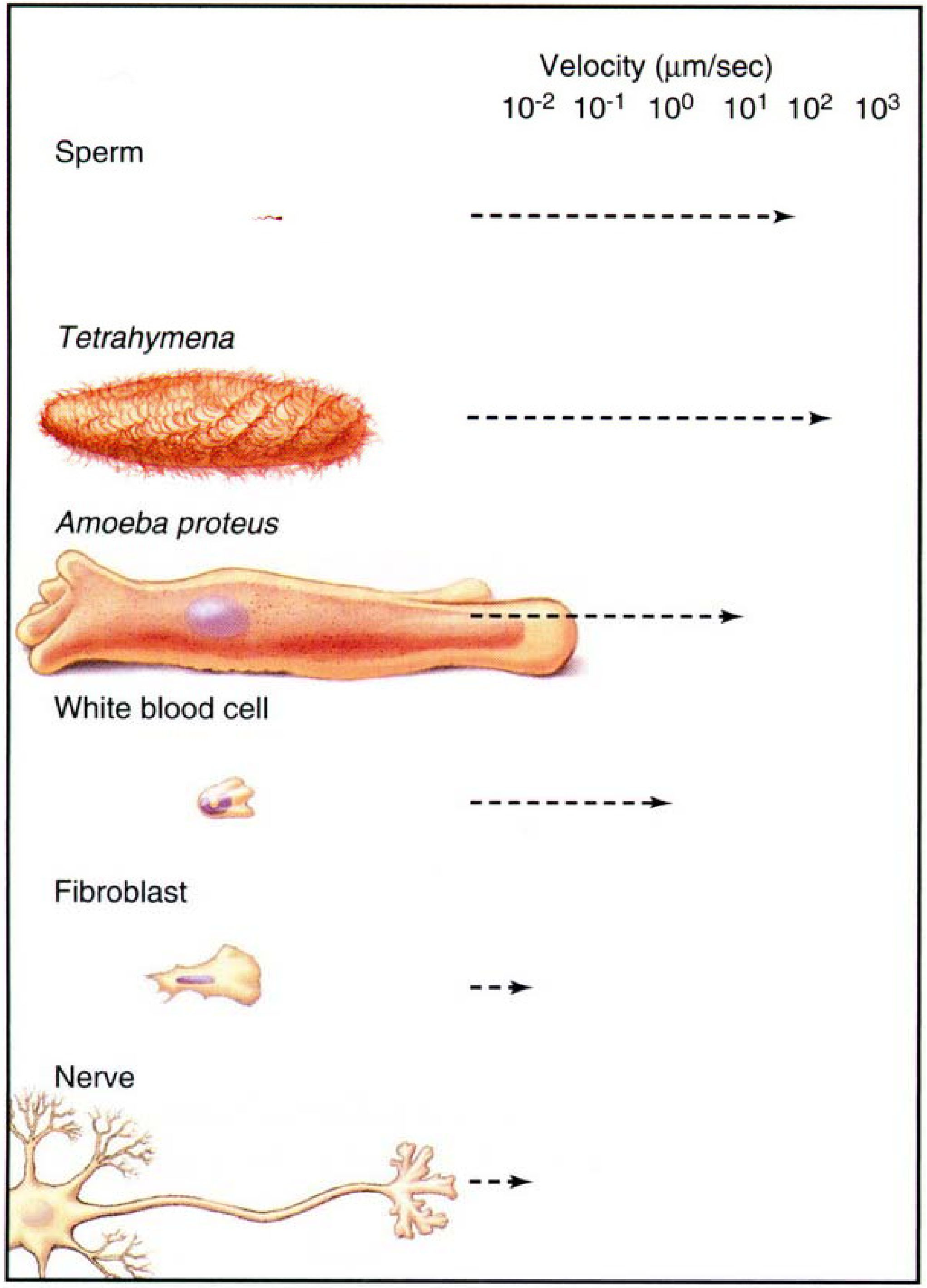
(close-packed)

2 nm tall, each
gets 1nm x 1nm

0.07 mm (0.003")

1/100
of a
Bacteria

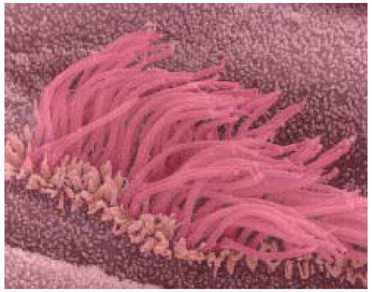
Purdue University



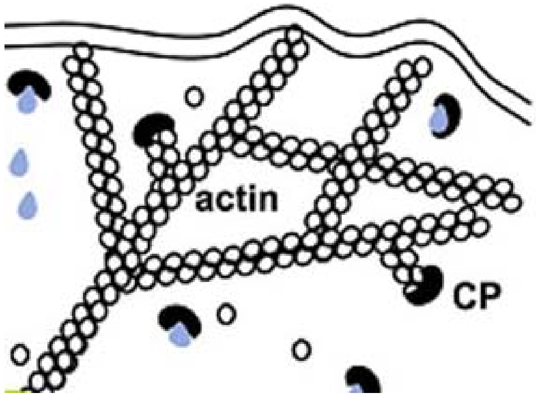
The cytoskeleton enables cells to move

- Microtubuli make **flagella** bend

- Microtubuli make **cilia** waving



- **Actin polymerization** pushes membrane forward

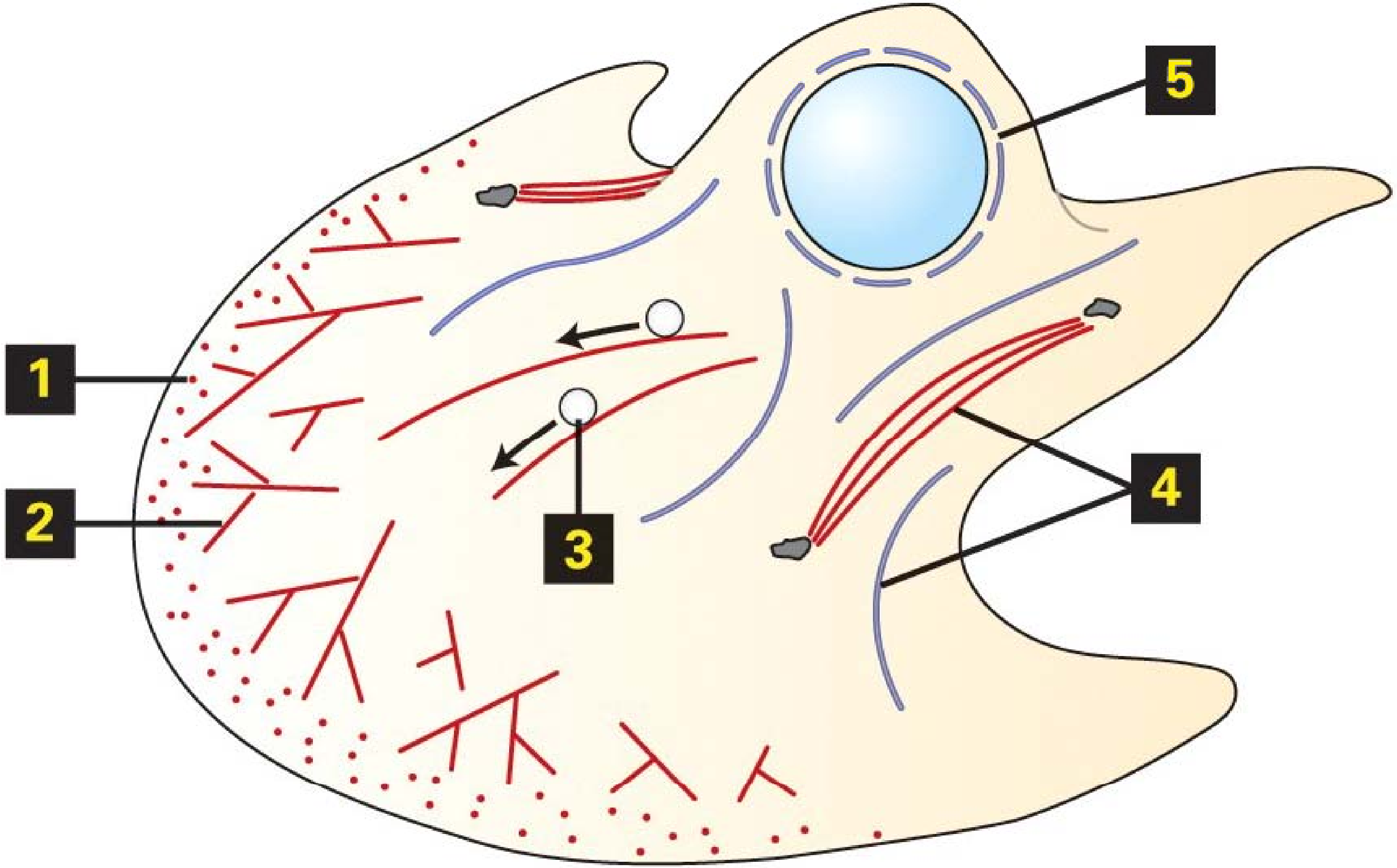


Pollard, 1st ed.

Movie
v19-04-keratinocyte.mov

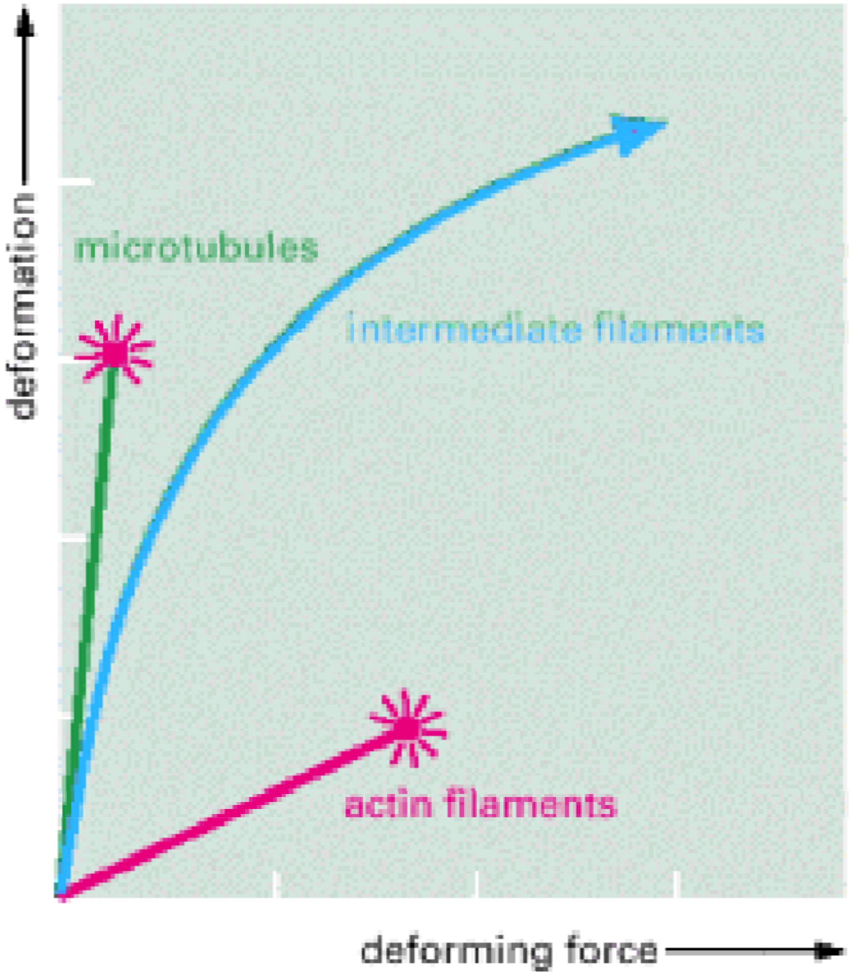
The cytoskeleton in motile cells

1+2 = **Actin polymerization** pushes the membrane forward
3 = Organelles, vesicles, mitochondria move on actin and microtubule tracks
4 = **Actin** helps to connect cells to substrate (“focal adhesions”)
5 = **Intermediate filaments** make a strong shell for the nucleus



Cytoskeletal fibers also differ in their mechanical properties

Based on their specific structures, the 3 types of cytoskeletal polymers exhibit also different elastic properties



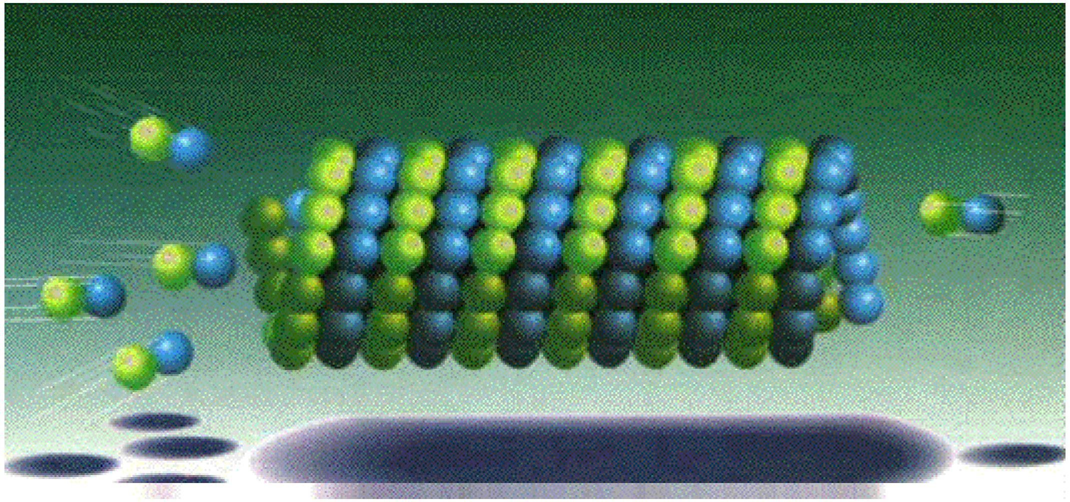
P. Janmey, *JCB*, 1991

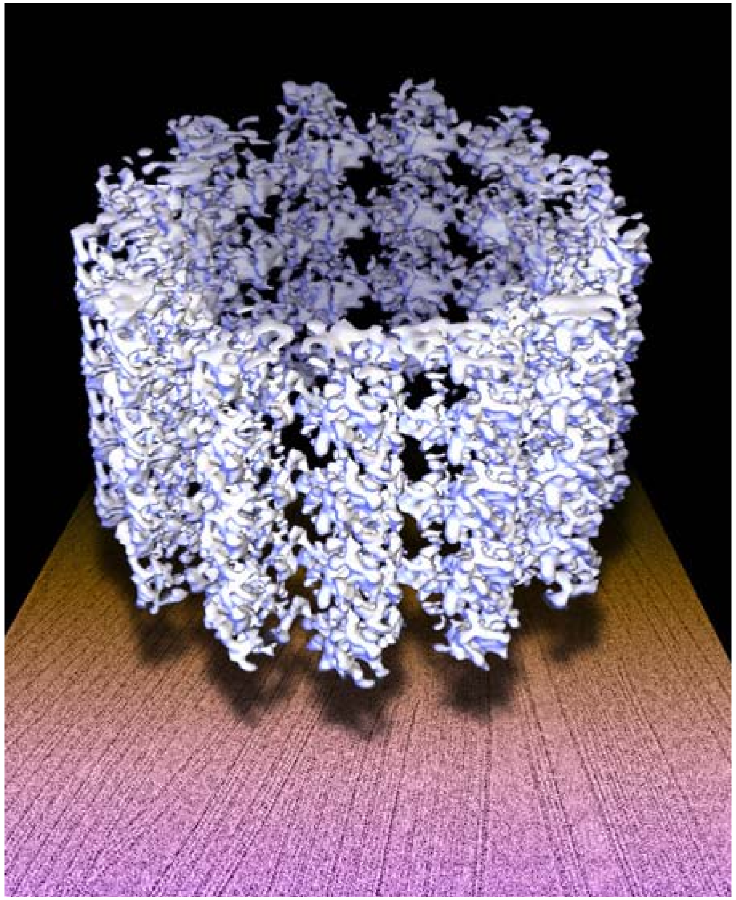
- **Microtubules**, **actin** and **intermediate filaments** (all the same concentrations) were exposed to shear force in a *elastometer* and the resulting degree of stretch was measured

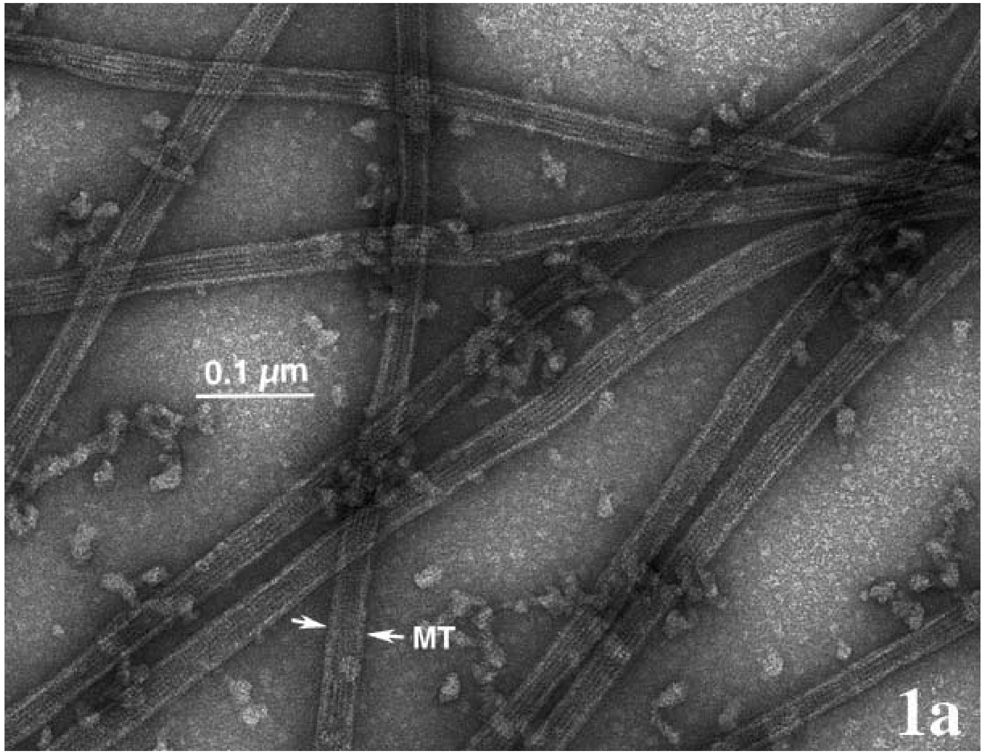
- With increasing deforming force, micro-tubules are the first which cannot resist the strain and start to break following actin

- IFs are the most flexible filaments which resist large deformations

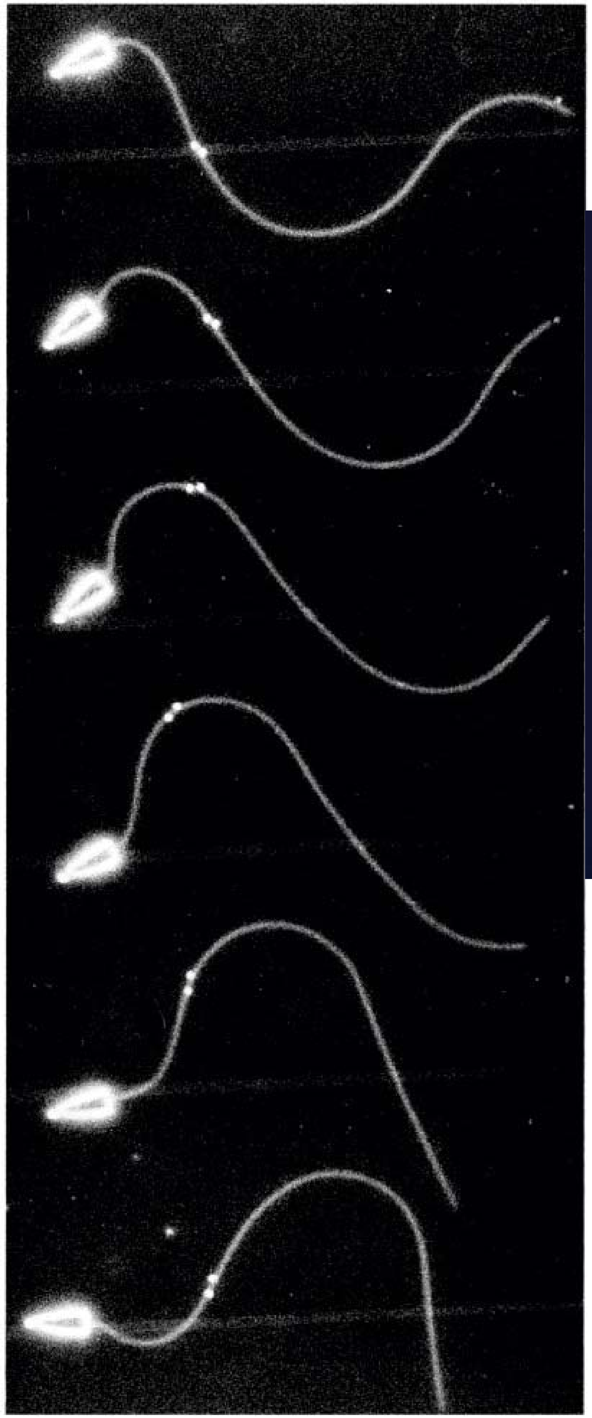
Microtubules





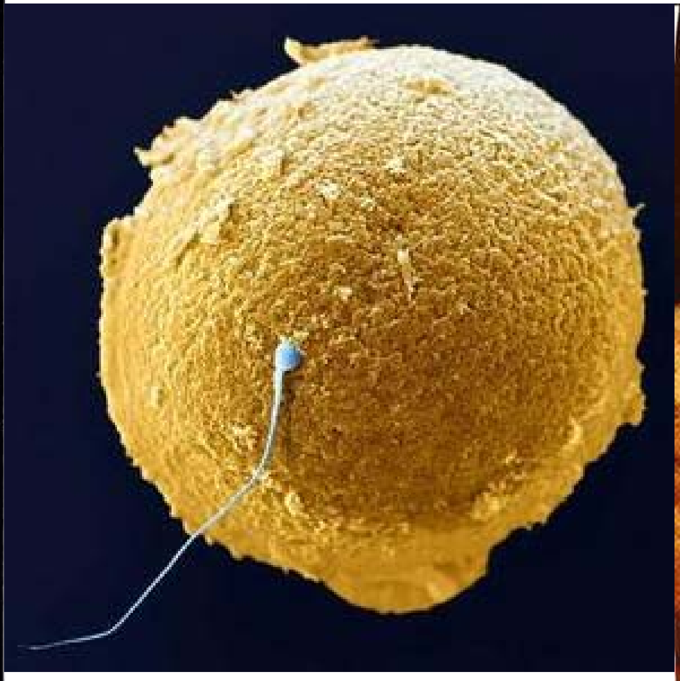


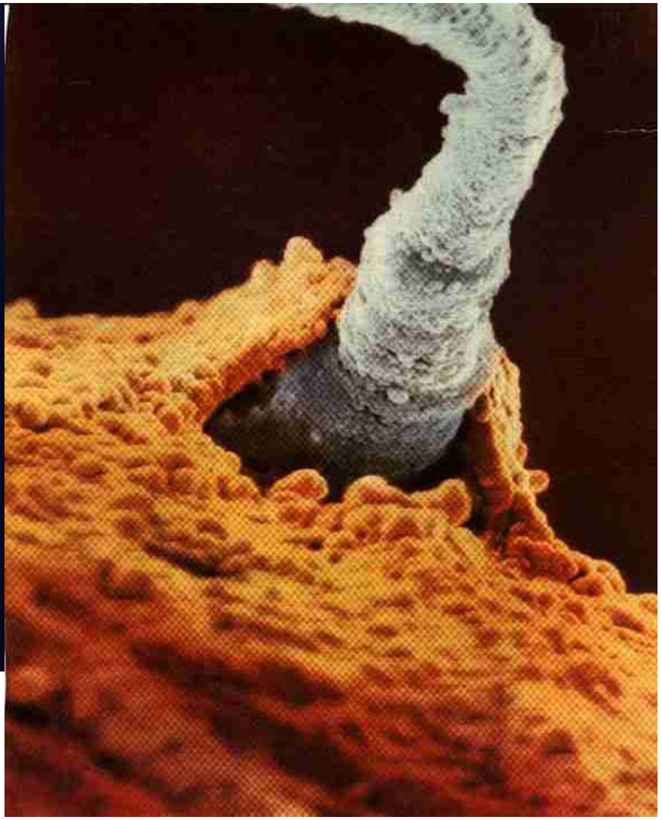
MTs are composed of tubulin dimers
MT diameter = 25 nm



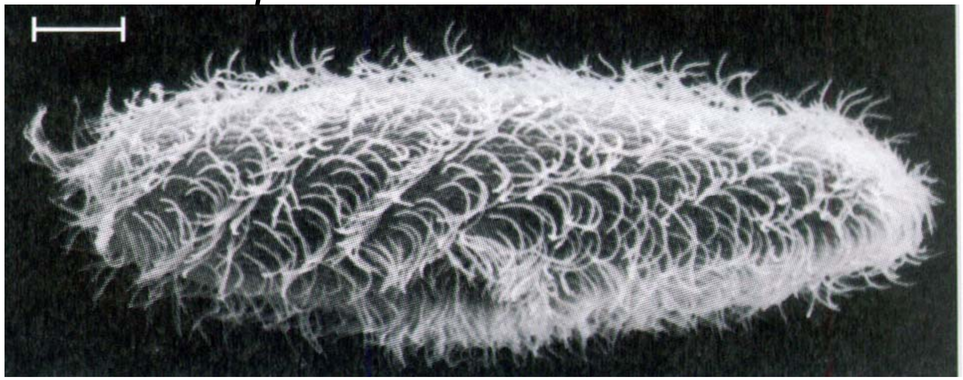
Microtubules form flagella and cilia

The bending sperm flagella pushes against the surrounding fluid propelling the cell forward





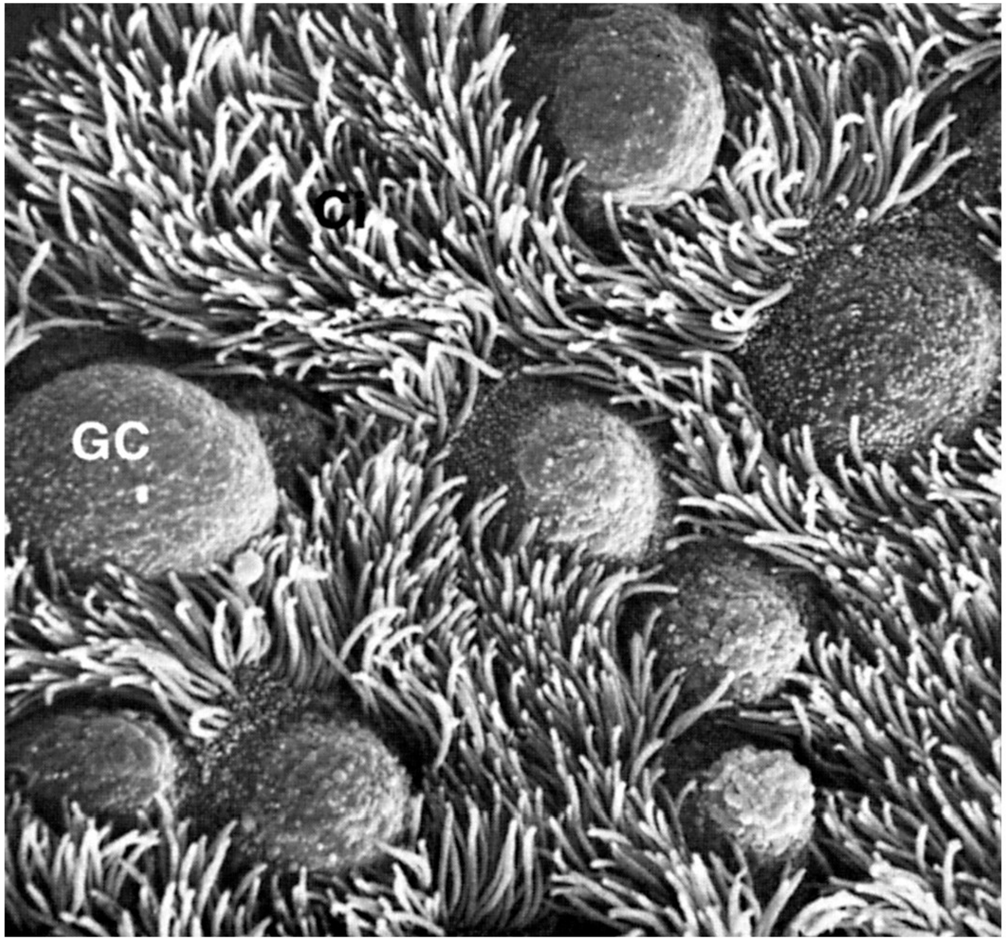
Cilia on a *paramecium*



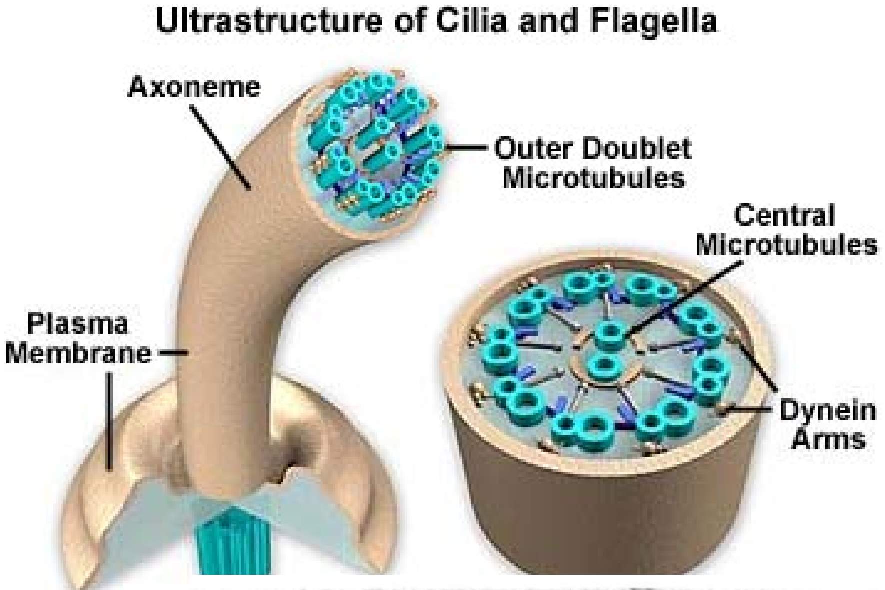
Protozoa are
single cells
that can live
autonomously

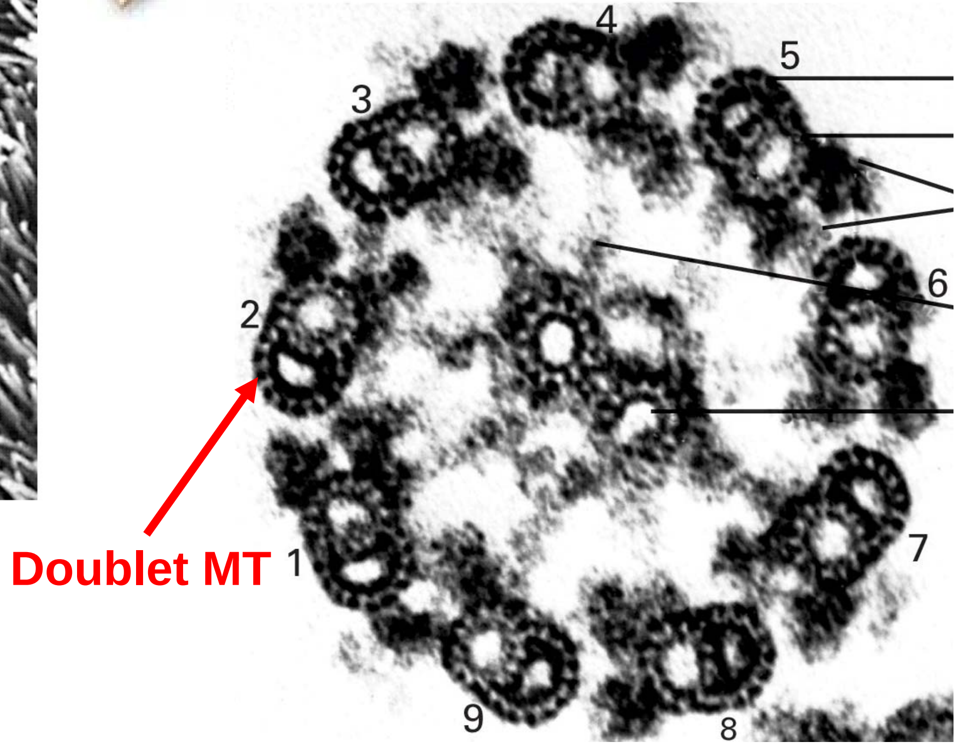
Cilia and flagella are thick bundles of microtubules which move rhythmically

- Flagella enable a **sperm** to swim
- Cilia move **eggs** thru an <u>oviduct</u> (egg canal)



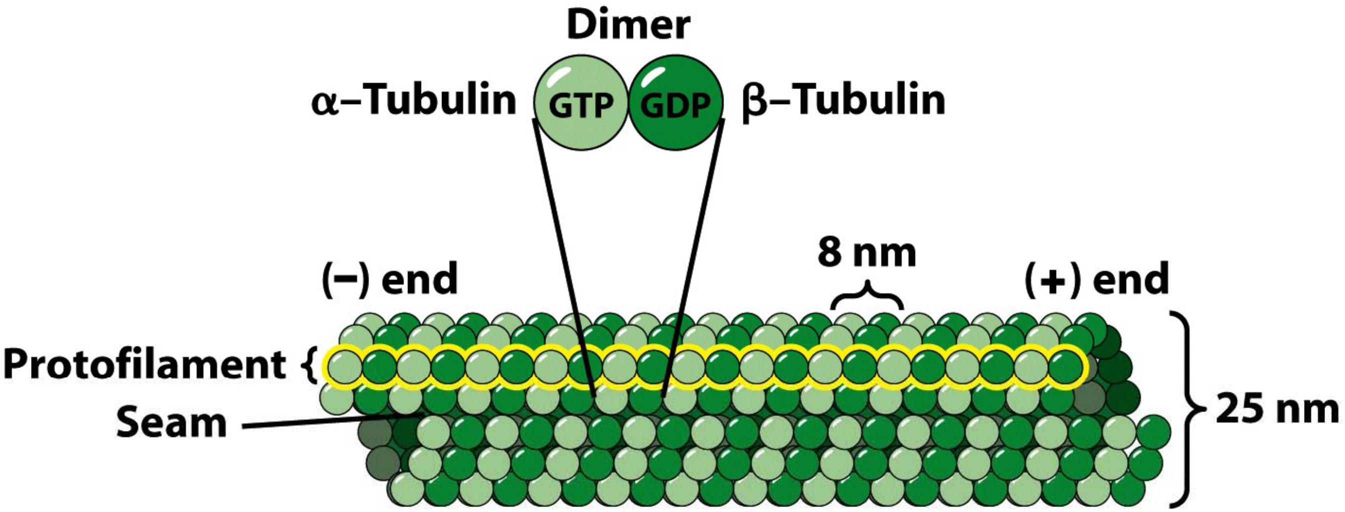
Eggs moved by cilia in an egg-canal

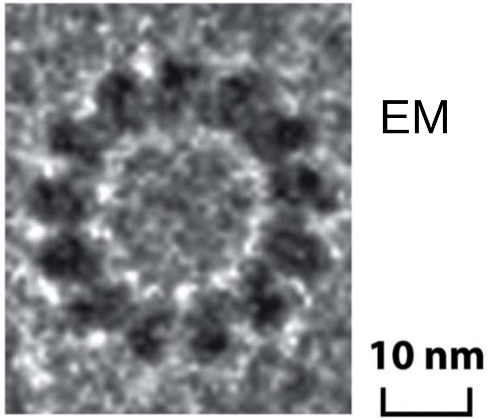


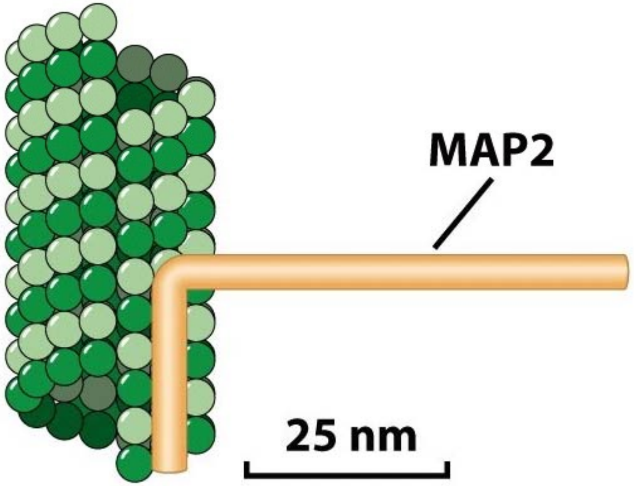


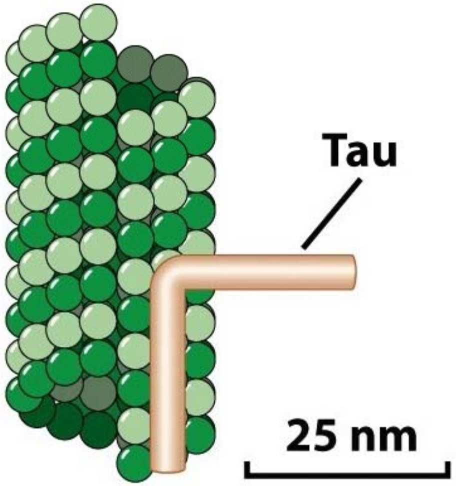
Polymer-Science: The detailed structure of microtubules

- Tubulin dimers polymerize into **protofilaments**
- 13 protofilaments then longitudinally associate to form the **hollow MT cylinder**
- The distance between the subunits is 8 nm





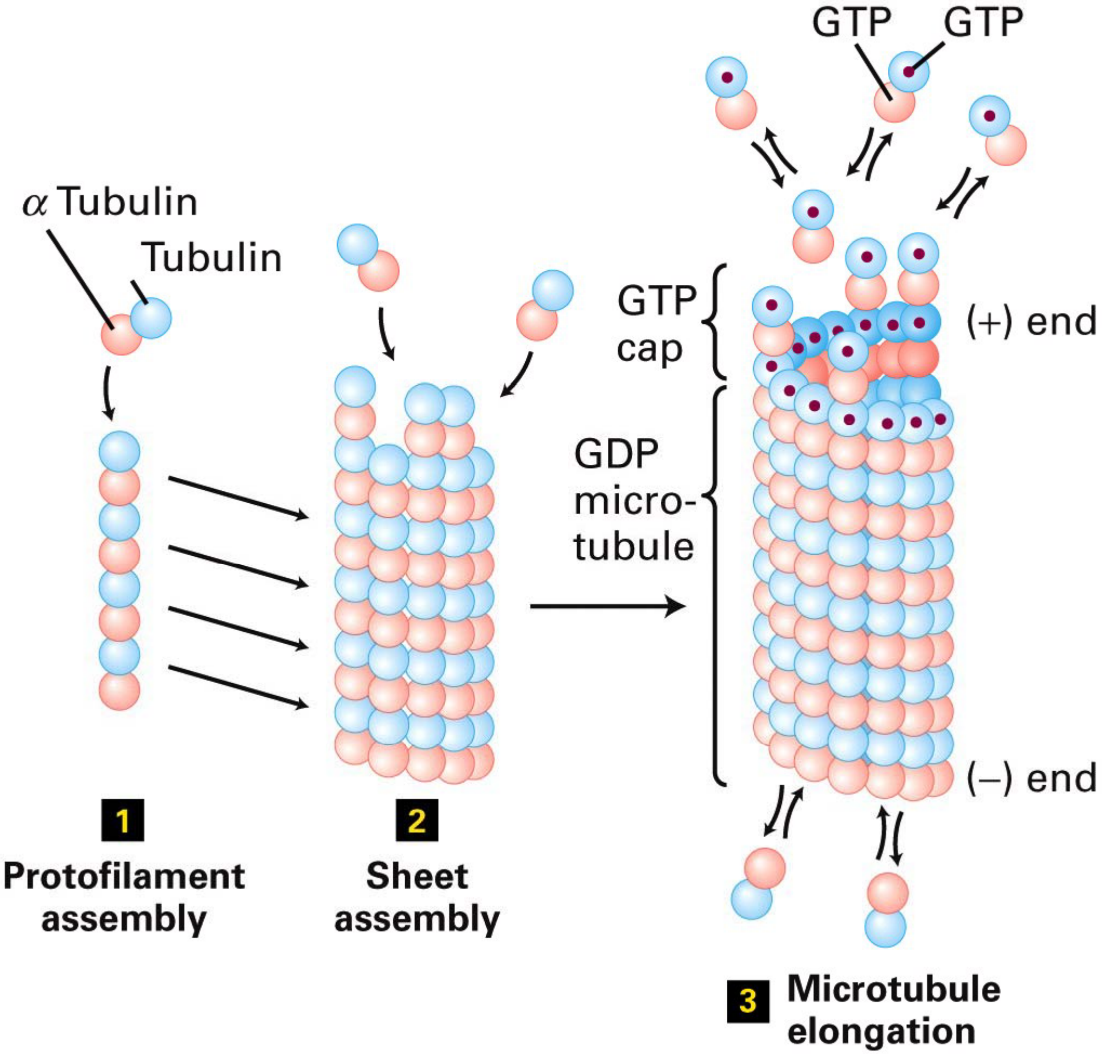




- In the cell, MTs are associated with different filamentous proteins
- Tau plays a role in **Alzheimer’s disease**

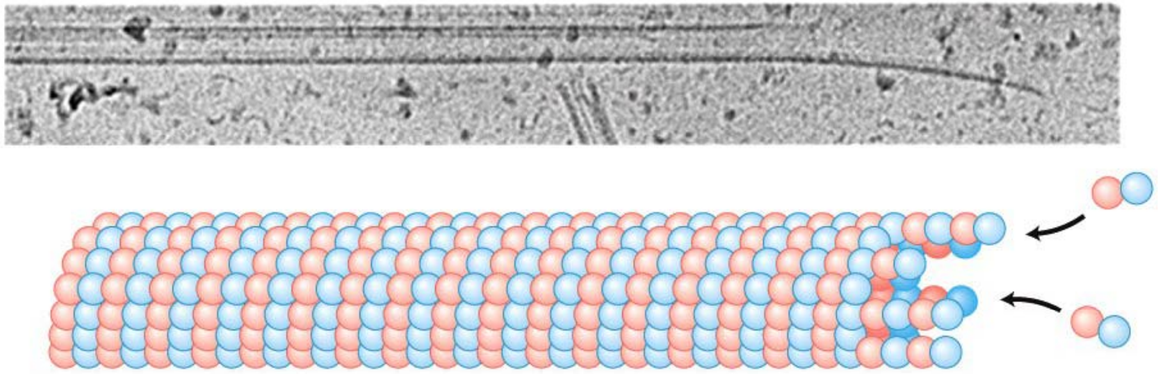
Polymer-Science: The 3 steps of microtubule assembly

1. Tubulin dimers polymerize *longitudinally* into **protofilaments**
2. Protofilaments associate *laterally* into more stable **sheets**
3. Sheet of 13 protofilaments closes to form a hollow MT cylinder

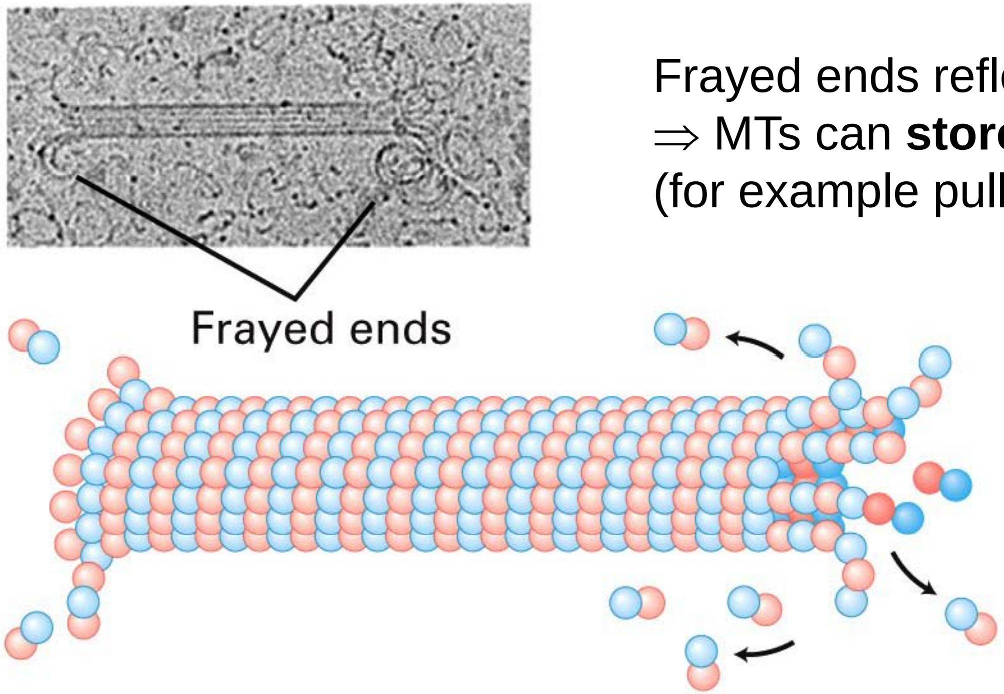


MT ends look different from each other upon polymerization/depolymerization

(a) Assembly (elongation)



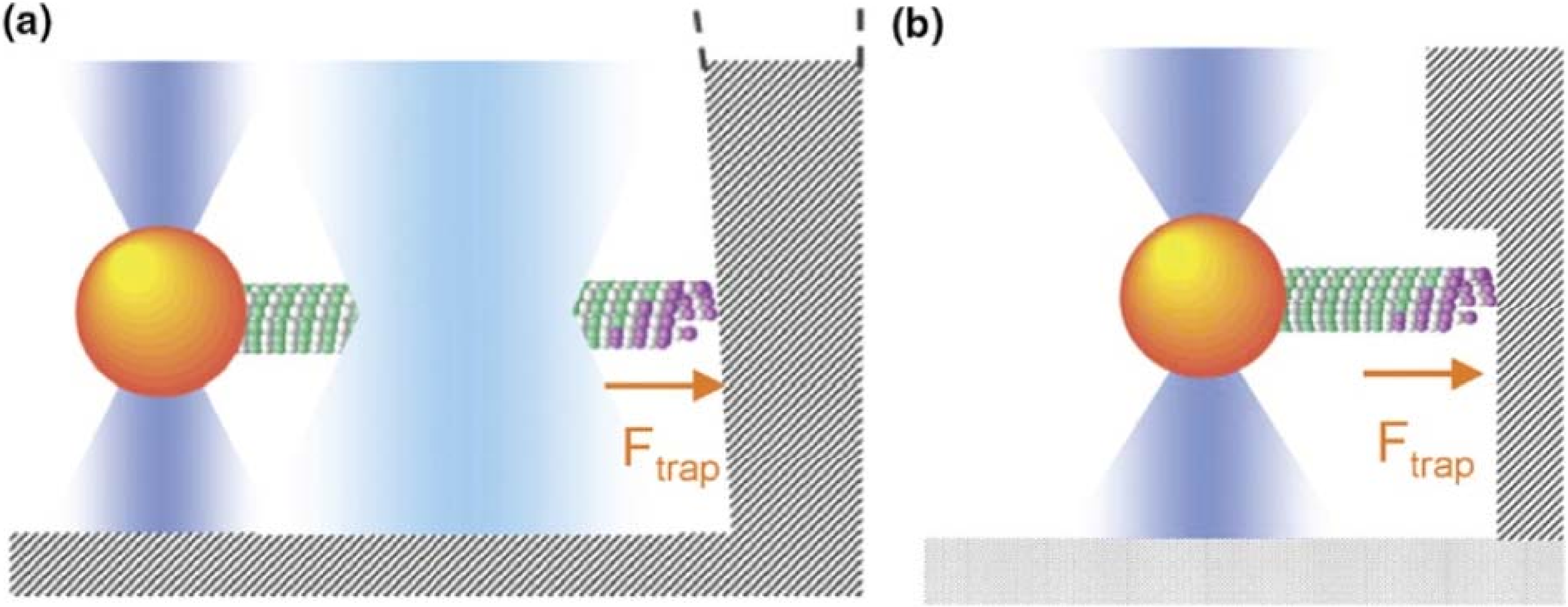
(b) Disassembly (shrinkage)



Frayed ends reflect the strong tension within the MT:
⇒ MTs can **store torsional energy** to work
(for example pulling on chromosomes)

Engineers meet Biologists: Measuring "nanoscale" microtubule dynamics

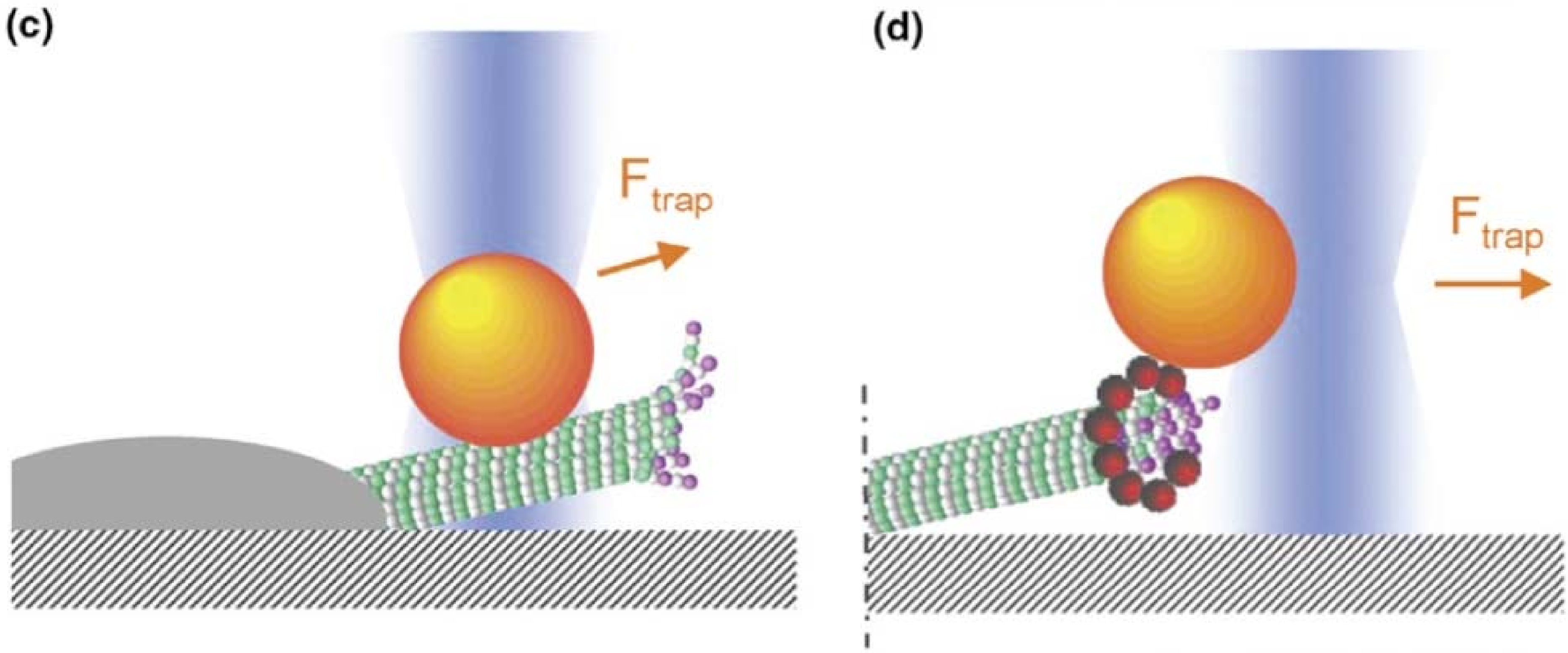
- MT-attached bead is centered via an **optical trap** (highly focused infrared laser beam) keeping the plus-end in contact with a **microfabricated barrier**
- The light blue trap serves to orient the MT perpendicular to the barrier wall
- Deflection of the bead reflects protofilament length fluctuations at the MT plus-end
- A different setup similarly measures MT plus-end fluctuations via bead deflections
- Here the MT is held in position by the **microfabricated structure**



Gardner et al., 2008, Curr. Opin. Cell Biol.

Engineers meet Biologists: Measuring “nanoscale” microtubule dynamics

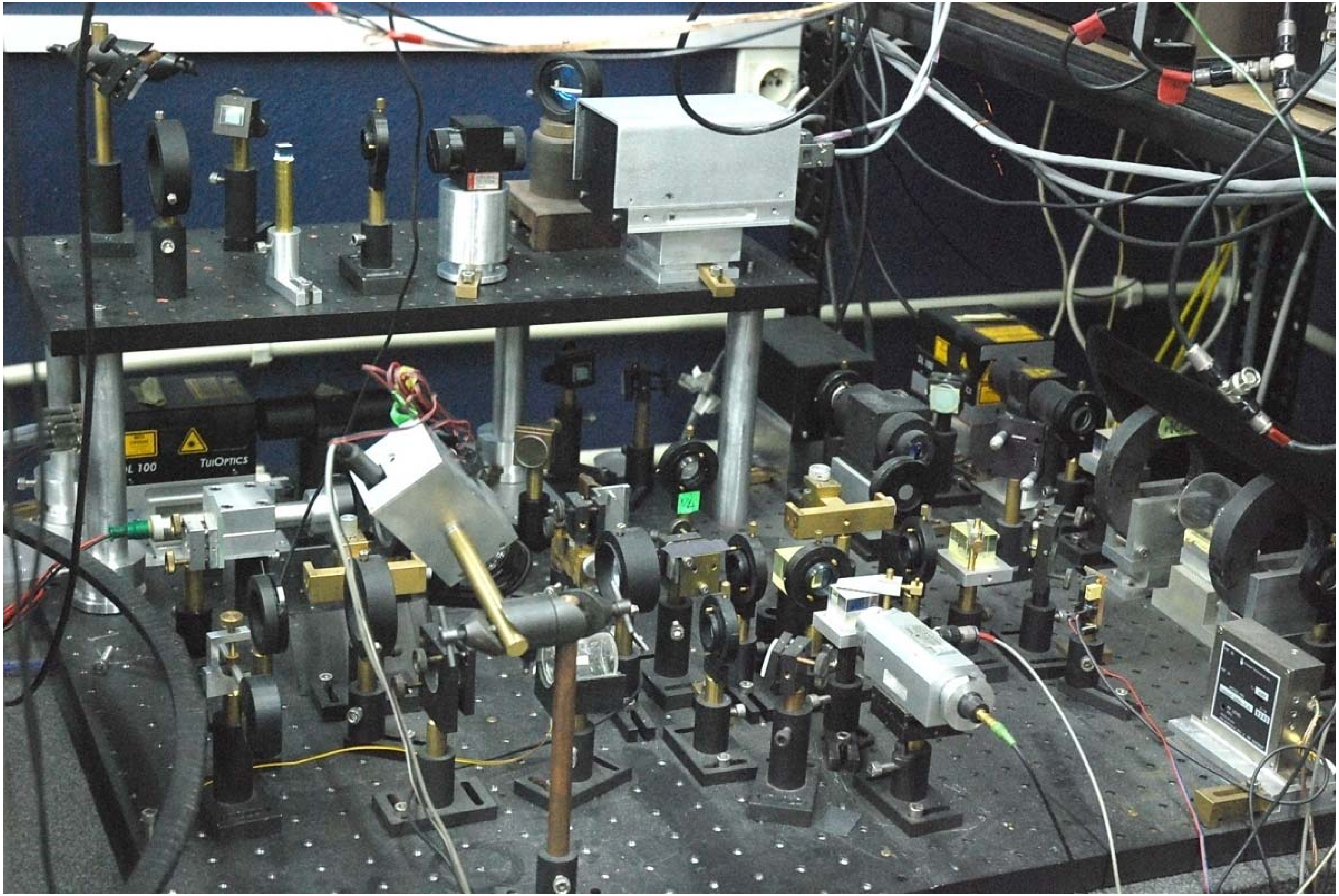
- Bead attached to the **microtubule lattice**
- As MT depolymerizes bead deflection is measured (arrow: resisting force of bead)
- Bead linked to the plus-end via a **specific MT-binding protein**
- During MT fluctuations, the bead is pulled away while bead deflection is measured



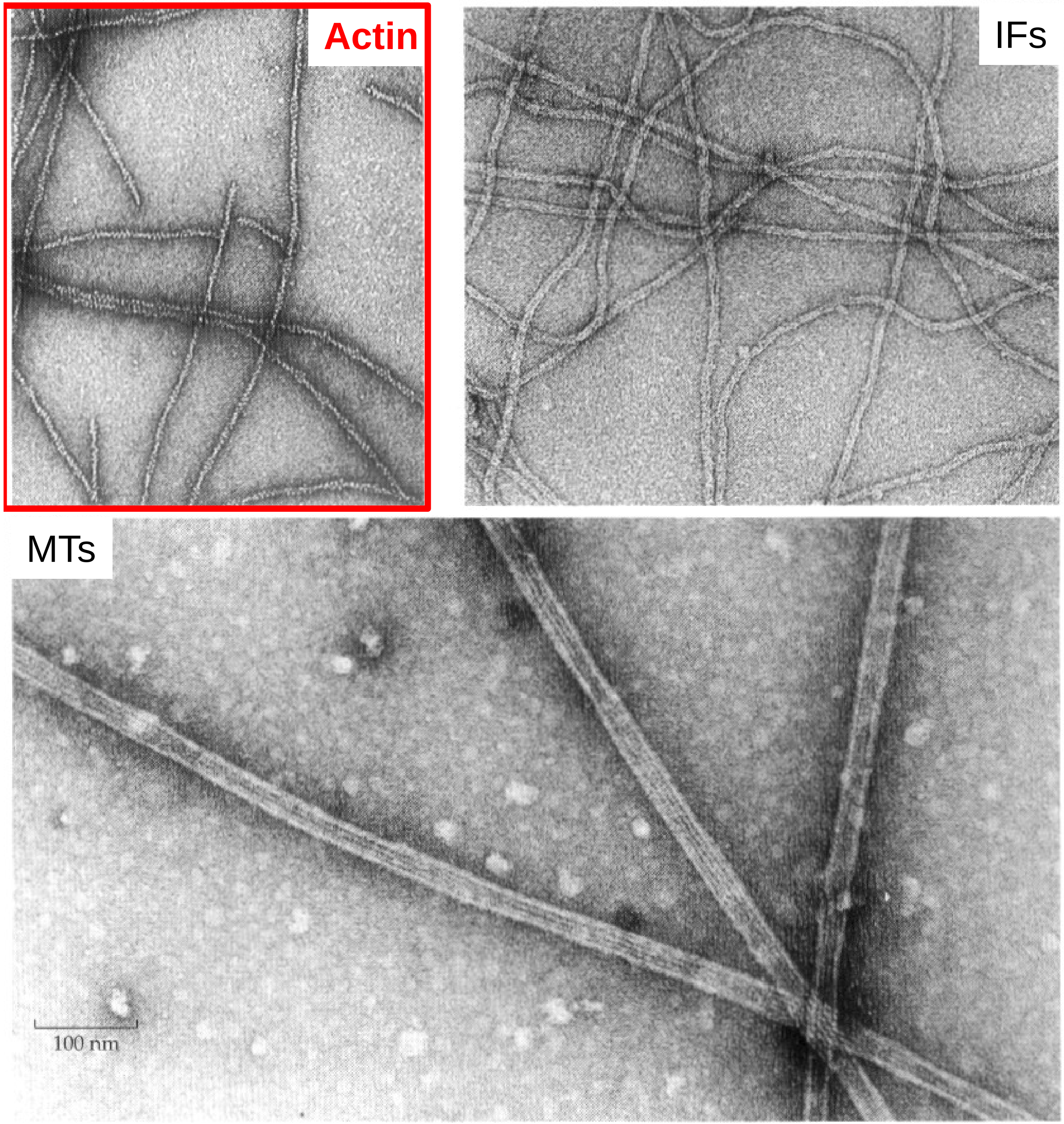
Gardner et al., 2008, Curr. Opin. Cell Biol.

Optical trap setups are complicated and tricky

Optical trap setups are complicated and tricky



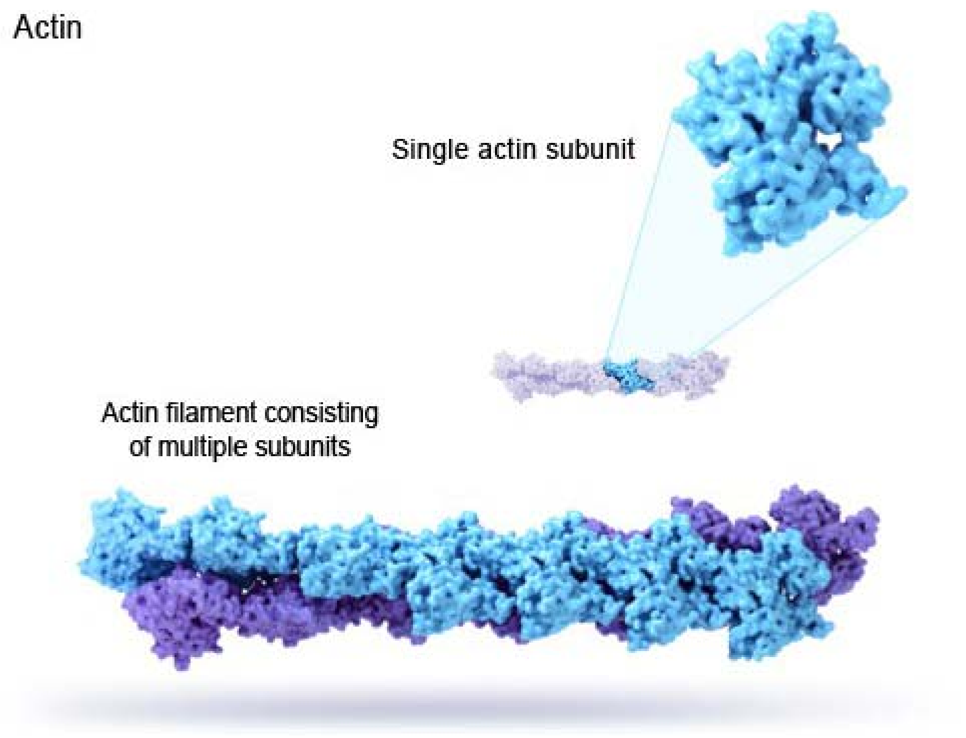
Cytoskeletal elements drawn to scale

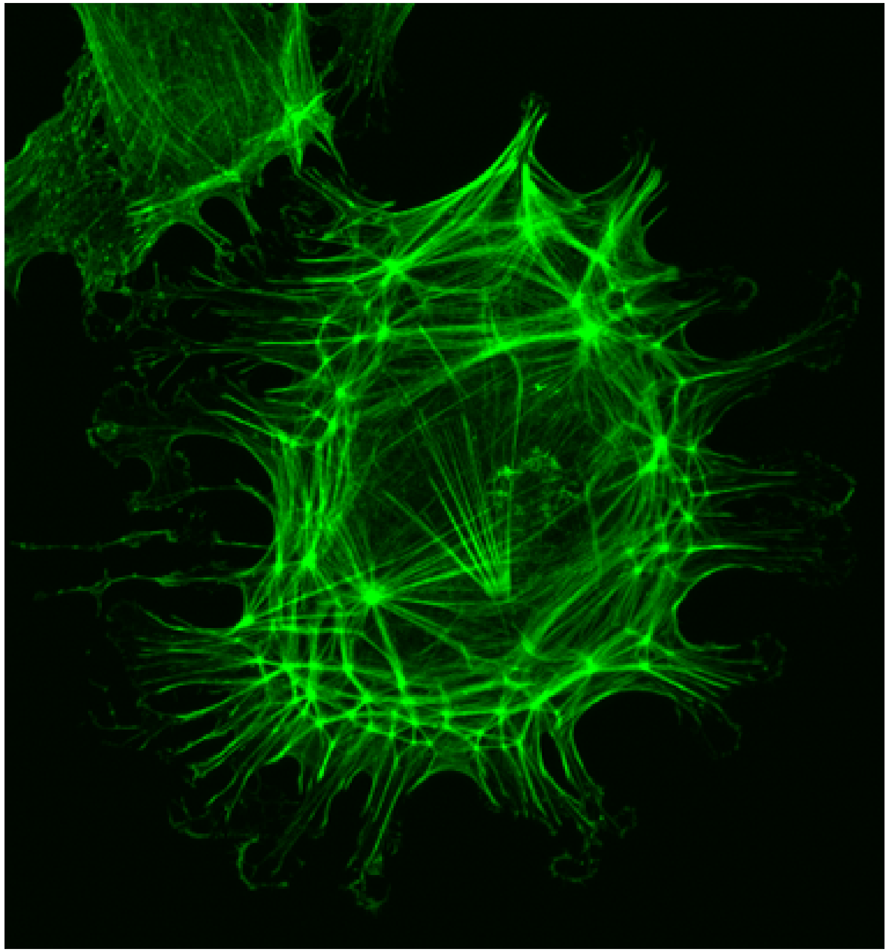


Howard, Mechanics of Motor Proteins, 1st Ed.

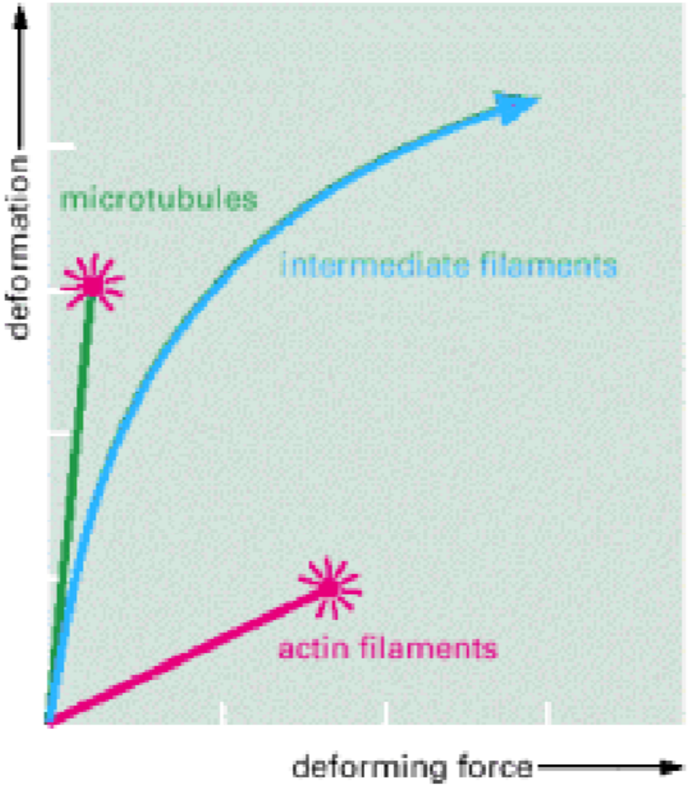
Actin polymers are important for cell movements

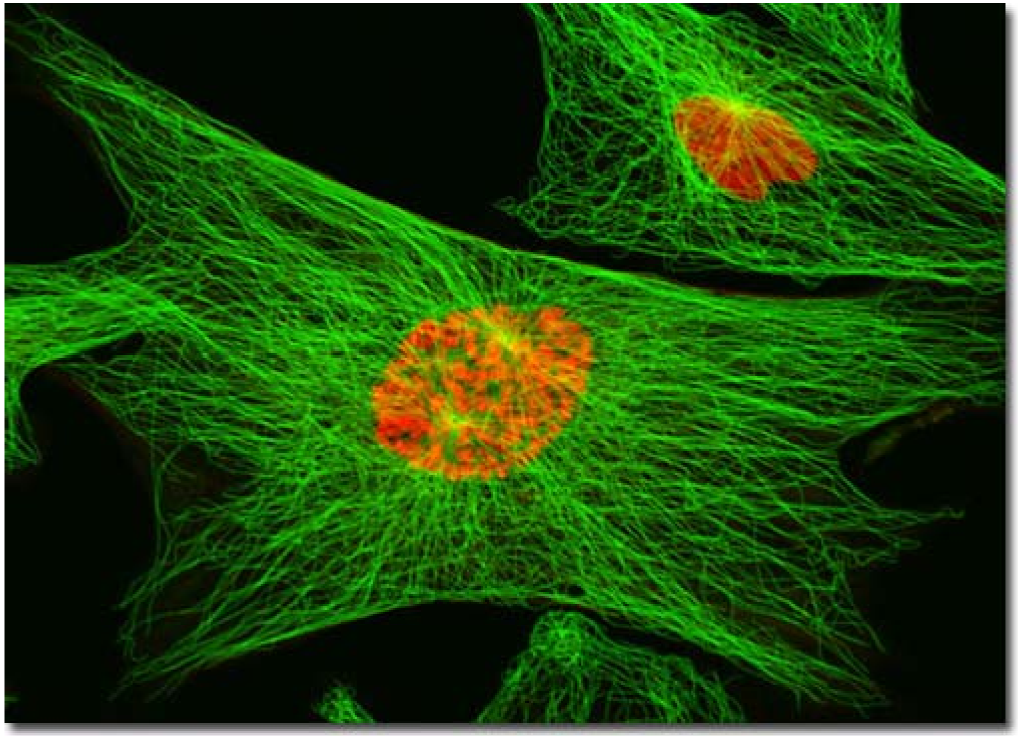
- **Highway for** molecular motors type **myosins**
- Stabilizing cells and fixing them to the substrate
- Polymerization and depolymerization **drives cells forward** (cell motility)





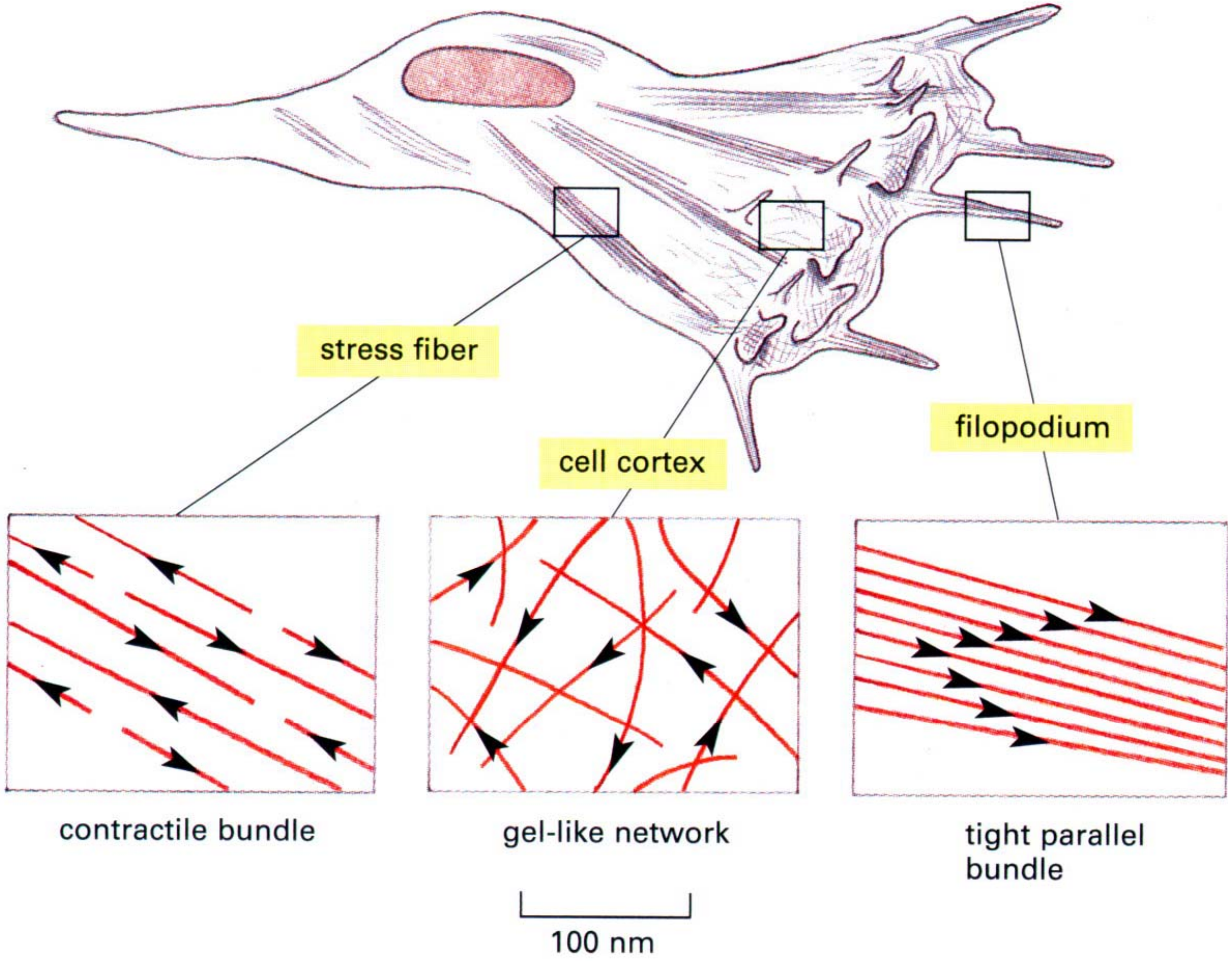
Actin filaments are more resistant to deformation than MTs

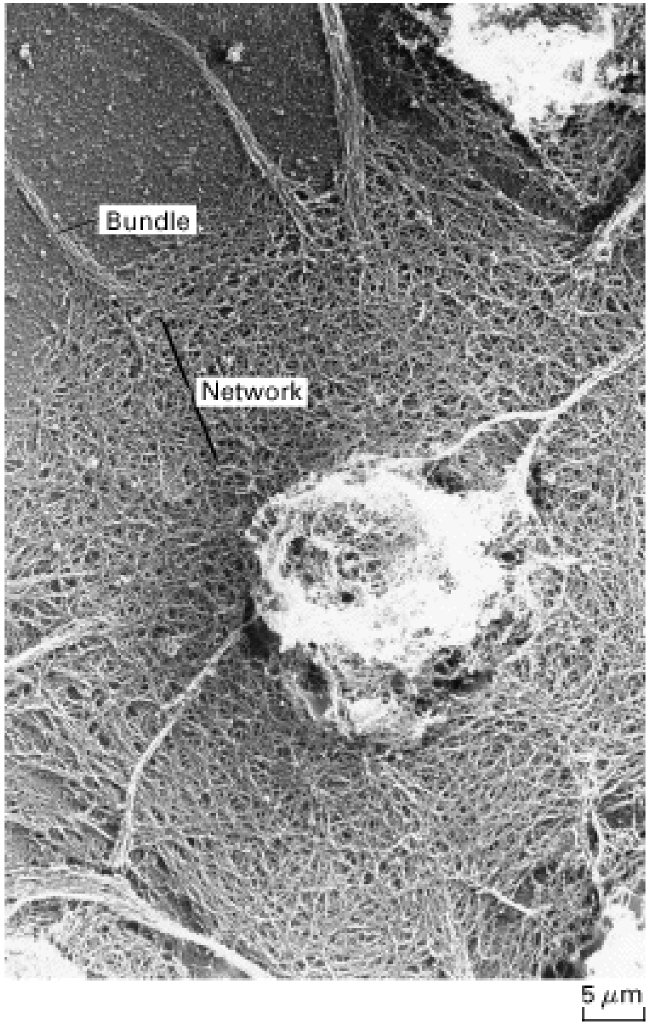




P. Janmey, *JCB*, 1991

Cellular actin organization





Stress fibers: provide cell-strength and some can contract

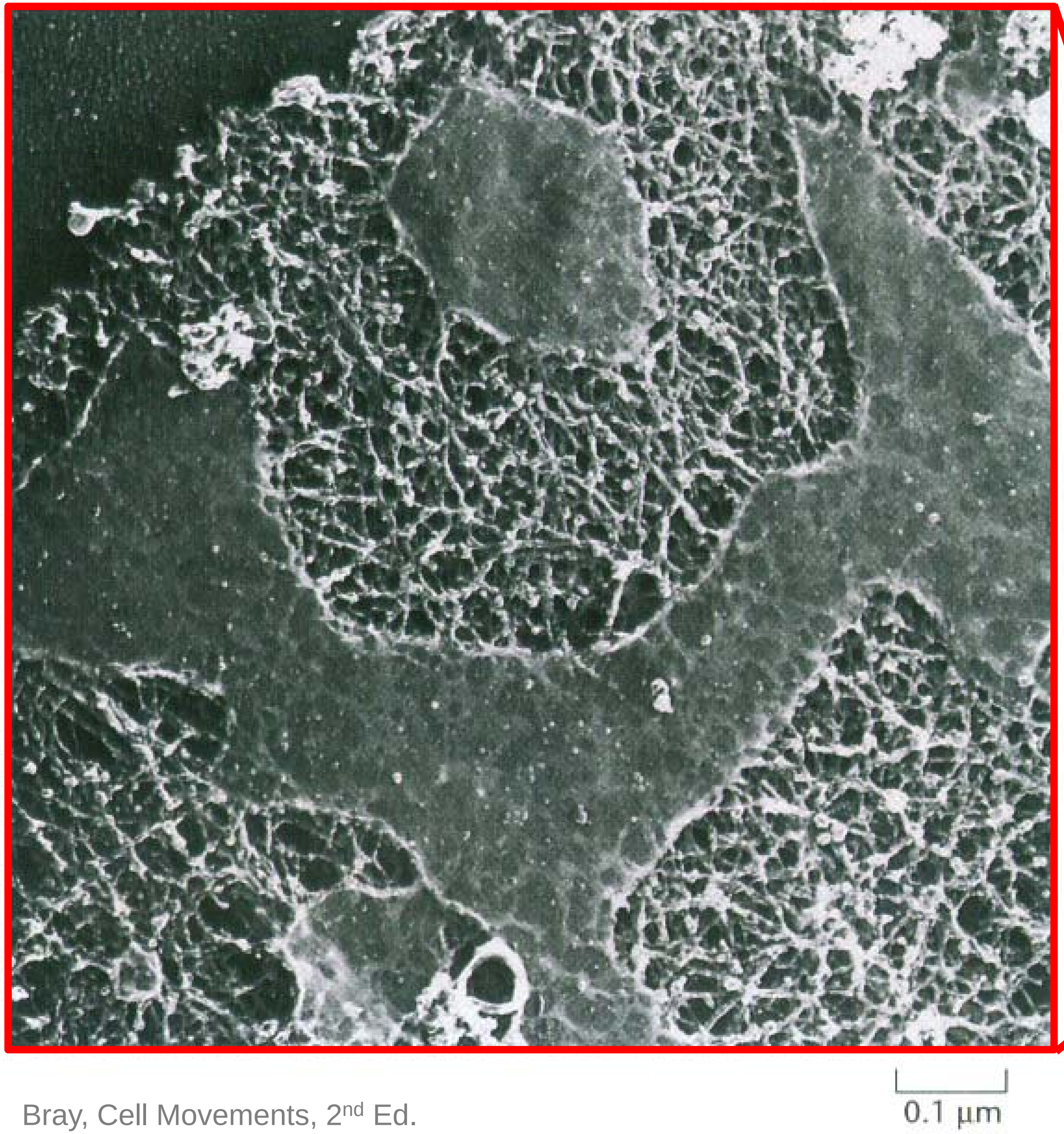
Cell cortex: fast-acting gel-sol transition

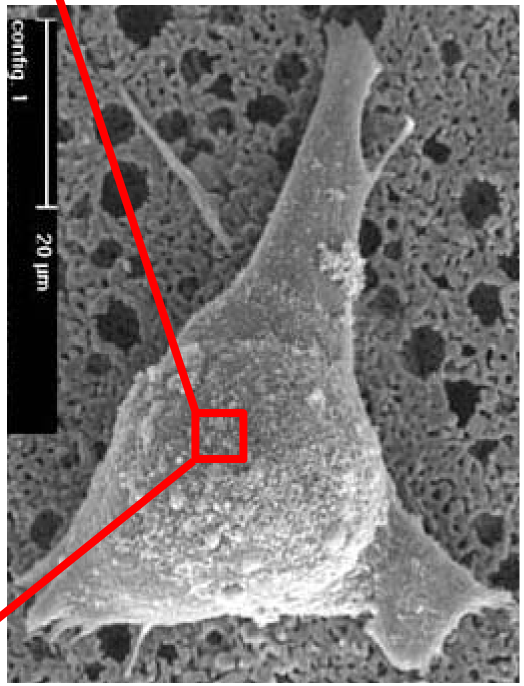
Filopodia: sensing the environment

Alberts, 4th ed.

Cell cortex: gel-like and highly elastic

- Fibroblast treated with **mild detergent** to partial remove plasma membrane.
- <u>Fast-freeze</u>, <u>deep-etch electron microscopy</u> reveals actin-network

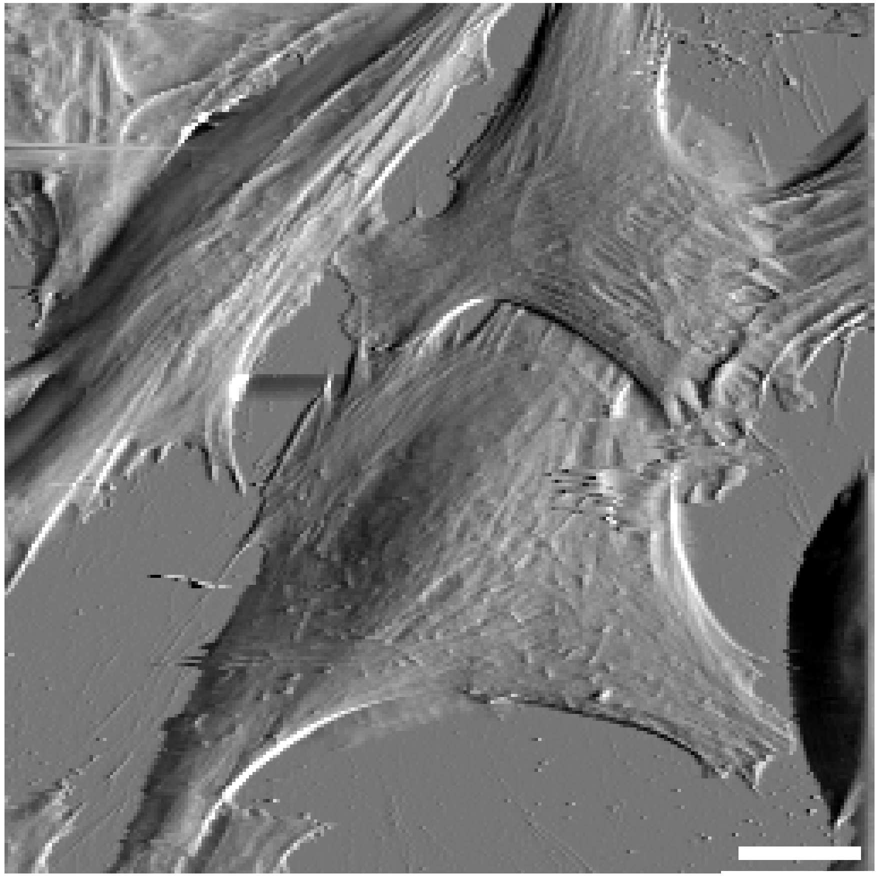


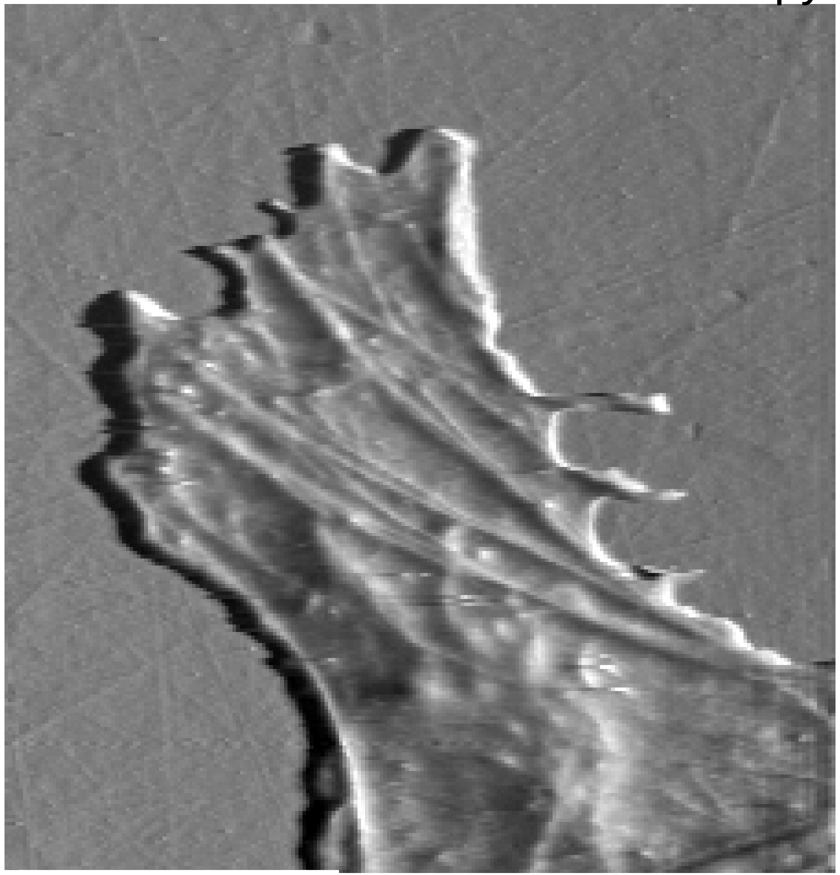


Bray, Cell Movements, 2nd Ed.

Actin-bundles in fibroblasts made visible by AFM

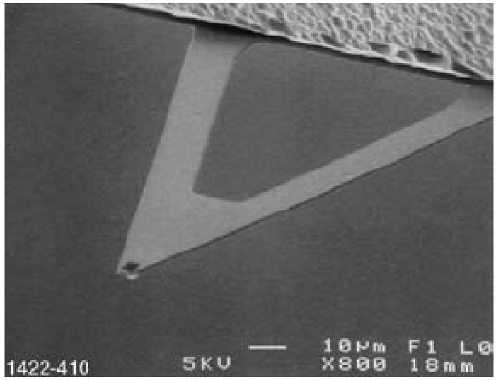
Atomic Force Microscopy

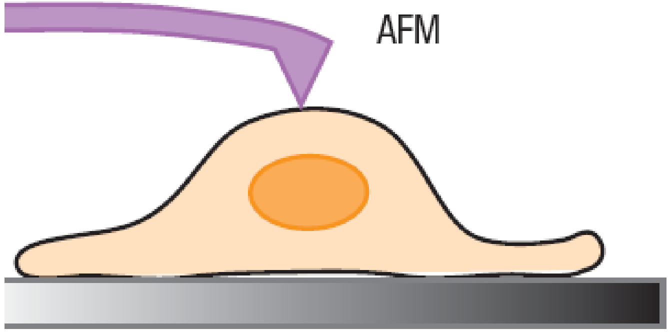




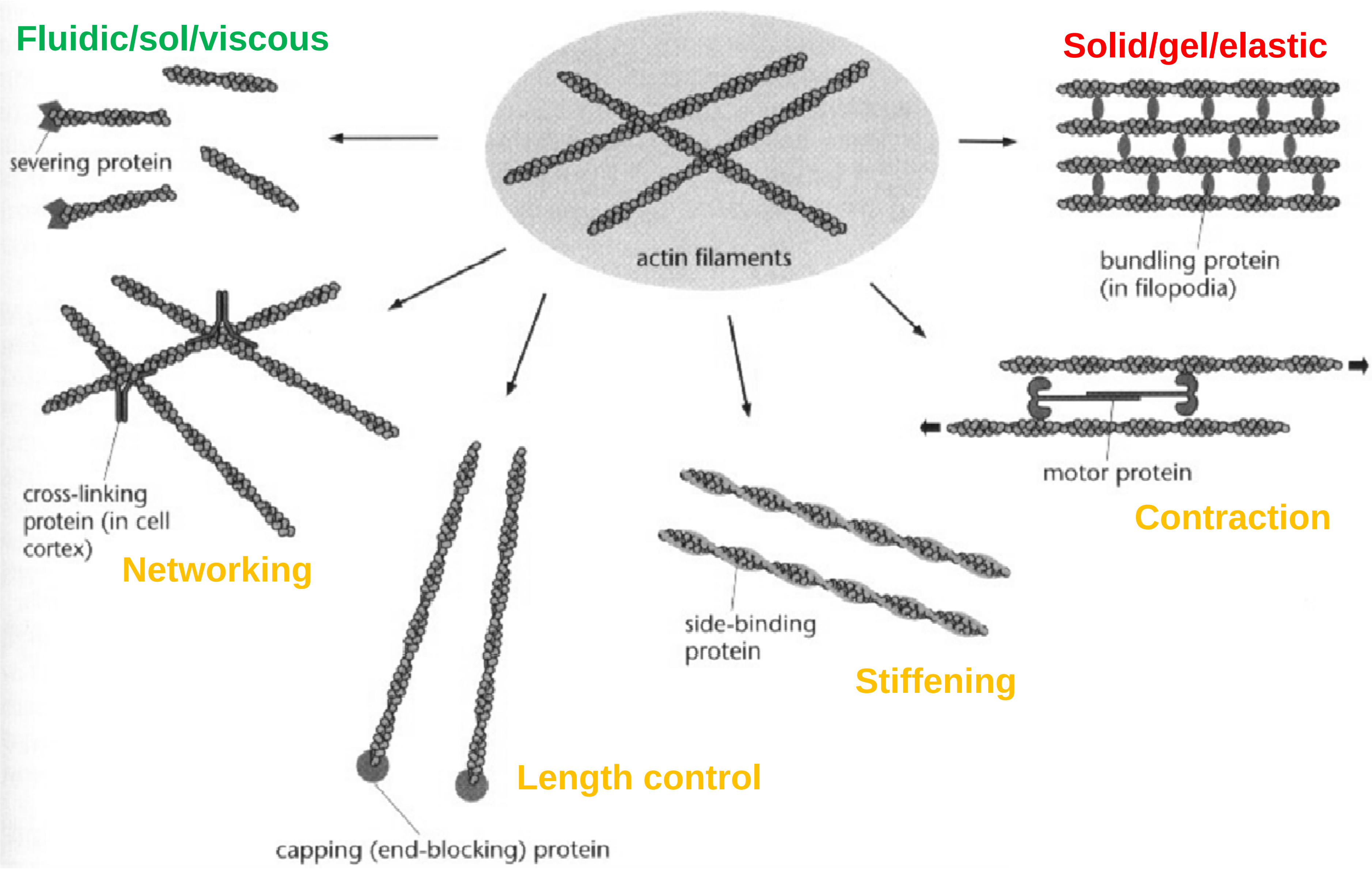
Parak et al., *Biophys. J.*, 1999

Tip radius: 20-60 nm



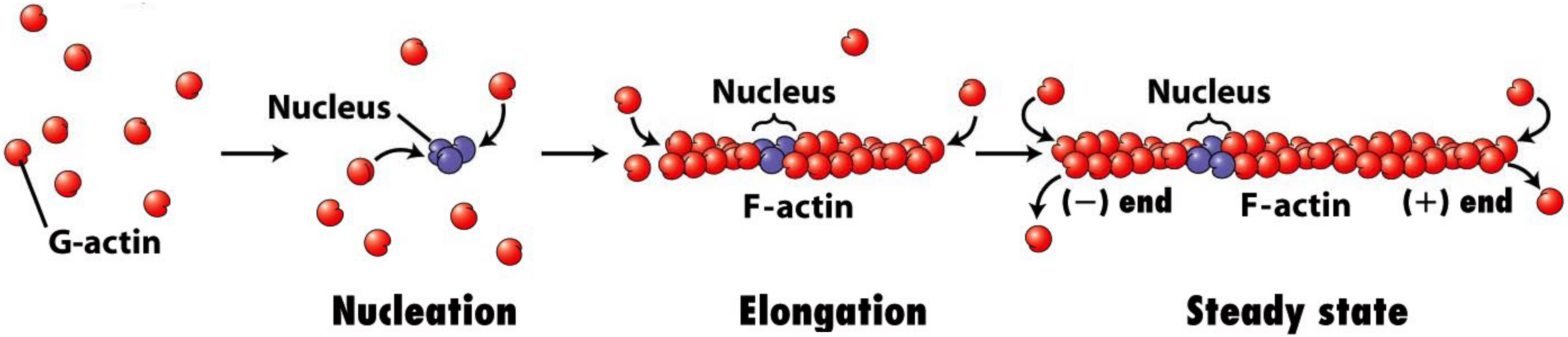


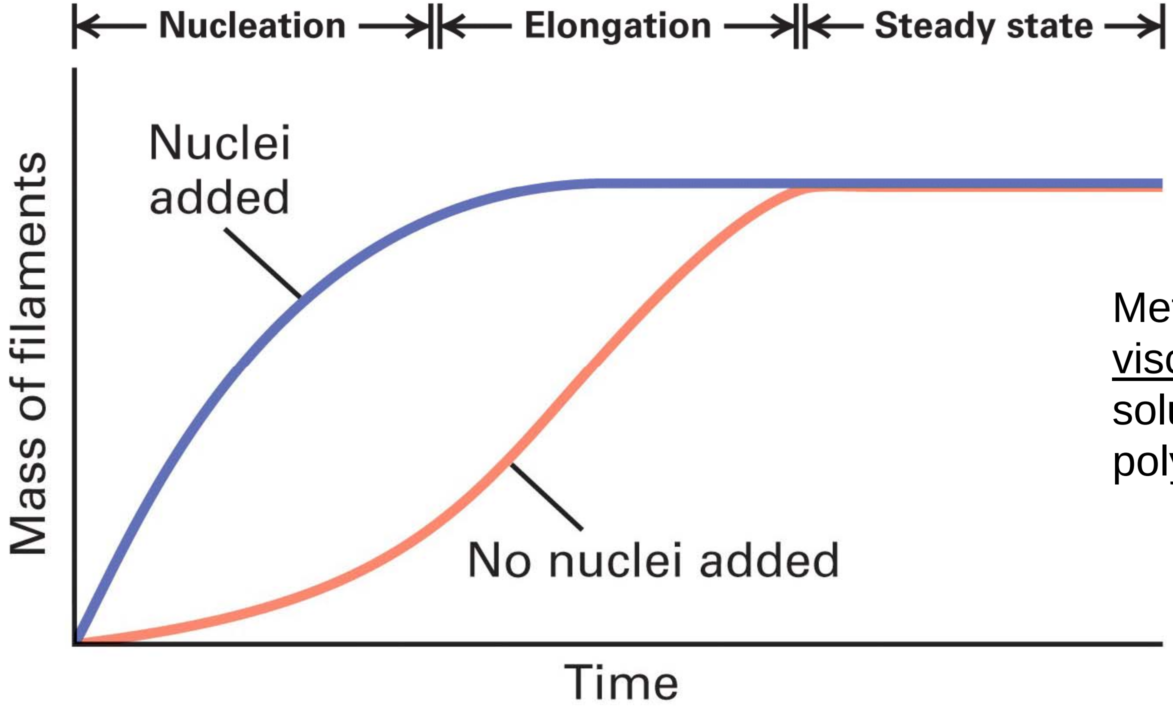
Polymer-Science: Cellular control of different actin arrangements



Polymerization of actin proceeds in three steps

1) **Nucleation phase**: G-actin **slowly** aggregates into short oligomers (nuclei/seeds)
2) **Elongation phase**: To both ends of the seed, G-actin monomers **rapidly** added
3) **Steady-state phase**: **Equilibrium** is reached between filaments and monomers





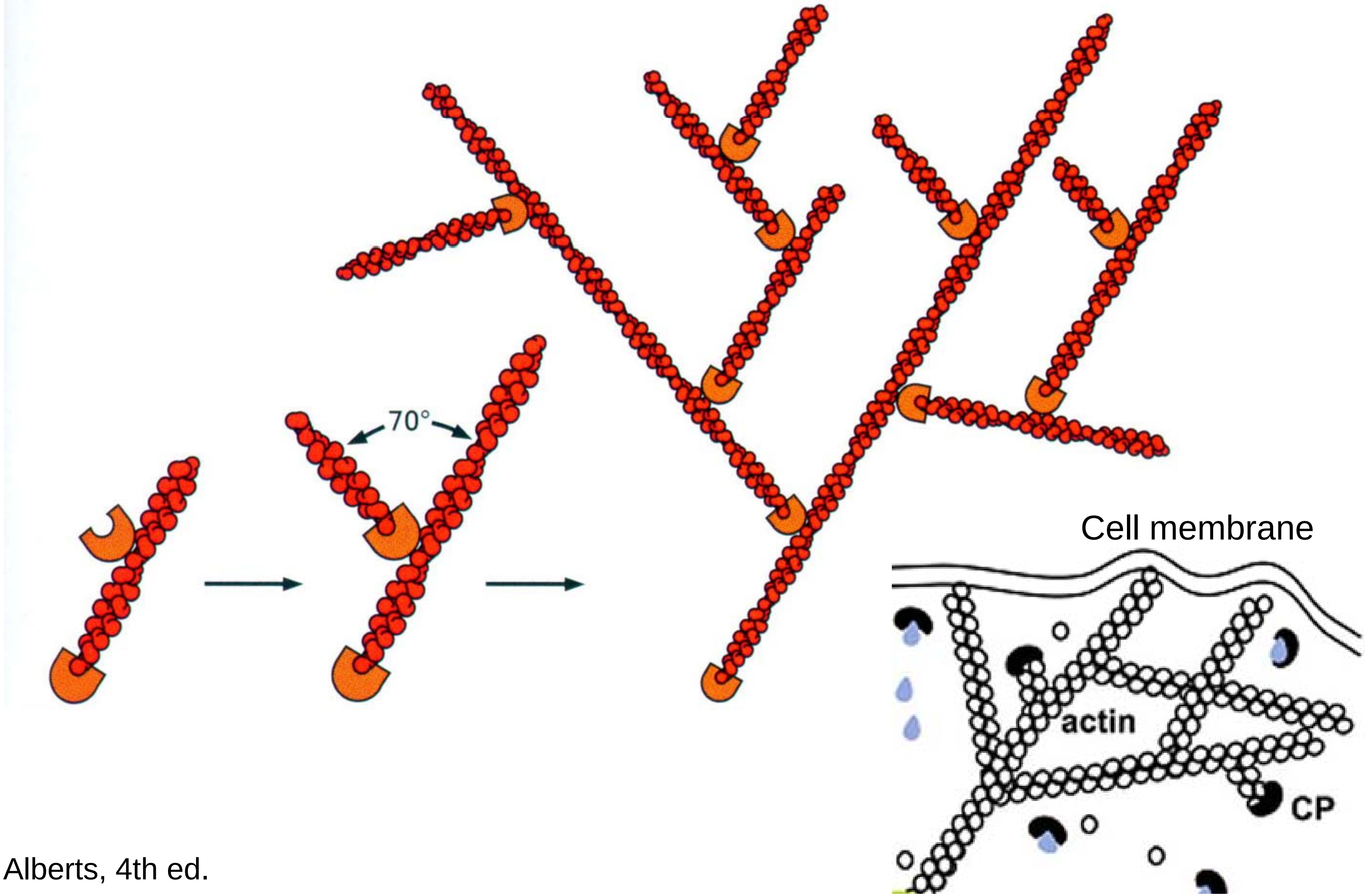
Method: Measuring viscosity increase of solution during polymerization

Animation

a19-02-actin_polymerization.swf

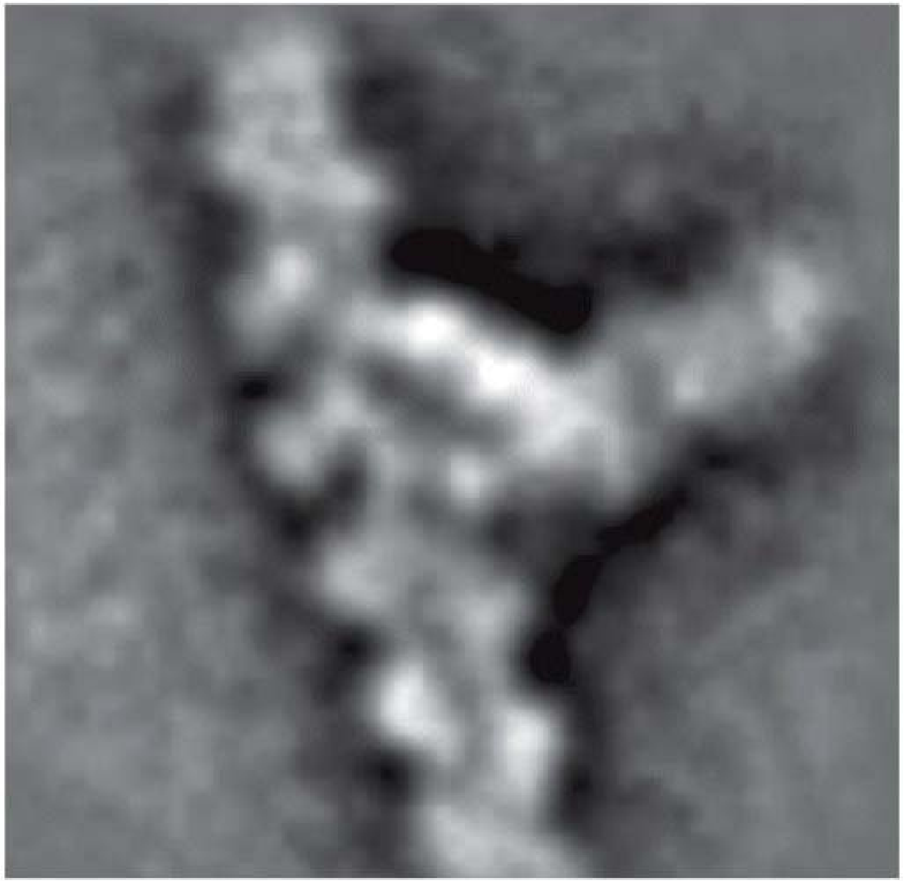
Arp2/3 is a protein that branches actin filaments

Arp 2/3 **binds to the side** of an actin filament and **branches** them at an angle of 70°

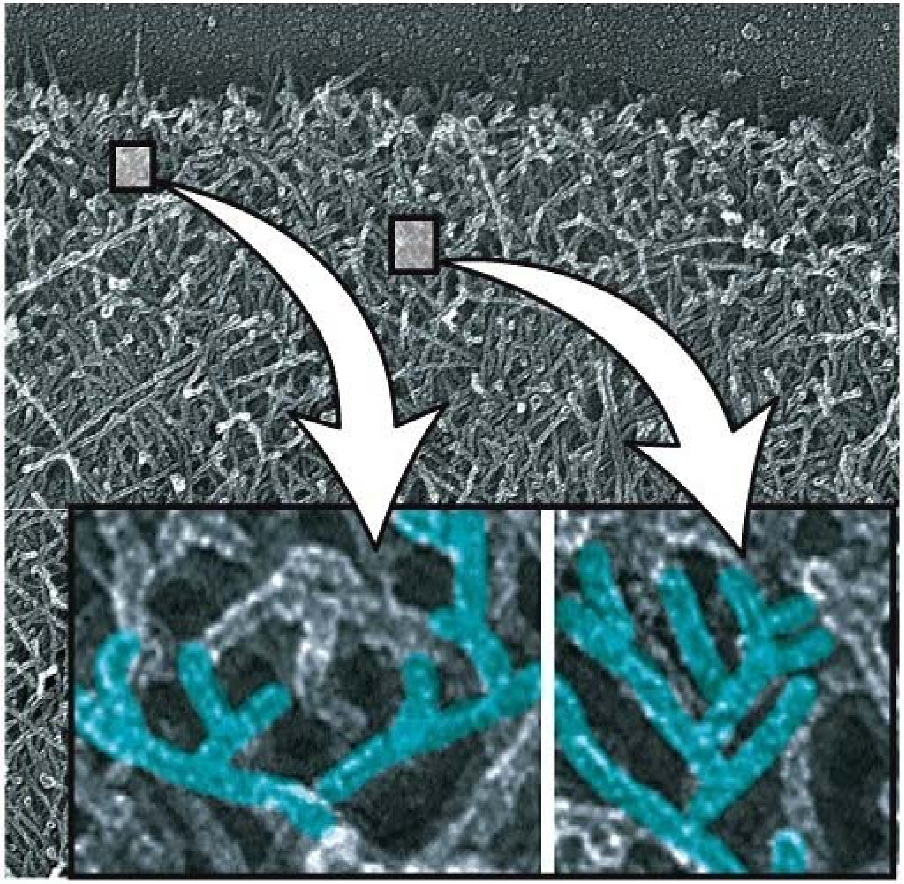


Alberts, 4th ed.

Arp2/3 is a protein that branches actin filaments



High magnification EM of actin branched by Arp2/3



Actin branches at the cell cortex (border)

Actin filament elongation visualized by fluorescence microscopy

Movie: polymerization of G-actin

v19-02-actin_filaments_a.mov

Movie: polymerization of G-actin + Arp2/3

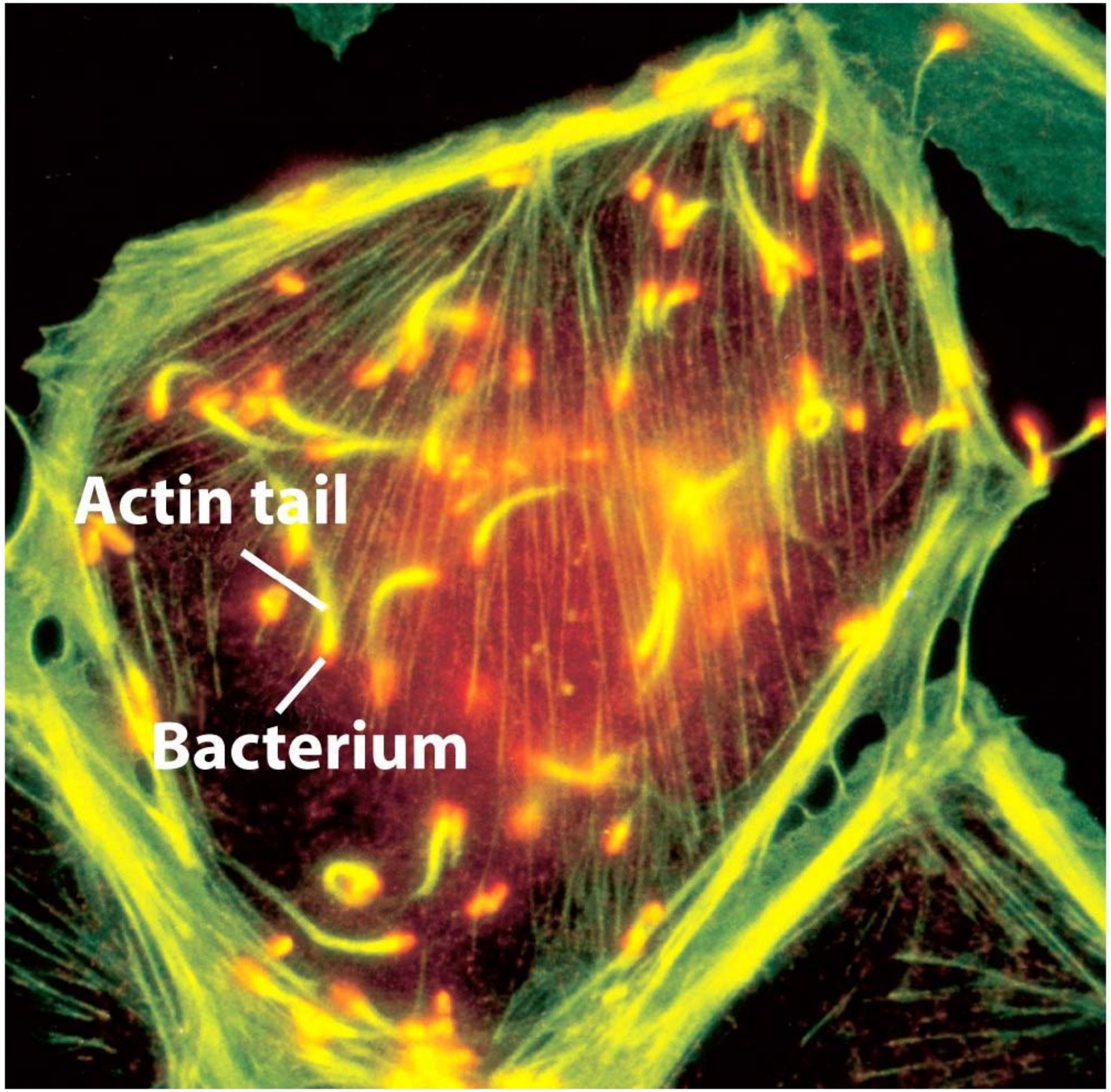
v19-02-actin_filaments_c.mov

Movie: polymerization of G-actin + Arp2/3 (magnification)

v19-02-actin_filaments_b.mov

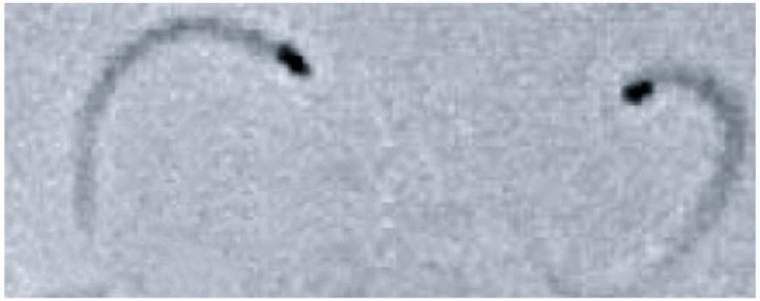
Arp2/3 is needed for *Listeria* movement in infected cells

- *Listeria monocytogenes* is a bacterium which propels thru the cytoplasm using the **power of actin polymerization** stimulated by Arp2/3
- Actin polymerizes into filaments at the base of the bacterium pushing it forward

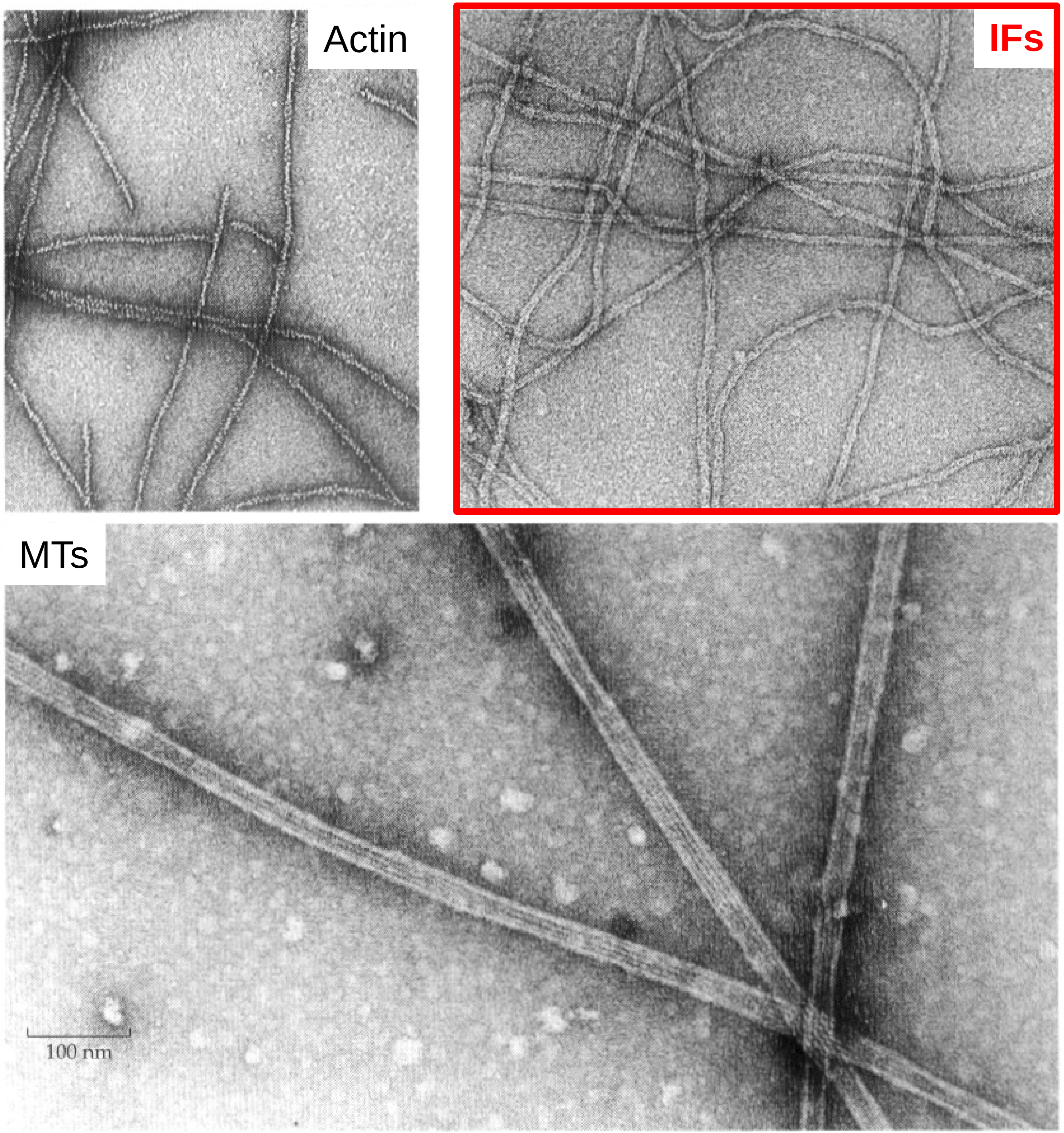


"Actin rocket tails" might also push endosomes thru the cytoplasm

Movie
25_2.mov



Electron micrograph of listeria

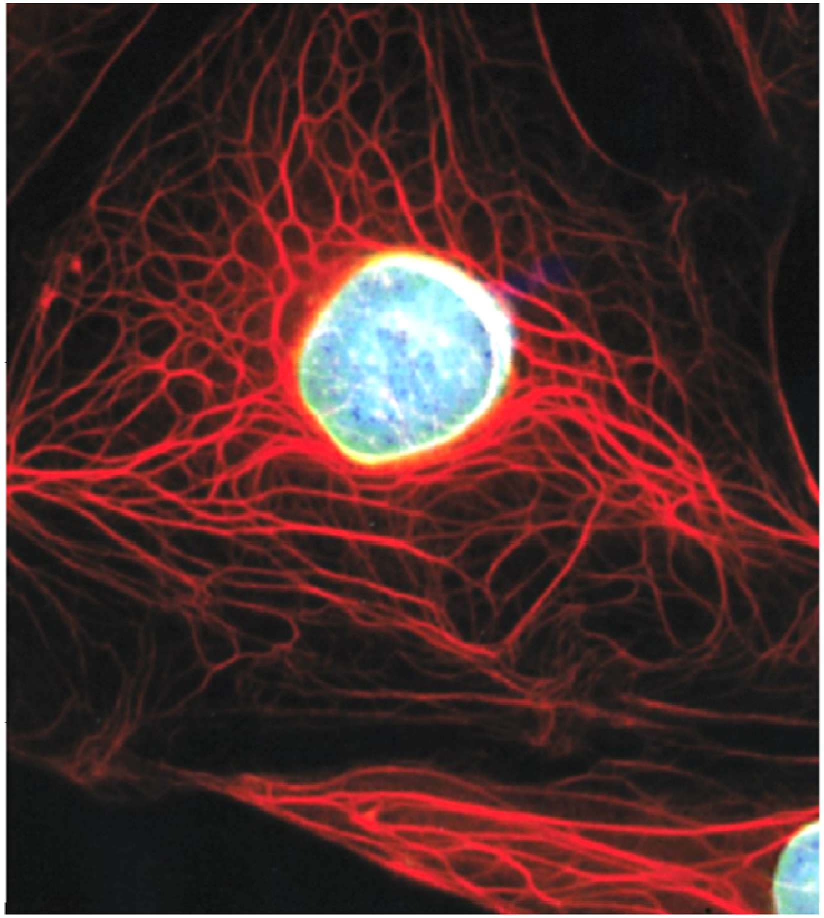


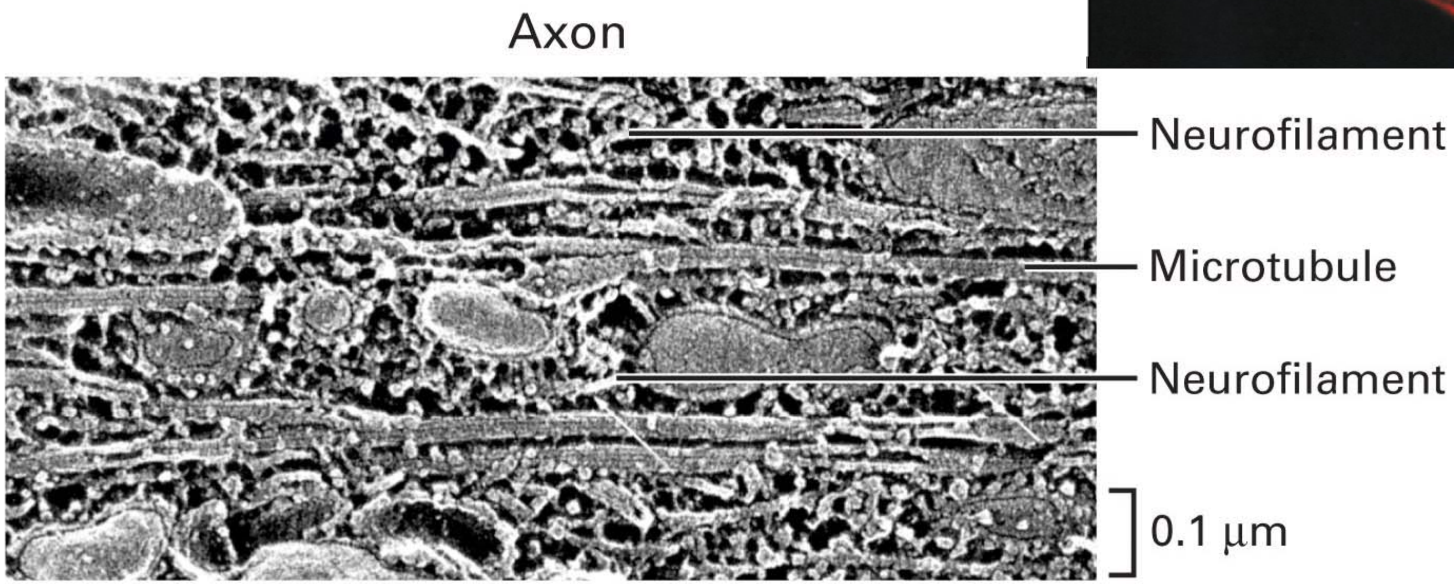
Cytoskeletal elements drawn to scale

Howard, Mechanics of Motor Proteins, 1st Ed.

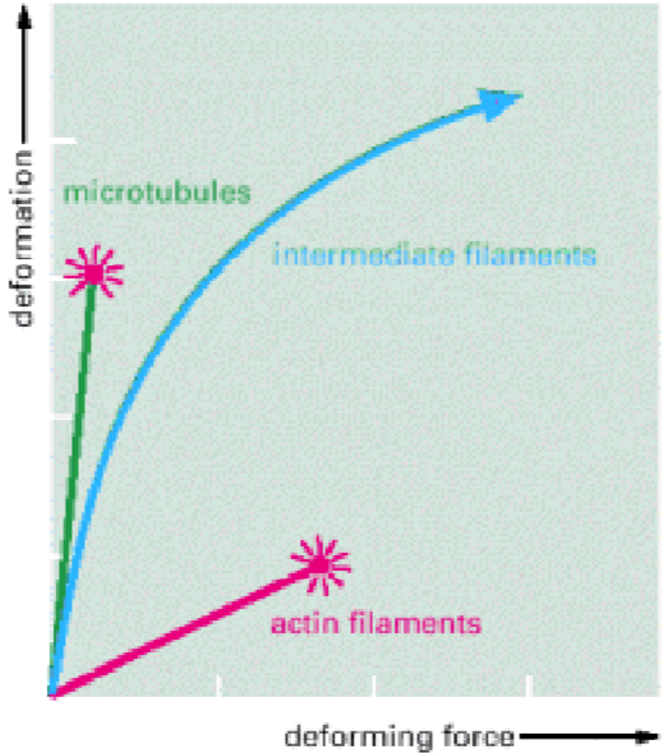
INTERMEDIATE FILAMENTS

Different from actin or microtubules:
- No motors attached
- No need for ATP or GTP to polymerize
- No globular subunits

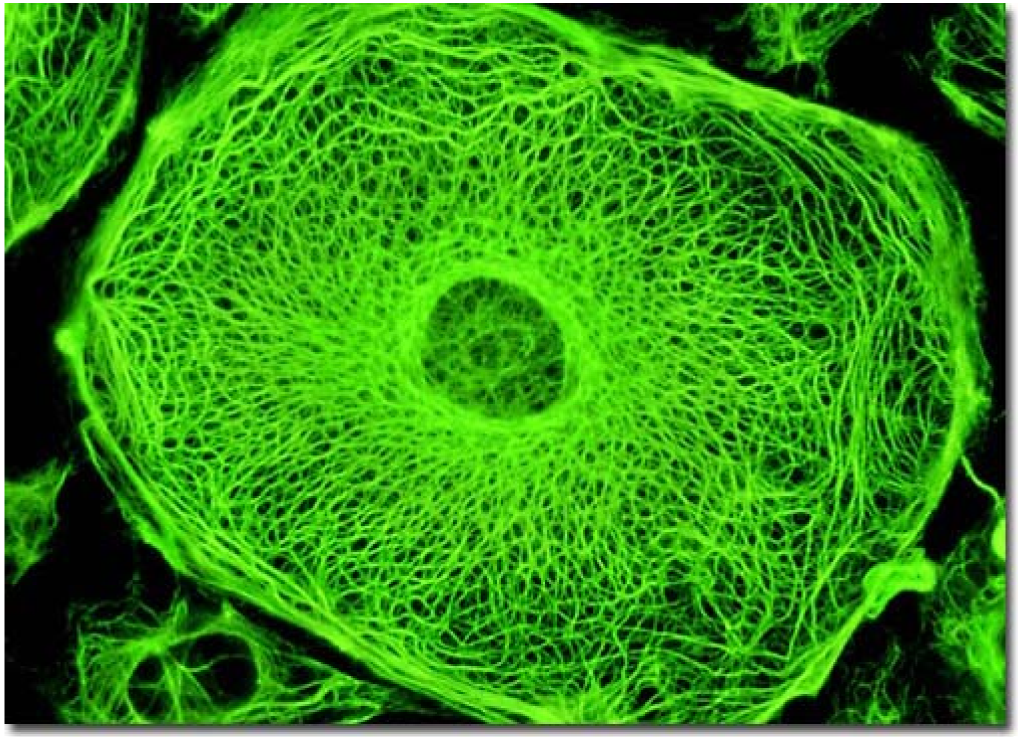


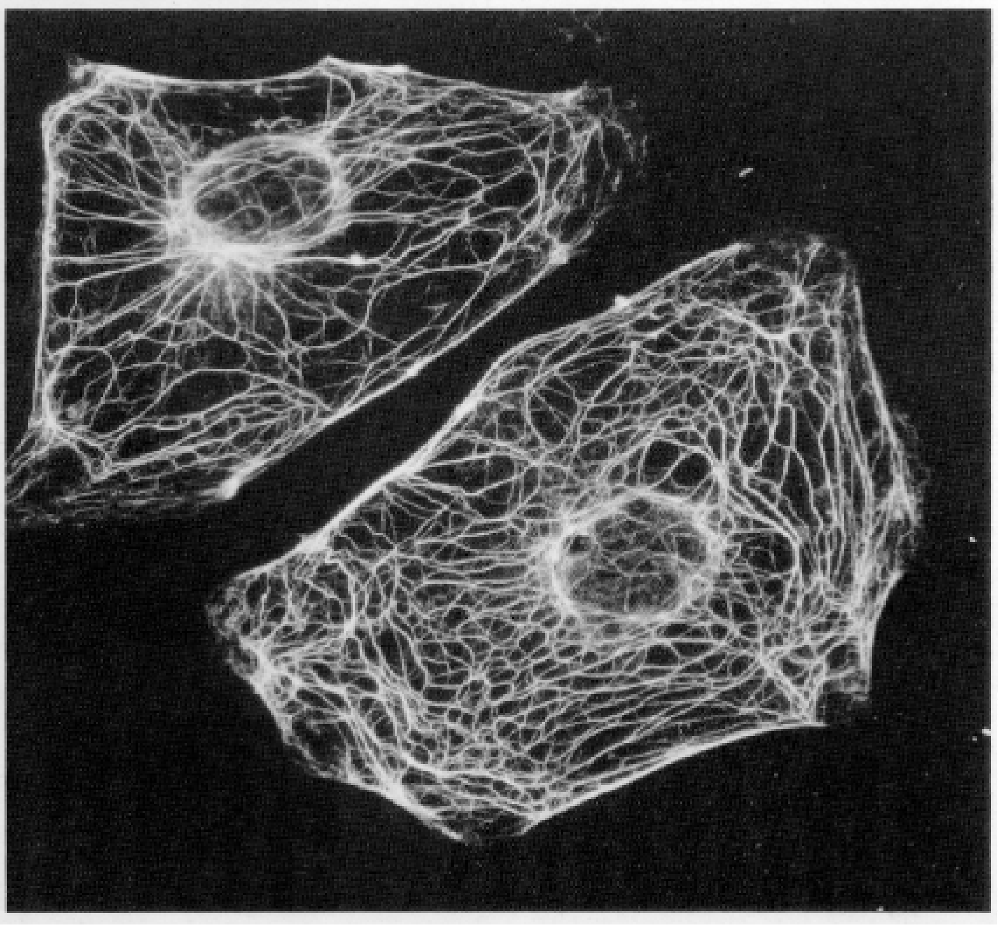


Intermediate filaments: size is *intermediate* that of actin and microtubules



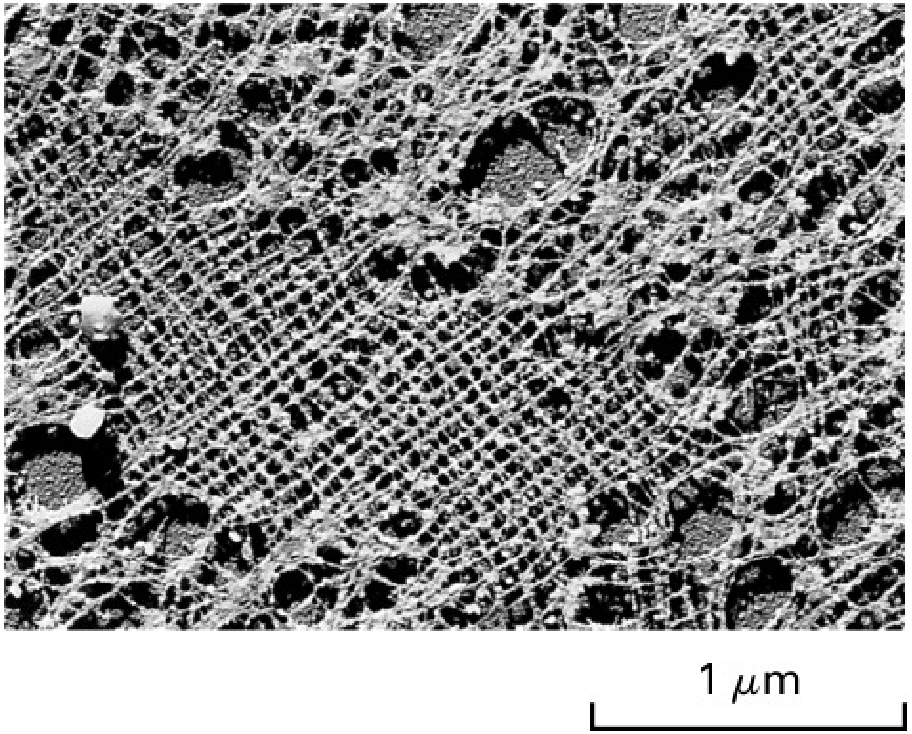
The **most flexible filaments (highly elastic)** due to a very complex structure





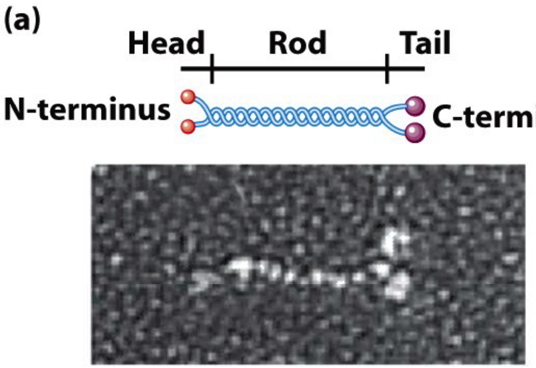
Cytokeratin in epithelial cells as present in blood vessels or **nails**, **hair**, **wool**

Nuclear envelope made by intermediate filament named **lamin**

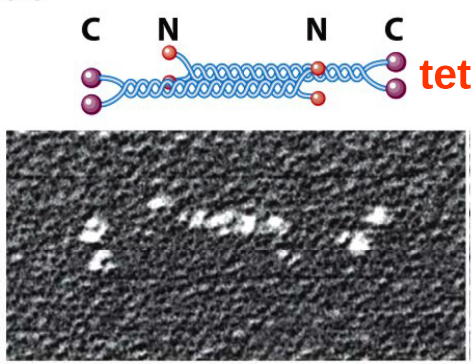


Intermediate filaments look like a rope

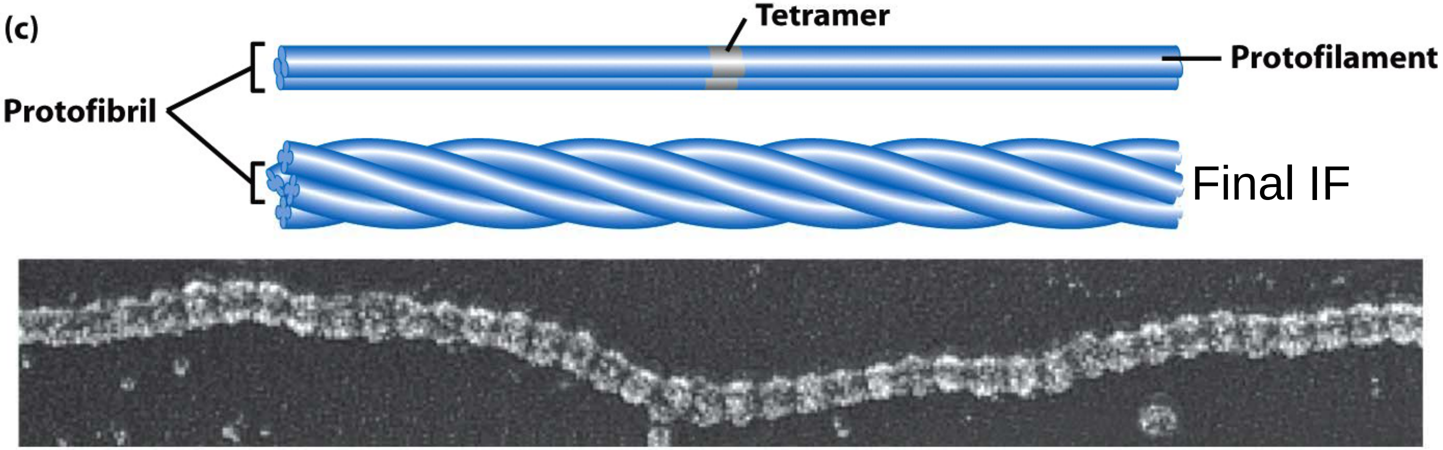
- IFs assemble from monomers into **dimers**
- Two dimers assemble into <u>tetramers</u>
- Tetramers form the proto**filament**
- 4 protofilaments form a proto<u>fibril</u>
- 4 protofibrils form the **final IF**



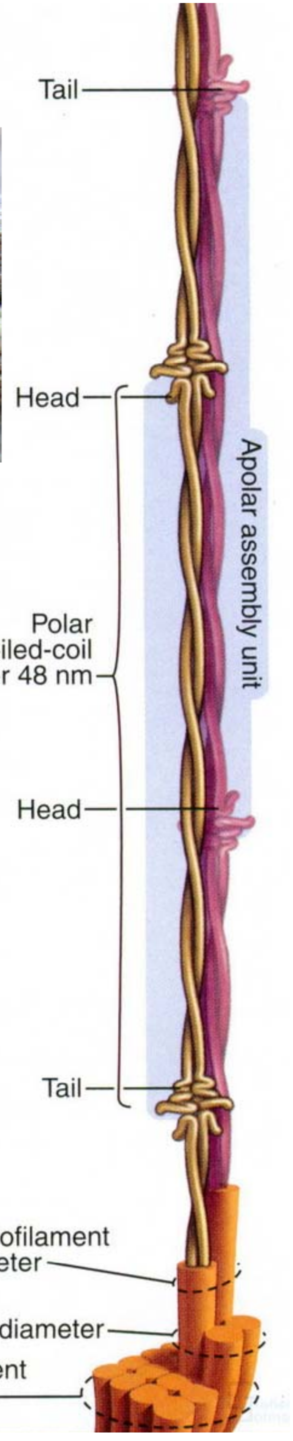
coiled-coil dimer



tetramer

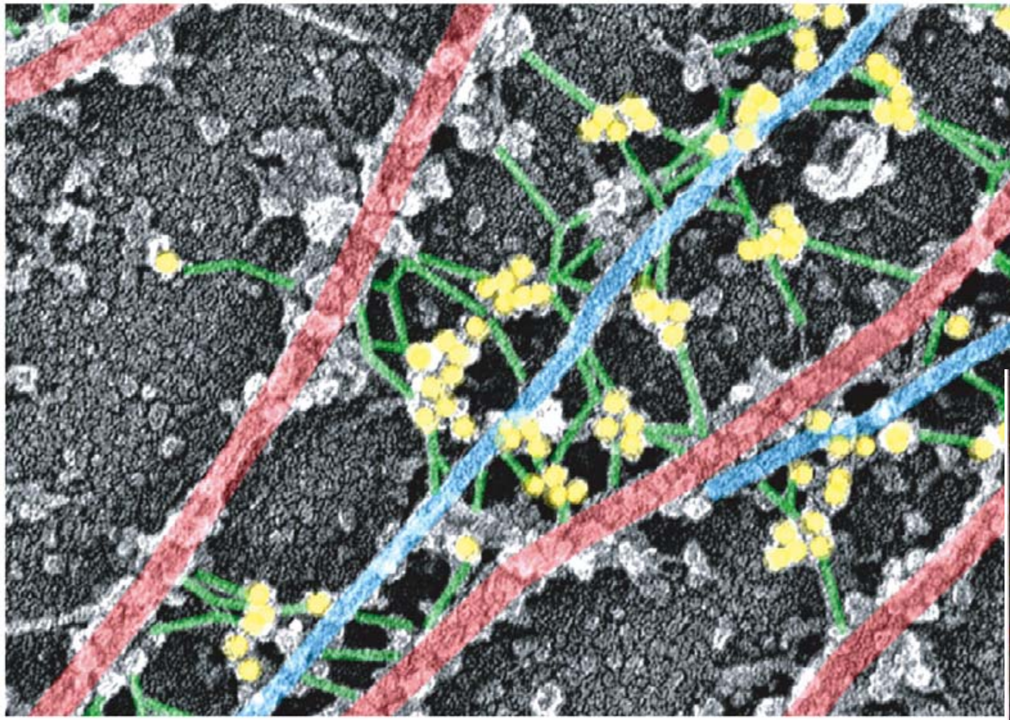


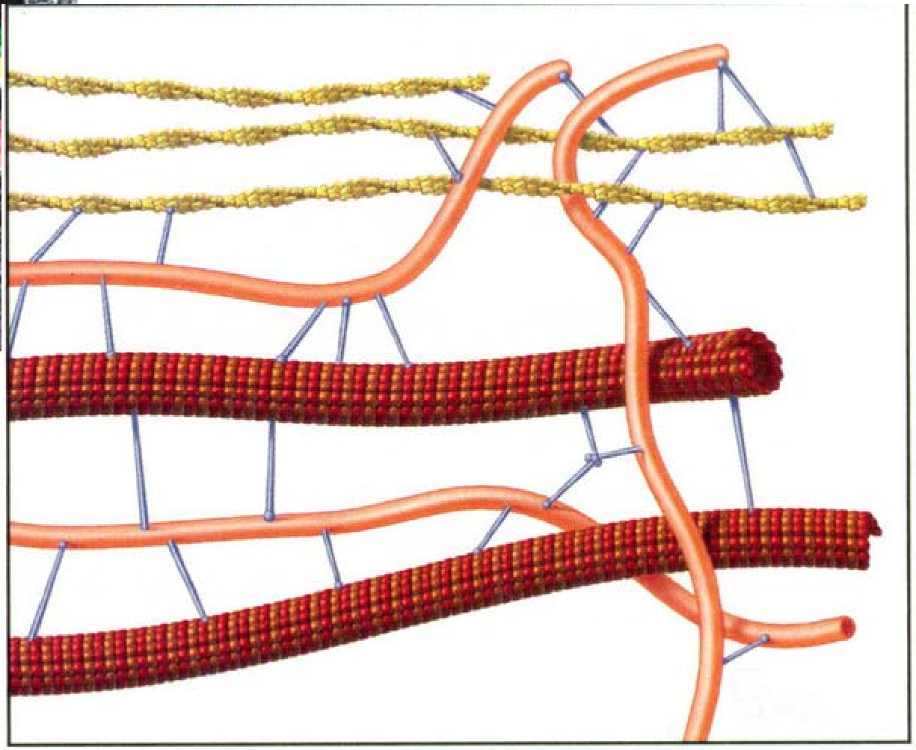
Final IF



Intermediate filament-*associated* proteins (IFAPs)

Cross-link and bundle IFs, connect all 3 major cytoskeletal elements (actin, microtubules, intermediate filaments) with each other

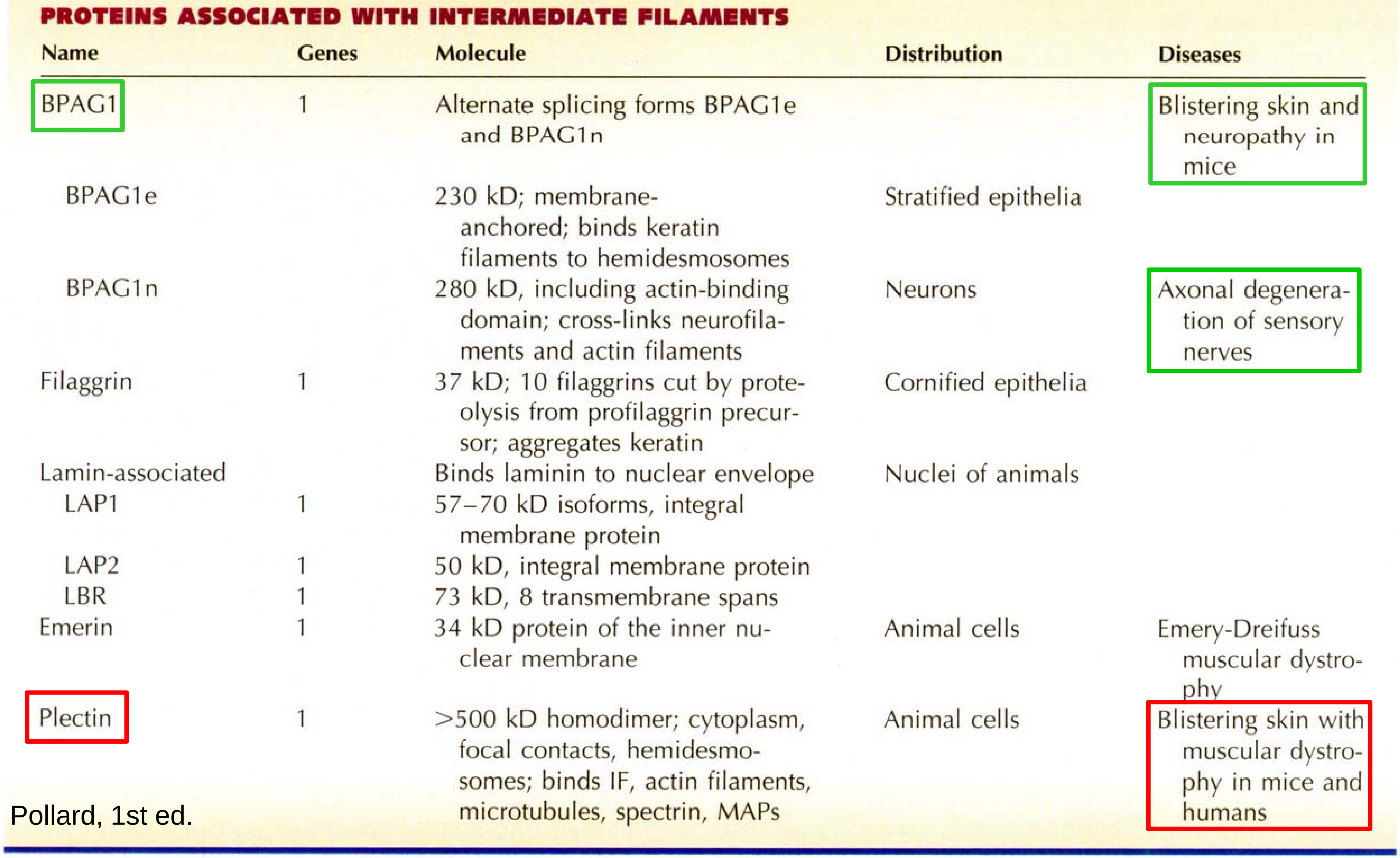




The crosslinker **plectin** integrates the 3 major cytoskeletal elements

Pollard, 1st ed.

IFAPs and diseases



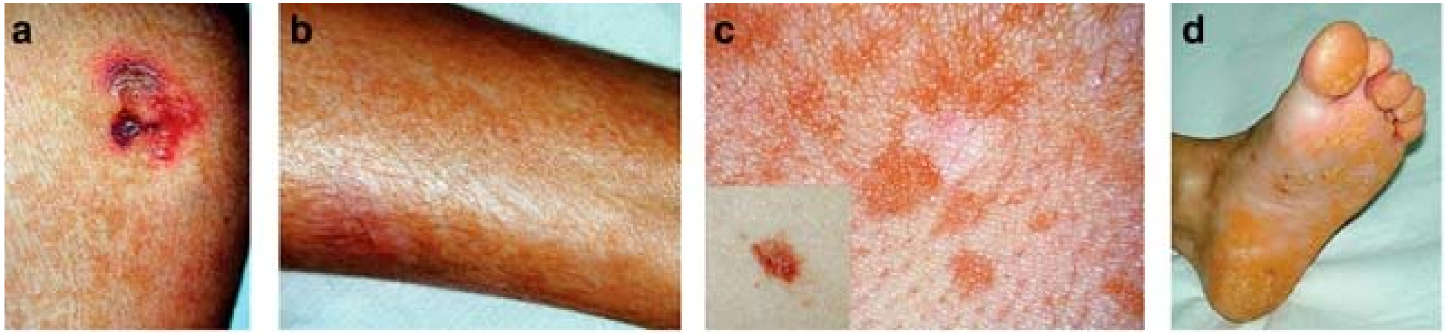
PROTEINS ASSOCIATED WITH INTERMEDIATE FILAMENTS

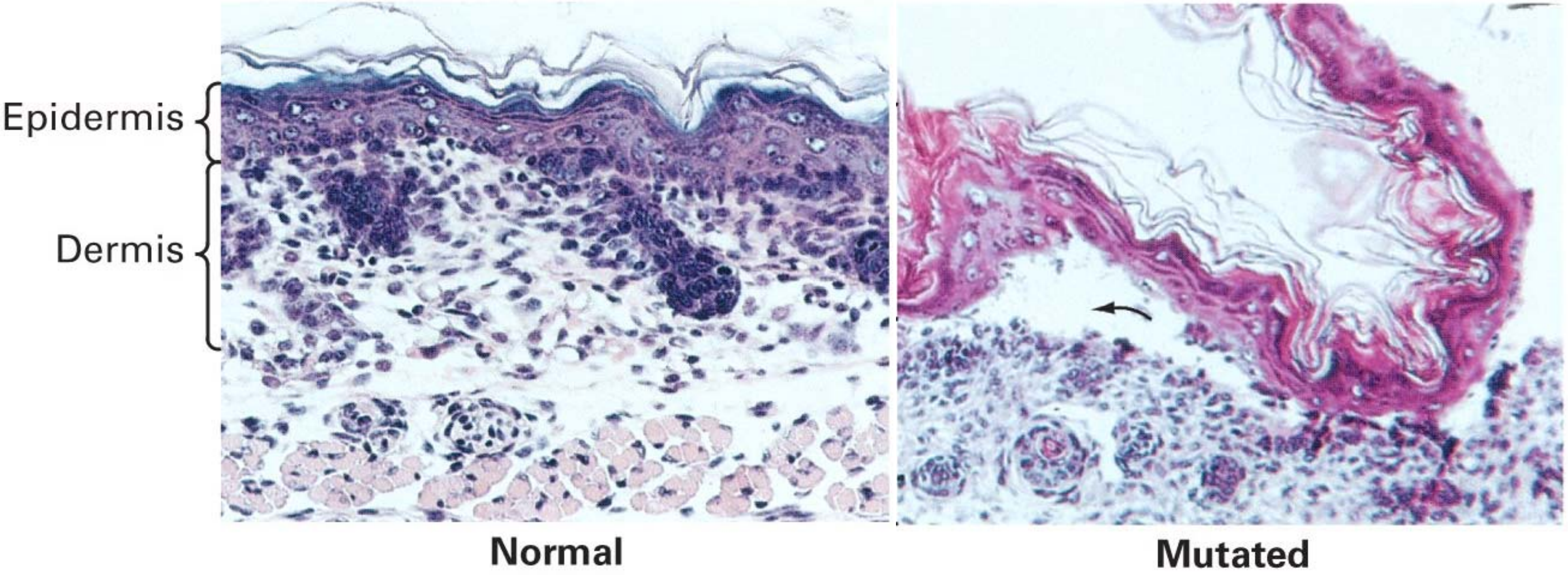
Name	Genes	Molecule	Distribution	Diseases
BPAG1	1	Alternate splicing forms BPAG1e and BPAG1n		Blistering skin and neuropathy in mice
BPAG1e		230 kD; membrane-anchored; binds keratin filaments to hemidesmosomes	Stratified epithelia	
BPAG1n		280 kD, including actin-binding domain; cross-links neurofilaments and actin filaments	Neurons	Axonal degeneration of sensory nerves
Filaggrin	1	37 kD; 10 filaggrins cut by proteolysis from profilaggrin precursor; aggregates keratin	Cornified epithelia	
Lamin-associated		Binds laminin to nuclear envelope	Nuclei of animals	
LAP1	1	57–70 kD isoforms, integral membrane protein		
LAP2	1	50 kD, integral membrane protein		
LBR	1	73 kD, 8 transmembrane spans		
Emerin	1	34 kD protein of the inner nuclear membrane	Animal cells	Emery-Dreifuss muscular dystrophy
Plectin	1	>500 kD homodimer; cytoplasm, focal contacts, hemidesmosomes; binds IF, actin filaments, microtubules, spectrin, MAPs	Animal cells	Blistering skin with muscular dystrophy in mice and humans

Pollard, 1st ed.

Intermediate filaments and disease

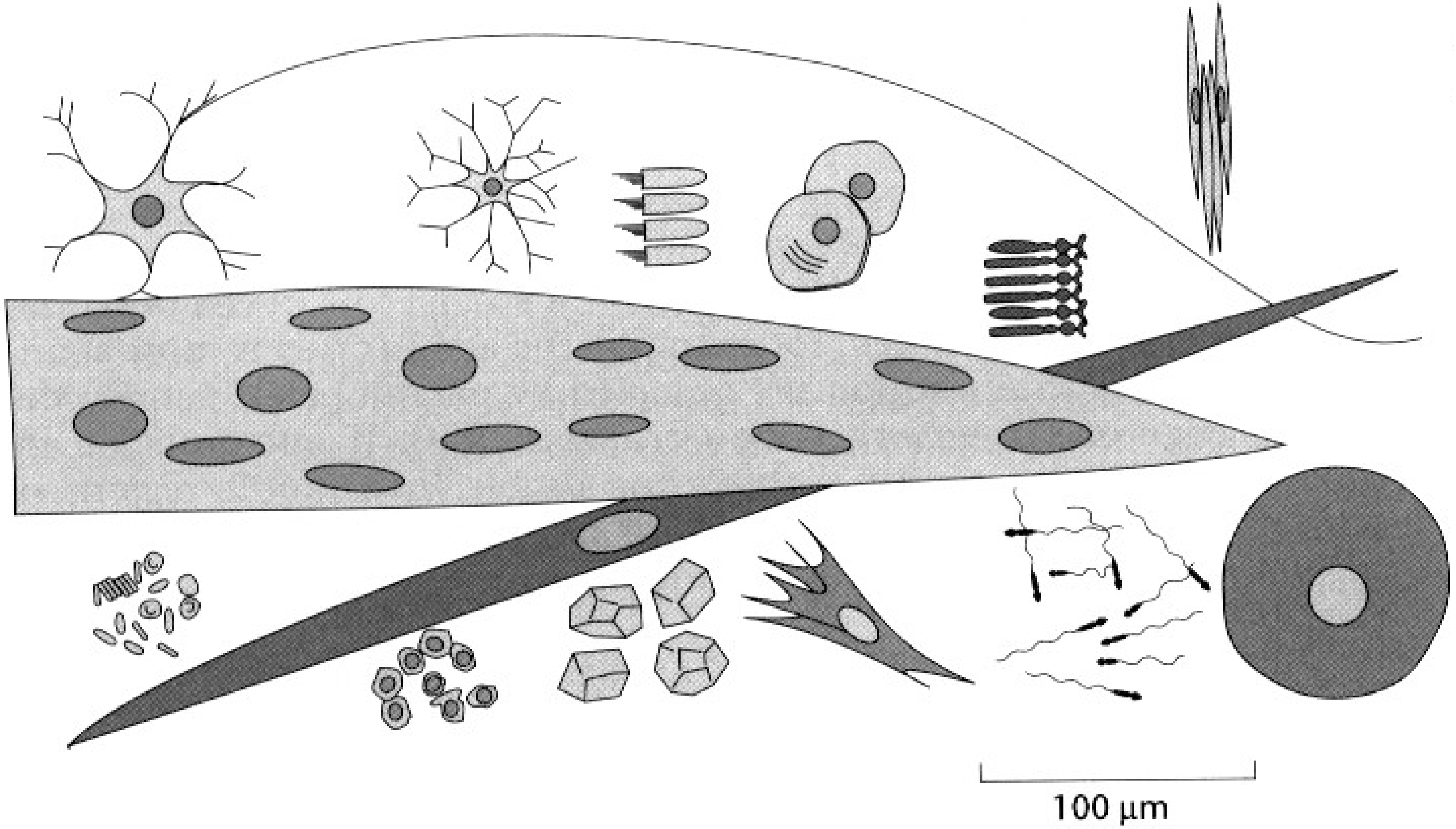
- Non-functional **keratin K14** leads to **EBS** (***epidermolysis bullosa simplex***)
- **Neurofilament NF-L** is involved in **amyotrophic lateral sclerosis** (**ALS**) and **Parkinson disease**





K14 mutation

Cell mechanics: physical forces that maintain cell shape



Muscle cells, fibroblast, red blood cells, neurons, egg, sperm, hair cell, retinal cells...
... drawn to same scale.

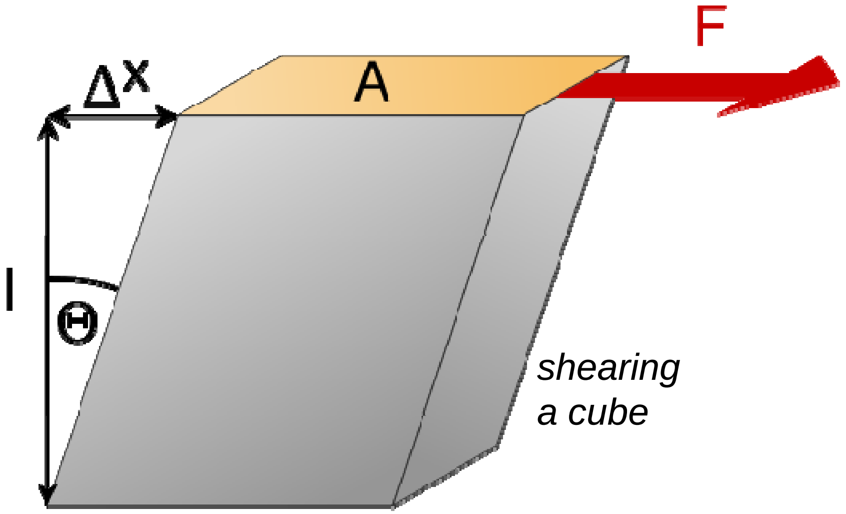
Bray, Cell Movements, 2nd Ed.

The quantities of cell mechanics: Physics meets Biology

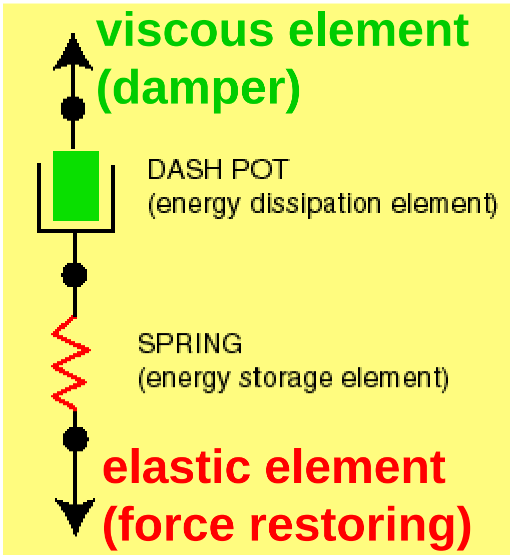
Cells have both, viscous and elastic properties, they behave **viscoelastic**

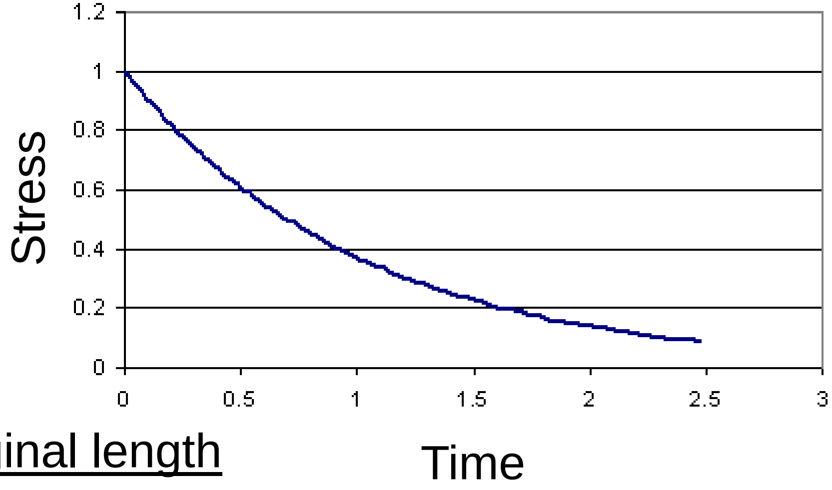
Shear stress δ = **F**orce per **A**rea or: $\sigma = \frac{F}{A}$ [Pa]

Strain γ = Deformation = $\Delta x / x_0$



Maxwell model of viscoelastic materials





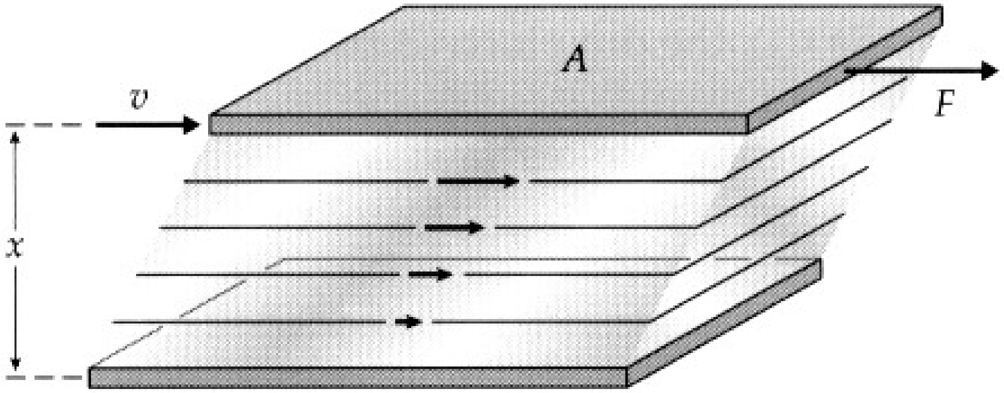
- If a Maxwell material is suddenly strained (deformed): stresses decay with time
- If we suddenly free the deformation:

elastic element = spring back

viscous element = does not return to its original length

=> Problem: **irreversible deformation component**

Anatomy of the viscous dashpot: viscous damping



Sheared liquid

Velocity gradient

Shear stress proportional to velocity gradient:

$$\frac{F}{A} = \eta \frac{dv}{dx}$$

- When a fluid is placed between to plates and the upper plate is moved while the lower plate is stationary a **velocity gradient** is observed
- The shear stress (F/A) is proportional to this velocity gradient (dv/dx)
- The **constant** η (êta) of this relation **is called** the **coefficient of viscosity**
- Because the unit for shear stress is Pa and the unit for the velocity gradient (= shear rate) is s^{-1}, the unit for the viscosity is Pa · s

The quantities of cell mechanics

Problems of the Maxwell model

- If a Maxwell material is **suddenly released** from stress:

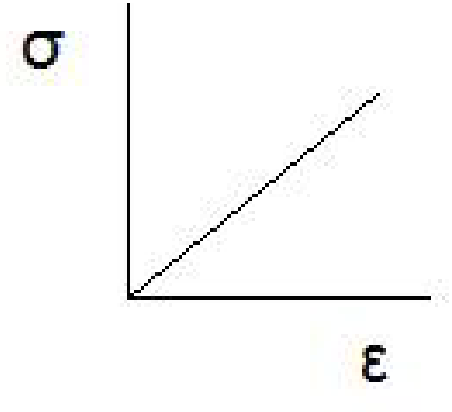
elastic element: spring-back to its original value

viscous element: no change in deformation

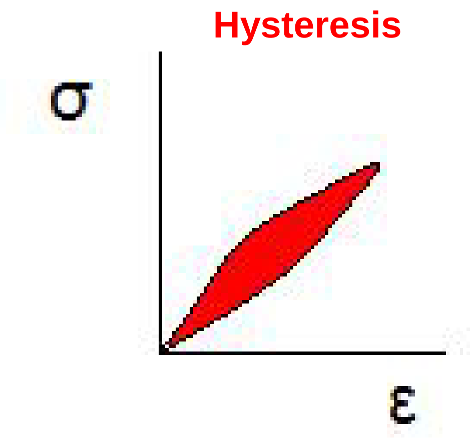
- Further problem: Maxwell model not ideal for predicting **creep behavior** (because it describes the strain relationship with time as linear)

> “**Creep** is the tendency of a solid material to slowly move or deform permanently under the influence of stresses”

Stress/Strain curves



Elastic material

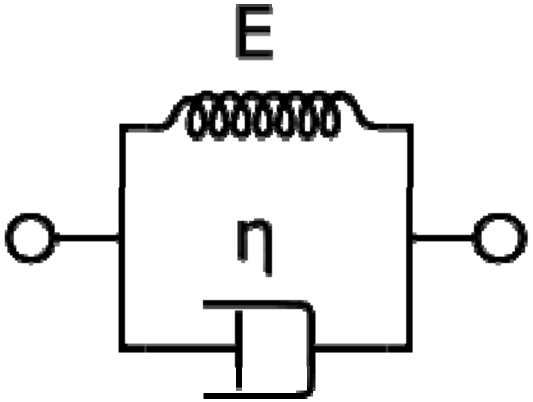


Viscoelastic material

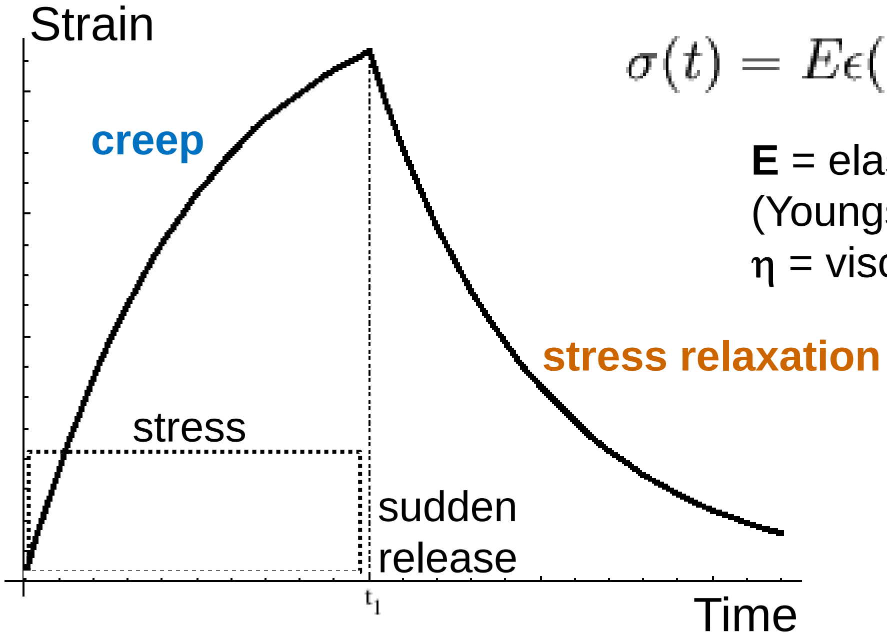
The quantities of cell mechanics

Kelvin-Voigt model describes well the creep behavior of viscoelastic materials

Kelvin-Voigt model of viscoelastic materials (solid-type)



Spring and dashpot in parallel



$$\sigma(t) = E\epsilon(t) + \eta\frac{d\epsilon(t)}{dt}$$

E = elastic modulus (Youngs modulus)
η = viscous modulus

- If we suddenly free the material from strain:

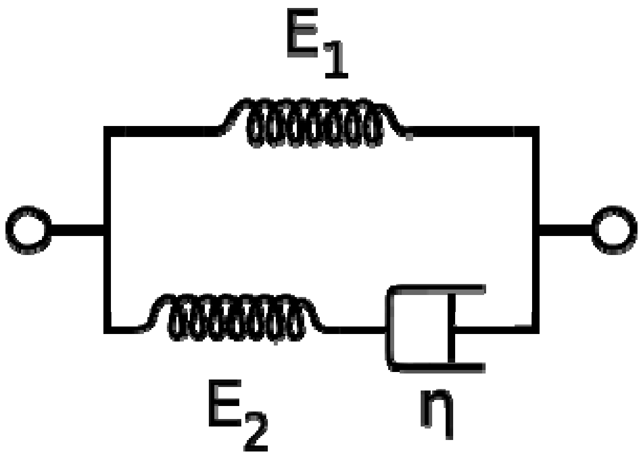
elastic element retard the material back until the deformation become zero
⇒ **elastic element *resets*** **dash-pot** = deformation is reversible

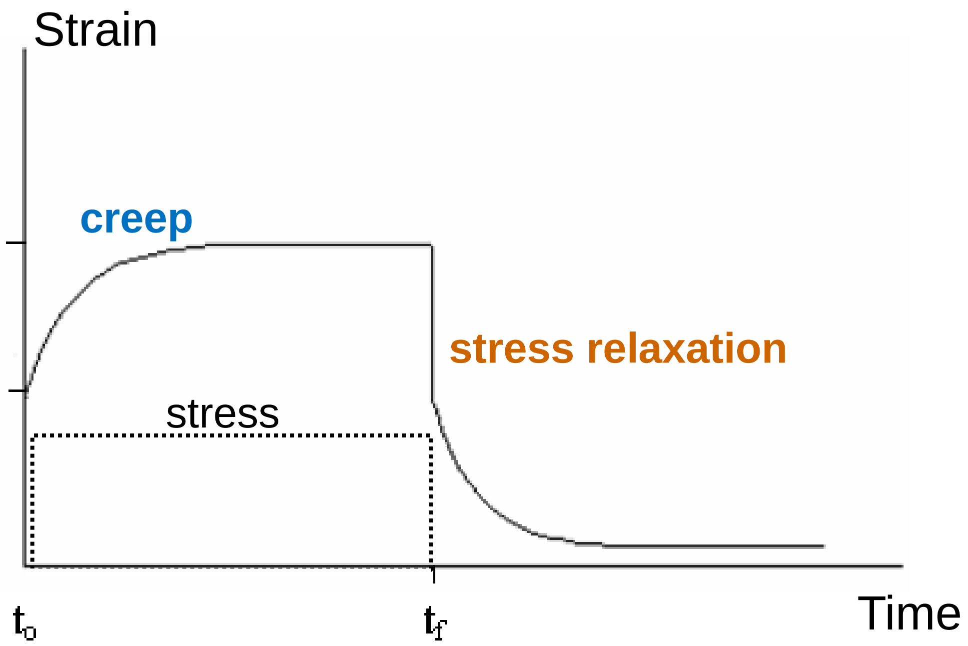
- Further: model better for describing **creep** behavior
- Problem: model not good to describe **stress relaxation** (here too continuous)

The quantities of cell mechanics

SLS model describes well the creep and stress relaxation of viscoelastic materials

Standard-Linear-Solid (SLS) model of viscoelastic materials



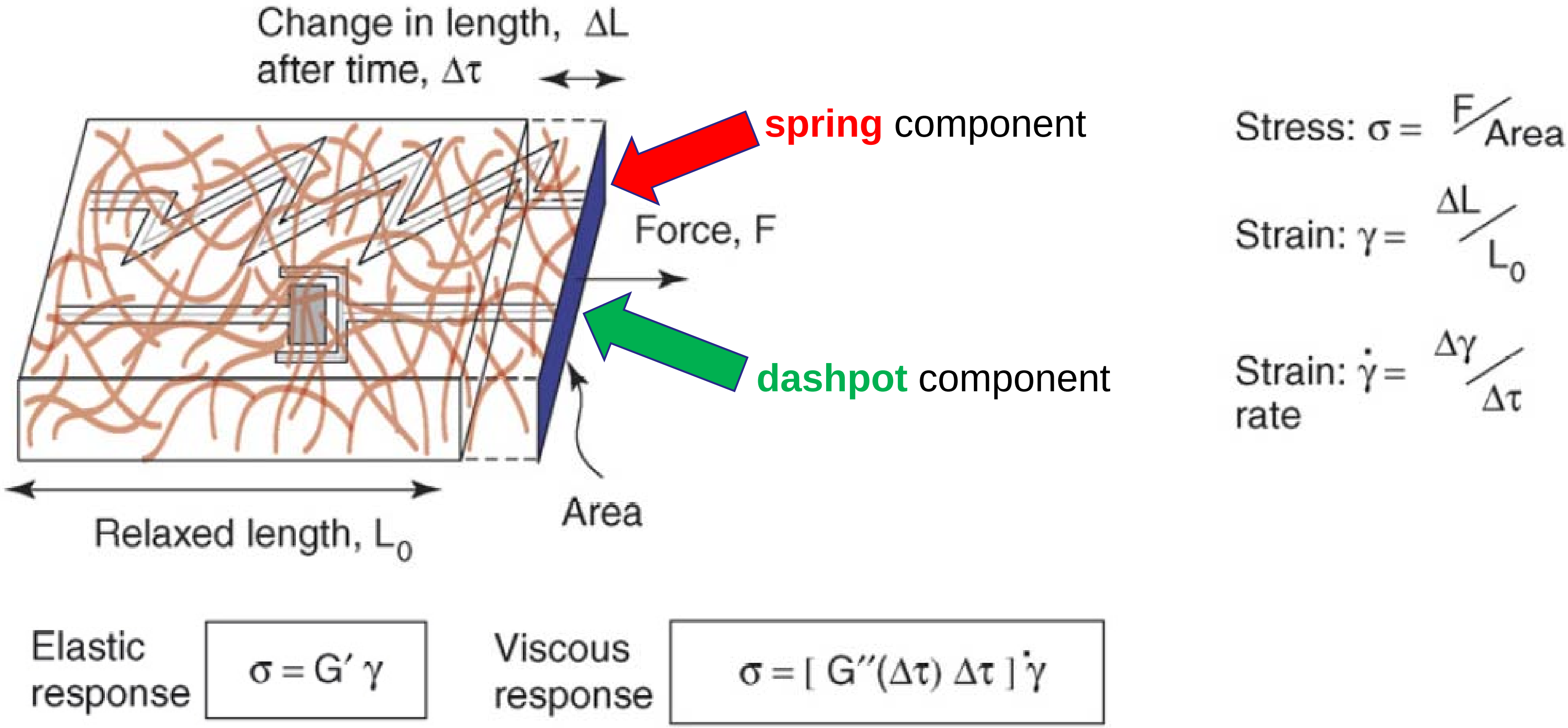


SLS model describes well **creep** and (discontinuous) **stress relaxation**

Is the cell a solid or a liquid?

Cell mechanics: *Example from your reading material!*

Storage and loss modulus describing elastic and viscous behavior of cells



spring component

dashpot component

Stress: $\sigma = F/\text{Area}$

Strain: $\gamma = \Delta L / L_0$

Strain: $\dot{\gamma} = \Delta\gamma / \Delta\tau$
rate

Elastic response $\boxed{\sigma = G'\gamma}$

Viscous response $\boxed{\sigma = [\, G''(\Delta\tau)\, \Delta\tau \,]\, \dot{\gamma}}$

- **Elasticity** of **biopolymer** networks allows them to <u>resist deformation</u> **like a spring**

⇒ energy of deformation is stored <u>regardless of time</u>: **storage modulus G´**

- **Viscous behavior** of **biopolymer** networks allows them to **flow as a fluid**:

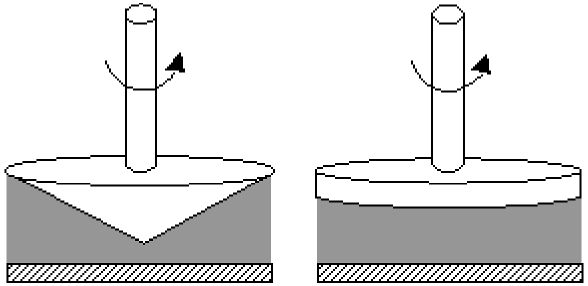
⇒ <u>resistance depends</u> on the <u>rate of deformation</u> (like in a **dashpot**)

⇒ <u>energy</u> put into deformation: <u>dissipated or lost</u>: **loss modulus G´´**

Rheology: determination of viscoelastic properties of liquids

- Rheo = flow (Greek) = measuring the flow of liquids
- Most popular: cone-plate or plate-plate **rheometer** = liquid placed between 2 plates
- Upper plate rotates at defined speed and angle = **shear rate** (velocity per distance)
- Upper plate also measures the resistance (response) of the fluid to applied shear by measuring the **torque** (= twisting force) = **shear stress** (F/A)

Oscillating cone or plate

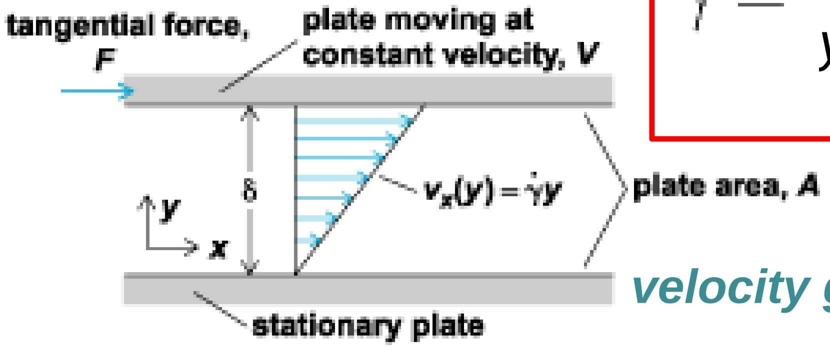


Fixed plate

Shear rate:

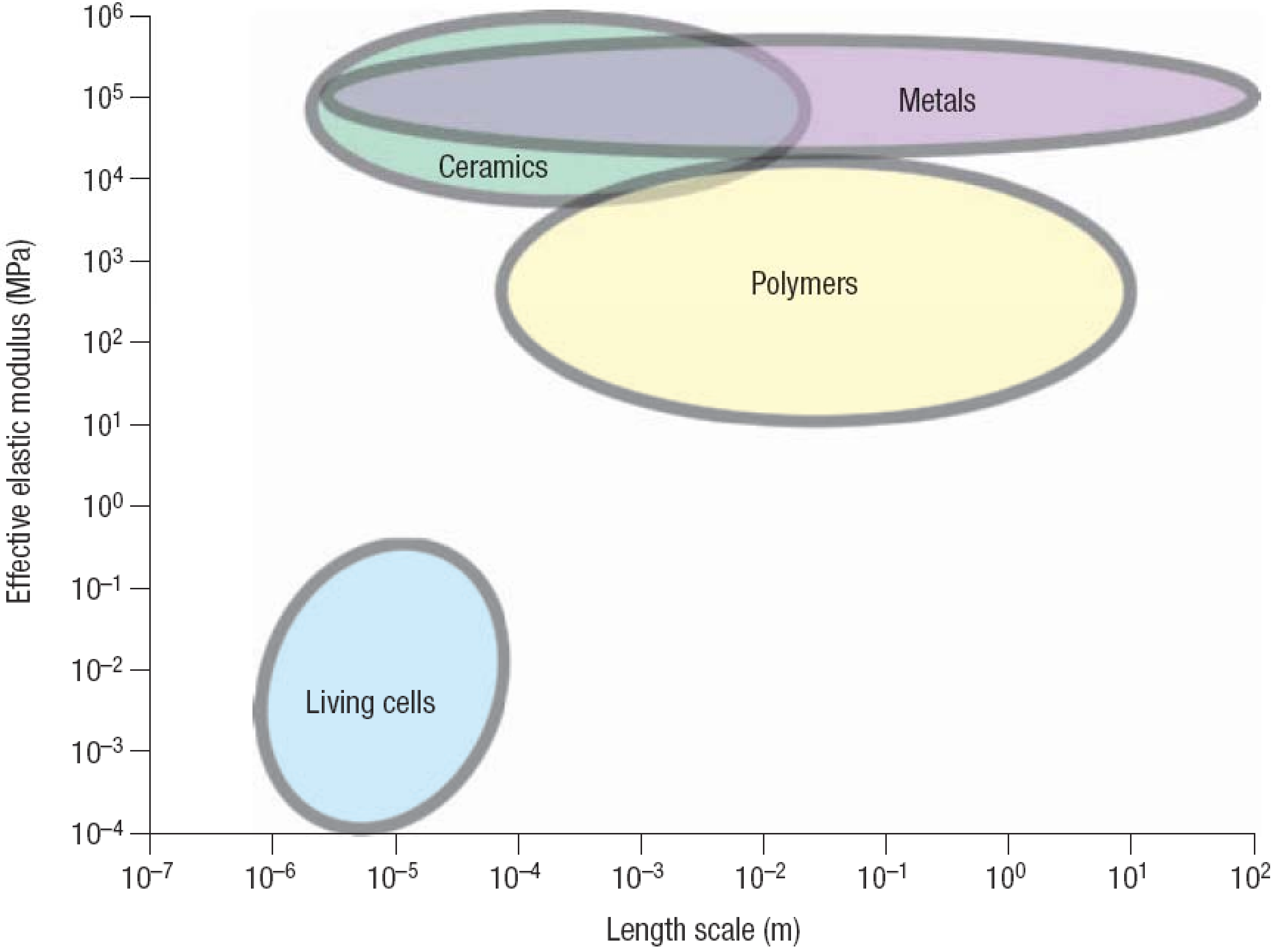
$$\dot{\gamma} = \frac{v}{y}$$

velocity per distance (distances between plates)



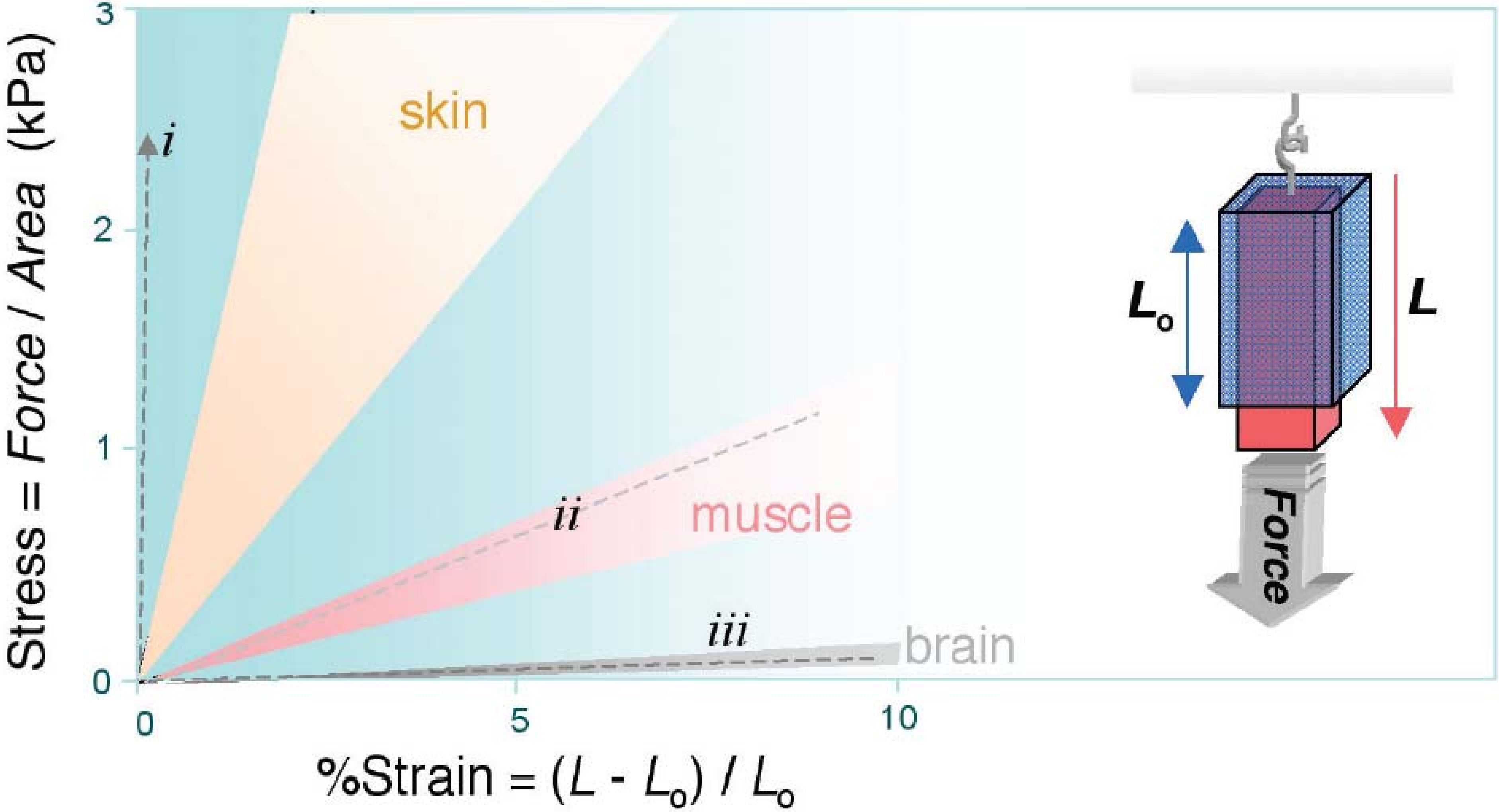
velocity gradient

Range of elastic moduli of cells compared with metals, ceramics and polymers



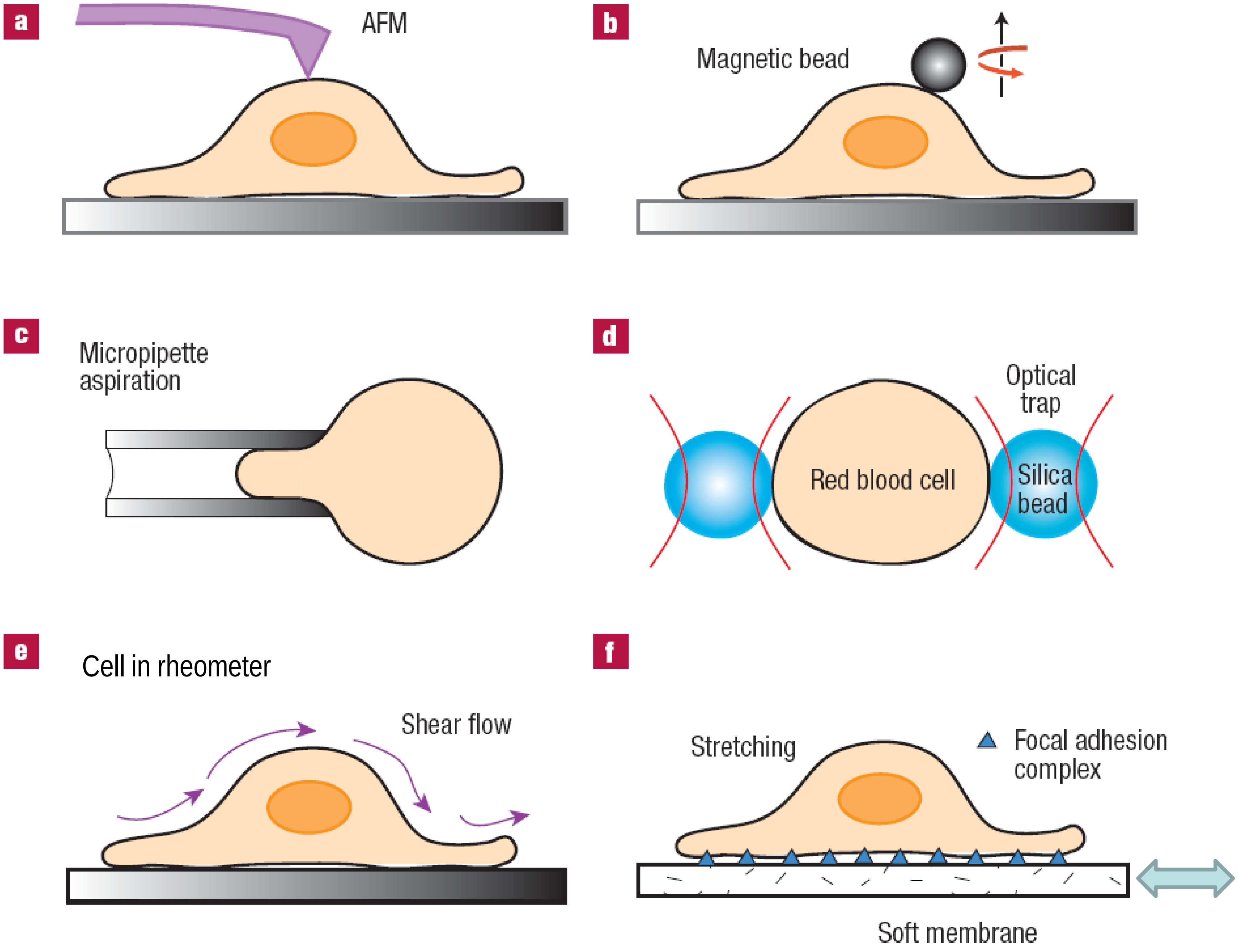
Strain/stress plot for different tissues

- To stretch (strain) **skin tissue**, a considerable amount of force (stress) is needed
- **Muscle tissues** can be deformed (strain) easily using only low forces (stress)
- **Brain tissue** does not show any elastic behavior (negligible strain/stress features)

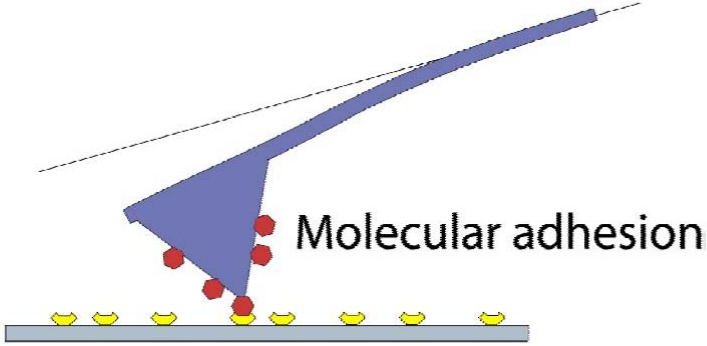


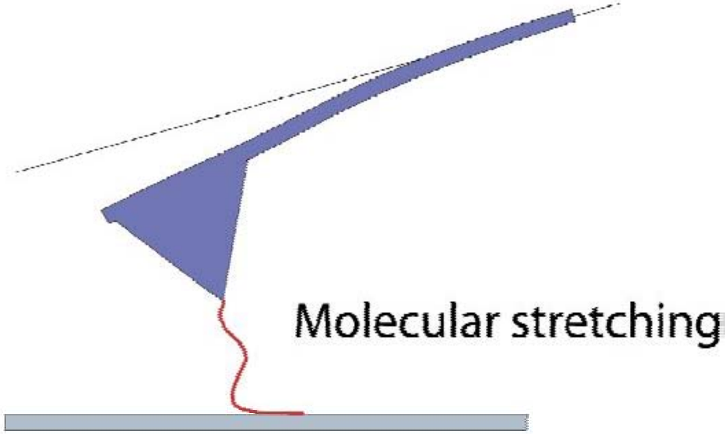
Discher et al., Science, 2005

Methods to measure the mechanical properties of cells

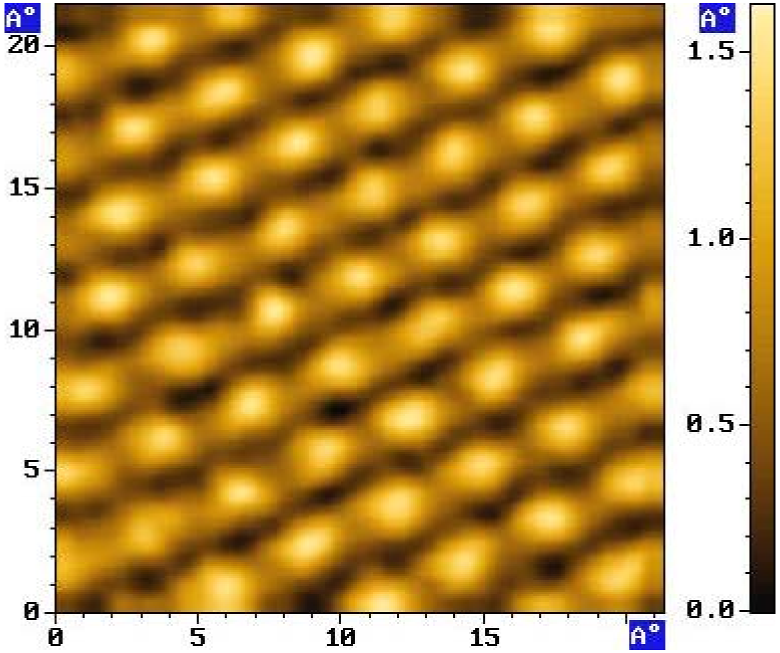


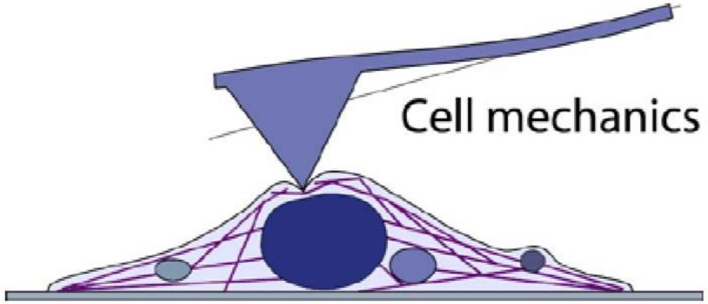
Nano-manipulation of cells and biopolymers using AFM (atomic force microscopy)

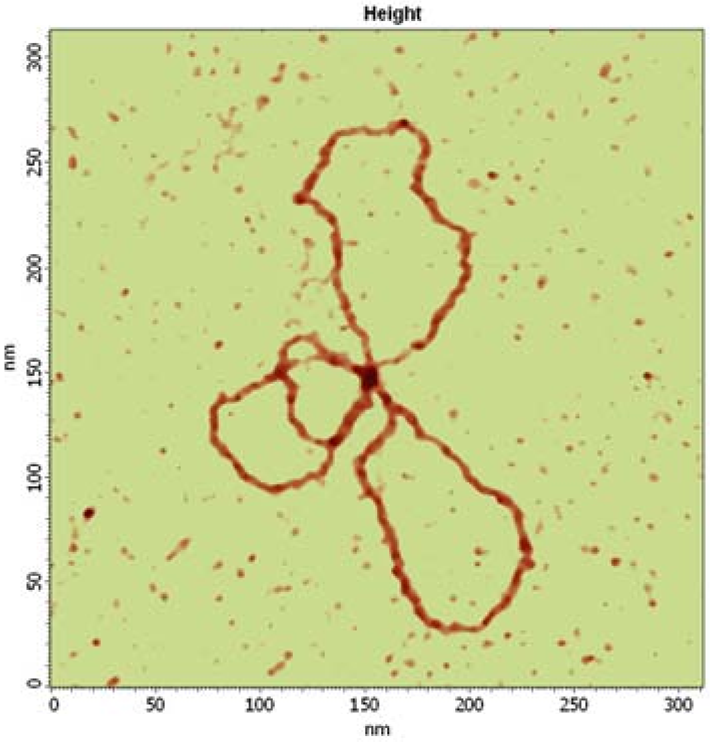




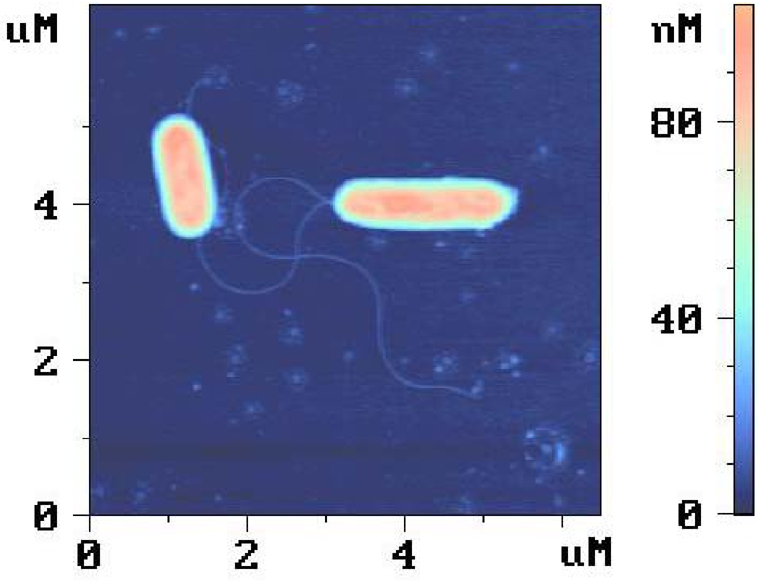
1980s scanning probe microscope (SPM) presented the first atomic-scale image of a gold-surface



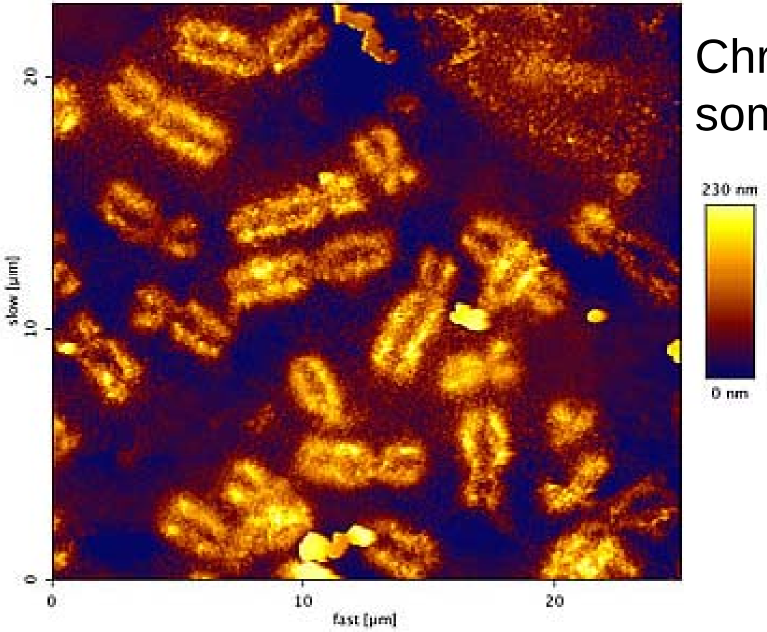




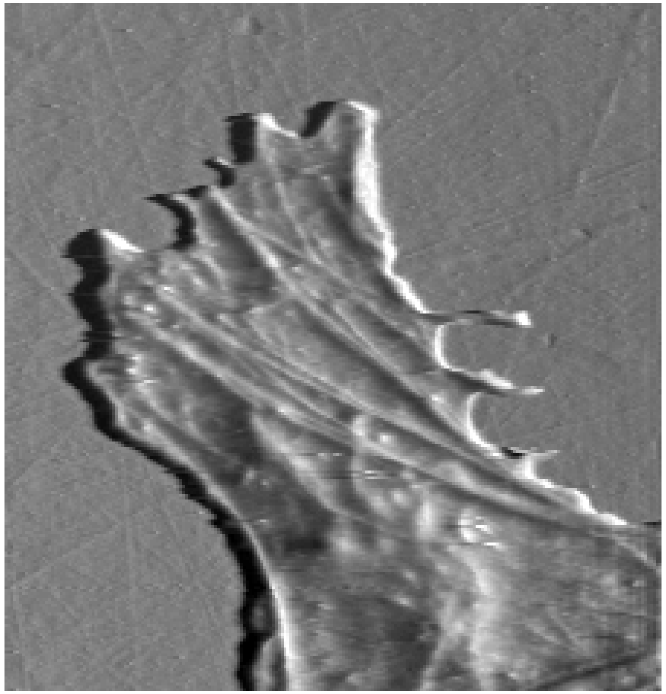
DNA



Bacteria

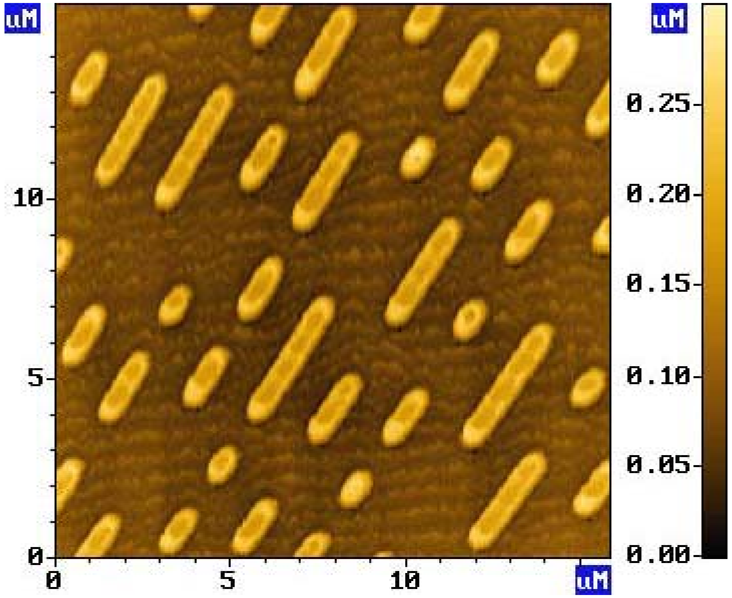


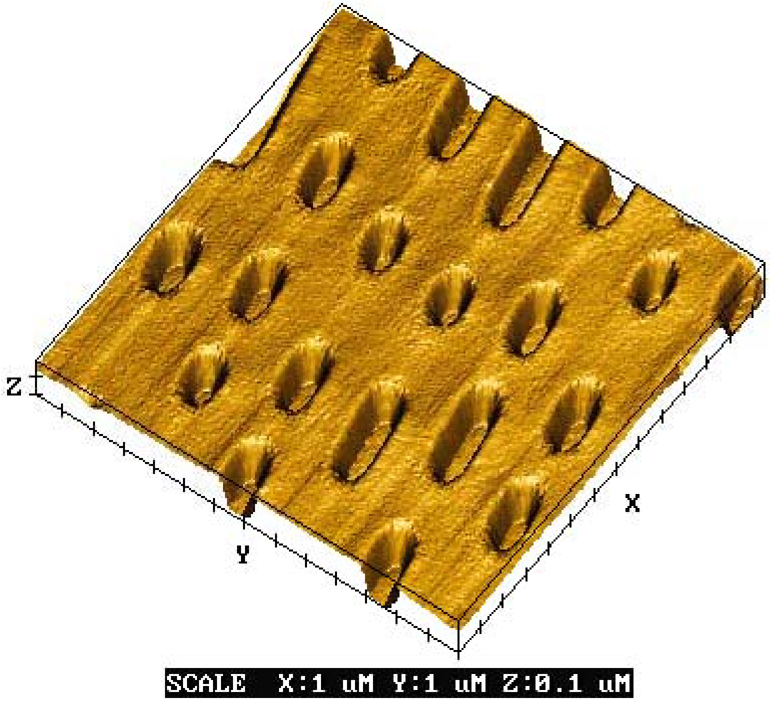
Chromosomes



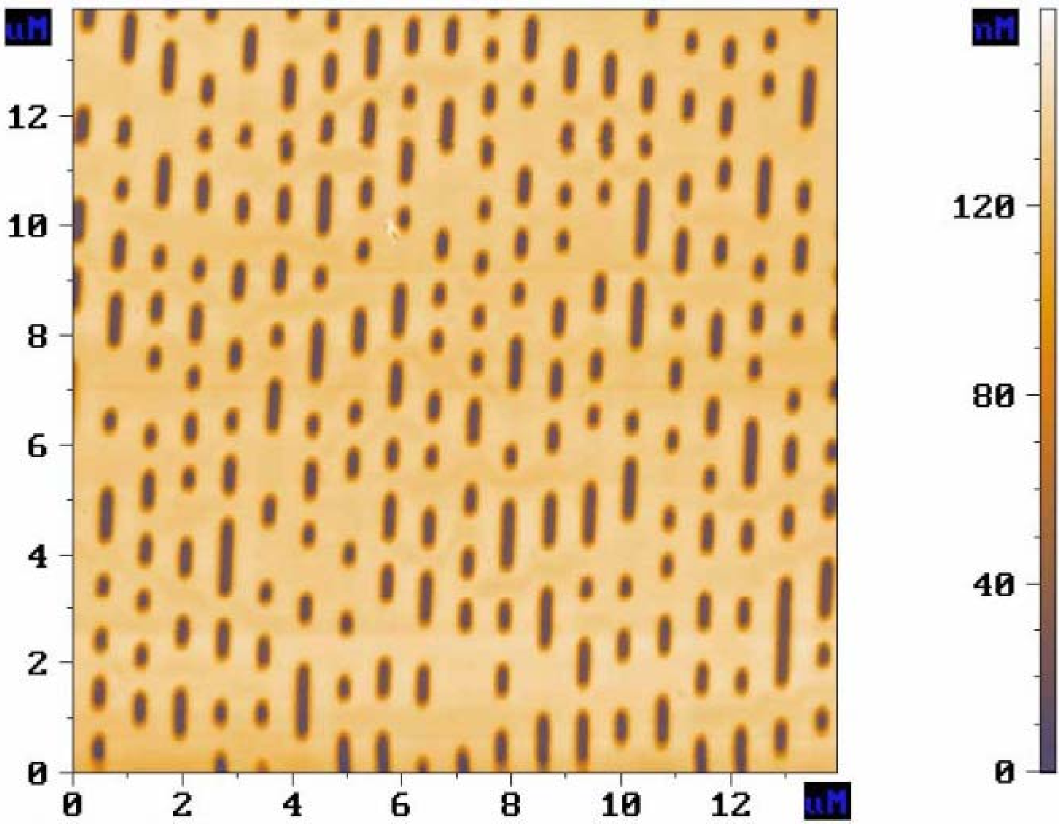
Cells (cytoskeleton)

CD Disk

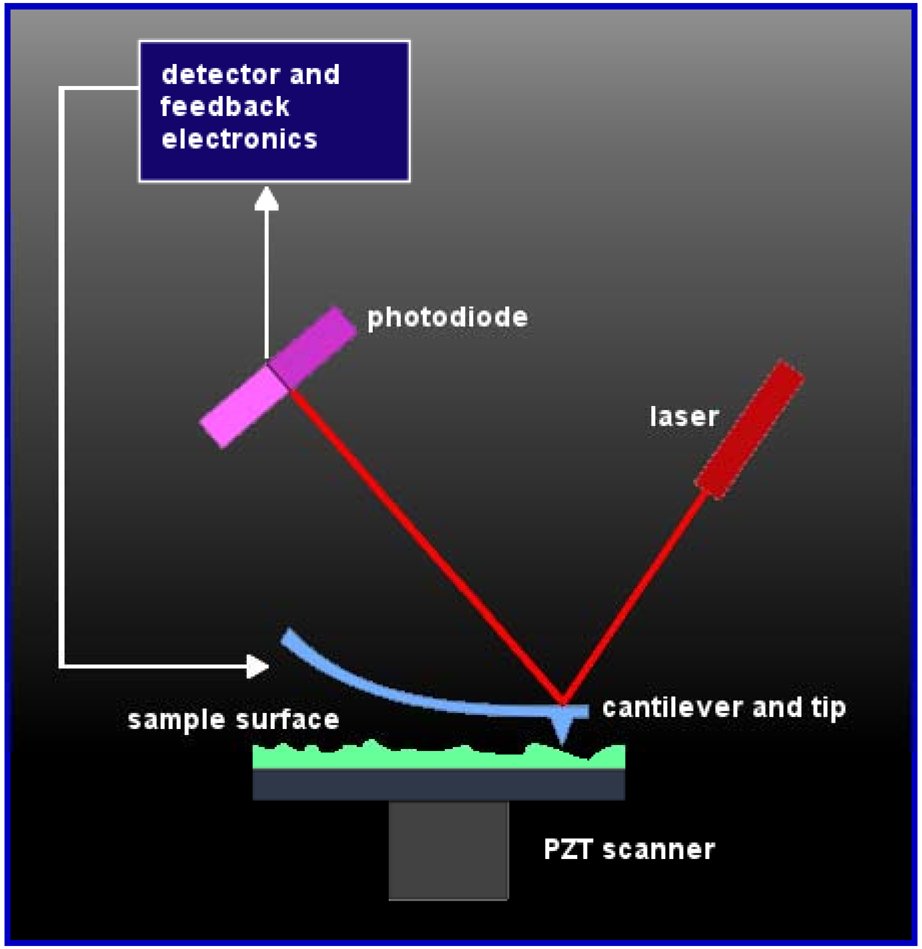




DVD Disk



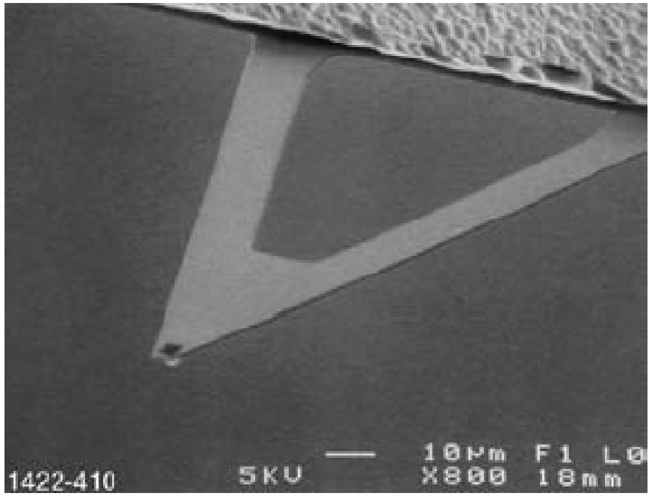
How does it work?

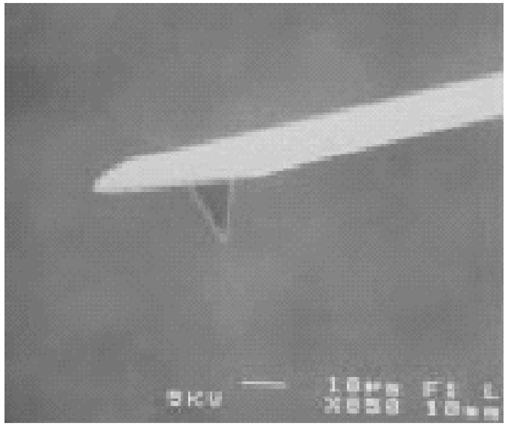


Animation
AFMModel.exe

Animation
pi.swf

Spring constant (k): 0.6-0.06 N/m
Tip radius: 20-60 nm
Cantilever length: 100-200 µm

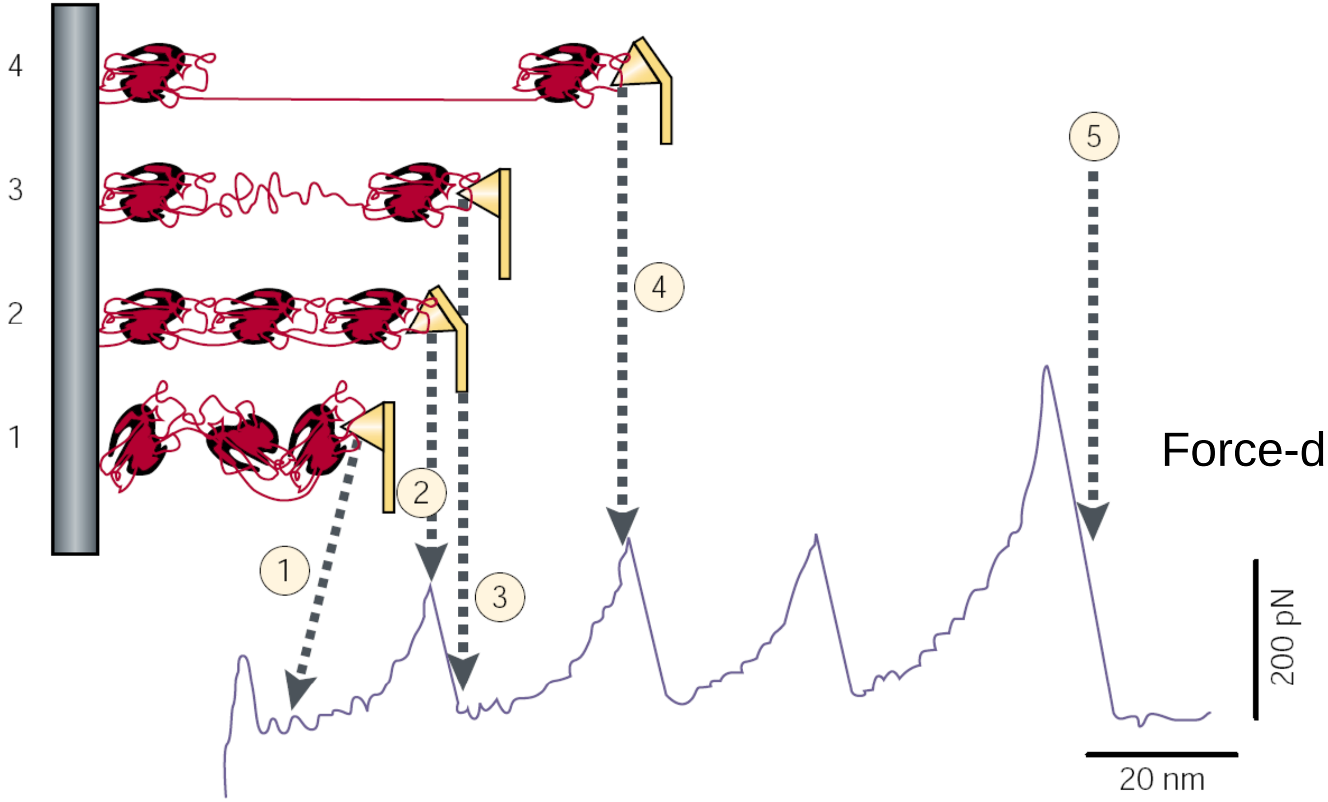




SEM of AFM tip

Advanced Force Spectroscopy

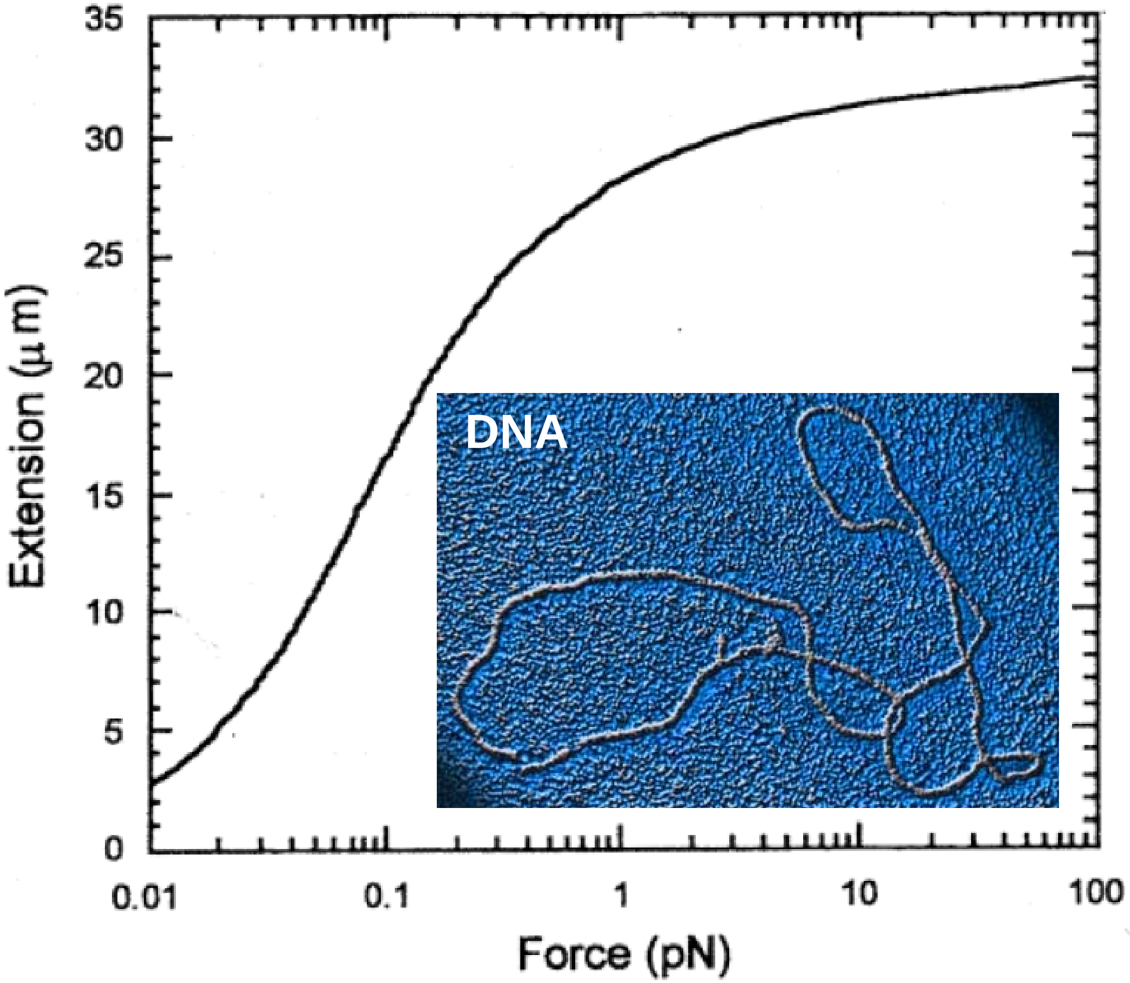
- **Protein unfolding**: AFM tip grabs the end of a protein (attached to a surface) => **protein unfolds in its several domains**
- Resulting **force-distance curve** shows a series of **snap-back points** each representing the breaking of a chemical bond

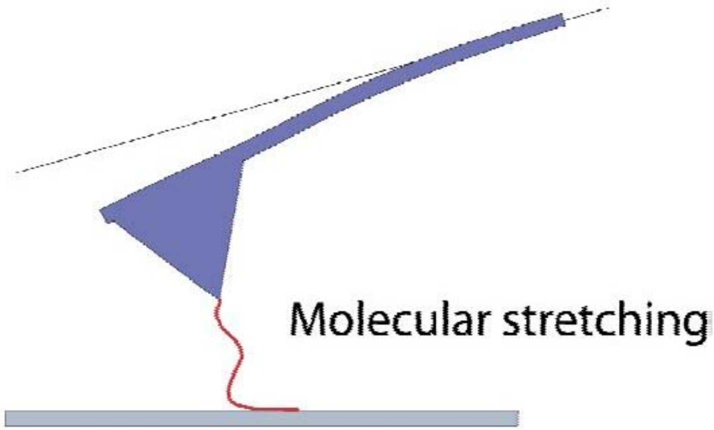


Force-distance curves

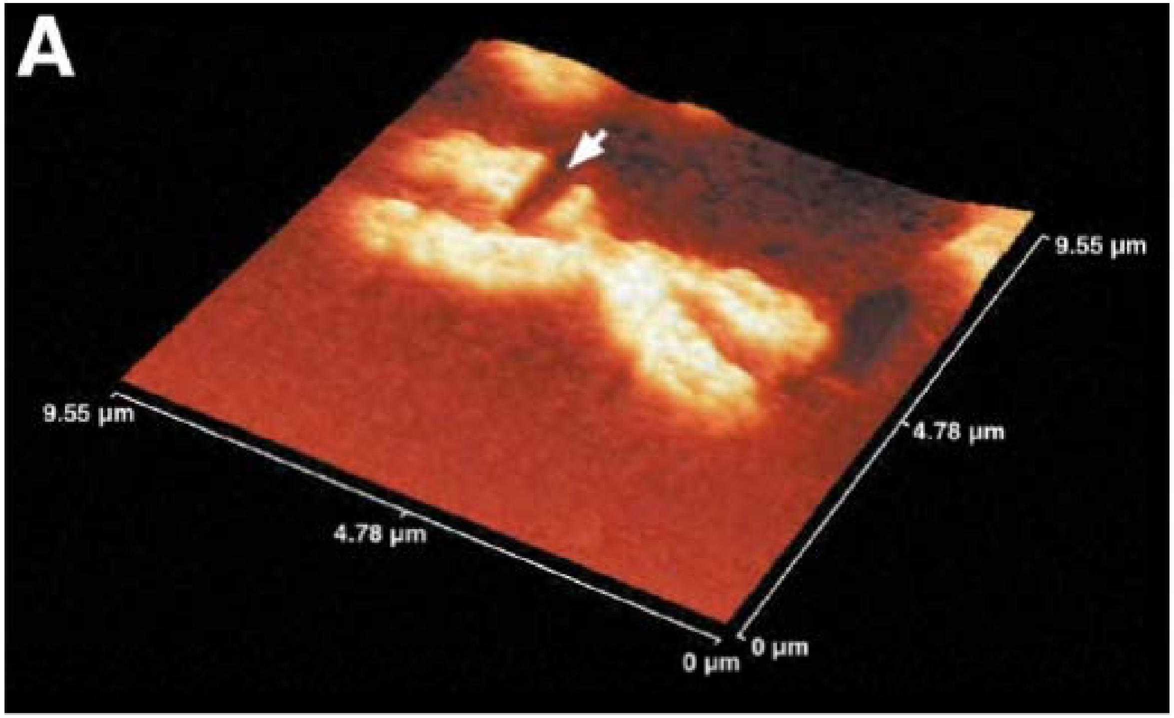
Domain unfolding of repeating immunoglobulin-like domains

How much force needed to stretch DNA?

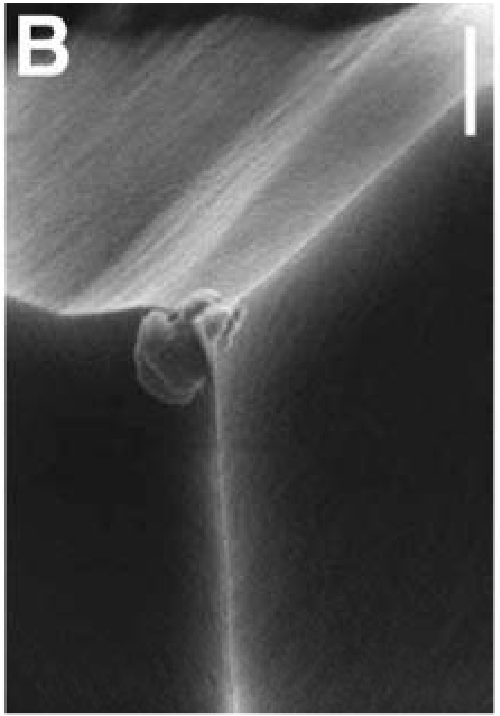




Nano-dissection

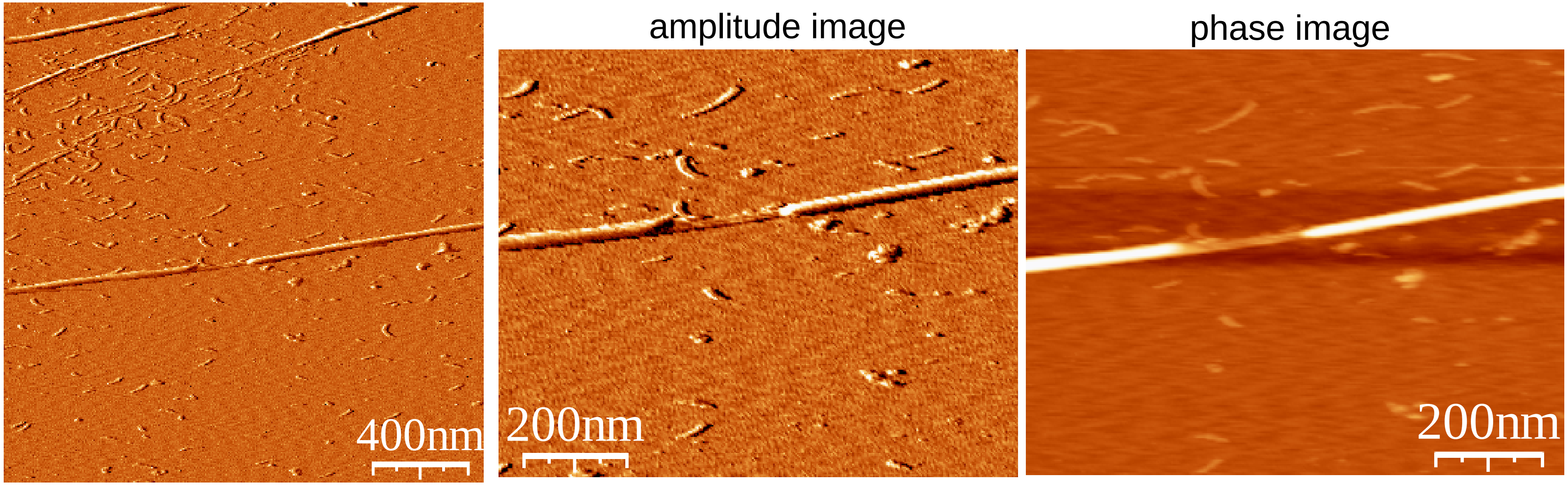


DNA extraction from a human chromosome



SEM image of the tip shows the piece of DNA

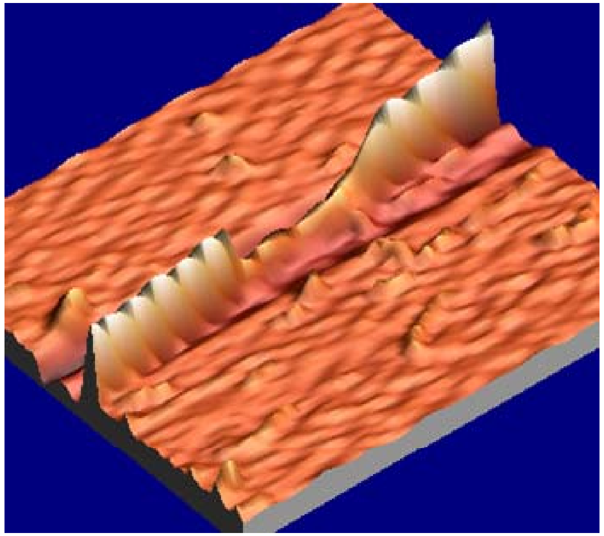
Nano-dissection of microtubules using AFM


amplitude image

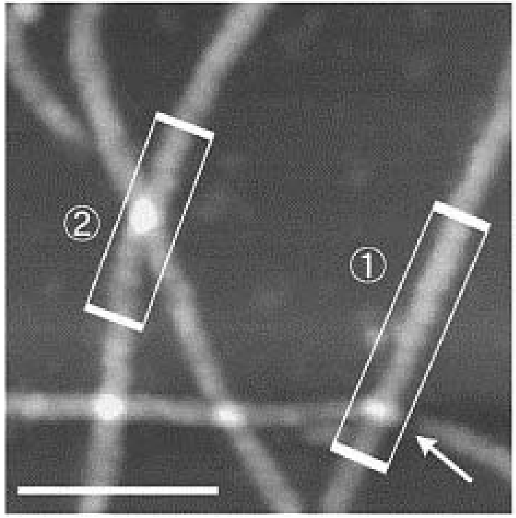
phase image

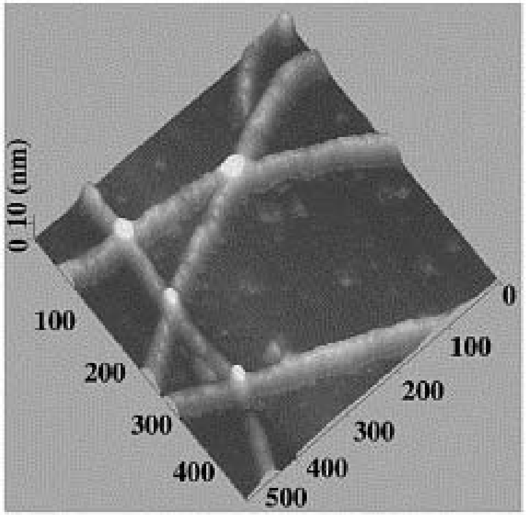
Single microtubule in buffer dissected by AFM tip

⇒ AFM to cut and shorten microtubules
to desired length for MEMS application
(e.g. MTs of defined length served as motor tracks)

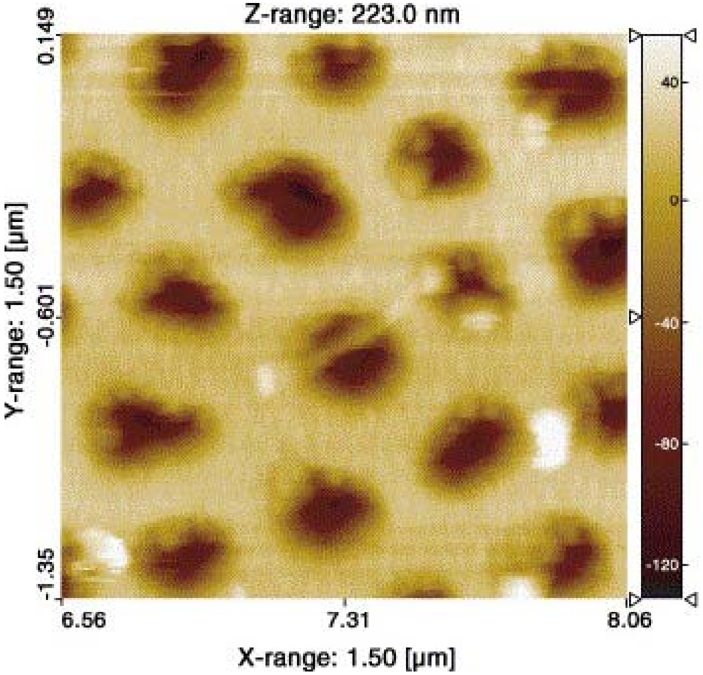

3 D image

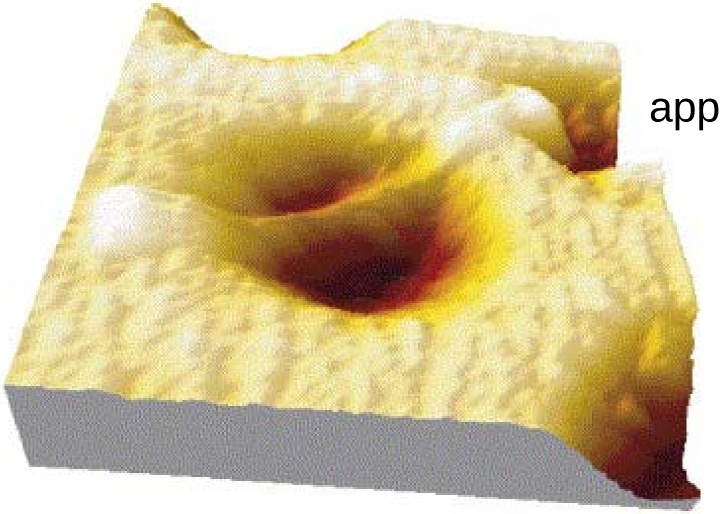
Nano-indentation: Squeezing an intermediate filament thru a nano-hole





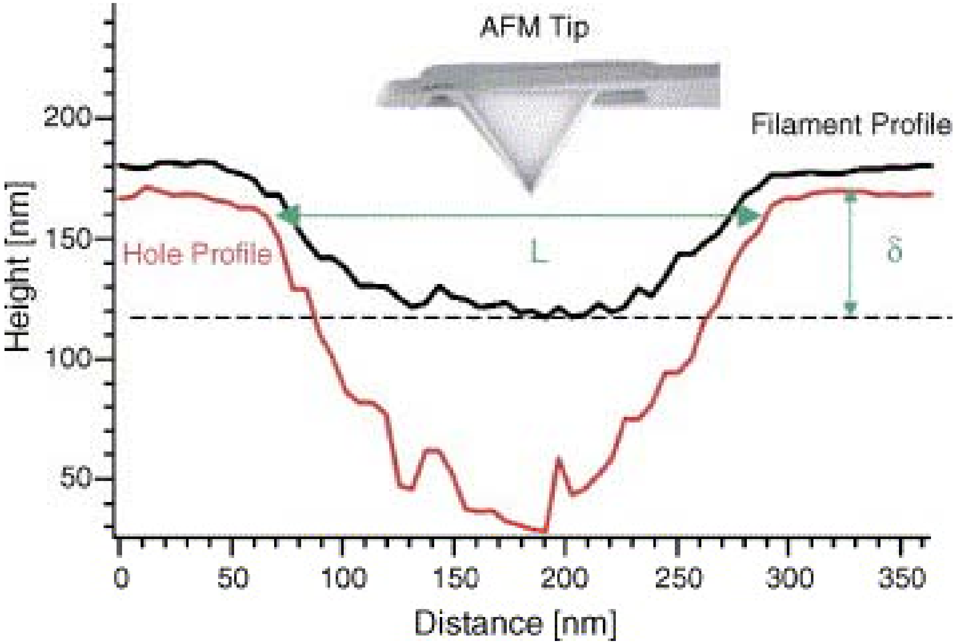
- Measuring the bending properties of a single **intermediate filament** using an AFM
- Tip elastically **deforms single filaments** hanging over a porous membrane



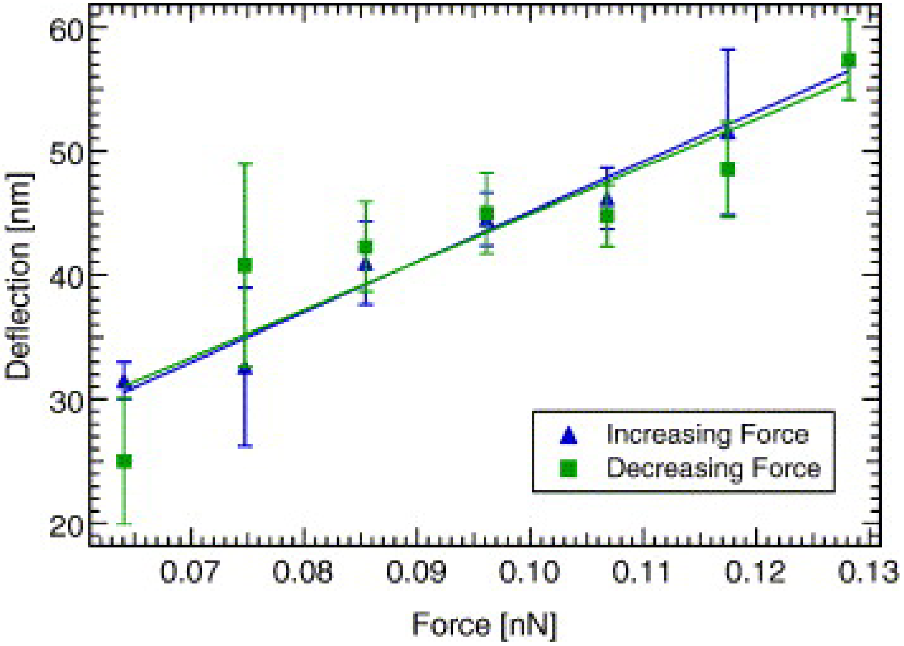


applied force **0.11 nN**

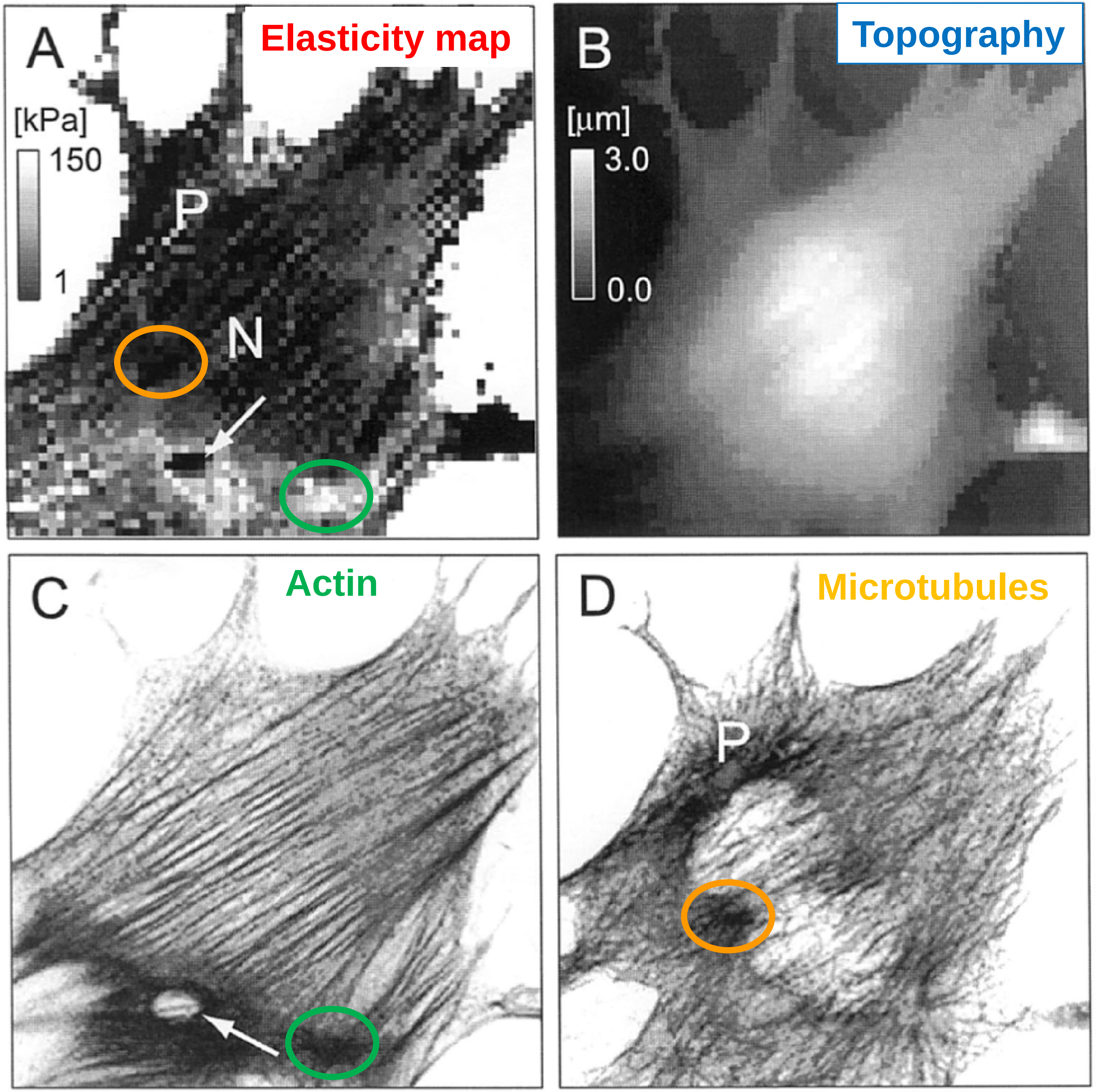
Nano-indentation: Squeezing an intermediate filament thru a nano-hole



- AFM tip pushes the IF into the hole
- From the height difference between the IF's lowest point (L) and the substrate around the hole, the **deflection** can be **calculated**



- **Deflection** of one IF as a function of the applied **force**
- E_{Bending} = 300 MPa determined from the slope of the linear fit
- Graph shows that the **filament is elastic** (i.e. it returns to its original position after the force is decreased)



Elasticity Map:

White = Stiff

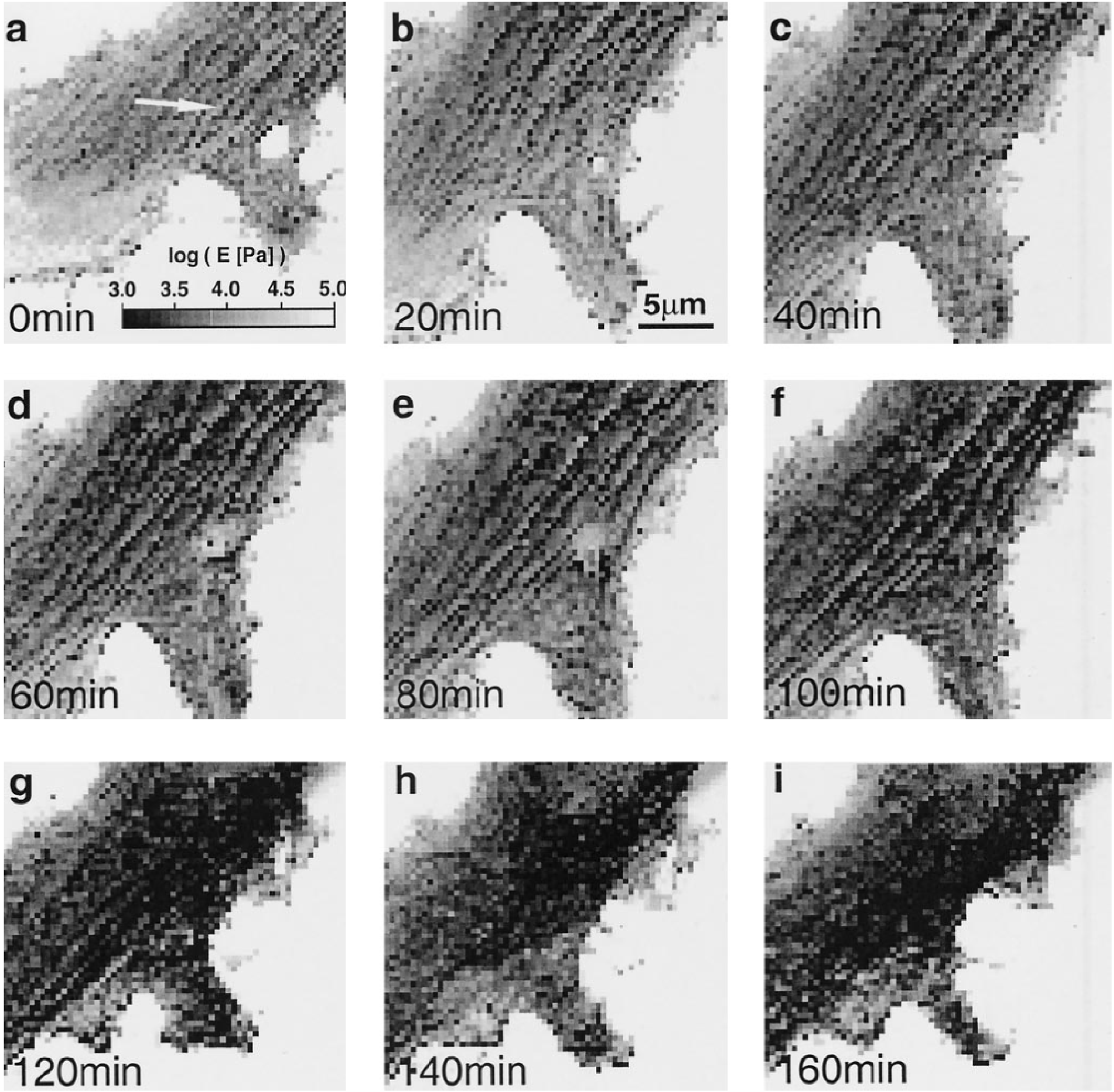
Black = Soft

Nucleus surprisingly soft (arrow A and C)

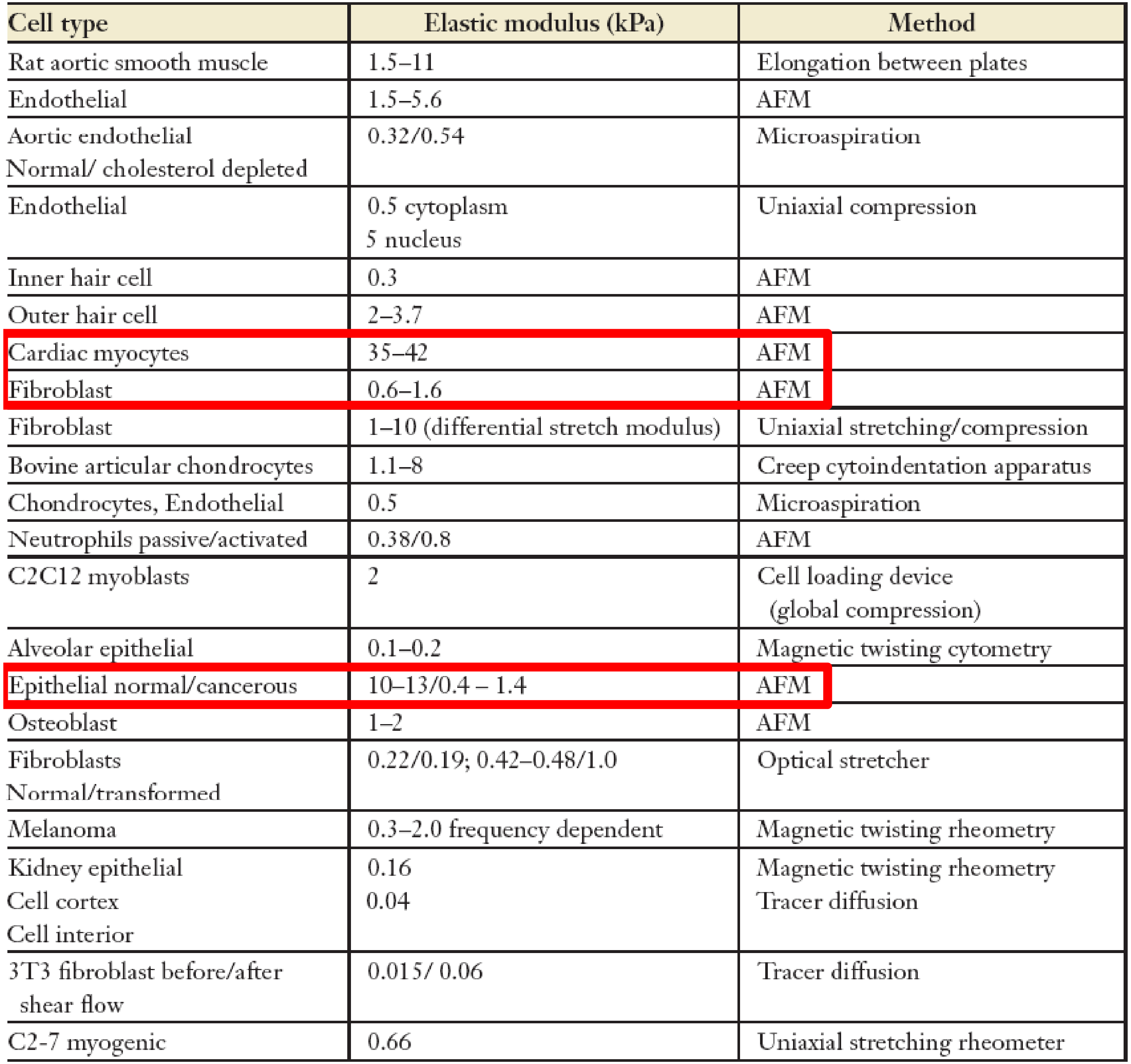
Occurrences of dense F-actin surprisingly **stiff** (A, C)

Occurrences of dense microtubules surprisingly **soft** (A, D)

Living fibroblast



The drug “cyto-chalasin” cuts actin filaments => the cell becomes softer



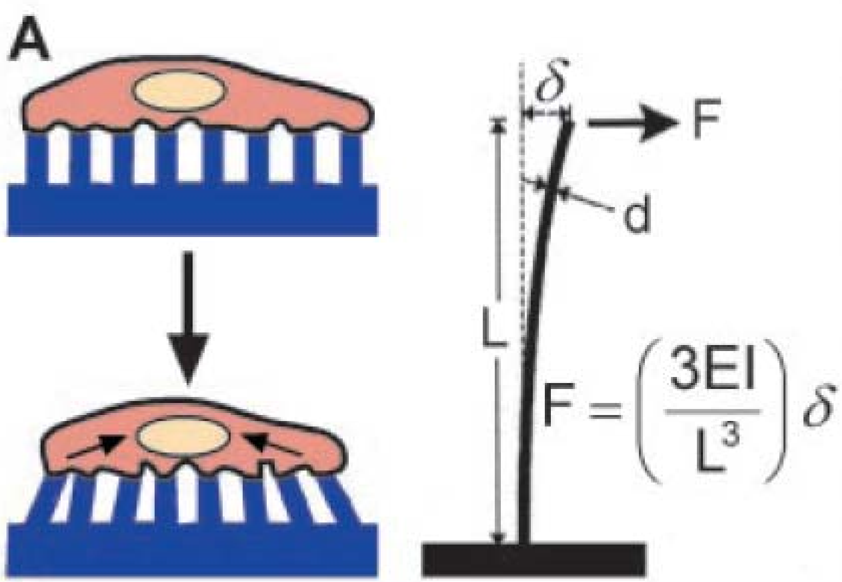
Cell type	Elastic modulus (kPa)	Method
Rat aortic smooth muscle	1.5–11	Elongation between plates
Endothelial	1.5–5.6	AFM
Aortic endothelial Normal/ cholesterol depleted	0.32/0.54	Microaspiration
Endothelial	0.5 cytoplasm 5 nucleus	Uniaxial compression
Inner hair cell	0.3	AFM
Outer hair cell	2–3.7	AFM
Cardiac myocytes	35–42	AFM
Fibroblast	0.6–1.6	AFM
Fibroblast	1–10 (differential stretch modulus)	Uniaxial stretching/compression
Bovine articular chondrocytes	1.1–8	Creep cytoindentation apparatus
Chondrocytes, Endothelial	0.5	Microaspiration
Neutrophils passive/activated	0.38/0.8	AFM
C2C12 myoblasts	2	Cell loading device (global compression)
Alveolar epithelial	0.1–0.2	Magnetic twisting cytometry
Epithelial normal/cancerous	10–13/0.4 – 1.4	AFM
Osteoblast	1–2	AFM
Fibroblasts Normal/transformed	0.22/0.19; 0.42–0.48/1.0	Optical stretcher
Melanoma	0.3–2.0 frequency dependent	Magnetic twisting rheometry
Kidney epithelial	0.16	Magnetic twisting rheometry
Cell cortex	0.04	Tracer diffusion
Cell interior		
3T3 fibroblast before/after shear flow	0.015/ 0.06	Tracer diffusion
C2-7 myogenic	0.66	Uniaxial stretching rheometer

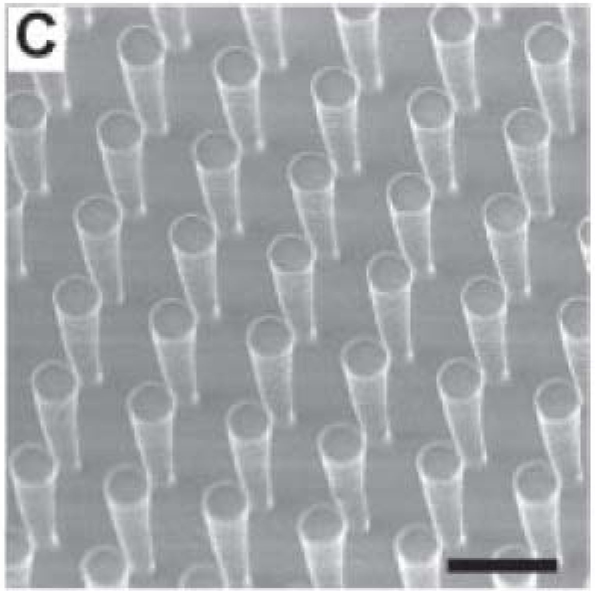
Heart cells have more actin and stress fibers

Cancer cells are less elastic

Janmey et al., 2007, Annu Rev Biomed Eng

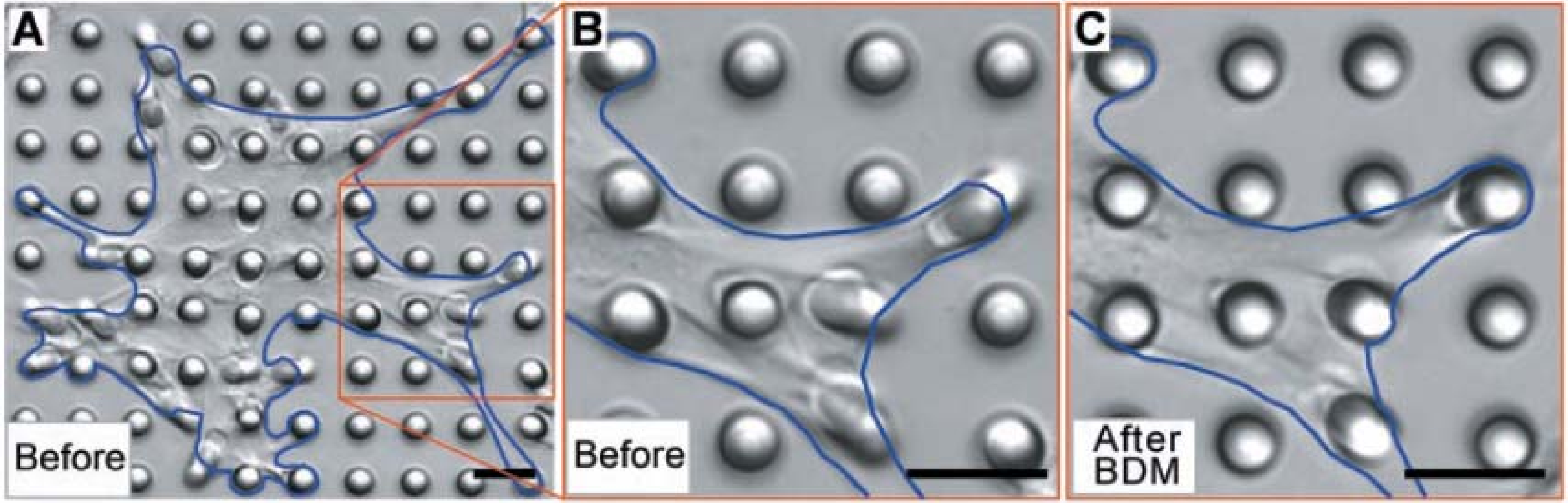
Microelectromechanical (MEMS) devices for measuring cytomechanics





Cells on microneedles

Exerted <u>force determined</u>
by <u>needle bending</u>
(need to know
spring constant)

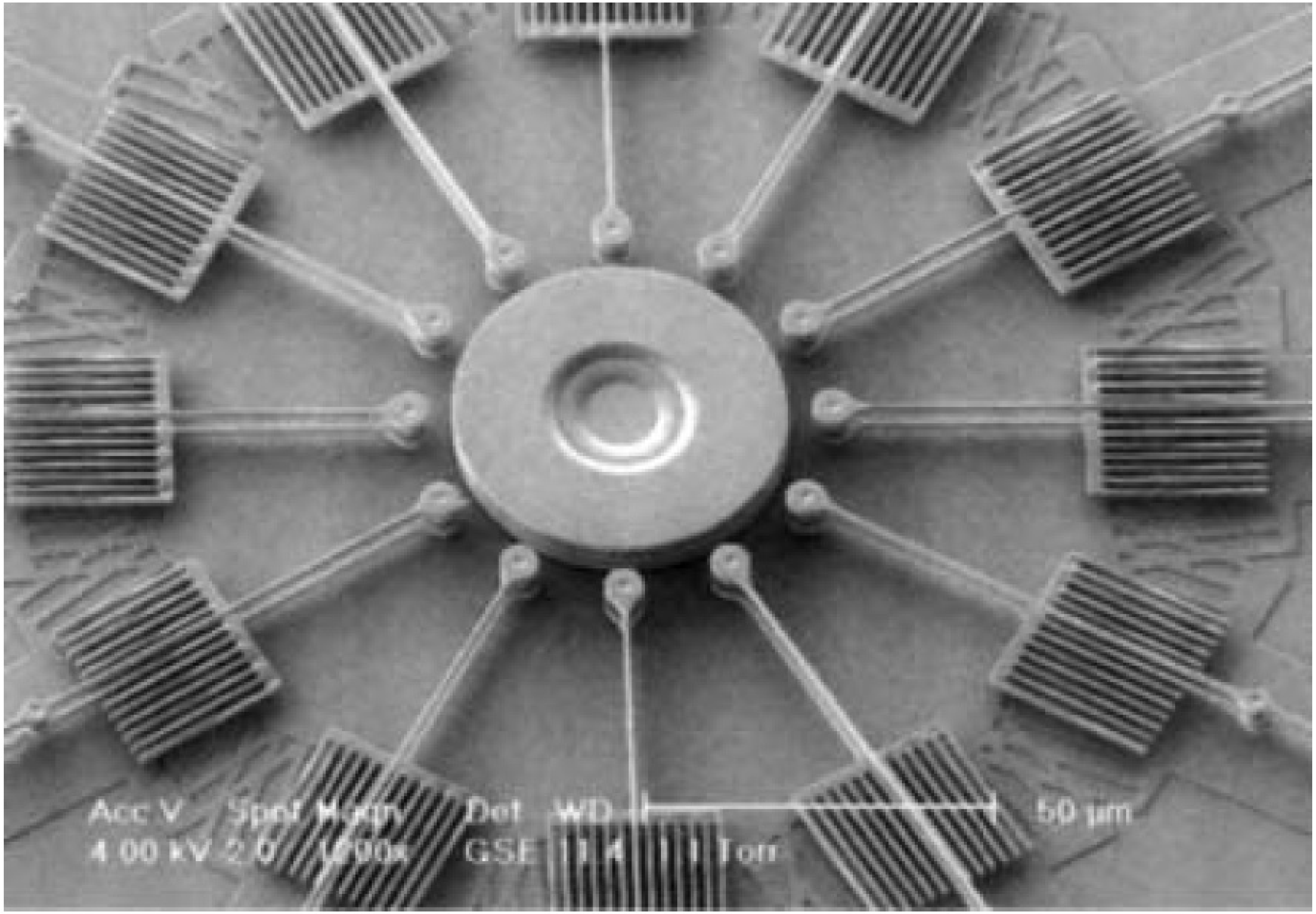


BDM inhibits actin-myosin interactions

Tan et al., PNAS, 2003

Microelectromechanical (MEMS) devices for measuring cytomechanics

- MEMS device with multiple **active and passive cantilevers** to measure forces generated by a cell at different locations
- **Localized shear forces** can be applied using the **electrostatic actuators**

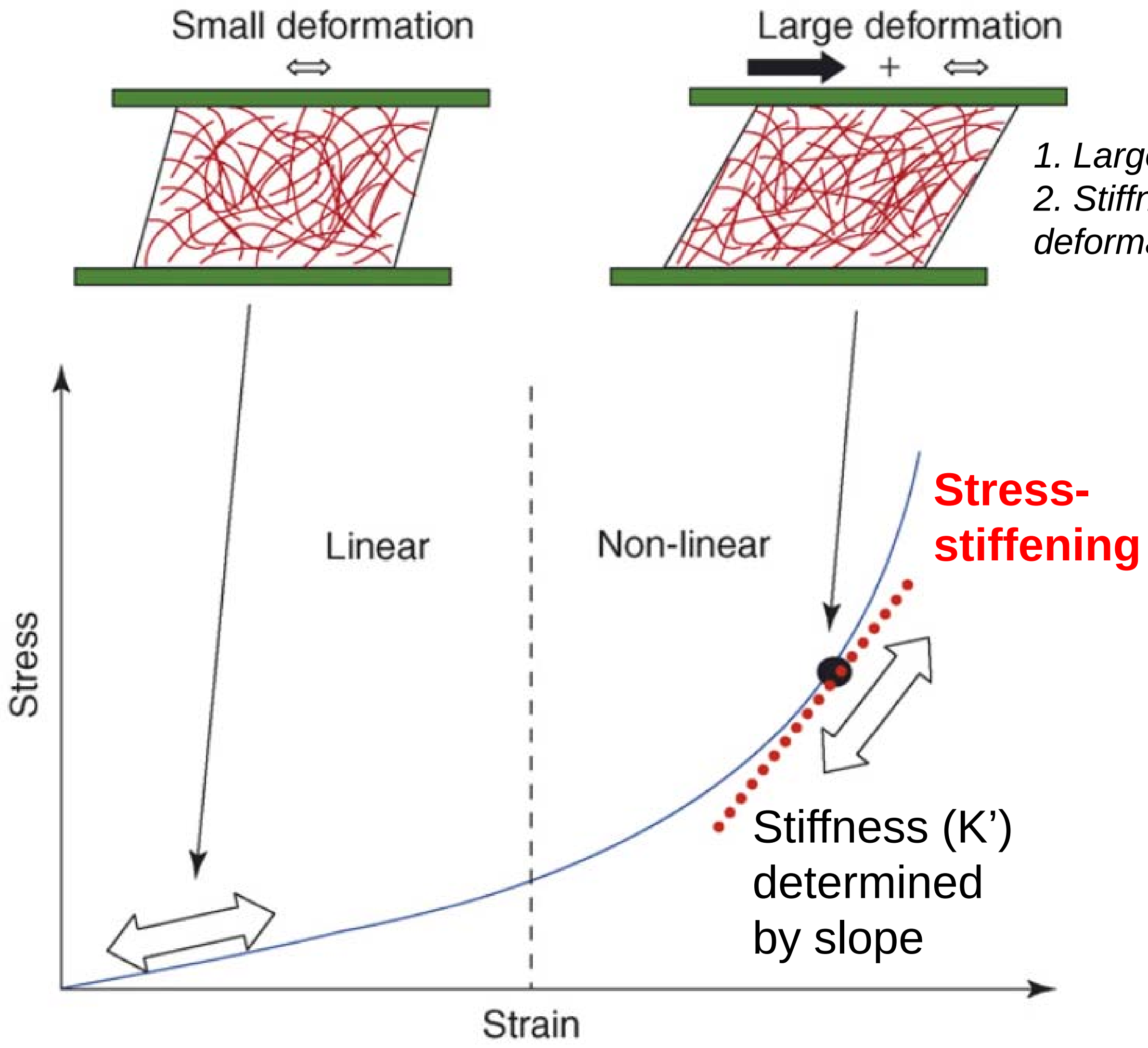


Bao and Suresh
Nat Mater., 2003

Newtonian and non-newtonian behavior of viscoelastic materials

Example from your reading material!

- Under small deformations, stress is proportional to strain: material is in **linear regime**
- Under large deformations, stress increase more rapidly: material is in **non-linear regime**

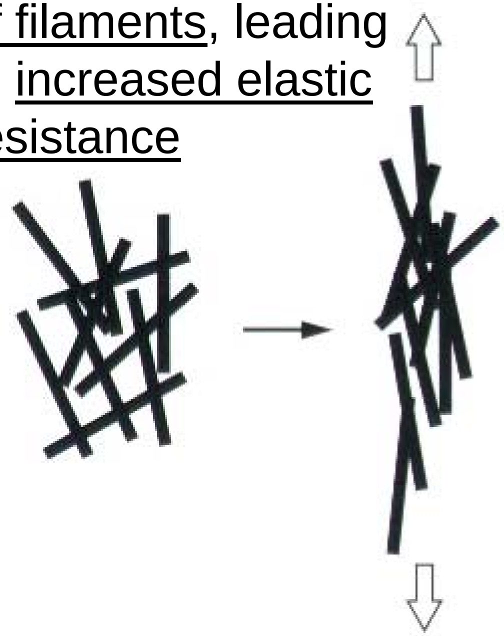


1. Large and steady deformation applied
2. Stiffness determined by applying small deformations

Stress-stiffening

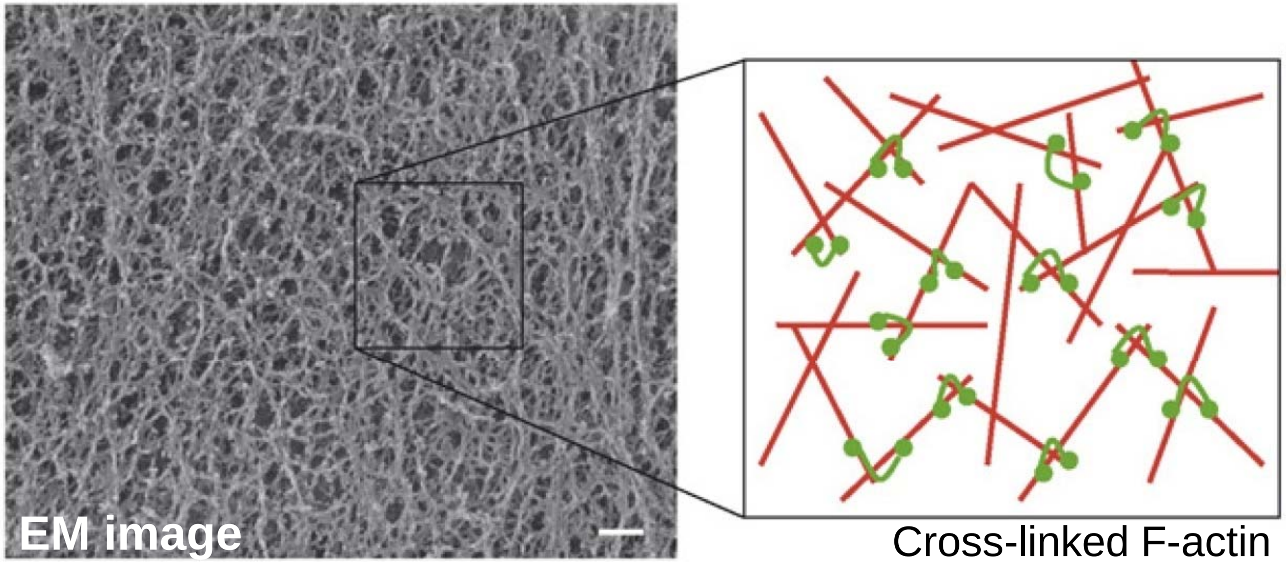
Stiffness (K’) determined by slope

Application of tension leads to alignment of filaments, leading to increased elastic resistance

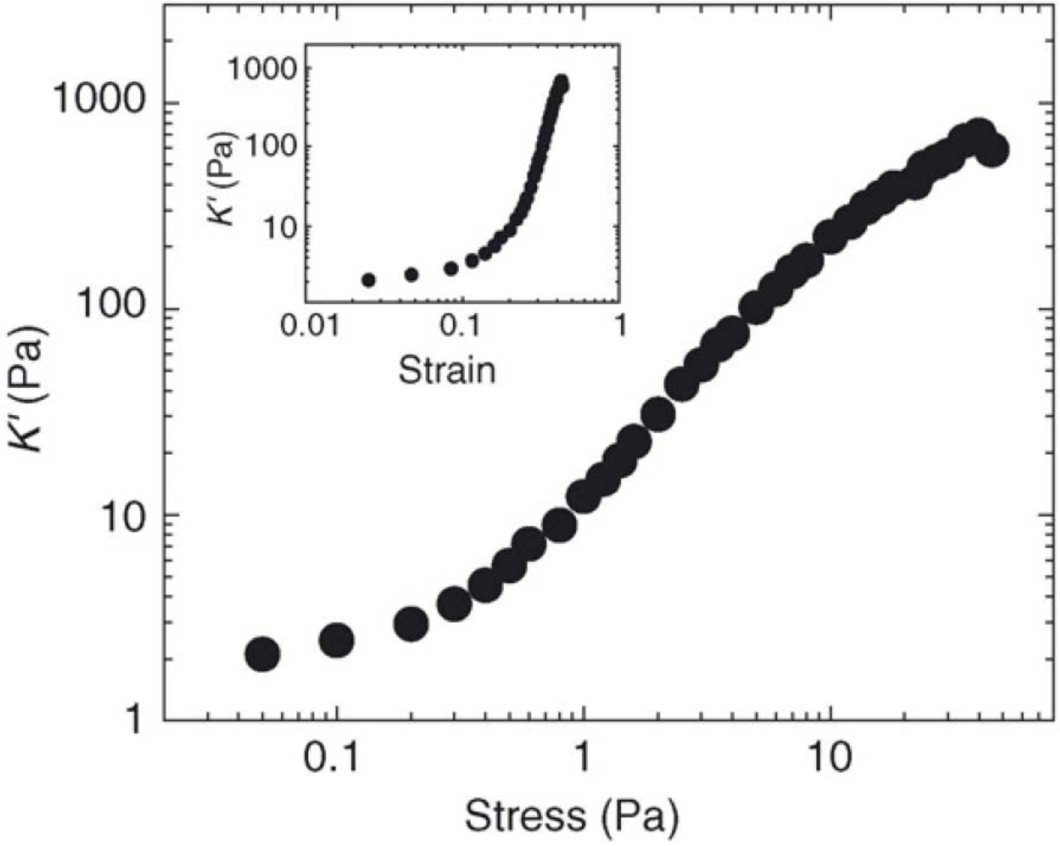


Strain-Stiffening of cross-linked actin-networks

Example from your reading material!



Cross-linked F-actin



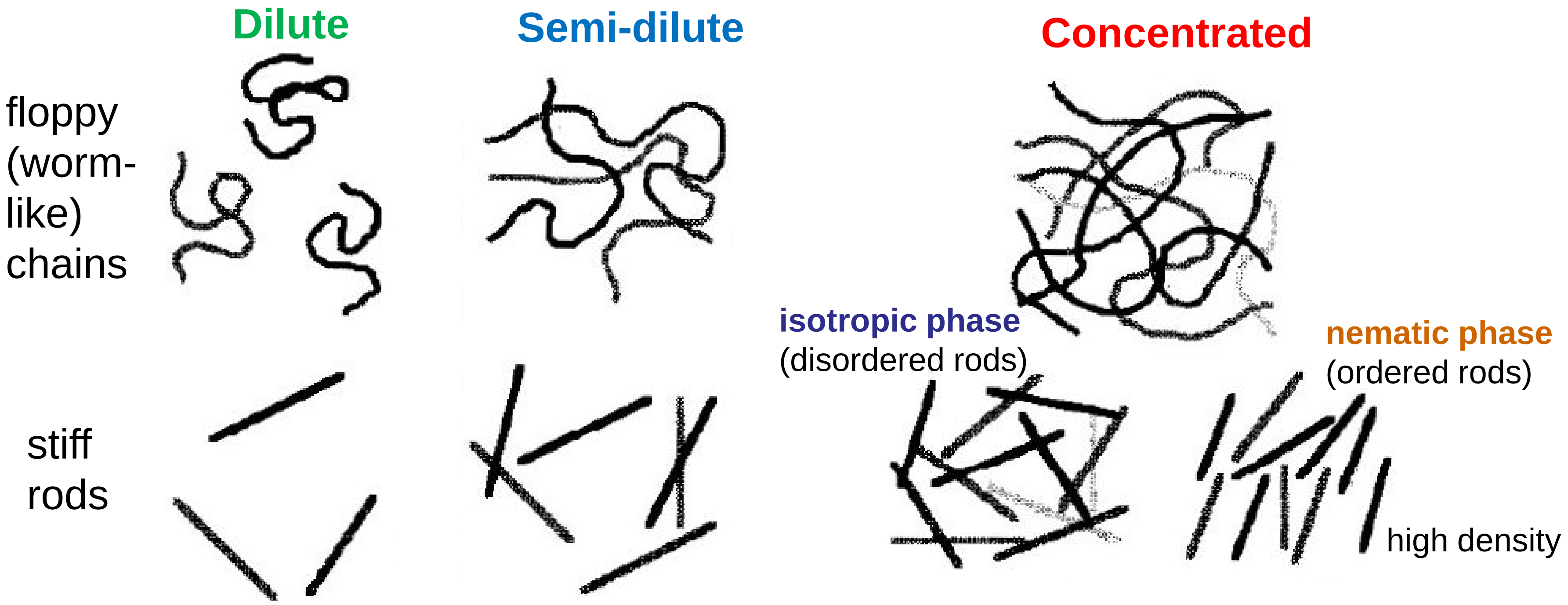
Experiment: Rheology of actin

Dramatic **stiffening per strain** (inset) of cross-linked f-actin

If stress keeps increasing, the whole **network brakes**

How does the polymer concentration affect the viscosity?

Polymer solutions can be classified based on their concentrations



Dilute

Semi-dilute

Concentrated

floppy (worm-like) chains

stiff rods

isotropic phase
(disordered rods)

nematic phase
(ordered rods)

high density

Polymer density affects viscoelastic properties:

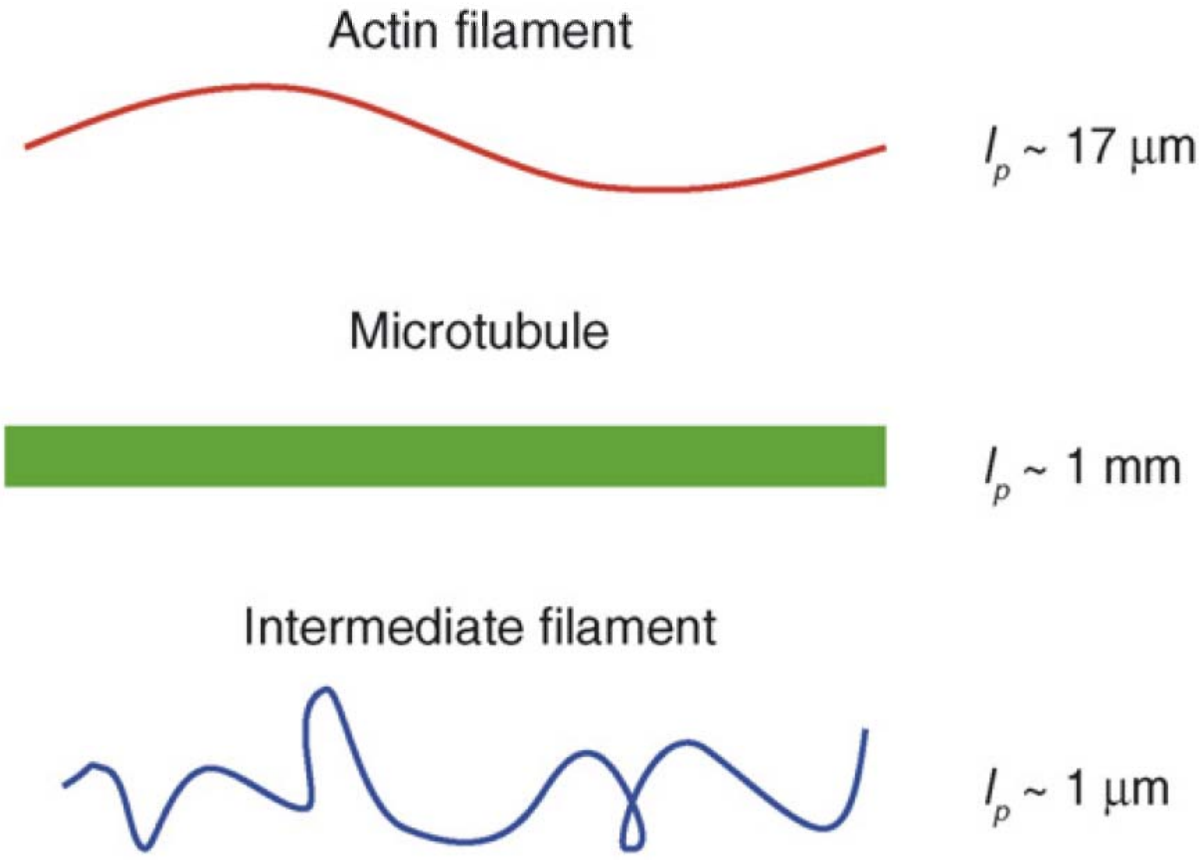
- **Dilute regime**, stiff filaments can rotate largely without colliding: viscosity is close to that of the solvent (buffer)
- **Highly concentrated solutions** do not allow filament to rotate:
isotropic phase: ***higher*** viscosity, **nematic phase**: ***lower*** viscosity
- In-between is the **semi-dilute regime** which is characteristic for many biopolymer solutions

David Boal, Mechanics of the Cell, 1st Ed.

Other ways to determine the stiffness or floppiness of polymers?

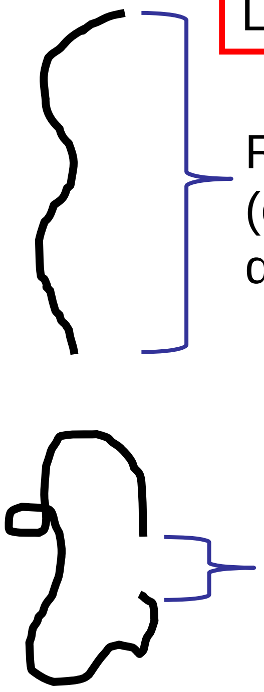
Example from your reading material!

- Stiffness/floppiness of polymers can vary to a large extent
- The persistence length (Lp) is related to a polymer’s flexibility
- **MT** are very **stiff** and have a large persistence length (1 mm)
- **IFs** are very **floppy** with a low persistence length (1 µm)
- Other examples: DNA = 50 nm / Spaghetti = 10 cm



Approximation of **persistence length** L_p

$$\boxed{L_p = R^2/L_c}$$



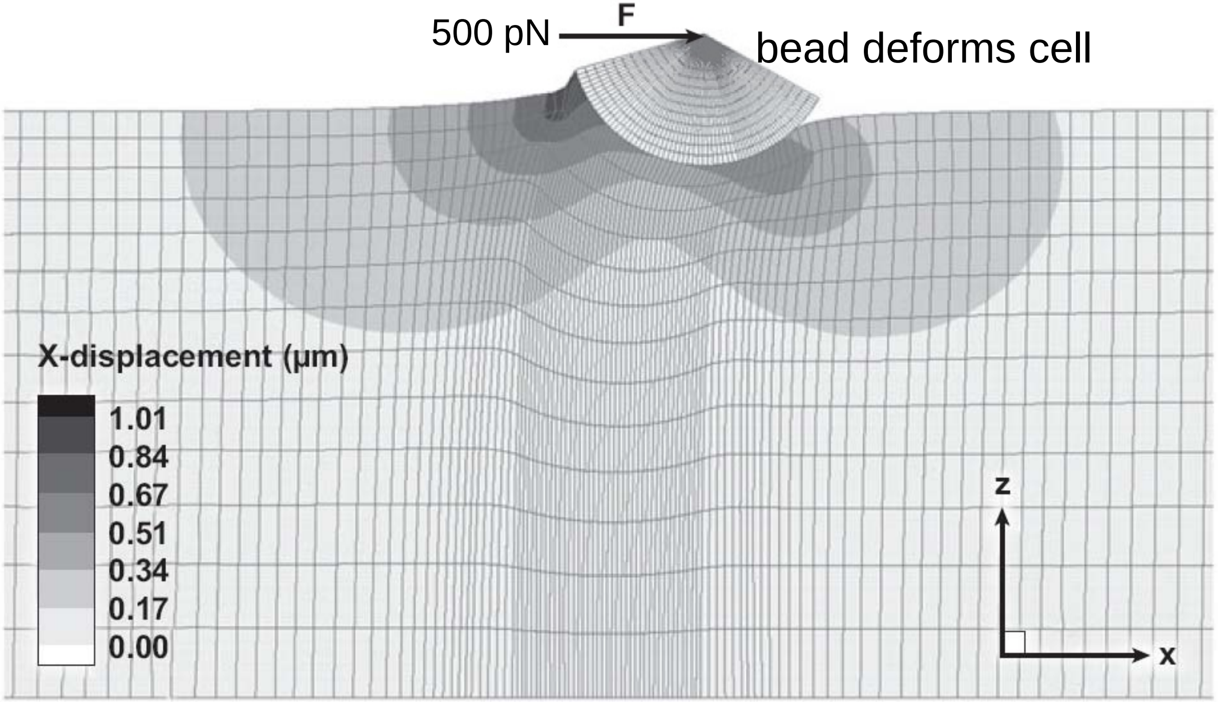
R = large (end-to-end distance)

R = small

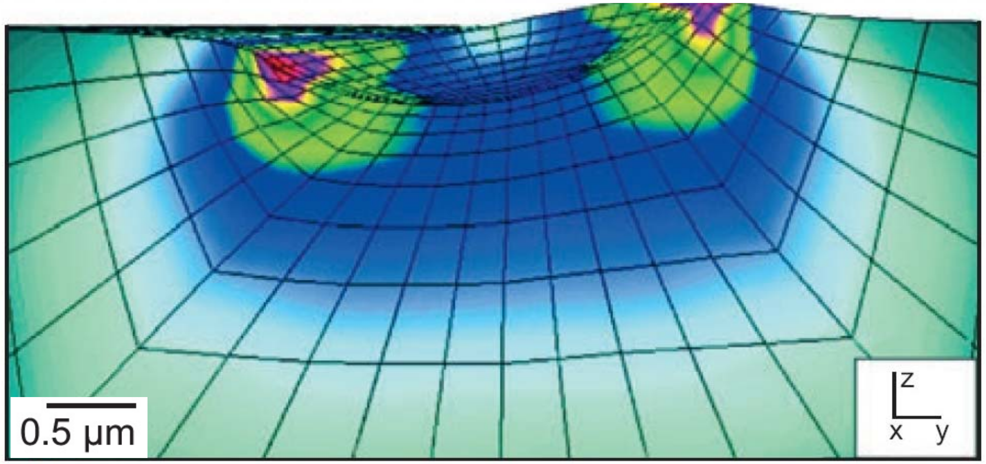
L_c = contour lenght

Mathematicians meet Biologists: Stress-field in a model cell

Finite element modeling: provides maps of how forces applied to a cell are transmitted through its interior


500 pN

bead deforms cell

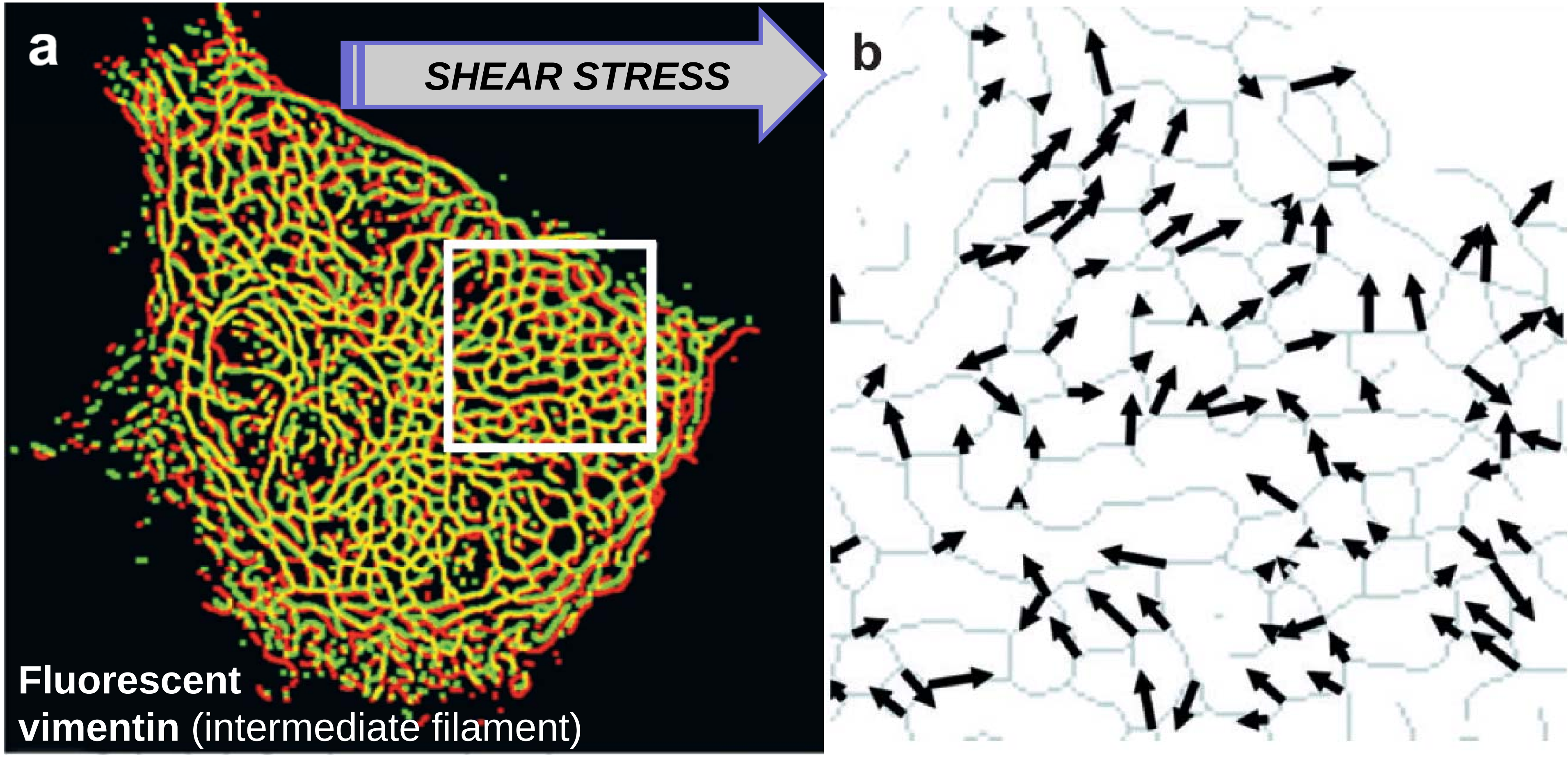


- **Forces** are **transmitted uniformly** but only a few microns away from the point of force application
- Conclusion: largest cytoskeletal deformation near the edges of the bead
- Does this **affine deformation** model really apply to the cell?

Theory and experiment: Response of the cytoskeleton to shear force

Non-affine deformation: because interior of the cell is **anisotropic**, cell deformation does not respond to shear stress as predicted for a homogenous viscoelastic material

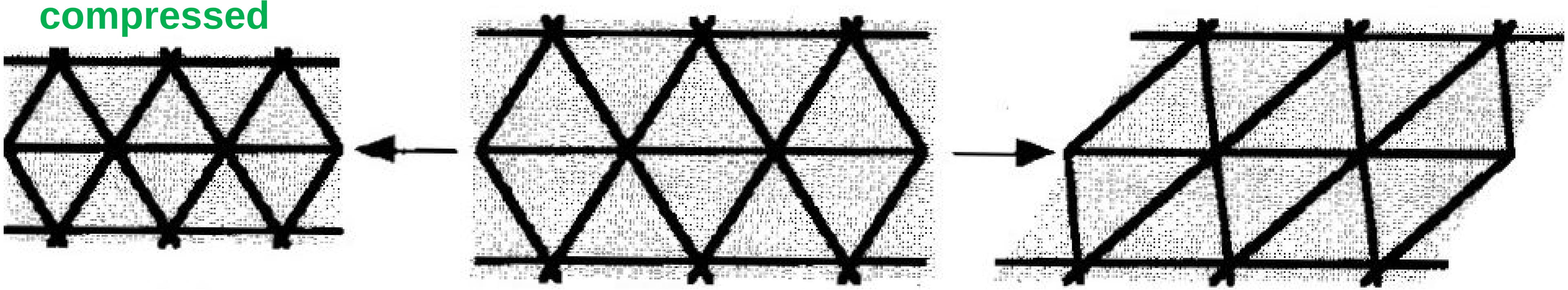
Microscopic displacements of vimentin do not follow the direction of applied shear stress



Fluorescent vimentin (intermediate filament)

RED = **BEFORE** stress, **GREEN** = **AFTER** stress, **YELLOW** = Zero displacement

Difference between shear stress and compression



compressed

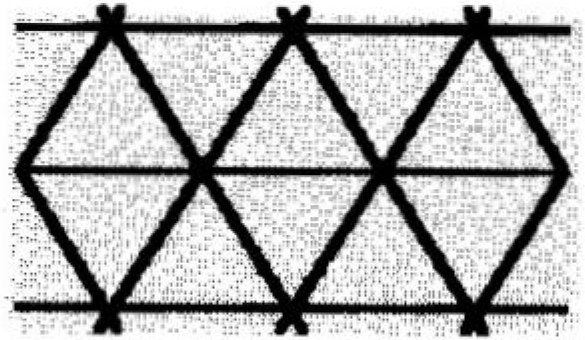
undeformed

sheared

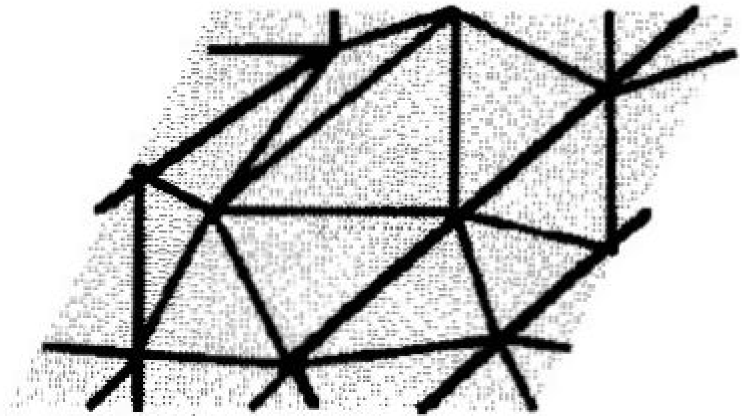
network area changed but <u>no changes in internal angles</u>

<u>internal network angles changes</u> but **area unchanged**

Effect of **thermal fluctuations**



Zero-temperature network

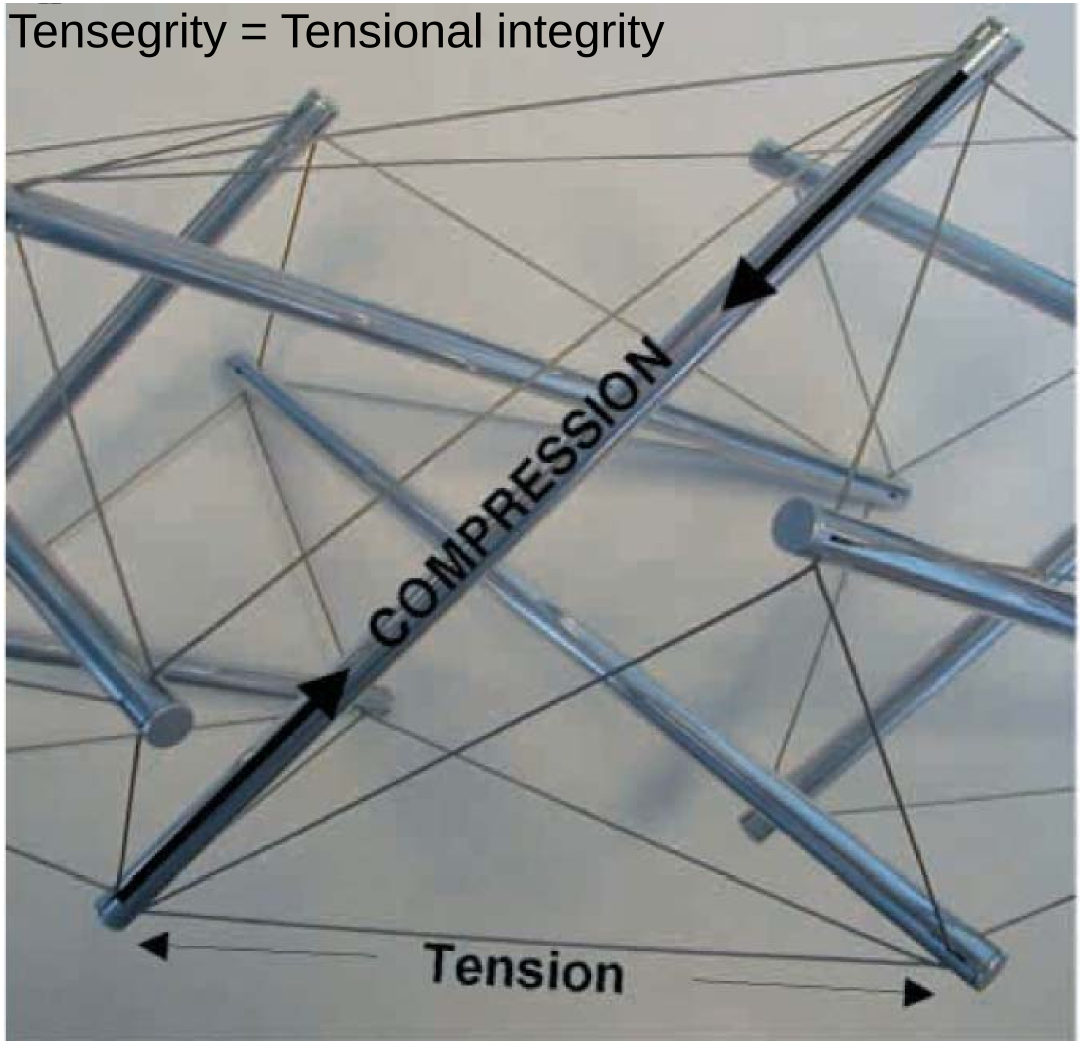


Network becomes more erratic similar after applying a two-dimensional stress

David Boal, Mechanics of the Cell, 1st Ed.

Tensegrity model: A balance between compression and tension

Experimental results on **non-uniform behavior of the cell** is consistent with the tensegrity model (**microtubules** = **compression** elements; **actin** = **tension** elements)


Tensegrity = Tensional integrity

- Tensegrity model **focus on the geometry of the network elements** and the interplay of tension and compression
- "**Tensegrity systems** keep their structure by **continuous tension** rather than by continuous compression (e.g., stone arc)"

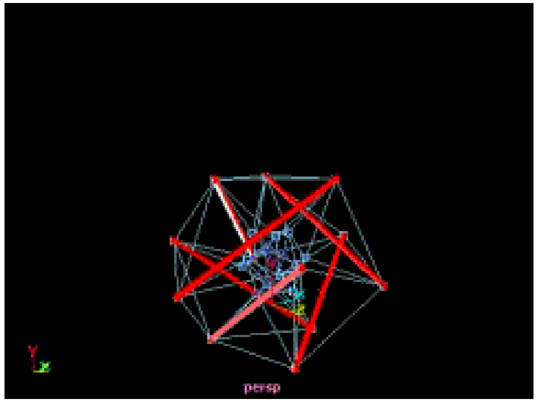
R. Buckminster Fuller, 1961


Stone arc

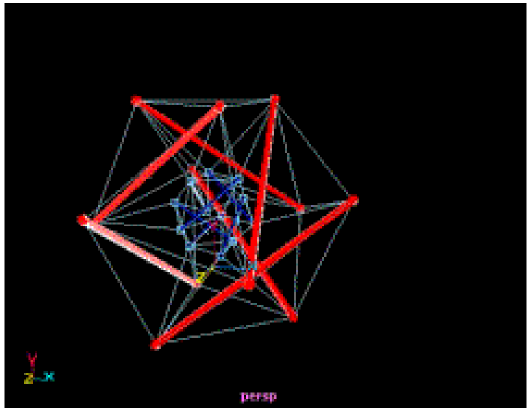
Computer model of cellular tensegrity

Computer model shows how hierarchical tensegrity structures, such as a cell with a nucleus behave when pulled, sheared and stretched

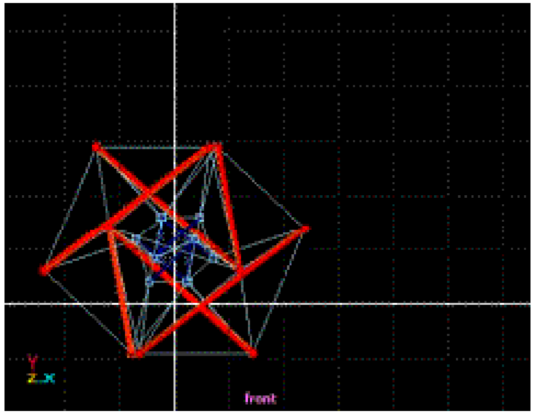
Pull



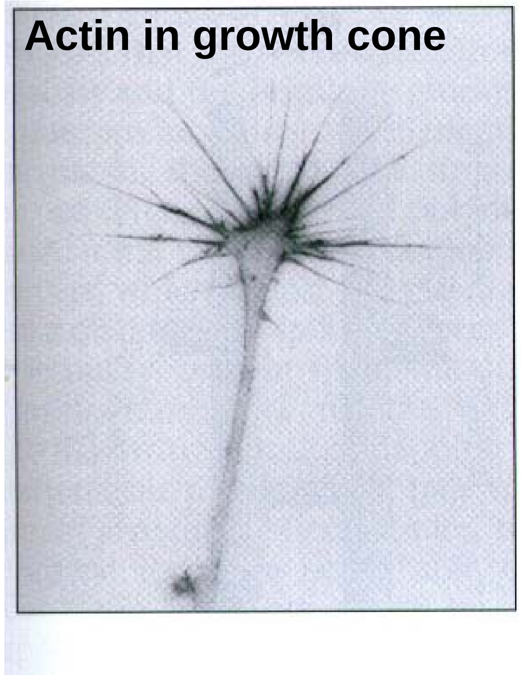
Shear

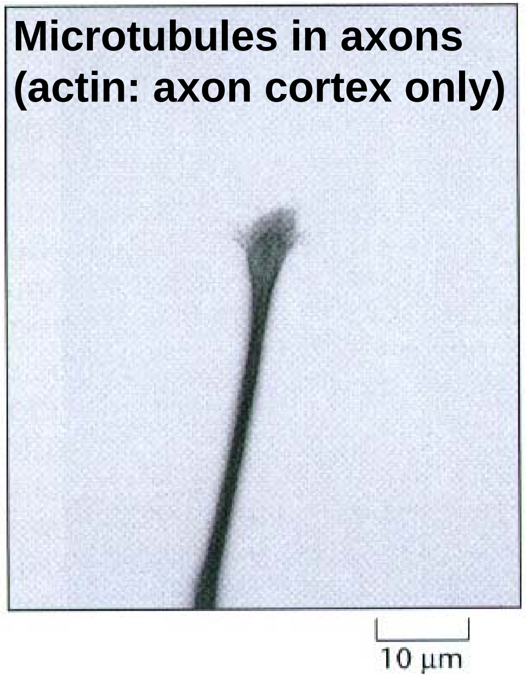


Stretch

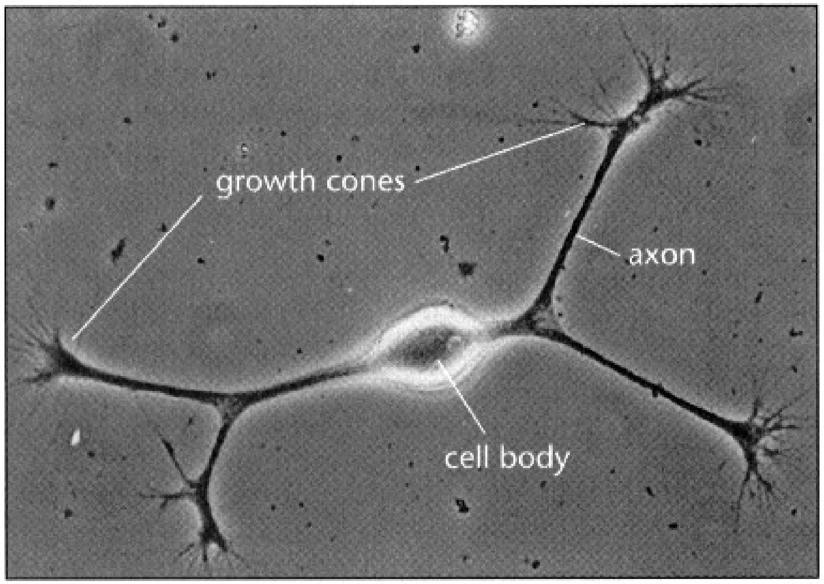


Tensegrity model explains retraction of neurons after drug treatment

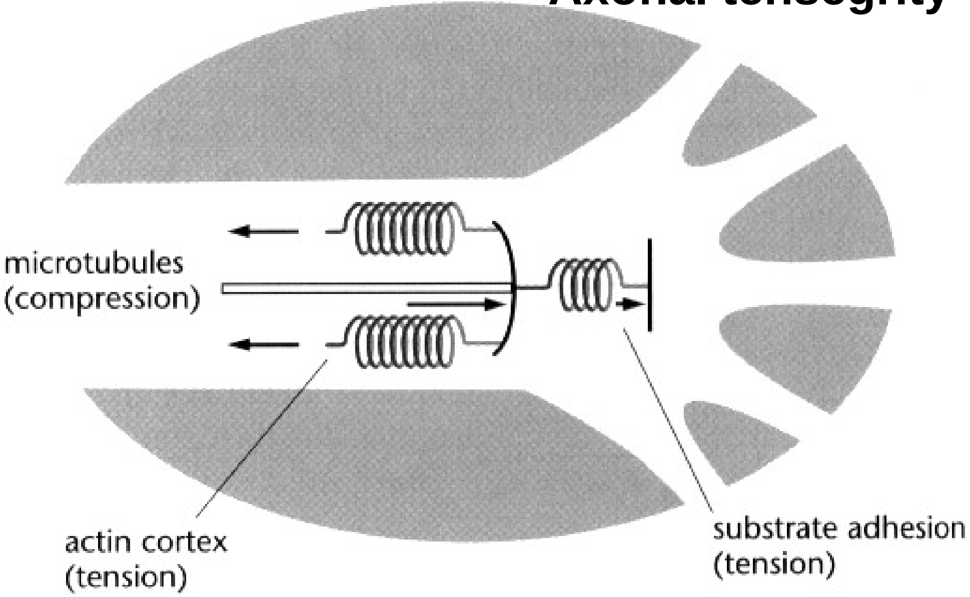

Actin in growth cone


Microtubules in axons (actin: axon cortex only)

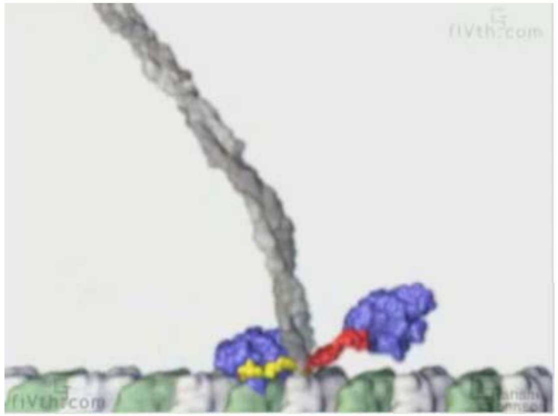
Abrupt <u>axon retraction</u> observed after <u>nocodazole treatment</u> (depolymerizes microtubules):
⇒ **Mechanical balance of the axon** is provided by the **tension** of **actin** and (antagonistic) **compression** forces provided by **microtubules**

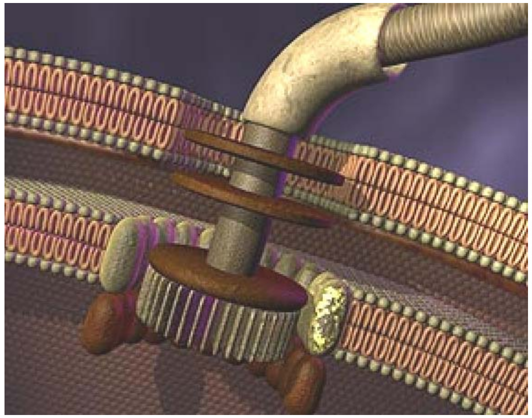


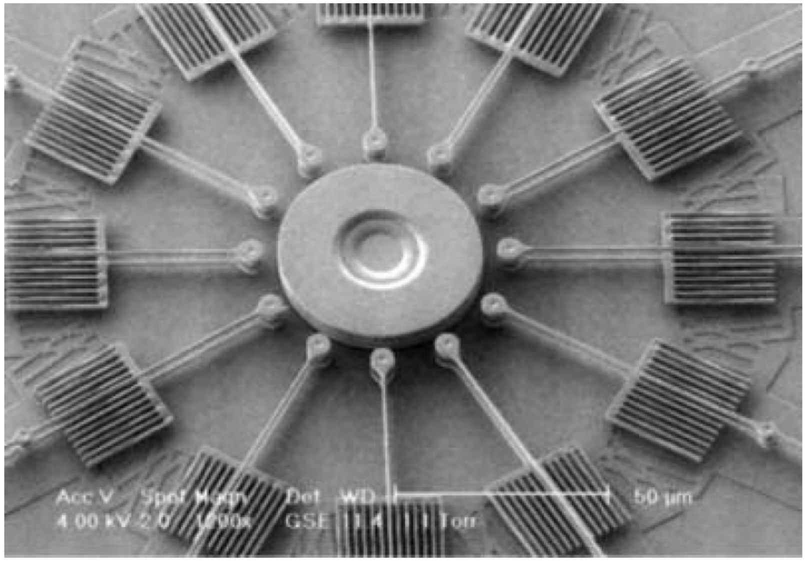
Axonal tensegrity



Thank you for your attention!







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