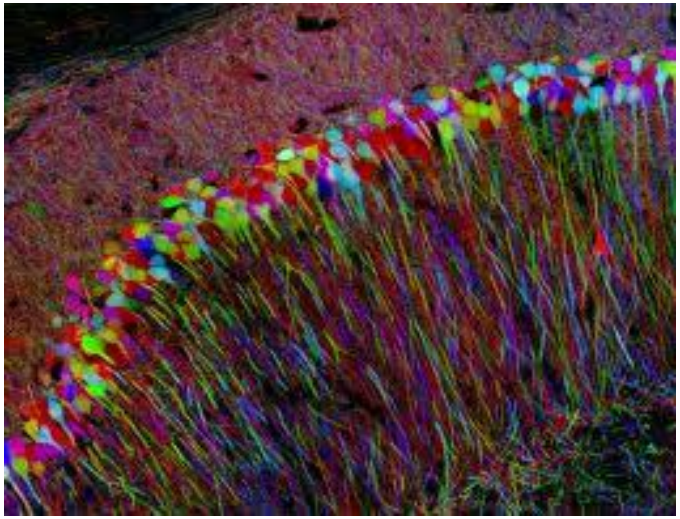
Lecture 01

Quantitative Methods for Biological Systems



Course Instructor

Halil Bayraktar

MBG Department

hbayraktar@itu.edu.tr

Office Hours: Tuesday 13:30-14:30 pm

MOBGAM 308

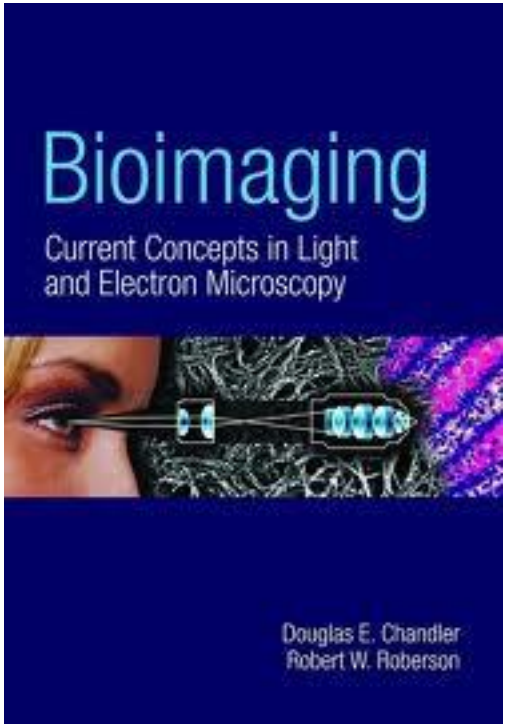
Course web site
Password : microscopy

https://b2lab.wordpress.com/

Course Texts

- Check online resources

- Readings will be assigned



1. Quatitative Bioimaging by Raimund J. Ober
2. Bioimaging: Current concepts in light and electron microscopy by Douglas E. Chandler
3. Physical Biology of the Cell by Rod Phillips
4. Imaging the future of bioimage analysis, Nature Biotechnology Perspective By Erik Meijering, Anne Carpenter, Hanchuan Peng, Fred Hamprecht and Jean-Christophe Olivo Marin
5. Optogenetics: the age of light, Michael Hausser

Weeks	Topics
1	The architecture of the cell and its organelles
2	Biology in the context of physics and mathematics; important problems in biology and discussion on solutions
3	Light, compounds of microscope, resolution, diffraction and its applications in microscopy
4	Fluorescence Microscopy (TIRF microscopy, confocal microscopy, epifluorescence)
5	High resolution microscope methods
6	Applications of fluorescence methods in biology (FRET imaging)
7	Functional imaging in biology, genetically encoded fluorescent proteins
8	Neuro imaging methods-Optogenetic methods
9	Introduction to digital image analysis
10	Quantitative methods for imaging and biology datasets
11	Image segmentation, counting and applications in bioimage analysis
12	Cell tracking techniques and algorithms in biology
13	Motility dynamics of cells and quantitative methods
14	Regression analysis in biology

Exams and grades

Exam Dates and Places:
To be announced later.

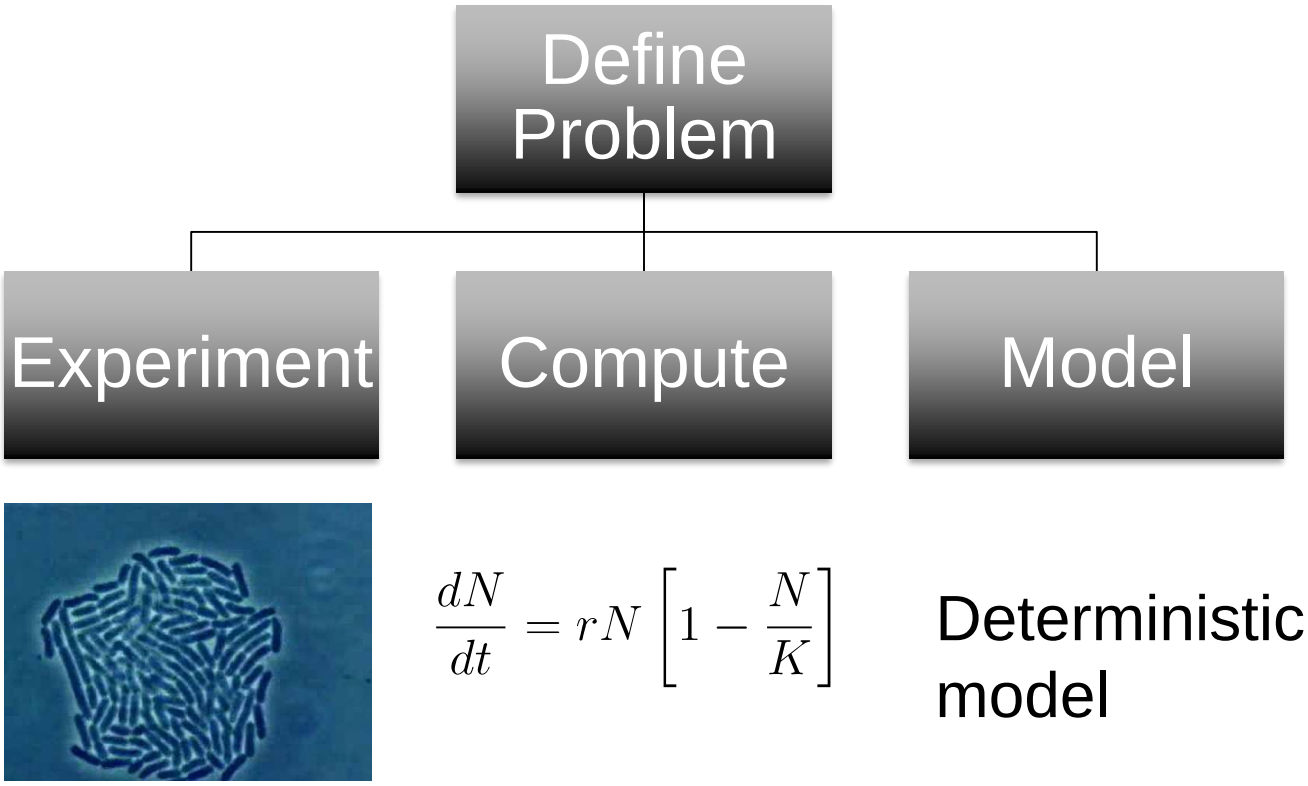
Your grade will be based on the following grading scheme

	Plan
midterm	60 %
final	40 %

Needle in a haystack problem in biology

How do we solve biological problems?
What tools do we need?

Microscopy and Computing



We will explore how scientists take images with microscopy and create unusual solutions, breakthroughs.

Microscopy is an important tool to study biology.

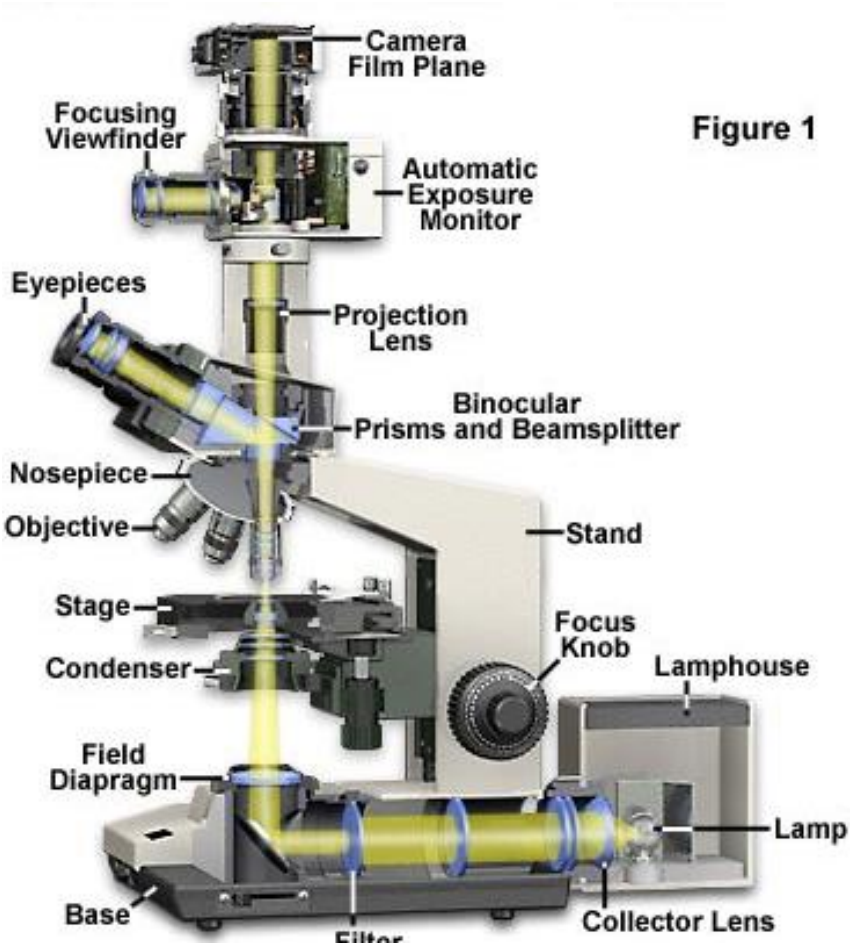
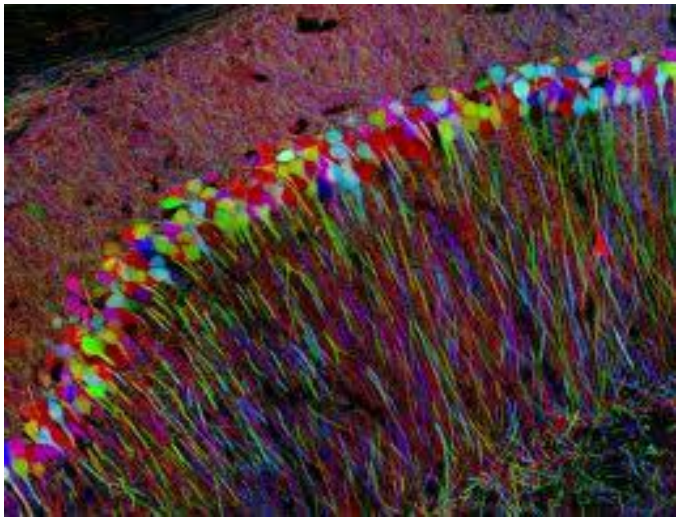
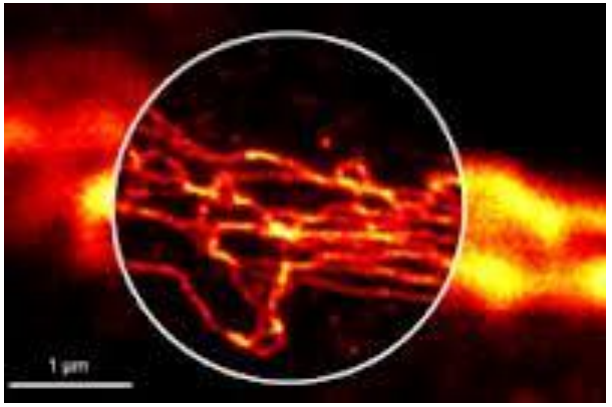


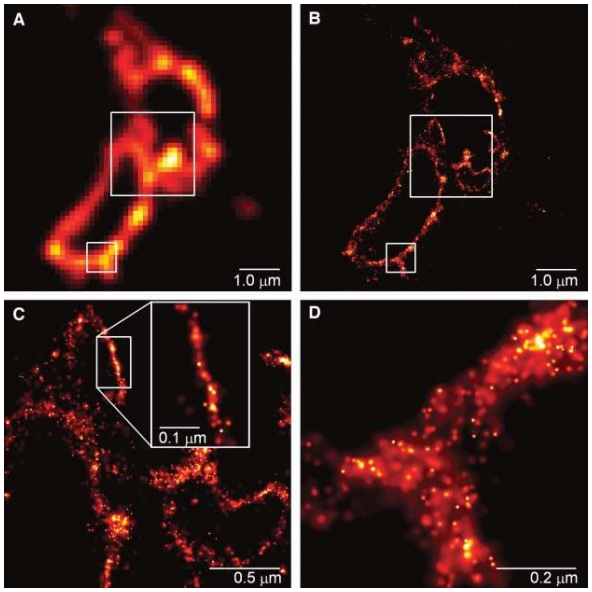
Figure 1



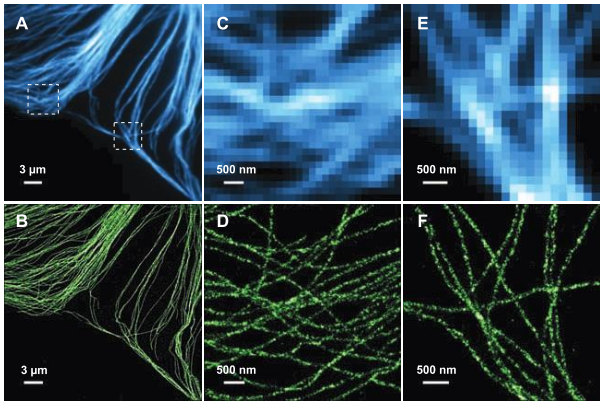


High resolution microscopy

1. Photoactivated localization microscopy (PALM),
Invented by Eric Betzig, Chemistry , Jenalia farm



with a brief laser pulse at 405 nm and then imaged at 561 nm until most are bleached. This process is repeated many times until the population of inactivated, unbleached molecules is depleted.



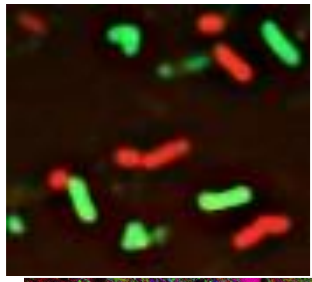
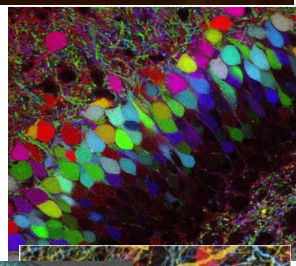
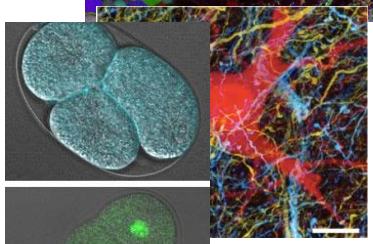
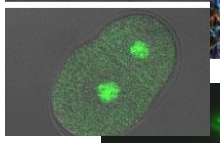
2. Stochastic optical reconstruction microscopy (STORM)
Invented by Xiaowei Zhuang, Chemistry , Harvard University

achieved 20- to 50-nm resolution in the far field and promise to preserve the inherent noninvasive imaging capability of optical microscopy.

Motivation

-Biological research depends on <u>preparation of various gene products, and their expression in cells and tissues</u>.

neurons
pancreatic islet beta cells
cardiomyocytes, kidney cells
E. coli, yeast
C-elegans, fruit fly's, mouse etc.

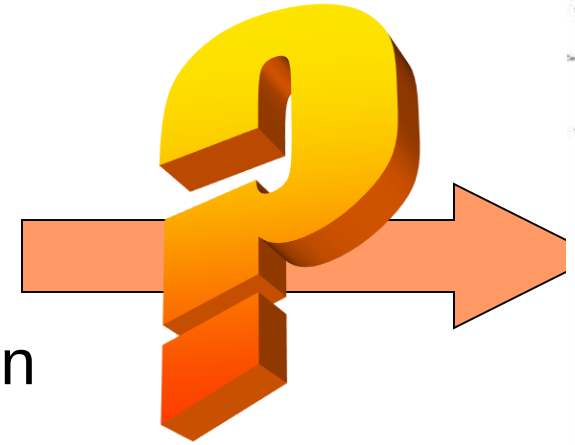
- We'd like to understand how protein activities are coupled to cellular function in cancer and brain disease.
- We can measure protein activity using various fluorescence imaging, molecular biology techniques etc. These methods provide a quantitative information about gene expression.
- The ability to optically monitor changes in protein expression levels, oxidative stress, genetic changes with a genetically encoded sensor is *incredibly* awesome!

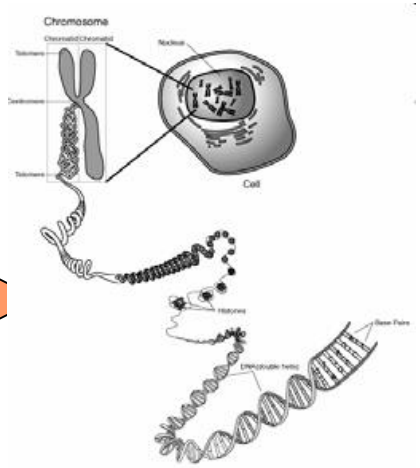
Biophysics : Quantitative tools

DNA

RNA

Protein





The Central Dogma of molecular biology:

Copy numbers of key molecules per cell:

	DNA	mRNA	Protein
Bacterial cell	**1 or 2**	**A few**	**1- 10^4**
Eukaryotic cell	**2**	**1- 10^3**	**1 - 10^6**

We seek a quantitative description of biological processes and phenomena. A description founded on fundamental physical laws.

Study of cell biology in the context of mathematics and physics.

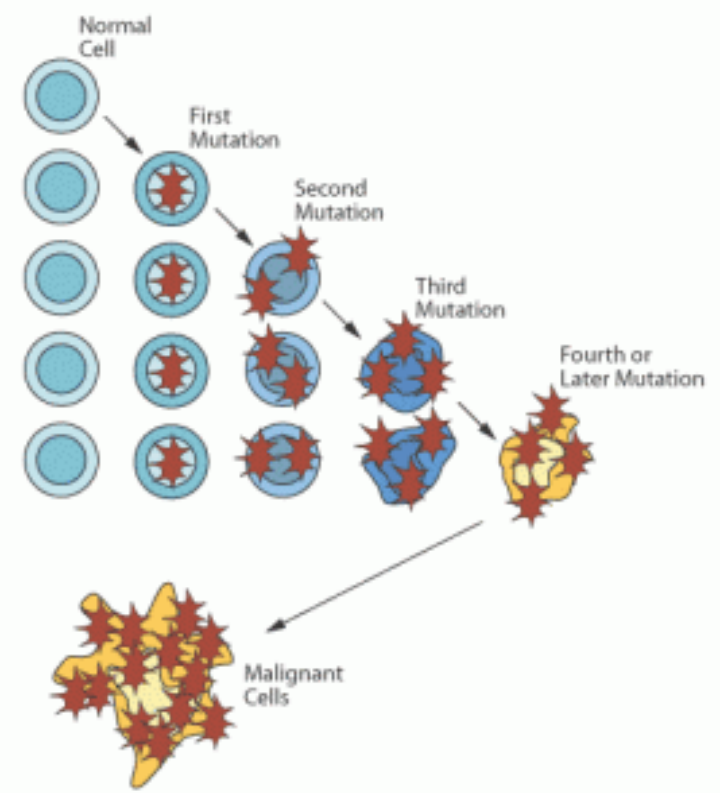
1. CANCER

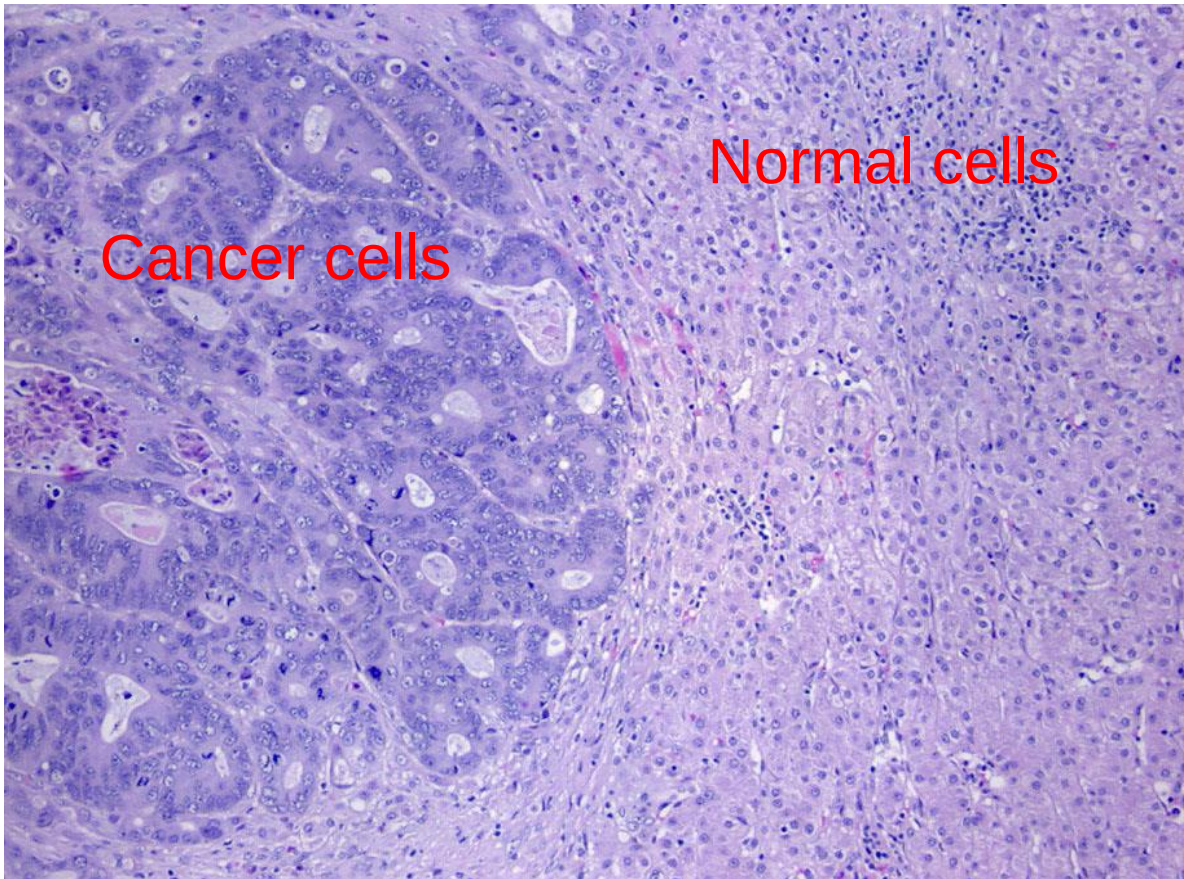
- It is the disease in which the cells acquire new capabilities
- It is the second leading cause of death in the Turkey. Half of men and one third of women in Turkey will develop cancer during their lifetimes.
- Skin, lung, colon and prostate cancers are the most common types.

Static data ⟶ Dynamics Information

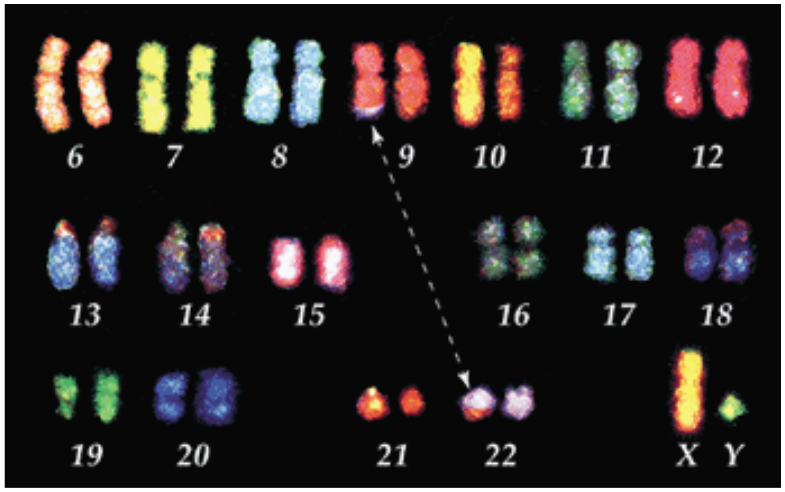
Past 50 years

Next 20 years





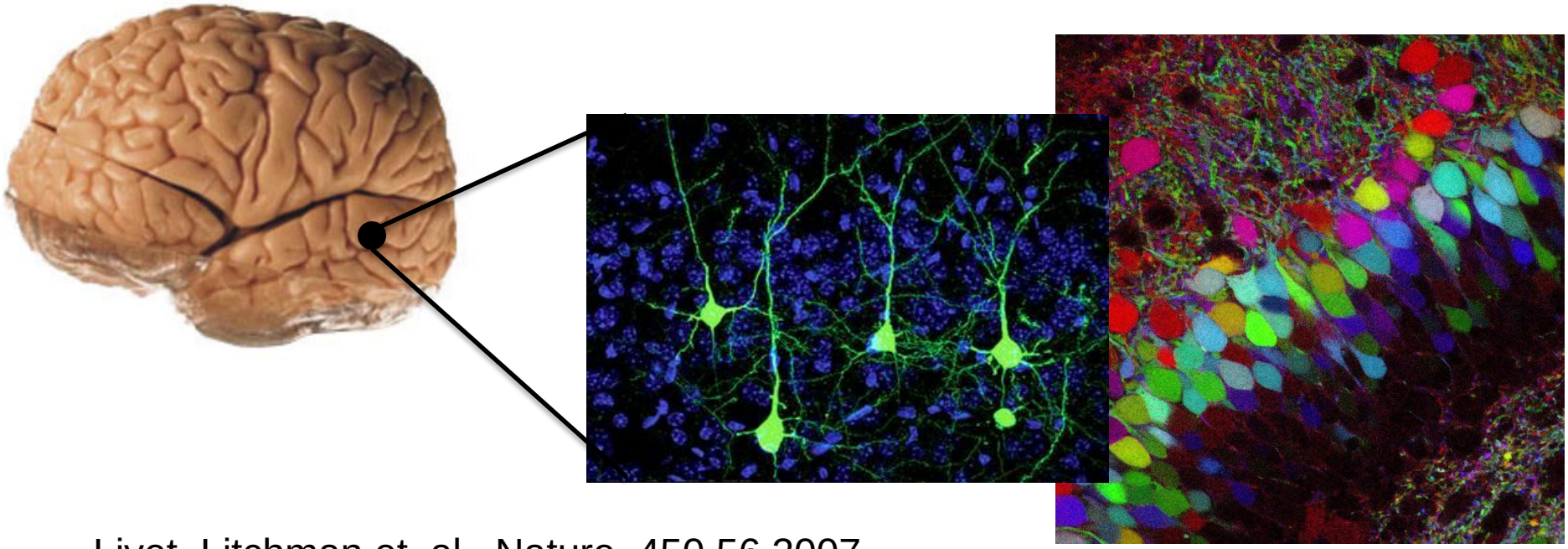
Microscopy to Identify chromosome translocation



a fragment of a chromosome moved ("trans-located") from one chromosome to another

2. Brain is a challenging problem

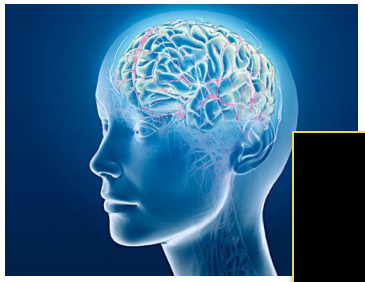
- Highly complex structure within a small volume
- Neurons regulates certain functions inside the brains
- These neurons are connected with junctions through axon dendrite links/



Livet, Litchman et. al., Nature, 450 56 2007

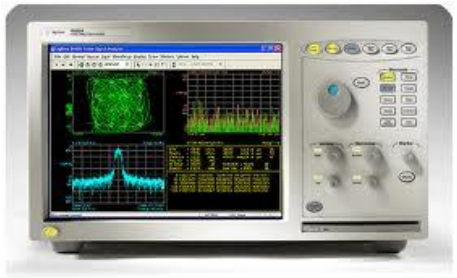
Two major problems to reveal secrets of our brain

1. Developing new tools to study brain signaling



?

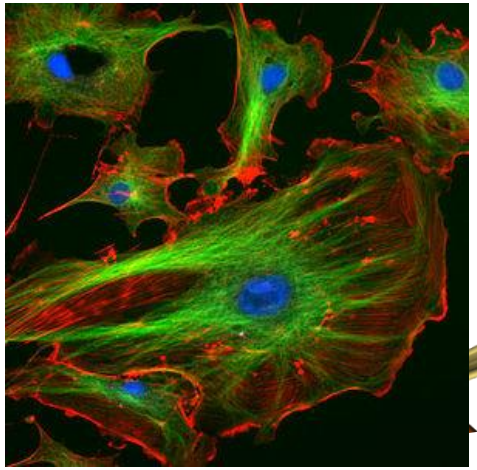
1. Developing new microscopy techniques and tools to image electrical signalling

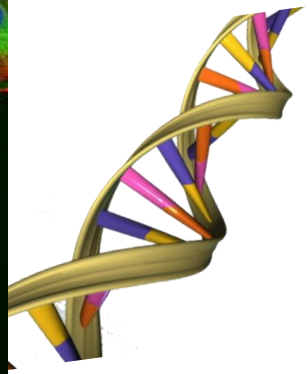


?

Disruptive Technology in Neuroscience: Optogenetics

“Optogenetics is the combination of genetic and optical methods to control and to monitor specific events in targeted cells of living tissue.”





Dr. Karl Deisseroth Lab - Stanford

Adamantidis, Deisseroth et. al, Nature, 450, 420, 2007

Light controlled cell morphology and motility

LETTERS

A genetically encoded photoactivatable Rac controls the motility of living cells

Yi I. Wu[1,3], Daniel Frey[4], Oana I. Lungu[1,2,3], Angelika Jaehrig[1,3], Ilme Schlichting[4], Brian Kuhlman[2,3] & Klaus M. Hahn[1,3]

The precise spatio-temporal dynamics of protein activity are often critical in determining cell behaviour, yet for most proteins they remain poorly understood; it remains difficult to manipulate protein activity at precise times and places within living cells. Protein activity has been controlled by light, through protein derivatization with photocleavable moieties[1] or using photoreactive small-molecule ligands[2]. However, this requires use of toxic ultraviolet wavelengths, activation is irreversible, and/or cell loading is accomplished via disruption of the cell membrane (for example, through microinjection). Here we have developed a new approach to produce genetically encoded photoactivatable derivatives of Rac1, a key GTPase regulating actin cytoskeletal dynamics in

abolish GTP hydrolysis and diminish interactions with nucleotide exchange factors, guanine nucleotide dissociation inhibitors (Q61L) and GTPase activating proteins (E91H and N92H) (Supplementary Fig. 2 and Supplementary text 'Characterization of Rac1 constructs'). This resulted in the photoactivatable analogue of Rac1 (PA-Rac1) used in the following studies. Pull-down assays showed that PA-Rac1 has greatly reduced affinity for its effector protein PAK in the dark, as does a PA-Rac1 construct containing a light-insensitive LOV2 mutation (C450A)[13]. Effector binding was restored in a PA-Rac1 construct containing a LOV2 mutant (I539E)[14] which mimics the unfolded 'lit state' (Fig. 1b and Supplementary Fig. 1b). Isothermal titration experiments indicated that the dark and lit state mutants of

http://www.nature.com/nature/journal/v461/n7260/extref/nature08241-s7.mov

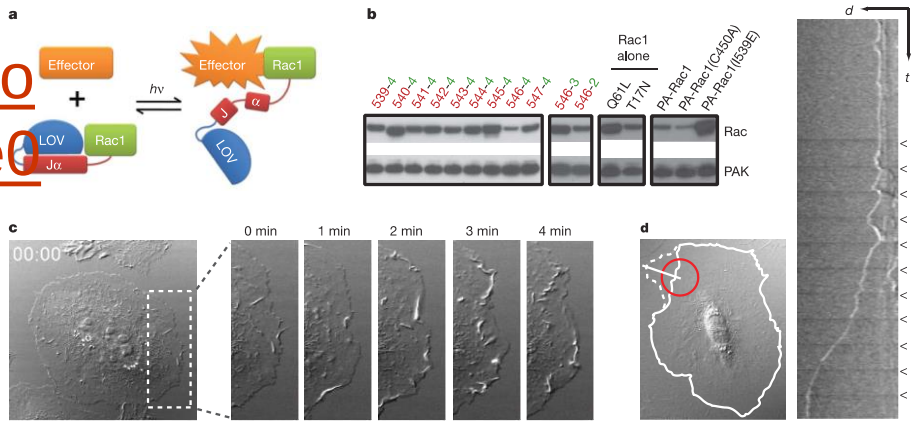
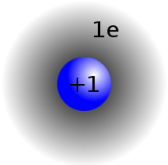


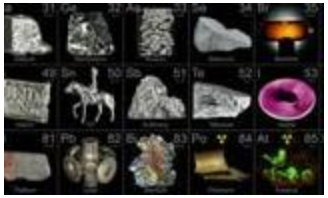
Figure 1 | Engineering and *in vivo* characterization of a photoactivatable Rac1 (PA-Rac1). **a**, Cartoon representation of PA-Rac1 design. *hν*, irradiation. **b**, Pull down of PA-Rac1 constructs with PAK in the dark. Truncations of LOV and Rac at their linkage point were tested: 539–547 in red, terminal amino acid of Jα; 2–4 in green, first residue of Rac1. 546-4 showed the strongest inhibition. PA-Rac1, 546-4 Q61L/E91H/N92H; PA-Rac1(C450A), light-insensitive mutant; PA-Rac1(I539E), lit state mutant. Pull down by constitutively active (Q61L) and dominant negative (T17N) mutants are included for comparison with PA-Rac1. **c**, Whole-cell irradiation of a HeLa cell expressing PA-Rac1 (minutes after irradiation, DIC, short axis of box = 20 μm). **d**, Spatial control of Rac1 activity. A 20-μm circle (red) was irradiated every 60 s in serum-starved MEF cells. Solid line, cell border at time 0; dotted line, 10 min after initial light pulse. Little movement of the cell border was detected, except adjacent to the point of irradiation. The kymograph (taken using white line, 20 μm), shows the initial formation of ruffles after each pulse, followed by protrusion (arrowheads indicate irradiation pulses).

Building up cells from its parts

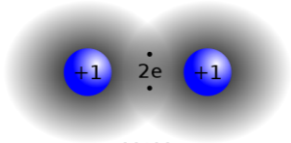
e^-, p^+, N, $h\nu$



H

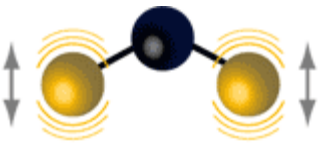


elements

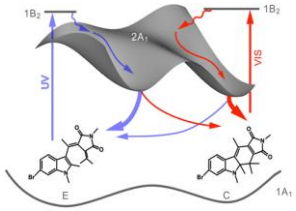


H:H
H – H

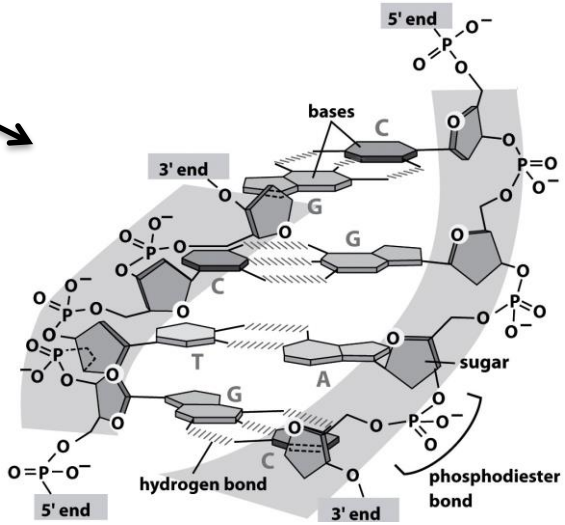
molecules

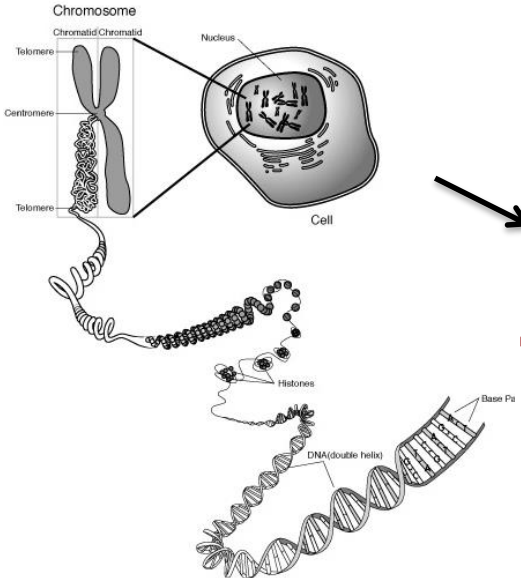


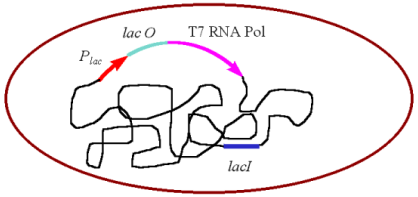
vibrations



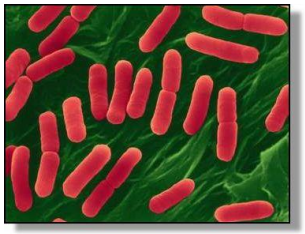
making and
breaking bonds



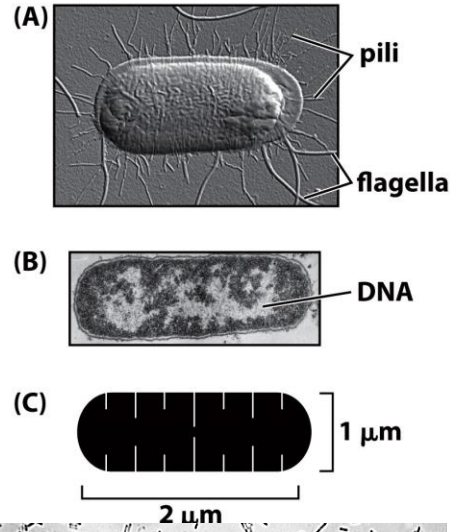


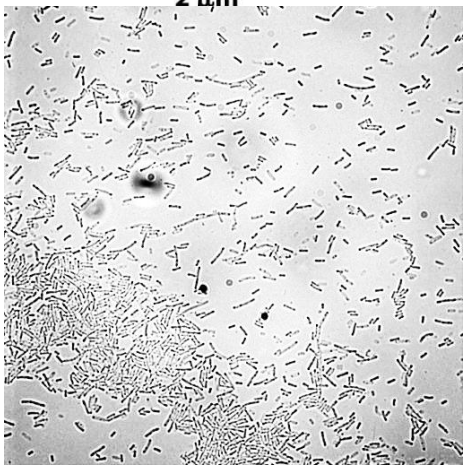


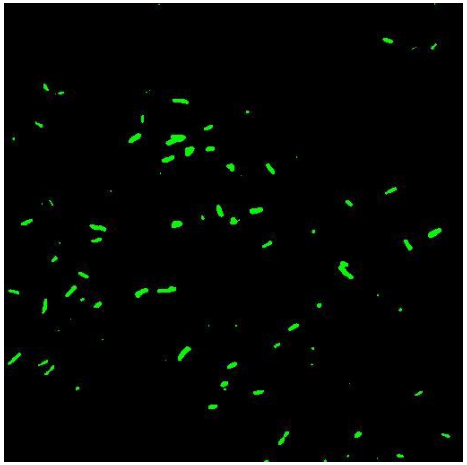
BL(DE3) Host Chromosome

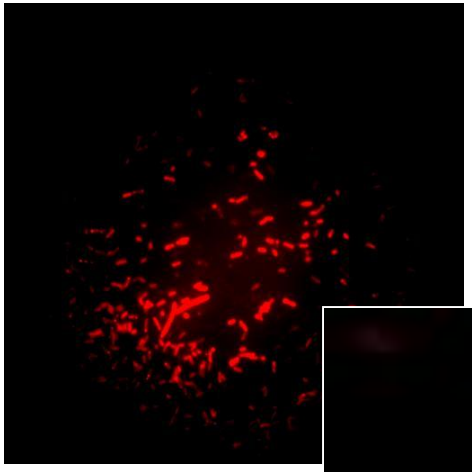


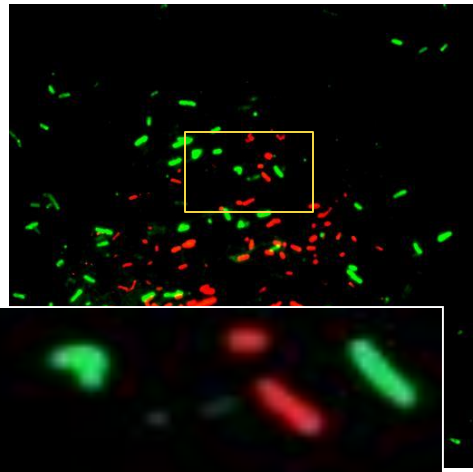
E coli has a machinery inside to produce $4x10^6$ proteins in less than 40-50 minutes



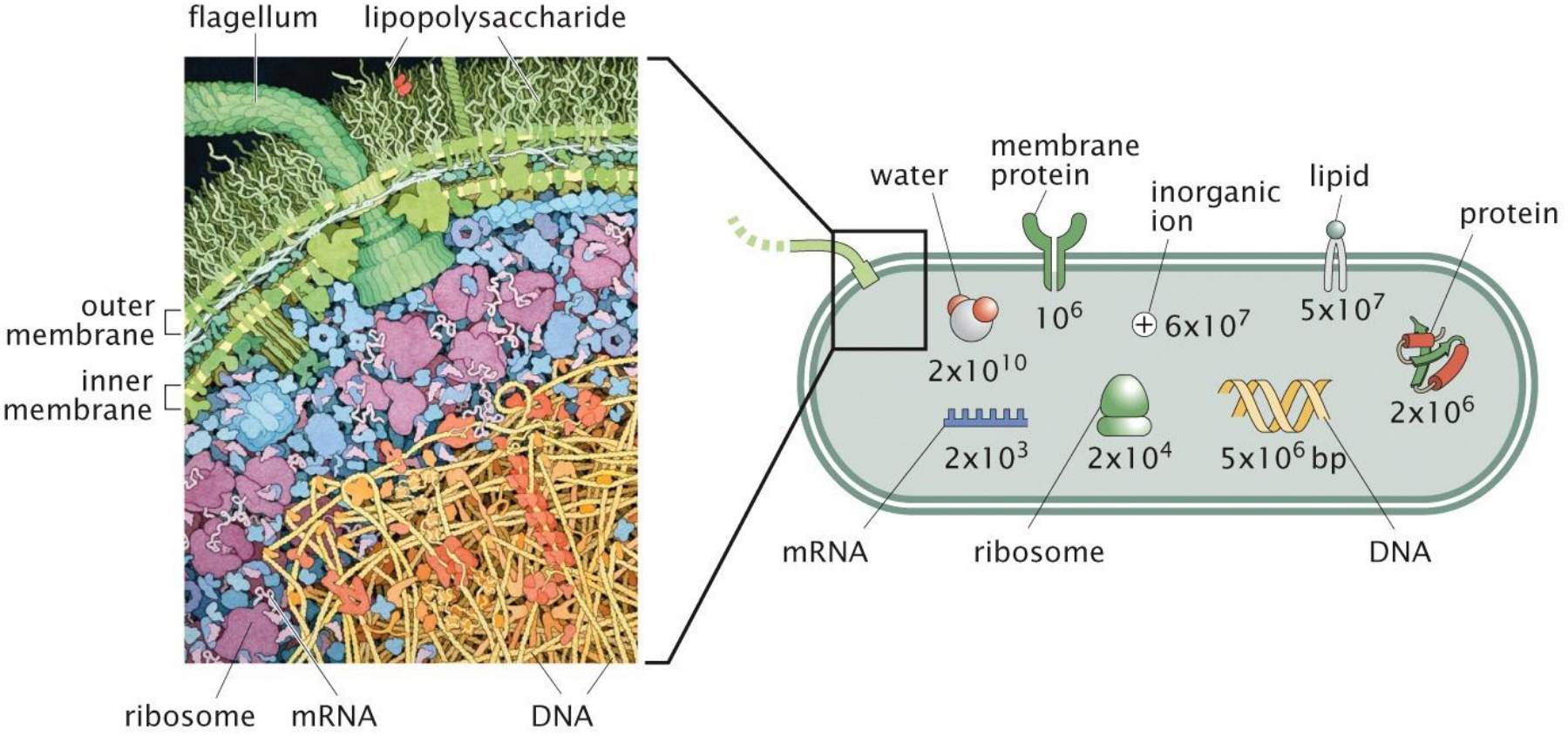








How you model the shape of the e.coli?


flagellum
lipopolysaccharide
outer membrane
inner membrane
ribosome
mRNA
DNA
water
membrane protein
inorganic ion
lipid
protein
2x10^10
10^6
6x10^7
5x10^7
2x10^6
2x10^3
2x10^4
5x10^6 bp
mRNA
ribosome
DNA

Cellular Size Scales

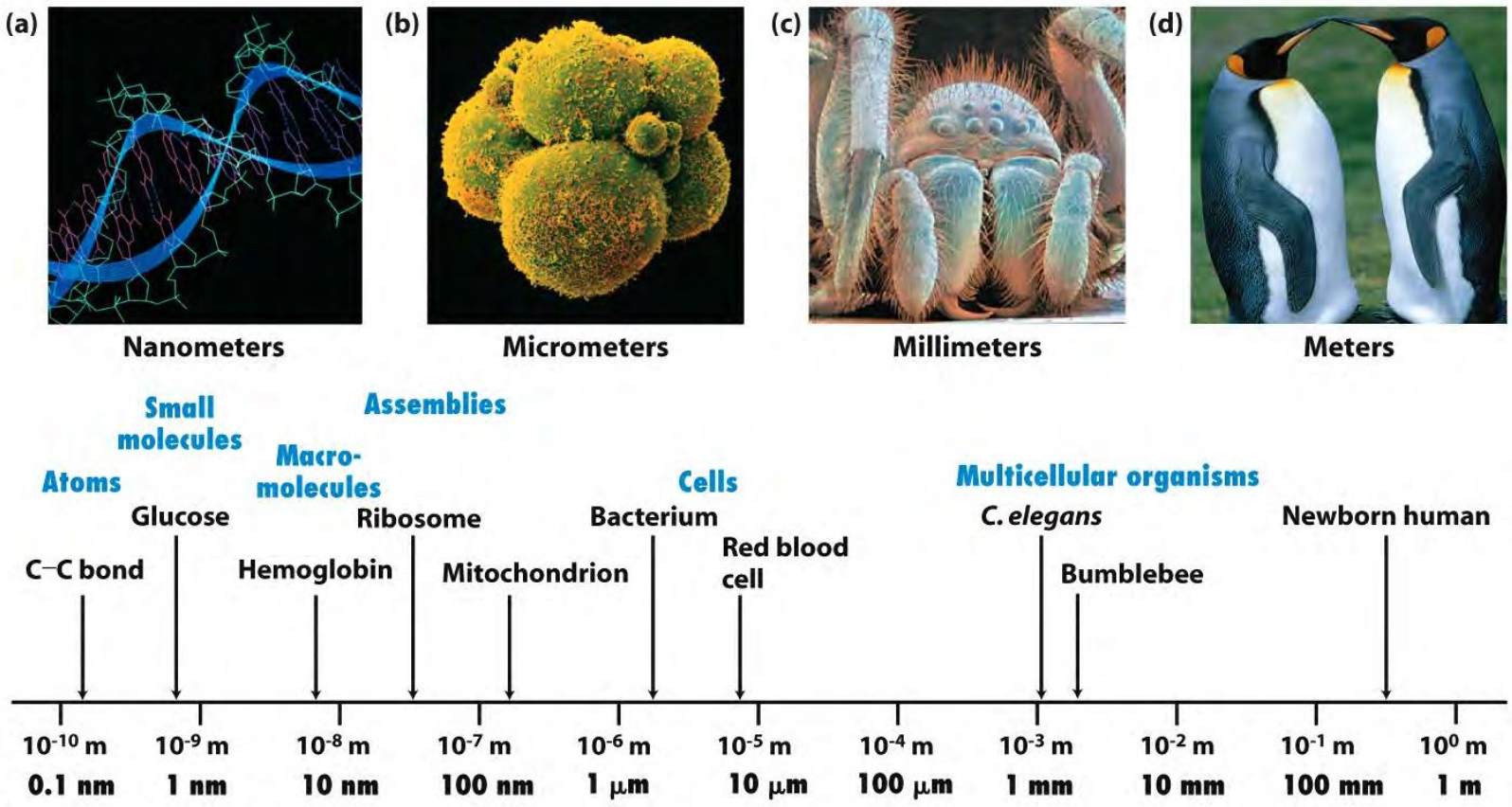


Table 1.1 Rules of thumb for biological estimates

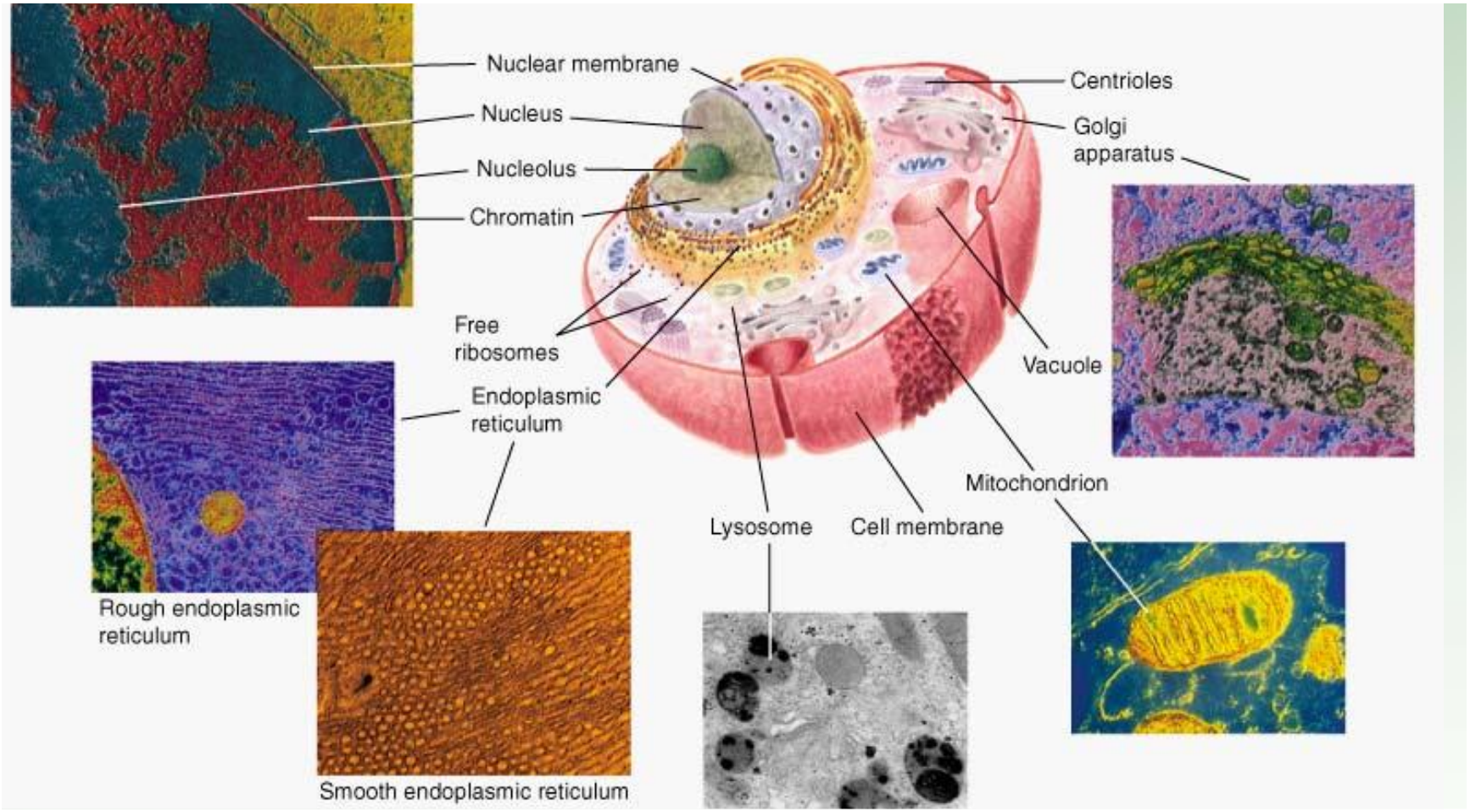
	Quantity of interest	Symbol	Rule of thumb
E. coli	Cell volume	$V_{E.\ coli}$	$\approx 1\ \mu m^3$
	Cell mass	$m_{E.\ coli}$	≈ 1 pg
	Cell cycle time	$t_{E.\ coli}$	≈ 3000 s
	Cell surface area	$A_{E.\ coli}$	$\approx 6\ \mu m^2$
	Genome length	$N_{bp}^{E.\ coli}$	$\approx 5 \times 10^6$ bp
	Swimming speed	$v_{E.\ coli}$	$\approx 20\ \mu m/s$
Yeast	Volume of cell	V_{yeast}	$\approx 60\ \mu m^3$
	Mass of cell	m_{yeast}	≈ 60 pg
	Diameter of cell	d_{yeast}	$\approx 5\ \mu m$
	Cell cycle time	t_{yeast}	≈ 200 min
	Genome length	N_{bp}^{yeast}	$\approx 10^7$ bp
Organelles	Diameter of nucleus	$d_{nucleus}$	$\approx 5\ \mu m$
	Length of mitochondrion	l_{mito}	$\approx 2\ \mu m$
	Diameter of transport vesicles	$d_{vesicle}$	≈ 50 nm
Water	Volume of molecule	V_{H_2O}	$\approx 10^{-2}\ nm^3$
	Density of water	ρ	$1\ g/cm^3$
	Viscosity of water	η	≈ 1 centipoise (10^{-2} g/(cm s))
	Hydrophobic embedding energy	$\approx E_{hydr}$	25 cal/(mol Å^2)
DNA	Length per base pair	l_{bp}	$\approx 1/3$ nm
	Volume per base pair	V_{bp}	$\approx 1\ nm^3$
	Charge density	λ_{DNA}	2 e/0.34 nm
	Persistence length	ξ_P	50 nm
Amino acids and proteins	Radius of "average" protein	$r_{protein}$	≈ 2 nm
	Volume of "average" protein	$V_{protein}$	$\approx 25\ nm^3$
	Mass of "average" amino acid	M_{aa}	≈ 100 Da
	Mass of "average" protein	$M_{protein}$	$\approx 30{,}000$ Da
	Protein concentration in cytoplasm	$c_{protein}$	≈ 300 mg/mL
	Characteristic force of protein motor	F_{motor}	≈ 5 pN
	Characteristic speed of protein motor	v_{motor}	≈ 200 nm/s
	Diffusion constant of "average" protein	$D_{protein}$	$\approx 100\ \mu m^2/s$
Lipid bilayers	Thickness of lipid bilayer	d	≈ 5 nm
	Area per molecule	A_{lipid}	$\approx \frac{1}{2}\ nm^2$
	Mass of lipid molecule	m_{lipid}	≈ 800 Da

Table 1.1 Physical Biology of the Cell (© Garland Science 2009)

Substance	% of total dry weight	Number of molecules
Macromolecule		
Protein	55.0	2.4×10^6
RNA	20.4	
23S RNA	10.6	19,000
16S RNA	5.5	19,000
5S RNA	0.4	19,000
Transfer RNA (4S)	2.9	200,000
Messenger RNA	0.8	1,400
Phospholipid	9.1	22×10^6
Lipopolysaccharide	3.4	1.2×10^6
DNA	3.1	2
Murein	2.5	1
Glycogen	2.5	4,360
Total macromolecules	**96.1**	
Small molecules		
Metabolites, building blocks, etc.	2.9	
Inorganic ions	1.0	
Total small molecules	**3.9**	

Table 2.1 Observed macromolecular census of an *E. coli* cell. (Data from F. C. Neidhardt et al., Physiology of the Bacterial Cell, Sunderland, Sinauer Associates Inc., 1990 and M. Schaechter et al., Microbe, Washington DC, ASM Press, 2006.)

Eukaryotic Cell



Inside of a cell is a crowded place.

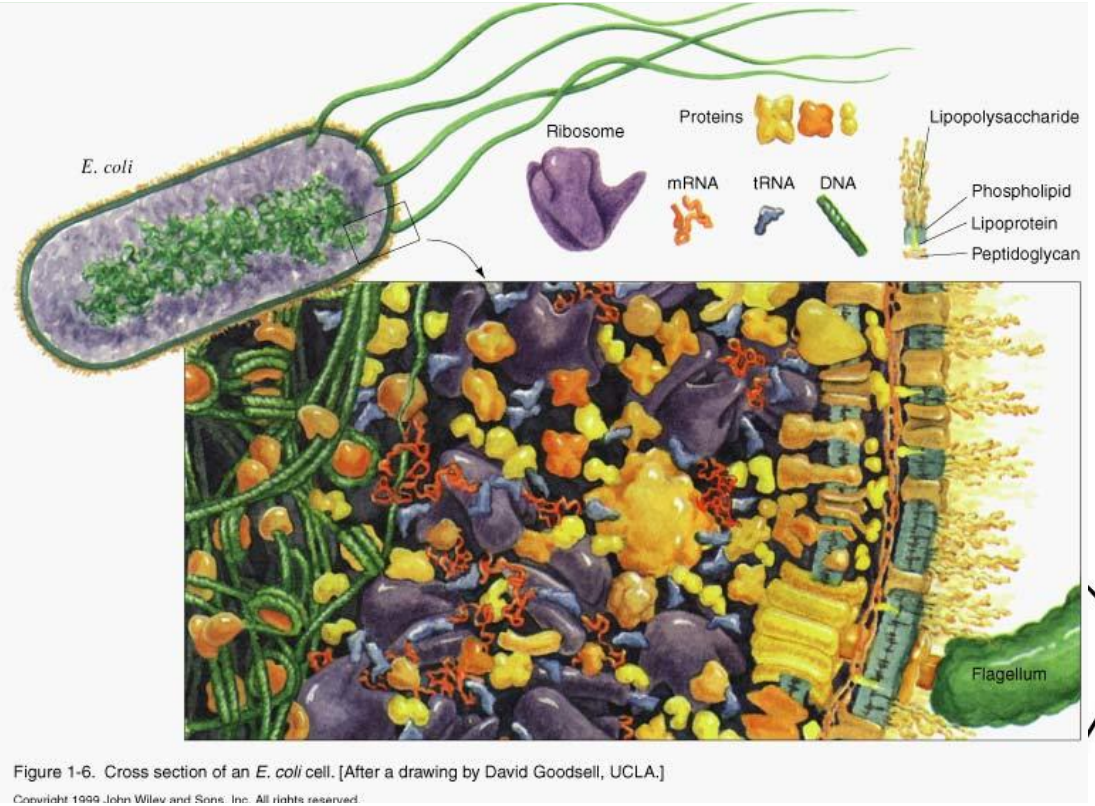
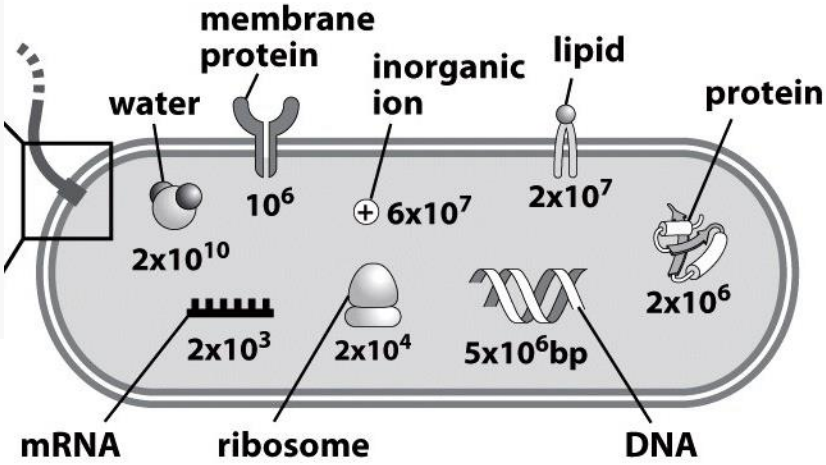
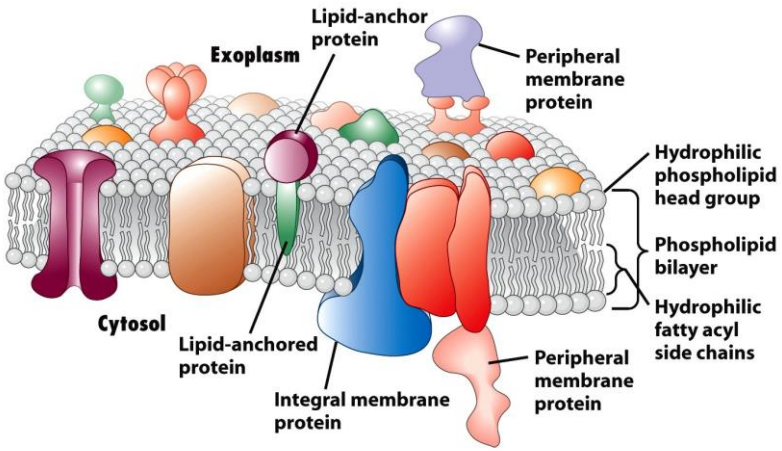
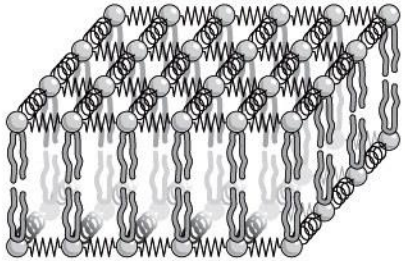


Figure 1-6. Cross section of an *E. coli* cell. [After a drawing by David Goodsell, UCLA.]

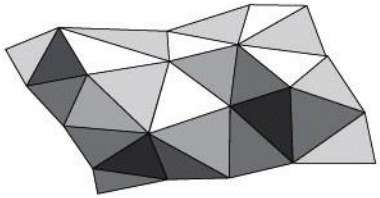




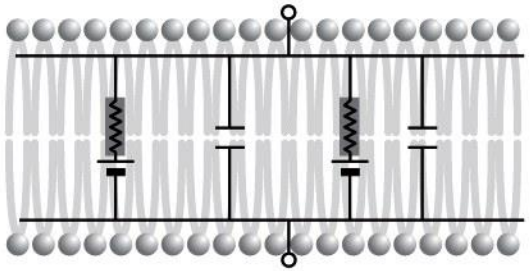
ARRAY OF SPRINGS



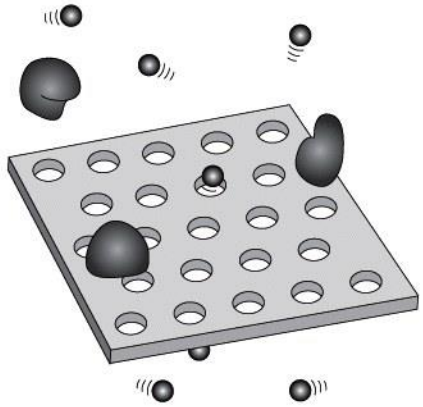
RANDOM SURFACE



RC CIRCUIT

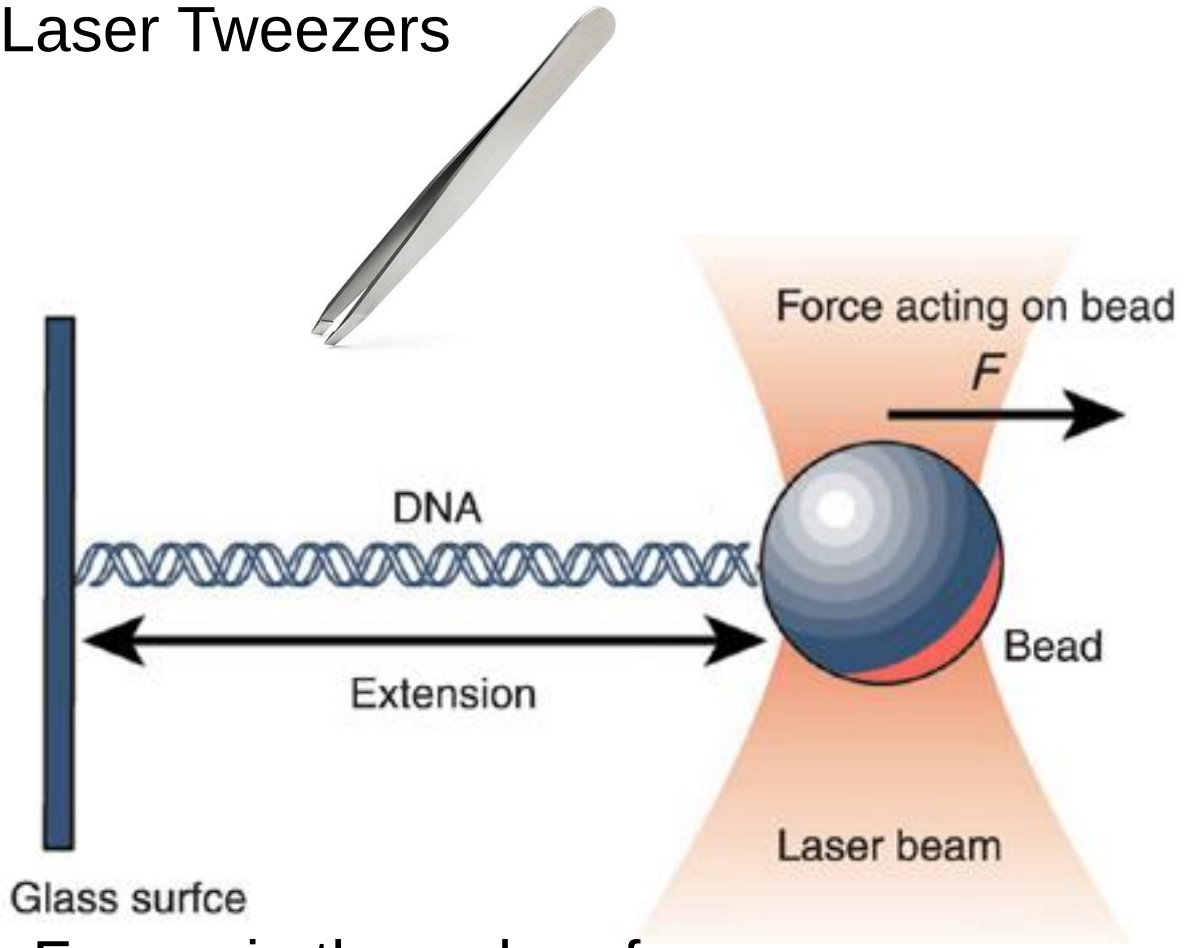


SEMI-PERMEABLE BARRIER



Photons has a momentum : Laser Tweezer technology

Laser Tweezers



Forces in the order of Piconewtons

Steven Chu
Nobel Prize 1997
Current secretary of energy

Laser Tweezers Tetris

See you next time!