BME 42-620 Engineering Molecular Cell Biology

Lecture 06:

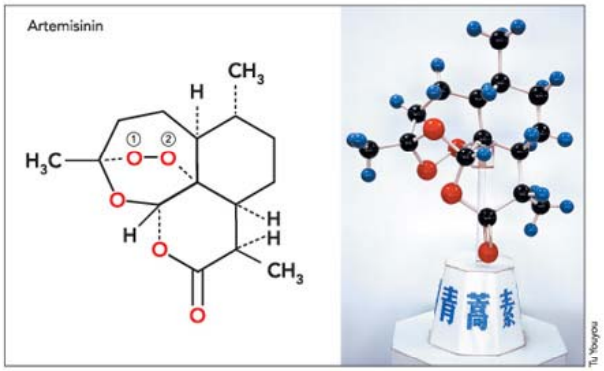
Basics of Cell Biology Literature Reading

Methods of Cell Biology (I): Imaging

Basics of the Diffusion Theory

A Case Study: Malaria & Artemisinin

- Malaria is a mosquito-borne infectious disease caused by parasite *Plasmodia*.
- 225 million cases each year; 1 million deaths in 2010 (World malaria report).
- 4000 recipes
- 380 extracts from 200 herbs
- Compound 191→ artemisinin



Artful herb, model drug

Artemisia annua (*left*), or sweet wormwood, contains the powerful antimalarial drug artemisinin, originally known as Qinghaosu. The three-dimensional diagram of artemisinin (*middle*) shows the endoperoxide bond, which is crucial for the compound's antimalarial effects, between the numbered oxygens. The ball model of artemisinin (*right*) shows that bond on the left-hand side (two red oxygen atoms linked to each other). The Chinese characters on the stand mean "Qing Hao Su."

http://www.laskerfoundation.org/

Youyou Tu

http://www.who.int/malaria/world_malaria_report_2010/en/index.html

Outline

- Basics of cell biology literature reading

- Imaging (I): light microscopy
- Imaging (II): electron microscopy
- Basics of the diffusion theory

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- Imaging (I): light microscopy
- Imaging (II): electron microscopy
- Basics of the diffusion theory

Why Focus on Literature Reading?

- Biology is a scientific discipline undergoing rapid development.
- For in-depth understanding of cell biology, it is essential to read primary research literature.
- The cell biology information that can be covered in our textbook is rather limited.
- Basic skills for effective reading of cell biology research literature can be learned.

An Overview of Cell Biology Literature

- Journals
  - Examples of general purpose journals
    - Science
    - Nature
    - PNAS
  - Examples of specialized journals
    - Cell and associated journals
    - Journal of Cell Biology
    - Nature associated journals
    - Plos journals
- Commercial versus noncommercial journals
    - subscription versus open access
- Review journals and review articles

How to Read Cell Biology Papers (I)

- It is essential to read and evaluate contemporary cell biology papers critically. - Why so critical?

- General guidelines
  - Fundamentally, it is about original data and ideas
  - Not that different from a mathematical proof:
    Logical coherence and rigor

- Highly stereotyped structures of biology papers

- Organization (I): biology papers are result-driven
  - Introduction: However, …
  - Results: To…, we did …
  - Discussion: We speculate …

How to Read Cell Biology Papers (II)

- Organization (II):
  - Every figure must tell
  - Logical flow: connection between result sections

- Our aims
  - To be able to effectively read papers in cell biology
  - To be able to effectively communicate cell biology results

General Process of Publication

- Journal selection
  - What are the messages: *short vs long format*
  - Usually several comparable journals to choose from
    Similar paper formats
    Similar review standards
  - Keep a rational perspective: *vanity journals*
  - Keep doing good science, the record will show

- Submission and review process
  - Pre-submission inquiry: usually for vanity journals
  - Editorial review
  - External review
  - Outcome I: preliminary acceptance
    Point-to-point response to reviews
  - Outcome II: rejection
  - Peer-review system is not perfect but generally works

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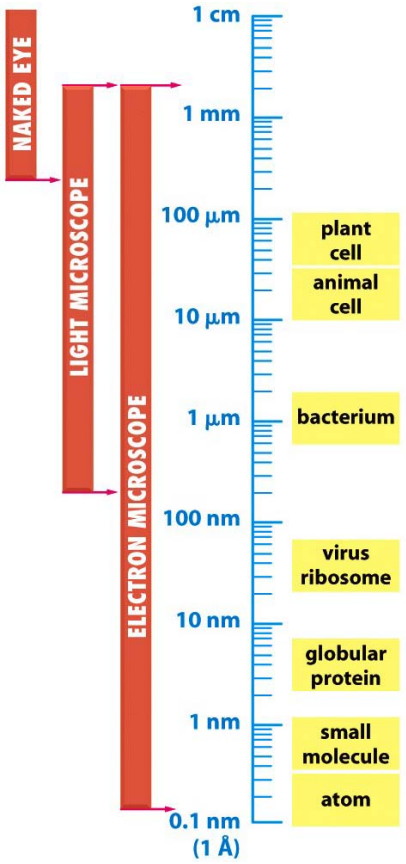
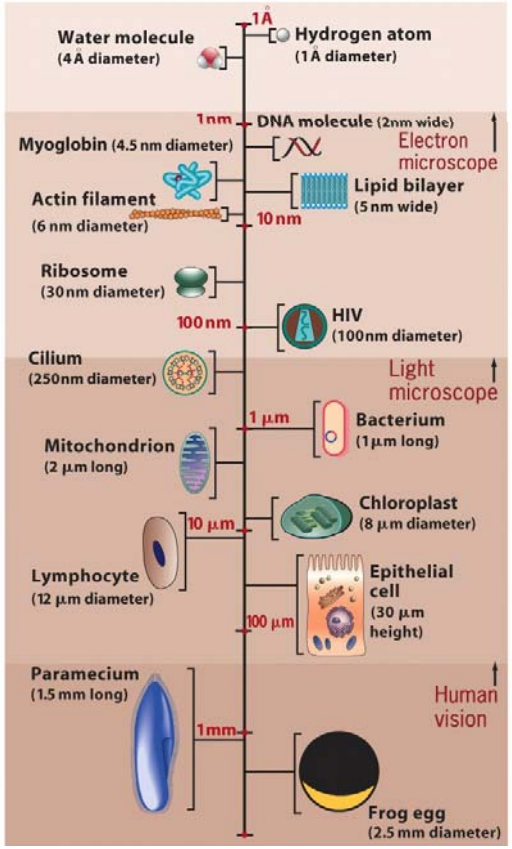
- Imaging (II): electron microscopy
- Basics of the diffusion theory

Visualizing Cellular Structure by Imaging

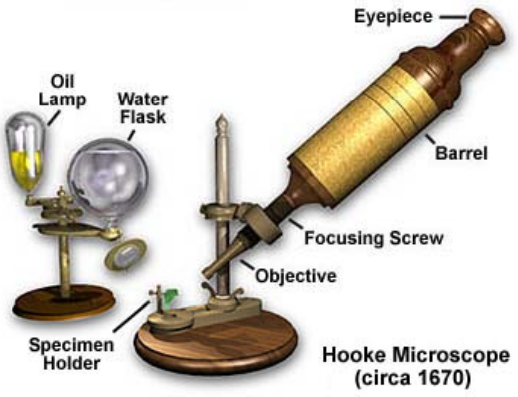
- Microscopy makes it possible to visualize cell structure and dynamics.
- Light microscopy permits live imaging of cellular processes.
- Below 100nm, electron microscopy
- Below 1 nm: crystallography, NMR, spectroscopy
- Imaging modalities are generally non-contact and non-invasive.

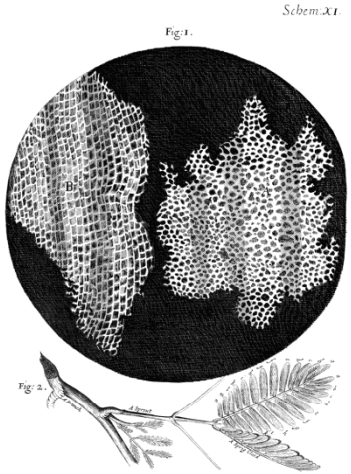




Invention of Light Microscopes

- Light microscope was invented more than three hundred years ago.
  (*Micrographia*, Robert Hooke, 1665)





http://micro.magnet.fsu.edu/index.html
Molecular expressions: microscopy world

Two Microscope Configurations

Upright

Inverted

http://www.olympusamerica.com/seg_section/seg_home.asp

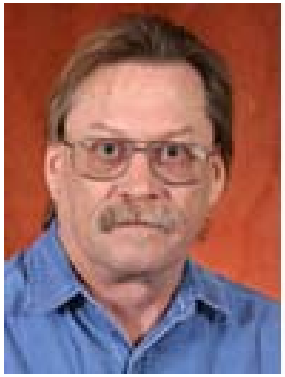
- Modern microscopes are computer-controlled.
- Modern microscopes are often configured to be highly automated.

Sources of Information (I)

- Major microscope manufacturers

- Basic microscope structures and performance from different suppliers are very similar.

Sources of Information (II)



Michael W. Davidson
Florida State University

MOLECULAR EXPRESSIONS™
Exploring the World of Optics and Microscopy

http://micro.magnet.fsu.edu/index.html
Molecular expressions: microscopy world

Microscopy Resource Center

http://www.olympusmicro.com/

MICROSCOPYU
THE SOURCE FOR MICROSCOPY EDUCATION

http://www.microscopyu.com/

ZEISS

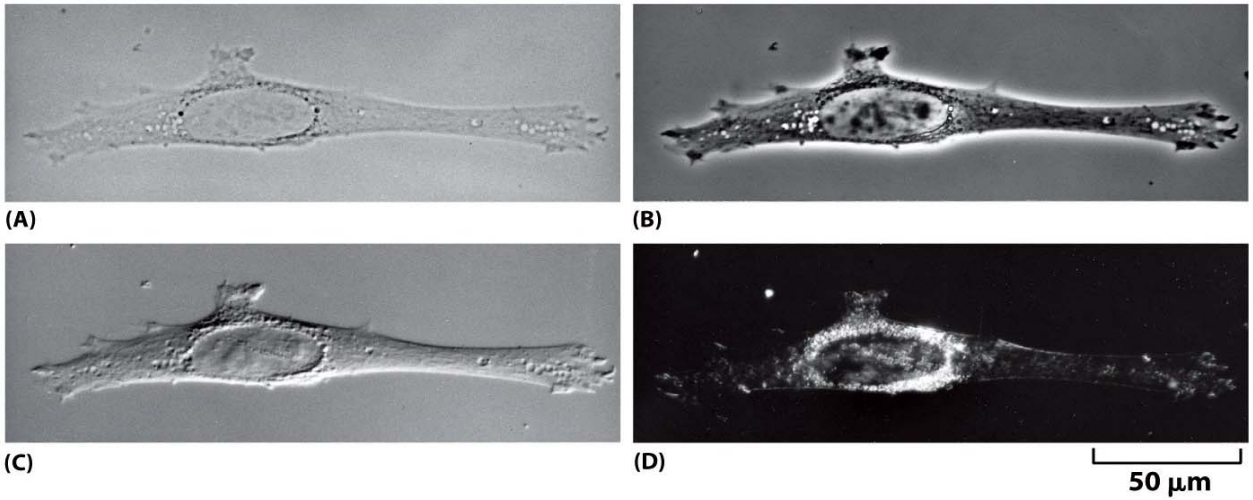
http://zeiss-campus.magnet.fsu.edu/index.html

Light Microscopy (I)

- The basic role of any microscope is to provide adequate contrast and resolution.

- Contrast generation
  - Bright-field vs dark-field
  - Phase contrast
  - Fluorescent microscopy

(A) Bright-field
(B) Phase
(C) DIC
(D) Dark-field



Light Microscopy (II)

- Under dark-field contrast, by using a special condenser, only the light scattered by the specimen can enter the objective lens.

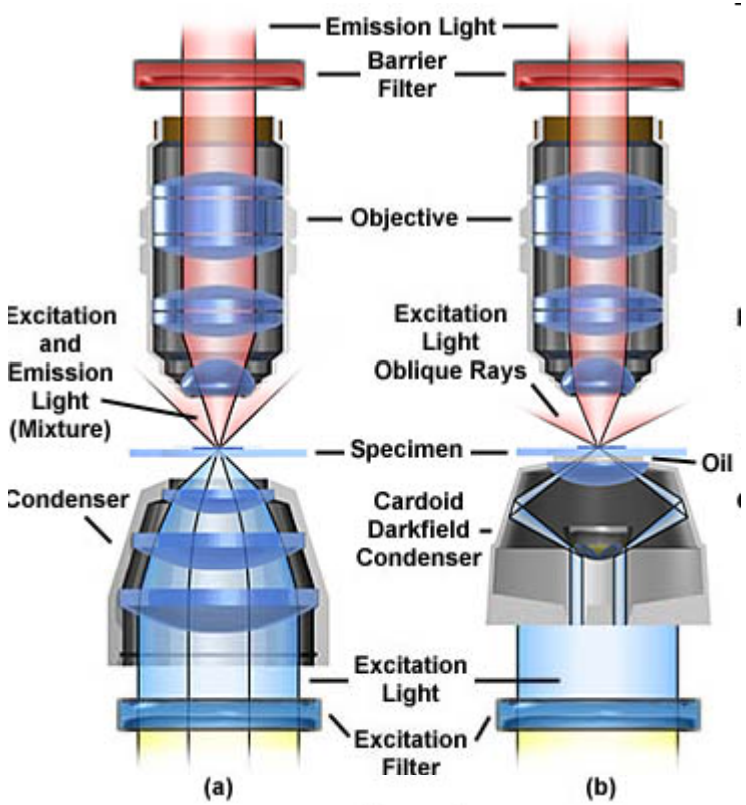
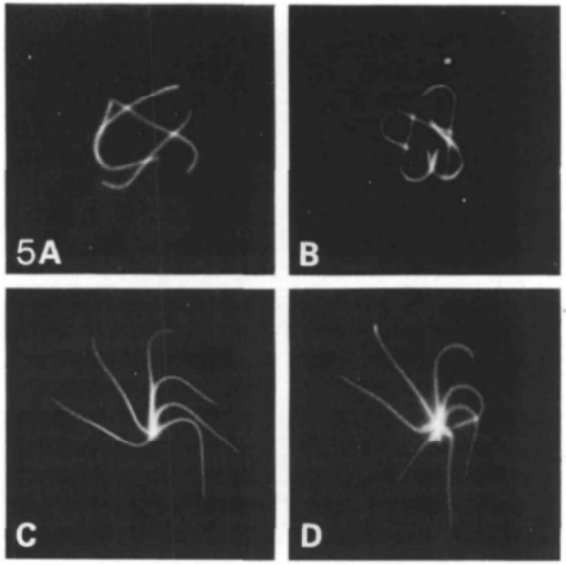
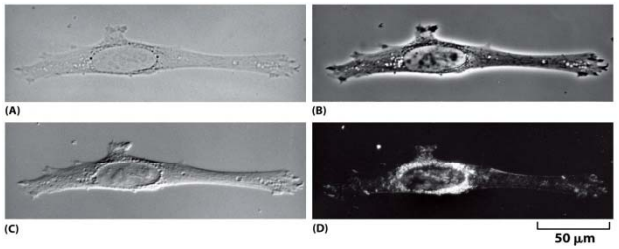


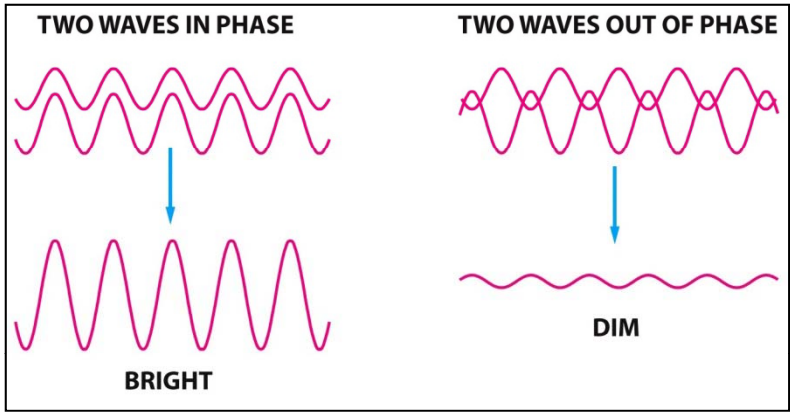
Figure 1

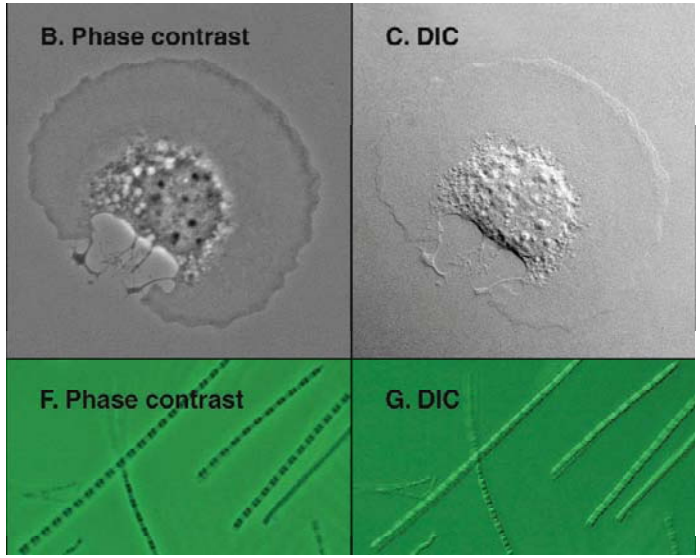




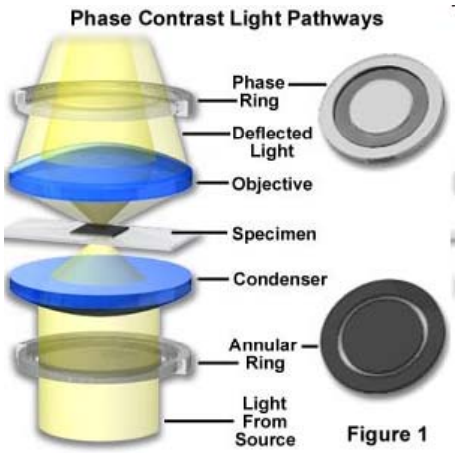
Phase Contrast & DIC (I)

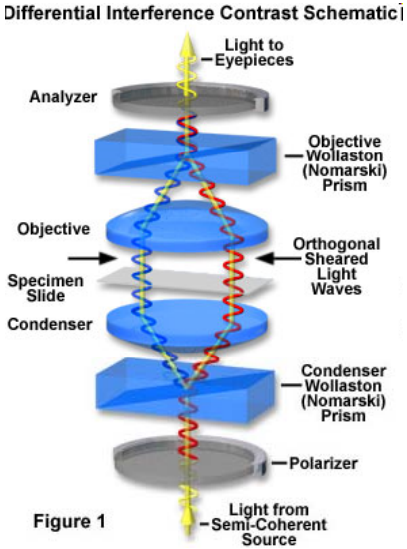
- Phase contrast is very useful in imaging transparent specimens, which do not change light magnitude.
- Contrast is generated due to the different refractive indices of the sample and the background.
- Phase contrast can generate artifacts.
  - Halos by boundary
  - Artificial shadows
- DIC significantly reduces but does not eliminate halos and shadows.

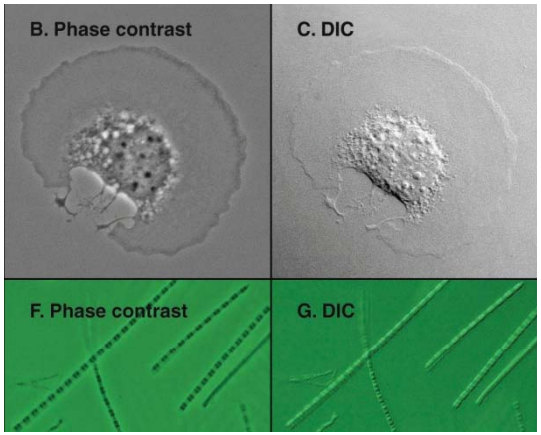


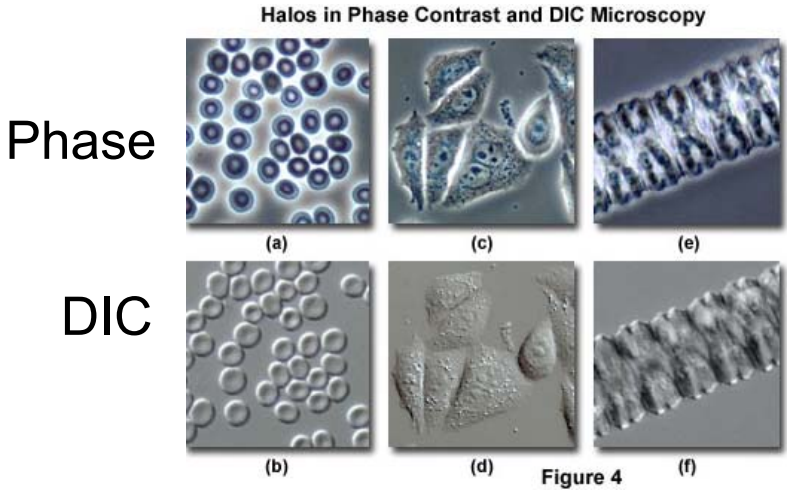


Phase Contrast & DIC (II)

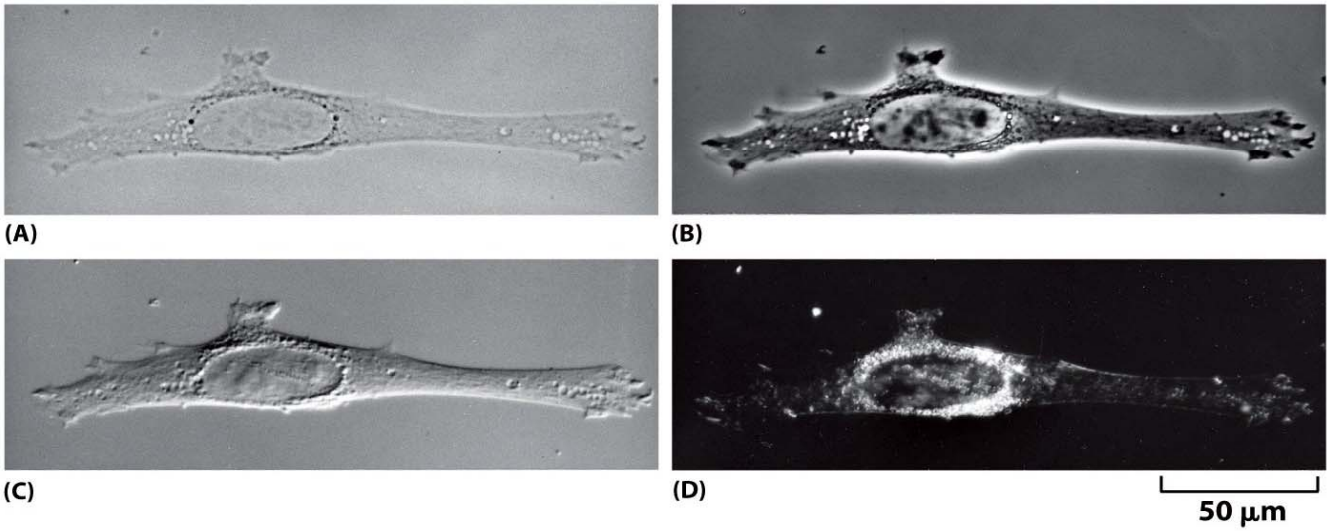


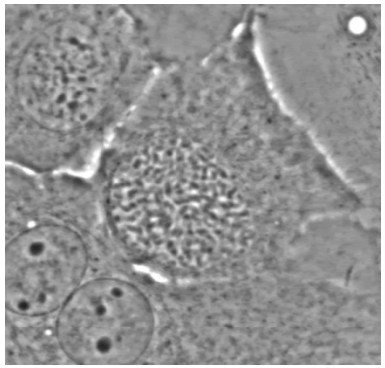






What is missing from these images?





The Nobel Prize in Physics 1953

"for his demonstration of the phase contrast method, especially for his invention of the phase contrast microscope"

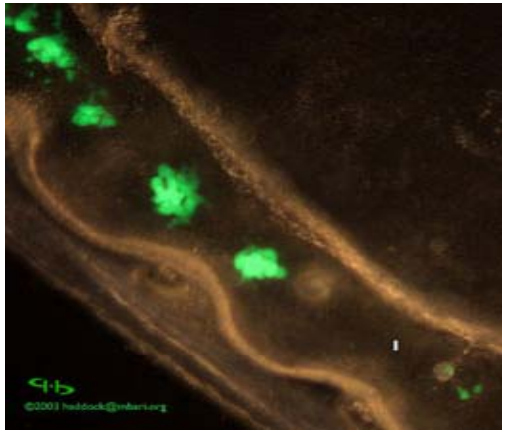
Frits (Frederik) Zernike

the Netherlands

Groningen University
Groningen, the Netherlands

b. 1888
d. 1966

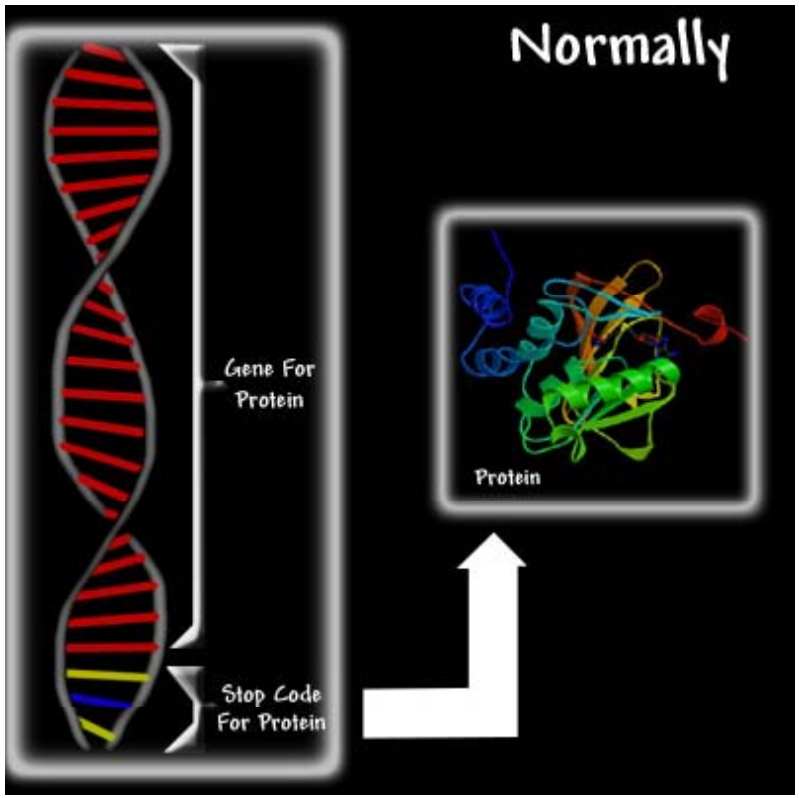
Green Fluorescence Protein

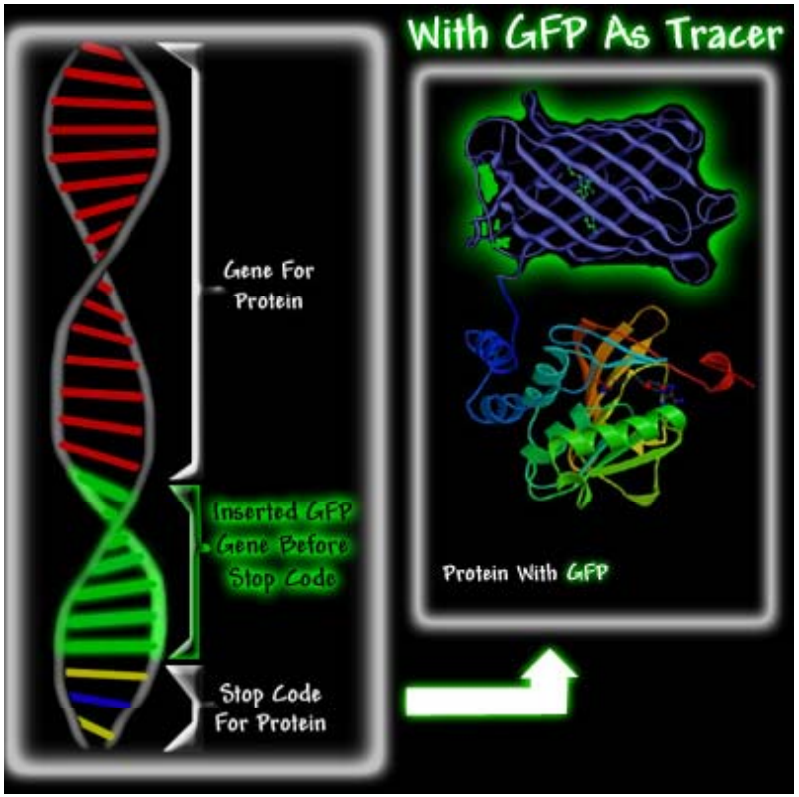


Jellyfish: *Aequorea victoria*

http://gfp.conncoll.edu/GFP-1.htm

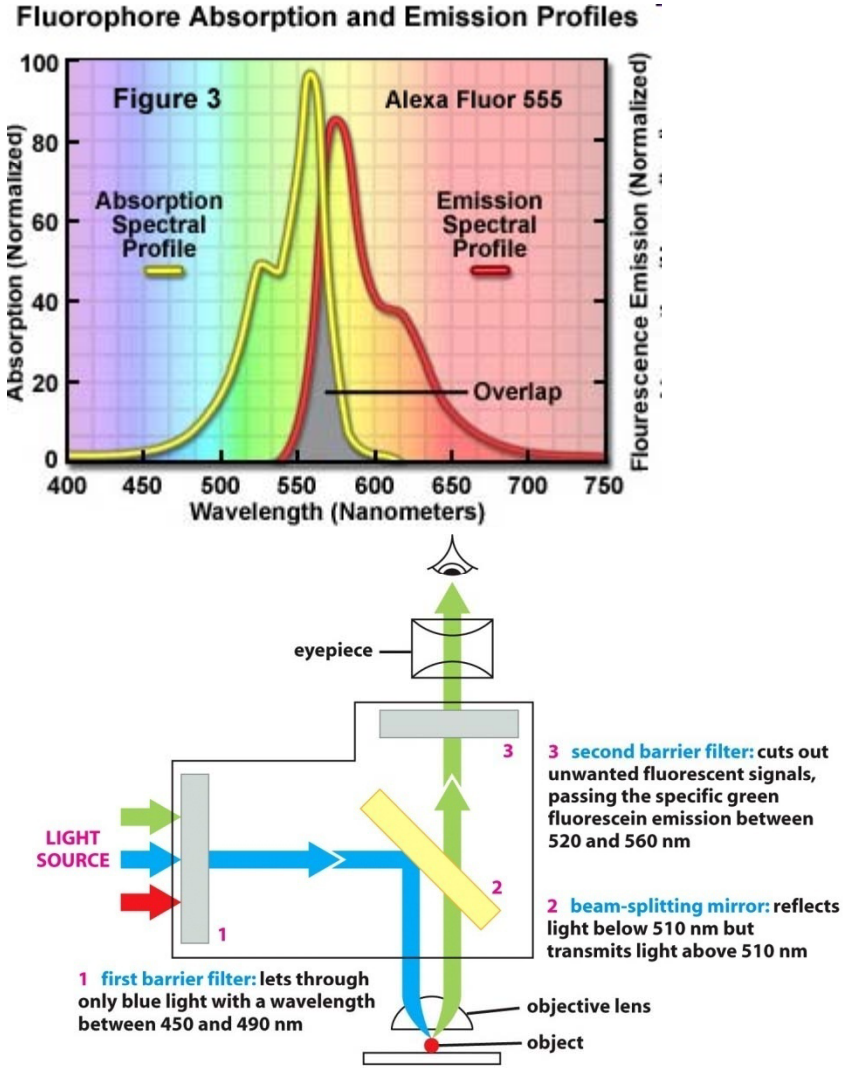
Tagging a Protein Using GFP

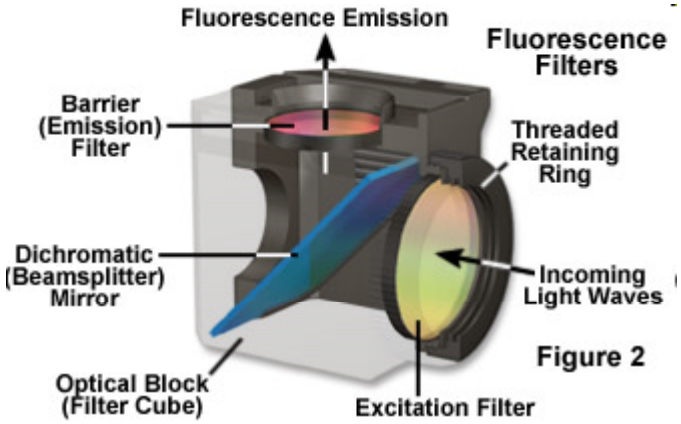




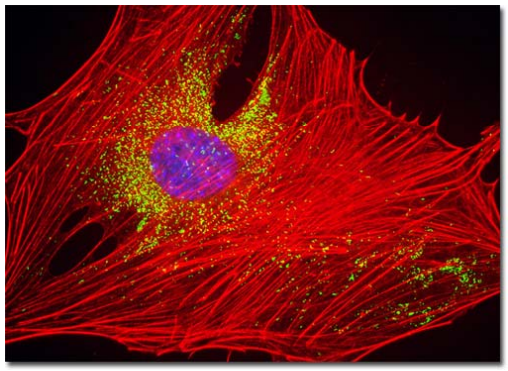
http://gfp.conncoll.edu/GFP-1.htm

Fluorescence Microscopy (I)

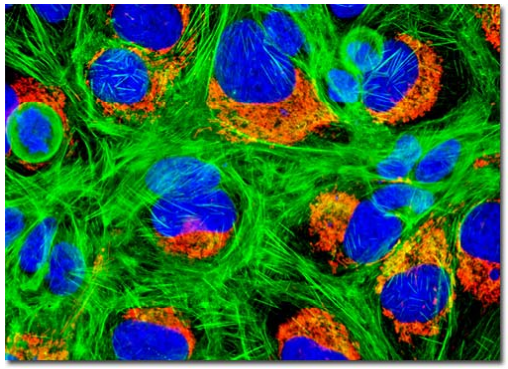




Fluorescence Microscopy (II)



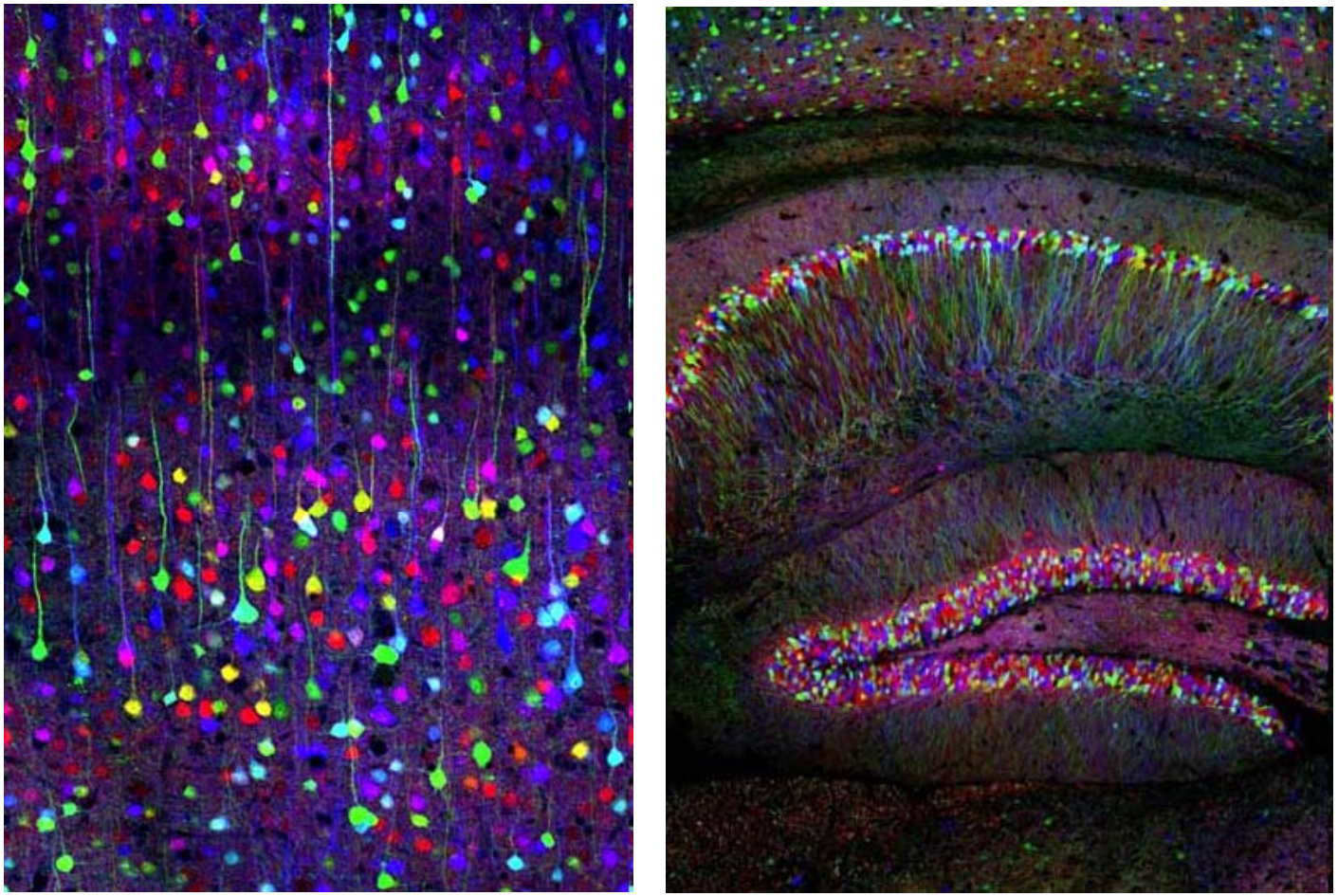
Embryonic Swiss Mouse Fibroblast Cells (3T3)



Human Bone Osteosarcoma Cells (U-2 OS)

http://micro.magnet.fsu.edu/primer/techniques/fluorescence/gallery/cells/3t3/3t3cellslarge.html

Fluorescence Imaging of Neurons in Cerebral Cortex Sections



Livet J, Weissman TA, Kang H, *et al. Nature* **450**: 56–62, 2007

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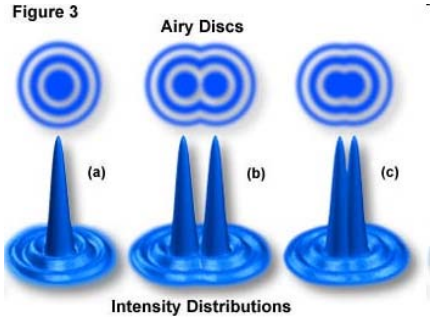
- Basics of cell biology literature reading
- Imaging (I): light microscopy
- Imaging (II): electron microscopy
- Basics of the diffusion theory

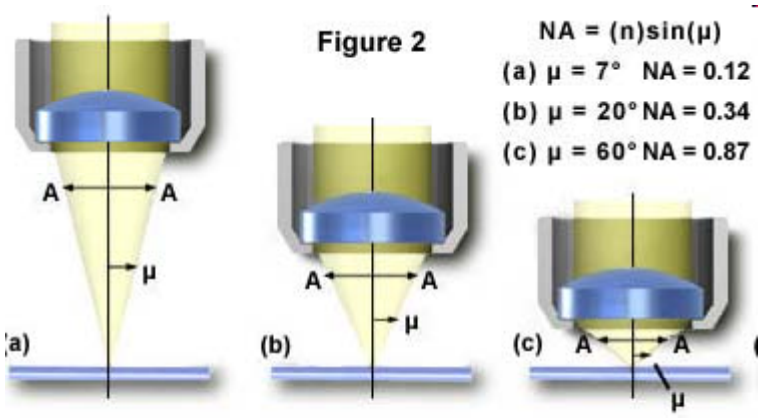
Resolution of Light Microscopy

- Airy pattern



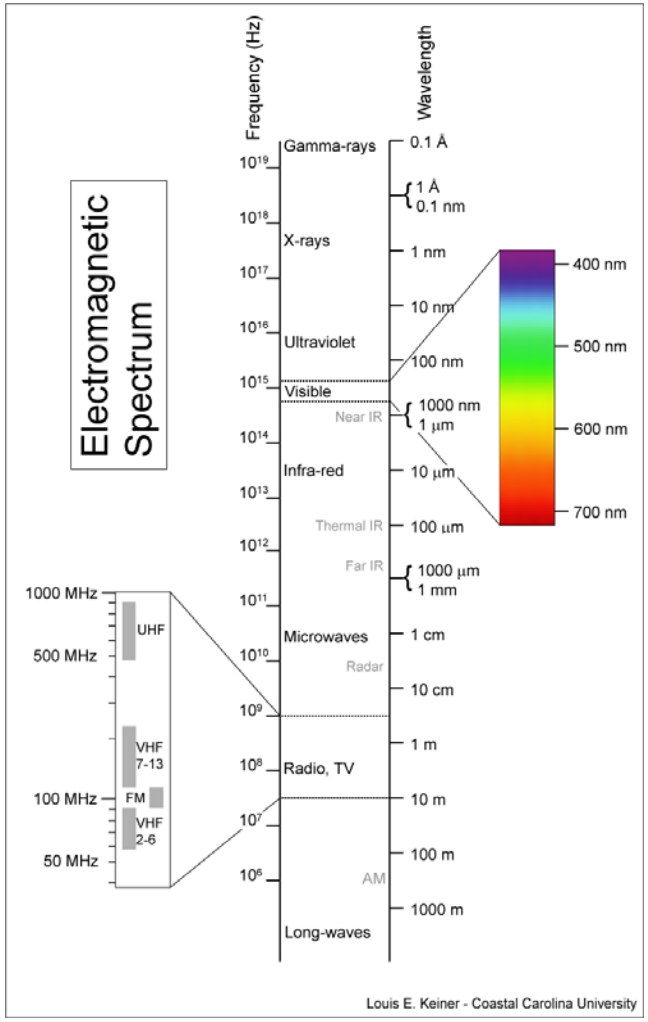
- Rayleigh limit $D = \frac{0.61\lambda}{NA}$

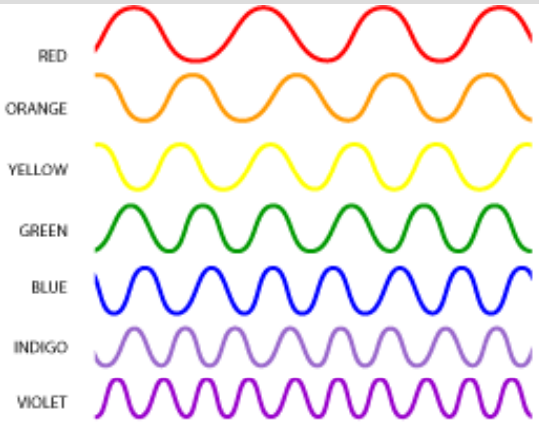
- Numerical aperture



http://www.microscopy.fsu.edu/primer/java/imageformation/rayleighdisks/index.html

Spectrum of Visible Light



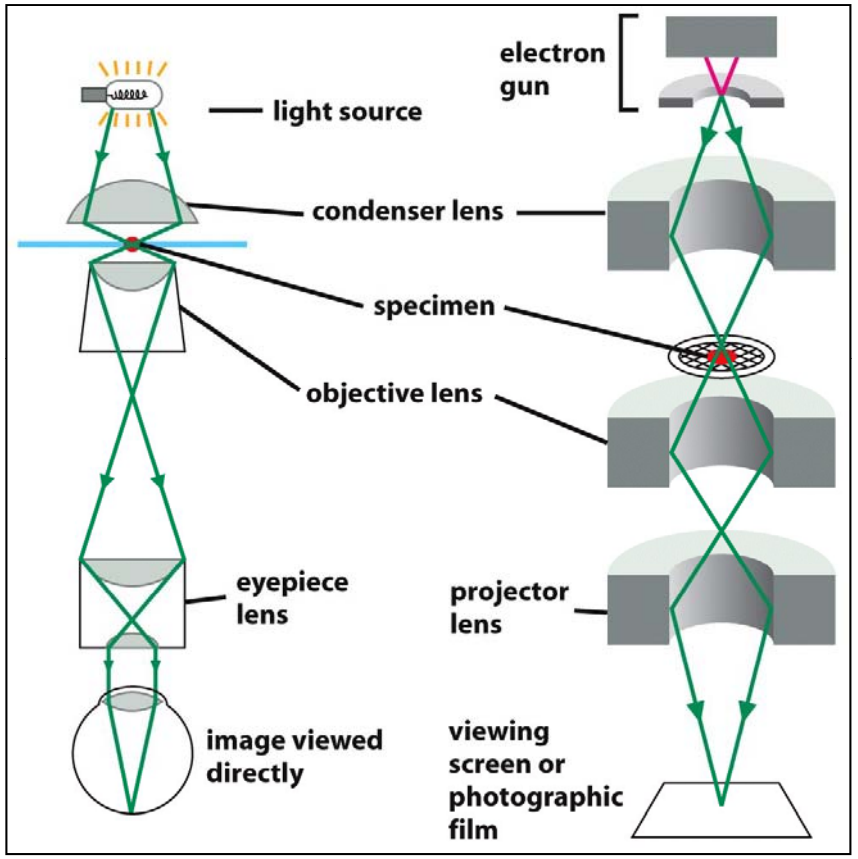


Color	Wavelength
violet	380–450 nm
blue	450–495 nm
green	495–570 nm
yellow	570–590 nm
orange	590–620 nm
red	620–750 nm

http://en.wikipedia.org/wiki/Visible_spectrum
http://science.hq.nasa.gov/kids/imagers/ems/visible.html

Electron Microscopy

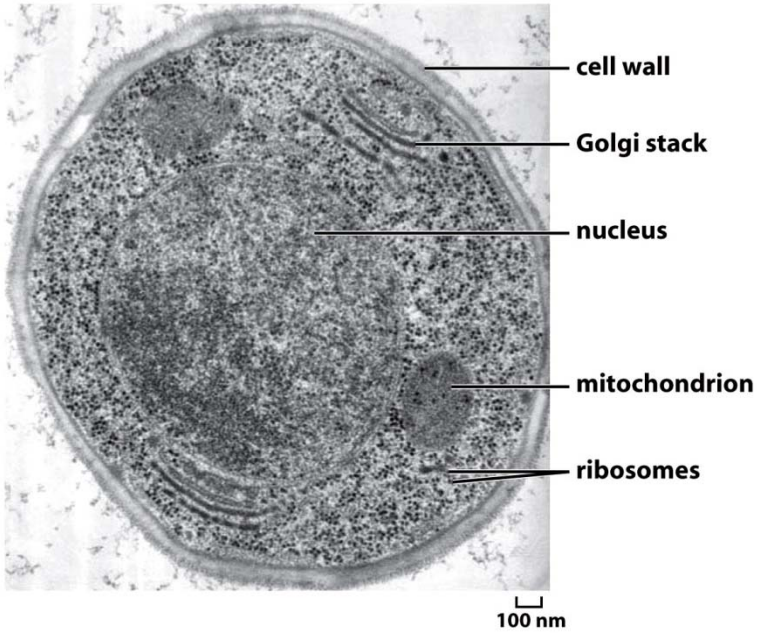
- The same principles of optics applies to light microscopy and electron microscopy.
- Wavelength of an electron beam: ~0.002-0.004nm
- Resolution:
  TEM: ~0.1-1nm or
  SEM: ~10nm
- Lower resolution due to the small NA achievable



Different Electron Microscopy Modalities

- Two major EM modalities
  - Transmission electron microscopy
  - Scanning electron microscopy

- Transmission electron microscopy
  - High resolution: up to ~1Å
  - Samples must be fixed
  - Samples must be sectioned into thin slices (~50-100 nm)

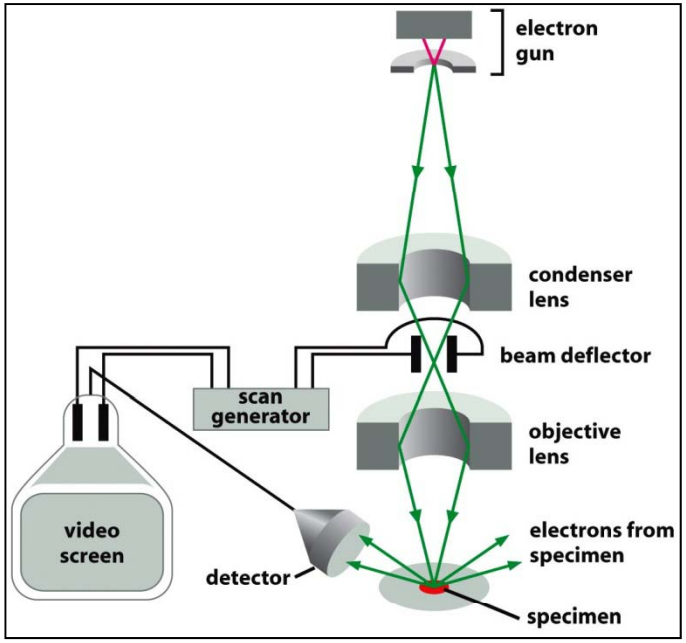


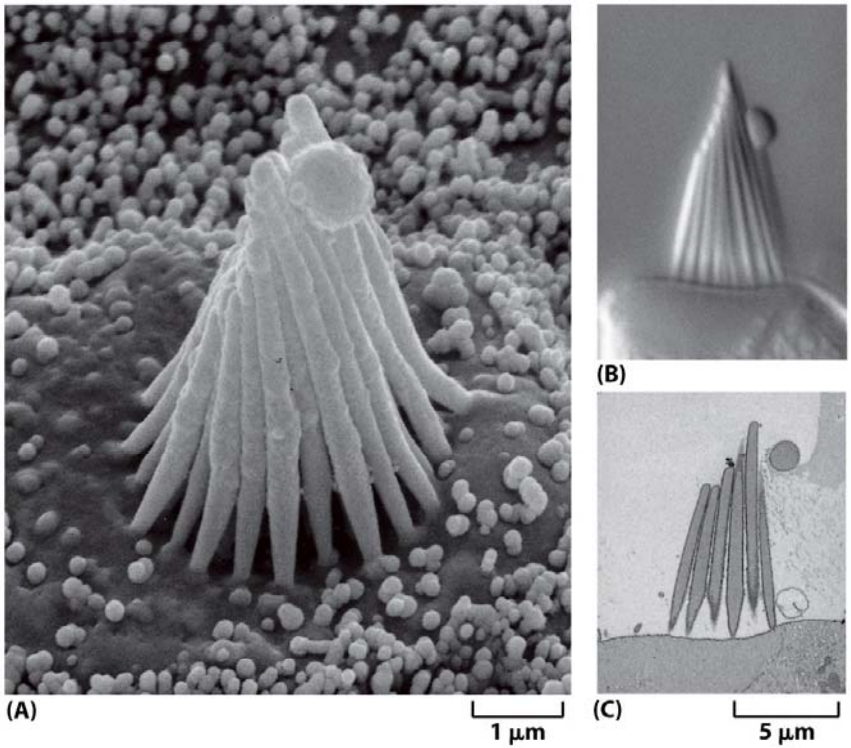
- Specific molecules can be identified using antibody conjugated gold particles.

TEM reveals internal structure.

Different Electron Microscopy Modalities

- Scanning electron microscopy
  - Lower resolution than TEM; typically 10nm
  - Often used to image large samples
  - Surface scan
  - 3D visualization





SEM reveals surface structure.

Cryo-EM; 3D Reconstruction

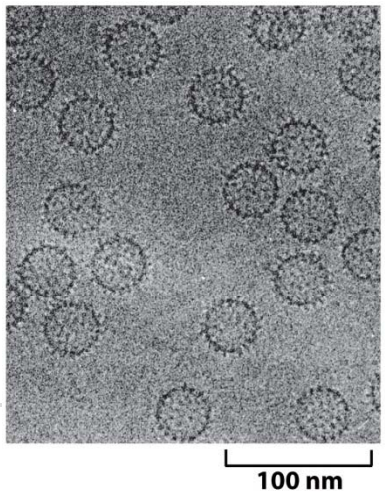
- Preservation of samples by rapidly freezing the sample to a very low temperature.

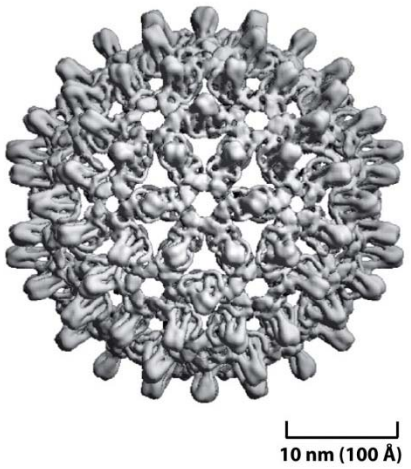
- Electron-tomography:

  - Images from different angles are integrated for 3D reconstruction in a tomography fashion.

  - The crystal structure, if available, can often facilitate this process.

Hepatitis B virus





Comments: Methods in Cell Biology

- Cell biology is experimental science and is strongly dependent on technological advancement.

- Importance of protocols.
  - It is common to see different or even conflicting results in different studies on the same subject.

- Importance of control experiments.

- Cultural differences from engineering

Outline

- Basics of cell biology literature reading

- Imaging (I): light microscopy
- Imaging (II): electron microscopy
- Basics of the diffusion theory

References

- Howard Berg, *Random Walks in Biology*, Princeton University Press, 1993.

- Jonathon Howard, *Mechanics of Motor Proteins and the Cytoskeleton*, Sinauer Associated, 2001.

Introduction

Table 2–2 The Approximate Chemical Composition of a Bacterial Cell

	PERCENT OF TOTAL CELL WEIGHT	NUMBER OF TYPES OF EACH MOLECULE
Water	70	1
Inorganic ions	1	20
Sugars and precursors	1	250
Amino acids and precursors	0.4	100
Nucleotides and precursors	0.4	100
Fatty acids and precursors	1	50
Other small molecules	0.2	~300
Macromolecules (proteins, nucleic acids, and	26	~3000

- Cellular molecules are subject to thermal force due to collisions with water and other molecules.

- The resulting motion and energy are called thermal motion and thermal energy.

Movement of a Free Molecule (I)

- The average kinetic energy of a particle of mass m and velocity v is

$$\left\langle \frac{1}{2} m v_x^2 \right\rangle = \frac{kT}{2}$$

Boltzmann constant=1.381×10^{-23} J/K

$t_K = t_C + 273.15$

where k is Boltzmann's constant and T is absolute temperature (Einstein 1905).

- Principle of equipartition of energy

$$\left\langle \frac{1}{2} m v^2 \right\rangle = \frac{3 \cdot kT}{2}$$

Movement of a Free Molecule (II)

- Molecular mass of GFP is 27 kDa. One atomic mass unit (Da) is 1.6606×10^{-24}g. So the mass of one GFP molecule is 4.48×10^{-20}g.

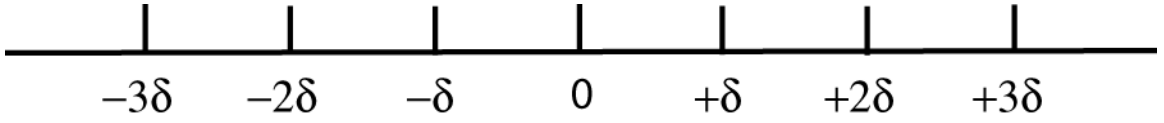
  At 27 degree C, kT is 4.14×10^{-14}g·cm^2/sec^2.

$$\sqrt{\langle v_x^2 \rangle} = \sqrt{\frac{kT}{m}} = 961.3 \text{ cm/sec}$$

1D Random Walk in Solution (I)

- Assumptions:
  (1) A particle *i* has equal probabilities to walk to the left and to the right.
  (2) Particle movement at consecutive time points are independent.
  (3) Movement of different particles are independent.
  (4) Each particle moves at a average step size of $\delta = v_x \cdot \tau$

$$x_i(n) = x_i(n-1) \pm \delta$$



$$\langle x(n) \rangle = \frac{1}{N}\sum_{i=1}^{N} x_i(n) = \frac{1}{N}\sum_{i=1}^{N}\left[x_i(n-1) \pm \delta \right]$$
$$= \frac{1}{N}\sum_{i=1}^{N} x_i(n-1) = \langle x(n-1) \rangle$$

- Property 1: The mean position of a particle undergoing random walk remains at the origin.

1D Random Walk in Solution (II)

- Property 2: The mean square displacement of a particle undergoing random walk increases linearly w.r.t. time.

$$\left\langle x^2(n)\right\rangle = \frac{1}{N}\sum_{i=1}^{N} x_i^2(n) = \frac{1}{N}\sum_{i=1}^{N}\left[x_i^2(n-1) \pm 2\delta x_i(n-1) + \delta^2\right]$$
$$= \left\langle x^2(n-1)\right\rangle + \delta^2$$

$$\left\langle x^2(n)\right\rangle = n\delta^2 = \frac{t}{\tau}\delta^2 = 2Dt \qquad \left\langle r^2(n)\right\rangle = \left\langle x^2(n) + y^2(n)\right\rangle = 4Dt$$

$$\left\langle r^2(n)\right\rangle = \left\langle x^2(n) + y^2(n) + z^2(n)\right\rangle = 6Dt$$

- Property 3: The displacement of a particle follows a normal distribution.

1D Random Walk in Solution (III)

$$p(k;n) = \frac{n!}{k!(n-k)!}\frac{1}{2^k}\frac{1}{2^{n-k}}$$

$$p(k) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(k-\mu)^2}{2\sigma^2}} \text{ where } \sigma^2 = \frac{n}{4} \text{ and } \mu = \frac{n}{2}$$

$$x(n) = \left[k - (n-k)\right]\delta = (2k-n)\delta \qquad \left\langle x(n)\right\rangle = \left(2\left\langle k\right\rangle - n\right)\delta = 0$$

$$\left\langle x^2(n)\right\rangle = \left(4\left\langle k^2\right\rangle - 4\left\langle k\right\rangle n + n^2\right)\delta^2 = \left(n^2 + n - 2n^2 + n^2\right)\delta^2 = n\delta^2$$

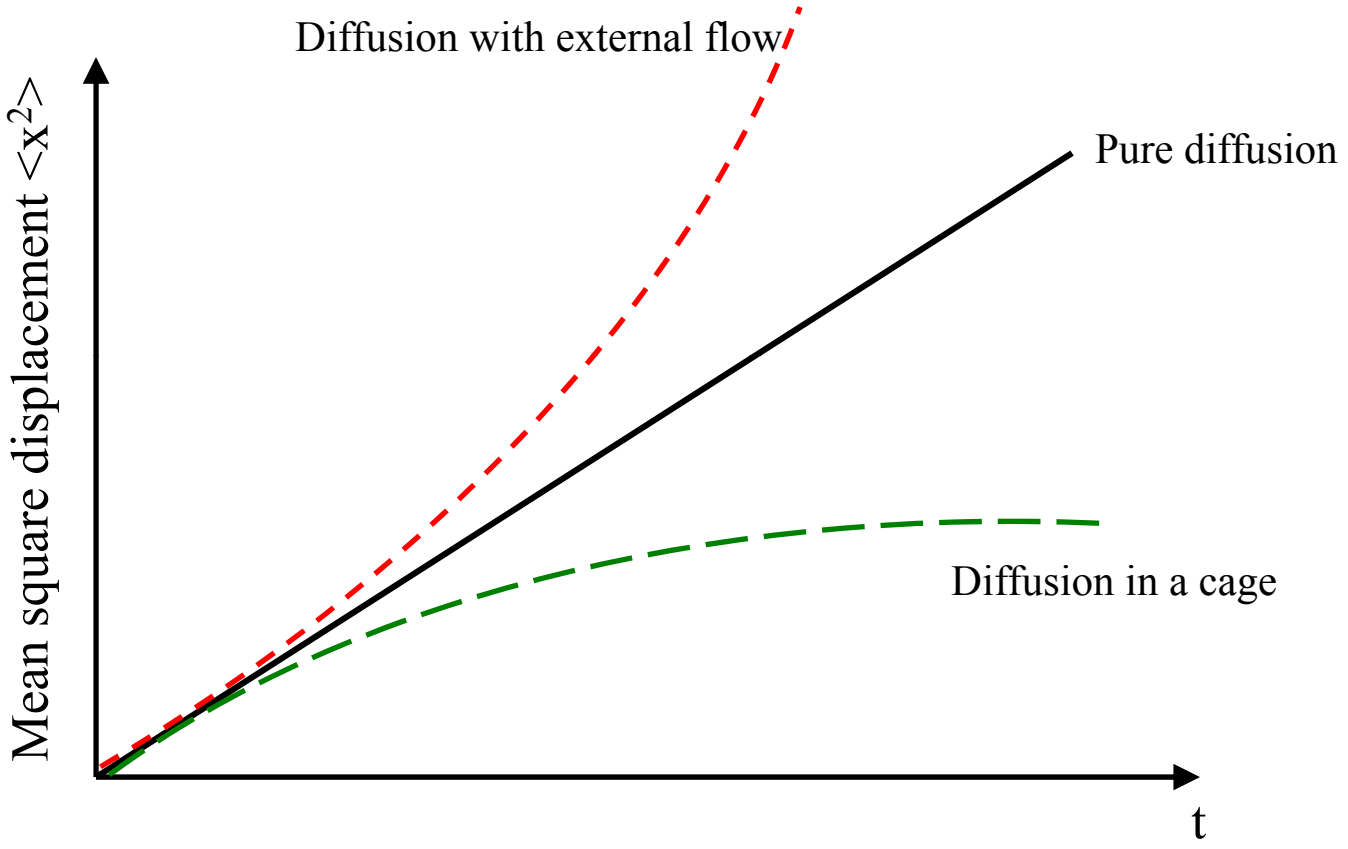
$$p(x) = \frac{1}{\sqrt{4\pi Dt}} e^{-\frac{x^2}{4Dt}} \text{ where } n\delta^2 = 2Dt$$

Application of the Microscopic Theory (I)

Object	Distance diffused			
	1 μm	**100 μm**	**1 mm**	**1 m**
K^+	0.25ms	2.5s	2.5×10^4s (7 hrs)	2.5×10^8s (8 yrs)
Protein	5ms	50s	5.0×10^5s (6 days)	5.0×10^9s (150 yrs)
Organelle	1s	10^4s (3 hrs)	10^8s (3 yrs)	10^{12}s (31710 yers)

K+: Radius = 0.1nm, viscosity = 1mPa·s^{-1}; T = 25°C; D=2000 μm^2/sec
Protein: Radius = 3nm, viscosity = 0.6915mPa·s^{-1}; T = 37; D = 100 μm^2/sec
Organelle: Radis = 500nm, viscosity = 0.8904mPa·s^{-1}; T = 25°C; D = 0.5 μm^2/sec

Application of the Microscopic Theory (II)

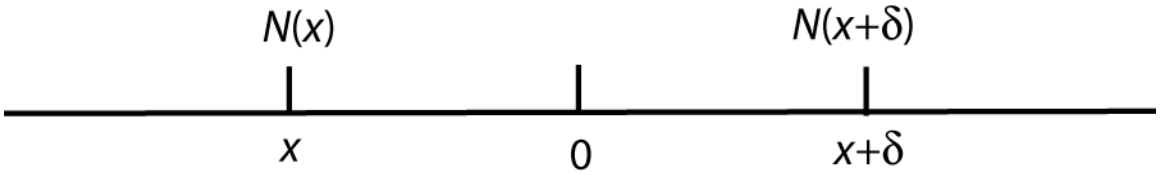


H. Qian, M. P. Sheetz, E. L. Elson, *Single particle tracking: analysis of diffusion and flow in two-dimensional systems*, Biophysical Journal, 60(4):910-921, 1991.

Questions ?

Macroscopic Theory of Diffusion (I)

- Fick's first equation

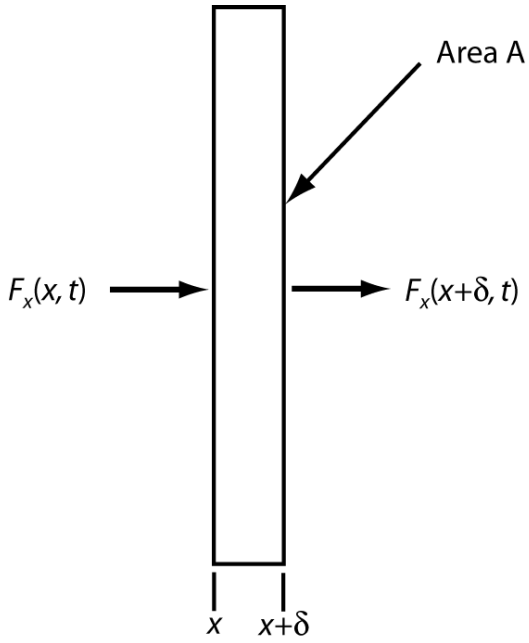


$$-\frac{1}{2}\left[N\left(x+\delta\right)-N\left(x\right)\right]$$

$$\begin{aligned} F_x &= \lim_{\delta\to 0} -\frac{1}{2}\left[N\left(x+\delta\right)-N\left(x\right)\right]/A\tau \\ &= \lim_{\delta\to 0} -\frac{\delta^2}{2\tau}\frac{1}{\delta}\left[\frac{N\left(x+\delta\right)}{A\delta}-\frac{N\left(x\right)}{A\delta}\right] \\ &= \lim_{\delta\to 0} -D\frac{1}{\delta}\left[C\left(x+\delta\right)-C\left(x\right)\right] \\ &= -D\frac{\partial C}{\partial x} \end{aligned}$$

Macroscopic Theory of Diffusion (II)

- Fick's second equation



$$\left[C\left(t+\tau\right)-C\left(t\right)\right]=-\frac{1}{A\delta}\left[F_x\left(x+\delta\right)-F_x\left(x\right)\right]A\tau$$

$$\frac{1}{\tau}\left[C\left(t+\tau\right)-C\left(t\right)\right]=-\frac{1}{\tau}\frac{1}{A\delta}\left[F_x\left(x+\delta\right)-F_x\left(x\right)\right]A\tau$$

$$=-\frac{1}{\delta}\left[F_x\left(x+\delta\right)-F_x\left(x\right)\right]$$

$$\frac{\partial C}{\partial t}=-\frac{\partial F_x}{\partial x}=D\frac{\partial^2 C}{\partial x^2}$$

Calculation of Diffusion Coefficient

- Einstein-Smoluchowski Relation

$$v_d = \frac{1}{2} a\tau = \frac{1}{2}\frac{F_x}{m}\tau \qquad f = \frac{F_x}{v_d} = \frac{2m}{\tau} = \frac{2m\frac{\sigma^-}{\tau^2}}{\frac{\delta^2}{\tau}} = \frac{mv_x^2}{D} = \frac{kT}{D}$$

$$D = \frac{kT}{f}$$

f: viscous drag coefficient

- Stokes' relation: the viscous drag coefficient of a sphere moving in an unbounded fluid

$$f = 6\pi\eta r$$

η: viscousity
r: radius

An example of D calculation

- Calculation of diffusion coefficient

$$D = \frac{kT}{6\pi\eta r}$$

- $k=1.381\times10^{-23}$J/k=1.381 $\times10^{-17}$ N·μm/k
- T = 273.15 + 25
- η=0.8904mPa·s=0.8904 $\times10^{-3}$ $\times10^{-12}$N·μm^{-2}·s
- r= 500nm=0.5μm
- D=0.5 μm^2/s

An example of direct measurement of D

M. B. Elowitz et al, *Protein mobility in the cytoplasm of E. coli*,
J. Bacteriology, 181:197-203, 1999