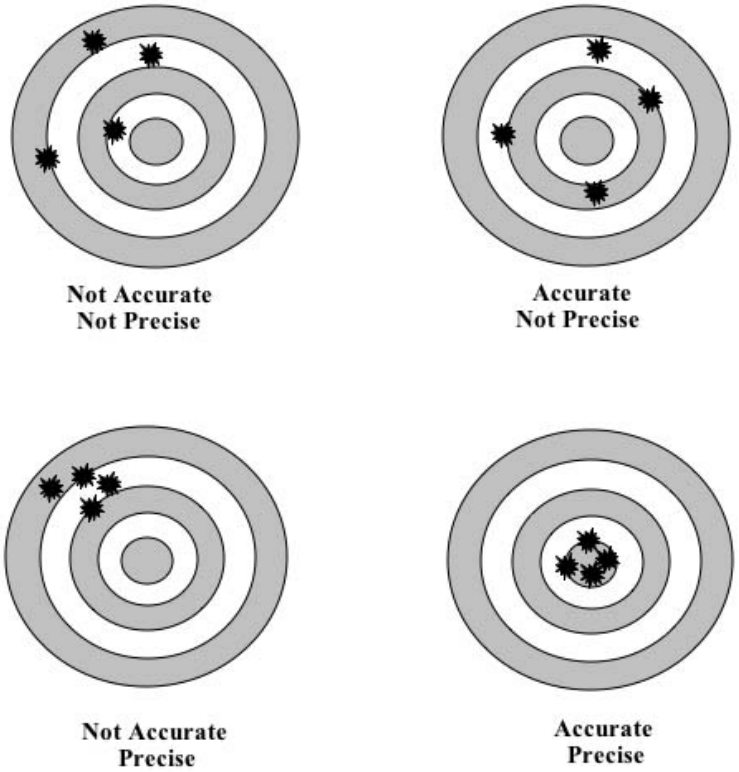
Bioimage Informatics

Lecture 2, Spring 2012

Fundamentals of Light Microscopy



http://celebrating200years.noaa.gov/magazine/tct/tct_side1.html

Outline

- Importance of understanding and optimizing image formation
- Some basic optics facts
- Light microscope structure
- Contrast generation in microscopy
- Practical considerations of microscopy

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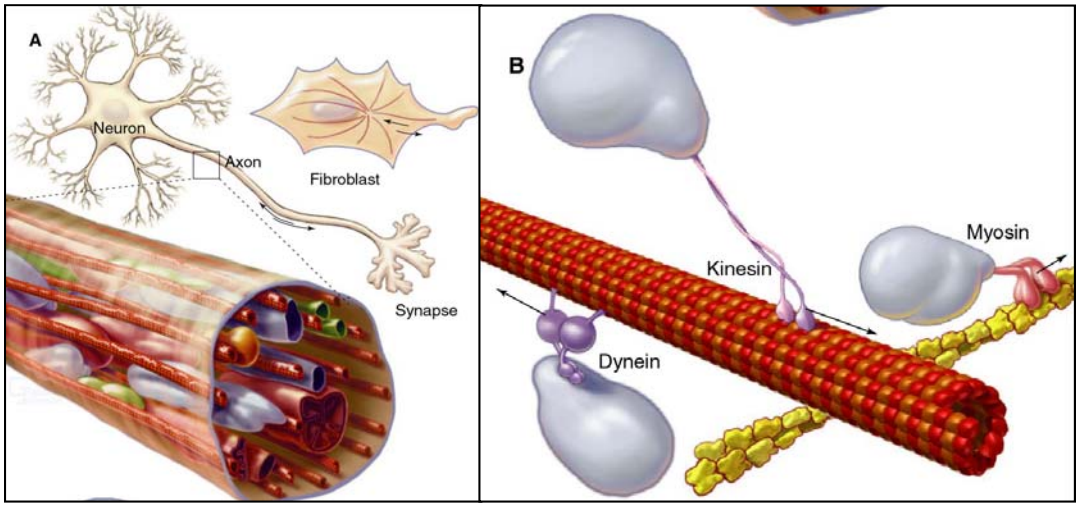
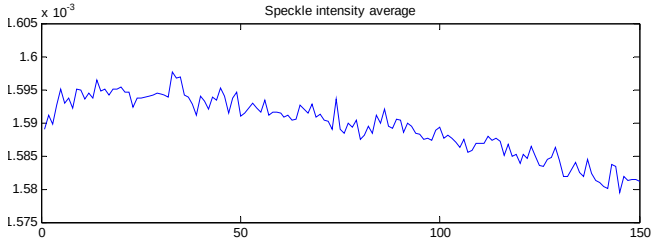
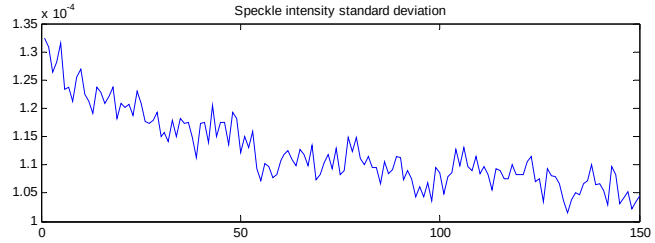
Understanding Image Formation







axonal transport of APP vesicles

http://micro.magnet.fsu.edu/primer/java/fluorescence/photobleaching/index.html

- Photobleaching results in gradual decreasing of image signal intensities over time under repetitive exposure.

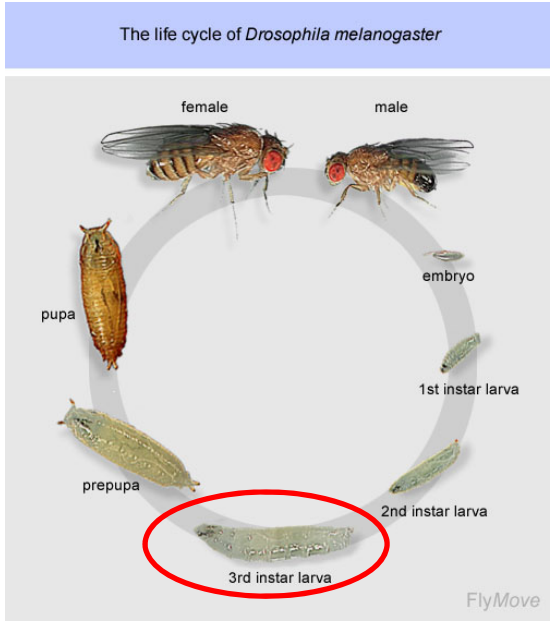
Optimizing Image Formation

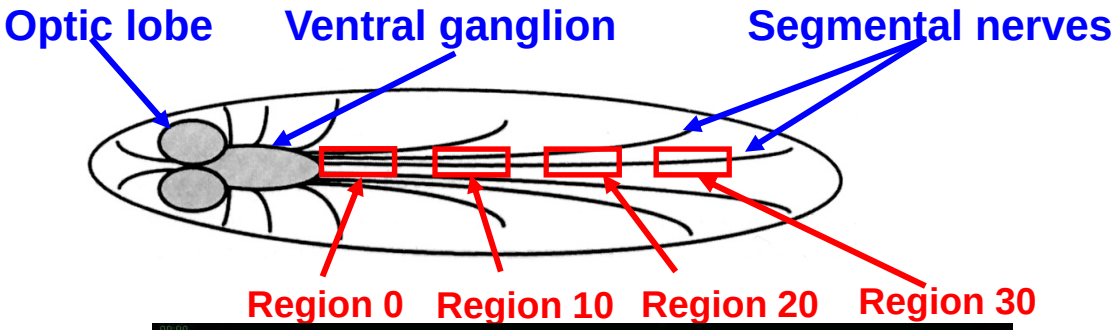
- Bioimage data collection and data analysis must be collaborative processes.

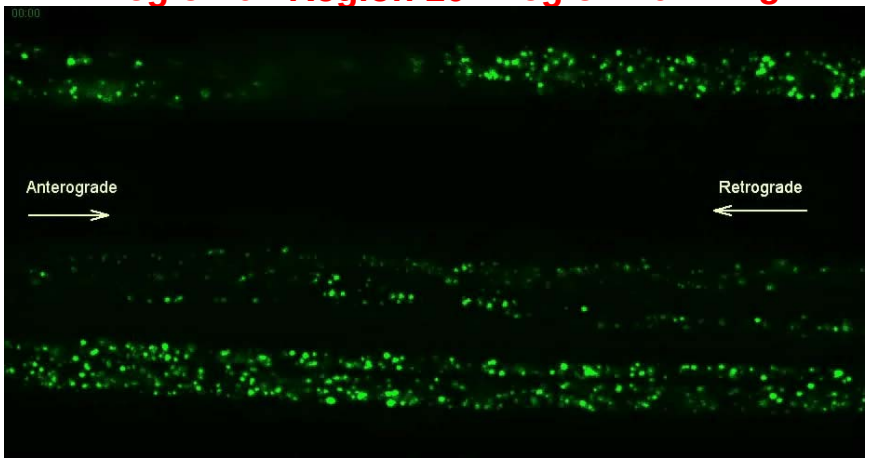
  → Images that are improperly collected cannot be analyzed.
  E.g. violation of Nyquist sampling

  → Optimization of image collection can significantly simplify data analysis.
  This requires concurrent design of imaging and data analysis.

Example: Imaging Axonal Cargo Transport







Saxton lab, UC Santa Cruz

axonal transport of human APP-YFP vesicles; 0.1 sec/frame 10 μm

Relation between Image Collection and Image Analysis

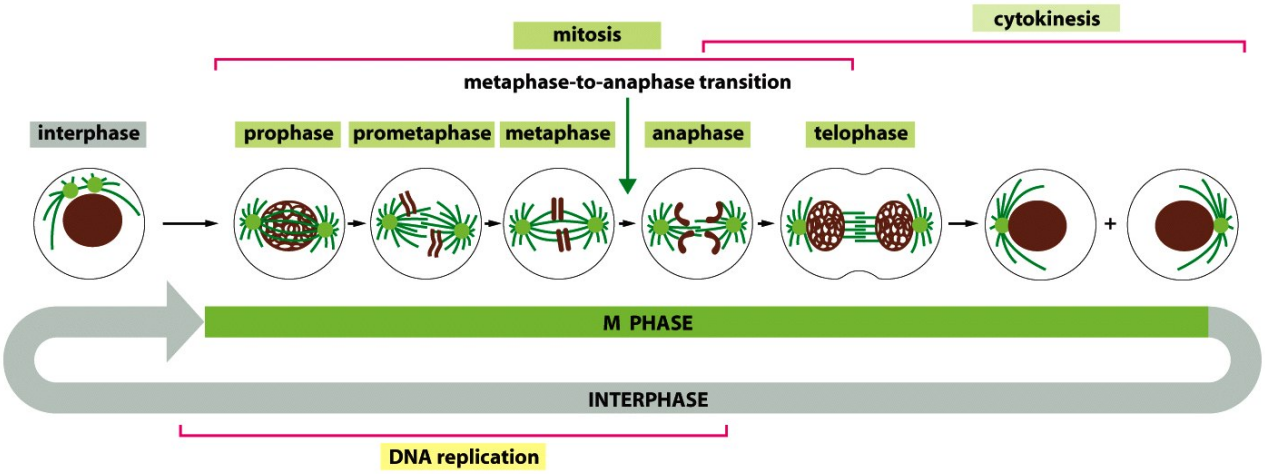
- Bioimage data collection and data analysis must be collaborative processes.

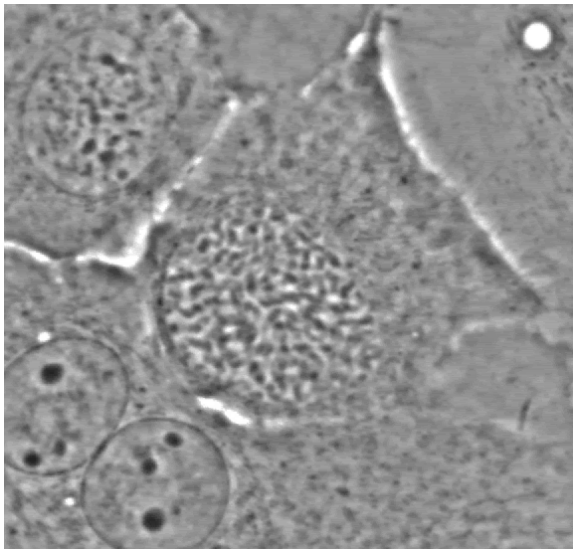
  → Image processing and computer vision can significantly reduce challenges in image collection.

  This is one of the purposes of this class.

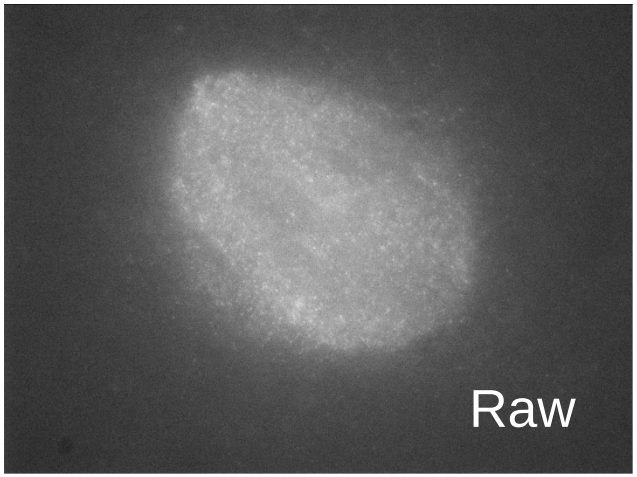
Example: Image Alignment/Registration

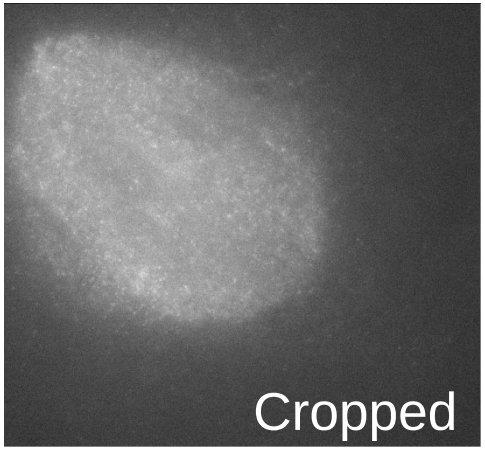
- Alignment of mitotic spindle images
- Introduction to the mitotic spindle

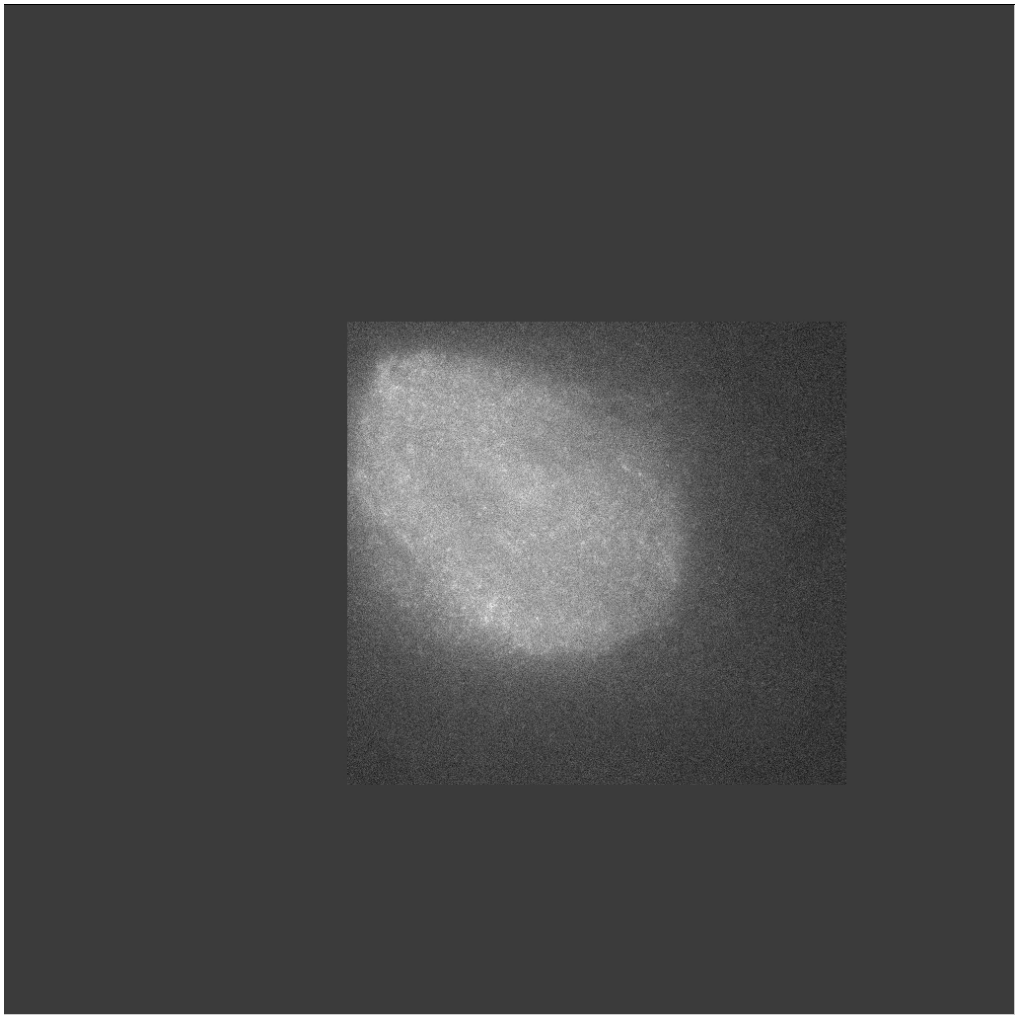




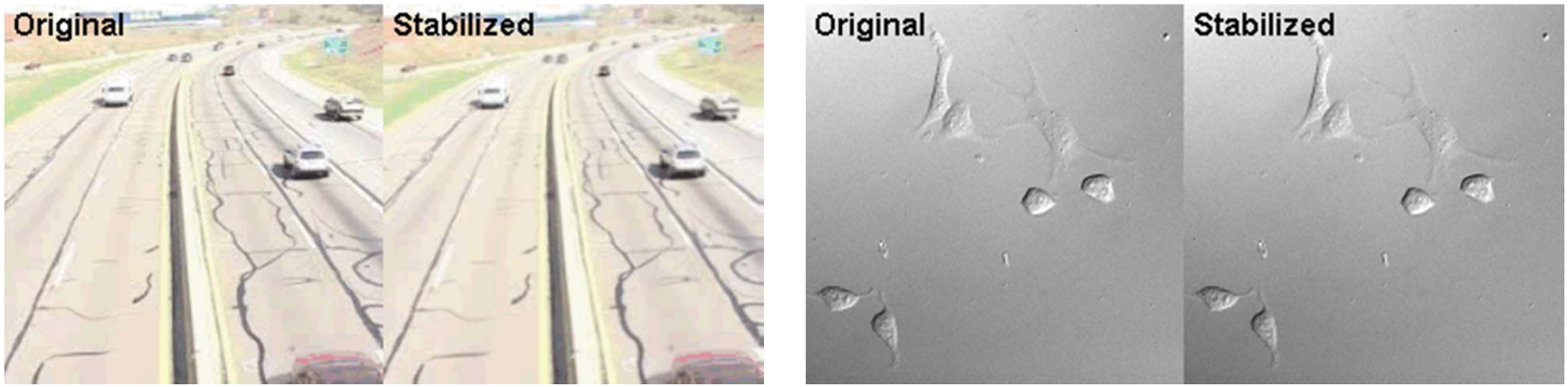
Demo I: Image Alignment







Demo II: Image Alignment



http://www.cs.cmu.edu/~kangli/code/Image_Stabilizer.html

Summary

- Image collection and image analysis should be collaborative processes.
- Correct collection of image data is essential to subsequent data analysis.
- Computational image analysis can help overcome some of the challenges in image collection.
- Understanding image formation is essential to image analysis.
- Proper design of image collection can significantly simplify subsequence.

- Importance of understanding and optimizing image formation
- Some basic optics facts
- Light microscope structure
- Contrast generation in microscopy
- Practical considerations of microscopy

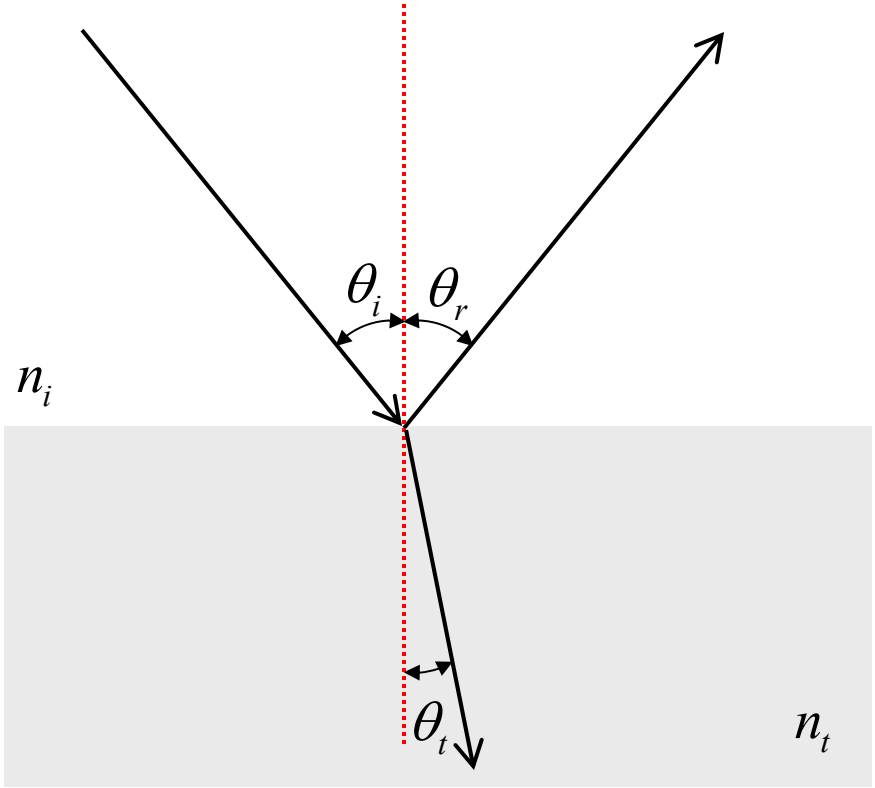
Reflection & Refraction

- Law of reflection

$$\theta_i = \theta_r$$

- Law of refraction (Snell's law)

$$n_i \sin\theta_i = n_t \sin\theta_t$$



Refractive Index

- Absolute refractive index of a material

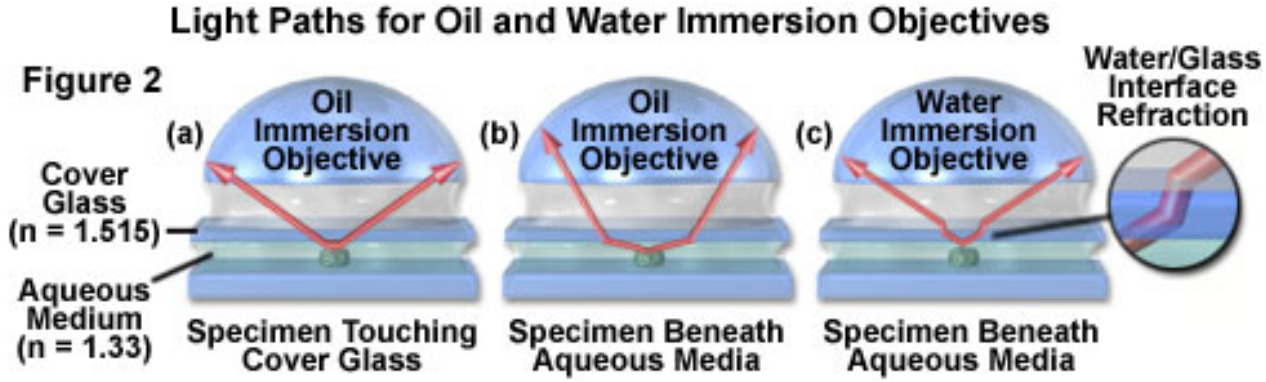
$$\frac{\text{velocity of electromagnetic wave in vacuum}}{\text{velocity of electromagnetic wave in the material}}$$

  - Air 1.000293

- Refractive index (also called relative refractive index)
  - Water 1.333
  - Glass ~1.50
  - Immersion oil 1.51

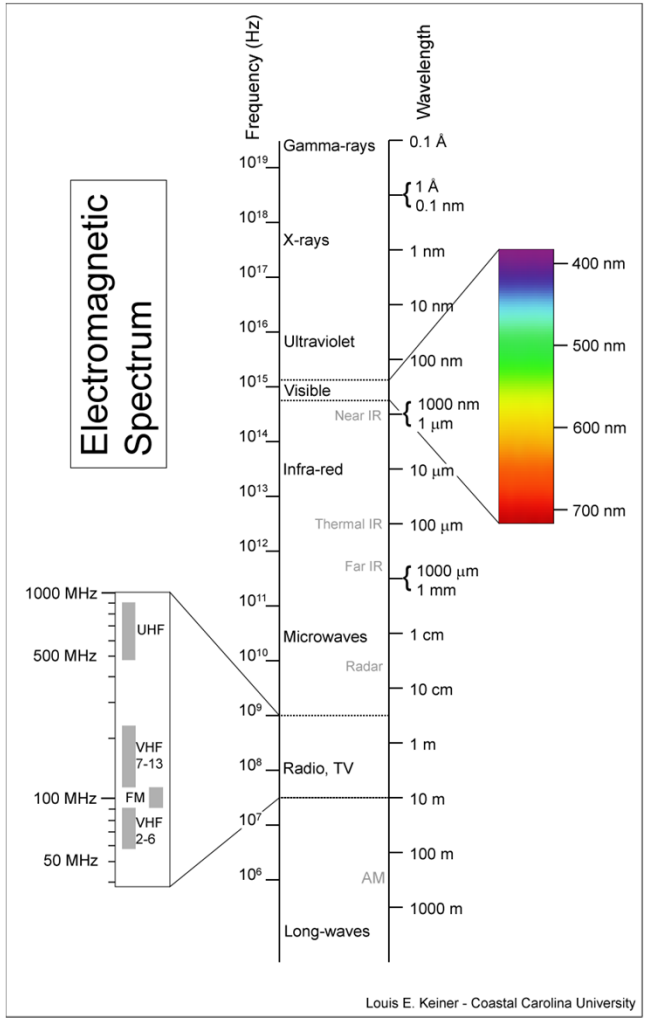
$$n_{12} = \frac{n_2}{n_1}$$

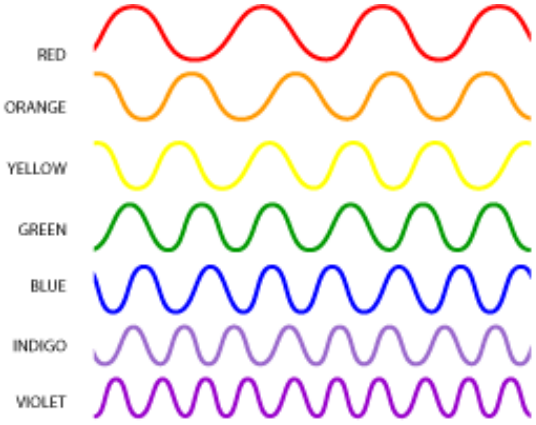
Minimizing Distortion by Matching Refractive Indices



http://www.microscopyu.com/articles/optics/waterimmersionobjectives.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

Photon Energy

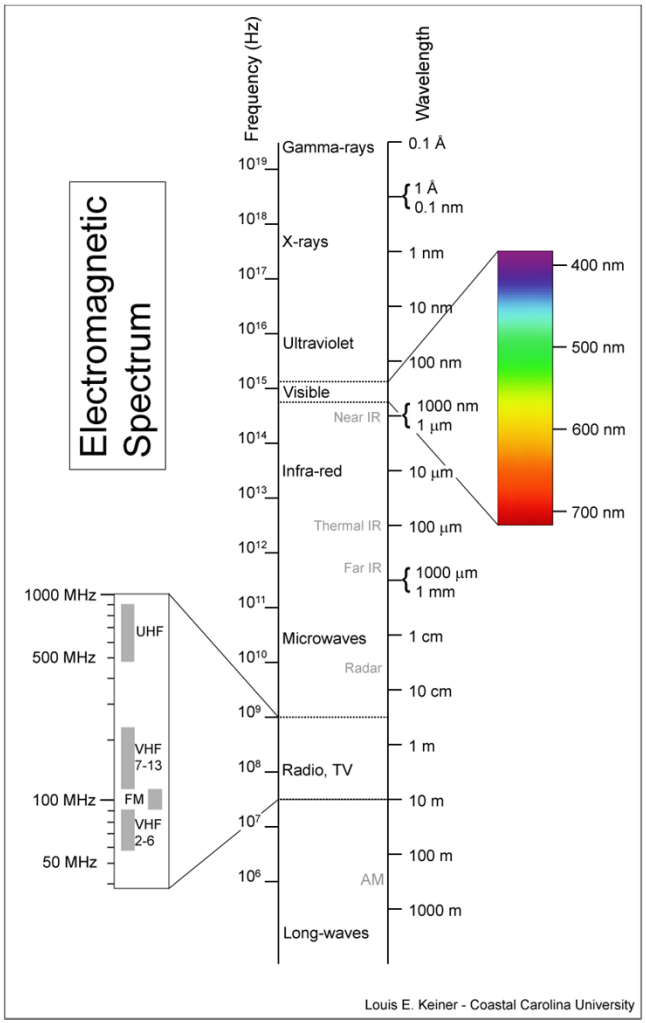
- Energy of a photon: Planck's law

$$E = h\nu = h\frac{c}{\lambda}$$

h: Planck's constant; 6.626×10^{-34}J·s
v: frequency of light;
λ: wavelength of light
c: speed of light

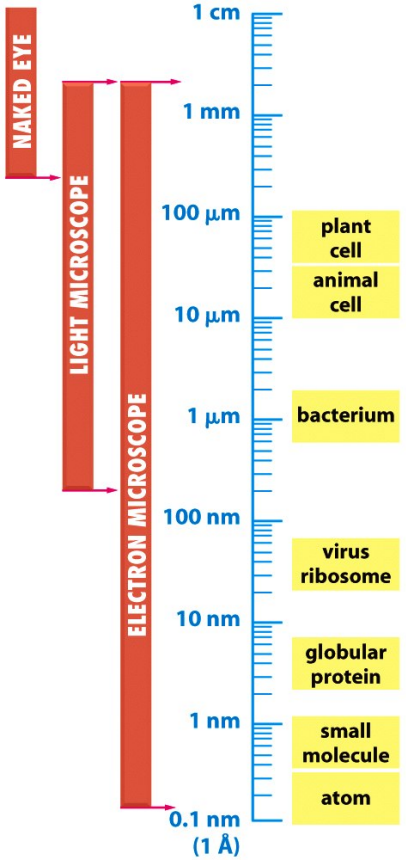
energy of a photon = 3.973×10^{-19}J at 500nm

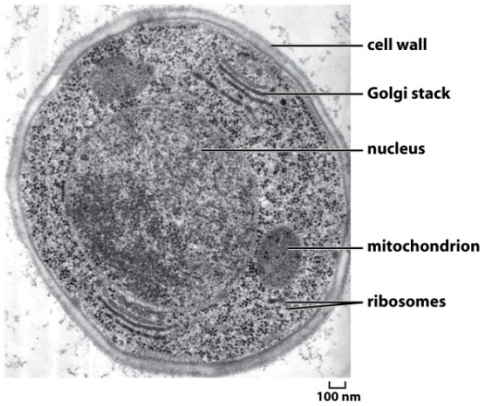
- Shorter waves have higher energy.



Why Use Light Microscopy?

- Microscopy makes it possible to visualize cell structure and dynamics.
- Light microscopy permits live imaging of cellular processes.
- Electron microscopy provides higher resolution but requires samples to be fixed.





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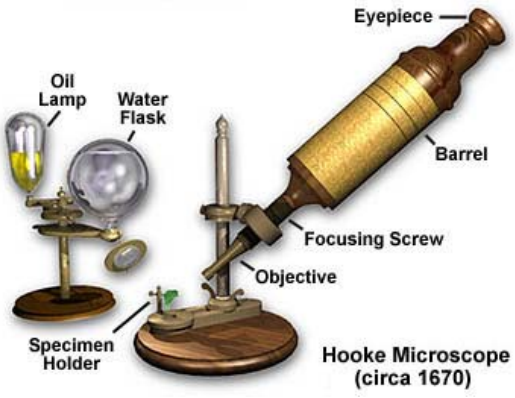
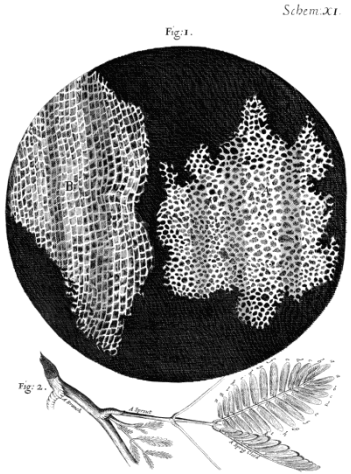
Origination of Light Microscopes

- 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

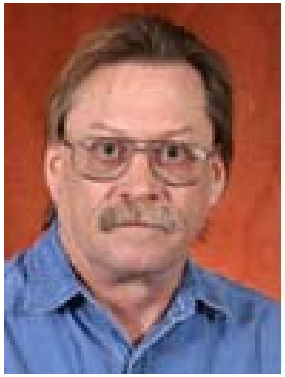
- Modern microscopes are computer-controlled .
- Modern microscopes can be configured to be highly automated.

Some Reference Information

- Major microscope manufacturers

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

Some Reference Information



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

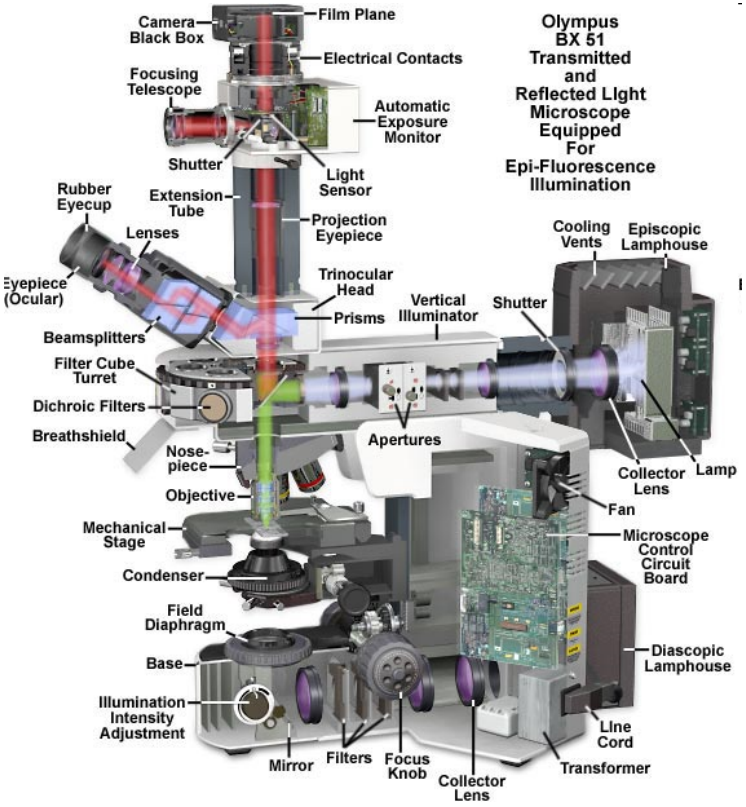
http://zeiss-campus.magnet.fsu.edu/index.html

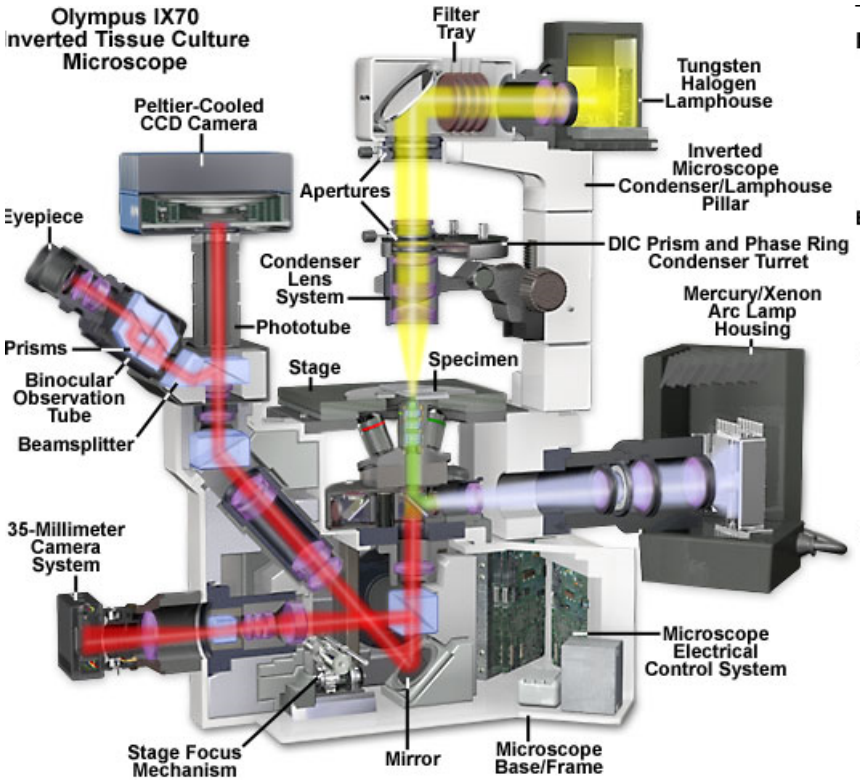
External Structure of Modern Microscopes

Upright

Inverted

Internal Structure of Modern Microscopes





Upright

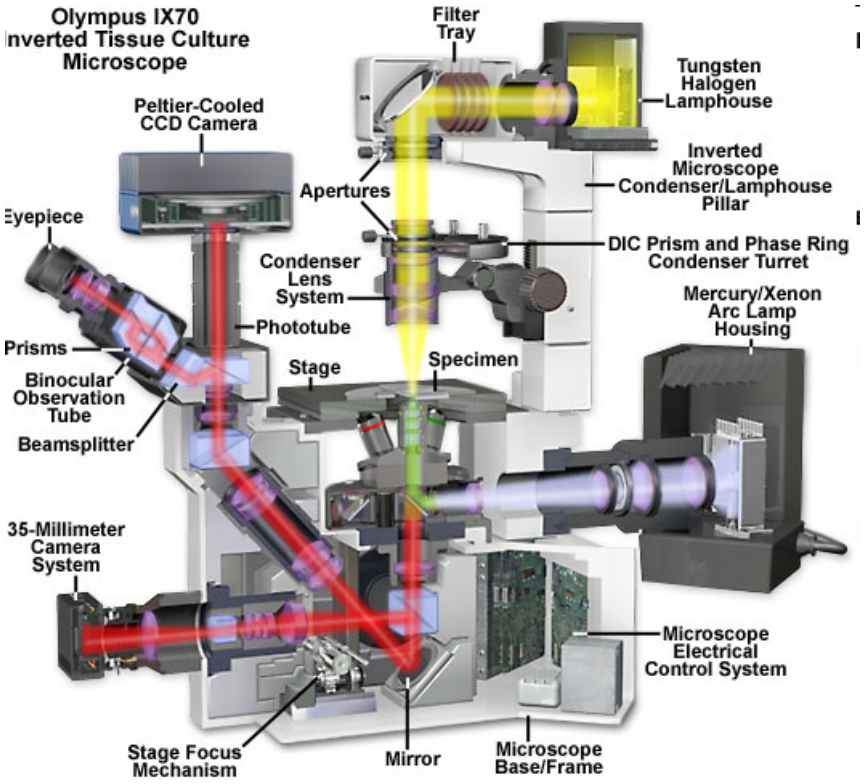
http://www.olympusmicro.com/

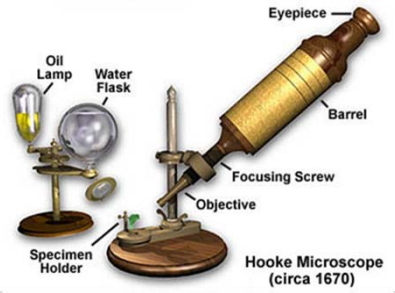
Inverted

Episcopic: reflected; Diascopic: transmitted

Light Path Components (I)

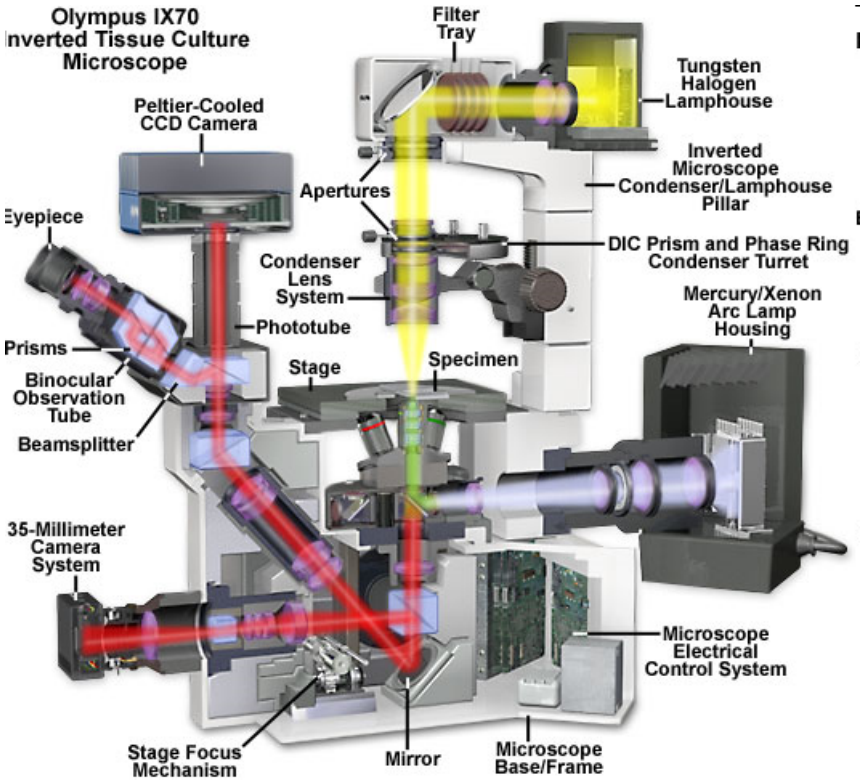
- Illumination source
- Illumination filters
- Condenser
- Specimen stage





Light Path Components (II)

- Objective lens
- Image filter
- Image sensor
- Eyepiece



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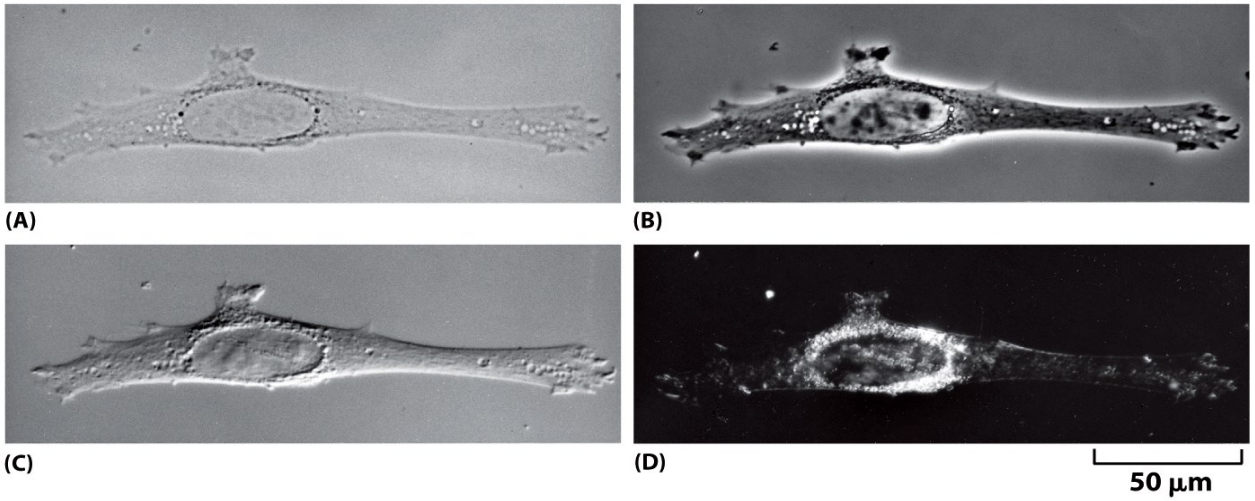
Contrast Generation in Light Microscopy

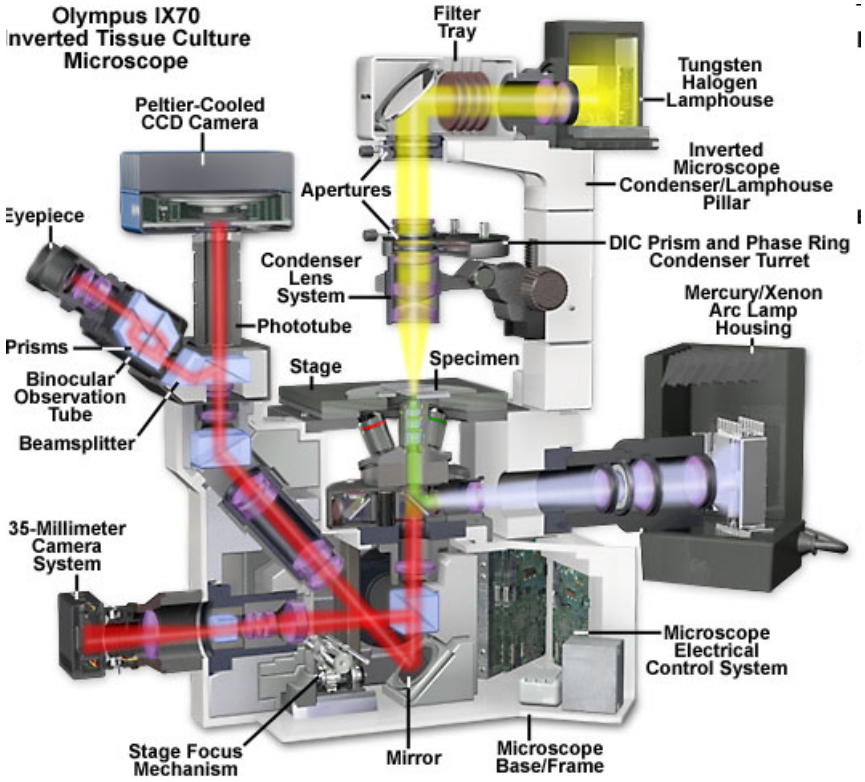
- Two fundamental roles of any microscope
  - To provide adequate contrast
  - To provide adequate resolution.

- Contrast generation
  - Transmitted light illumination vs reflected light illumination
  - Bright-field vs dark-field
  - Phase contrast
  - Fluorescent micros

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



Reflected Light vs Transmitted Light

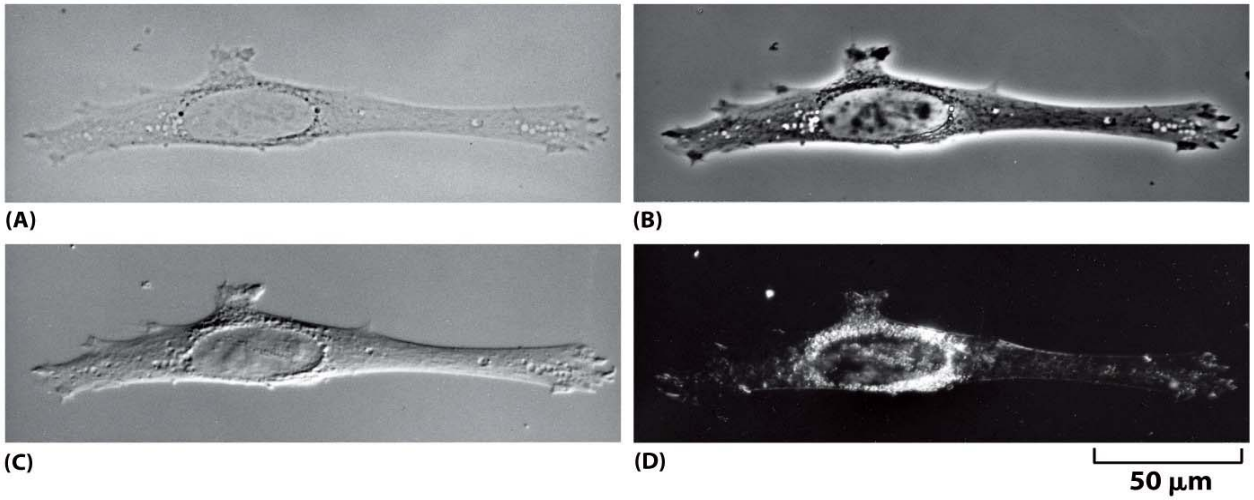


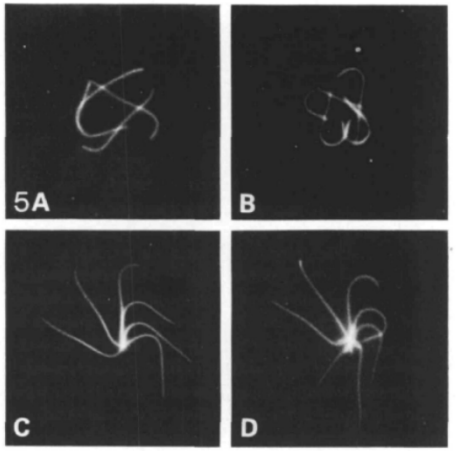
http://www.olympusmicro.com/primer/java/lightpaths/ix70fluorescence/ix70.html

Bright-field vs Dark-field (I)

- Under bright-field contrast, the specimen appears dark against a bright background.

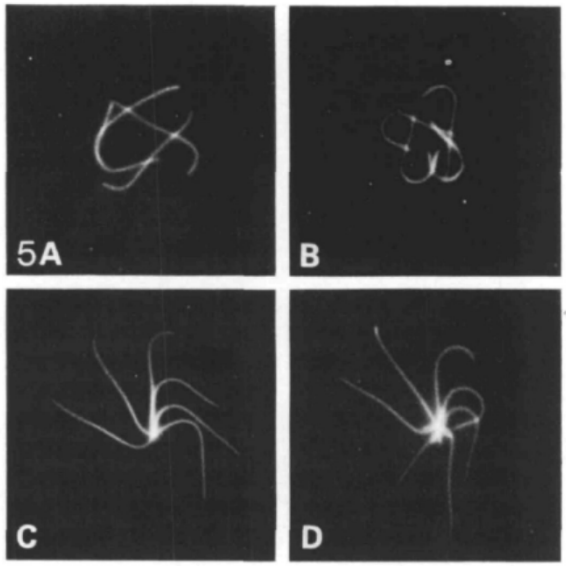
- Dark-field contrast is particularly useful when imaging thin filaments or small particles.





Bright-field vs Dark-field (II)

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



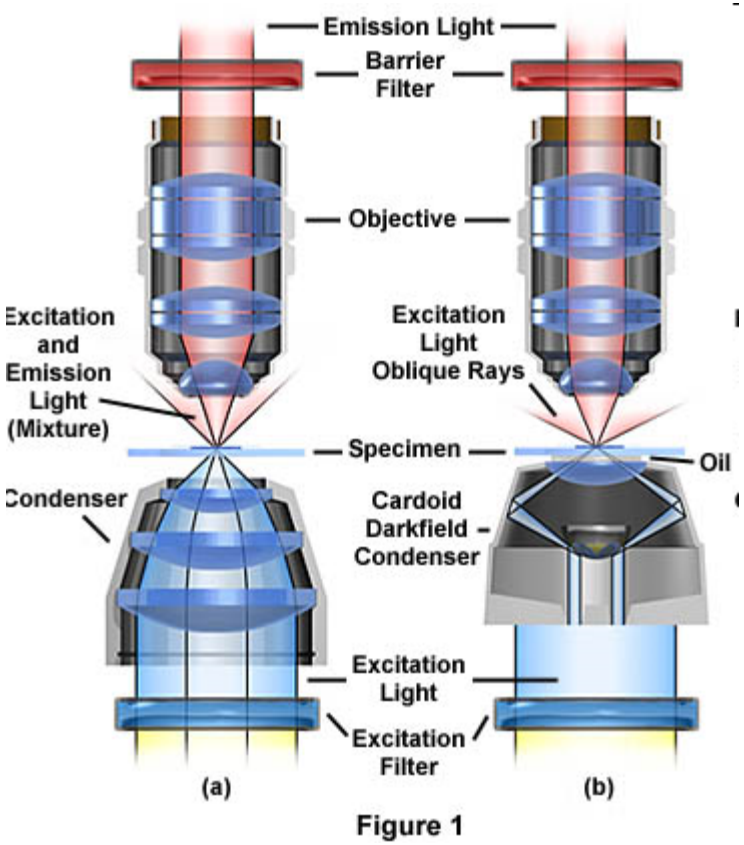
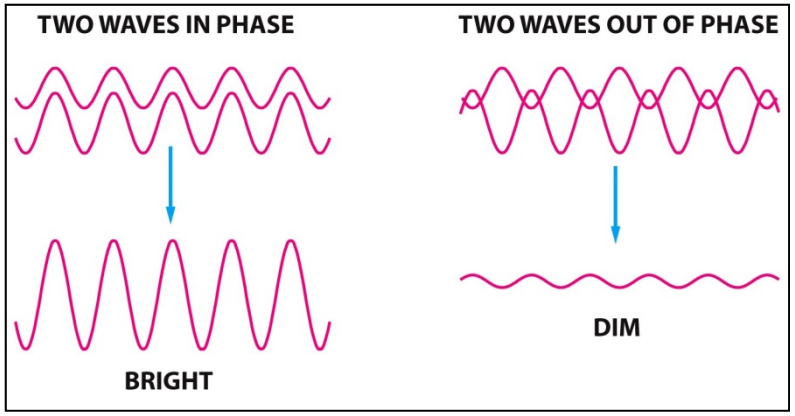
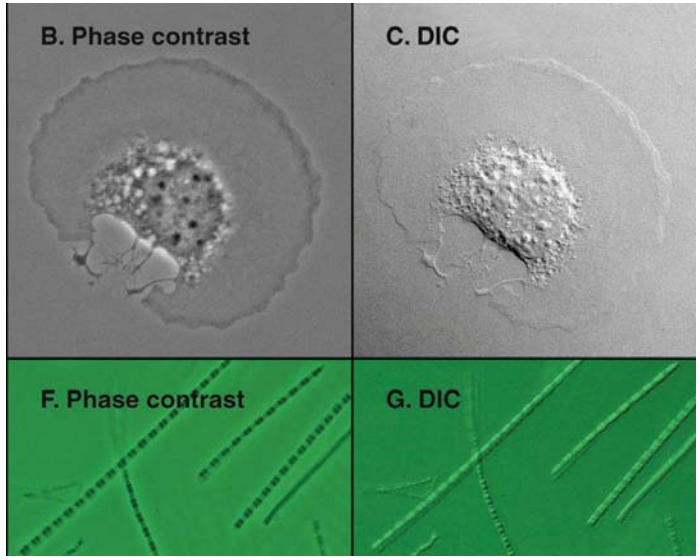


Figure 1

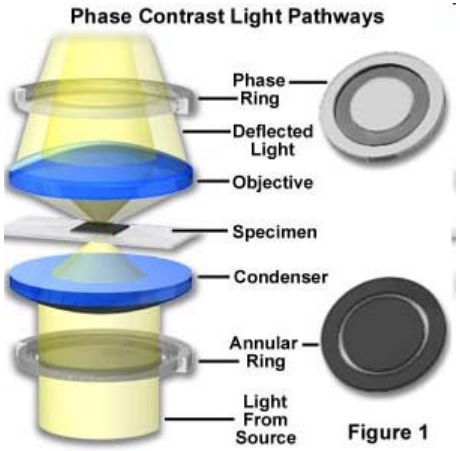
Phase Contrast & DIC (I)

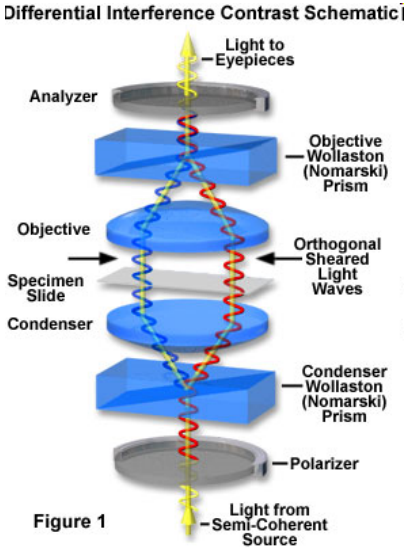
- Phase contrast is very useful in imaging transparent specimens, which do not change light magnitude.
- Contrast is generated due to the different refractive indices of the sample and the background.
- Phase contrast can generate artifacts.
  - Halos by boundary
  - Artificial shadows
- DIC significantly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

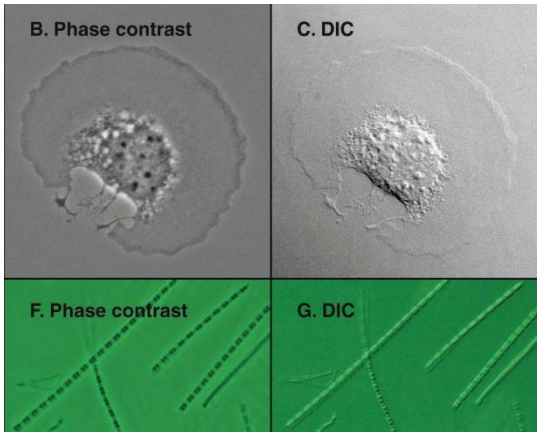


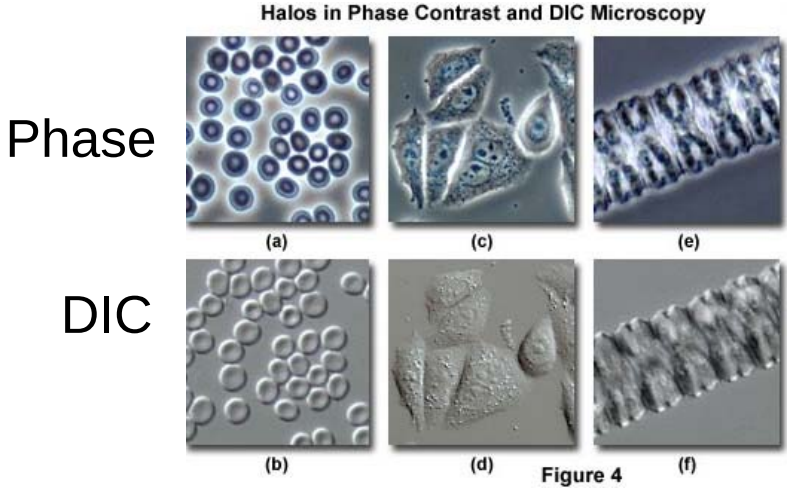


Phase Contrast & DIC (II)

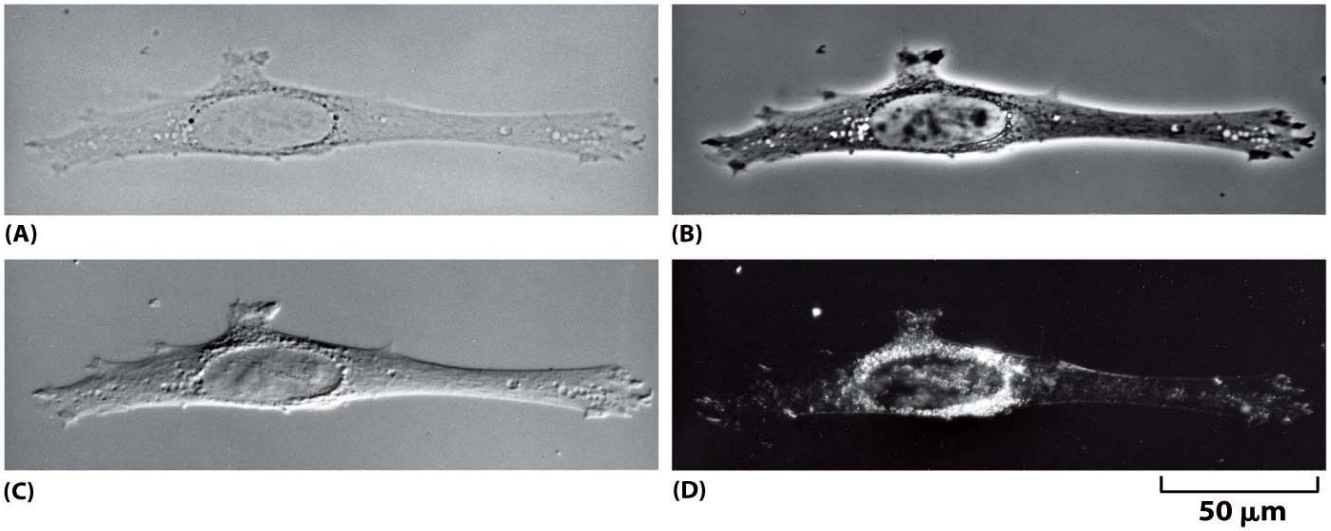


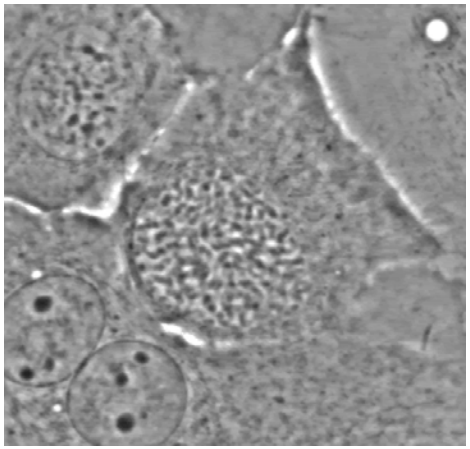






What is wrong with ALL the cell images?





The Nobel Prize in Physics 1953

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

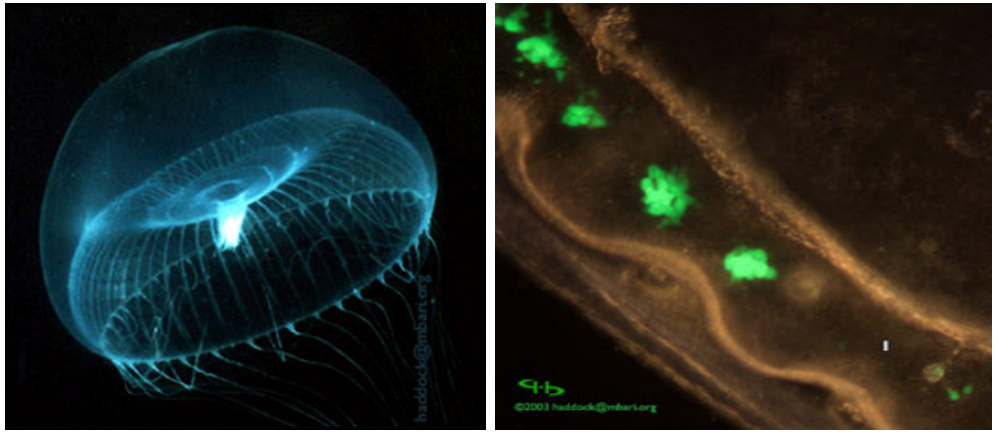
Frits (Frederik) Zernike

the Netherlands

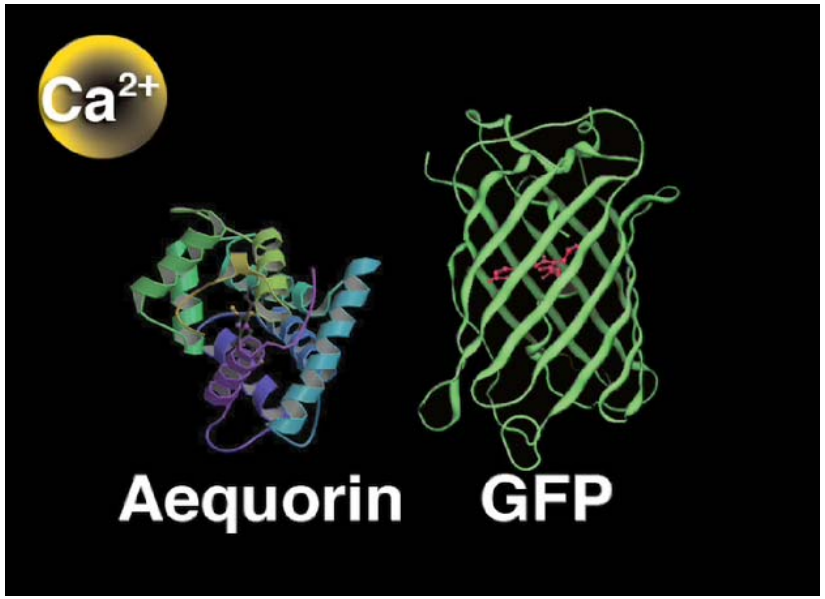
Groningen University
Groningen, the Netherlands

b. 1888
d. 1966

Green Fluorescence Protein

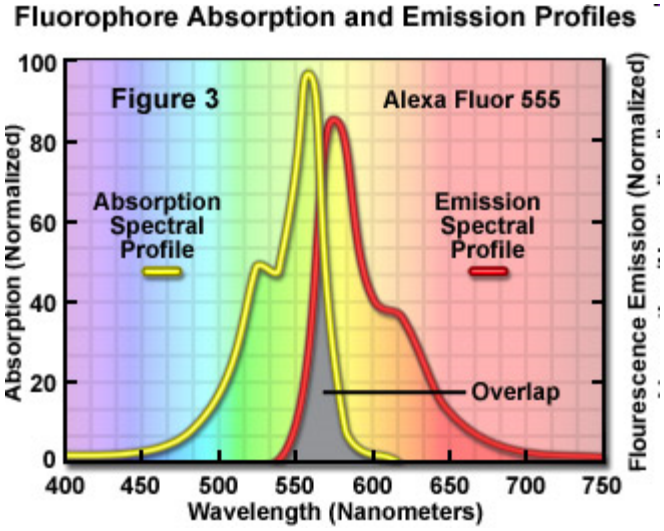


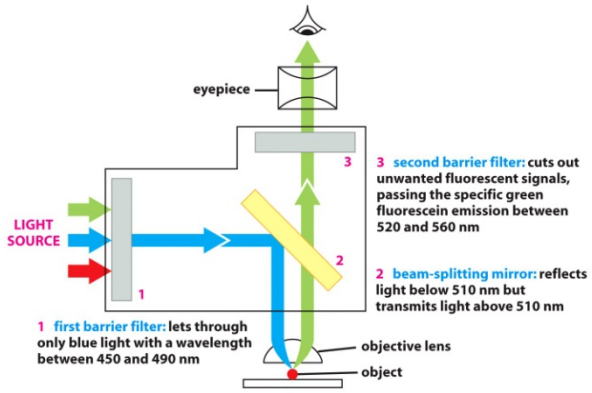
Jellyfish: *Aequorea victoria*

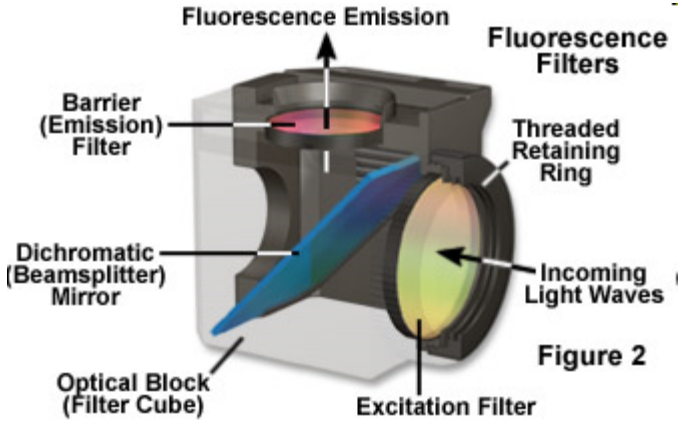


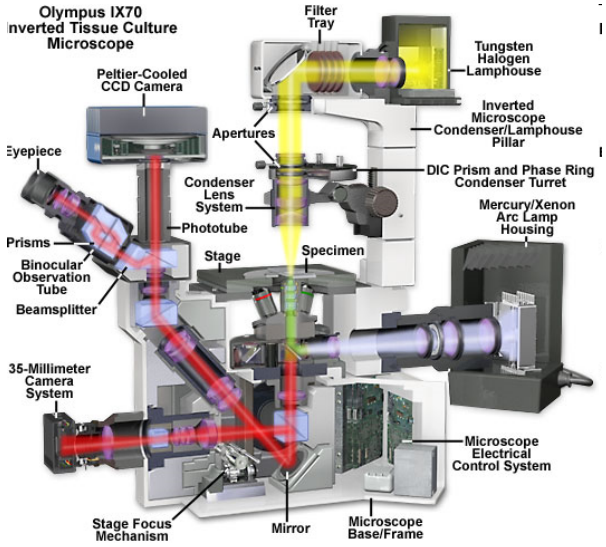
http://gfp.conncoll.edu/GFP-1.htm

Fluorescence Microscopy (I)



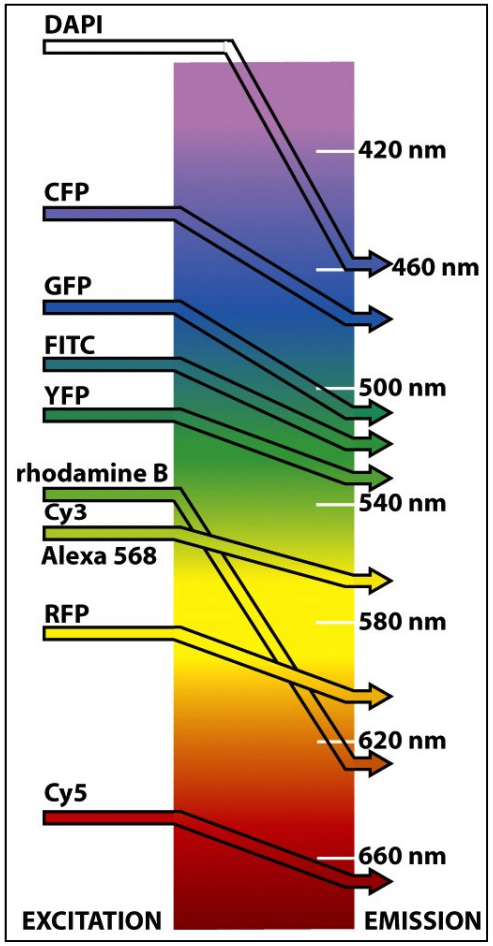


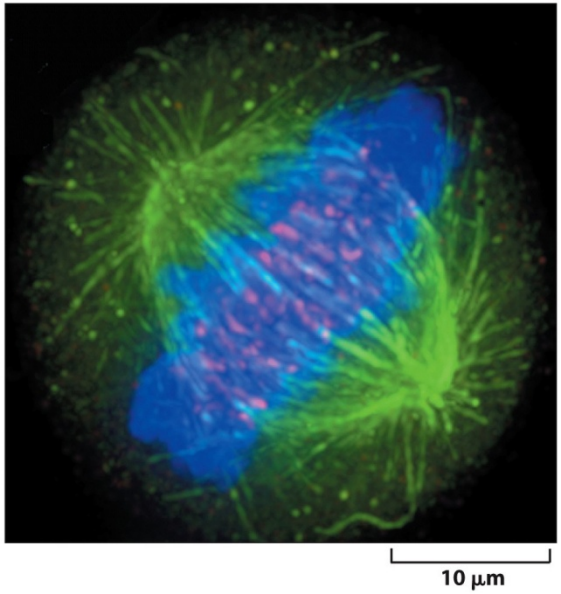




Fluorescence Microscopy (II)

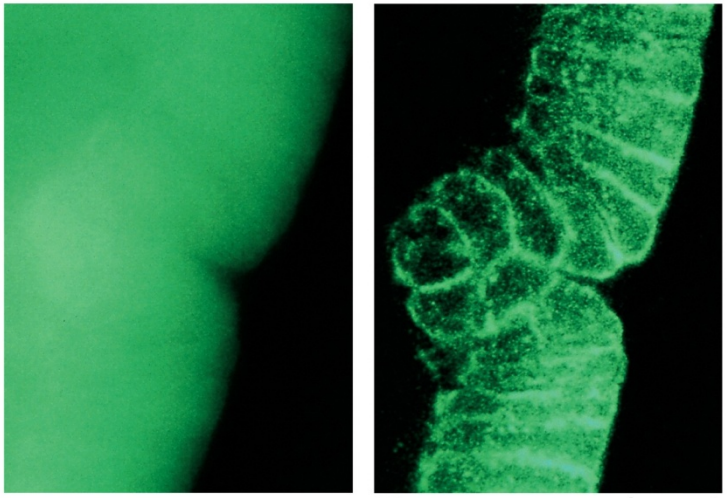
Fluorophores are available at many different colors

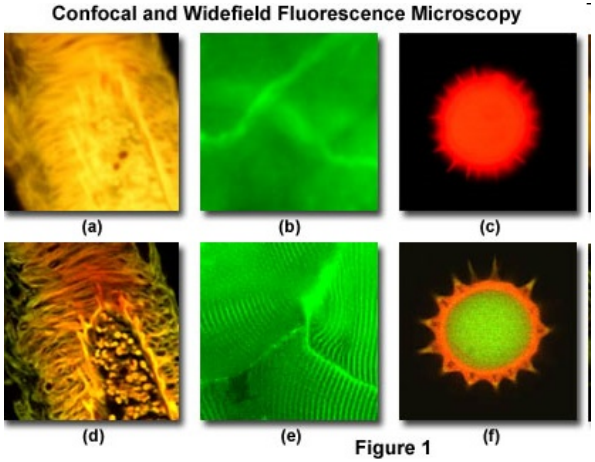


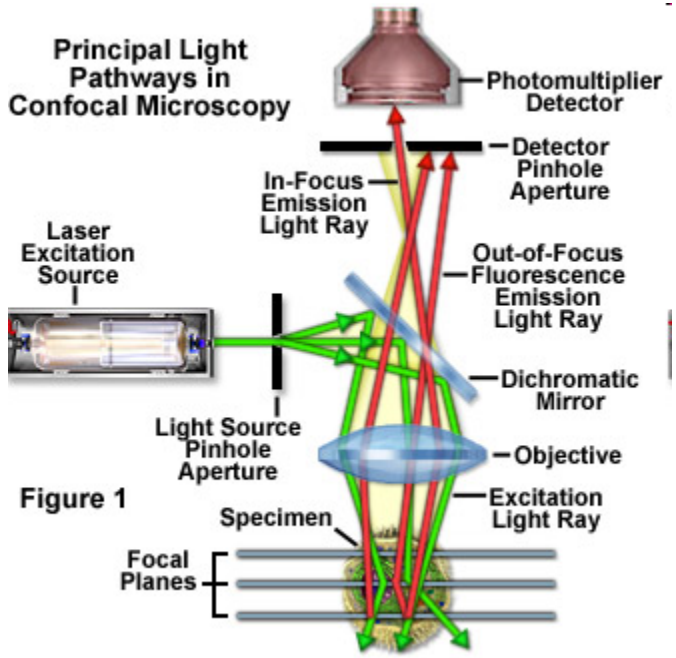


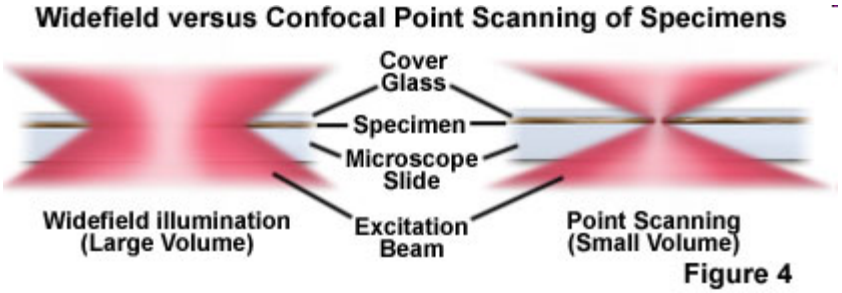
Blue: chromosome
Green: microtubules
Red: kinetochores

Widefield vs Confocal Microscopy







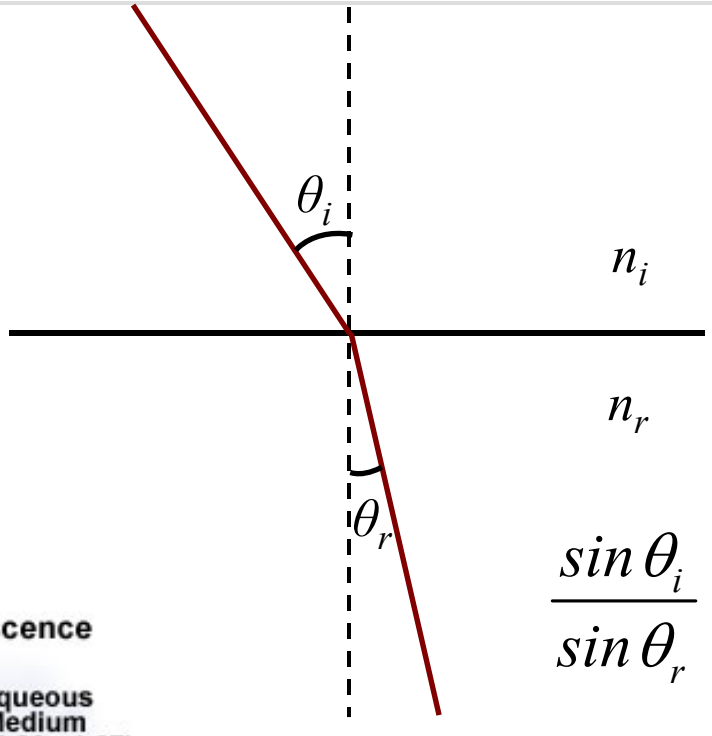


http://www.olympusfluoview.com/theory/confocalintro.html

Total Internal Reflection Microscopy (I)

- Typical thickness of the evanescent layer is less than 200nm

- Often used for imaging
  - membrane related cellular processes
  - single molecules



$$\frac{\sin\theta_i}{\sin\theta_r} = \frac{n_r}{n_i}$$

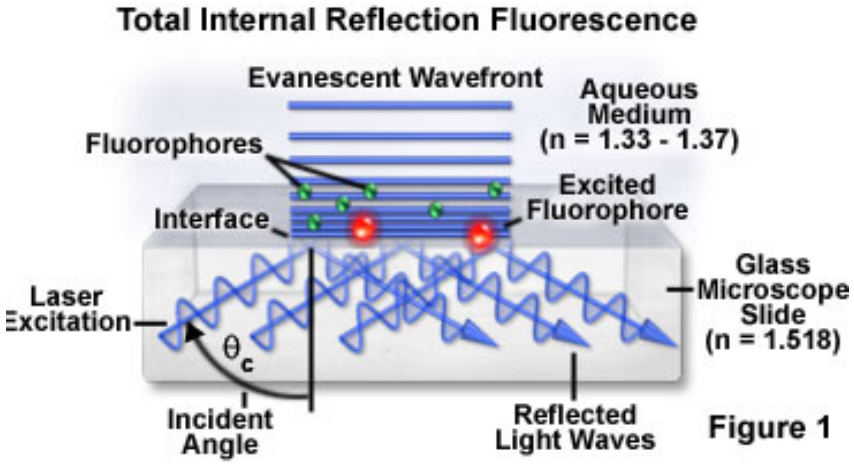
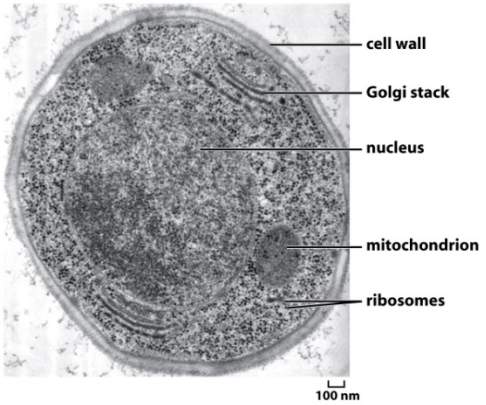
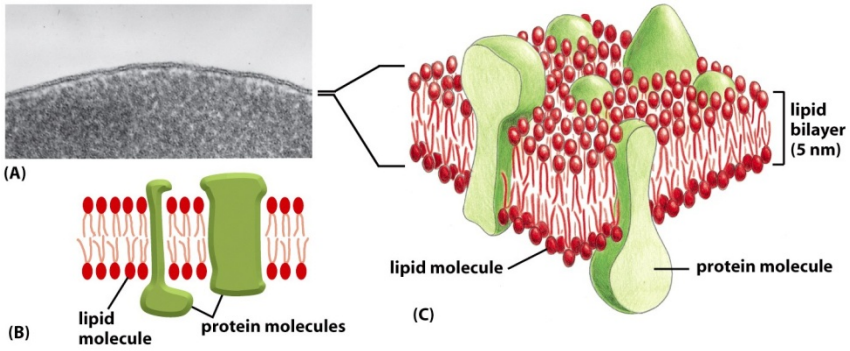


Figure 1

Total Internal Reflection Microscopy (II)

- TIRF is often used for imaging
  - membrane related cellular processes
  - single molecules





Fluorescence Microscopy Summary

- High specificity:
  - Chemical fluorophores
  - Fluorescent proteins

- High sensitivity: up to single molecules.

- Importance of understanding and optimizing image formation
- Some basic optics facts
- Light microscope structure
- Contrast generation in microscopy
- Practical considerations of microscopy

Practical Considerations

- Photobleaching

  - Fluorophores gradually lose their ability of light emission.

  - This results in a sustained decrease in image intensity.

- Phototoxicity

  - Constant illumination generates free radicals that cause cell death.

  - This places a fundamental limit on how many frame of images can be collected.

Questions?