生物图像处理与信息学

Biological Image Processing and Informatics

第四讲
Lecture 4

光学成像基础 (I)
Fundamentals of Light Microscopy (I)

Outline

- Contrast generation in microscopy
- Introduction to fluorescence microscopy
- Practical constraints in microscopy
- Basic metrics of a microscope

- Contrast generation in microscopy
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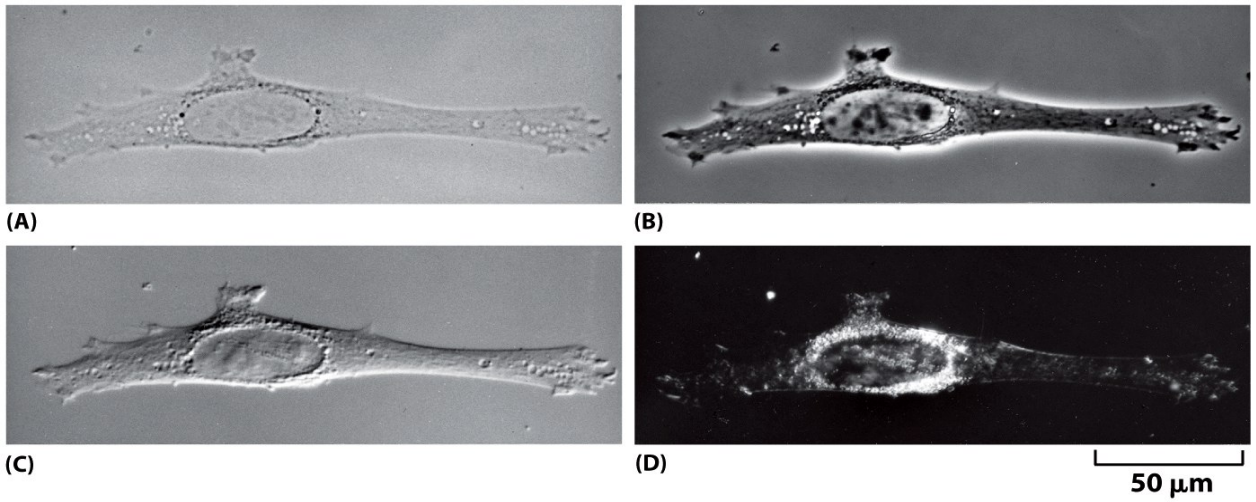
Contrast Generation in Light Microscopy

- 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

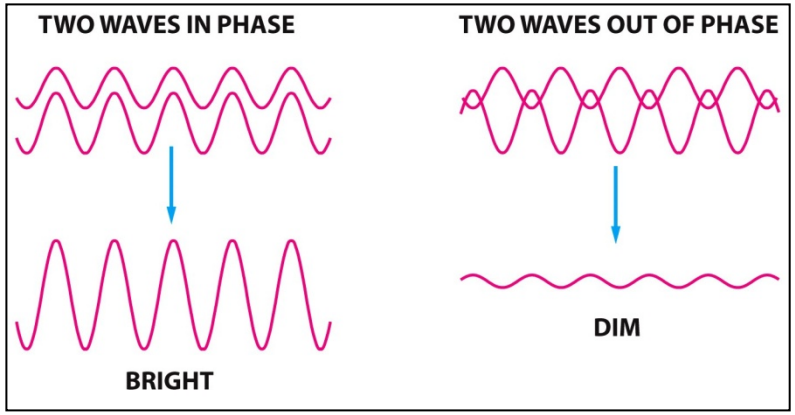
(A) Bright-field
(B) Phase
(C) DIC
(D) Dark-field



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.



The Nobel Prize in Physics 1953

Frits Zernike
Prize share: 1/1

The Nobel Prize in Physics 1953 was awarded to Frits Zernike *"for his demonstration of the phase contrast method, especially for his invention of the phase contrast microscope".*

Phase Contrast & DIC (III)

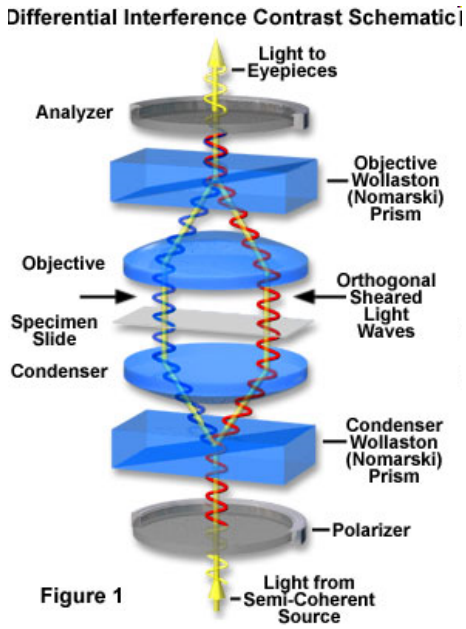
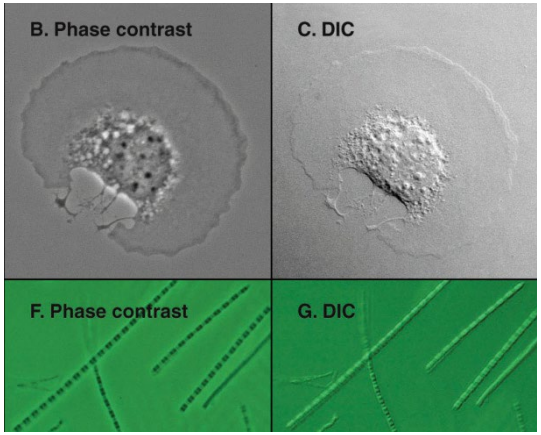


Figure 1




Georges (Jerzy) Nomarski (1919-1997)

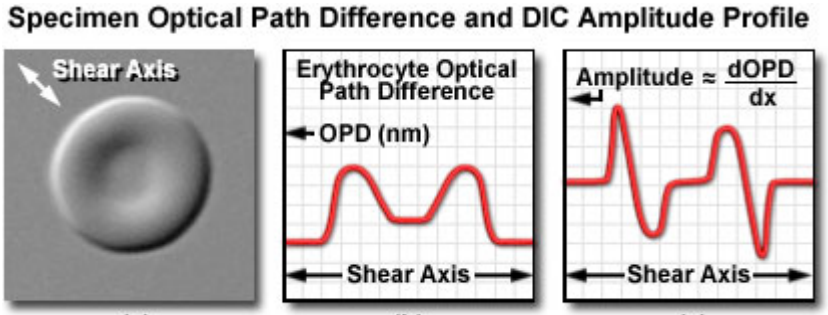


Figure 2

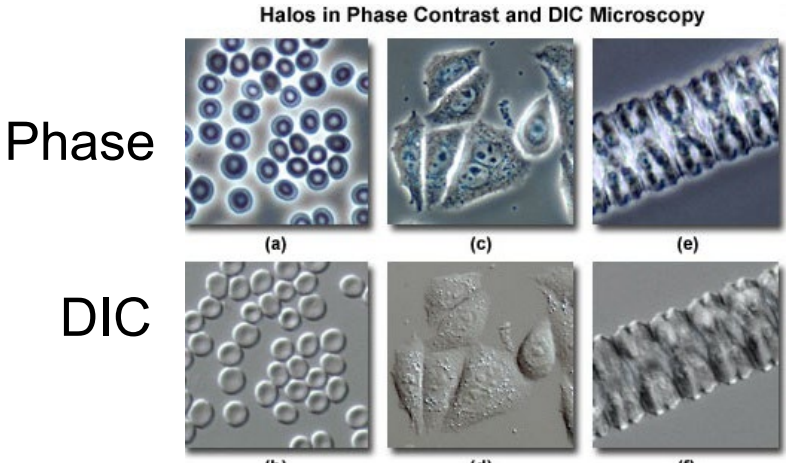
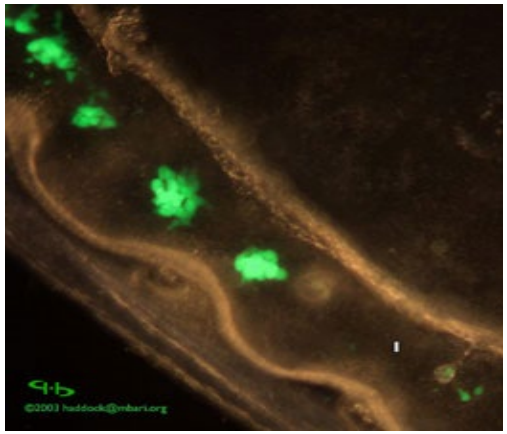


Figure 4

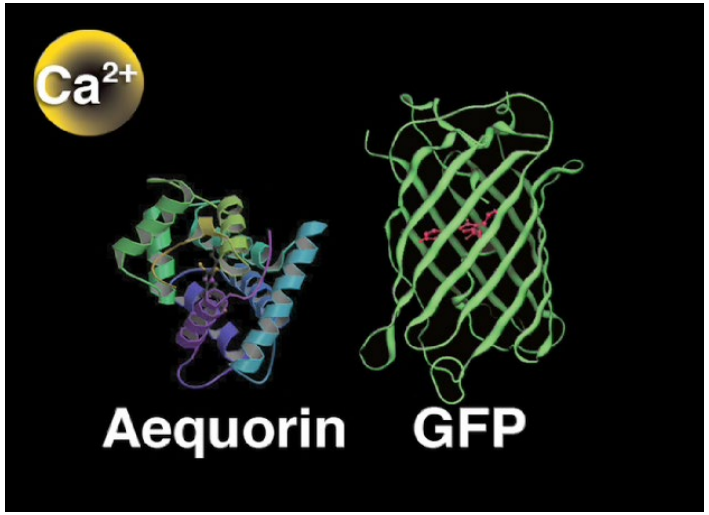
Why Color/Specificity is Important?

- Contrast generation in microscopy
- Introduction to fluorescence microscopy
- Practical constraints in microscopy
- Basic metrics of a microscope

Discovery of Green Fluorescence Protein



Jellyfish: *Aequorea victoria*



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

The Nobel Prize in Chemistry 2008

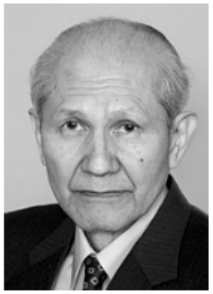


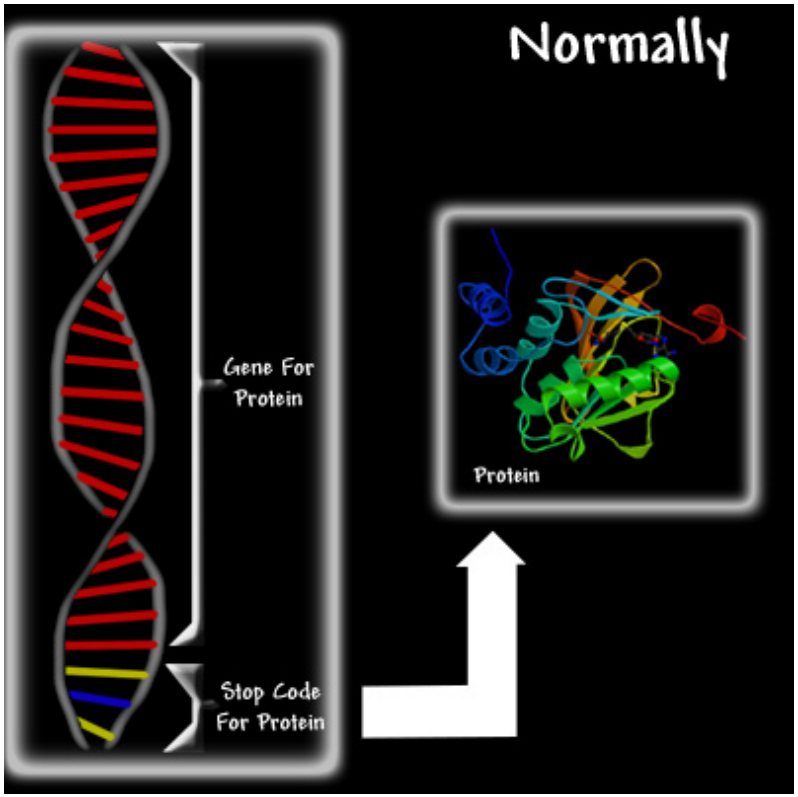
Photo: U. Montan
Osamu Shimomura
Prize share: 1/3

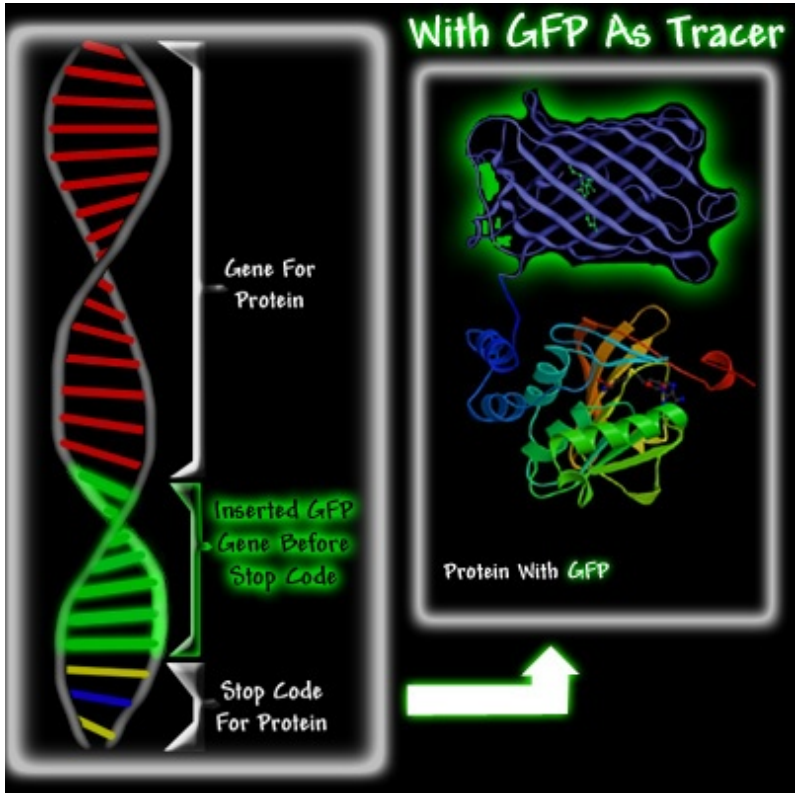
Photo: U. Montan
Martin Chalfie
Prize share: 1/3

Photo: U. Montan
Roger Y. Tsien
Prize share: 1/3

The Nobel Prize in Chemistry 2008 was awarded jointly to Osamu Shimomura, Martin Chalfie and Roger Y. Tsien *"for the discovery and development of the green fluorescent protein, GFP".*

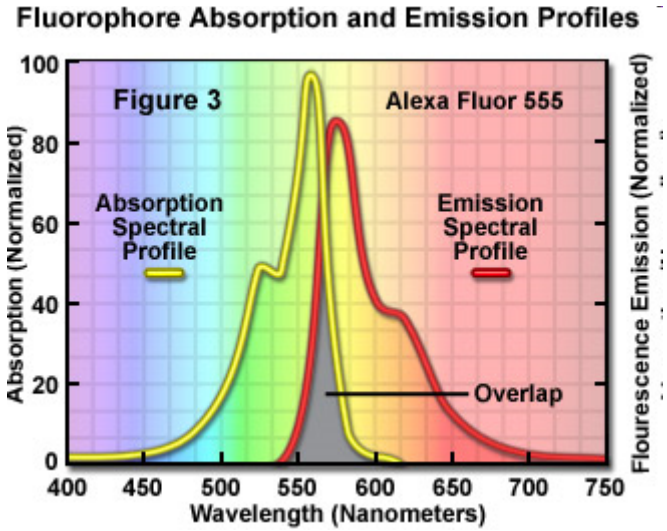
Labeling a Protein Using GFP

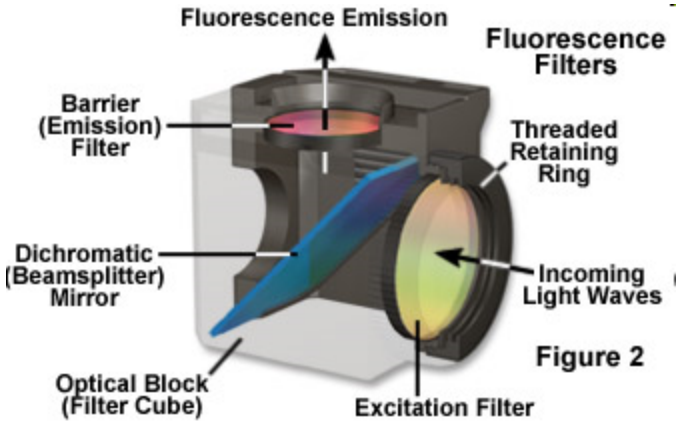


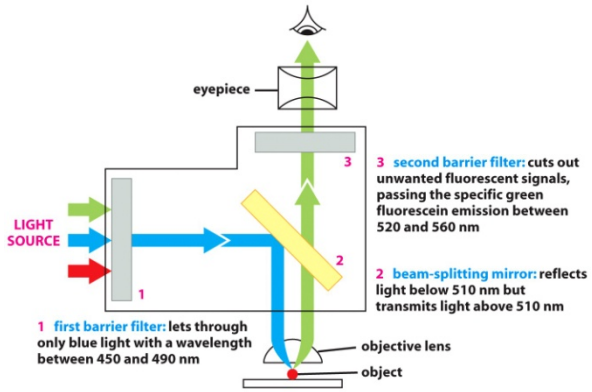


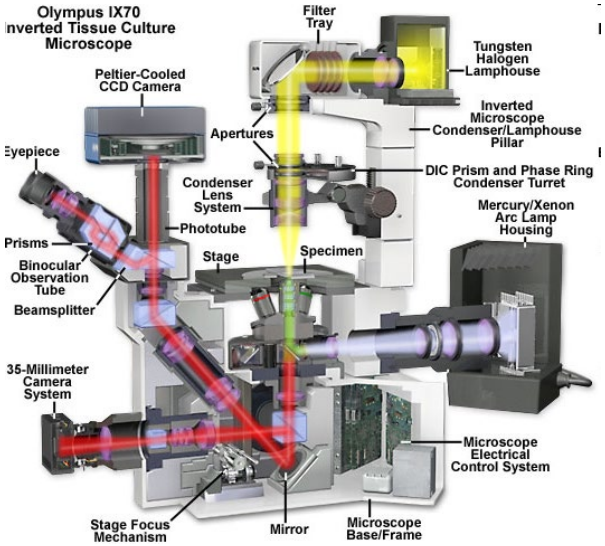
http://gfp.conncoll.edu/GFP-1.htm

Fluorescence Microscopy (I)

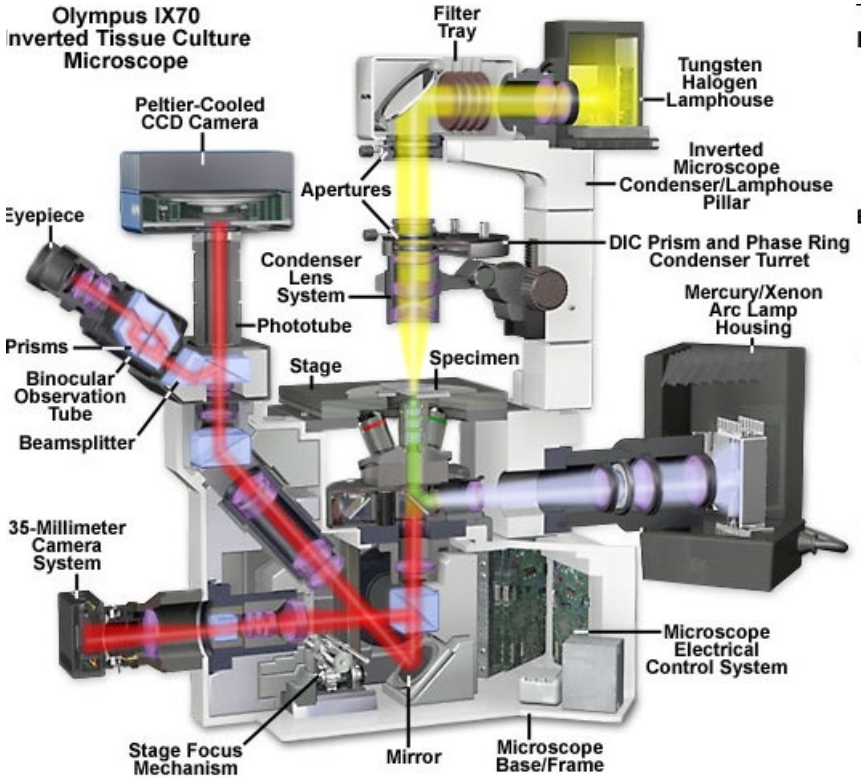






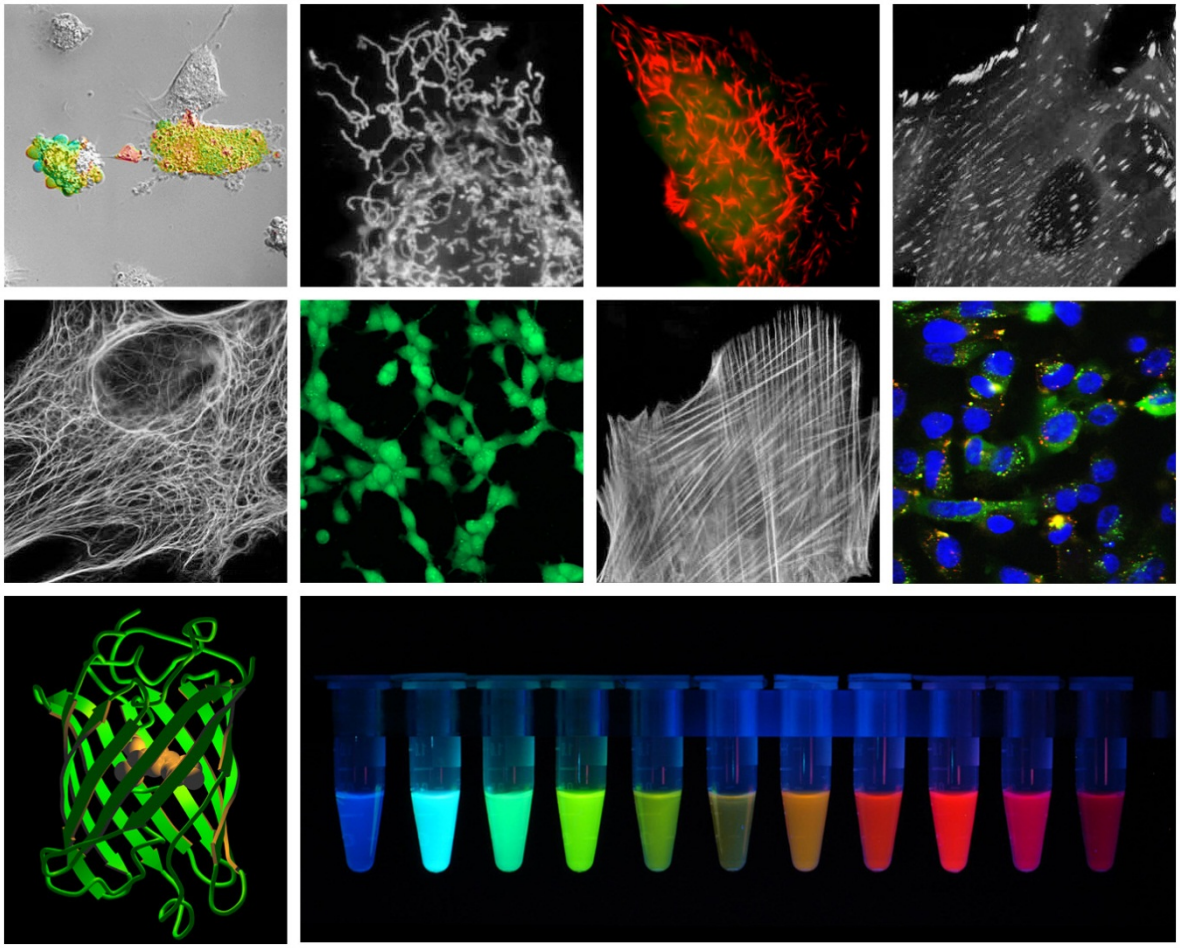


Reflected Light vs Transmitted Light



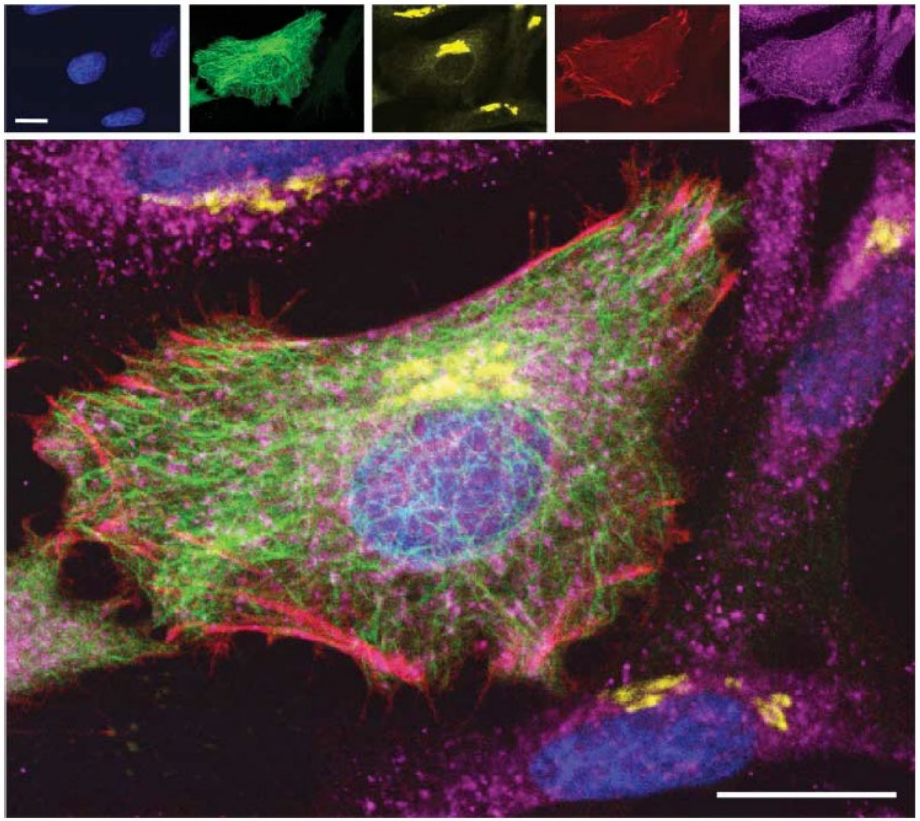
http://micro.magnet.fsu.edu/primer/java/tirf/ix70/ix70java.html

Fluorescence Microscopy (III)



A Multicolor Image of a HeLa Cell

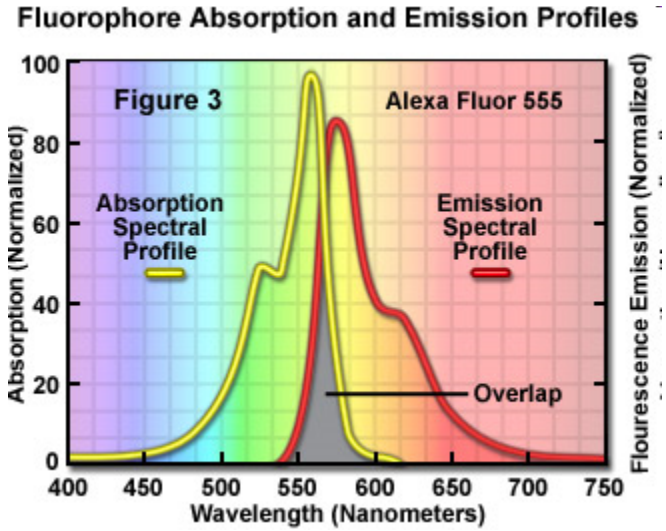
Excitation (nm):	800 (2 photon)	488	432	568	637
Emission (nm):	410-490	500-530	555-565	580-620	>660
Fluorophore:	Hoechst	GFP	QD565	ReAsH	Cy5
Targeting:	direct affinity	genetic	immuno	genetic	immuno
Target:	DNA	α-tubulin	giantin	β-actin	Cytochrome c
Structure:	nuclei	microtubules	golgi	stress fibers	mitochondria



Giepmans et al, *Science*, 312:217, 2006

Excitation & Emission Spectrum

- A fluorescent molecule can only absorb excitation light within a certain range of wavelengths (excitation spectrum).
- A fluorescent molecule can only emit light within a certain range of wavelengths (emission spectrum).
- Emission wavelengths are always longer due to internal energy loss.
- Emission spectrum is approximately a mirror image of excitation spectrum.



Fluorescence Microscopy Summary

- High specificity:
  - Chemical fluorophores
  - Fluorescent proteins

- High sensitivity: up to single molecules.

Useful References

- Lakowicz JR, *Principles of fluorescence spectroscopy*, Springer, 2006.

- Herman B, *Fluorescence microscopy*, 2nd ed., Taylor & Francis, 1998.

- Contrast generation in microscopy
- A more detailed introduction to fluorescence
- **Practical constraints in microscopy**
- Basic metrics of a microscope

Practical Constraints in Microscopy

- 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.

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

- Contrast generation in microscopy
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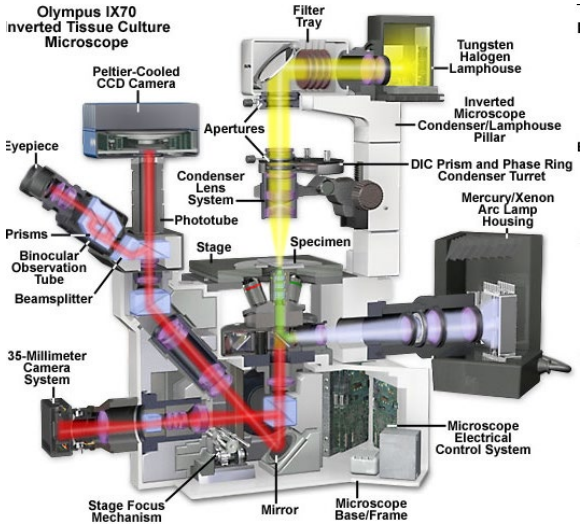
Microscope as a Linear System



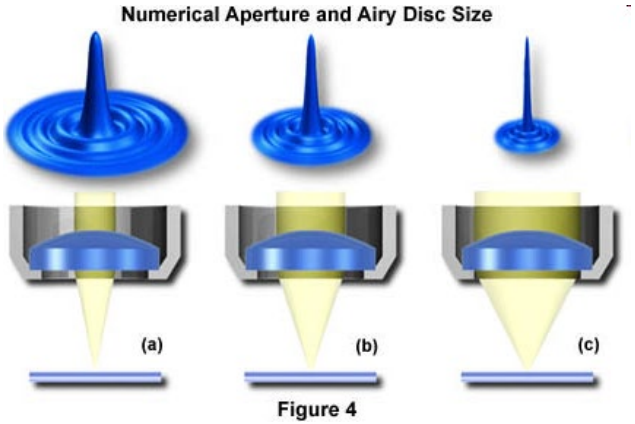


Figure 4

- A light microscope can be considered as a linear system.

http://micro.magnet.fsu.edu/primer/java/imageformation/airydiskformation/index.html

A Microscope as a Linear System

- A light microscope can be considered as a linear system.

- A linear system satisfies the following two conditions
  - Homogeneity
  - Additivity

- Homogeneity

$$x(t) \rightarrow y(t)$$
$$\Downarrow$$
$$k \cdot x(t) \rightarrow k \cdot y(t)$$

- Additivity

$$x_1(t) \rightarrow y_1(t)$$
$$x_2(t) \rightarrow y_2(t)$$
$$\Downarrow$$
$$x_1(t) + x_2(t) \rightarrow y_1(t) + y_2(t)$$

How to Characterize a Linear System

- A linear system can be characterized by
  - Impulse response
  - Frequency response

- Impulse response of a microscope: point spread function

$$I(x,y) = O(x,y) \otimes psf(x,y) = \int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} O(u,v)\cdot psf(x-u,y-v)\,dudv$$

- Frequency response of a microscope: optical transfer function

$$F\{I(x,y)\} = F\{O(x,y)\}\cdot F\{psf(x,y)\} = F\{O(x,y)\}\cdot OTF(\cdot)$$

Airy Disk

- Airy (after George Biddell Airy) disk is the diffraction pattern of a point feature under a circular aperture.

- It has the following form

$$y = \left[\frac{2J_1(x)}{x} \right]^2$$

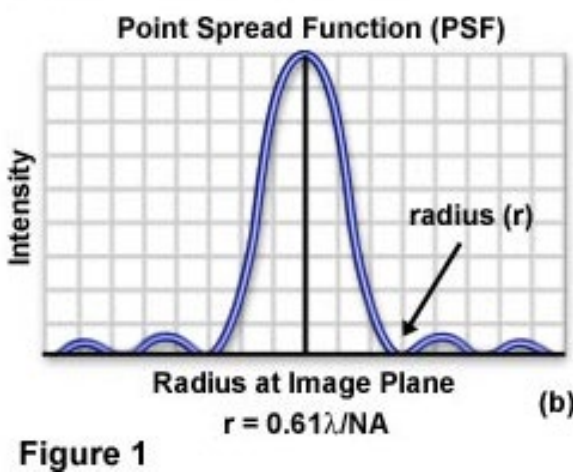


Figure 1

$J_1(x)$ is a Bessel function of the first kind.

- Detailed derivation is given in

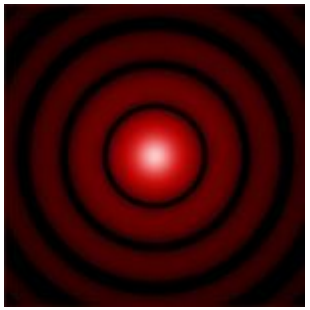
  Born & Wolf, *Principles of Optics*, 7th ed., pp. 439-441.

Microscope Image Formation (I)

- Microscope image formation can be modeled as a convolution with the PSF.

$$I(x,y) = O(x,y) \otimes psf(x,y)$$

$$F\{I(x,y)\} = F\{O(x,y)\} \cdot F\{psf(x,y)\}$$



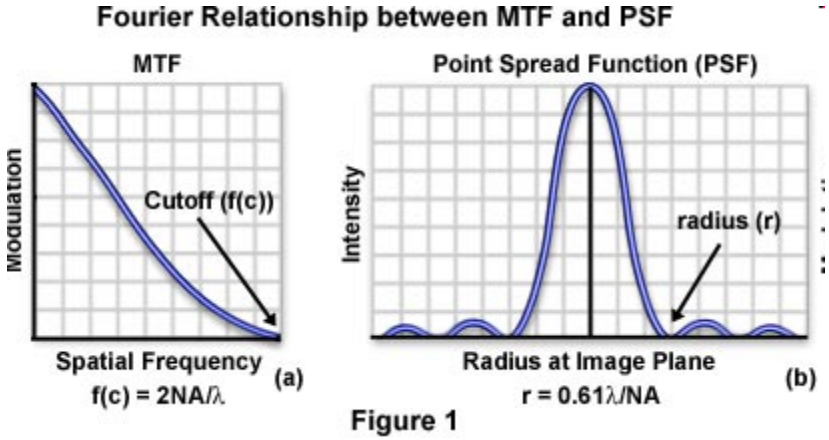


Figure 1

http://micro.magnet.fsu.edu/primer/java/mtf/airydisksize/index.html

Microscope Image Formation (II)

- The impulse response of the microscope is called its point spread function (PSF).
- The transfer function of a microscope is called its optical transfer function (OTF).
- The PSF has the shape of an Airy Disk.

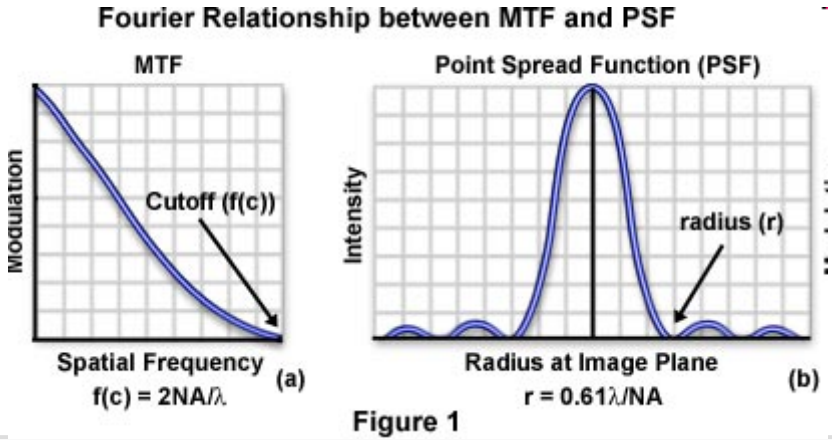
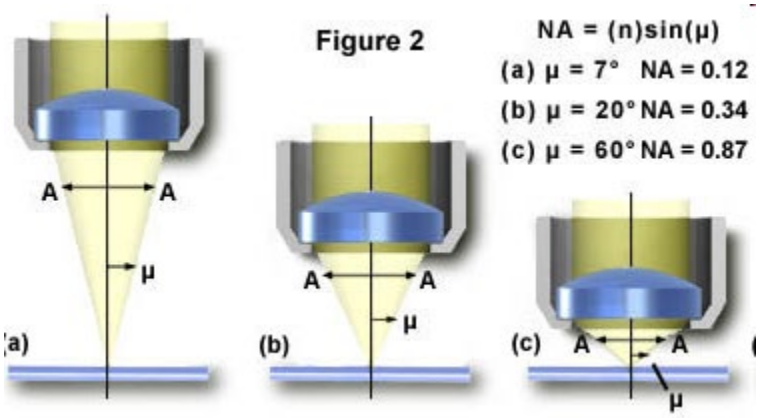


Figure 1

Numerical Aperture

- Numerical aperture (NA) determines microscope resolution and light collection power.



$$NA = n \cdot \sin \mu$$

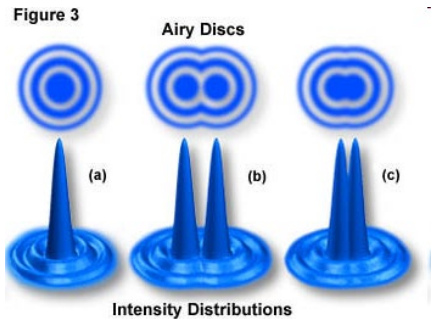
n: refractive index of the medium between the lens and the specimen

μ: half of the angular aperture

Different Definition of Light Microscopy Resolution Limit (Demo)

- Rayleigh limit

$$D = \frac{0.61\lambda}{NA}$$



- Sparrow limit

$$D = \frac{0.47\lambda}{NA}$$

https://micro.magnet.fsu.edu/primer/java/microscopy/airydiscs/index.html

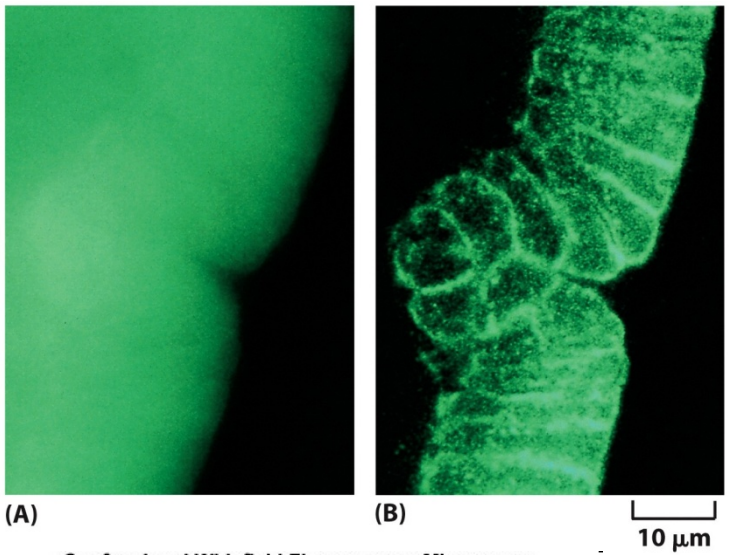
Summary: High Resolution Microscopy

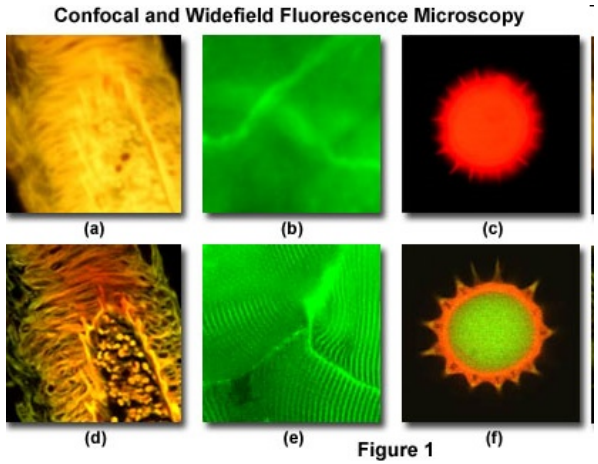
- Size of cellular features are typically on the scale of a micron or smaller.

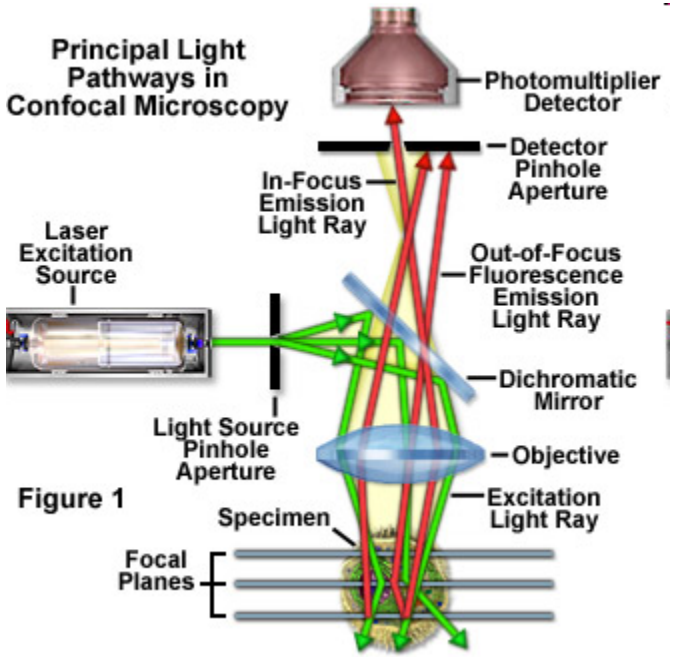
- To resolve such features require
  - Shorter wavelength (electron microscopy)
  - High numerical aperture (resolution)
  - High magnification (spatial sampling)

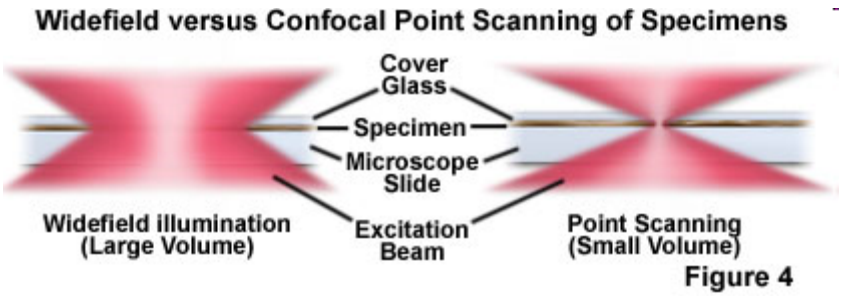
$$D = \frac{0.61\lambda}{NA}$$

Widefield vs Confocal Microscopy



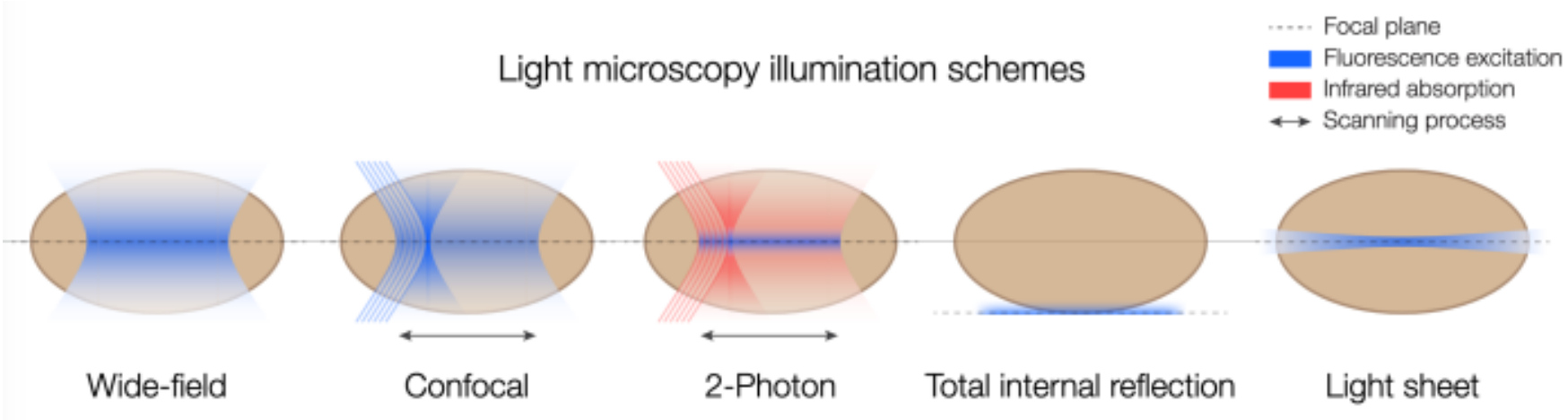






http://www.olympusfluoview.com/theory/confocalintro.html

Summary of Different Illumination Configurations



https://involv3d.org/principles/

Questions?