臺北醫學大學

Biomedical Imaging

生物醫學影像學

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2013/03/04

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Course Outline

1. Course Introduction
2. Basic Optics and Light Microscopes
3. Fluorescence/Confocal/TIRF Microscopes
4. FRET Techniques and Photo-Spectroscopic Imaging
5. Single Molecule Detection
6. Cell Imaging
7. Atomic Force Microscopy (AFM)
8. Scanning Electron Microscope (SEM)
9. Transmission Electron Microscopy (TEM)
10. Digital Image Processing Using MATLAB


Part I
The Fundamentals of Light Microscopy
Hubble Telescope—Deep Space Photo
http://www.hubblesite.org

The Eye and Optical Microscope

Diffraction Limited Resolution

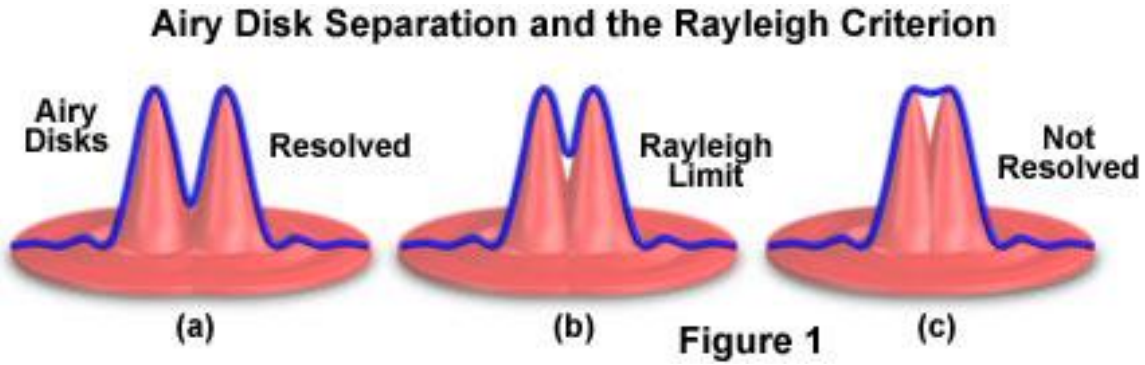
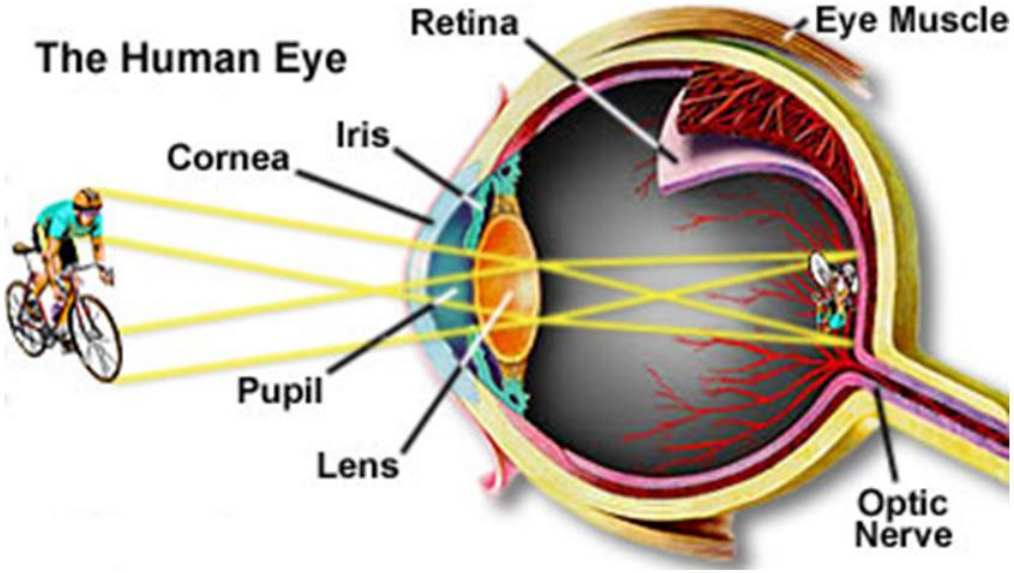
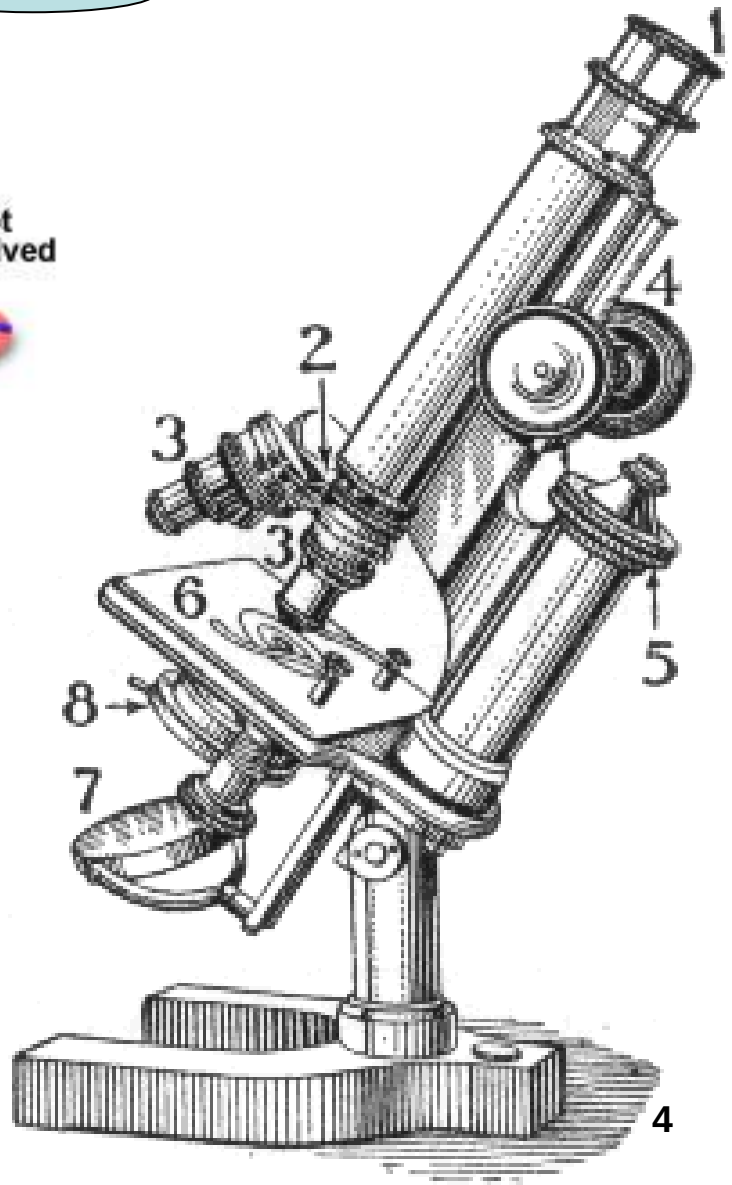


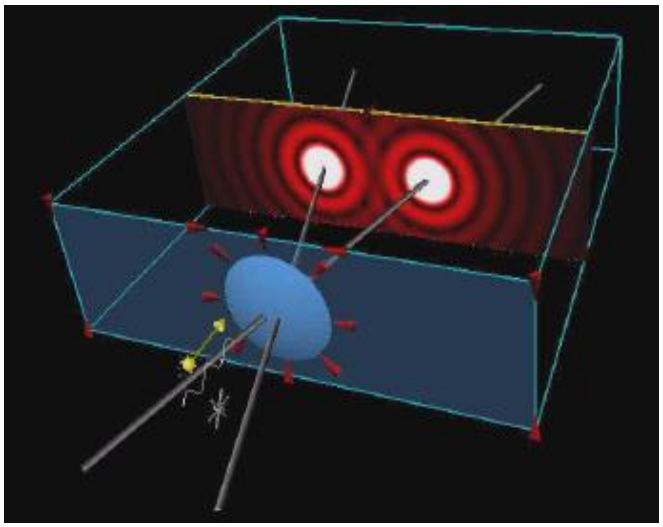
Figure 1

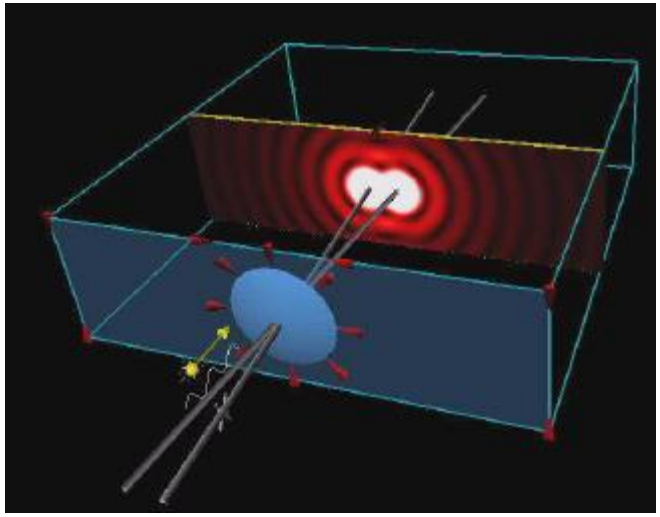


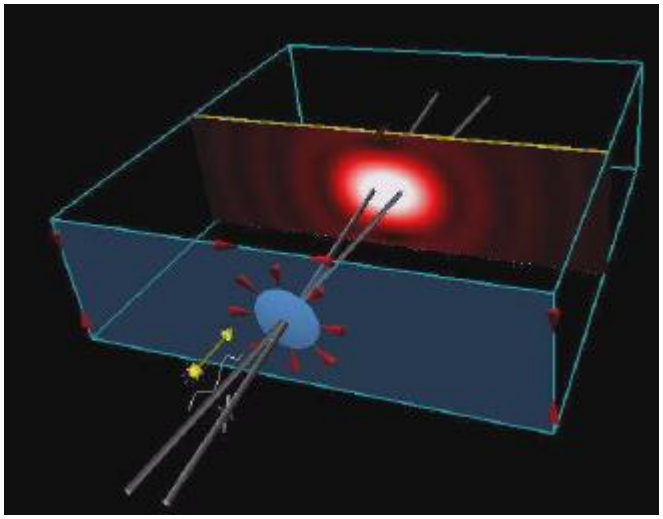


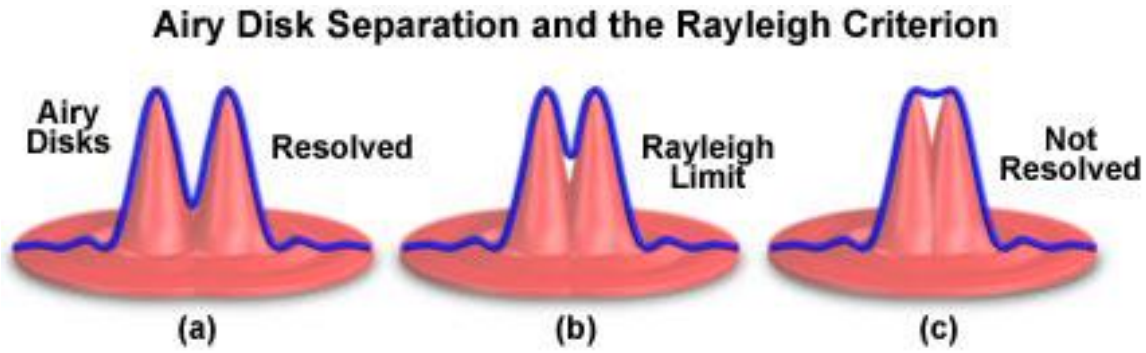
Airy Disks and Resolution

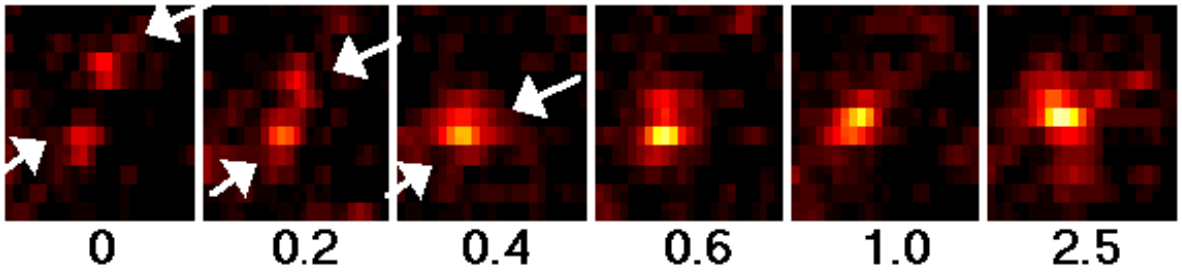
Diffraction Limited Resolution





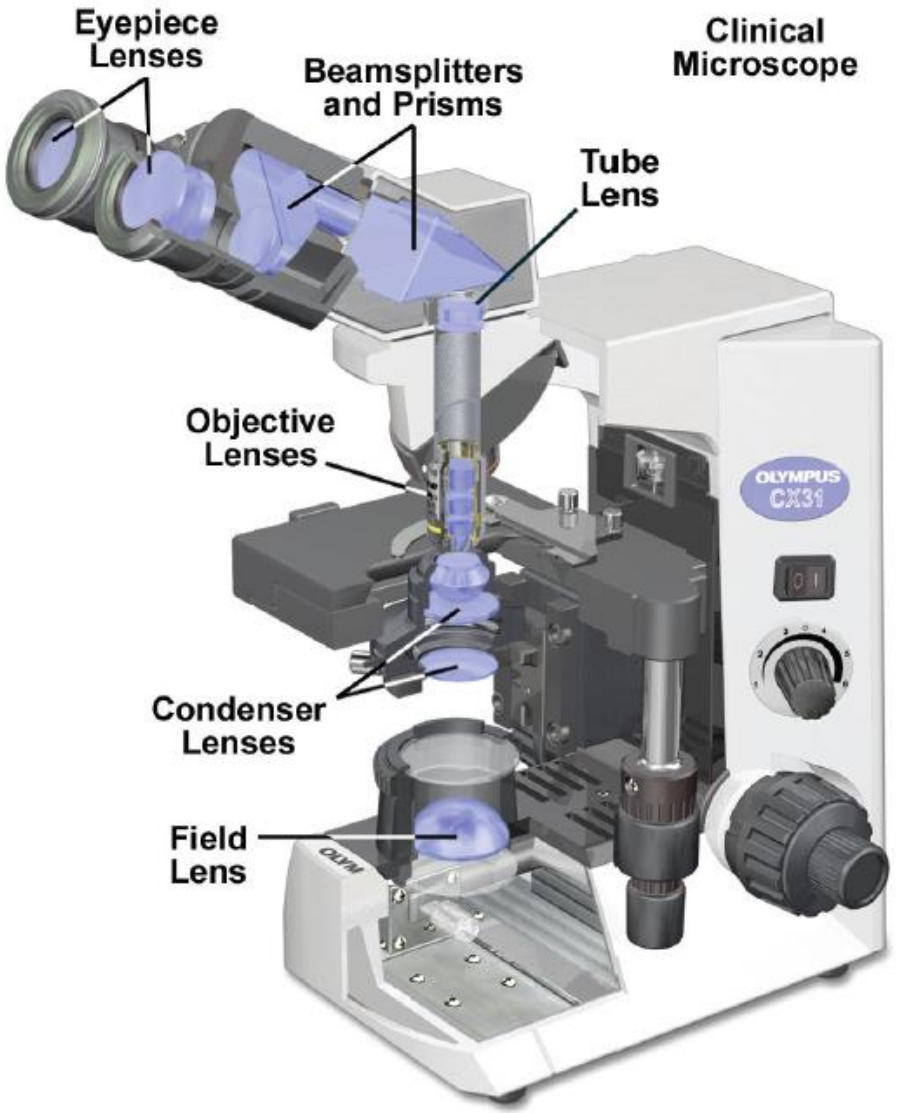






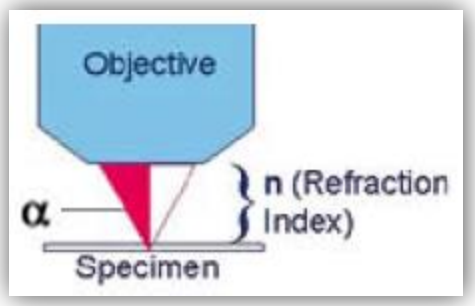
Transmitted Light Microscope: Upright vs. Inverted

正立式

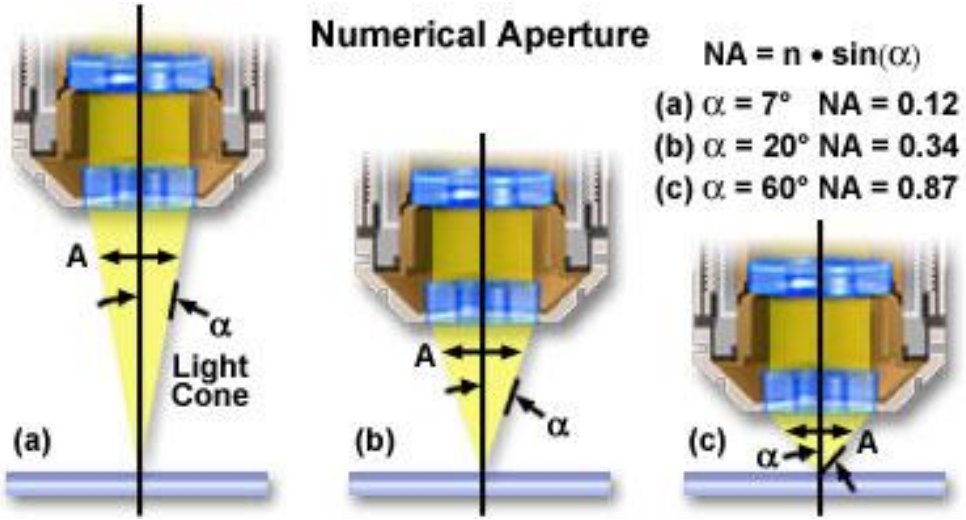


倒立式

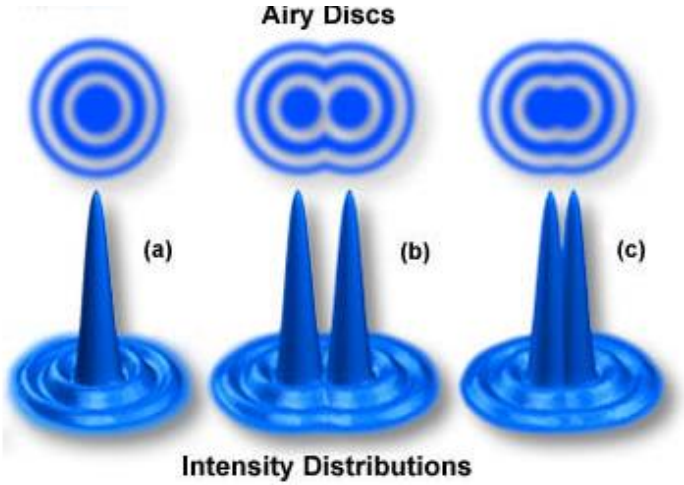
Airy Disks and Resolution

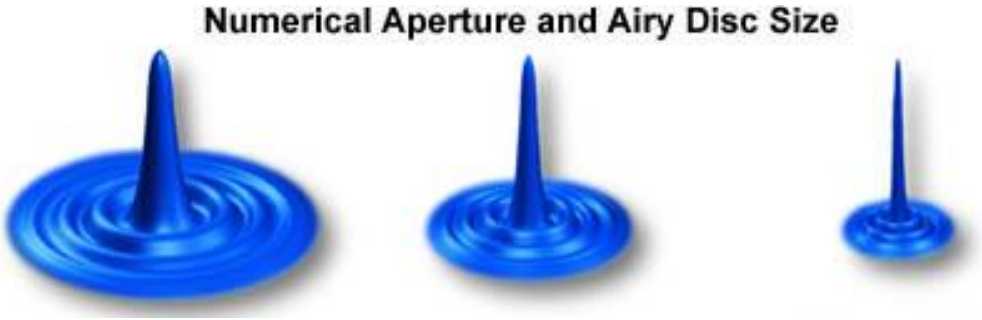


R=(1.22λf/D); NA=n × sin(α)
if both λ and D are the same,
∴NA↑⇒ resolution ↑



Overlapping images





http://micro.magnet.fsu.edu/primer/anatomy/numaperture.h

Depth of Field and Depth of Focus

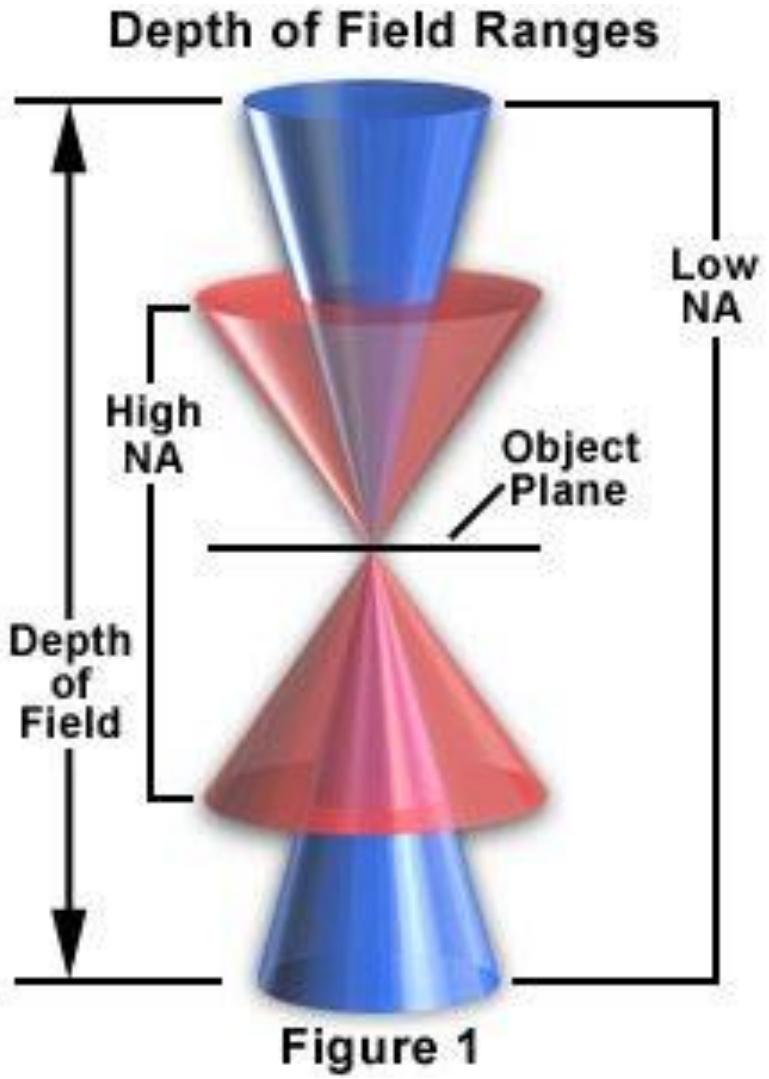


Figure 1

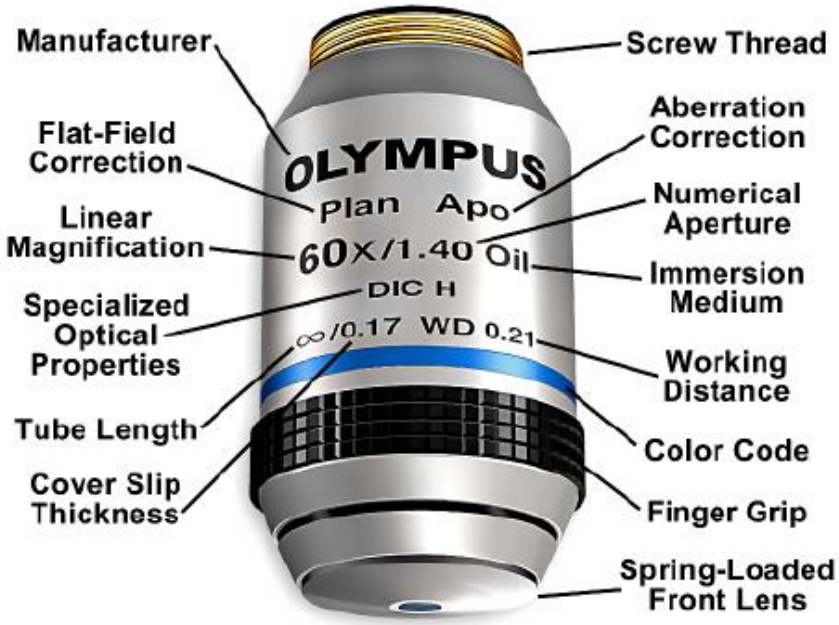
When considering resolution in optical microscopy, a majority of the emphasis is placed on point-to-point lateral resolution in the plane perpendicular to the optical axis (Figure 1). Another important aspect to resolution is the axial (or longitudinal) resolving power of an objective, which is measured parallel to the optical axis and is most often referred to as depth of field.

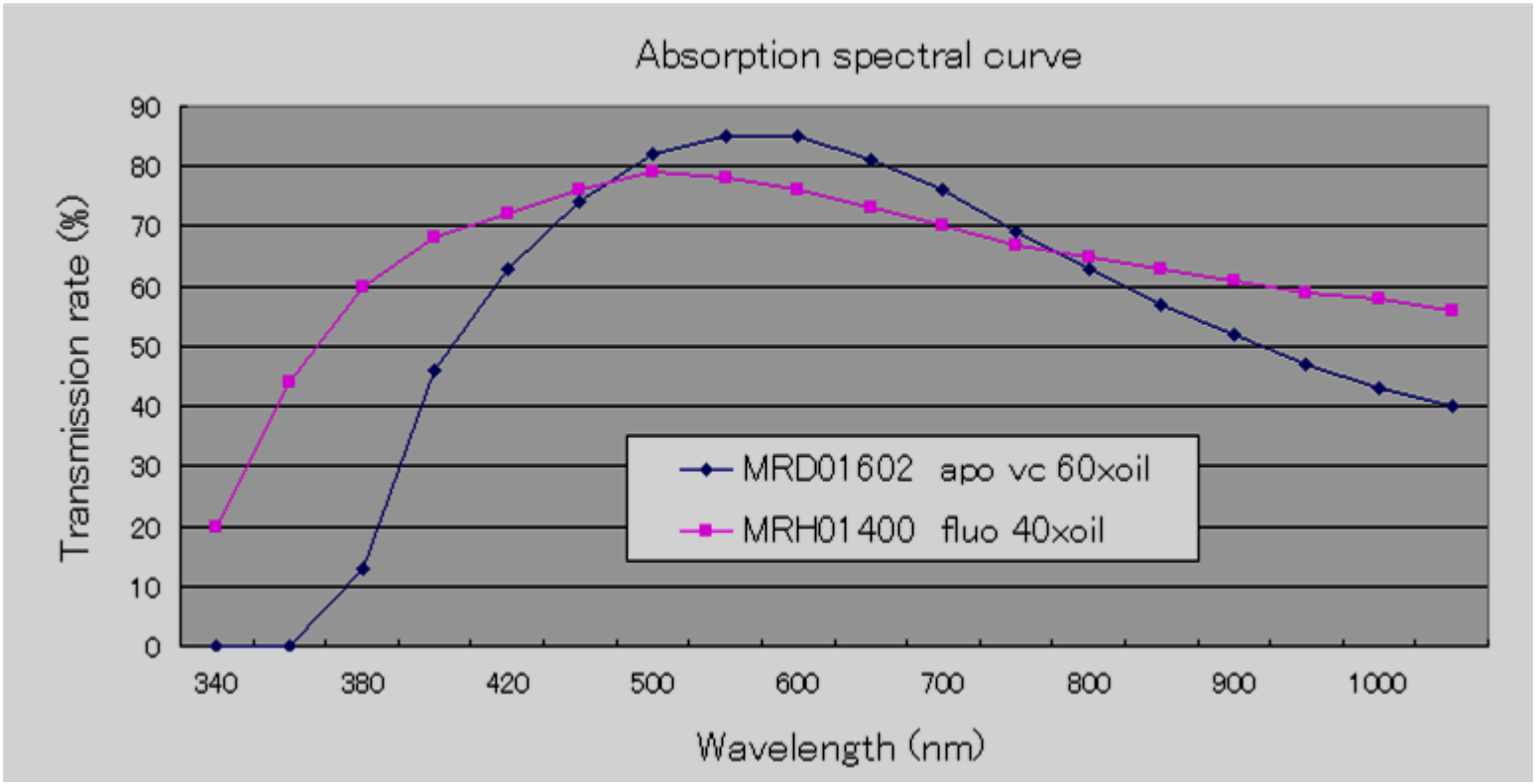
Common Objective Working Distances

Manufacturer	Correction	Magnification	Numerical Aperture	Working Distance
Nikon	PlanApo	10x	0.45	4.0 mm
Nikon	PlanFluor	20x	0.75	0.35 mm
Nikon	PlanFluor (oil)	40x	1.30	0.20 mm
Nikon	PlanApo (oil)	60x	1.40	0.21 mm
Nikon	PlanApo (oil)	100x	1.40	0.13 mm

Objective Working and Parfocal Distance

Figure 1



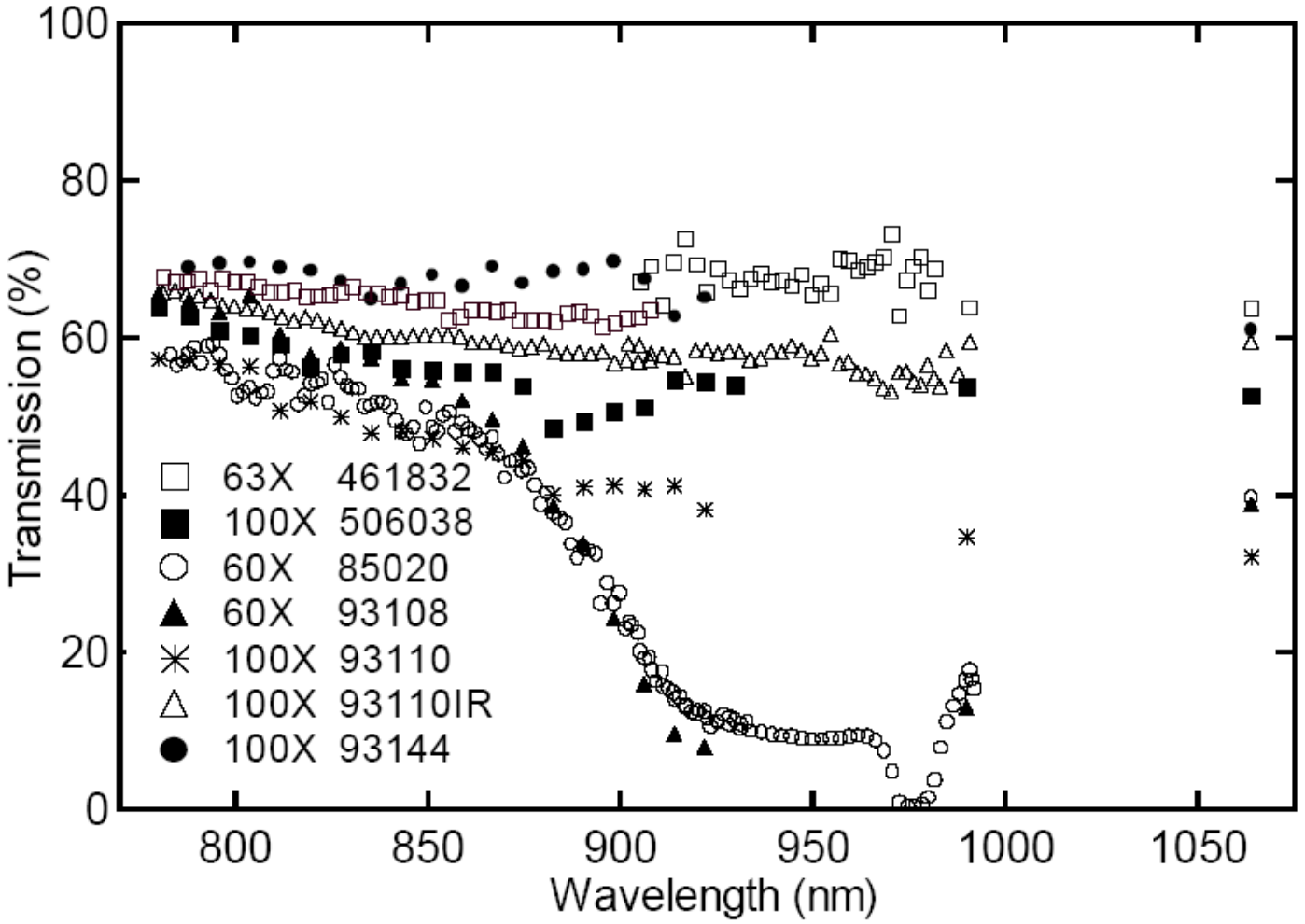


CFI Plan Apochromat VC Series

CFI Plan Apochromat VC 60X Oil, N.A. 1.40
CFI Plan Apochromat VC 60X WI, N.A. 1.20
CFI Plan Apochromat VC 100X WI, N.A. 1.40

- Chromatic aberrations have been thoroughly correct throughout the view field. Suitable for digital imaging.
- Perfect choice for multi-stained, fluorescence specimens and when using brightfield and DIC techniques.
- Axial chromatic aberration has been corrected up to the violet range (405nm), making these objectives highly effective for confocal applications.
- Excellent brightness throughout the view field.
- The 60X water-immersion type, in particular, features high spectral transmittance, even in the 360nm wavelength range.

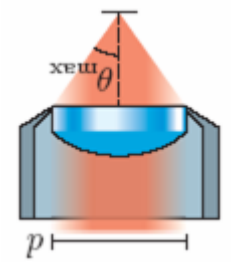
High N.A Objective



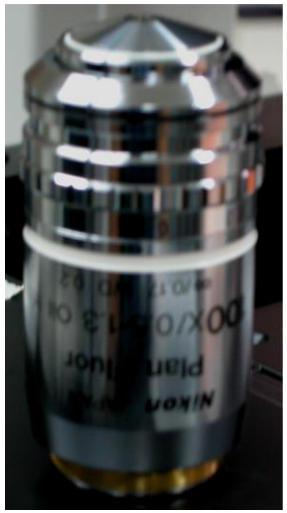
Keir Cajal Neuman, PhD Thesis (2002)

High N.A Objective

generate a highly focused laser beam



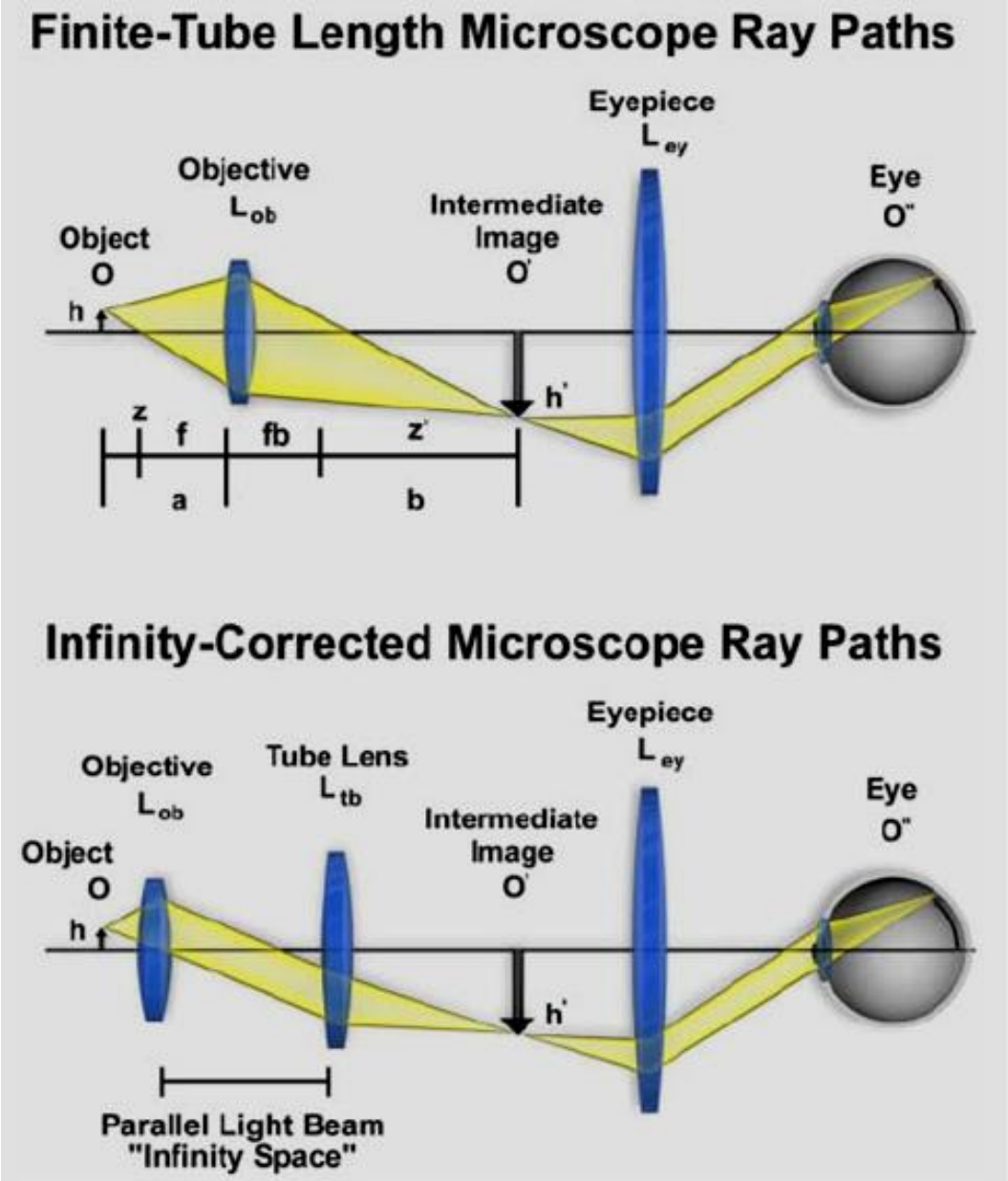
$$N.A. = n\sin\theta$$



Part Number	Manu-facturer	Magnification/ Tube length (mm)/ Numerical aperture	Type designation	Transmission (±5%) 830 nm	850 nm	990 nm	1064 nm
461832	Zeiss	63/160/1.2 Water	Plan NeoFluar	66	65	64	64
506038	Leica	100/∞/1.4-0.7 Oil	Plan Apo	58	56	54	53
85020	Nikon	60/160/1.4 Oil	Plan Apo	54	51	17	40
93108	Nikon	60/∞/1.4 Oil	Plan Apo CFI	59	54	13	39
93110	Nikon	100/∞/1.4 Oil	Plan Apo CFI	50	47	35	32
93110IR	Nikon	100/∞/1.4 Oil	Plan Apo IR CFI	61	60	59	59
93144	Nikon	100/∞/1.3 Oil	Plan Fluor CFI	67	68	-	61

Keir Cajal Neuman, PhD Thesis (2002)

Infinity-corrected Microscope Systems

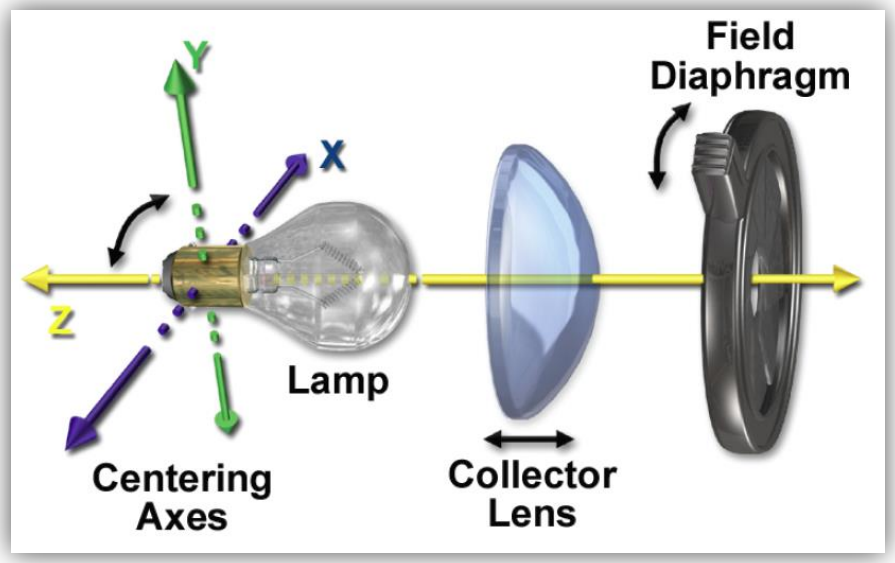


1) Note the **infinity space** that is defined by parallel light beams in every azimuth (方位) between the objective and the tube lens.

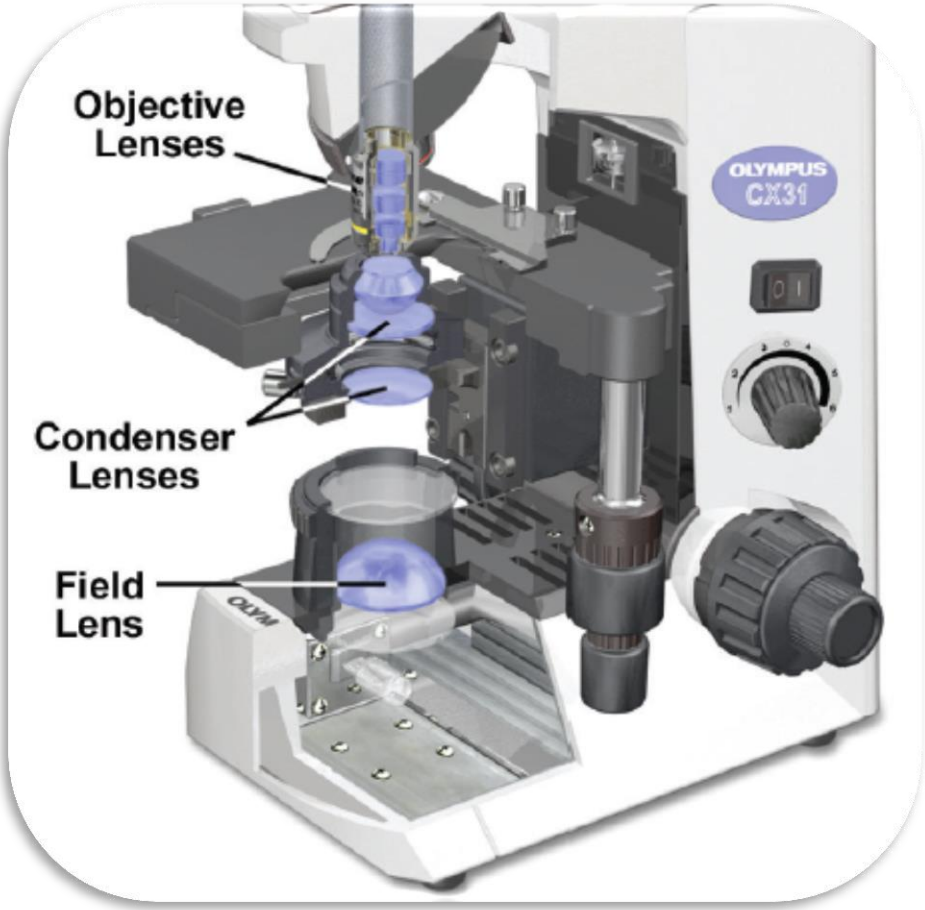
2) This is the space used by microscope manufacturers to **add accessories** with much simpler designs and with little distortion of the image.

Microscope Illuminator

The essential elements of the illuminator are the lamp, a collector lens, and the field diaphragm. The diaphragm is adjustable.



正立式

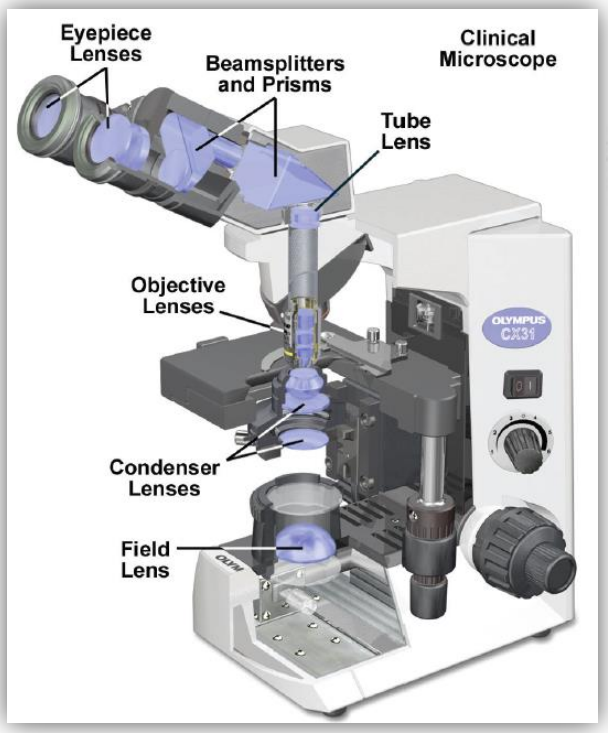


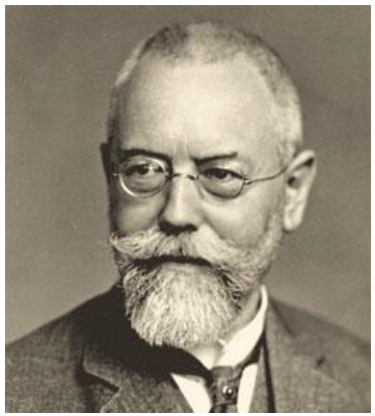
Koehler Illumination (柯氏照明)

Conjugate Planes in the illumination path and in the image path:

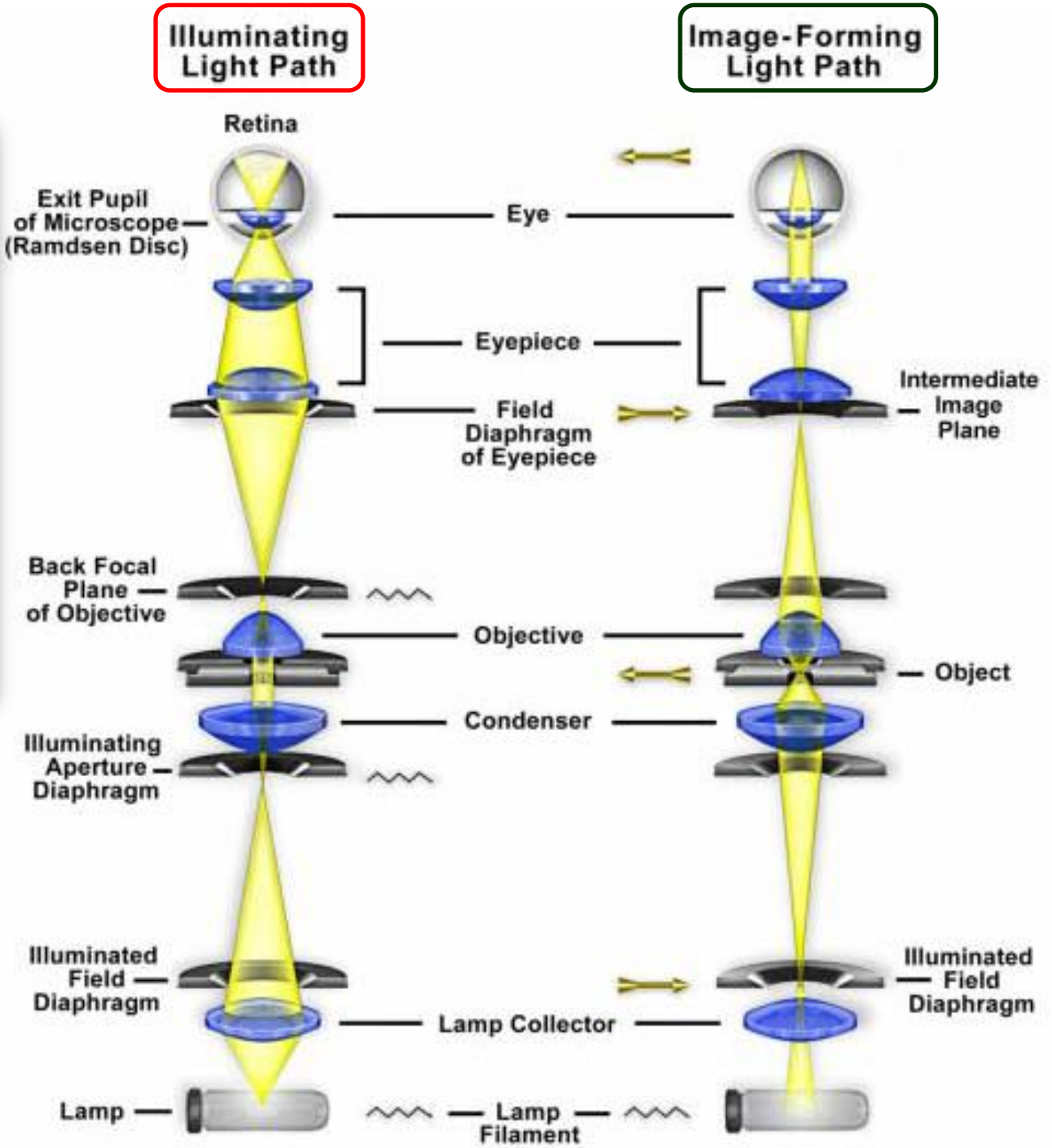
1) Conjugated Planes: set of planes such that an image focused on one plane is automatically focused on all other conjugate planes.
2) Light ray path produces focused images of the lamp filament at the plane of the condenser aperture, back focal plane of the specimen and at the eye point of the eyepiece.
3) These planes called conjugated planes.
4) Provides an evenly illuminated field of view with a bright image, without glare (刺眼) and minimum heating of the specimen.
5) Very common in transmission microscopes.

正立式

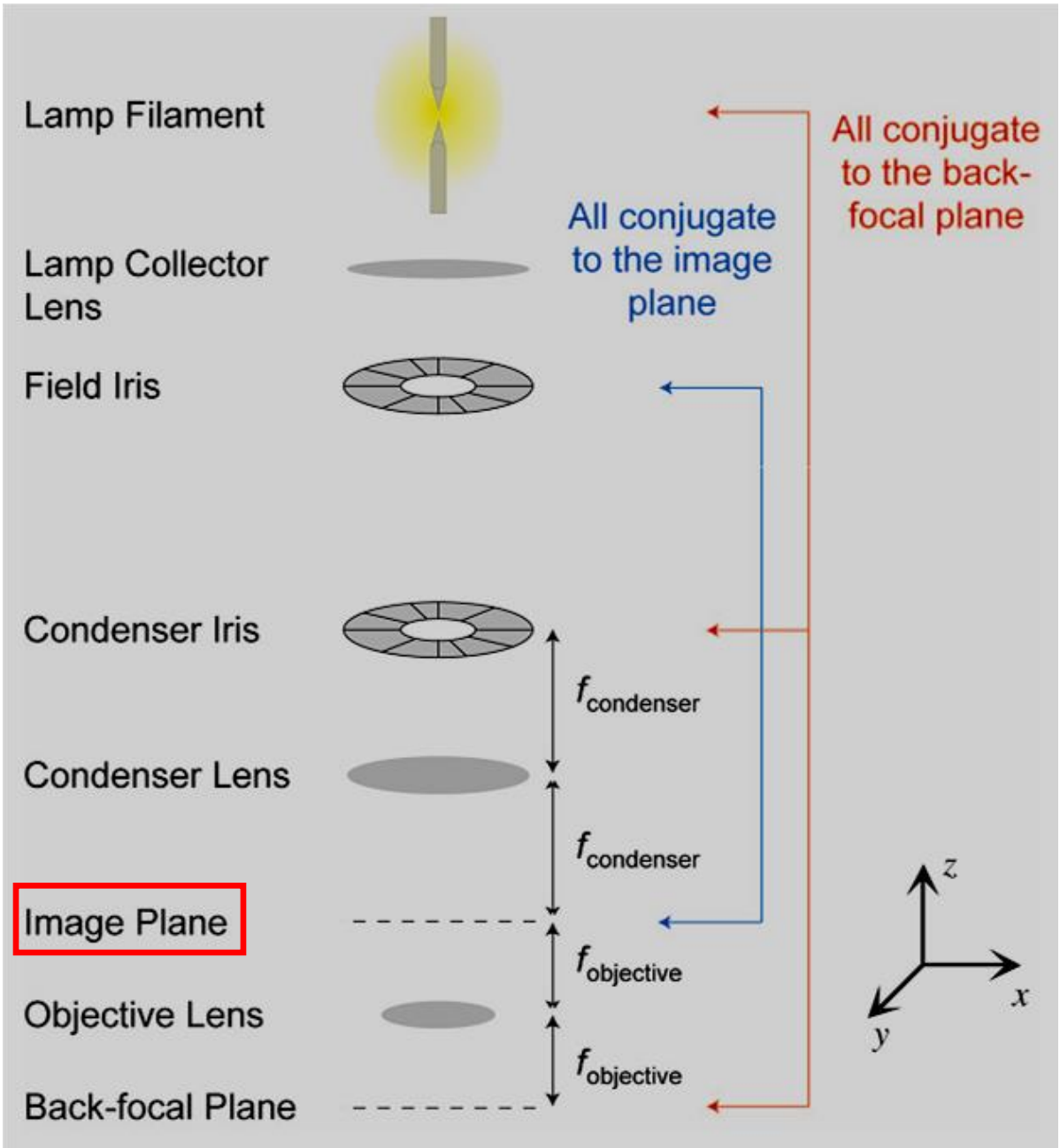




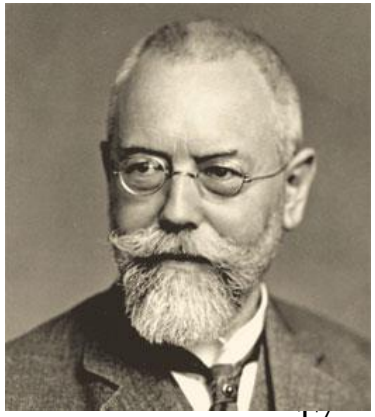
August Koehler



Koehler Illumination (柯氏照明)



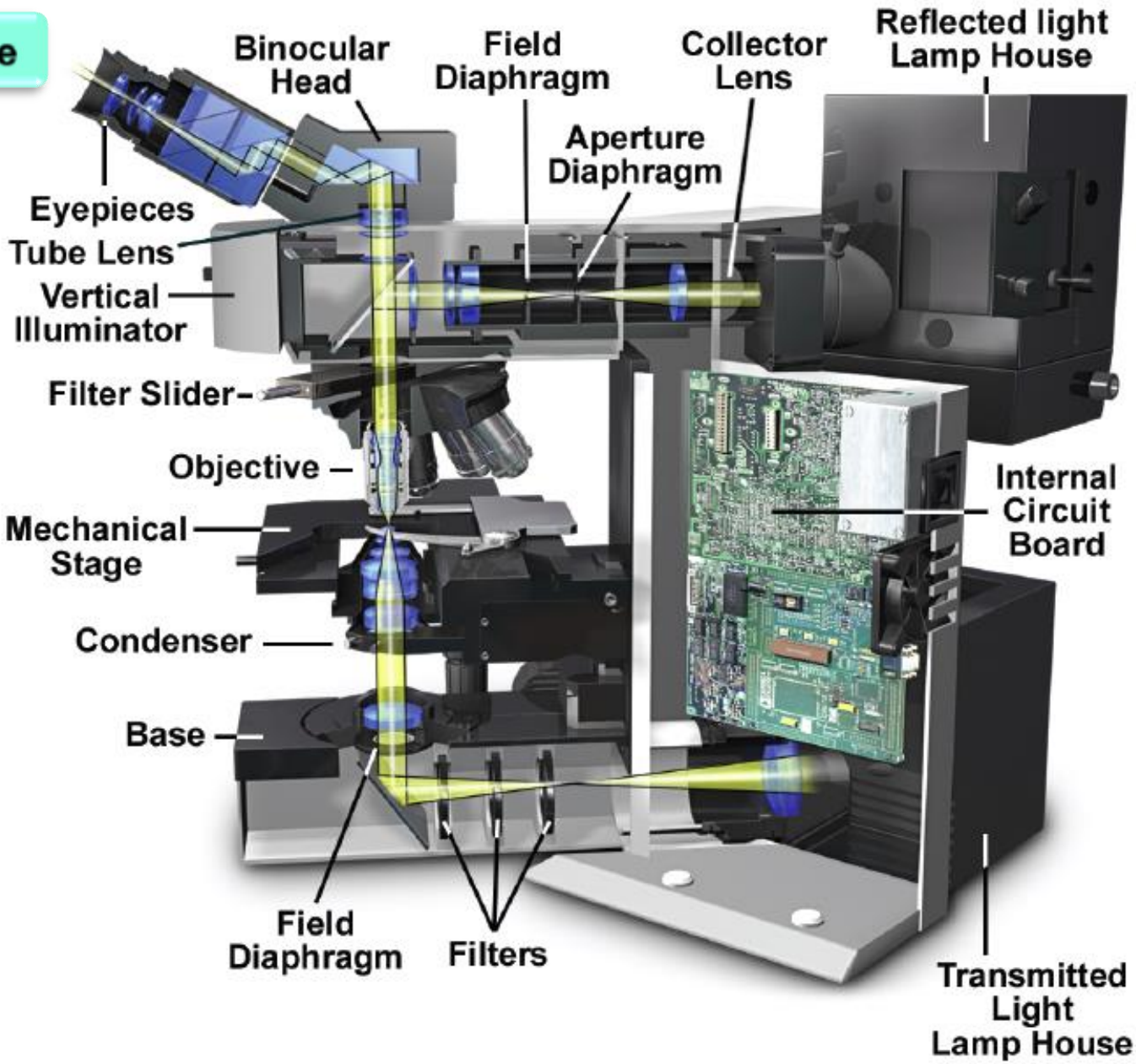
倒立式



August Koehler

Reflected Light Microscope

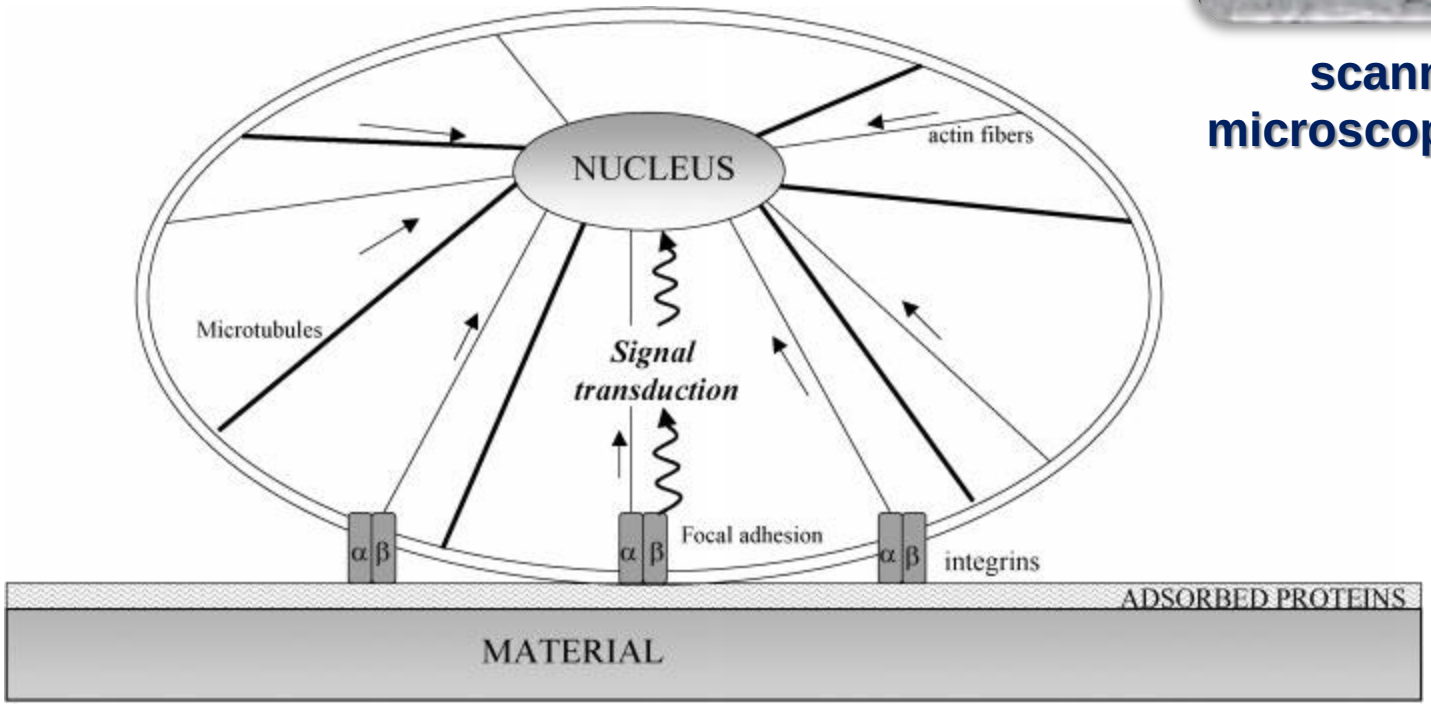
Research Microscope



Reflected Light Microscope

➢Epithelial cells attachment on five different dental implant abutment surface candidates

scanning electron microscope (SEM) imaging

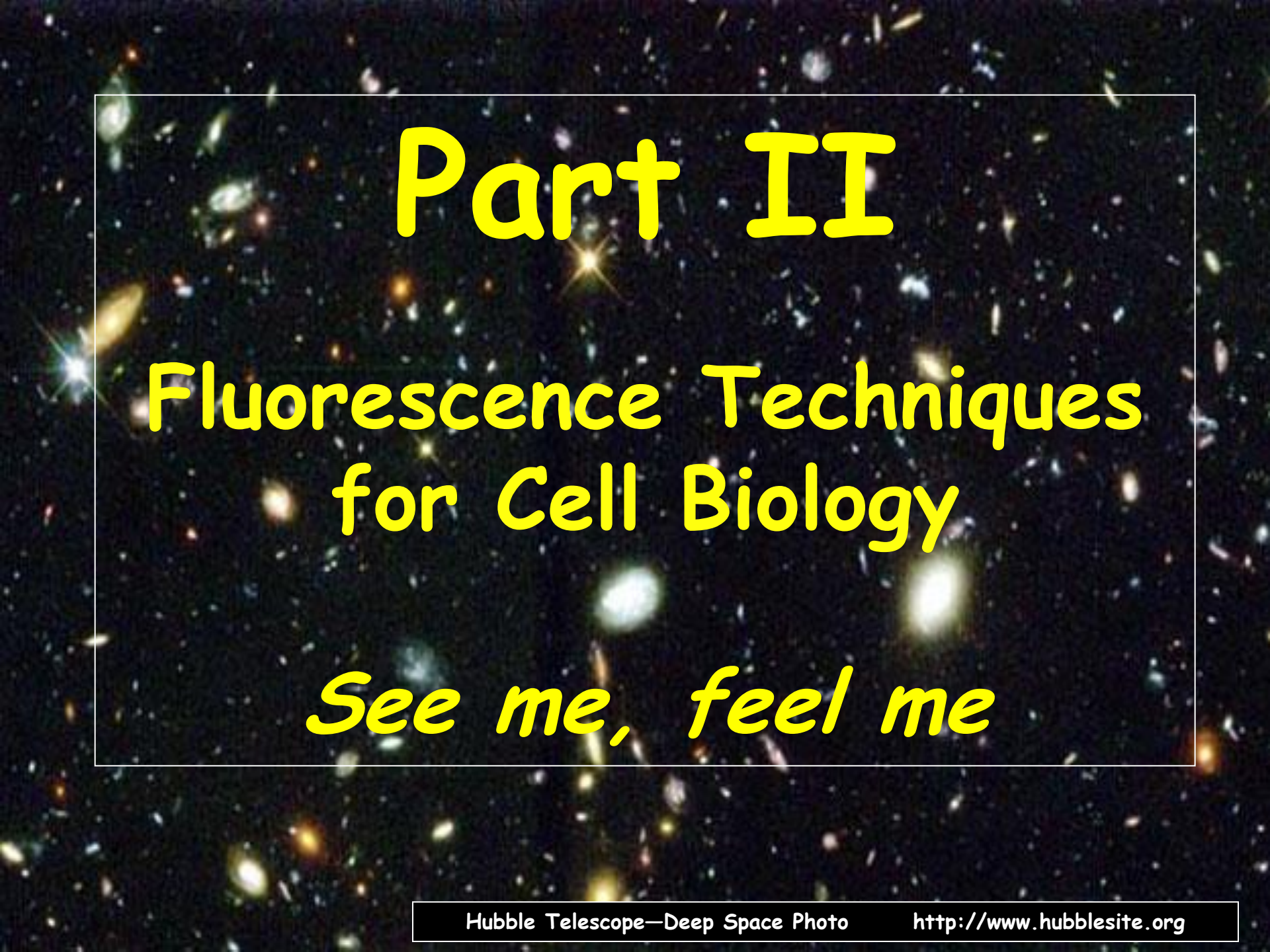


http://ir.uiowa.edu/cgi/viewcontent.cgi?article=1451&context=etd

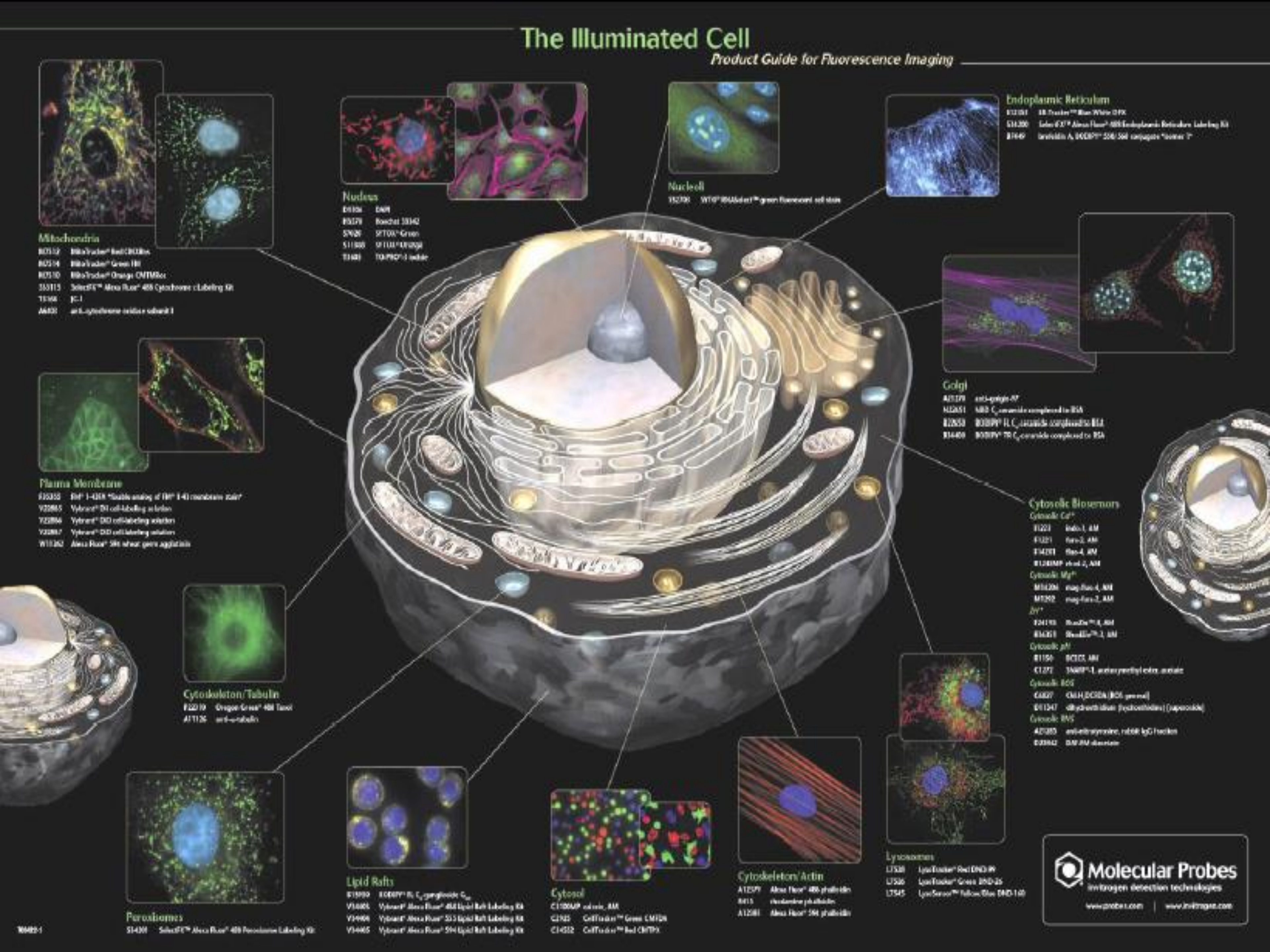
Part II

Fluorescence Techniques for Cell Biology

See me, feel me



Hubble Telescope—Deep Space Photo http://www.hubblesite.org



The Illuminated Cell
Product Guide for Fluorescence Imaging
Mitochondria
Nucleus
Nucleoli
Endoplasmic Reticulum
Golgi
Plasma Membrane
Cytosolic Biosensors
Cytoskeleton/Tubulin
Peroxisomes
Lipid Rafts
Cytosol
Cytoskeleton/Actin
Lysosomes
Molecular Probes
invitrogen detection technologies
www.probes.com
www.invitrogen.com

Fluorophore	Laser (nm)	Emission (nm)
Alexa Fluor® 405	405	421
Pacific Blue™	405	455
Pacific Orange™	405	551
Qdot® 565	405	565
Qdot® 605	405	605
Qdot® 655	405	655
Qdot® 705	405	705
Alexa Fluor® 488	488	519
FITC	488	525
Cy3	488	570
R-PE	488	575
PE-Texas Red®	488	615
PE-Alexa Fluor® 610	488	628
TRI-COLOR® (TC, PE-Cy5)	488	670
PerCP	488	675
PE-Cy5.5	488	694
PerCP-Cy5.5	488	710
PE-Alexa Fluor® 700	488	723
PE-Cy7	488	767
Texas Red®	595	615
APC	633/635	660
Alexa Fluor® 647	633/635	668
Cy5	633/635	670
APC-Cy5.5	633/635	694
Alexa Fluor® 700	633/635	723
APC-Cy7	633/635	767
APC-Alexa Fluor® 750	633/635	775

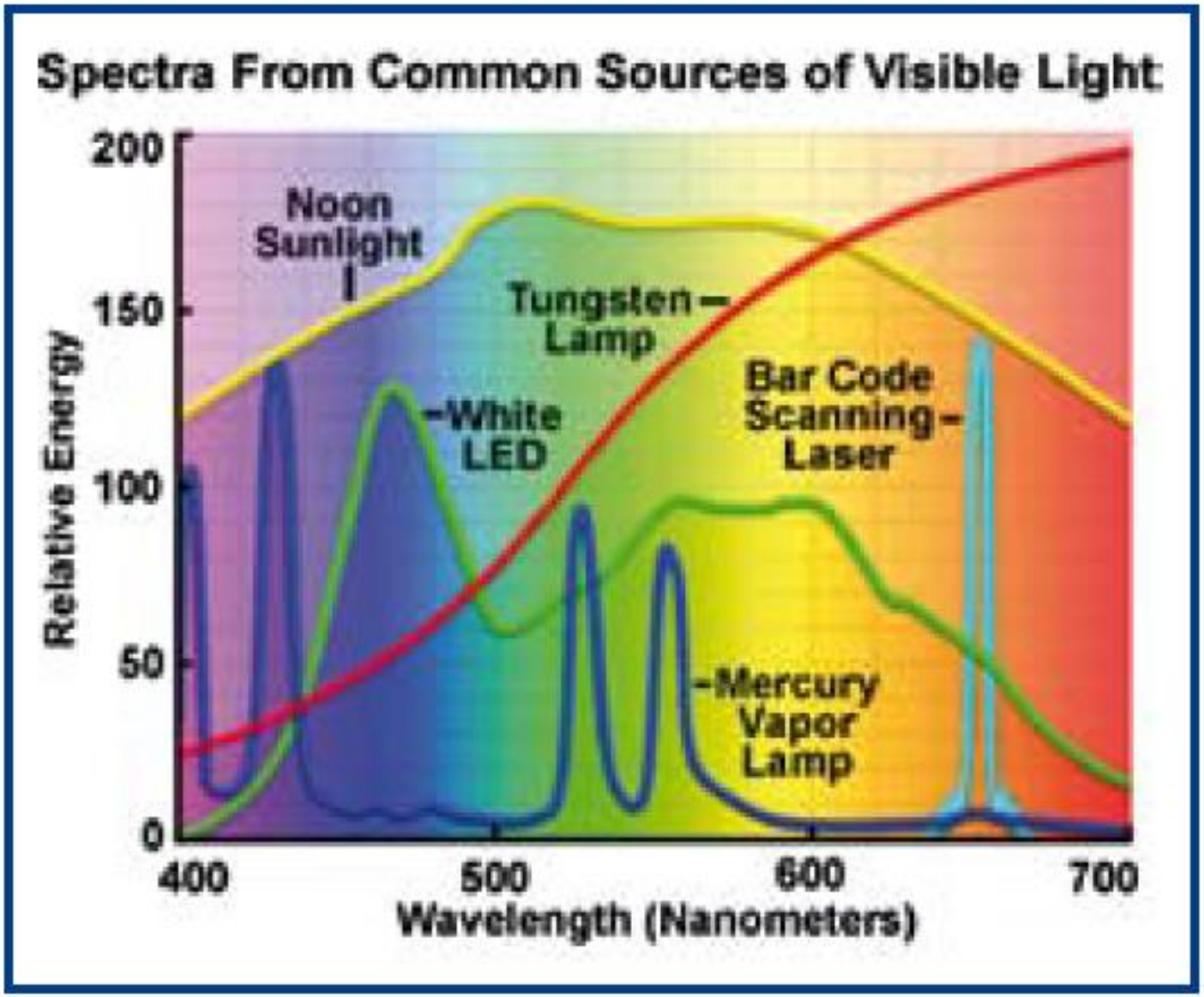
Violet Laser Tools

Blue Argon Laser Tools

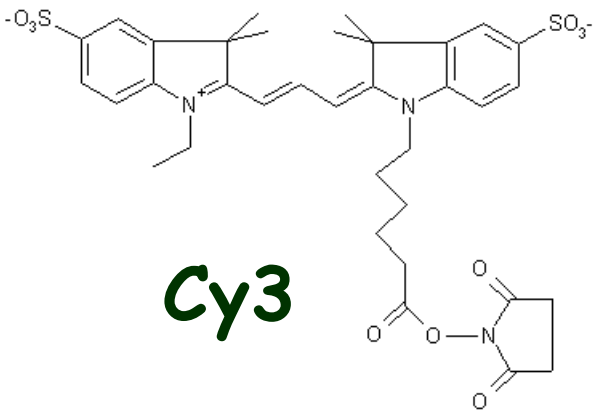
Red Diode Laser Tools

http://www.ssct.net/bauer2.pdf

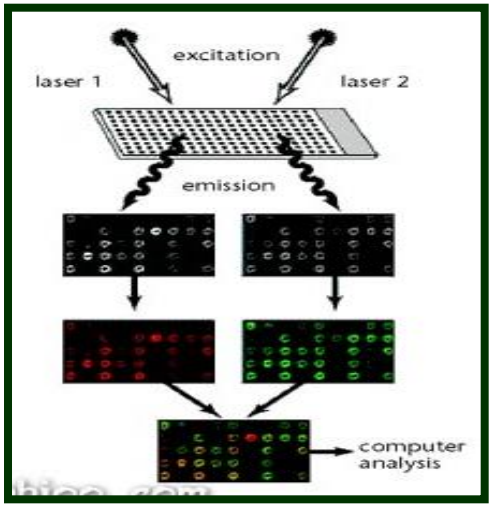
Spectrum of the sun, and spectra of common sources of visible light

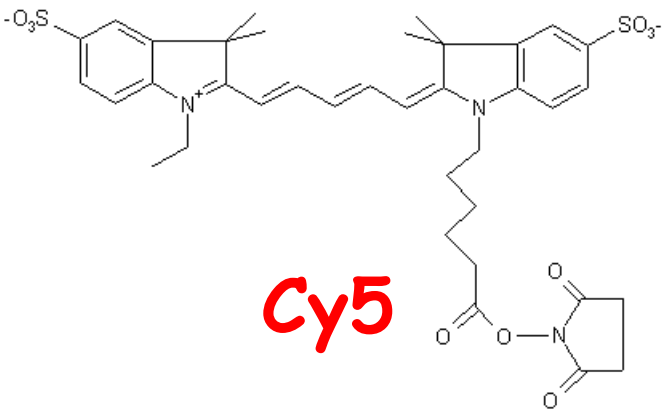


I. Organic Dye
Excitation and Emission Spectra

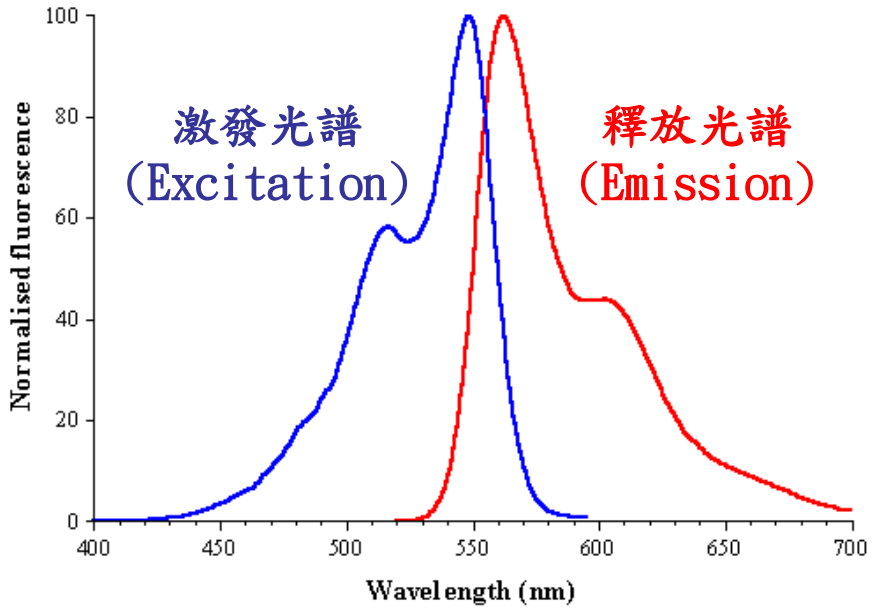


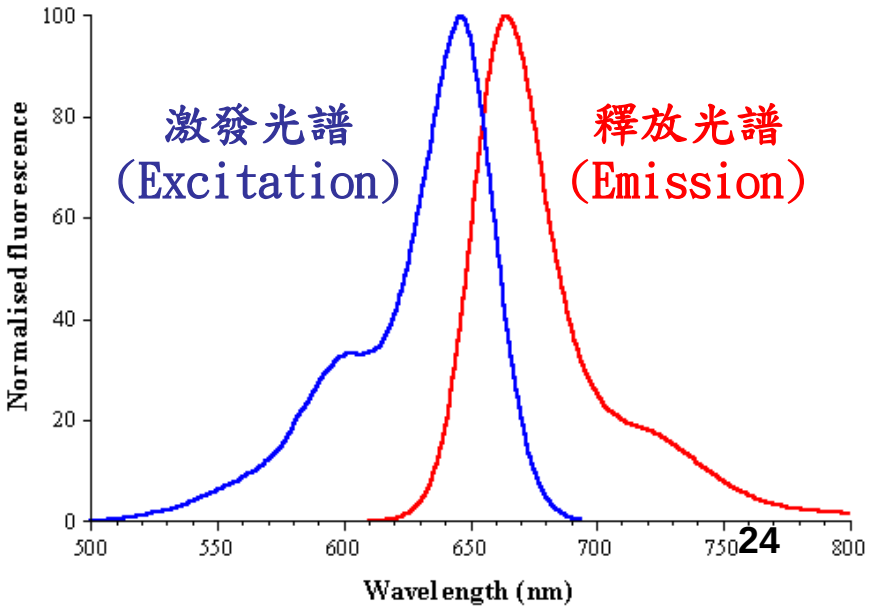
Cy3



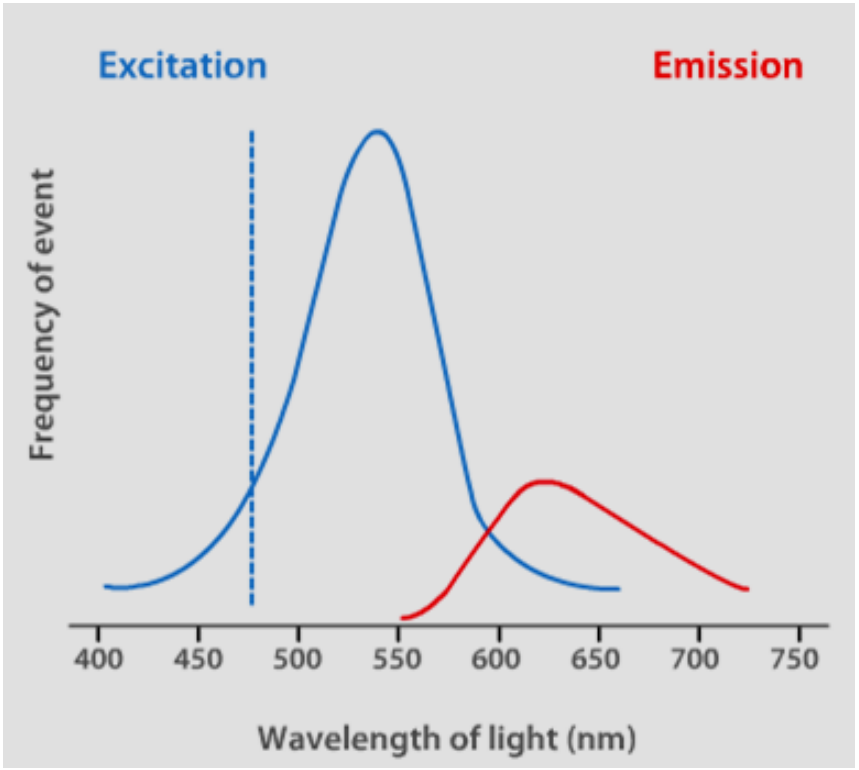


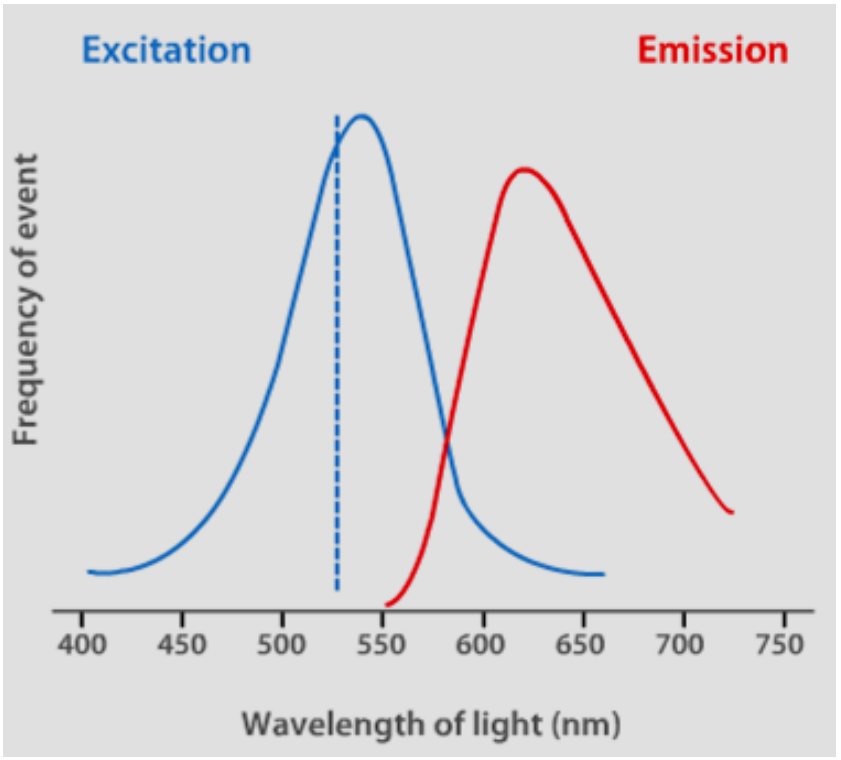
Cy5





Excitation and Emission Spectra of Organic Dyes

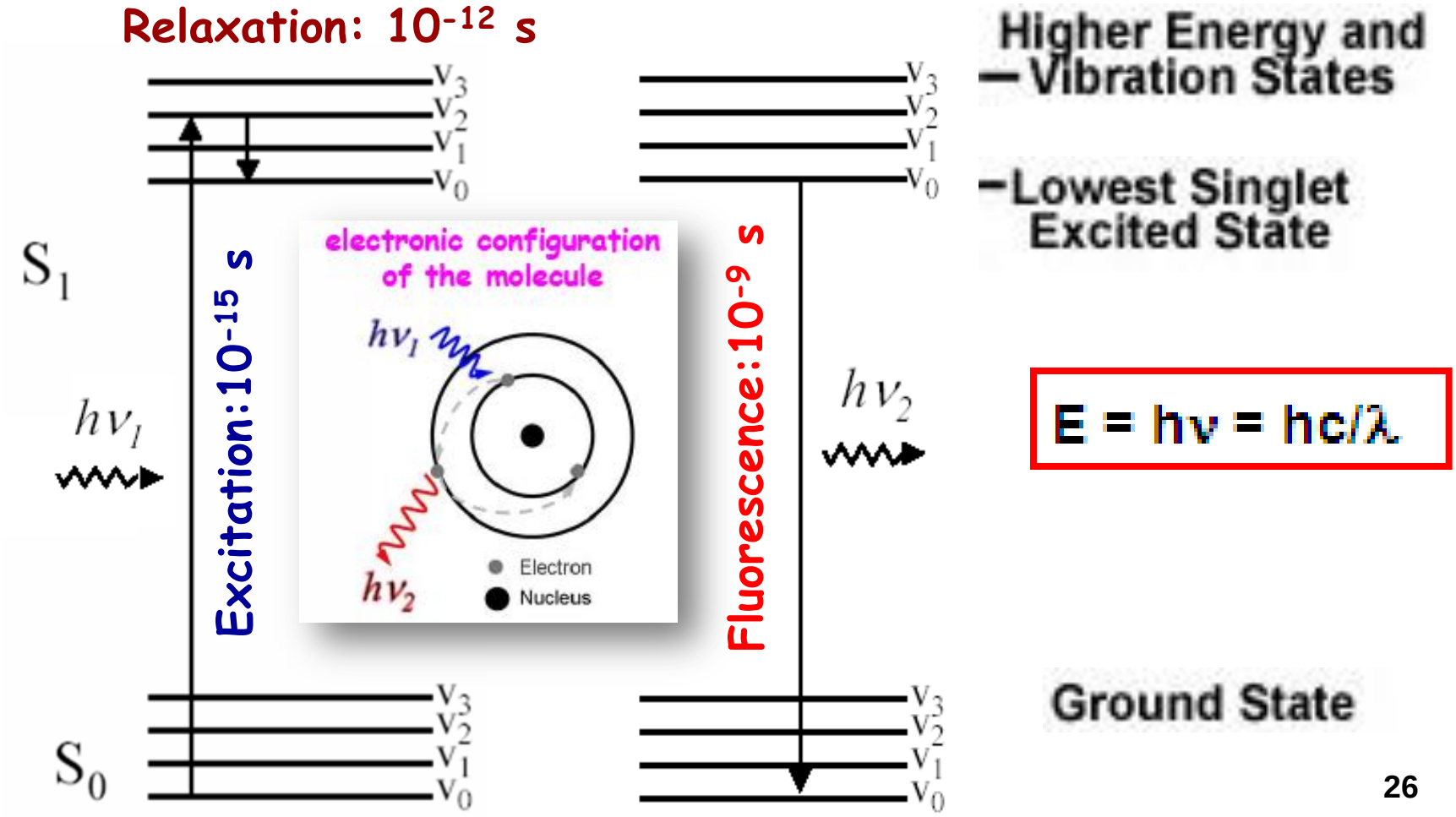




http://probes.invitrogen.com/resources/education/tutorials/2Spectra/player.html

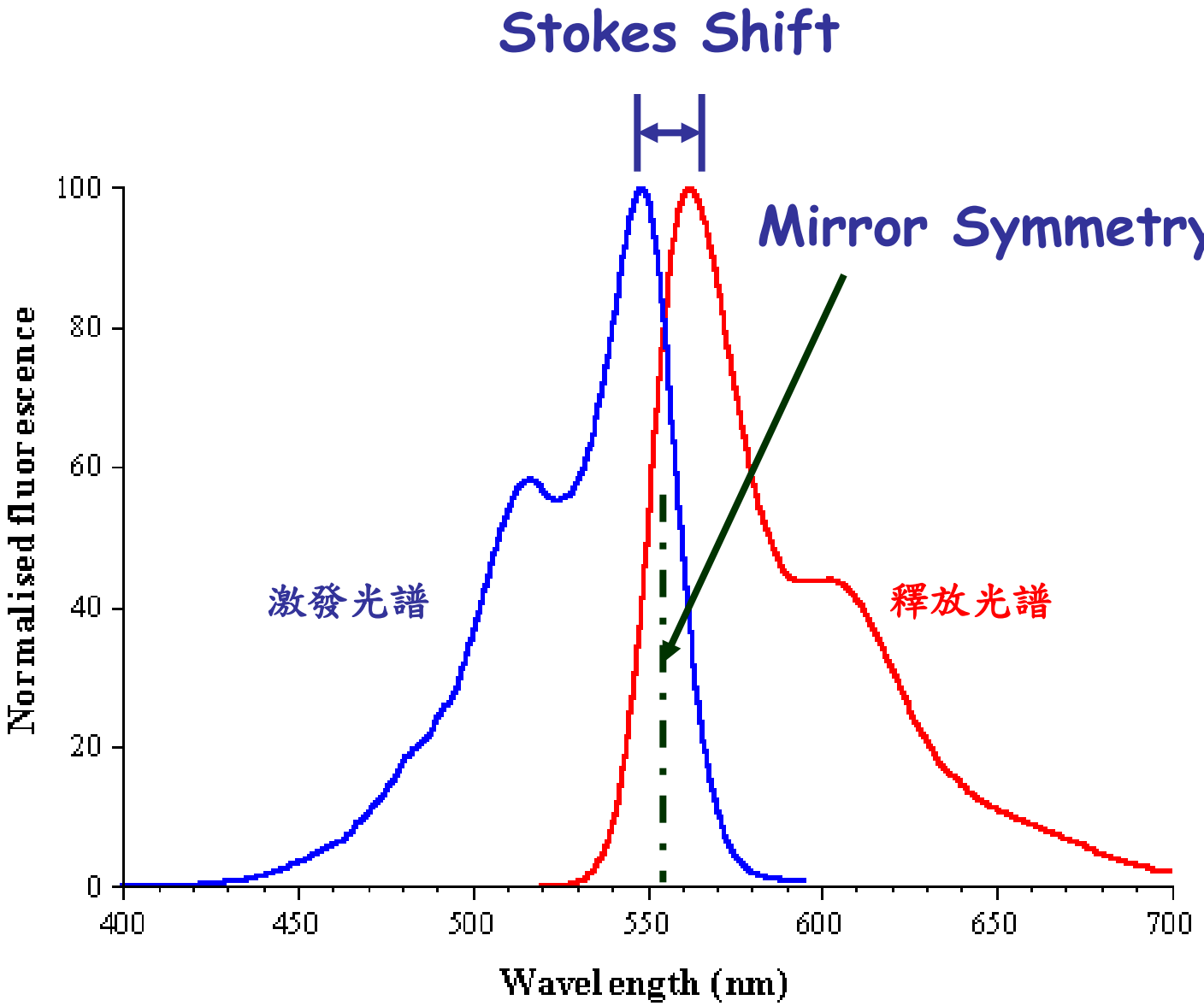
Fluorescence Energy-Level Diagram

Jablonski Energy Diagram for fluorescent organic dyes

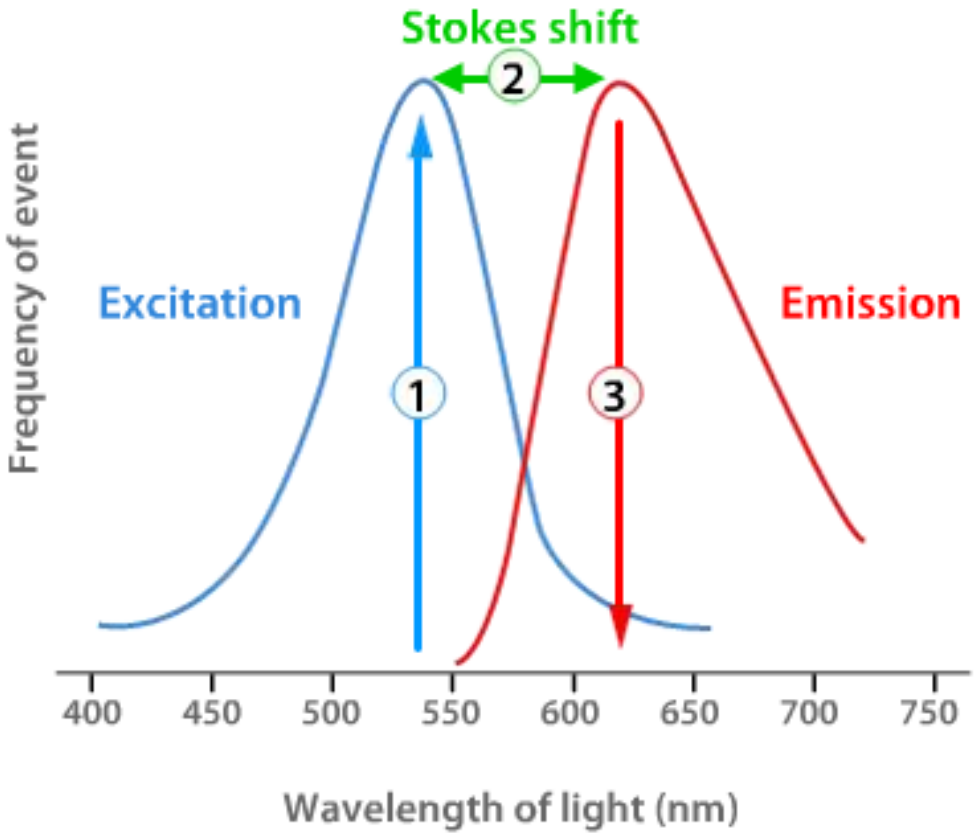


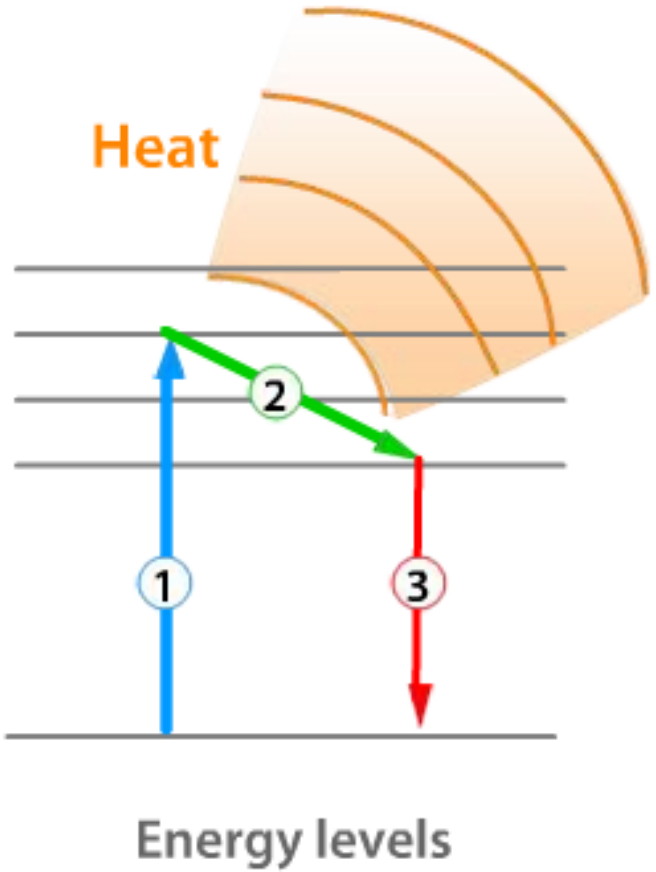
http://www.olympusmicro.com/primer/java/jablonski/jabintro/

Stokes Shift and Mirror Symmetry



Stokes Shift Explained





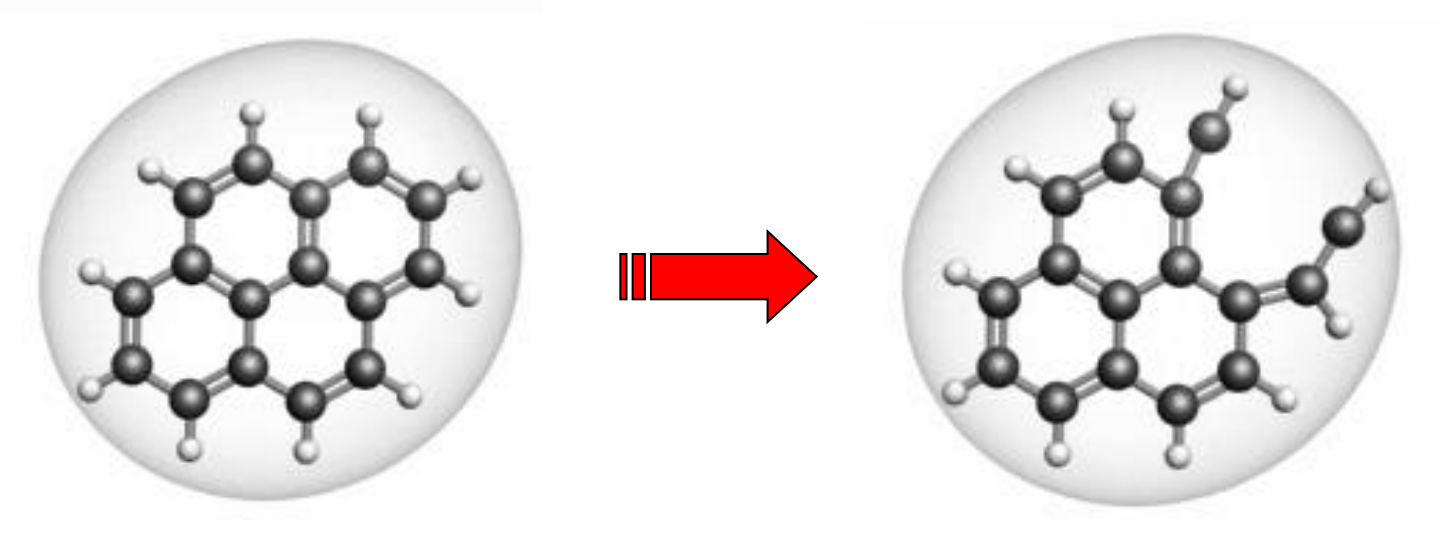
http://www.invitrogen.com/site/us/en/home/support/Tutorials.html

Photobleaching (光漂白)

photostability

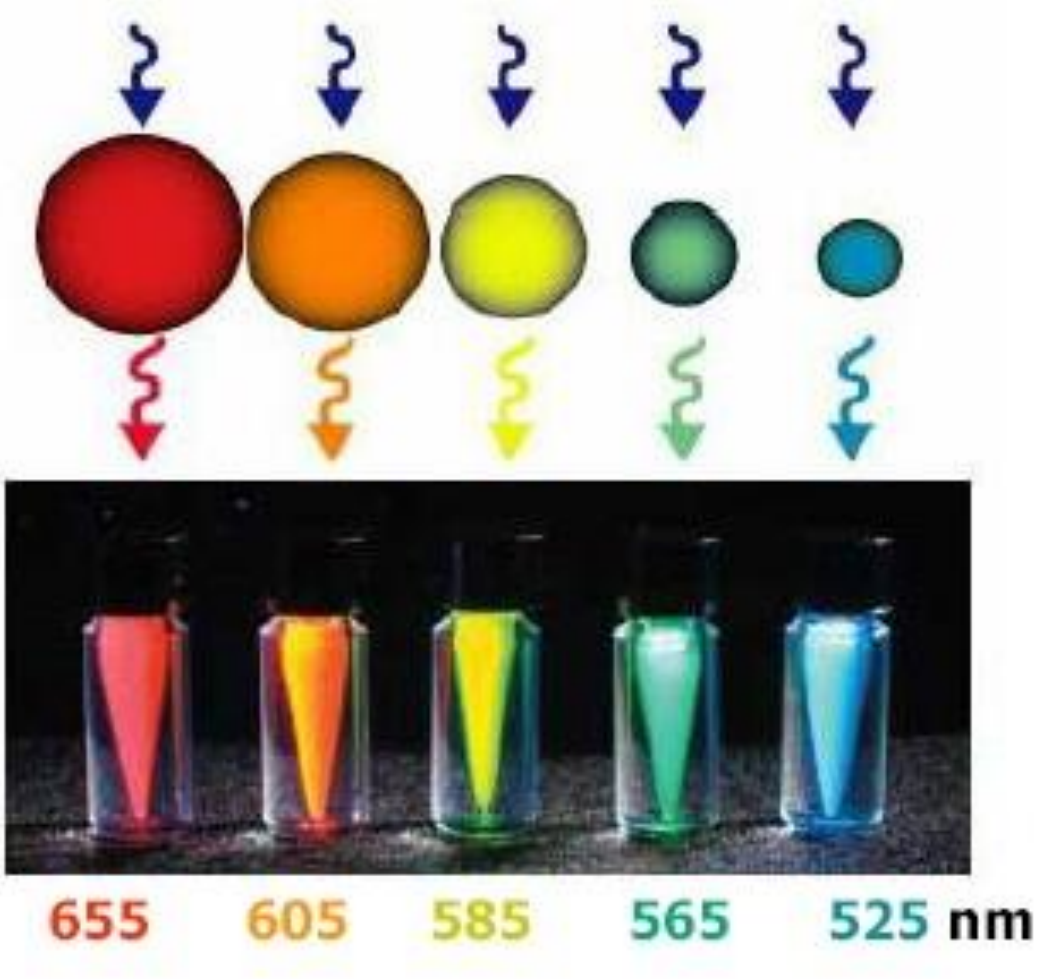
cycling of fluorescence

photobleaching

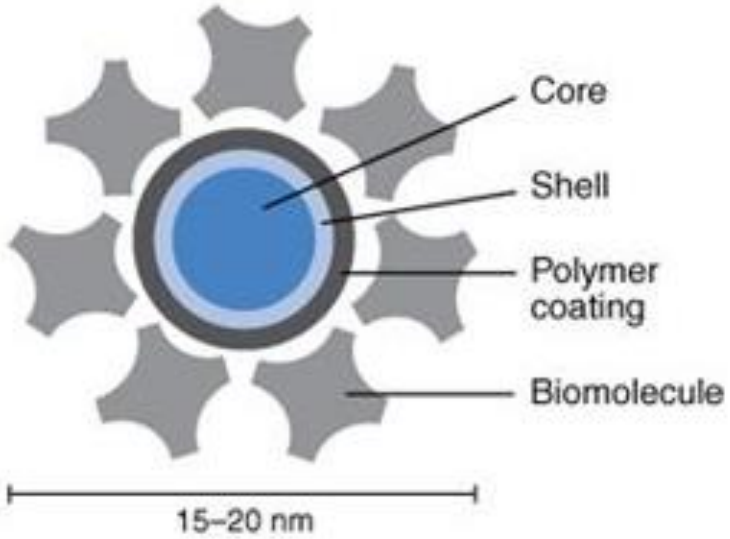


http://www.invitrogen.com/site/us/en/home/support/Tutorials.html

II. Quantum Dots (量子點)

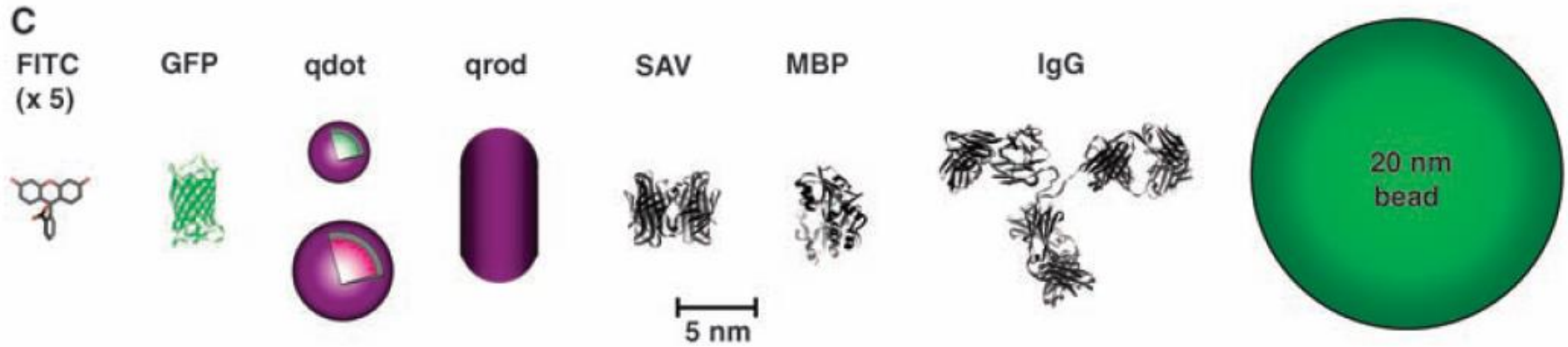


Highly fluorescent
Nanometer-sized
Single crystals
Semiconductor materials



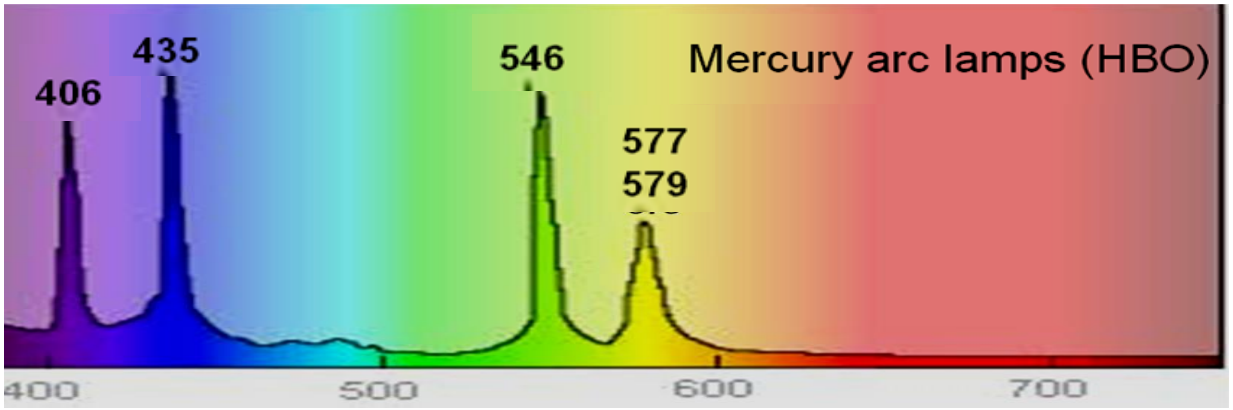
Tuneability of Qdot® nanocrystals. Five different nanocrystal solutions are shown excited with the same long-wavelength UV lamp; the size of the nanocrystal determines the color.

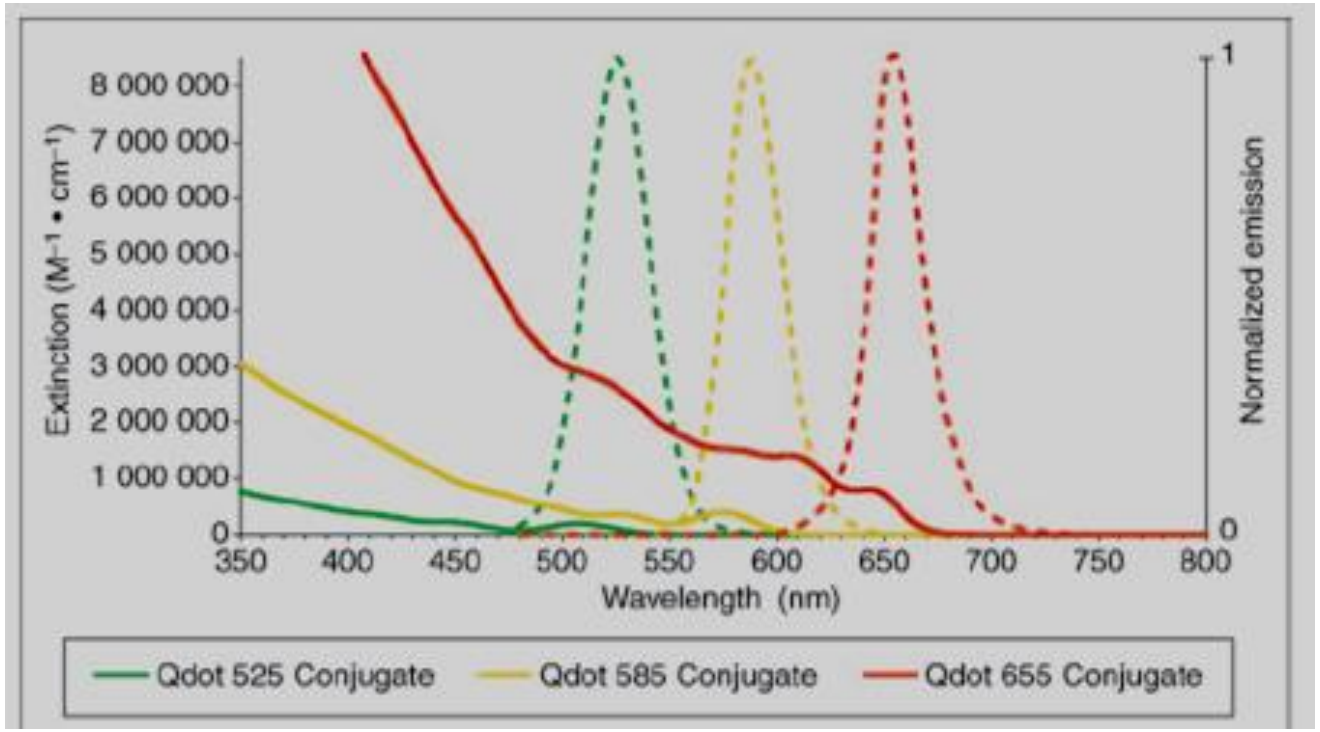
Size Comparison of Qdots and Comparable Objects



FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; qdot, green (4 nm, top) and red (6.5 nm, bottom) CdSe/ZnS qdot; qrod, rod-shaped qdot (size from Quantum Dot Corp.'s Web site). Three proteins—streptavidin (SAV), maltose binding protein (MBP), and immunoglobulin G (IgG)—have been used for further functionalization of qdots and add to the final size of the qdot, in conjunction with the solubilization chemistry.

Turning all the lights on: quantum dots in cellular assays

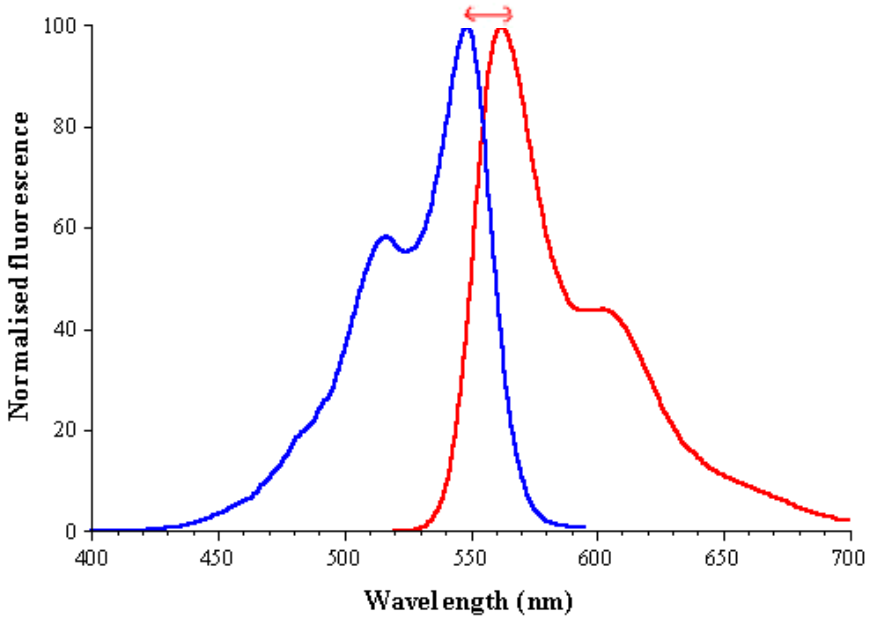




Absorbance (solid) and emission (dashed) spectra of different color Qdot® conjugates. Note that the colors of the lines for each conjugate represent the approximate color that they appear in fluorescence detection.

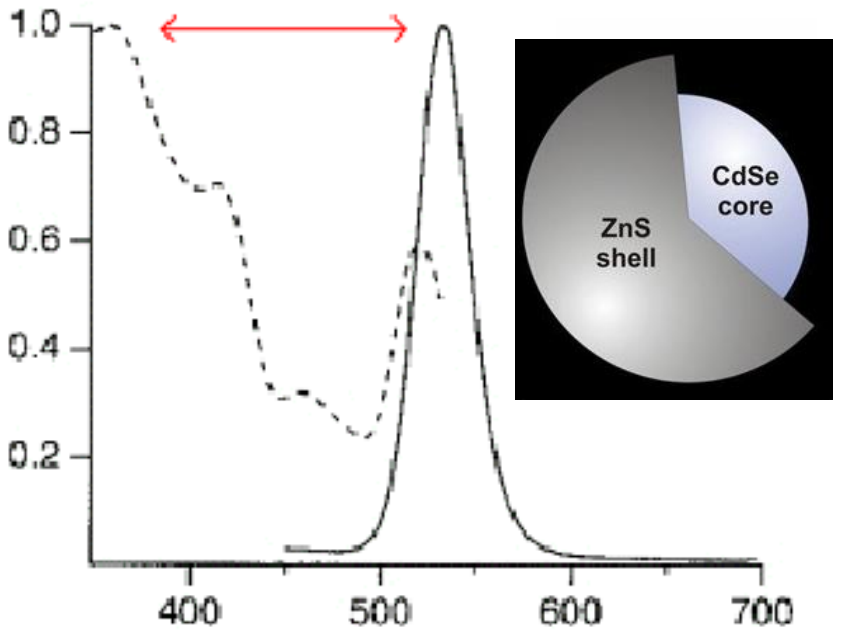
Spectral Properties

Organic dye



- **Small Stokes shift**
- **Multiple source excitation req'd.**
- **Broad emission**
- **Poor photostability**

Qdot® Conjugate (525)



- Large "Stokes shift"
- Single-source excitation
- Narrow emission
- Excellent photostability

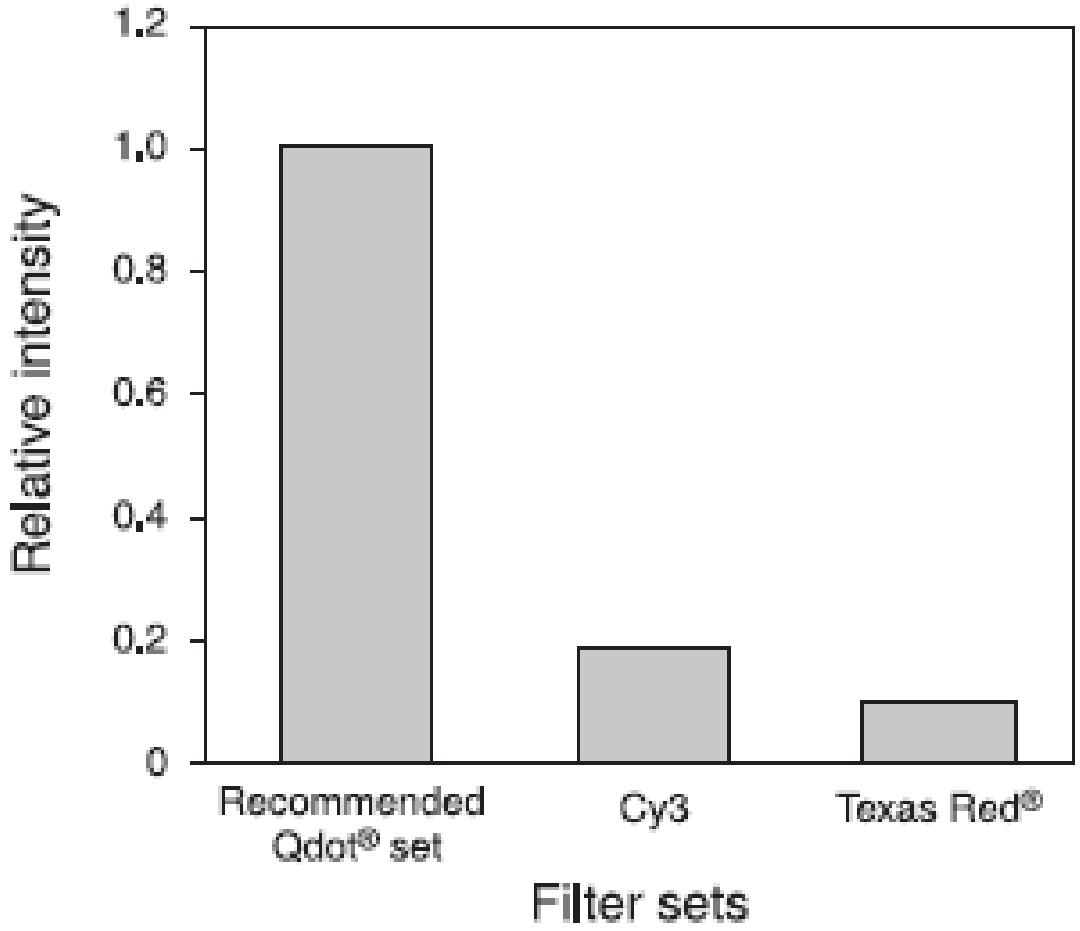
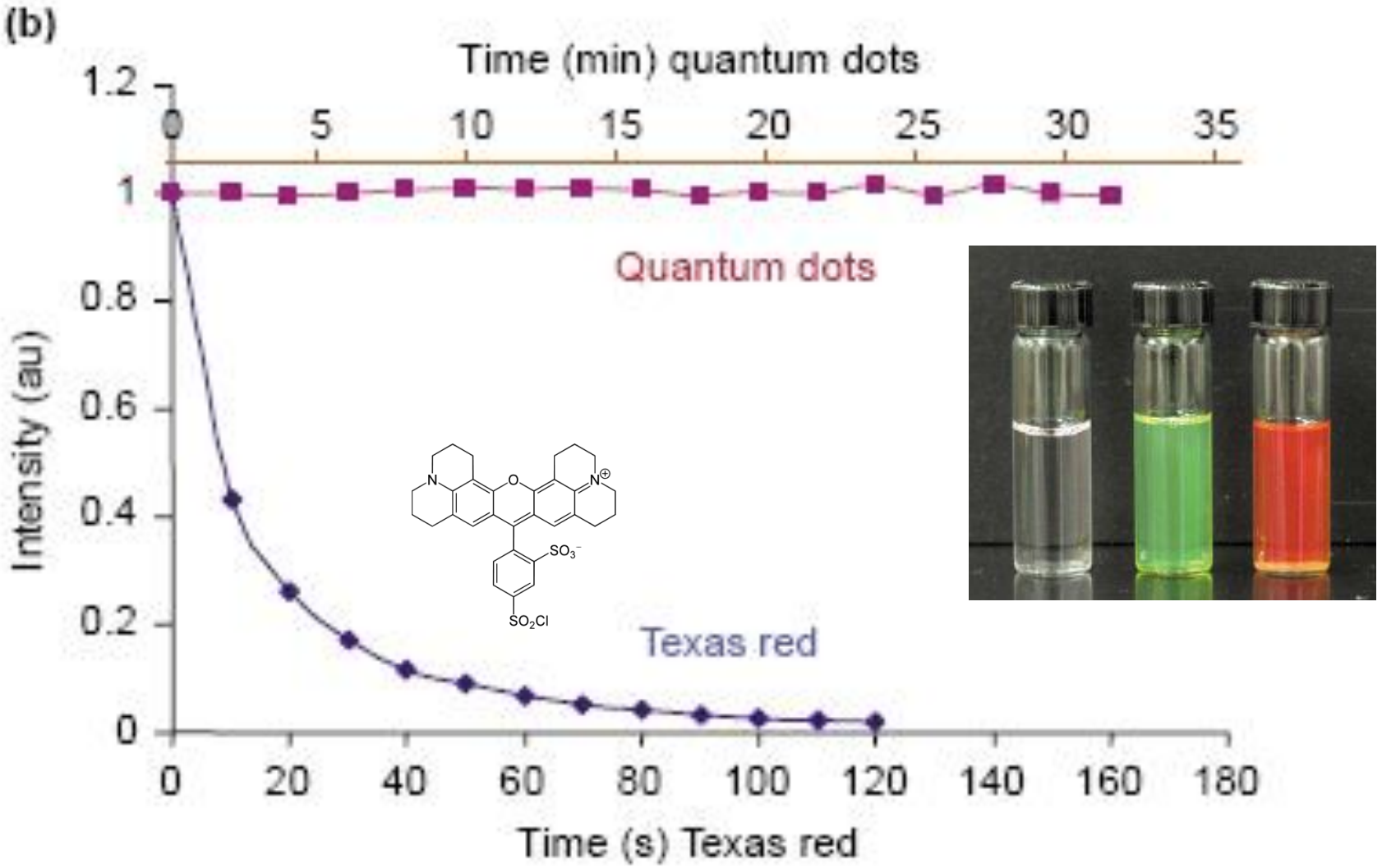


Figure 4. Detection of Qdot® conjugates on tissue sections with recommended and standard filter sets. Mouse kidney sections were stained with Qdot® 605 streptavidin conjugate, and then images were collected on a Nikon epi-fluorescence microscope in 16 bit capture mode. The mean fluorescence of positively stained samples was extracted using Scion Image software. The recommended Qdot® filter set included a 460 nm short pass exciter, a 475 nm dichroic, and a 605/20 nm band pass emitter. The Cy3 filter set included a 545/30 nm exciter, a 570 nm dichroic, and a 610/75 nm emitter. The Texas Red® filter set included a 560/40 nm exciter, a 595 nm dichroic, and a 630/60 nm emitter.

http://probes.invitrogen.com/media/pis/mp19000.pdf

Photostability



Current Opinion in Biotechnology 2005, 16:63–72

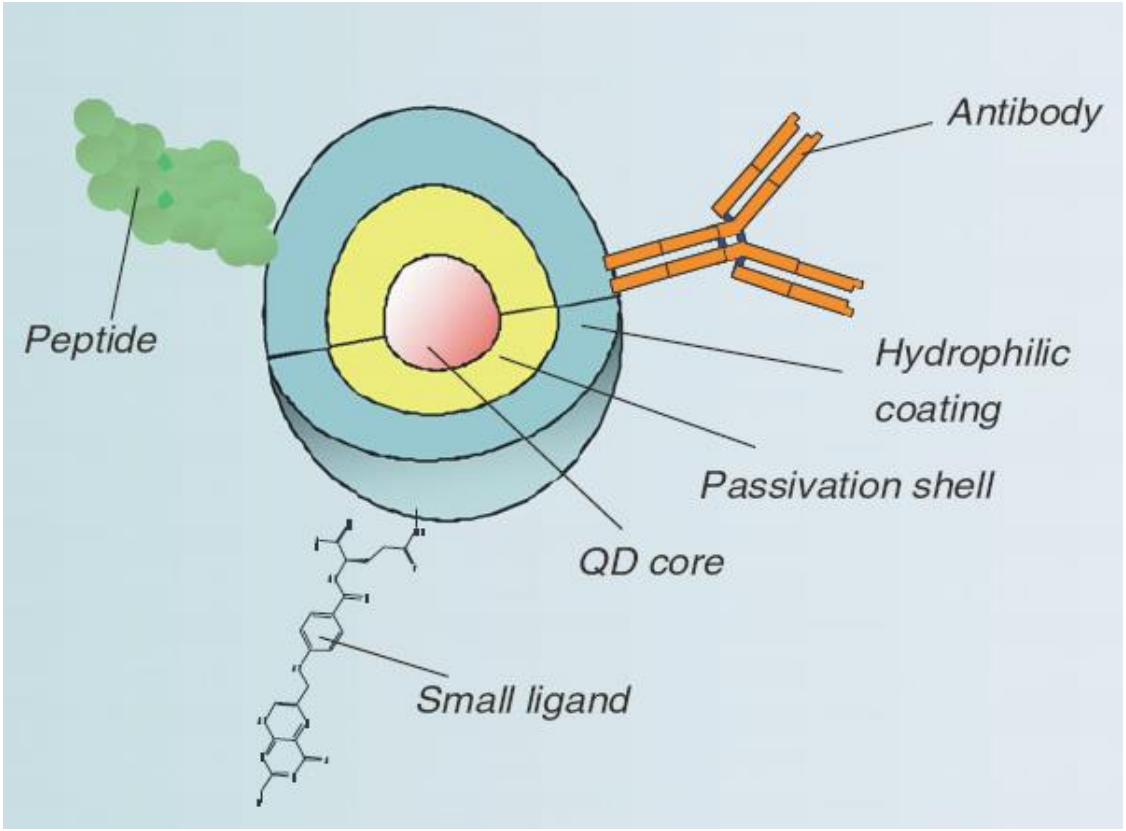
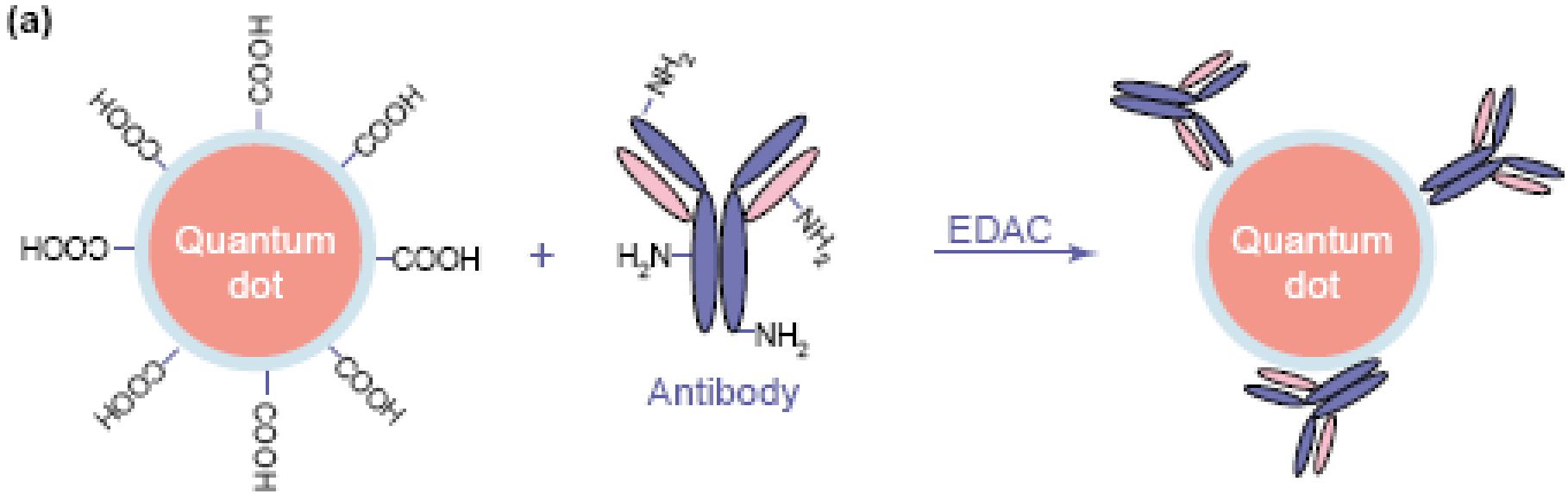


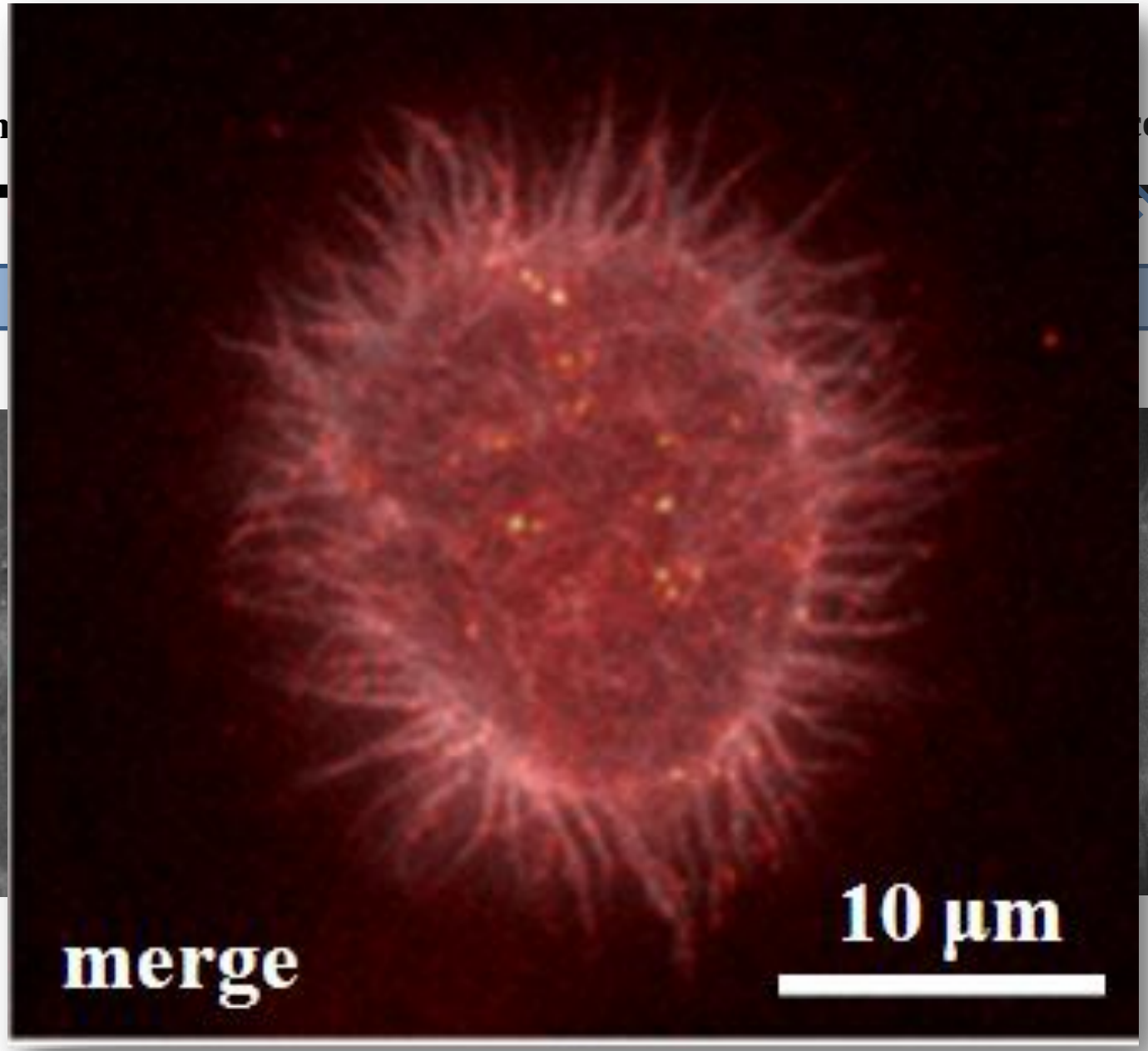
Fig. 1. Scheme of a QD for biological application. The nanocrystal core (e.g. CdSe) is passivated by another semiconductor shell (e.g. ZnS). The QD-surface is covered by a hydrophilic coating which enables conjugation to biological active compounds (e.g. antibodies, peptides or small ligands, here depicted for folic acid).

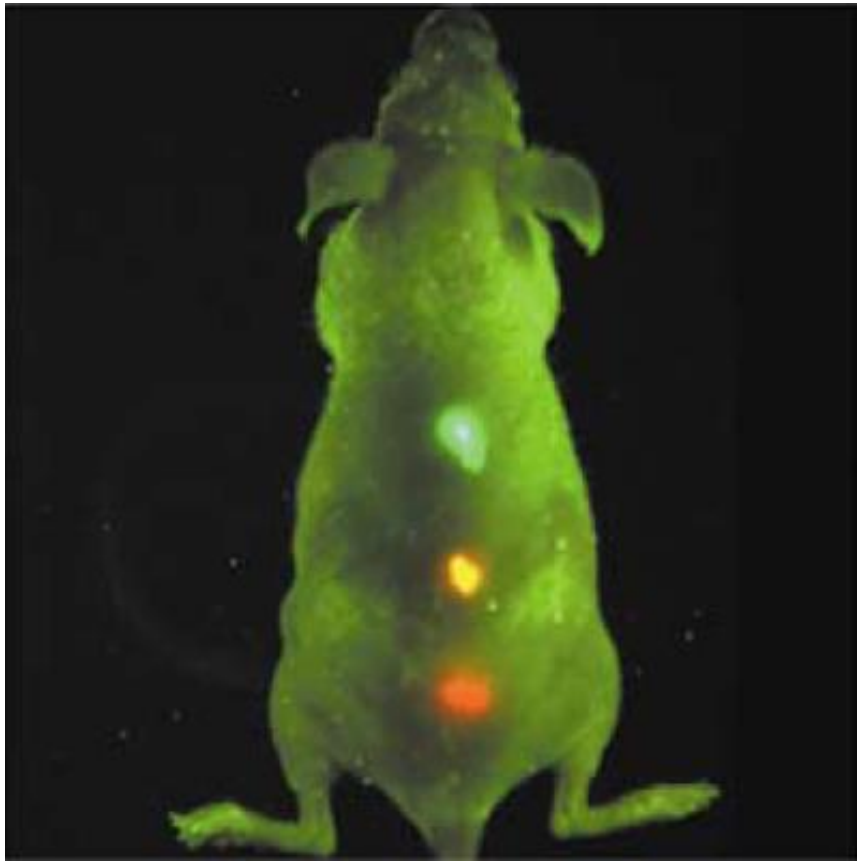
利用活化劑EDC及sulfo-NHS活化量子點表面羧基再加入protein（具有NH_2官能基），使protein 能夠共價鍵結在量子點表面。



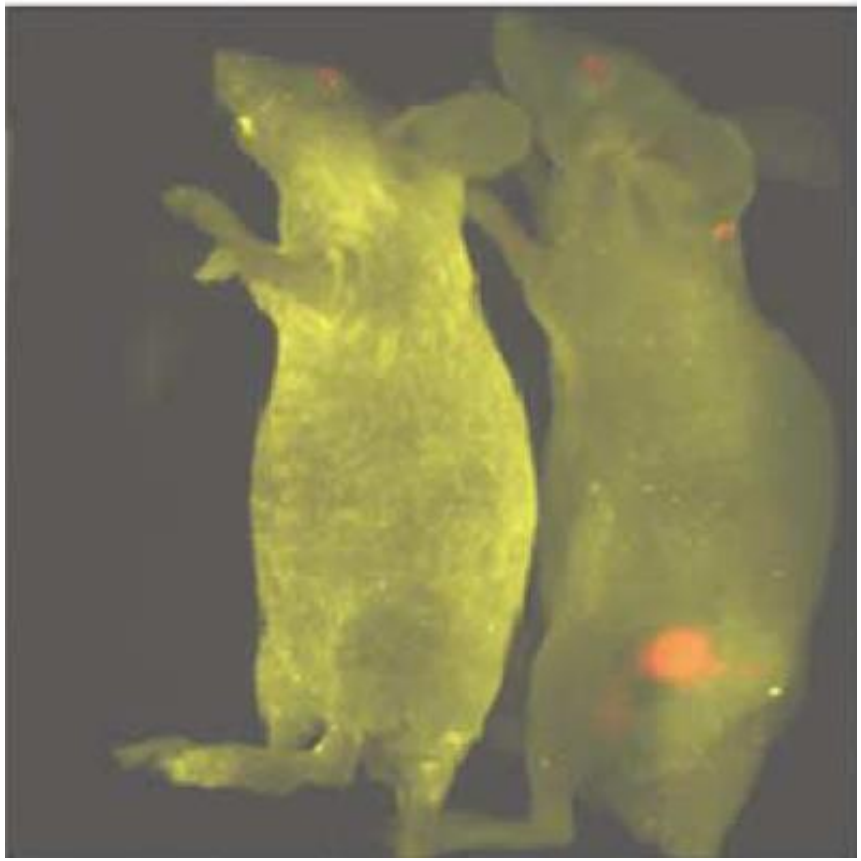
Current Opinion in Biotechnology 2005, 16:63–72

Investigate the QD-EGF Trafficking Pathway



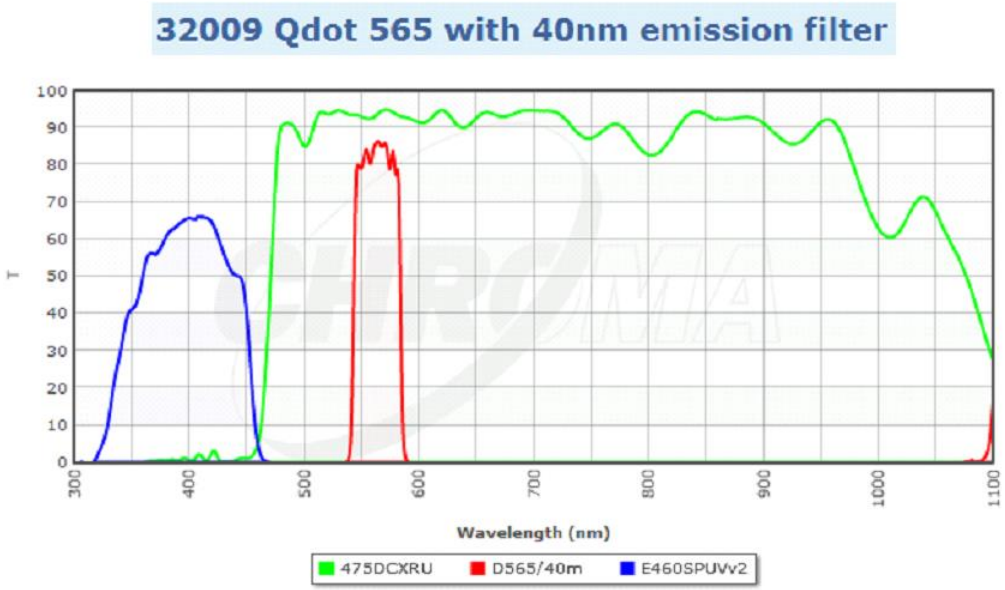


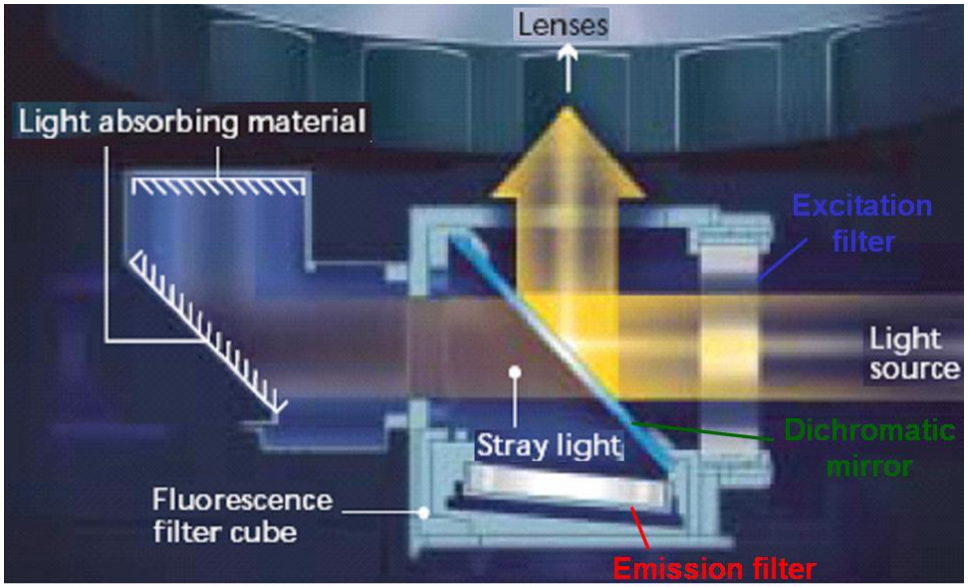
In vivo simultaneous imaging of multicolor QD-encoded microbeads injected into a live mouse.



Molecular targeting and in vivo imaging of a prostate tumor in mouse using a QD–antibody conjugate (red).

Inverted Microscope with Epi-Fluorescence

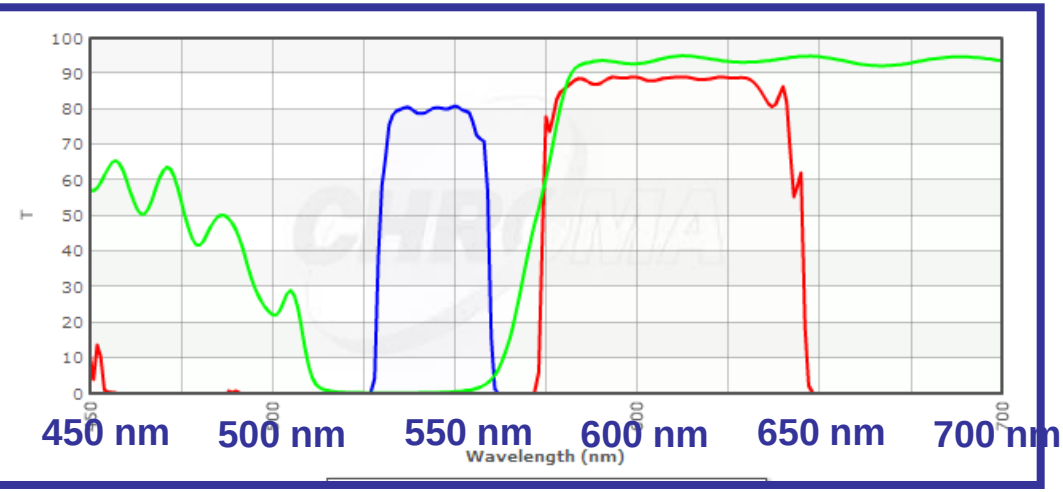


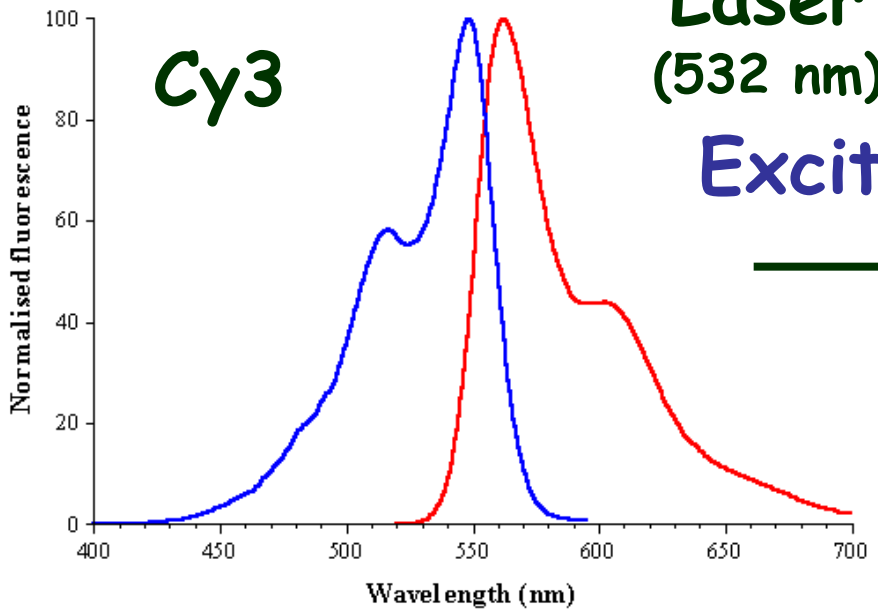


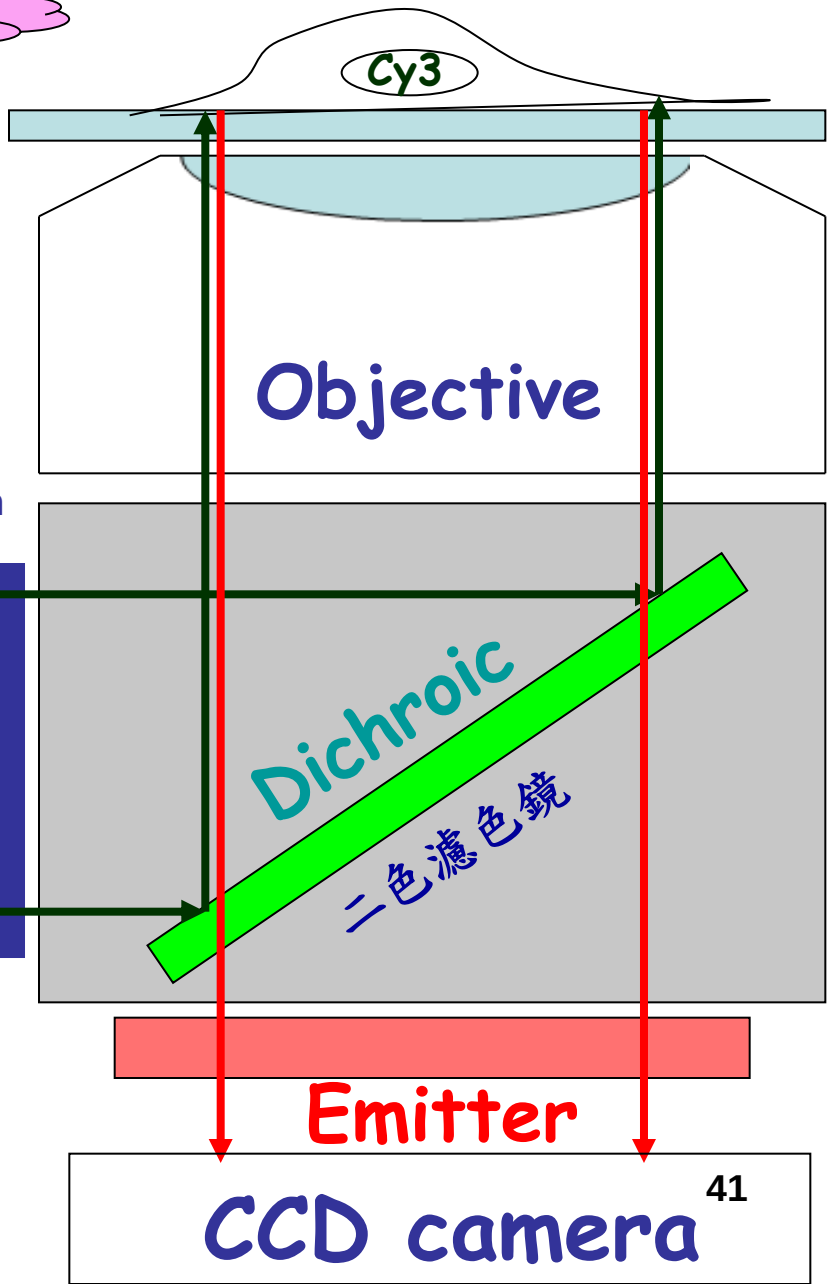
CCD camera

Inverted Microscope with Epi-Fluorescence

This is the recommended Cy3™ filter set





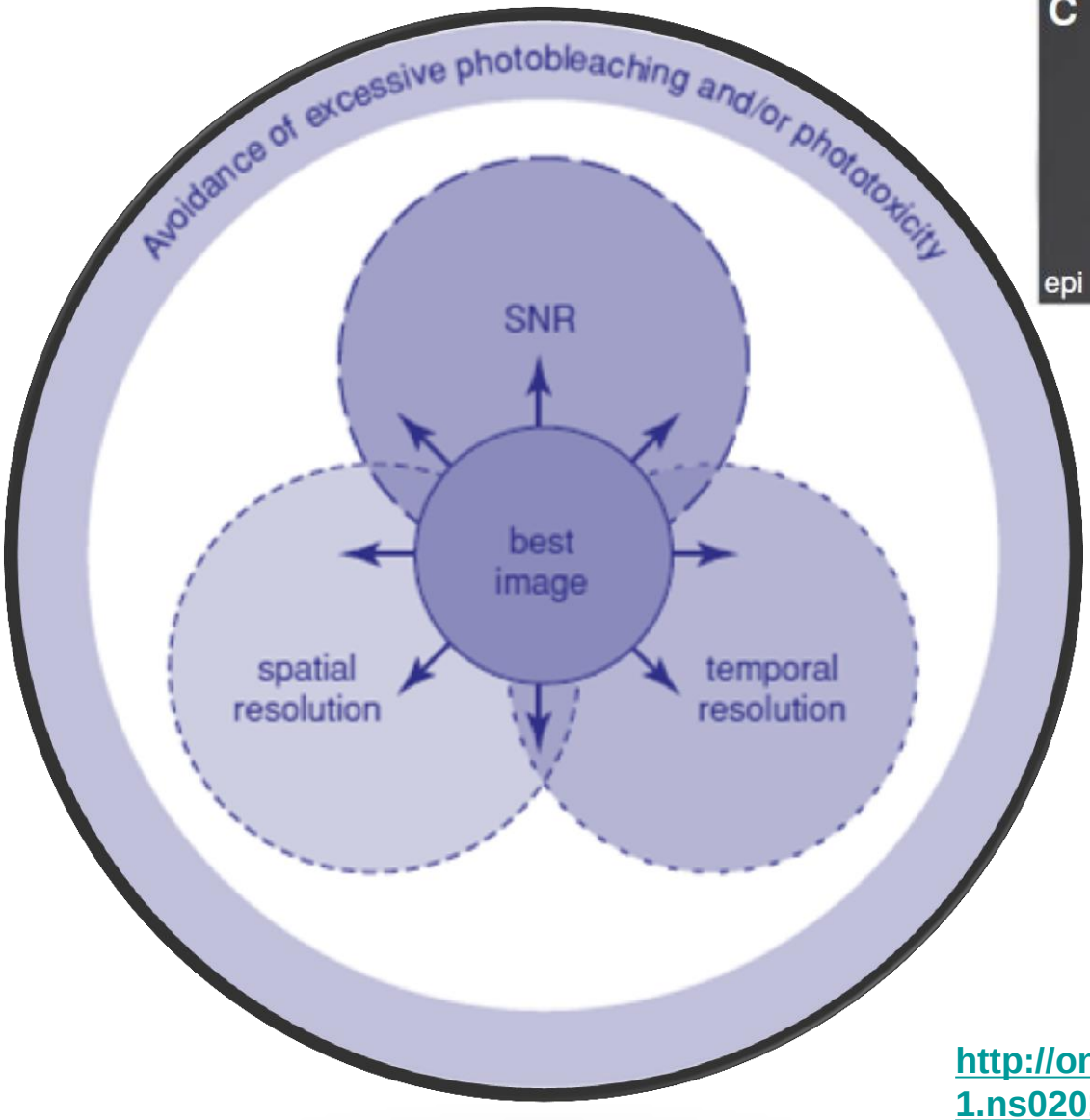


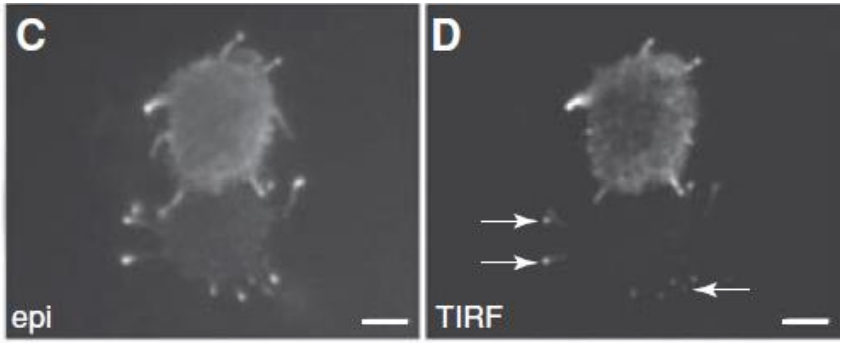
Part III

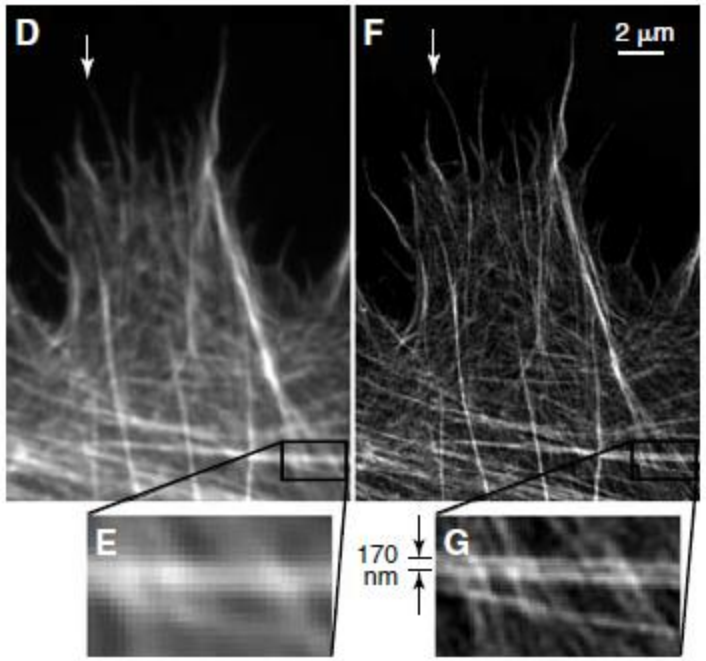
The Resolving Power of a Microscope

Hubble Telescope—Deep Space Photo http://www.hubblesite.org

Diagram of Some of the Critical Opposing Factors in an Imaging Experiment

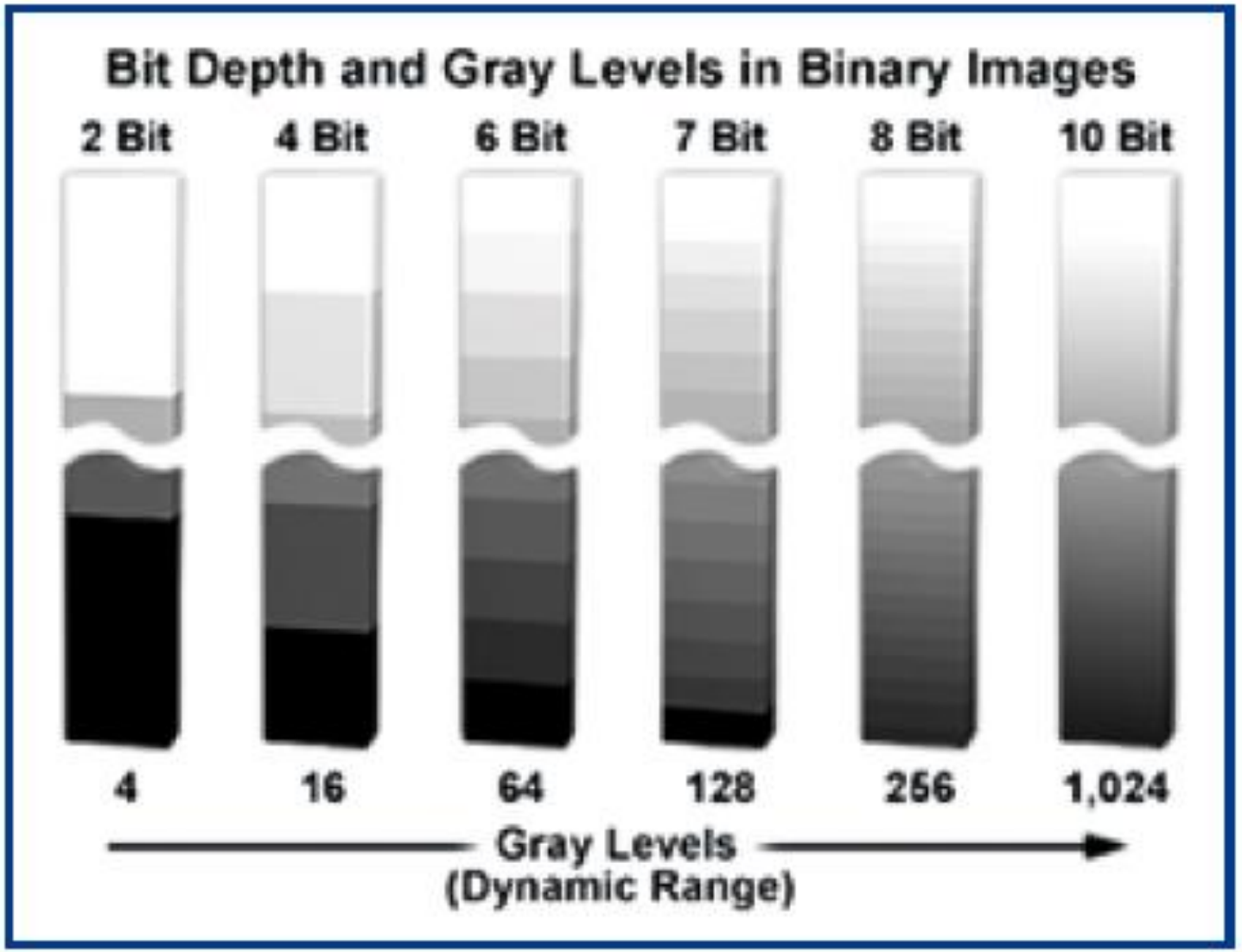




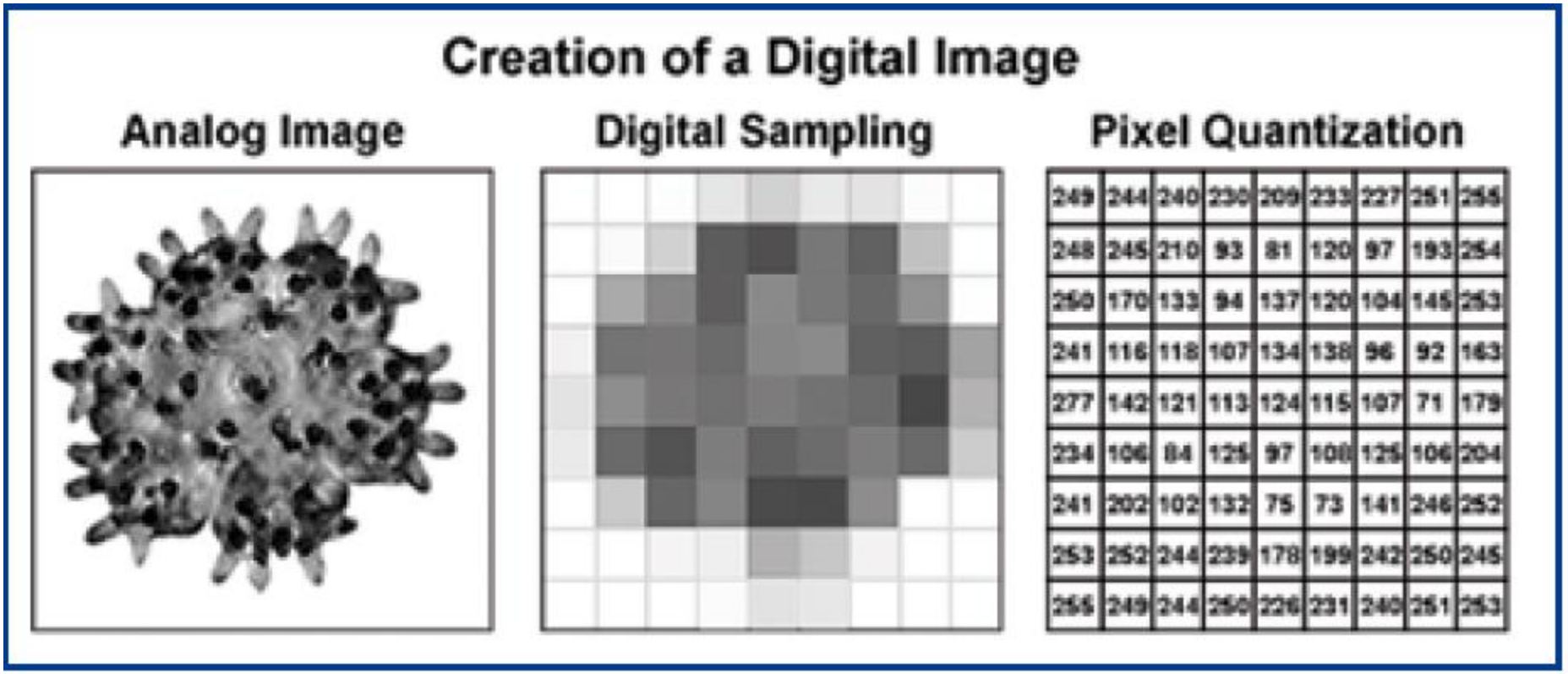


http://onlinelibrary.wiley.com/doi/10.1002/047114230 1.ns0201s50/figures

Bit depth and grey levels in digital images



Creation of a digital image



Resolution in digital images – is it important?

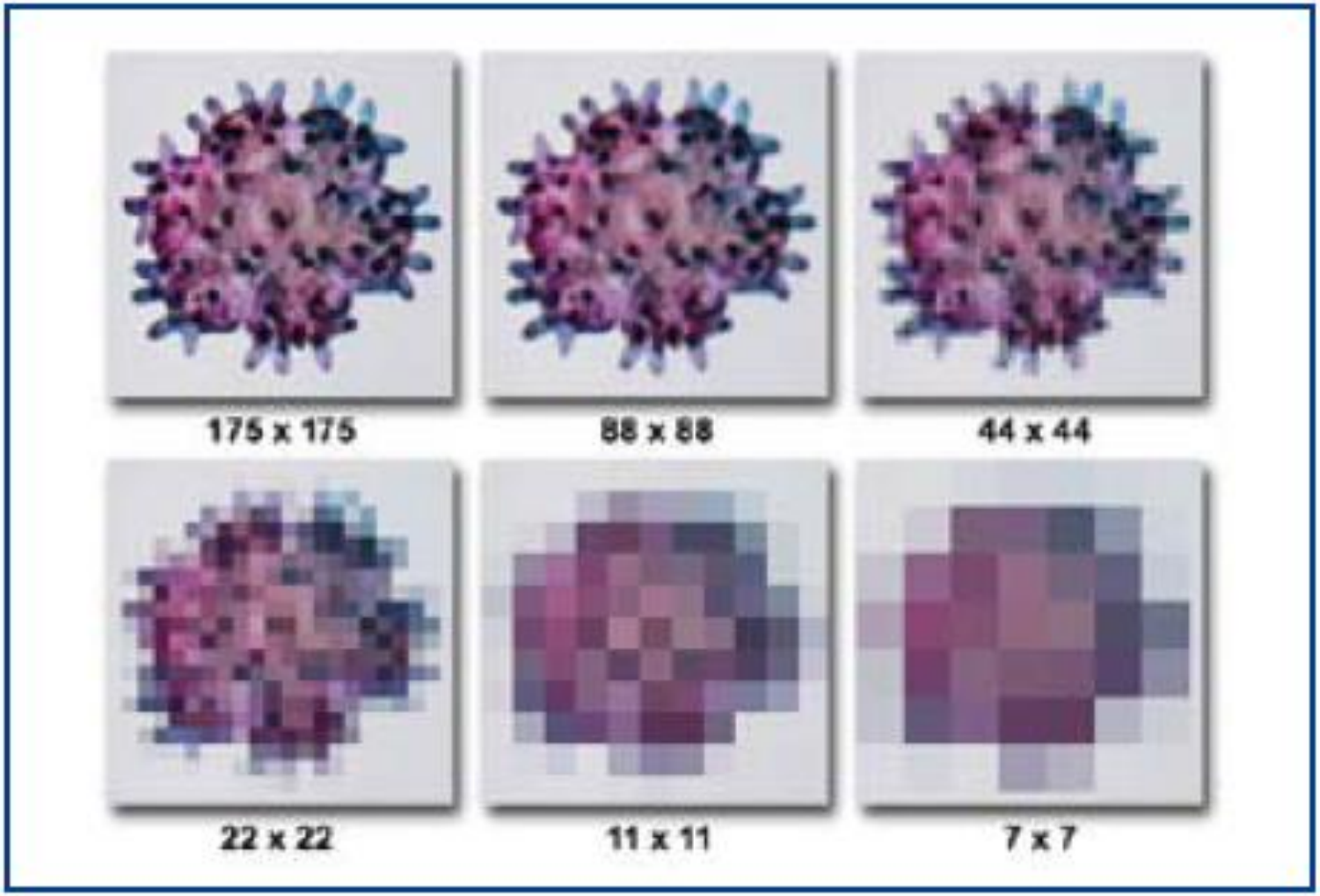
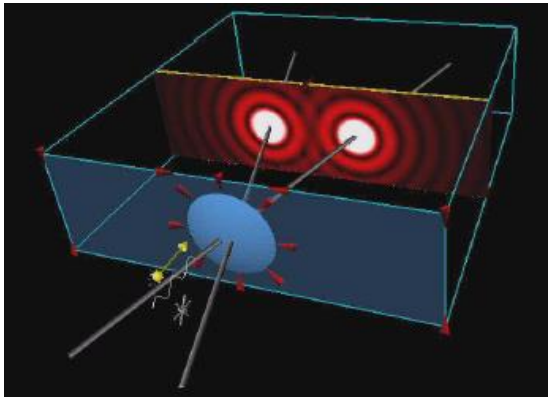
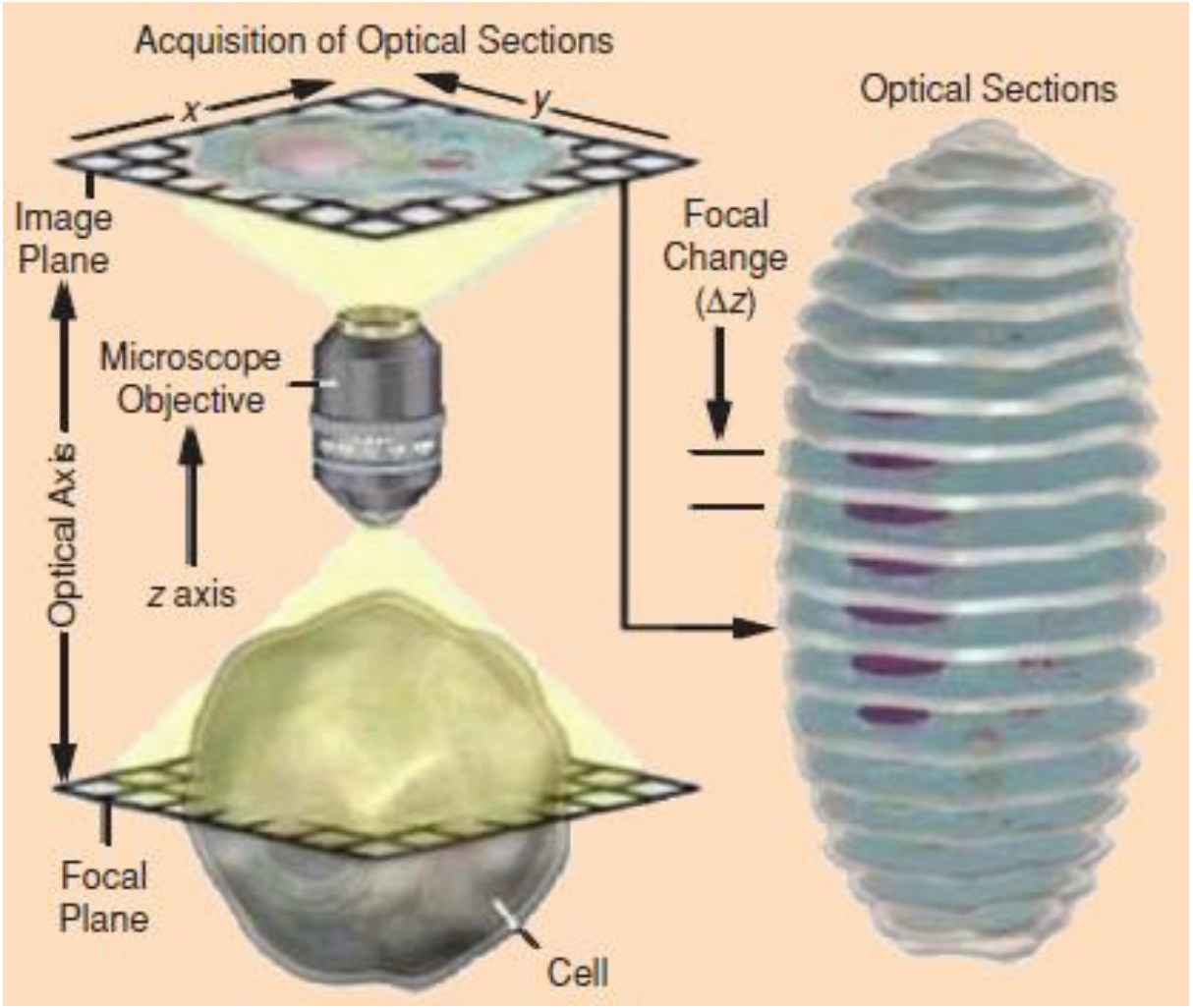


Fig. 17: Four representations of the same image, with different numbers of pixels used. The numbers of pixels is written below each image.

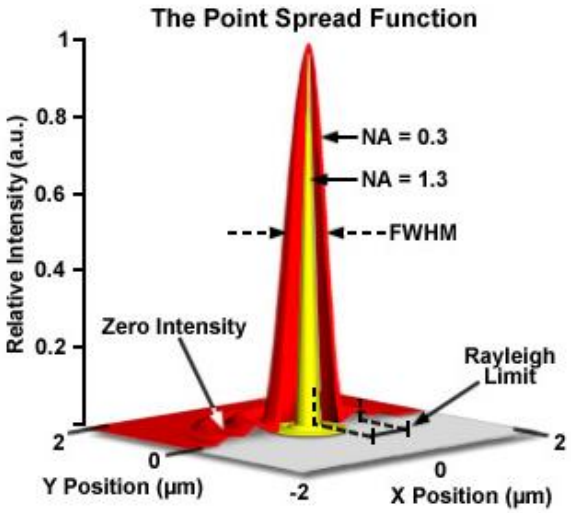
Physical limits and methods to overcome

An example of the acquired 3-D image of a cell, captured by a fluorescence microscope.



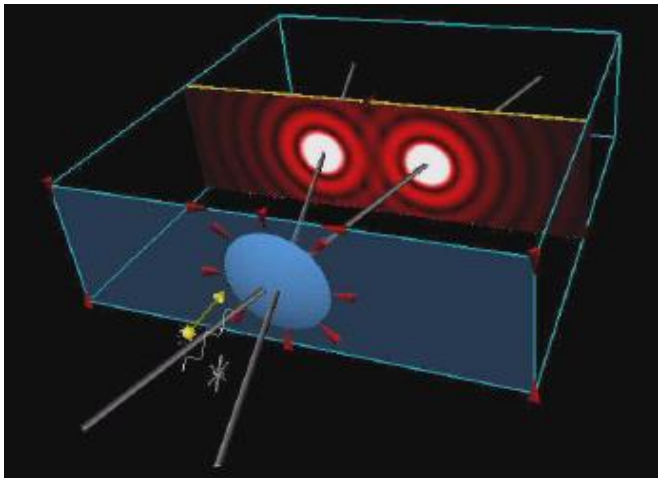


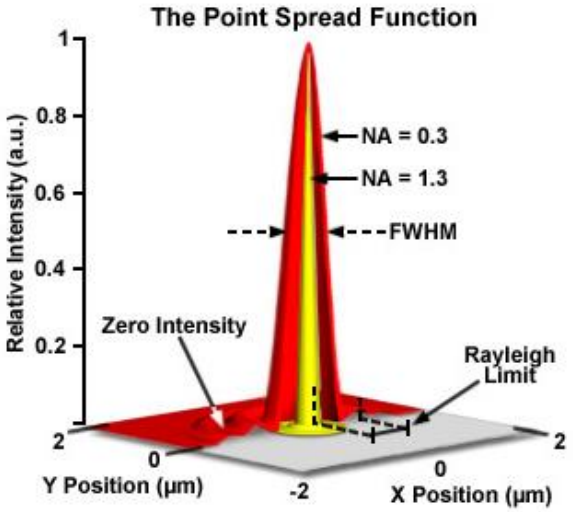
Point Spread Function (PSF)

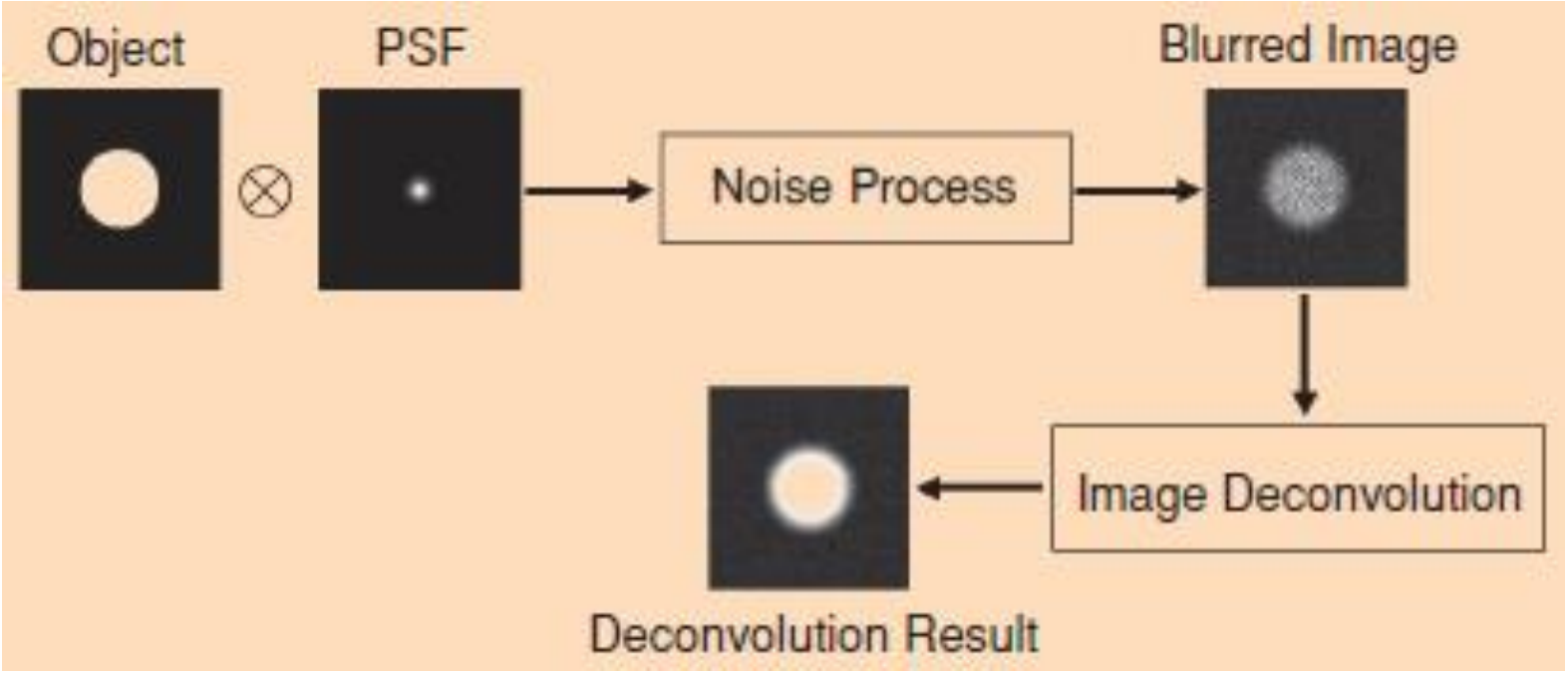


http://www.ese.wustl.edu/~nehorai/paper/deconvolutions1.pdf
http://www.olympusmicro.com/primer/digitalimaging/deconvolution/deconintro.html
http://zeiss-campus.magnet.fsu.edu/articles/basics/psf.html

Physical limits and methods to overcome

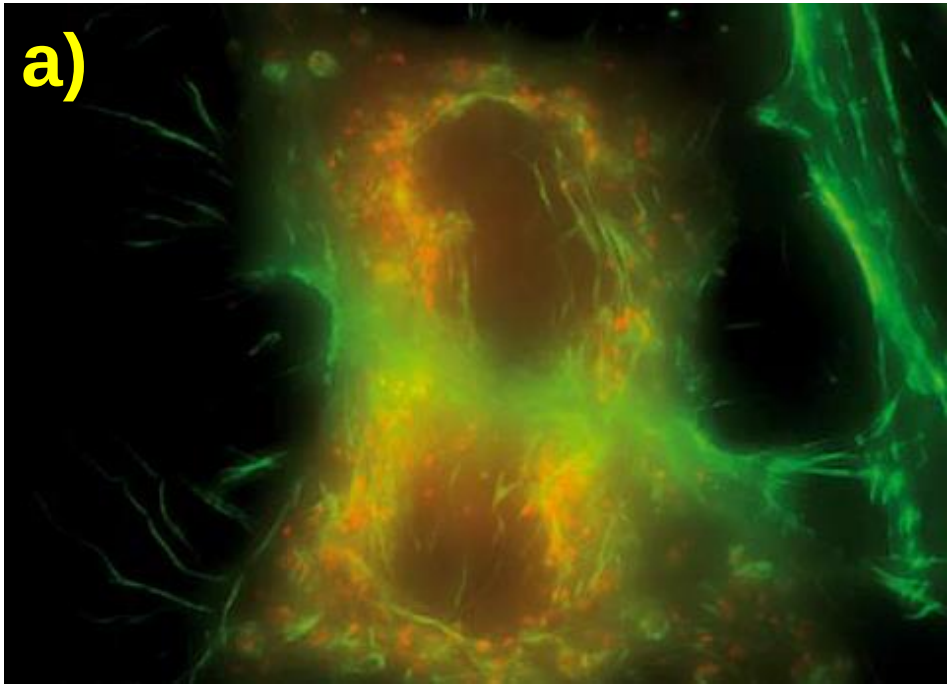


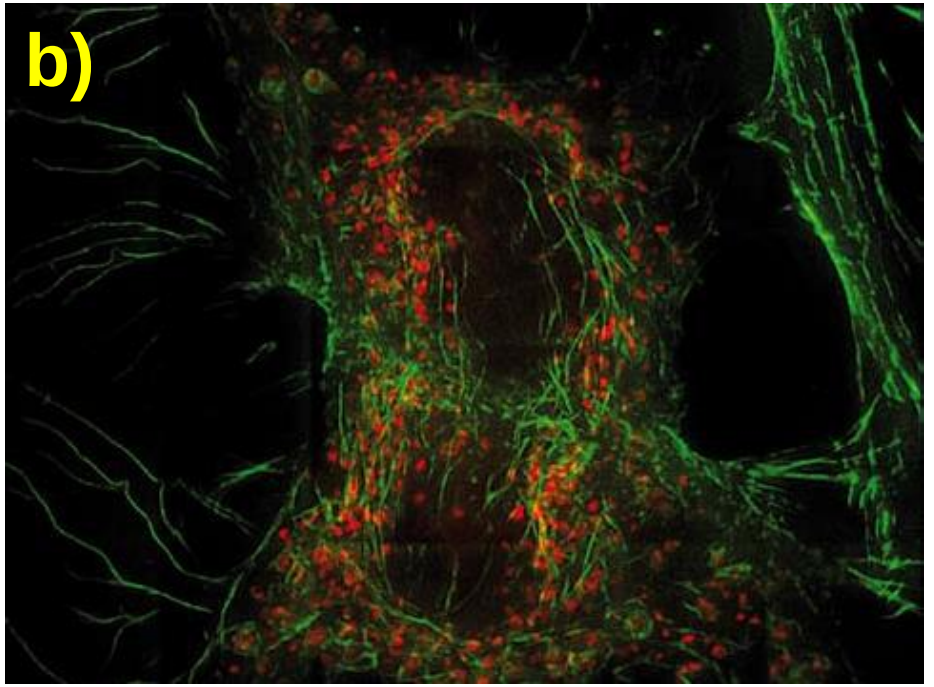




http://www.ese.wustl.edu/~nehorai/paper/deconvolutions1.pdf
http://www.mathworks.com/help/images/examples/deblurring-images-using-the-blind-deconvolution-algorithm.html

Physical limits and methods to overcome





Via deconvolution artefacts can be computed out of fluorescence images.

a) These artefacts are caused by the stray light from non-focused areas above and below the focus level. These phenomena, referred to as convolution, result in glare (螢光訊號過亮), distortion and blurriness (模糊).

b) Deconvolution is a recognised mathematical procedure for eliminating such artefacts. The resulting image displayed is sharper with less noise and thus at higher resolution. This is also advantageous for more extensive analyses.

http://www.mathworks.com/help/images/ref/deconvlucy.html

Thanks For Your Attention