臺北醫學大學

Biomedical Imaging

生物醫學影像學

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牙體技術學系

2013/03/11

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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

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.

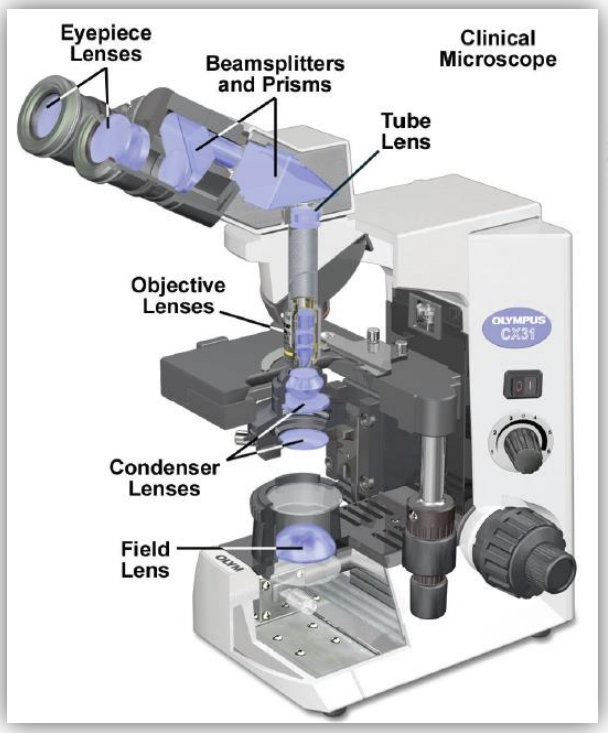
2) Light ray path produces focused images of the lamp filament at the plane of the condenser aperture, back focal plane of the specimen and at the eye point of the eyepiece.

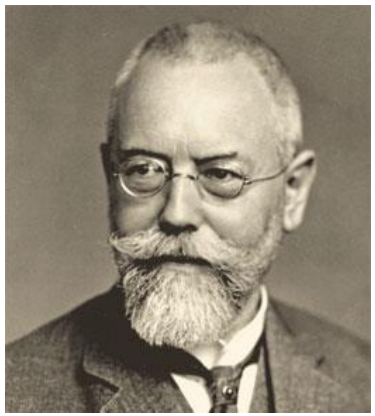
3) These planes called conjugated planes.

4) Provides an evenly illuminated field of view with a bright image, without glare (刺眼) and minimum heating of the specimen.

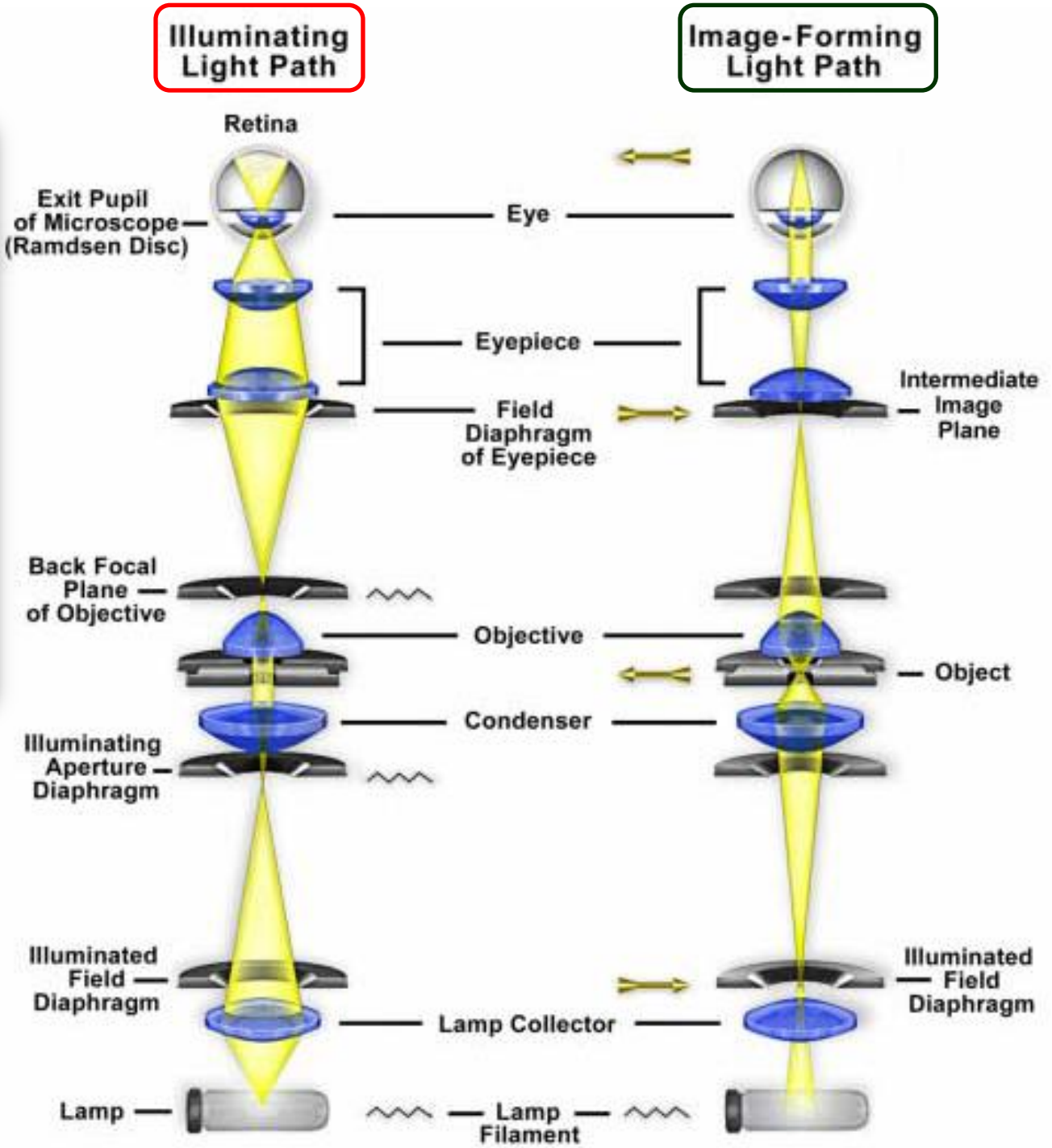
5) Very common in transmission microscopes.

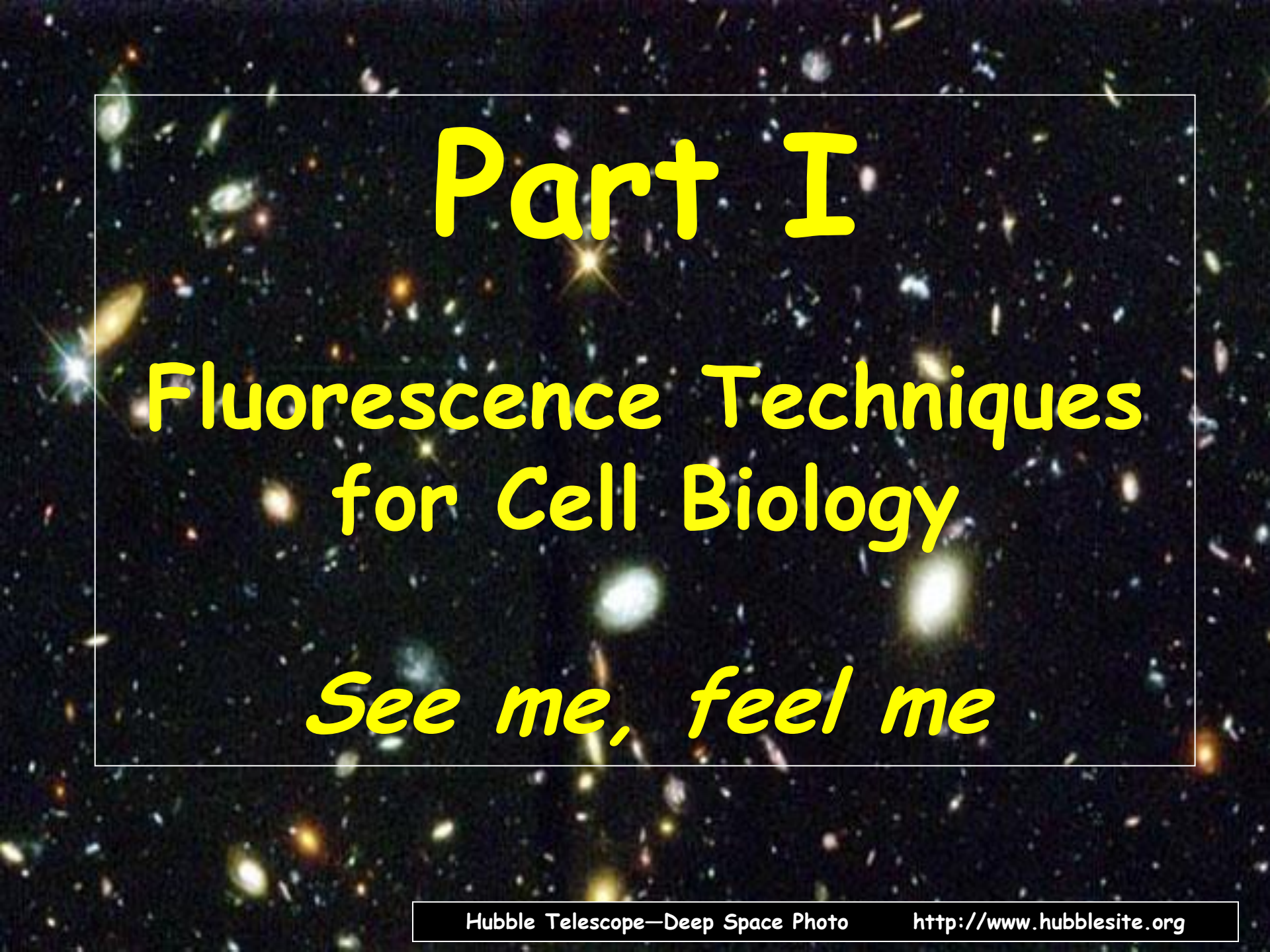
正立式





August Koehler





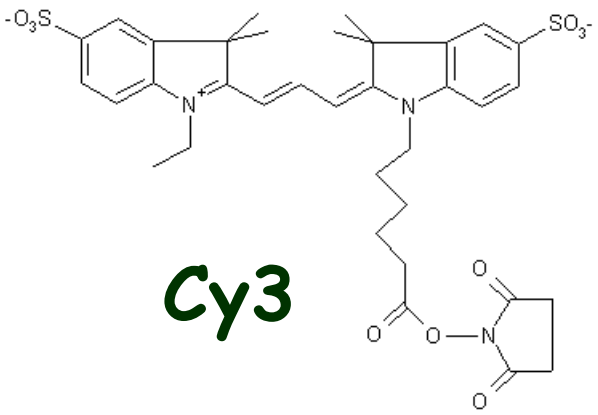
Part I

Fluorescence Techniques for Cell Biology

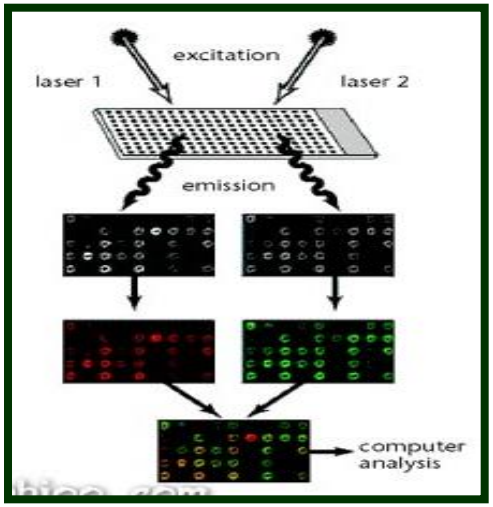
See me, feel me

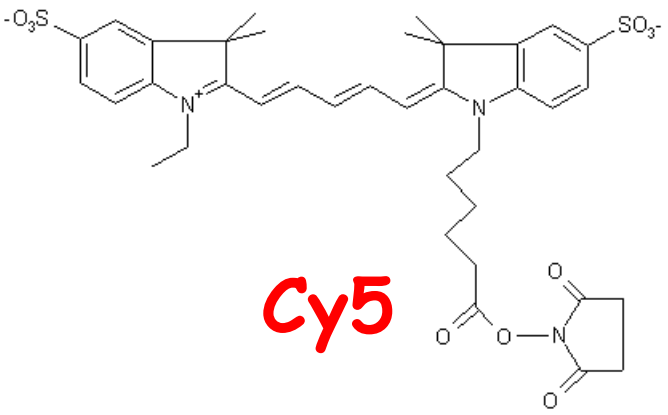
Hubble Telescope—Deep Space Photo http://www.hubblesite.org

I. Organic Dye
Excitation and Emission Spectra

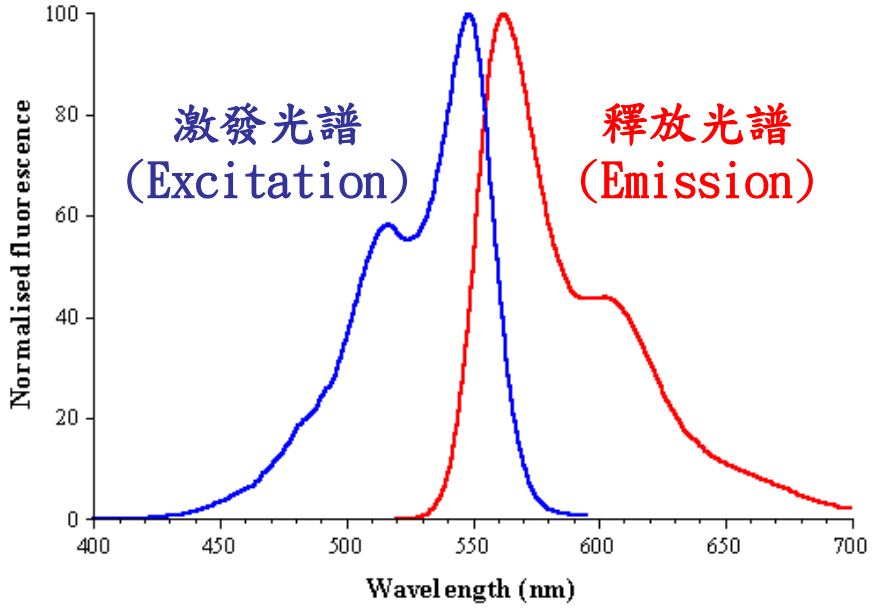


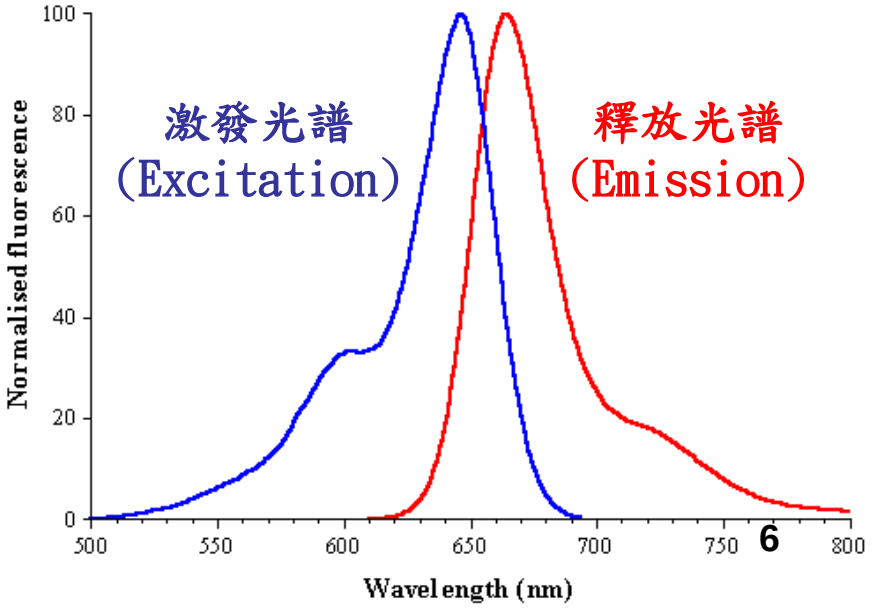
Cy3





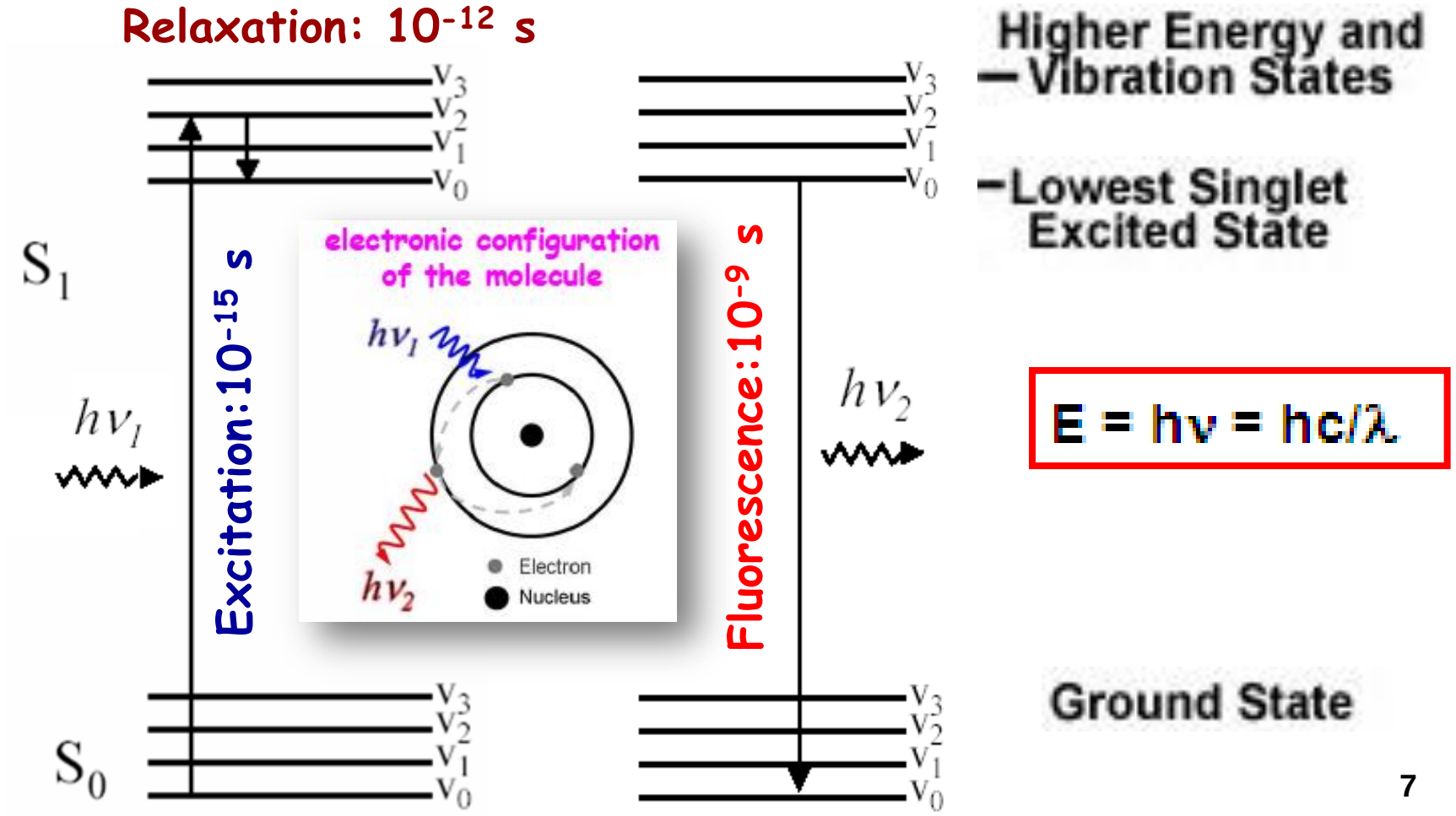
Cy5





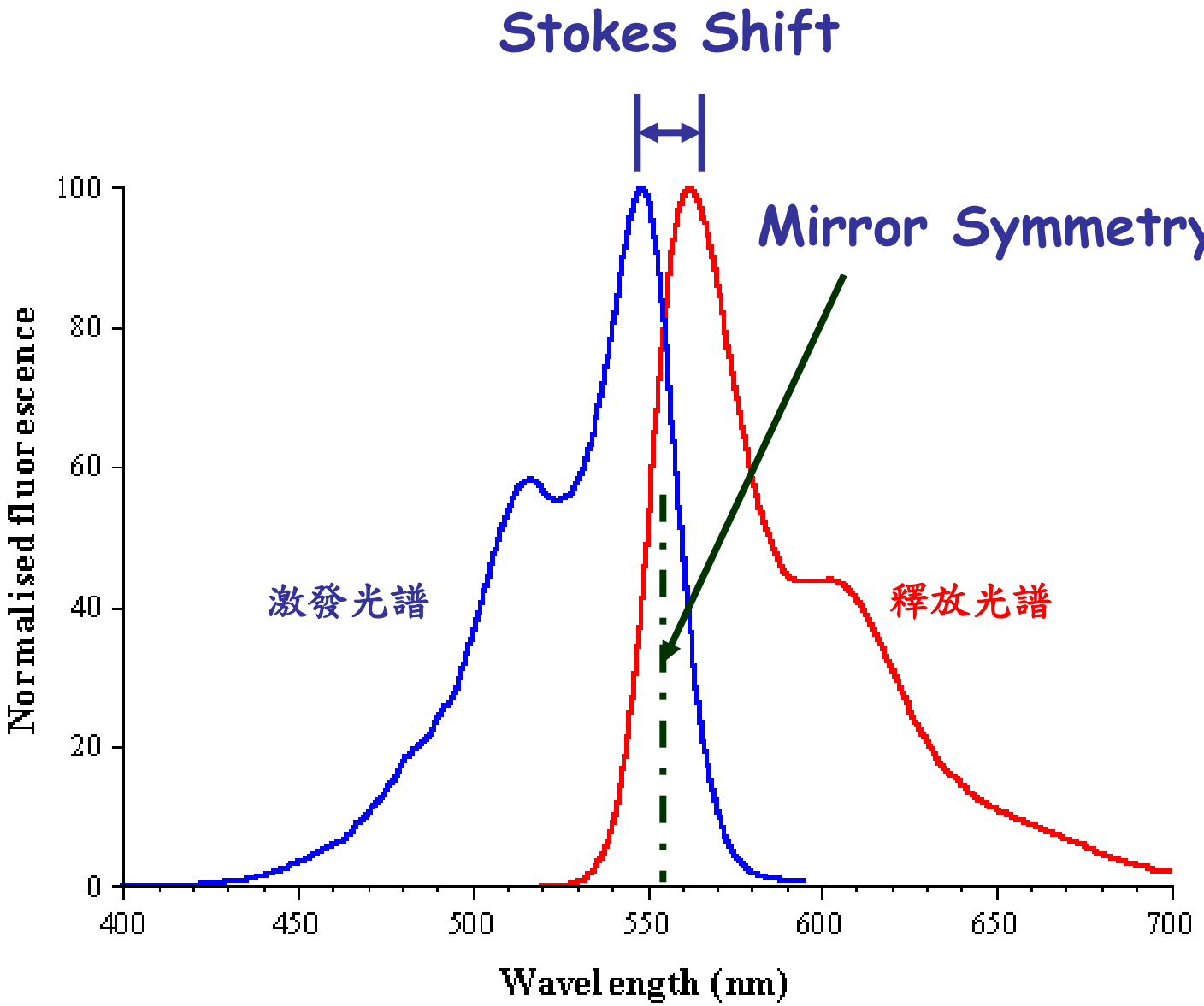
Fluorescence Energy-Level Diagram

Jablonski Energy Diagram for fluorescent organic dyes

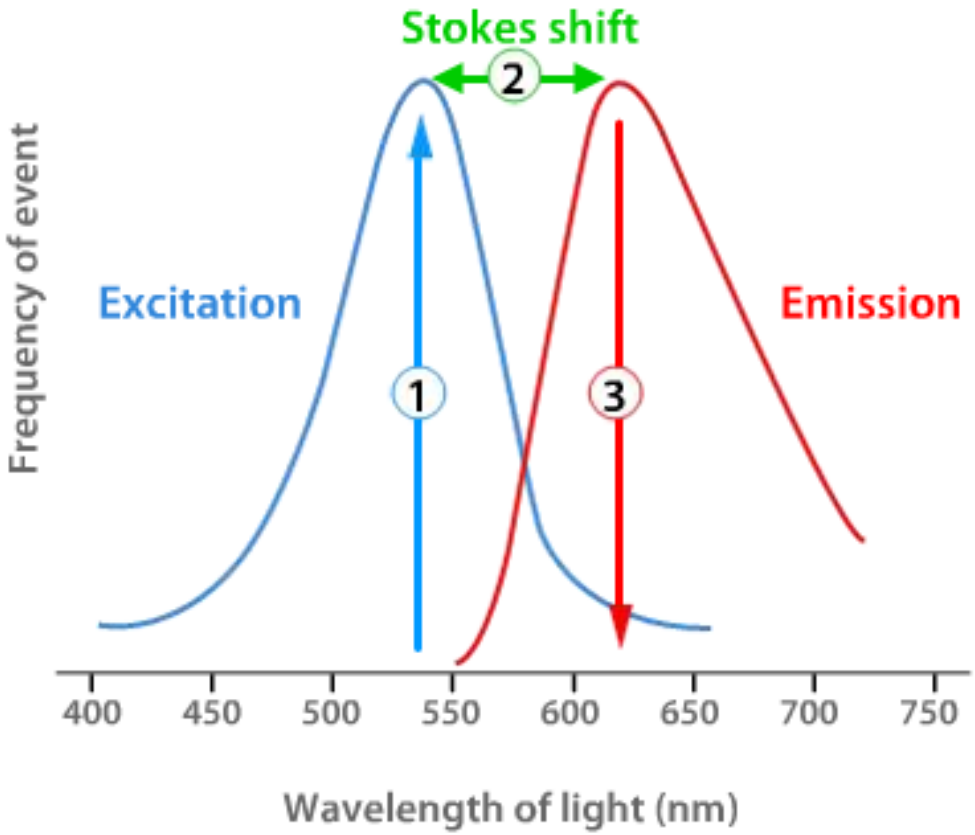


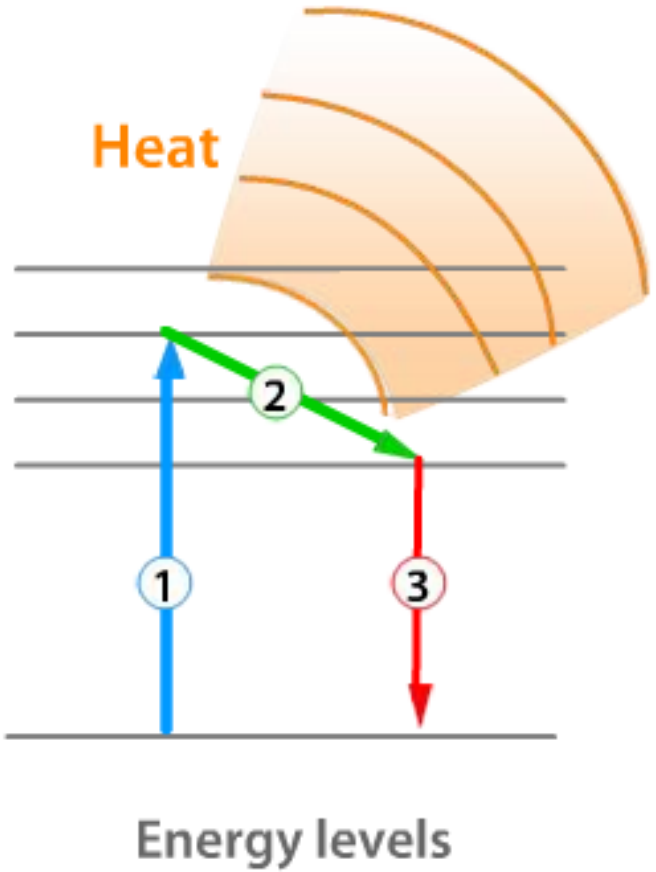
http://www.olympusmicro.com/primer/java/jablonski/jabintro/

Stokes Shift and Mirror Symmetry



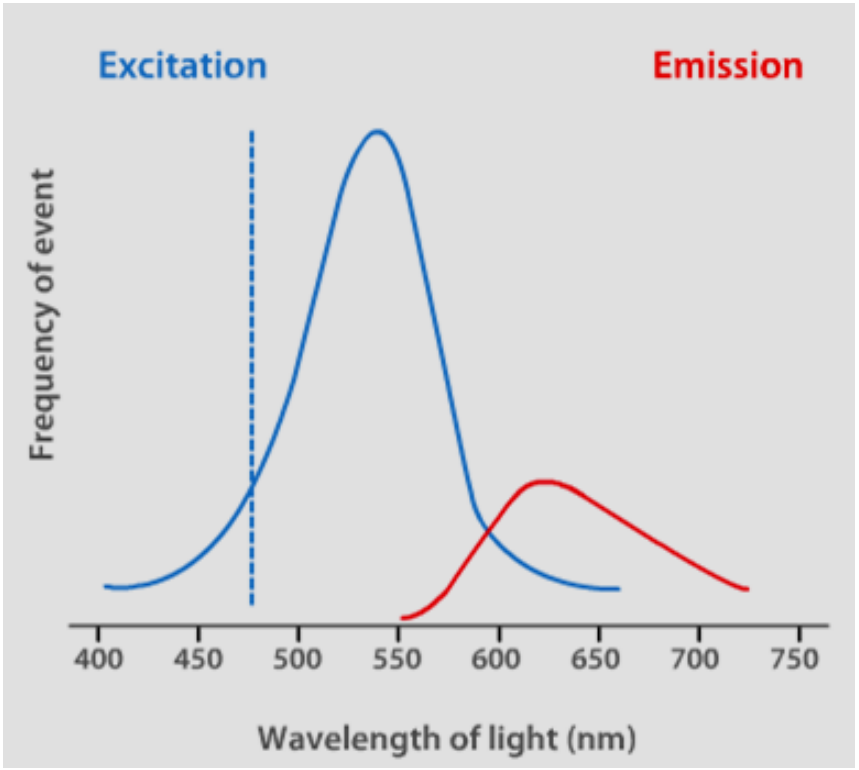
Stokes Shift Explained

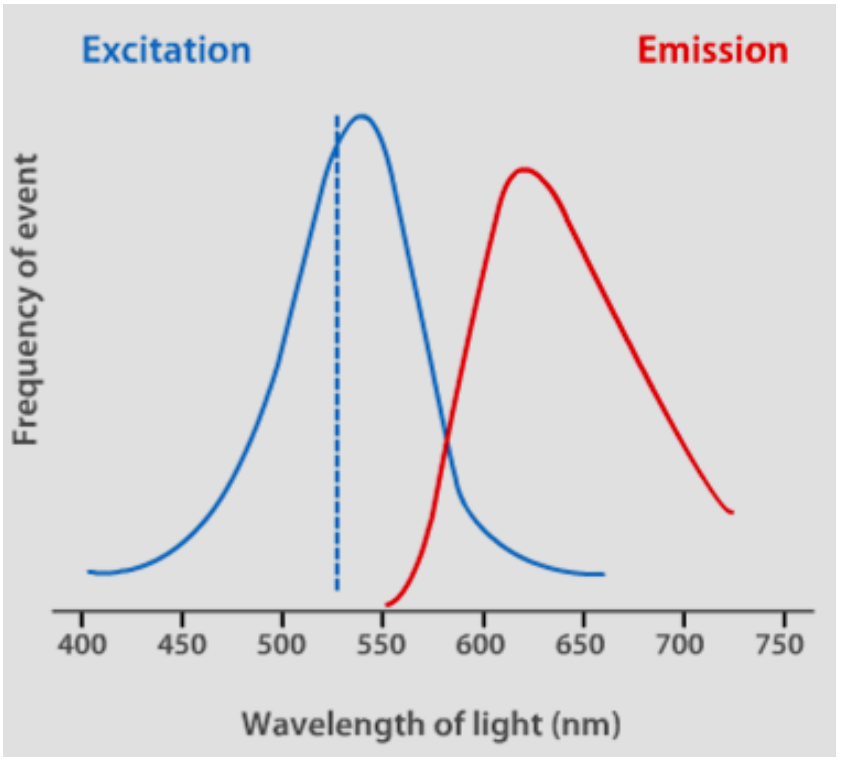




http://www.invitrogen.com/site/us/en/home/support/Tutorials.html

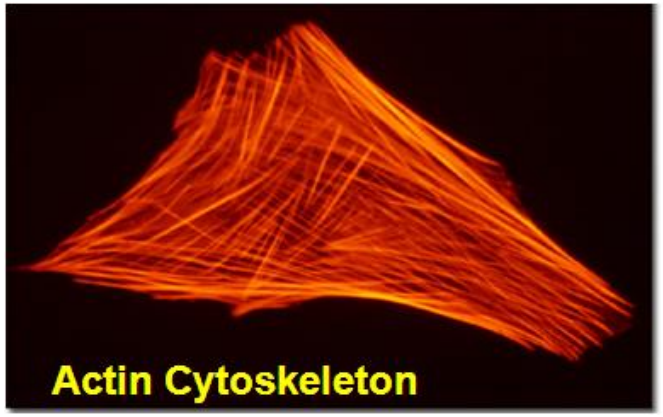
Excitation and Emission Spectra of Organic Dyes





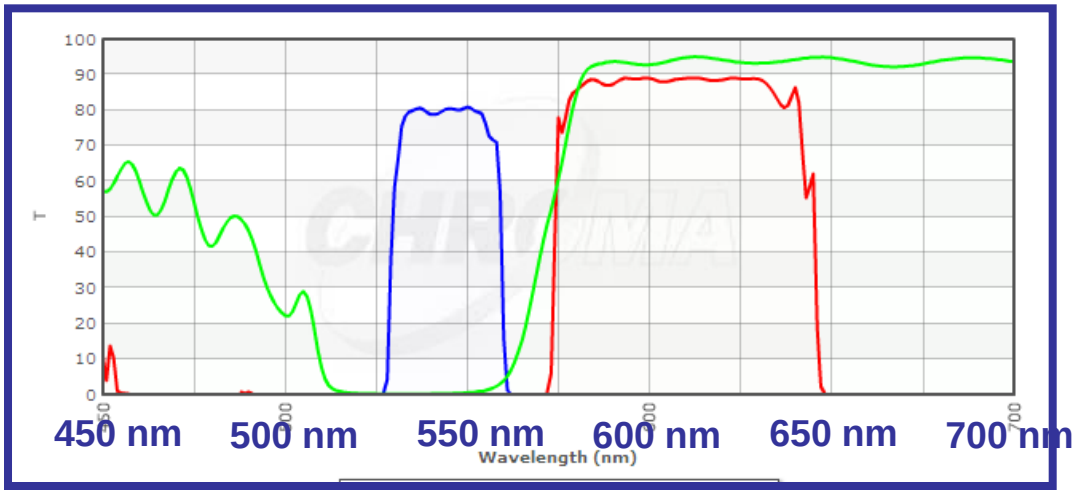
http://probes.invitrogen.com/resources/education/tutorials/2Spectra/player.html

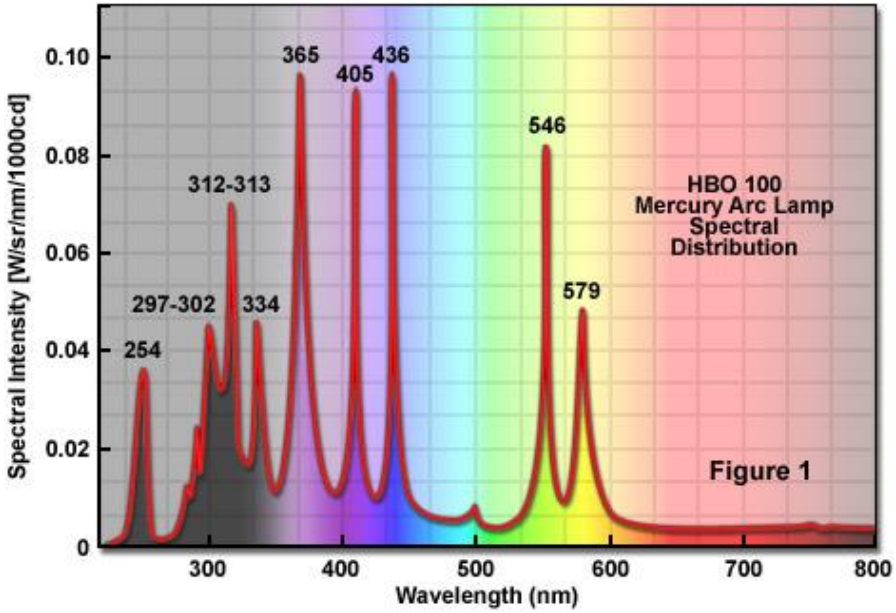
Fluorescence Microscope Equipped with Highly Specific Fluorescence Filter Sets

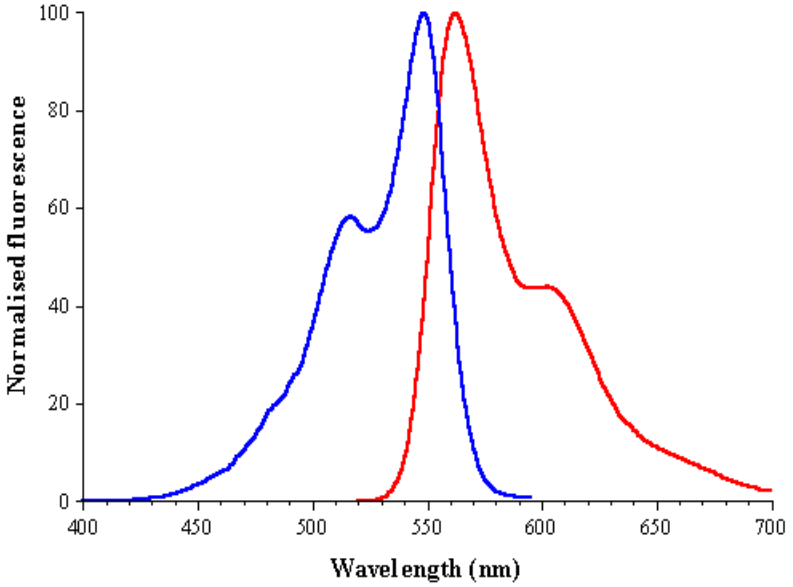


http://www.microscopyu.com/articles/fluorescence/filtercubes/green/cy3/cy3muntjacactinlarge.html

This is the recommended Cy3™ filter set

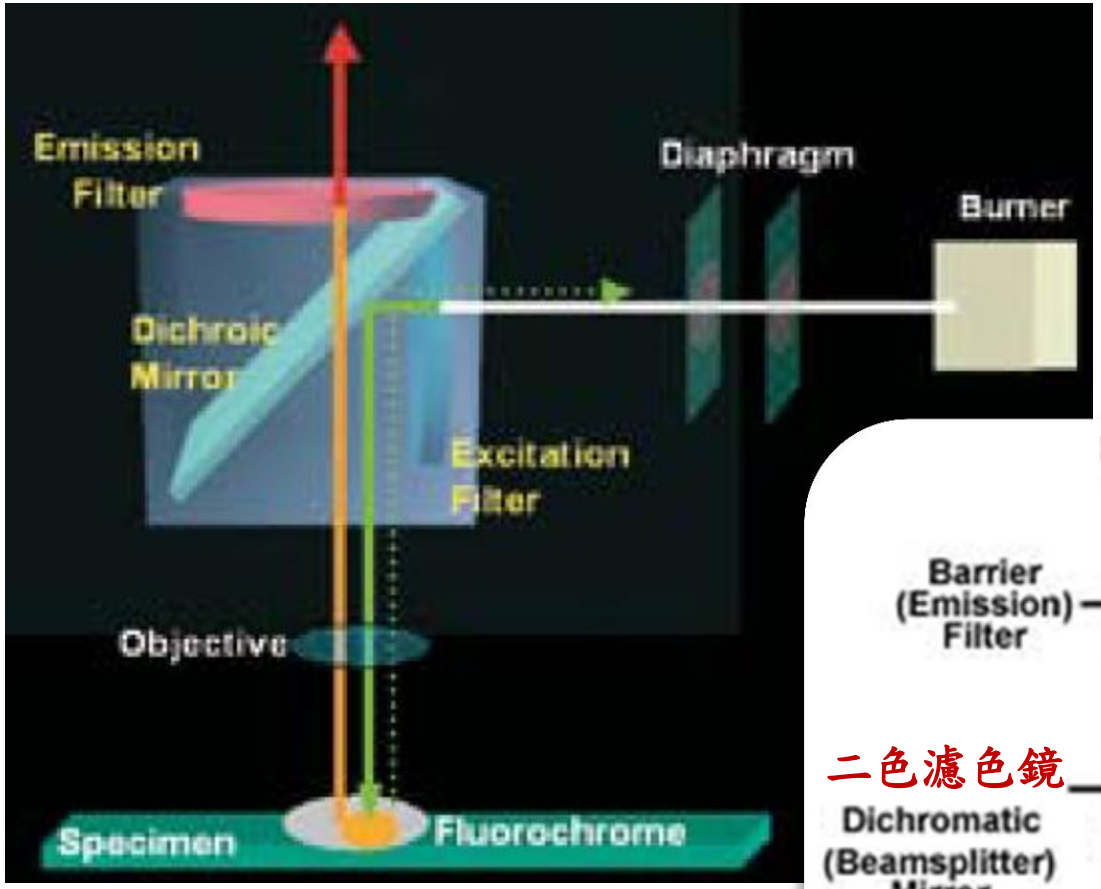


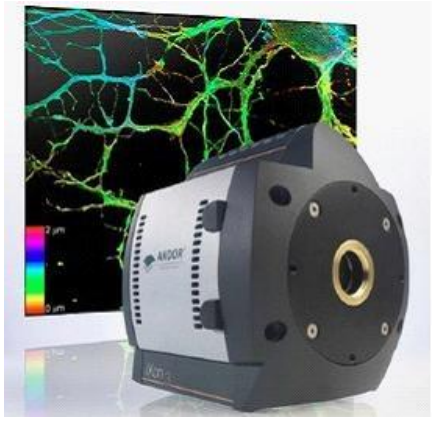


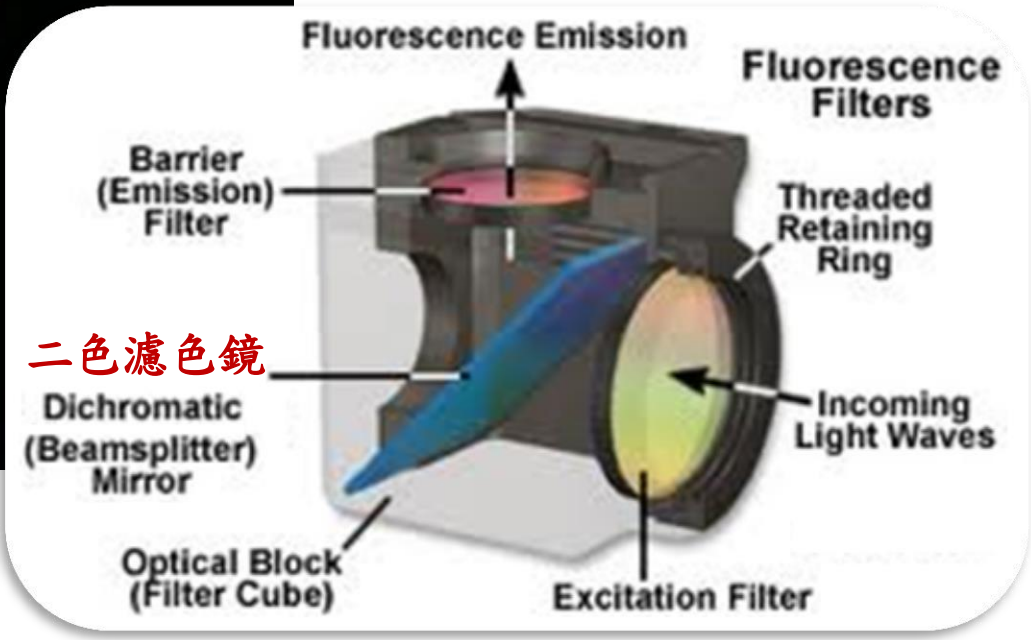


Light Path on a Microscope Equipped for Fluorescence

CCD camera





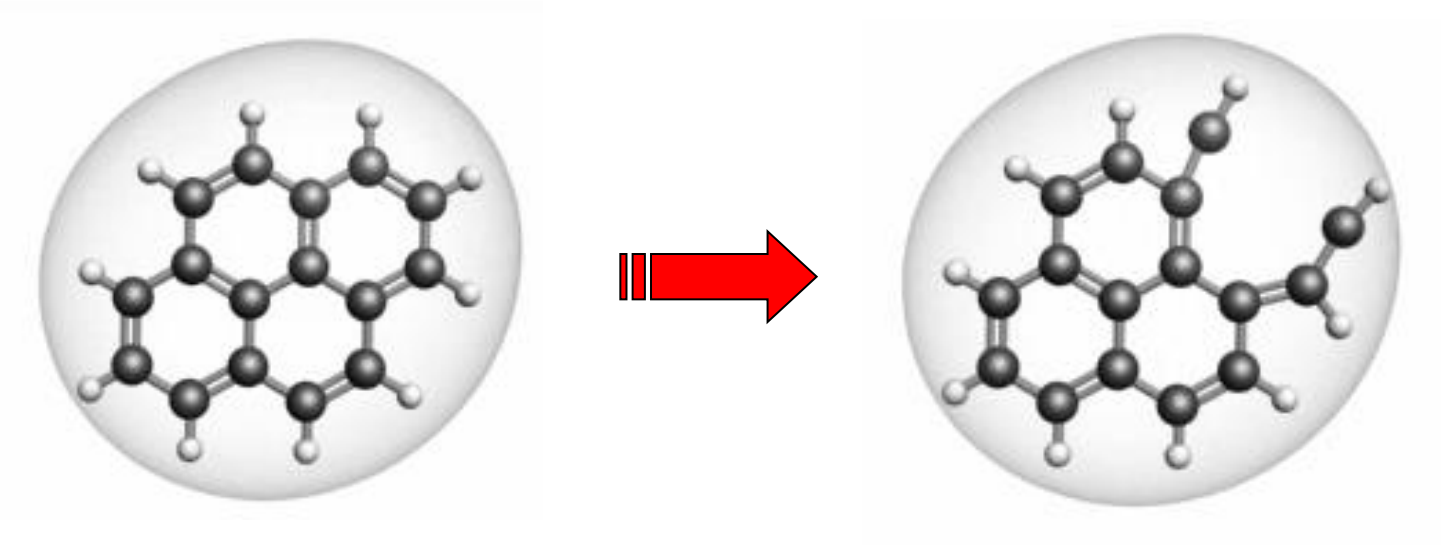


Photobleaching (光漂白)

photostability

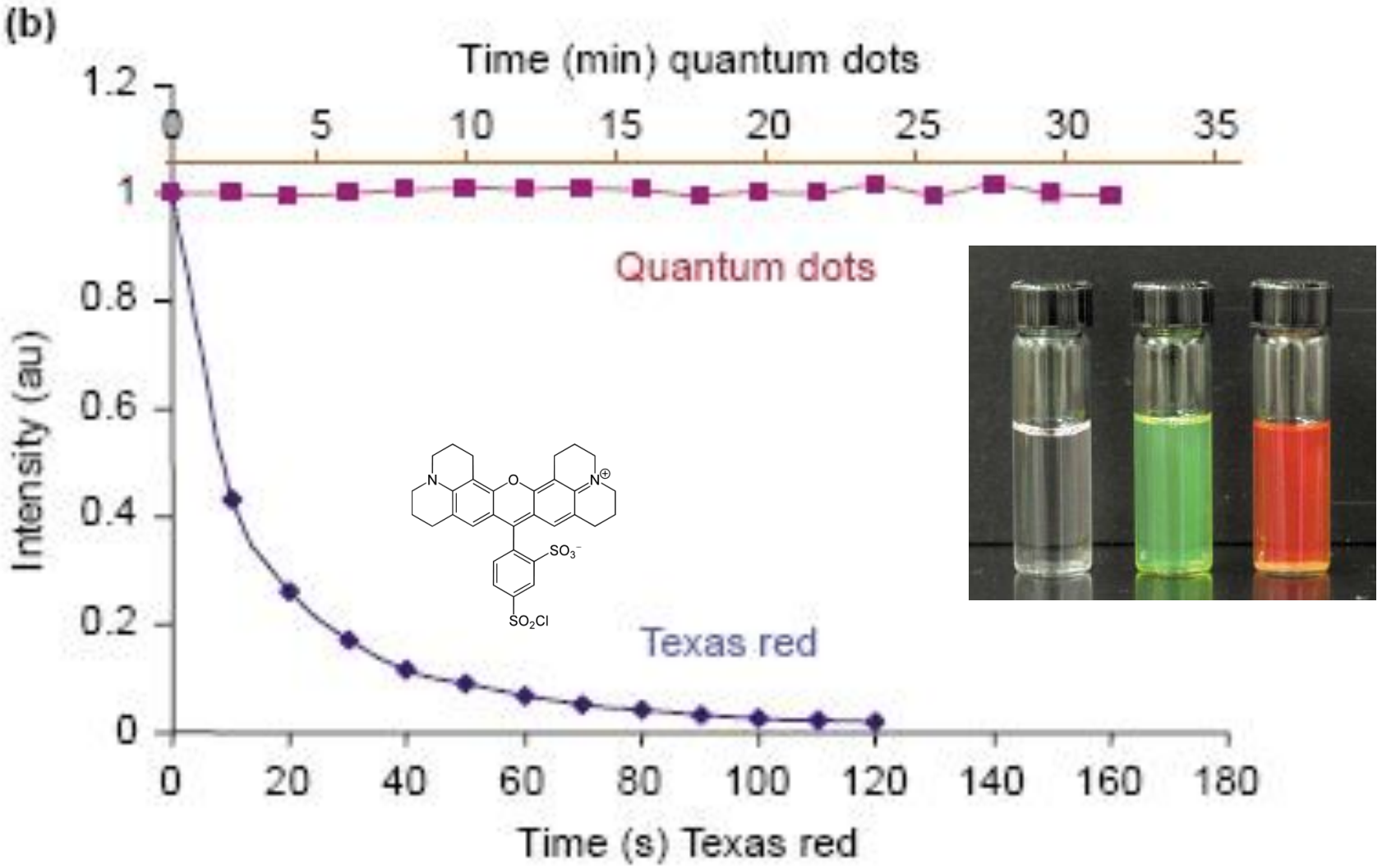
cycling of fluorescence

photobleaching



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

Photostability



Current Opinion in Biotechnology 2005, 16:63–72

How to Use The Modern Single-Molecule Toolkit

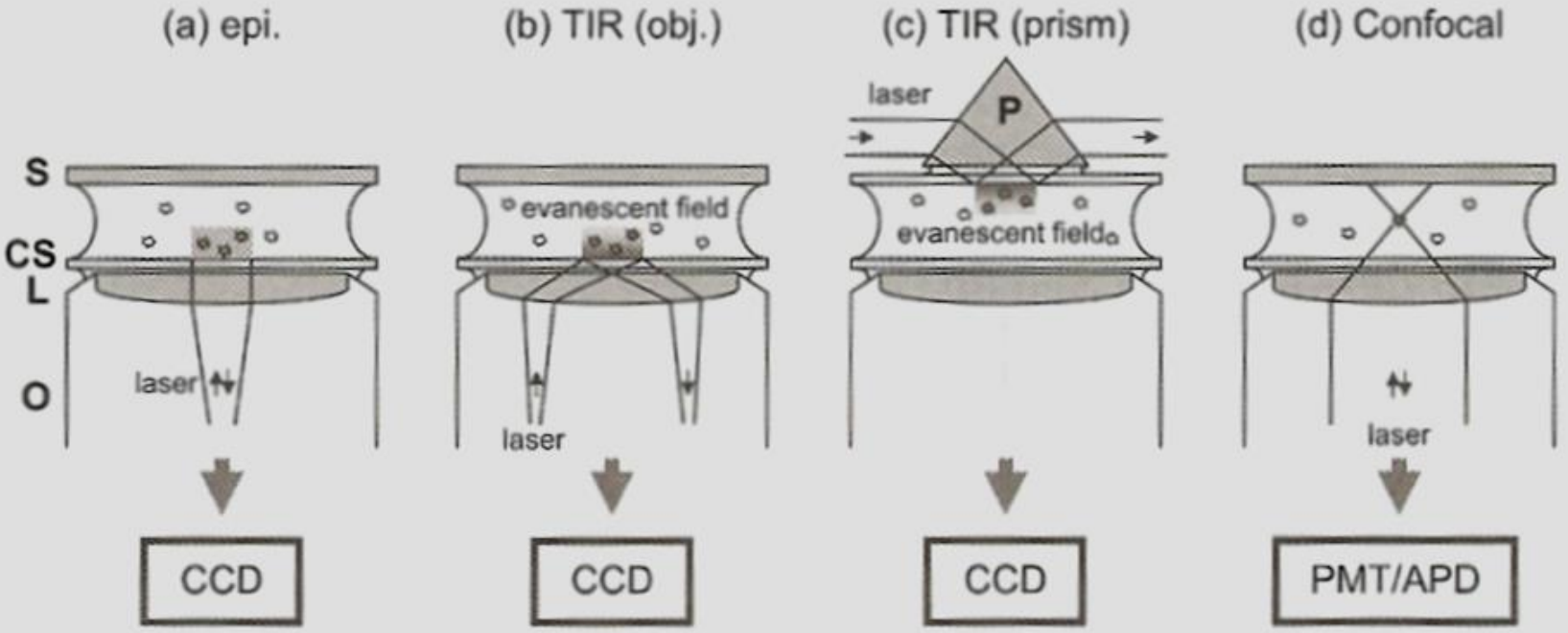


Figure 3.4 Different excitation/detection geometries utilized in single-molecule microscopy: (**a**) epifluorescence, (**b**) through-the-objective TIR, (**c**) prism TIR, and (**d**) confocal microscopy (laser scanning beam or scanning stage). S: slide; CS: coverslip; L: lens; O: objective, typically high NA oil-immersion (>1.4 for TIR); P: prism. Area (CCD) or point (PMT or APD) detectors are shown. The filled and empty circles represent fluorescent molecules that are visible and invisible, respectively. Figure is not to scale. (Adapted from Moerner (2003) and Kellermayer (2005))

Select a suitable single-molecule technique to study a given biological problem

LSCM

Epifluorescence

TIRFM

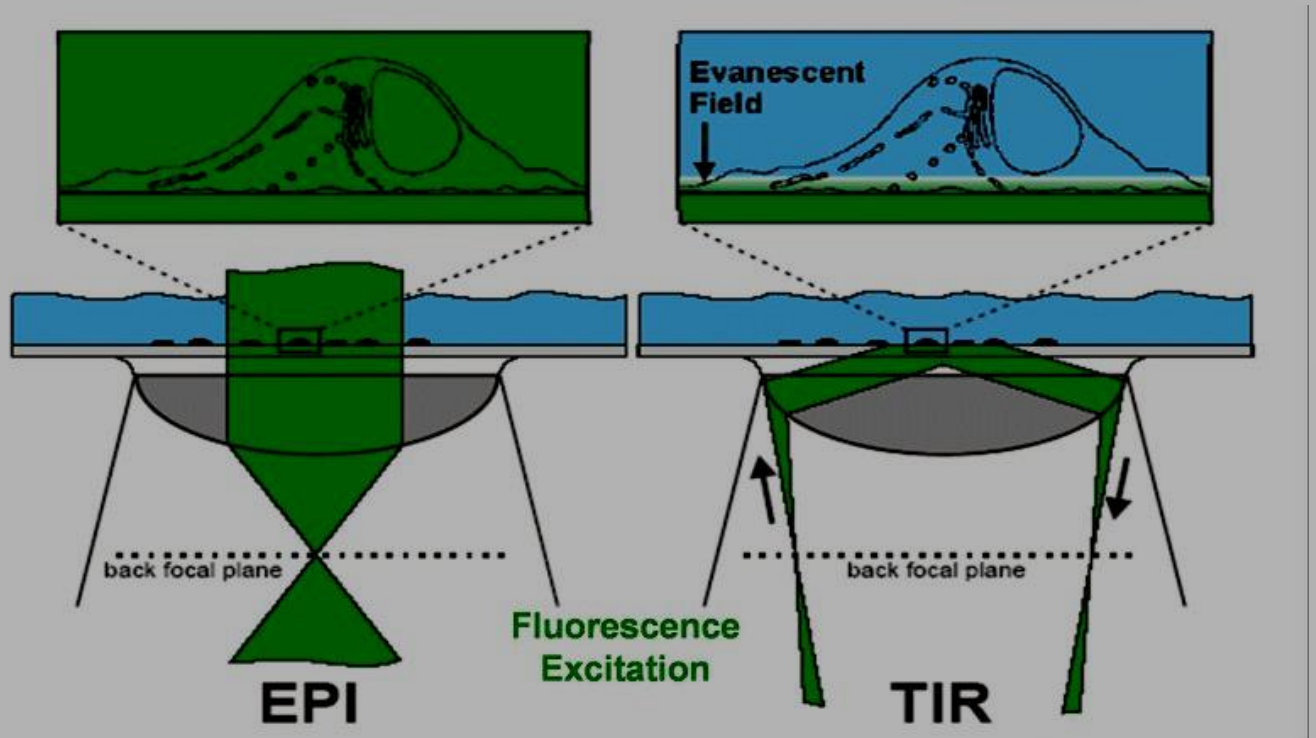
Good for measuring concentration, location, shape, intermediate to slow dynamics

Sample requirement: surface containment (particularly TIRFM)

雷射共軛焦顯微鏡（Laser Scanning Confocal Microscope；LSCM）

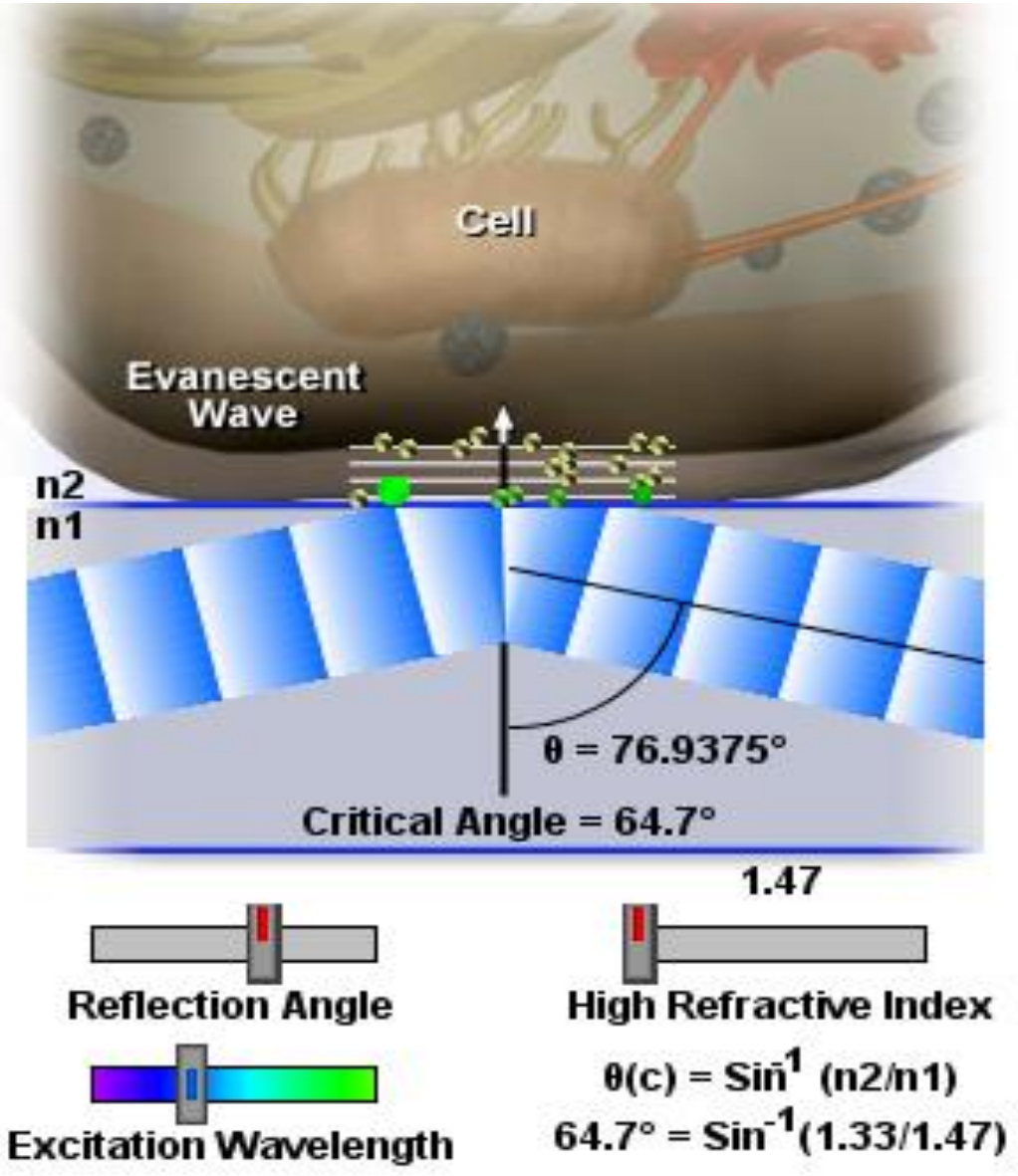
落射螢光顯微鏡（Epi-fluorescence Microscopy；EFM）

全內反射螢光顯微鏡（Total Internal Reflection Fluorescence Microscope；TIRFM）



http://www.umich.edu/~rnapeopl/Walter_group_NatureMethods2008.pdf

Total Internal Reflection Fluorescence Microscope



http://www.olympusmicro.com/primer/java/tirf/reflect/index.html

Evanescent Waves in Microscopy

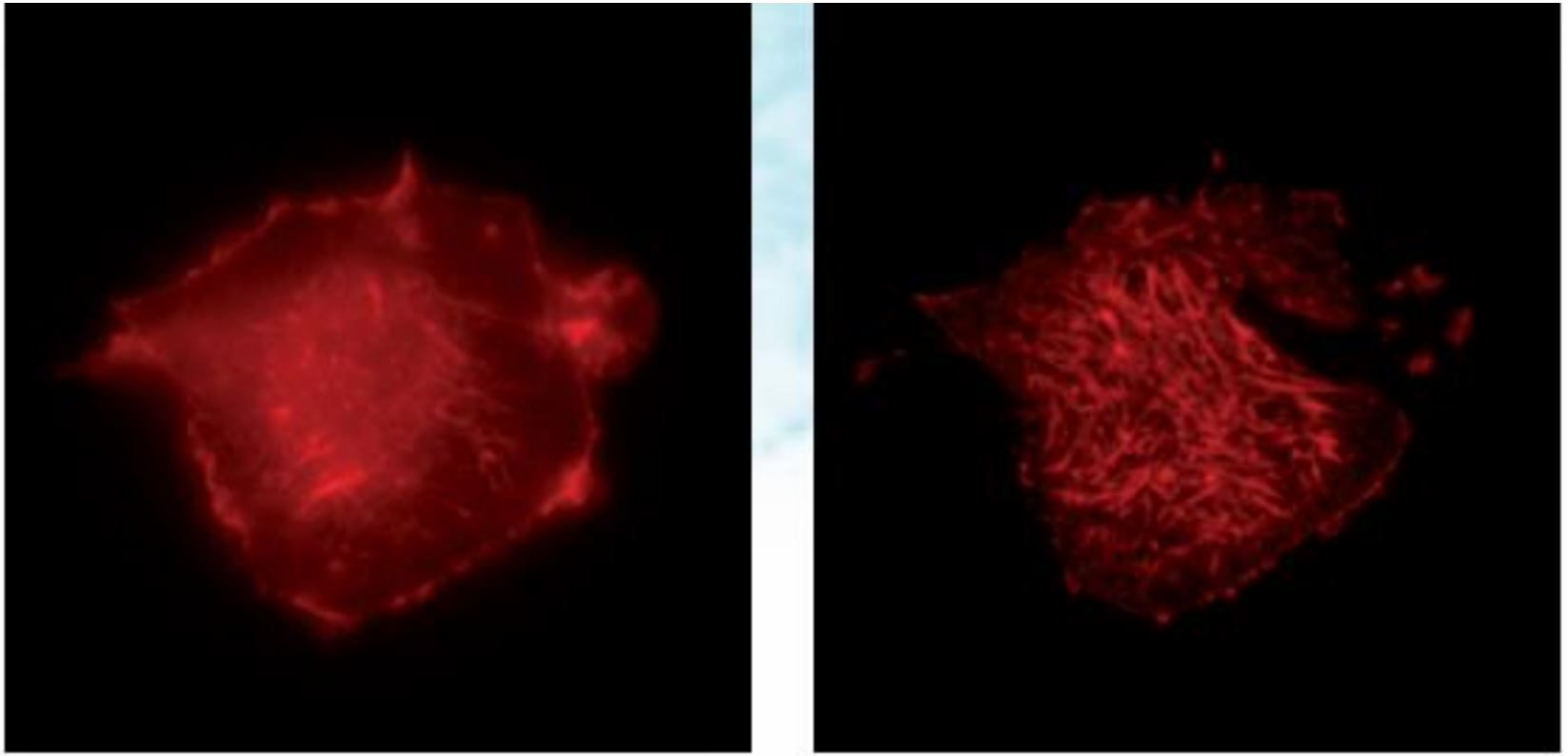


***Figure 1**: Phalloidin f-Actin protein coupled with Alexa (546), actin filaments, Cytoskeleton within the range of the Submembrane cortex in cattle cells, left in Epifluorescence, right in TIRF (Dr. J. Klingauf, MPI for biophysical chemistry, Goettingen)*

www.zeiss.co.kr/.../photonik_2004_02_42_e1.pdf

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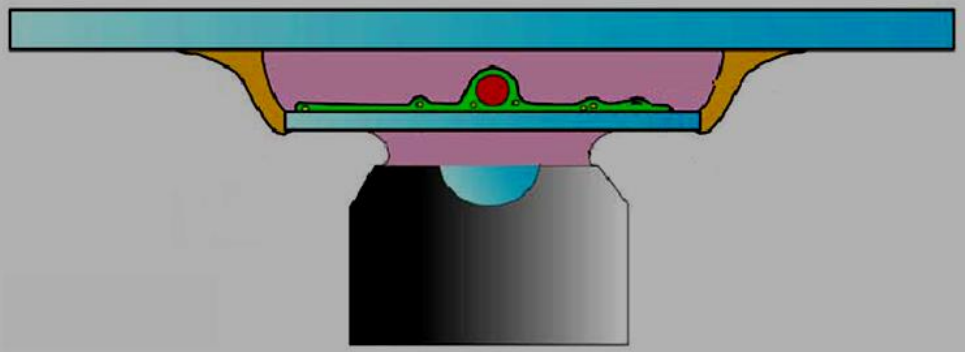
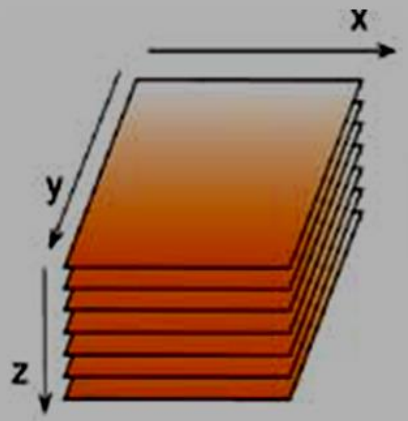
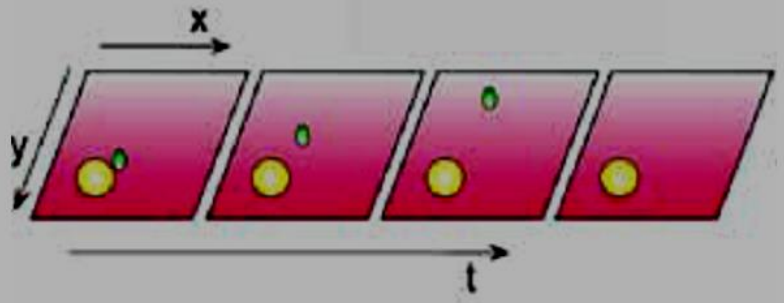






http://www.umich.edu/~rnapeopl/Walter_group_NatureMethods2008.pdf

The Confocal Principle

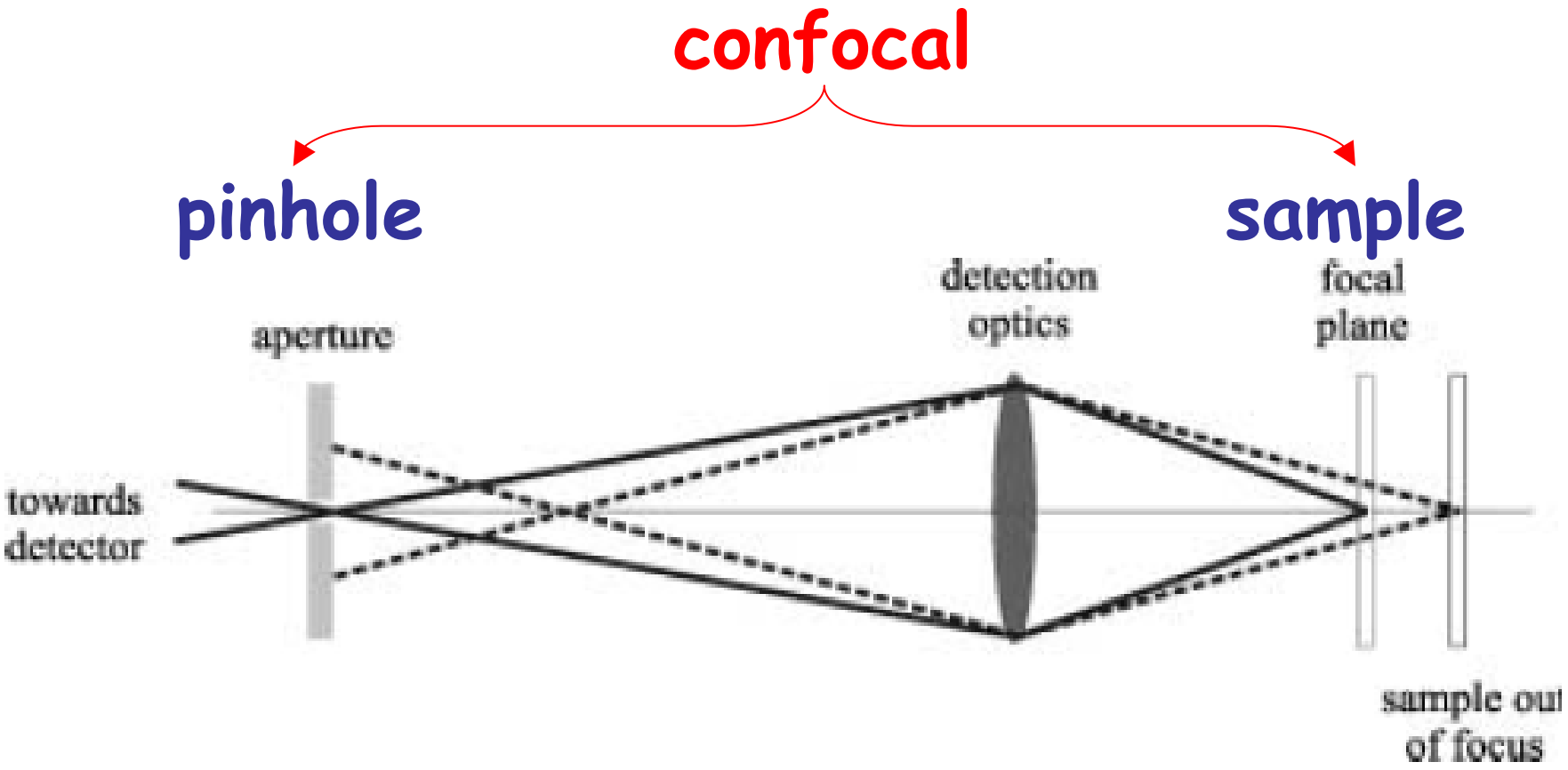
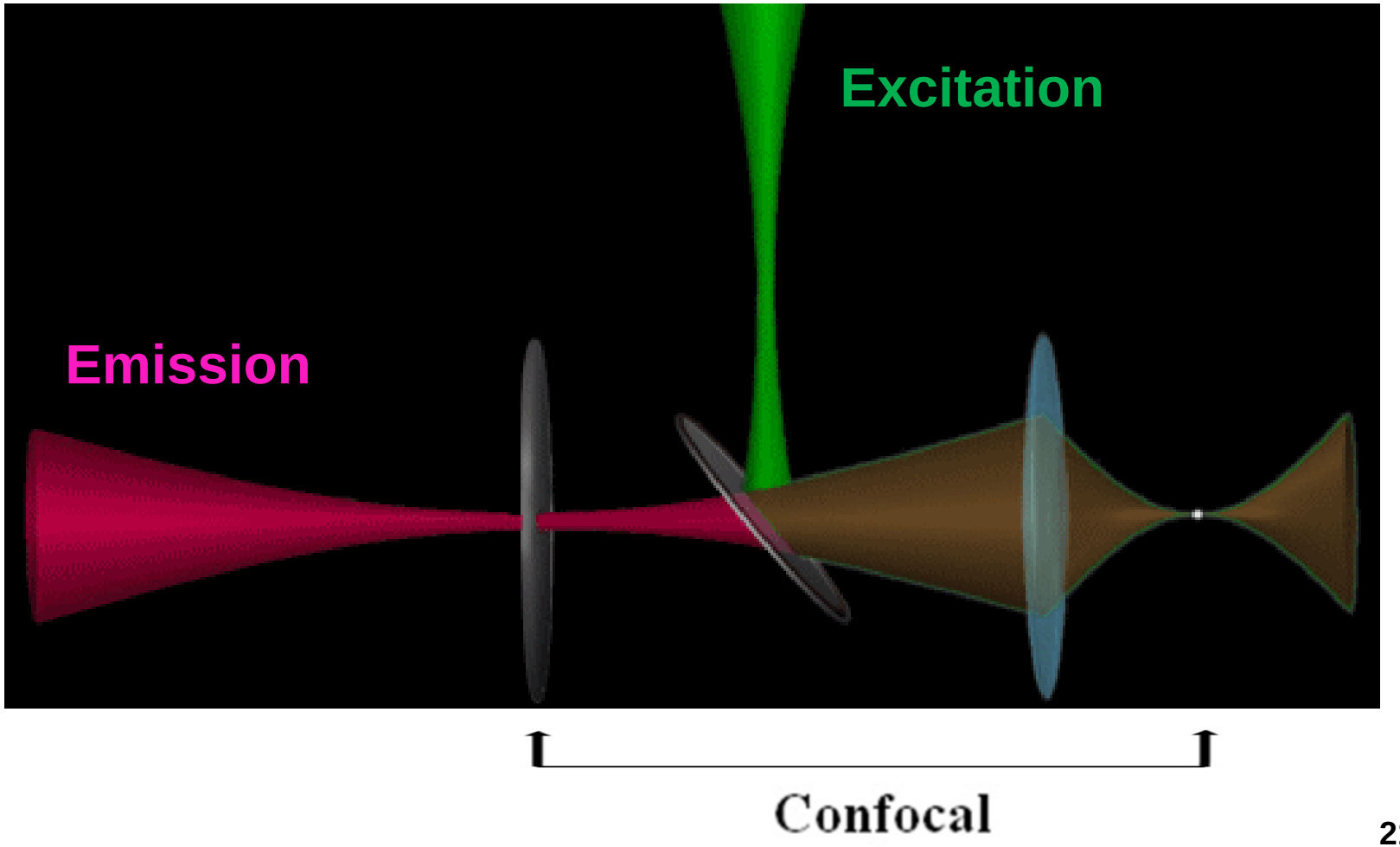


Figure 4. Schematic of confocal detection: Only light emerging near the optical axis and the focal plane is focused onto the opening of the aperture that is positioned at the conjugate focal plane of the imaging optics. Light that comes from points off the optical axis or the focal planes is mostly blocked by the aperture.

CHEMPHYSCHEM **2003**, 4, 792 – 808

The Confocal Principle

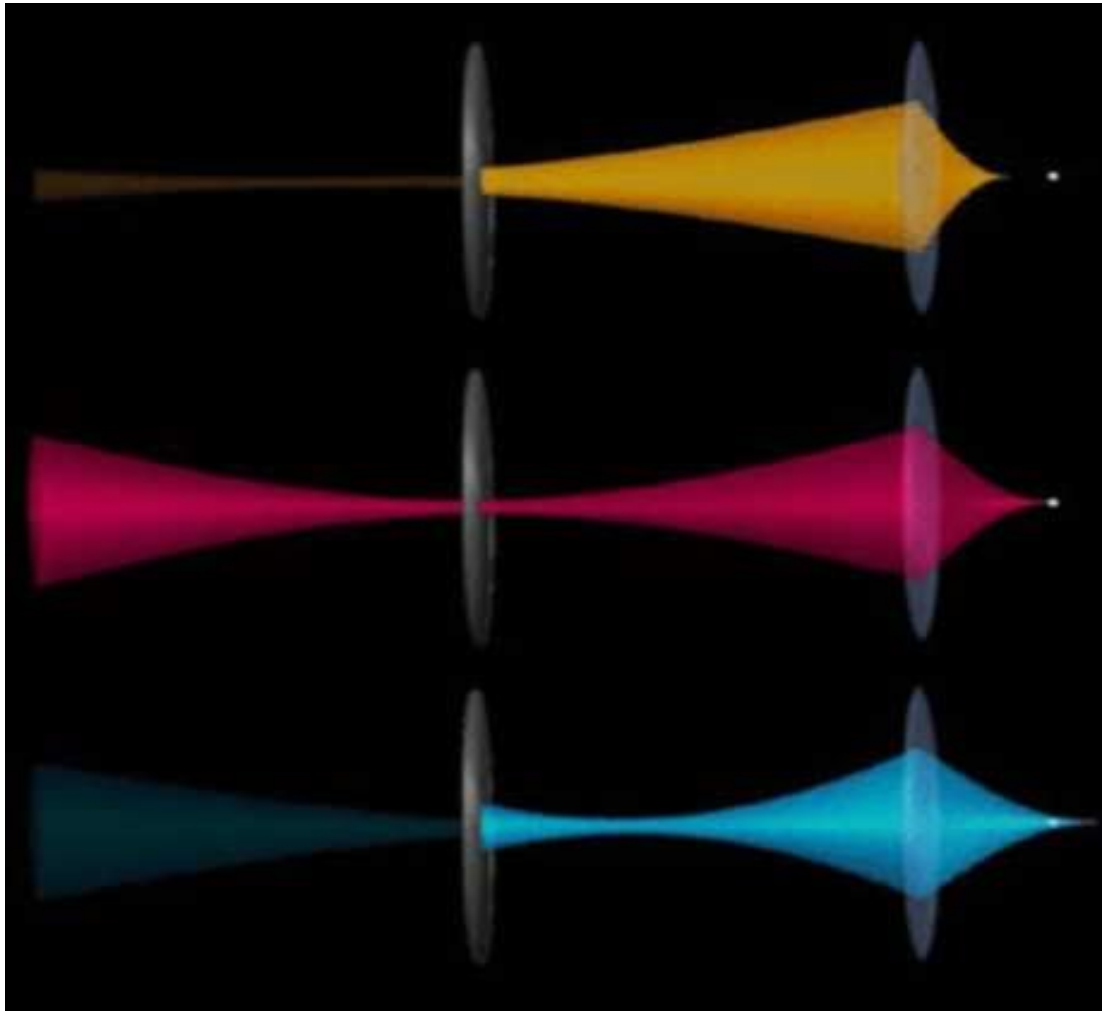
Confocal Illumination & Signal Collection



http://www.fcsxpert.com/classroom/theory/what-is-confocal-volume.html

The Confocal Principle

Efficient Signal Collection from the Object Plane



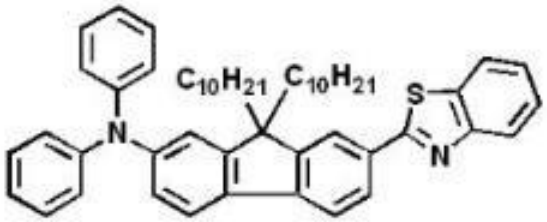
The white dot indicates the object plane. Light emitted from this plane (center diagram) is efficiently collected by the detector. Light from below (left diagram) or above (right diagram) is poorly collected.

One vs Two-photon Excitation

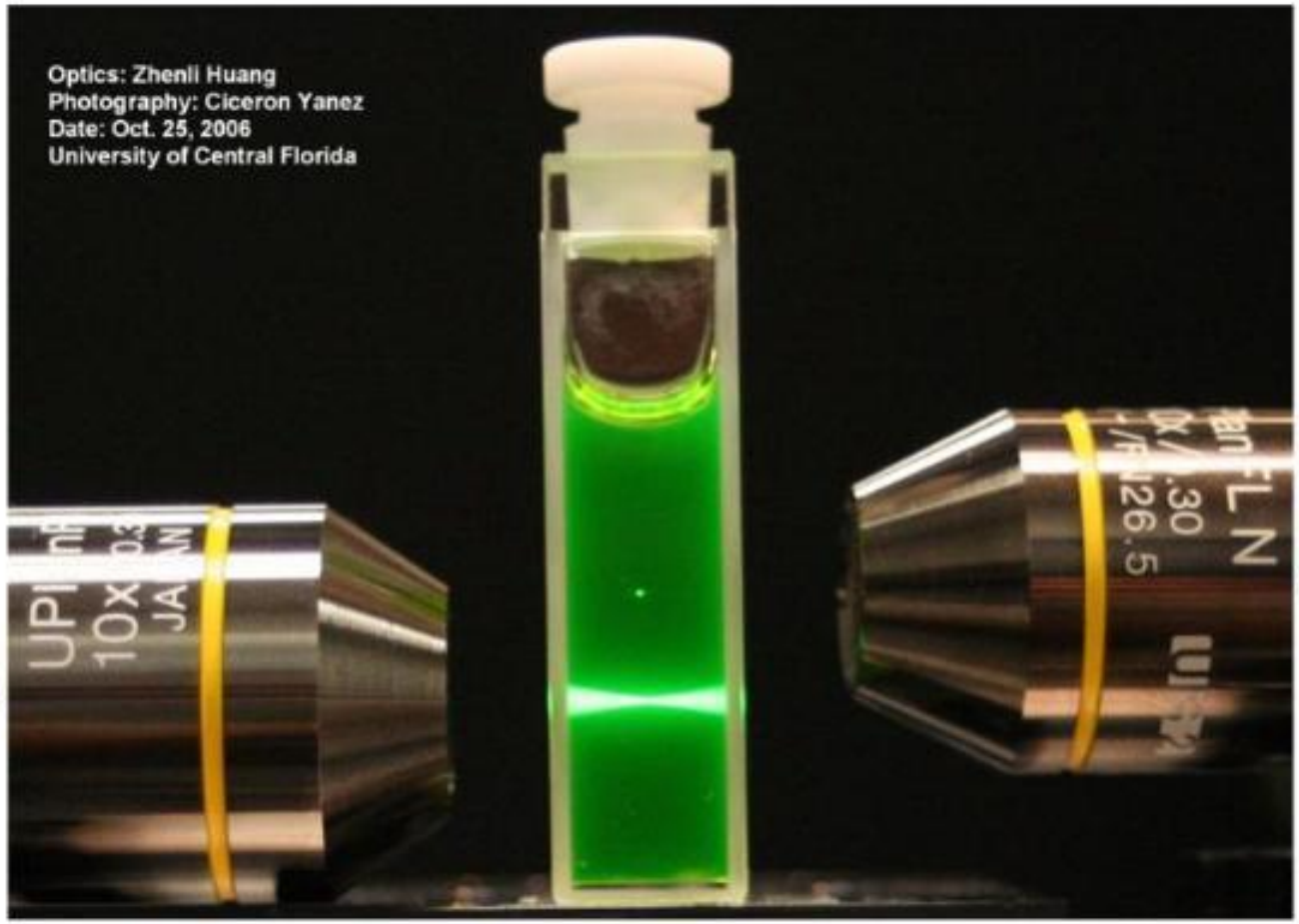
380nm,200fs

760nm,200fs

Fluorene 3



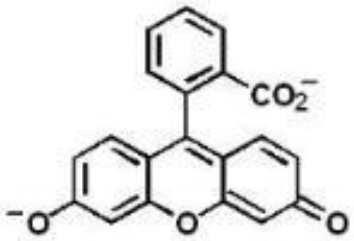
One vs Two-photon Excitation



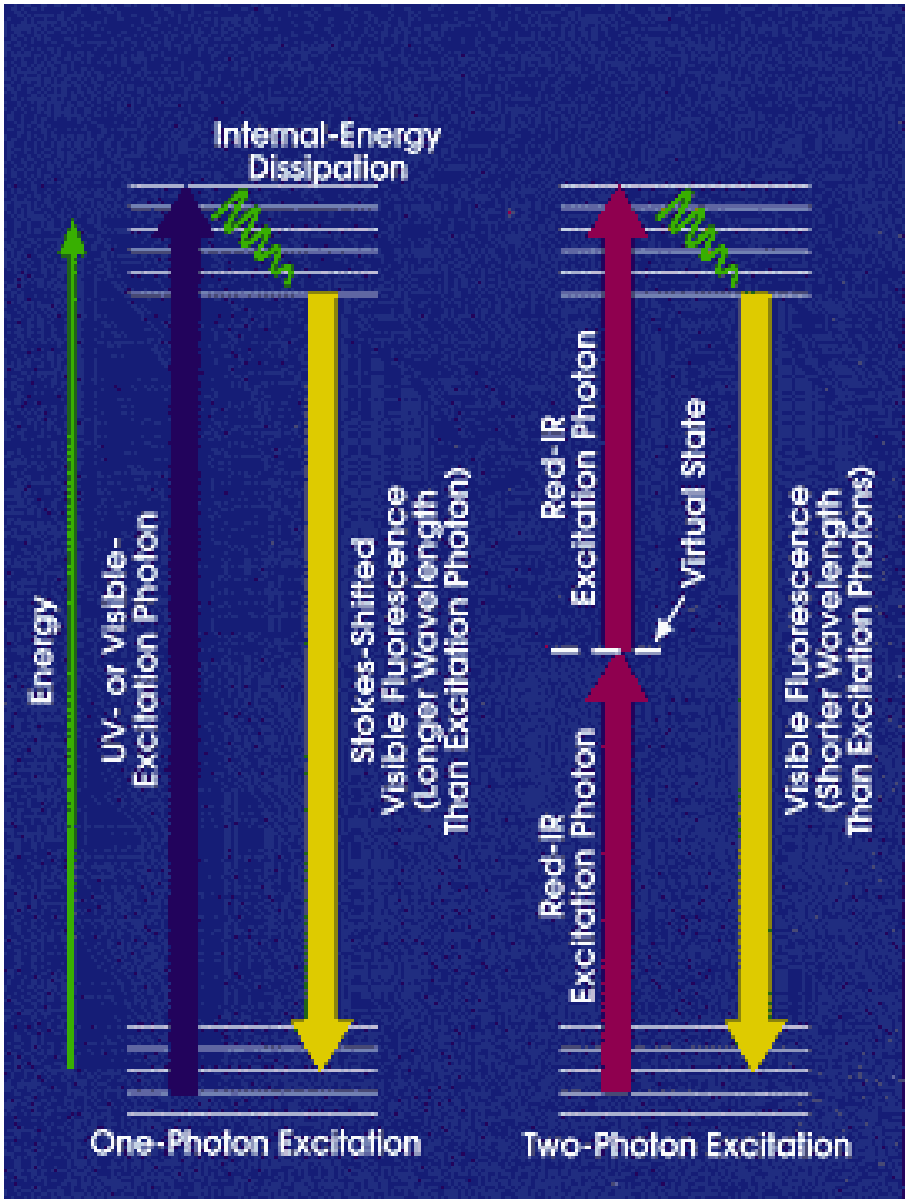
380nm,200fs

760nm,200fs

Fluorescein

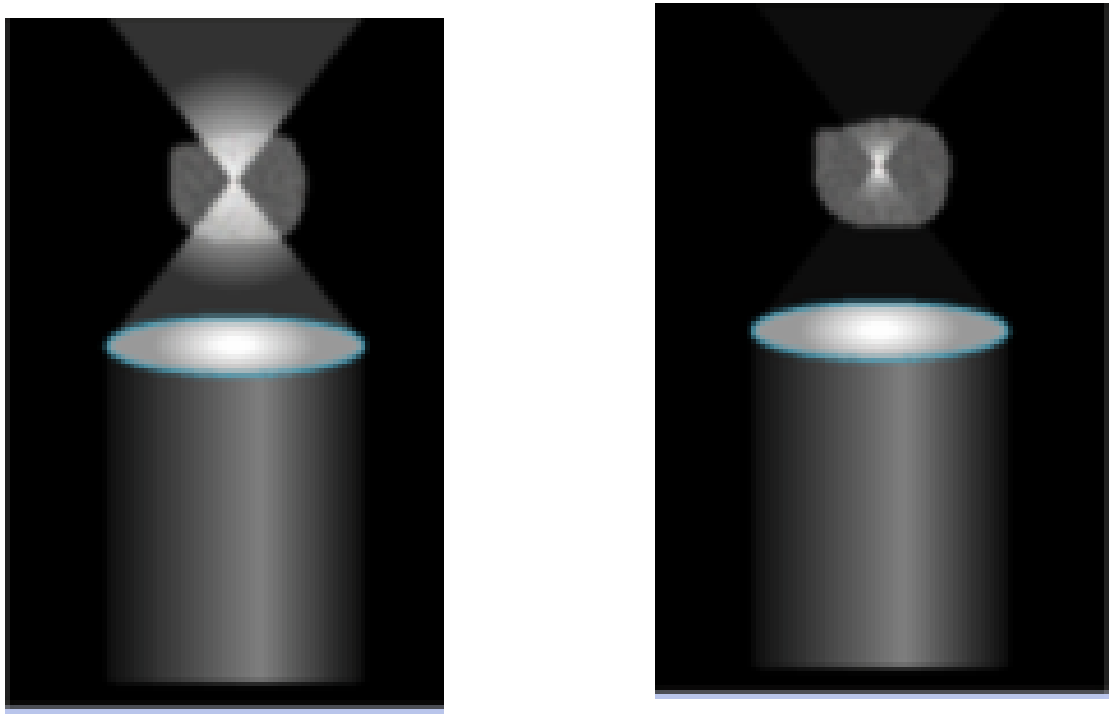


One vs Two-photon Excitation

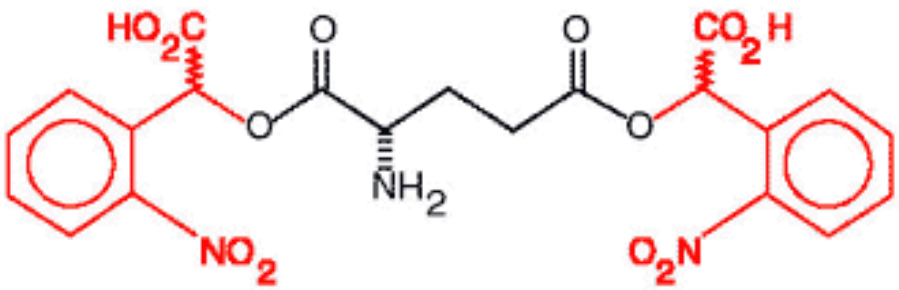


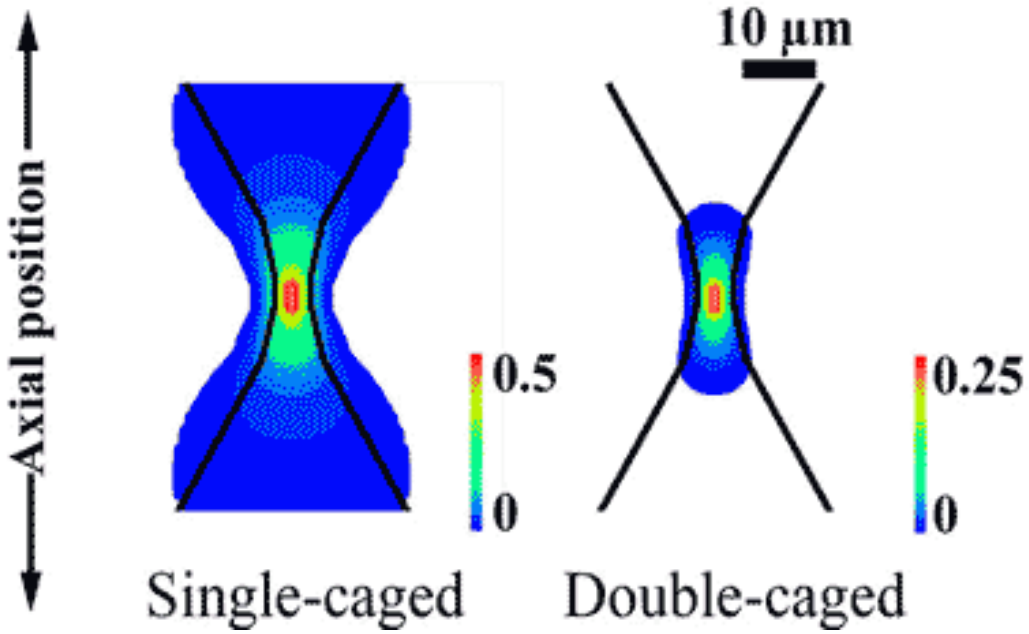
Advantages of Two-photon Excitation

Reduce photobleaching



In TPE fluorescence processes (right-hand figure) the bleaching is restricted to a small regions surrounding the focus.



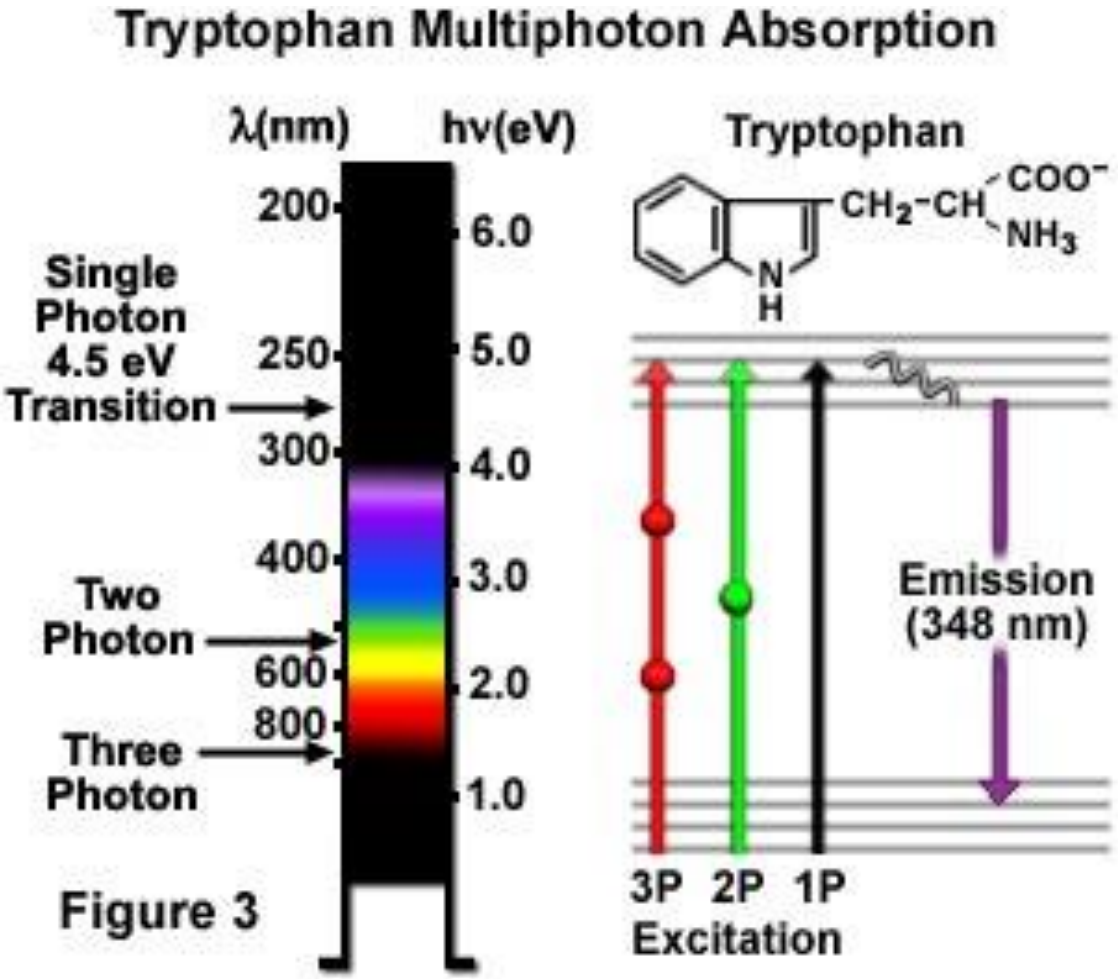


Chemical two-photon uncaging: the use of specially designed compounds that achieve a two-photon effect by having multiple caging groups attached. This approach is approximately a billion-fold more efficient in its use of light. Adapted from Pettit et al. (1997).

http://www.csbmb.princeton.edu/2photon.html

Advantages of Two-photon Excitation

Separate EX/EM



Excitation by the two-photon mechanism is accomplished with greenish-yellow light centered at 580 nanometers, while three-photon excitation occurs when the amino acid is illuminated with 840-nanometer radiation in the near-infrared region.

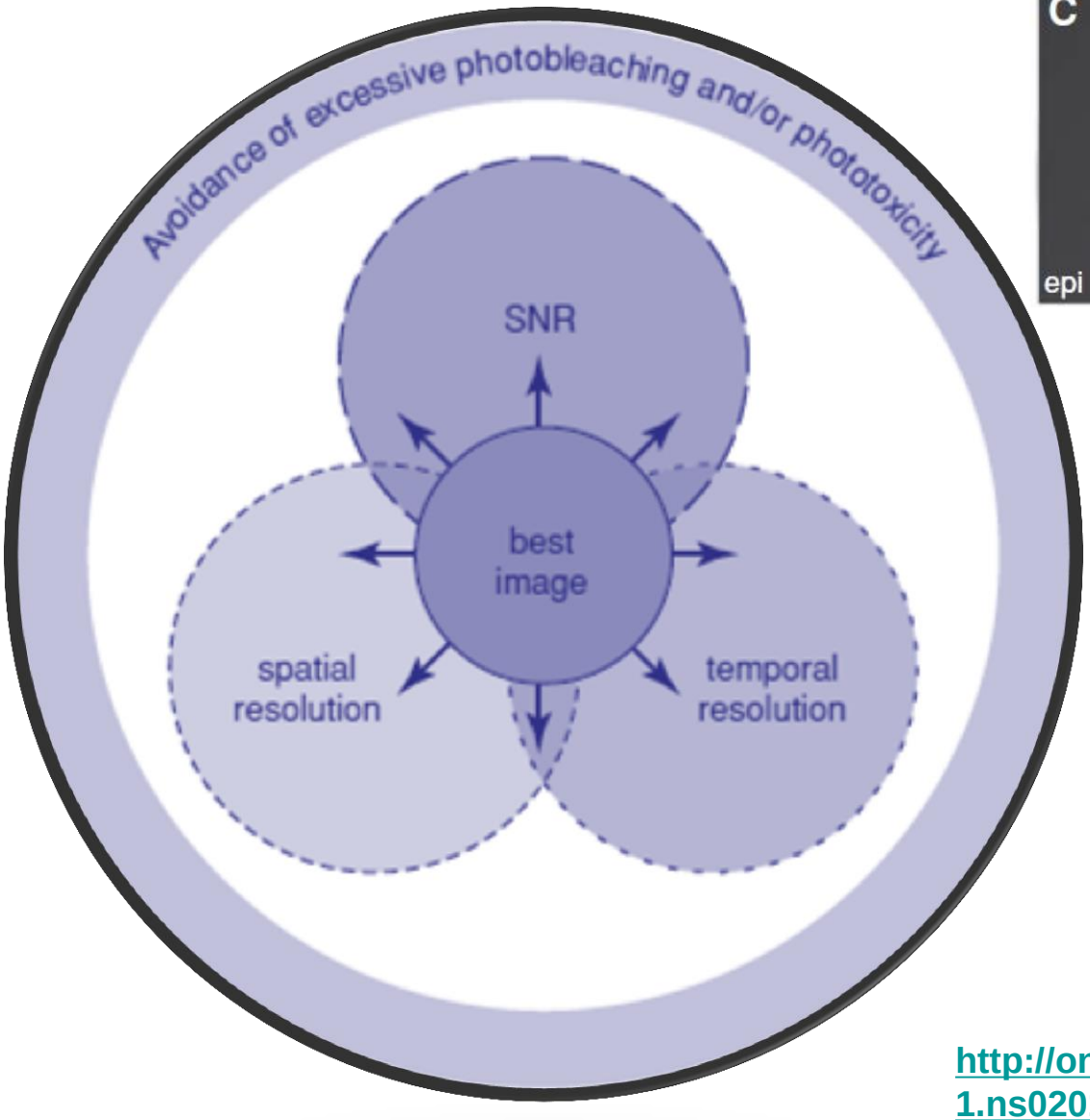
http://micro.magnet.fsu.edu/primer/techniques/fluorescence/multiphoton/multiphotonintro.html

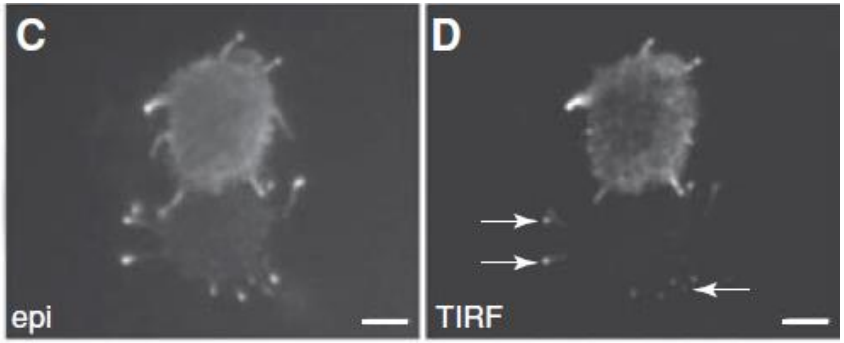
Part II

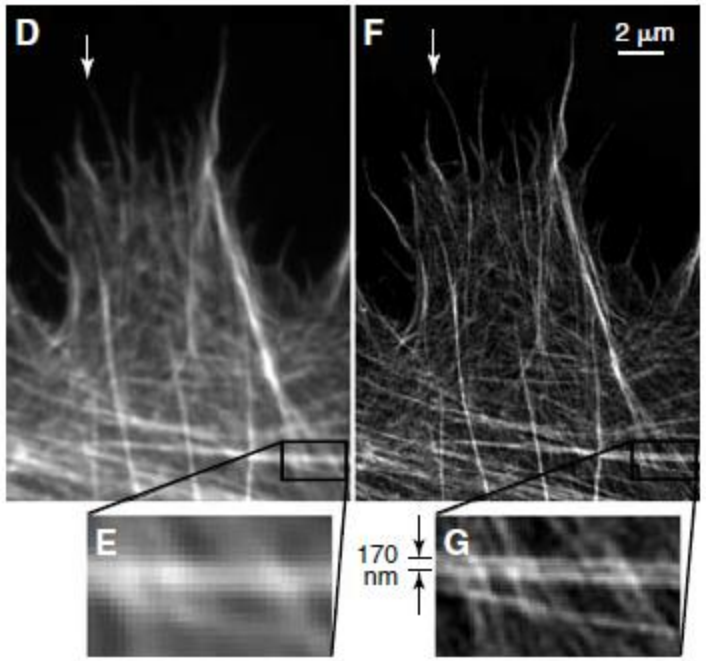
The Resolving Power of a Microscope

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

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

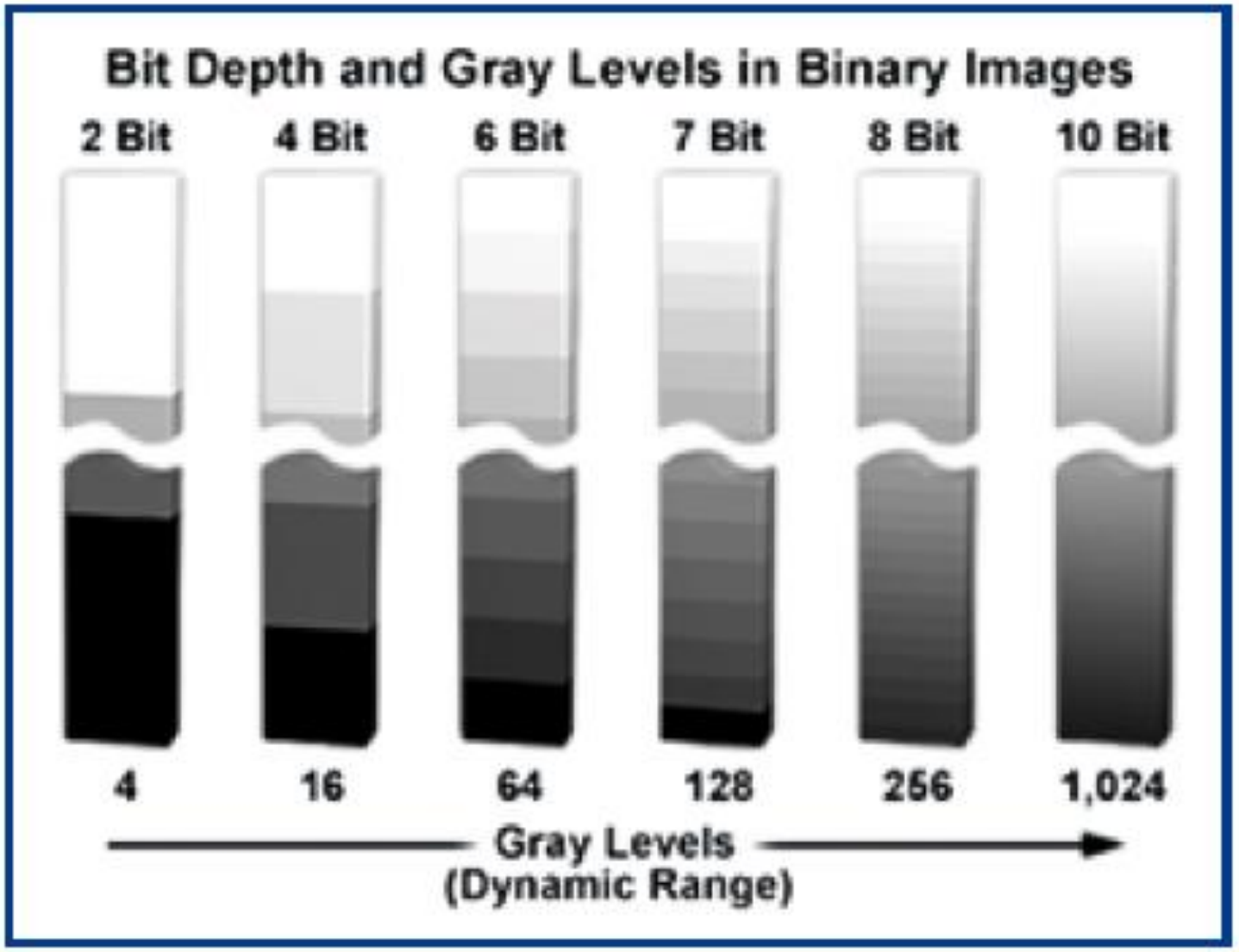




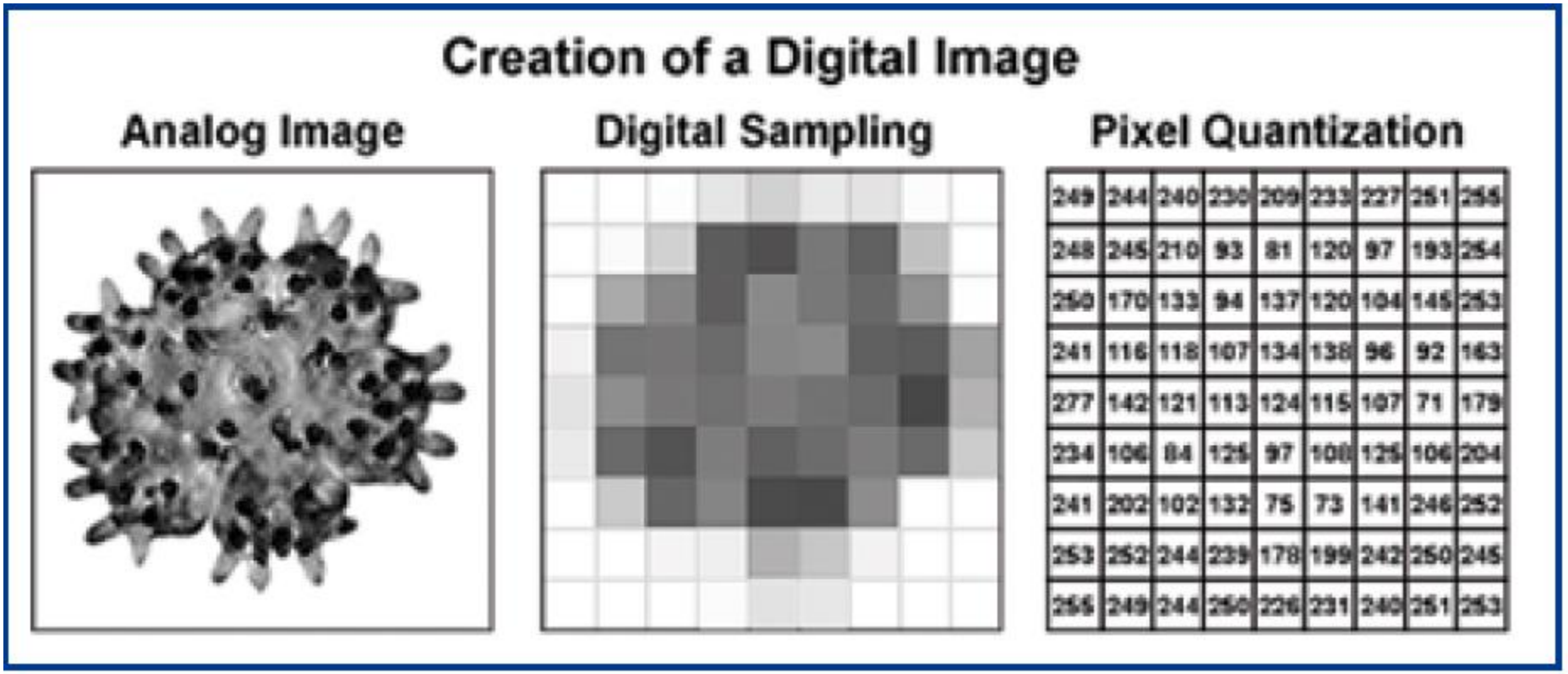


http://onlinelibrary.wiley.com/doi/10.1002/047114230 1.ns0201s50/figures

Bit depth and grey levels in digital images



Creation of a Digital Image



Resolution in Digital Images – is it important?

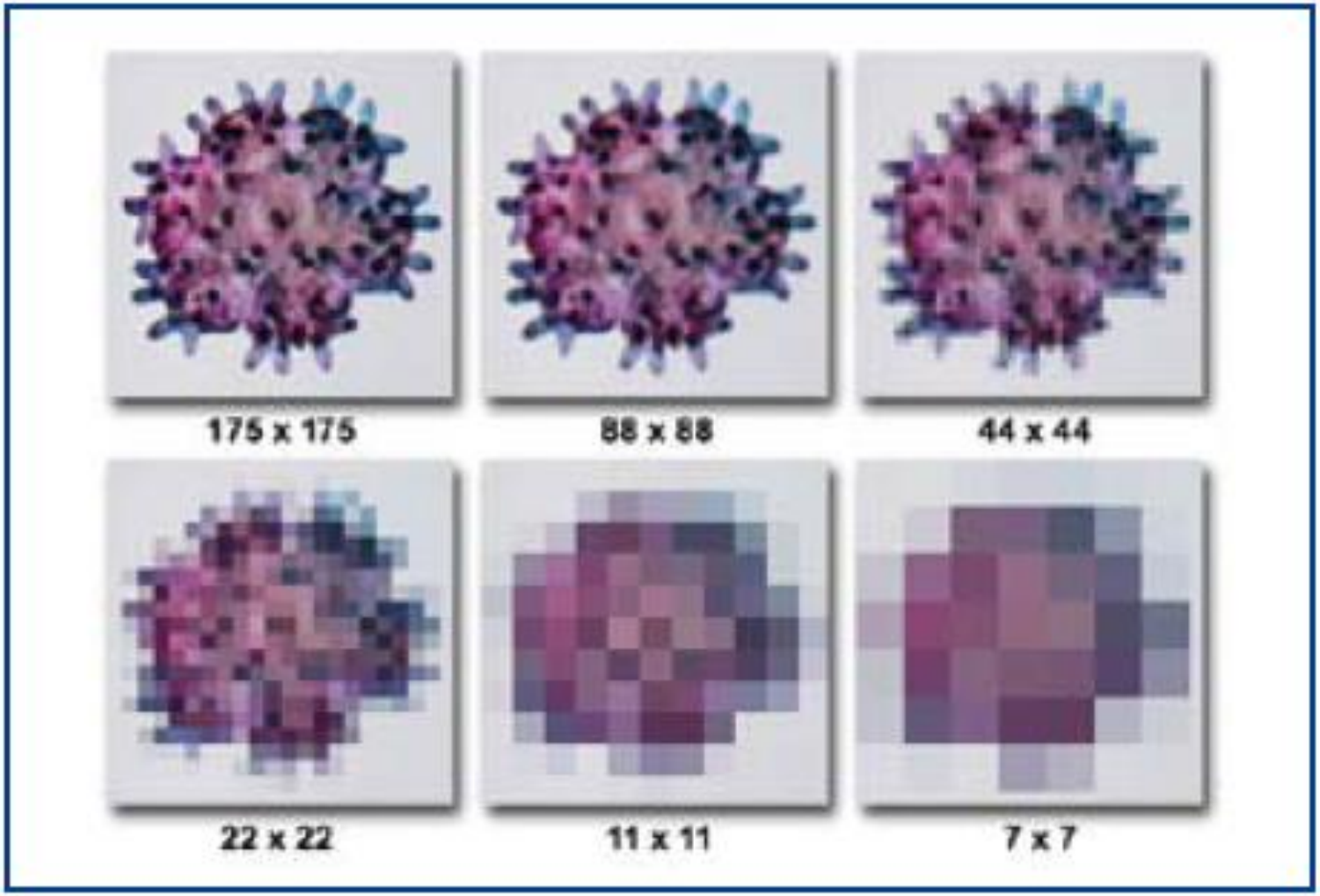
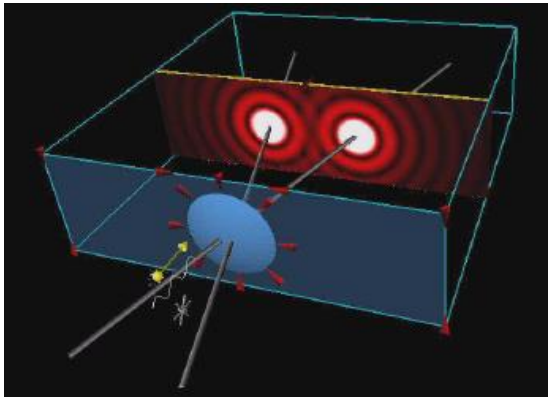
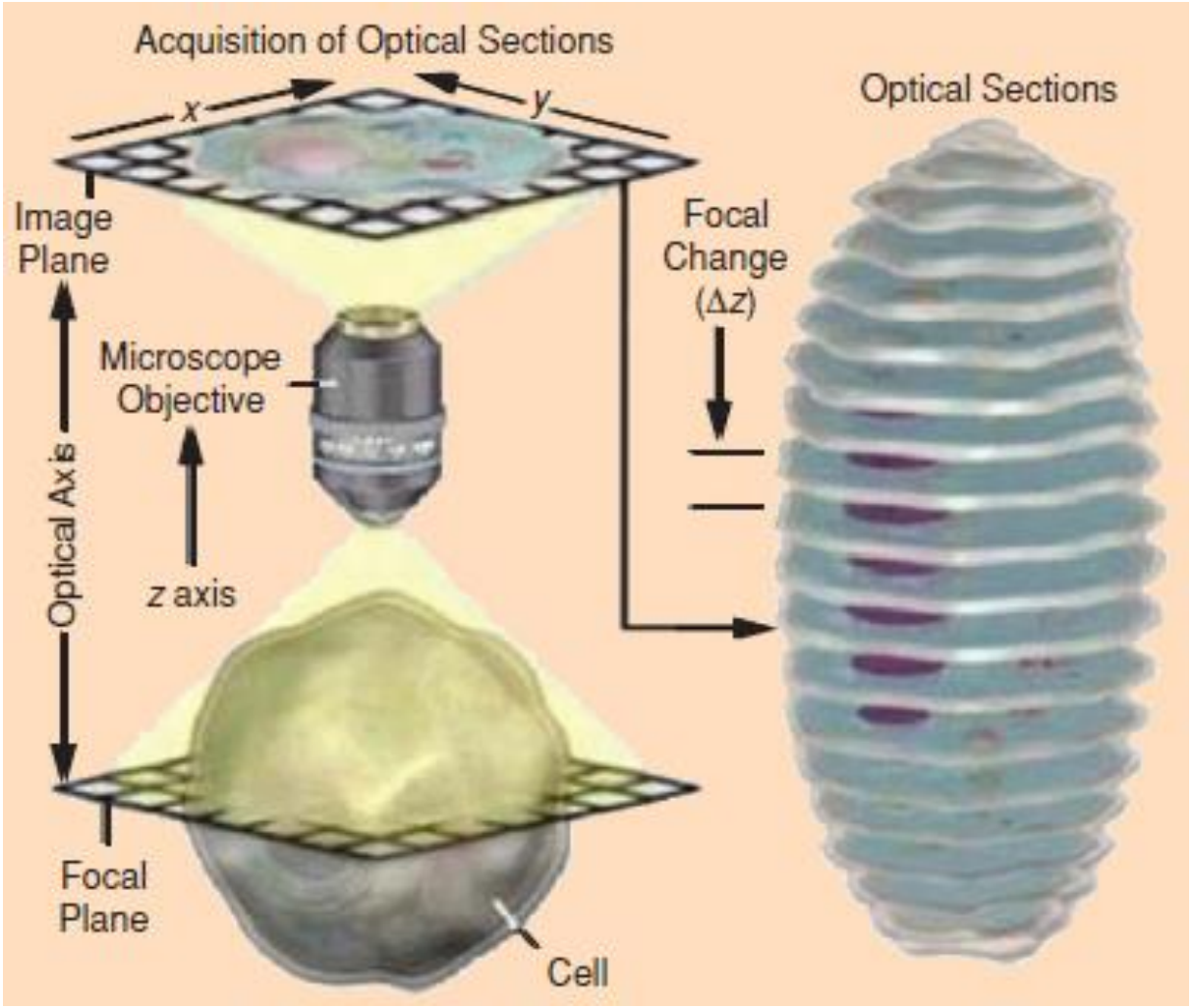


Fig. 17: Four representations of the same image, with different numbers of pixels used. The numbers of pixels is written below each image.

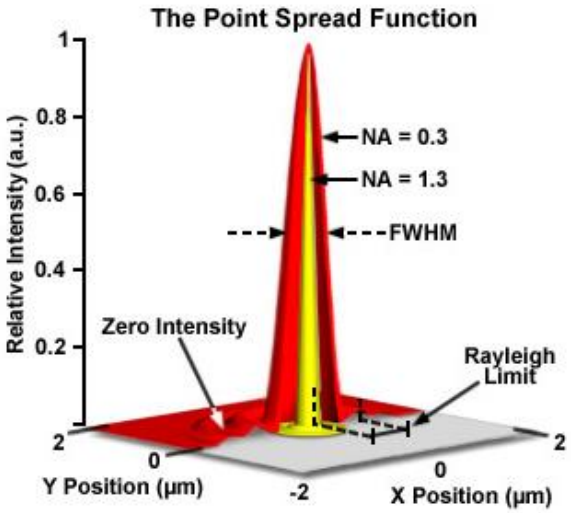
Physical Limits and Methods to Overcome

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



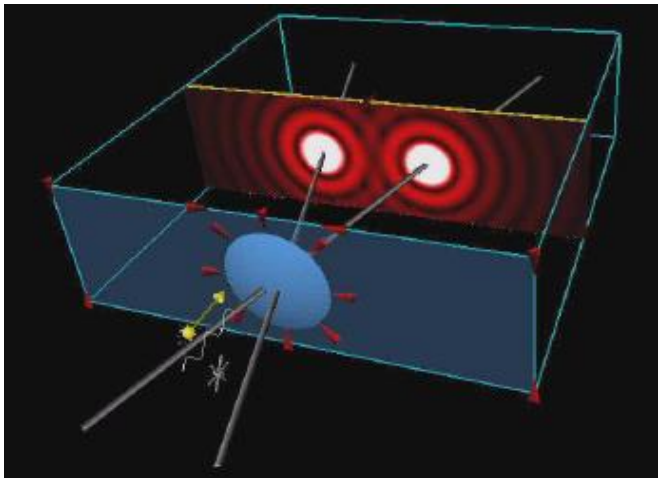


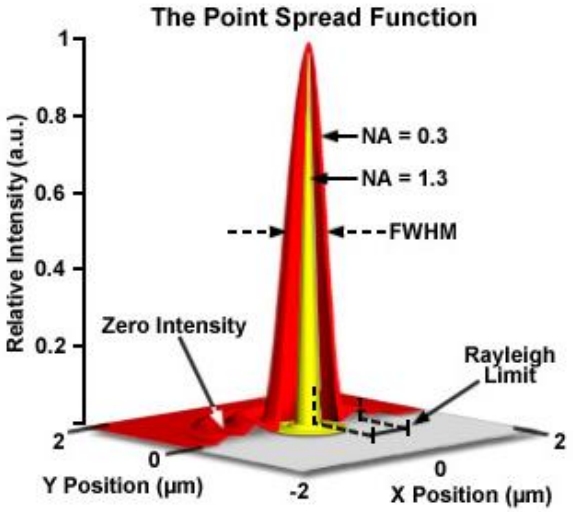
Point Spread Function (PSF)

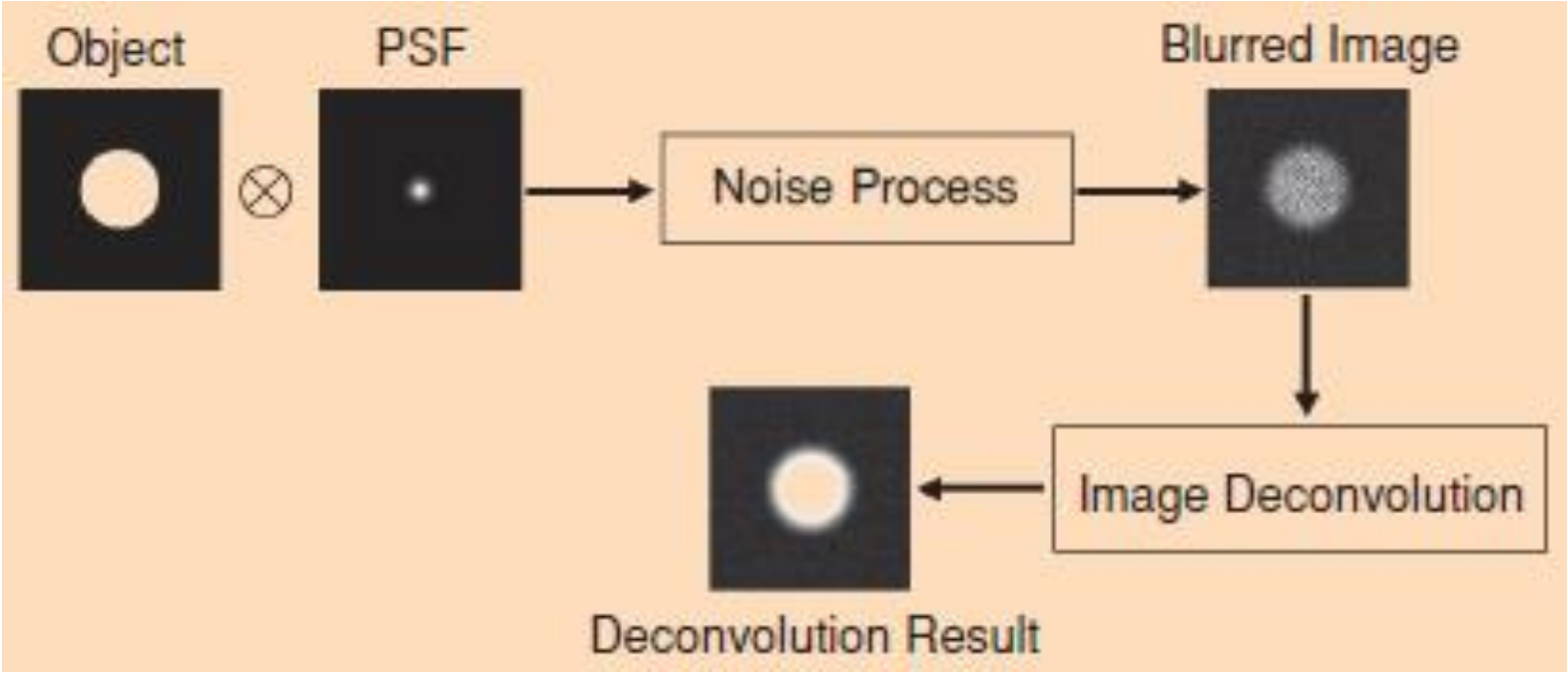


http://www.ese.wustl.edu/~nehorai/paper/deconvolutions1.pdf
http://www.olympusmicro.com/primer/digitalimaging/deconvolution/deconintro.html
http://zeiss-campus.magnet.fsu.edu/articles/basics/psf.html

Physical limits and methods to overcome

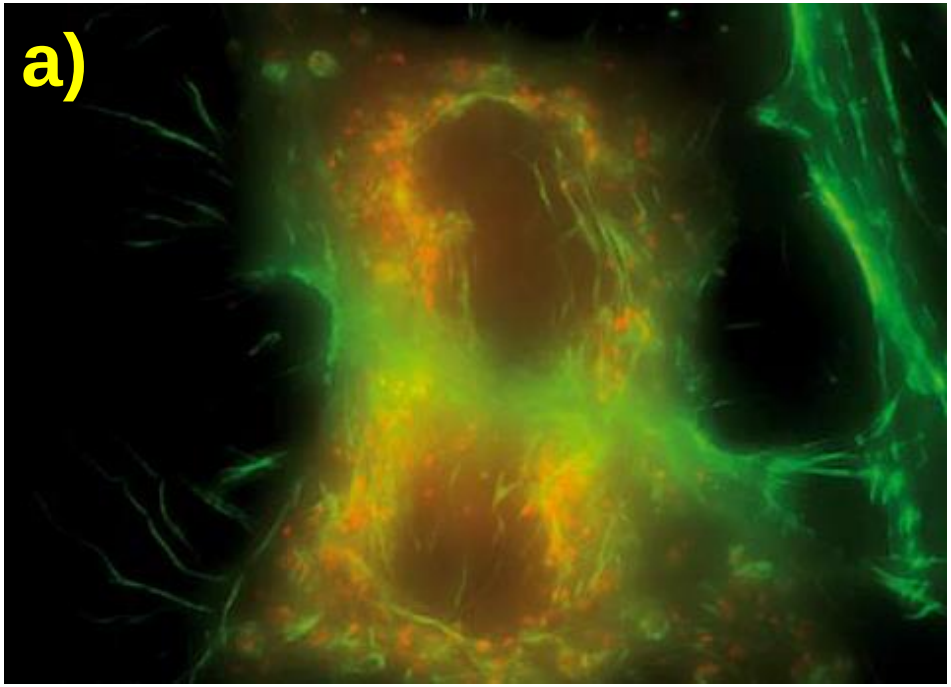


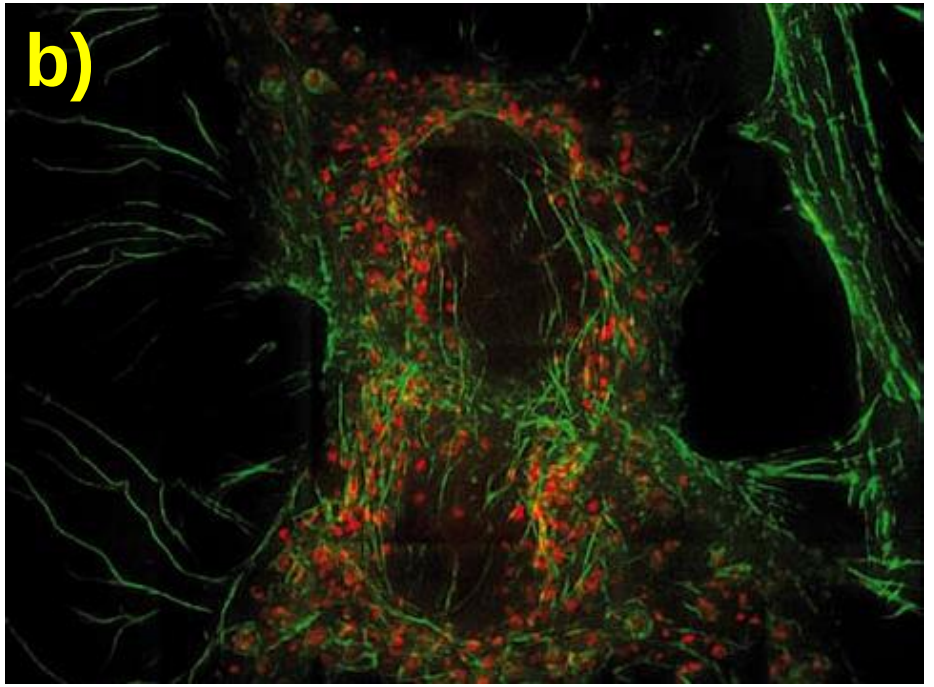




http://www.ese.wustl.edu/~nehorai/paper/deconvolutions1.pdf
http://www.mathworks.com/help/images/examples/deblurring-images-using-the-blind-deconvolution-algorithm.html

Physical limits and methods to overcome



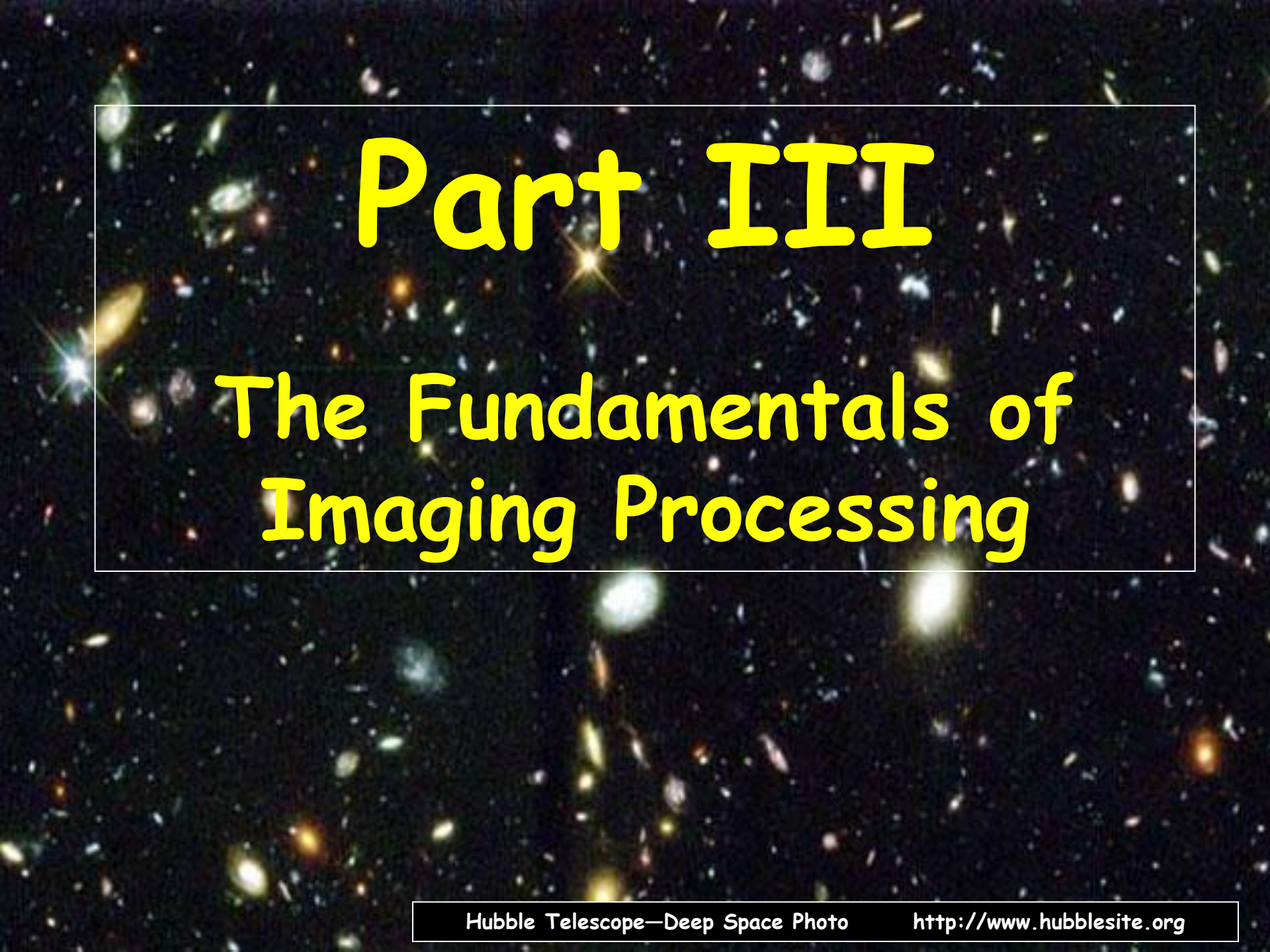


Via deconvolution artefacts can be computed out of fluorescence images.

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).

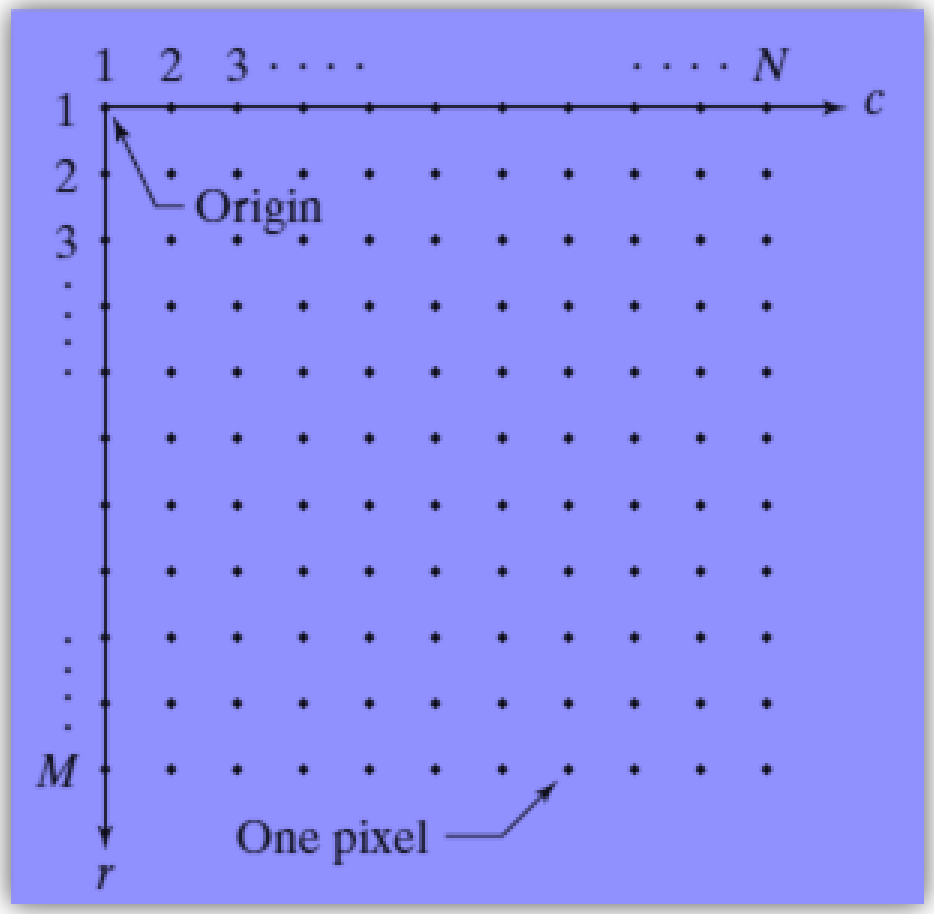
b) Deconvolution is a recognised mathematical procedure for eliminating such artefacts. The resulting image displayed is sharper with less noise and thus at higher resolution. This is also advantageous for more extensive analyses.

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

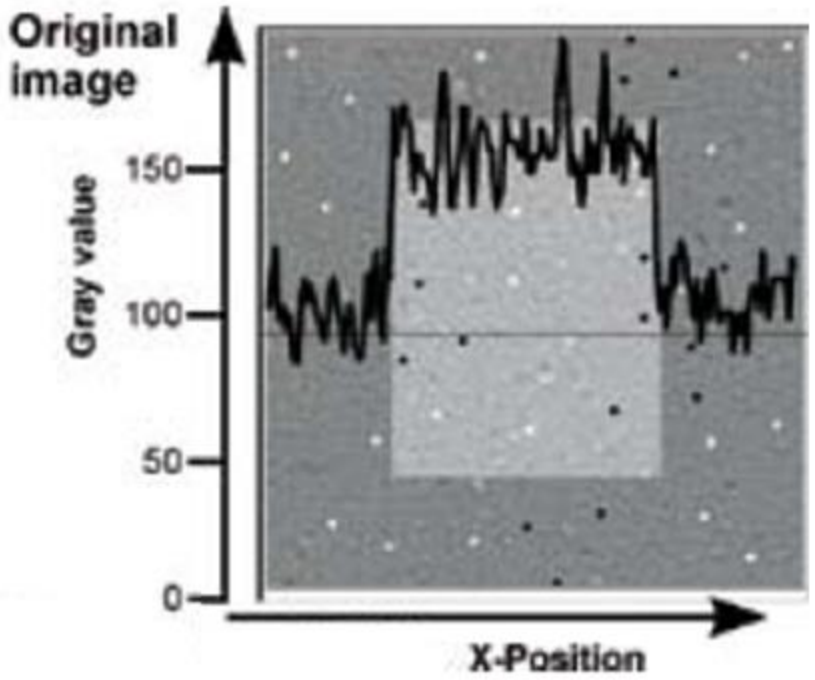

Part III
The Fundamentals of Imaging Processing
Hubble Telescope—Deep Space Photo
http://www.hubblesite.org

Coordinate Convention in the Image Processing

Array Indexing




```
>> f = imread('chestxray.jpg');
>> f = imread('D:\myimages\chestxray.jpg');
>> imshow(f);
>> imwrite(f, 'DT.bmp')
```



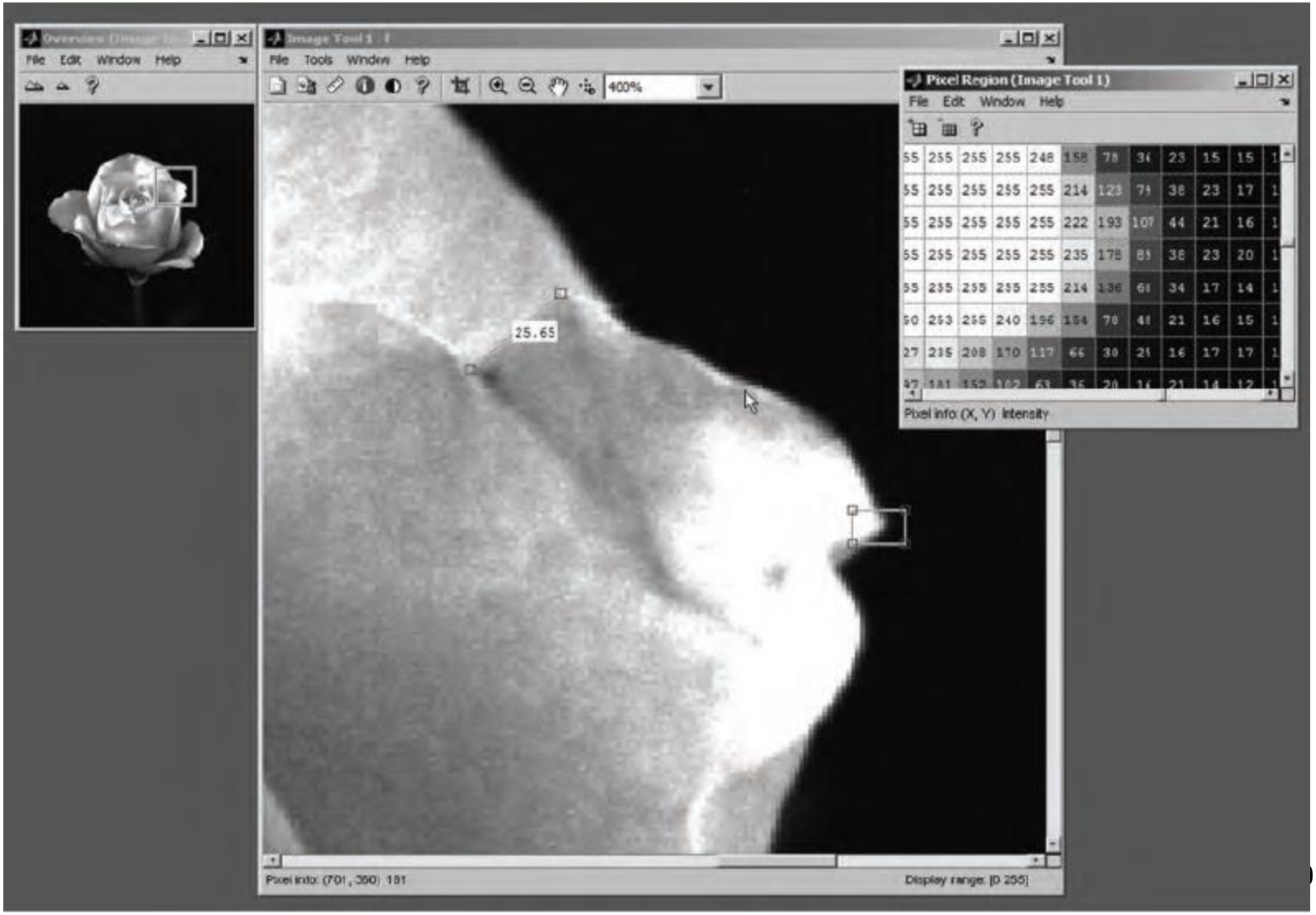
Coordinate Convention in the Image Processing

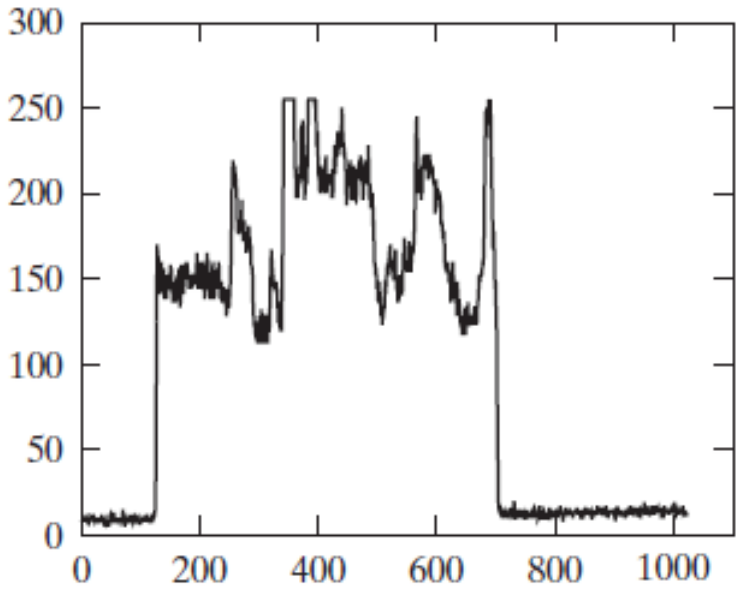
Example

```
A =
     1     2     3
     4     5     6
     7     8     9
```

```
>> T2 = A(1:2, 1:3)
T2 =
     1     2     3
     4     5     6
```

Coordinate Convention in the Image Processing

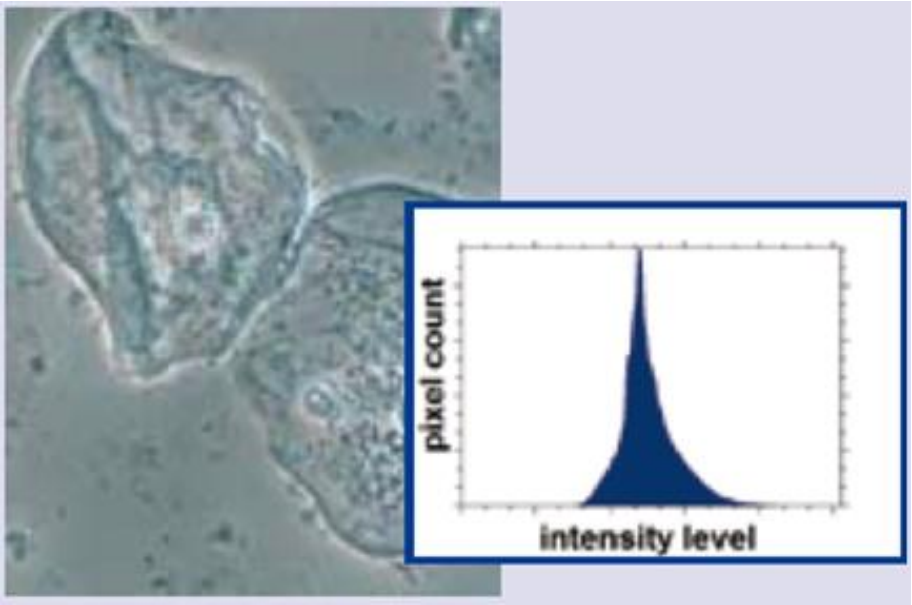


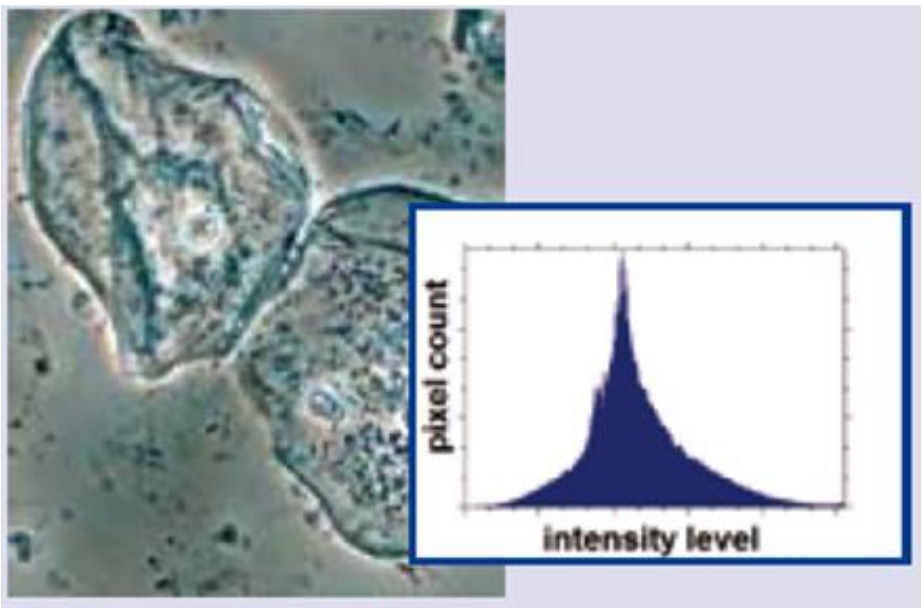


a b c
d
e

Results obtained using array indexing. (a) Original image. (b) Image flipped vertically (垂直翻轉). (c) Cropped image (裁剪). (d) Subsampled image. (e) A horizontal scan line through the middle of the image in (a).

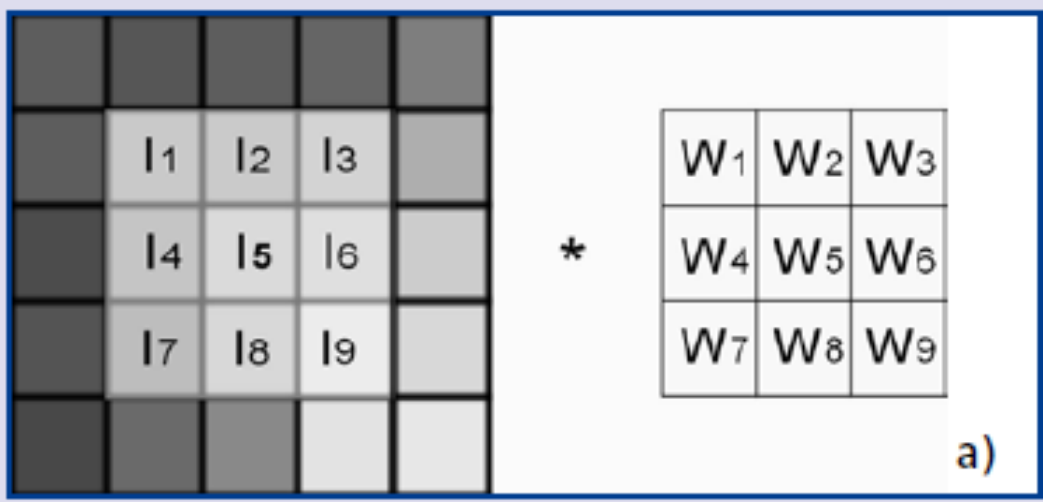
Histogram Optimisation During Acquisition





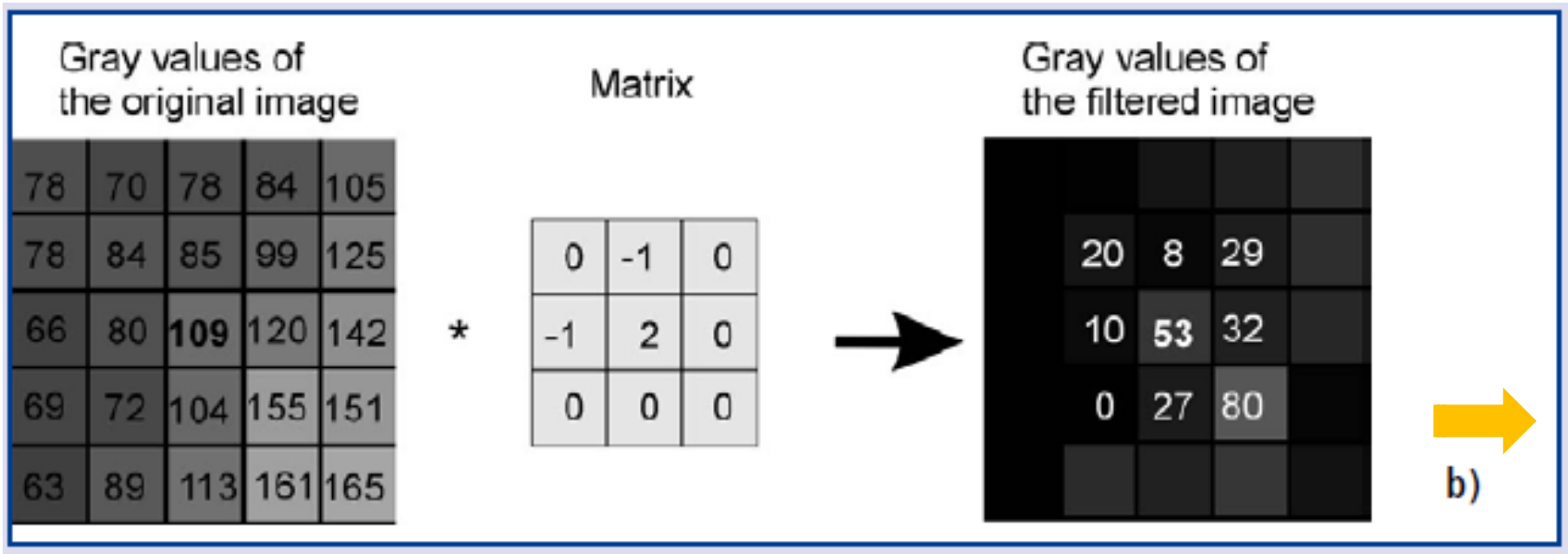
Left side with low contrast setting, right side with contrast optimisation of microscope system and camera setting.

How to describe image processing operations mathematically



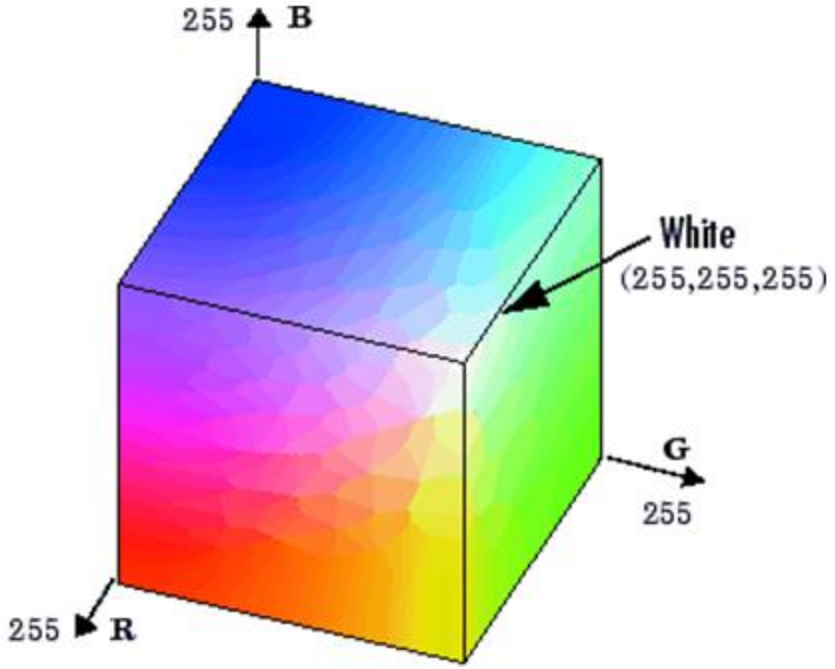
Local Operations:

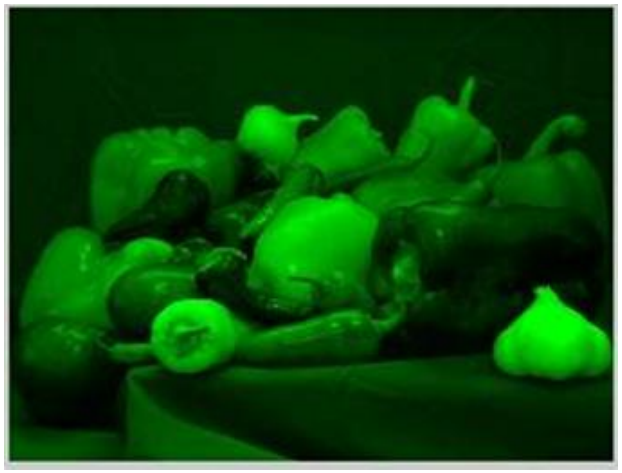
Many well known noise reduction, sharpening and edge enhancing filters are convolution filters.



http://www.fmhs.auckland.ac.nz/sms/biru/_docs/Olympus_Sonderheft.pdf

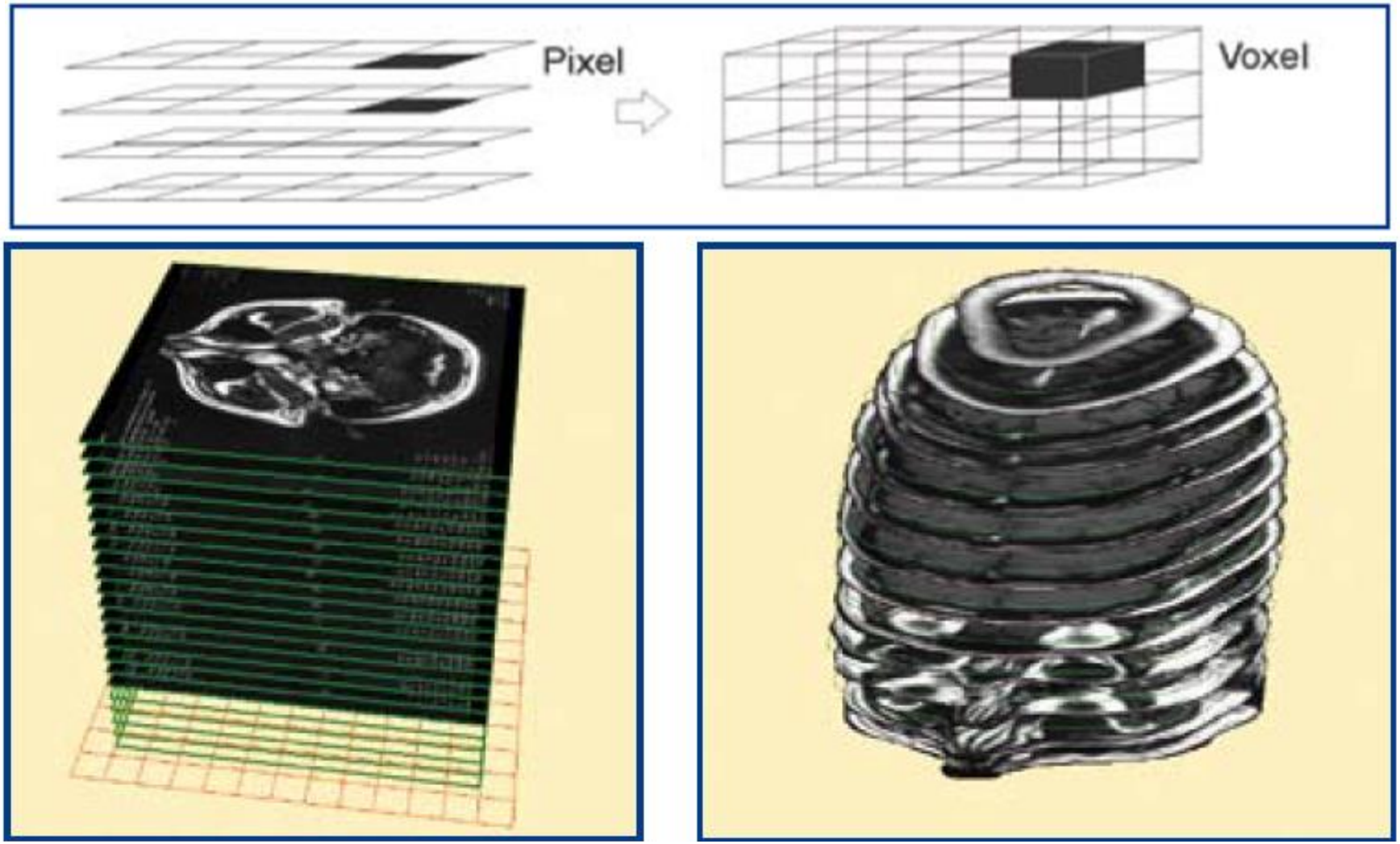
RGB Image Decomposition





http://www.mathworks.com/matlabcentral/fileexchange/18125-rgb-image-decomposition

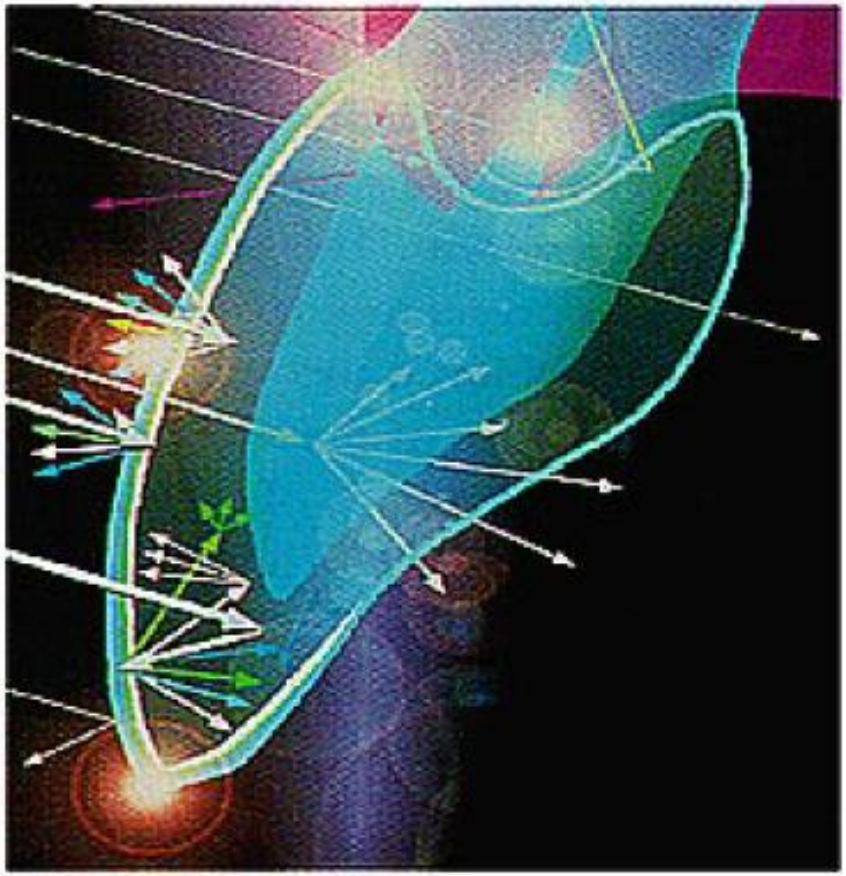
3-D Reconstruction using an image series of specimen sections



This is how a two-dimensional **pixel** (像素/picture element) is expanded to a three-dimensional **voxel** (體素/volume element).

http://www.fmhs.auckland.ac.nz/sms/biru/_docs/Olympus_Sonderheft.pdf

Visual Shade Matching



The appearance of a tooth is the summation of light that is reflected, transmitted, and fluoresced.

http://www.dentalproductsreport.com/dental/article/video-review-dr-john-flucke-gendex-dental-imaging-solutions

Thanks For Your Attention

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