臺北醫學大學

Biomedical Imaging

生物醫學影像學

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2013/03/18

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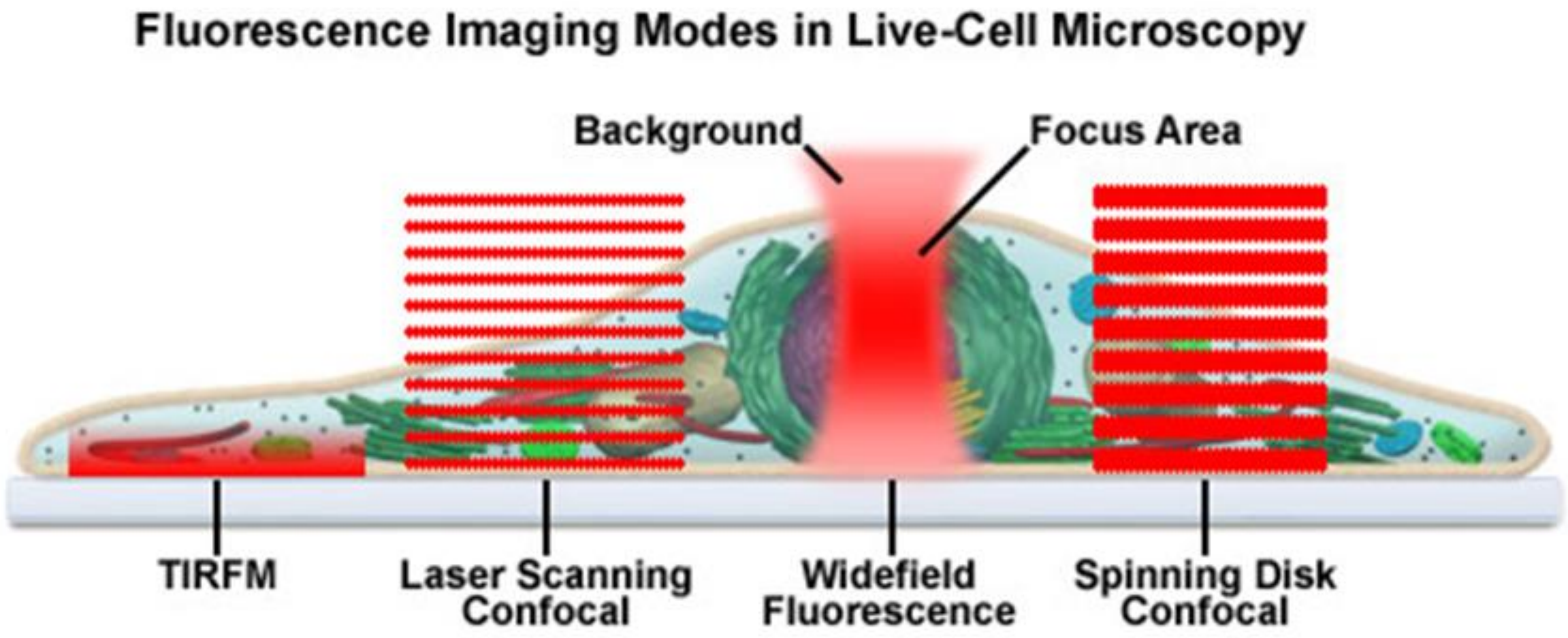
Course Outline

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

Imaging Modes in Optical Microscopy

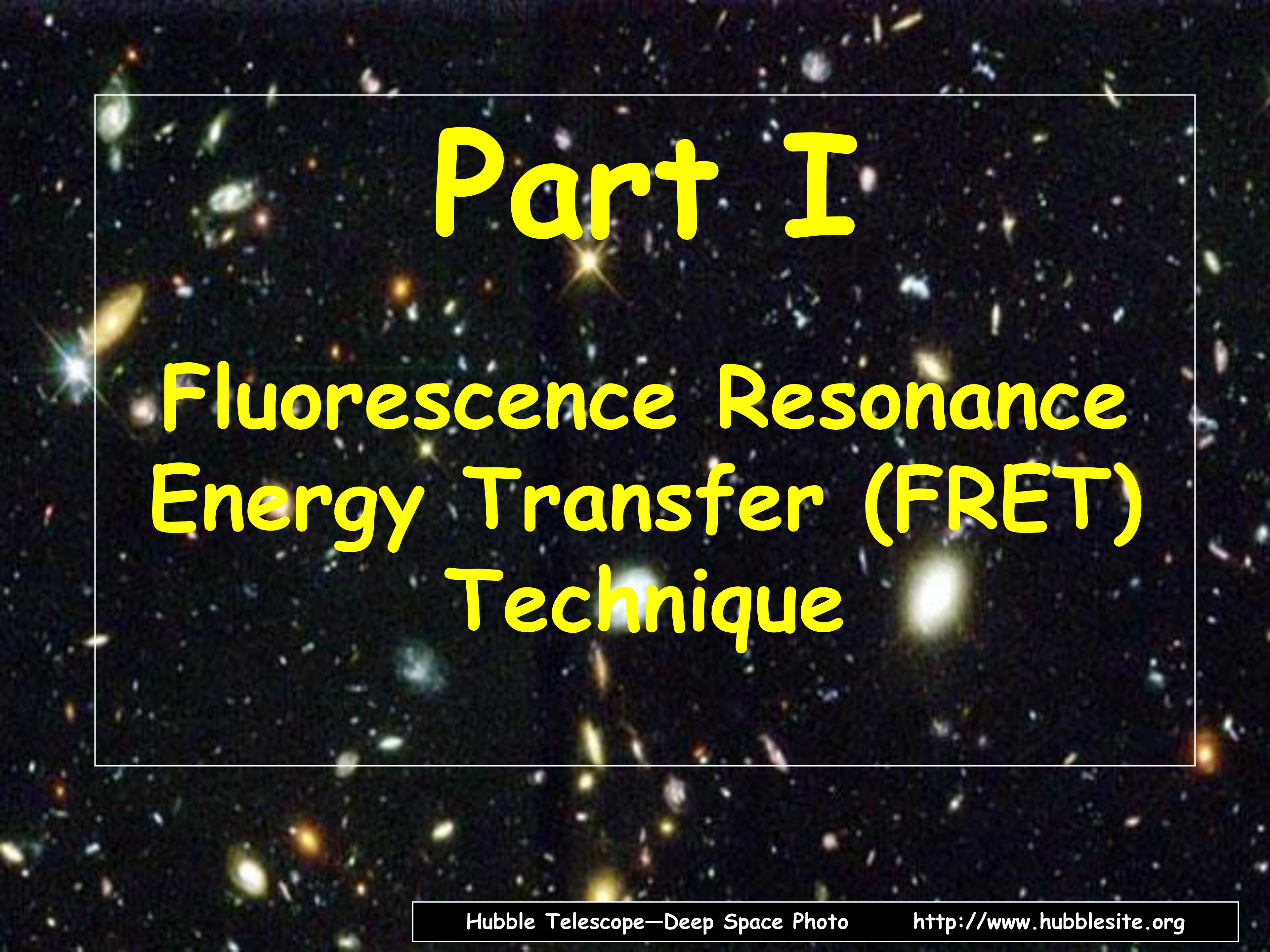


A majority of the live-cell imaging investigations are conducted with adherent mammalian cells, which are positioned within 10 micrometers of the coverslip-medium interface.

http://learn.hamamatsu.com/articles/microscopyimaging2.html

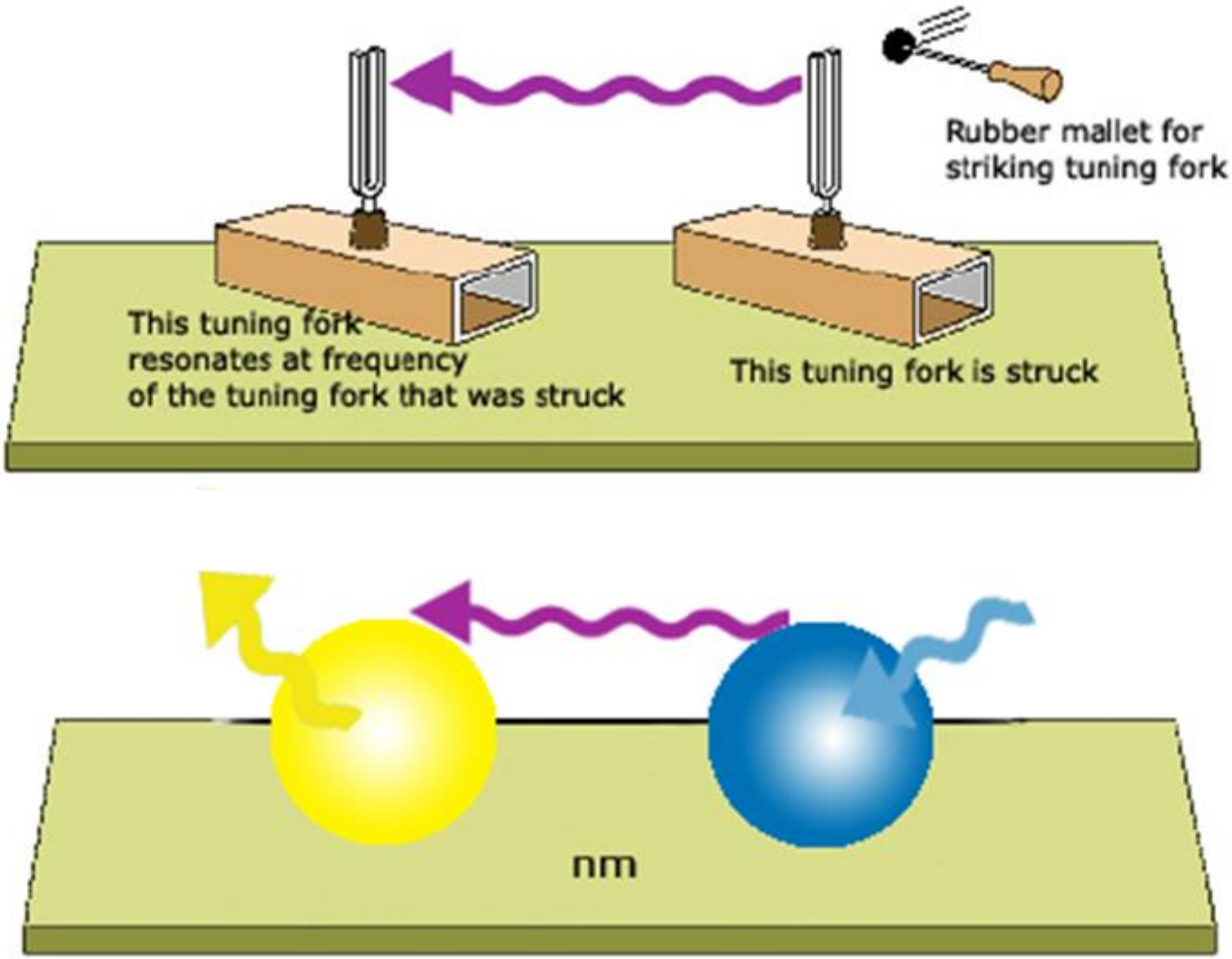
Part I

Fluorescence Resonance Energy Transfer (FRET) Technique



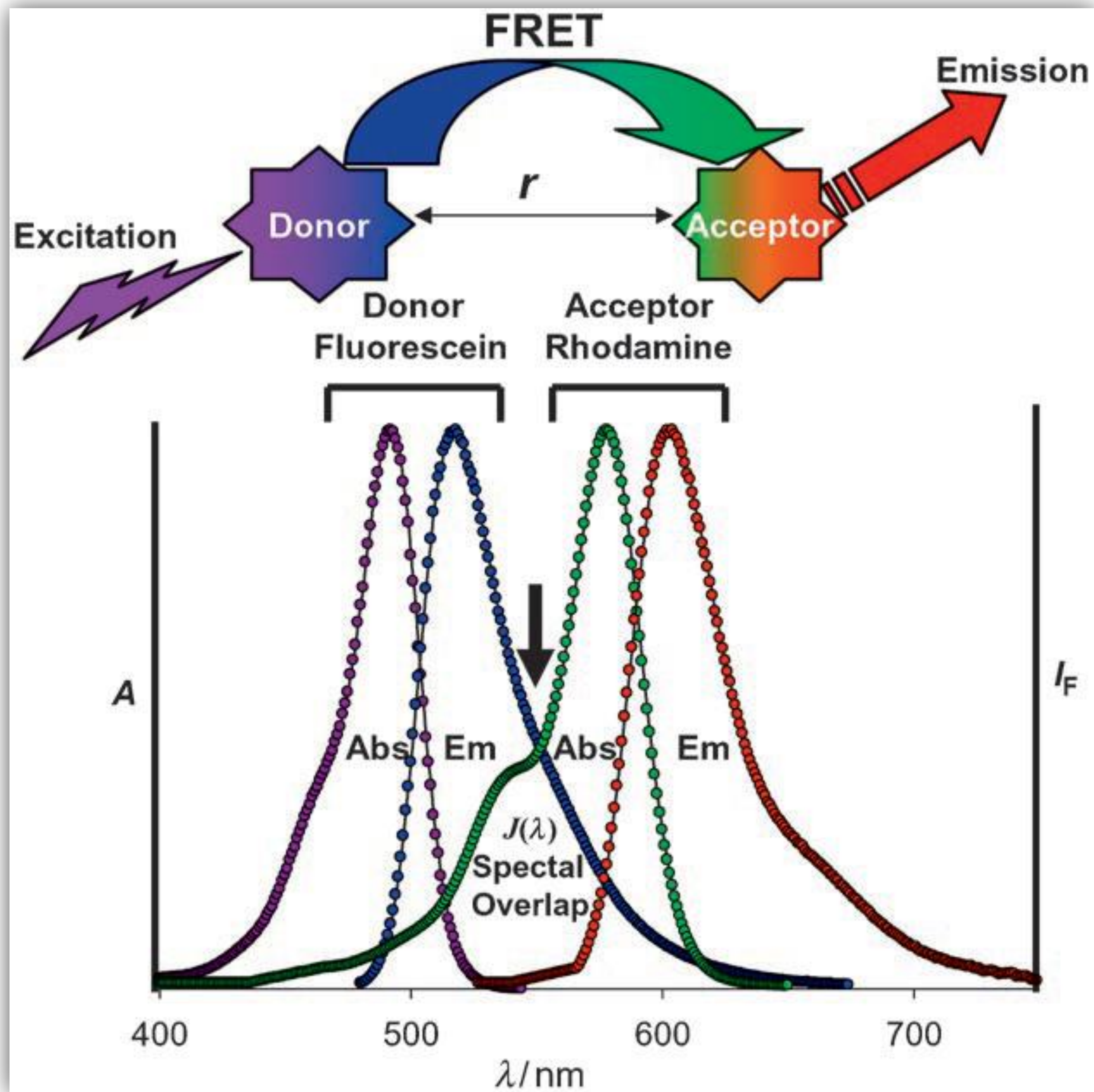
Hubble Telescope—Deep Space Photo http://www.hubblesite.org

Tuning Fork Analogy for Resonance Energy Transfer



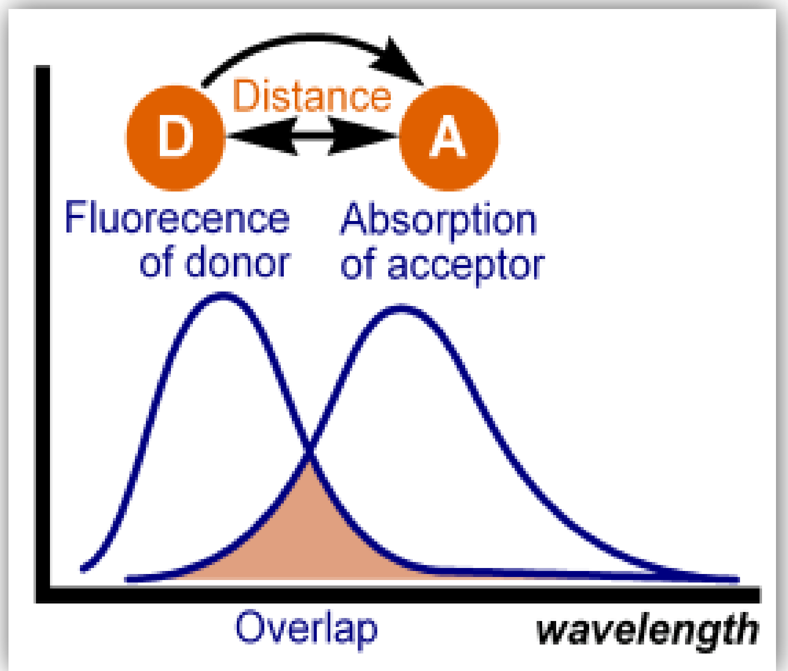
Theodore Förster in 1948

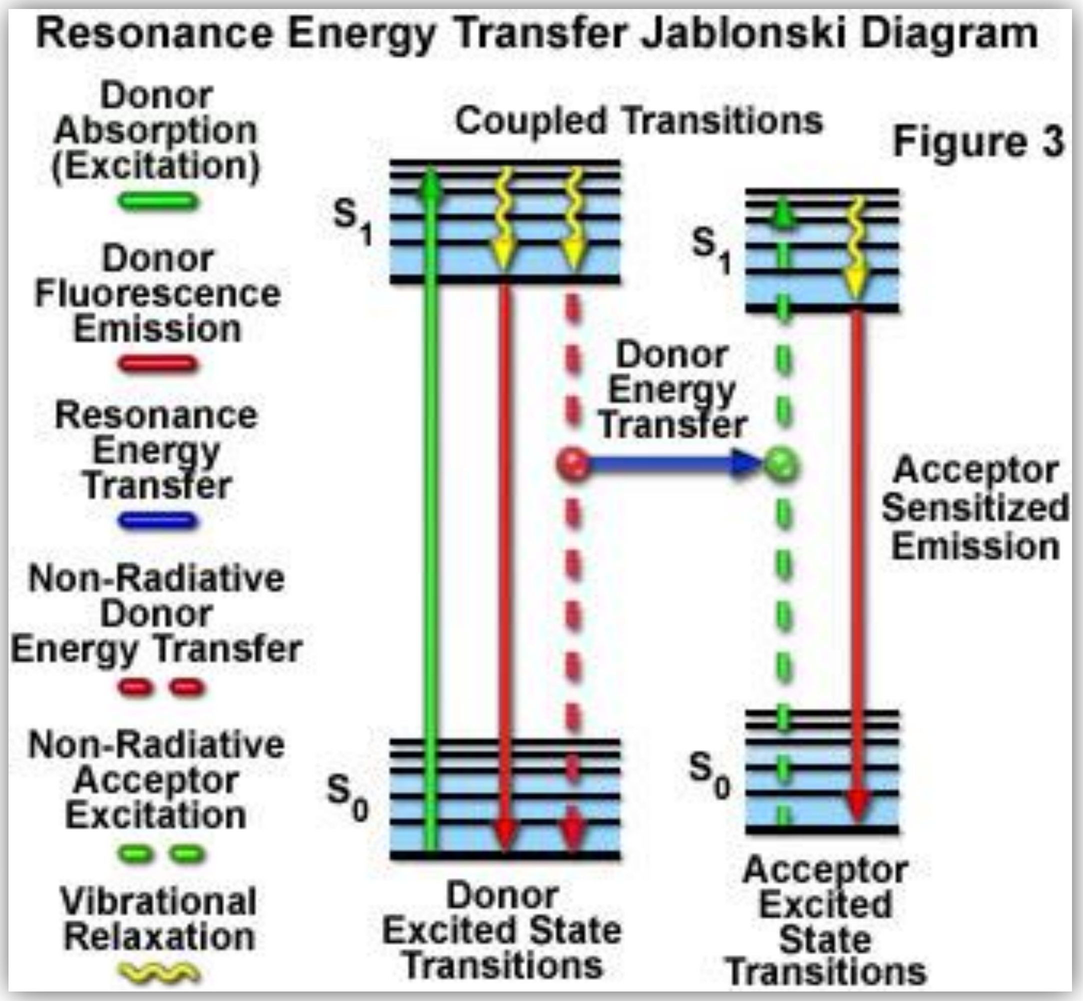
Fluorescence Resonance Energy Transfer (FRET)



Förster Resonance Energy Transfer (FRET)

- **Based on the principles published by Theodore Förster in 1948**
- **FRET involves the transfer of energy between oscillating dipoles of similar resonance frequency**

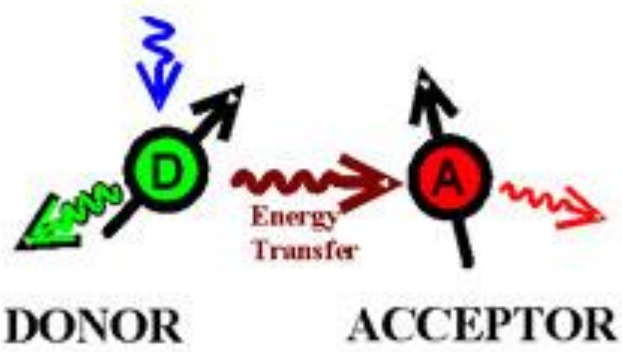




Förster Resonance Energy Transfer (FRET)

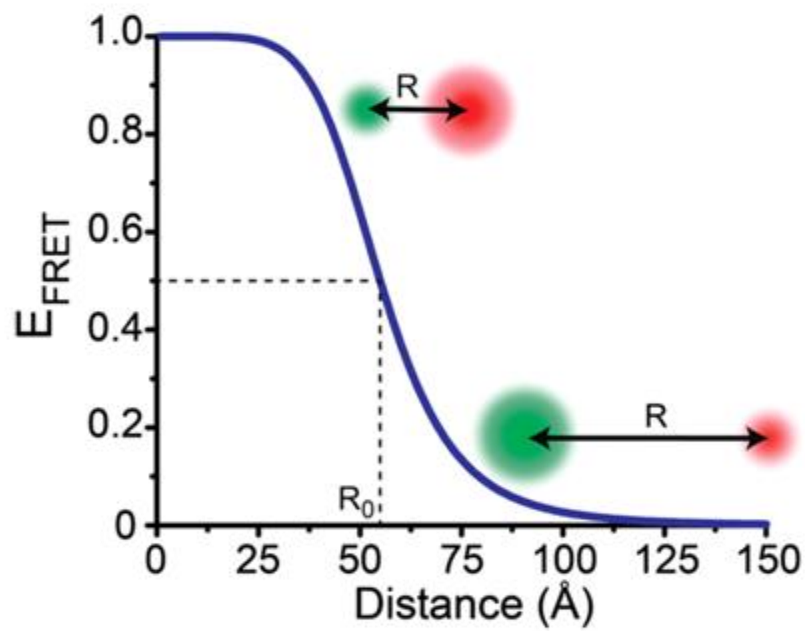
- **Energy transfer efficiency :**
  - Degree of spectral overlap between donor fluorescence emission and acceptor absorption
  - Inversely proportional to 6th power of the distance between fluorophores
  - ~ 10 nm

"Spectroscopic Ruler"

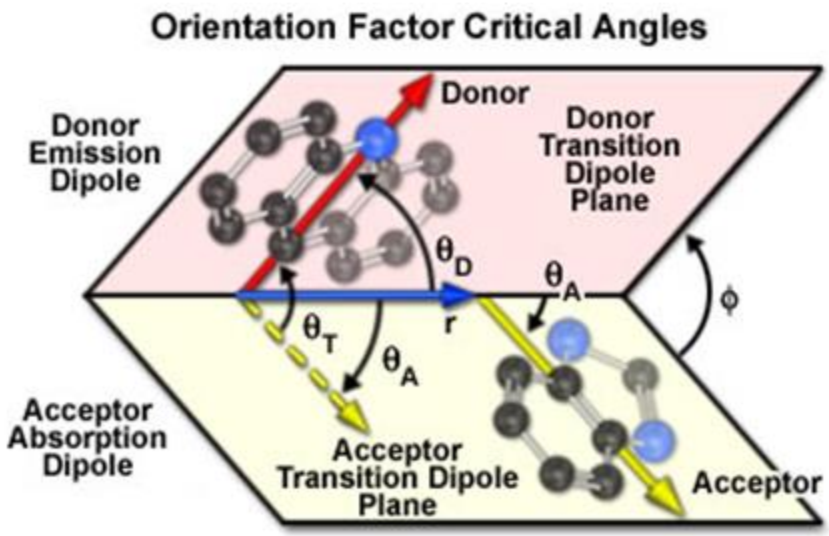


$$E = \frac{1}{1 + \left(\frac{R}{R_0}\right)^6}$$

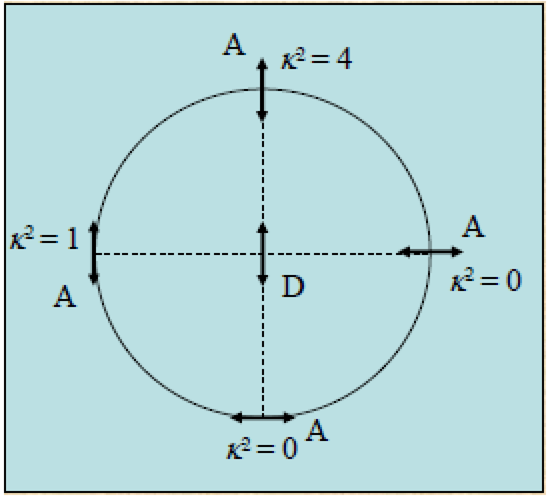
R_0=50% transfer efficiency distance
3nm~7nm

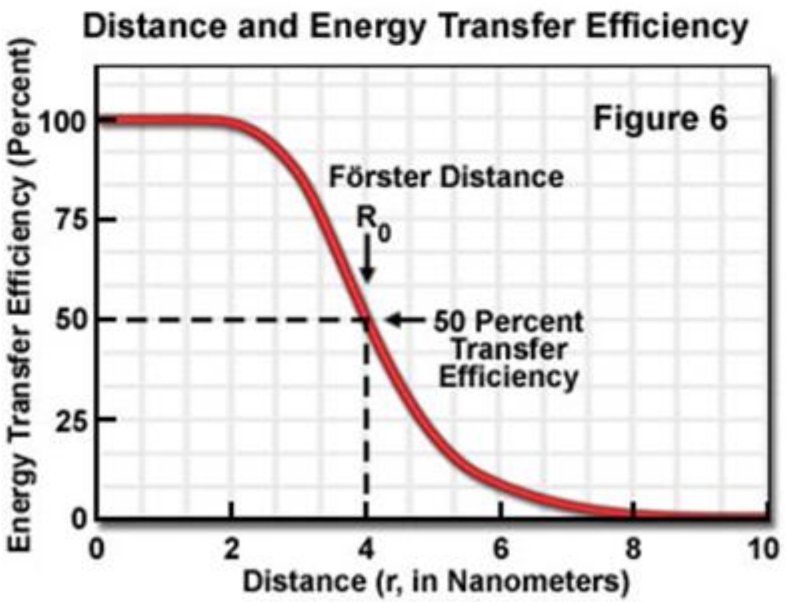


Principles of Fluorescence Resonance Energy Transfer



Dipole – dipole coupling





FRET efficiency is given by

$$E = \frac{1}{1 + \left(\frac{R}{R_0}\right)^6}$$

http://www.olympusfluoview.com/applications/fretintro.html
http://fluorescence-foundation.org/lectures%5Cchicago2009%5Clecture3.pdf

Förster Resonance Energy Transfer (FRET)

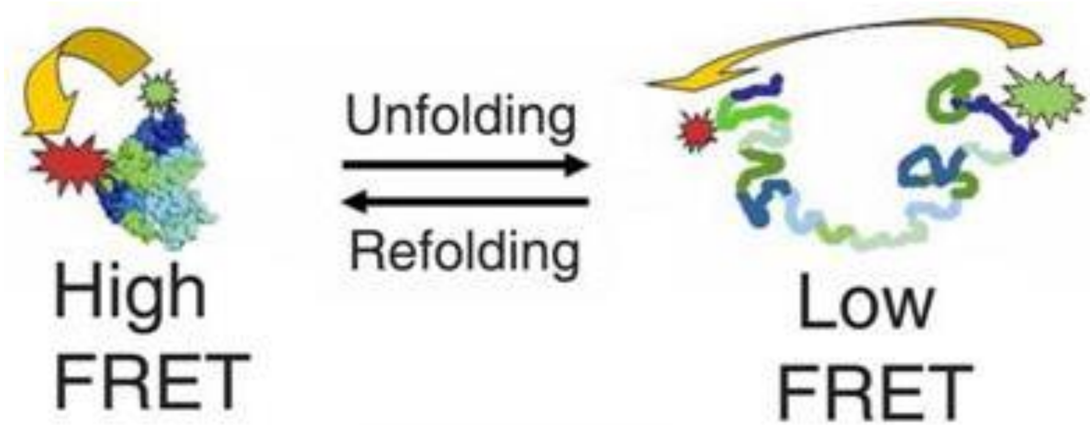
Calculation of the distance between fluorophores

- Detection of target analytes
- Analysis of biomolecular interactions
- Single molecule analysis
  - Protein folding/unfolding
  - Protein dynamics
- Living cell analysis

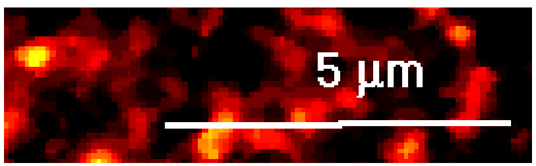
FRET efficiency is given by

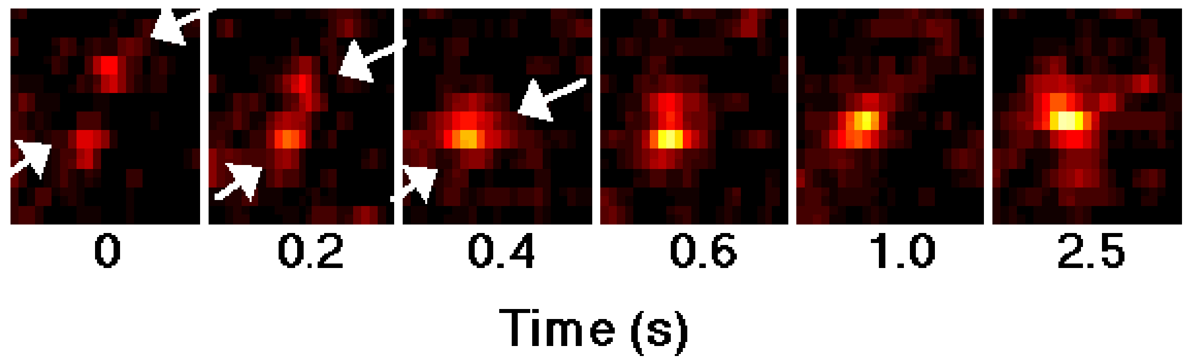
$$E = \frac{1}{1 + \left(\frac{R}{R_0}\right)^6}$$

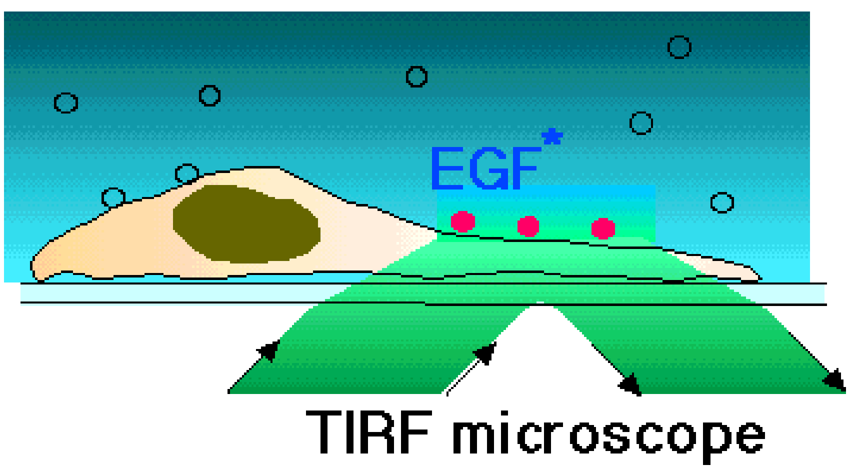
$$E_{FRET} = \frac{I_A}{(I_D + I_A)}$$

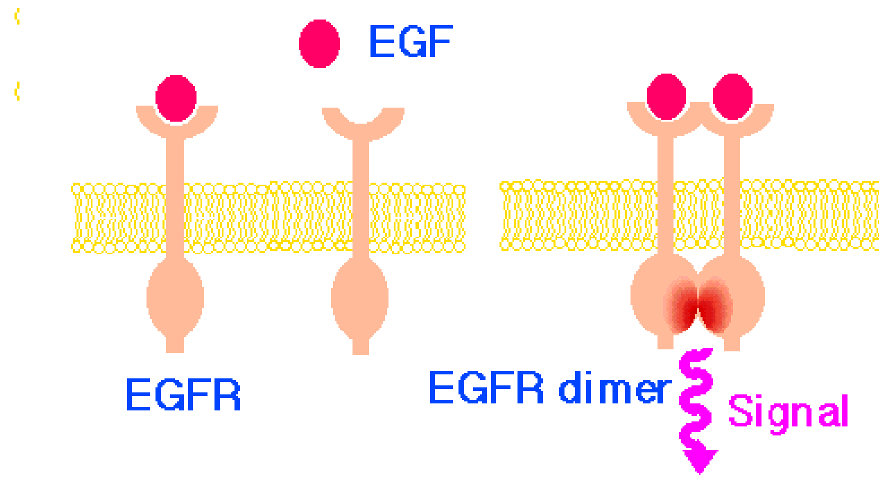


Cell signaling. Fluorescently labeled epidermal growth factor (EGF) was visualized on the cell surface. A single EGF molecule can be seen as a spot and a time sequence of images shows dimerization of the EGF/EGF receptor, triggering transmission of signal.







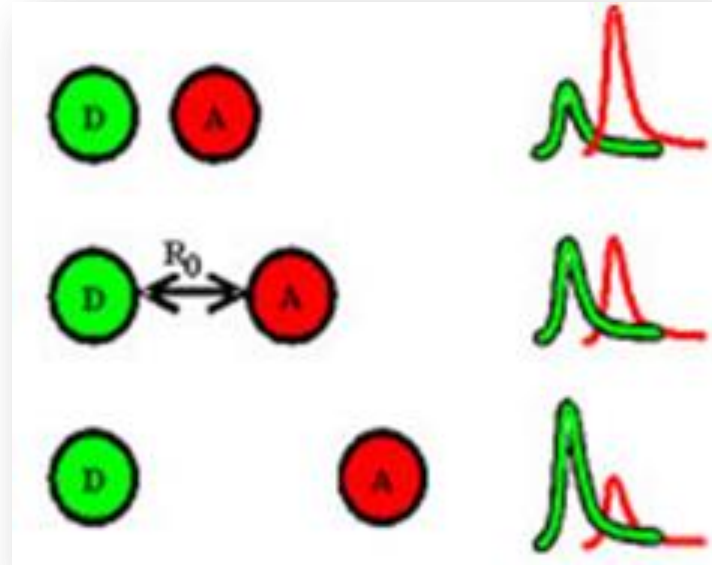


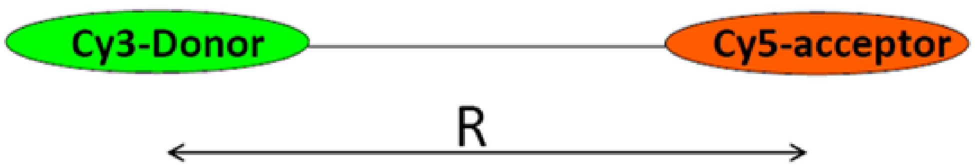
Single Mol. **1** (2000) 1

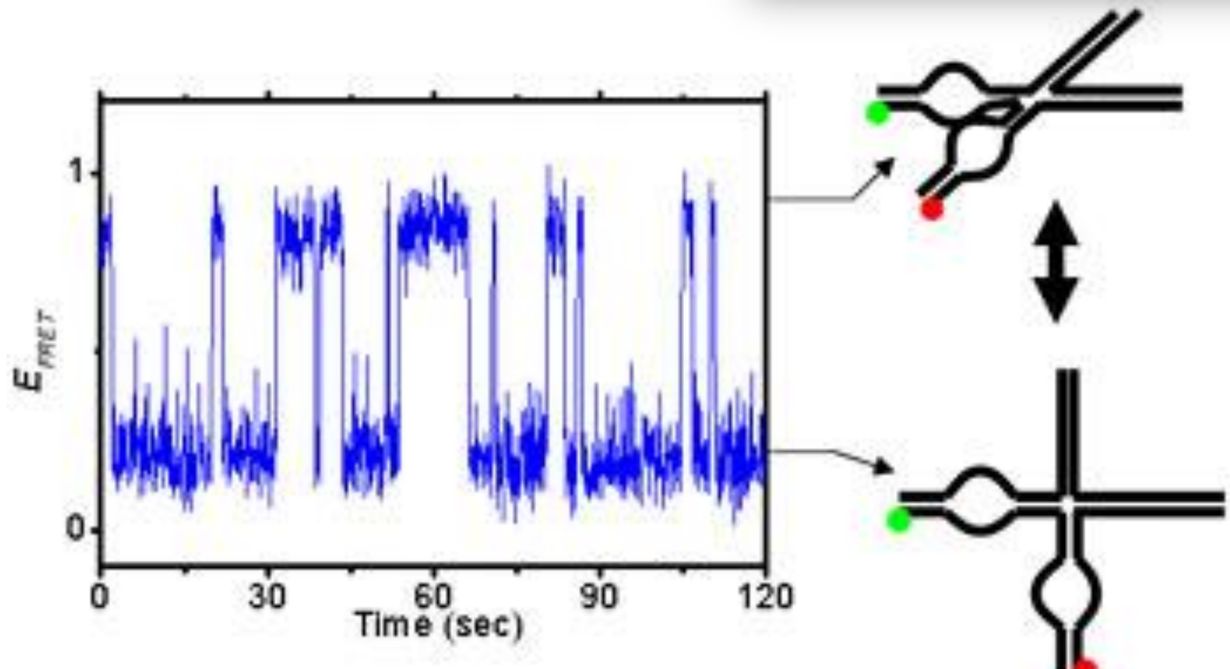
Quantitative analysis using FRET

FRET efficiency is given by

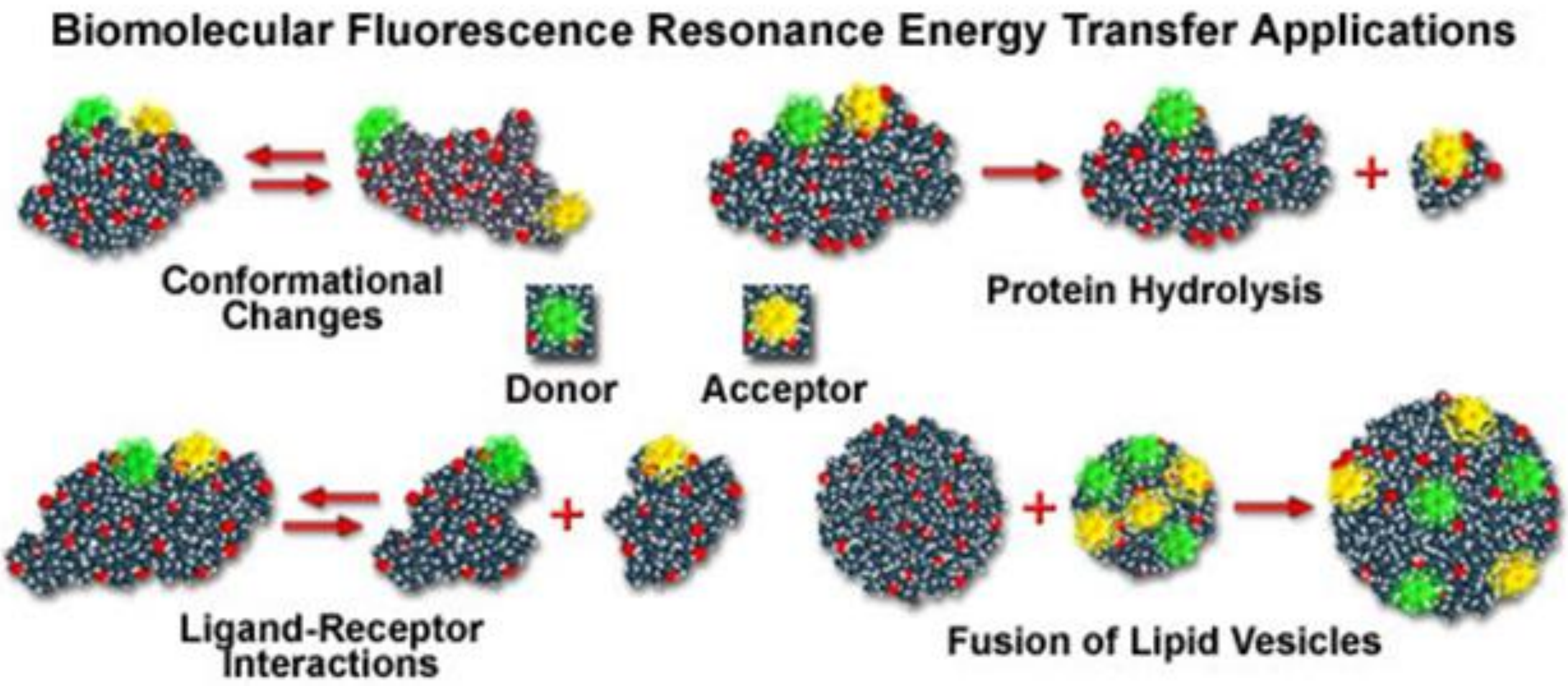
$$E = \frac{1}{1 + \left(\frac{R}{R_0}\right)^6}$$





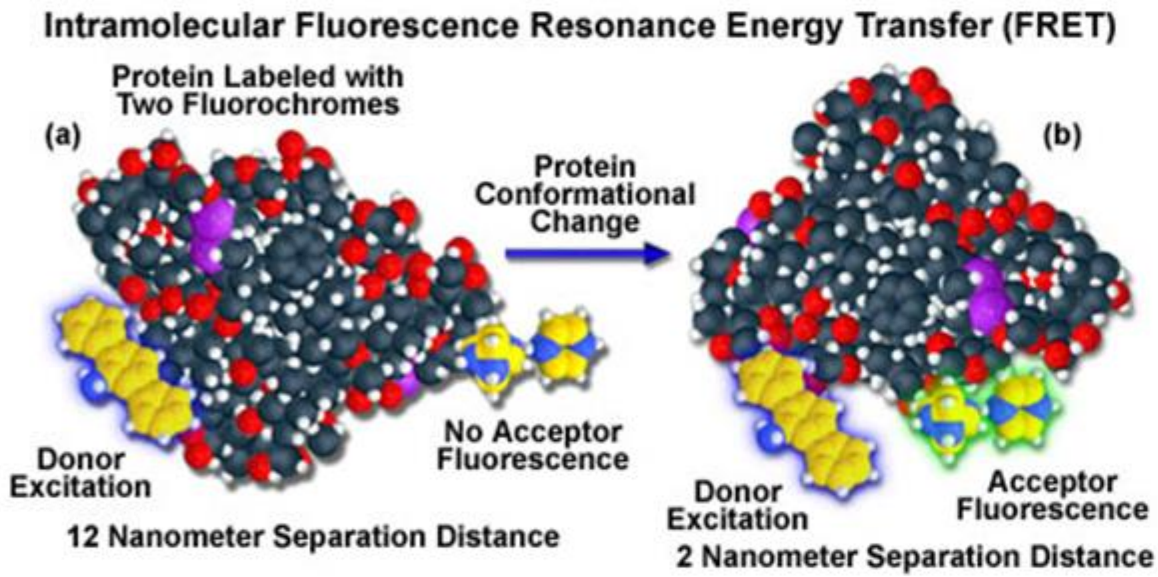


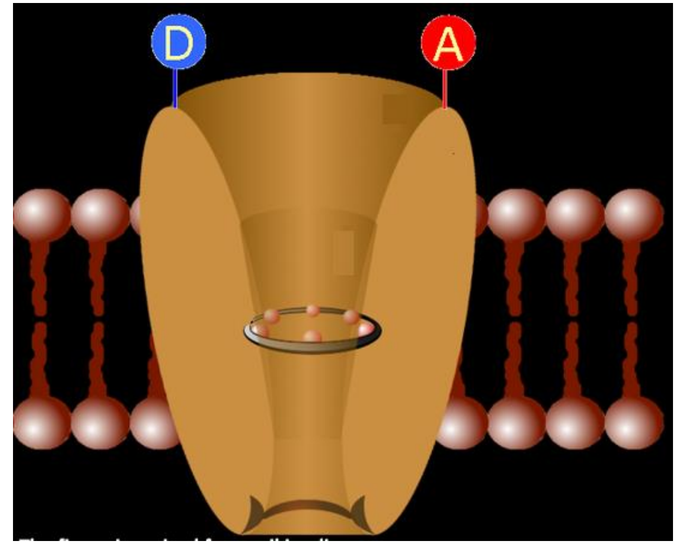
Quantitative analysis using FRET

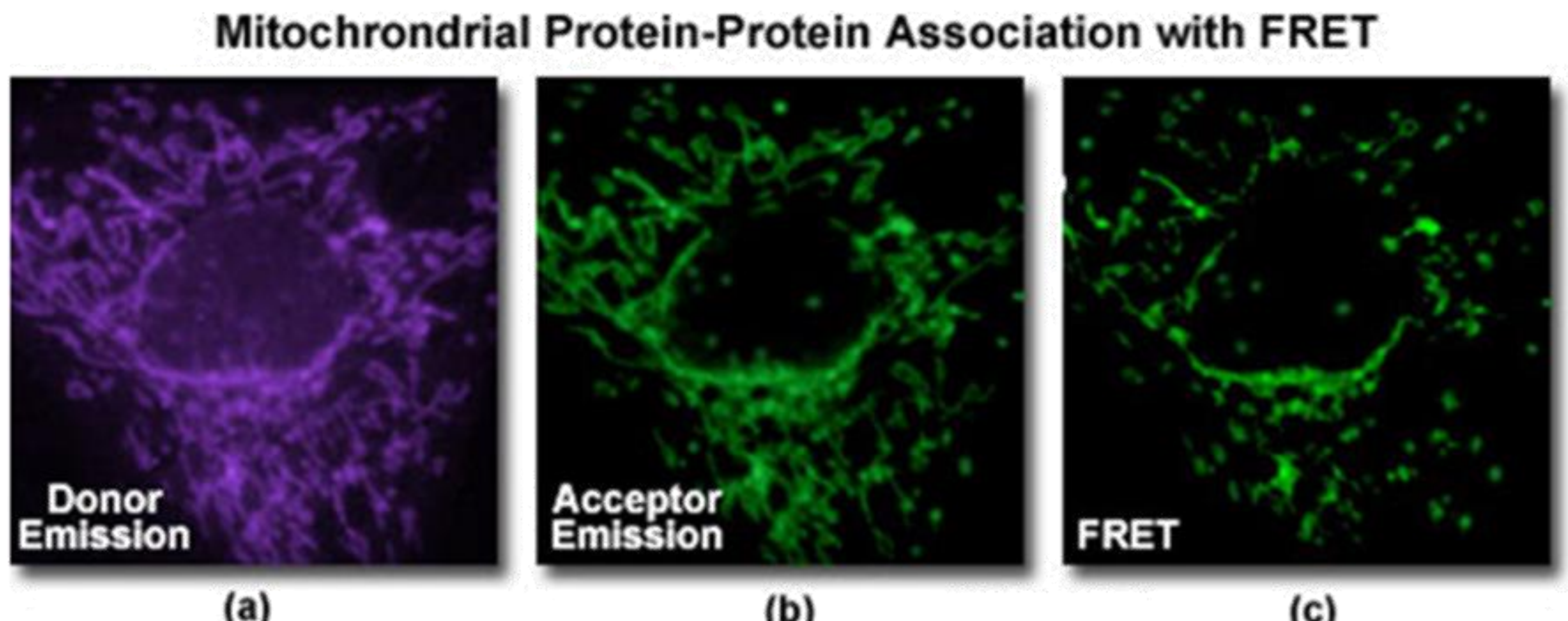


http://www.olympusfluoview.com/applications/fretintro.html

Quantitative analysis using FRET







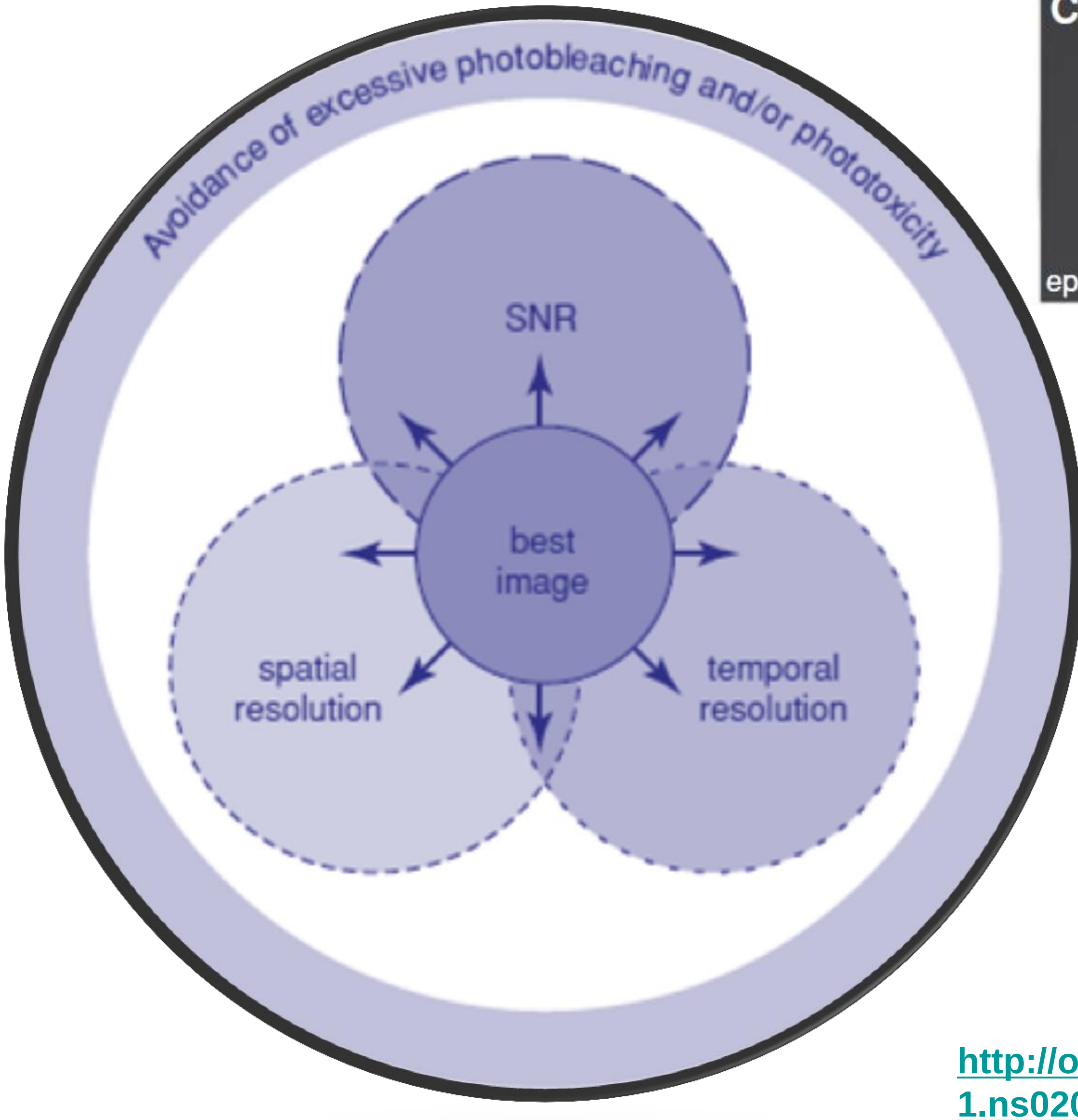
http://chemwiki.ucdavis.edu/Wikitexts/UCD_Chem_115/Chemwiki_Module_Topics/Fluorescence_Resonance_Energy_Transfer

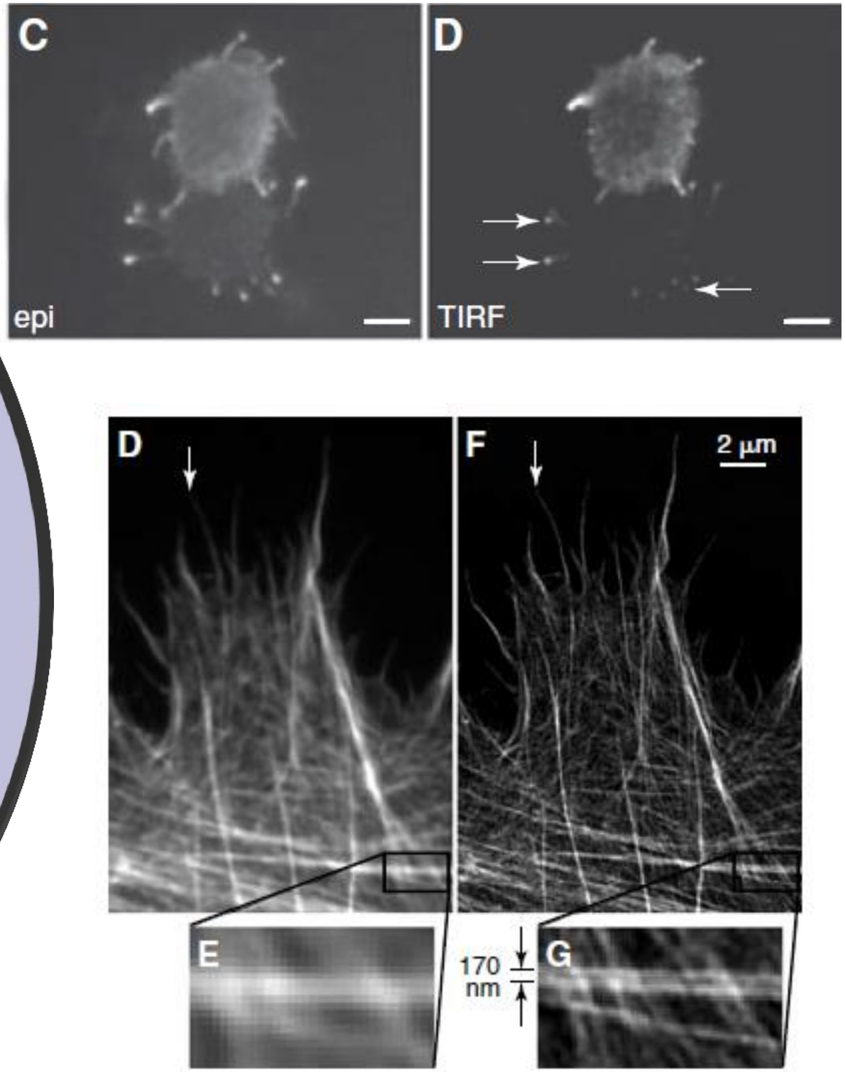
Part II

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

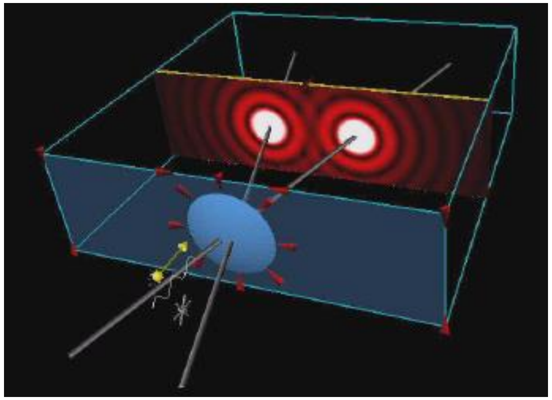




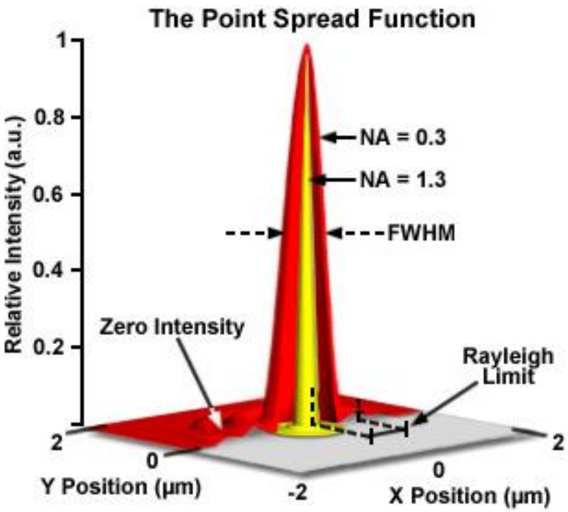
http://onlinelibrary.wiley.com/doi/10.1002/0471142301.ns0201s50/figures

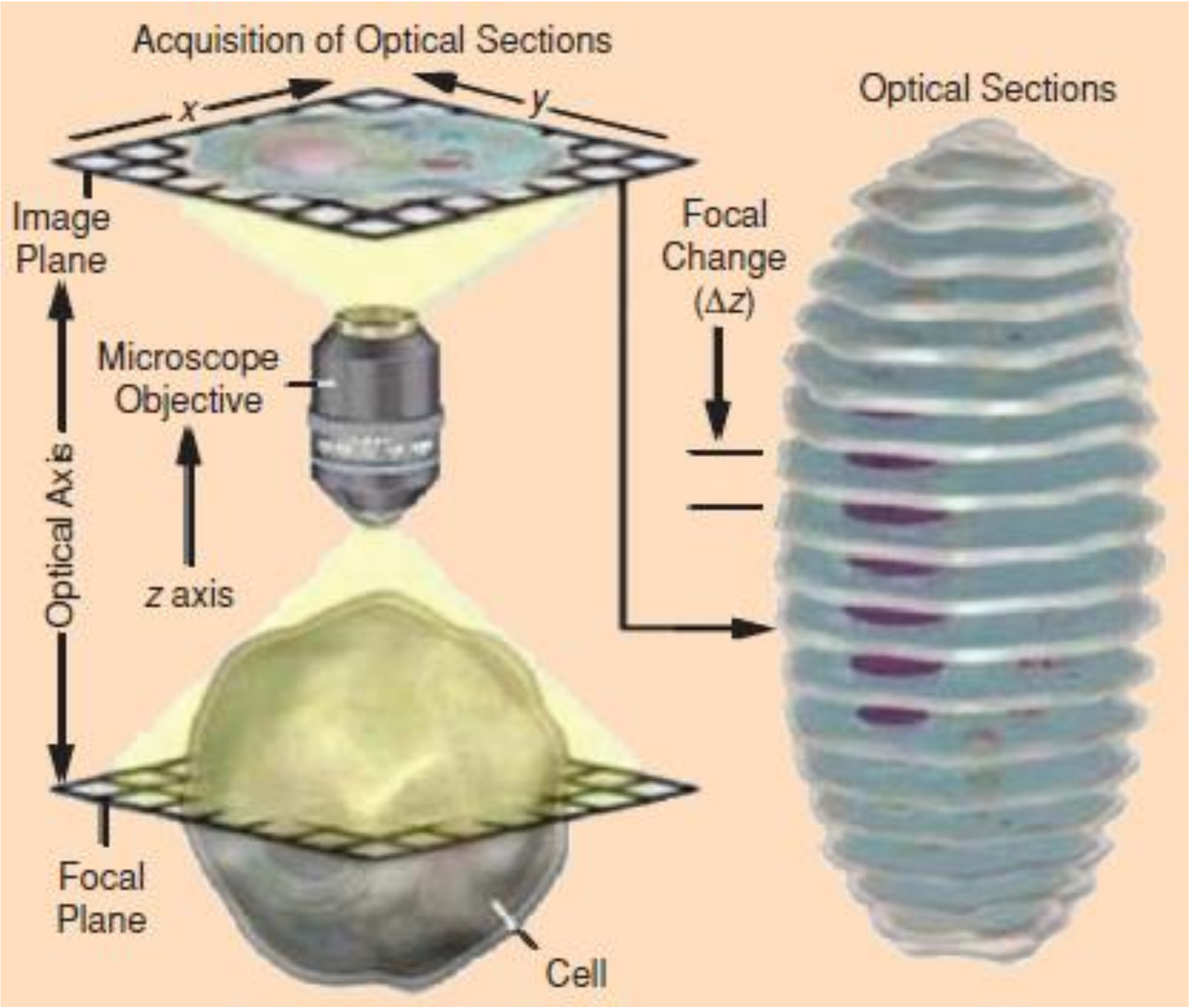
Physical Limits and Methods to Overcome

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



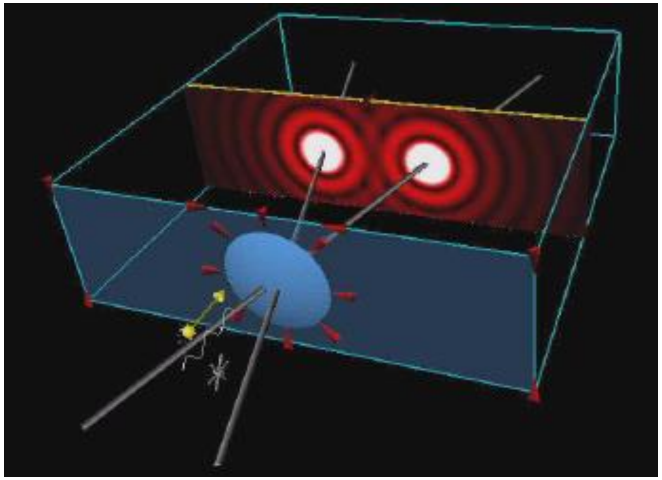
Point Spread Function (PSF)

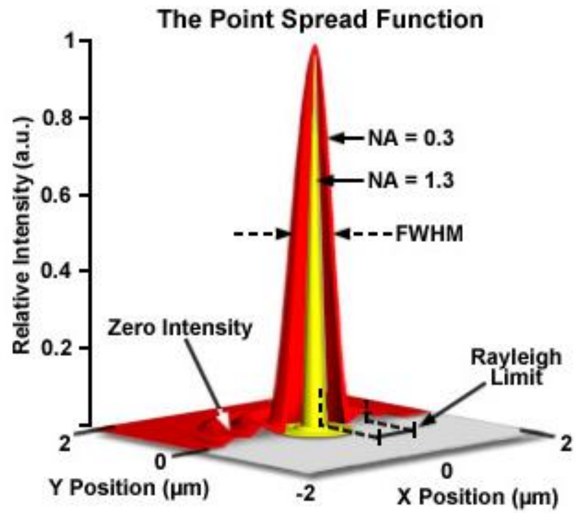


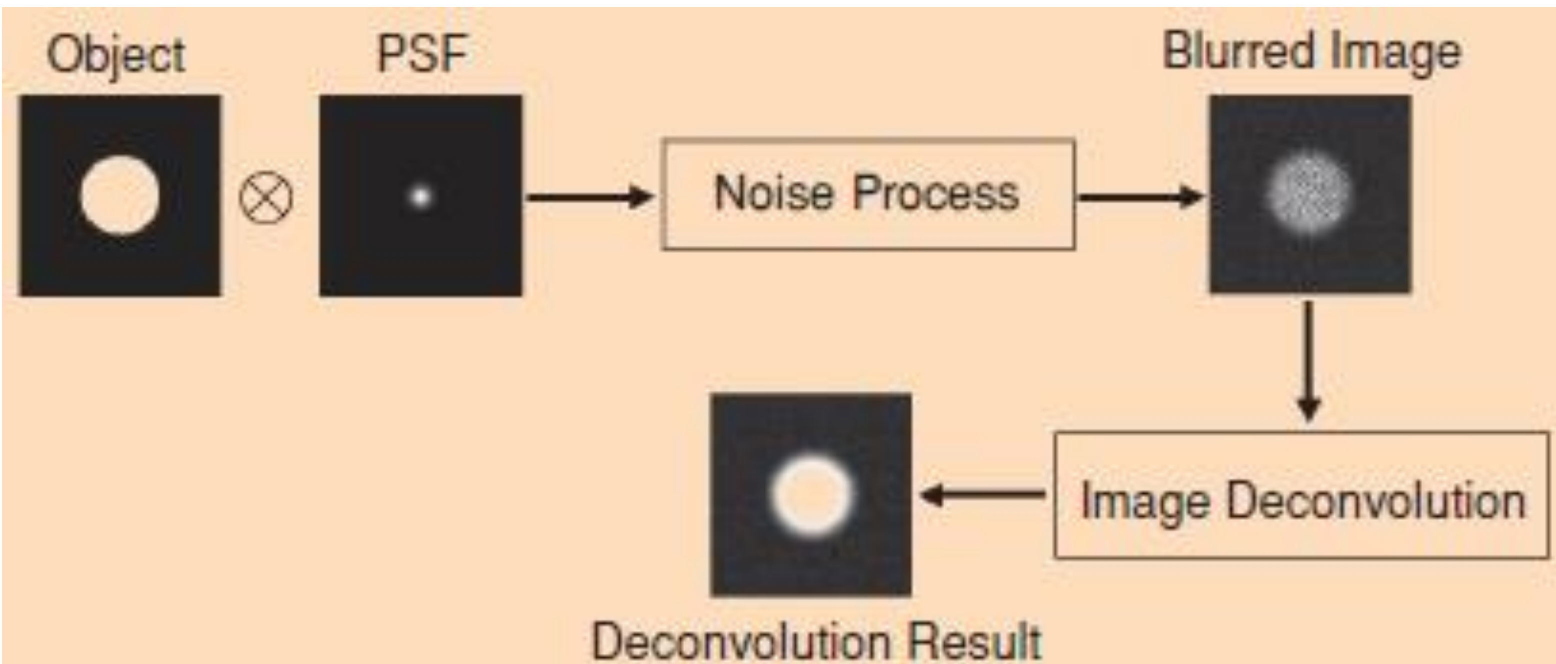


http://www.ese.wustl.edu/~nehorai/paper/deconvolutions1.pdf
http://www.olympusmicro.com/primer/digitalimaging/deconvolution/deconintro.html
http://zeiss-campus.magnet.fsu.edu/articles/basics/psf.html

Physical limits and methods to overcome

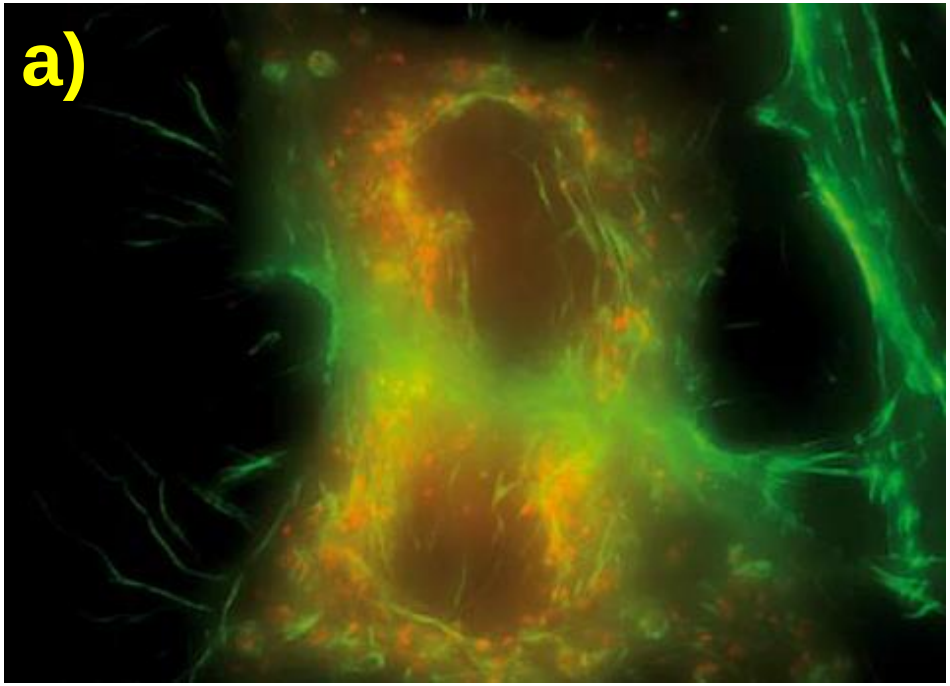


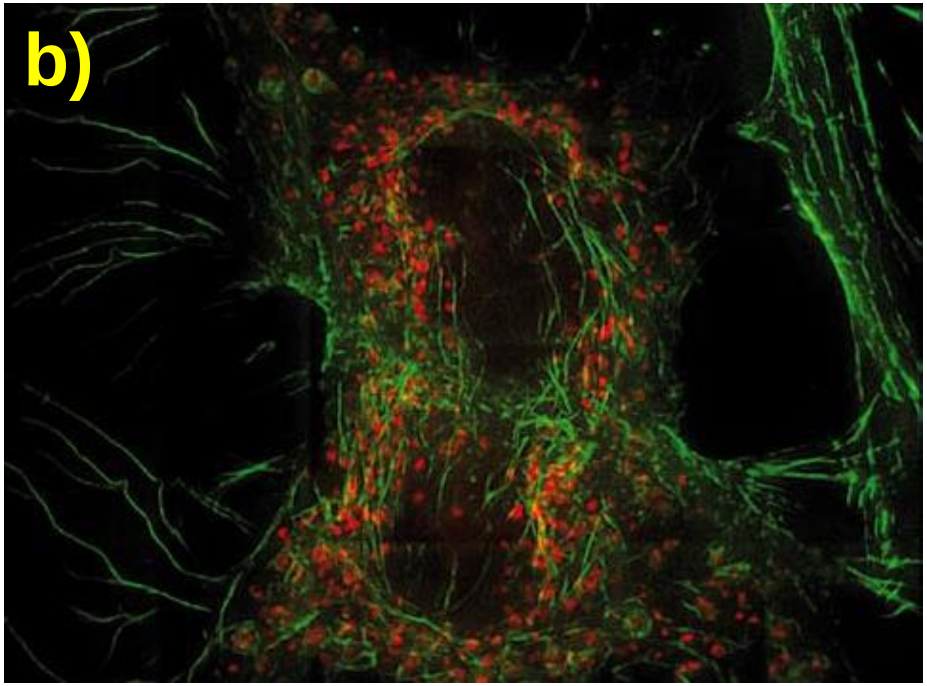




http://www.ese.wustl.edu/~nehorai/paper/deconvolutions1.pdf
http://www.mathworks.com/help/images/examples/deblurring-images-using-the-blind-deconvolution-algorithm.html

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

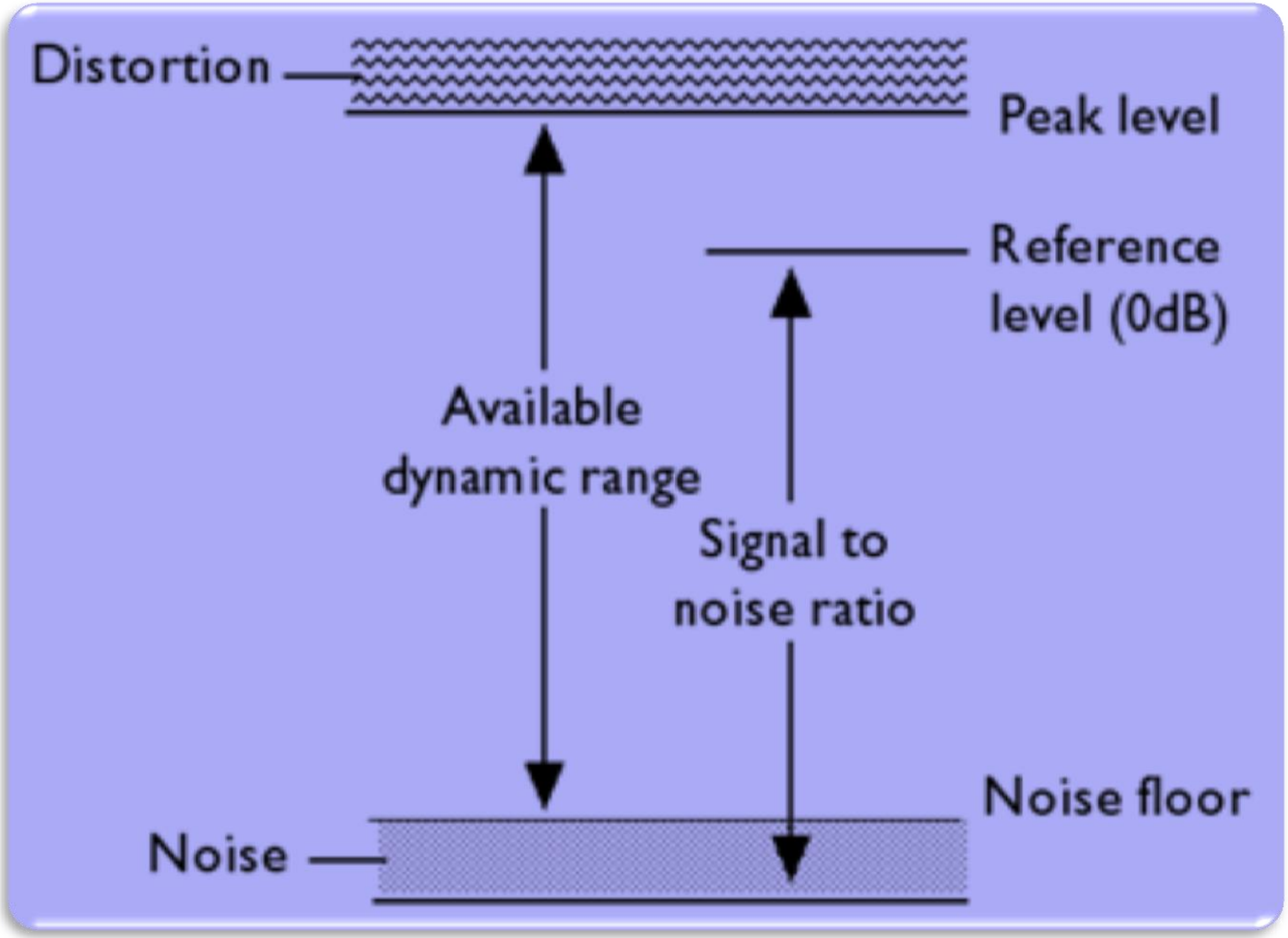
Part III

Digital Imaging in Microscope

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

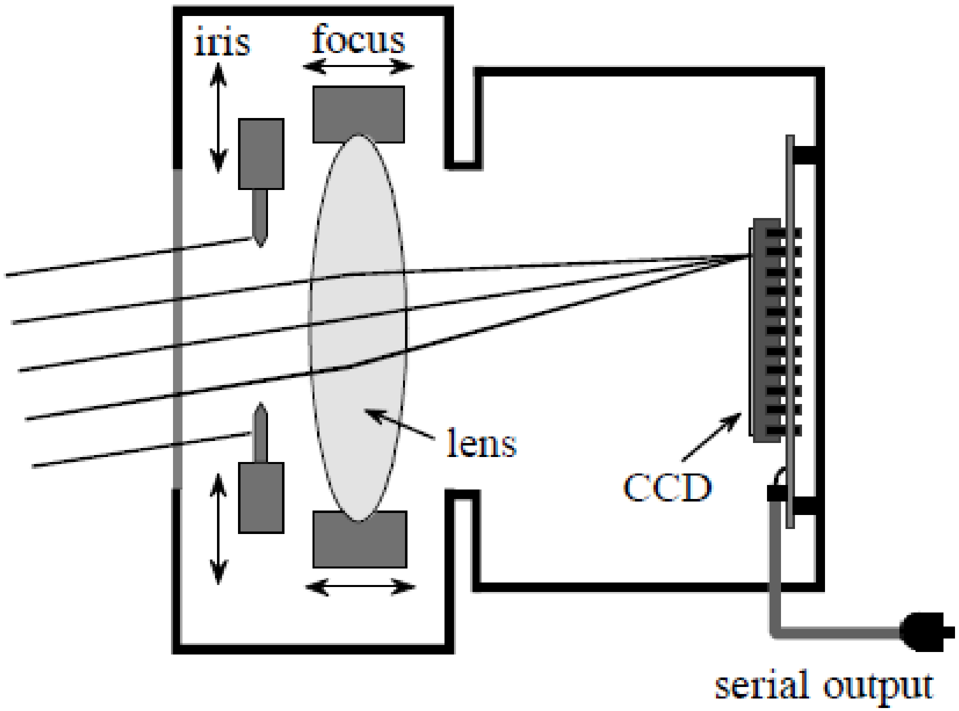
Dynamic Range vs. SNR

The dynamic range (動態範圍) of a charge-coupled device (CCD): the maximum achievable signal divided by the camera noise.



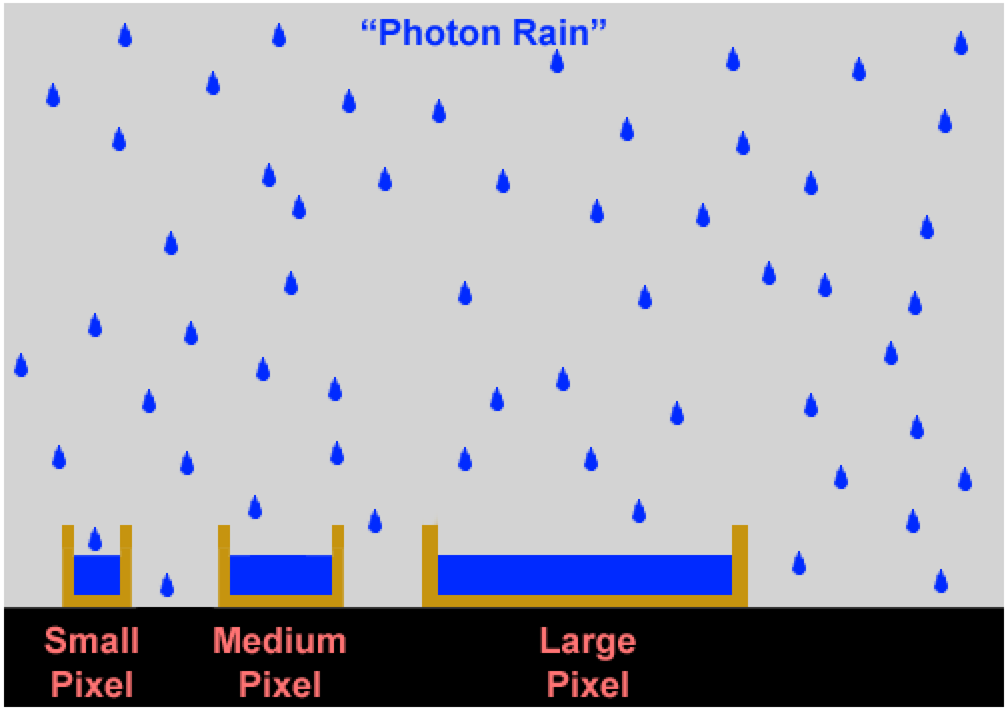
http://learn.hamamatsu.com/articles/dynamicrange.html
http://www.planetoftunes.com/record/dynamic_range.htm
http://pro.sony-asia.com/article/508446/section/articles

Diagram of an Electronic Camera



- ➢ The most common **imaging sensor** in present day cameras is the **CCD**, **a two-dimensional array of light sensitive elements**.
- ➢Focusing is achieved by moving the lens toward or away from the imaging sensor.
- ➢ The amount of light reaching the sensor is controlled by the iris, a mechanical device that changes the effective diameter of the lens.

Digital Cameras: Does Pixel Size Matter?



泊松分佈 (Poisson distribution)

$\therefore SNR = n/n^{1/2} = n^{1/2}$

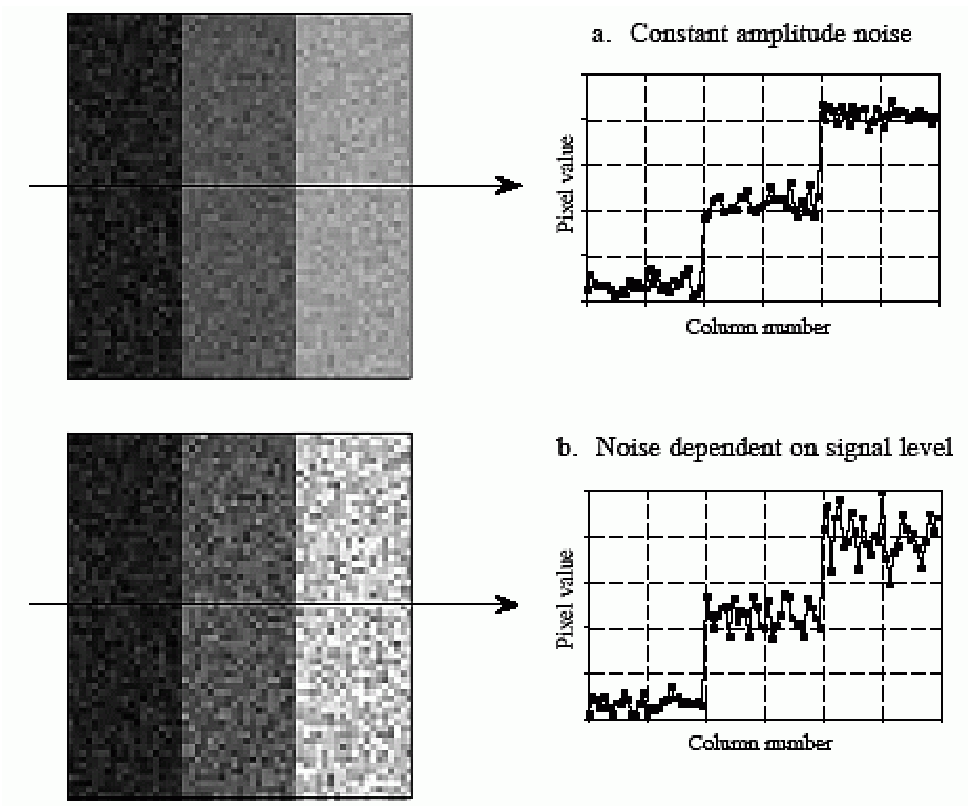
Photons	Noise	signal-to-noise
9	3	3
100	10	10
900	30	30
10000	100	100
40000	200	200

- In the physics of **photon counting**, the **noise** in the signal is equal to **the square root of the number of photons**.

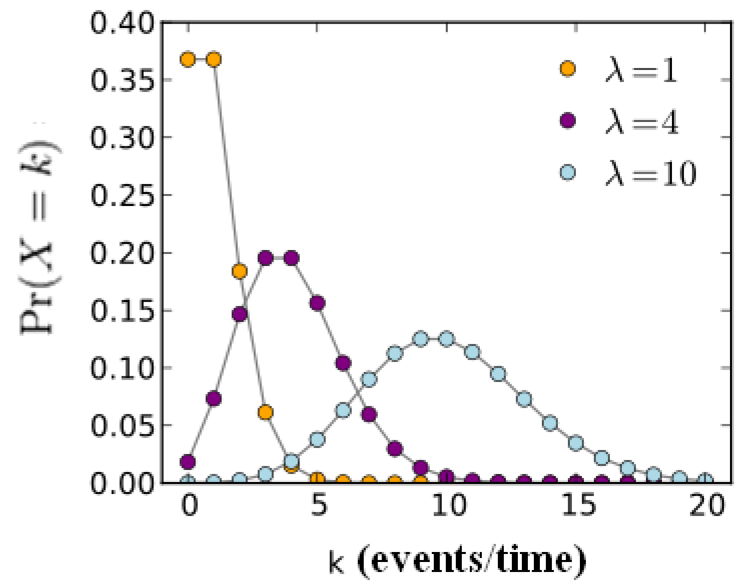
- The larger the bucket, the more drops that can be collected in a given amount of time.
- The accuracy of the signal measured is directly proportional to the size of the signal.

http://www.clarkvision.com/articles/does.pixel.size.matter/

Image Noise: shot noise (electronic noise)



泊松分佈 (Poisson distribution)



$$\Pr(X=k)=\frac{\lambda^k e^{-\lambda}}{k!},$$

λ:平均發生率(mean rate)
→mean events/time

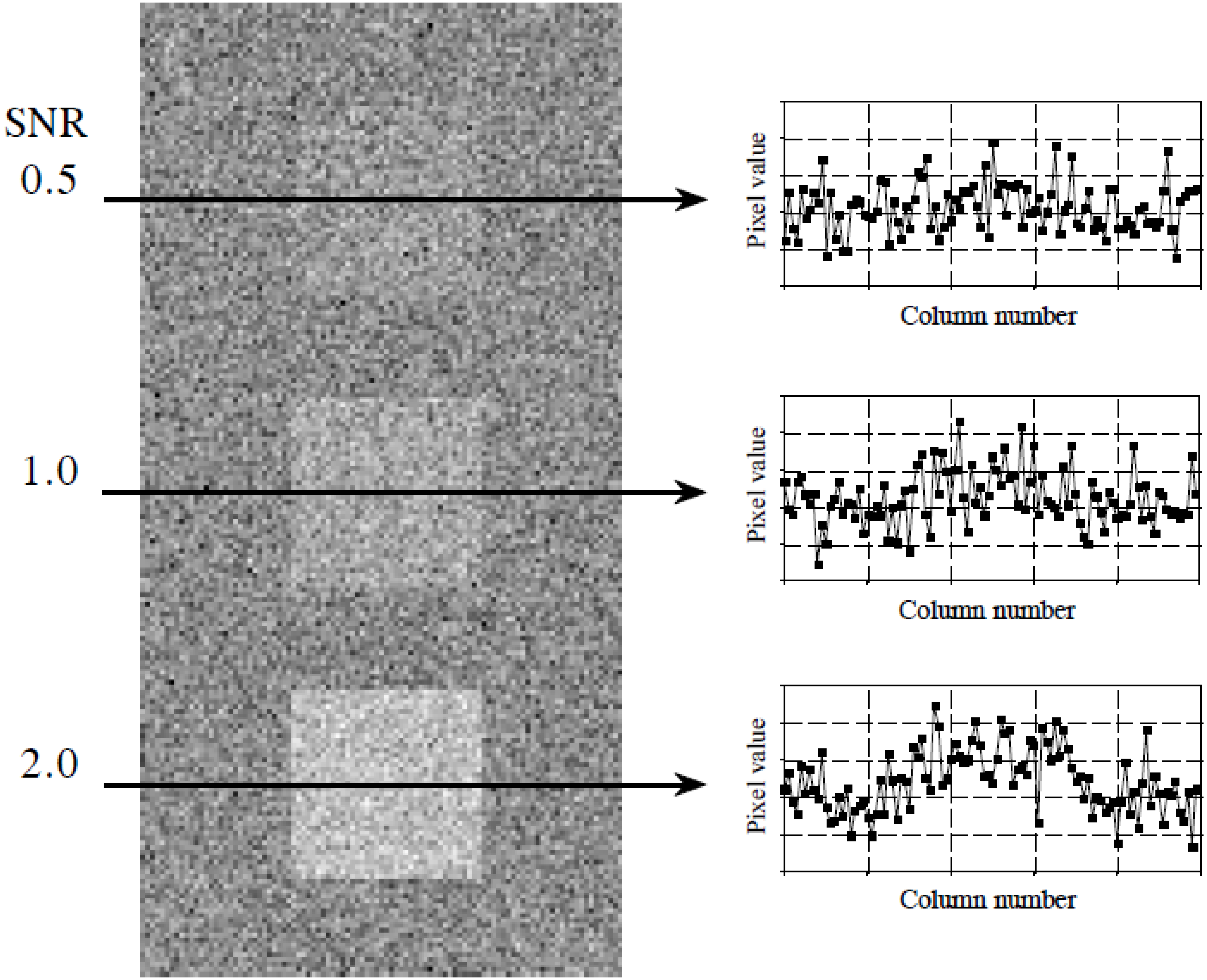
Random noise in images takes two general forms.

➢**In (a), the amplitude of the noise remains constant as the signal level changes. This is typical of electronic noise.**

➢**In (b), the amplitude of the noise increases as the square-root of the signal level. This type of noise originates from the detection of a small number of particles, such as light photons, electrons, or x-rays.**

http://en.wikipedia.org/wiki/Shot_noise

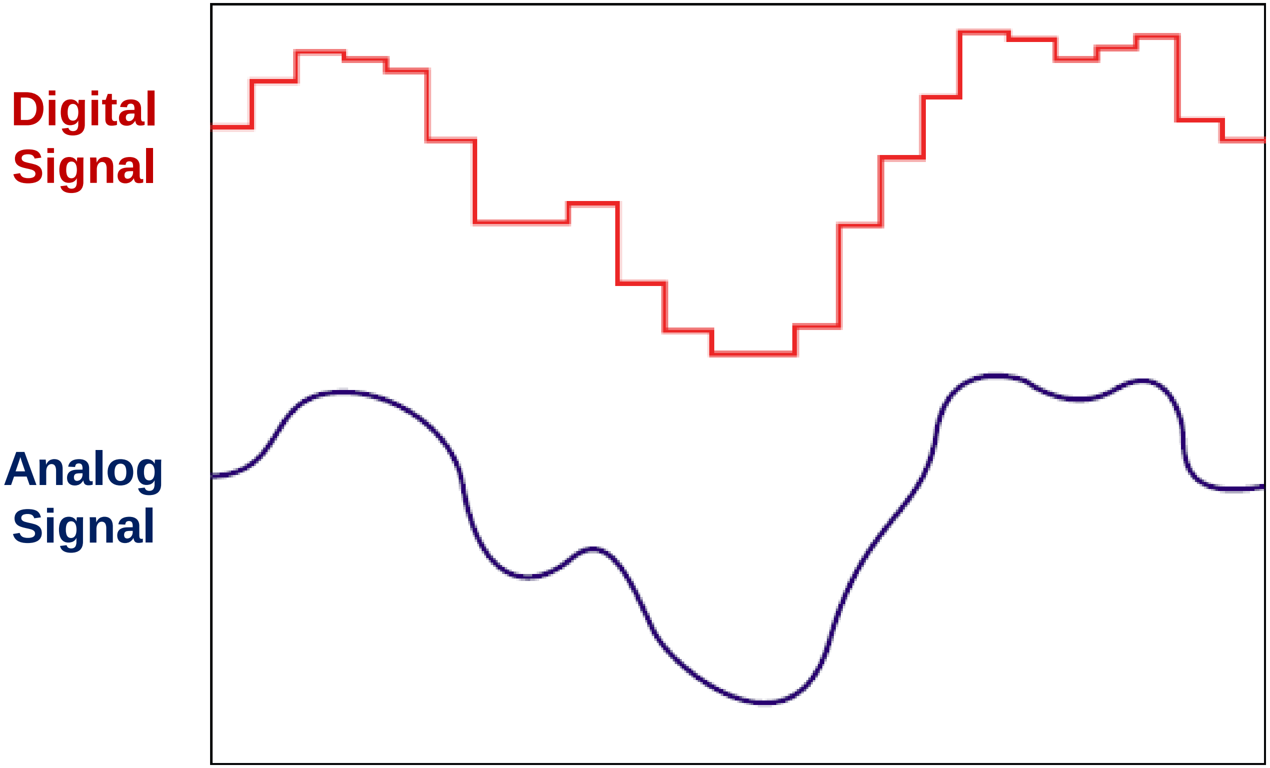
Signal-to-noise ratio (SNR)



- An object is visible in an image only if its contrast is large enough to overcome the random image noise.

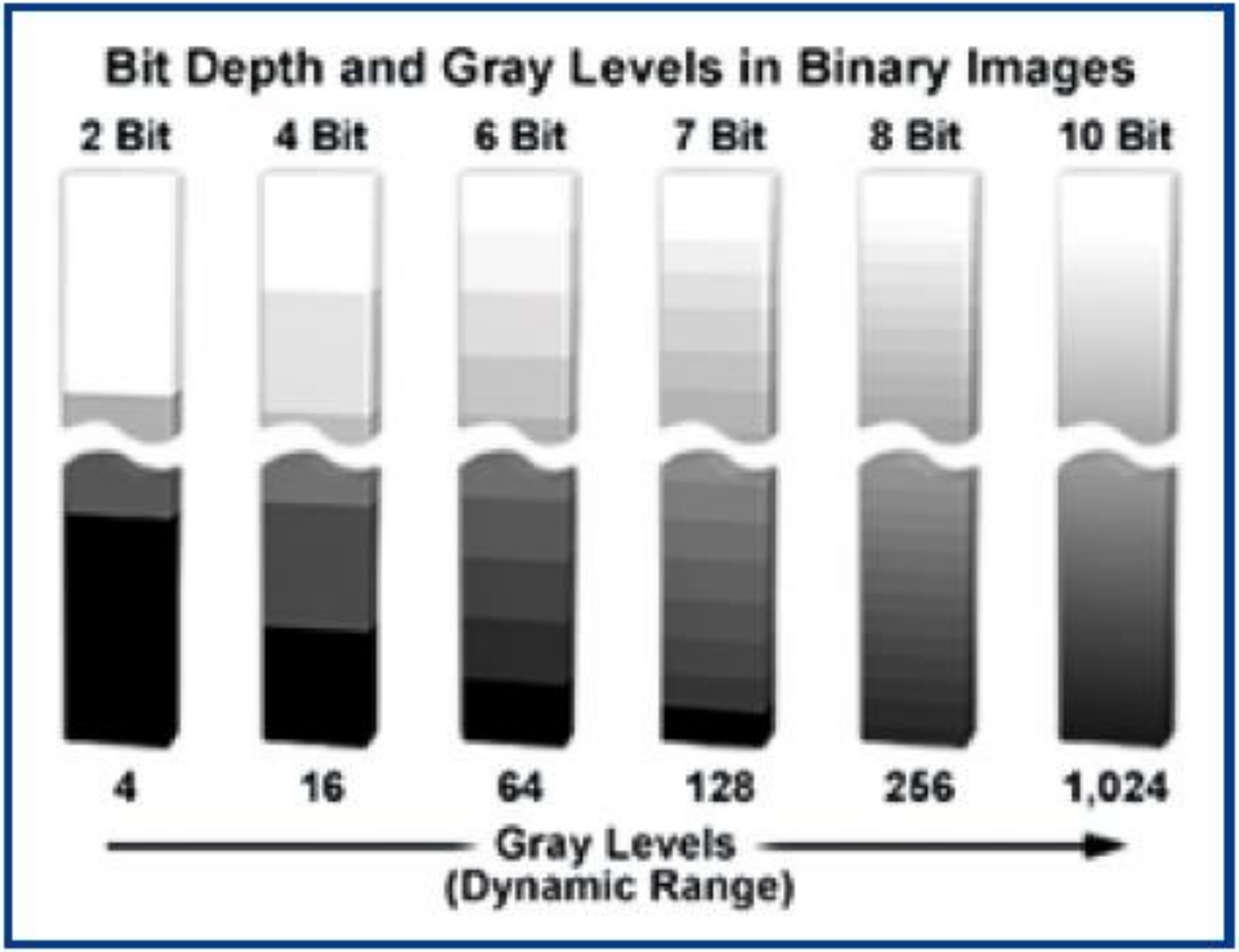
Basic Imaging Concepts

Digital Image Acquisition: Analog to Digital Conversion

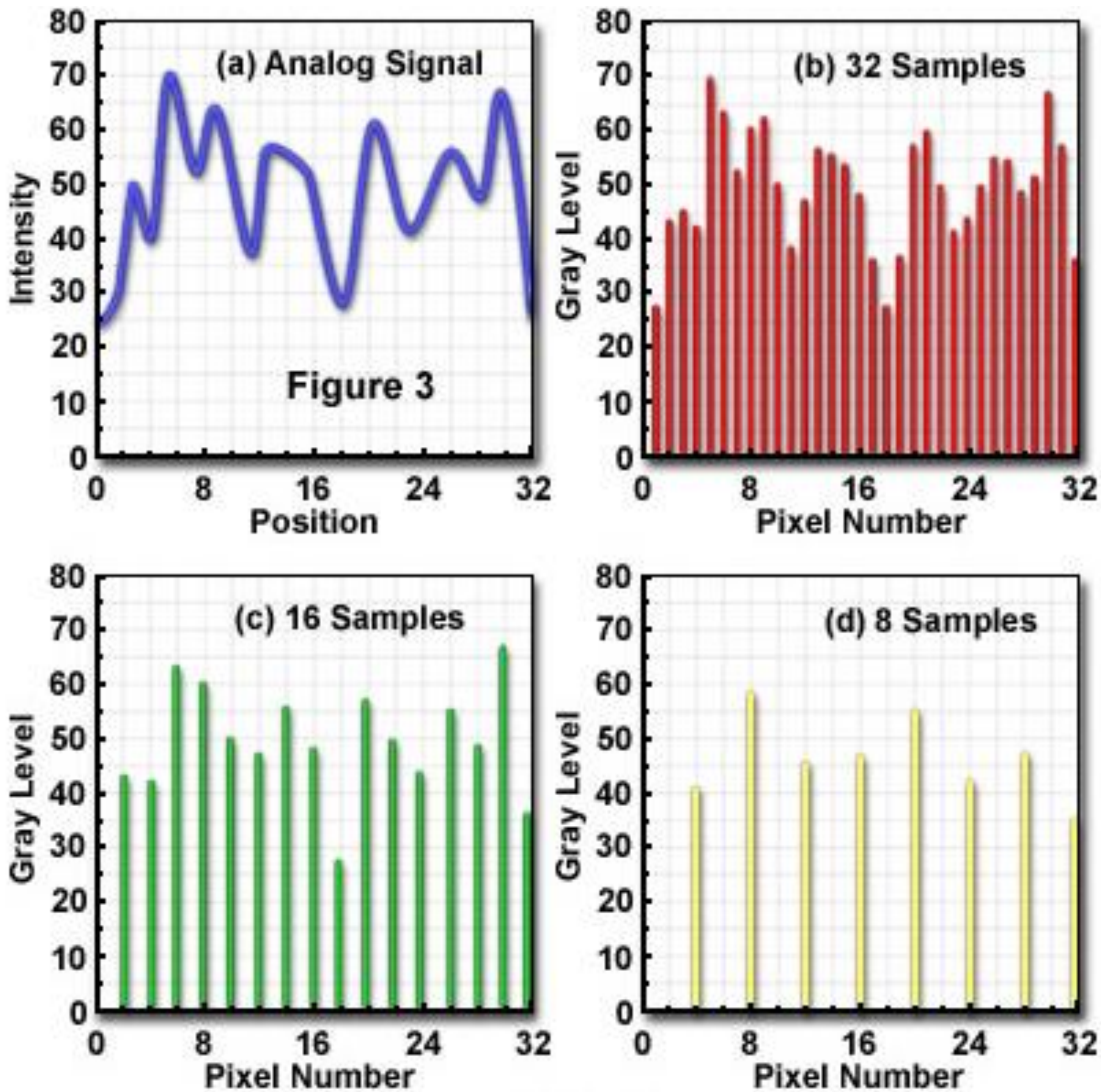


Digitization of an analog voltage signal along one line in an image (blue) produces a series of values that correspond to a series of steps (red) equal in time and rounded to integral multiples of the smallest measurable increment.

Bit depth and grey levels in digital images

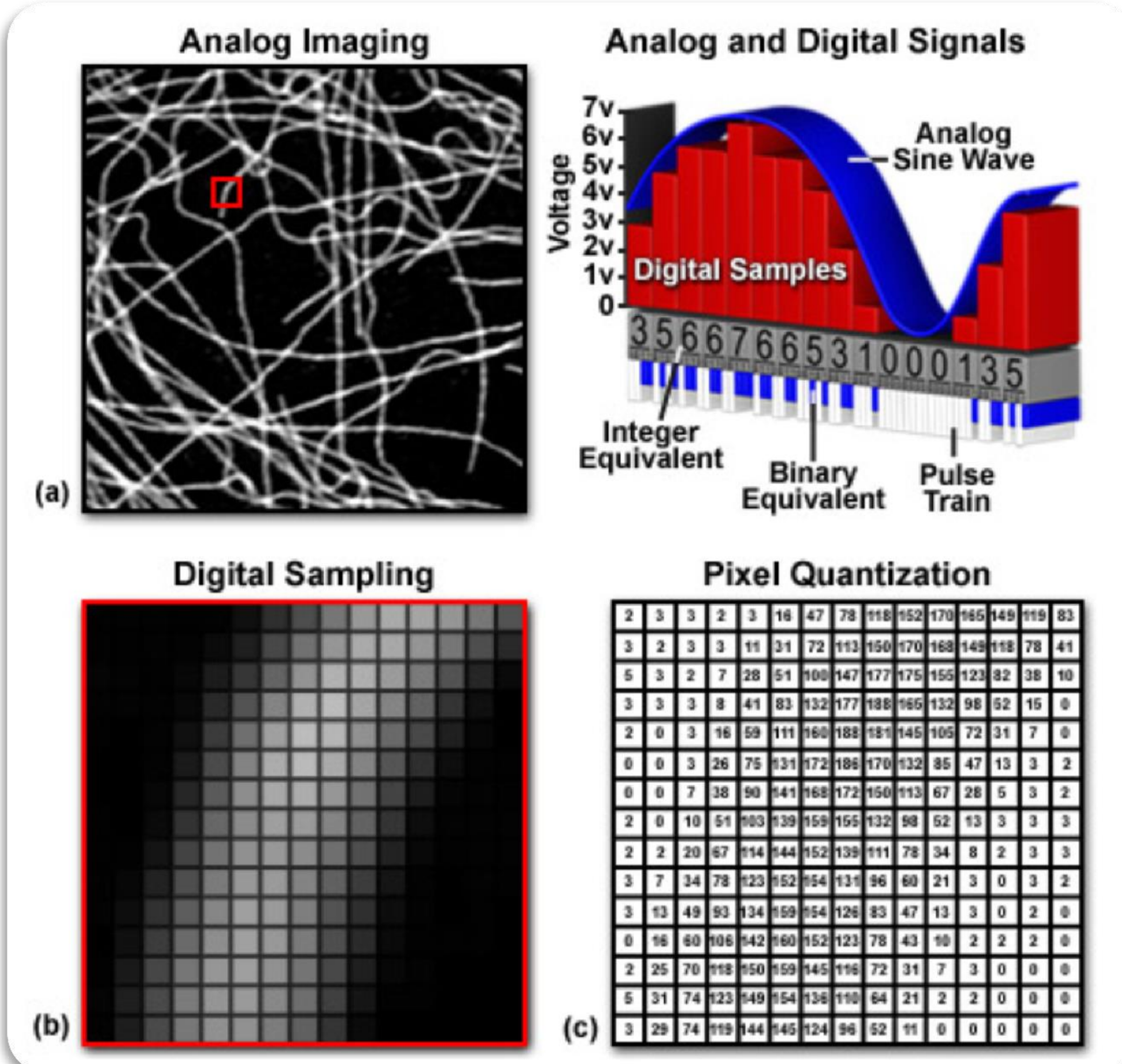


Bit depth and grey levels in digital images



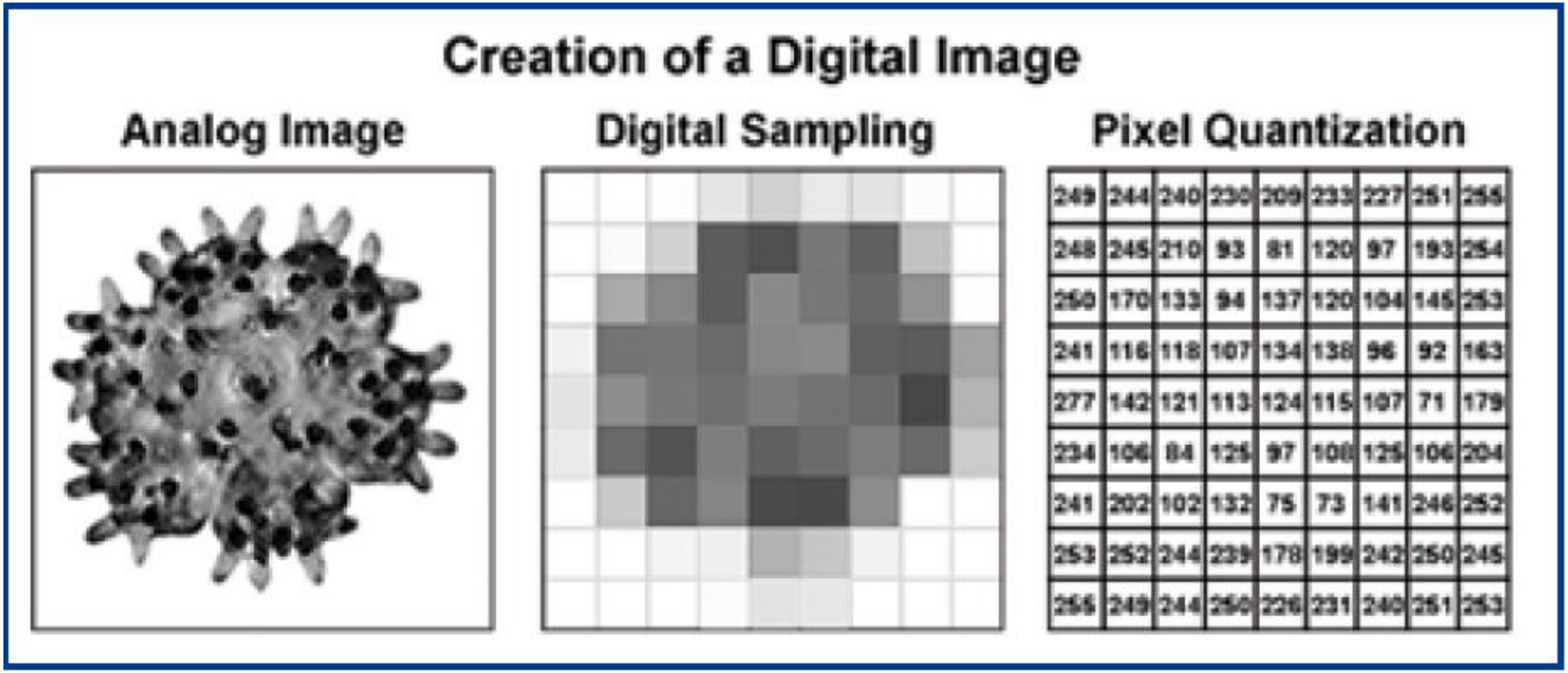
Basic Imaging Concepts

Digital Image Acquisition: Analog to Digital Conversion



2	3	3	2	3	16	47	78	118	152	170	165	149	119	83
3	2	3	3	11	31	72	113	150	170	168	149	118	78	41
5	3	2	7	28	51	100	147	177	175	155	123	82	38	10
3	3	3	8	41	83	132	177	188	166	132	98	52	15	0
2	0	3	16	59	111	160	188	181	145	105	72	31	7	0
0	0	3	26	75	131	172	186	170	132	85	47	13	3	2
0	0	7	38	90	141	168	172	150	113	67	28	5	3	2
2	0	10	51	103	139	159	155	132	98	52	13	3	3	3
2	2	20	67	114	144	152	139	111	78	34	8	2	3	3
3	7	34	78	123	152	154	131	96	60	21	3	0	3	2
3	13	49	93	134	159	154	126	83	47	13	3	0	2	0
0	16	60	106	142	160	152	123	78	43	10	2	2	2	0
2	25	70	118	150	159	145	116	72	31	7	3	0	0	0
5	31	74	123	149	154	136	110	64	21	2	2	0	0	0
3	29	74	119	144	145	124	96	52	11	0	0	0	0	0

Creation of a Digital Image



Resolution in Digital Images – is it important?

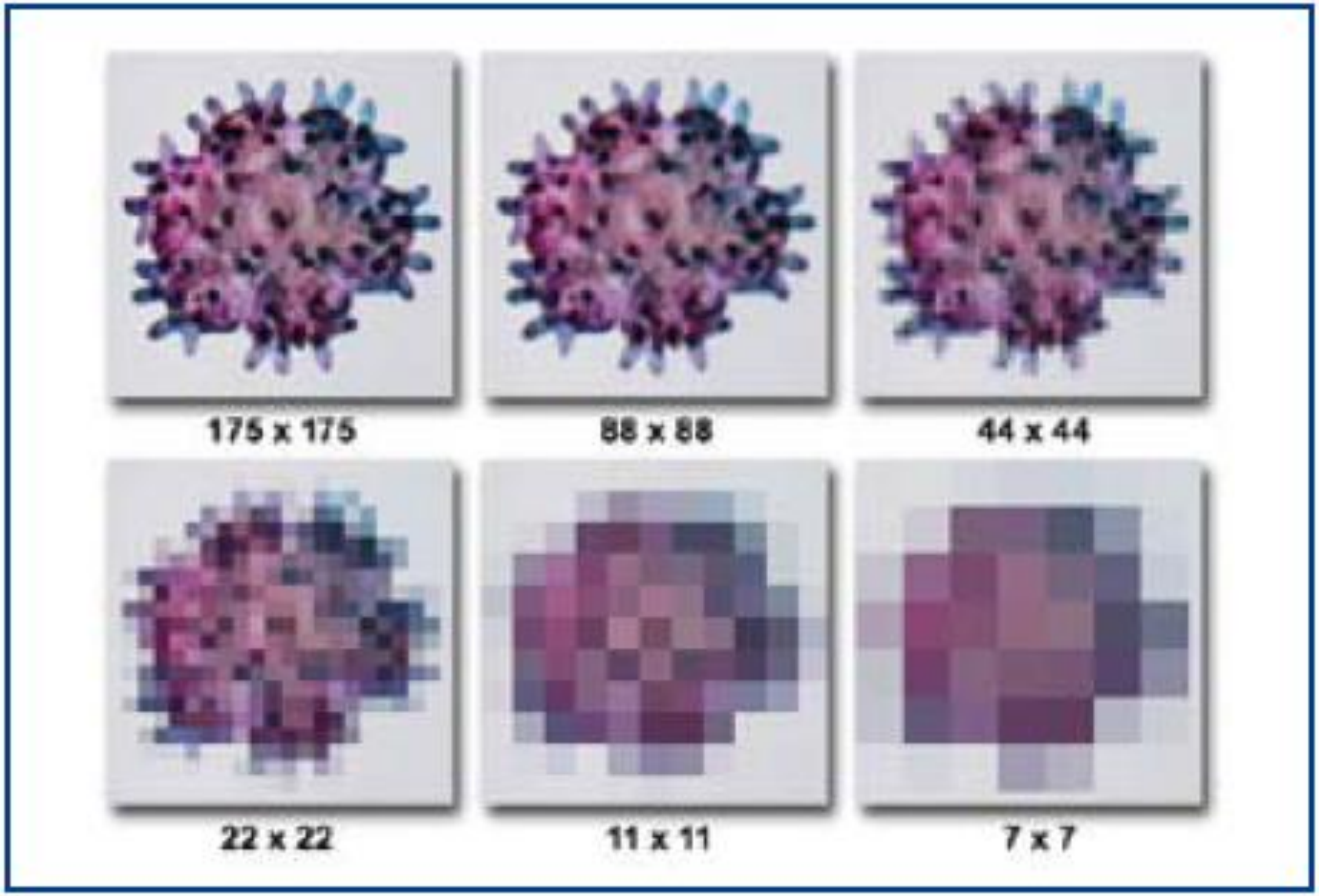


Fig. 17: Four representations of the same image, with different numbers of pixels used. The numbers of pixels is written below each image.

Four representations of the same image, showing a variation in the number of pixels used. In all cases, a full 256 gray values are retained. Each step in coarsening of the image is accomplished by averaging the brightness of the region covered by the larger pixels.

Four representations of the same image, with variation in the number of gray levels used. From the upper left: 32; 16; 8; 4. In all cases, a full 256 × 256 array of pixels is retained. Each step in the coarsening of the image is accomplished by rounding the brightness of the original pixel value.

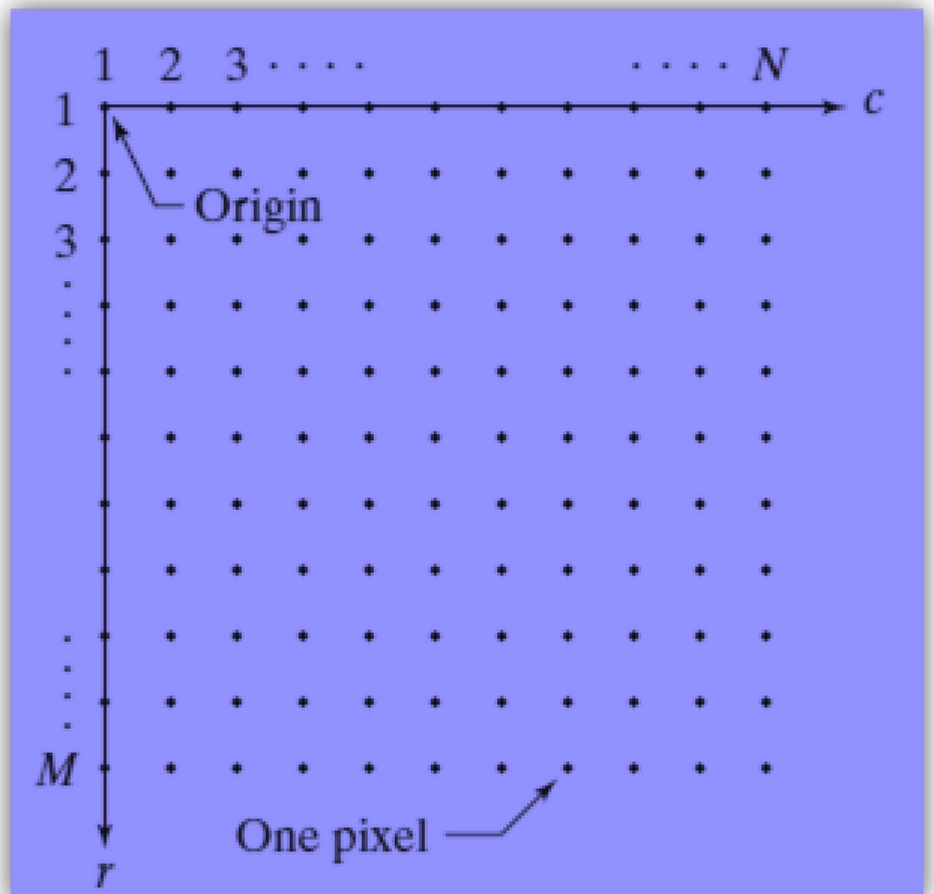
Part IV

The Fundamentals of Imaging Processing

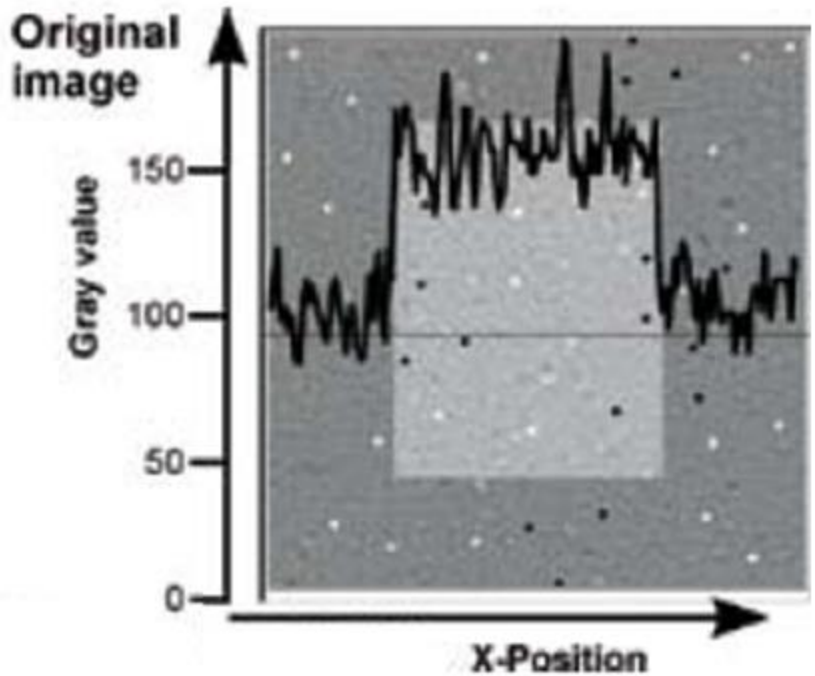
Hubble Telescope—Deep Space Photo http://www.hubblesite.org

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

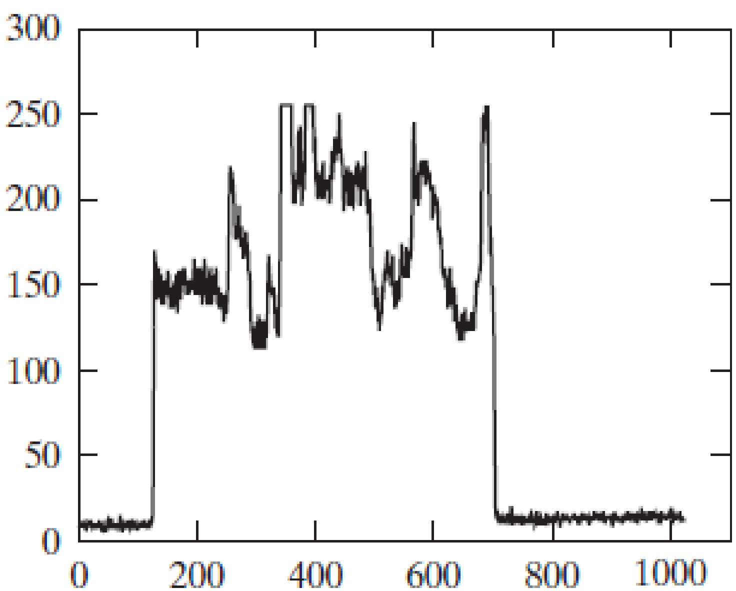
```
A =

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

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

     1     2     3
     4     5     6
```

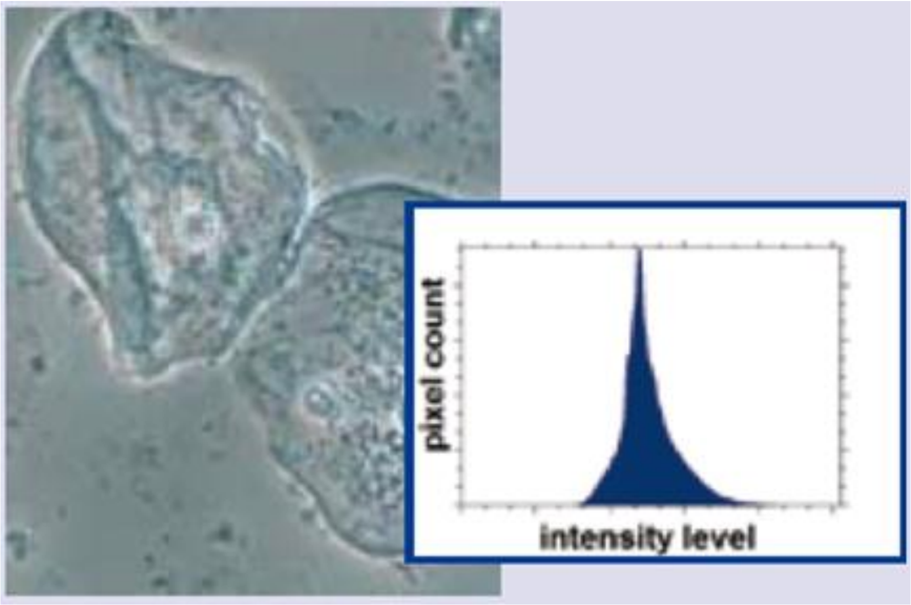
Coordinate Convention in the Image Processing

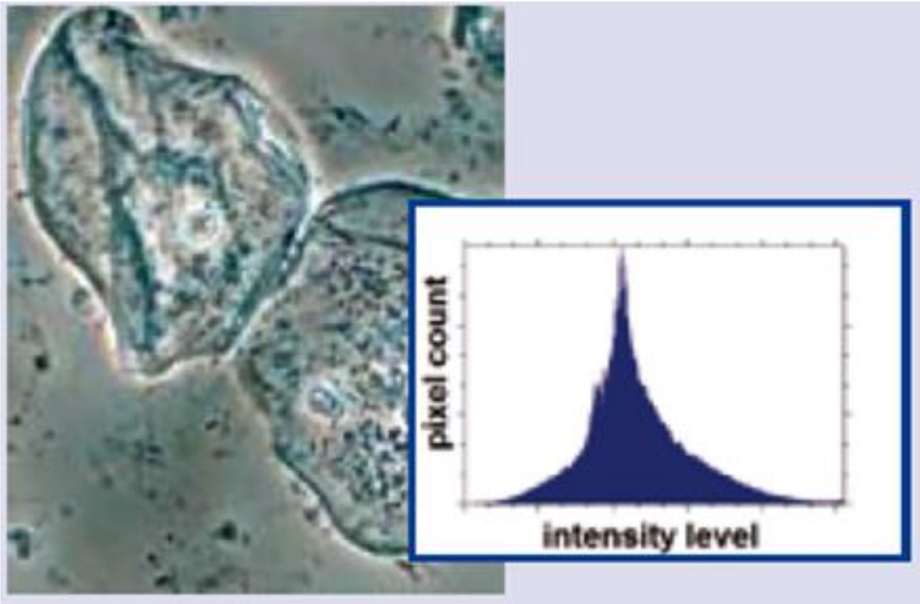


a b c
d
e

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).

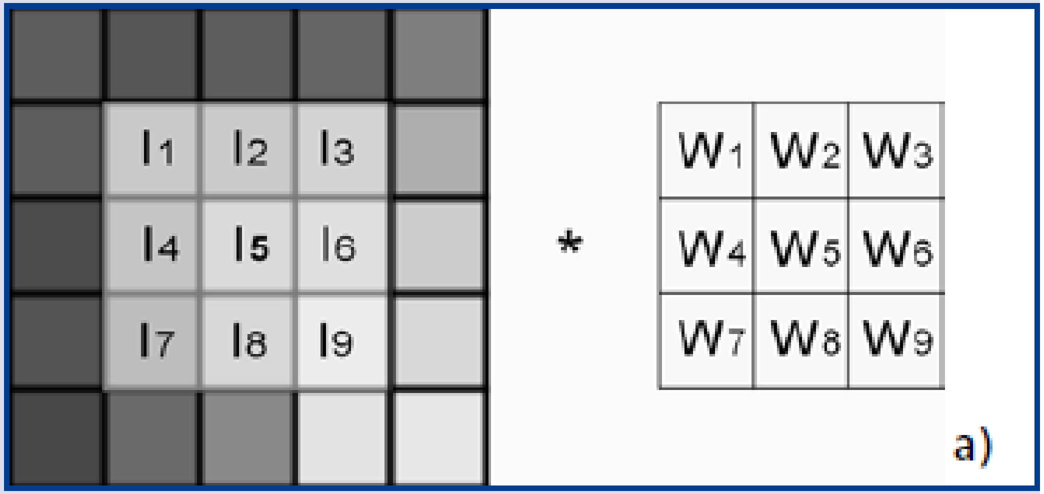
Histogram Optimisation During Acquisition





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

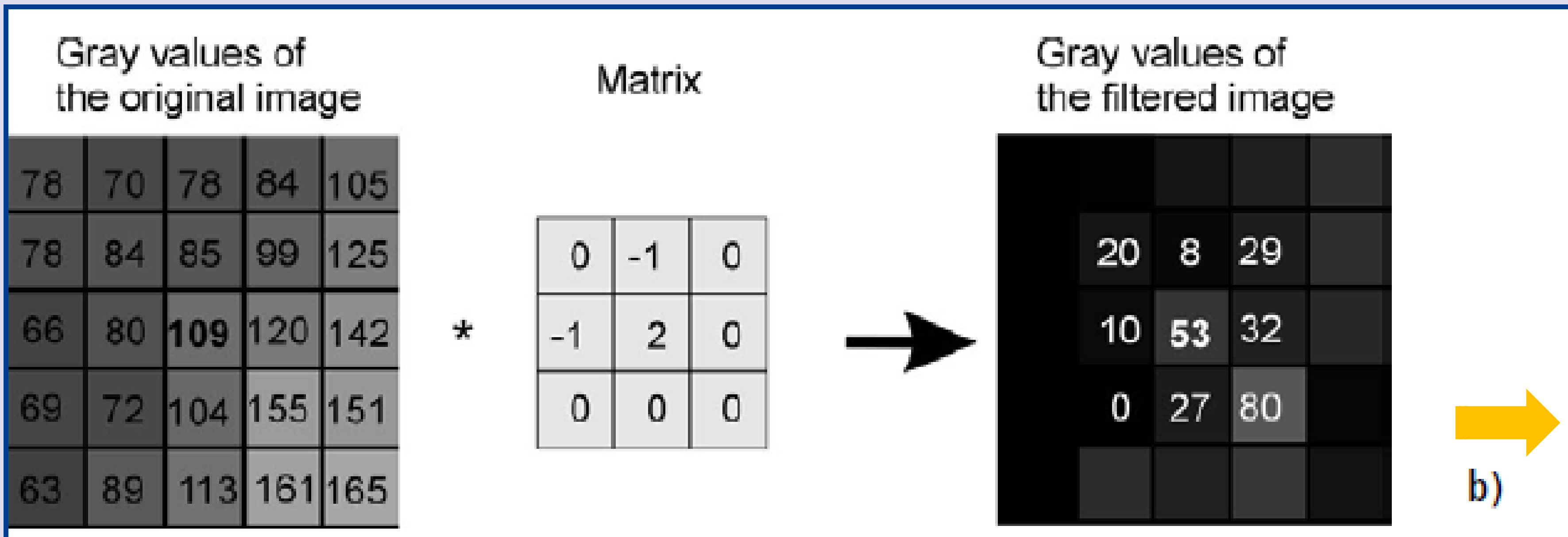
How to describe image processing operations mathematically



Local Operations:

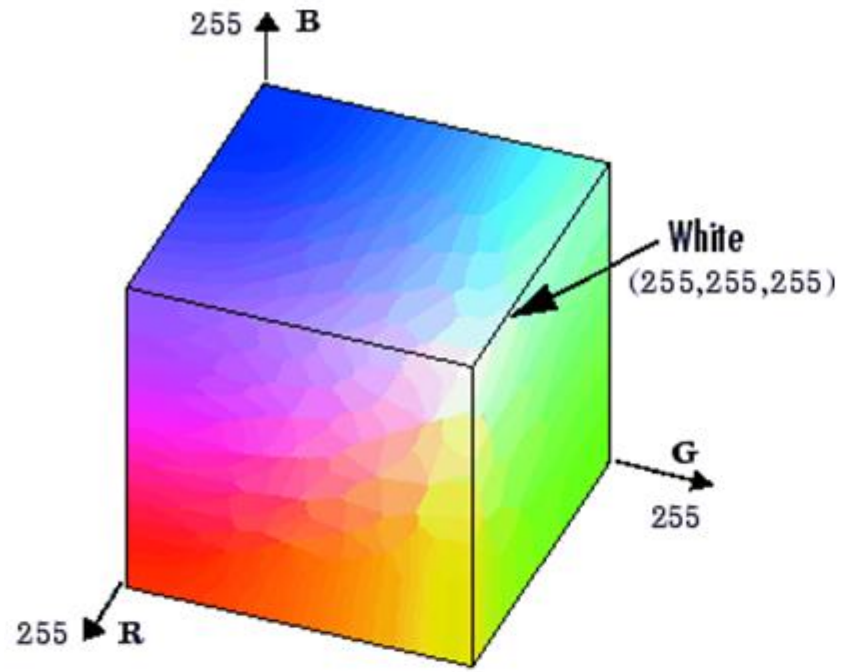
Many well known noise reduction, sharpening and edge enhancing filters

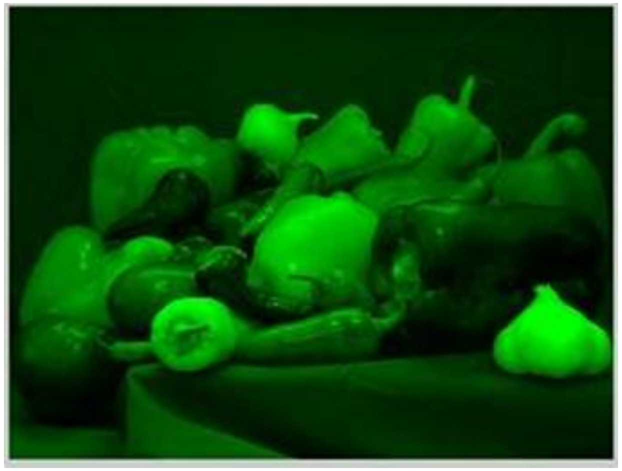
are convolution filters.



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

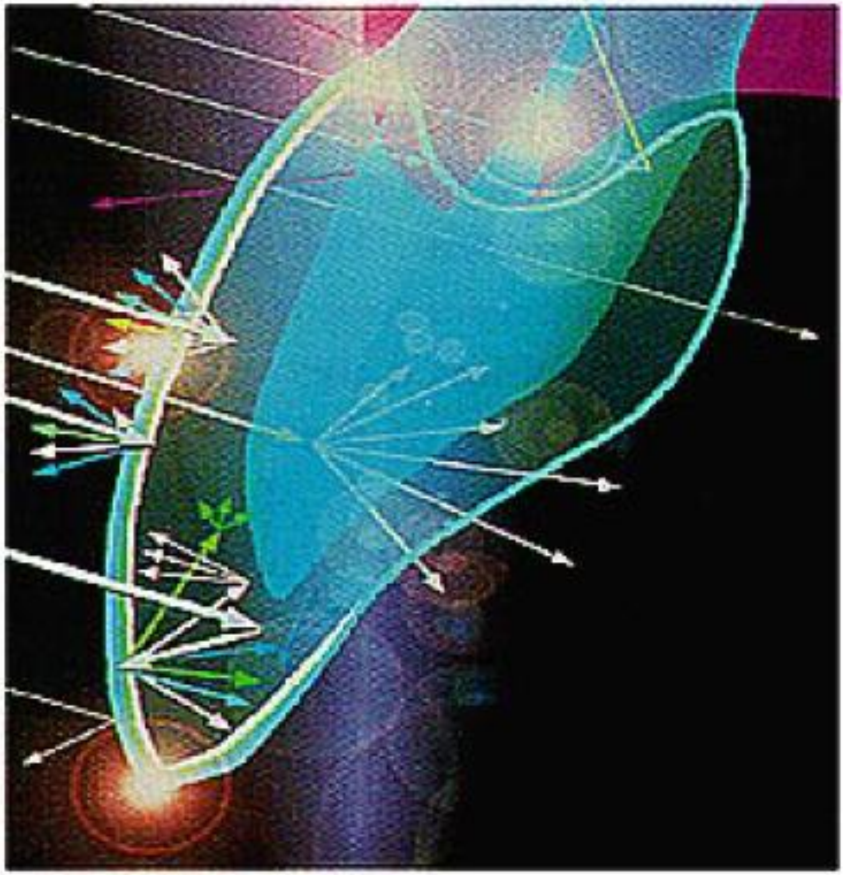
RGB Image Decomposition





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

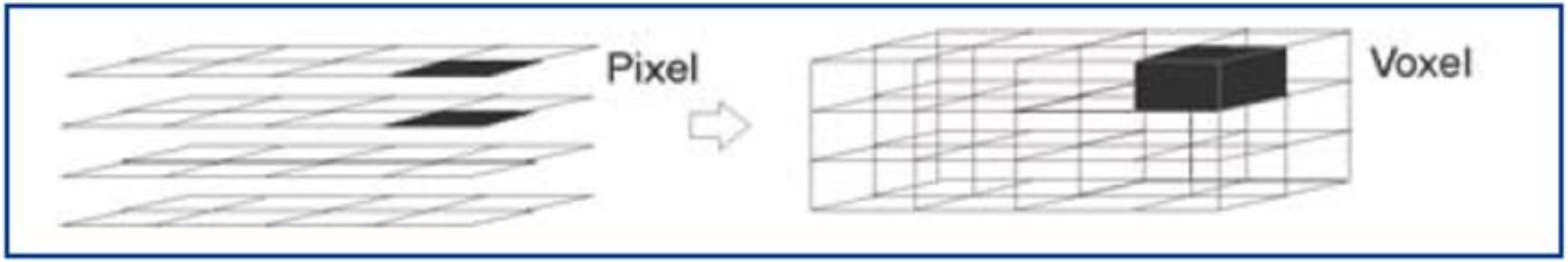
Visual Shade Matching

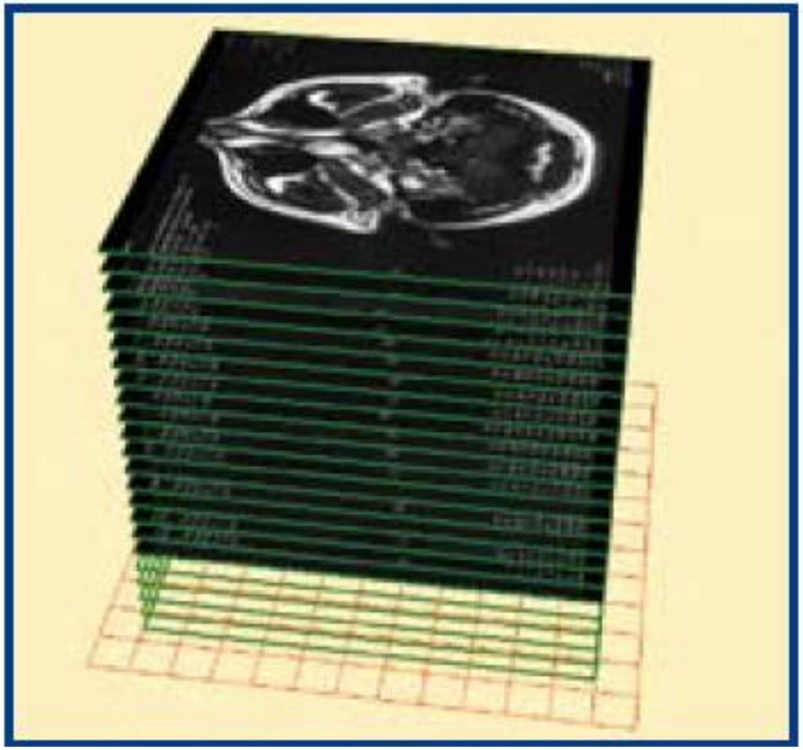


The appearance of a tooth is the summation of light that is reflected, transmitted, and fluoresced.

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

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

Thanks For Your Attention

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