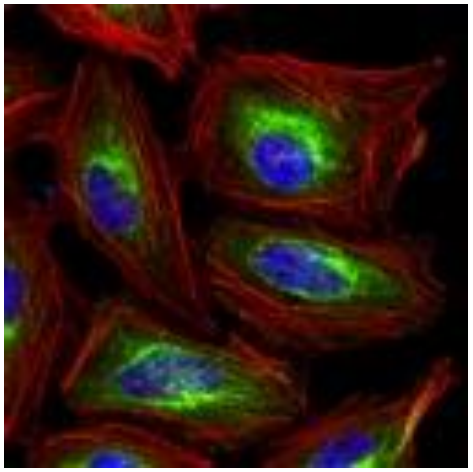
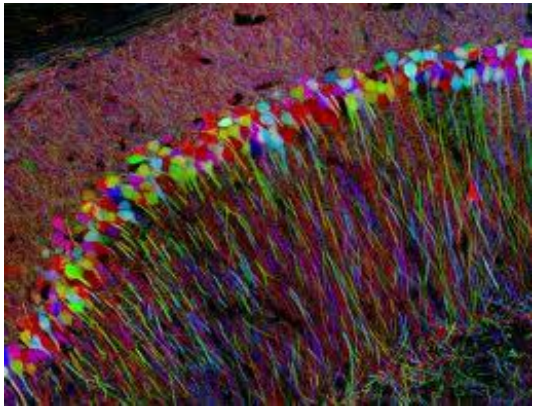
Lecture 03

Quantitative Methods for Biological Systems





Matlab - Matrix Laboratory

- It is numerical computing program.
- Used for data analysis, plotting, building functions, simulation etc.
- Easy to use

```
a = [7 8 9]

b = [1;2;3]

c = [1 2 3;4 5 6]
```

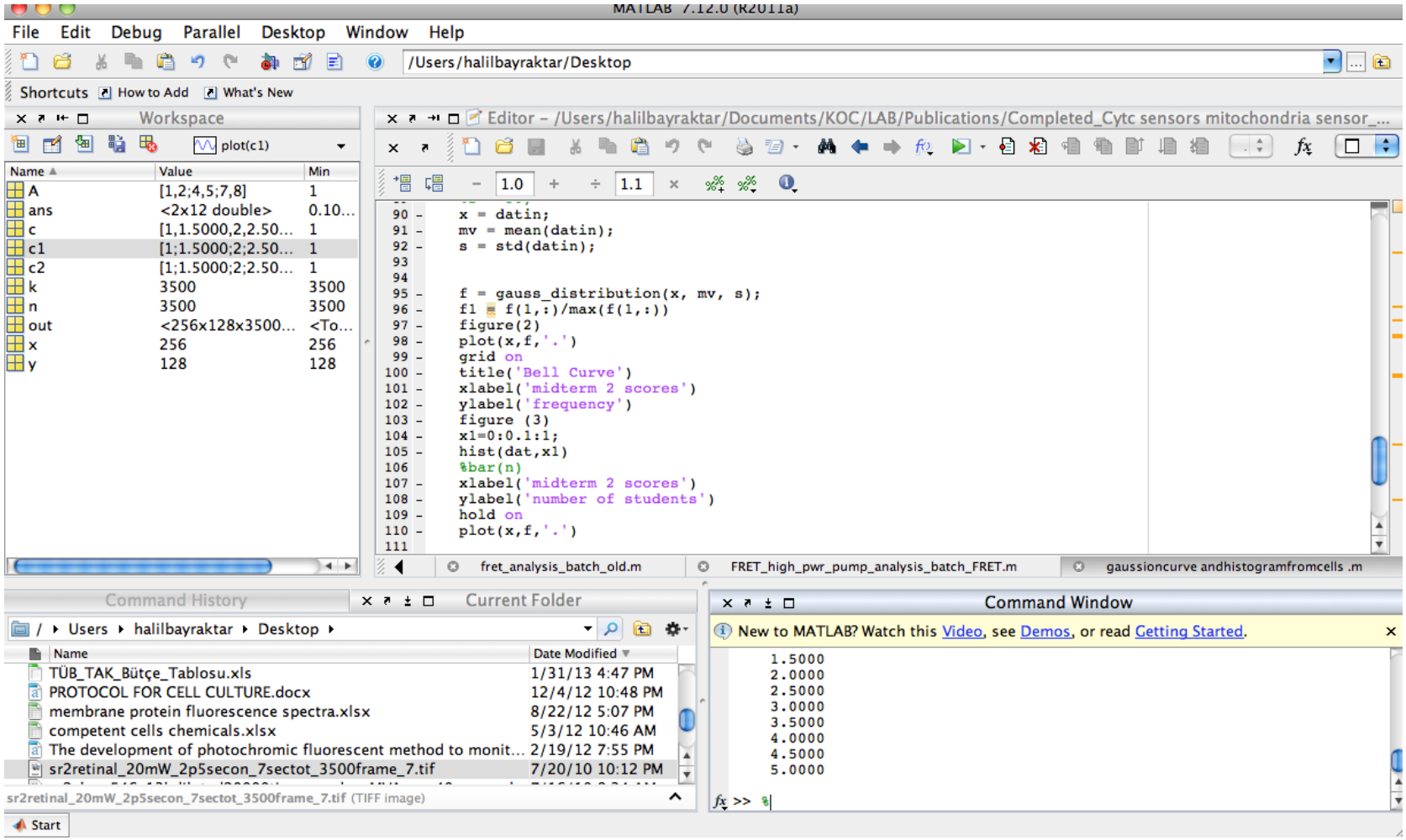

MATLAB 7.12.0 (R2011a)
File Edit Debug Parallel Desktop Window Help
/Users/halilbayraktar/Desktop
Shortcuts How to Add What's New
Workspace
plot(c1)
Name Value Min
A [1,2;4,5;7,8] 1
ans <2x12 double> 0.10...
c [1,1.5000,2,2.50... 1
c1 [1;1.5000;2;2.50... 1
c2 [1;1.5000;2;2.50... 1
k 3500 3500
n 3500 3500
out <256x128x3500... <To...
x 256 256
y 128 128
Editor - /Users/halilbayraktar/Documents/KOC/LAB/Publications/Completed_Cytc sensors mitochondria sensor_...
1.0
1.1
90 - x = datin;
91 - mv = mean(datin);
92 - s = std(datin);
95 - f = gauss_distribution(x, mv, s);
96 - f1 = f(1,:)/max(f(1,:))
97 - figure(2)
98 - plot(x,f,'.')
99 - grid on
100 - title('Bell Curve')
101 - xlabel('midterm 2 scores')
102 - ylabel('frequency')
103 - figure (3)
104 - xl=0:0.1:1;
105 - hist(dat,xl)
106 - %bar(n)
107 - xlabel('midterm 2 scores')
108 - ylabel('number of students')
109 - hold on
110 - plot(x,f,'.')
fret_analysis_batch_old.m
FRET_high_pwr_pump_analysis_batch_FRET.m
gaussioncurve andhistogramfromcells .m
Command History
Current Folder
/ Users halilbayraktar Desktop
Name Date Modified
TÜB_TAK_Bütçe_Tablosu.xls 1/31/13 4:47 PM
PROTOCOL FOR CELL CULTURE.docx 12/4/12 10:48 PM
membrane protein fluorescence spectra.xlsx 8/22/12 5:07 PM
competent cells chemicals.xlsx 5/3/12 10:46 AM
The development of photochromic fluorescent method to monit... 2/19/12 7:55 PM
sr2retinal_20mW_2p5secon_7sectot_3500frame_7.tif 7/20/10 10:12 PM
sr2retinal_20mW_2p5secon_7sectot_3500frame_7.tif (TIFF image)
Command Window
New to MATLAB? Watch this Video, see Demos, or read Getting Started.
1.5000
2.0000
2.5000
3.0000
3.5000
4.0000
4.5000
5.0000
>> %
Start

Image Processing in Matlab

- Many functions are available for computation in matlab
- dat = imread(’neuron.tif');
- Size(dat)
- Imshow(dat,[10 50])
- Imwrite(dat, 'test', 'jpeg’)

Microscopy is an instrument to see small objects

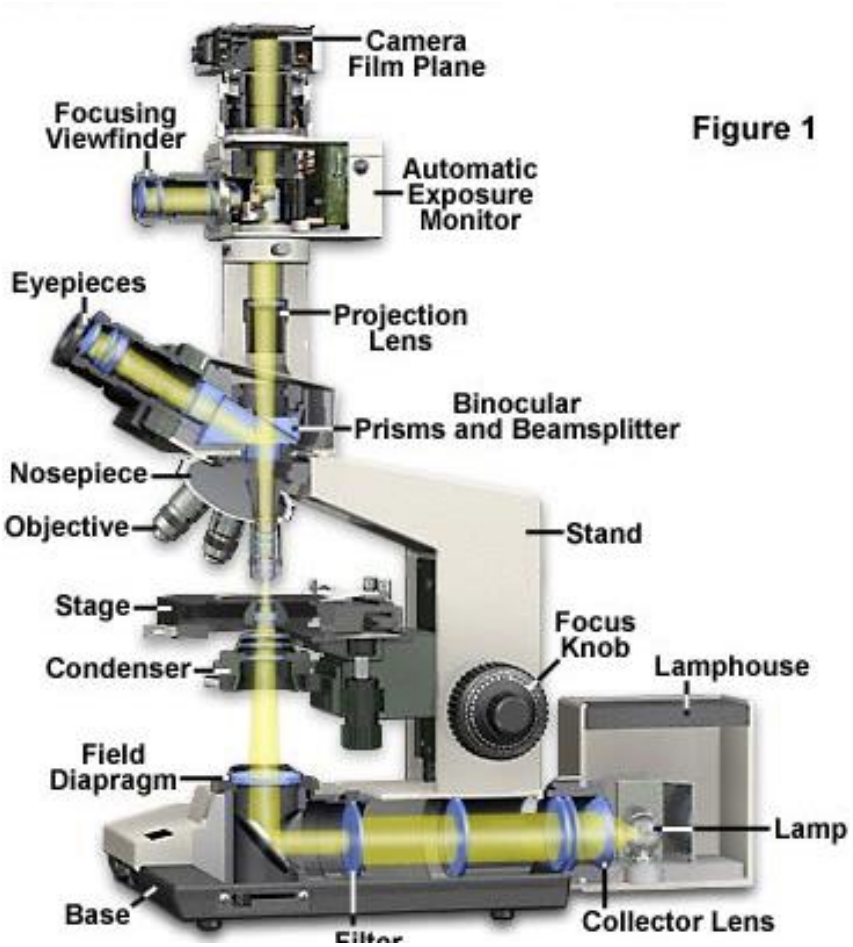
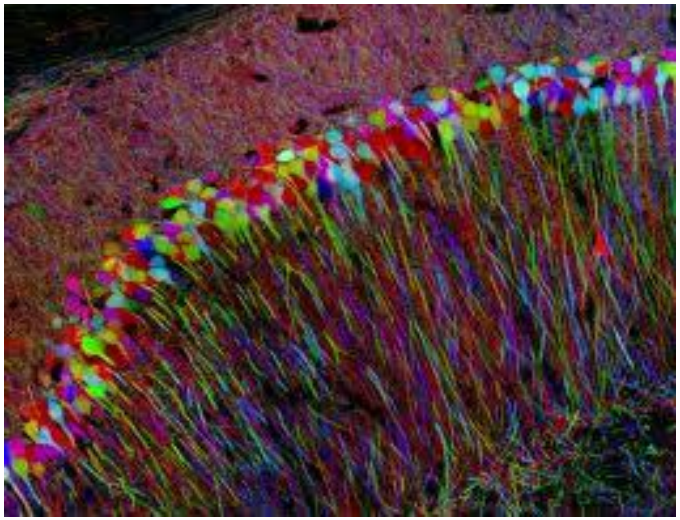
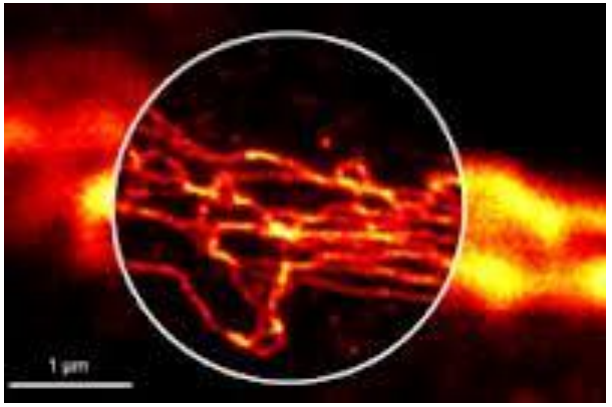


Figure 1

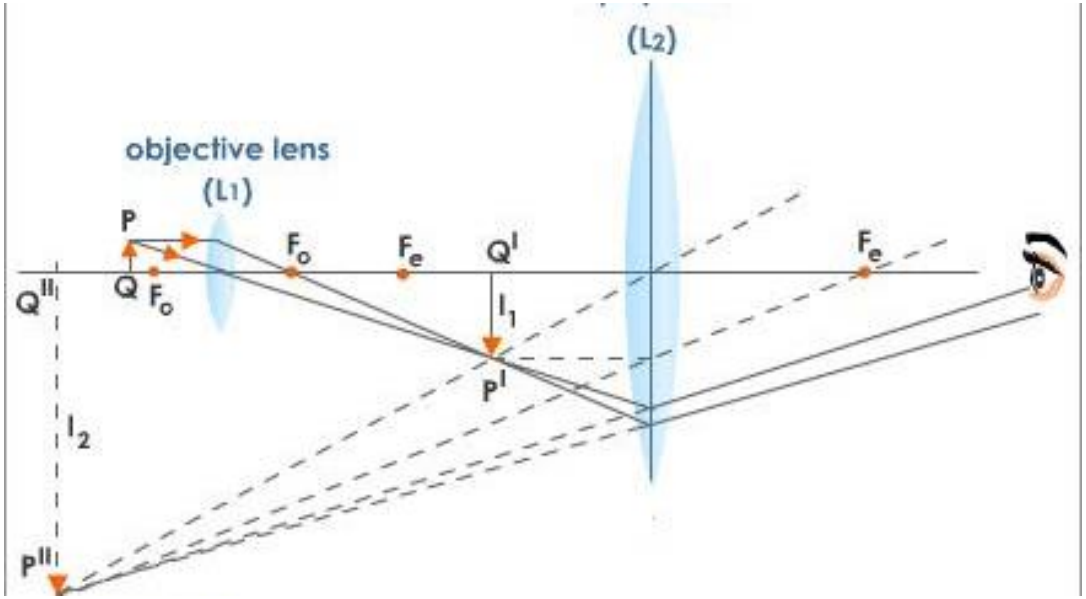


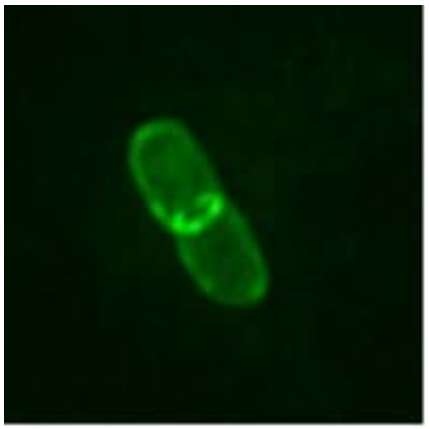


How does microscope work?

- How do they magnify the image?
- What causes the image formation?
- Why determines the brightest in the images?
- Why point like objects looks a shape of airy disc?
- What is the difference between magnification and resolution?

1. Magnification of an image





Virtual Image

Mag = Length of the object from eye piece / Real length of the object

$Mag = Mag_{objective} \times Mag_{eyepiece}$

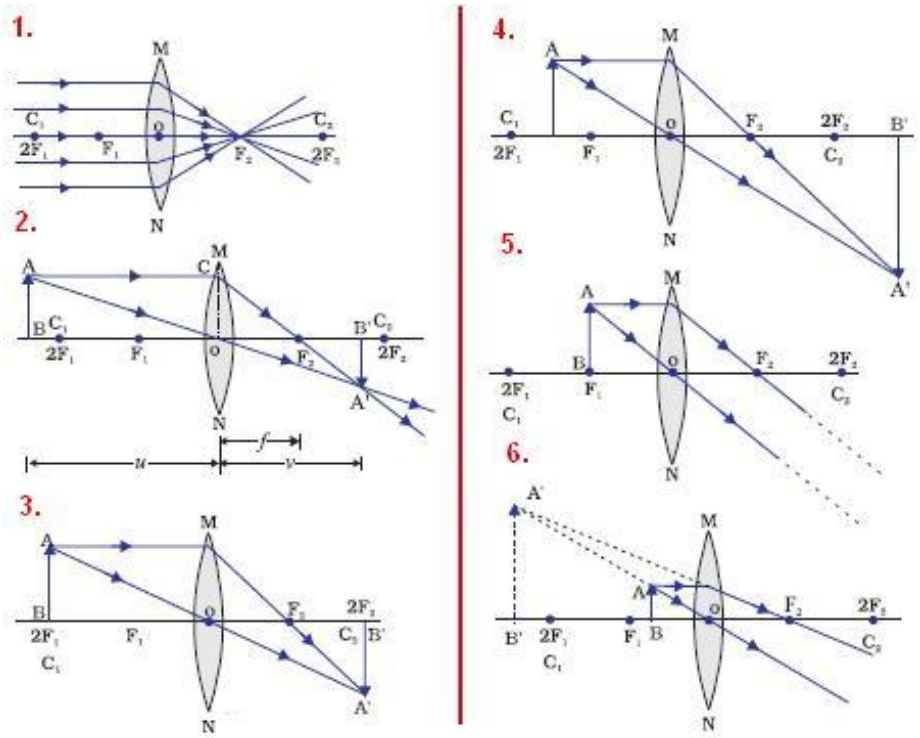
The e.coli has to be magnified to be observed by the eye piece on the microscope

Image formation by a a convex lens

- As you remember, image formation depends on

a) Focal length

b) Distance of the object to the lens



Virtual image

Real vs. Virtual Image

- Image on a cinema screen coming from projector
- Image produced on a detector on the back of the camera
- Image on the back of retina

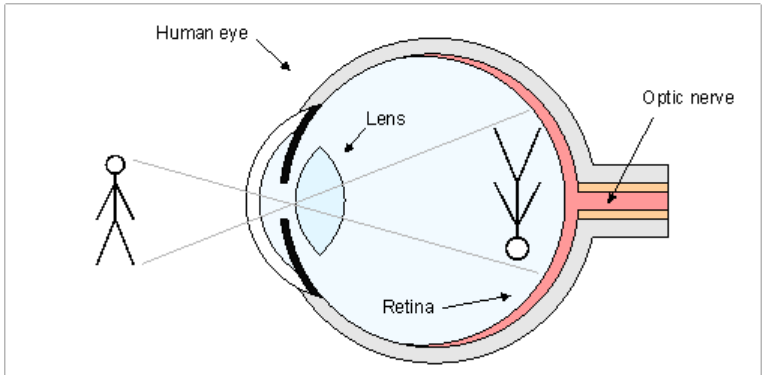
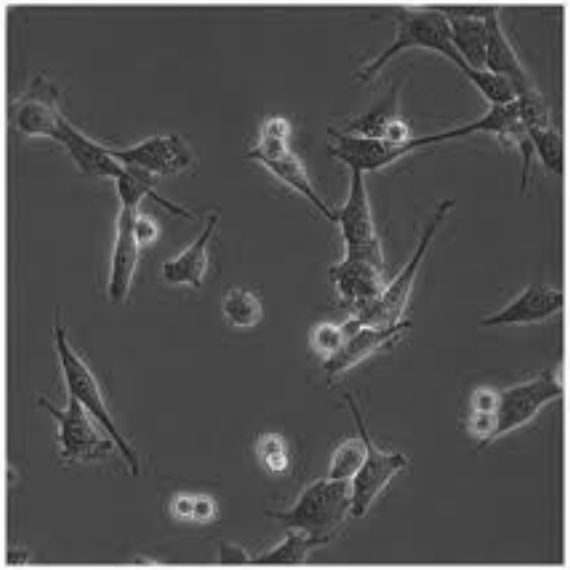


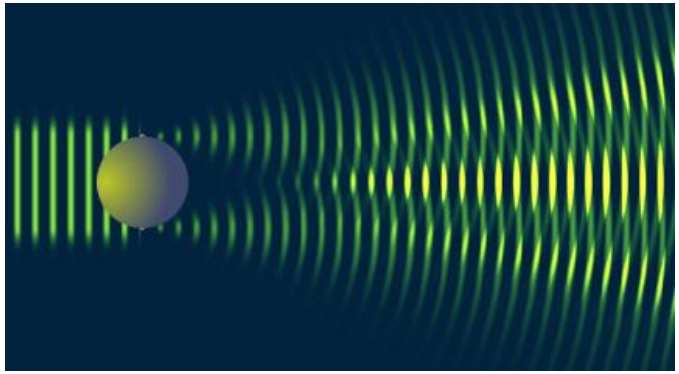
Image on mirror is the virtual image, you see as if the objects stands behind the mirror.

2. Diffraction and interference is required for image formation

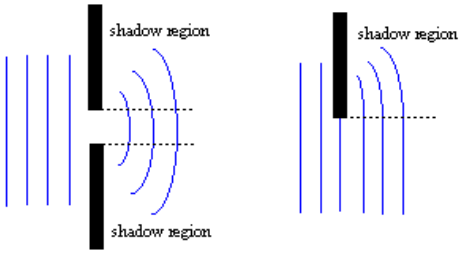
- If no light is diffracted or absorbed by the the specimen, there is no information in the light comes from the specimen.
- For example glass is so smooth and no irregularities or smaller than the wavelength of light, then it is completely transparent.



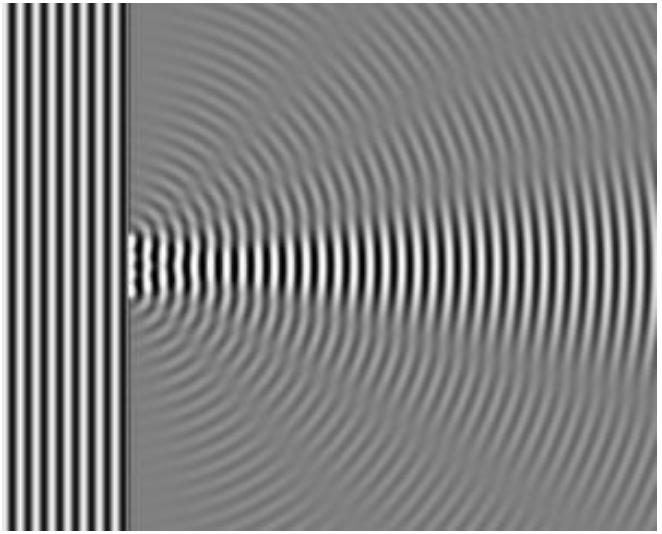
Diffraction of light: Bending light with materials

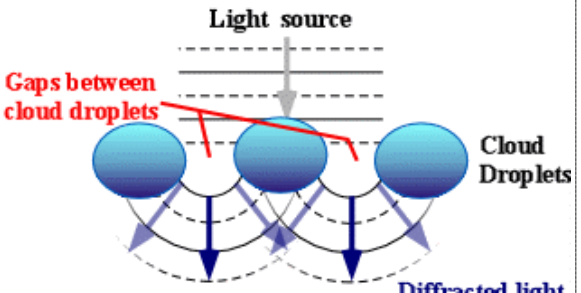


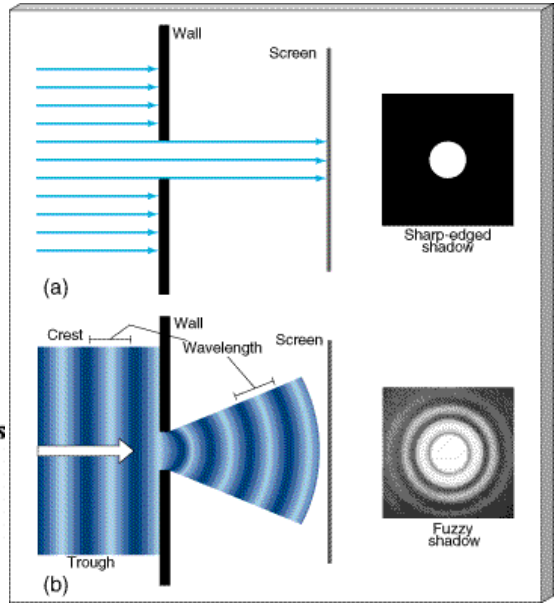
When light passes around a small object it appears to bend



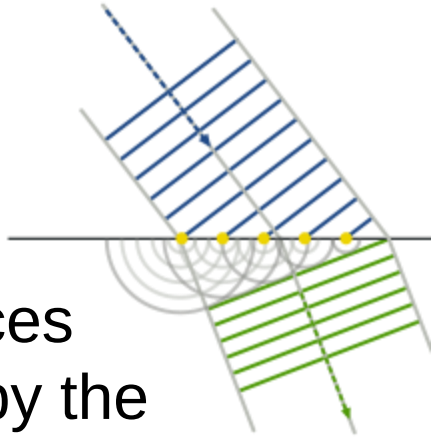
When the light passes through a small opening it spreads out.
If the wavelength is comparable to or bigger than the size of an aperture or an obstacle then significant diffraction takes place.



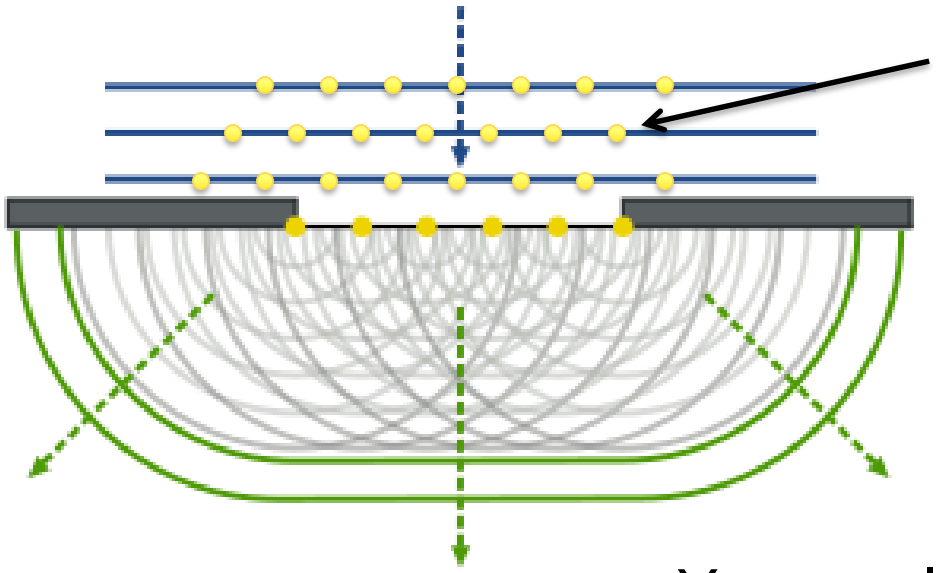




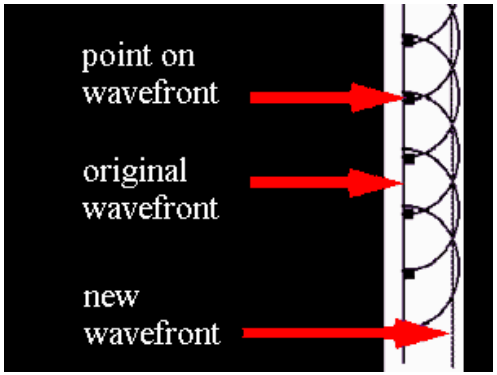
Huygens' Principle



Every wave front acts like a new source.



This point sources are eliminated by the slit, therefore can not contribute as a constructive wave on the screen.



You see light as if it is bending where the slit are. Point sources at edges are removed because of slit. Point sources at the corners of slit do not have any contribution to form the constructive patterns on the screen.

Fluorophore behave like a point source emitting a photon regularly

All optical instruments have circular apertures, for example the pupil of an eye or the circular diaphragm and lenses of a microscope.

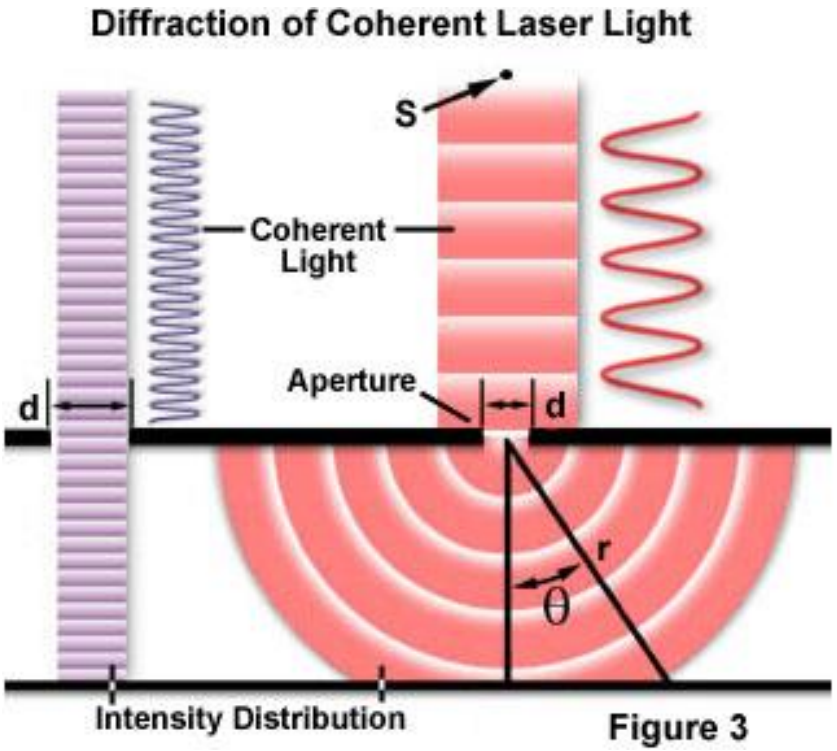


Figure 3

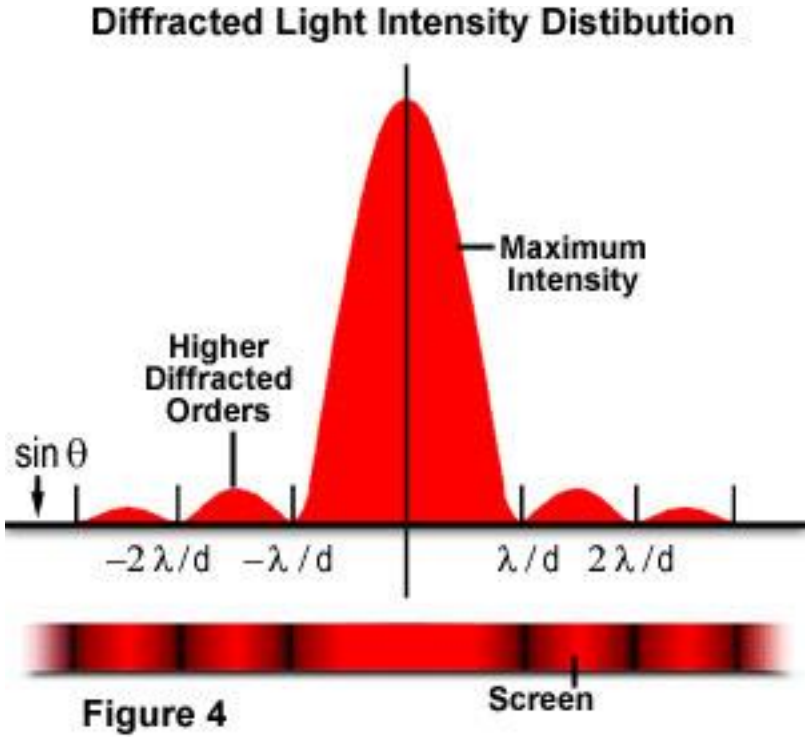


Figure 4

http://www.cabrillo.edu/~jmccullough/Applets/More_applets.html
http://www.walter-fendt.de/ph14e/singleslit.htm

Objective lenses

Figure 2

Figure 1

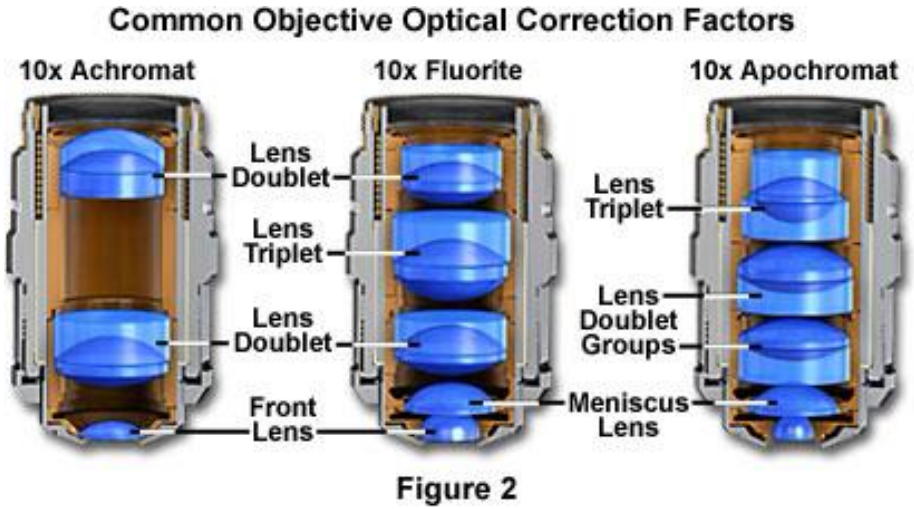
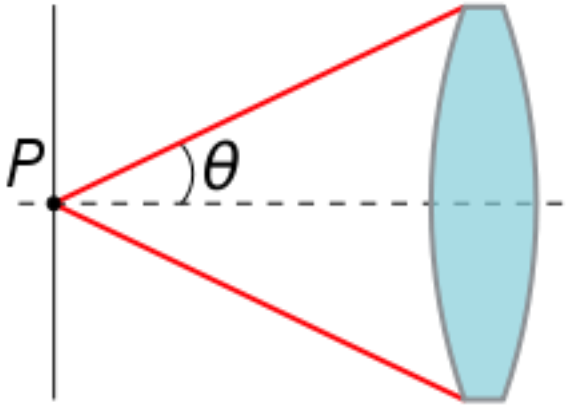


Figure 2

Plan Fluorite Correction		
Magnification	Numerical Aperture	Working Distance (mm)
4x	0.13	17.10
10x	0.30	16.00
20x	0.50	2.10
40x	0.75	0.72
40x (oil)	1.30	0.2
60x	0.85	0.3
100x (dry)	0.90	0.30
100x (oil)	1.30	0.20
100x (oil with iris)	0.5-1.3	0.20
Plan Apochromat Correction		
Magnification	Numerical Aperture	Working Distance (mm)
2x	0.10	8.50
4x	0.20	15.70
10x	0.45	4.00
20x	0.75	1.00
40x	0.95	0.14
40x (oil)	1.00	0.16
60x	0.95	0.15
60x (oil)	1.40	0.21
60x (Water Immersion)	1.20	0.22
100x (oil)	1.40	0.13

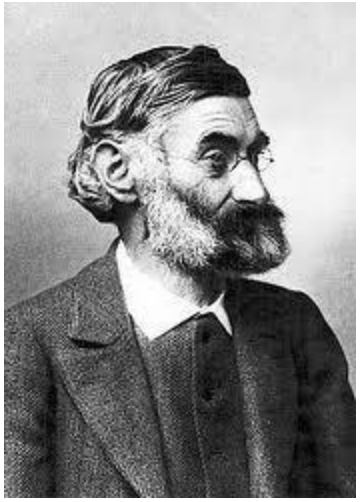
Numerical aperture

- ***NA= nsin(θ)***



R = λ/2n(sin(θ))

R is the separation distance
λ is the illumination wavelength
n is the imaging medium refractive index
θ is one-half of the objective angular aperture



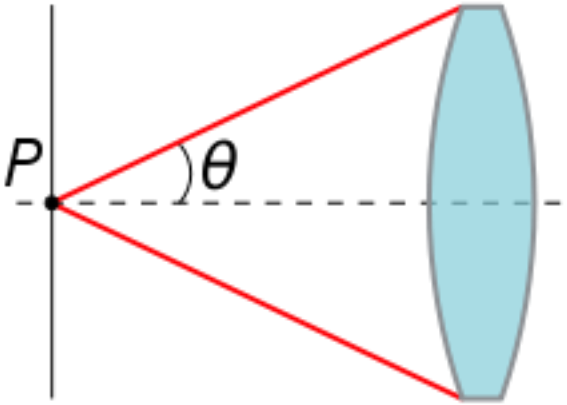
Abbe's theory

Ernst Abbe is the person who first established the theoretical framework for image formation in a microscopy. Any imaging system has a finite limit of resolution that is the capacity to generate distinguishable images of two close objects.

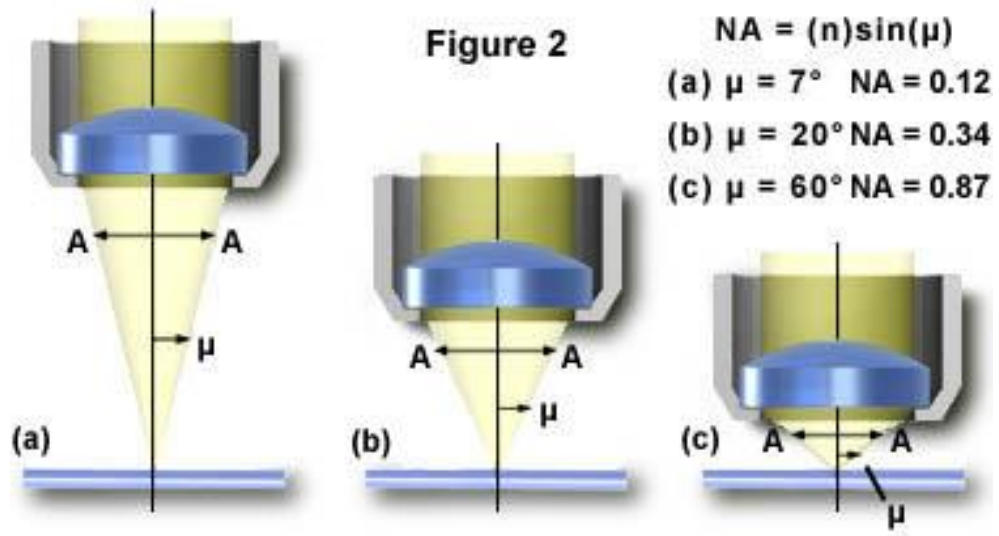
Diffraction of light waves is the principal reason limiting the resolution.

diaphragms and lens edges restricts light rays, leading to each infinitely small point being imaged as a diffraction spot of a finite size. Diffraction spots from nearby points may overlap with each other and become indistinguishable. The present experiment studies the diffraction resolution limit of a microscope objective.

$$d = \frac{\lambda}{2NA}$$

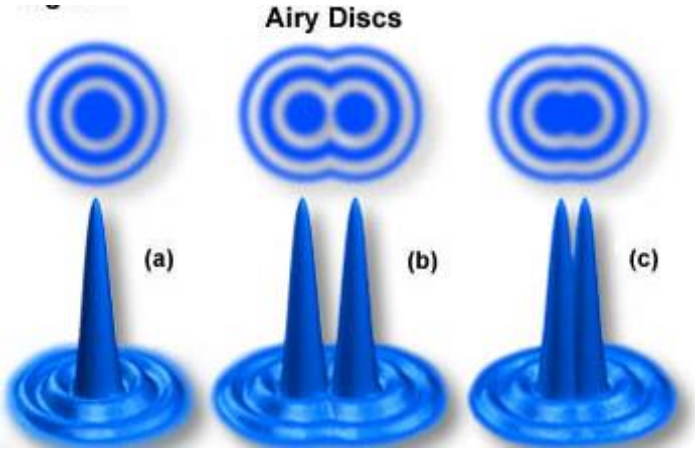


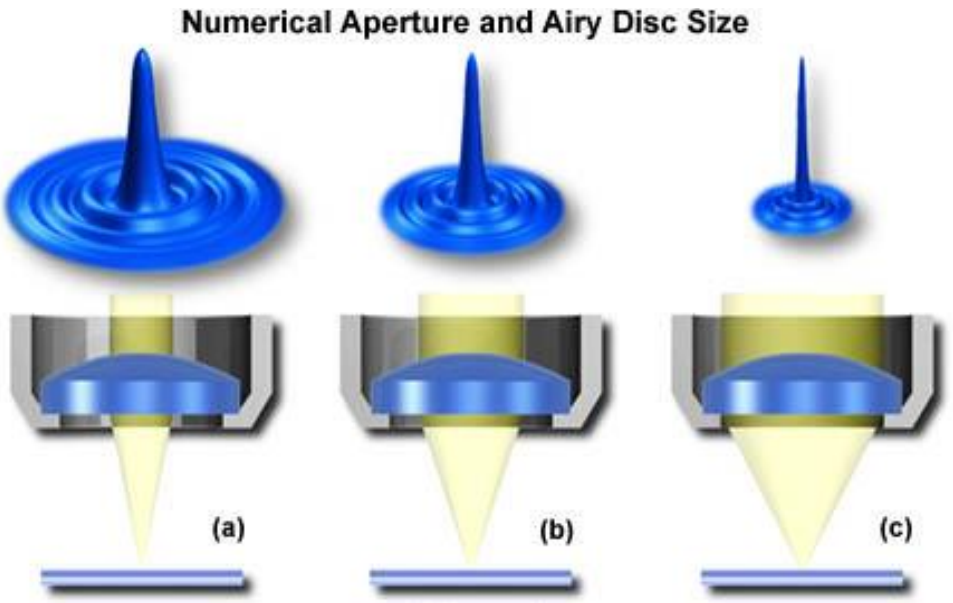
Objectives with different NA



Airy Disc

This phenomenon is caused by diffraction or scattering of the light as it passes through the minute parts and spaces in the specimen and the circular back aperture of the objective.



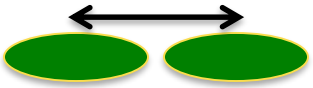


You can think of less resolution in small NA as the beam pass through a small slit therefore very wide zero order peak at the center of the airy disc.

http://www.walter-fendt.de/ph14e/singleslit.htm

Nyquist sampling theorem in microscopy

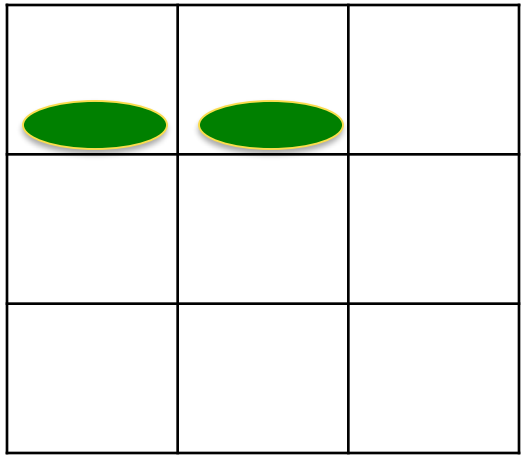
- Consider the smallest resolvable distance in the image is 200 nm, if you use 60x objective the distance is 12 um. The frequency is 1/12.
- The pixel sampling frequency should be 1/6, twice the ratio of distance frequency.



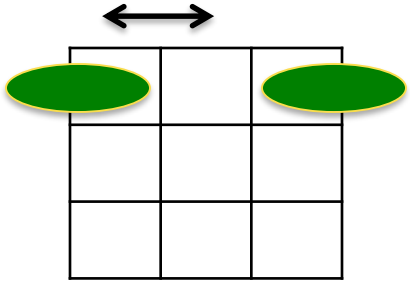
200 nm

60 x objective = 12000 nm = 12 um

12 um

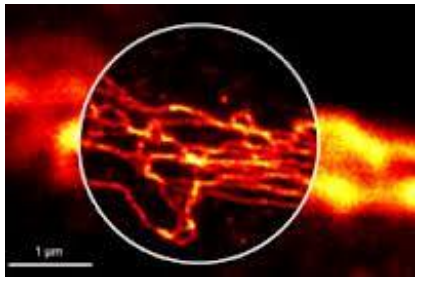


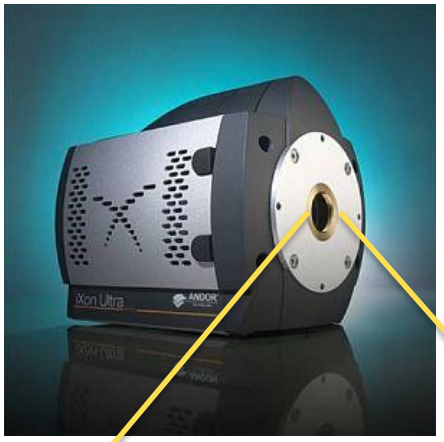
6 um

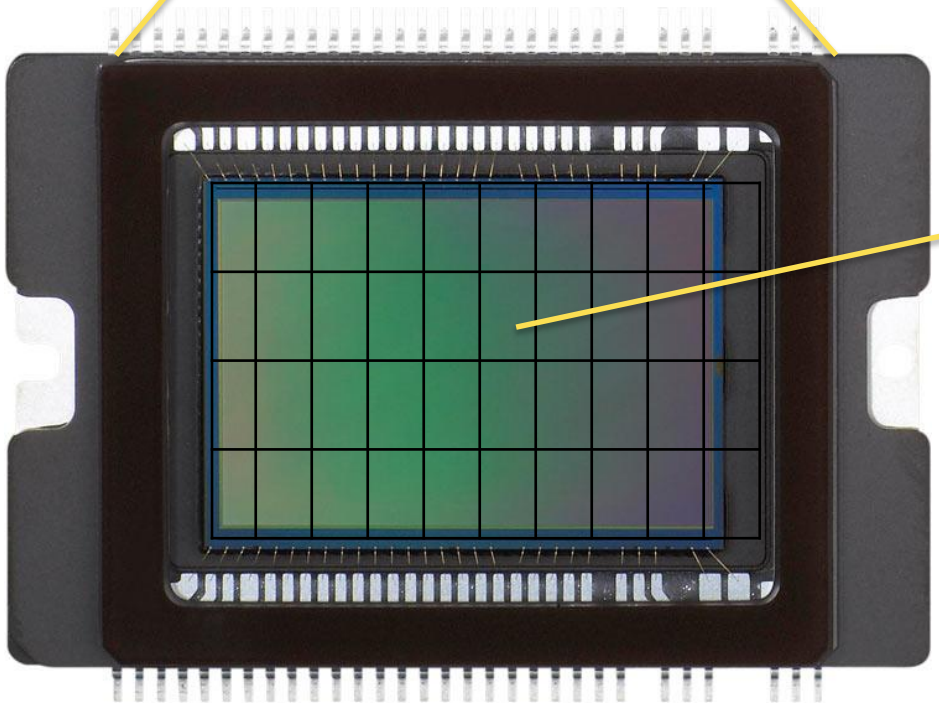


Can be resolved

Can not be resolved!







Pixels on the camera

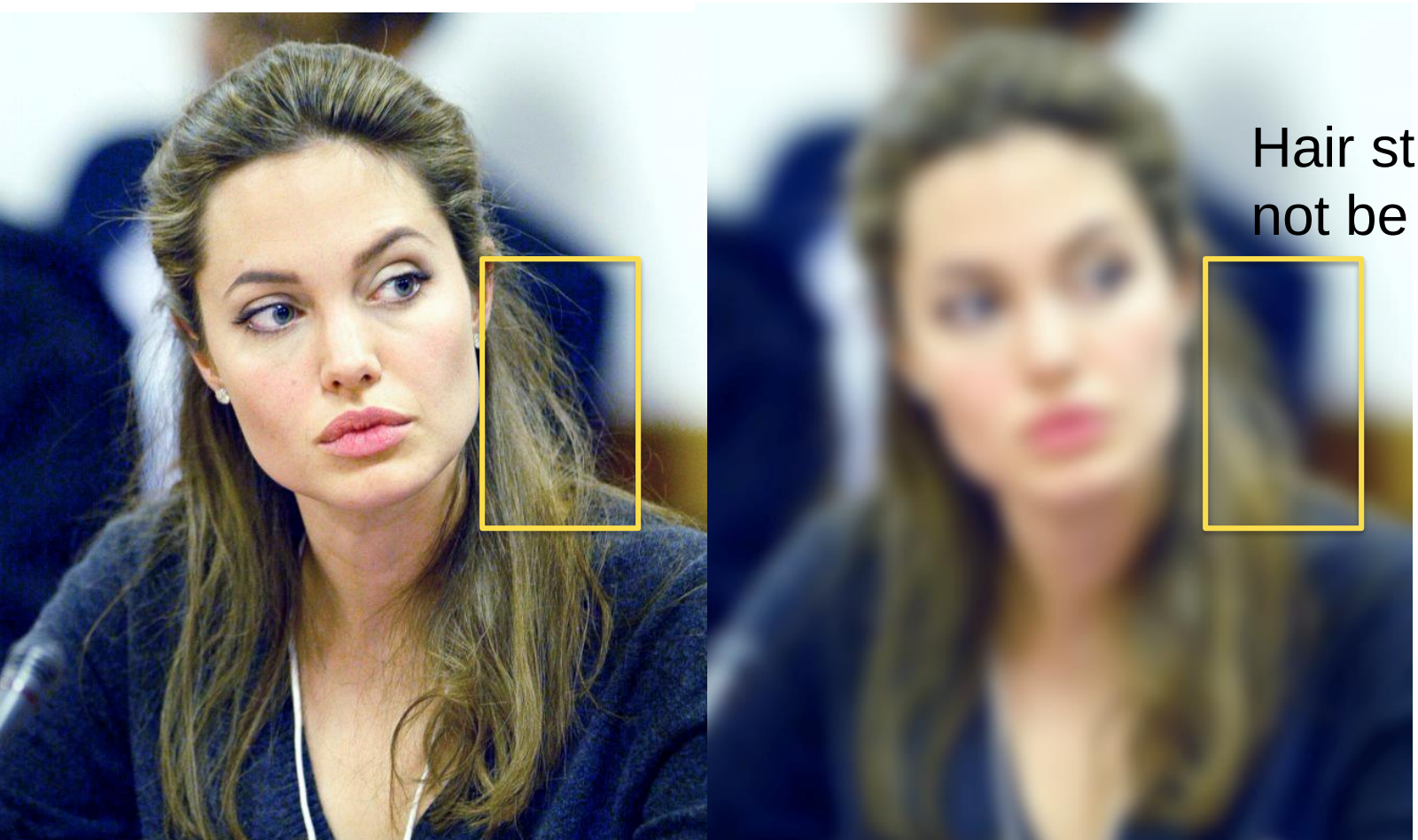
10 Megapixel CCD,
Each pixel is 10 um.

Pixel size and resolution

Small pixel size

vs.

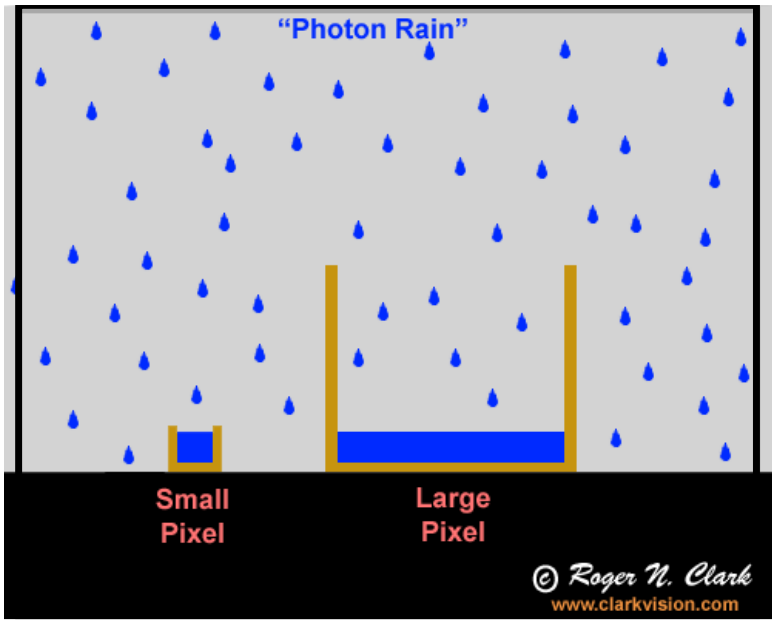
Large Pixel size



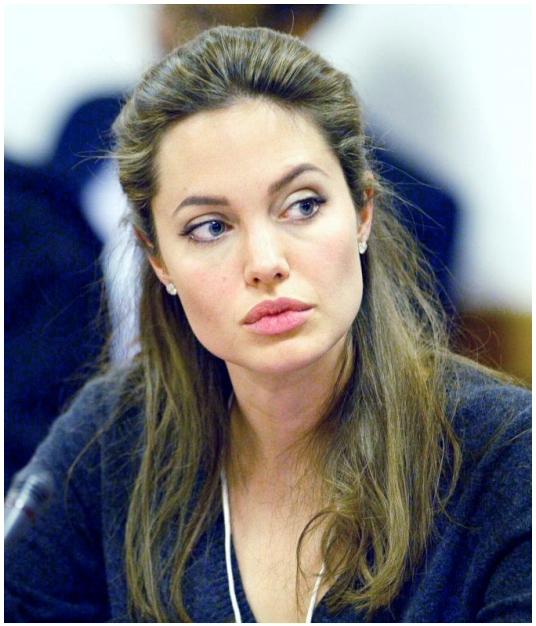
Hair strands can not be resolved!

what if the camera has a very small pixel size?

- Small pixel size will collect less photons, So you will need a longer exposure times for the brightness of image.
- There is a trade-off between the resolution and image brightness.



2 um pixel size > 1 um pixel size



200 ms exposure = 200 ms exposure

Lens Aberrations

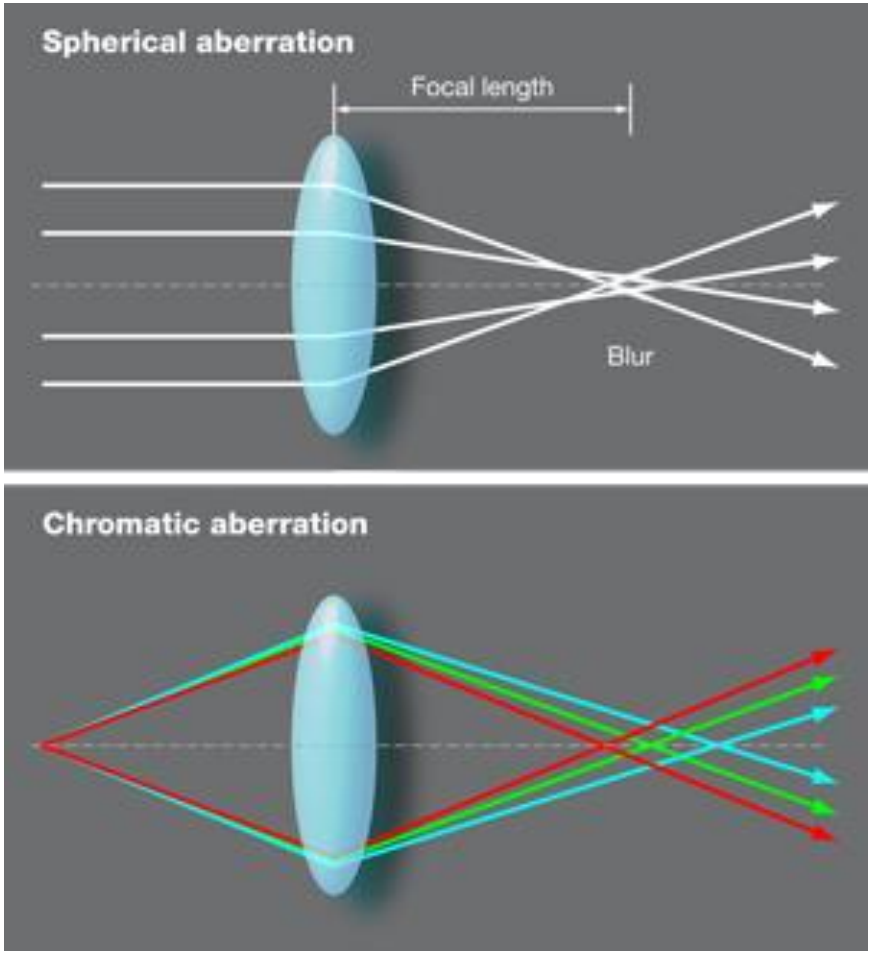
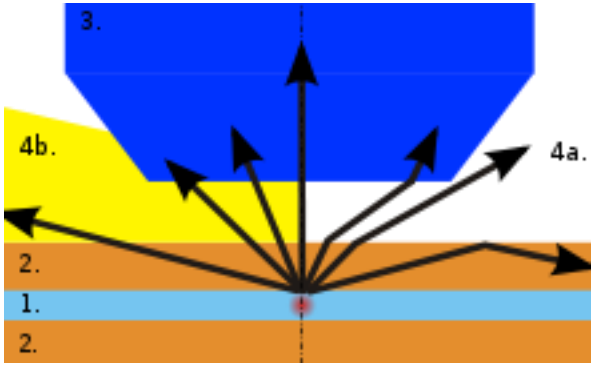


Figure 1

Corrected for spherical and chromatic aberration as well as planar aberration.

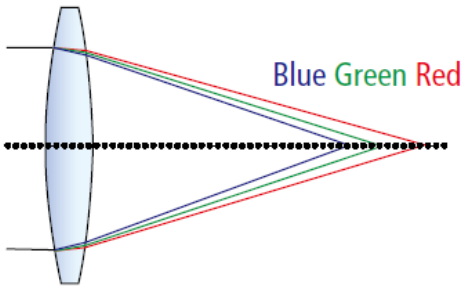
Immersion objectives

Immersion objectives operate with a liquid medium of defined refractive index between the front lens element and the coverslip, are more restricted in working distance lengths.

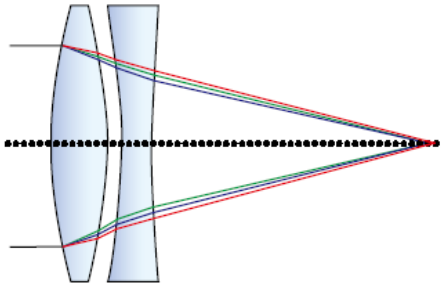


1. Oil immersion, refractive index 1.51
2. Water immersion, refractive index 1.33

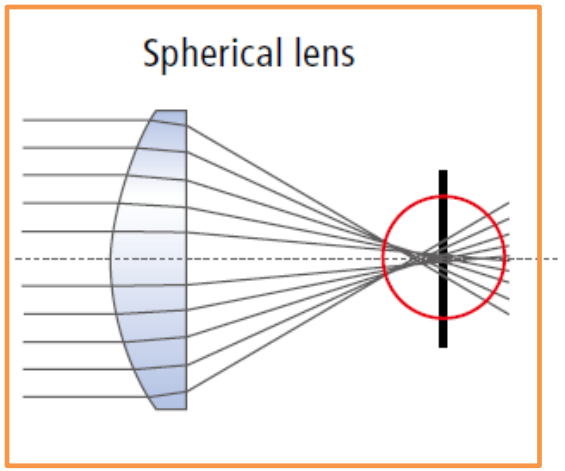
Chromatic and spherical aberration



The focal point shifts depending on the color.



The focal points are aligned by combining lenses with different dispersion.



Objective working distance

It is defined as the distance from the front lens element of the objective to the closest surface of the coverslip when the specimen is in sharp focus.

Working distance decreases in objectives as the magnification and numerical aperture increase.