Basic Optics

Initial Training for Optical Microscope Users

- Data Storage
- Fluorescence Imaging
- IGB Core Instrument LSM 880
- IGB Core Instrument LSM 900
- IGB Core Instrument V16
- IGB Core Instruments Axiovert 200M
- IGB Core Instruments LSM 700
- IGB Core Instruments Zeiss LSM 710
- Objectives
- Optics
- Sampling
- Working in the IGB Core

Data Storage

Store data on the data drive on the local machine:

D\Data may be deleted at anytime. Do not leave your data here!

The core staff will clean the computer drives occasionally. This means that your profile, desktop and data from the data drive will all be removed. We will also remove other files that look like they are not needed.

Core-Server:

The core-server was set up by the IGB Computer Network and Research Group (CNRG) as a place for core users to store their data long term and move it back to your office. You do not need to bring thumb drives or other devices that could bring a computer virus into the core when you come to collect data. Move your data off of the local machine onto the core server at the end of each imaging session. Your PI will have a folder on the core-server and you can make a sub folder for your work. you will have access to all of the sub folders in your PI's folder but not other PI's folders. All of the data in your PI's folder was paid for by your PI and belongs to him/her.

Your PI will be charged $8.75/terabyte every month Email help@igb.illinois.edu for information on charges and tape backup for

long term storage.

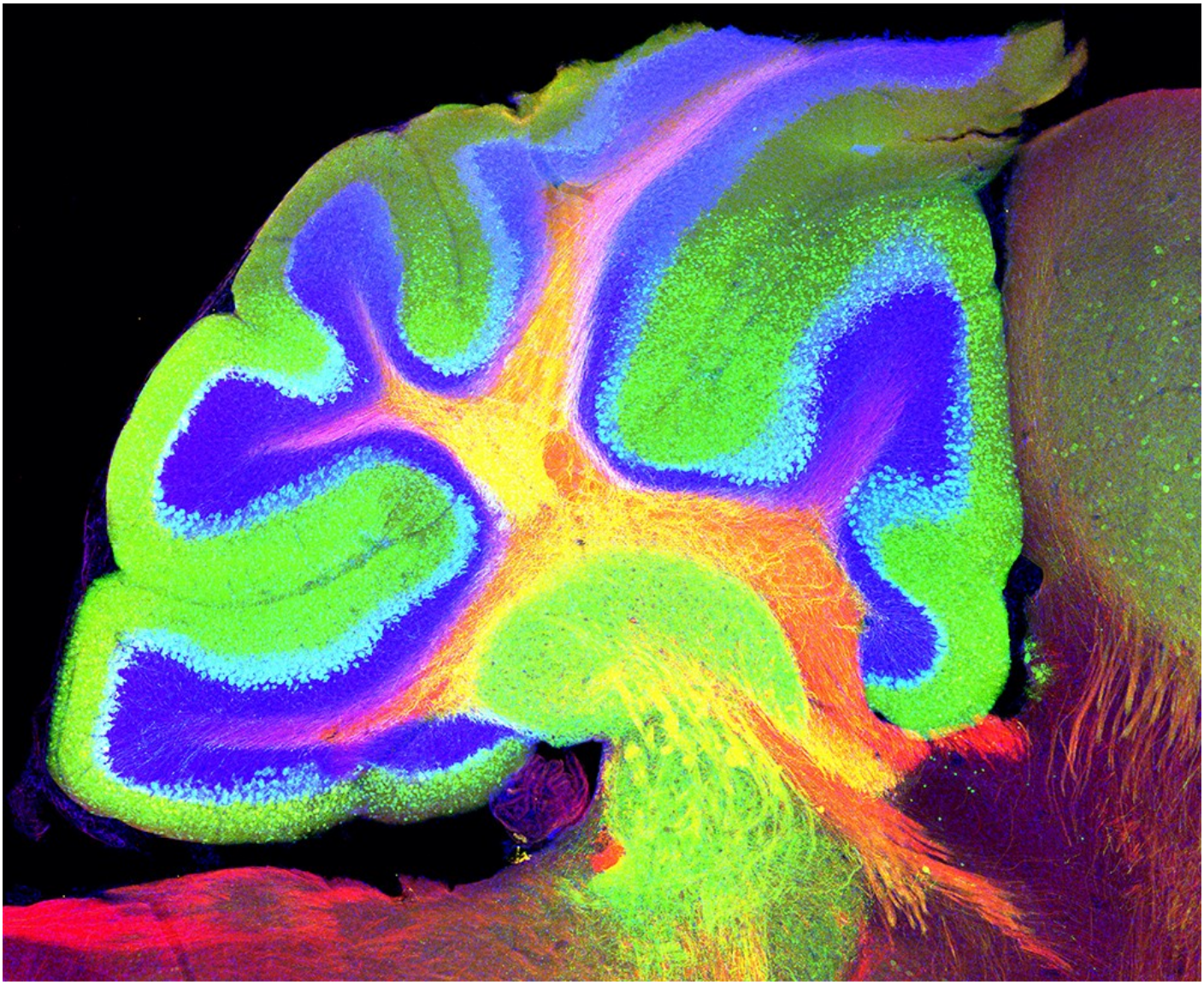
Long Term Storage

CNRG provides tape backup for long term storage for $200/ terabyte

Fluorescence Imaging

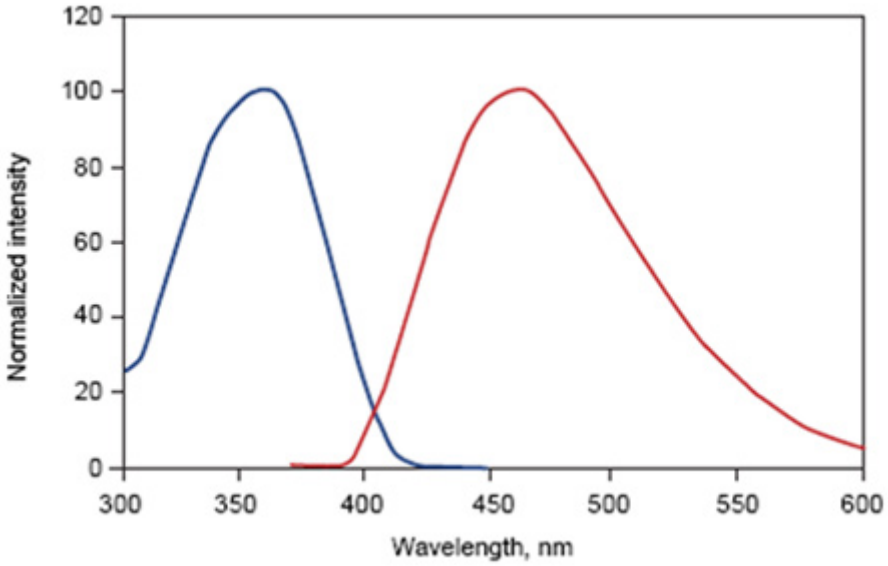
Why Fluorescence:

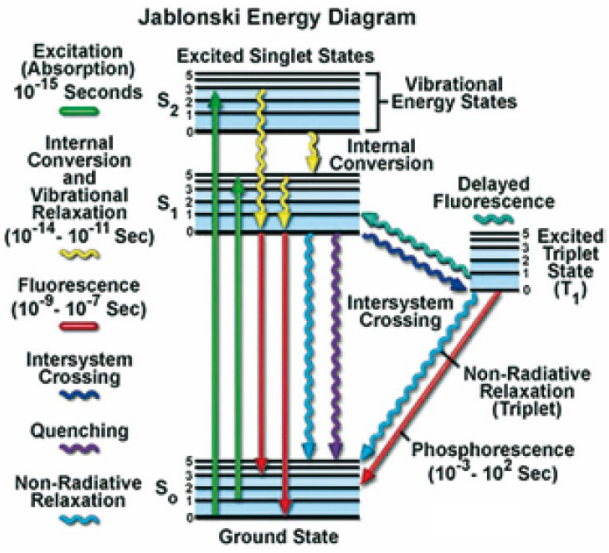
We can label what we want to see

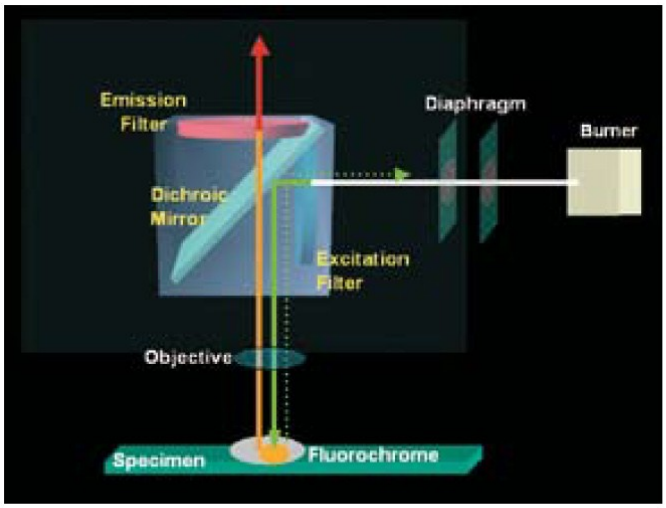


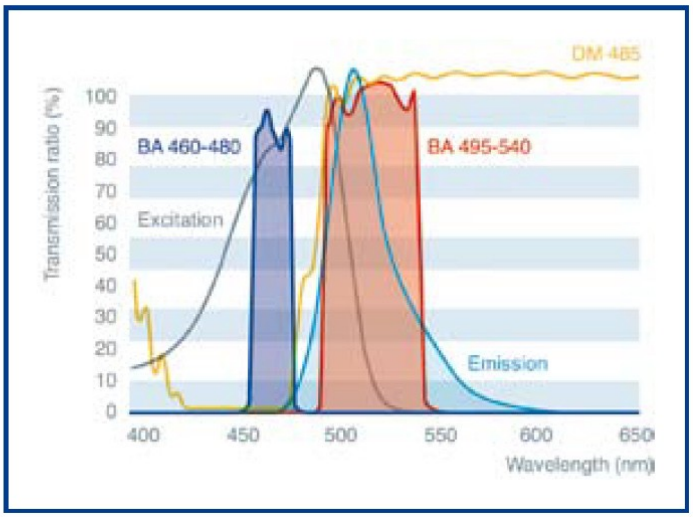
Excitation and Emission

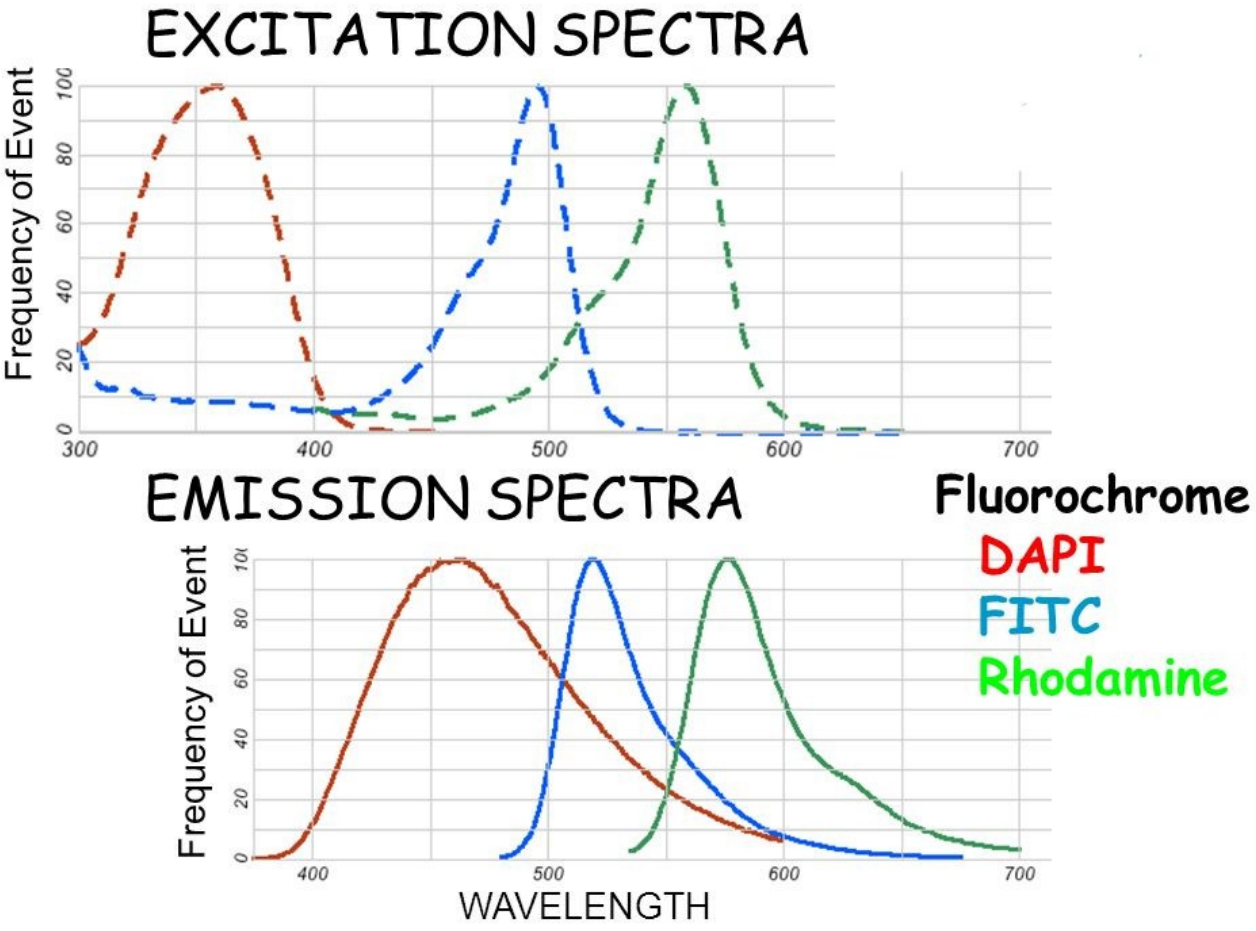
Dapi


Normalized intensity
120
100
80
60
40
20
0
300
350
400
450
500
550
600
Wavelength, nm

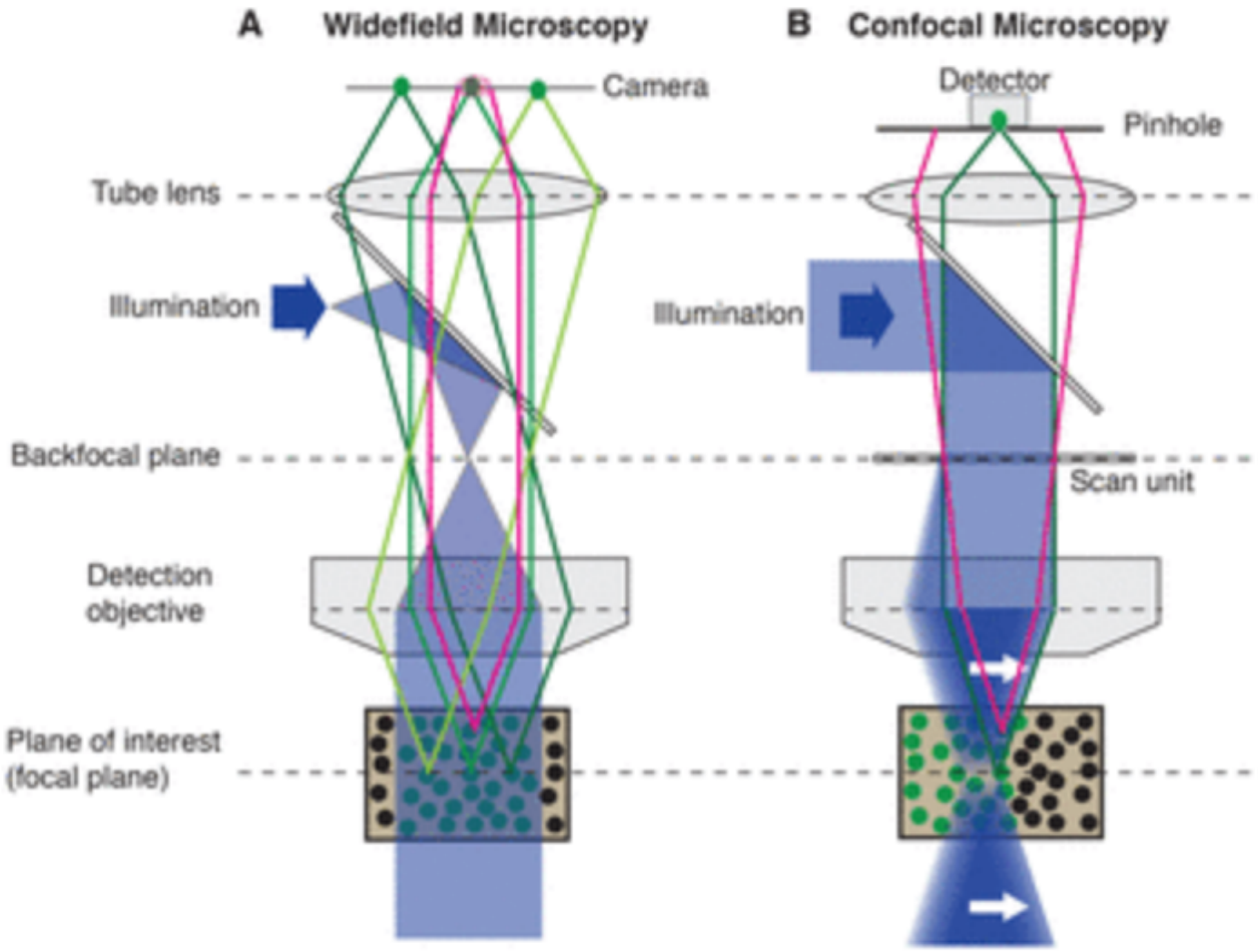

Jablonski Energy Diagram
Excitation (Absorption) 10^{-15} Seconds
Excited Singlet States
S_2
Vibrational Energy States
Internal Conversion
Internal Conversion and Vibrational Relaxation (10^{-14} - 10^{-11} Sec)
S_1
Delayed Fluorescence
Excited Triplet State (T_1)
Fluorescence (10^{-9} - 10^{-7} Sec)
Intersystem Crossing
Intersystem Crossing
Non-Radiative Relaxation (Triplet)
Quenching
Phosphorescence (10^{-3} - 10^{2} Sec)
Non-Radiative Relaxation
S_o
Ground State





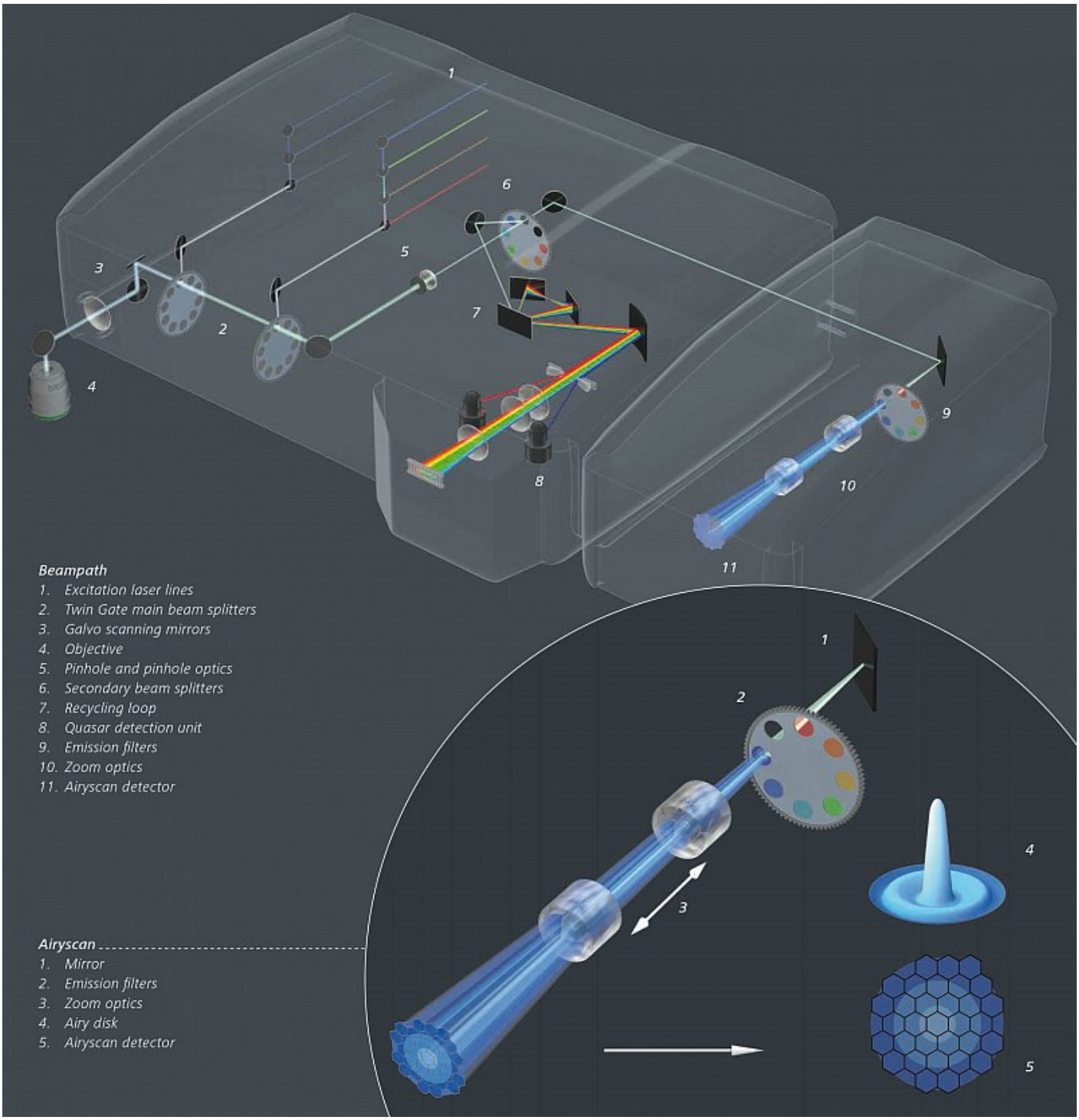


Widefield vs Confocal



https://www.journals.uchicago.edu/doi/full/10.1086/689588

IGB Core Instrument LSM 880



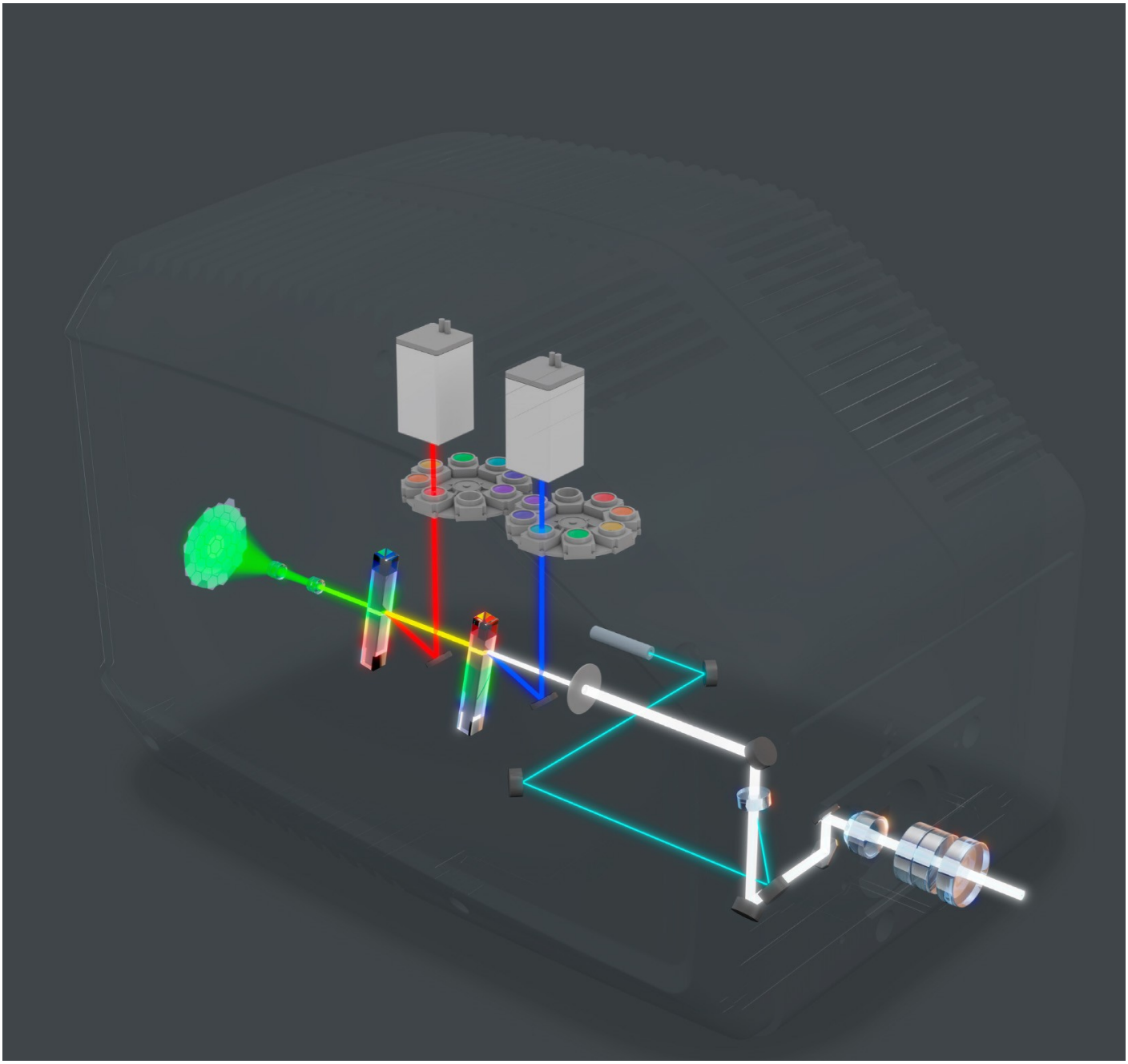
Beampath

1. Excitation laser lines
2. Twin Gate main beam splitters
3. Galvo scanning mirrors
4. Objective
5. Pinhole and pinhole optics
6. Secondary beam splitters
7. Recycling loop
8. Quasar detection unit
9. Emission filters
10. Zoom optics
11. Airyscan detector

Airyscan

1. Mirror
2. Emission filters
3. Zoom optics
4. Airy disk
5. Airyscan detector

IGB Core Instrument LSM 900



405nm, 488nm, 561nm, 640nm excitation.

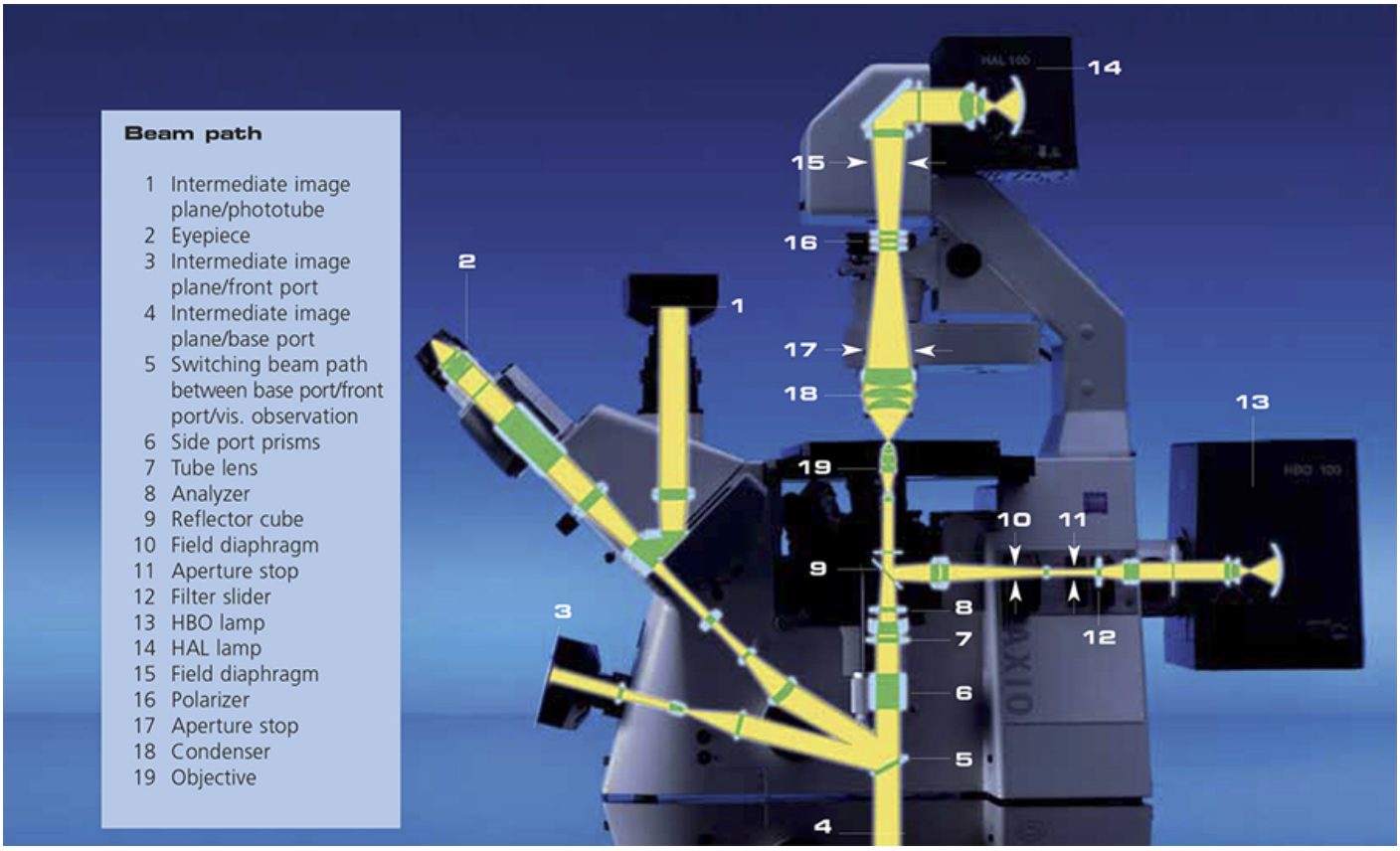
Zen Blue

IGB Core Instrument V16

IGB Core Instruments

Axiovert 200M

Axiovert 200M

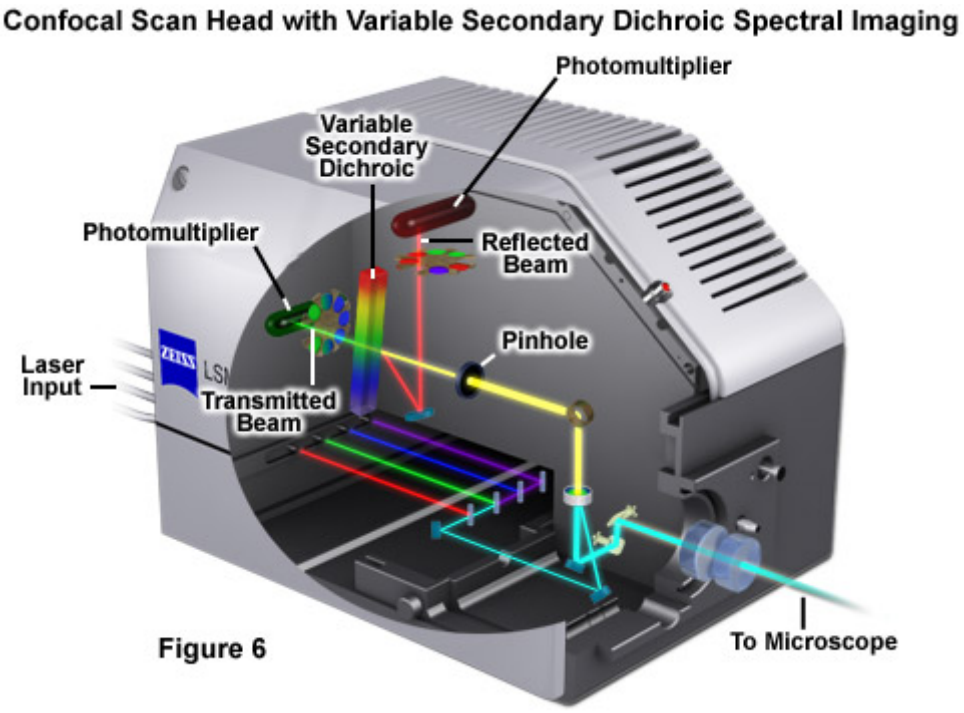


Cameras

cMOS

IGB Core Instruments LSM 700

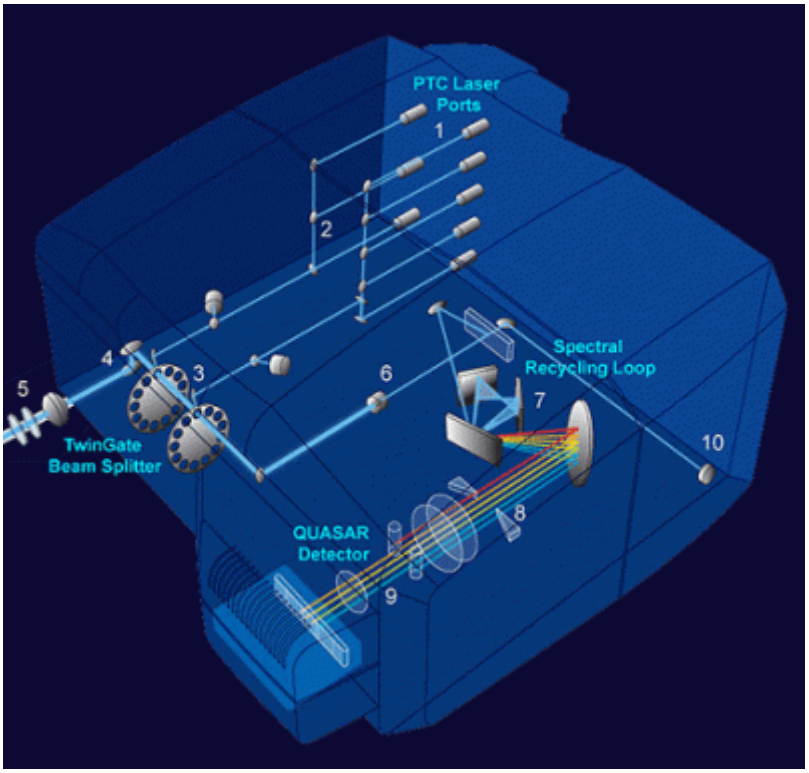
LSM 700



http://zeiss-campus.magnet.fsu.edu/tutorials/spectralimaging/lsm700/indexflash.html

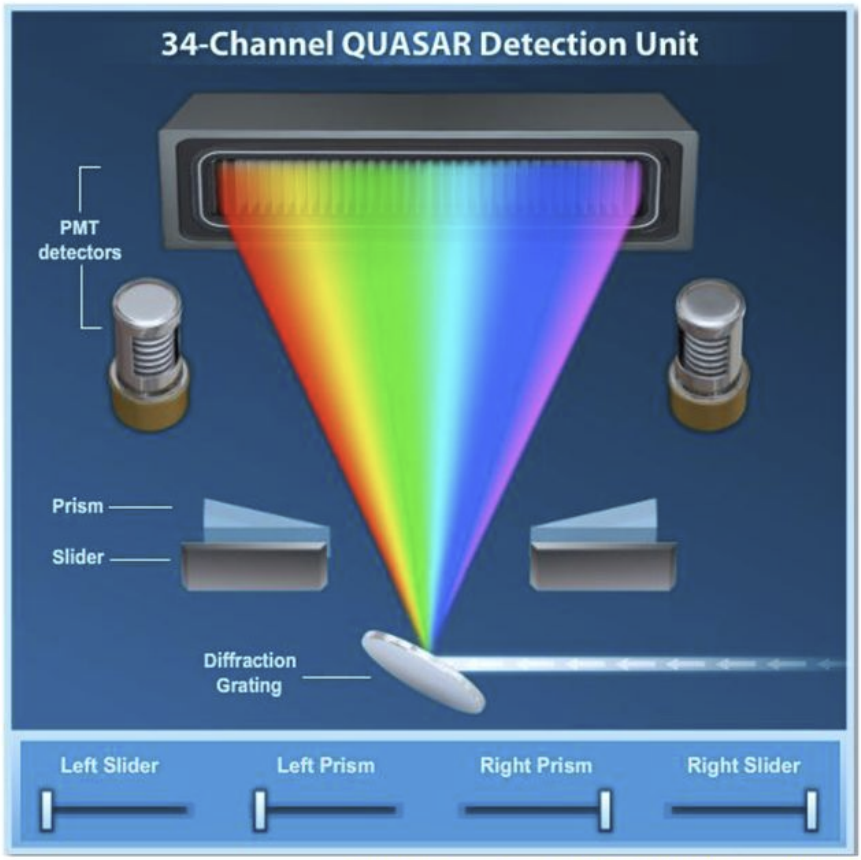
IGB Core Instruments Zeiss LSM 710

Light Path



https://www.gu.se/en/core-facilities/lsm-710-nlo

Zeiss 710 spectral system



http://zeiss-campus.magnet.fsu.edu/tutorials/spectralimaging/quasar34ch/index.html

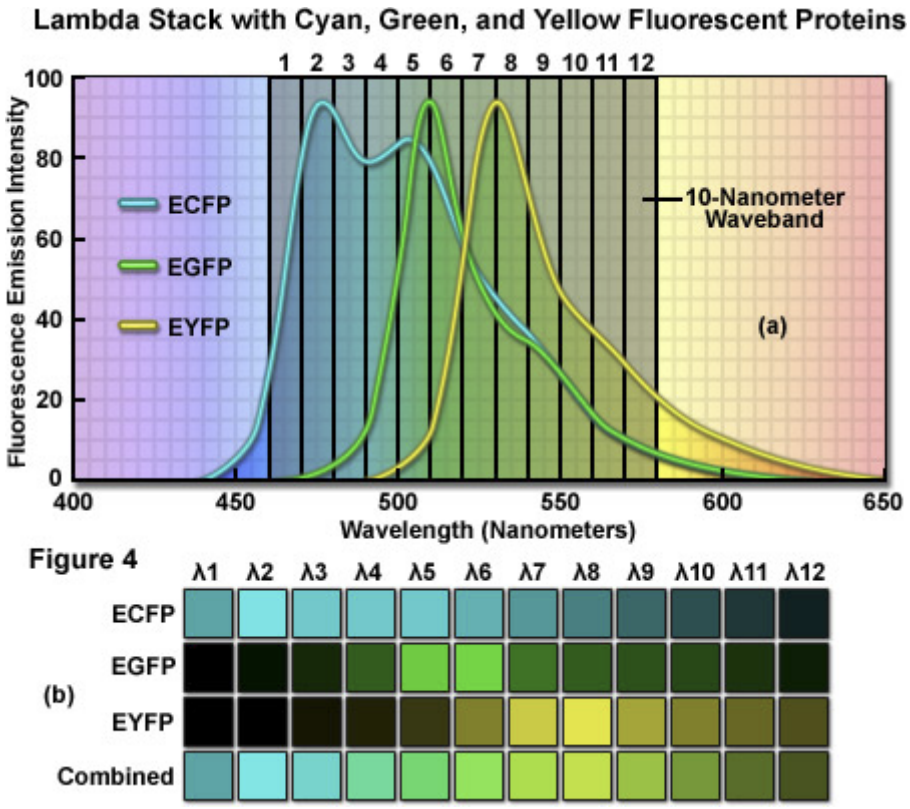


Figure 4

Seven visible excitation lines: 405nm, 458nm, 488nm, 514nm, 561nm, 594nm, 633nm.

Tisaphire laser 700nm to 980nm

> Spectral Unmixing http://zeiss-campus.magnet.fsu.edu/articles/spectralimaging/introduction.html

> Multiphoton Microscopy http://zeiss-campus.magnet.fsu.edu/referencelibrary/multiphoton.html

> Fluorescence Lifetime Imaging Microscopy (FLIM)
> http://www.iss.com/microscopy/components/FastFLIM.html

Objectives

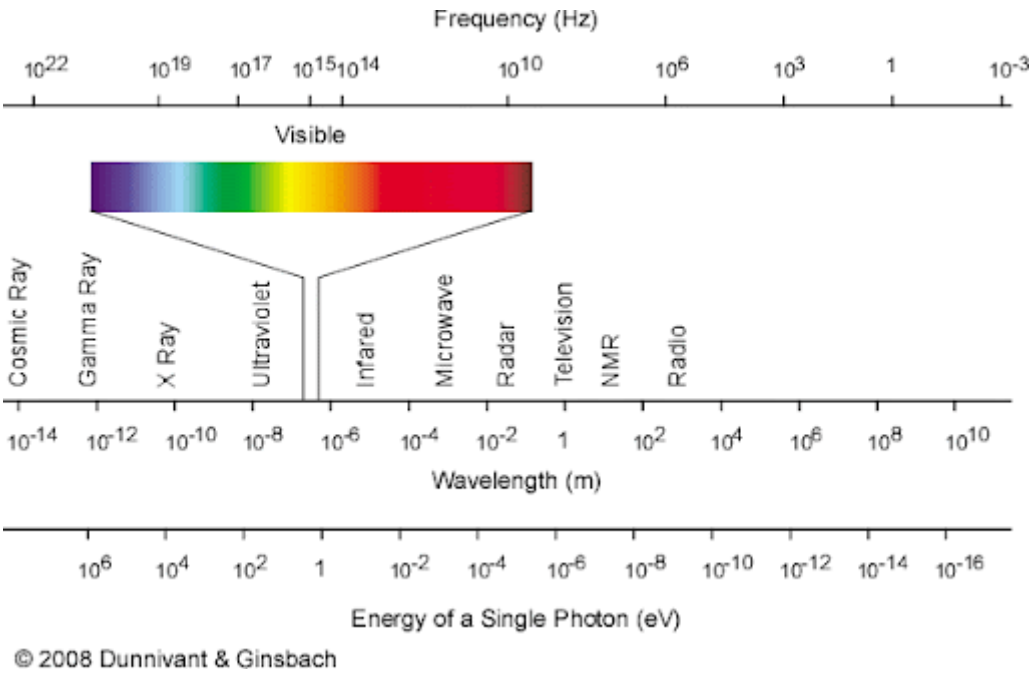
Optics

Properties of light

Wave particle duality:

Light is a wave

Image not found or type unknown



Absorption and Emission

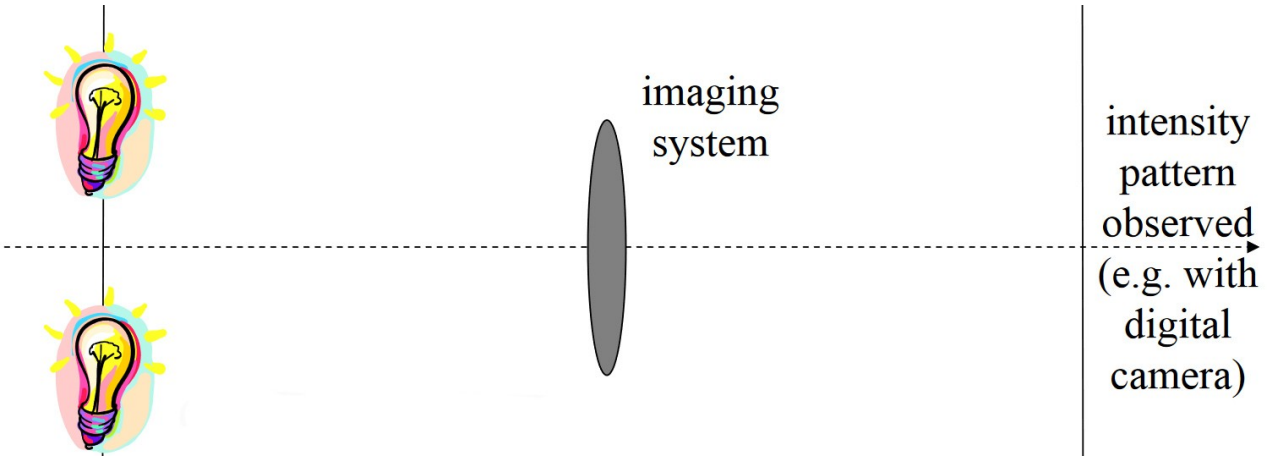
Beer's law

Refraction

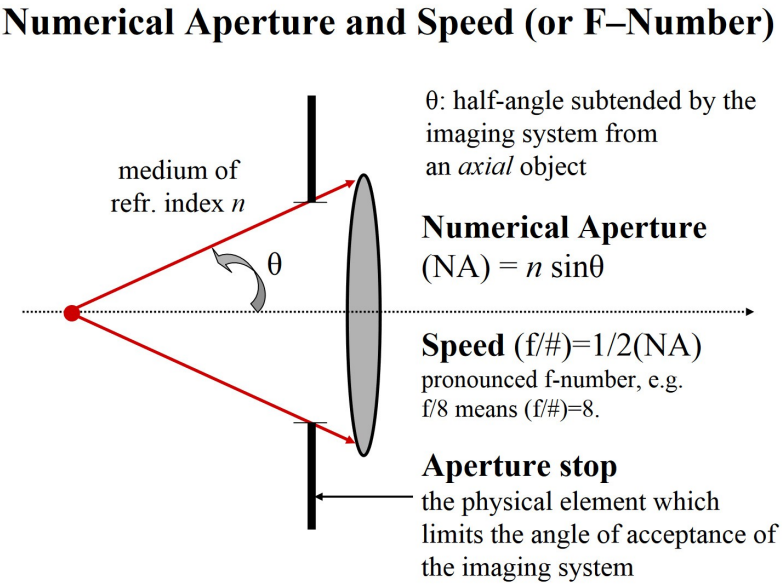
Ray tracing

Resolution

Resolution: The ability to separate two objects

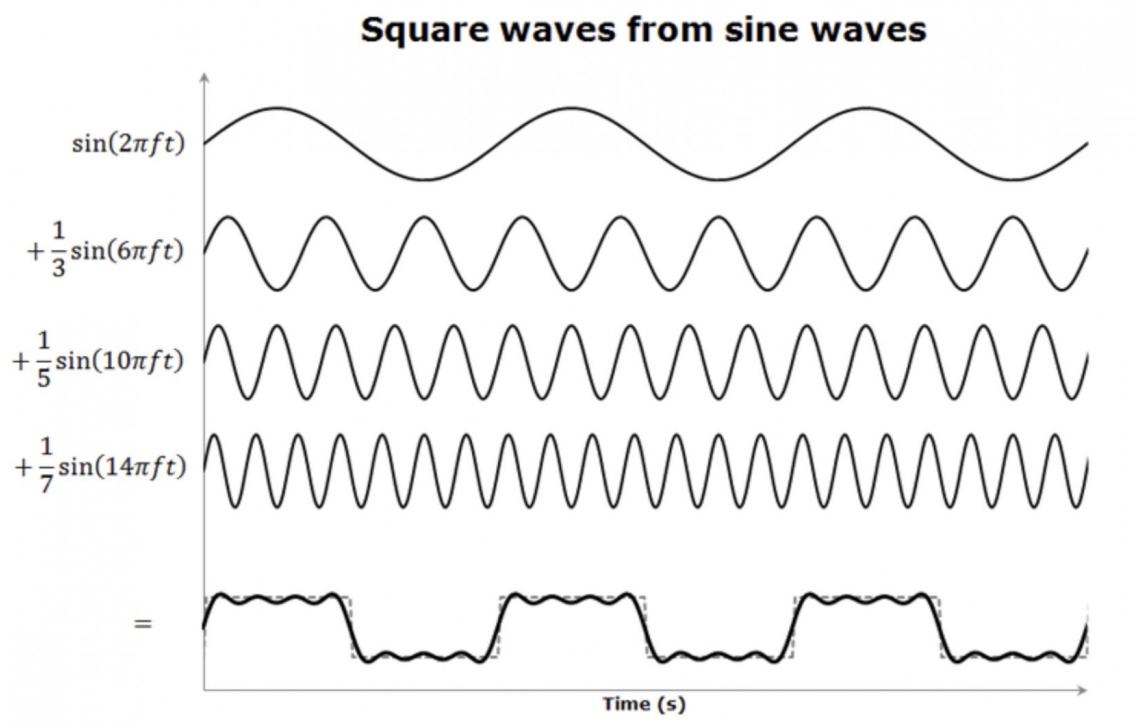


A definition of Numerical Aperture

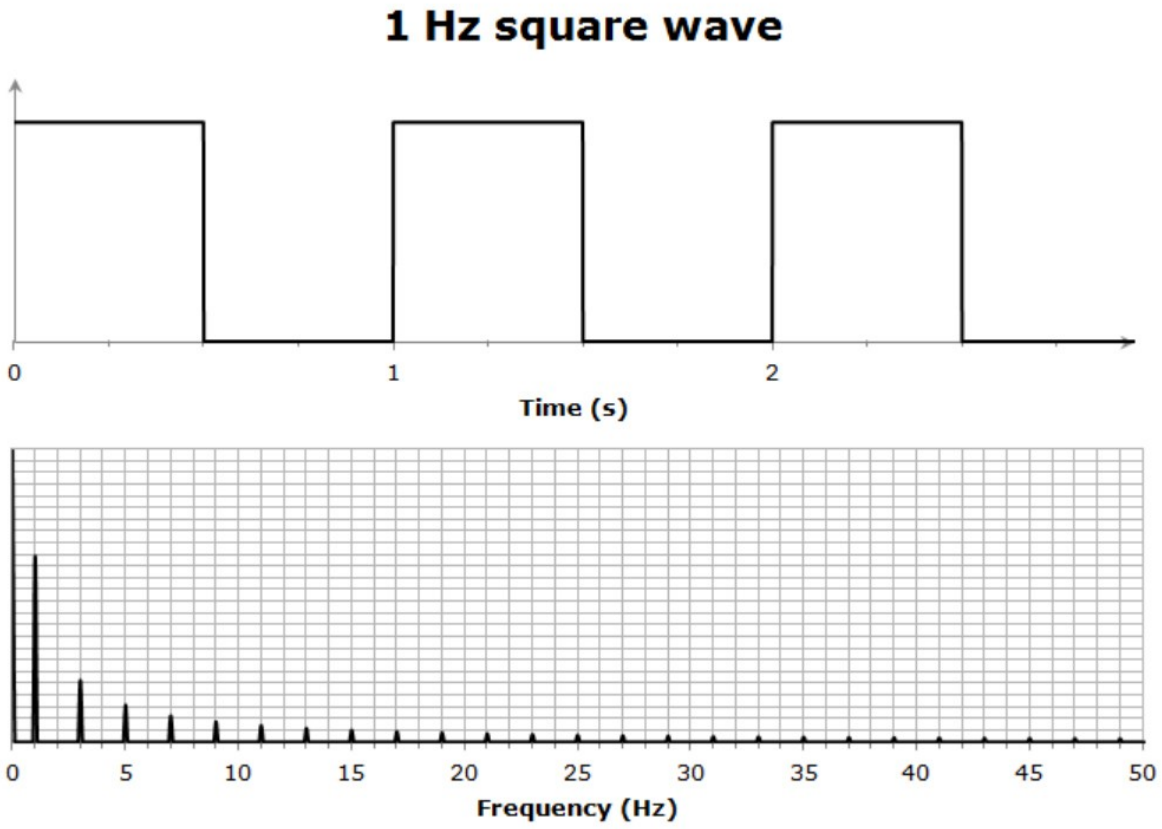


Fourier transform:

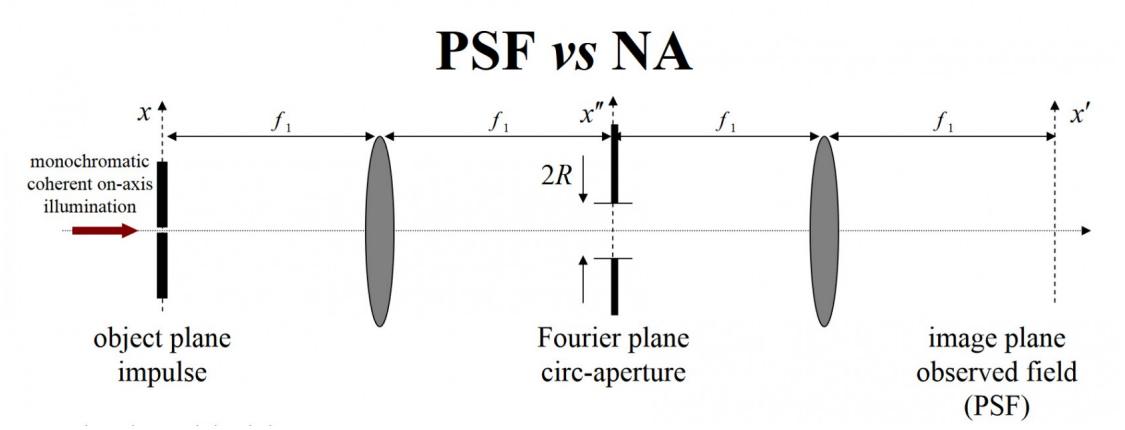
Transform from real space to frequency space



Now we look at a real square wave and frequency space



Look at a simple optical system:



Mathematical prediction of the Point Spread Function (PSF)

on the left we have the mathematical point source know as a delta function.

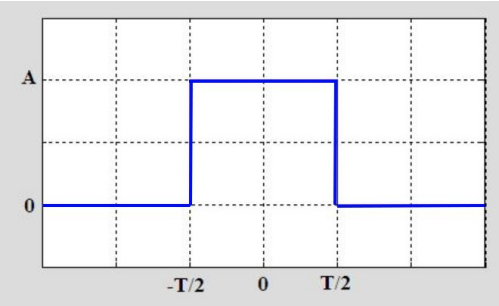
$$g_{\text{in}}(x,y) = \delta(x)\delta(y)$$

were

$$\delta(x) = \begin{cases} 0, & x \neq 0, \\ \infty, & x = 0, \end{cases}$$

the intensity at the Fourier plain can be found by taking the Fourier transform of this function.

$$F(\omega) = \mathcal{F}(\delta(x)) = \frac{1}{2\pi}\int_{-\infty}^{\infty} \delta(x) e^{i\omega x}\, dx = \frac{1}{2\pi}.$$ or

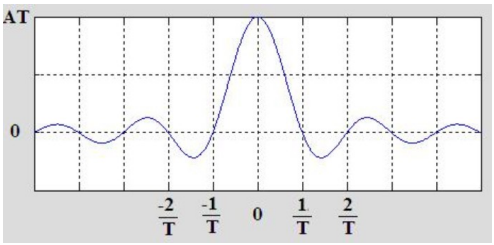


this has the same intensity at all points inside the aperture and zero outside. The second lens is now taking a Fourier transform on a box function the width of the aperture.

$$\text{jinc}(.,.) \equiv 2\frac{\mathrm{J}_1\left(2\pi \frac{R}{f_1}\frac{r'}{\lambda}\right)}{2\pi \frac{R}{f_1}\frac{r'}{\lambda}}$$

(unit magnification)

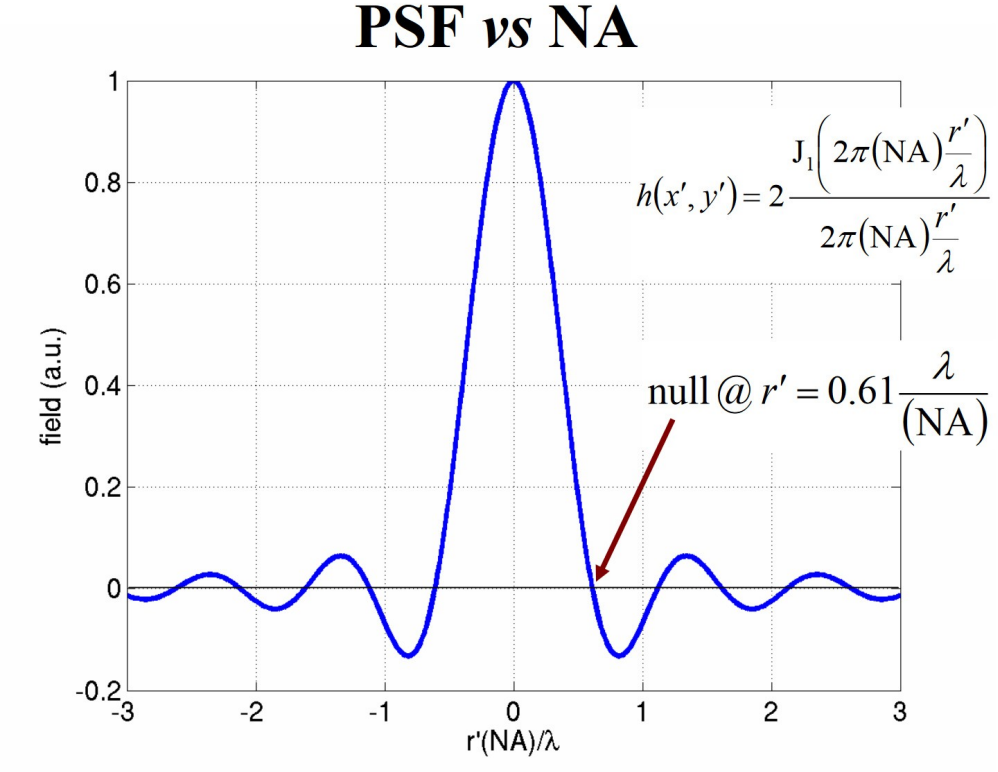
or

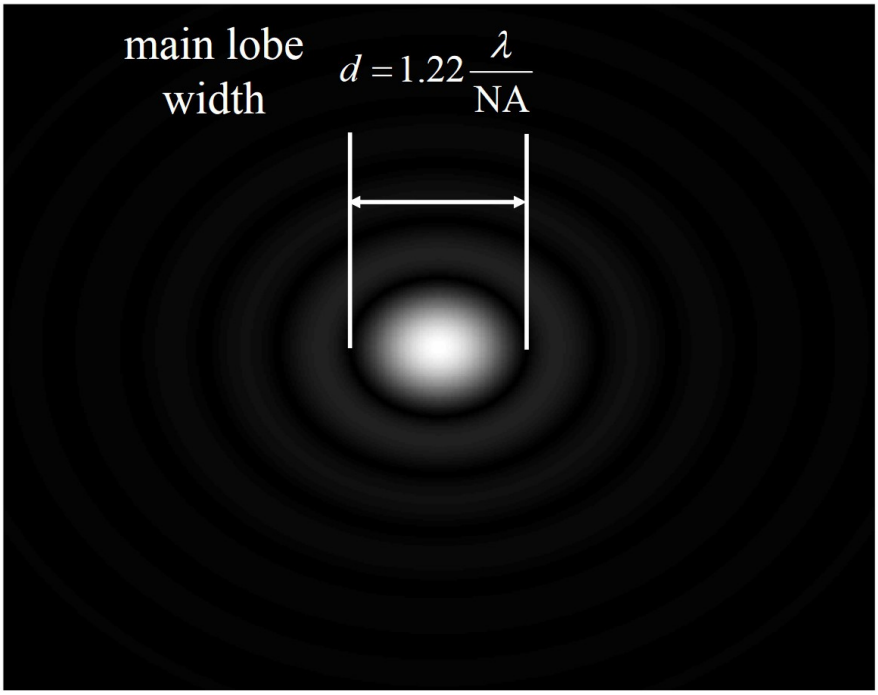


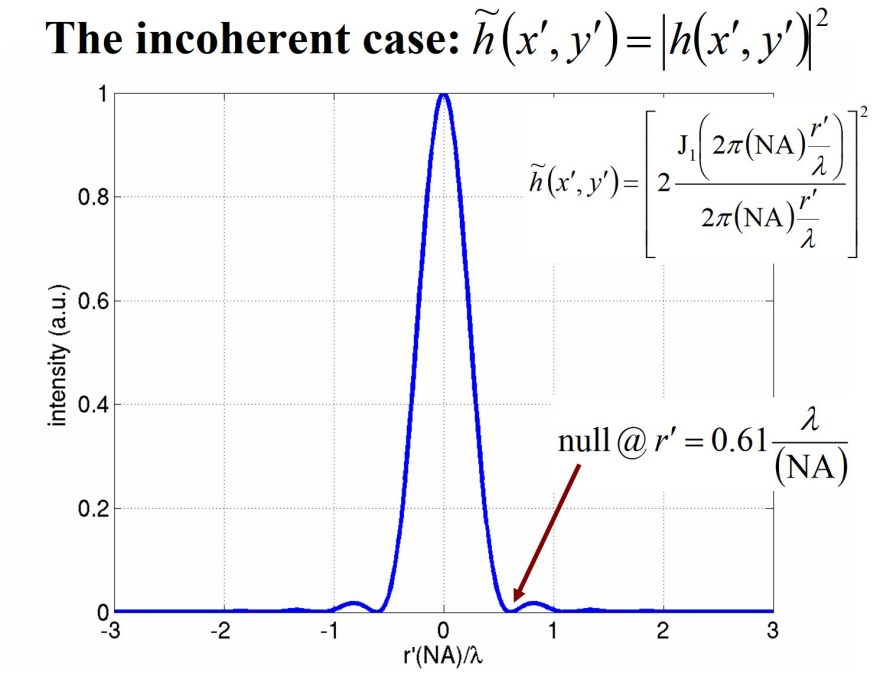
Substituting in the definition for NA

Numerical Aperture (NA) by definition: $(\text{NA}) \equiv \frac{R}{f_1}$

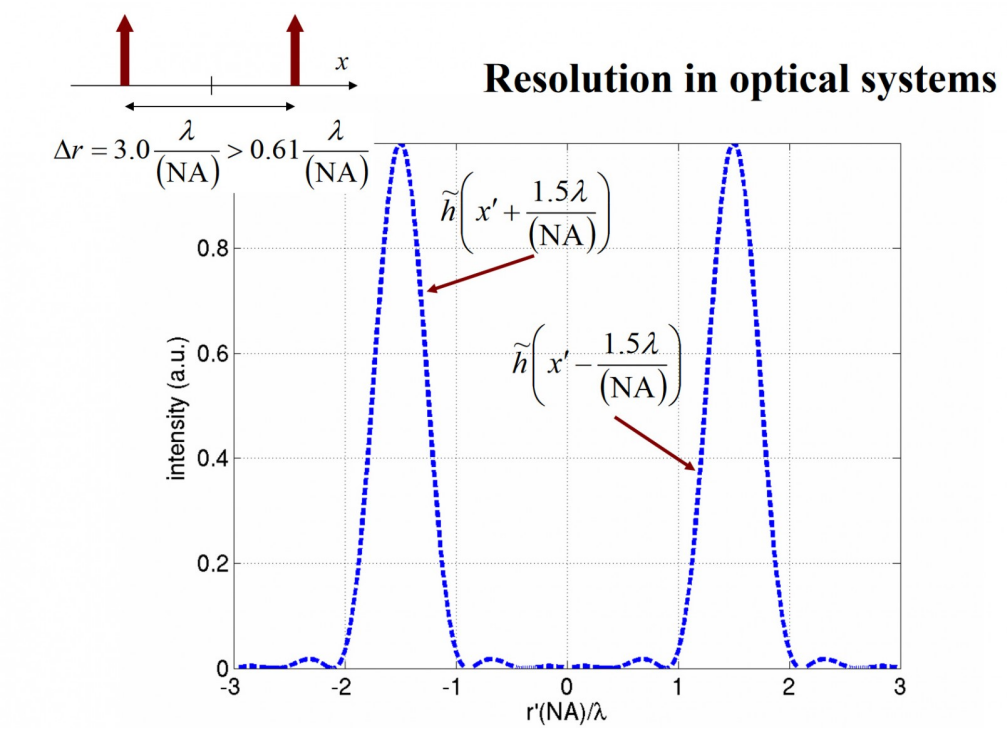
PSF *vs* NA


$h(x', y') = 2\frac{J_1\left(2\pi(\mathrm{NA})\frac{r'}{\lambda}\right)}{2\pi(\mathrm{NA})\frac{r'}{\lambda}}$
$\text{null} @ r' = 0.61\frac{\lambda}{(\mathrm{NA})}$
field (a.u.)
r'(NA)/λ
1
0.8
0.6
0.4
0.2
0
-0.2
-3
-2
-1
0
1
2
3


main lobe width
$d = 1.22\frac{\lambda}{\mathrm{NA}}$

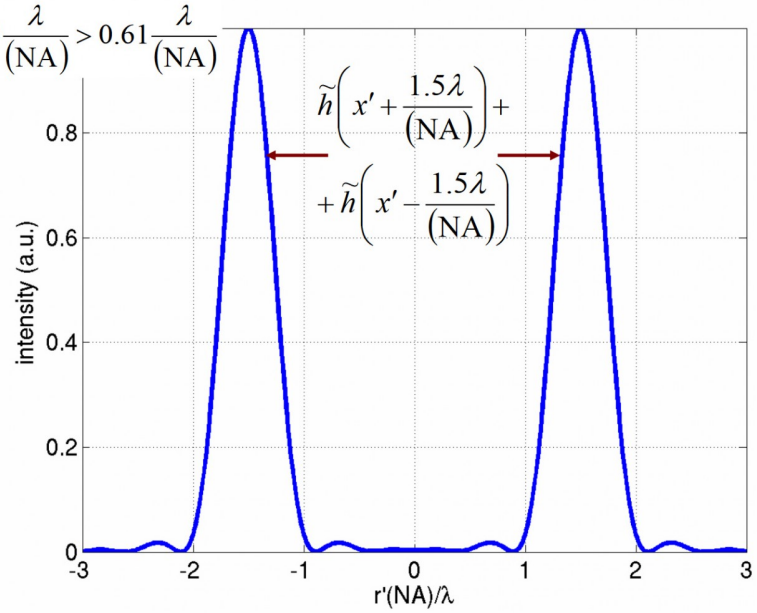


Now we go back to Resolution. How close together we can position two points and still distinguish them

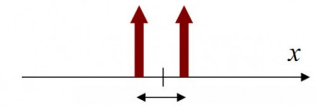


Resolution in optical systems

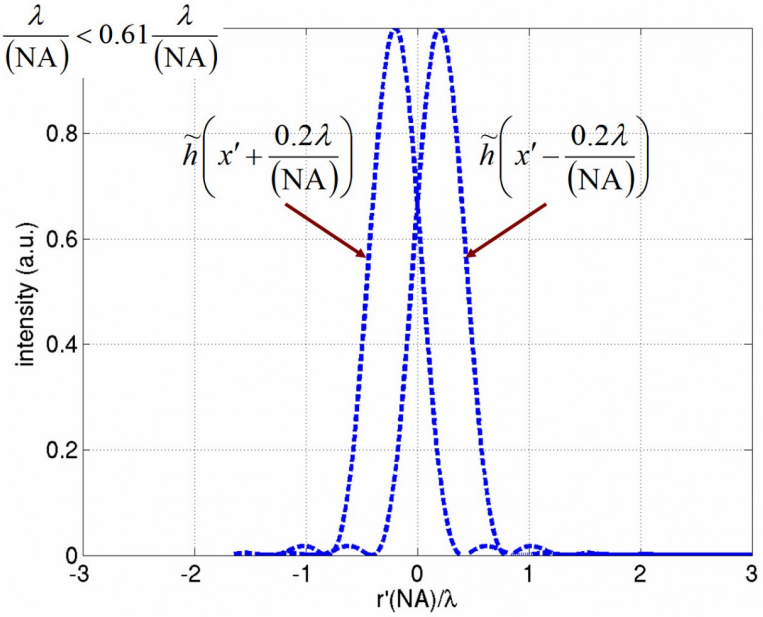
$$\Delta r = 3.0\frac{\lambda}{(\mathrm{NA})} > 0.61\frac{\lambda}{(\mathrm{NA})}$$



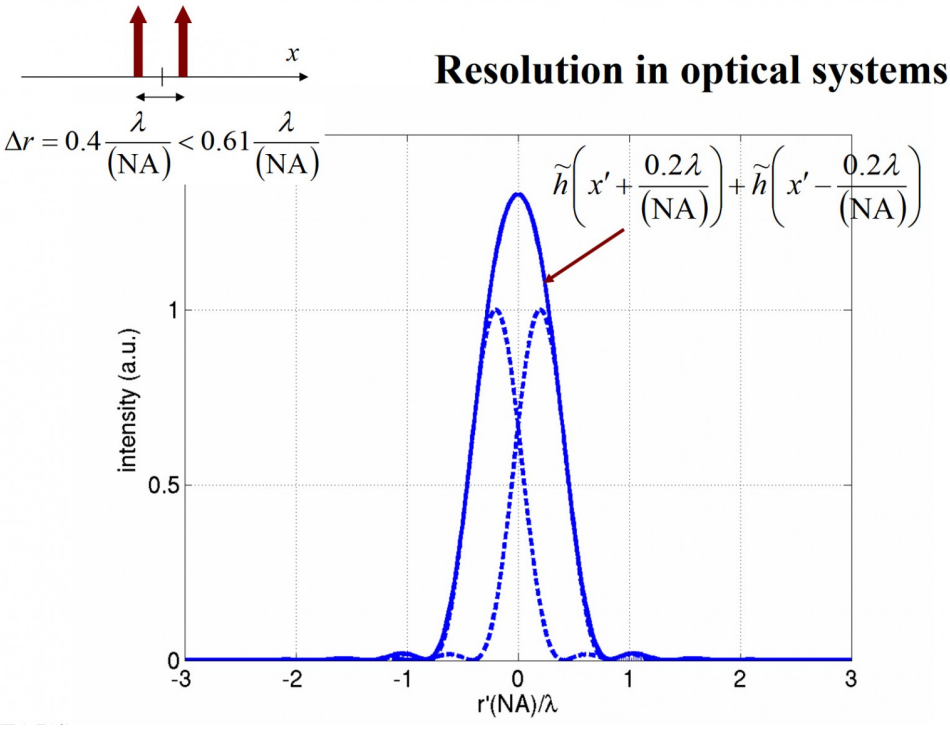
Resolution in optical systems



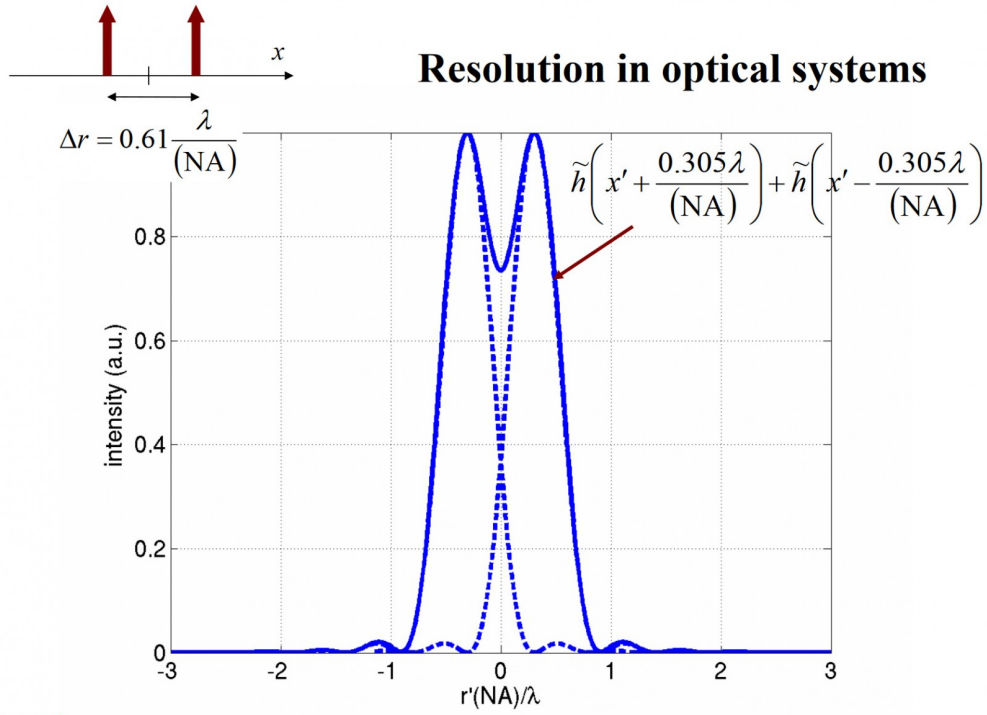
$$\Delta r = 0.4\frac{\lambda}{(\mathrm{NA})} < 0.61\frac{\lambda}{(\mathrm{NA})}$$



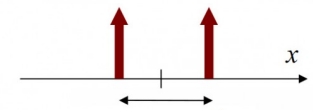
Resolution in optical systems



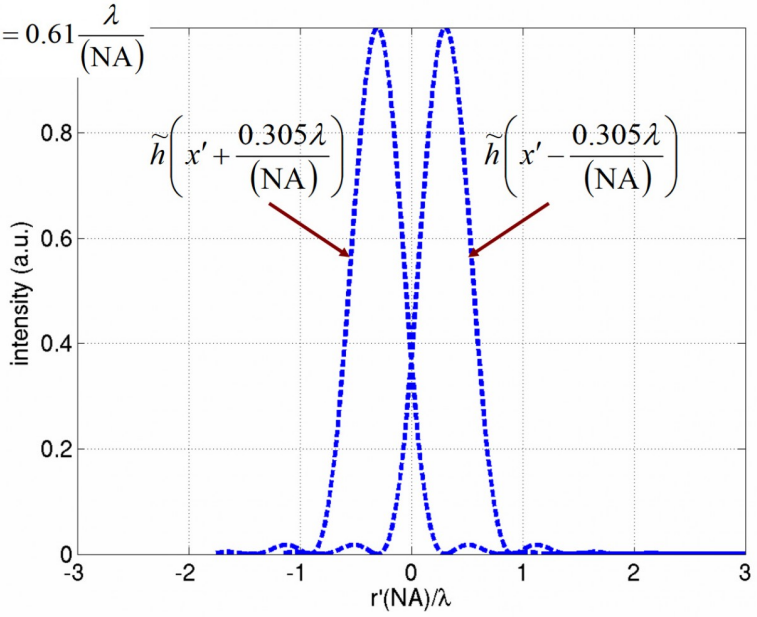
Resolution in optical systems



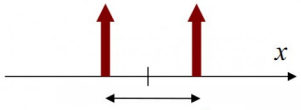
Resolution in optical systems



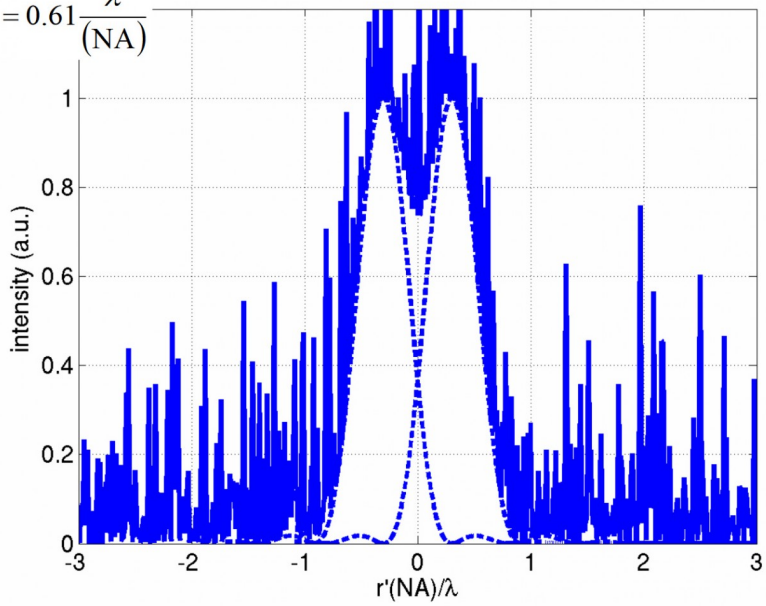
$$\Delta r = 0.61\frac{\lambda}{(\mathrm{NA})}$$

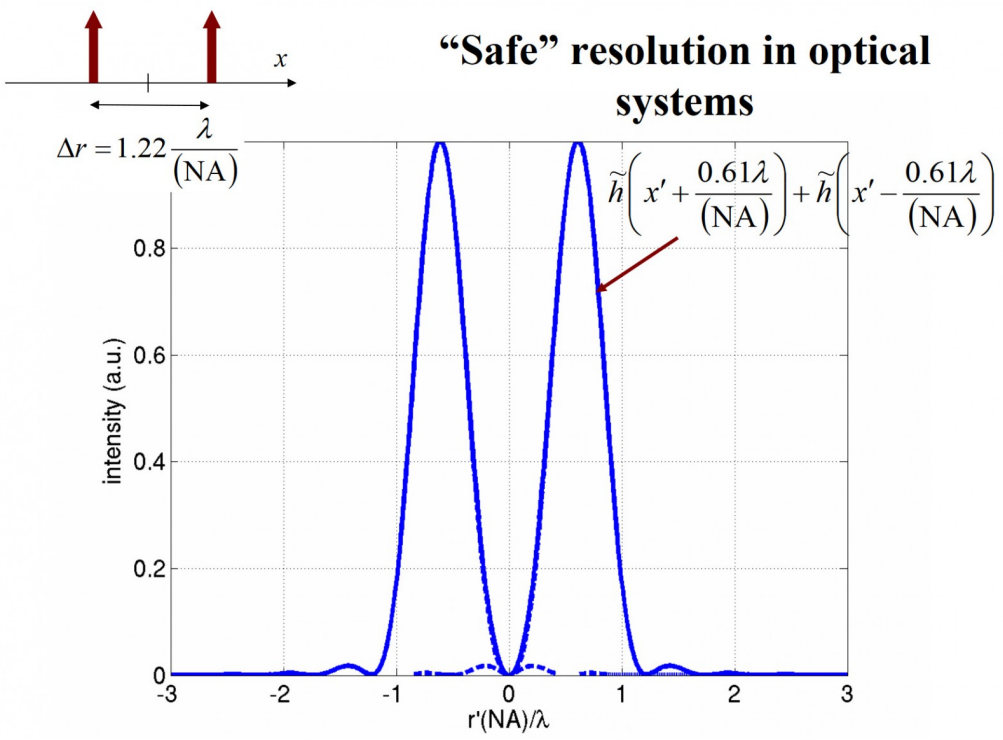


Resolution in noisy optical systems



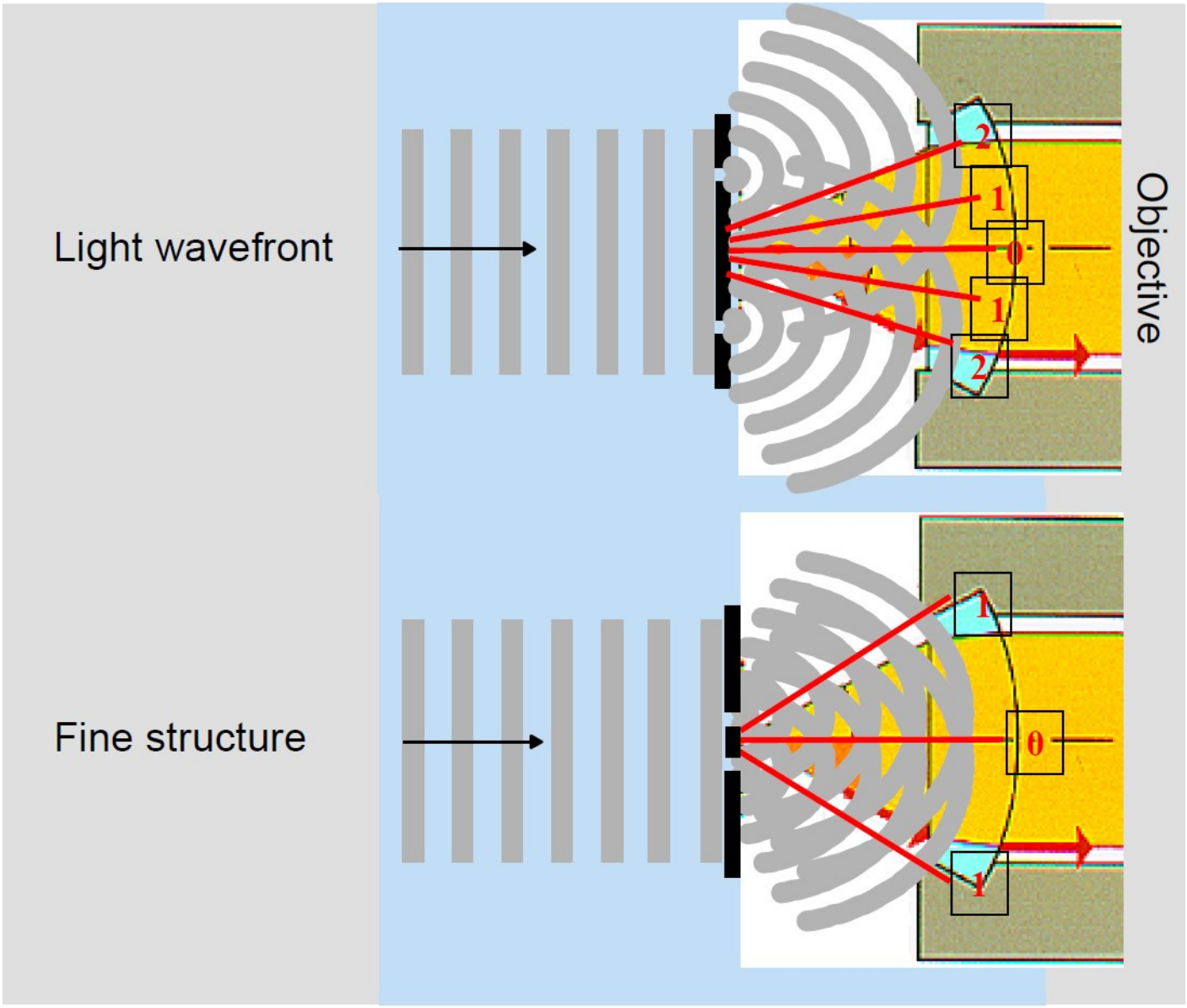
$$\Delta r = 0.61\frac{\lambda}{(\mathrm{NA})}$$





Theoretical maximal resolution d_0	$d_0 = \frac{\lambda}{\text{n. A.}_{\text{Objective}} + \text{n. A.}_{\text{Condenser}}}$
Simplified formula (wo condensor) for resolution d_0	$d_0 = \frac{\lambda}{2\ \text{n. A.}_{\text{Objective}}}$
Maximal resolution d_0 in reality	$d_0 = \frac{1.22 \times \lambda}{2\ \text{n. A.}_{\text{Objective}}}$
Example	**Green light λ = 550 nm, n. A. = 1.4 (Oil immersion)** **d_0 = 671 nm / (2 x 1.4) = 239 nm = 0.239 µm**

More intuitive approach



Notes from:

http://web.mit.edu/2.710/Fall06/2.710-wk12-b-sl.pdf

https://links.uwaterloo.ca/amath353docs/set11.pdf

https://www.thefouriertransform.com/pairs/box.php

http://www.phys.unm.edu/msbahae/Optics%20Lab/Fourier%20Optics.pdf

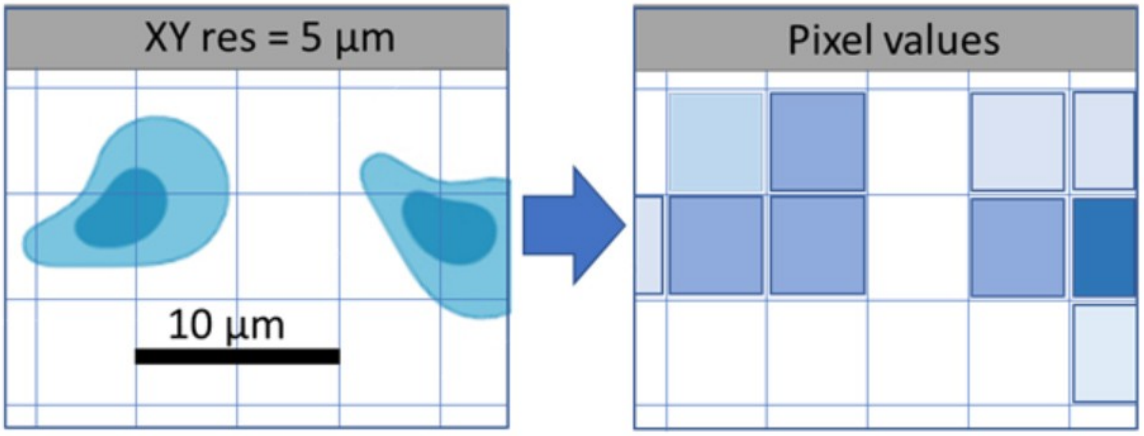
Super Resolution Techniques

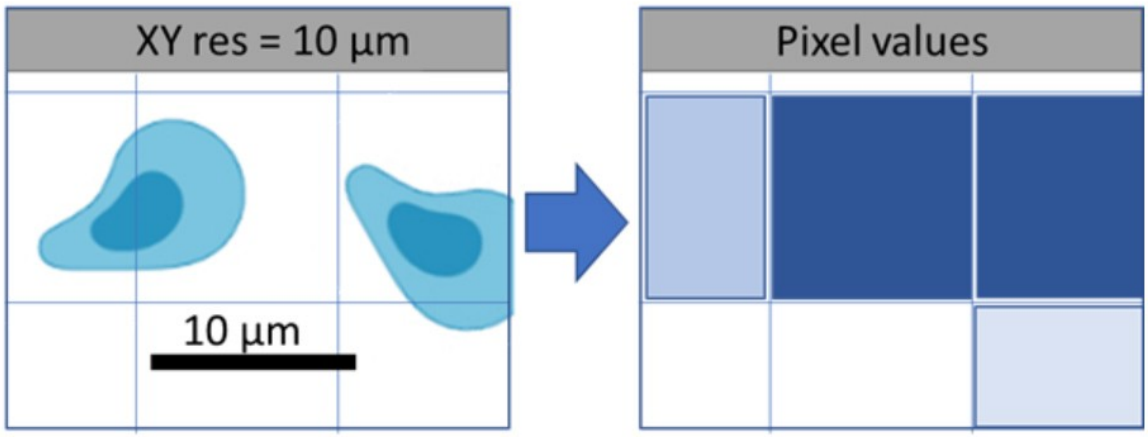
Sampling

How does digital sampling affect resolution

Look at imaging these object with a digital camera

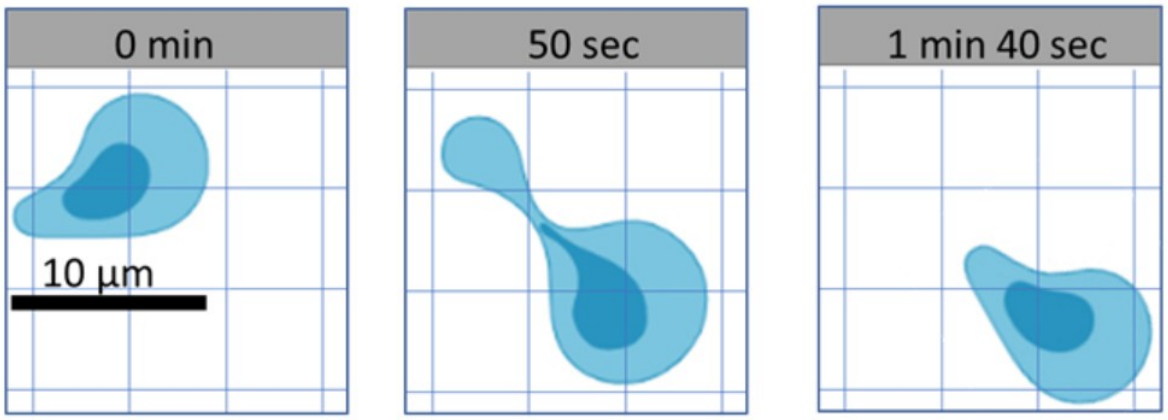
How close together do pixels need to be?





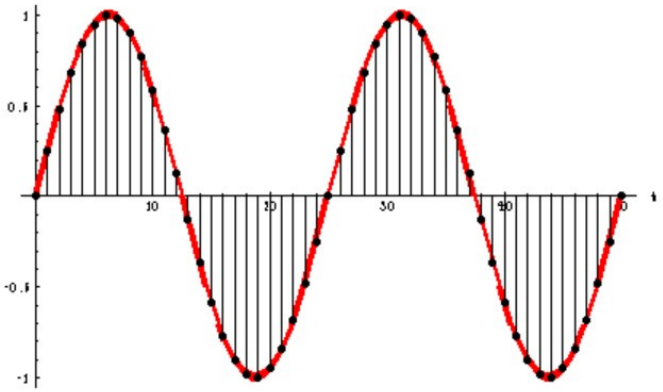
Sampling over time:

How often do you need to image a moving sample

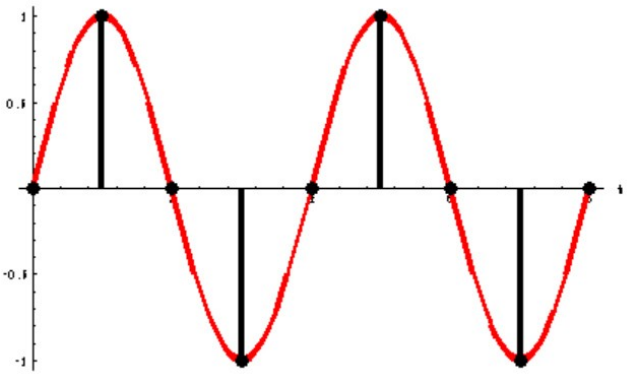


Nyquist theory states that you should sample more than 2 X the frequency that you expect.

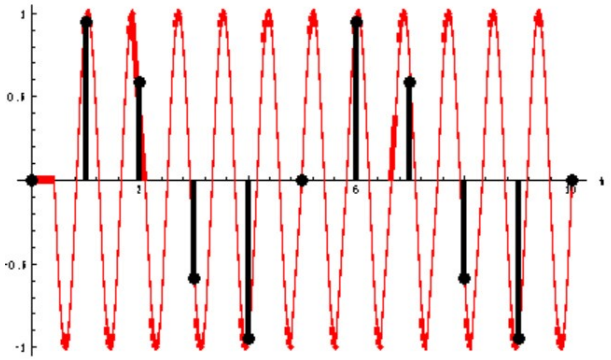
Over sampling



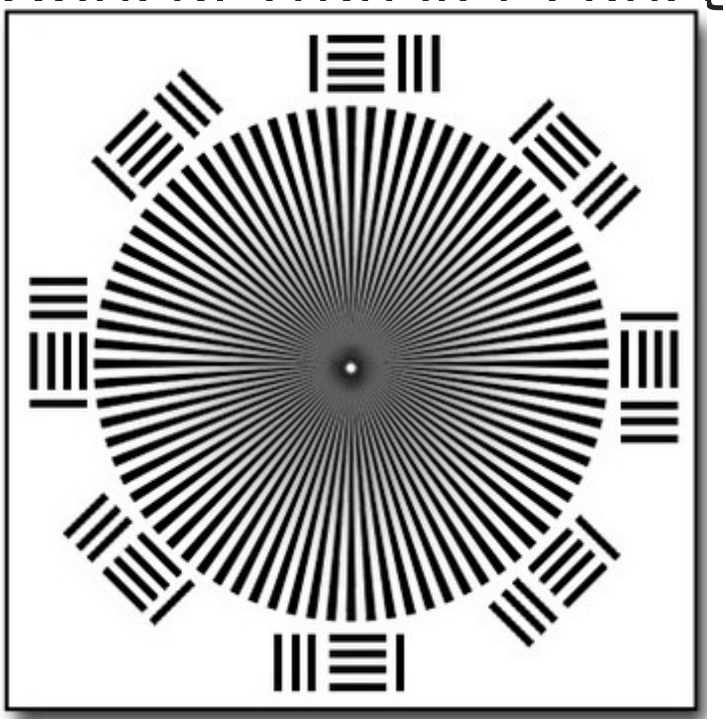
Nyquist sampling

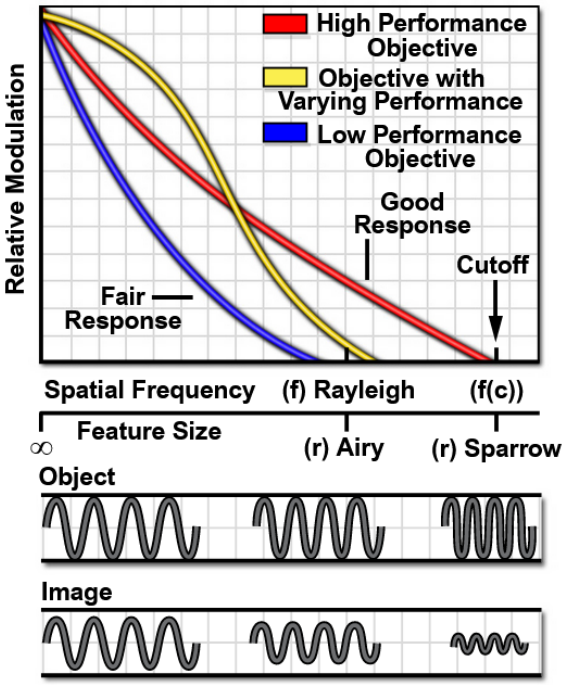


Under sampling causes aliasing



Optical Transfer Function MTF





When objects get close together the contrast decreases.

MTF = Image Modulation/Object Modulation

$MTF = 2(\varphi - \cos\varphi\sin\varphi)/\pi$ and

$$\varphi = \cos^{-1}(\lambda\nu/2NA)$$

The Optical Transfer function is the Modulation transfer function times a phase component.

$OTF = MTF \times e^{i\varphi(f)}$

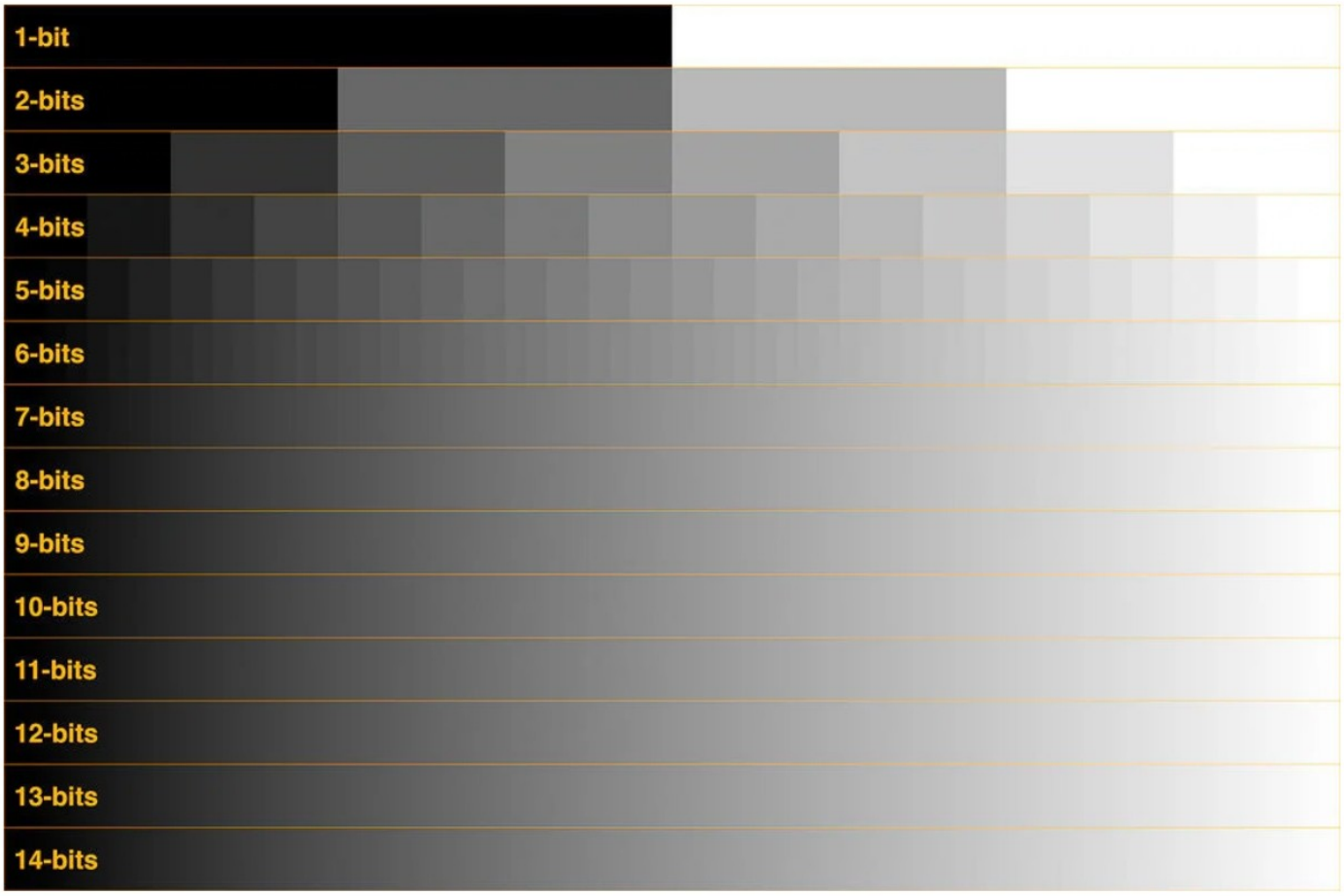
Camera bit depth

0 or 1

00 or 01 or 10 or 11

000 or 001 or 010 or 011 or 100 or 101 or 110 or 111

and so on



Jpg is 8 bit Tiff can be 16 bit

references

https://imb.uq.edu.au/research/facilities/microscopy/training-manuals/microscopy-online-resources/image-capture/nyquist-conditions

https://microscopy.berkeley.edu/courses/dib/sections/02images/sampling.html

https://ocw.mit.edu/courses/mechanical-engineering/2-71-optics-spring-2009/video-lectures/lecture-22-coherent-and-incoherent-imaging/MIT2_71S09_lec22.pdf

Working in the IGB Core

Expect to walk into a room with a fully functional instrument

Let a core staff person know if you see a problem

Clean up when you leave

Acknowledge the IGB Core as:

“Core Facilities at the Carl R. Woese Institute for Genomic Biology"

Let us know when you publish

Collaborations with the core facilities staff can be beneficial in the development of unique methods or capabilities.