

Basic Optics

Initial Training for Optical Microscope Users

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- [Fluorescence Imaging](#)
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- [IGB Core Instrument LSM 900](#)
- [IGB Core Instrument V16](#)
- [IGB Core Instruments Axiovert 200M](#)
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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.](#)

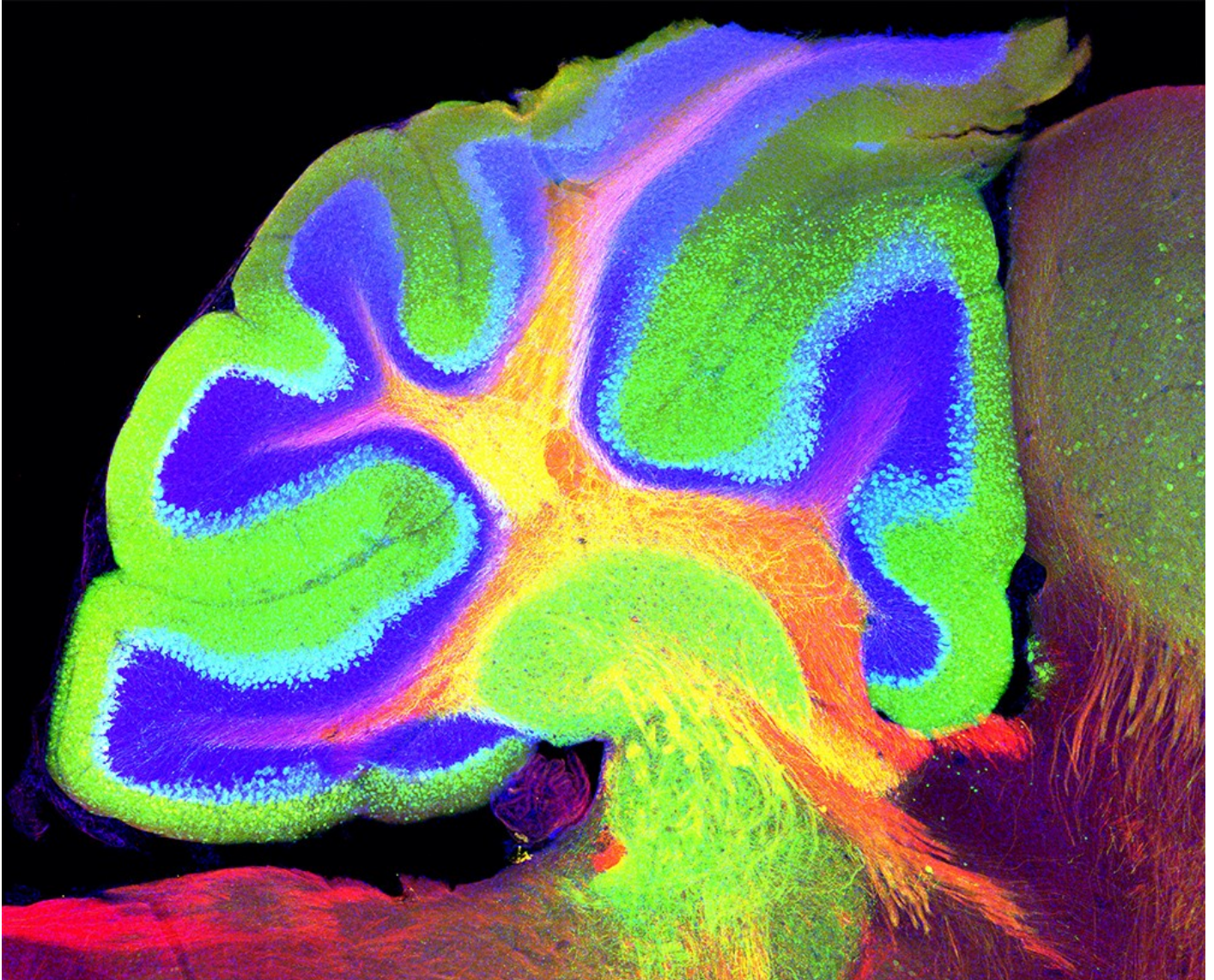
Long Term Storage

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

Fluorescence Imaging

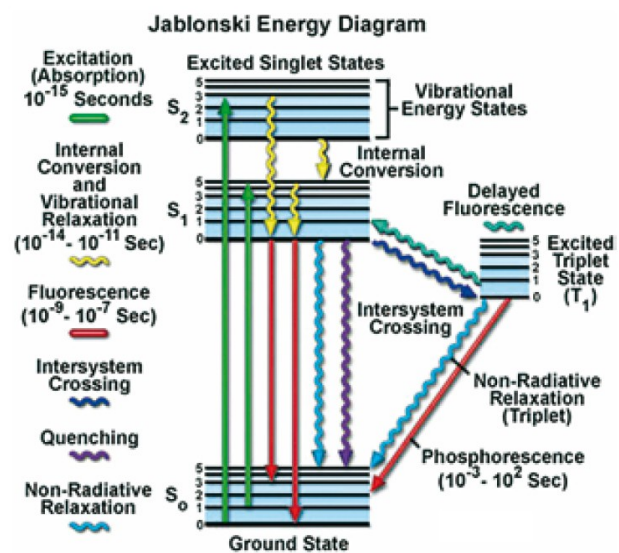
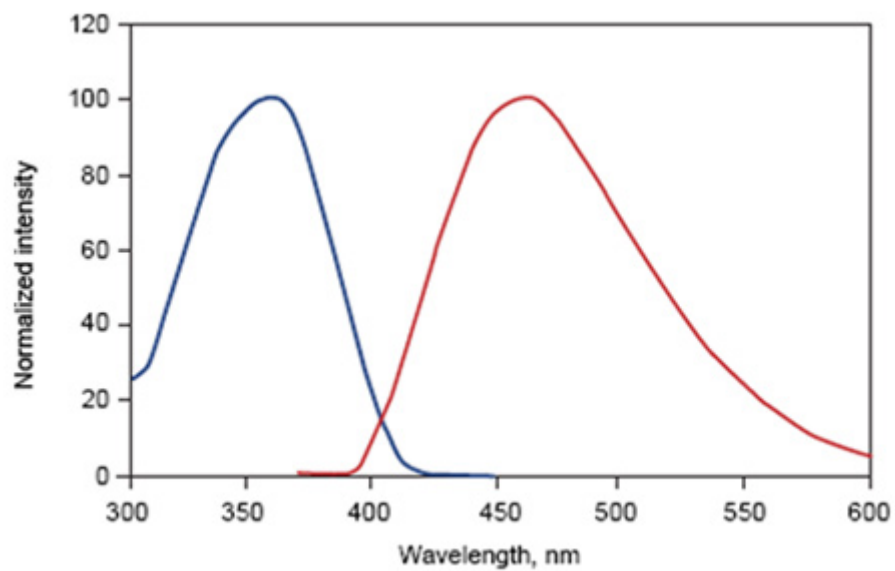
Why Fluorescence:

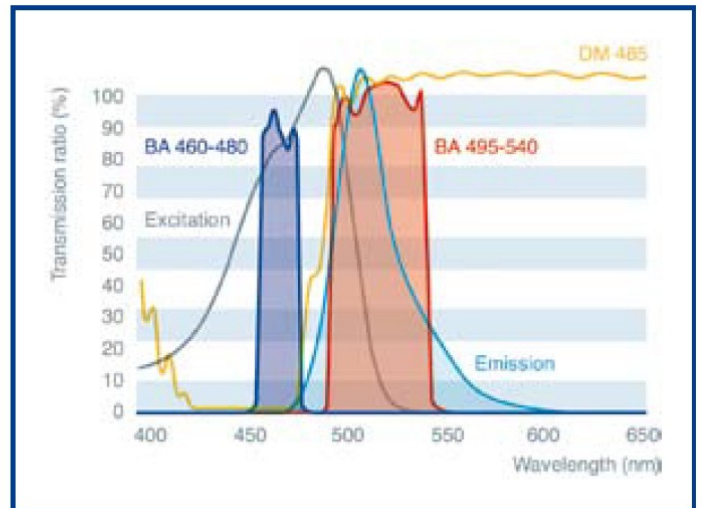
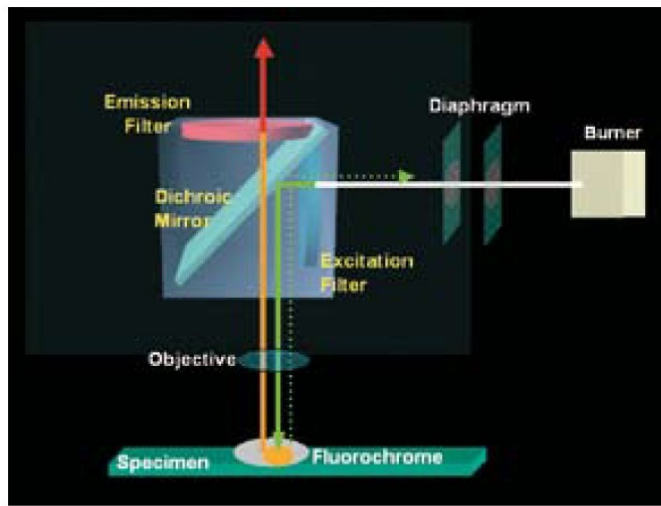
We can label what we want to see



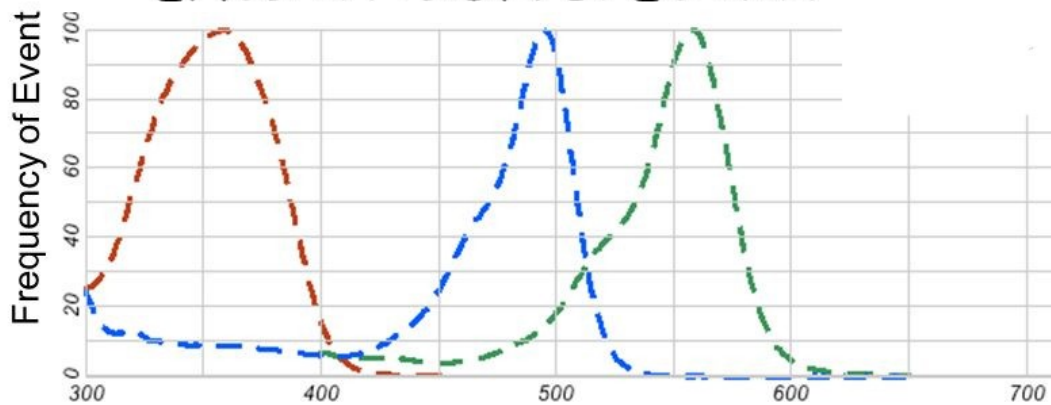
Excitation and Emission

Dapi

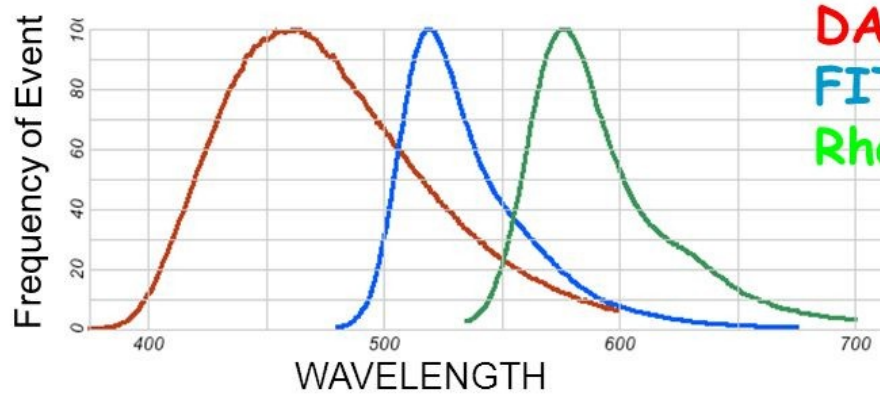




EXCITATION SPECTRA



EMISSION SPECTRA



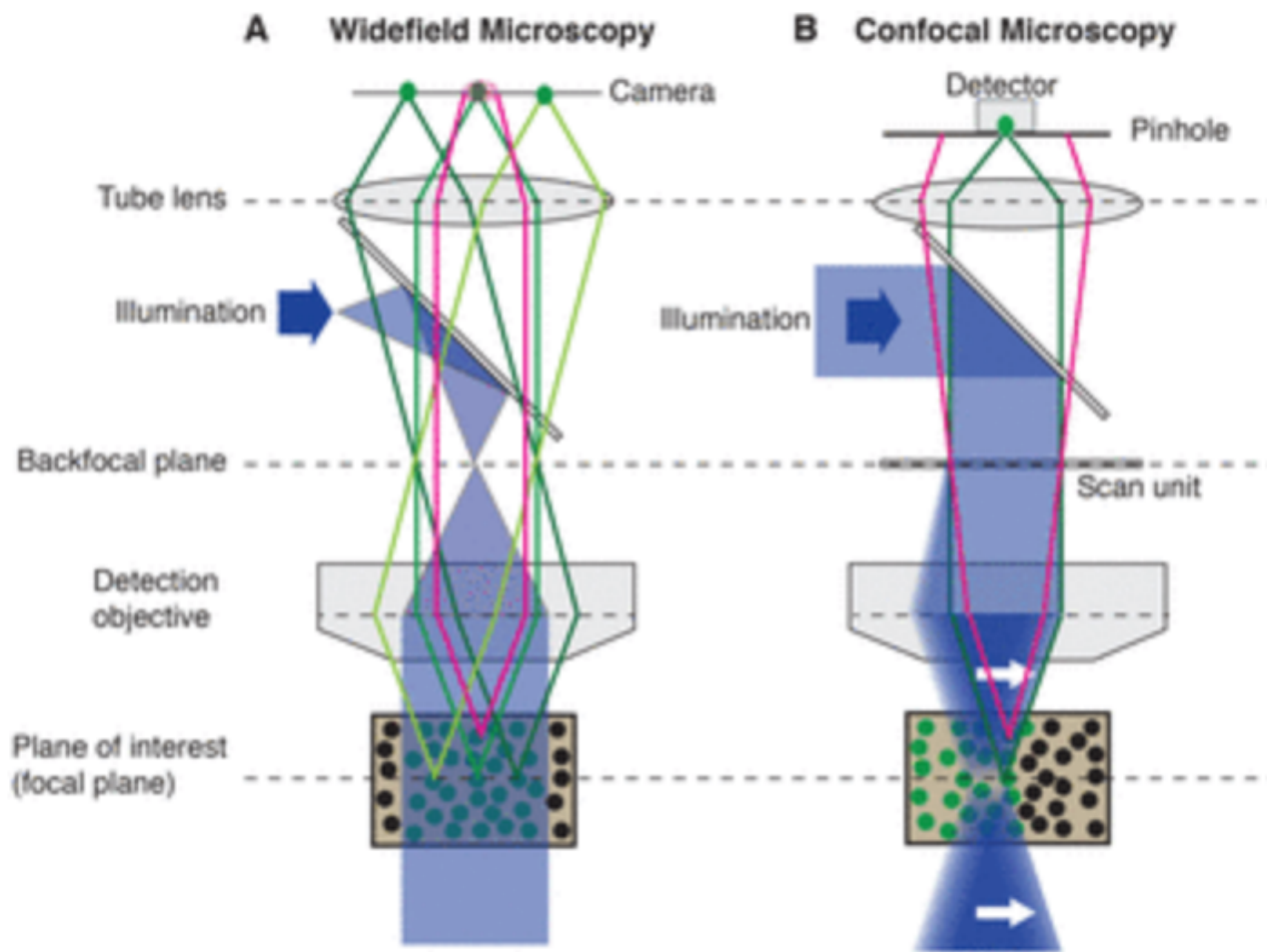
Fluorochrome

DAPI

FITC

Rhodamine

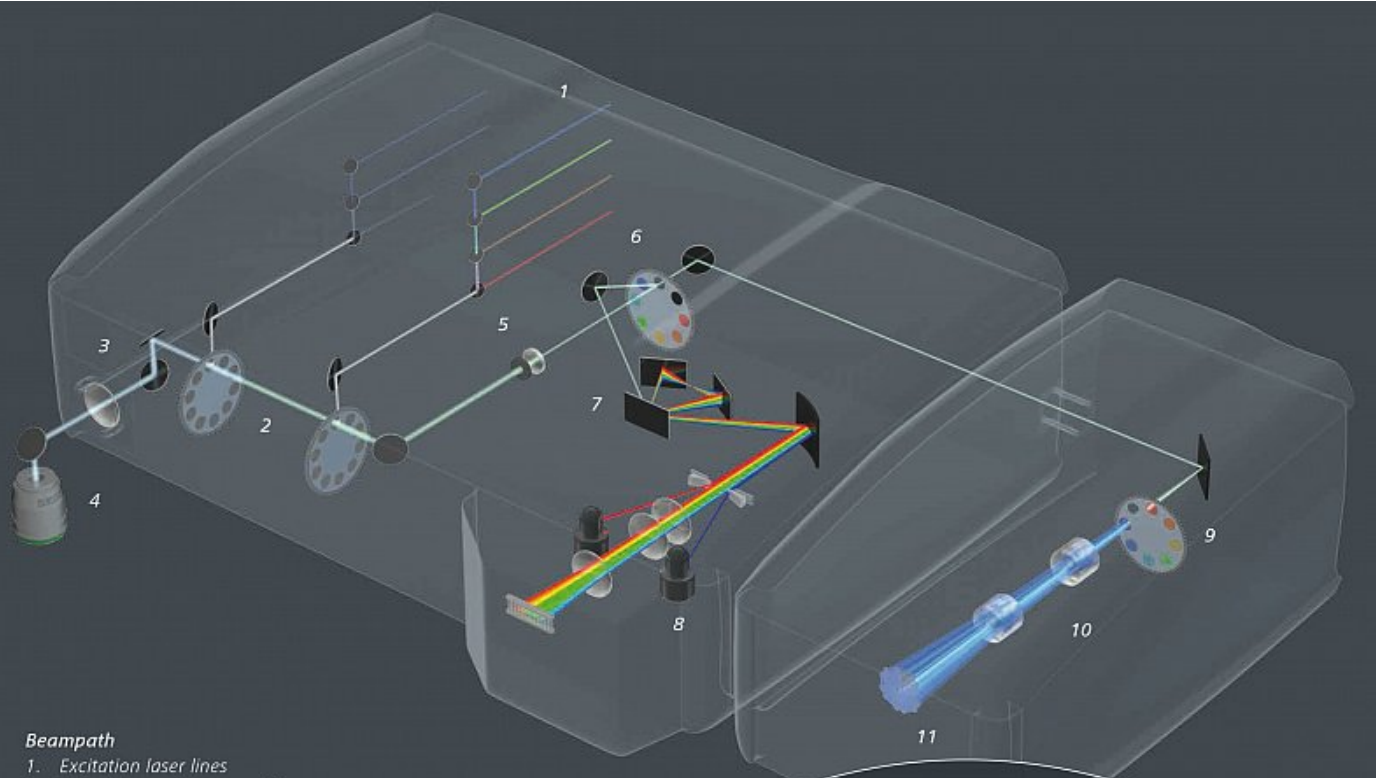
Widefield vs Confocal



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

IGB Core Instrument LSM

880

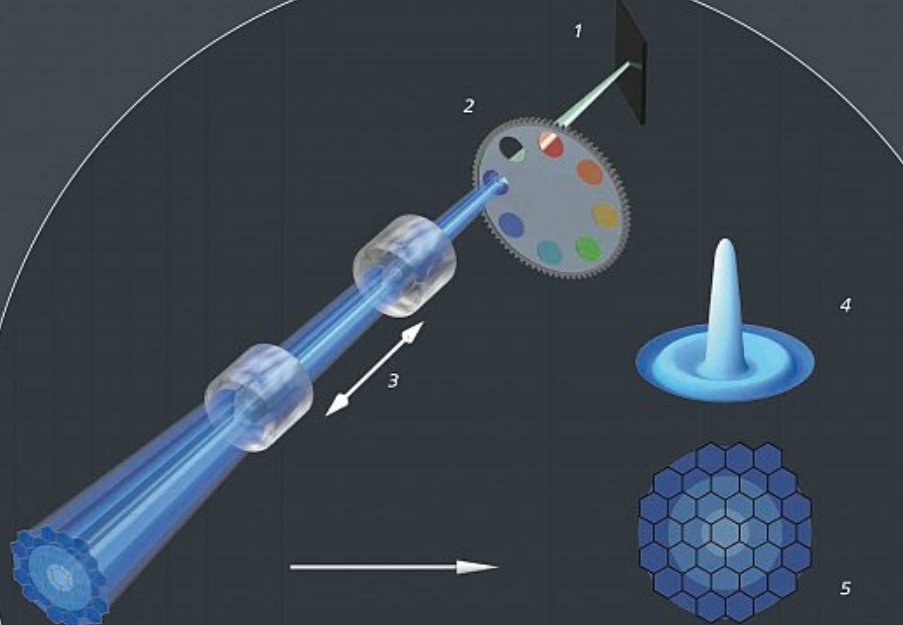


Beampath

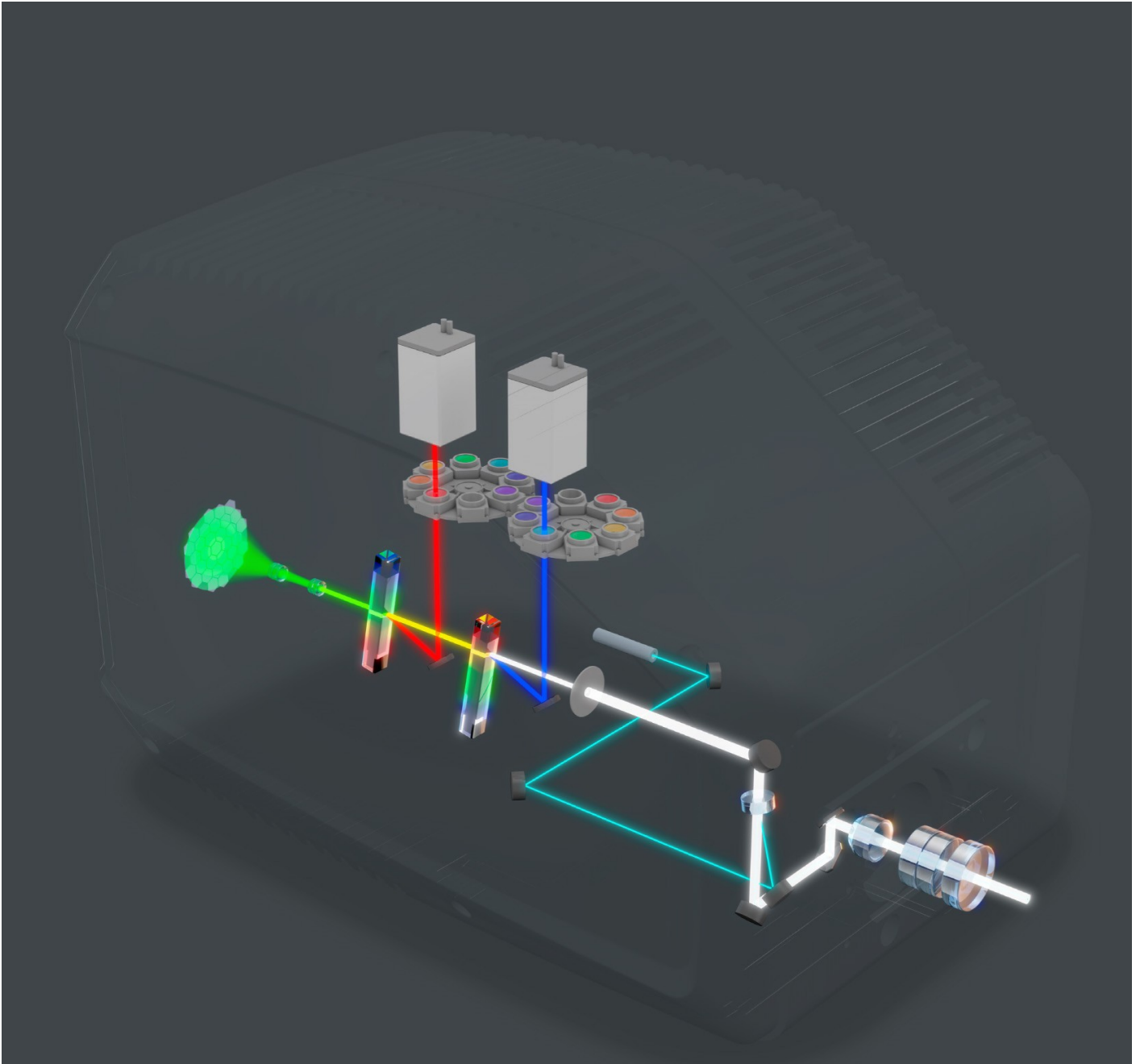
1. Excitation laser lines
2. Twin Gate main beam splitters
3. Galvo scanning mirrors
4. Objective
5. Pinhole and pinhole optics
6. Secondary beam splitters
7. Recycling loop
8. Quasar detection unit
9. Emission filters
10. Zoom optics
11. Airyscan detector

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

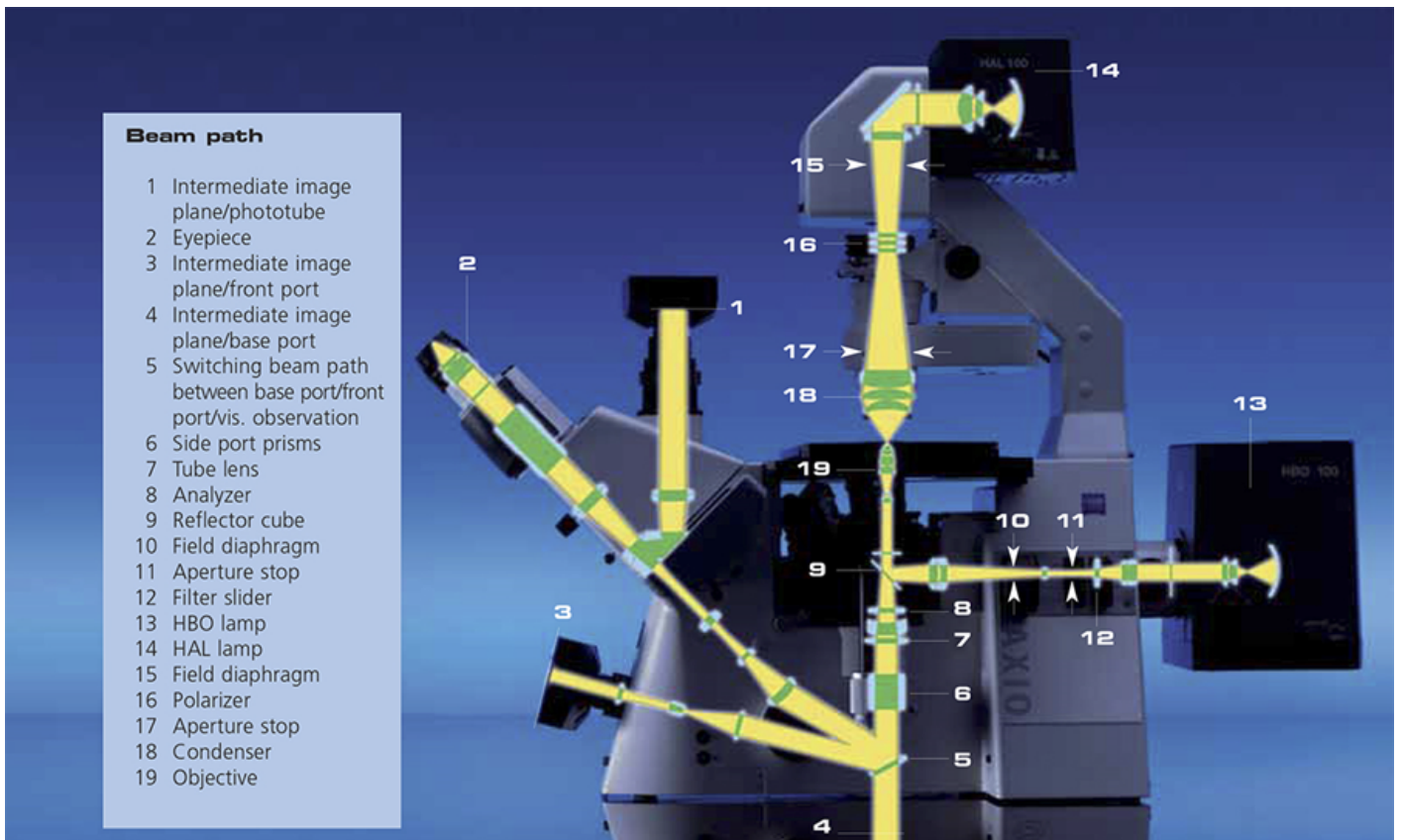
file:///C:/Users/gfried/Downloads/EN_poster_Beampath-LSM-900_A1.pdf

IGB Core Instrument V16

IGB Core Instruments

Axiovert 200M

Axiovert 200M



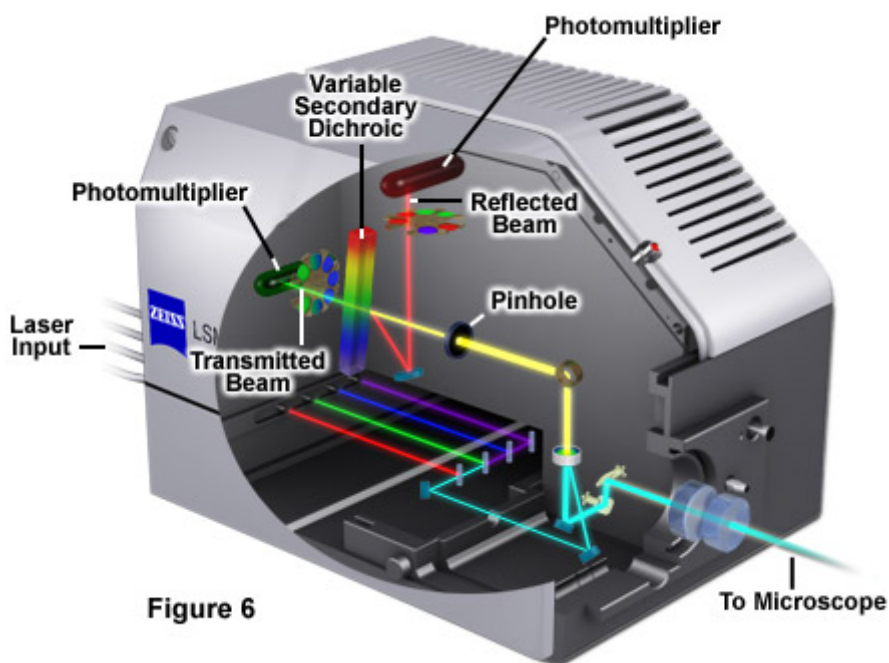
Cameras

cMOS

IGB Core Instruments LSM 700

LSM 700

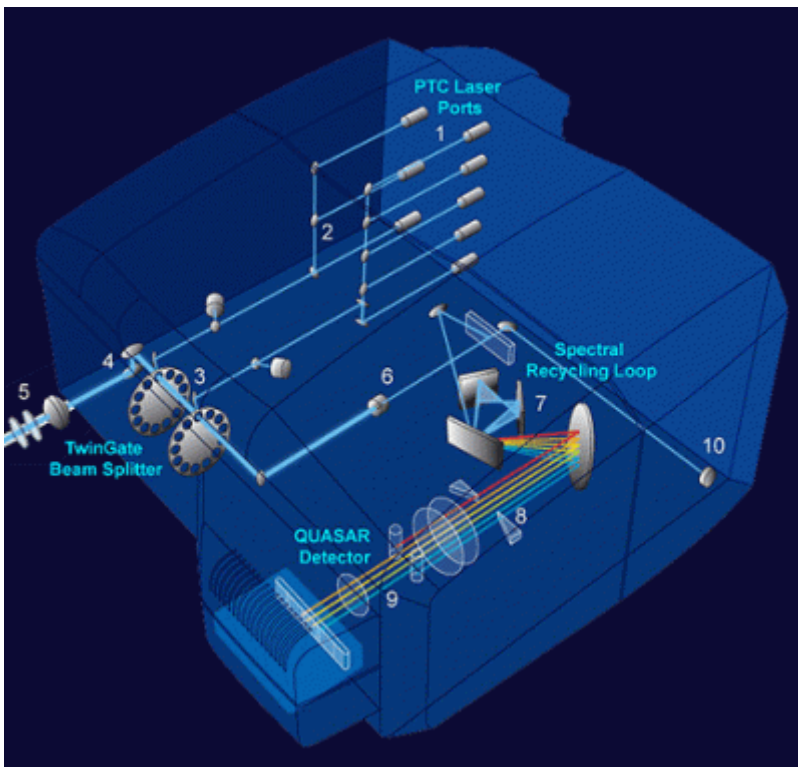
Confocal Scan Head with Variable Secondary Dichroic Spectral Imaging



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

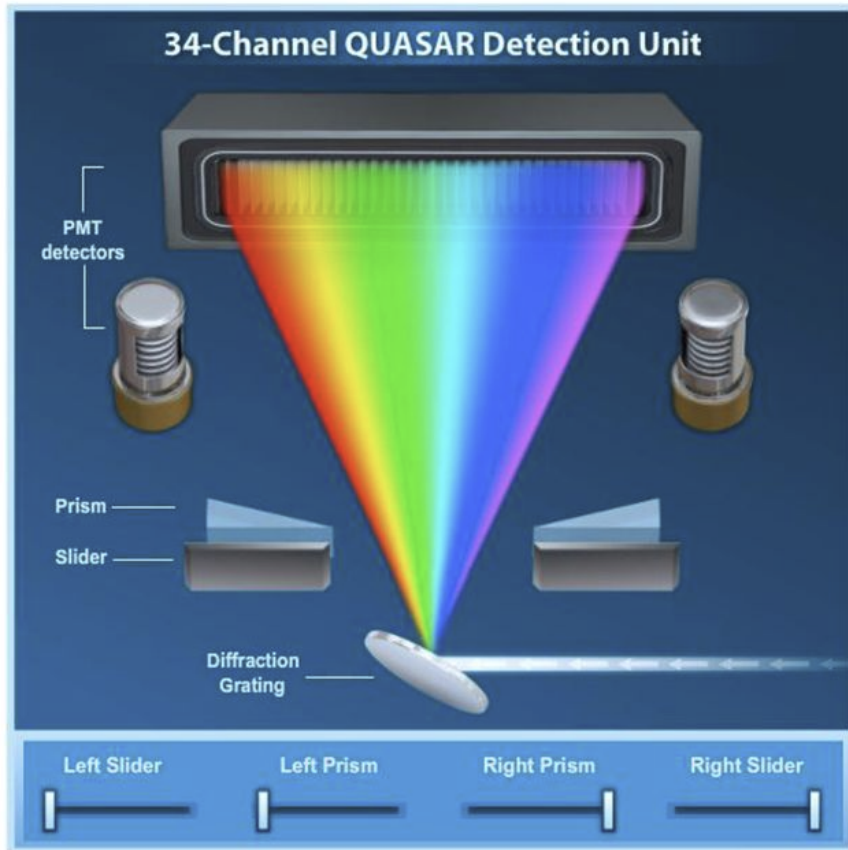
IGB Core Instruments Zeiss LSM 710

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>

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Slide 23 t:/powerpnt/course/524lect4.ppt

Lambda Stack with Cyan, Green, and Yellow Fluorescent Proteins

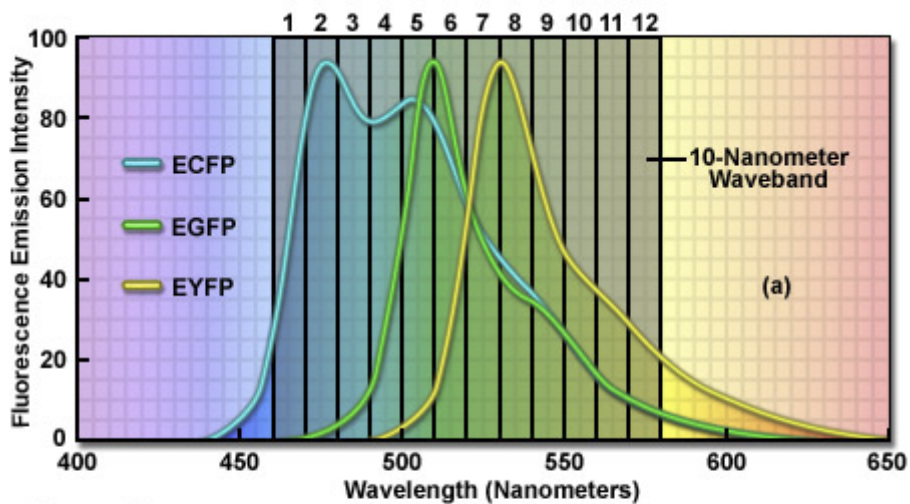


Figure 4



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>

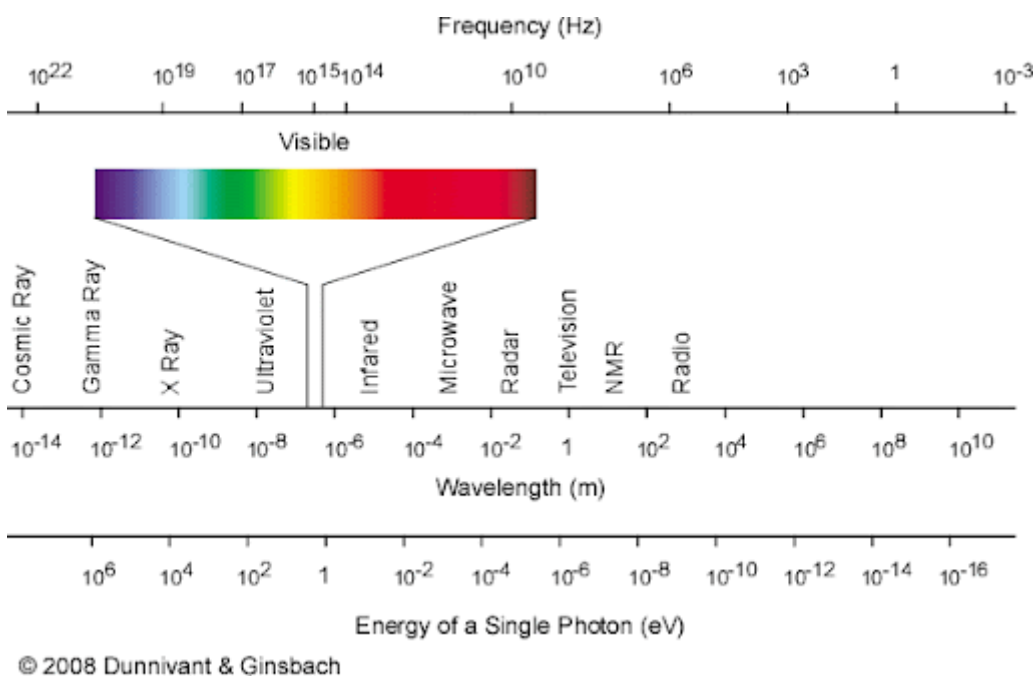
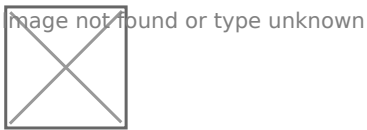
Objectives

Optics

Properties of light

Wave particle duality:

Light is a wave



Absorption and Emission

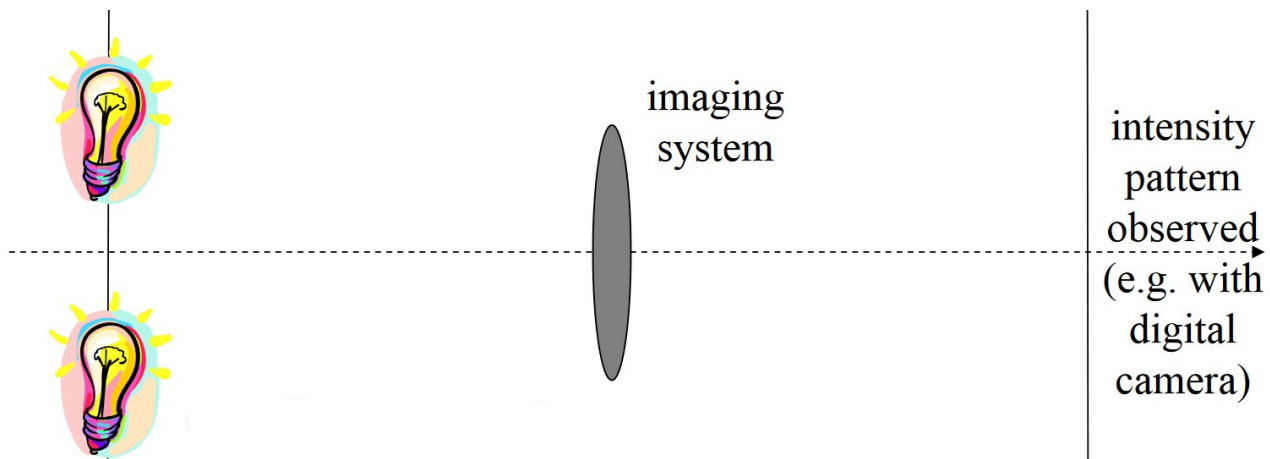
Beer's law

Refraction

Ray tracing

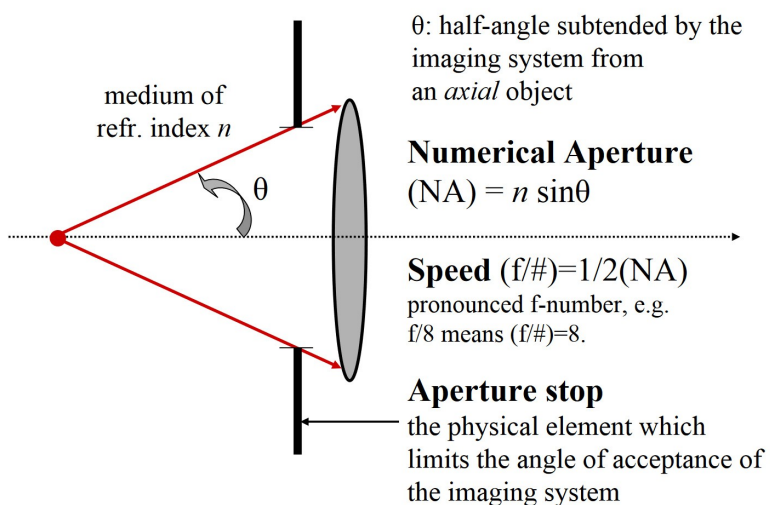
Resolution

Resolution: The ability to separate two objects



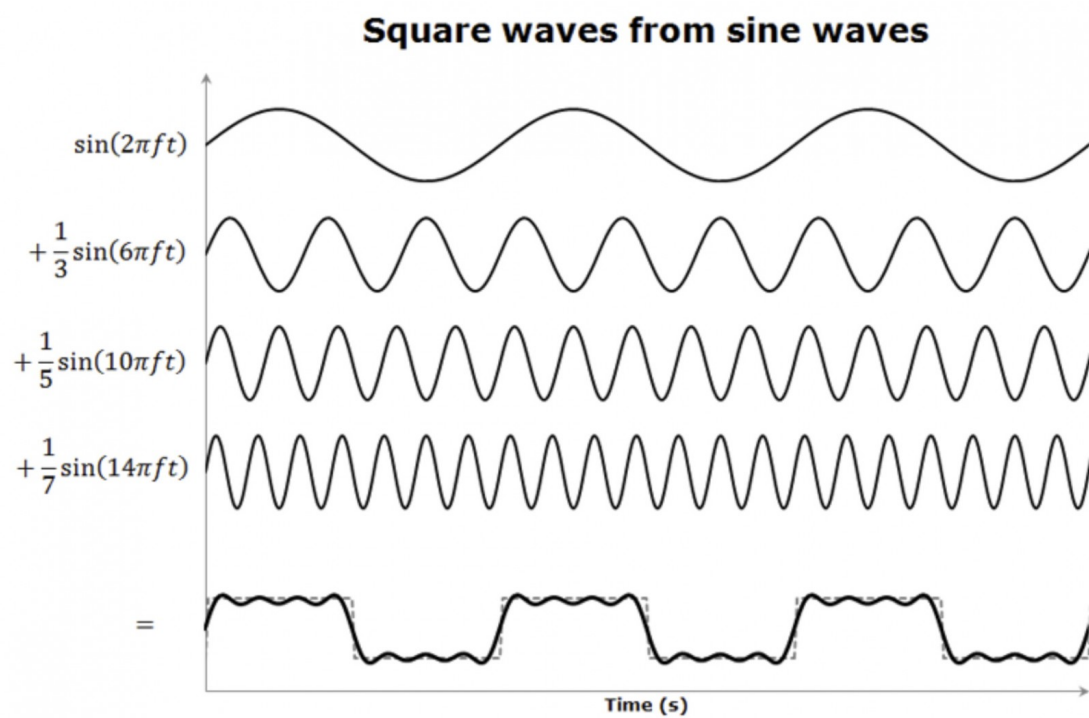
A definition of Numerical Aperture

Numerical Aperture and Speed (or F-Number)



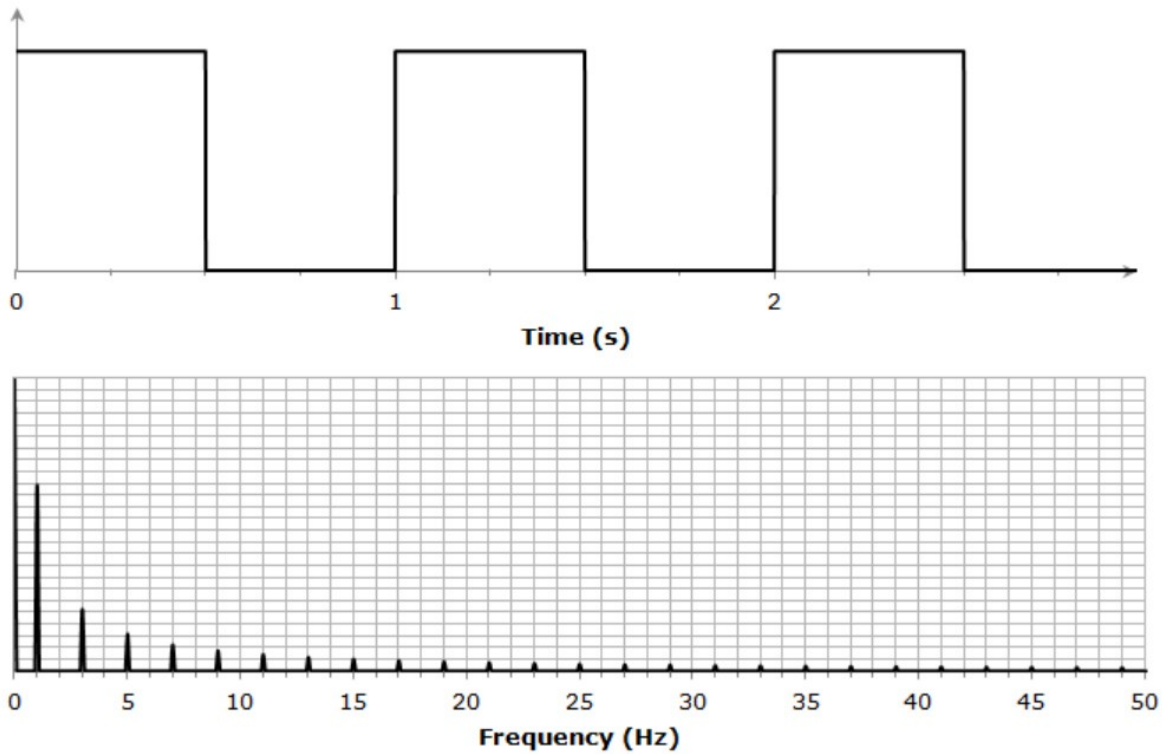
Fourier transform:

Transform from real space to frequency space



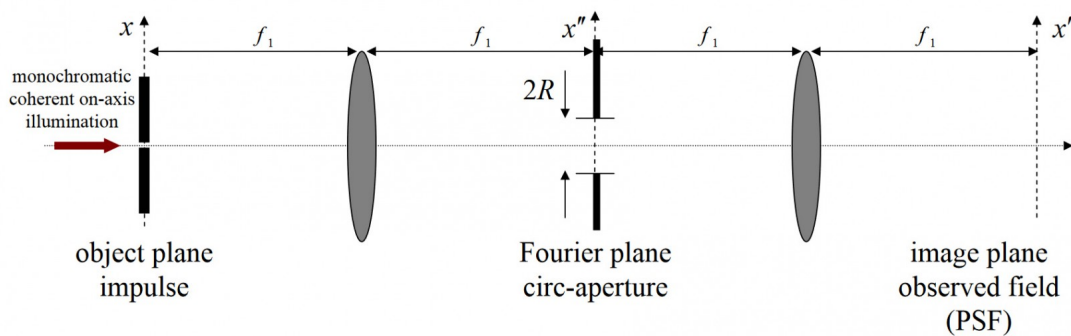
Now we look at a real square wave and frequency space

1 Hz square wave



Look at a simple optical system:

PSF vs NA



Mathematical prediction of the Point Spread Function (PSF)

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

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

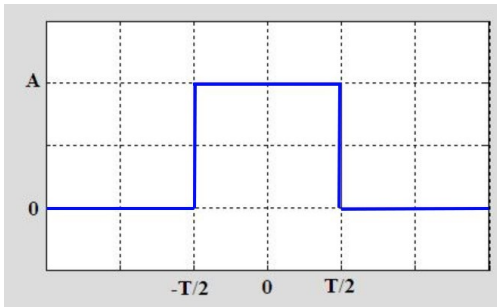
were

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

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

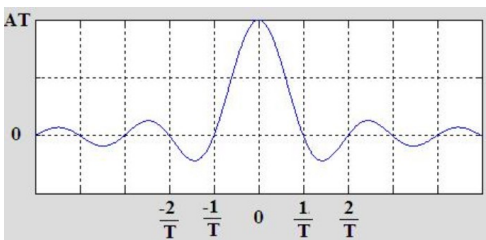


this has the same intensity at all points inside the aperture and zero outside. The second lens is now taking a Fourier transform on a box function the width of the aperture.

$$\text{jinc}(.,.) \equiv 2 \frac{J_1\left(2\pi \frac{R}{f_1} \frac{r'}{\lambda}\right)}{2\pi \frac{R}{f_1} \frac{r'}{\lambda}}$$

(unit magnification)

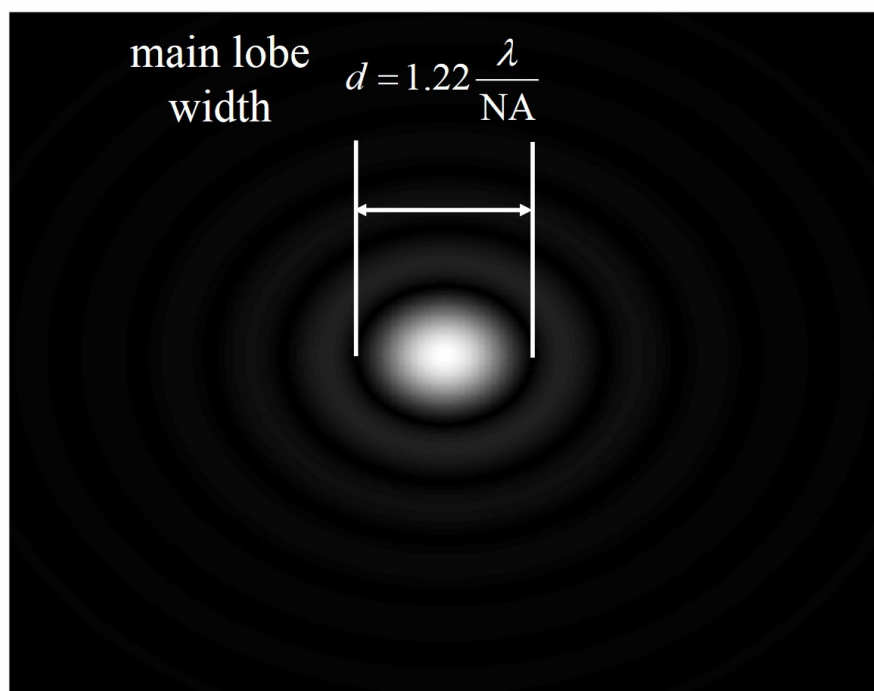
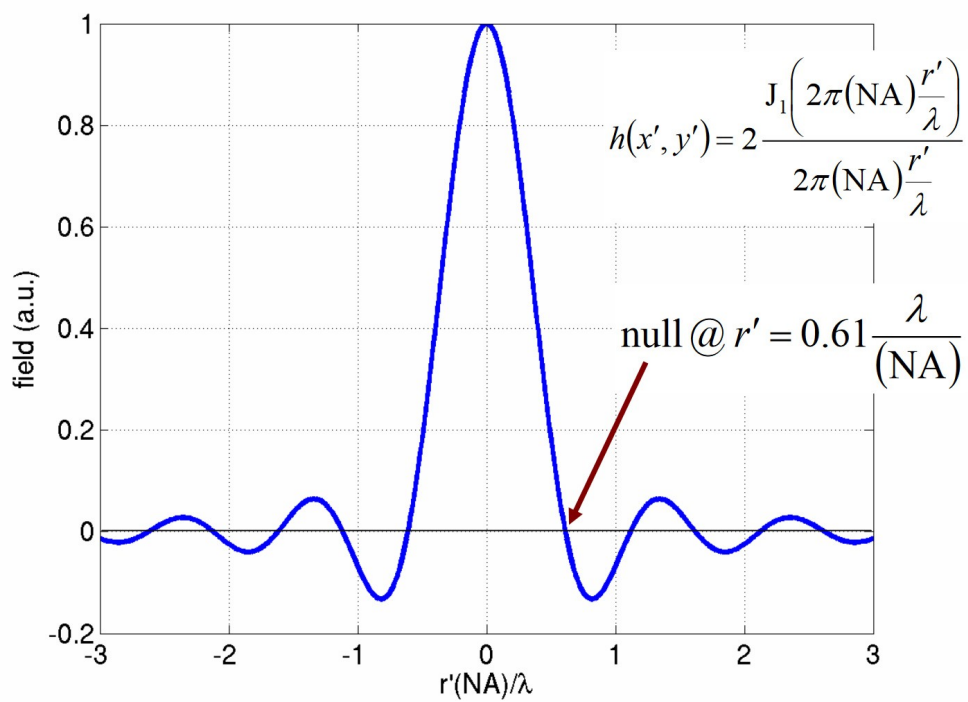
or



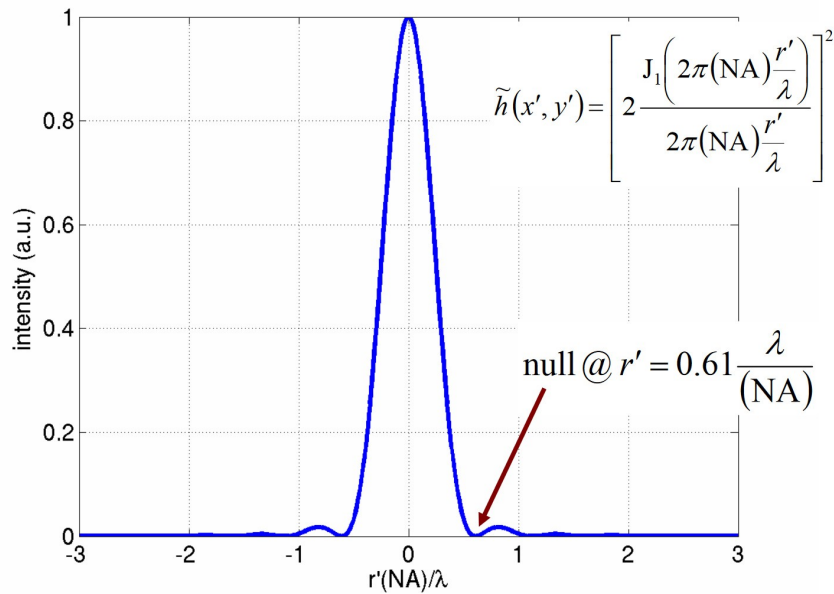
Substituting in the definition for NA

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

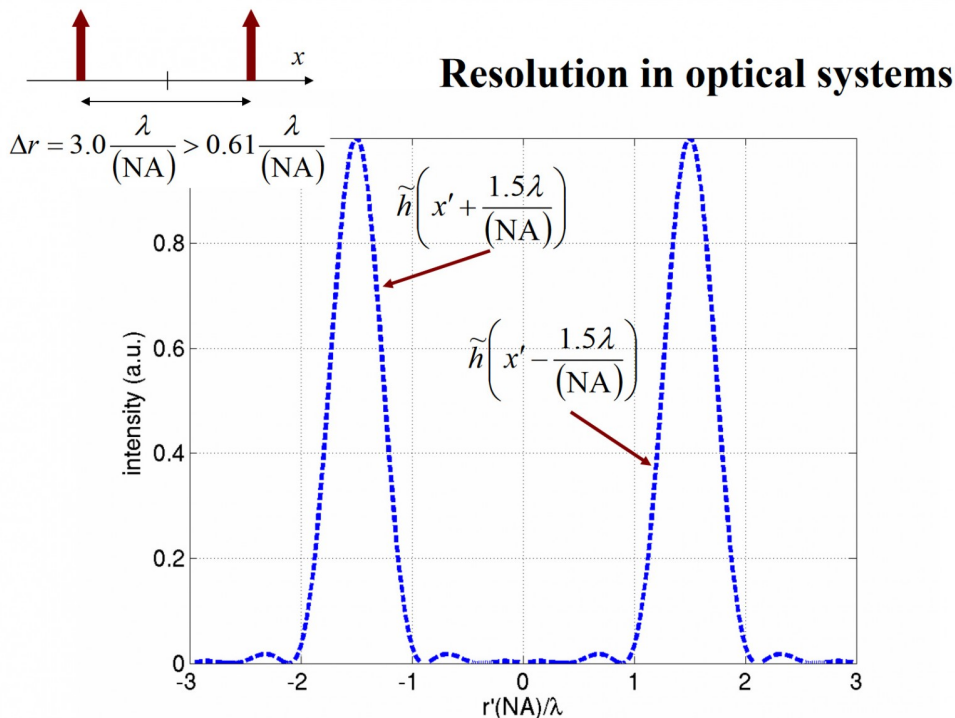
PSF *vs* NA

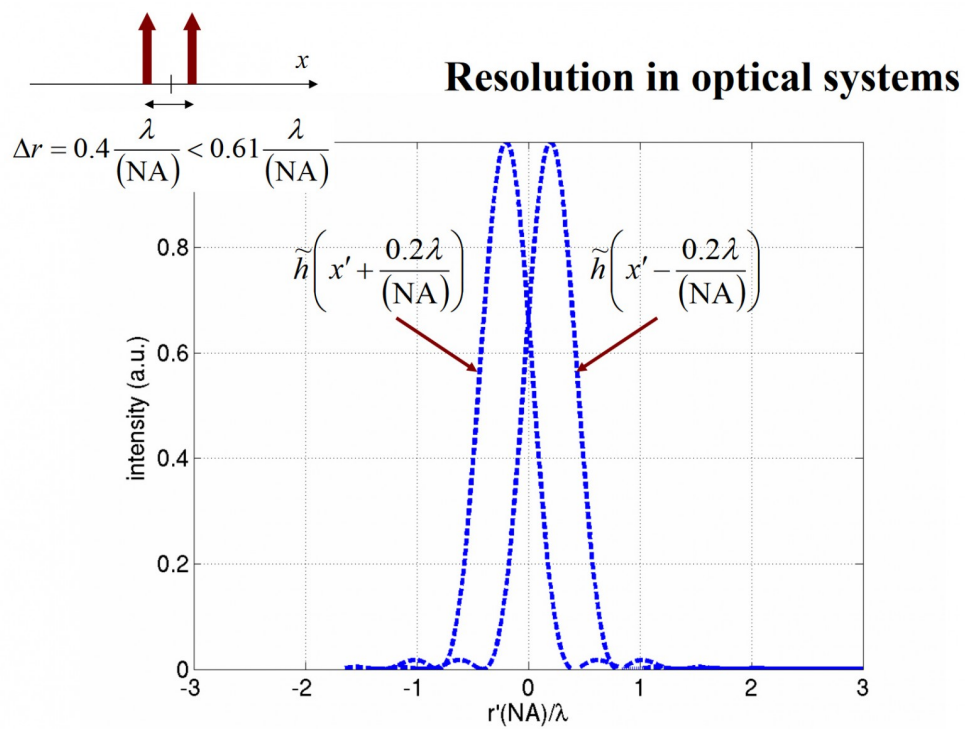
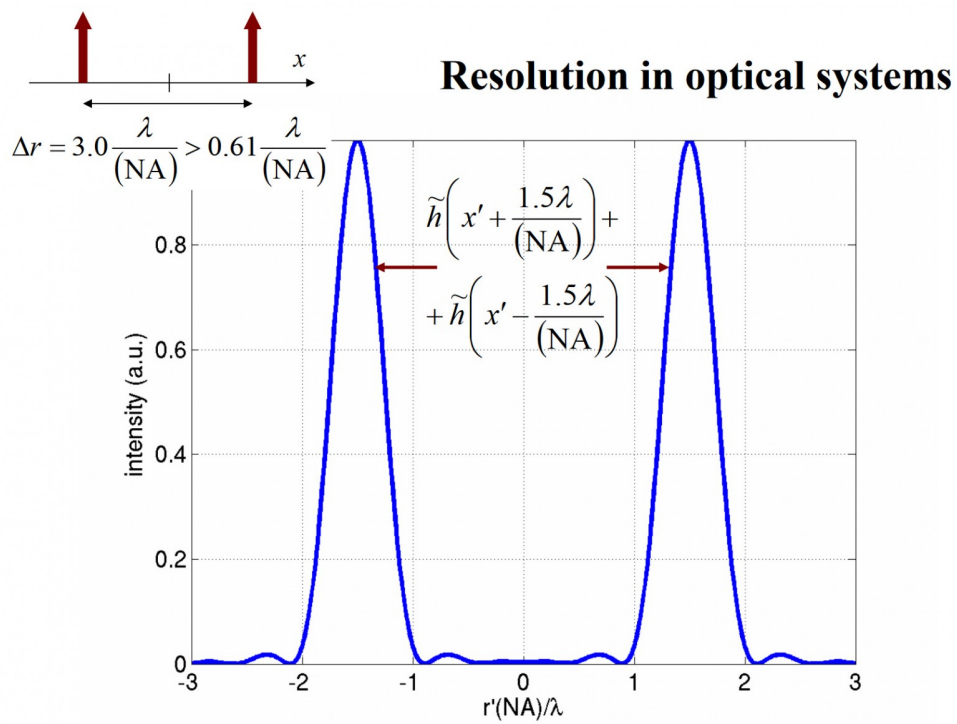


The incoherent case: $\tilde{h}(x', y') = |h(x', y')|^2$

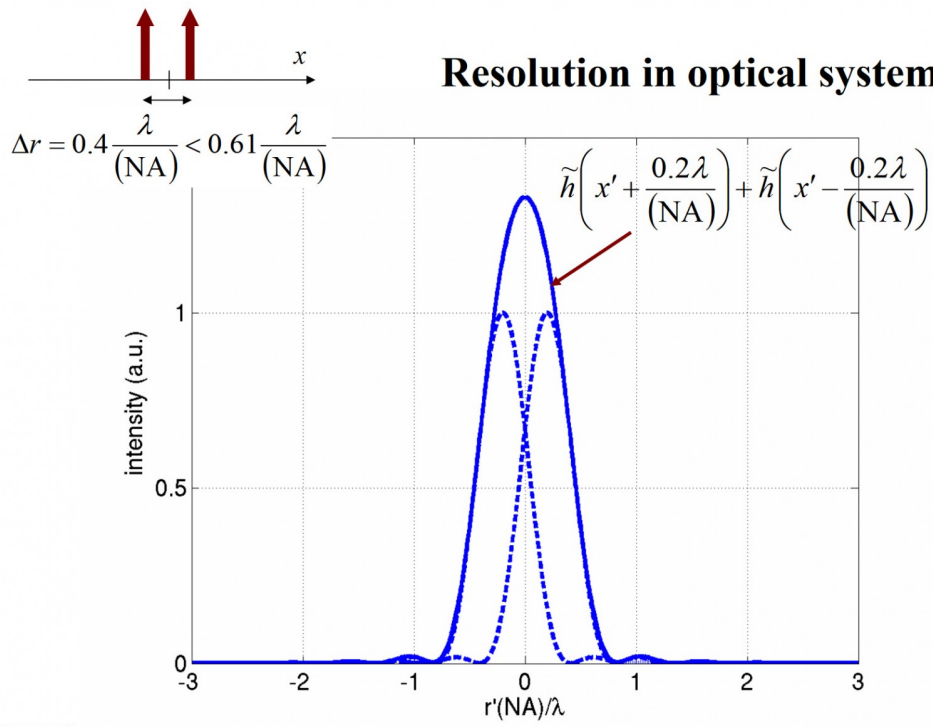


Now we go back to Resolution. How close together we can position two points and still distinguish them

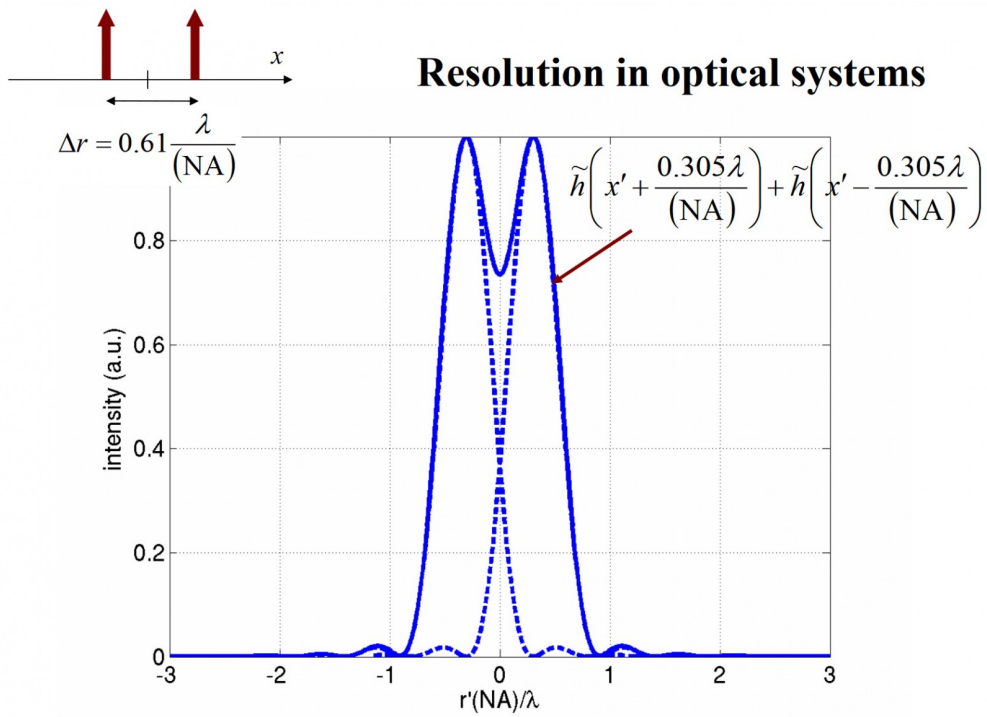


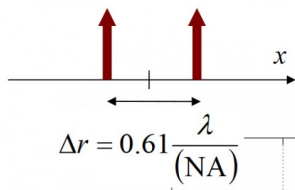


Resolution in optical systems

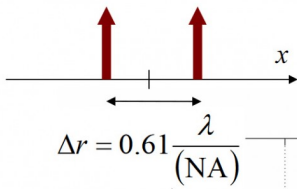
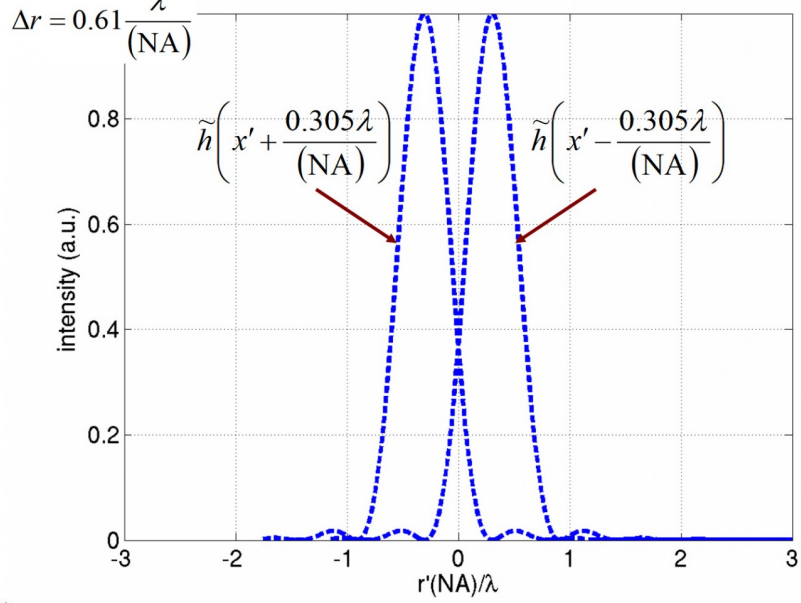


Resolution in optical systems

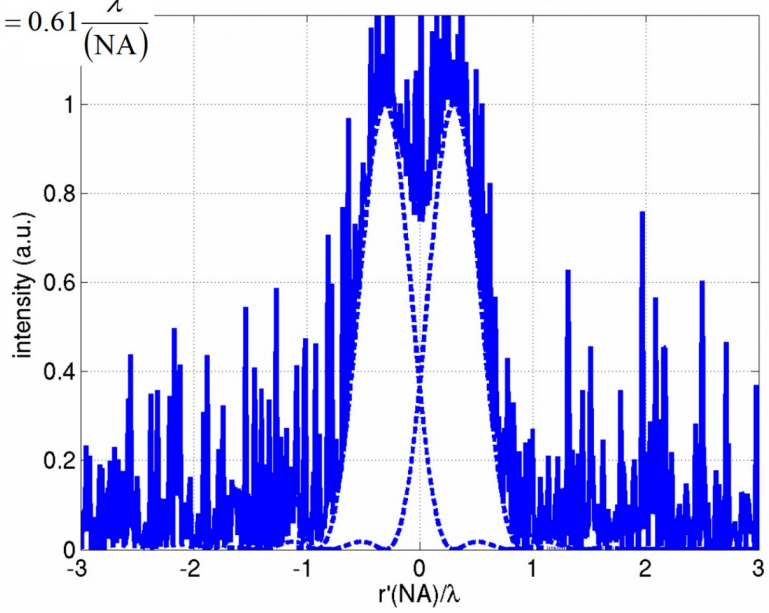


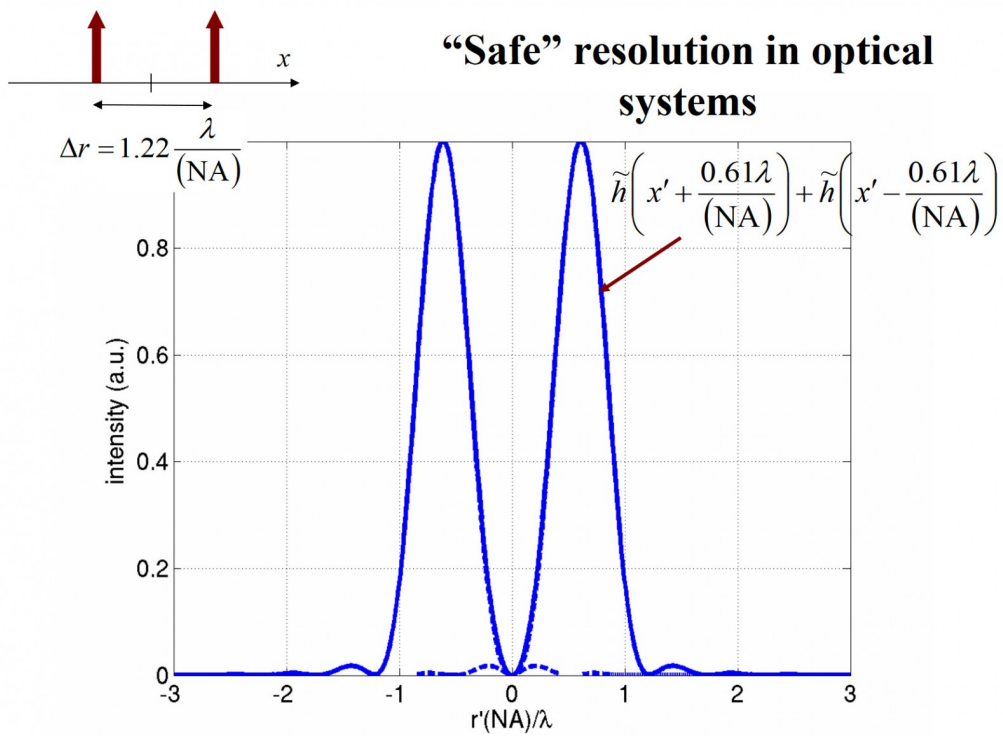


Resolution in optical systems



Resolution in noisy optical systems





Theoretical maximal resolution d_0

$$d_0 = \frac{\lambda}{n \cdot A_{\text{Objective}} + n \cdot A_{\text{Condenser}}}$$

Simplified formula (wo condensor) for resolution d_0

$$d_0 = \frac{\lambda}{2 n \cdot A_{\text{Objective}}}$$

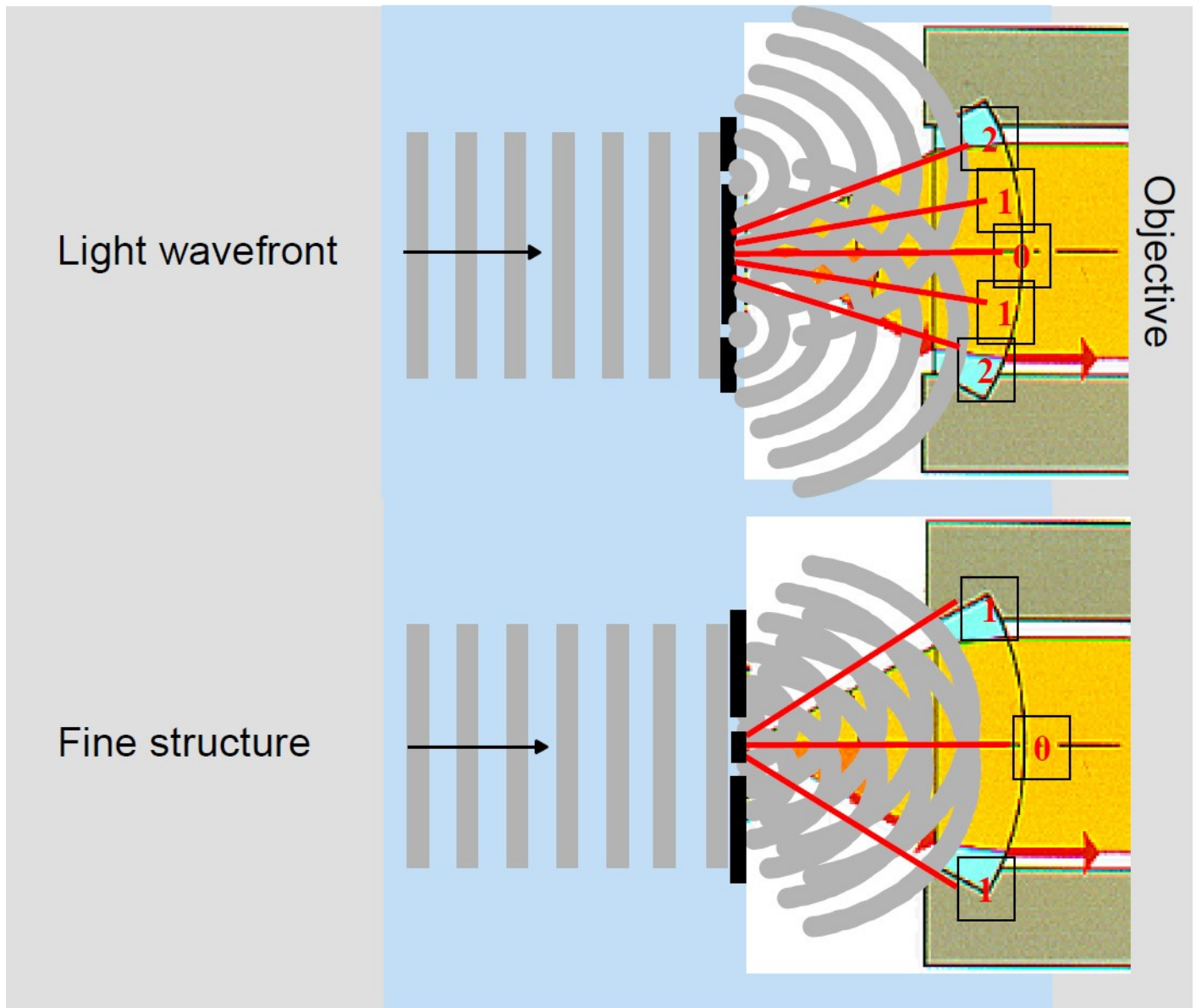
Maximal resolution d_0 in reality

$$d_0 = \frac{1.22 \times \lambda}{2 n \cdot A_{\text{Objective}}}$$

Example

Green light $\lambda = 550 \text{ nm}$, $n \cdot A = 1.4$ (Oil immersion)
 $d_0 = 671 \text{ nm} / (2 \times 1.4) = 239 \text{ nm} = 0.239 \mu\text{m}$

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>

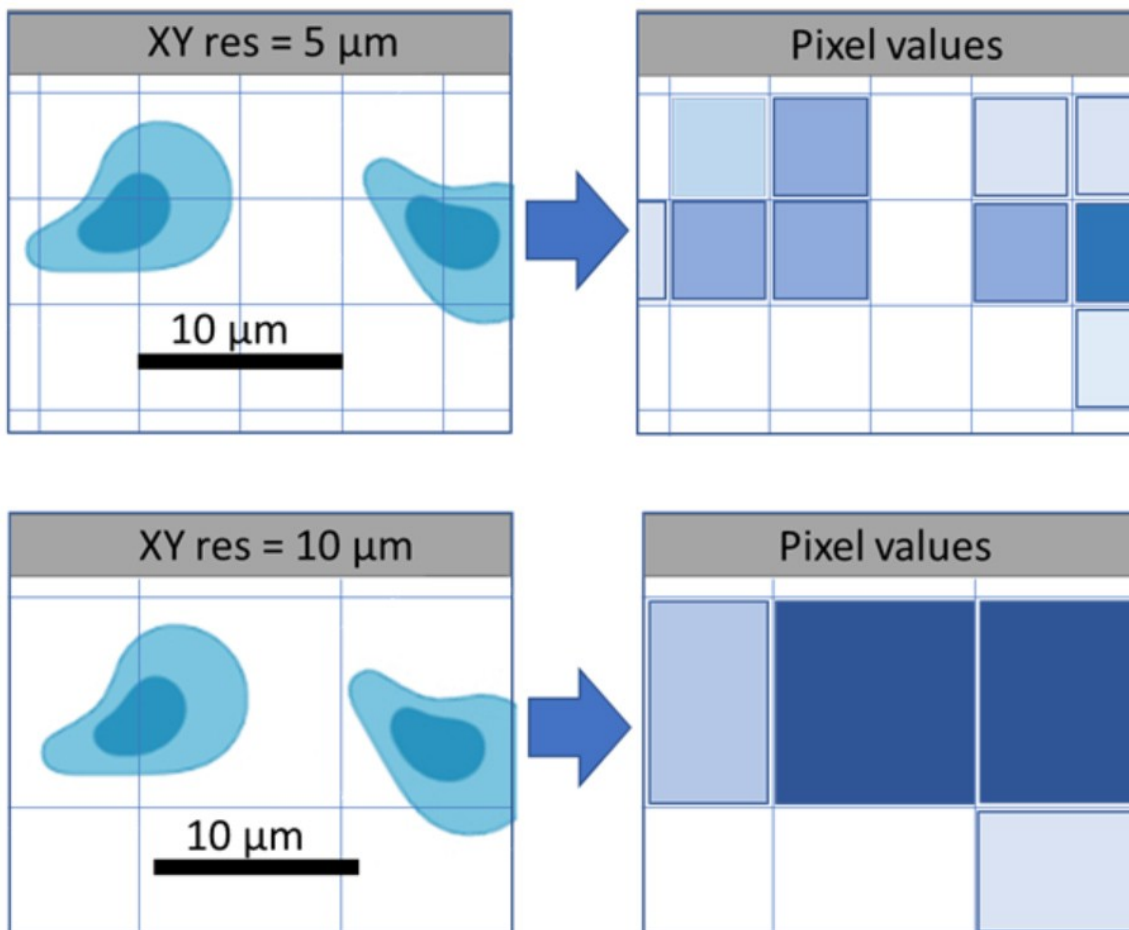
Super Resolution Techniques

Sampling

How does digital sampling affect resolution

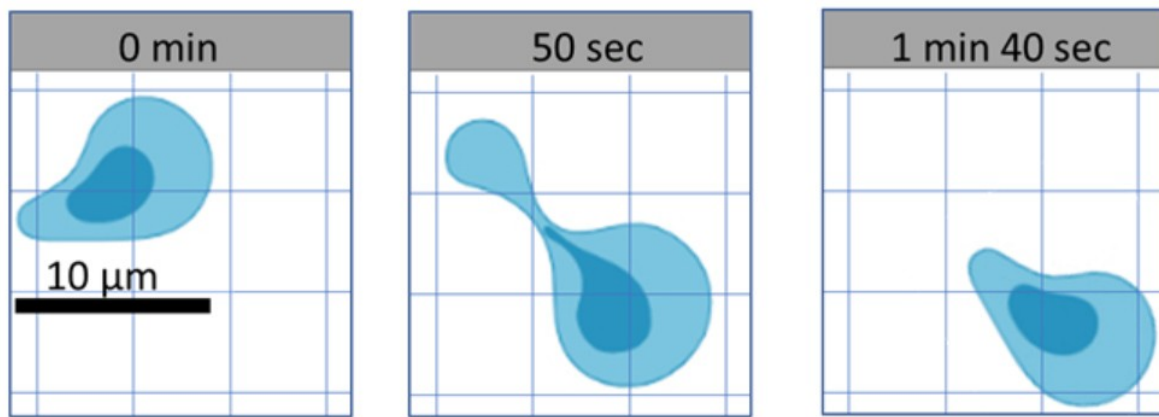
Look at imaging these object with a digital camera

How close together do pixels need to be?



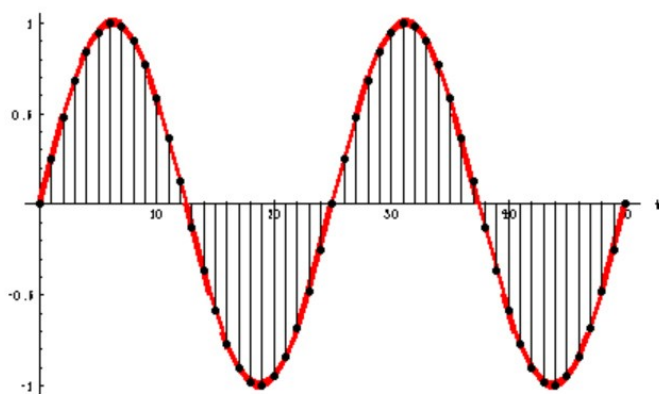
Sampling over time:

How often do you need to image a moving sample

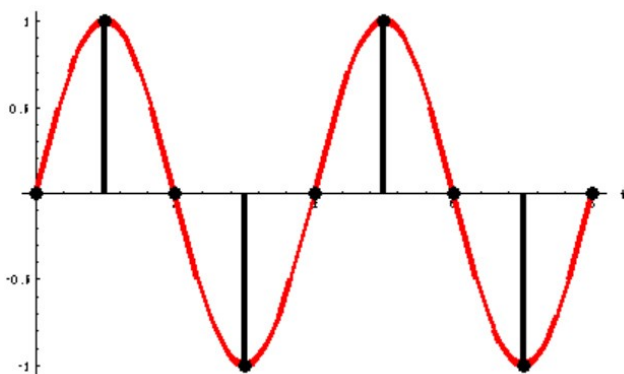


Nyquist theory states that you should sample more than 2 X the frequency that you expect.

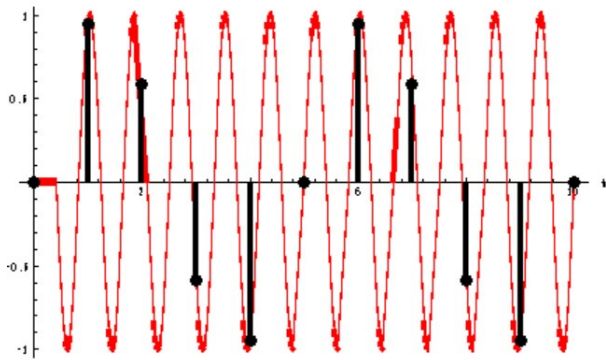
Over sampling



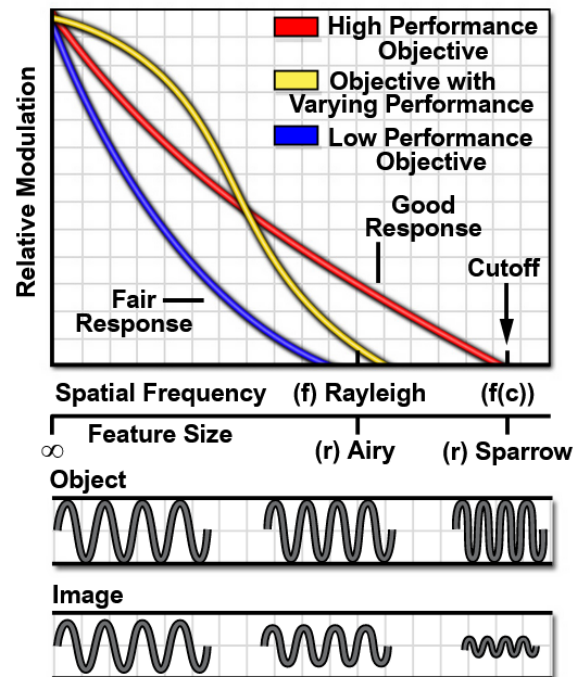
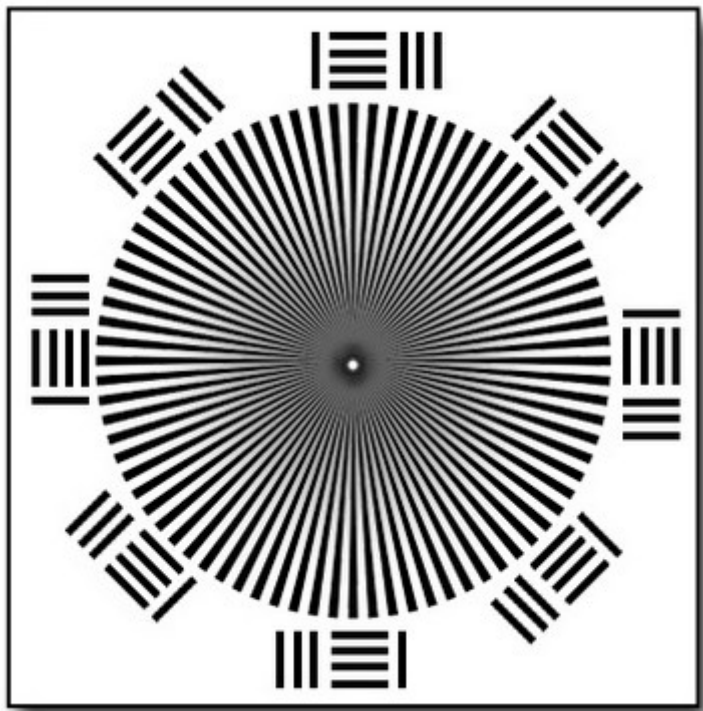
Nyquist sampling



Under sampling causes aliasing



Optical Transfer Function MTF



When objects get close together the contrast decreases.

$$\text{MTF} = \text{Image Modulation} / \text{Object Modulation}$$

$$\text{MTF} = 2(\varphi - \cos\varphi \sin\varphi)/\pi \quad \text{and}$$

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

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

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

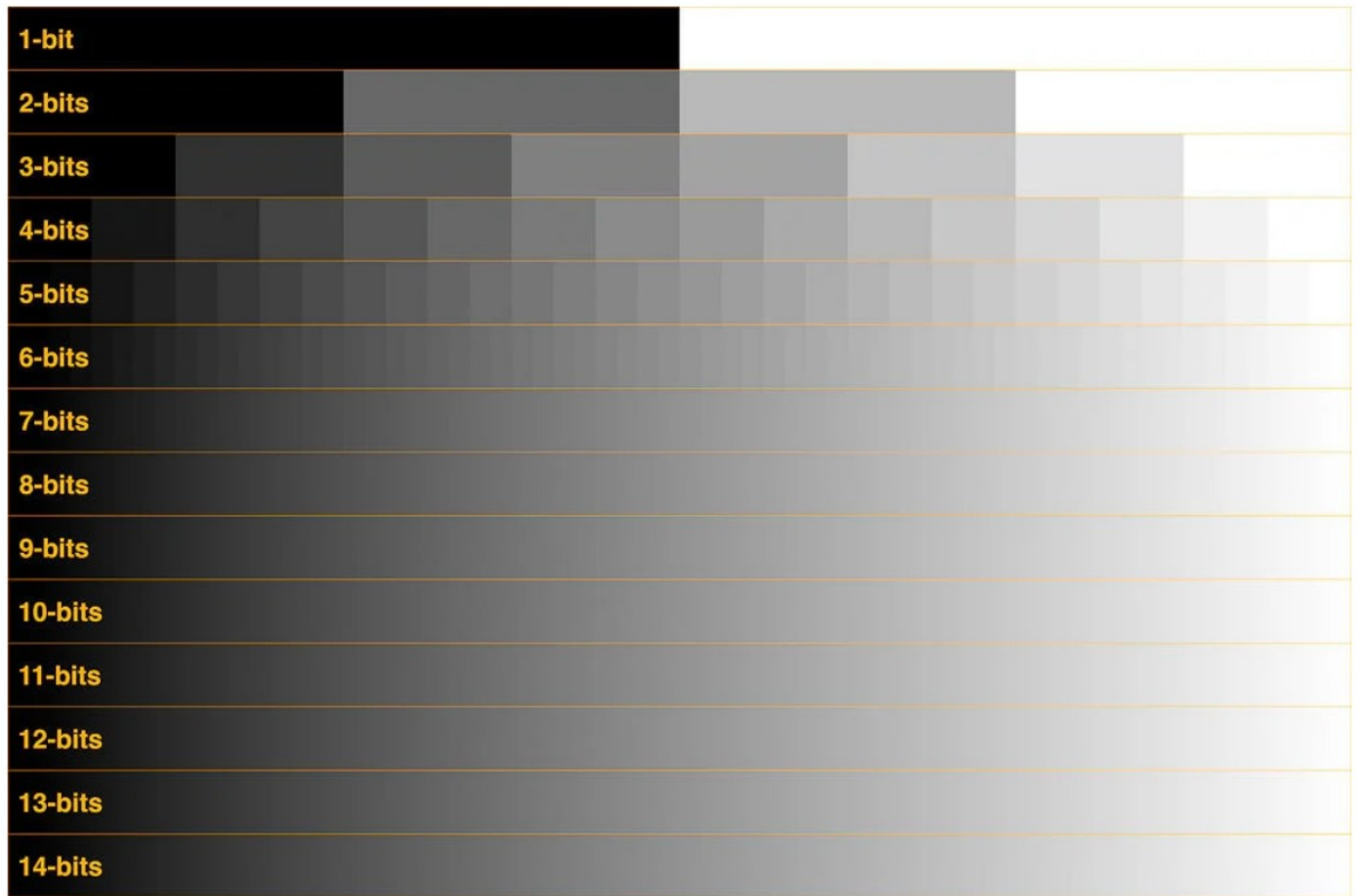
Camera bit depth

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.