Confocal microscopy course

7 HEC!

Alyona Minina

Organism Biology School

Uppsala BioCenter

2014

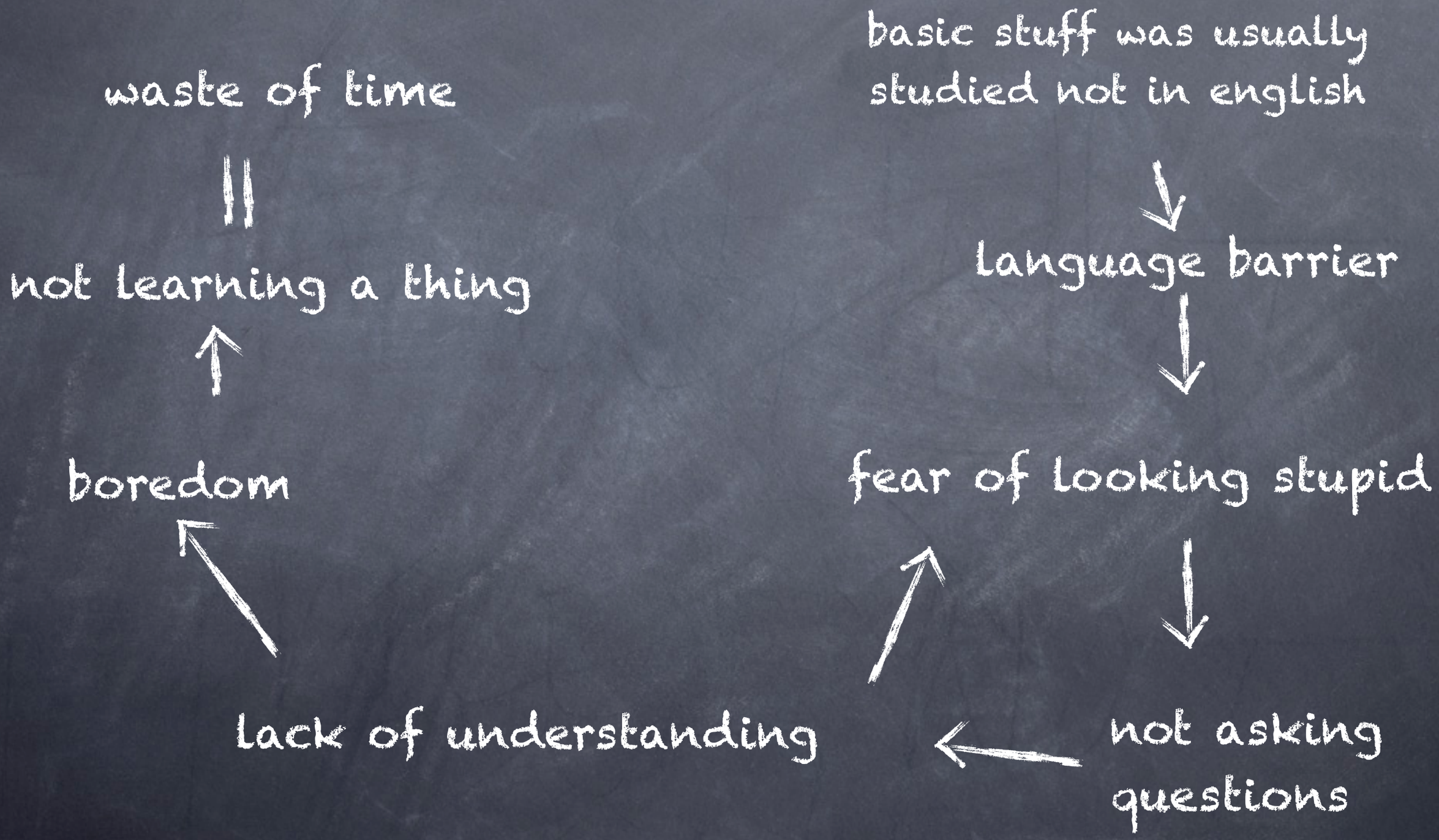
challenges of teaching

I. fear of looking stupid

II. phantom knowledge

III. language

A scientific representation of the first challenge to be met during this course



please ask questions!

has proven itself to be a useless request

phantom knowledge

How do you know that you know stuff?

please draw a bicycle

will your bicycle actually bike?

if no, can you fix it?

do not take anything for granted!

has proven itself to be another
useless request

The language problem

english as a second language

the language we use
influences WHAT WE THINK

who knows how to pronounce this:

$Log_2 8$

Language REALLY influences thinking

In zuñi language orange and yellow are the same word.

Zuñi speakers have trouble distinguishing these two colours.

In russian language light blue is "goluboi" and dark blue is "sinij"

Russian speakers are better than English speakers at distinguishing light/dark blue colours.

Blog.TED

Language REALLY influences thinking

English speaker would often say that
someone broke a vase by accident

Spanish and Japanese speakers tend to
say that the vase **broke itself**

English speakers were much more likely to remember who accidentally popped balloons, broke eggs, or spilled drinks in a video than Spanish or Japanese speakers who saw the same video.

Blog.TED

maybe there are so many definitions in science
for a reason

Masterplan

- find out what you want from the course
- adjust your expectations to the morbid reality
- find out what you need from the course
- adjust the course to your needs (includes me asking you a lot of nosy questions)

what is knowledge?

Zeiss 510 Zeiss 700 Zeiss 780

SP2 SP5 SP8

Zeiss Leica Olympus Nikon whatever else

basics of confocal microscopy

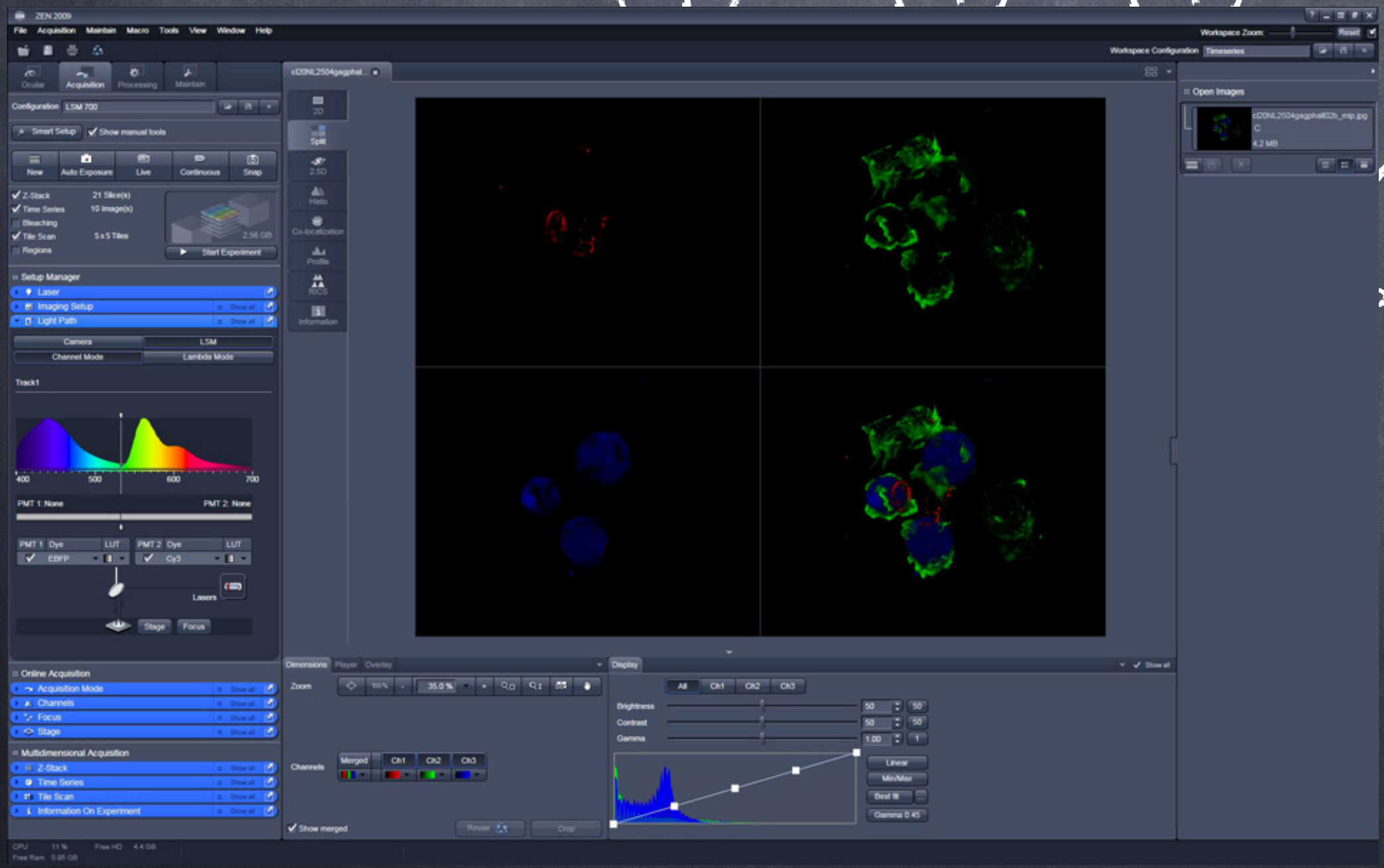
basics of light microscopy

Axiomes

Zeiss 510 Zeiss 700 Zeiss 780

SP2 SP5 SP8

whatever else

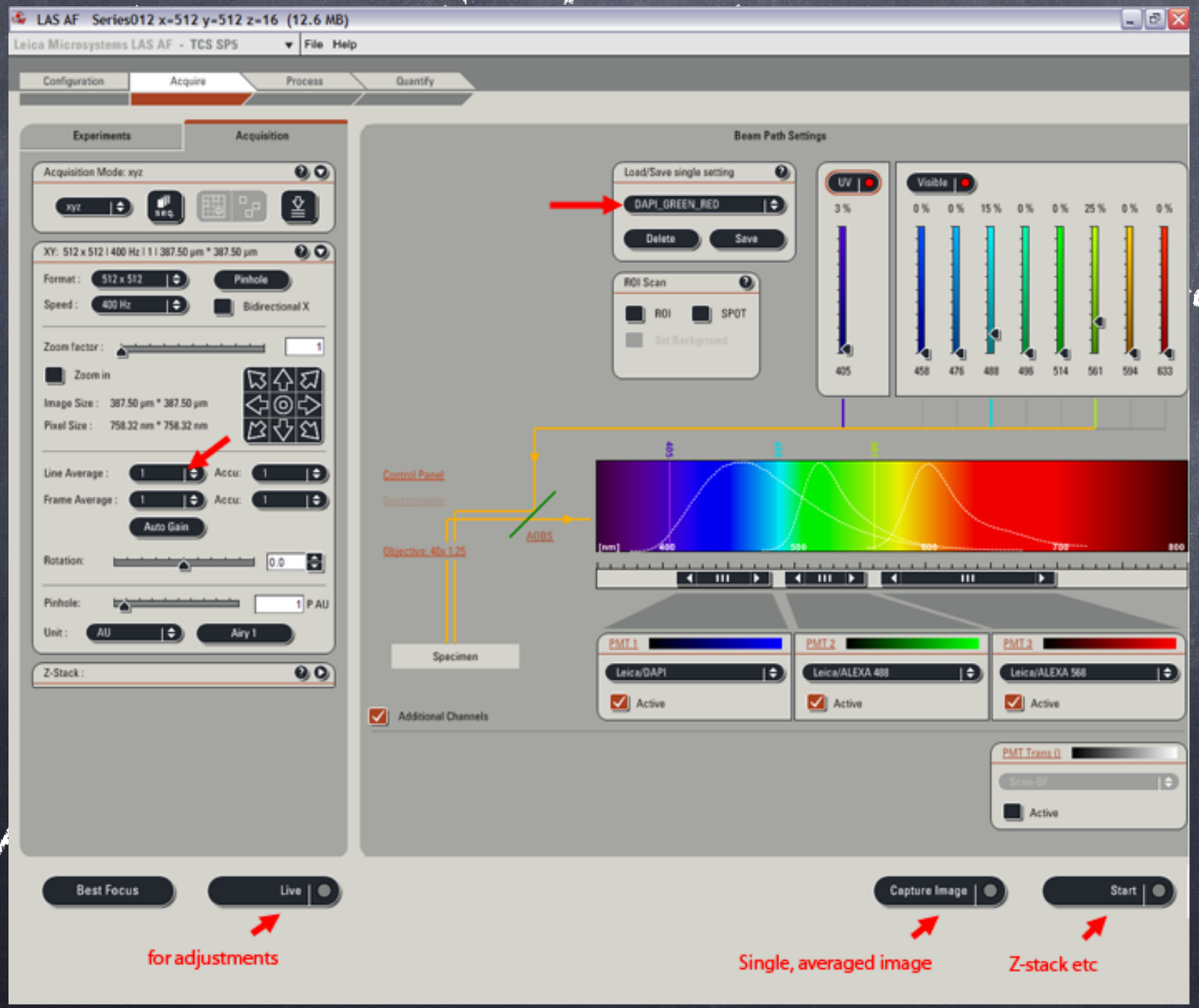


Zeiss 510 Zeiss 700 Zeiss 780

?

SP2 SP5 SP8

whatever else



for adjustments

Single, averaged image

Z-stack etc

oscopy

Zeiss 510

Zeiss 700

Zeiss 780

Zeiss

SP2

SP5

SP8

Leica

Olympus

Nikon

whatever else

basics of confocal microscopy

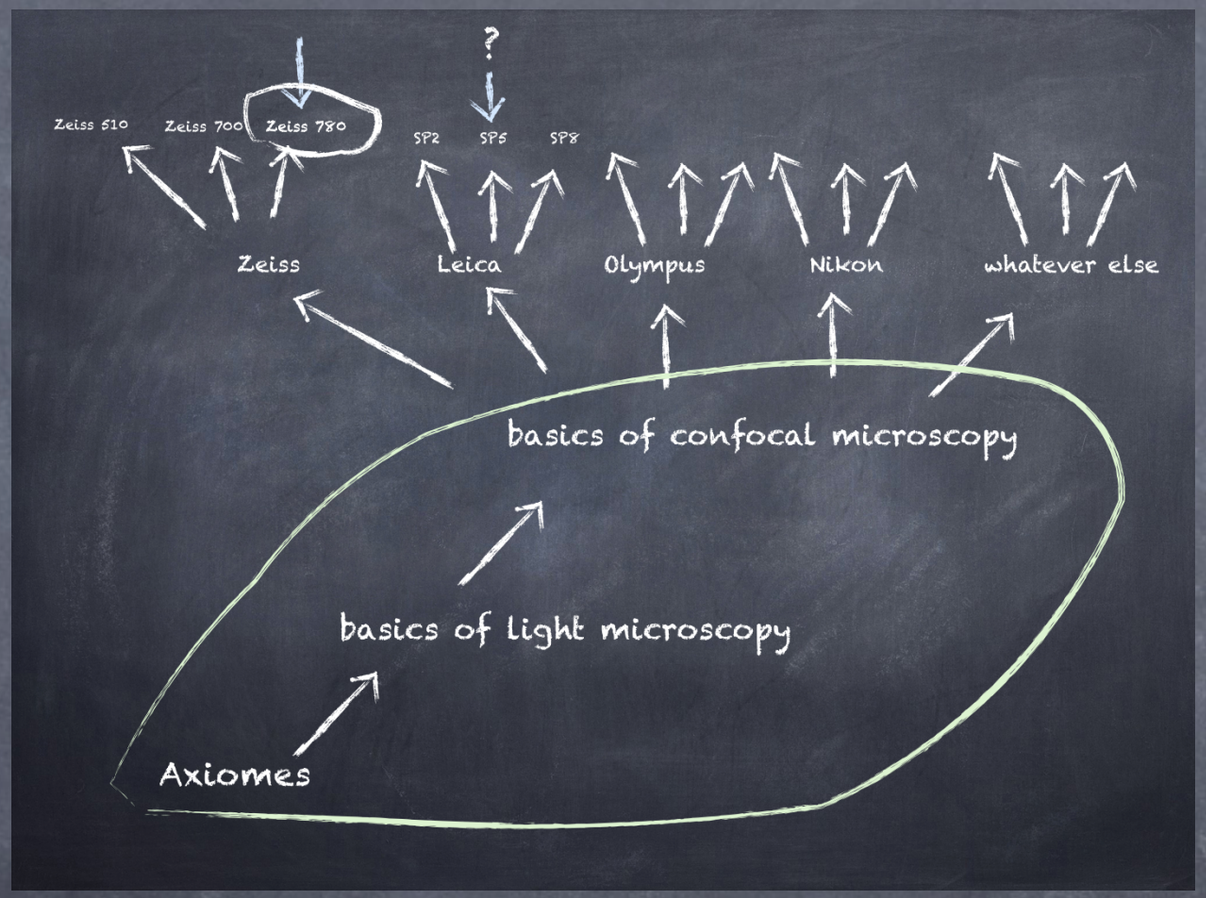
basics of light microscopy

Axiomes

There is SO much more

Super resolution microscopy

Light-Sheet



Structured-illumination microscopy

2-photon microscopy

CARS

etc.

and there is more and more new techs appearing all the time

Course structure

Theoretical part

- Basic theory
- Intros into fancy technologies
- Intros for the softwares

Practical part

- You'll work in pairs, Alyona will provide you with:
  - ✓a plate with transgenic Arabidopsis plants
  - ✓a list of tasks and instructions
  - ✓4 hours access to our Zeiss 780 confocal
- you will perform imaging by yourself
- analyse your data by yourself
- discuss problems during a seminar

this will be scary →

Report

- report your results as they would look in a manuscript ready for submission
  - Results
  - Figure
  - Legend to the figure
  - Materials and methods
  - Supplementary material
  - At each step please mention all the info, which you do not have, but it should be also included into a real manuscript
- discuss your reports during a seminar

← this is VERY weird will explain during the seminar

Basics of light microscopy

Light microscopy is ANY microscopy which deals with light

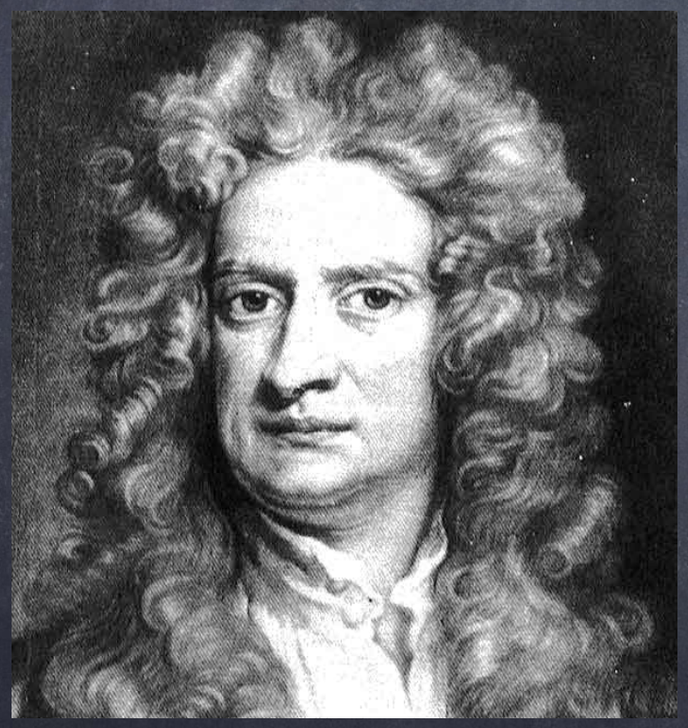
What is Light?

...

but we know how it behaves!

Light BEHAVES like a

a particle



Isaac Newton

a wave

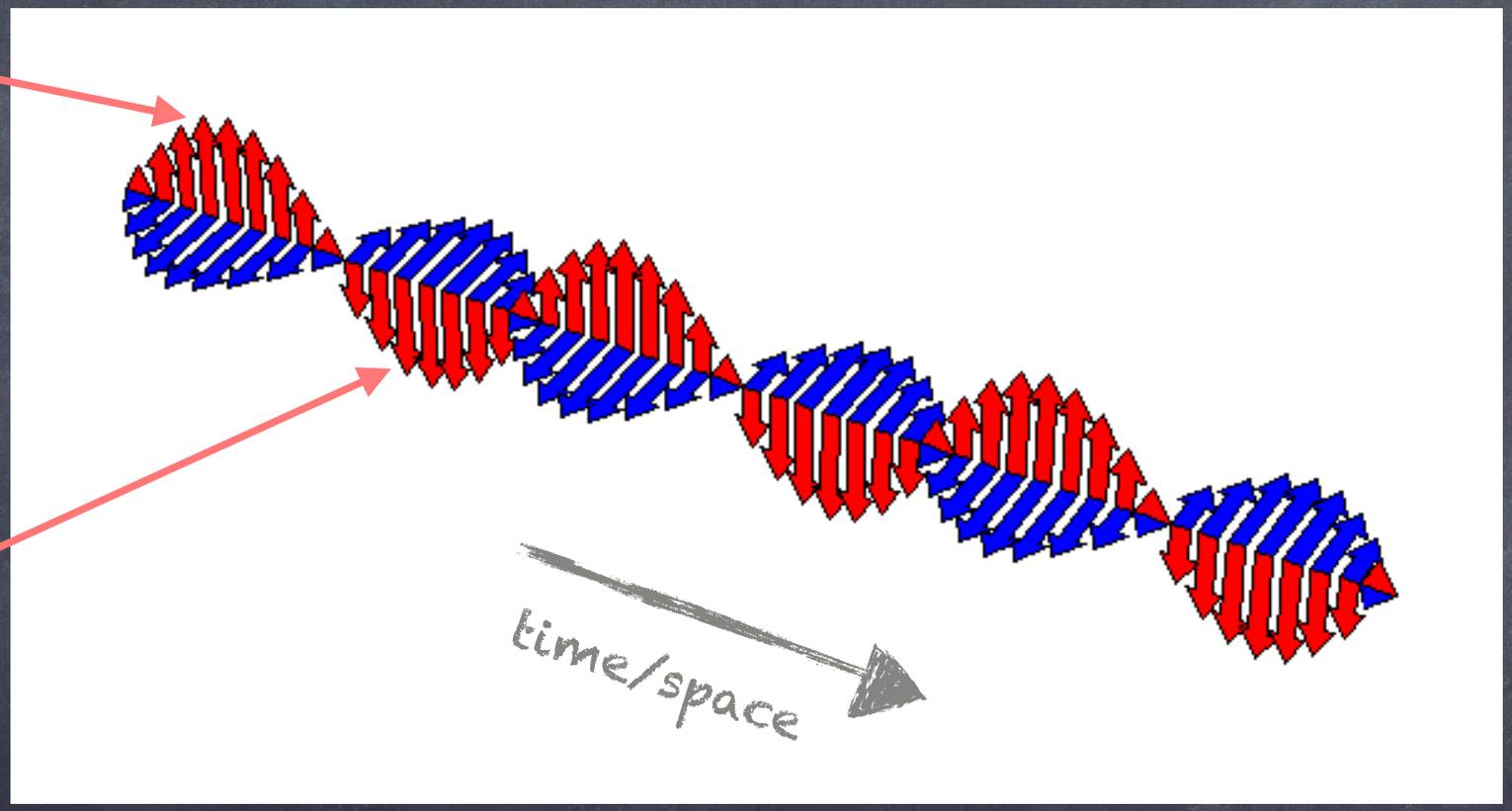
Christian Huygens

/ˈhaɪgənz/

What is light?

electromagnetic wave

crest



trough

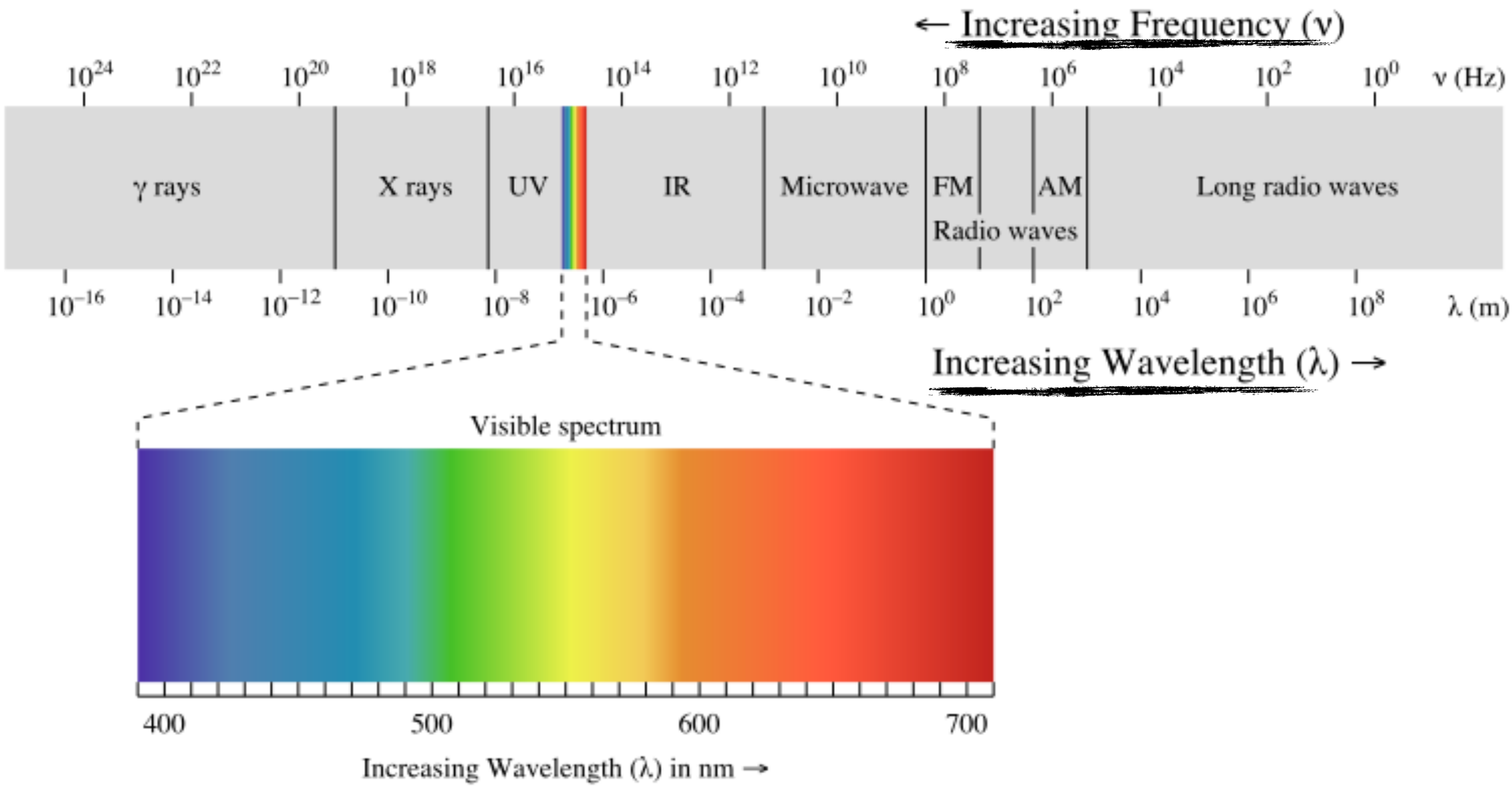
$v = \lambda * \nu$

$C = 3*10^9$ m/s

$\vec{E}$ electric field

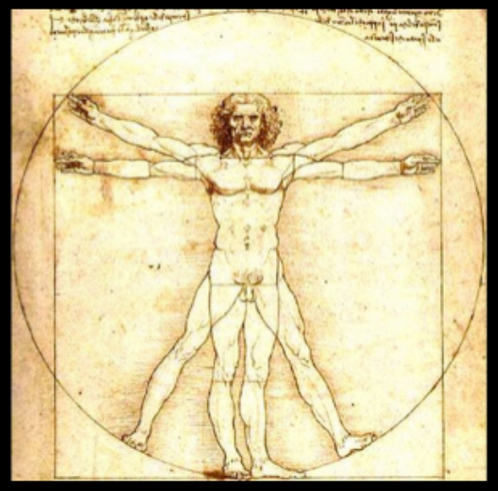
$\vec{B}$ magnetic field

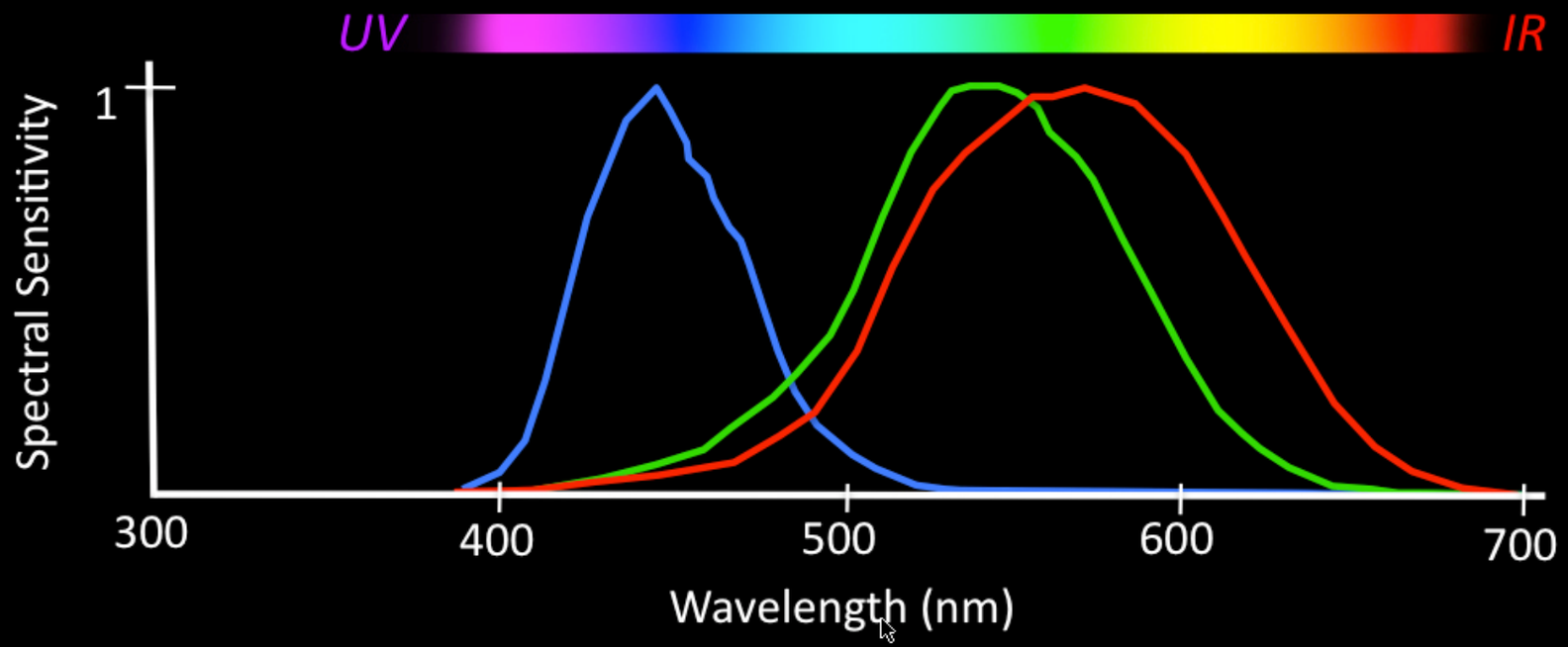
Electromagnetic Spectrum



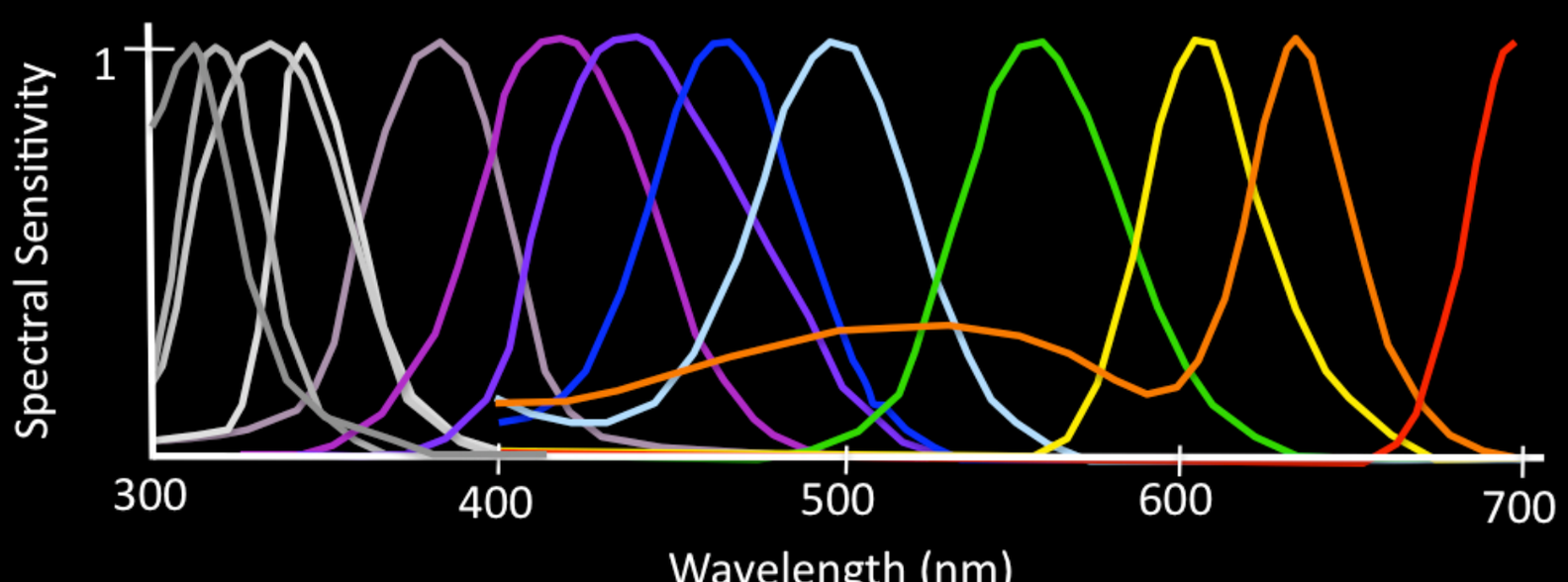
Mantis Shrimp: Extraordinary Eyes

Homo sapiens





Neogonodactylus oestedii



Marshall *et al.,* 2007; Marshall and Oberwinkler, 1999

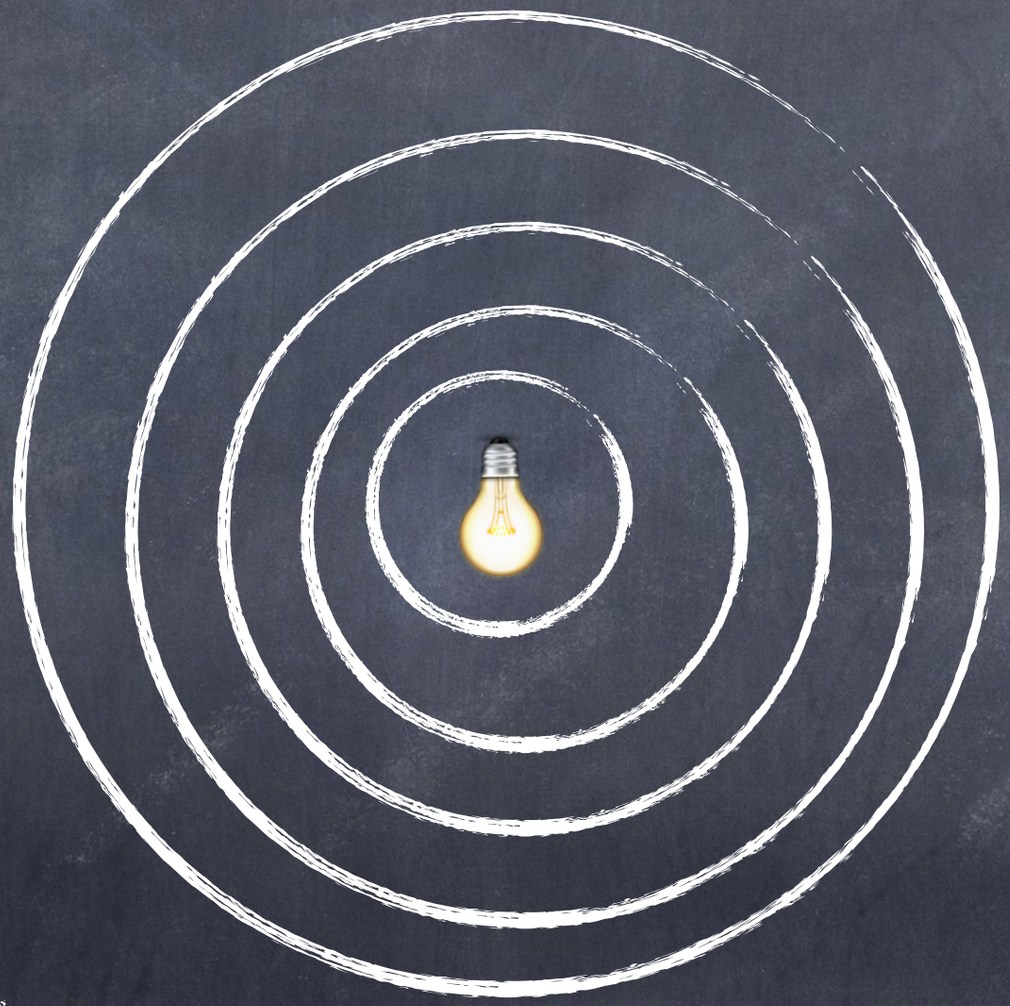
http://arthropoda.southernfriedscience.com/?p=1

Light features

please draw a light wave and mark each feature

- wavelength λ
- frequency ν or f
- phase ϕ
- speed v or c
- intensity I or A
- polarity
- energy E

Spherical light wave



please draw a spherical light wave and mark each feature on it

Light rays can interfere

- please draw interference between two coherent flat light waves

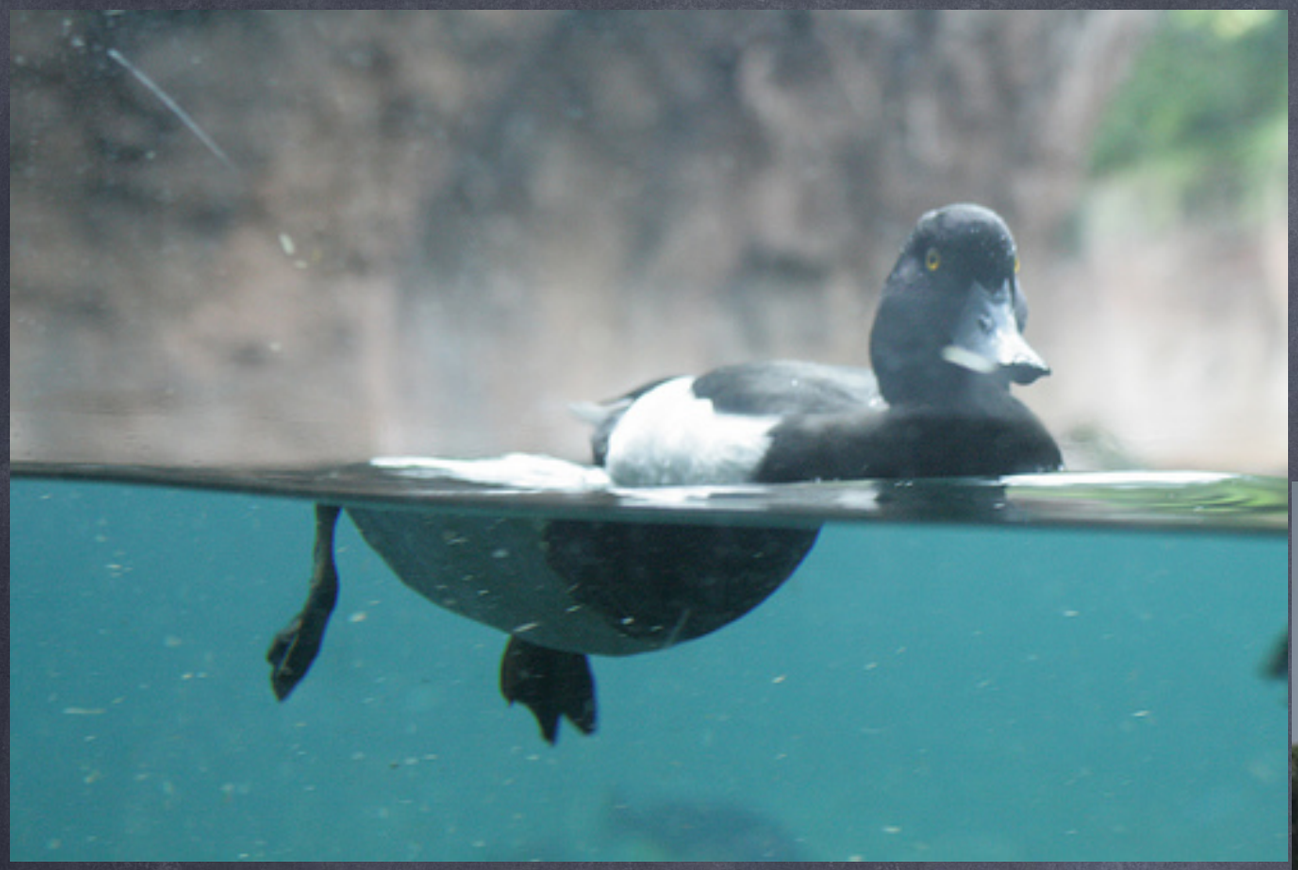
- please draw interference between two incoherent flat light waves

Light can be

- reflected
- refracted
- dispersed
- diffracted
- scattered
- transmitted
- absorbed
- emitted

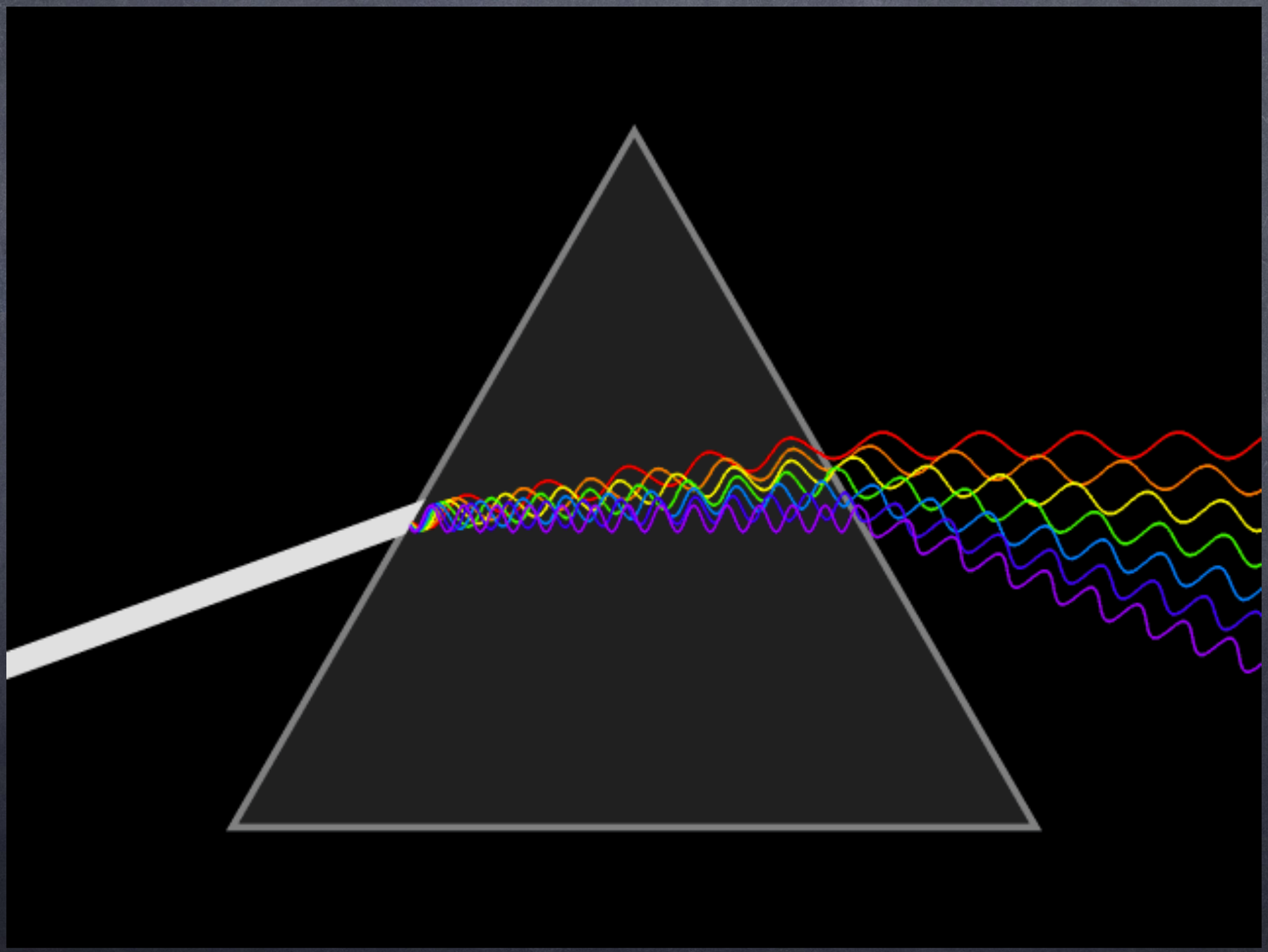
please draw a cube on a way of a light beam and a schematic representation of each process

Refraction



$$n = c/v$$

Dispersion



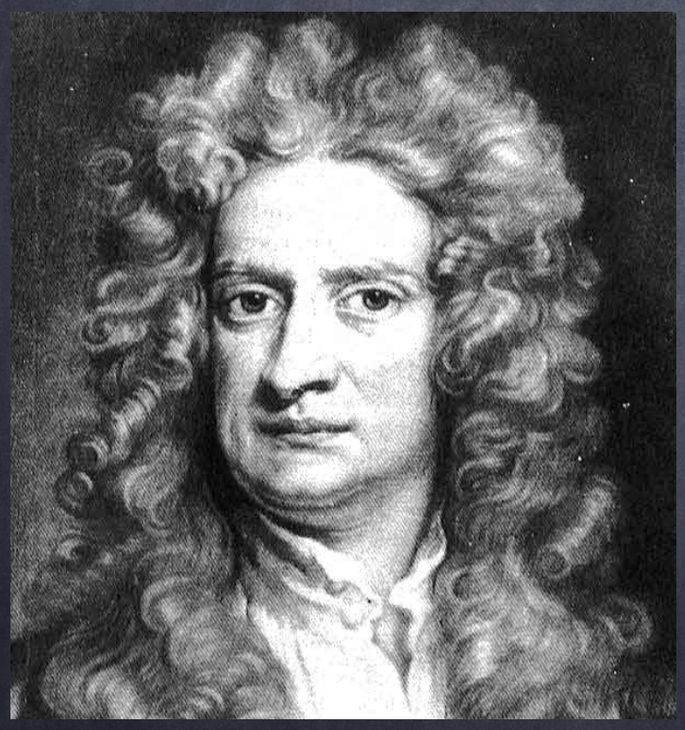
Diffraction

please draw a pattern you would expect to see on the wall

two tiny slits open

Light BEHAVES like a

a particle



Isaac Newton

!!!!!!

if being observed ⟷ when nobody is watching

dual slit + camera experiment

a wave

Christian Huygens

/ˈhaɪgənz/

Diffraction

please draw a pattern you would expect to see on the wall

tiny slit open

Numerical Aperture

sample

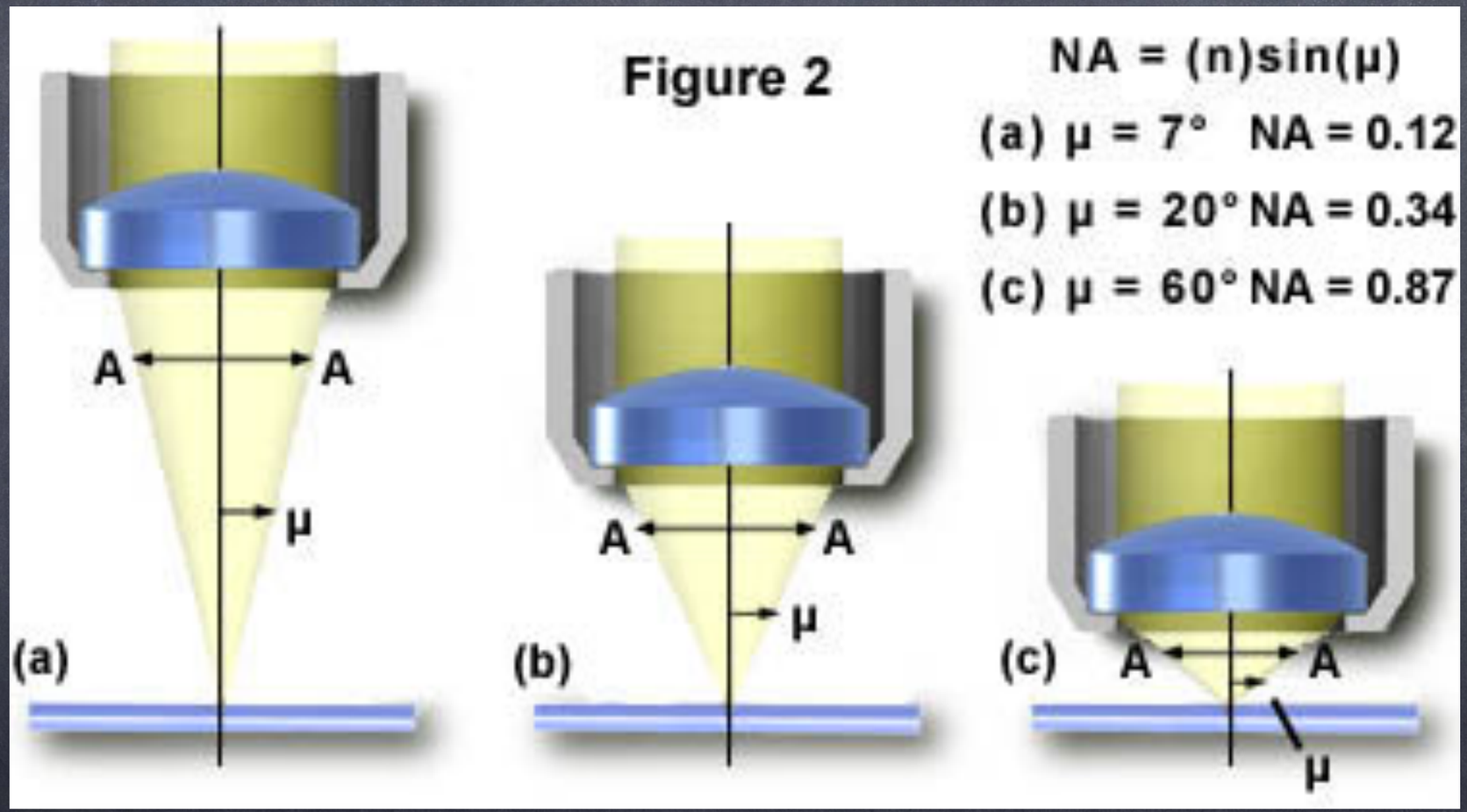
lens

detector

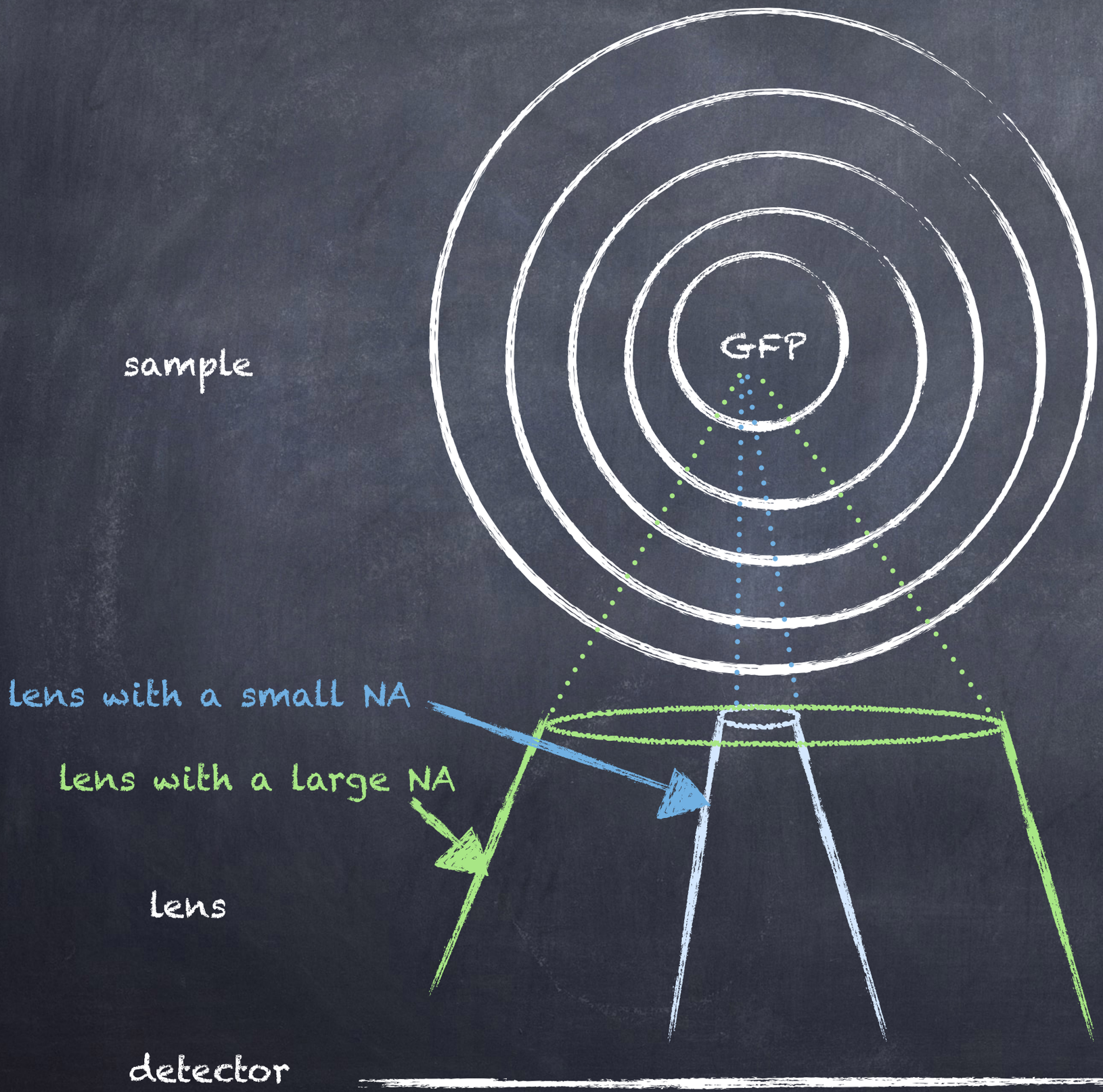
NA defines, how much of the spherical wave will make it to the detector

Numerical Aperture

$NA = n * \sin\mu$



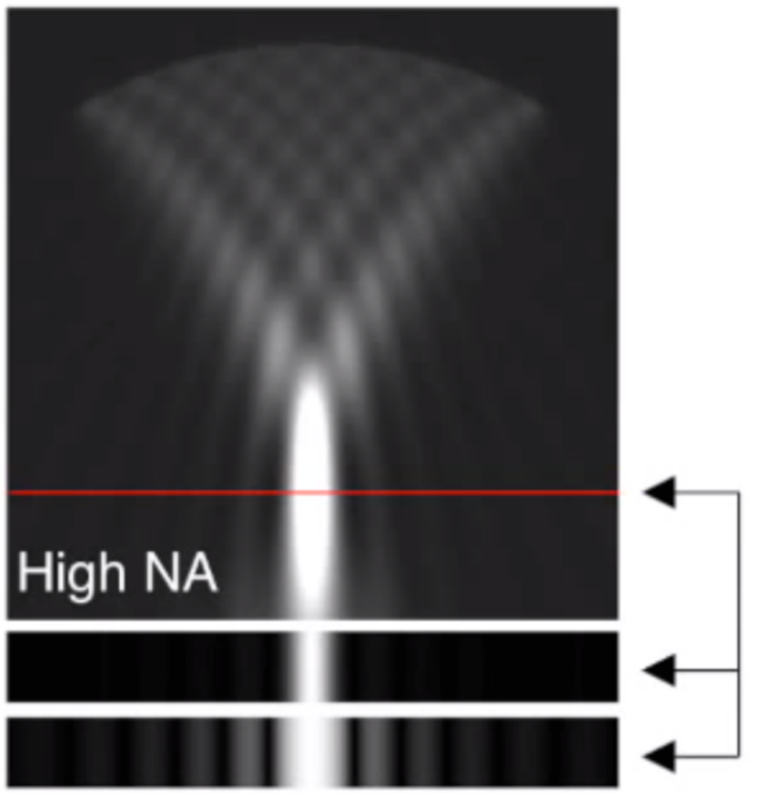
Numerical Aperture

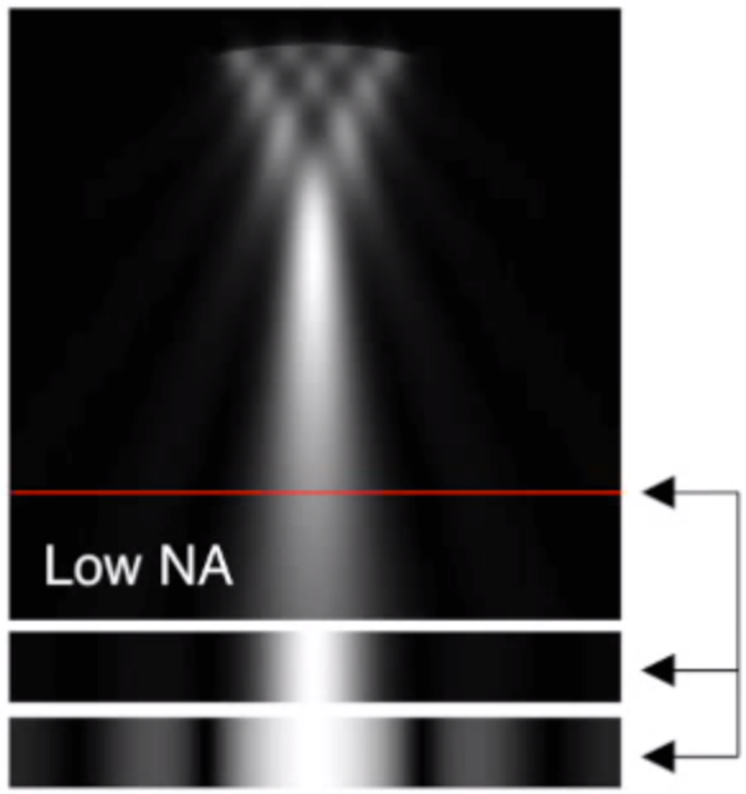


NA defines, how much of the spherical wave will make it to the detector

than further apart were interfering wavelets, than sharper and narrower will be the fringes on the diffraction pattern

Numerical Aperture

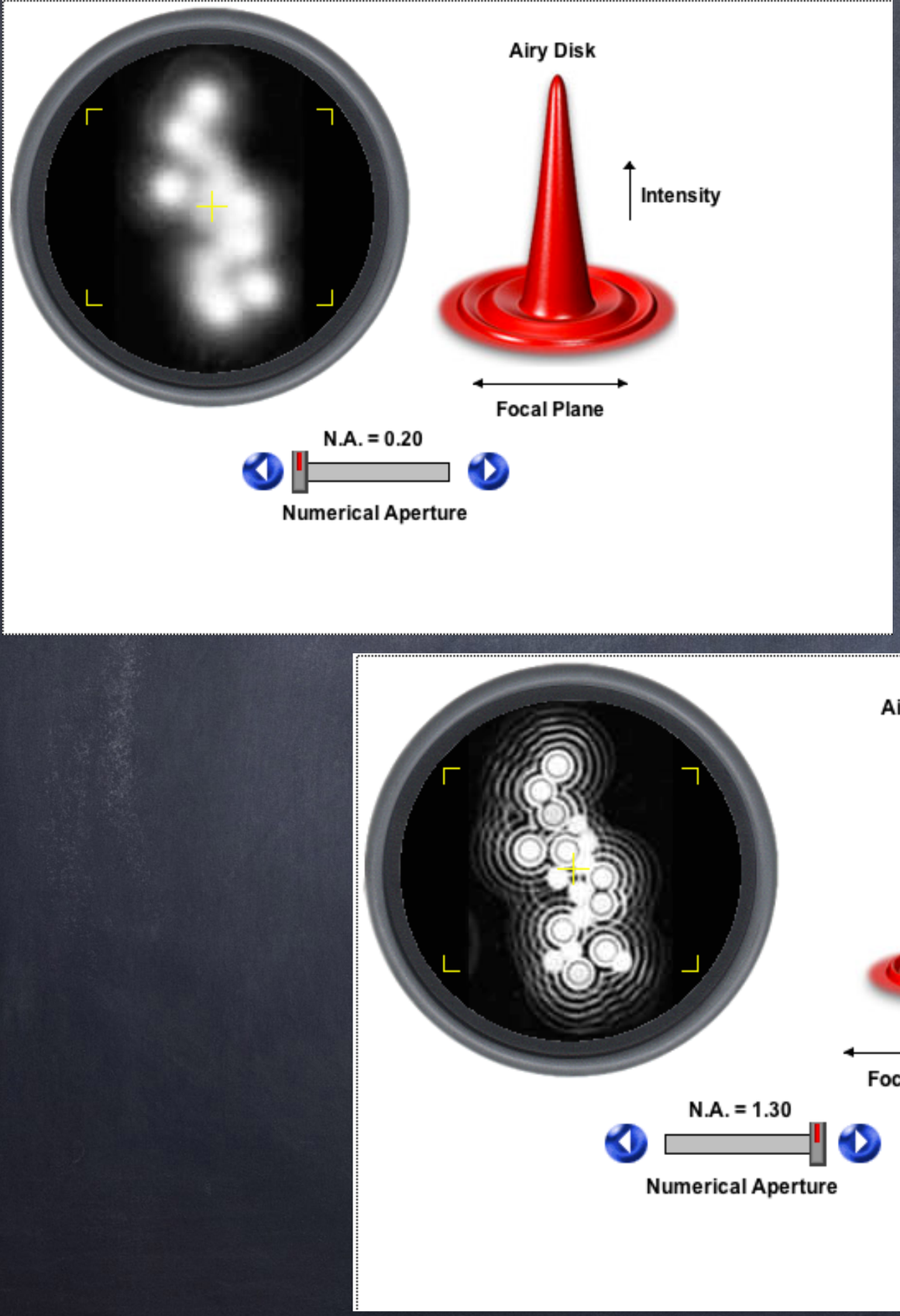


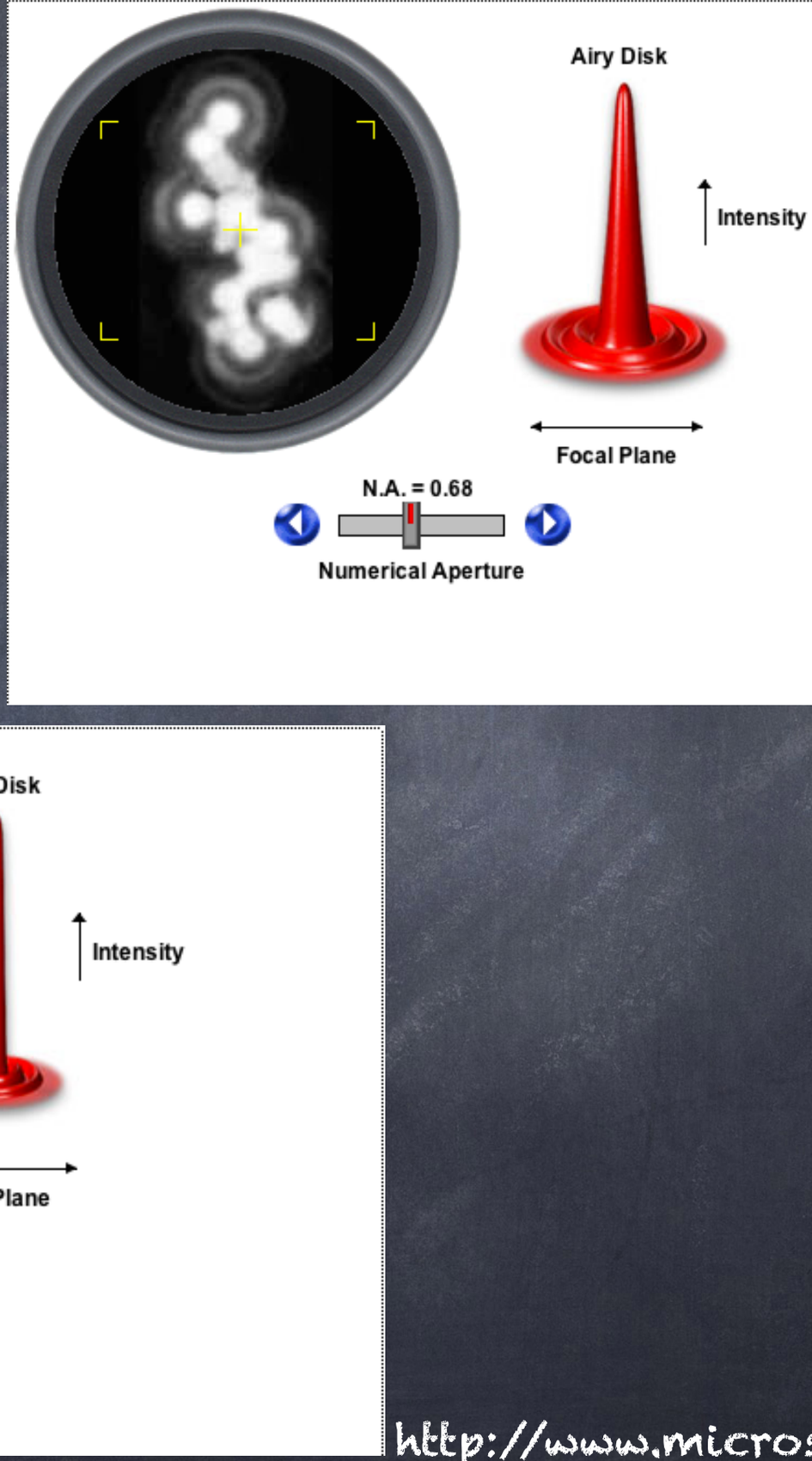


- High NA
  - Wider separation between wavelets possible
  - As a result small central peak
- Low NA
  - Only narrow separation between wavelets
  - Broad central peak

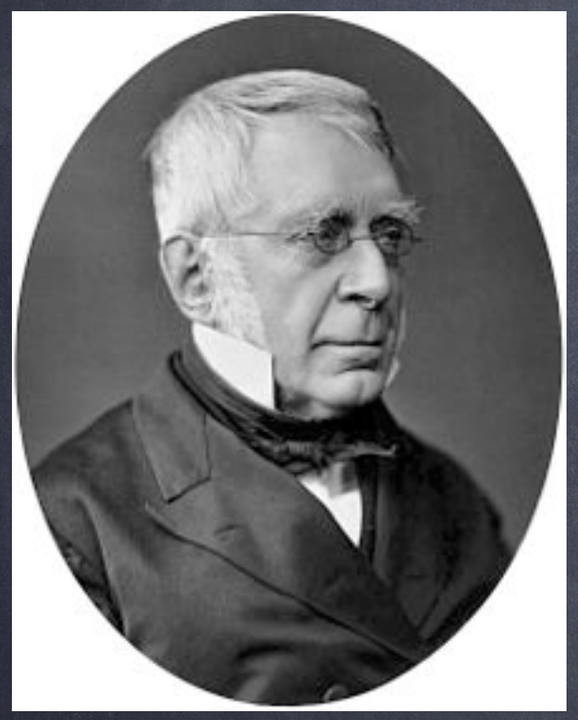
Microscopy: Point Spread Function (Jeff Lichtman)

Numerical Aperture

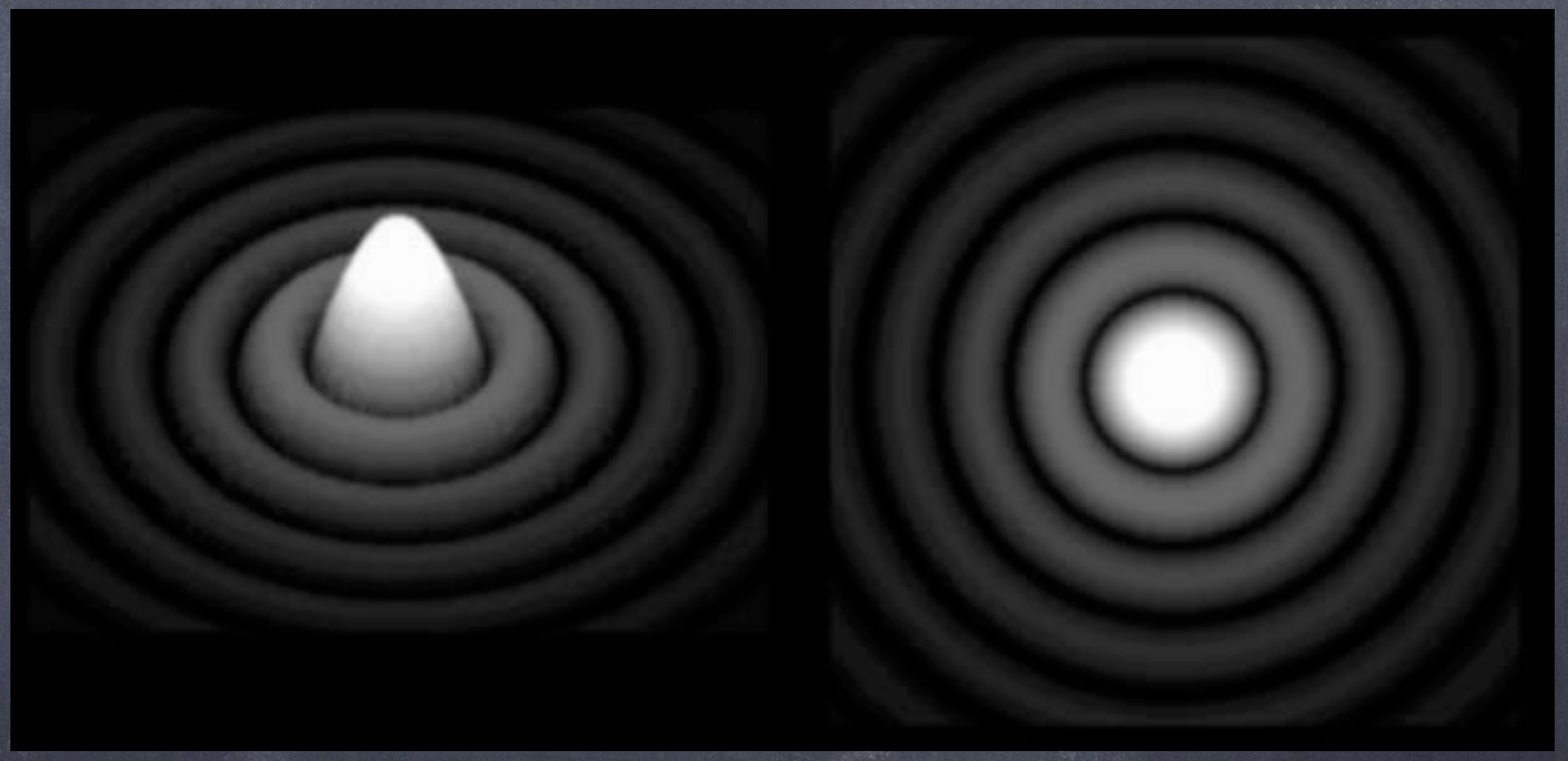




Airy disk



Sir George Biddell Airy



Airy unit = AU = diameter of the intensity peak $\approx 1.22\,\lambda/NA$

Rayleigh parameter for resolution $d \approx 0.61\,\lambda/NA$

Fluorescence

why fluorescence?

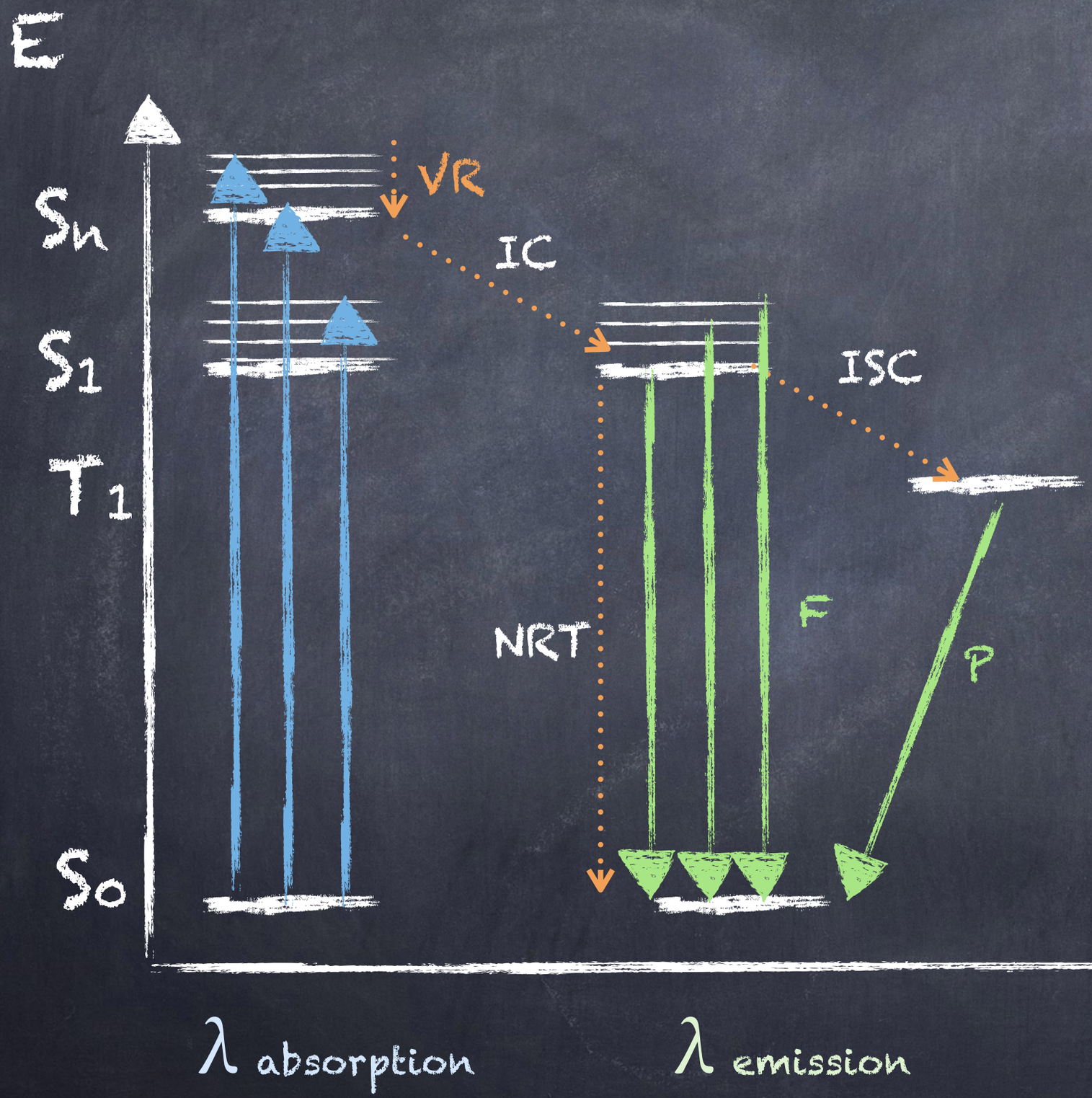
- high contrast
- high resolution
- quantitative
- specific
- broad range of fluorophores

- Life imaging
- FRET/FRAP/colocalization
- pH-sensors
- Ca^{2+}-sensors
- etc. etc

Light can be

- reflected
- refracted
- dispersed
- diffracted
- scattered
- transmitted
- absorbed
- emitted

Jablonski diagram



Aleksander Jabłoński

VR vibrational relaxation

IC internal conversion

NRT non radiative transition

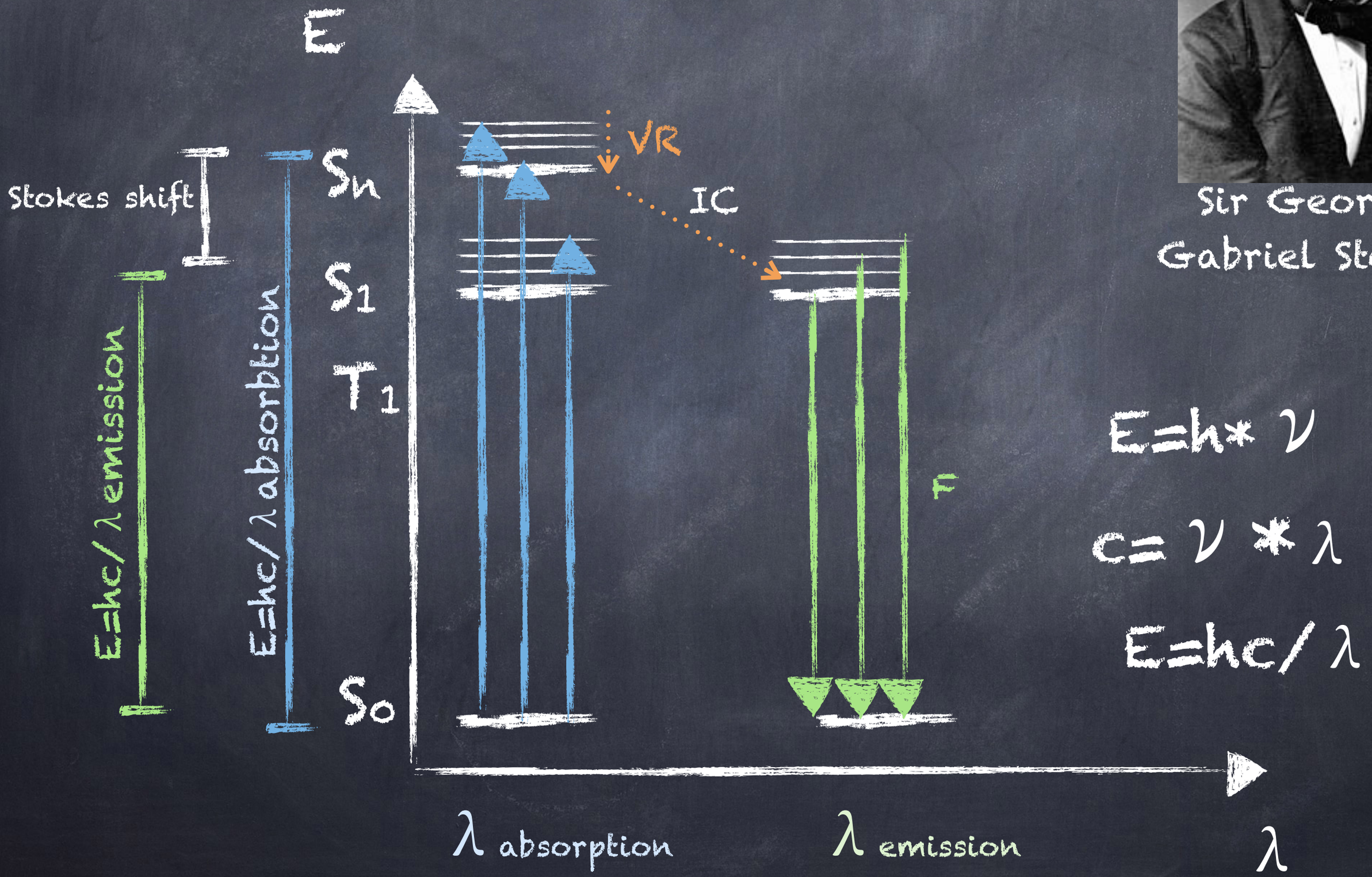
F fluorescence

ISC intersystem conversion

P phosphorescence

Stokes shift

Sir George Gabriel Stokes

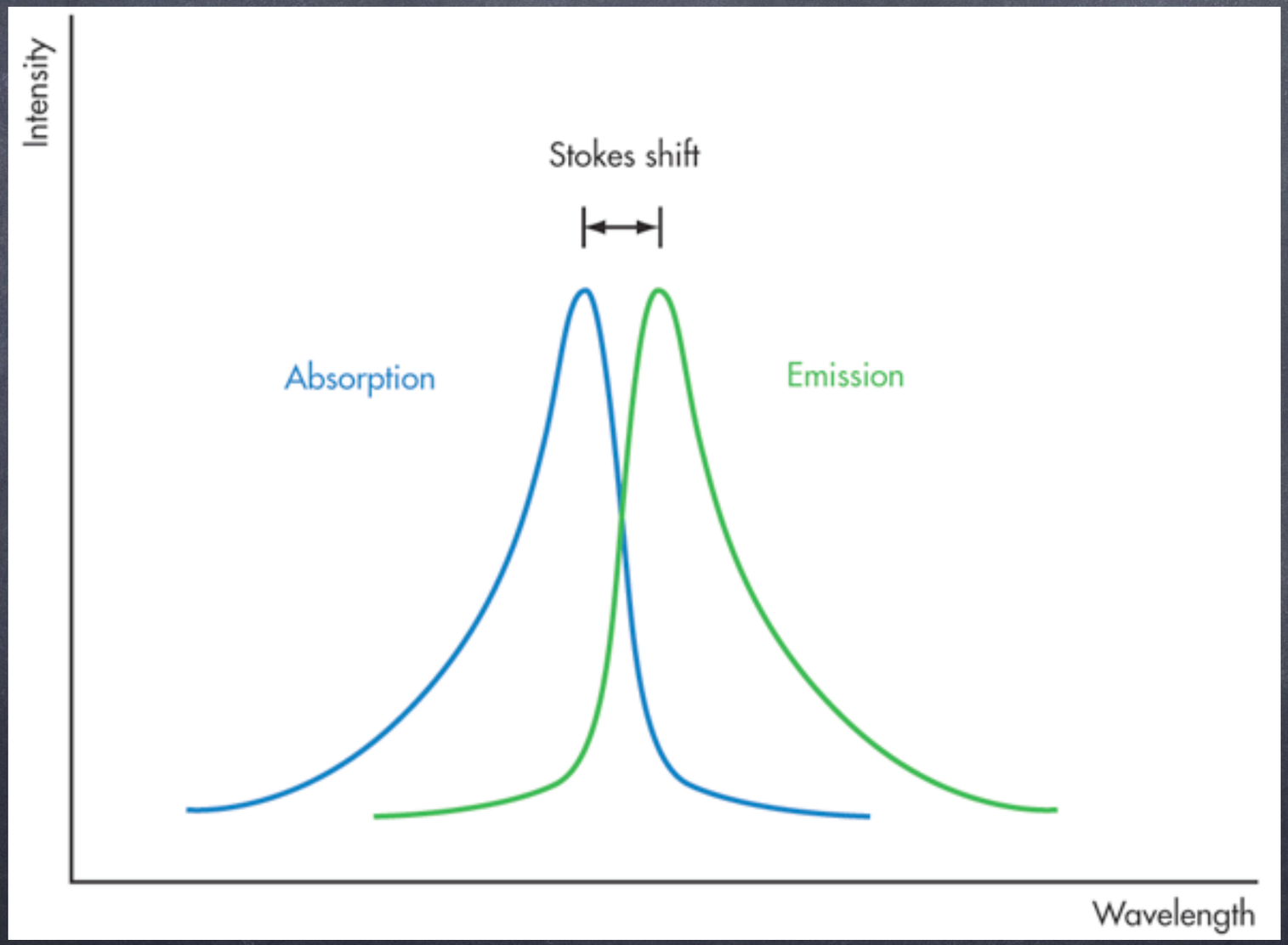


$E = h * \nu$

$c = \nu * \lambda$

$E = hc / \lambda$

Stokes shift



Aequorea victoria

1960 Osamu Shimomura purified aequorin and GFP

2008 Martin Chalfie, Osamu Shimomura, and Roger Y. Tsien received the Nobel Prize

GFP

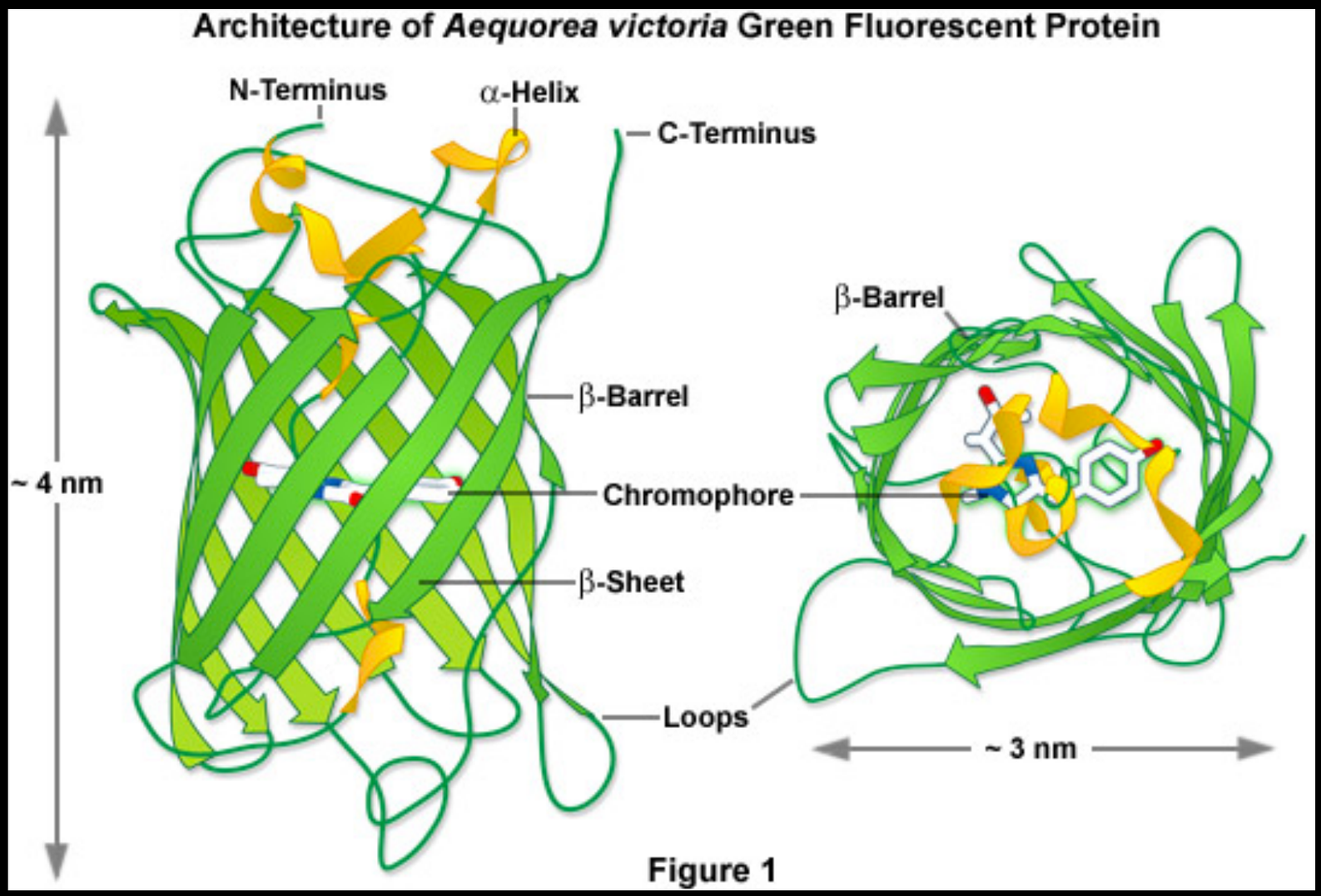

Architecture of *Aequorea victoria* Green Fluorescent Protein
N-Terminus
α-Helix
C-Terminus
β-Barrel
~ 4 nm
Chromophore
β-Sheet
Loops
β-Barrel
~ 3 nm

Figure 1

Acropora Millepora

mRFP

Discosoma

DsRed

modifications of fluorescent proteins

GFP-derived

mRFP1-derived

Evolved by SHM

Exc.	380	433/452	488	516	487/504	540	548	554	568	574	587	595	596	605	590	nm
Em.	440	475/505	509	529	537/562	553	562	581	585	596	610	620	625	636	648	nm