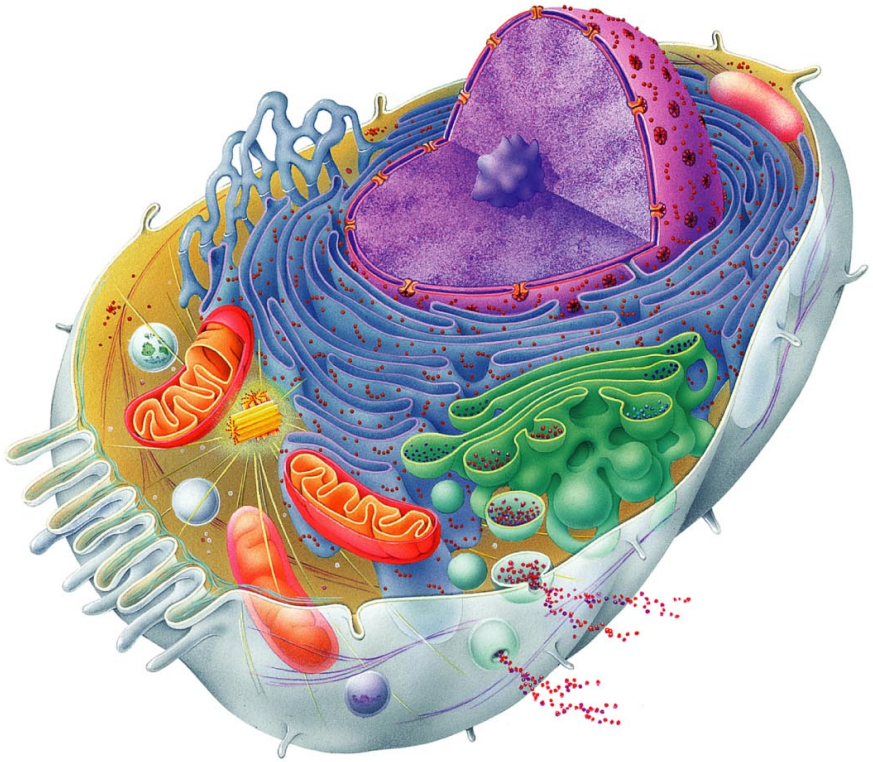
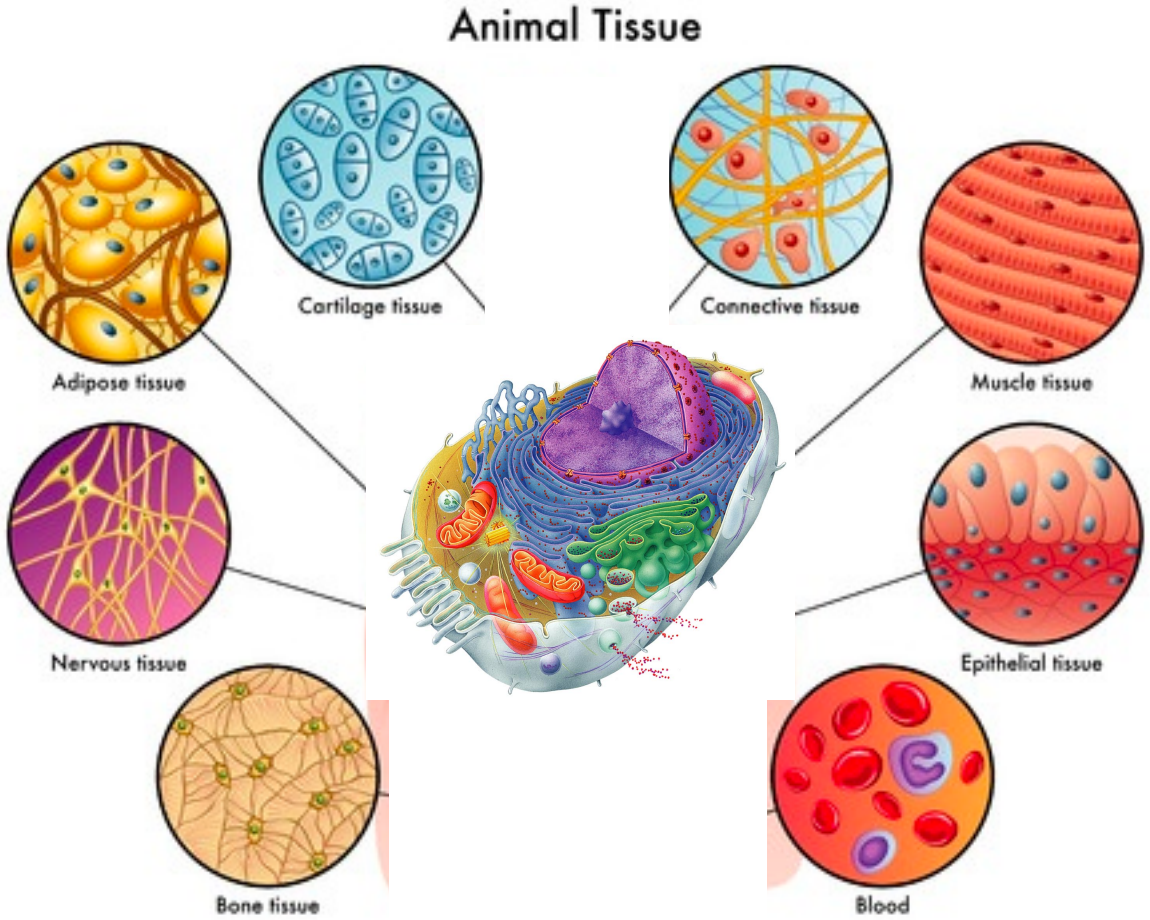
Cells – the autonomous unit of life

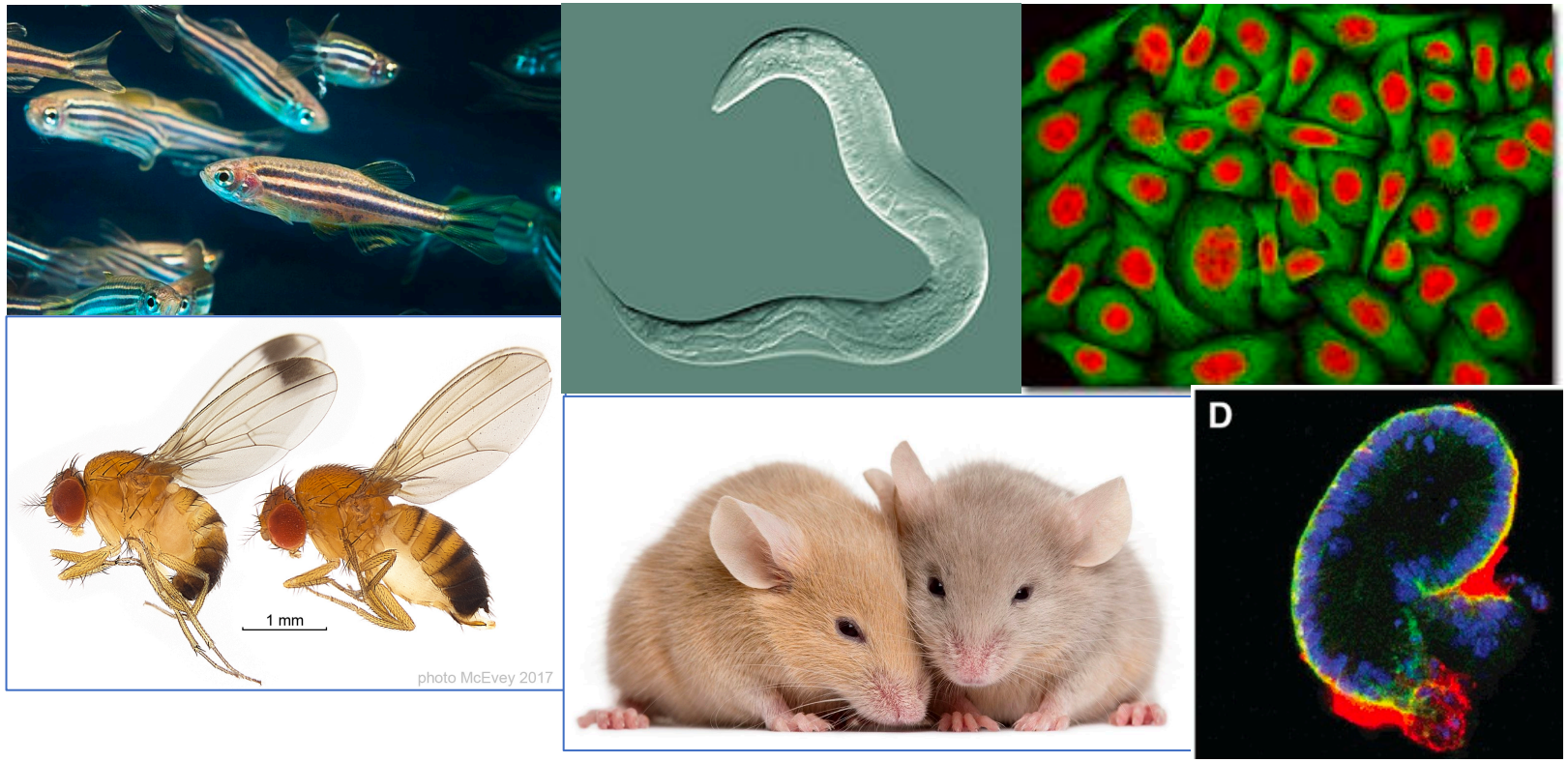


The mission - understanding how cells function

Cells come in different flavors



Model systems in cell biology

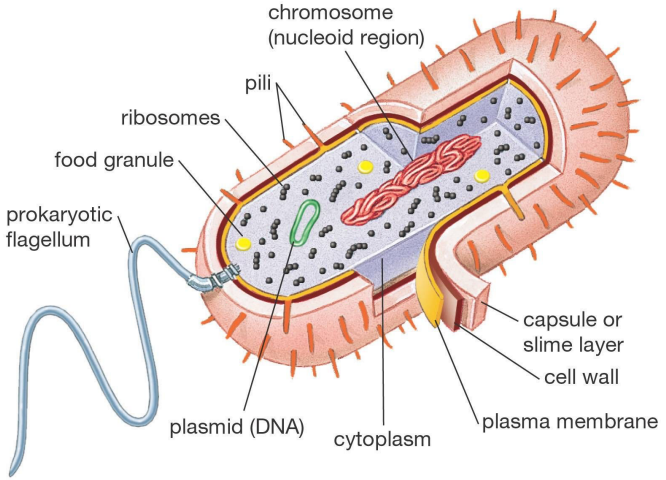


Advantages:

1. *Easy to grow.*
2. *Powerful genetics.*
3. *Suitable for experimental techniques.*

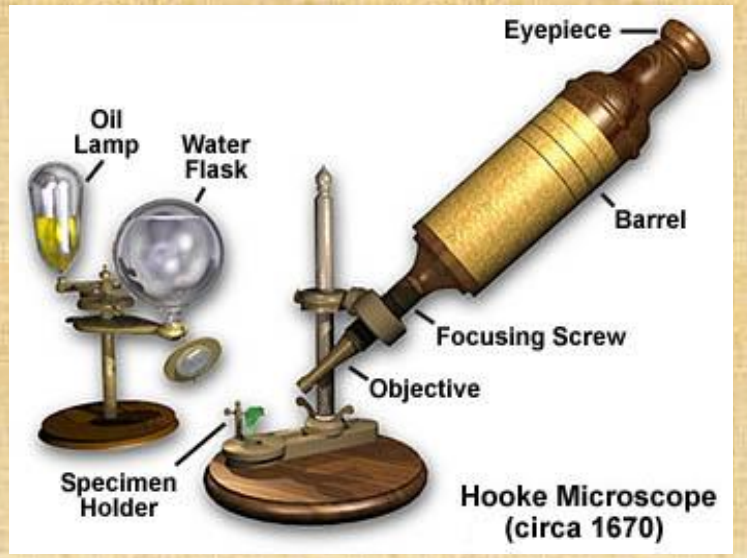
Documentation of cellular processes

1. All **three dimensions** (x, y, z) should be documented simultaneously.
2. Image acquisition **speed** has to be greater than the speed of the biological process.
3. Spatial **resolution** of the microscope should match the scale of the object examined.
4. It must be possible to **track individual components**.
5. Process should be imaged under **physiological conditions**.

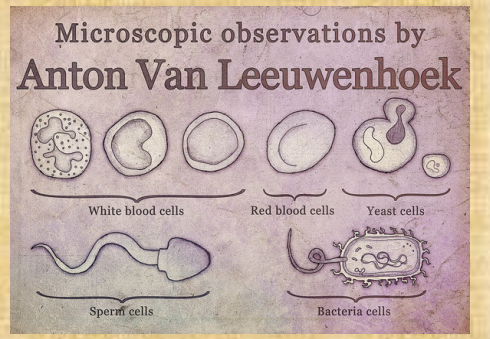


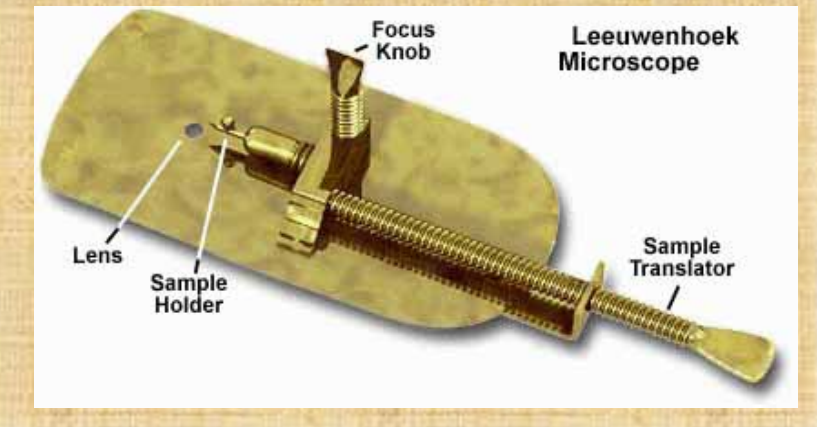
Cells and microscopes

Robert Hooke



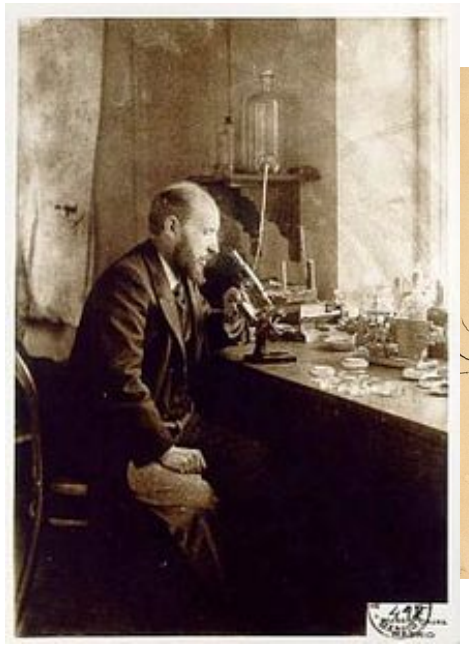
Anton Van Leeuwenhoek

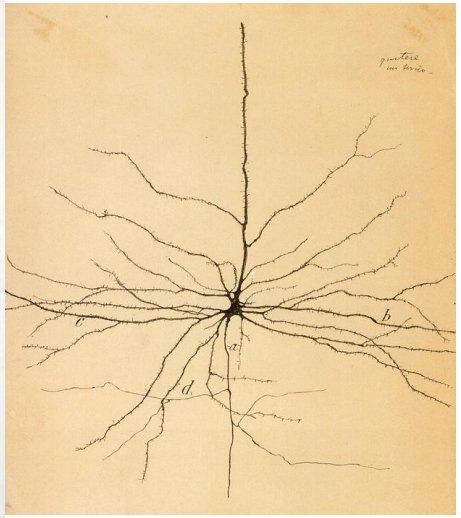




Cells and microscopes

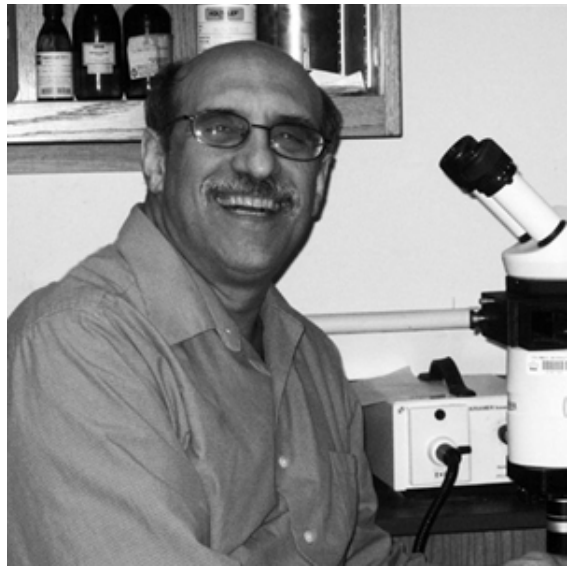
Ramon y cajal

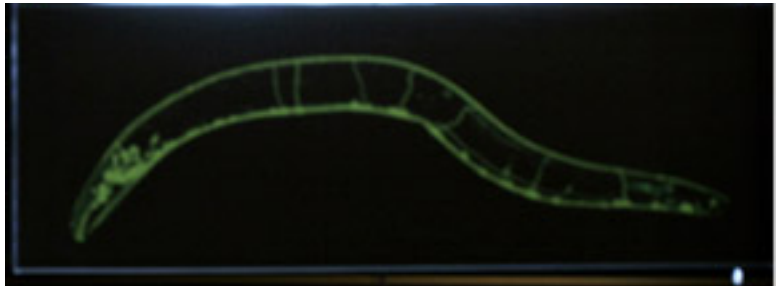




1890 – Describing the nervous system using specific staining (golgi staining).

Martin Chalfie





1995 – GFP in C. elegans

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry for 2008 jointly to:

Osamu Shimomura, Martin Chalfie, and
Roger Y. Tsien,

"for the discovery and development of the green fluorescent protein, GFP".

The discovery and development of the green fluorescent protein (GFP) have radically changed the scientific agenda. Improved variants of GFP and GFP-like proteins in synergy with high-resolution microscopes, computational techniques and powerful theoretical approaches are currently fuelling a scientific revolution focused on quantitative analyses of complex biological systems. **The gradual appearance of a world of hitherto unseen structures and dynamic principles is now impacting virtually all aspects of biological, medical and pharmaceutical research.** (presentation speech, 2008)

Fluorescence super resolution microscopy

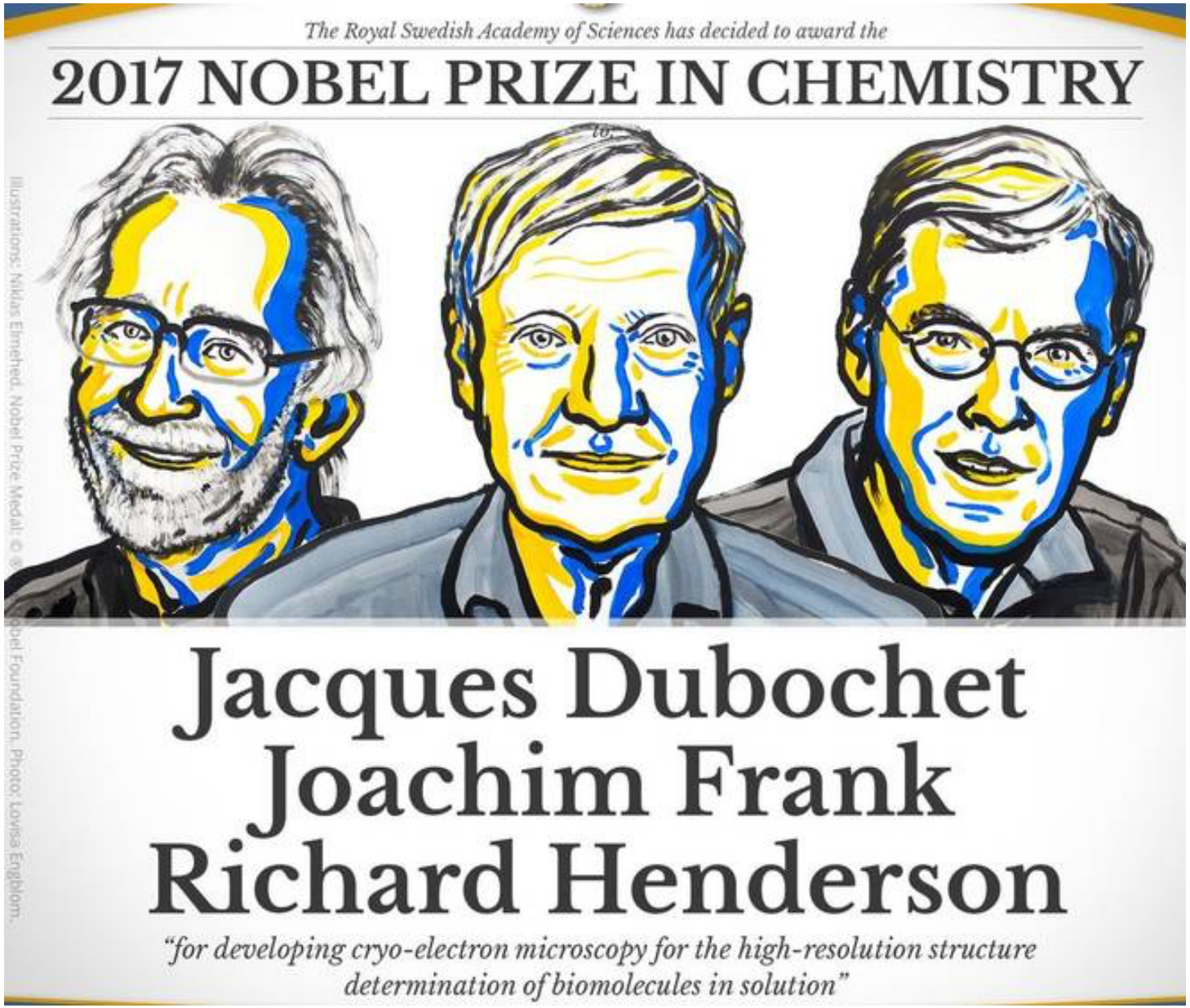
Nobel Prize 2014, Chemistry

Eric Betzig

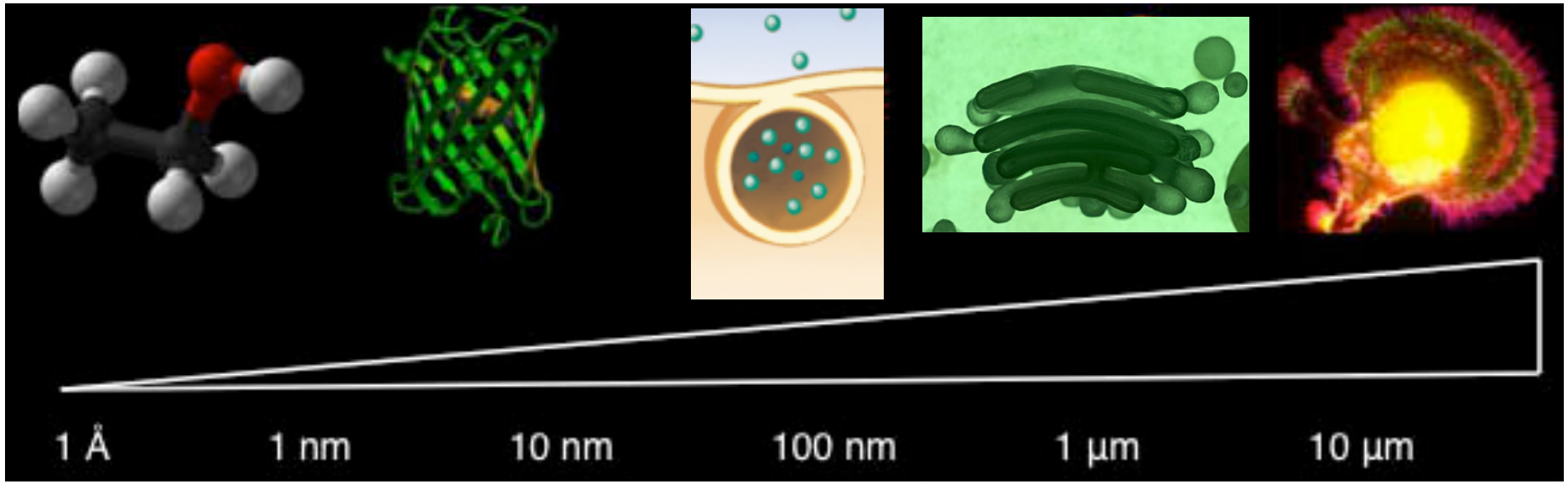
William Moerner

Stefan Hell

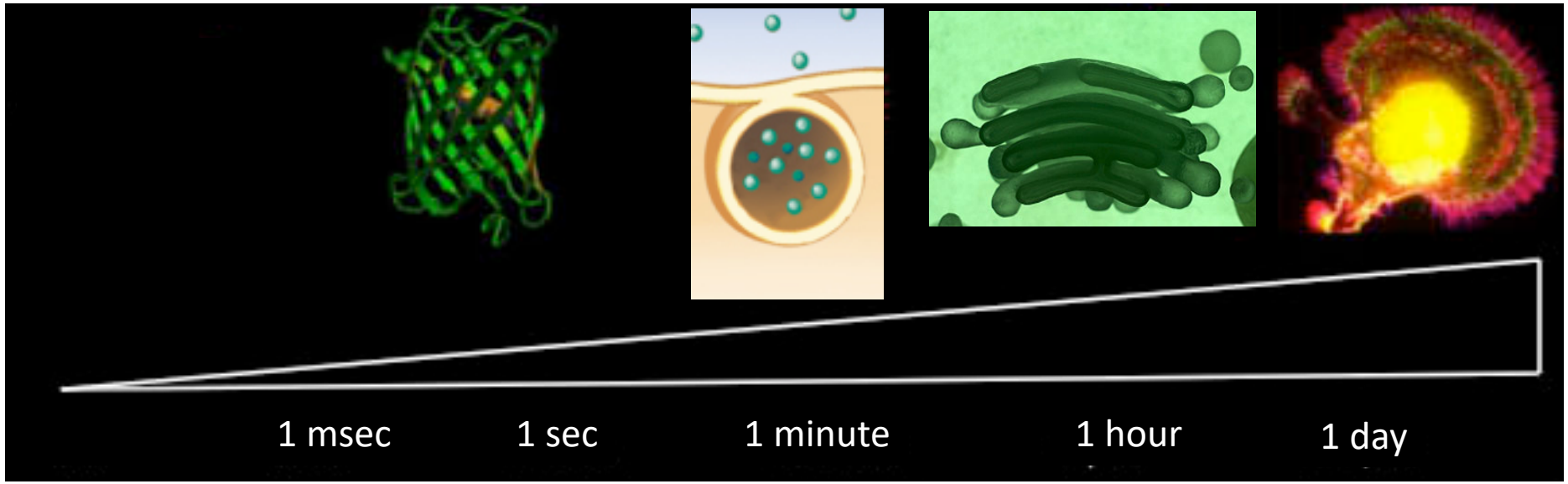
"for the development of super-resolved fluorescence microscopy".


The Royal Swedish Academy of Sciences has decided to award the
2017 NOBEL PRIZE IN CHEMISTRY
to
Illustrations: Niklas Elmehed. Nobel Prize Medal: © Nobel Foundation. Photo: Lovisa Engblom.
Jacques Dubochet
Joachim Frank
Richard Henderson
"for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution"

The cellular ruler - size

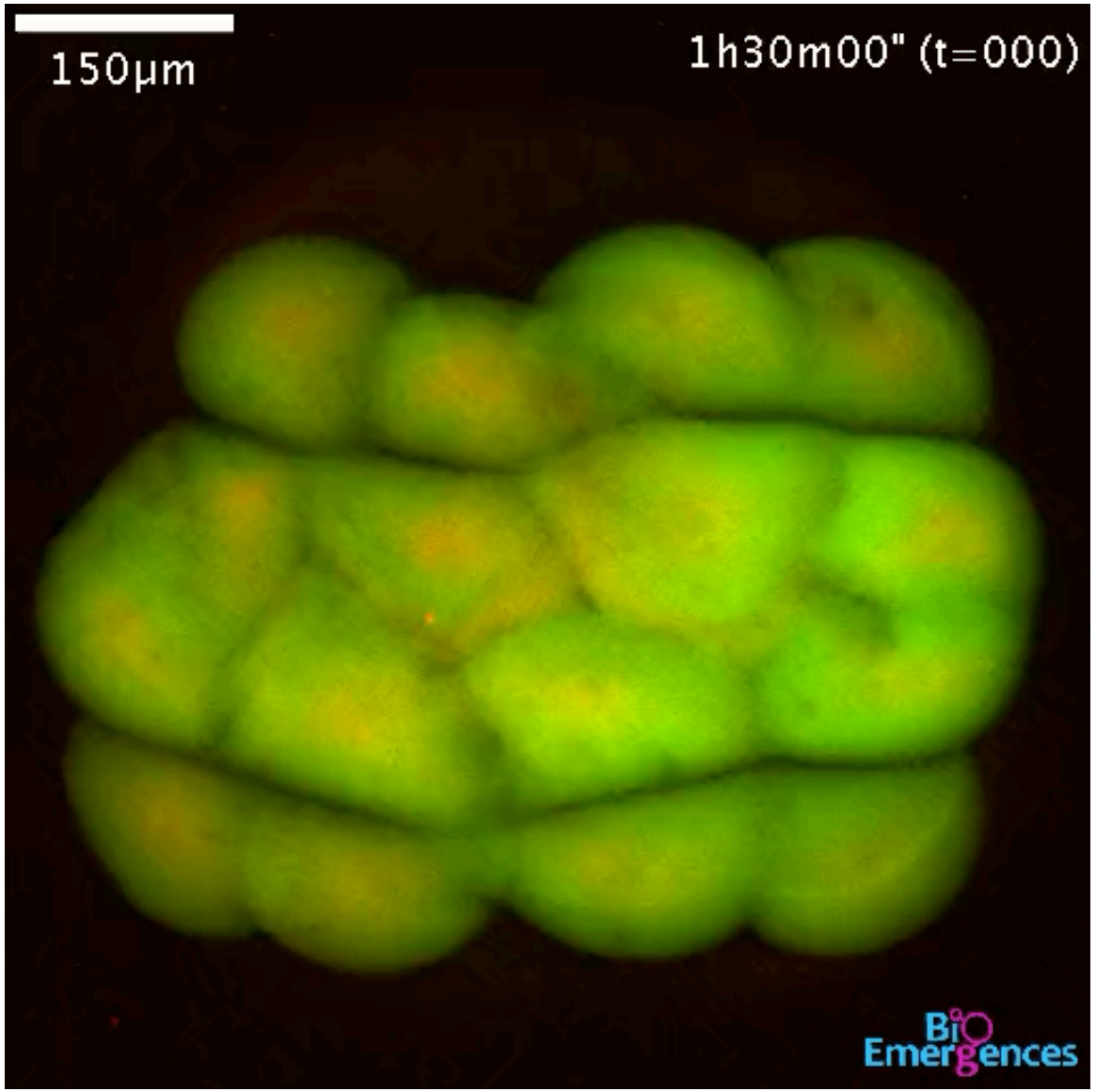


The cellular ruler - time

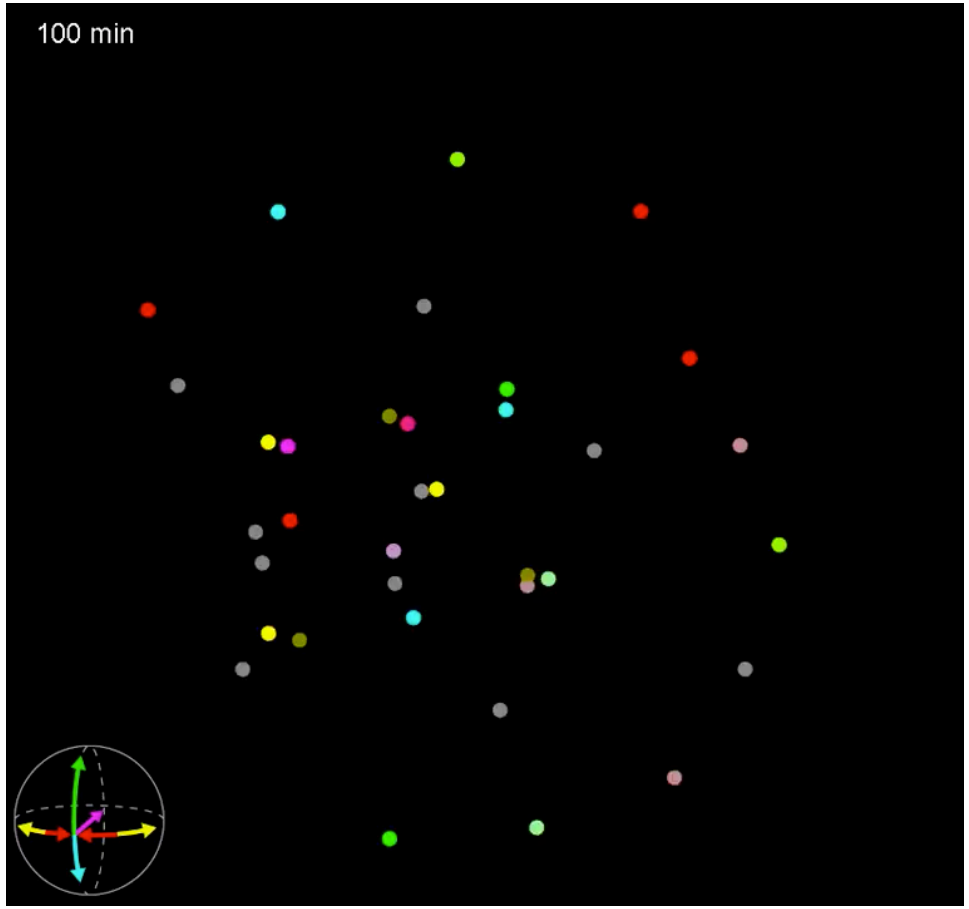


Diffusion of cytosolic proteins: msec
Diffusion of TM proteins: sec
Endocytosis: minute
Trafficking in the secretory pathway Glogi-PM: hour
Cell cycle: day

Fish embryos

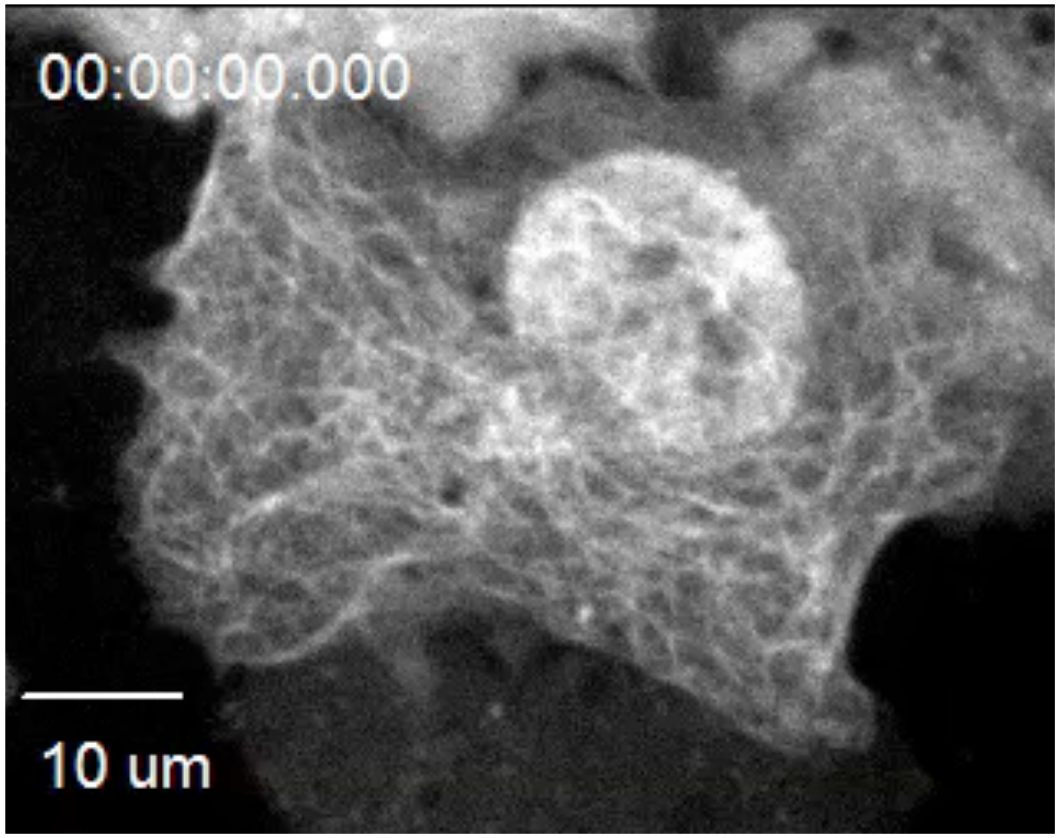


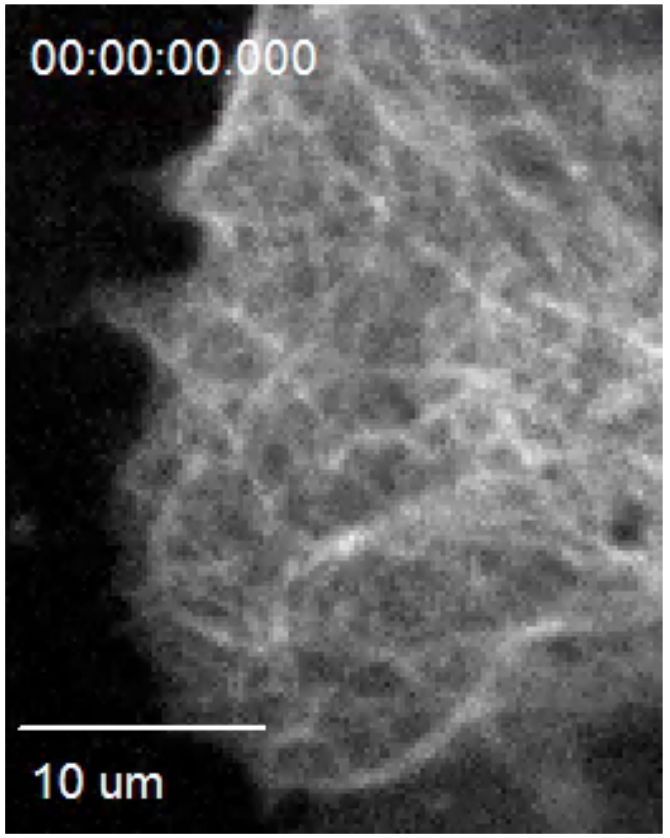
Fish embryos



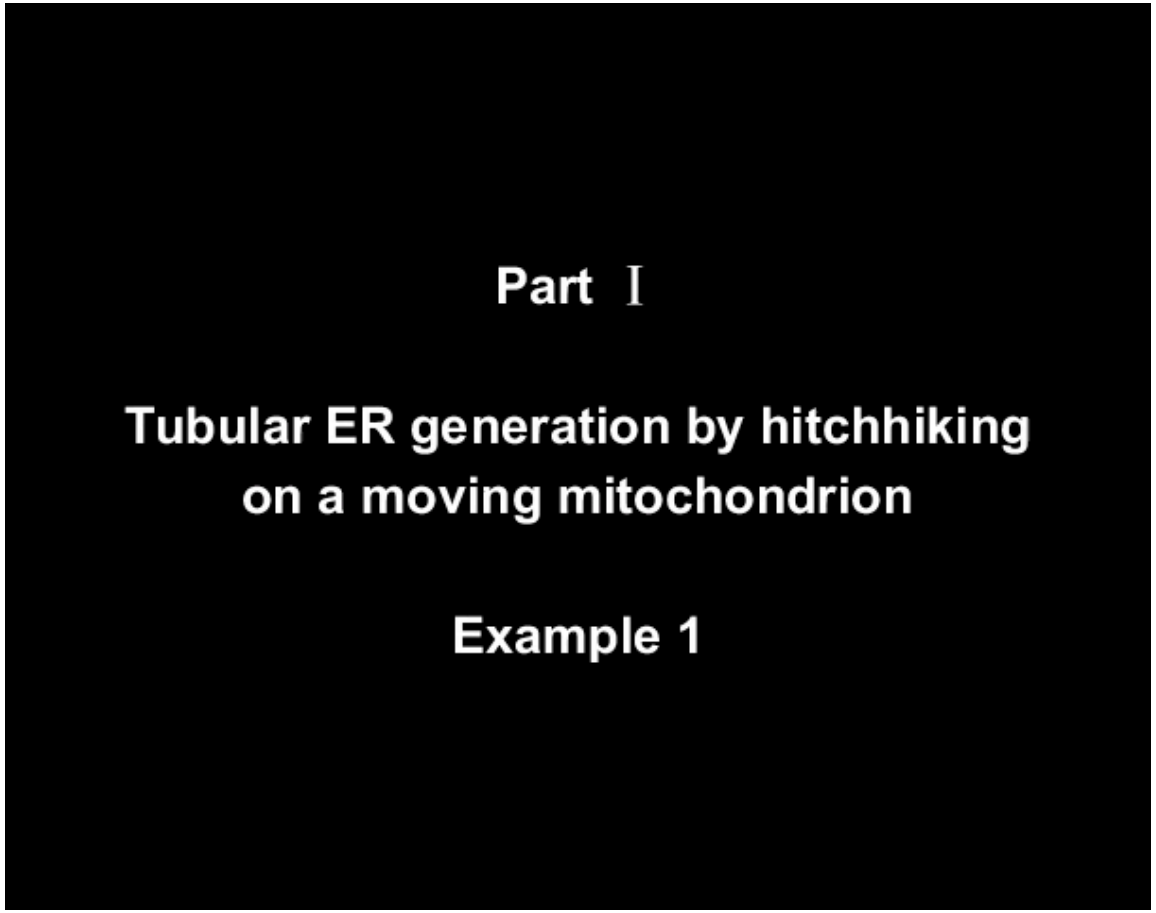
Zebrafish develoment, cell tracking, janelia farms

Intracellular dynamics - microtubules





Intracellular dynamics

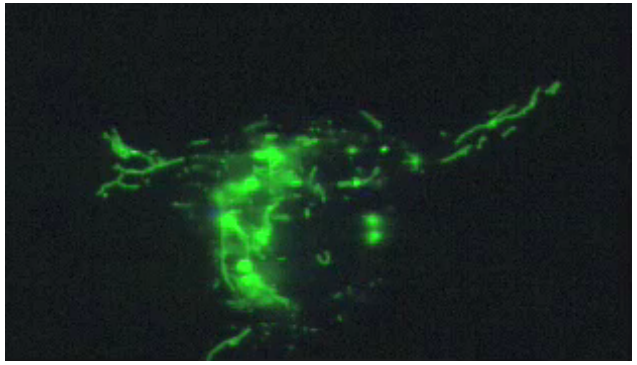


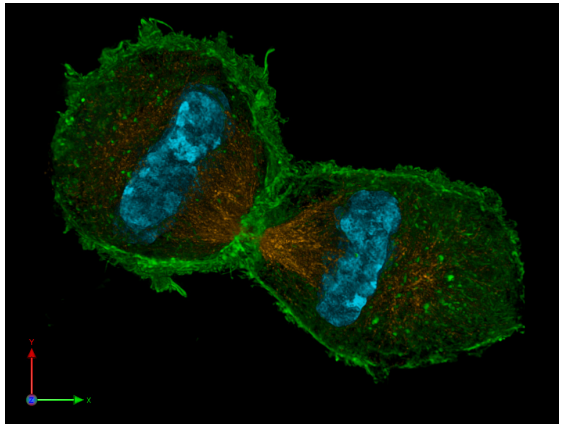
97-nm resolution and 266 frames/s over thousands of time points

Cell, 2018

Imaging the structure of a cell

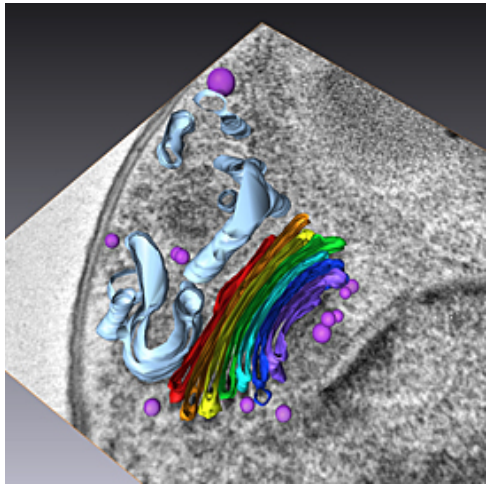
Fluorescence microscopy





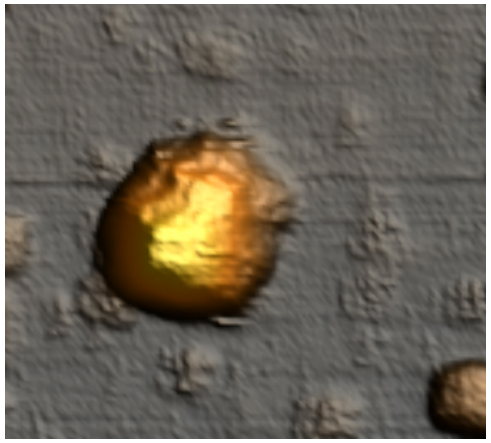
Structure and dynamics of selected protein complexes in cells in 3D.
Limitations: results depend on the fluorescent probe.
Resolution: up to 30 nm.

Electron microscopy



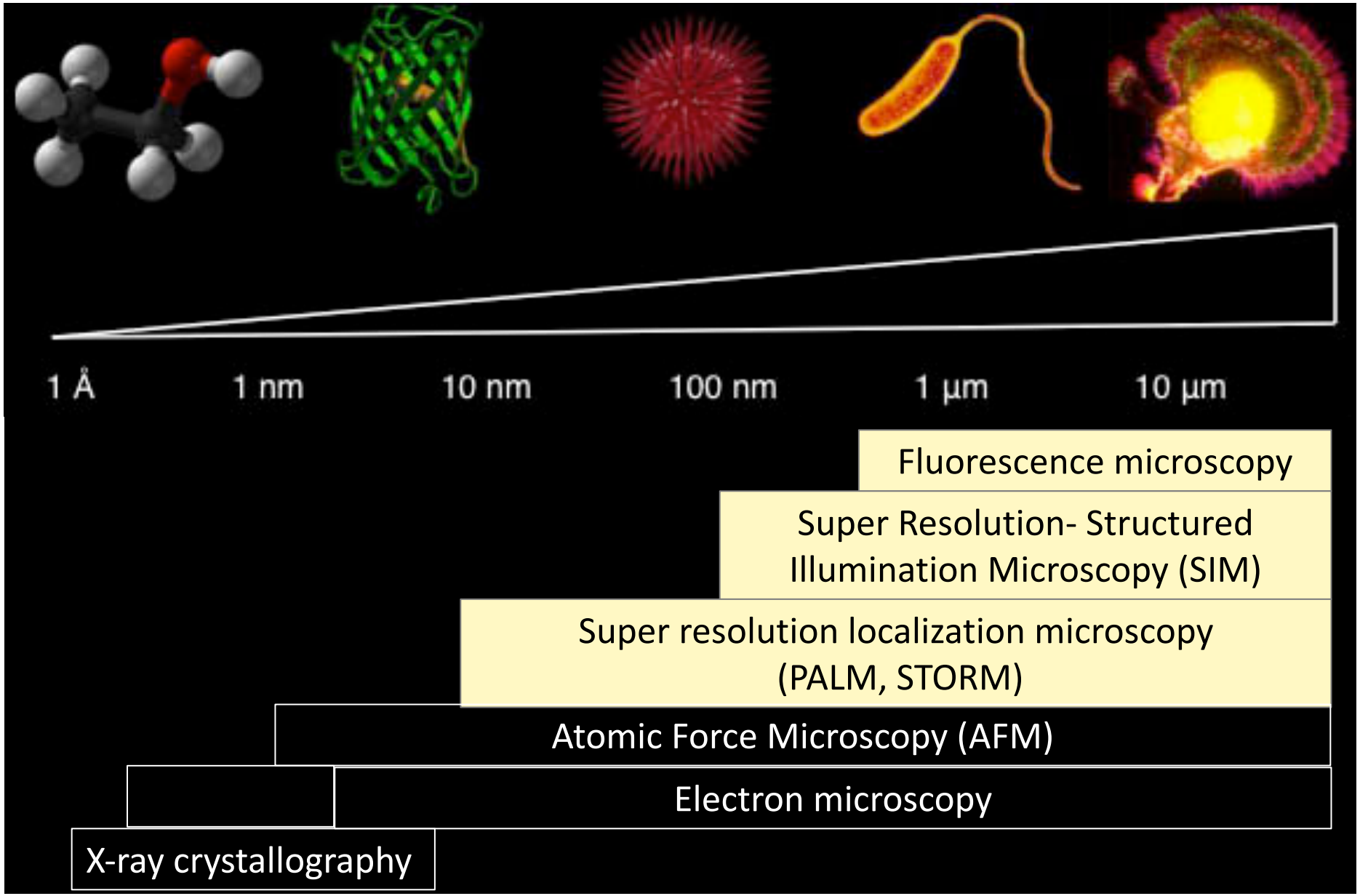
3D reconstruction of a cell and of large protein complexes.
Limitations: Difficult to label specific proteins, no dynamics.
Resolution: 0.1 nm in cells

AFM

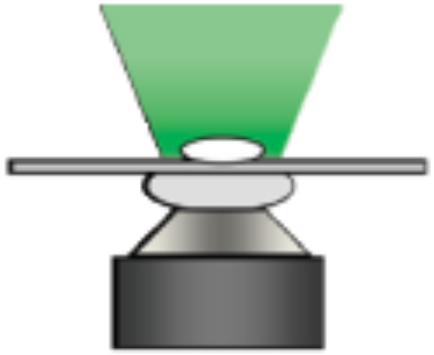


Mechanical properties of cells and proteins in living systems.
Limitations: limited to cell surface.
Resolution: sub nm

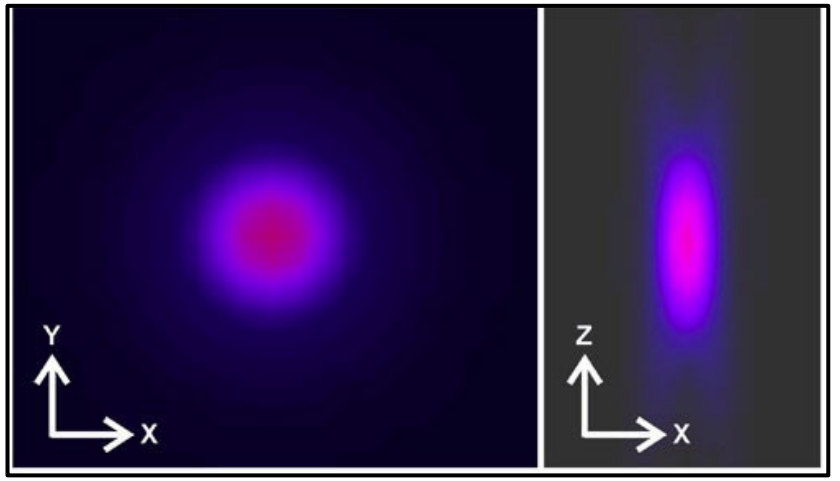
Bridging the resolution gap



PSF (point spread function)



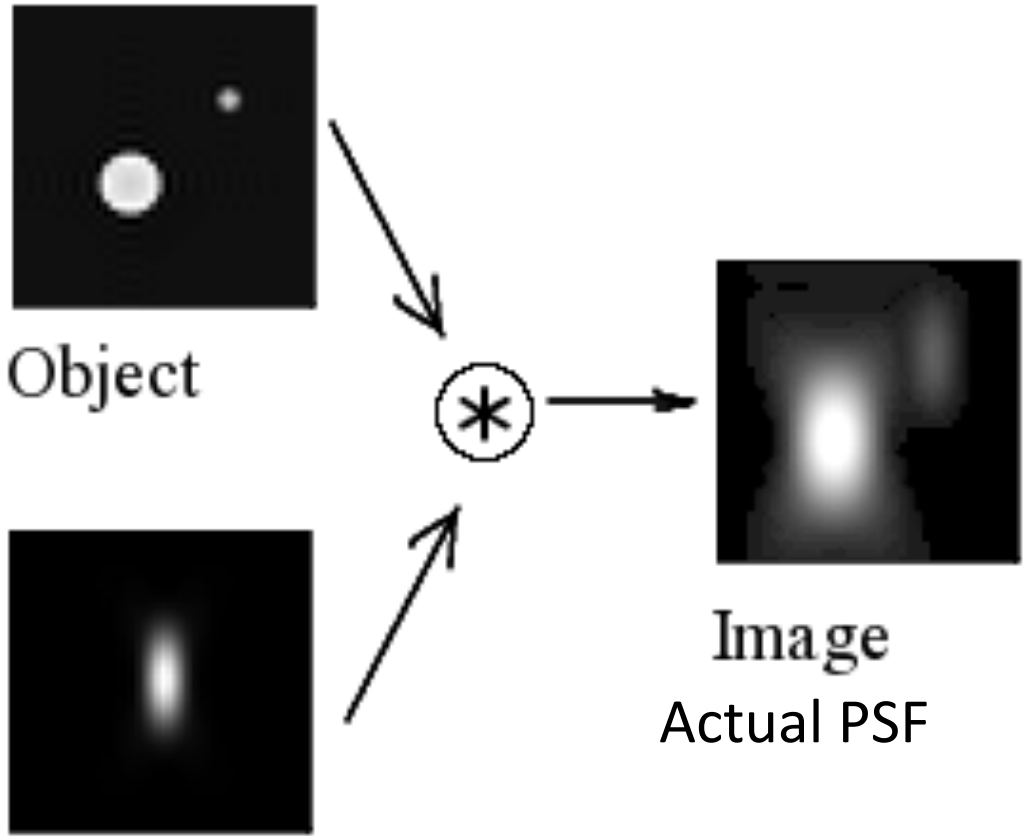
Wide Field



The objective lens does not focus the emitted light to an infinitely small point in the image plane. Light waves converge and interfere at the focal point.

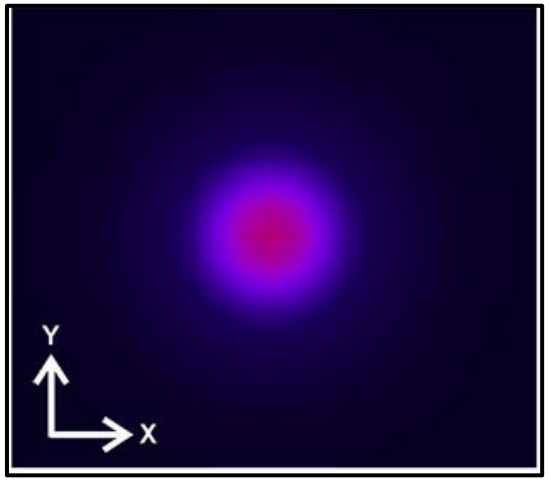
PSF depends on the NA of the objective.
The higher the NA the smaller the PSF.

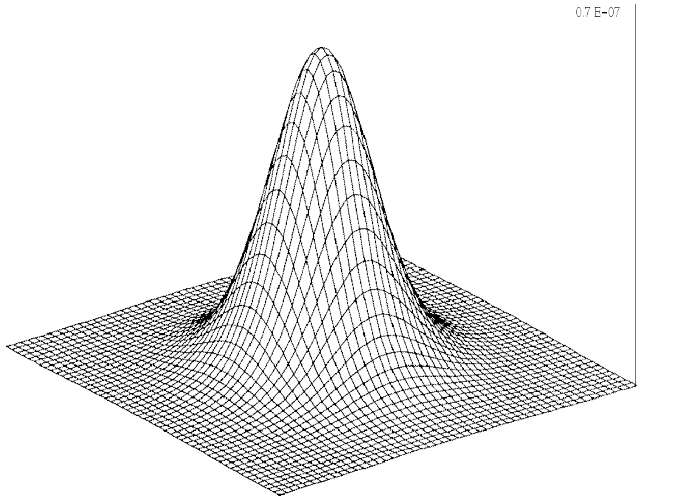
PSF

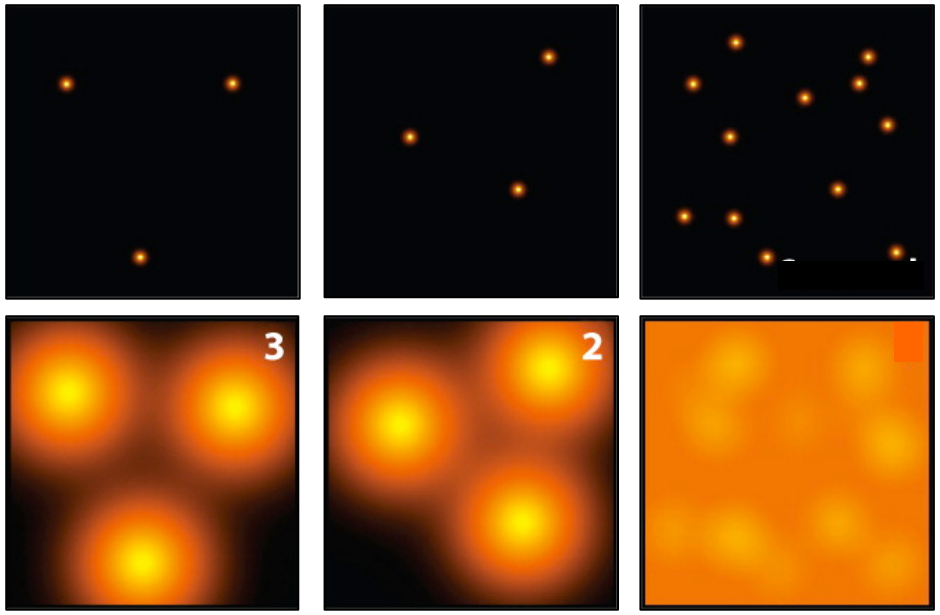


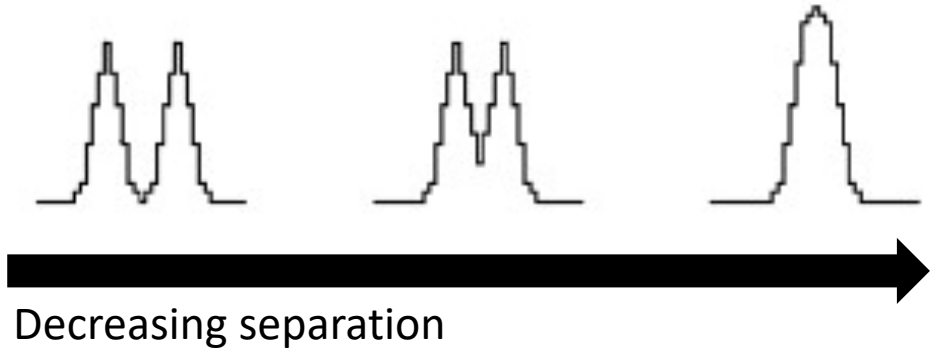
It is important to measure the actual PSF for each objective

Resolution



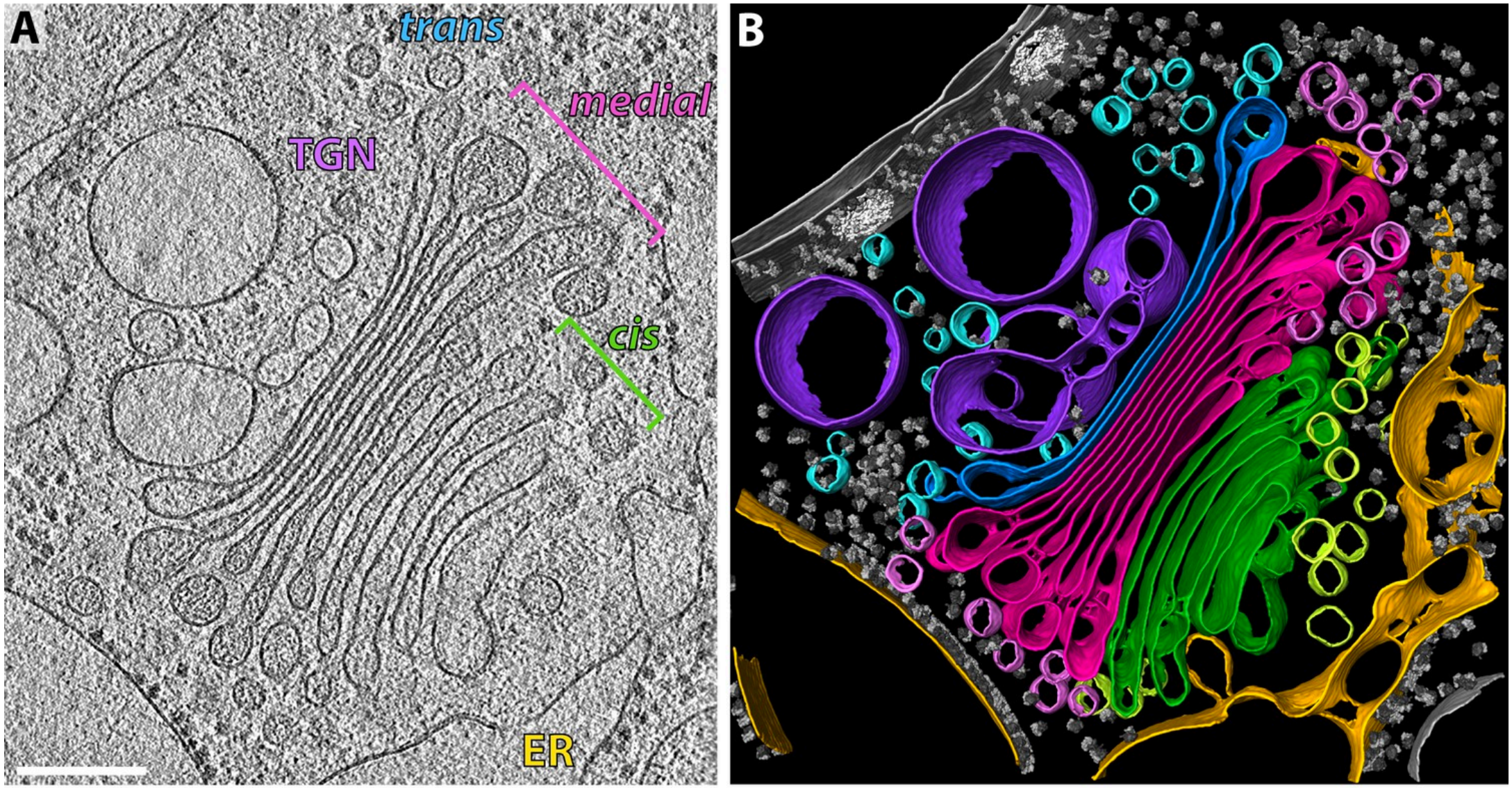




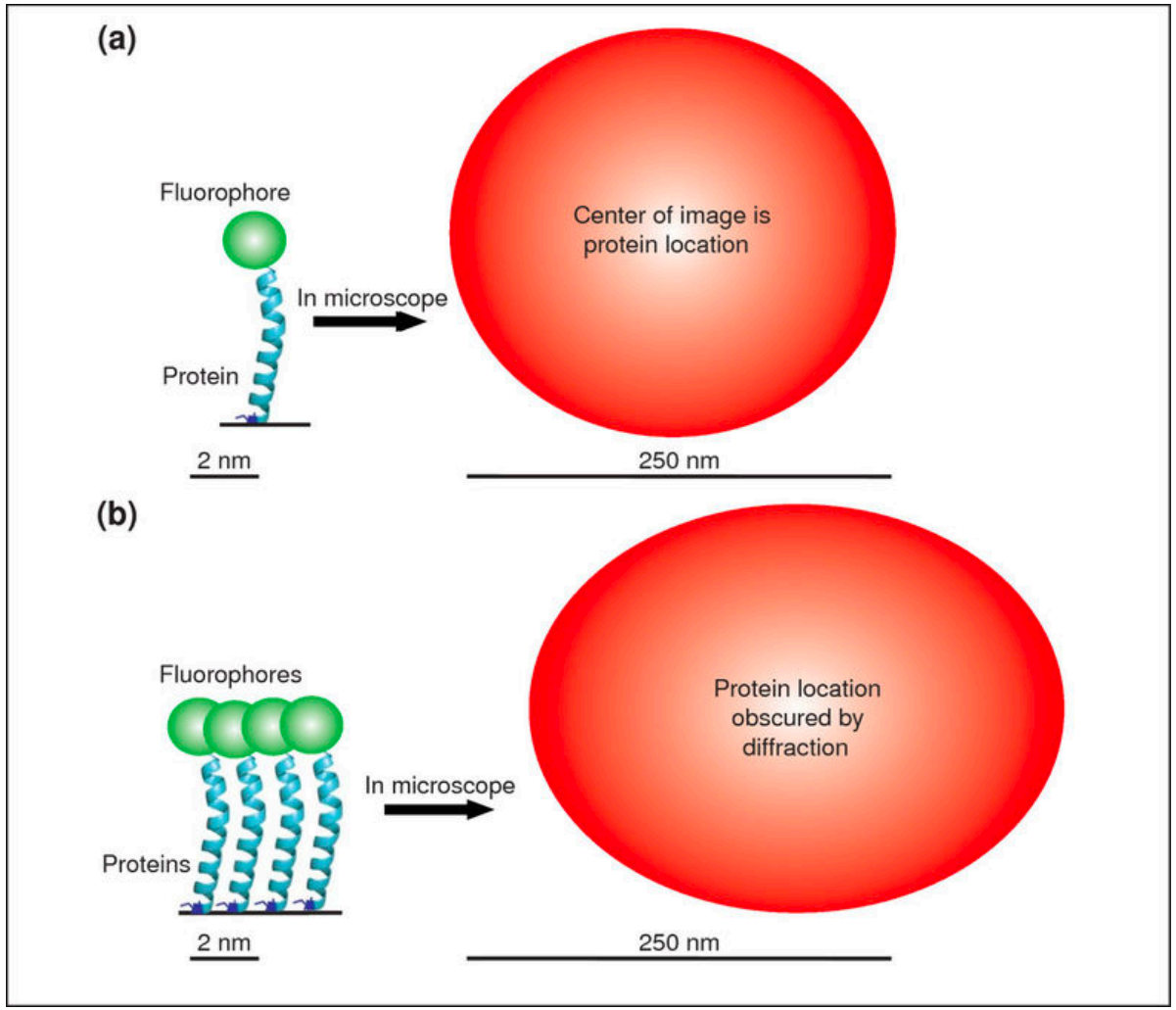


Resolution = λ/2NA

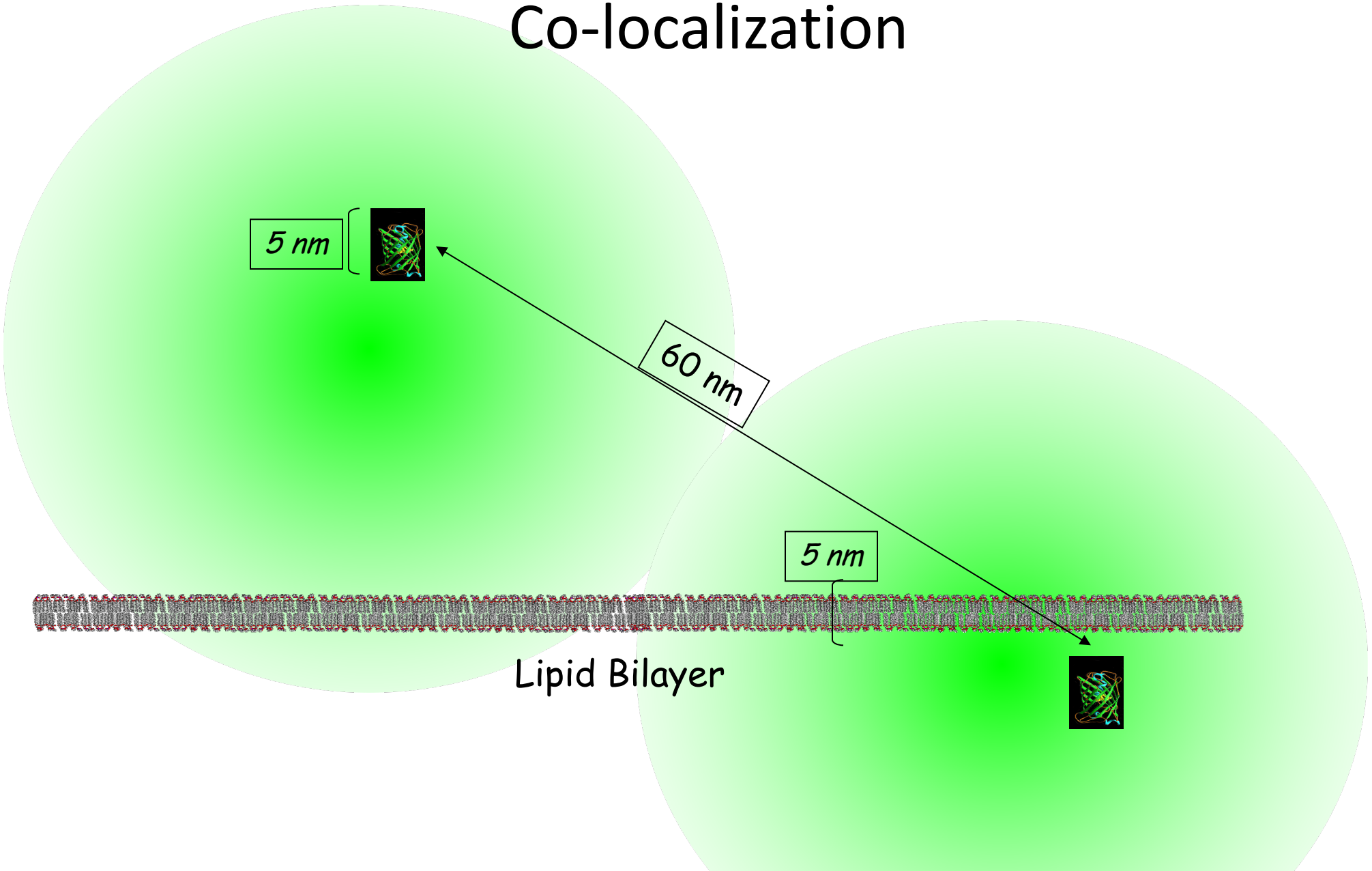

WINNER!
2010 TONY AWARD
BEST
MUSICAL
2011
TOSHIBA
HAPPY
NEW
YEAR!
TDK
NEW YORK
Happy
New Year!
SONY
Kodak
RESOLUTION
LEBRON
STAUDE
SATURDAY JAN 1
LIVE ON PAY-PER-VIEW
Bank of America
NIVEA
PREPARE TO BE KISSED
NIVEA
NIVEA


A
trans
medial
TGN
cis
ER
B

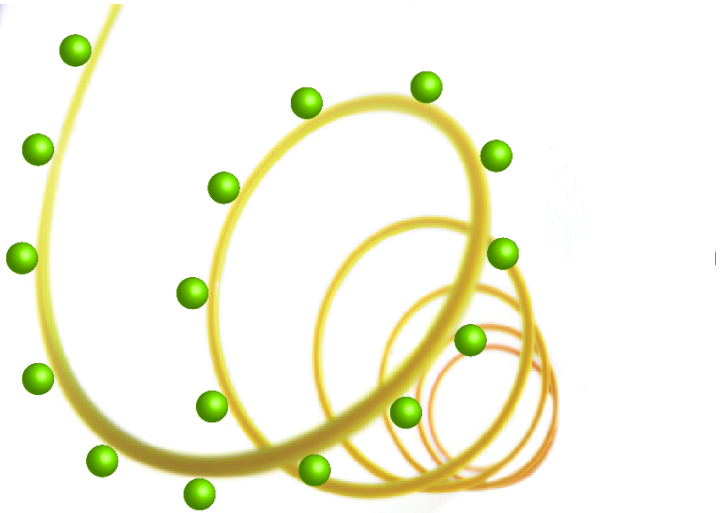
Issues associated with limited resolution: Quantitation

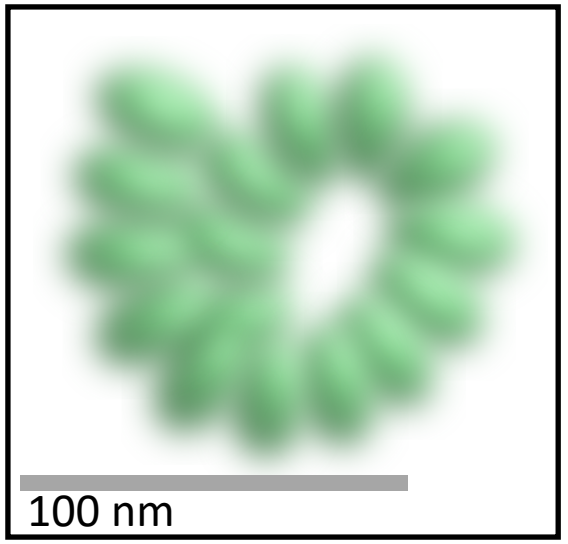


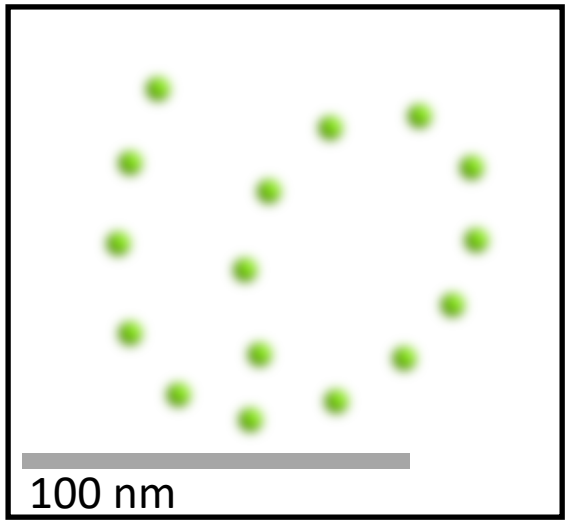
Issues associated with limited resolution: Co-localization



Issues associated with limited resolution: Resolving a structure

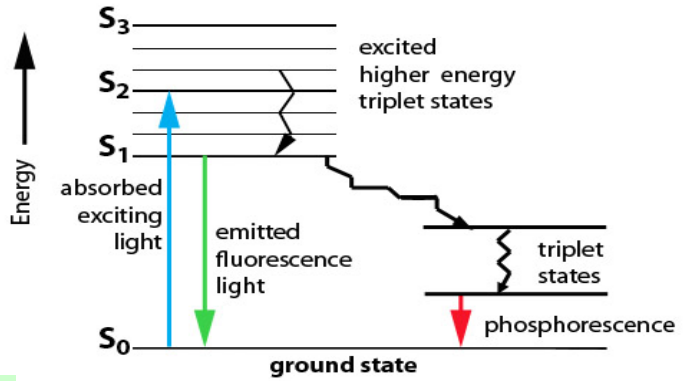






Fluorescence Microscopy: How does it work?

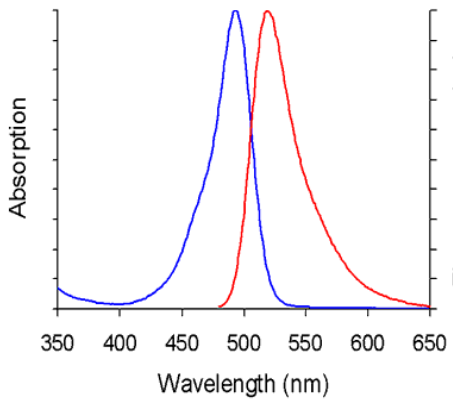
The high energy absorbed in the exited state is being released as fluorescent light.



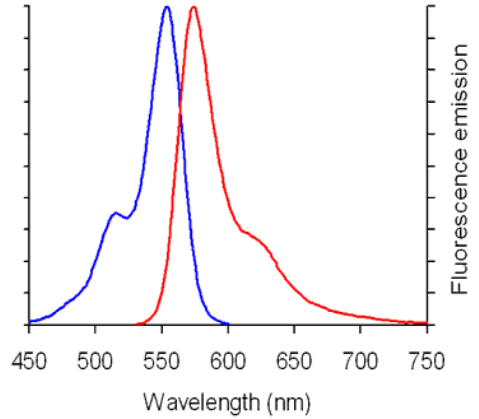
Fl- dyes

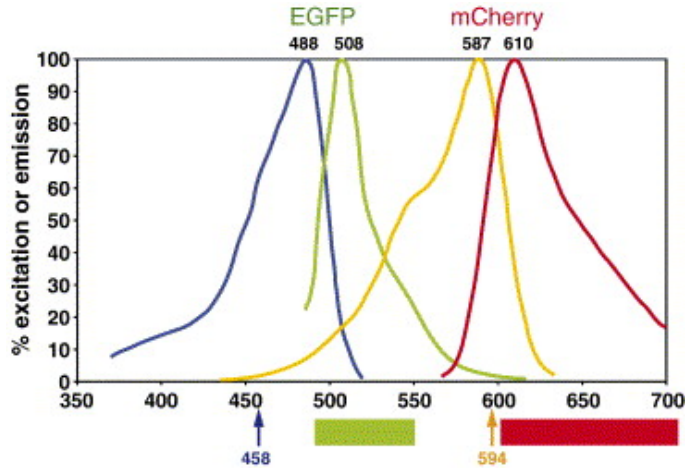
Fl- Proteins

Alexa 488

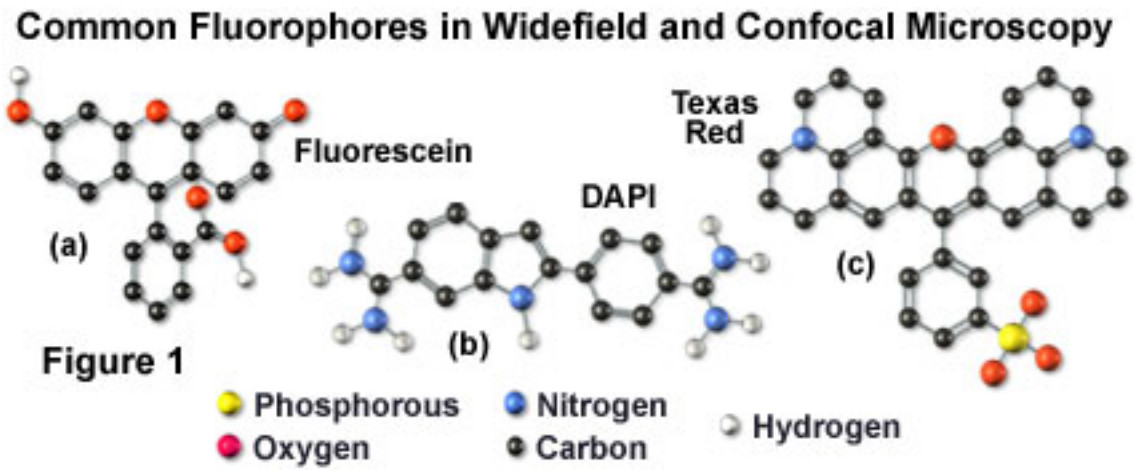


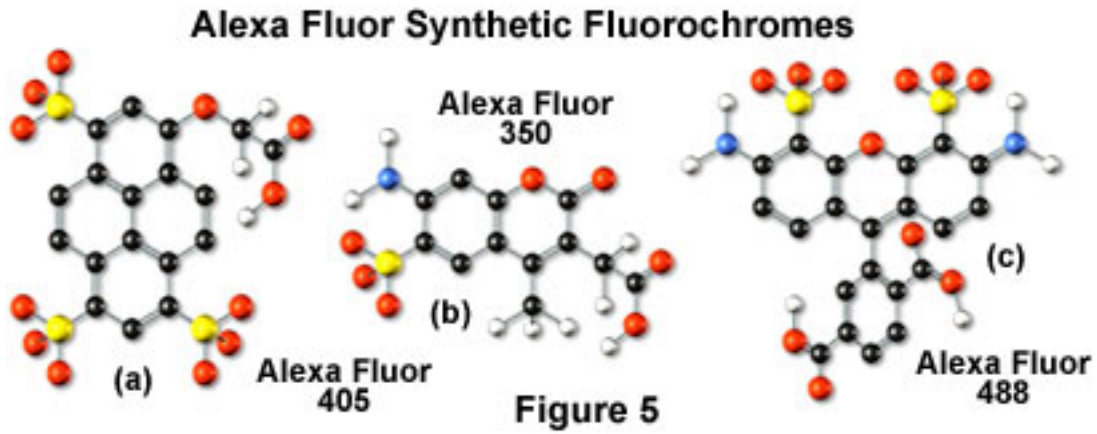
Alexa 546



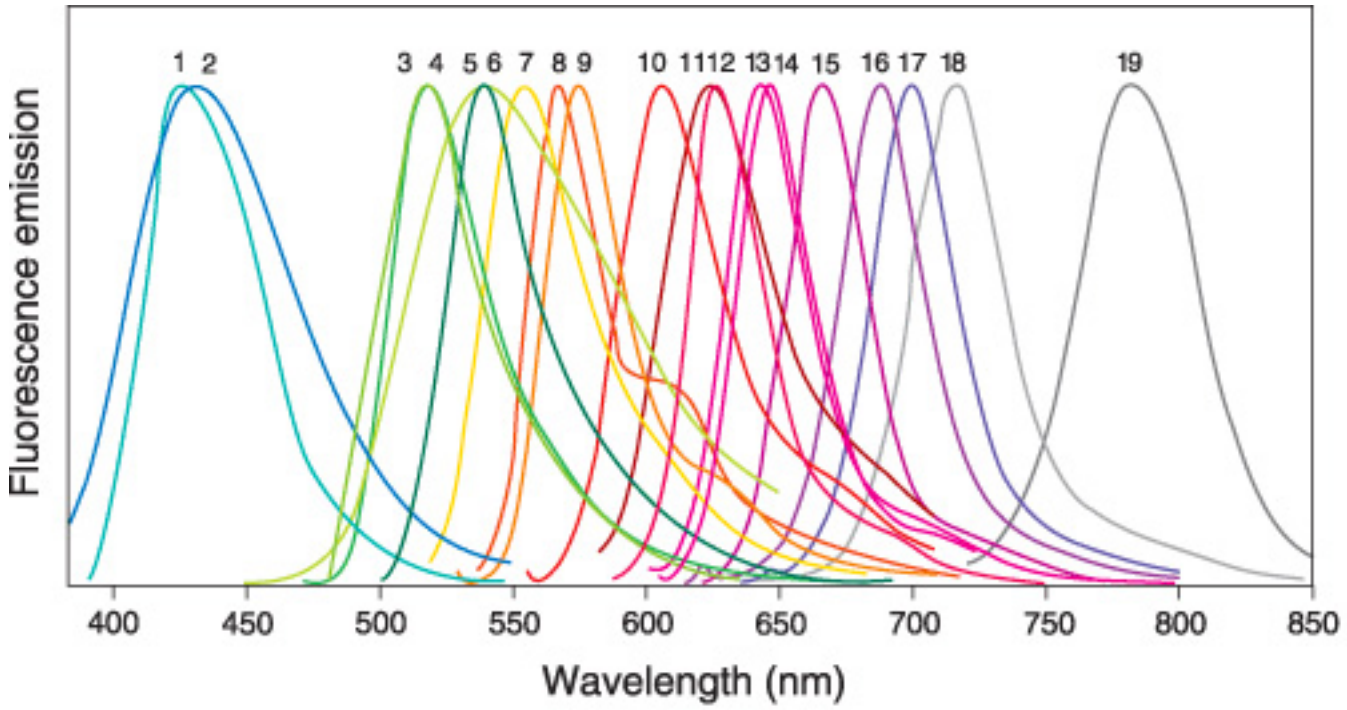


Fluorophores





Fluorophores



1. Alexa Fluor 405
2. Alexa Fluor 350
3. Alexa Fluor 500
4. Alexa Fluor 488
5. Alexa Fluor 430
6. Alexa Fluor 514
7. Alexa Fluor 532
8. Alexa Fluor 555
9. Alexa Fluor 546
10. Alexa Fluor 568
11. Alexa Fluor 594
12. Alexa Fluor 610
13. Alexa Fluor 633
14. Alexa Fluor 635
15. Alexa Fluor 647
16. Alexa Fluor 660
17. Alexa Fluor 680
18. Alexa Fluor 700
19. Alexa Fluor 750

Fluorescent proteins

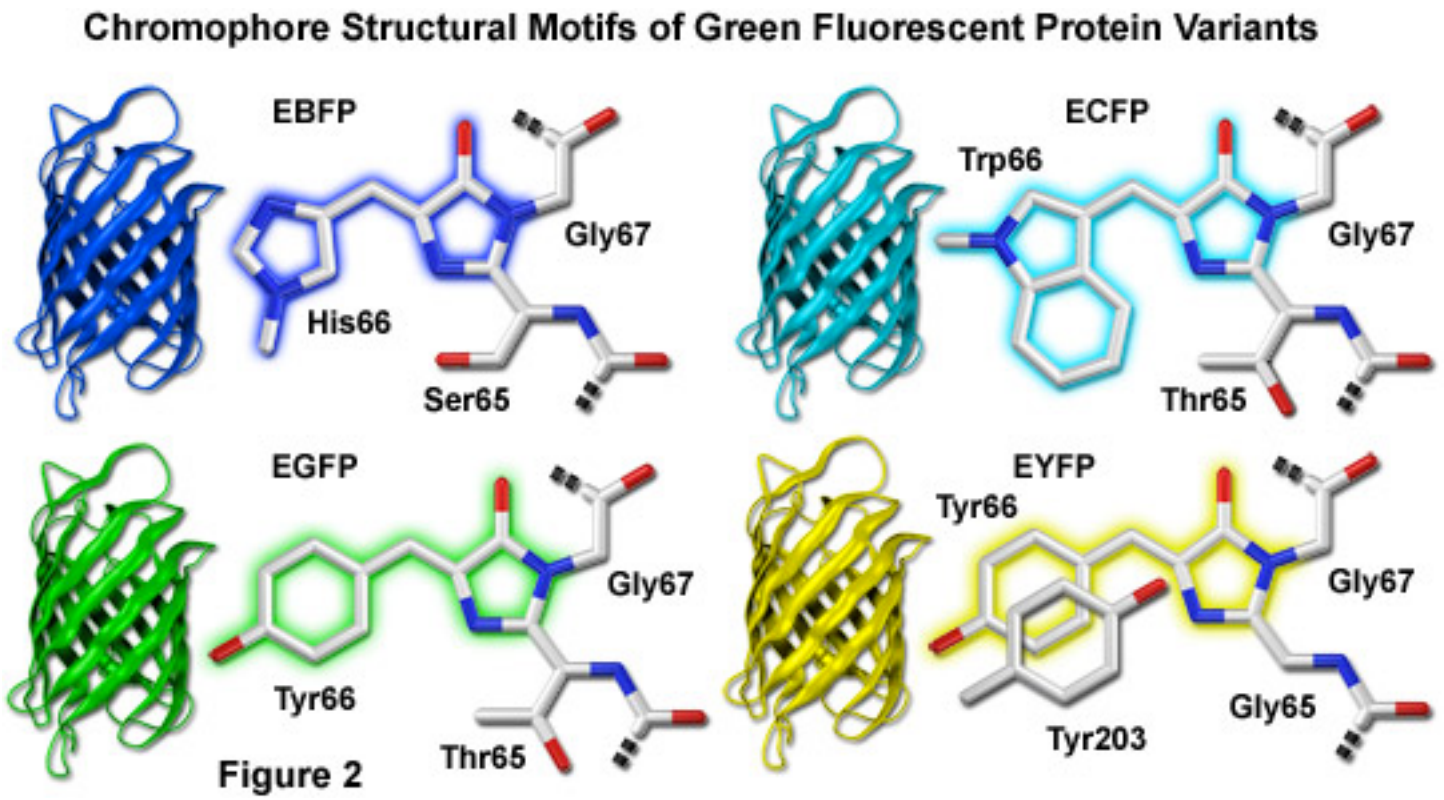
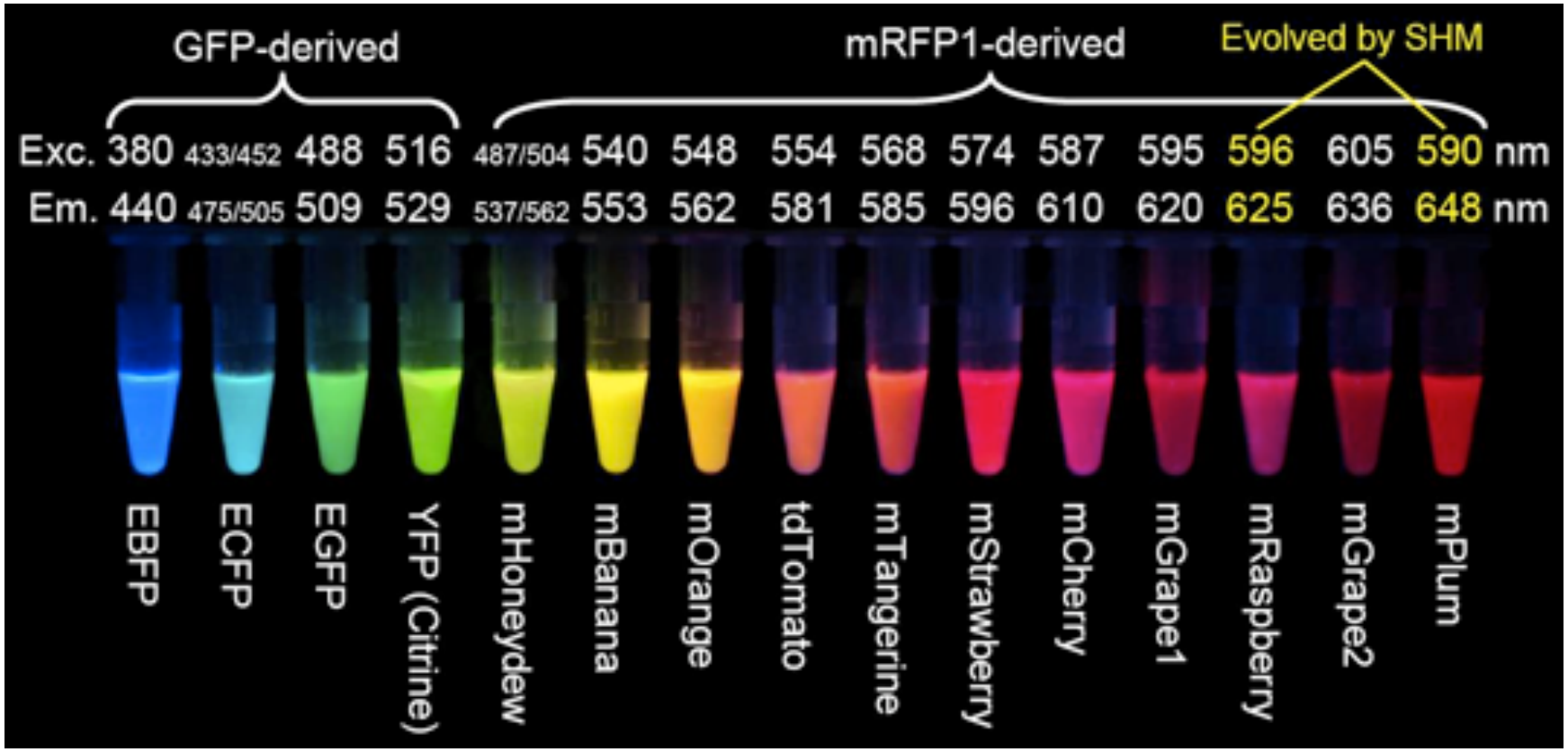


Figure 2

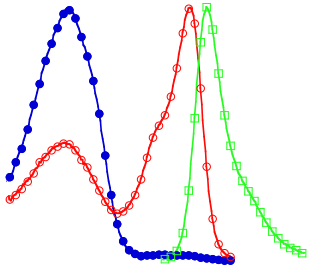
Fluorescent proteins



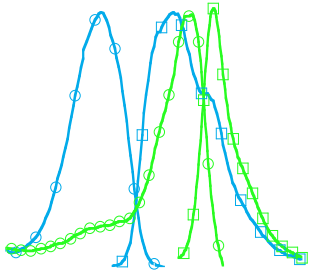
Photoactivatable Fluorescent Proteins

Irreversible

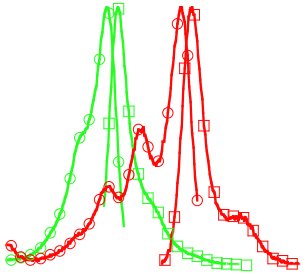
PAGFP



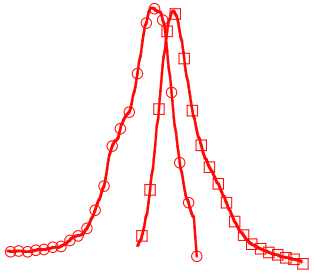
PS-CFP



Kaede
KikGR
EosFP
Dendra

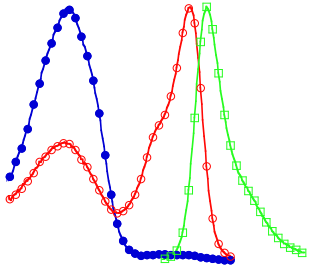


PAmRFP

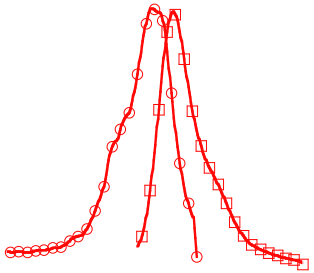


Reversible

Dronpa
rsFastlime
Padron

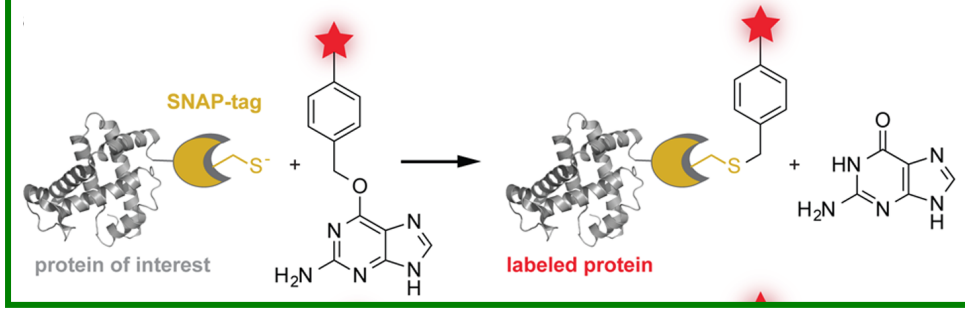


KFP1
rsCherry
rsCherryRev



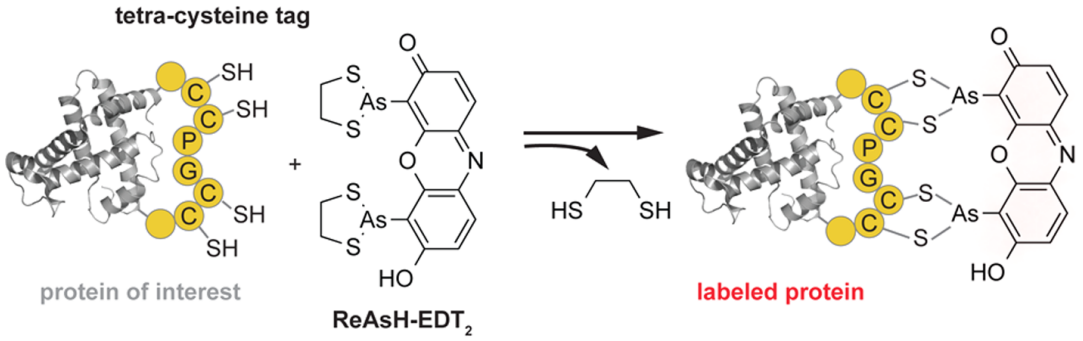
Labeling proteins in live cells with Fl-dyes

Self Labeling Enzymes



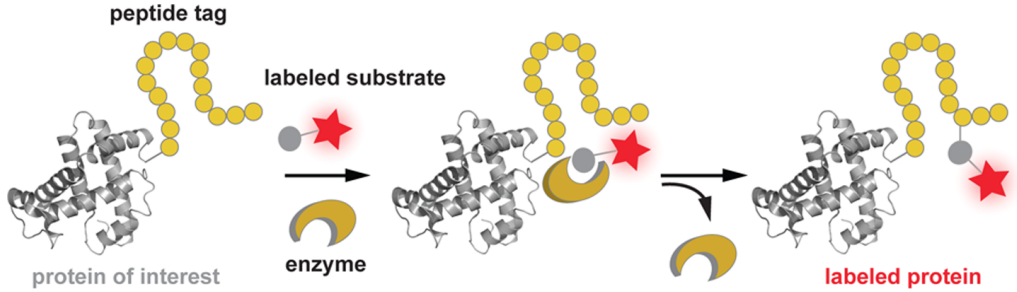
Disadvantages:
- tag is large: 20 - 34 kDa

Self Labeling tags



Disadvantages:
- cell toxicity
- non specific binding.

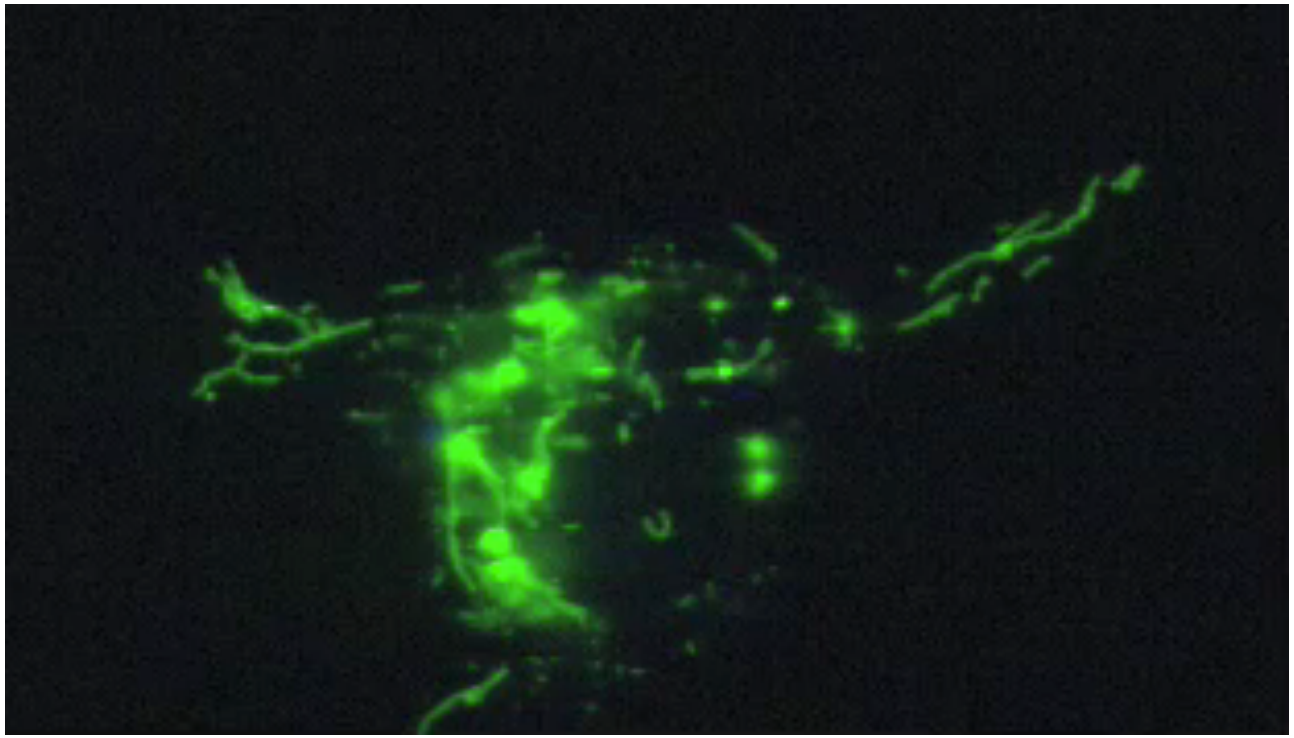
Enzyme Mediated Labeling



Disadvantages:
- cell permeability
- non specific enzymatic activity

Lang & Chin, 2014

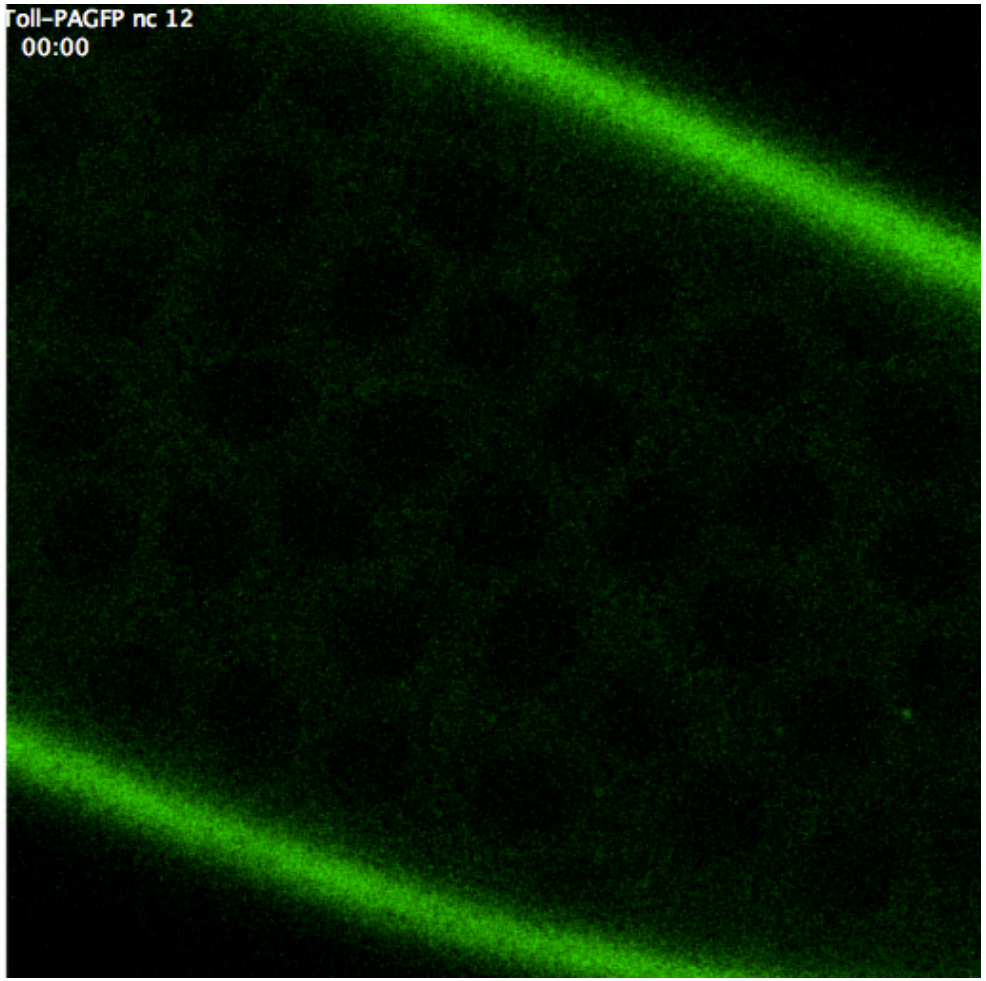
Photoconversion – getting 2 in 1



Using a targeted laser to *repetitively photoconvert mEos from green to red in the mitochondrial network.*

We can assess the degree of connectivity while tracking very dynamic cellular structures.

Photoactivation and photoconversion in Drosophila embryos



Comparison of available labeling techniques for live cell imaging

	Fl-proteins	techniques using Fl-dyes
Brightness	+	+++
stability	+	+++
Applicability to SR techniques	+	+++
Size of tag	+	+
Flexibility in choosing the tagging site	+	+
SNR	++	++
Over expression artifacts	++	++

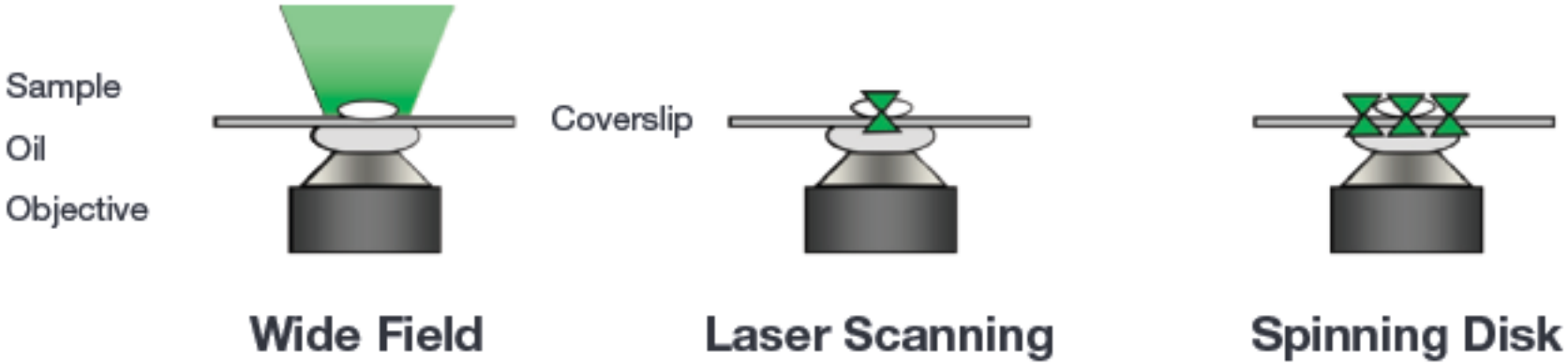
Live cell imaging - considerations

- Cells are sensitive to laser illumination in the blue-green range – laser exposure should be minimized. This means that imaging parameters should be compromised in order to maintain cells in physiological conditions.

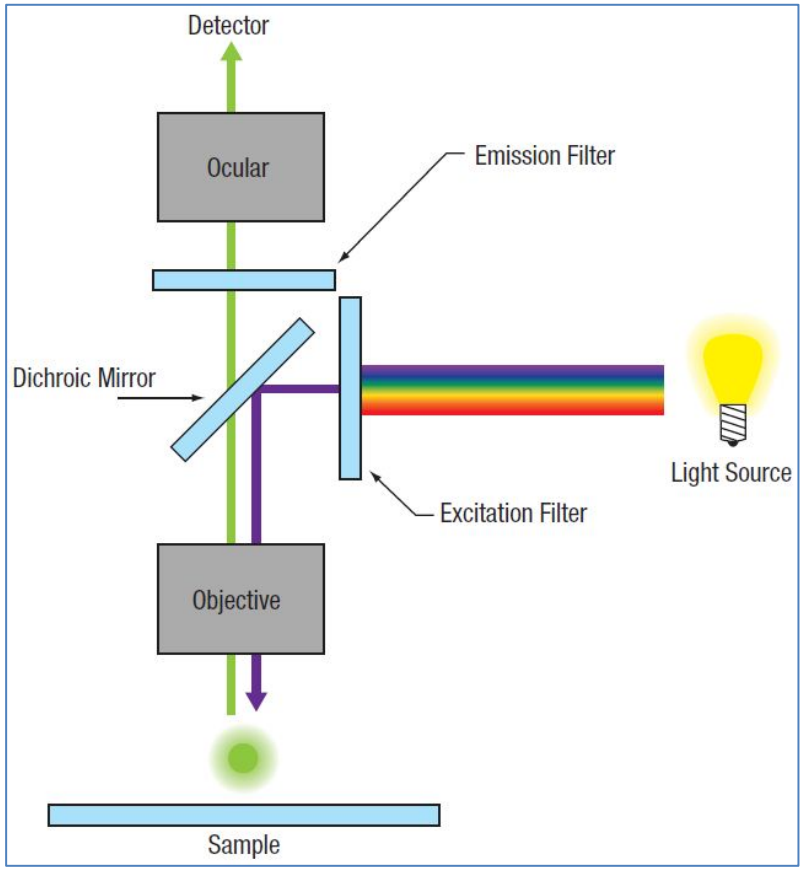
- Temporal resolution should be determined based on the process studied.

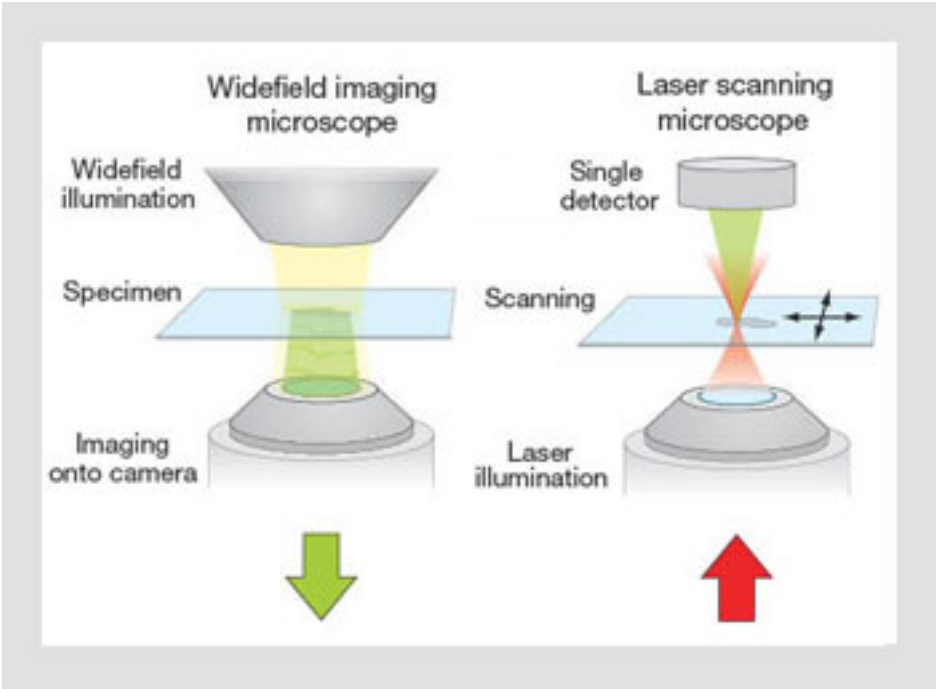
- Temp. and CO_2 levels - incubation system. Good for cells but introduce heat to the optical system.

Method comparison

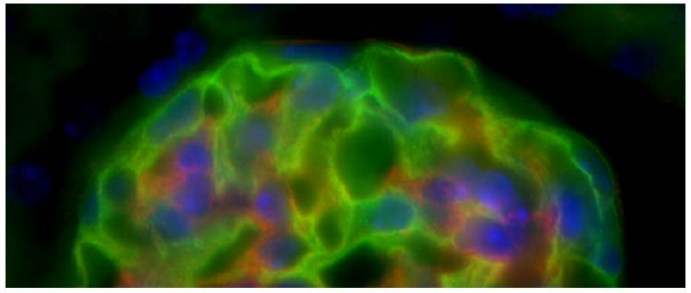


Widefield Microscope

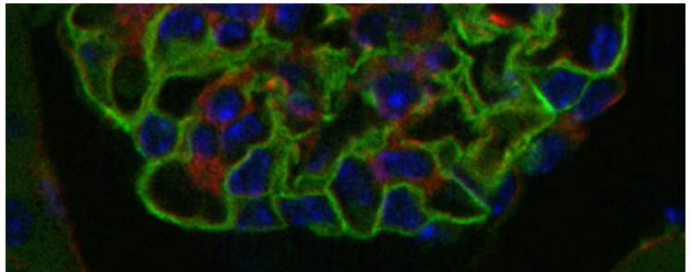




Widefield

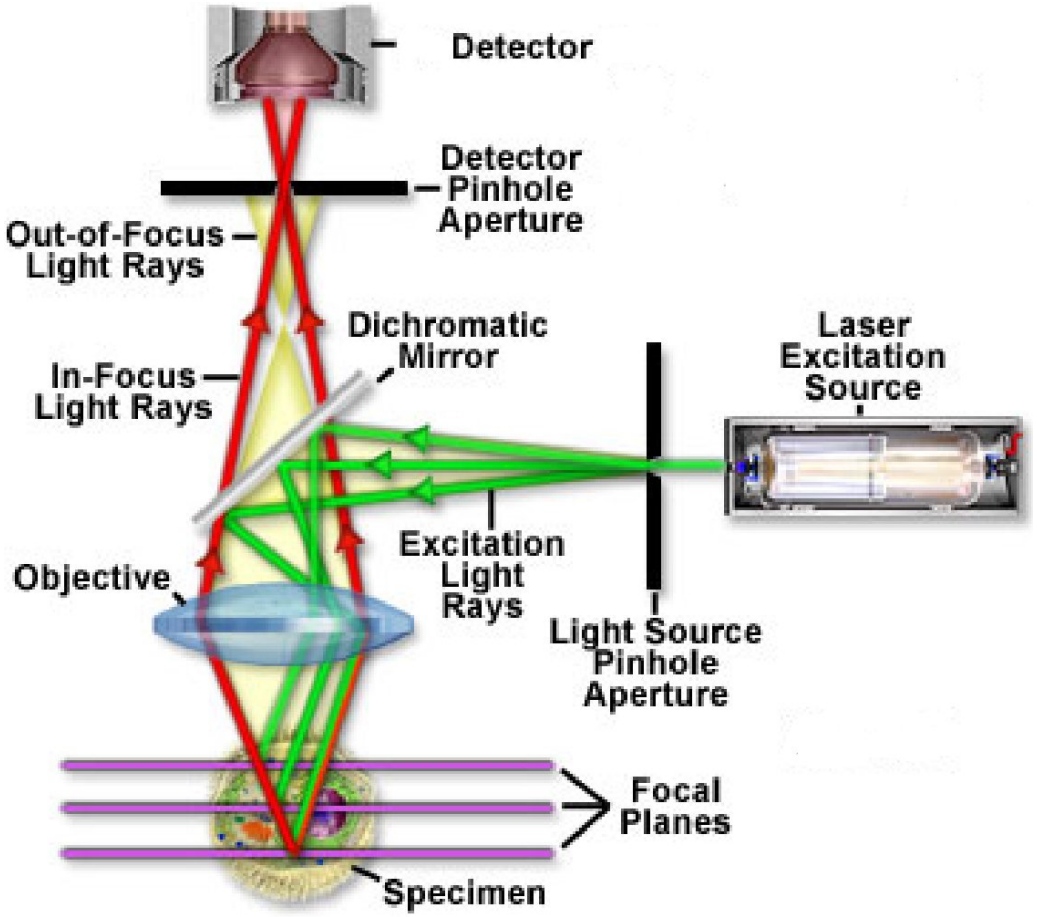


Confocal



Delivering light energy to a region of interest in a cell

Targeted laser illumination typically uses the same technology as scanning laser confocal microscopy.



Spinning disk

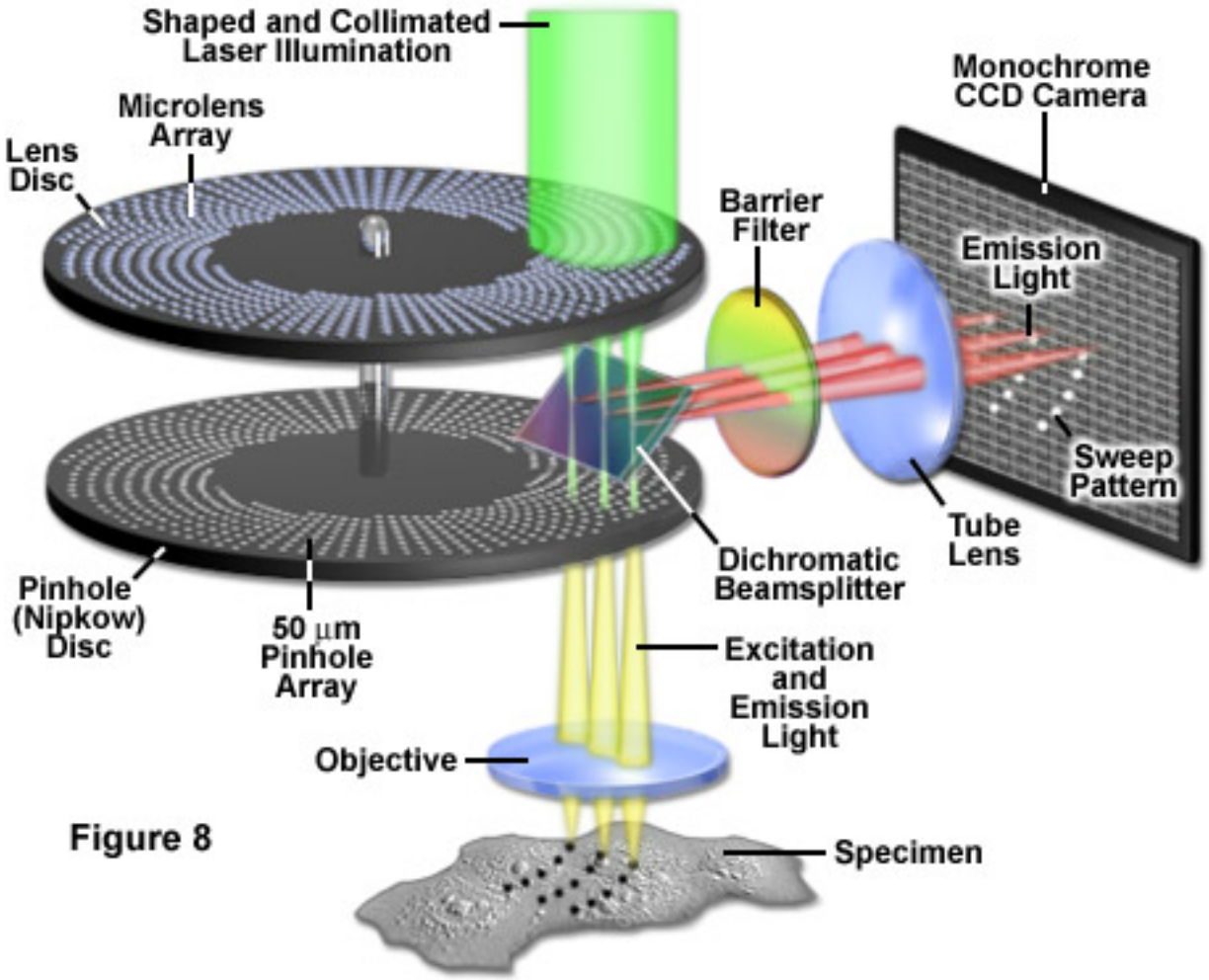
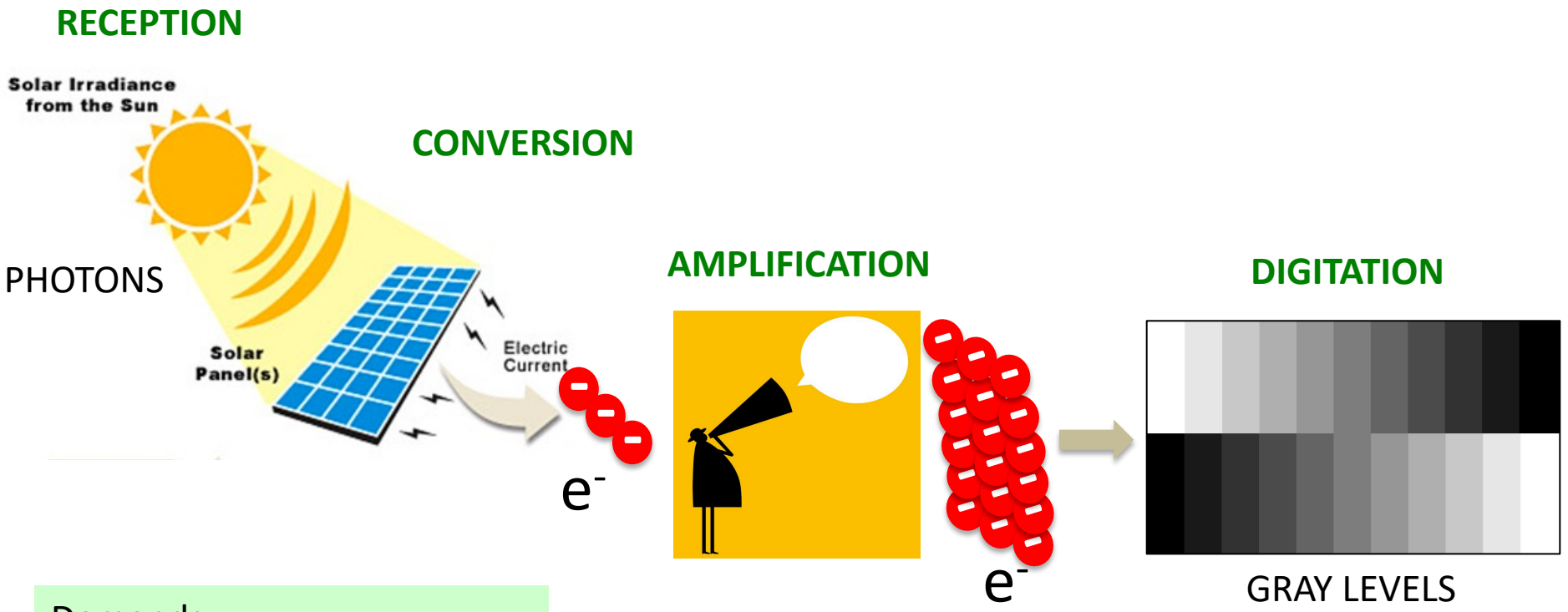


Figure 8

Detectors

Detect photons -> convert to electrons -> amplify signal -> Convert to digital signal



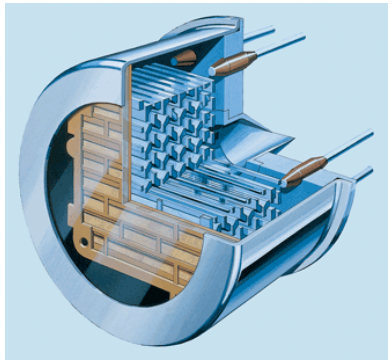
Demands:

- Speed
- Wide range of wavelength
- Good dynamic range
- High resolution
- Low noise (high SNR)

Types of detectors

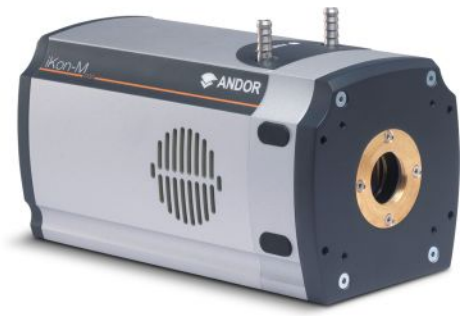
Spot Detectors:

- PMT
- APD



Area Detectors:

- CCD
- EMCCD
- sCMOS



Detection: Photomultiplier (PMT)

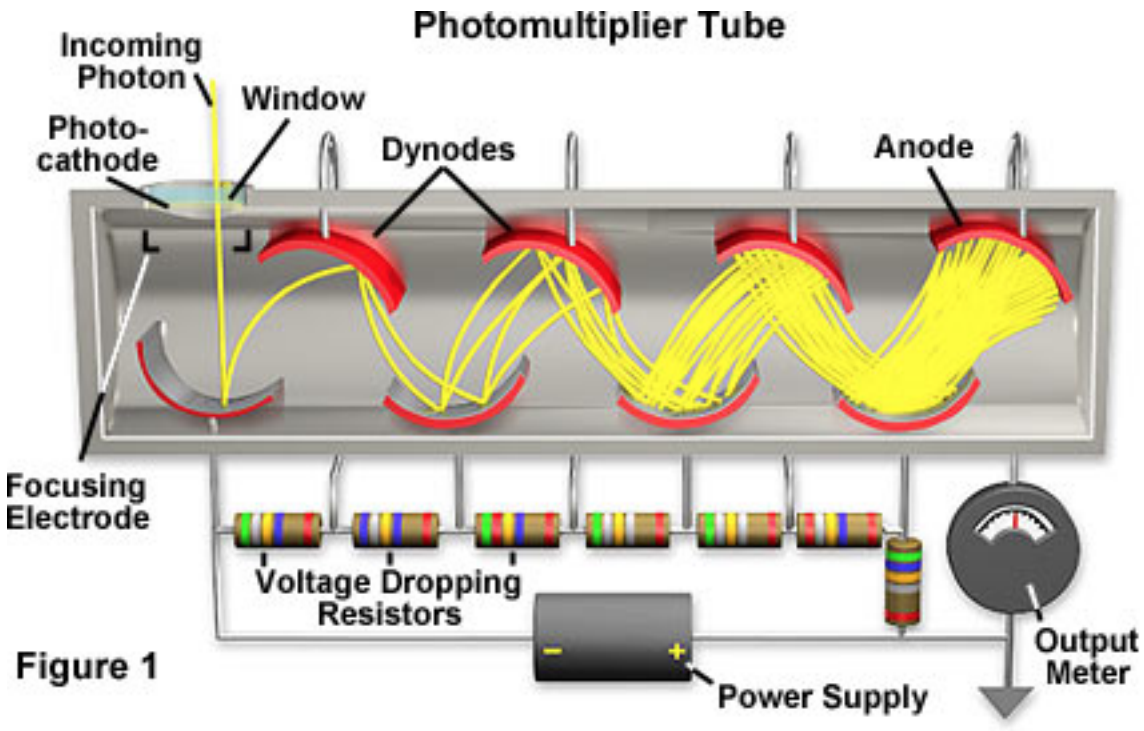


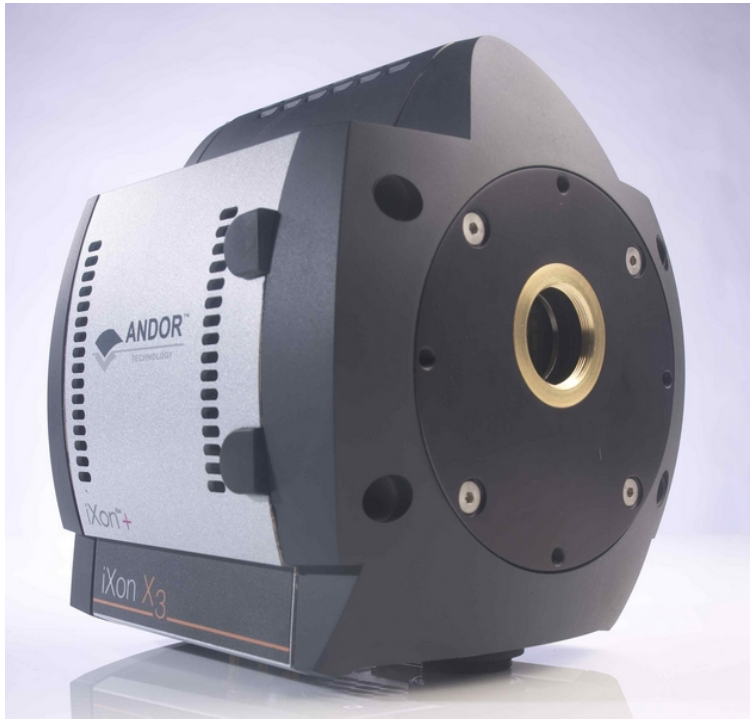
Figure 1

Laser scanning confocal

The Detector – EMCCD camera

* The whole field of view is imaged at the same time

- Camera is monochromatic – black and white – all colored images are artificial
- Pixel size is different in different cameras.



How does it work?

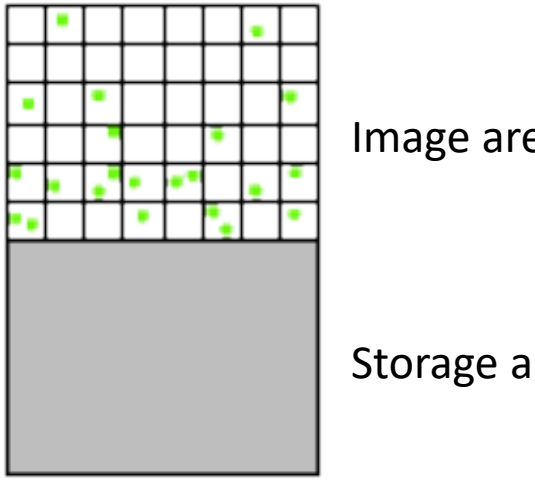
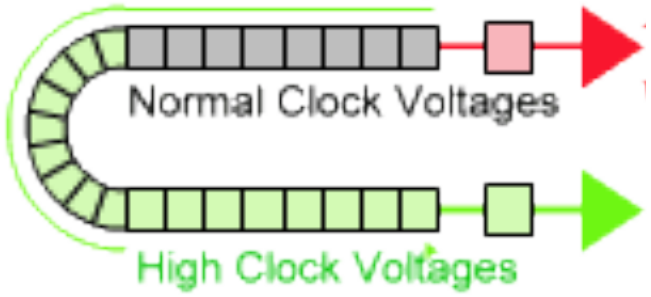


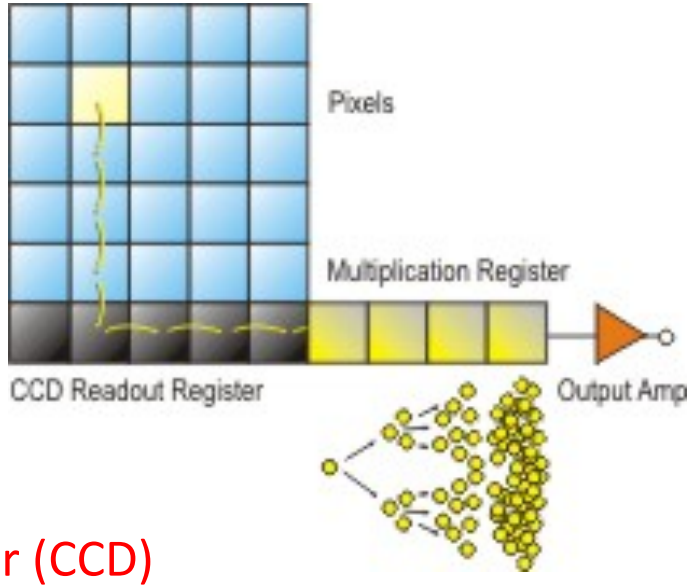
Image area

Storage area

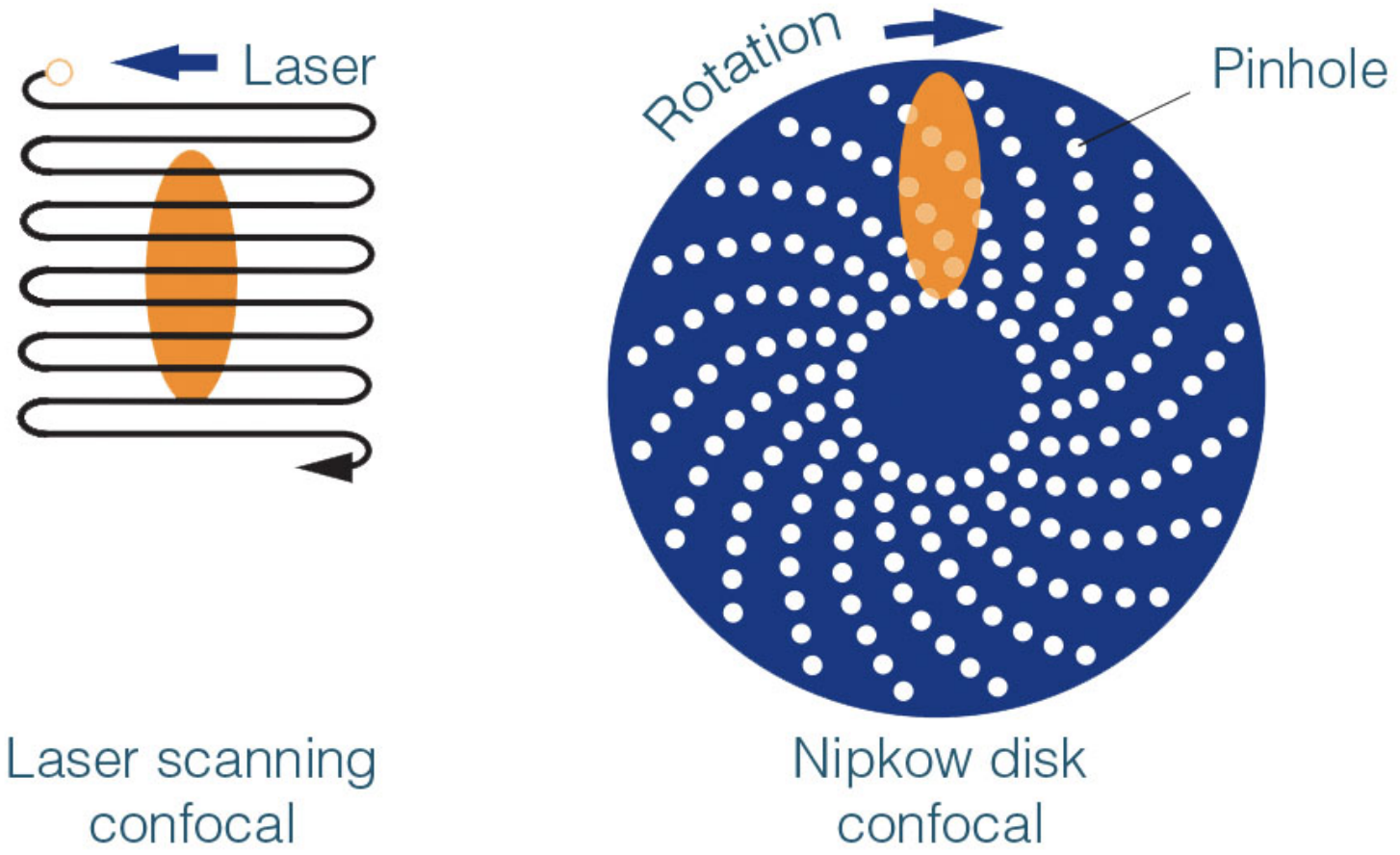


Traditional Amplifier (CCD)
Wide dynamic range

EMCCD Amplifier
High sensitivity



Detection confocal / Spinning Disk



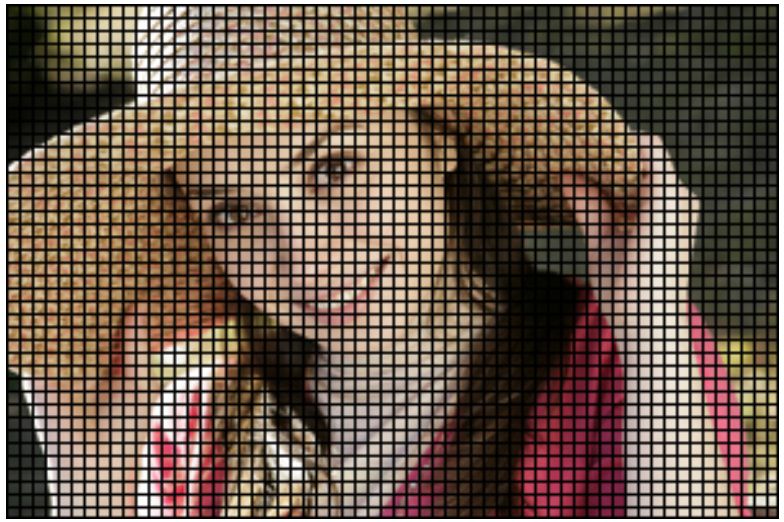
Spinning Disk: Faster acquisition + faster detection – Ideal for live cell imaging

Area detectors - cameras

Pixel - the smallest component, sensor unit

Large Pixel:
Lower resolution
Higher sensitivity

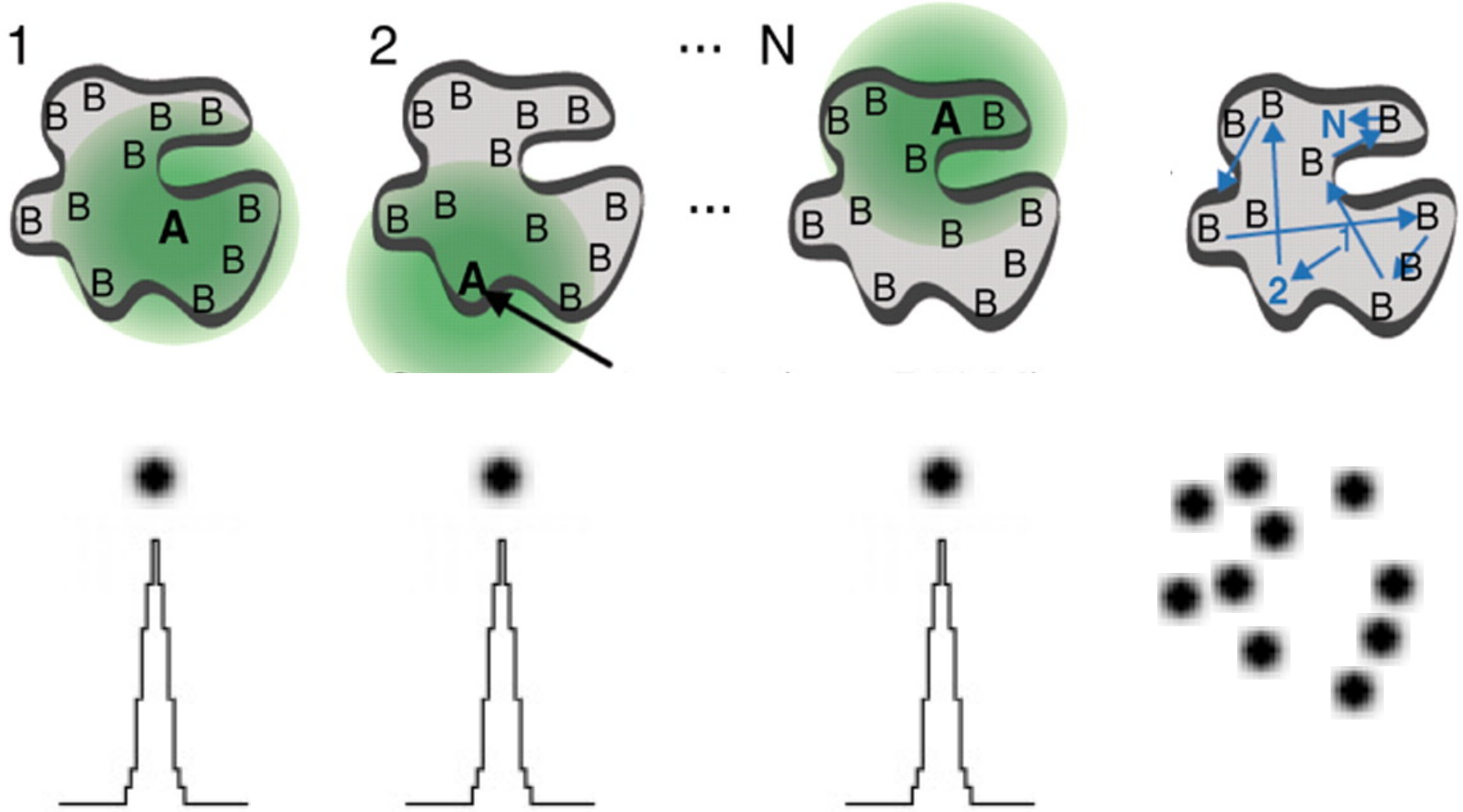
Small Pixel:
Higher resolution
Lower sensitivity



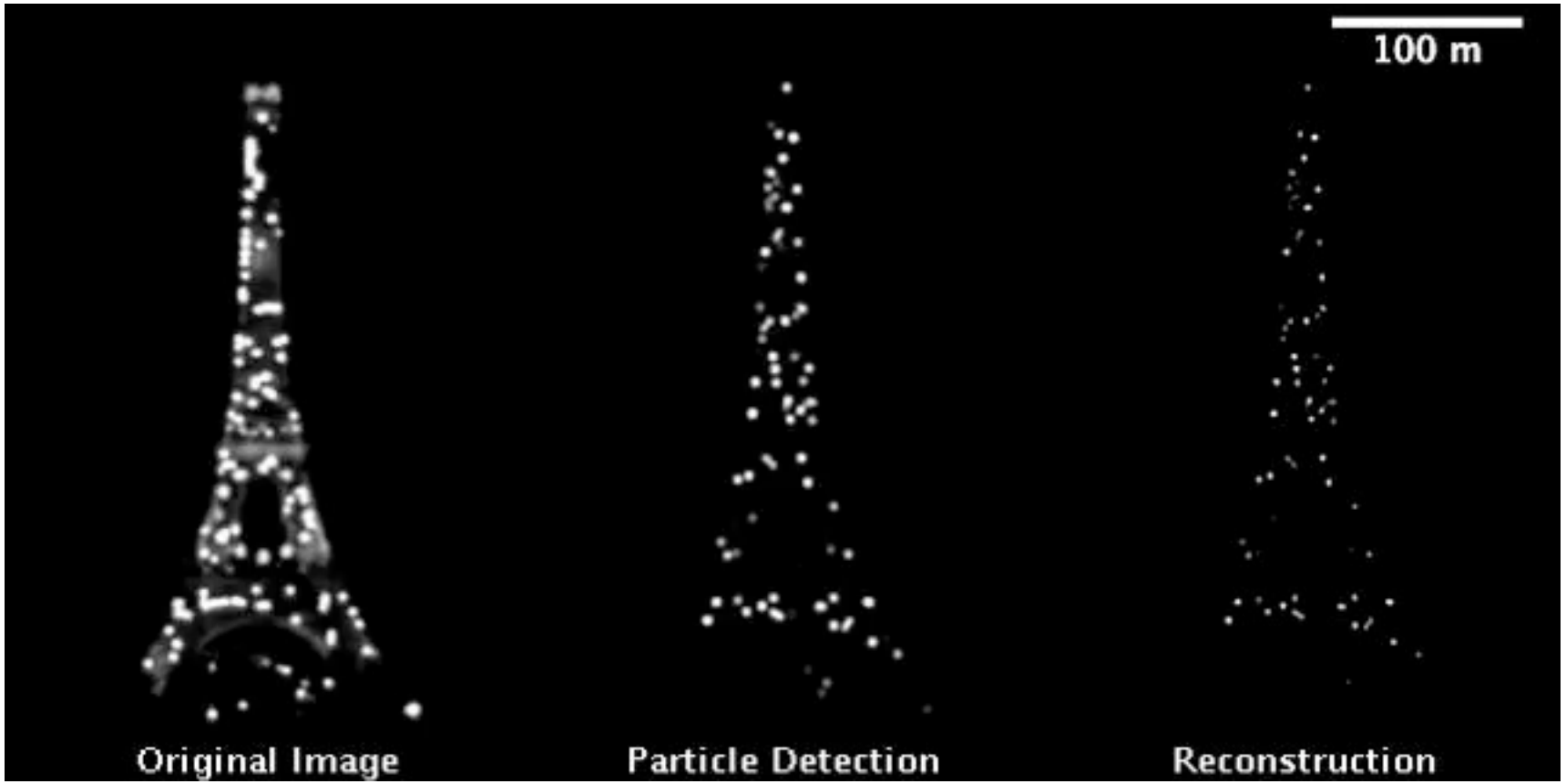
Detector comparison

	PMT	APD	CCD	EM-CCD	sCMOS
Quantum Efficiency	40%	80%	95%	95%	70%
Speed	Slow (pixel by pixel)	Slow (pixel by pixel)	Fast area scan (56 fps)	Fast area scan (26 fps)	Fastest (100fps)
Pixel size			Small (µm)	Normally Larger than CCD	Smallest
noise	low			Added noise factors. Low readout noise <1e	Low 1.6e
Sensitivity	High	High	Lower than EMCCD	High	High but small pixels
Saturation		Easy to saturate	Depends on pixel size	Depends on pixel size	Smaller pixel size lower FWC
Detector lifetime		Limited		Limited - detector aging	

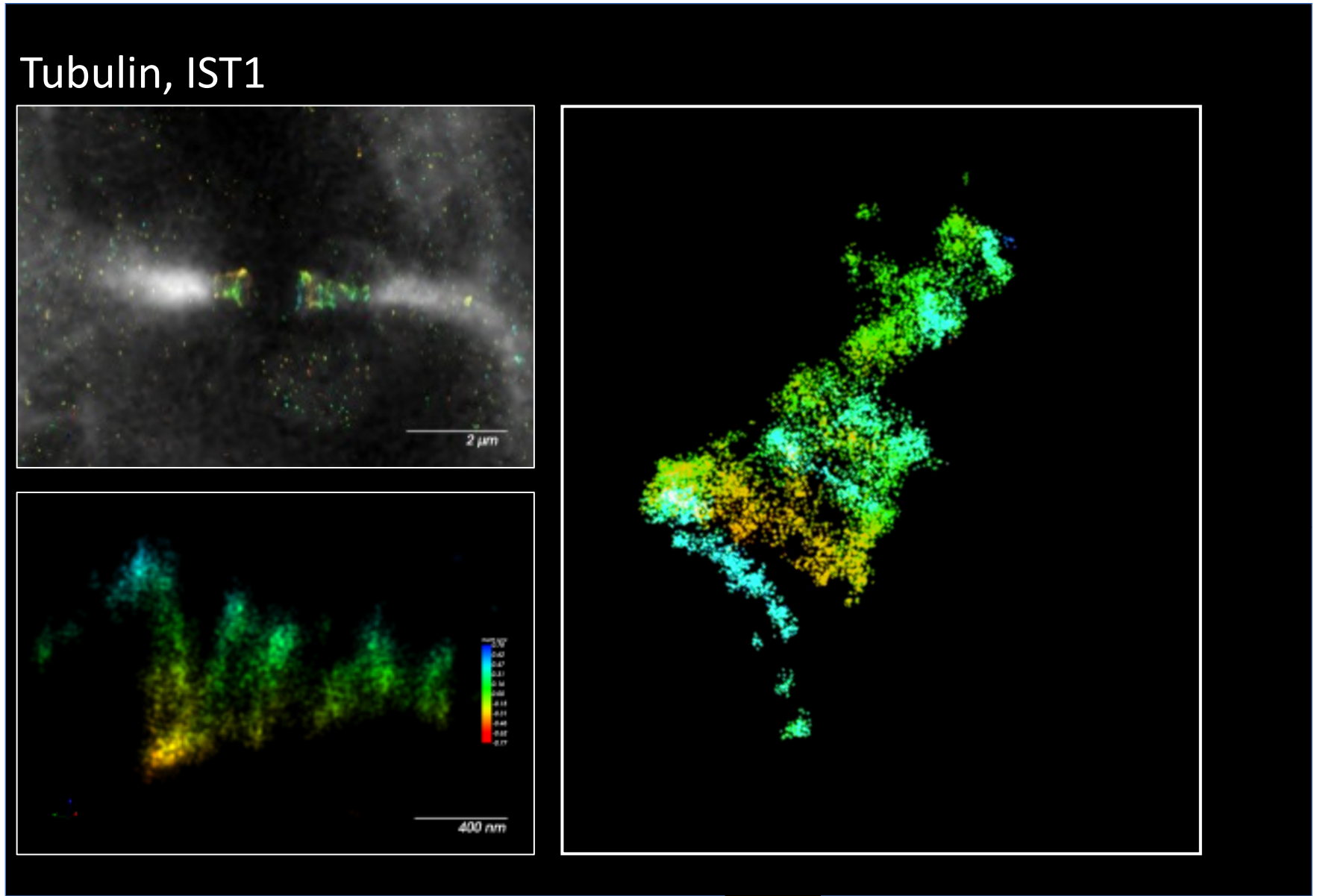
Super resolution microscopy - Localization Microscopy

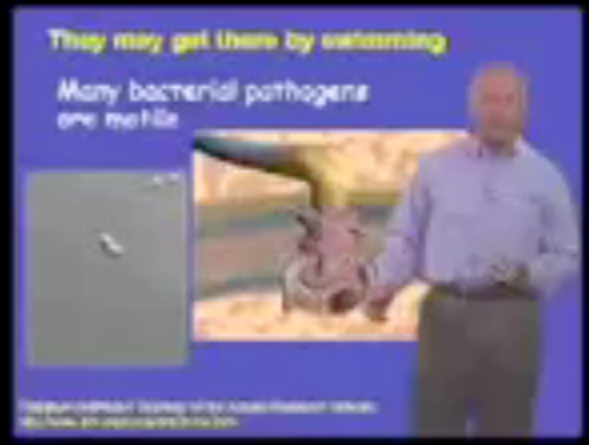


Super resolution microscopy - Localization Microscopy

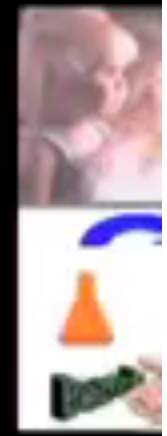


Visualizing ESCRTs in STORM (30 nm resolution)




They may get there by swimming
Many bacterial pathogens
are motile


THE CELL
THE CELL

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