Bioimaging Day
Introduction to modern microcopy

Joel Ryan

Leonhardt lab

June 2nd 2015

Summary

- Introduction to microscopy in biology
- Intro to “modern microscopy”
- Intro to resolution and the point spread function

Microscopy in biology

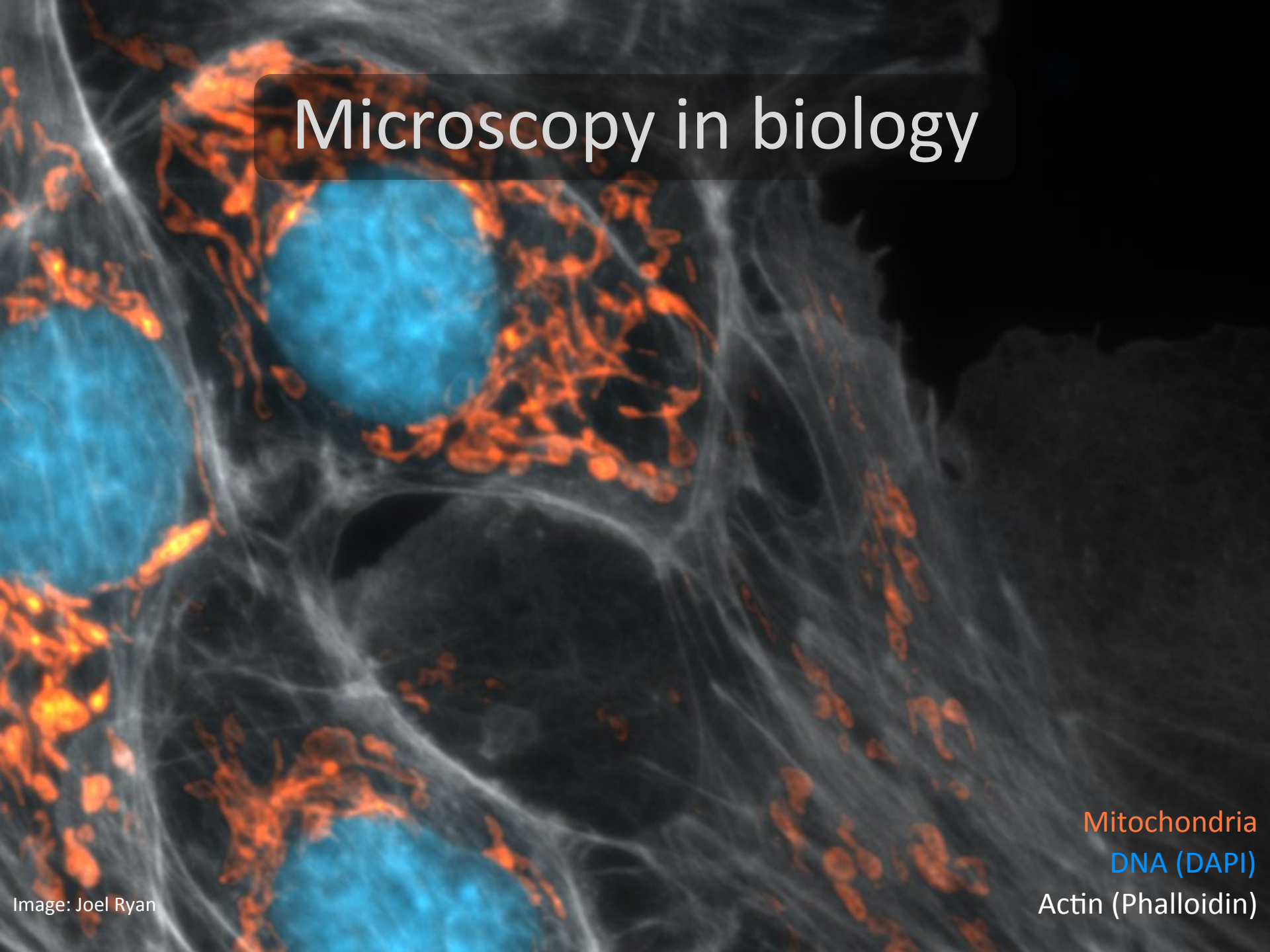


Image: Joel Ryan

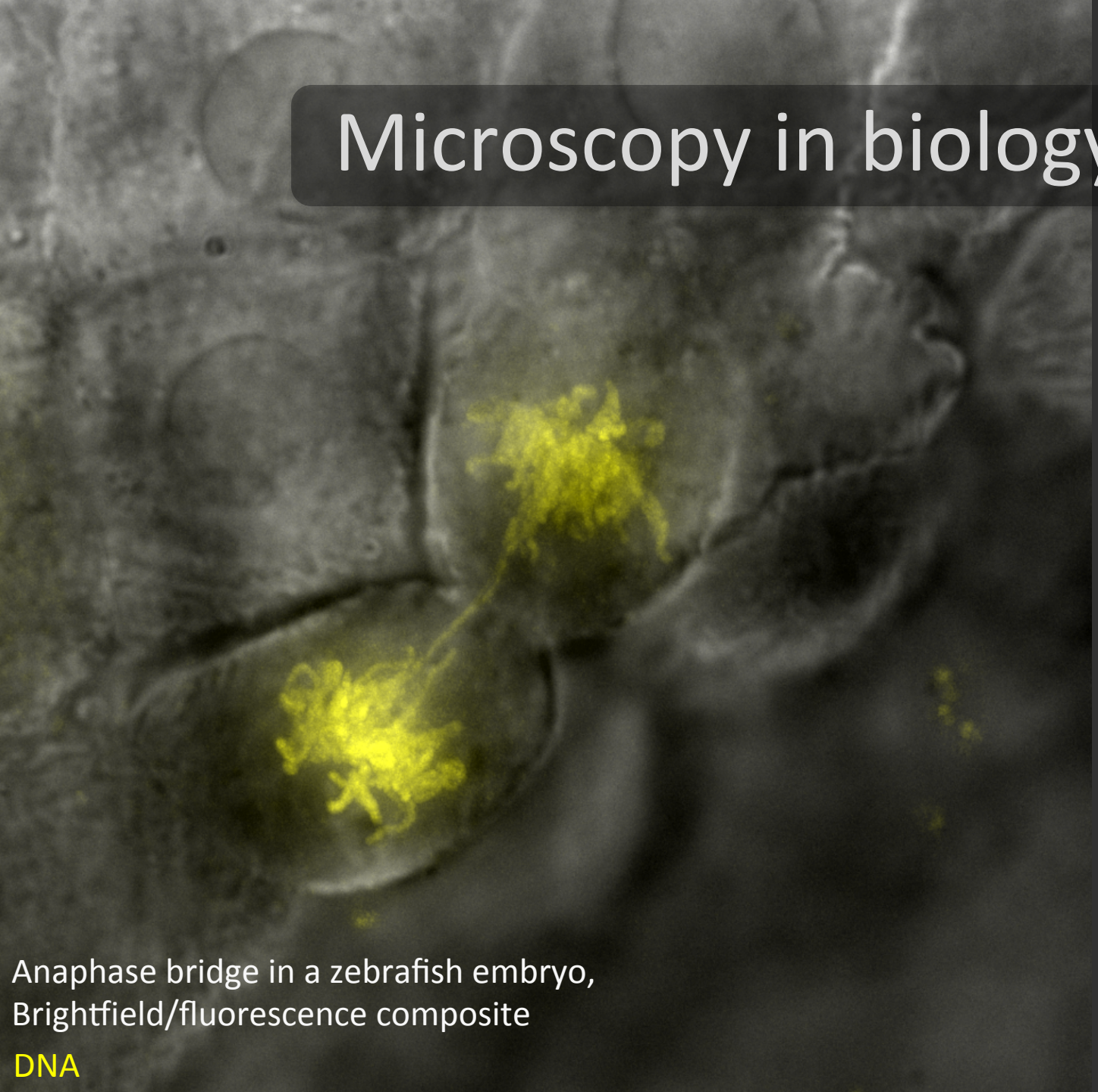
Microscopy in biology

C2C12 fused myoblasts

DIC - DAPI

Image: Joel Ryan

Microscopy in biology



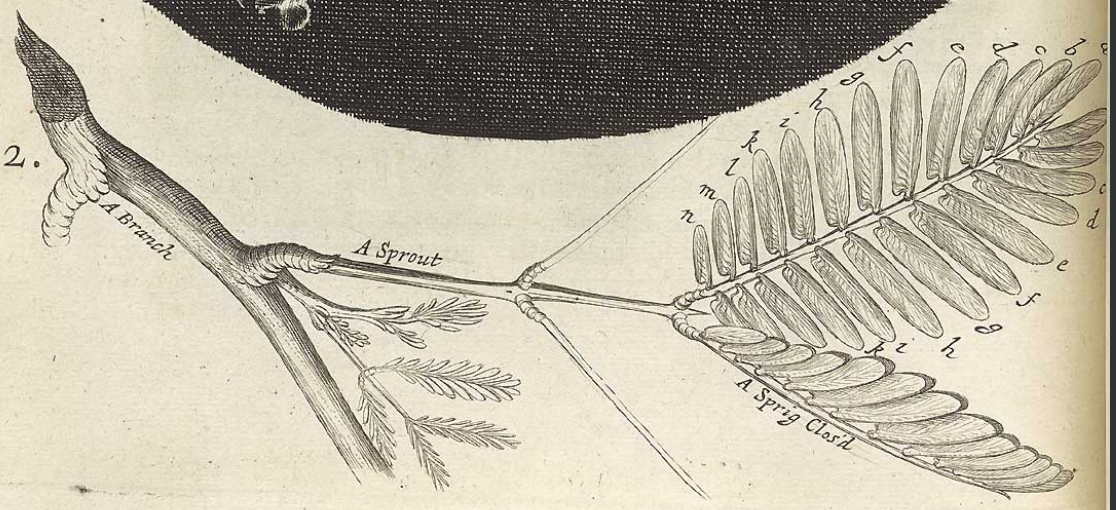
Anaphase bridge in a zebrafish embryo,
Brightfield/fluorescence composite

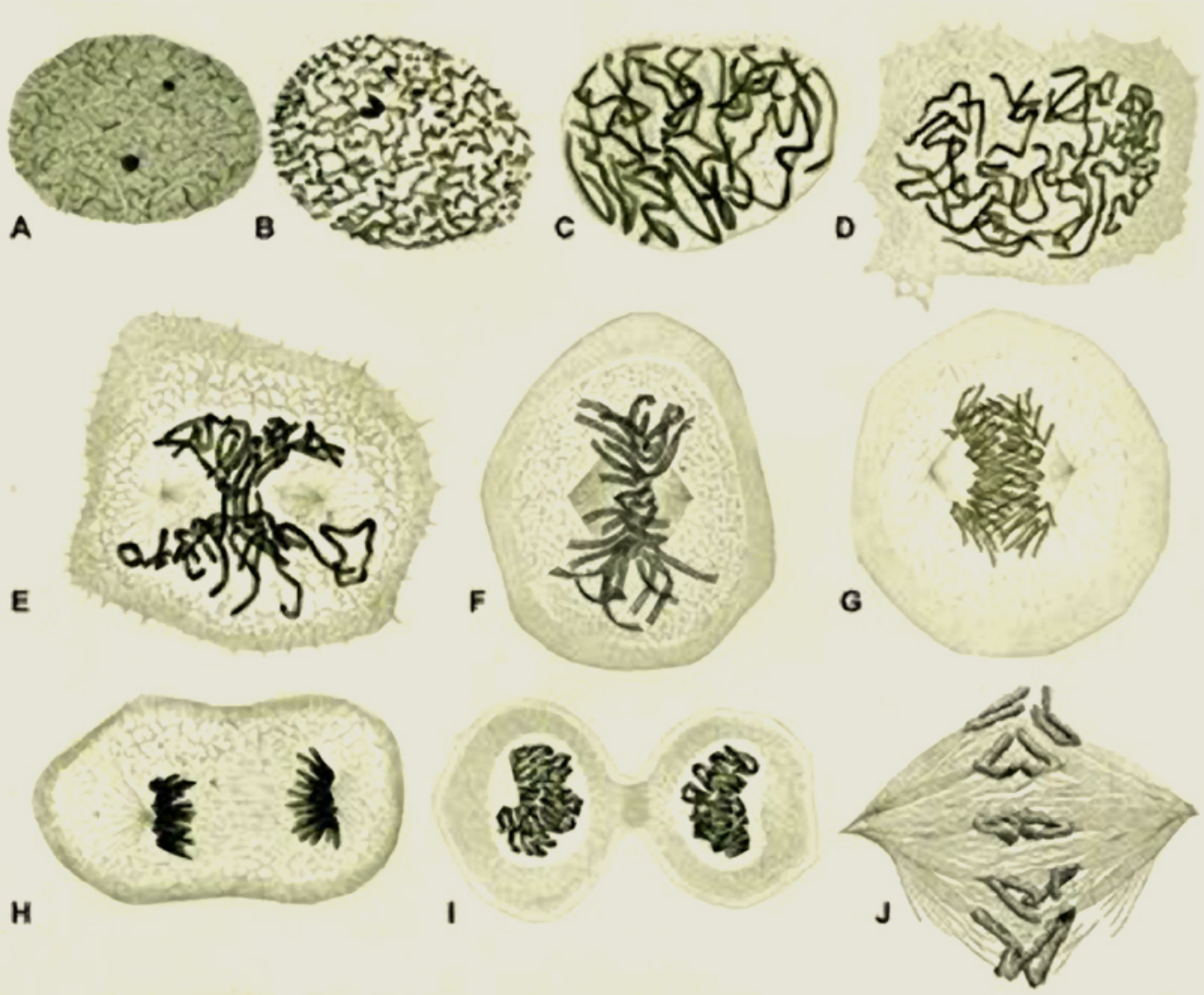
DNA

Image: Joel Ryan

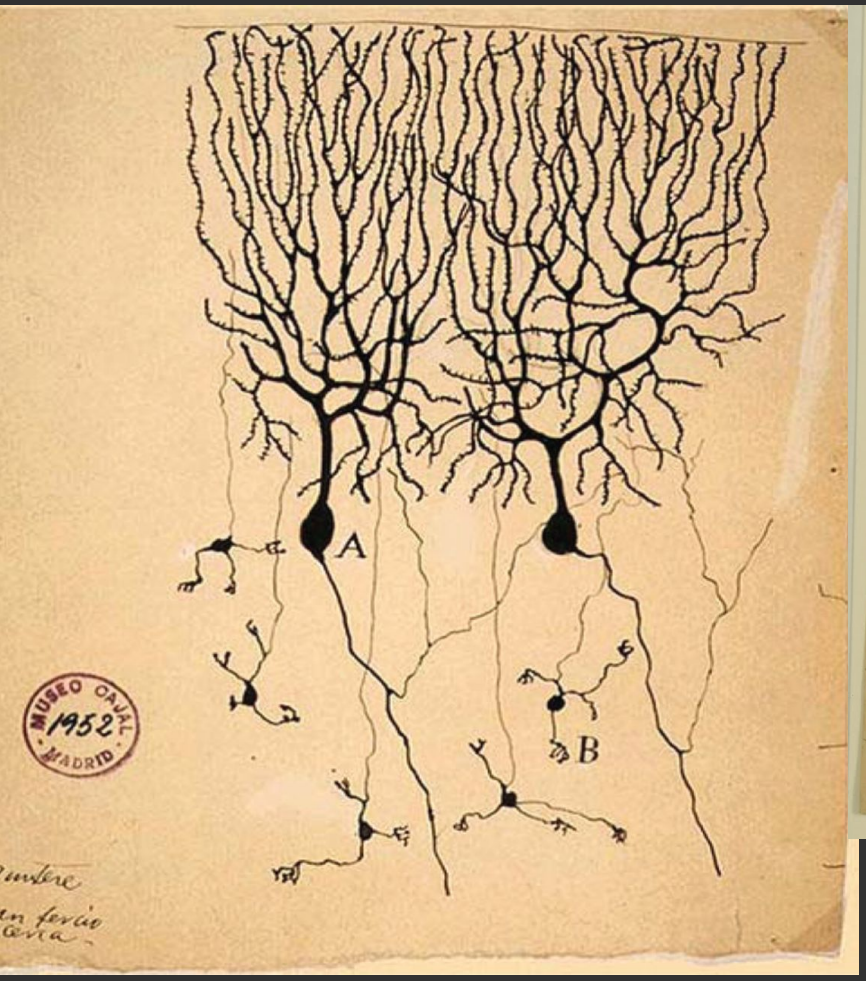
Fig: 1.

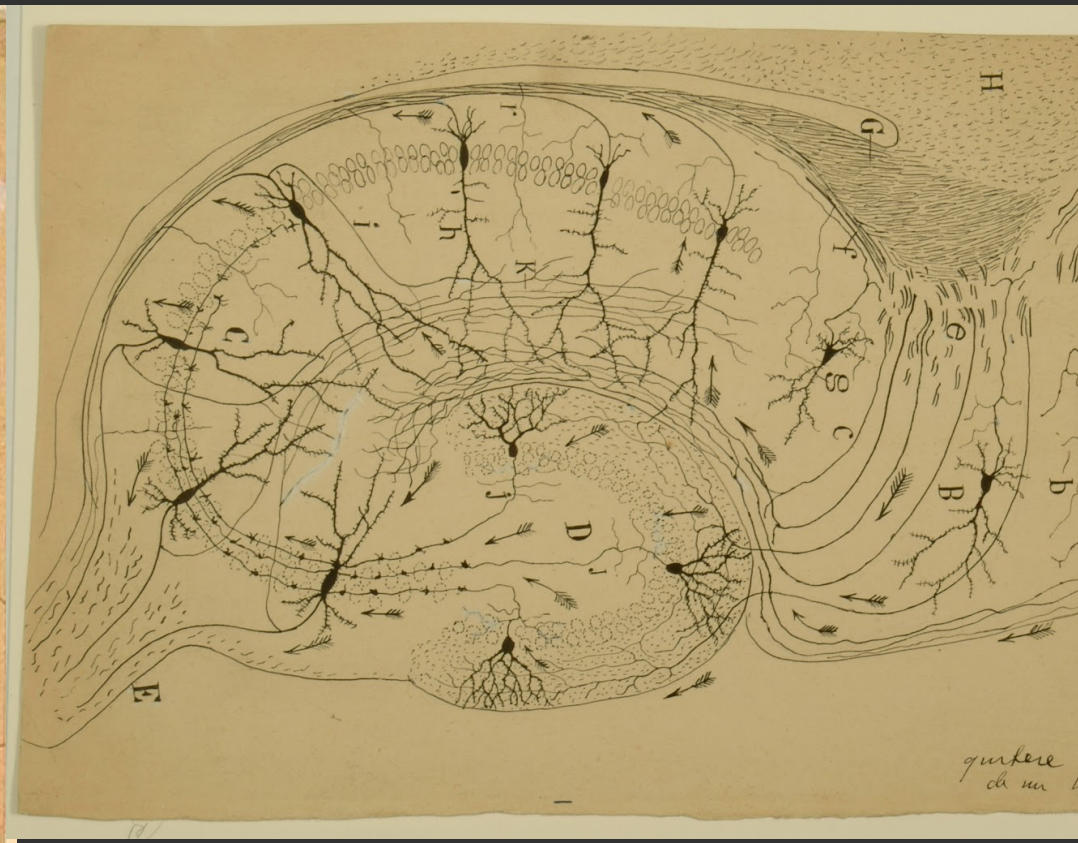
Fig: 2.





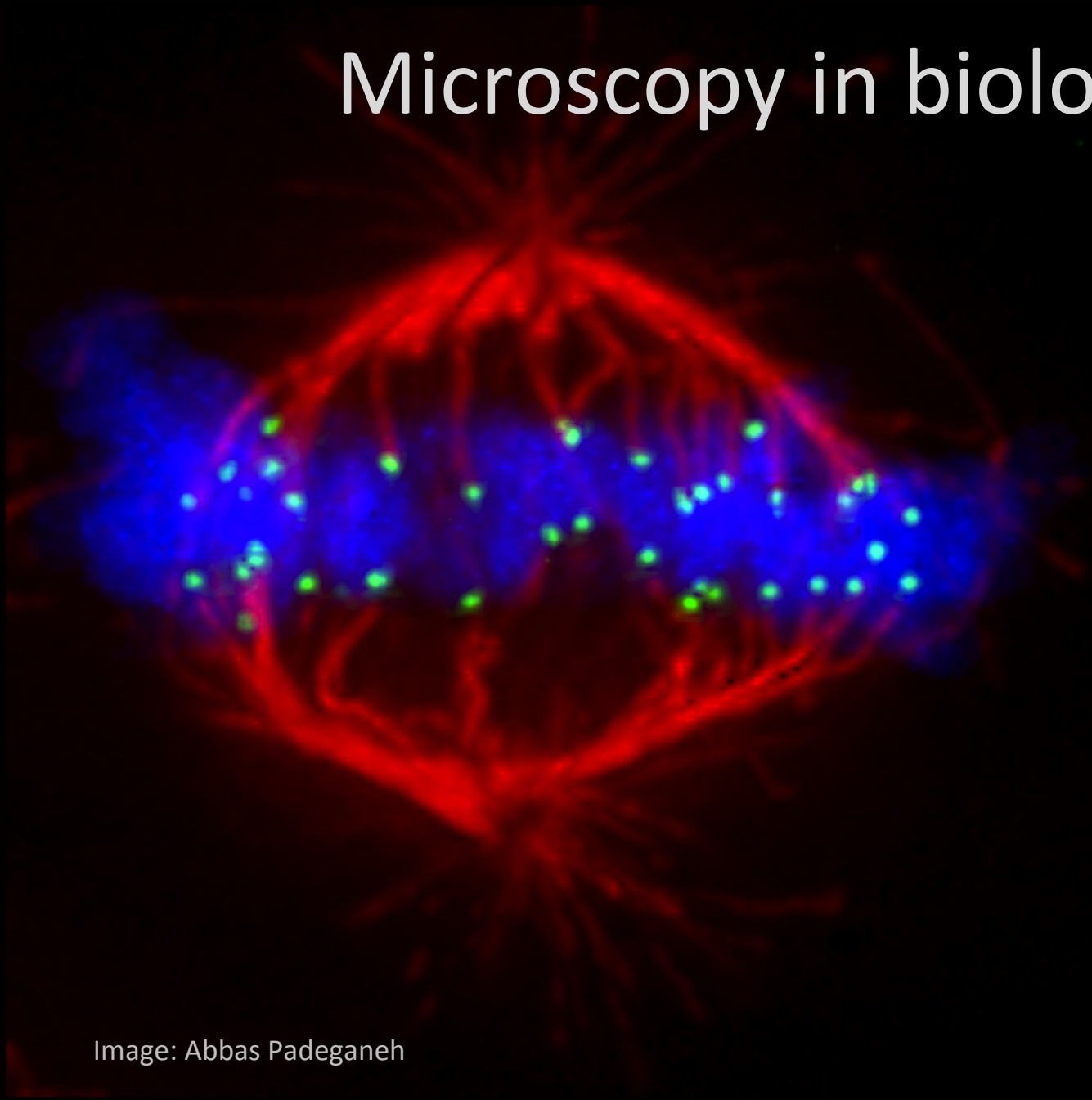
Walther Flemming, chromosome staining, 1878





Santiago Ramon y Cajal, neural structures, c. 1890

Microscopy in biology



Tubulin
DNA (DAPI)
Centromere Protein A

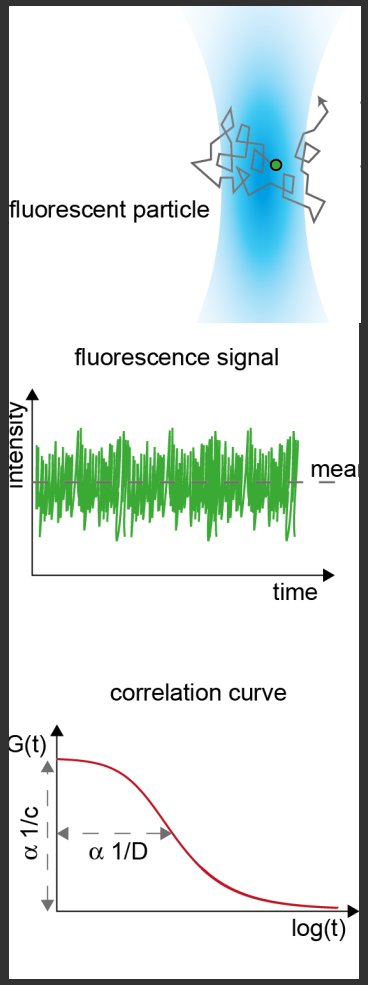
Image: Abbas Padeganeh

Microscopy in biology

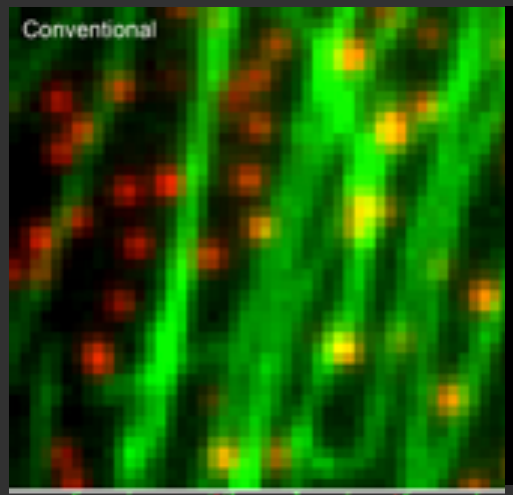
Image: Joel Ryan

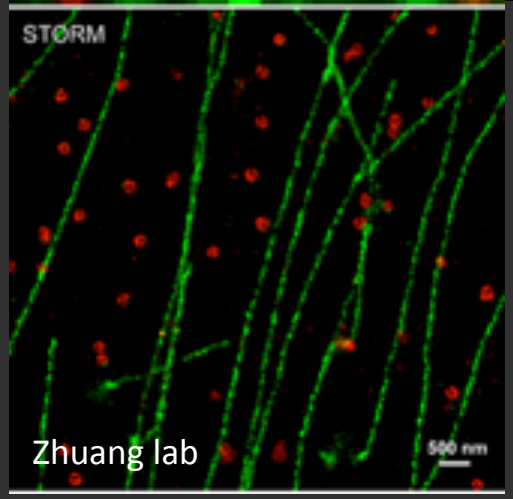
“Modern microscopes”

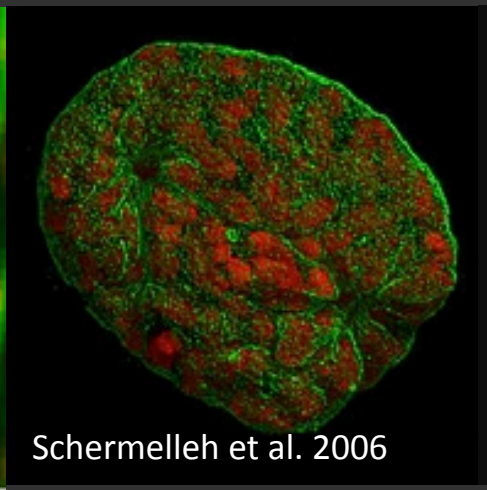
“Modern microscopy”

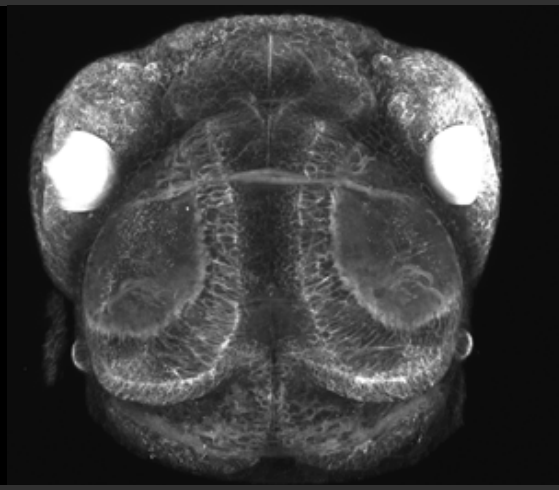


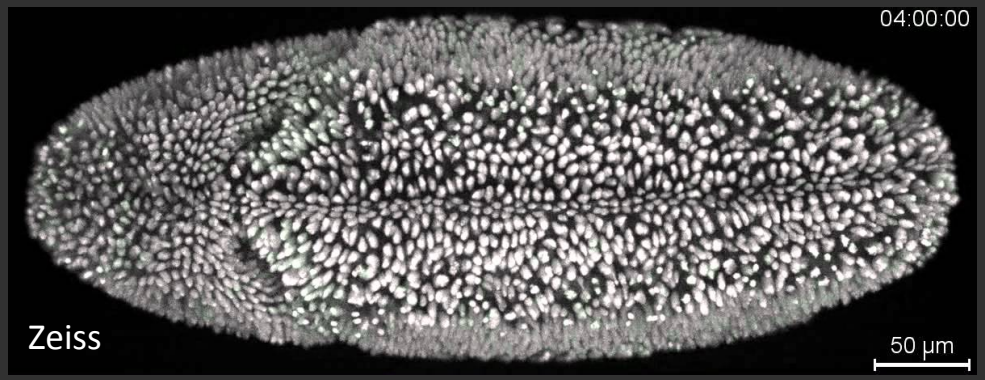
BioQuant – Uni Heidelberg





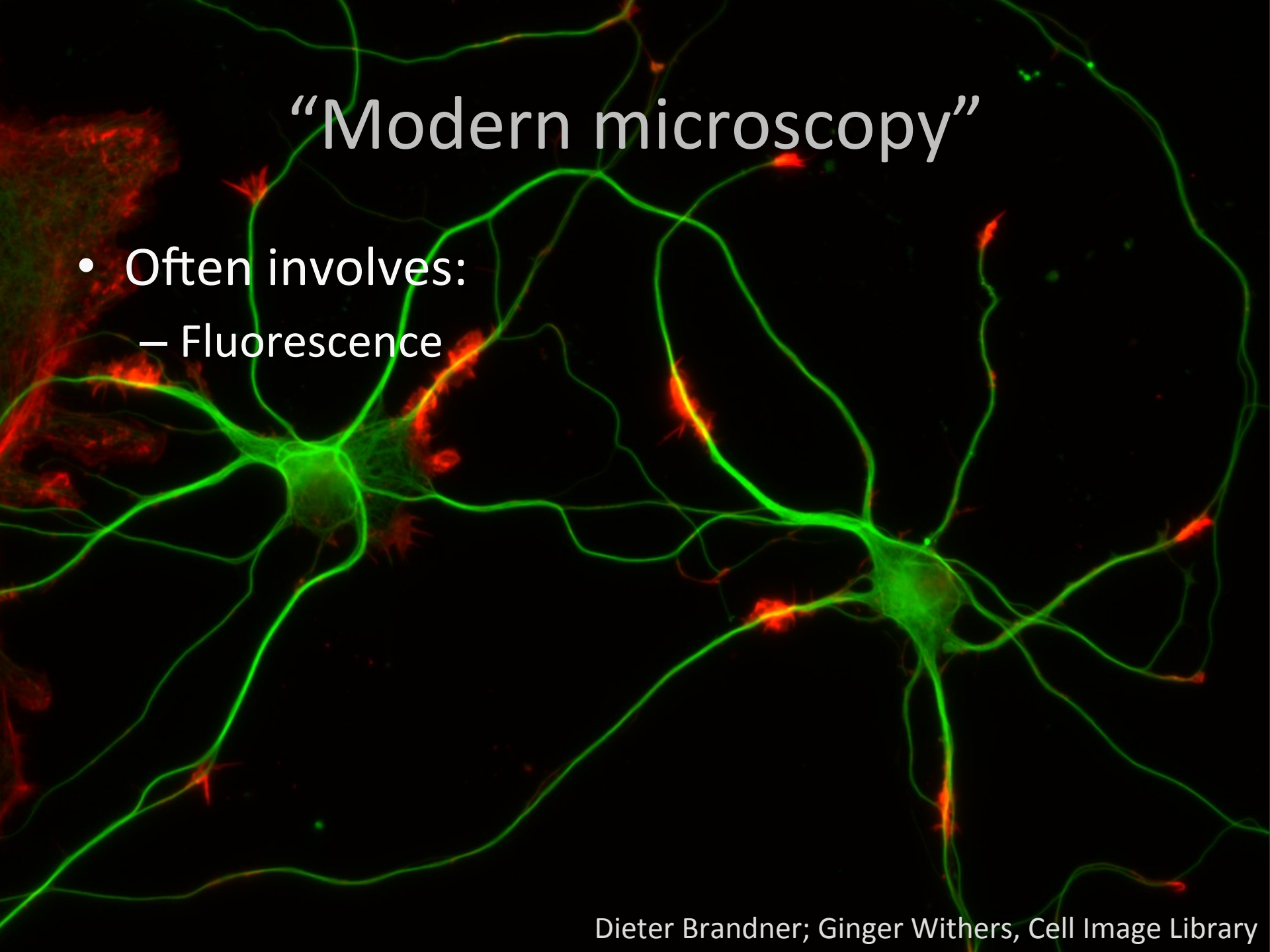

Schermelleh et al. 2006





“Modern microscopy”

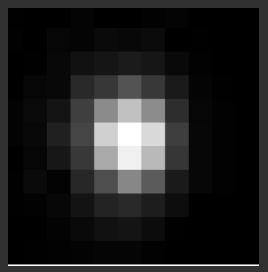
- Often involves:
  - Fluorescence



Dieter Brandner; Ginger Withers, Cell Image Library

“Modern microscopy”

- Digital acquisition
  - Using a digital detector to convert light signal into pixel intensities



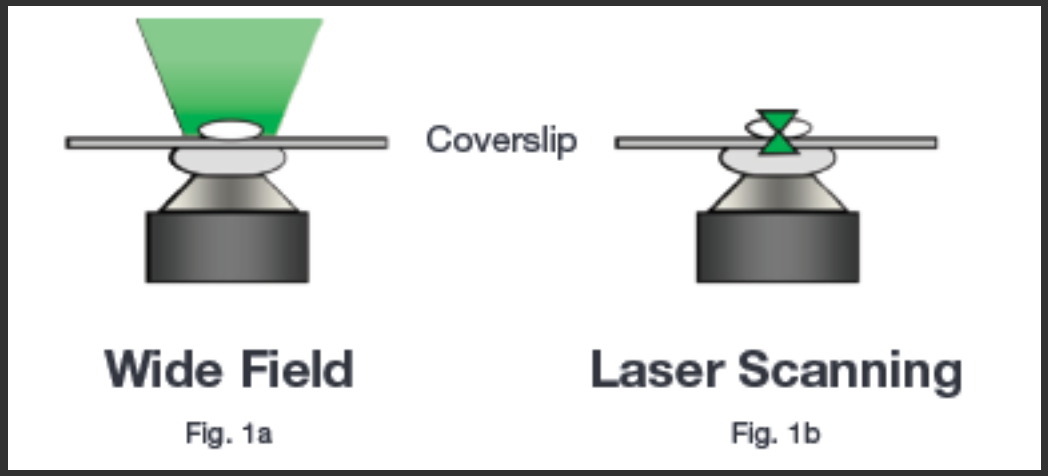
4106	4255	4198	4253	4395	4309	4277	4221	4188
4220	4280	4404	4487	4572	4586	4532	4446	4309
4262	4370	4549	4784	5076	5185	4879	4730	4404
4335	4480	4772	5404	6137	6635	6038	5066	4606
4347	4637	5067	6206	8438	10595	8548	5769	4599
4449	4612	5103	6472	10578	15650	11162	6041	4548
4376	4648	4985	6018	9530	13004	9980	5964	4581
4346	4456	4726	5370	6855	8213	6962	5261	4597
4230	4353	4627	4900	5444	5733	5288	4747	4438

“Modern microscopy”

Digital acquisition:

-Camera – a whole imaging plane at once

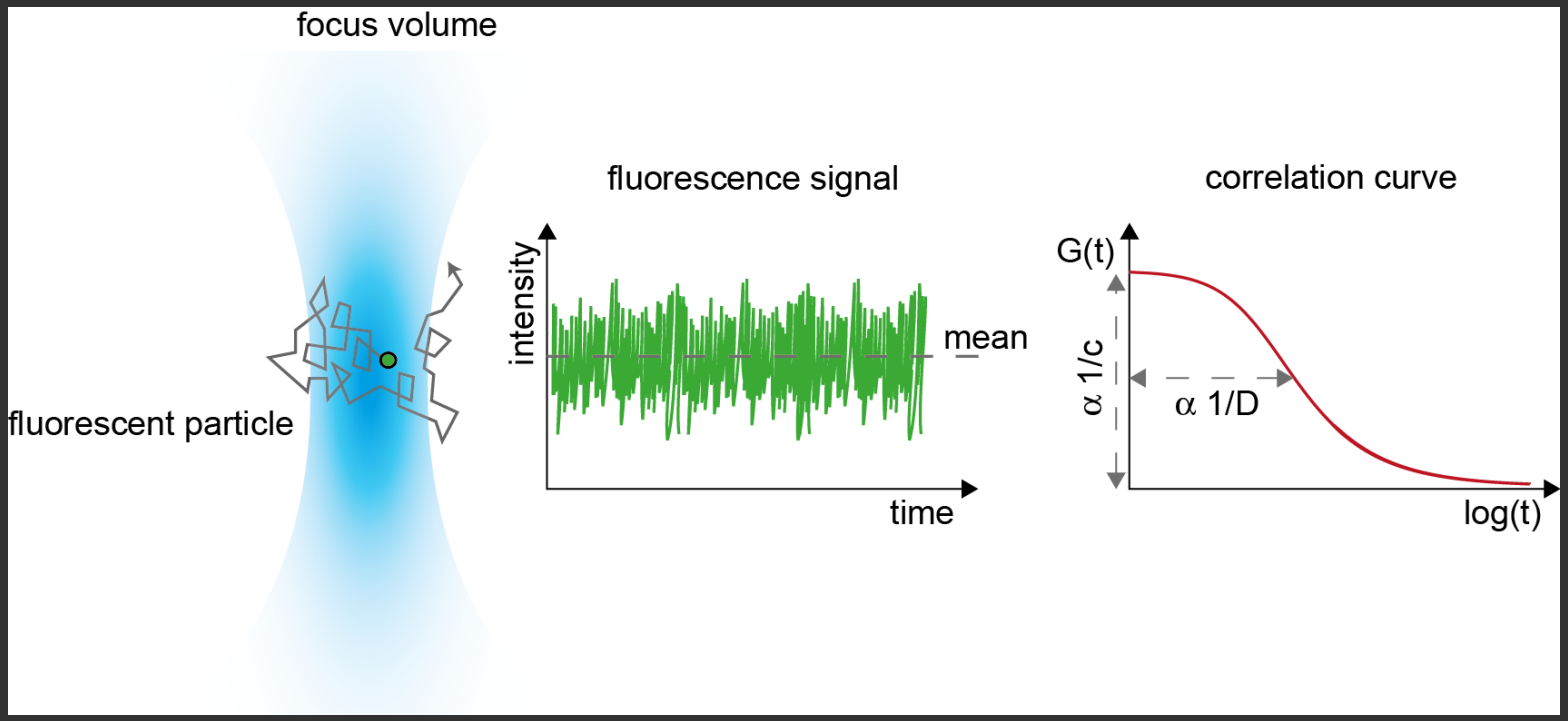
-Photomultiplier tube – one point at a time



Andor

“Modern microscopy”

- Often involves:
  - Computational analysis and/or reconstruction



“Modern microscopy”

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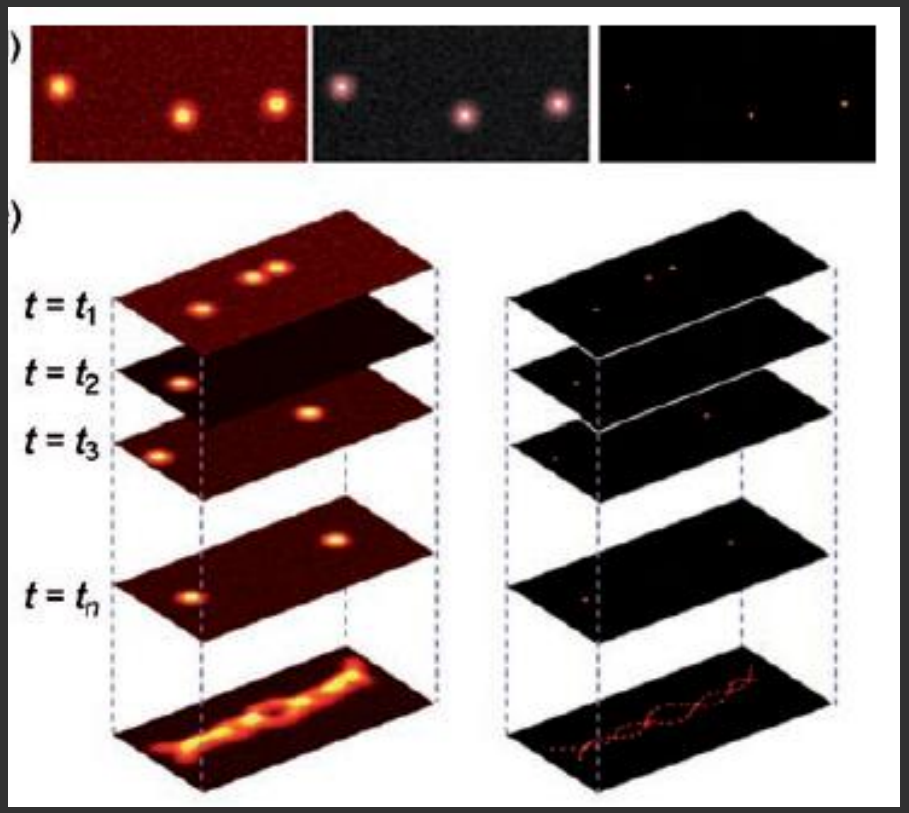


Bates et al. *Science*, 2007

“Modern microscopy”

Usually invovles:

- Fluorescence
- Digital acquisition
- Computational analysis or reconstruction

“Modern microscopy”

- Generates multidimensional dataset:
  - Pixel intensities in x y z t and c

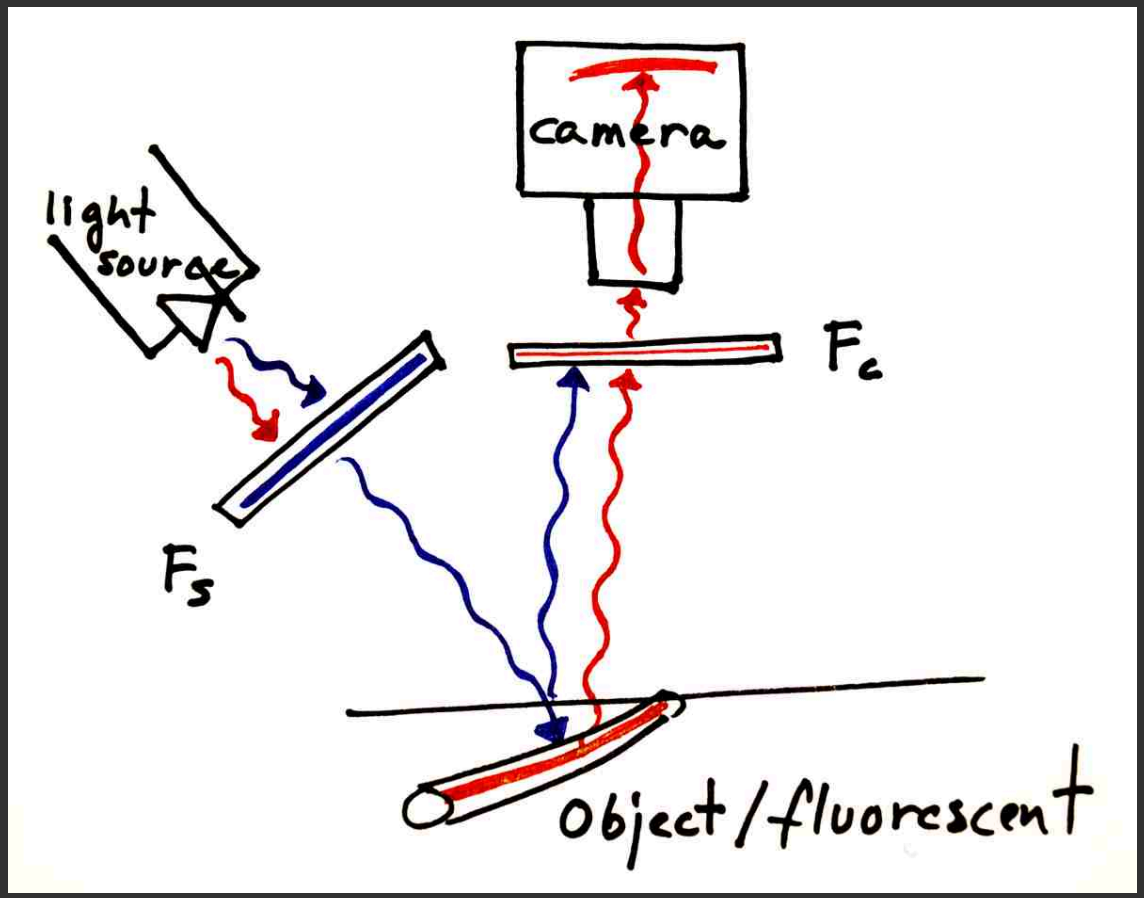
- Quantitative image analysis and reconstruction

Today!

- All techniques discussed today involve fluorescence…

Fluorescence

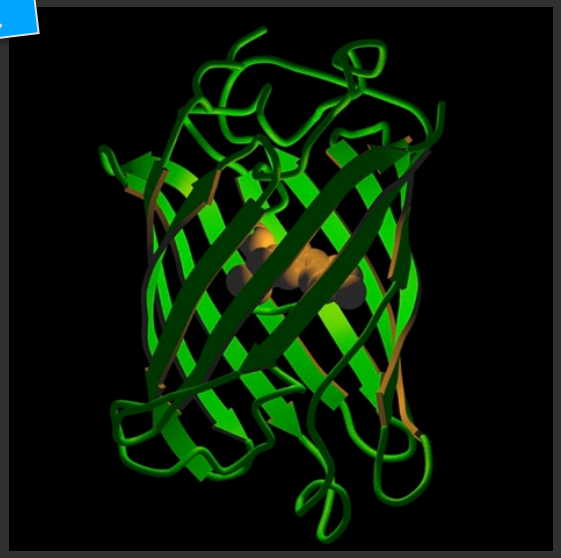
- Physical property of some materials



Fluorophores

- Small fluorescent molecules, label proteins or structures of interest

Excitation – 488 nm

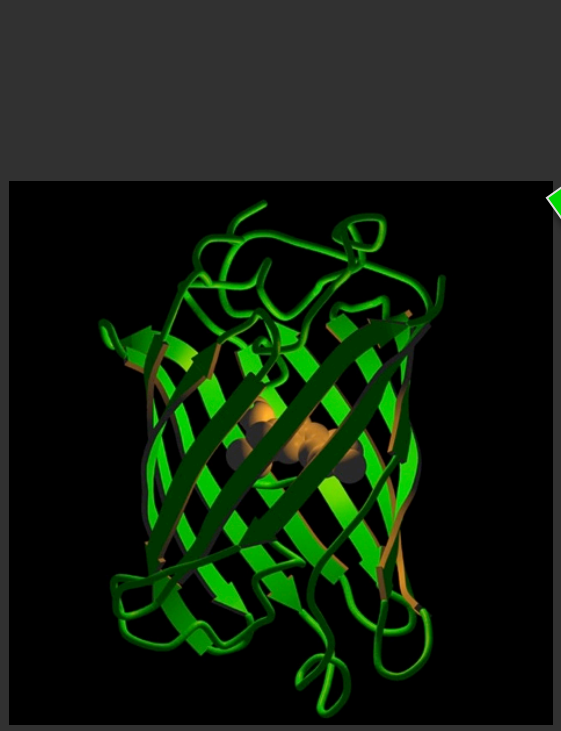


eGFP molecule

Image: Tsien lab

Fluorophores

- Small fluorescent molecules, label proteins or structures of interest



eGFP molecule

Emission – around 525 nm

Image: Tsien lab

Fluorophores

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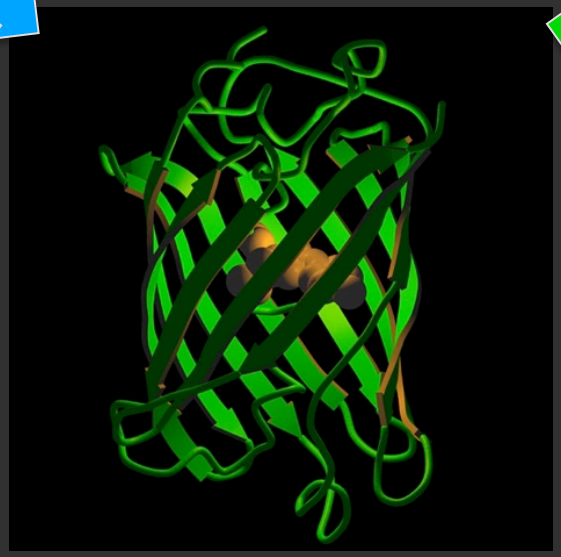

Emission – around 525 nm

eGFP molecule

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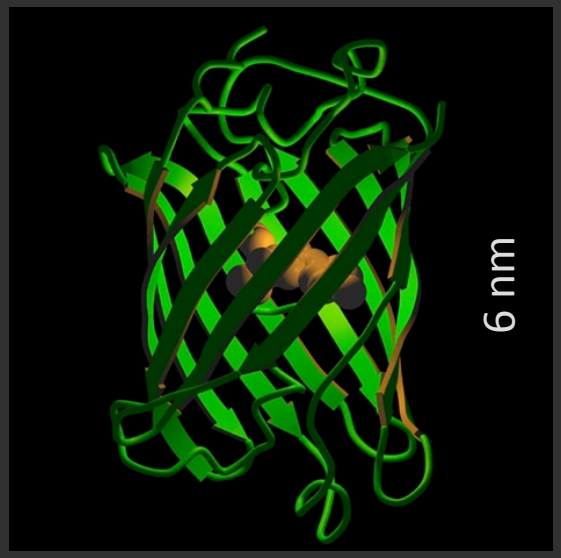
Fluorophores

- Small fluorescent molecules…



eGFP molecule

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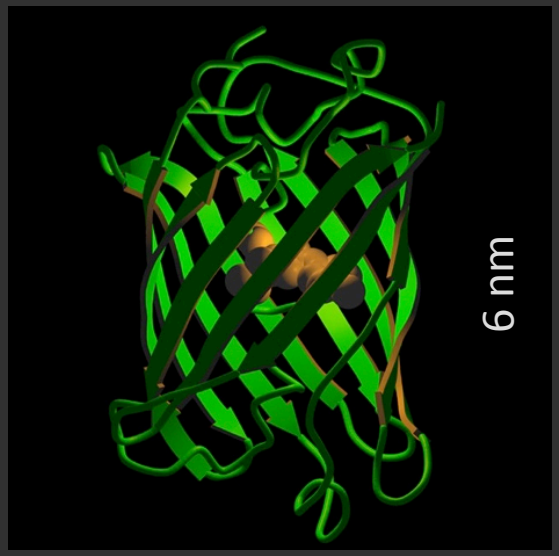
Fluorophores

- Small fluorescent molecules…



eGFP molecule

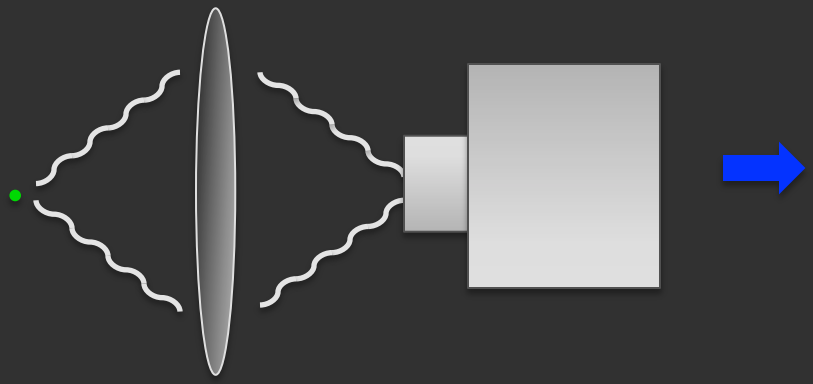
- Biological structures
  - Microtubules: width 25 nm
  - Nucleosomes: height 15 nm
  - Synaptic cleft: thickness 20nm

Resolution

- Minimal distance required between two objects allowing us to distinguish them

Resolution

- Our capacity to distinguish two objects depends on the point spread function



Point source Lens Camera

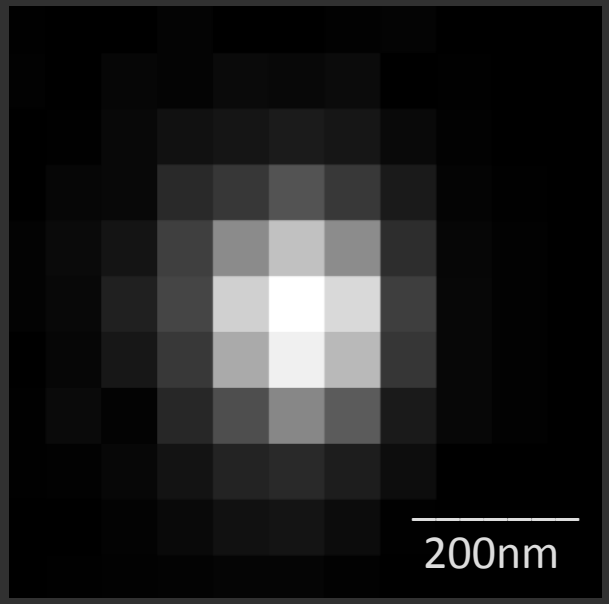
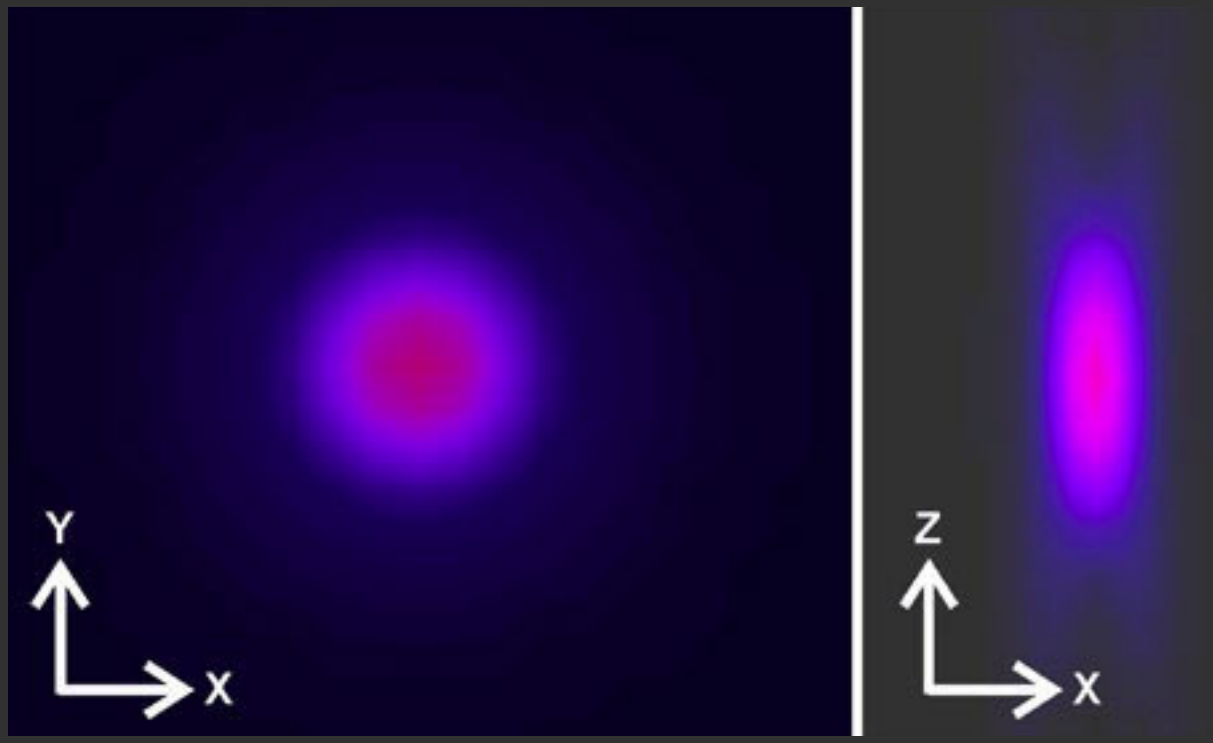


Image through optical system:
Point Spread Function

Point spread function

- Image representation of a “point source”, such as a single fluorescent molecule



- Size of the PSF: depends on wavelength, and optics objective numerical aperture

Image: ImageSurfer Tutorial

Point spread function

- Image representation of a “point source”, such as a single fluorescent molecule

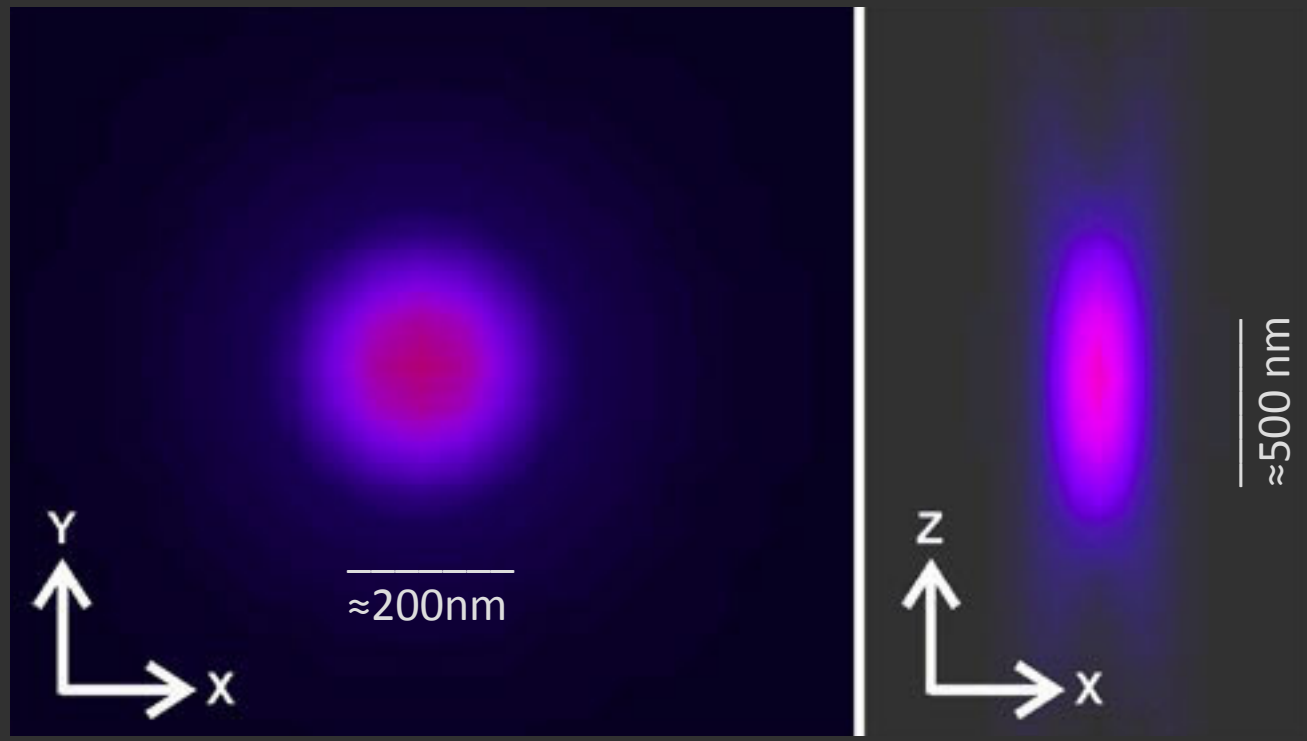
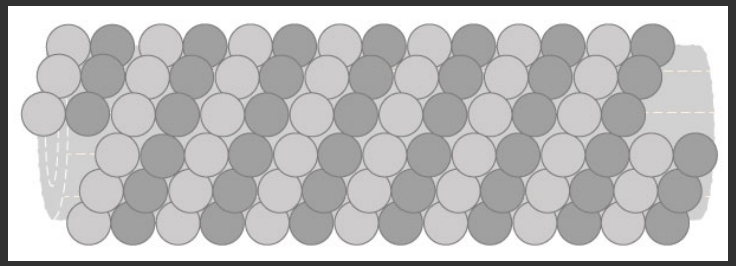


Image: ImageSurfer Tutorial

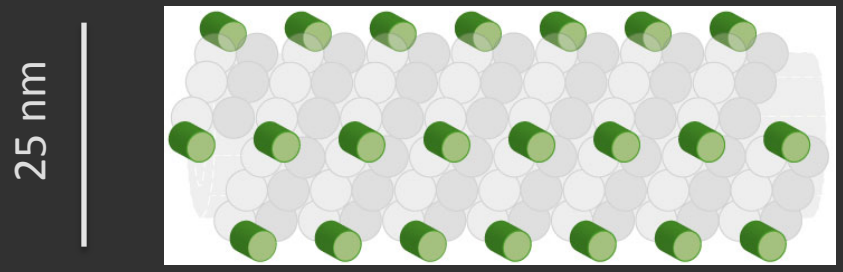
Point spread function

- Picture a microtubule…



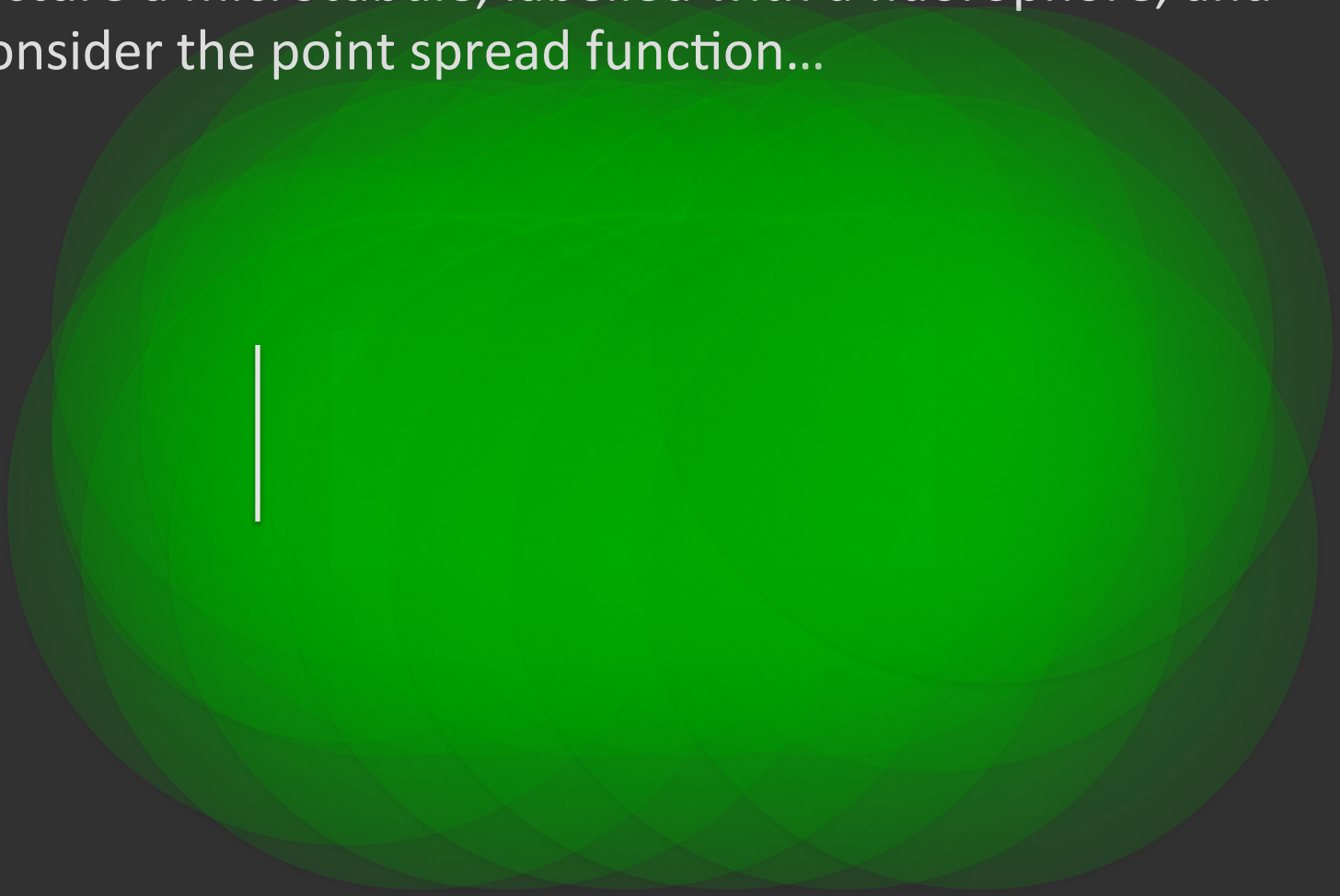
Point spread function

- Picture a microtubule… labelled with a fluorophore



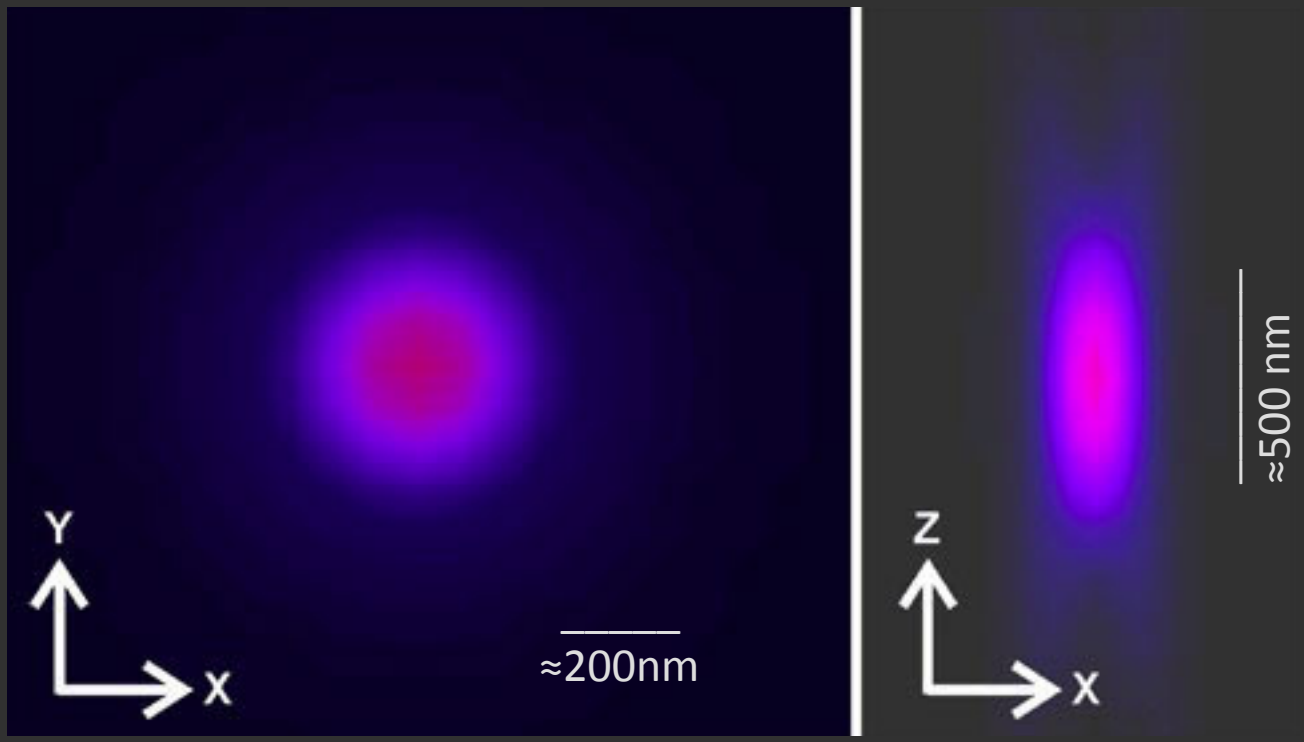
Point spread function

- Picture a microtubule, labelled with a fluorophore, and consider the point spread function…



Point spread function

- Resolution in z (“3D”) is worse than resolution in the xy (“2D”)



Point spread function

- Resolution in z ("3D") is worse than resolution in the xy ("2D"): this can result in blurry images…

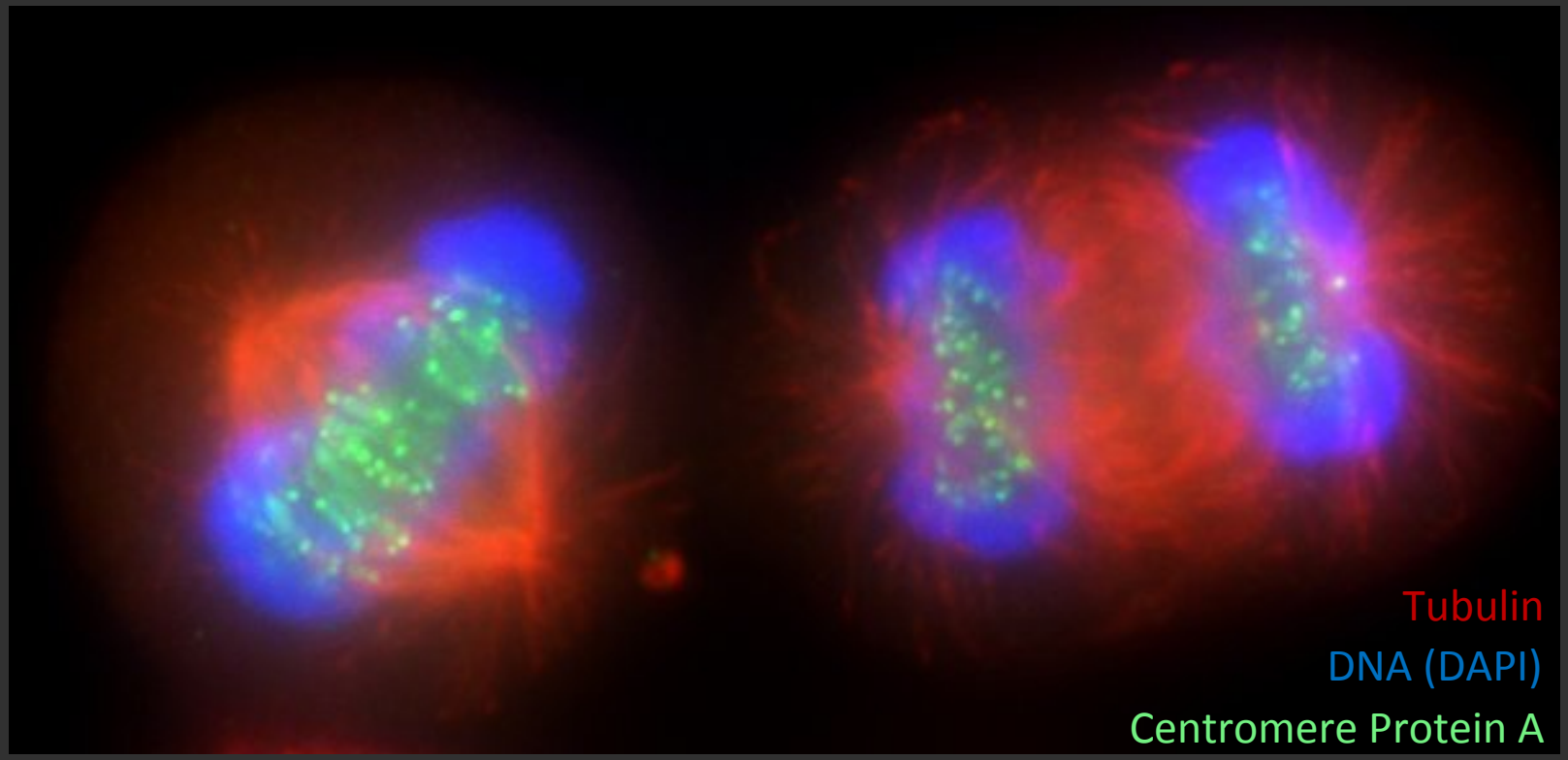


Image: Joel Ryan

Point spread function

- Resolution in z (“3D”) is worse than resolution in the xy (“2D”): this can result in blurry images…

- Solutions: … ?

Point spread function

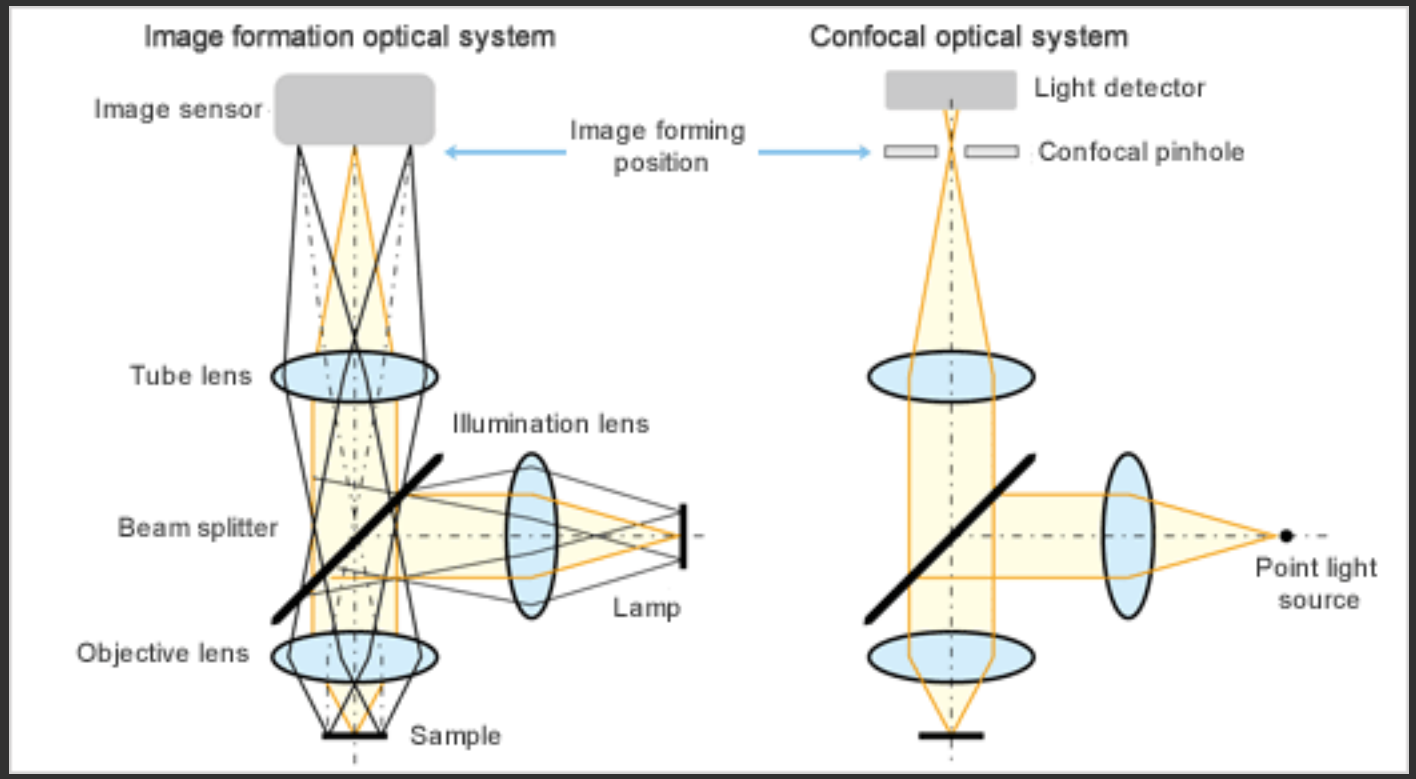
- Resolution in z (“3D”) is worse than resolution in the xy (“2D”): this can result in blurry images…

- Solutions: … ?
  - Illuminate fewer fluorophores within a sample
  - Acquire from a smaller volume multiple times

Confocal microscopy

Optical sectioning…

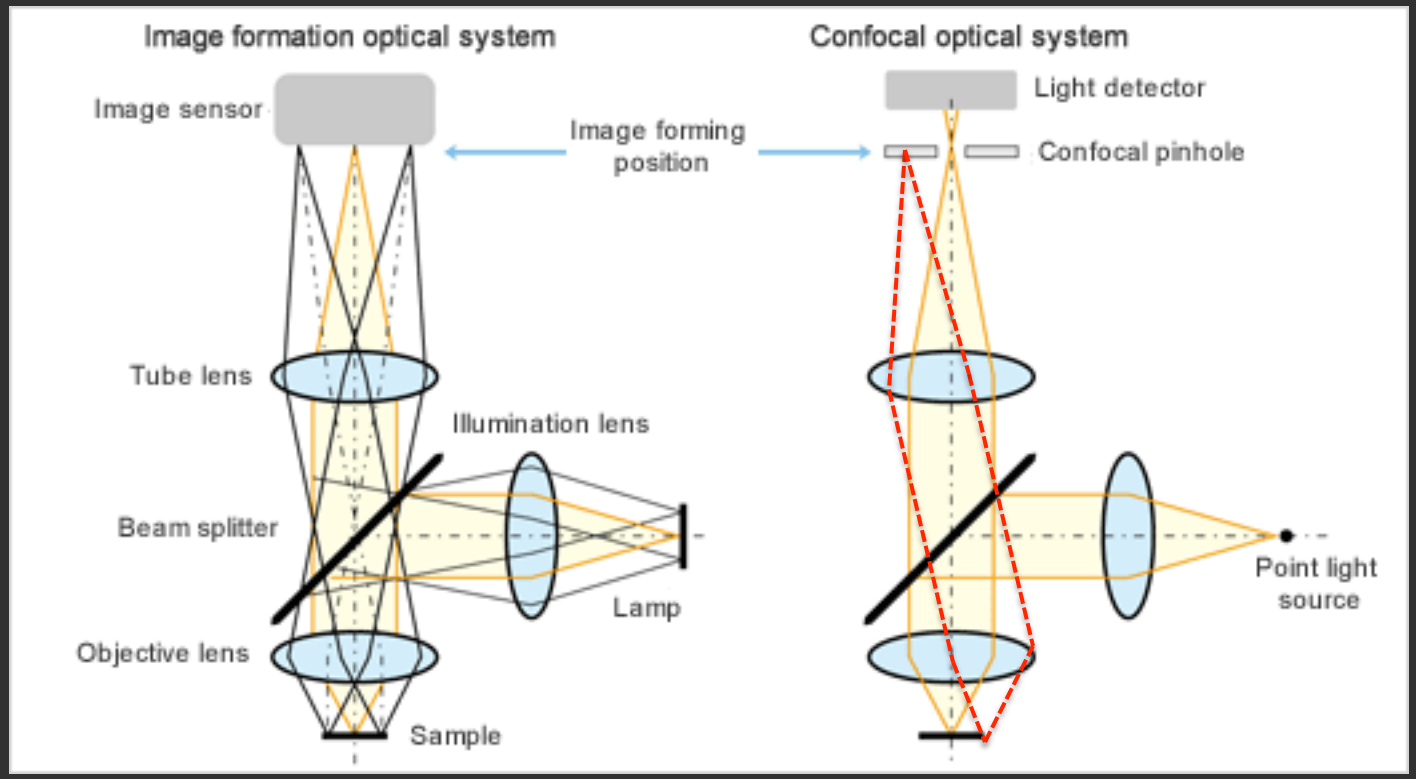
- Confocal microscope: using a pinhole to block out-of-focus light



Olympus

Optical sectioning…

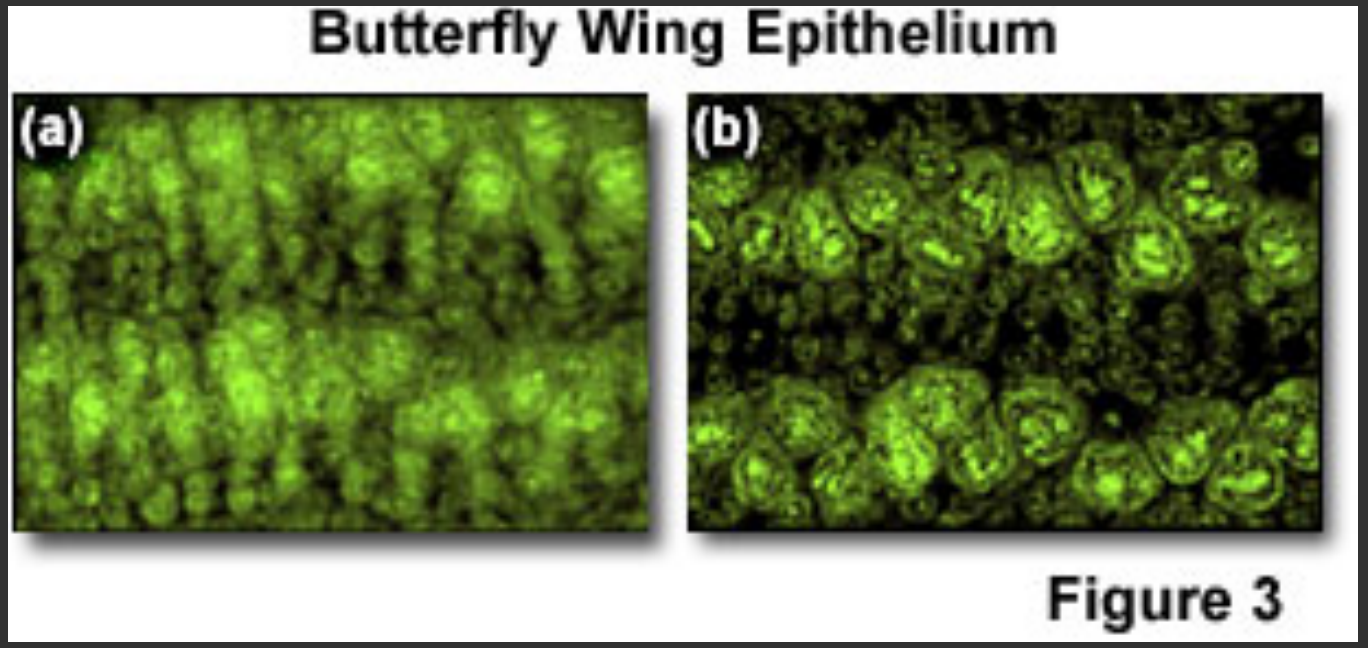
- Confocal microscope: using a pinhole to block out-of-focus light



Olympus

Optical sectioning…

- Pinhole aperture blocks out-of-focus light



Nikon

Optical sectioning…

- Other methods, ideas?

Along those lines….

- The point spread function is predictable

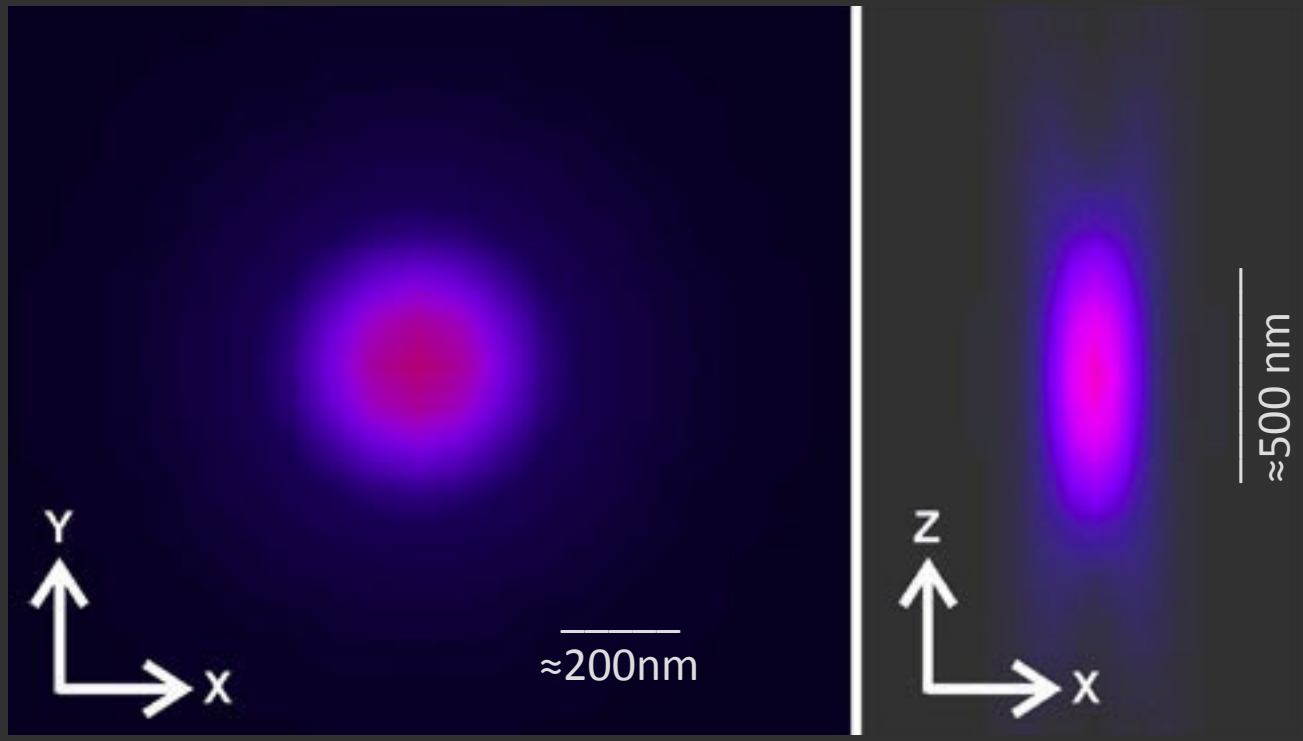
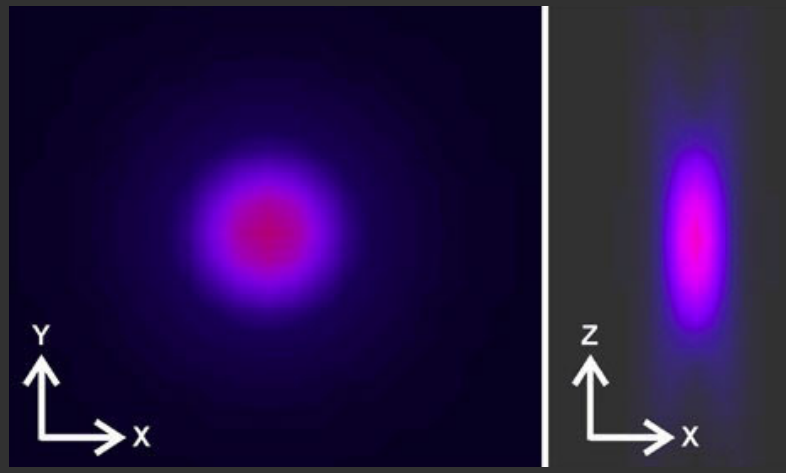


Image: ImageSurfer Tutorial

Along those lines….

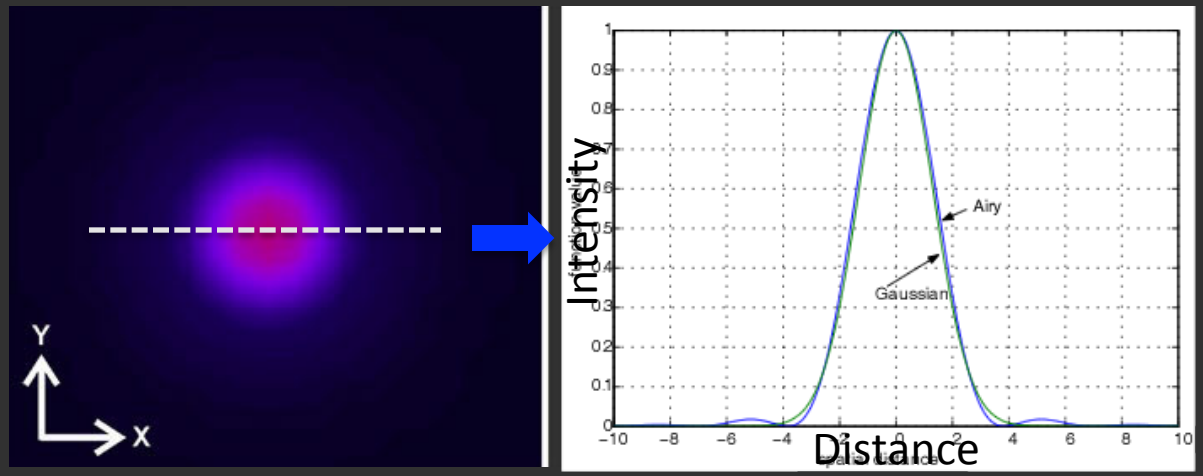
- The point spread function is predictable



- Its intensity profile fits a Gaussian curve

Along those lines….

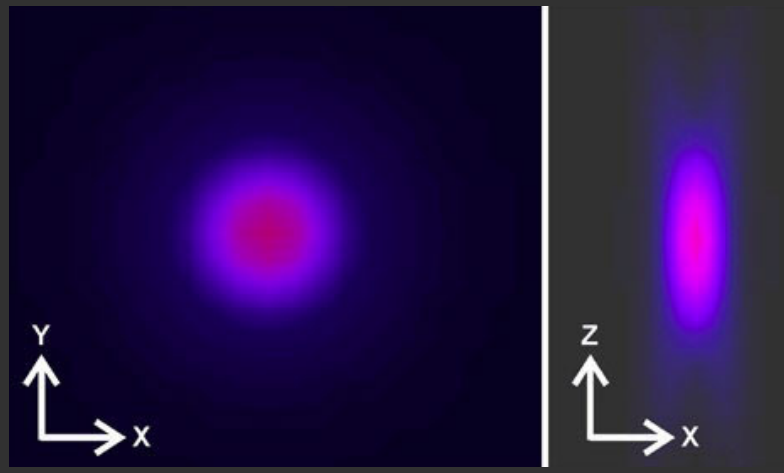
- The point spread function is predictable



- Its intensity profile fits a Gaussian curve:

Along those lines….

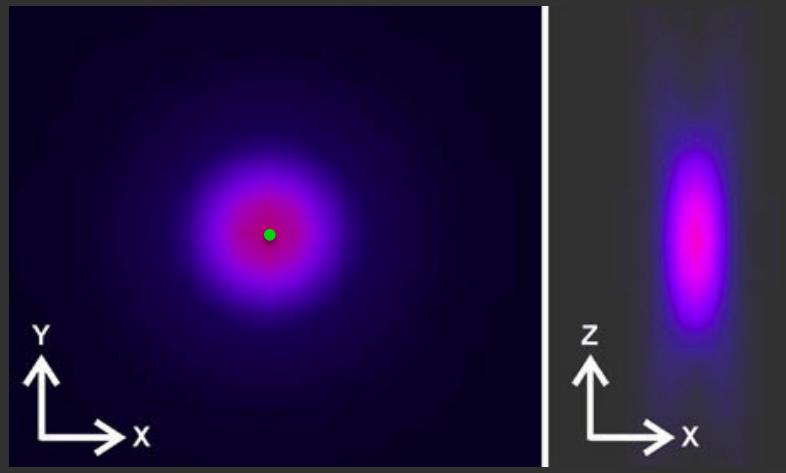
- The point spread function is predictable



- Its intensity profile fits a Gaussian curve:
  - Reliably easy to detect separate spots

Along those lines….

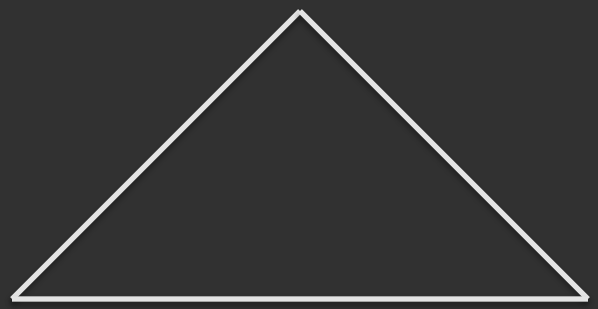
- The point spread function is predictable



- Its intensity profile fits a Gaussian curve:
  - Reliably easy to detect
  - Reliably easy to predict the localization of the source

Final thoughts

- Microscopy and life is a series of compromises



Adapted from PS Maddox, JF Dorn, and G Danuser

Final thoughts

- **Microscopy** and life is a series of compromises

Spatial resolution

Signal to noise

Temporal resolution and speed

vs.

-Cell Viability
-Fluorophore Stability

Final thoughts

- **Microscopy** and life is a series of compromises

Spatial resolution

Signal to noise

Temporal resolution and speed

vs.

-Cell Viability
-Fluorophore Stability

Summary

- Modern microscopy techniques typically generate quantitative datasets
- The Point Spread Function is the image of a point source through an optical system
- Microscopy is a series of compromises

Today

- Talks about current microscopy techniques
  - Here in Martinsried / Großhadern
- Ask questions !!!
- Interact with imaging experts and enthusiasts

Microscopy information and education online and free!

- iBiology Microscopy Course

- Zeiss Microscopy Online Campus
- Nikon Microscopy U
- Olympus Microscopy Resource Center

GFP molecule vs PSF