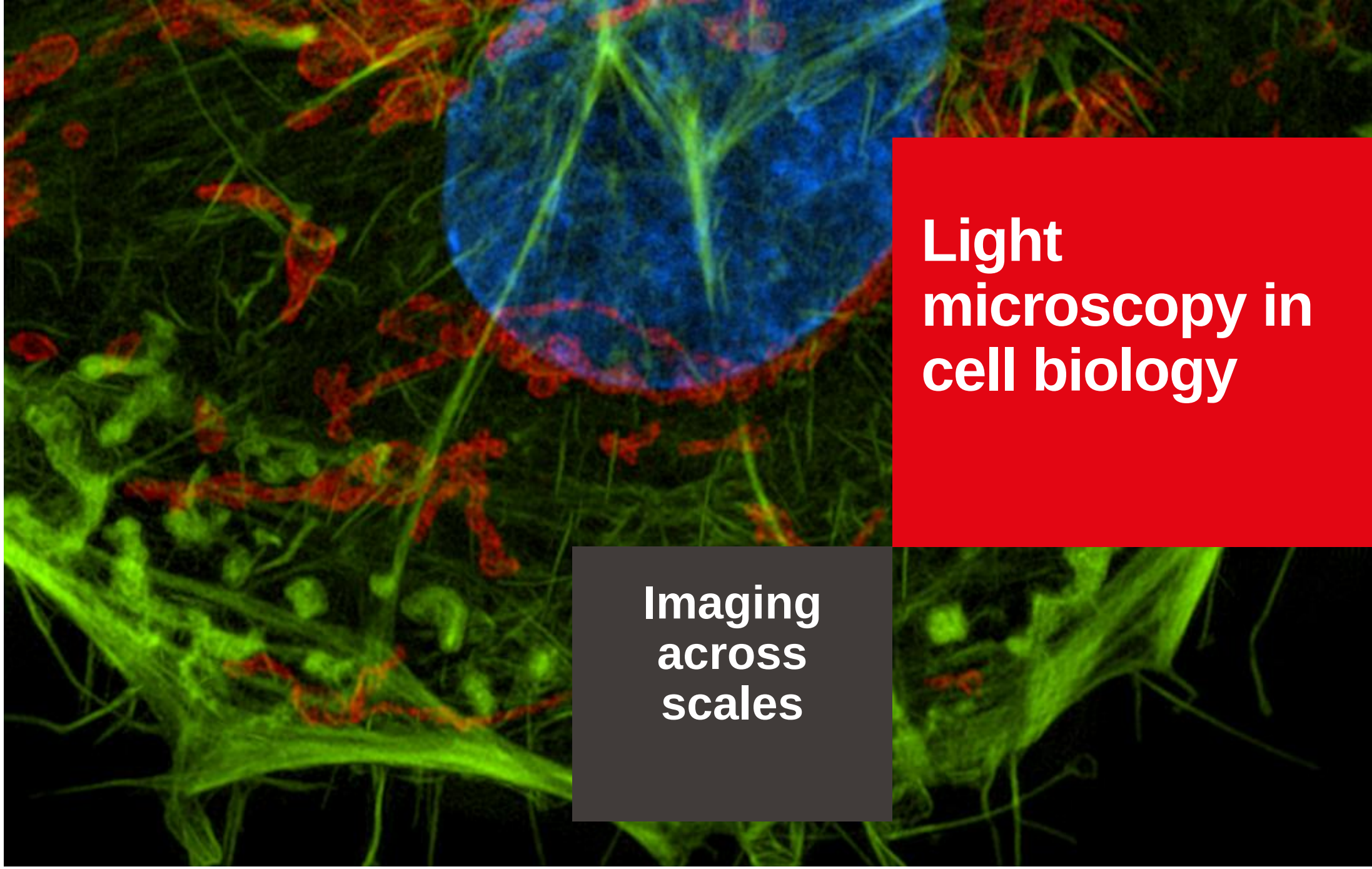
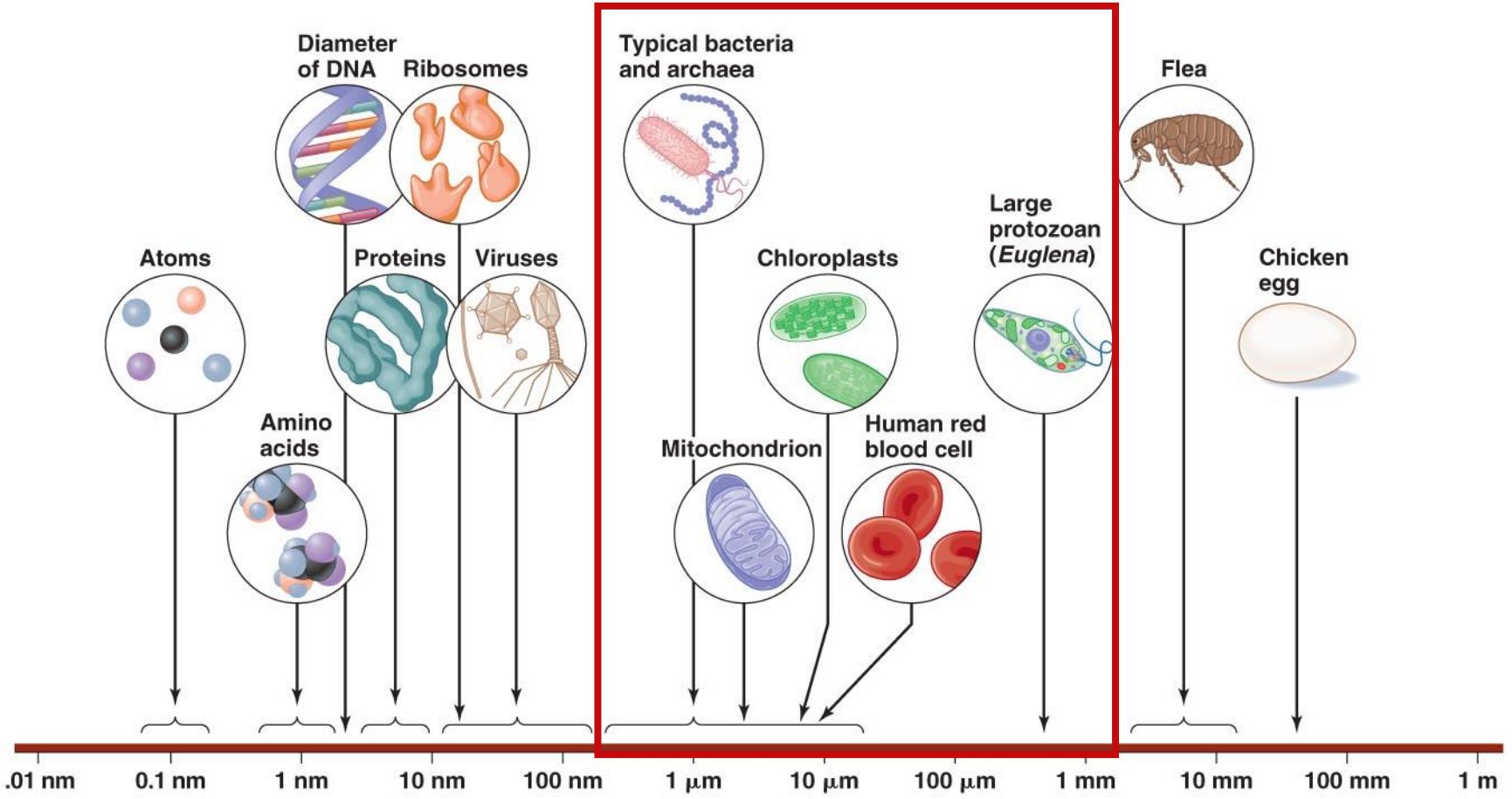


Light microscopy in cell biology

Imaging across scales

École polytechnique fédérale de Lausanne

Light microscopy in Life Science



At a standard viewing distance (~ 250 mm), the smallest object we can see with naked eye is ~80 μm.

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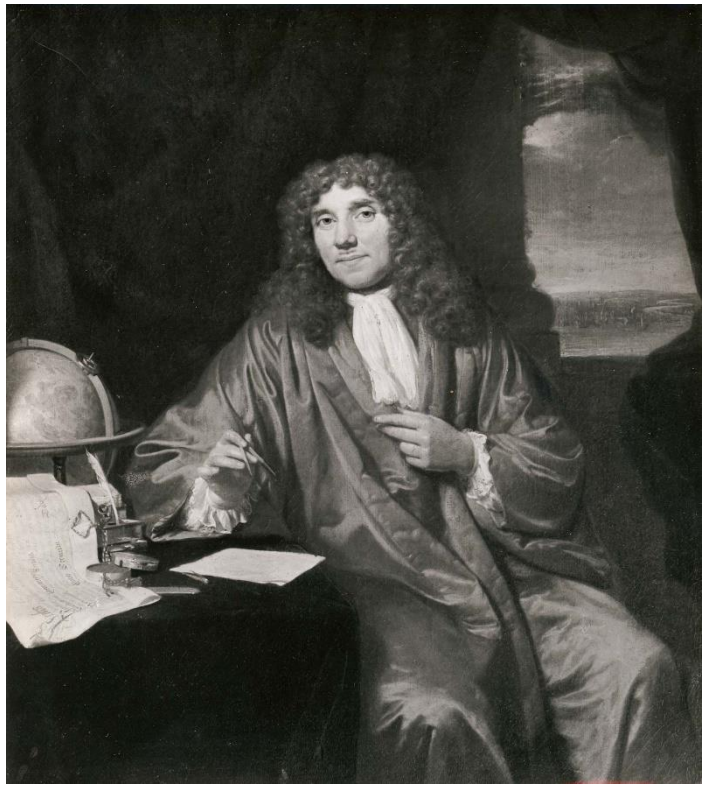
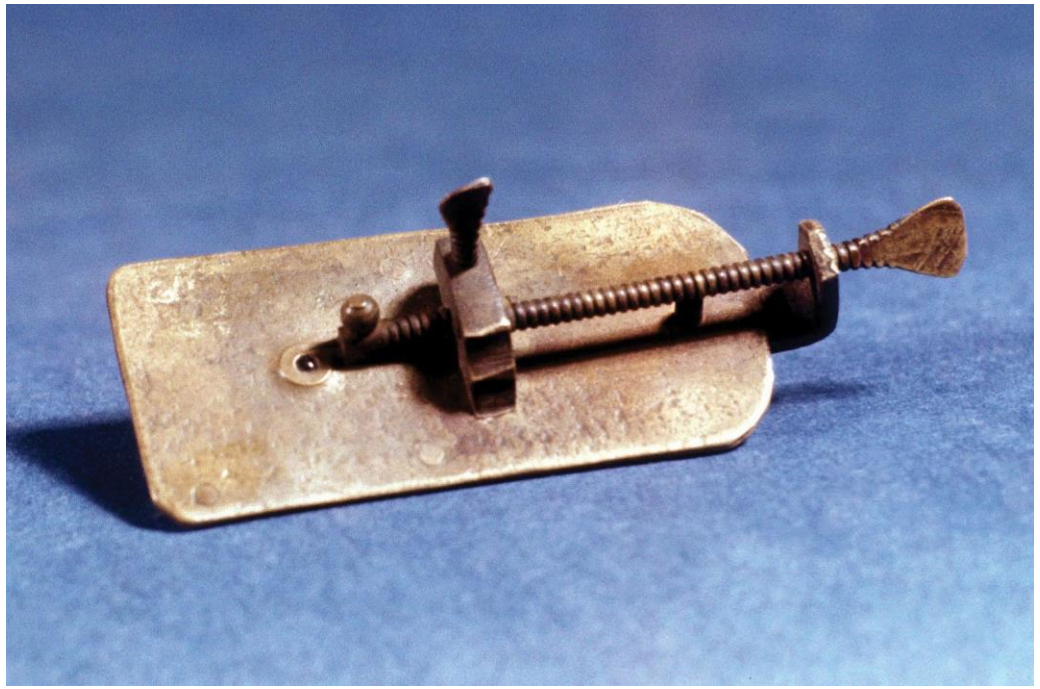
Microscopy meets Biology

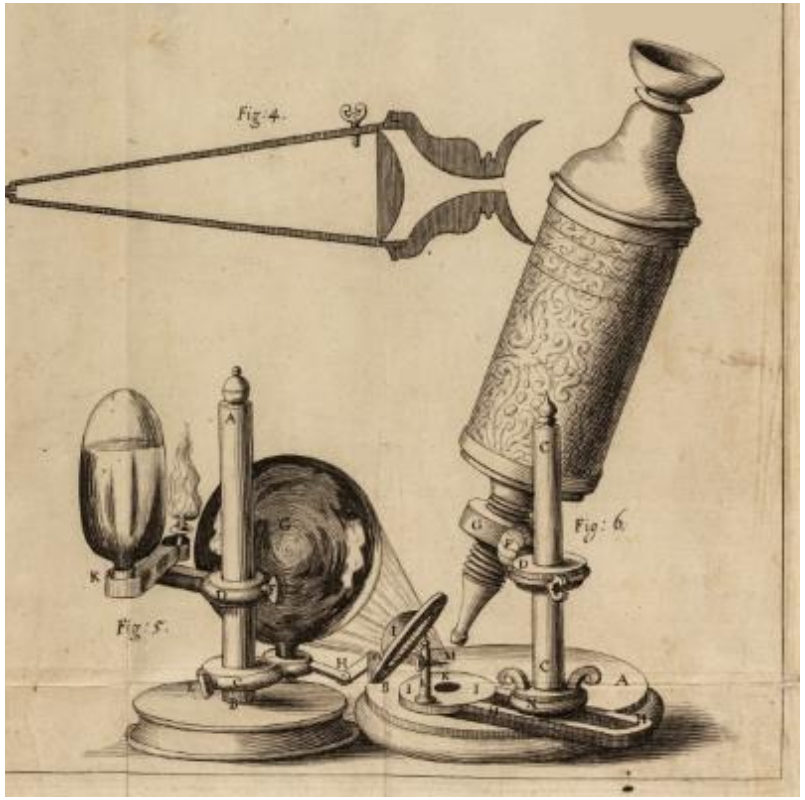


Antonie van Leeuwenhoek
October 24th, 1632 - August 26th, 1723

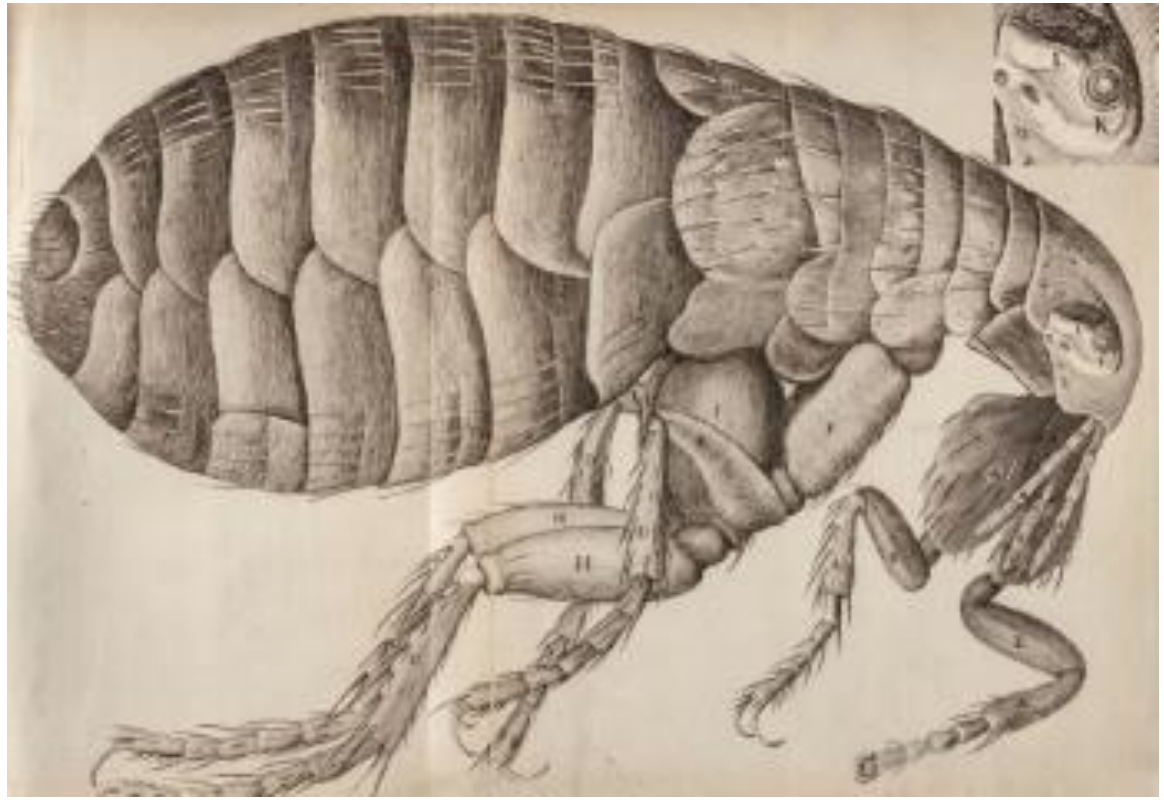


Philosophical Transactions 1683.
The first representation of bacteria is to be found in a drawing by Leeuwenhoek.

Microscopy meets Biology



Robert. Hooke
July 18th, 1635 - March 3rd, 1703



1665 Micrographia
https://royalsociety.org/blog/2020/07/micrographia-online/

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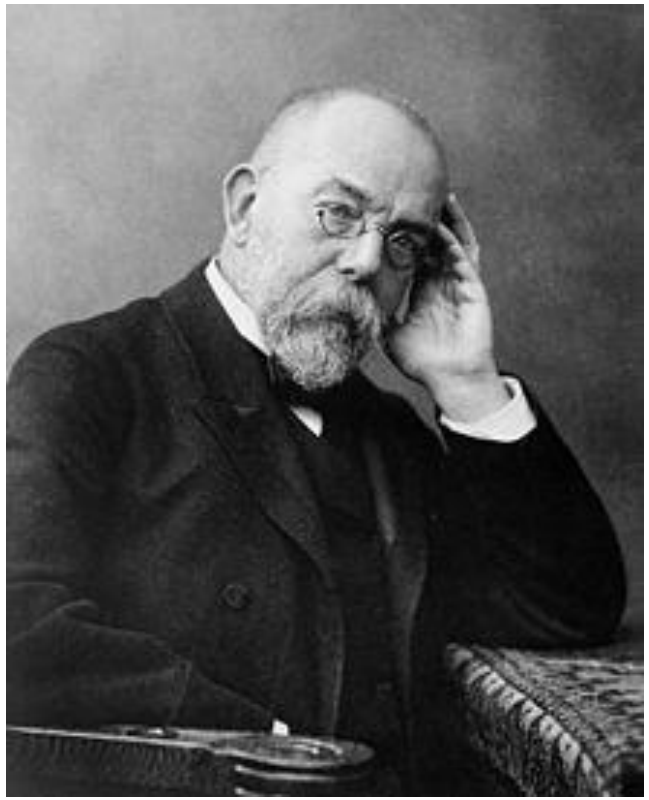
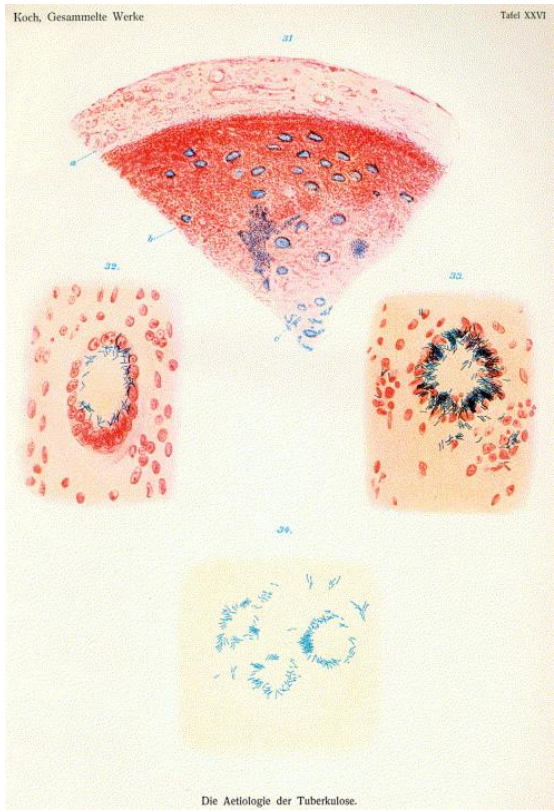
Microscopy meets Biology



Heinrich Hermann Robert Koch
Dezember 11th 1843 - Mai 27th 1910
Nobel Prize in Physiology and Medicine 1905

Mikroskop
Carl Zeiss 1879



Koch's drawing of tuberculosis bacilli in 1882
(from Die Ätiologie der Tuberkulose)

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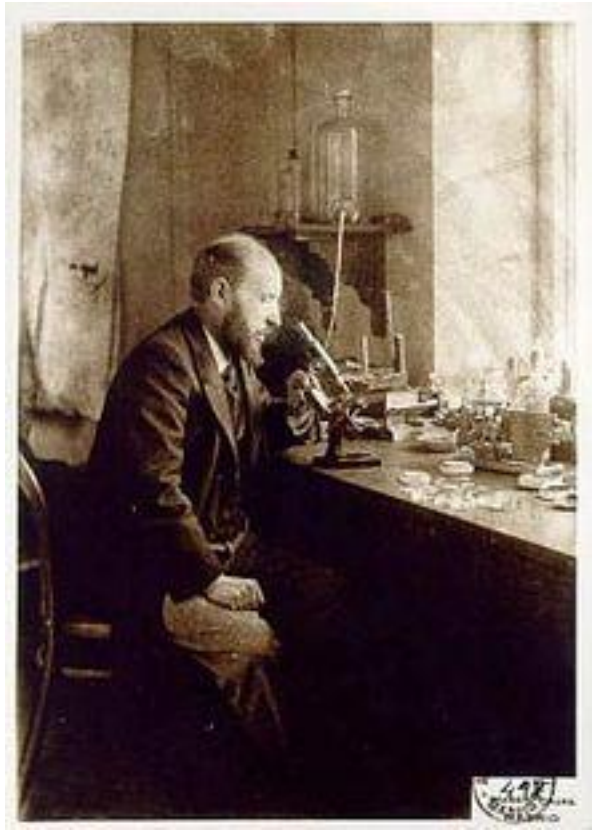
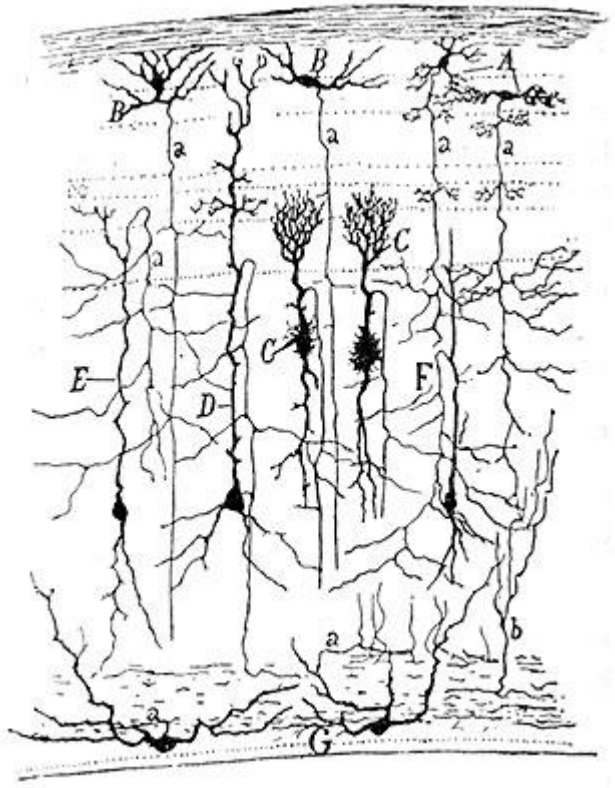
Microscopy meets Biology



Santiago Ramón y Cajal
May, 1st 1852 –October 17th 1934
Nobel Prize in Physiology and Medicine 1906



Drawing of a section through the optic tectum of a sparrow, from "Structure of the nervous centres of the birds", by Santiago Ramon y Cajal, 1905.

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Microscopy meets Electrical Engineering

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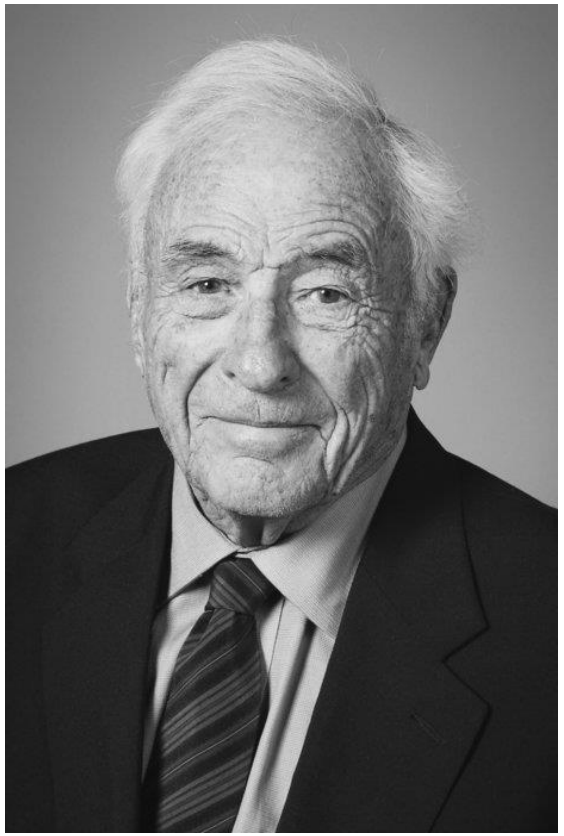
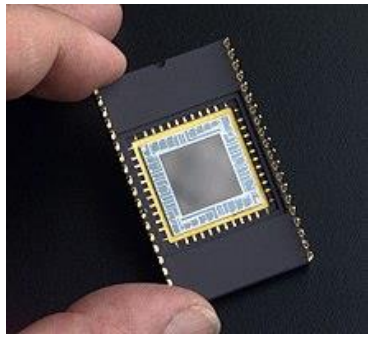
Nobel Price in Physics 2009



Willard S. Boyle
August 19th 1924 –May 7th 2011

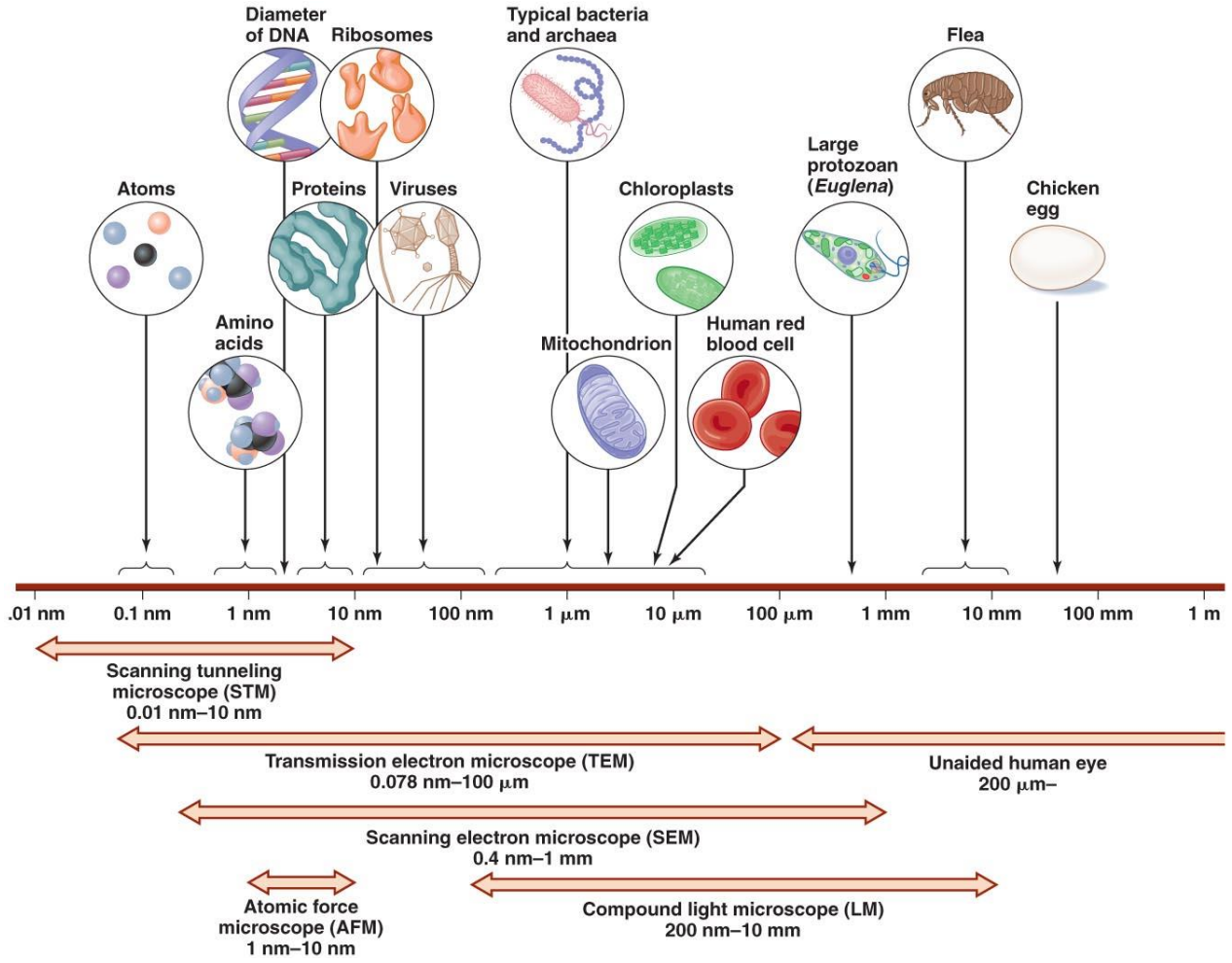
George E. Smith
May 10th 1930 -

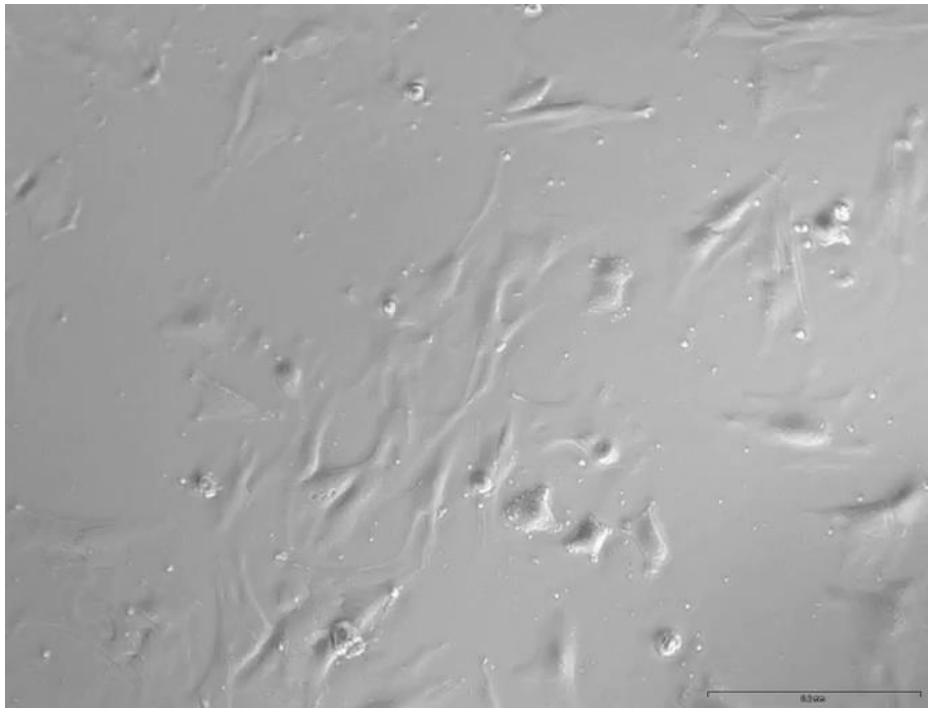
Charge-coupled device (CCD)



The basis for the CCD is the metal–oxide–semiconductor (MOS) structure, with MOS capacitors being the basic building blocks of a CCD, and a depleted MOS structure used as the photodetector in early CCD devices.

Resolution vs. dynamics





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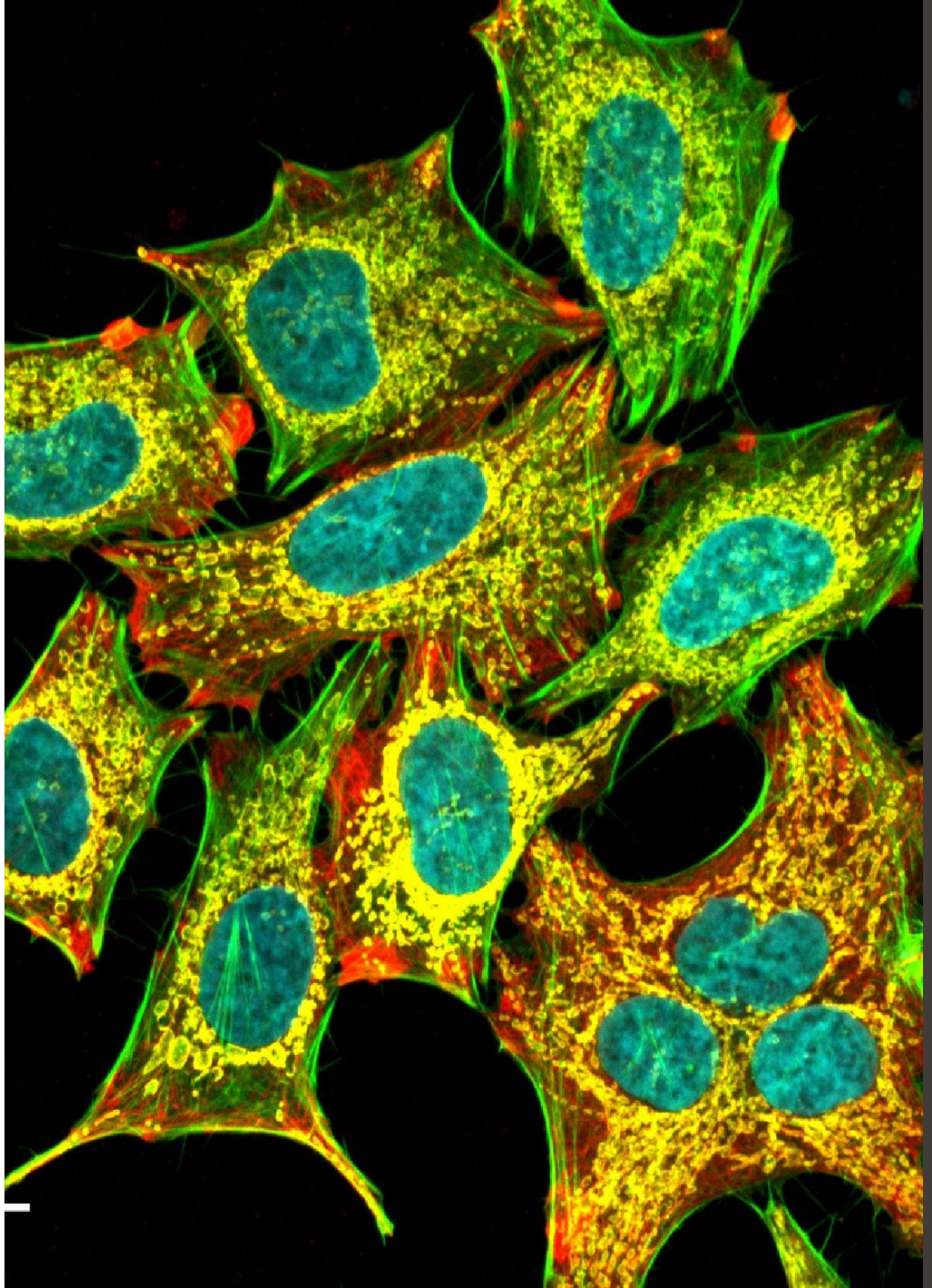

Labelling cellular components

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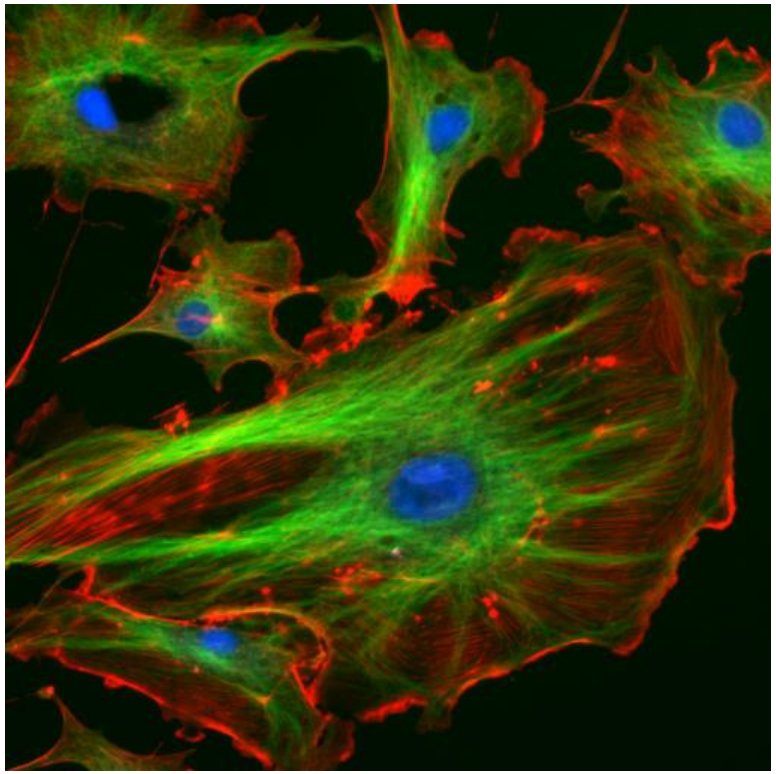
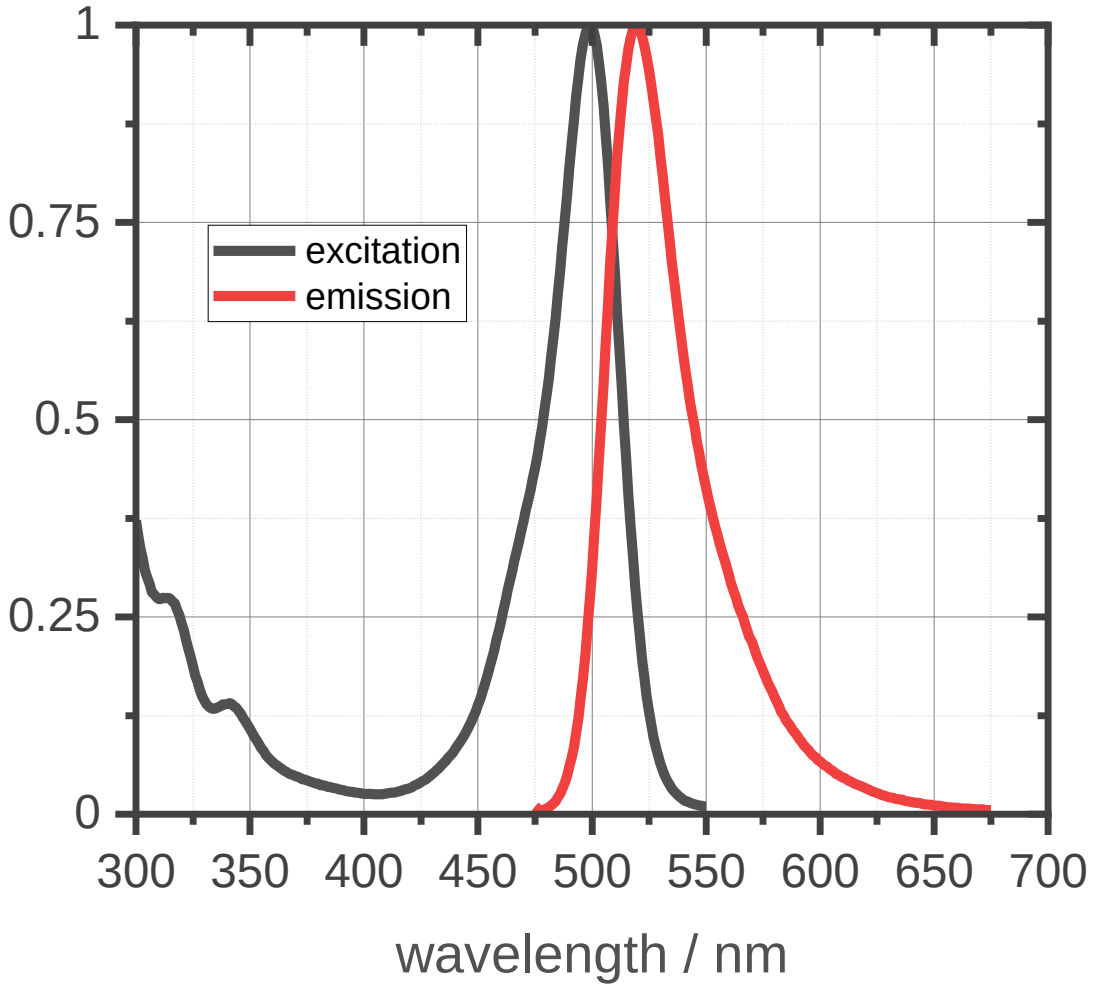
Fluorescence for organelle detection



Fluorescent dyes subcellular organelles



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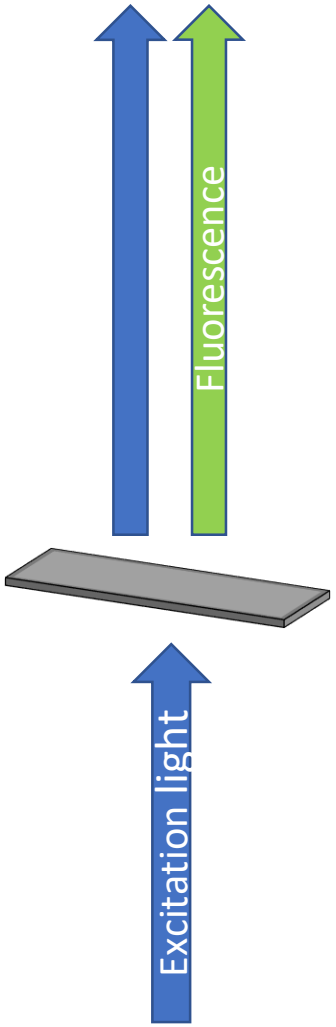
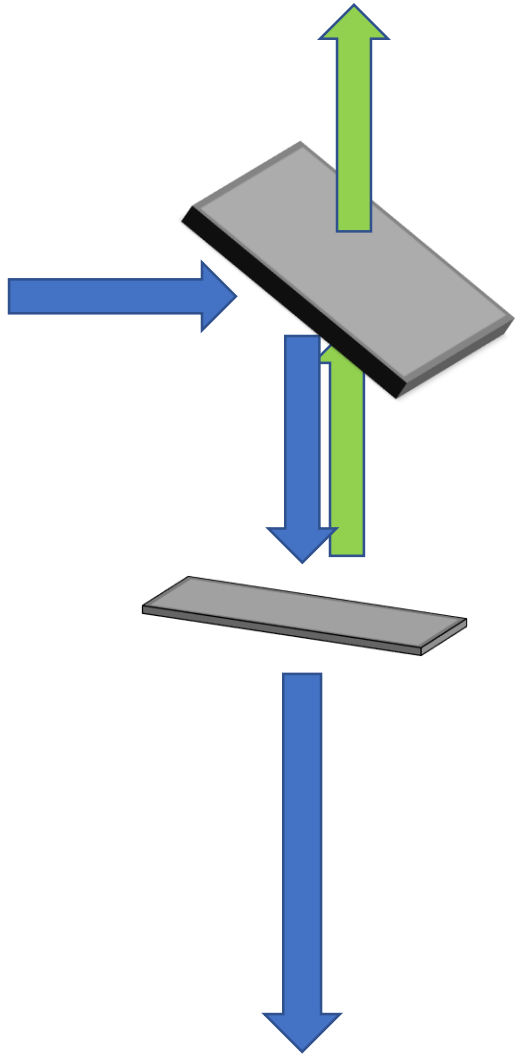
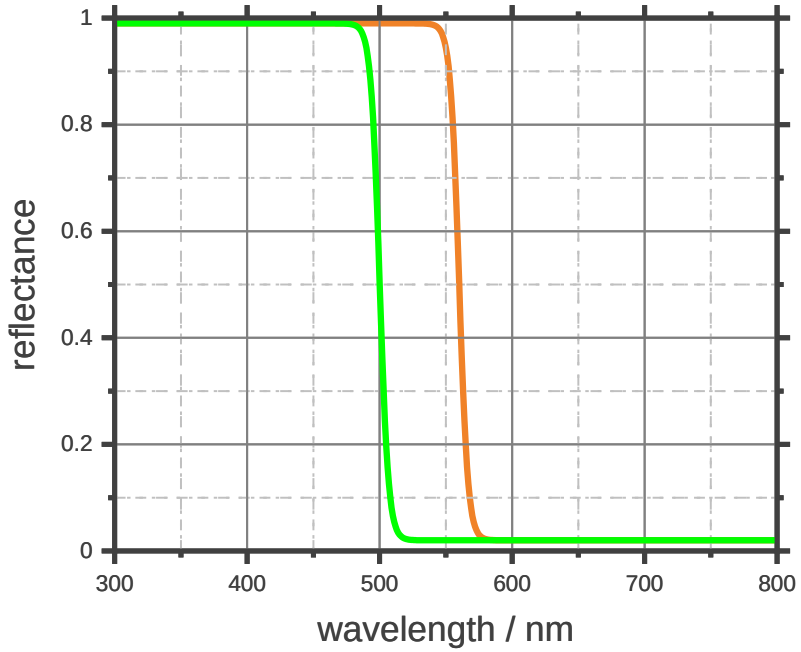
Epi fluorescence microscopy







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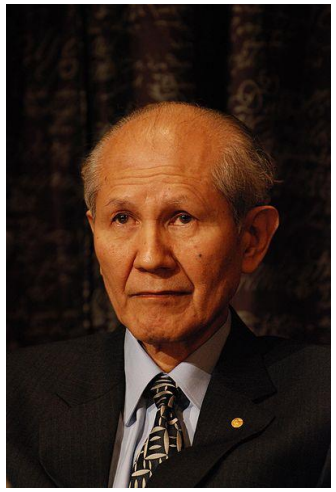
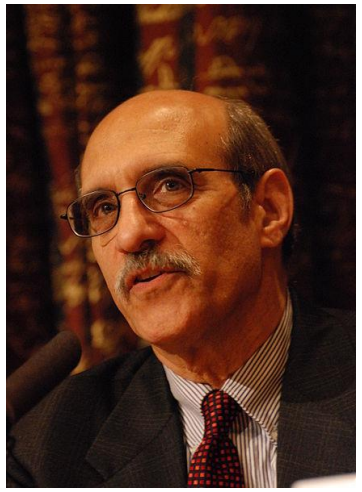
Green Fluorescent Protein

- 1962 GFP discovery/isolation
- 2008 Nobel Prize in Chemistry



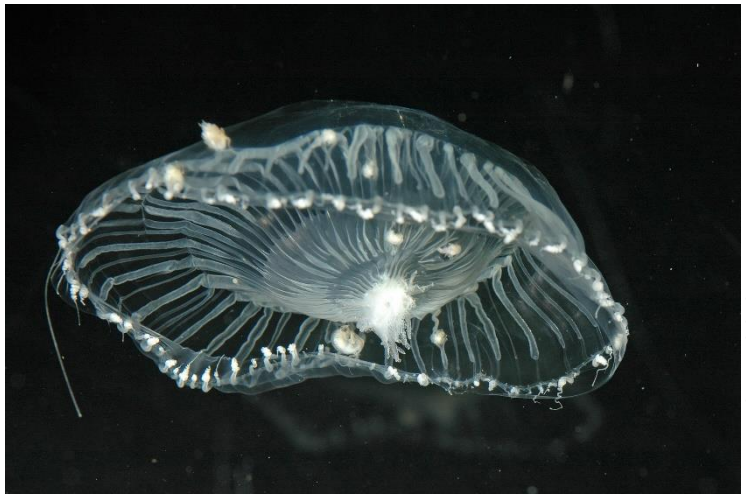
Osamu Shimomura



Martin Chalfie

Roger Y. Tsien

CC-BY Prolineserver



Aequorea victoria

CC-BY Sierra Blakely

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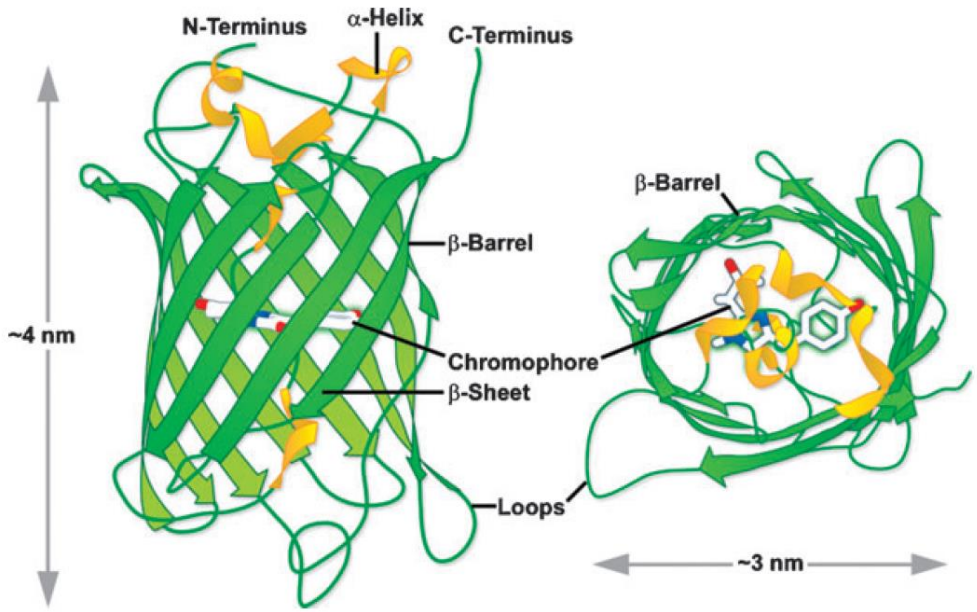
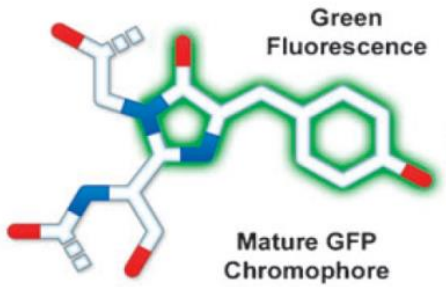
Green Fluorescent Protein

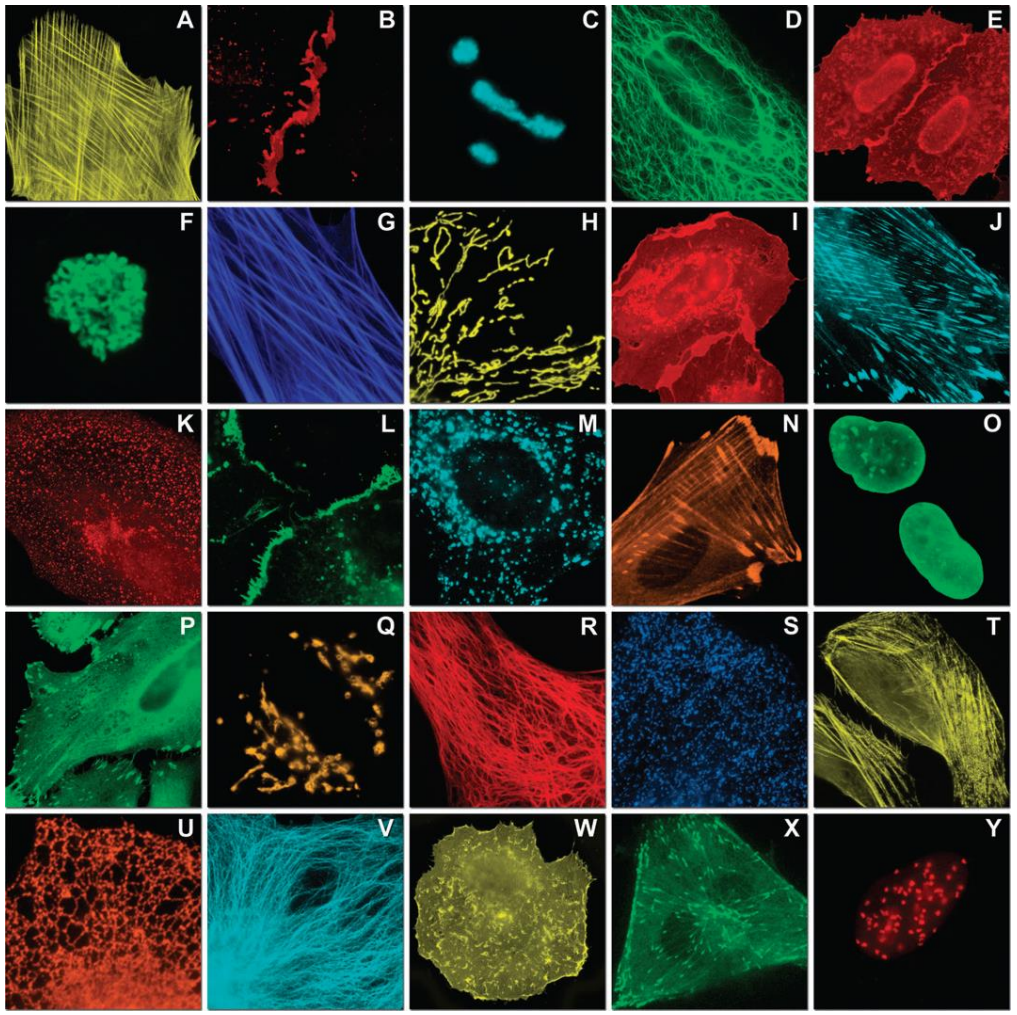




The fluorescent protein palette: tools for cellular imaging.
Richard N. Day and Michael W. Davidson
Chem. Soc. Rev., 2009, 38, 2887–2921.

Arne Seitz, EPFL-BIOP, 2024

Applications of Fluorescent Proteins

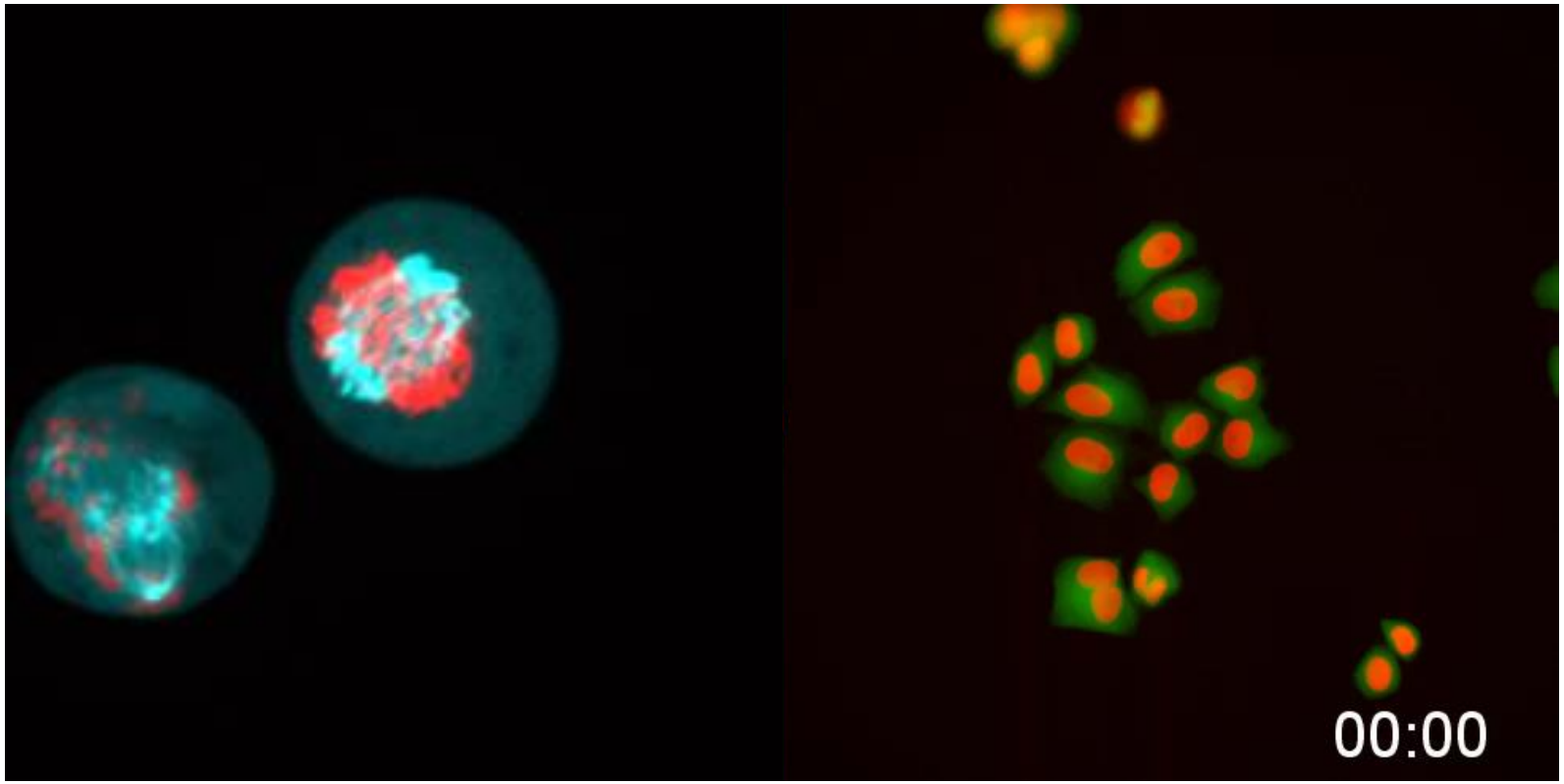


(A) mOrange2-b-actin-C-7.
(B) mApple-Cx43-N-7.
(C) mTFP1-fibrillarin-C-7.
(D) mWasabi-cytokeratin-N-17.
(E) mRuby-annexin (A4)-C-12.
(F) mEGFP-H2B-N-6.
(G) EBFP2-b-actin-C-7.
(H) mTagRFP-T-mitochondria-N-7.
(I) mCherry-C-Src-N-7.
(J) mCerulean-paxillin-N-22.
(K) mKate-clathrin (light chain)-C-15.
(L) mCitrine-VE-cadherin-N-10.
(M) TagCFPlysosomes-C-20.
(N) TagRFP-zyxin-N-7.
(O) superfolderGFP-lamin B1-C-10.
(P) EGFP-a-v-integrin-N-9.
(Q) tdTomato-Golgi-N-7.
(R) mStrawberry-vimentin-N-7.
(S) TagBFP-Rab-11a-C-7.
(T) mKO2-LC-myosin-N-7.
(U) DsRed2-endoplasmic reticulum-N-5.
(V) ECFP-atubulin-C-6.
(W) tdTurboRFP-farnesyl-C-5.
(X) mEmerald-EB3-N-7.
(Y) mPlum-CENP-B-N-22.

The fluorescent protein palette: tools for cellular imaging.
Richard N. Day and Michael W. Davidson, *Chem. Soc. Rev.*, 2009, 38, 2887–2921.

Arne Seitz, EPFL-BIOP, 2024

Timelapse microscopy



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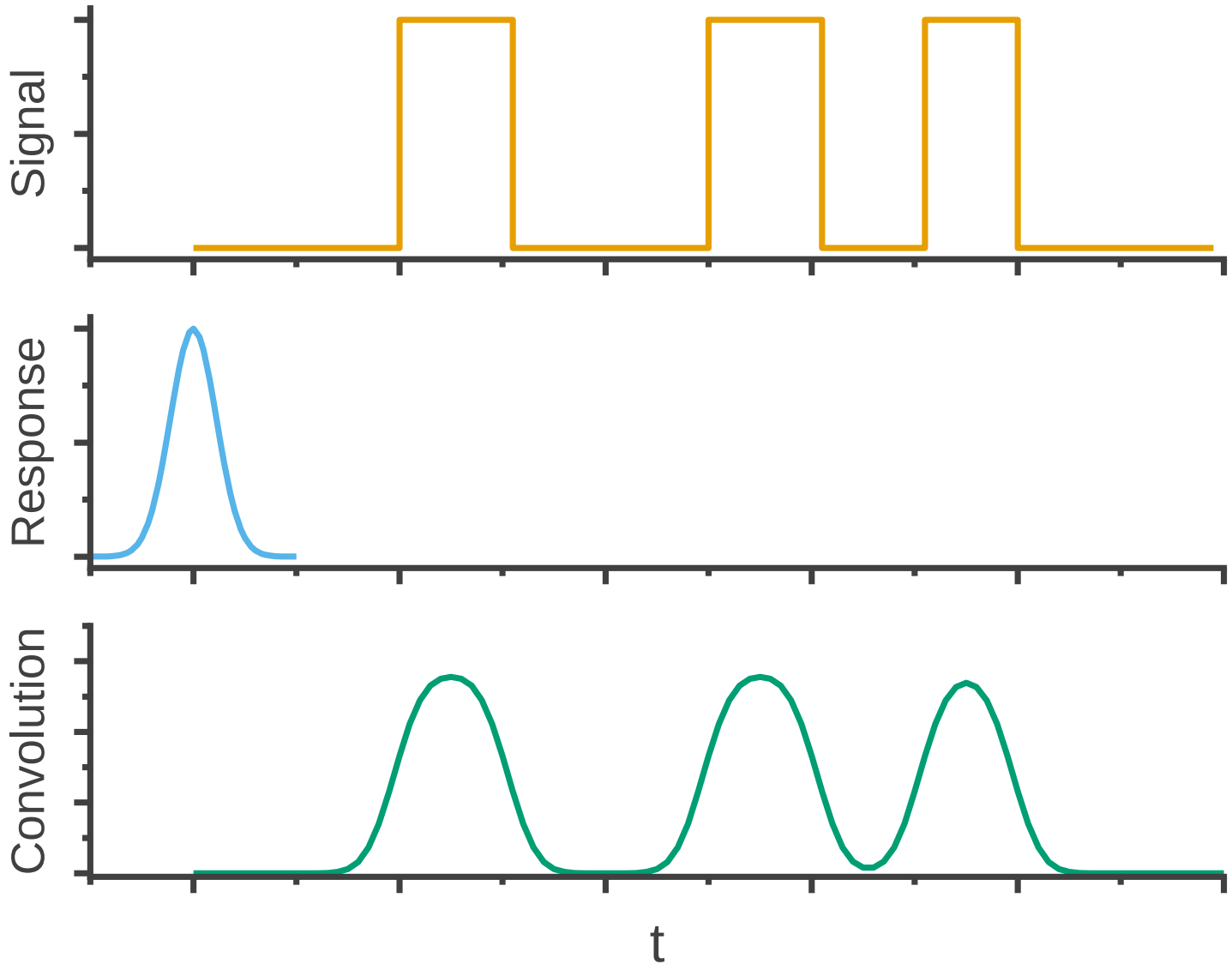
Basics and limitations of image formation

Arne Seitz, EPFL-BIOP, 2024

Convolution



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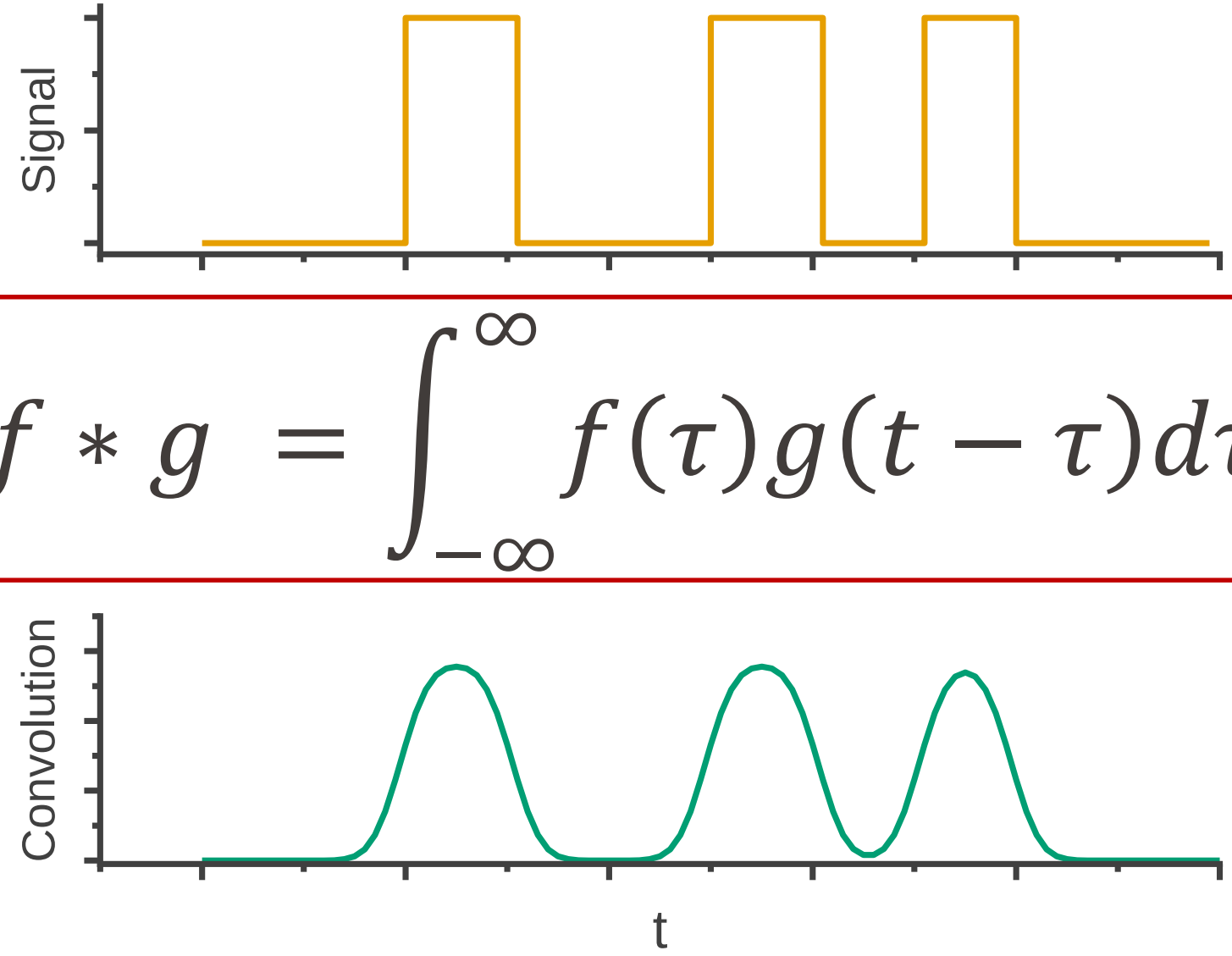
Convolution



$$f * g = \int_{-\infty}^{\infty} f(\tau) g(t - \tau) d\tau$$

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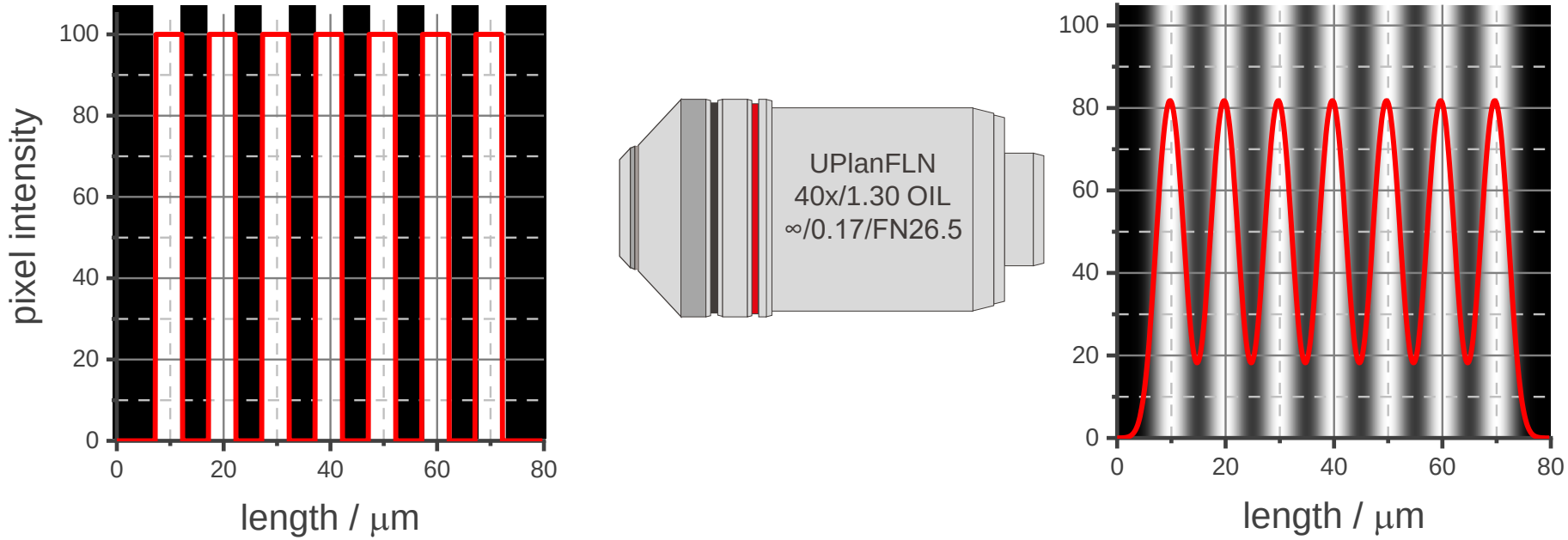
Low pass filtering



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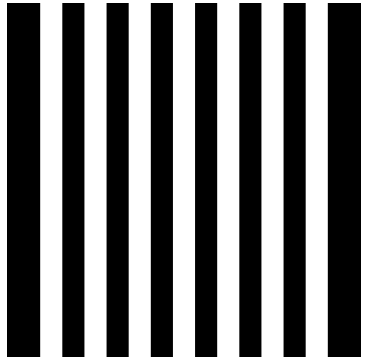
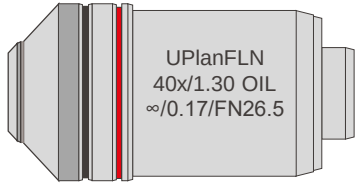
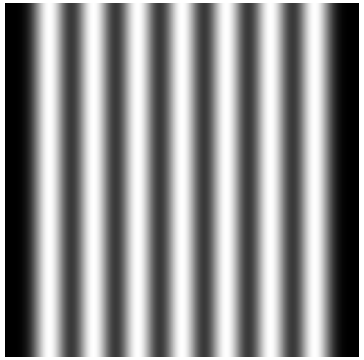
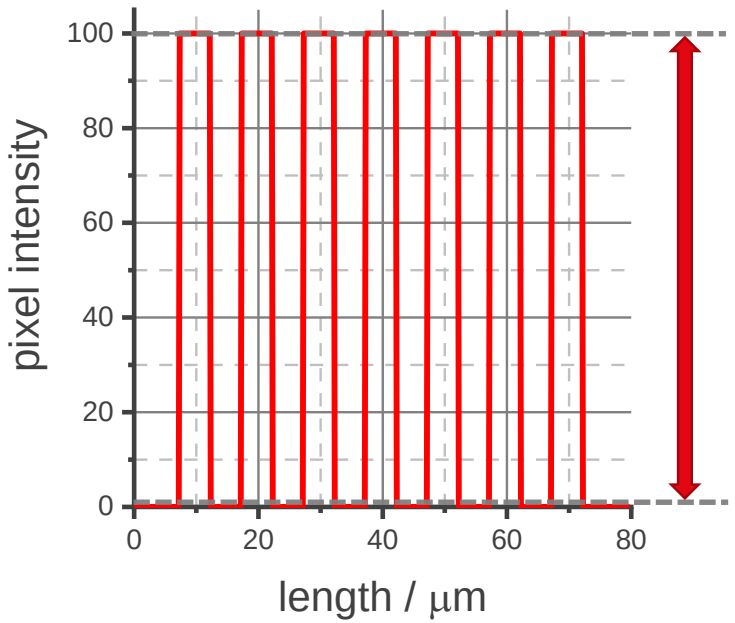
Contrast







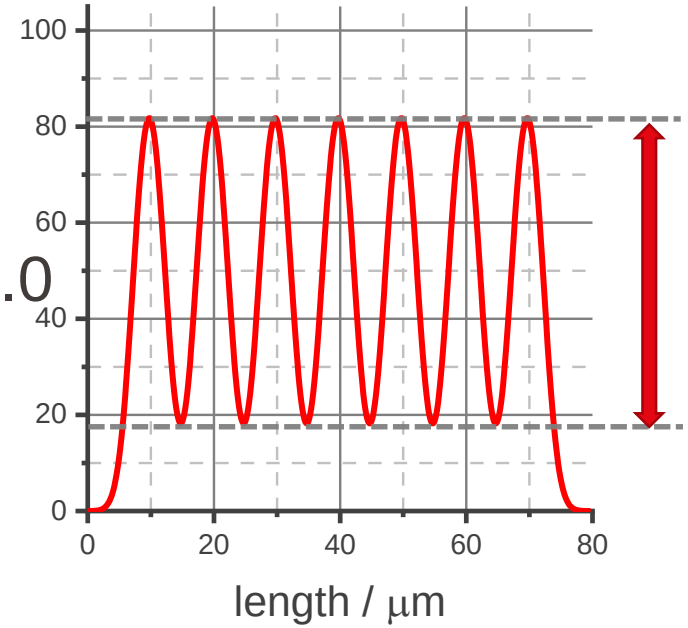
$$modulation = \frac{I_{max} - I_{min}}{I_{max} + I_{min}}$$



I_{max}

m = 1.0

I_{min}



I_{max}

m = 0.62

I_{min}

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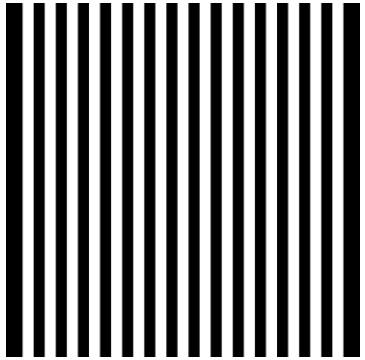
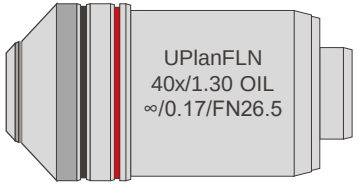
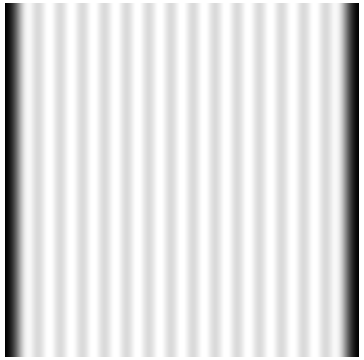
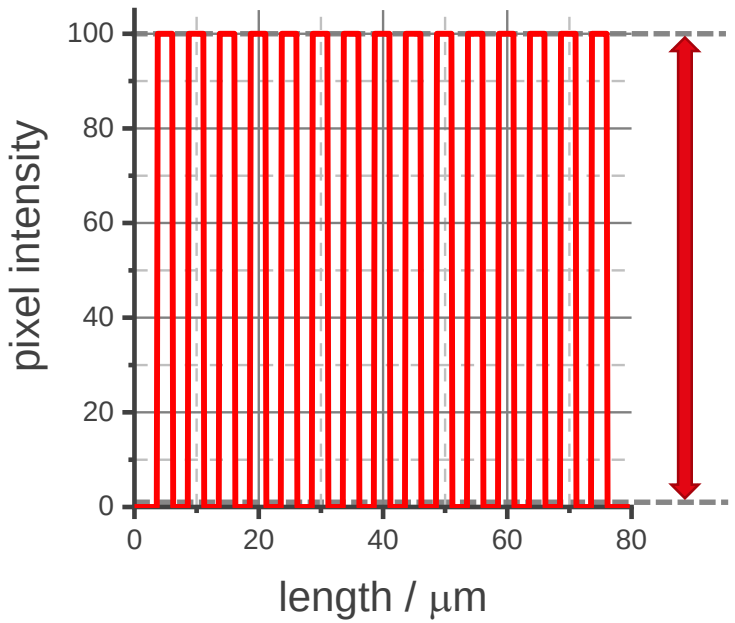
Contrast







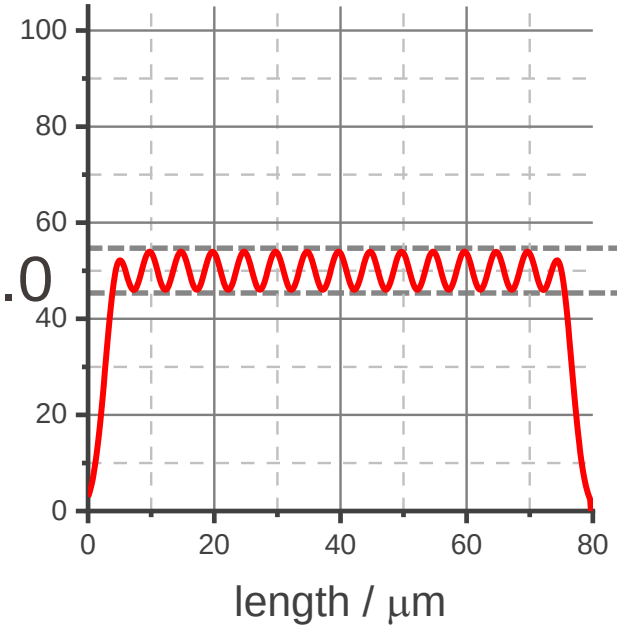
$$modulation = \frac{I_{max} - I_{min}}{I_{max} + I_{min}}$$



I_{max}

m = 1.0

I_{min}



I_{max}

m = 0.08

I_{min}

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Modulation Transfer Function

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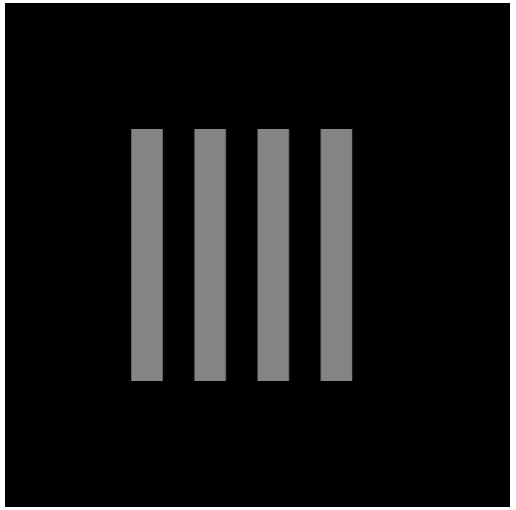
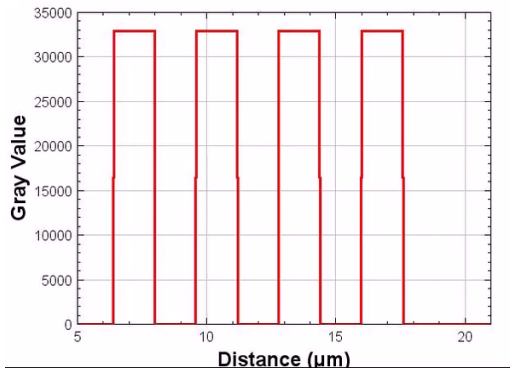
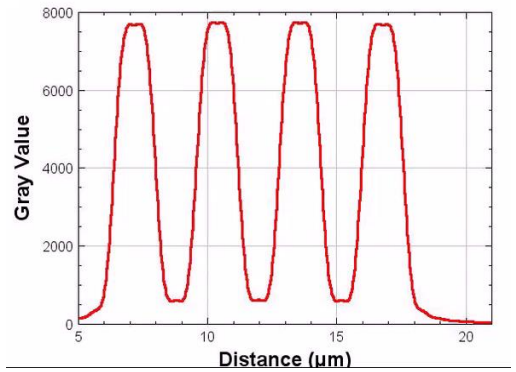
Object

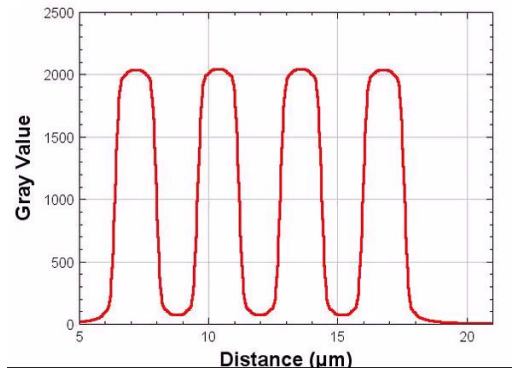




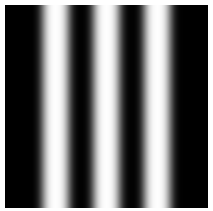
NA 0.4



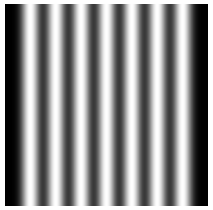
NA 0.8



Modulation Transfer Function

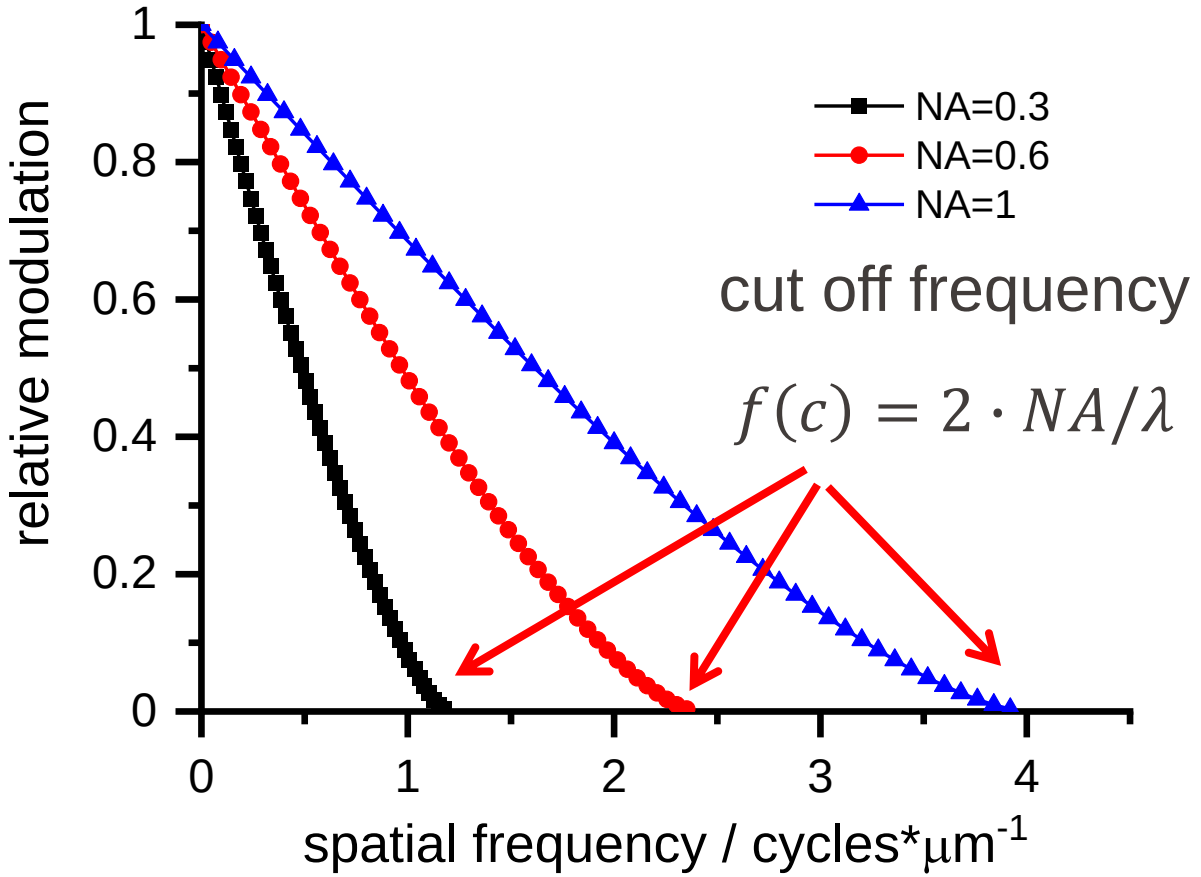


low spatial frequencies
high contrast



medium spatial frequencies
medium contrast

high spatial frequencies
low contrast



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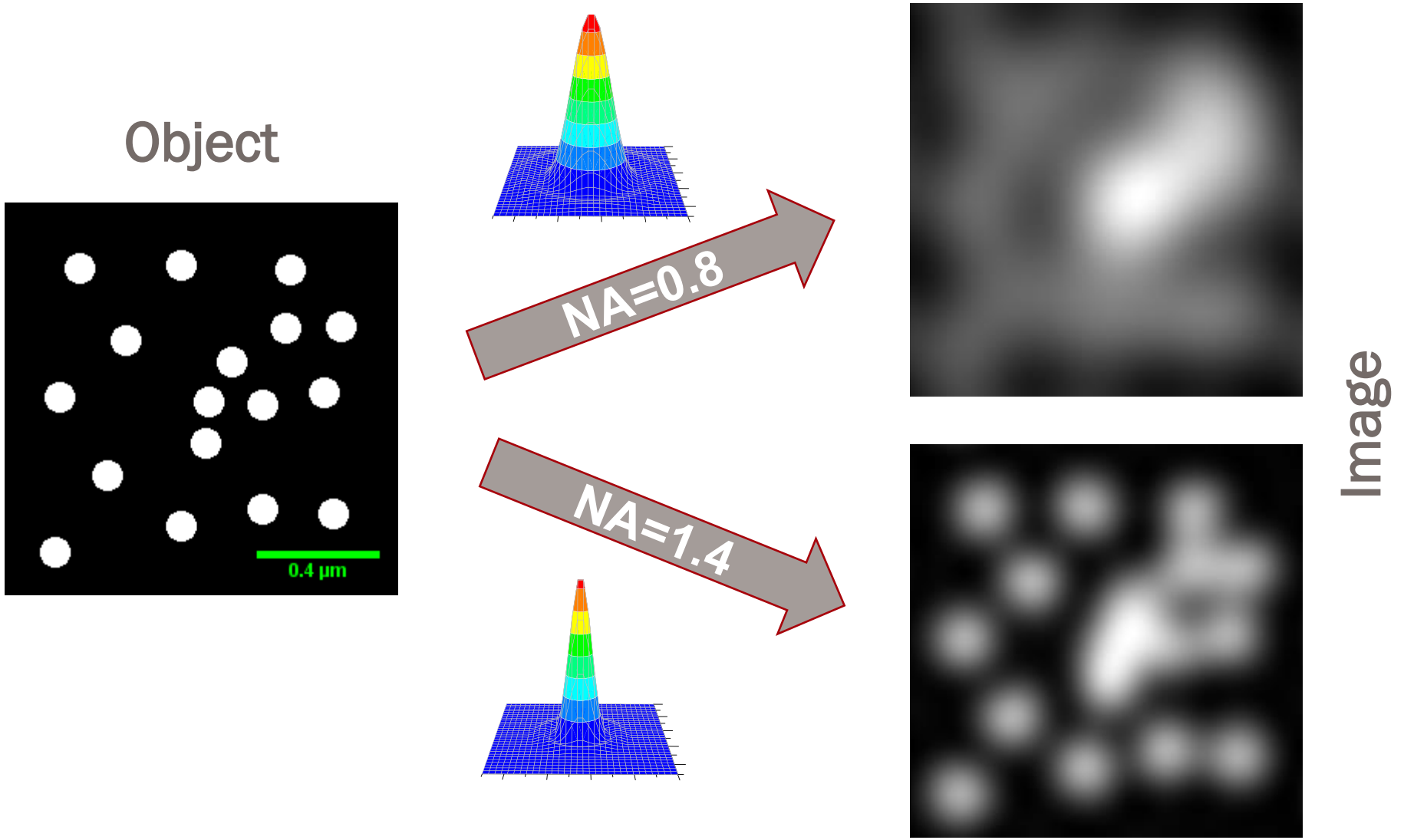
Low pass Filtering



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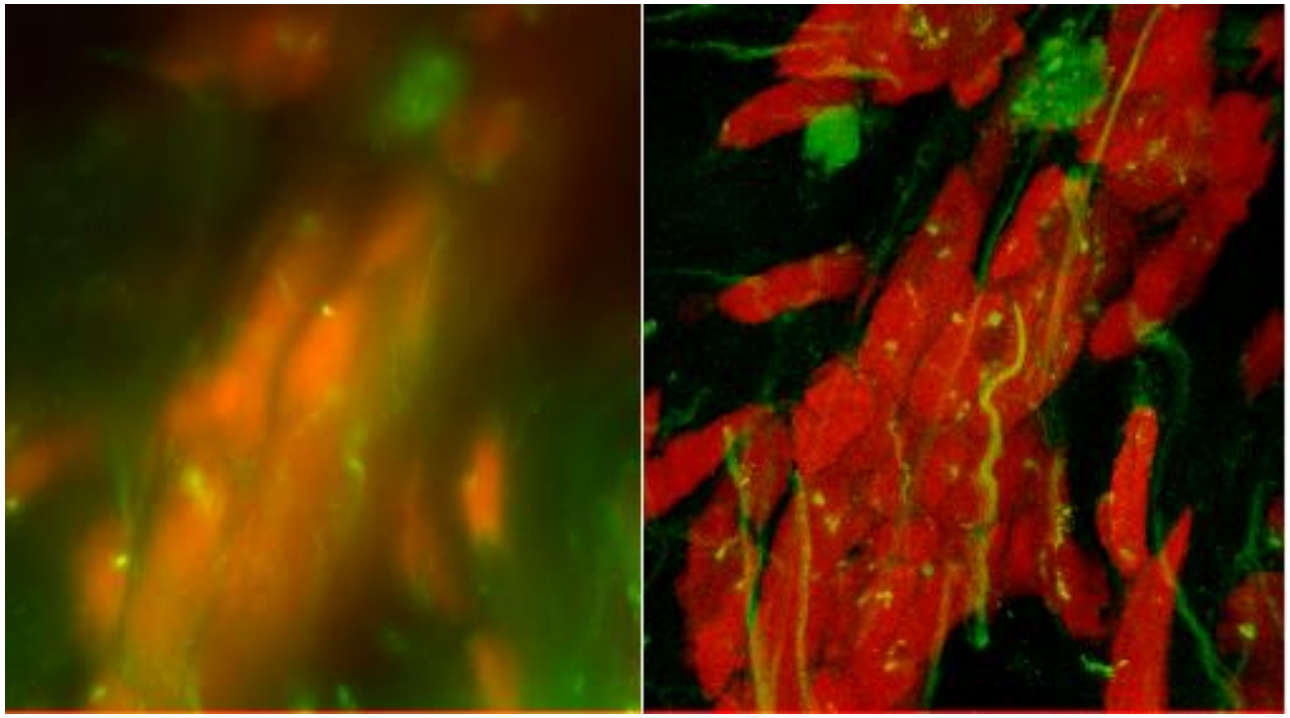
Imaging of thick specimen



wide-field confocal

→ „out of focus“ light is bluring the image and thus reducing the contrast/resolution

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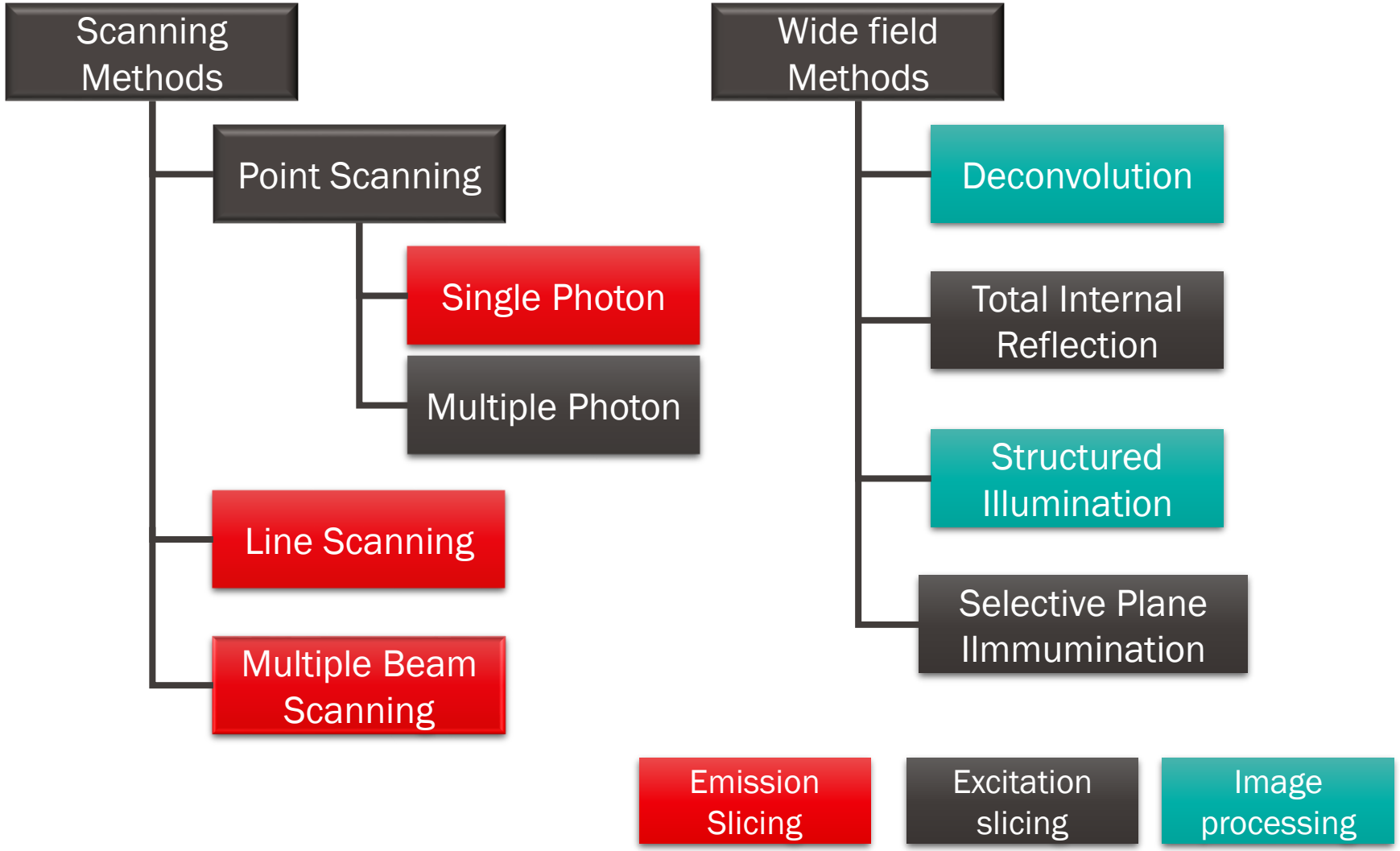
Optical slicing Methods



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Imaging point by point

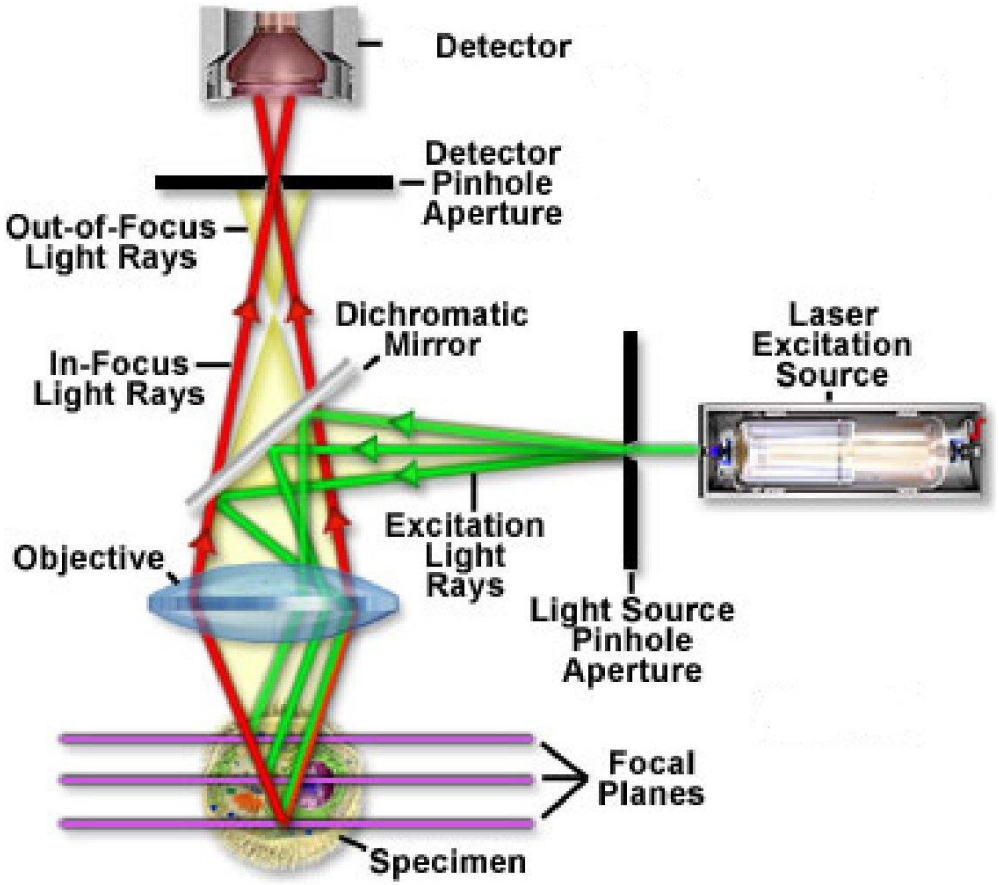
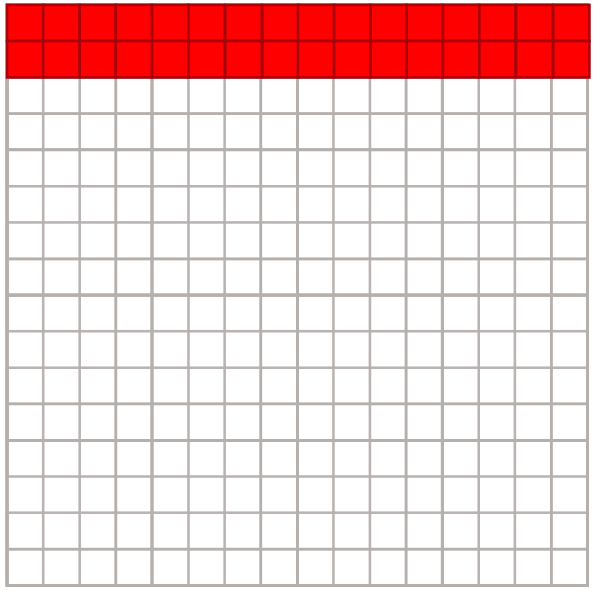


Image formation by reconstruction



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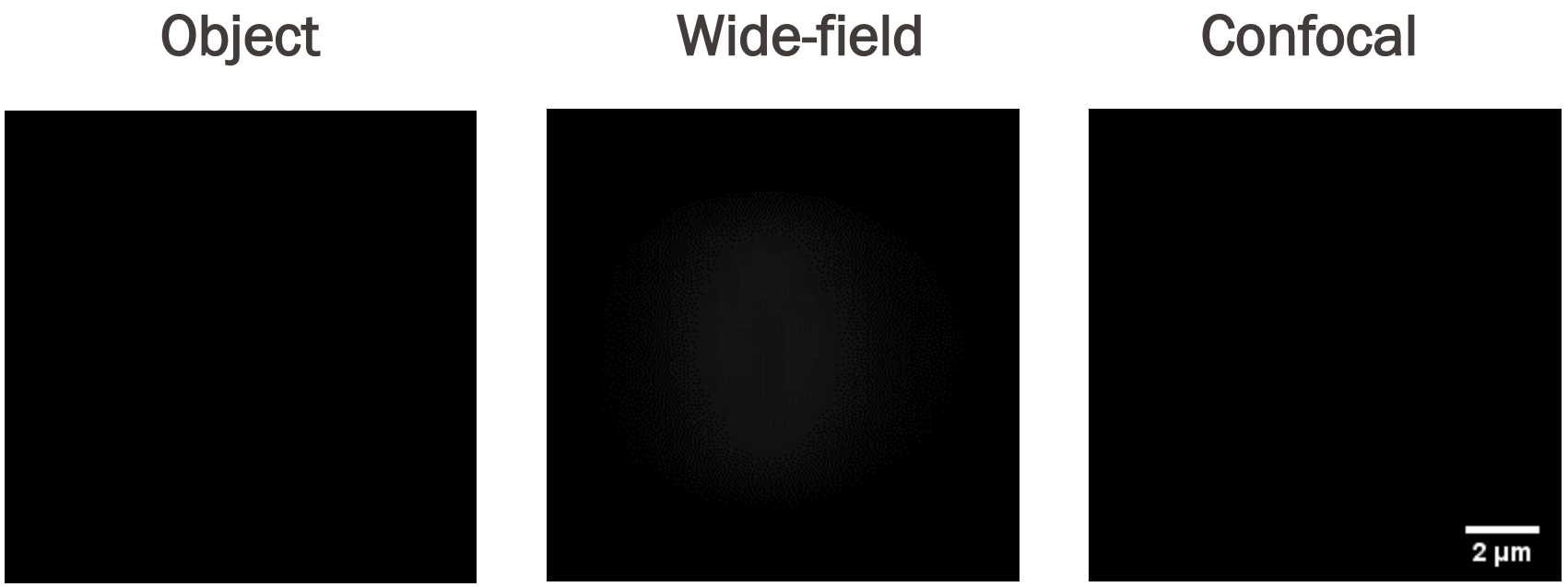
Optical slicing



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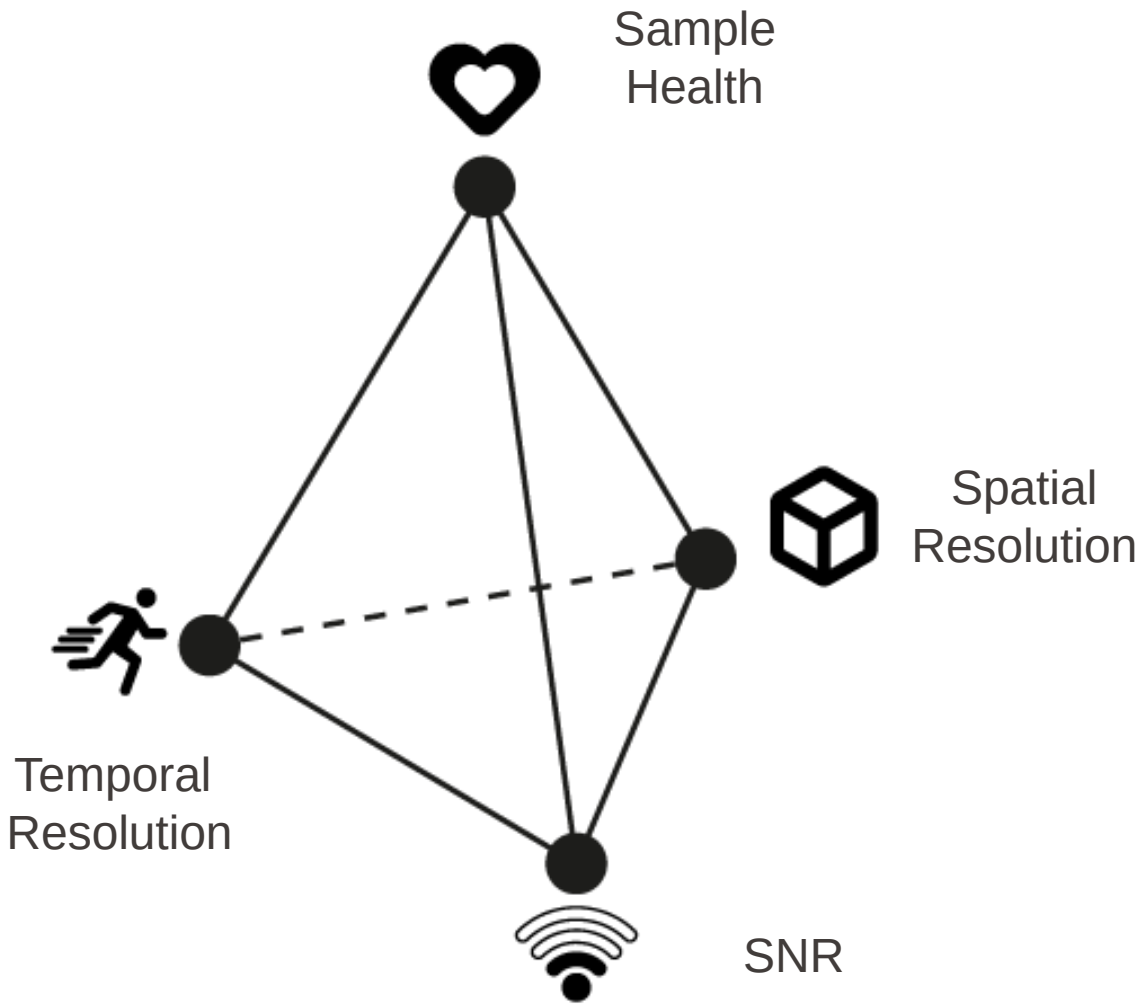
Imaging Frustration Pyramid



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Multiple beam confocal microscopy

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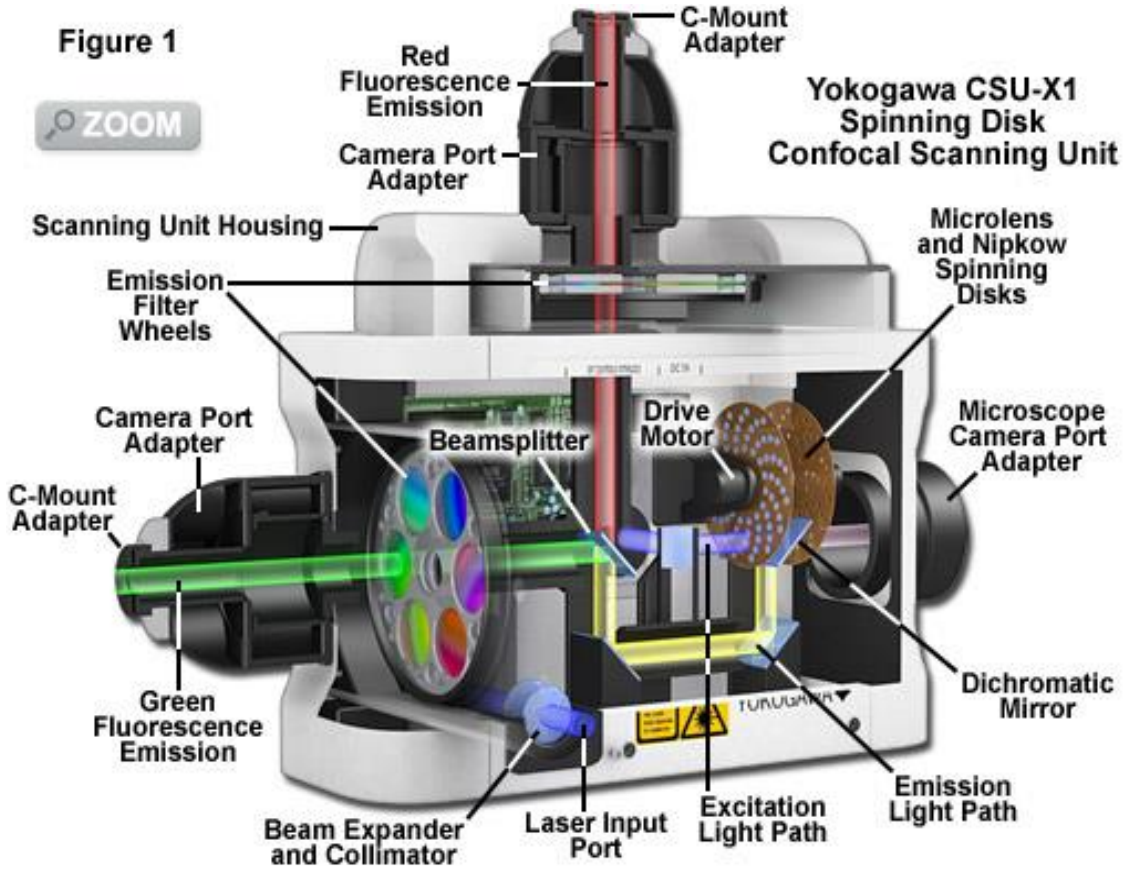
Spinning Disk confocal Microscope



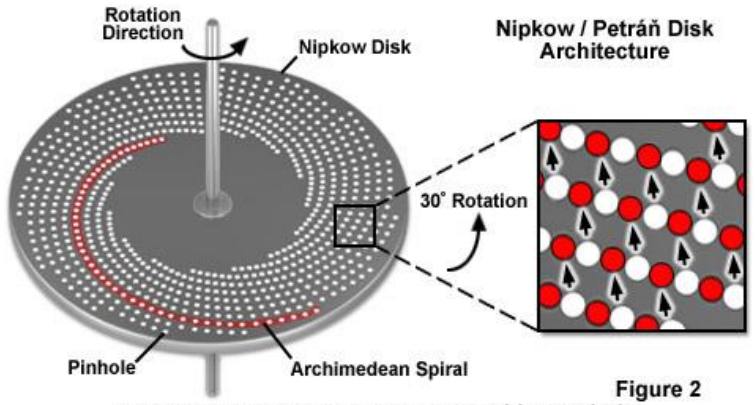
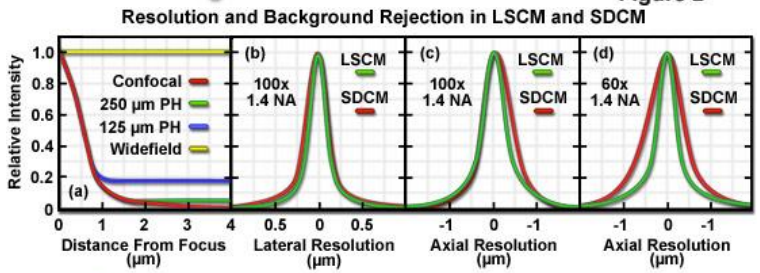
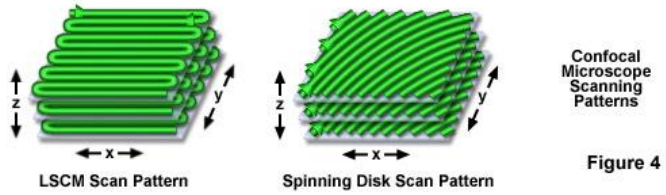


Figure 2

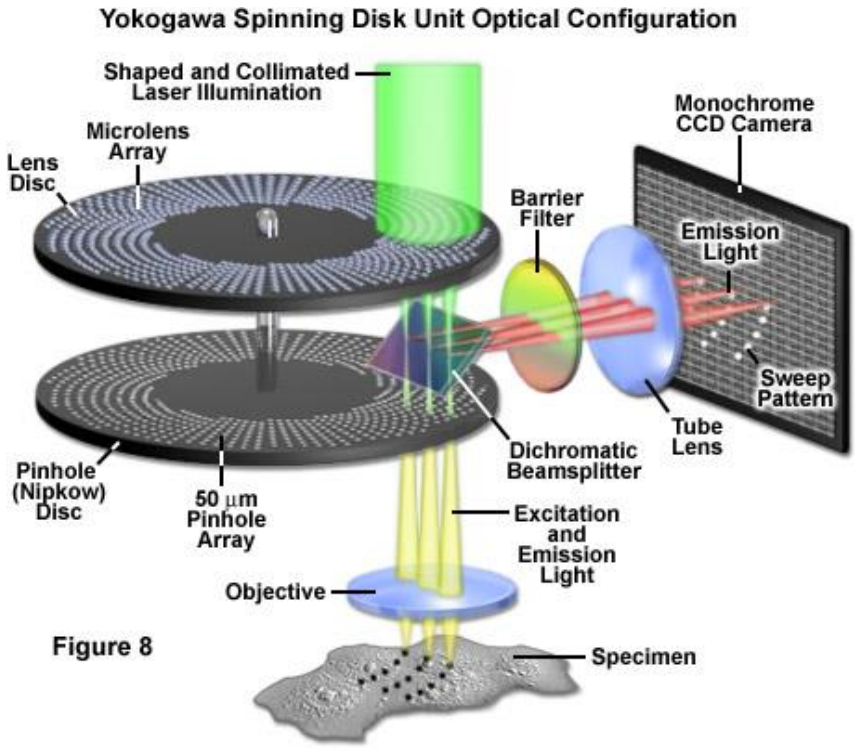




Confocal Microscope Scanning Patterns

Figure 4

Multibeam Confocal Microscopy



- **High quantum yield by CCD camera**
- **High frame rate possible**
- **Low photobleaching/ phototoxicity**

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Light sheet microscopy

Optical Slicing methods

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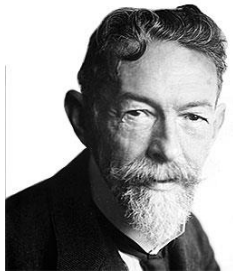
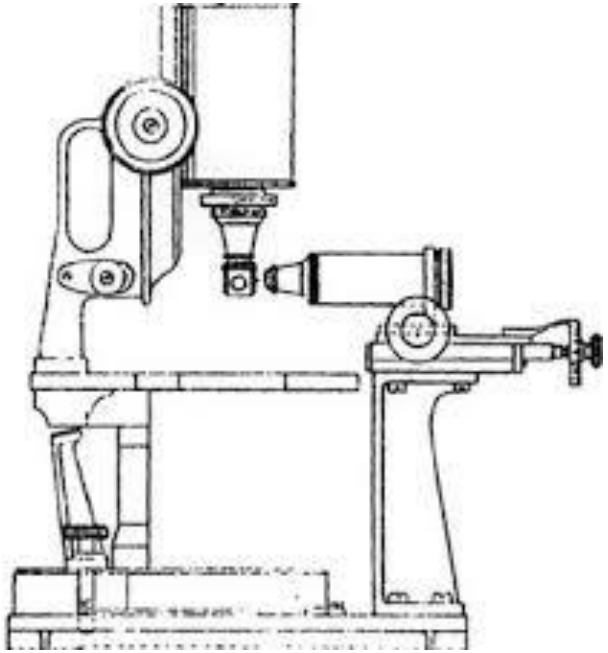
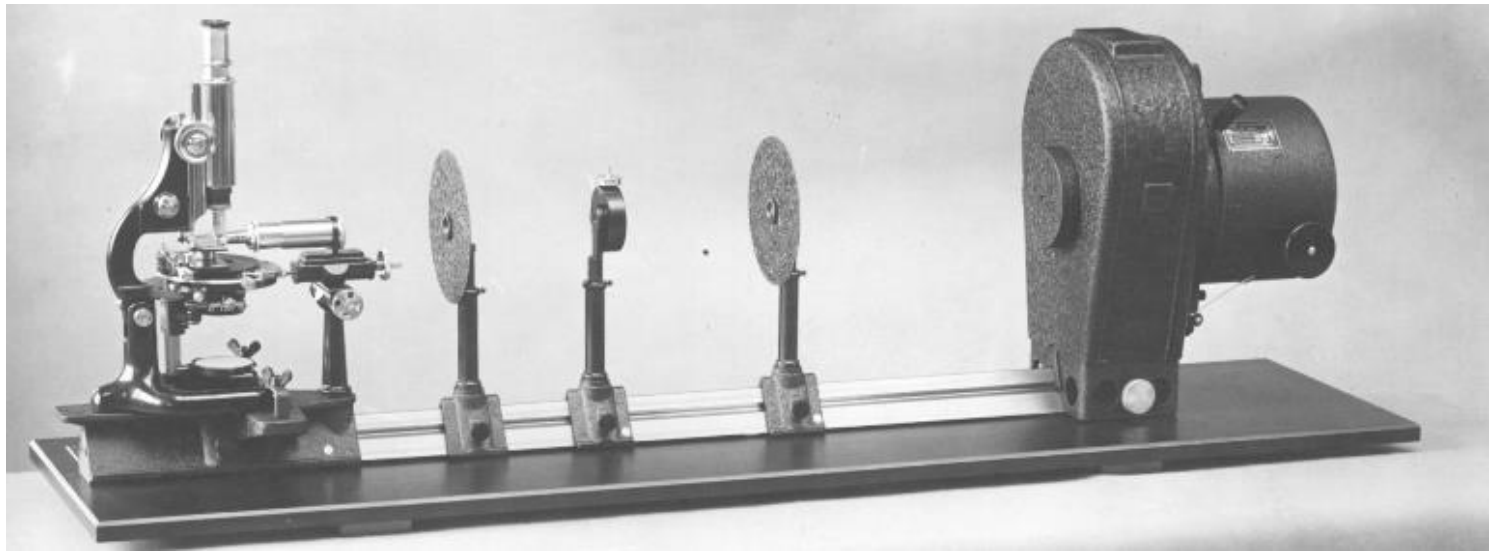
Ultramicroscopy







Siedentopf, H. and Zsigmondy, R. (1902). Über Sichtbarmachung und Größenbestimmung ultramikoskopischer Teilchen, mit besonderer Anwendung auf Goldrubingläser. *Ann. Phys.* 10, 1-39.

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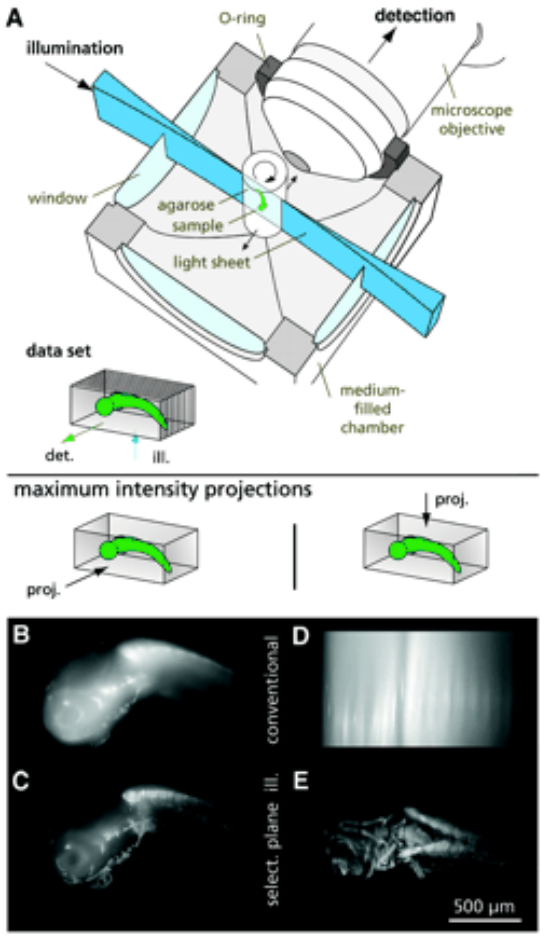
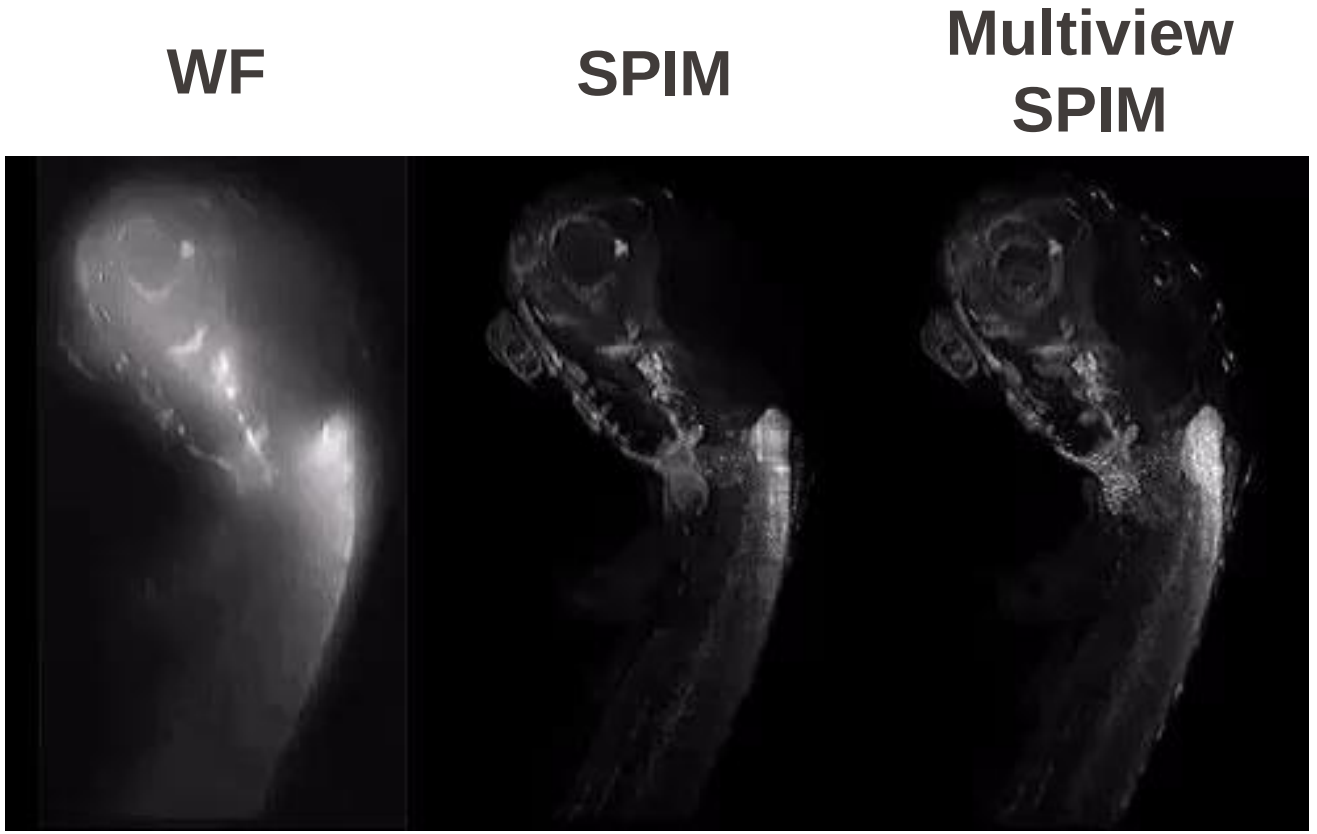
Selective plane illumination microscopy





Huisken, J., Swoger, J., Del Bene, F., Wittbrodt, J. and Stelzer, E. H. K. (2004). Optical sectioning deep inside live embryos by selective plane illumination microscopy. *Science* **305**, 1007-1009.

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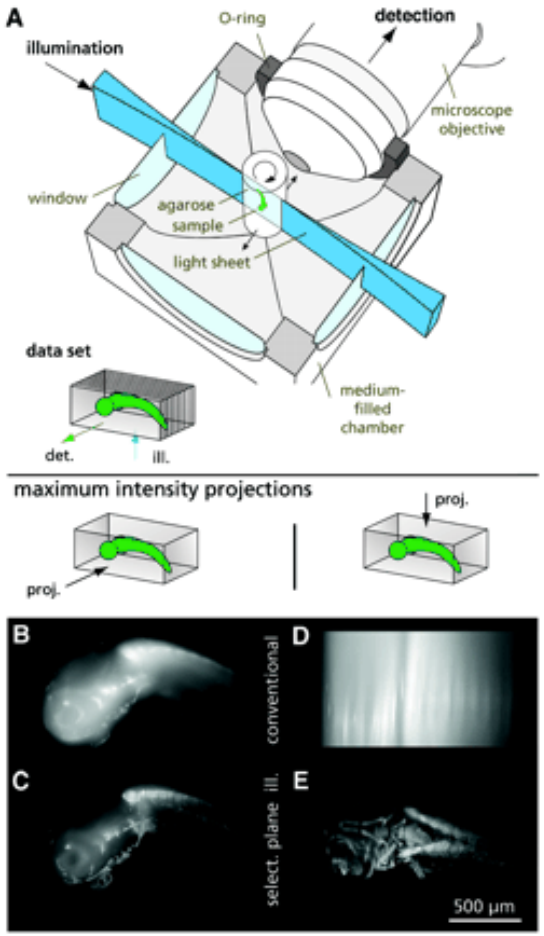
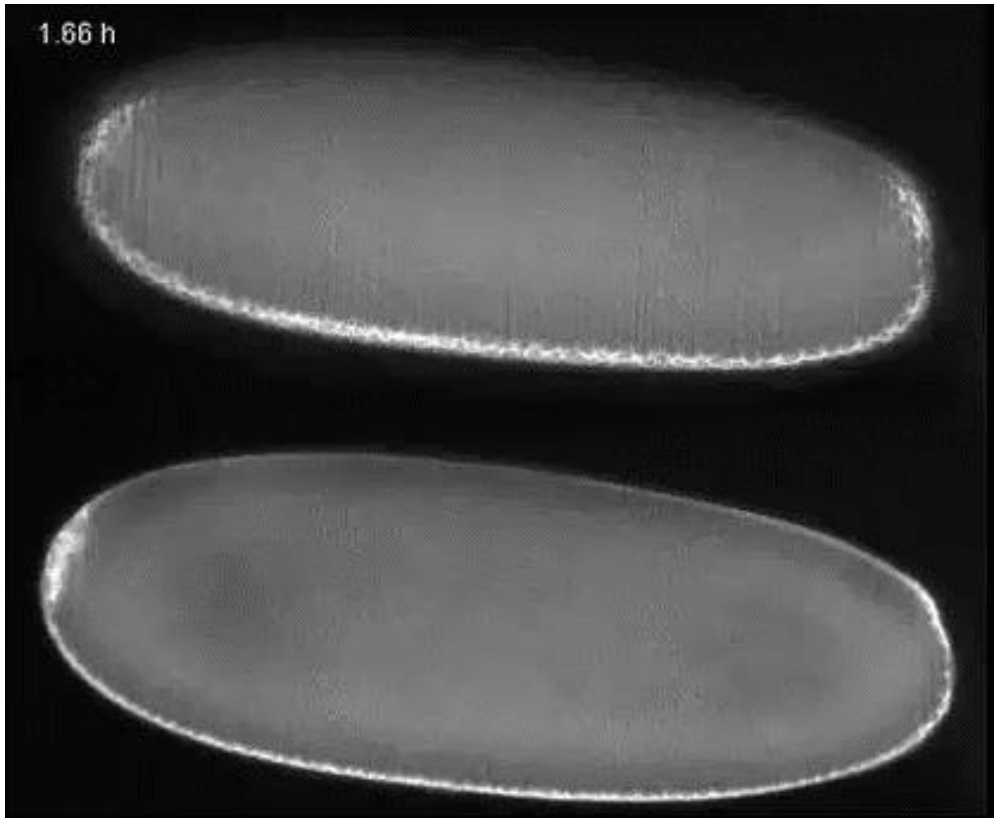
Selective plane illumination microscopy





Huisken, J., Swoger, J., Del Bene, F., Wittbrodt, J. and Stelzer, E. H. K. (2004). Optical sectioning deep inside live embryos by selective plane illumination microscopy. *Science* **305**, 1007-1009.

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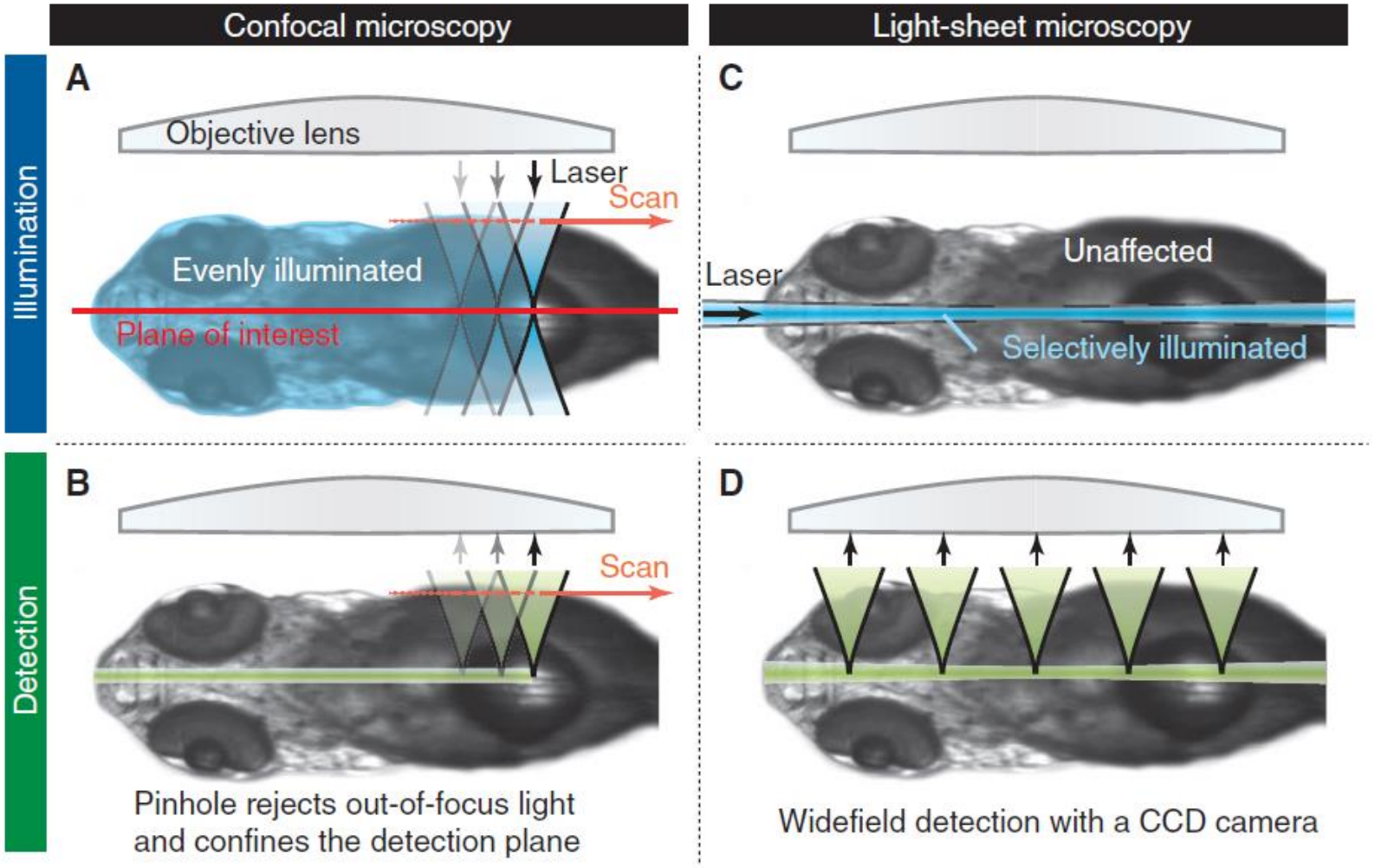
Light Sheet Microscopy



Huisken J, Stainier DY. (2009) Selective plane illumination microscopy techniques in developmental biology. *Development*. **136**(12):1963-75.

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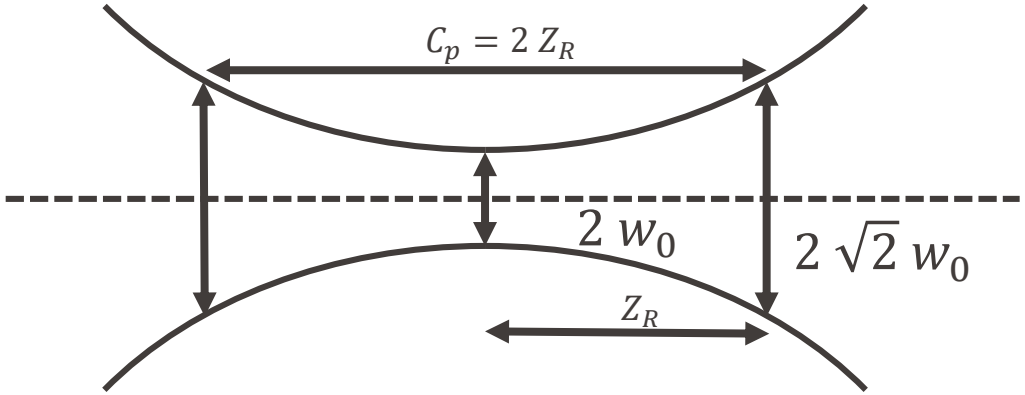
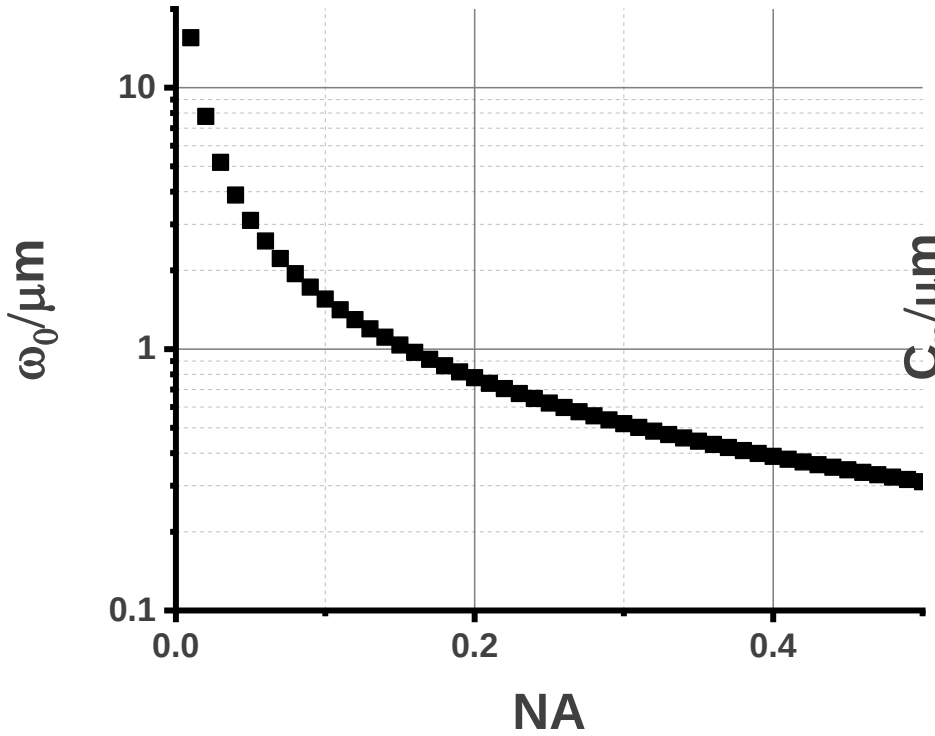
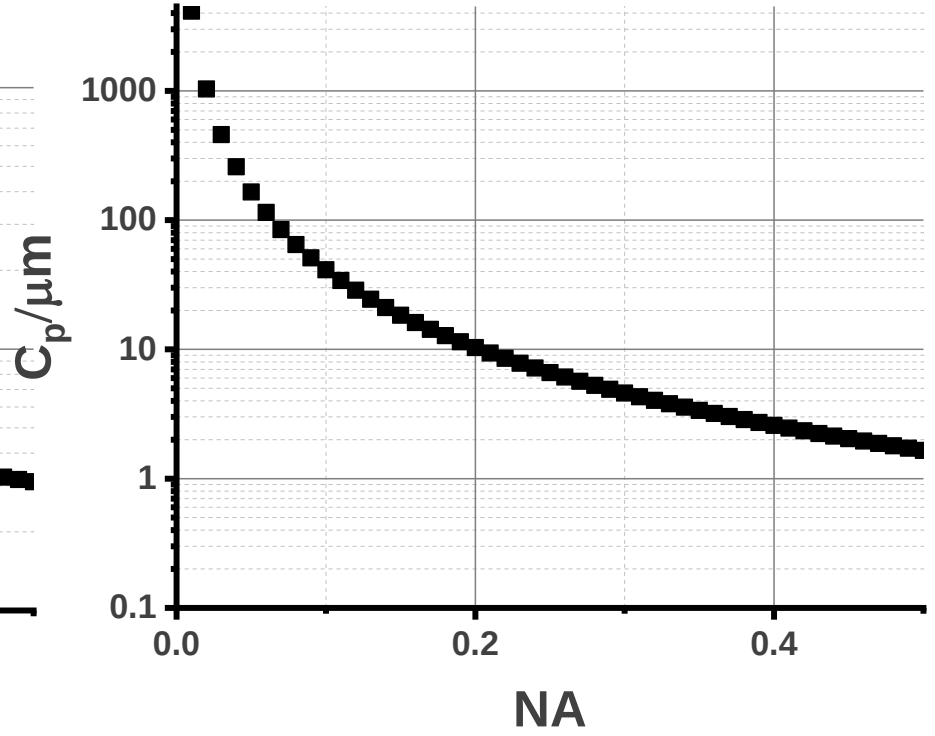
Gaussian Beam







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Advanced Light Sheet Microscopy

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Bessel beam

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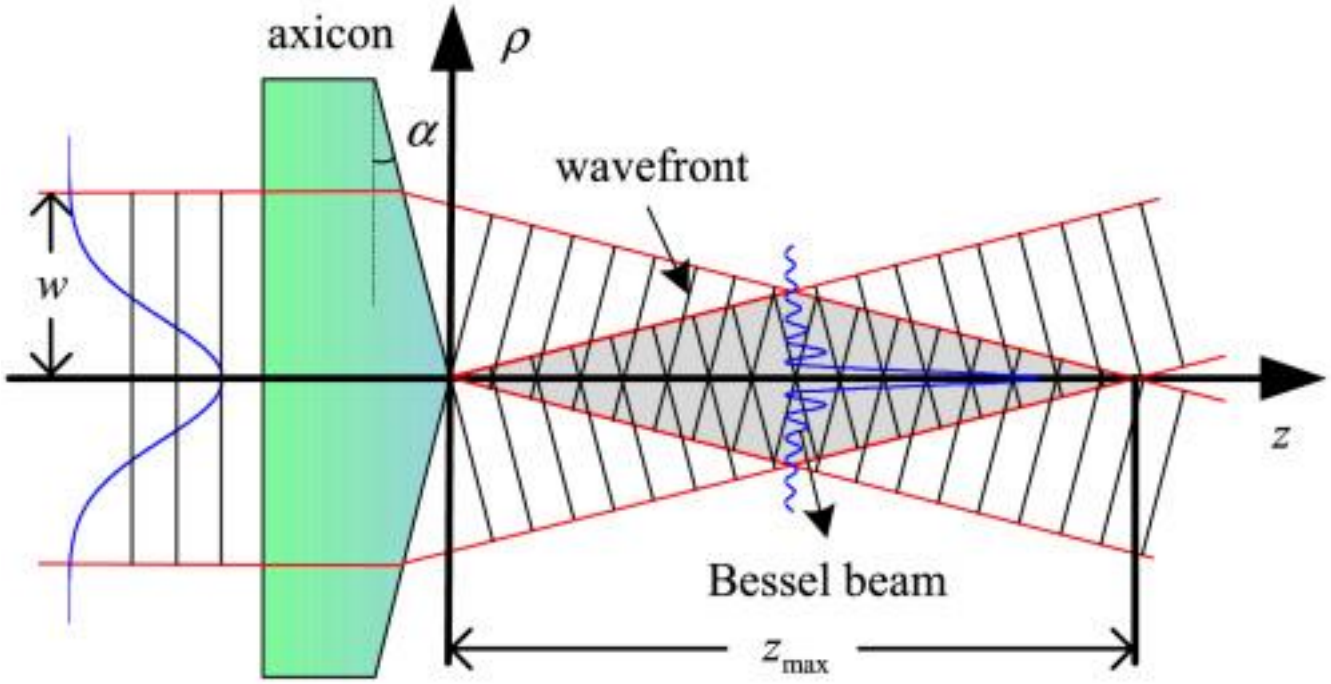
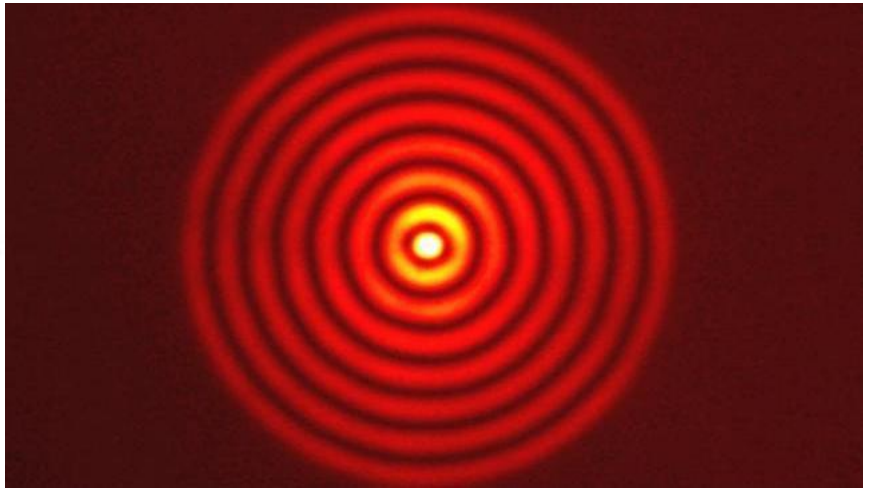


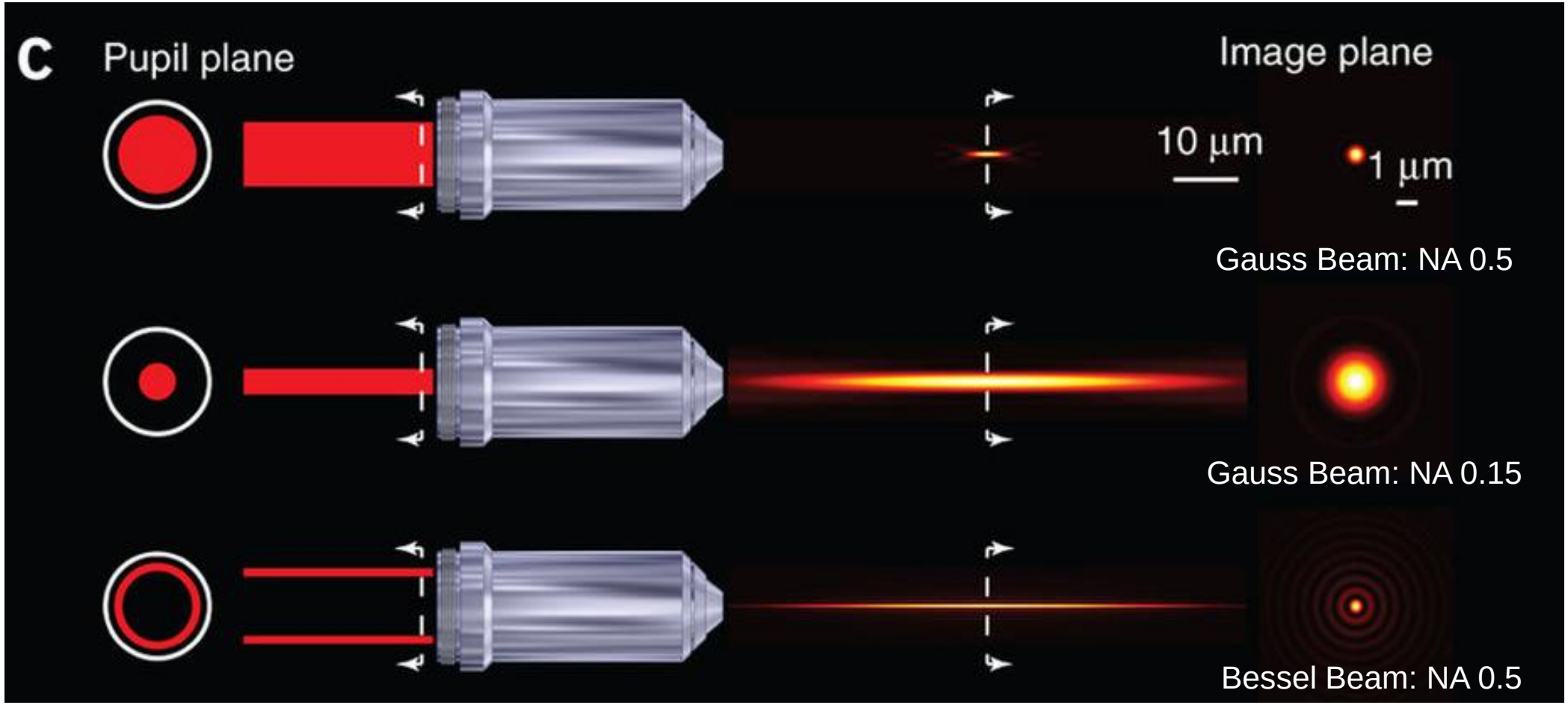


Photonics Research Vol. 2, Issue 3, pp. 82-86 (2014)
https://doi.org/10.1364/PRJ.2.000082

Ideal Bessel beam:

- non-diffractive
- self-healing

Gauss beam vs. Bessel beam



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Rapid Isotropic Imaging

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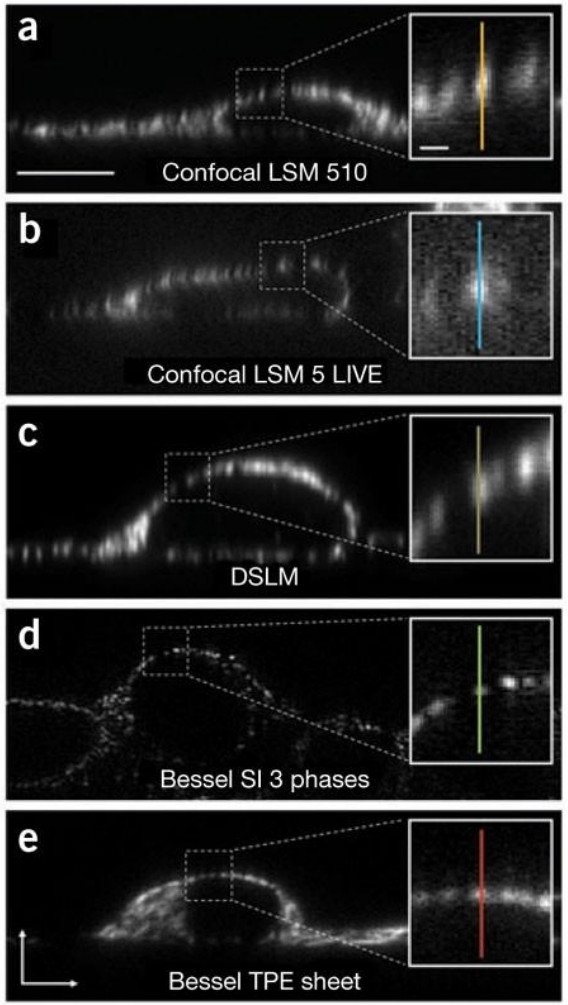
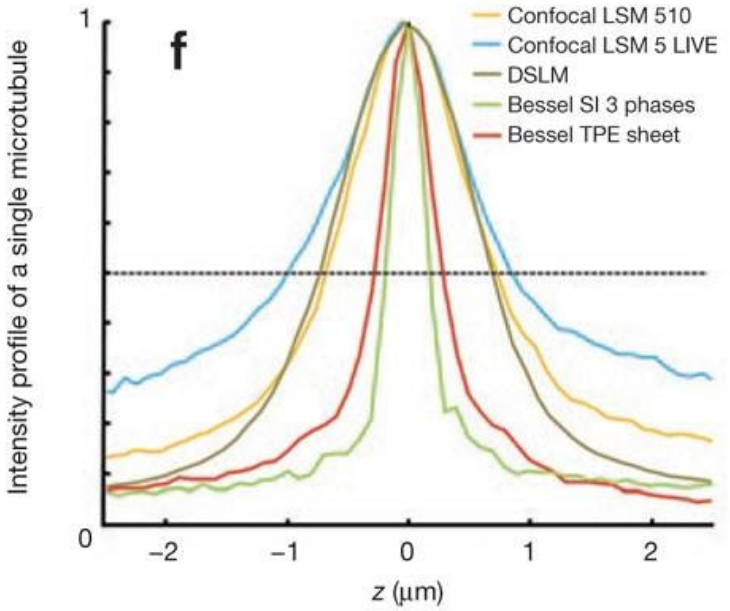
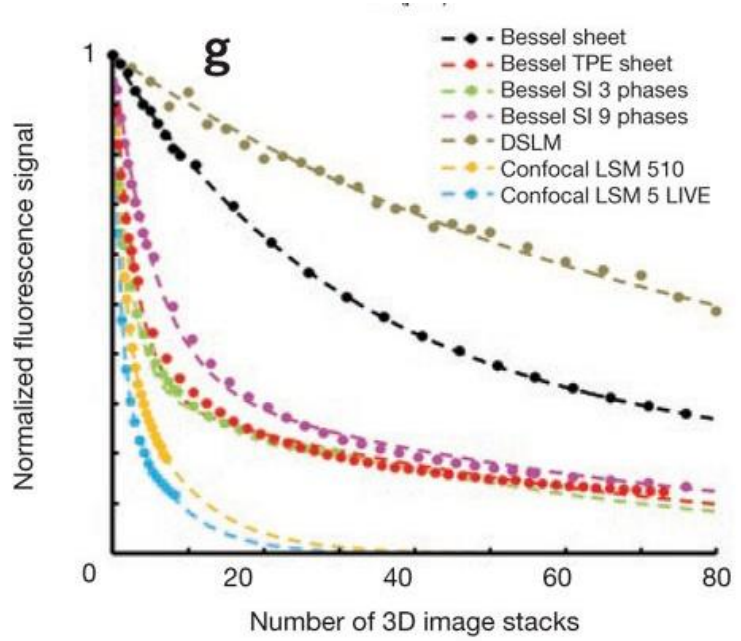
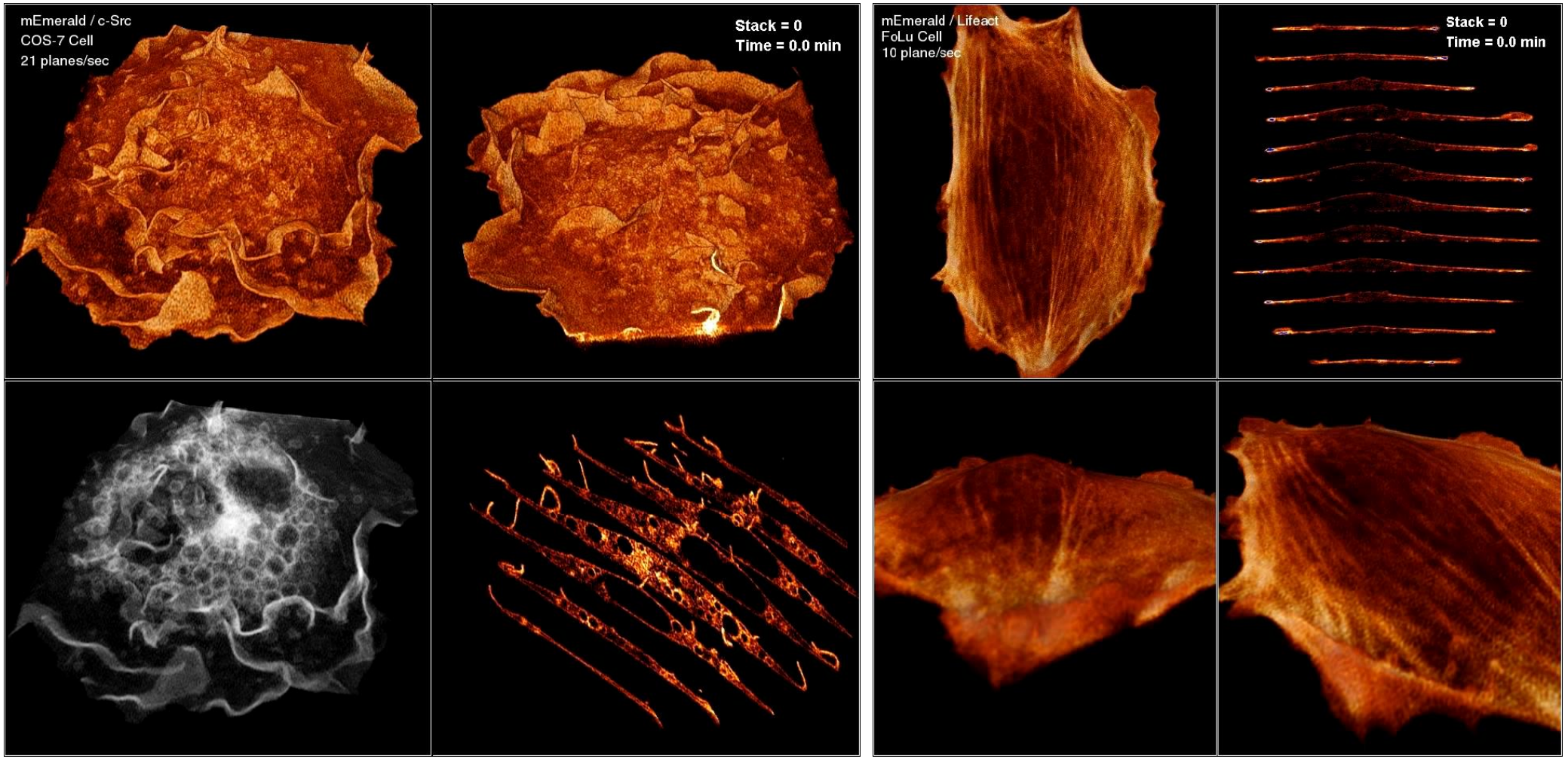






Thomas A Planchon, Liang Gao, Daniel E Milkie, Michael W Davidson, James A Galbraith, Catherine G Galbraith & Eric Betzig (2011) Rapid three-dimensional isotropic imaging of living cells using Bessel beam plane illumination *Nature Methods* (8): 417–423.

Need for Speed



Gao L, Shao L, Higgins CD, Poulton JS, Peifer M, Davidson MW, Wu X, Goldstein B, Betzig E. (2012) Noninvasive imaging beyond the diffraction limit of 3D dynamics in thickly fluorescent specimens. *Cell* 151(6):1370-85.

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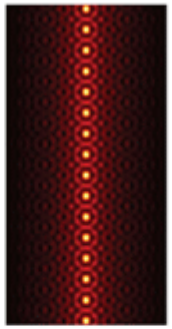
Bessel beam light sheet microscopy

Operation modes	Bessel beam plane illumination	Bessel SIM	TP Bessel beam plane illumination	TP Bessel SIM
Illumination pattern (*xz* cross-section)				
Typical spatial resolution	*x* ~230 nm	*x* ~180 nm	*x* ~230 nm	*x* ~180 nm
	y ~230 nm	*y* ~230 nm	*y* ~230 nm	*y* ~230 nm
	z ~600 nm	*z* ~350 nm	*z* ~450 nm	*z* ~450 nm
Optical sectioning capability	Fair	Good	Excellent	Excellent
Typical imaging speed	~40 planes/s	10–20 planes/s	~40 planes/s	10–20 planes/s
Multicolor imaging	Yes	Yes	No	No
Fluorophore brightness requirement	Low	Low	High	High
Photodamage	Low	Fair	Low	High

Liang Gao, Lin Shao, Bi-Chang Chen & Eric Betzig (2014) 3D live fluorescence imaging of cellular dynamics using Bessel beam plane illumination microscopy *Nature Protocols* (9): 1083–1101.

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Extending the resolution

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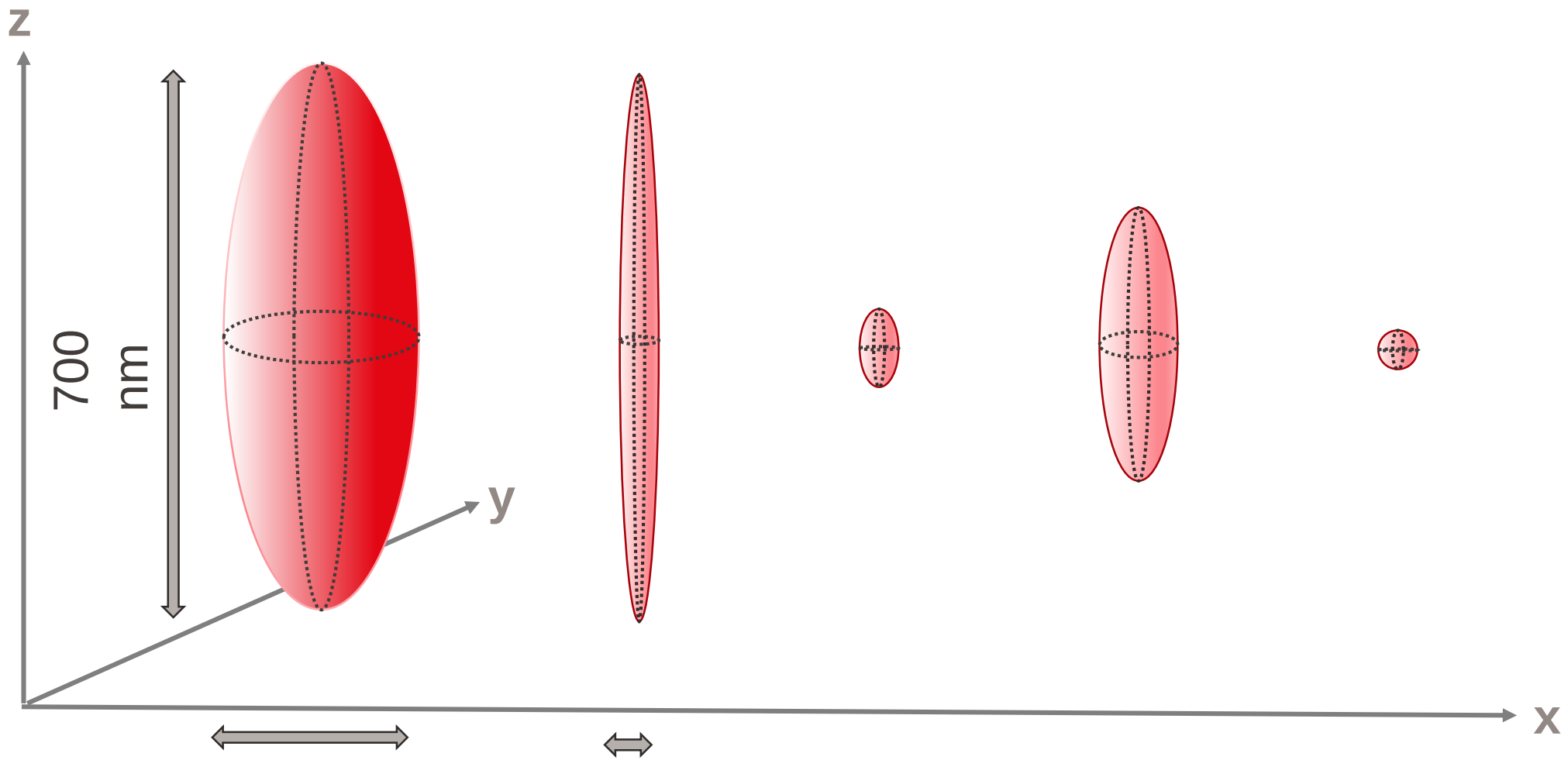
Resolution in light microscopy



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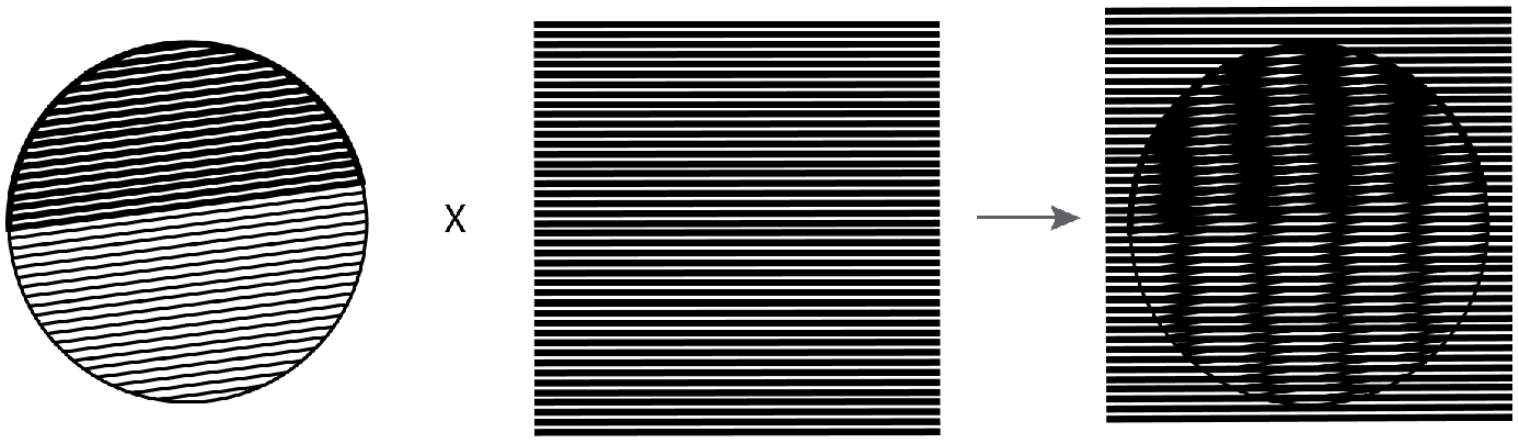
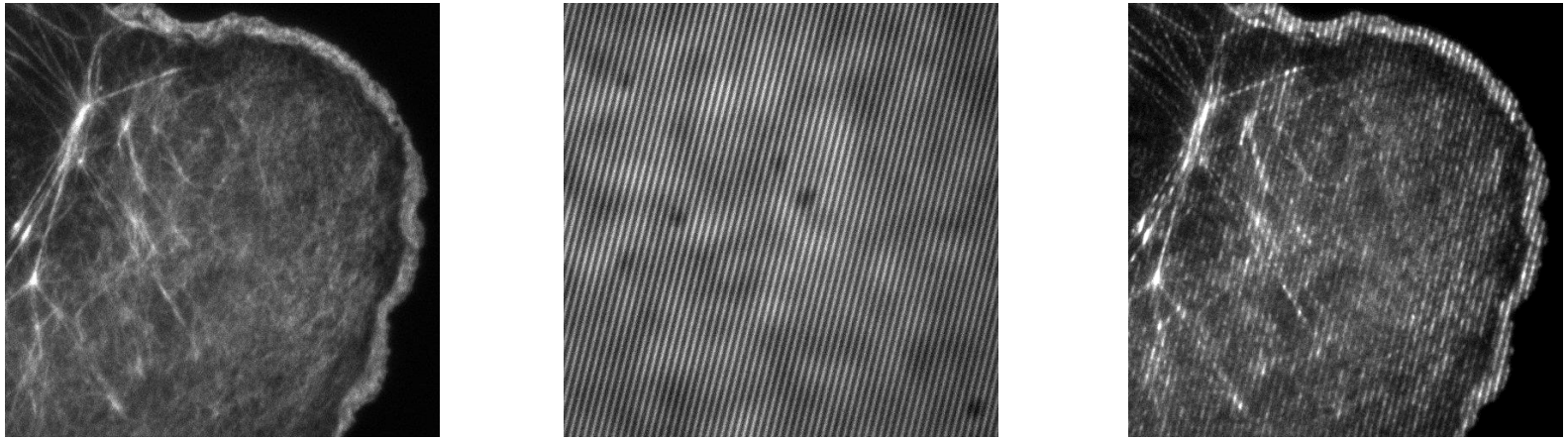
How to overcome the diffraction limit



Sample Illumination pattern Moiré fringes



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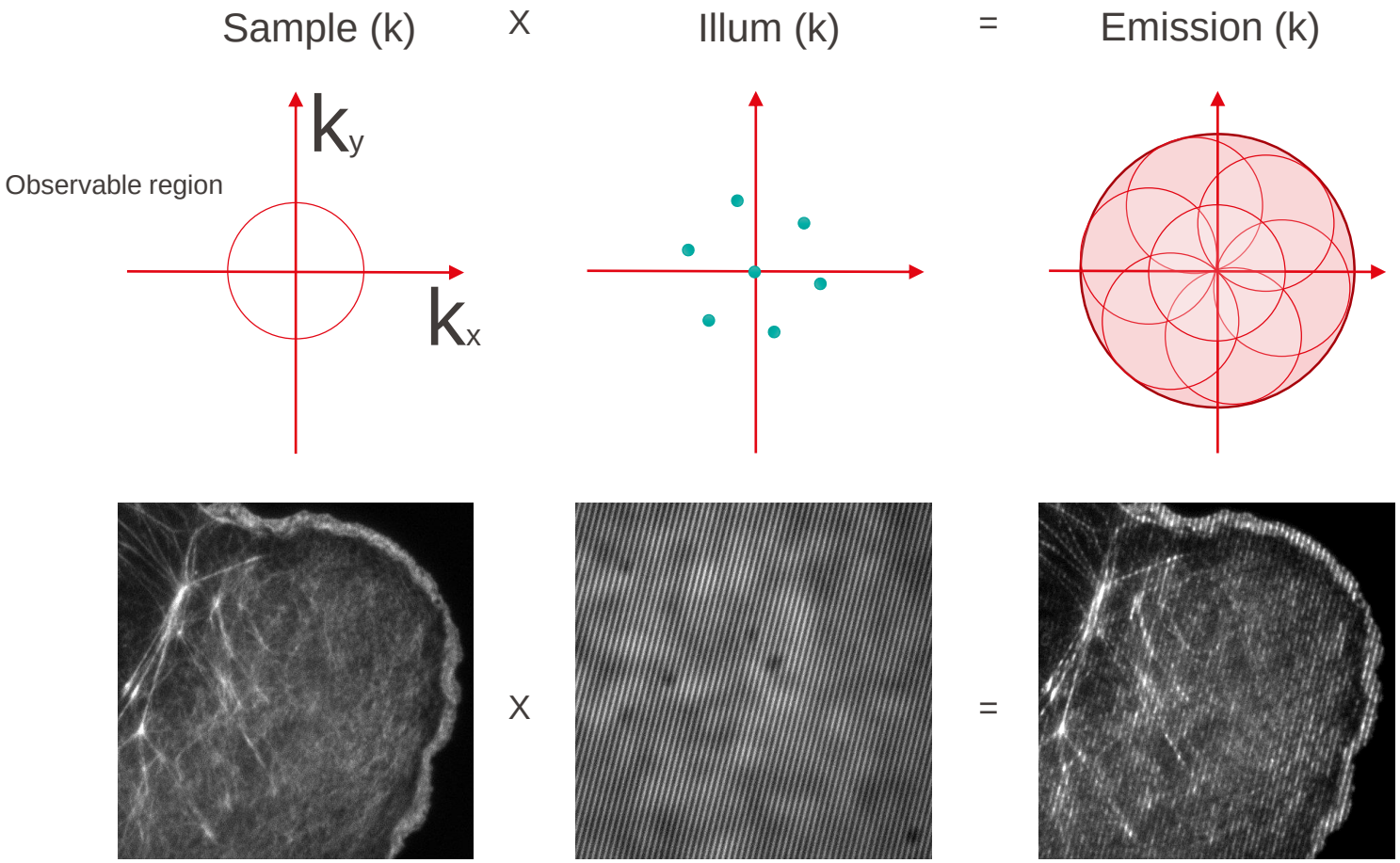
The Frequency Domain



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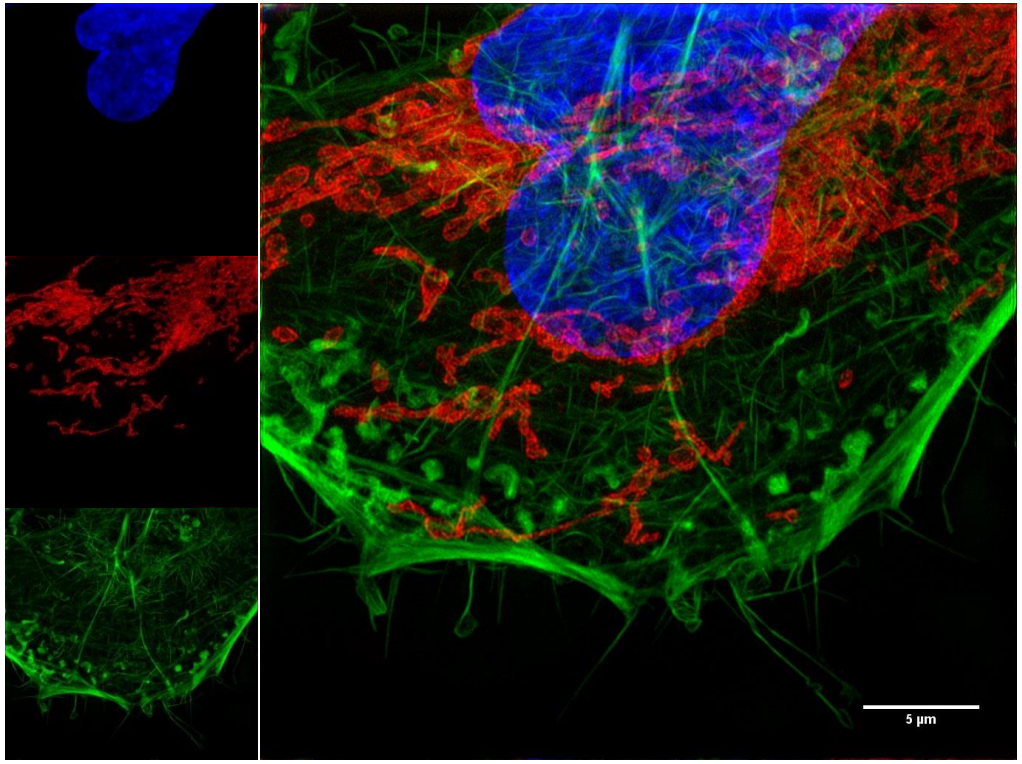
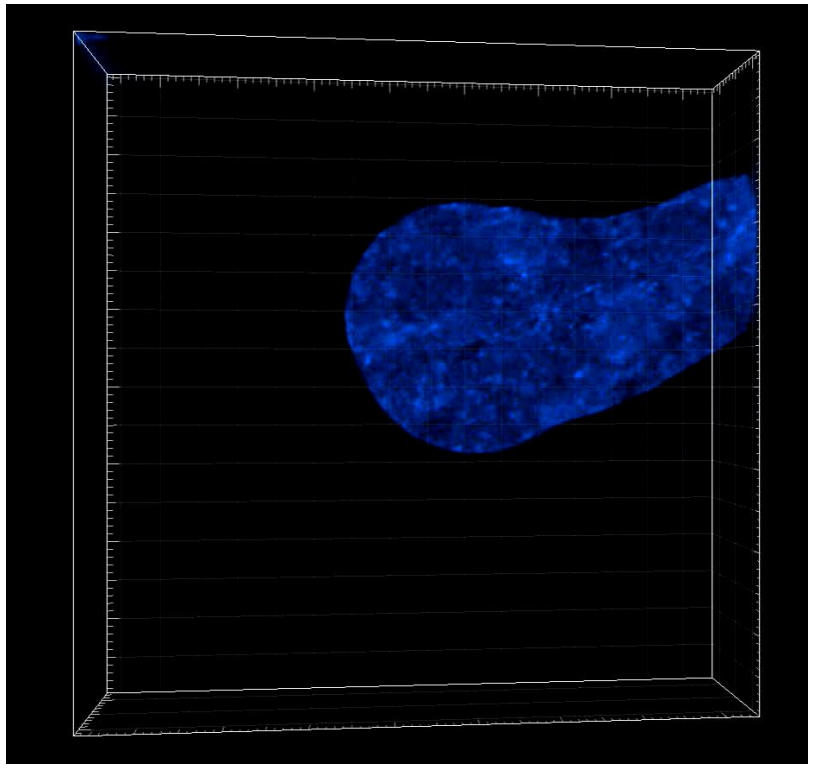
MultiColor 3D-SIM





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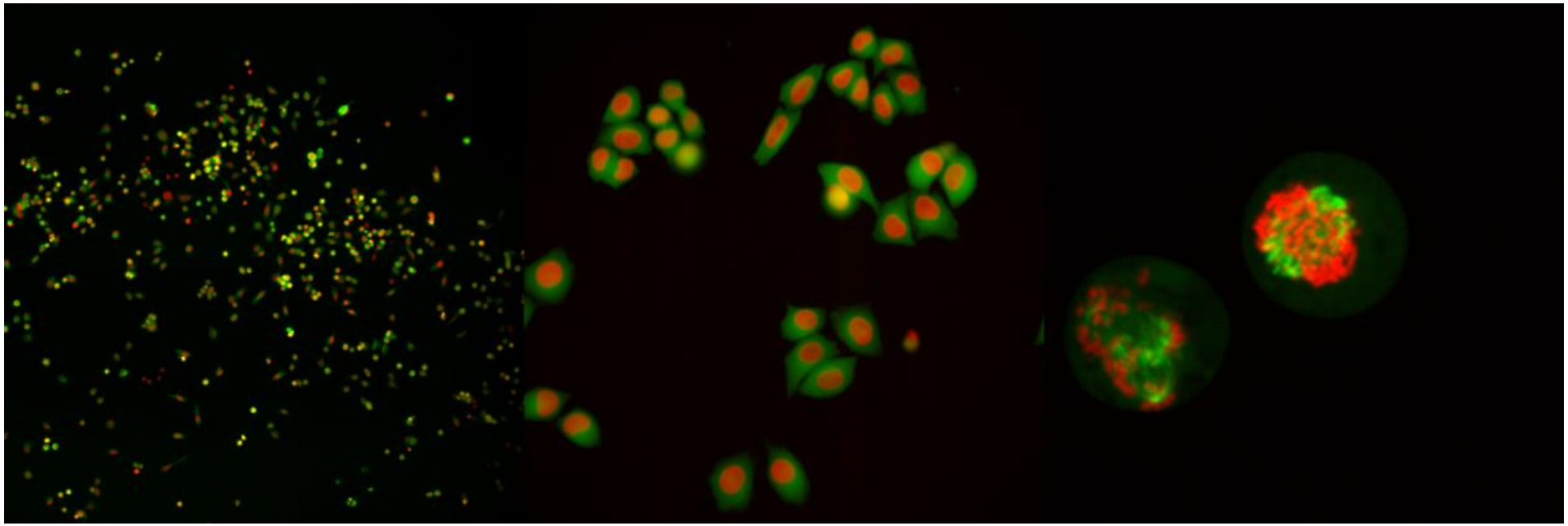
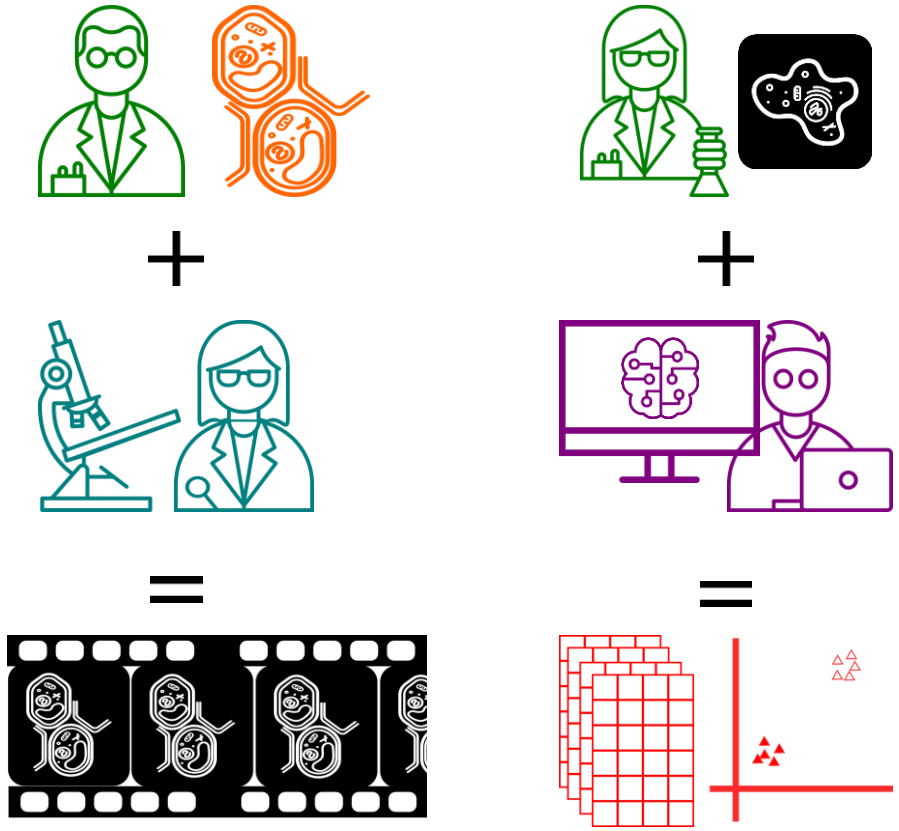
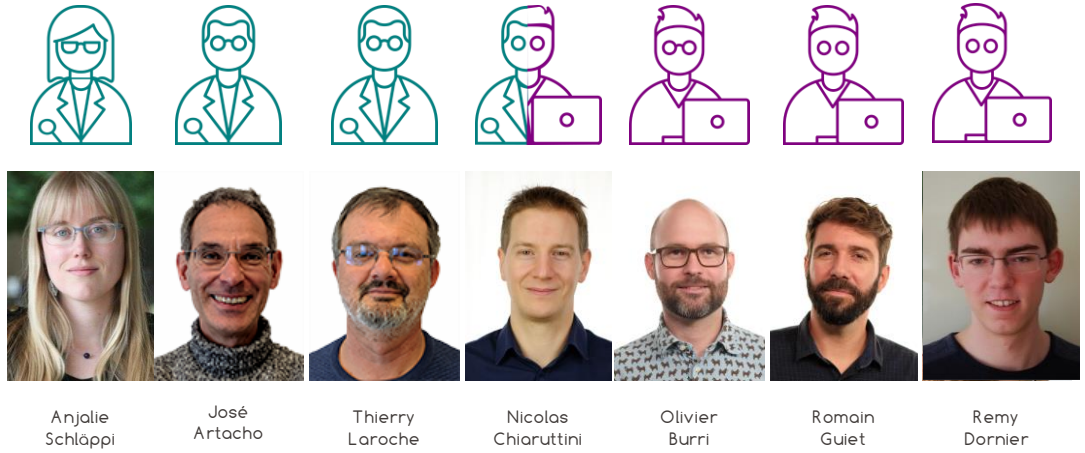
Live cell imaging



Balancing sample integrity, resolution, noise, temporal and spatial resolution.

BioImaging & Optics Platform (BIOP)





Anjalie Schläppi | José Artacho | Thierry Laroche | Nicolas Chiaruttini | Olivier Burri | Romain Guiet | Remy Dornier

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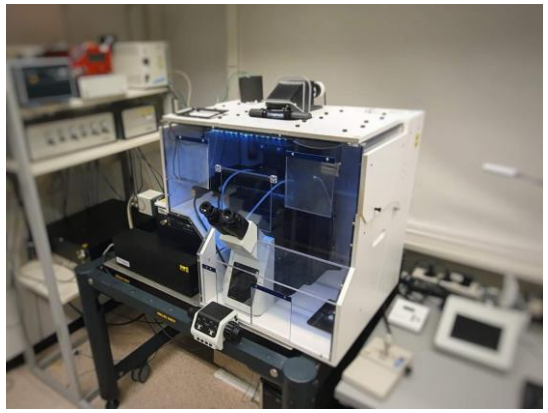
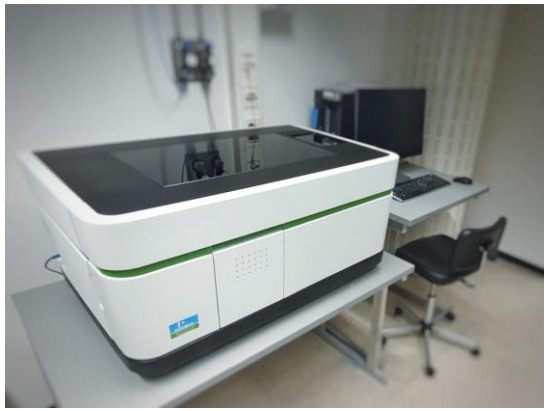
BioImaging & Optics Platform

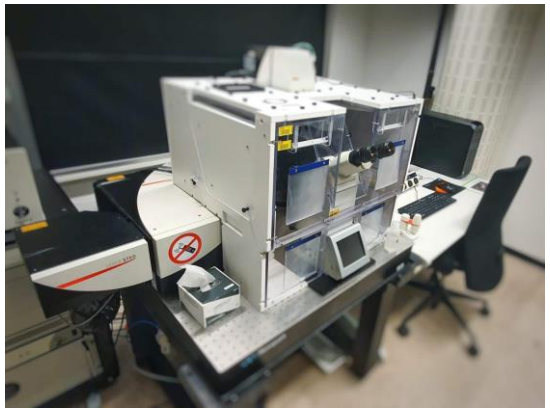
a.k.a BIOP

Microscope	Technique	Light Source	Detector	Characteristics	Contrast	Tutorial
Leica DM5500						
Leica SP8 FLIM						
Leica SP8 STED 3X						
Leica SP8 IN1						
Leica SP8 UP1						
Leica SP8 UP2						
Leica Stereomicroscope						
Nikon SIM/STORM						SIM STORM Calib
Nikon Stereology						
Olympus Cell xCellence						
Olympus Slide Scanner 1						
Olympus Slide Scanner 2						
PerkinElmer Operetta						
Labview CSU Microdissection						
Nikon CSU W1						
Visitron CSU-W1						
Zeiss Axioplan						
Zeiss LSM 700 IN1						
Zeiss LSM 700 IN2						
Zeiss LSM 700 UP2						
Zeiss LSM 710						
Zeiss LSM 980						
Zeiss Lattice Lightsheet 7						
Zeiss Lightsheet Z1						
Zeiss PALM Microbeam						

Microscopes







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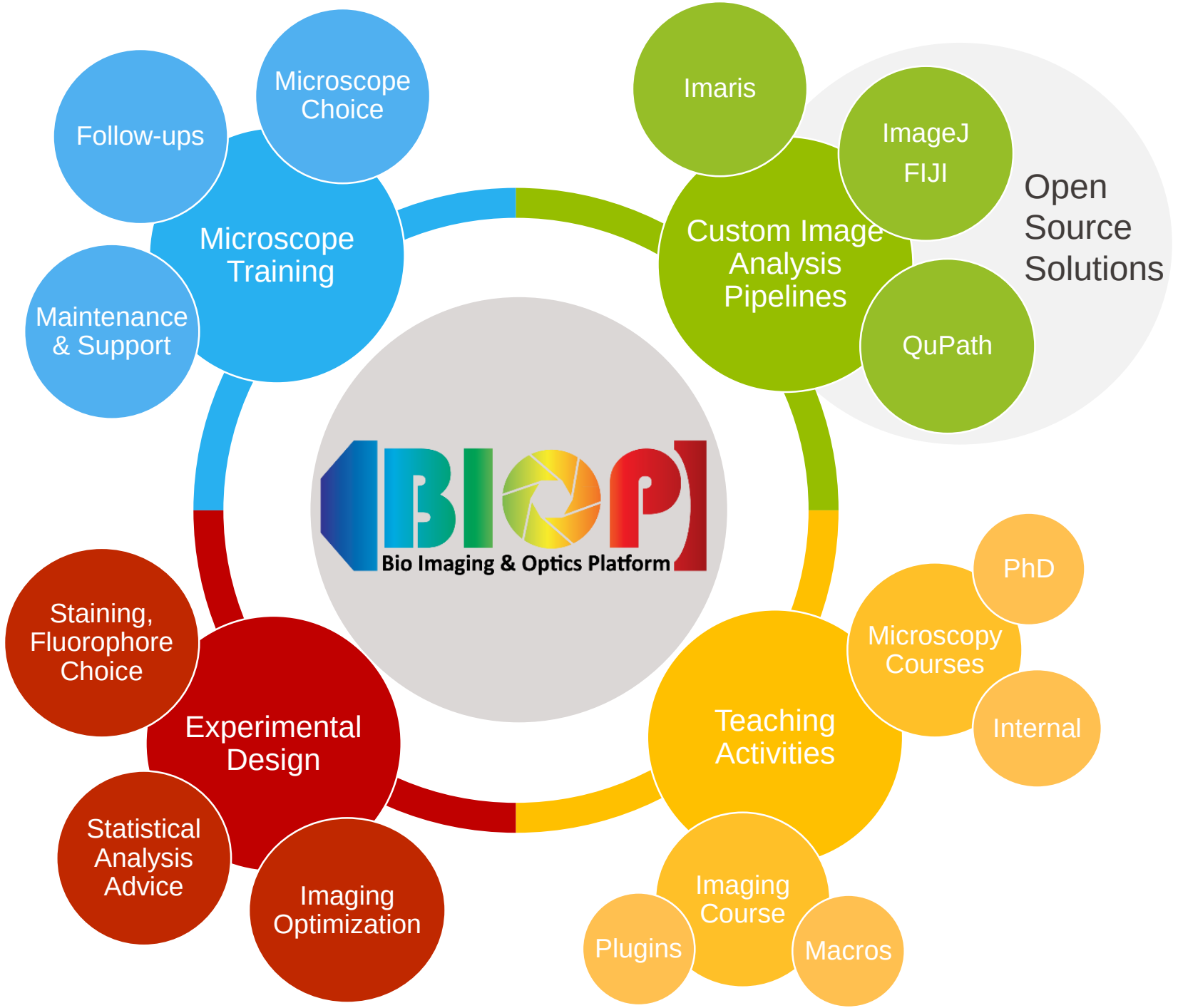

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