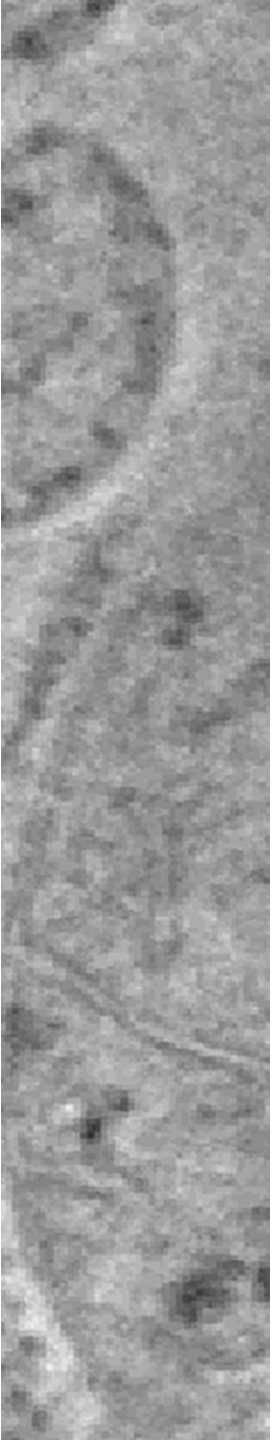


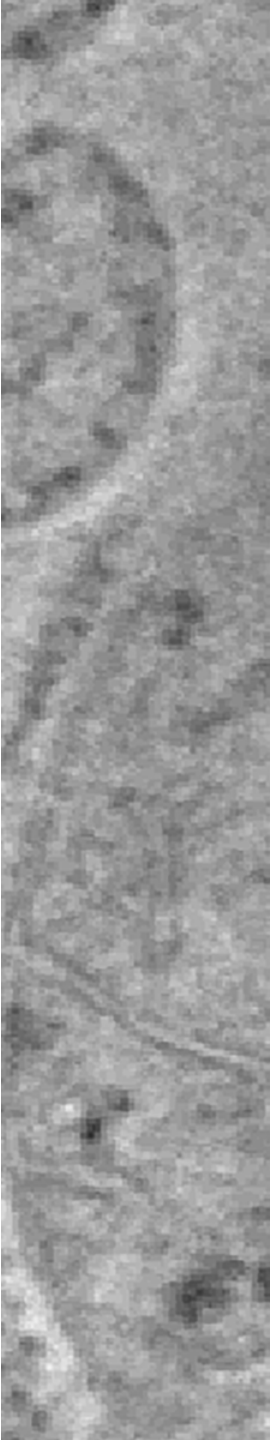
IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

Light Microscopy, Super Resolution Microscopy and Correlative Light and Electron Microscopy

Dr. Tali Dadosh
Electron Microscopy Unit
Weizmann Institute of Science

26 November 2020

מכון ויצמן למדע
WEIZMANN INSTITUTE OF SCIENCE



Outline

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

- Introduction to light microscopy
- Fluorescence microscopy
- Super resolution microscopy
- Correlative light and electron microscopy

Robert Hooke, “the father of microscopy” (1665)

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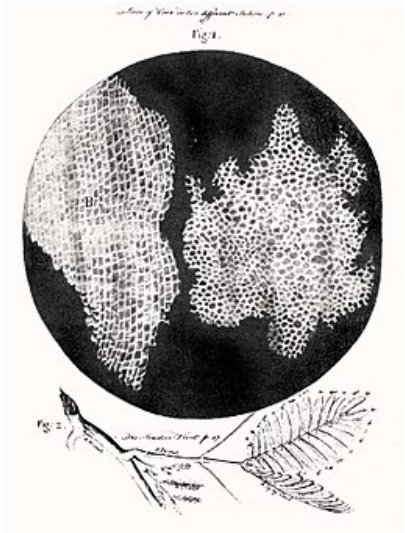
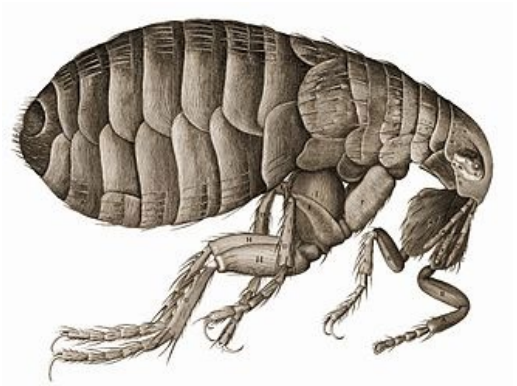
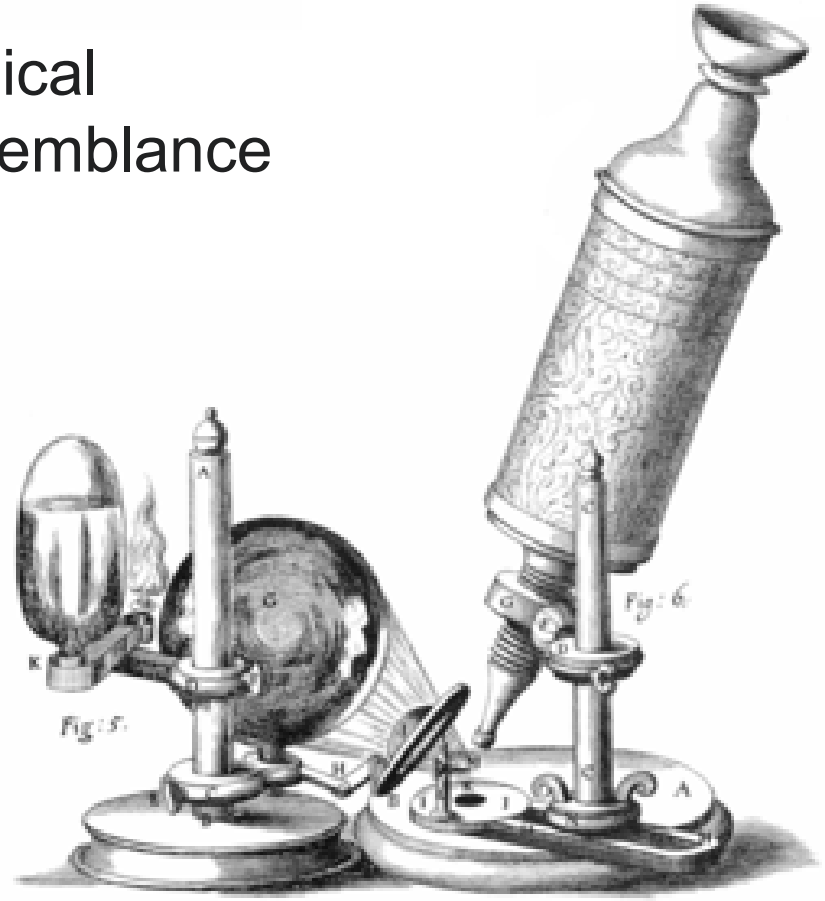
In 1665 Hooke published *Micrographia*, a book describing observations made with microscopes and telescopes, as well as some original work in biology.
Hooke coined the term ***cell*** for describing biological organisms, the term being suggested by the resemblance of plant cells to cells of a honeycomb.



Cell structure of cork by Hooke



Hooke's drawing of a flea



Wikipedia

Bio imaging length scale

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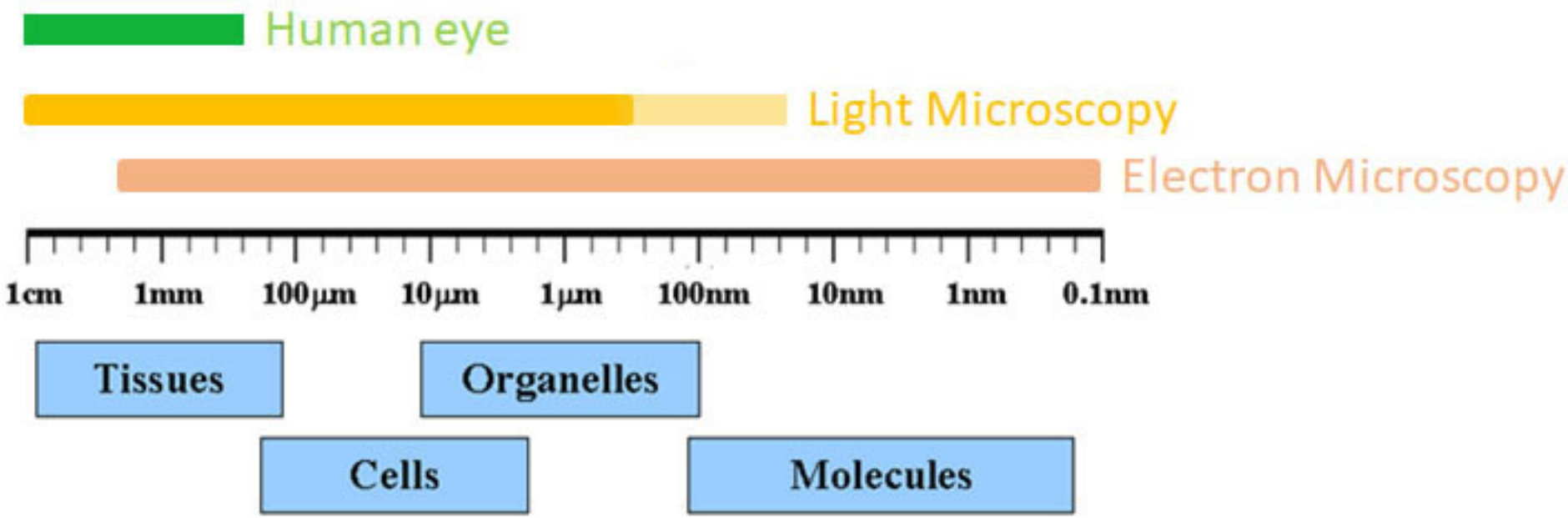

Light microscopy:
Lower resolution
natural environment
molecular specificity
Dynamics
Wavelength 400-700nm

Electron microscopy:
High resolution
Vacuum
Contrast
Identification
Wavelength 2-4 nm

Light Microscopy

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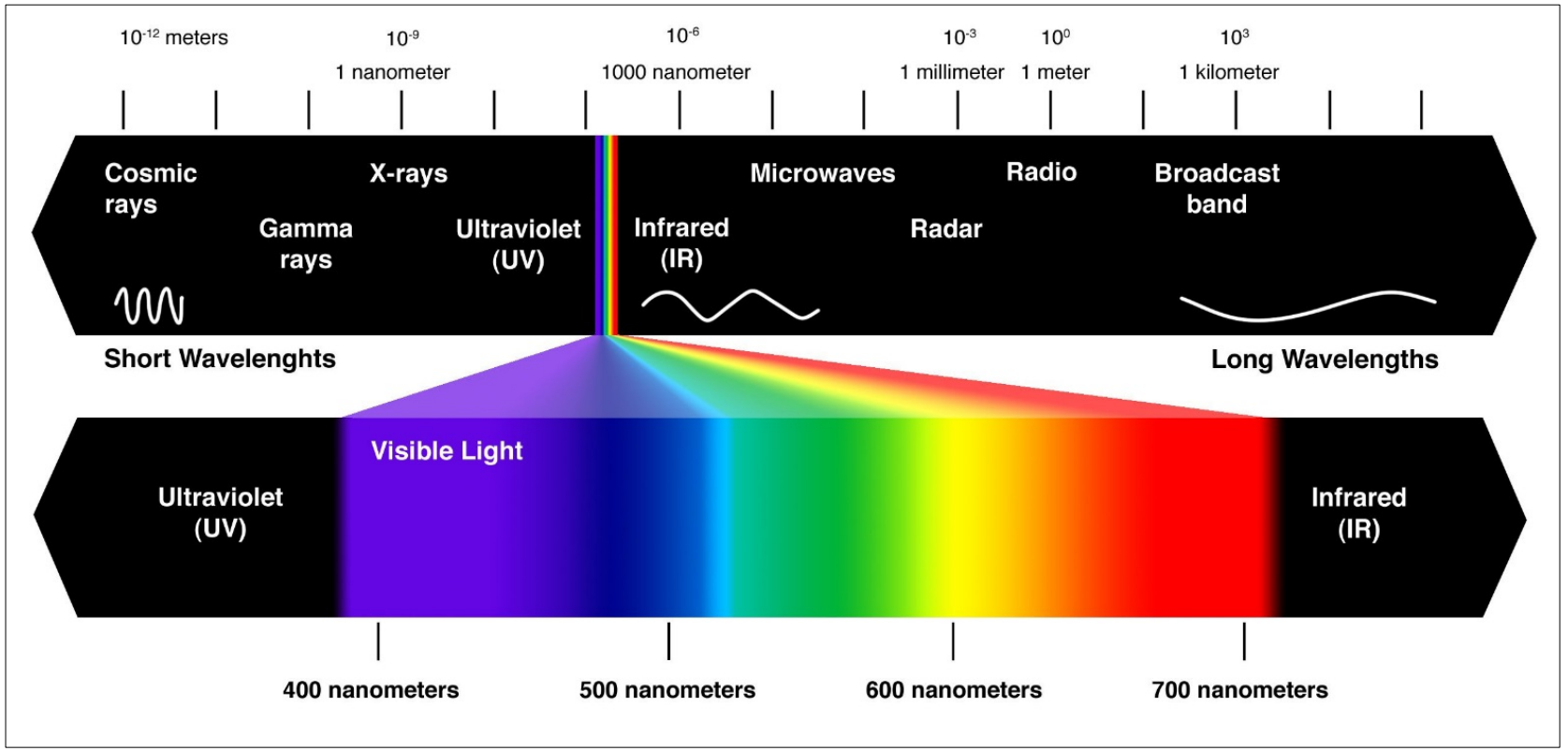
TO CELLS



Compound Microscope

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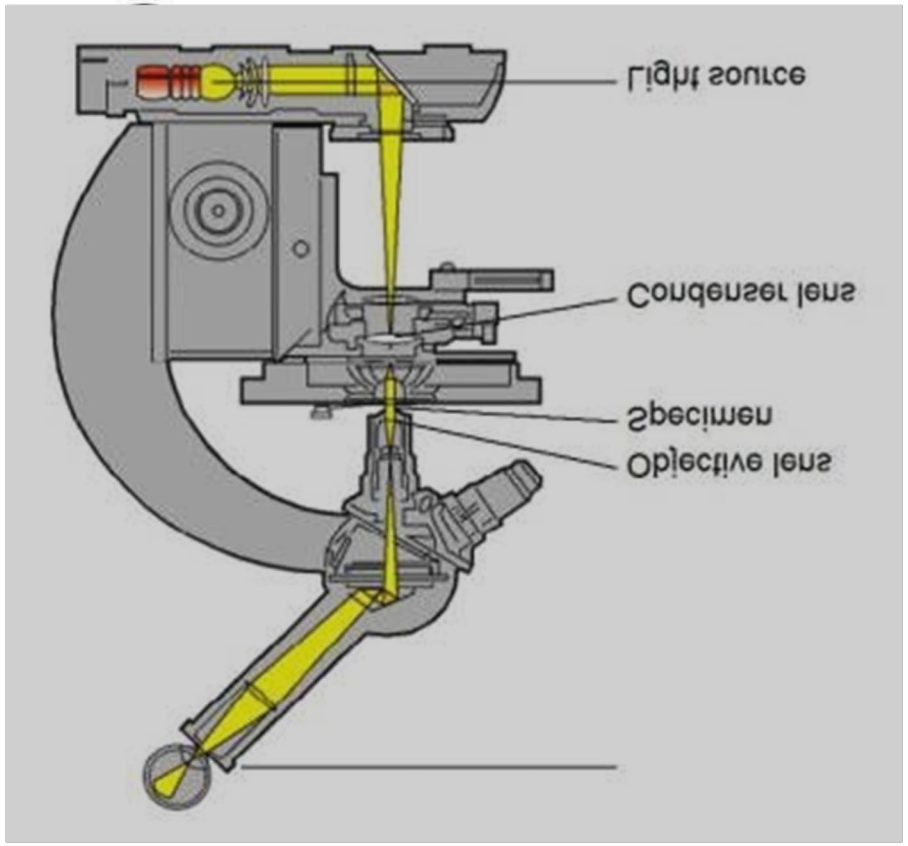
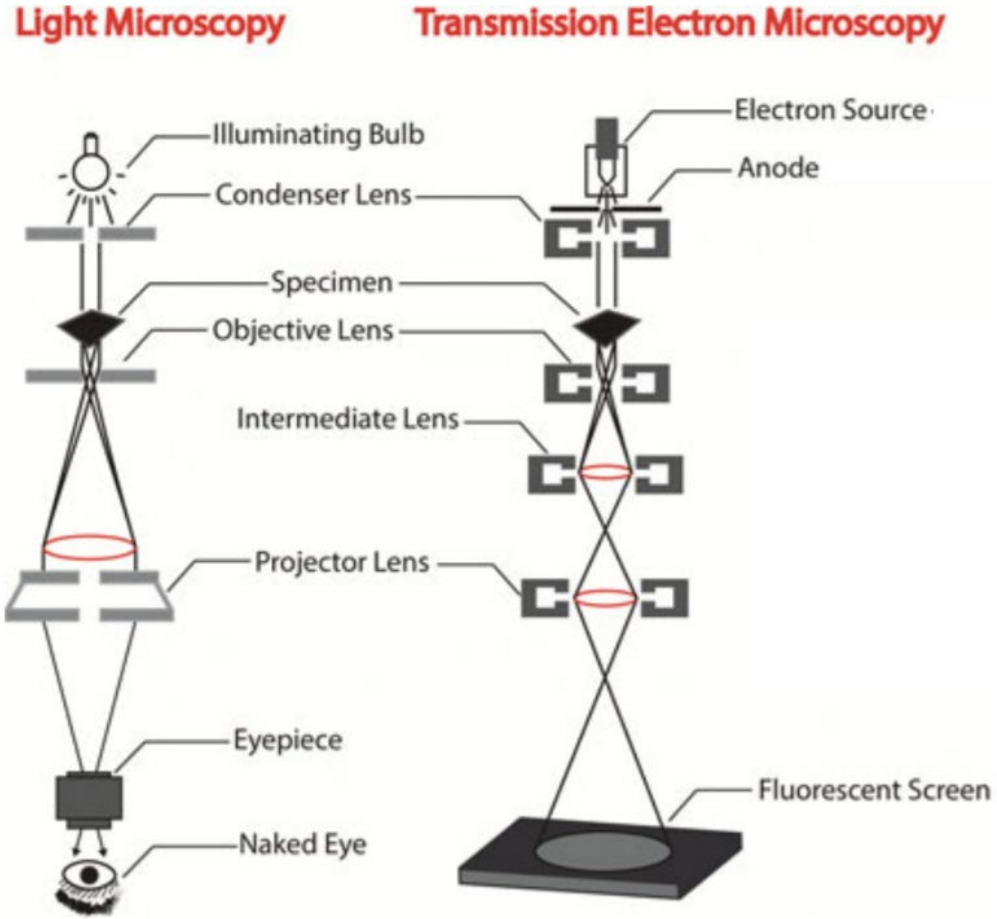
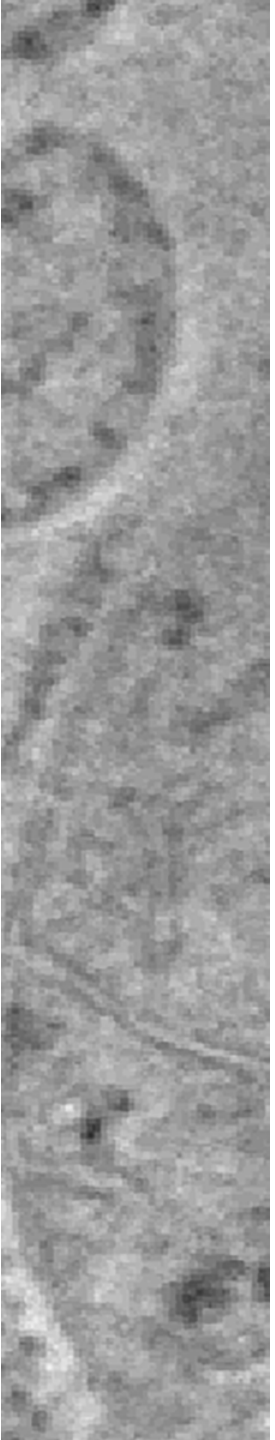
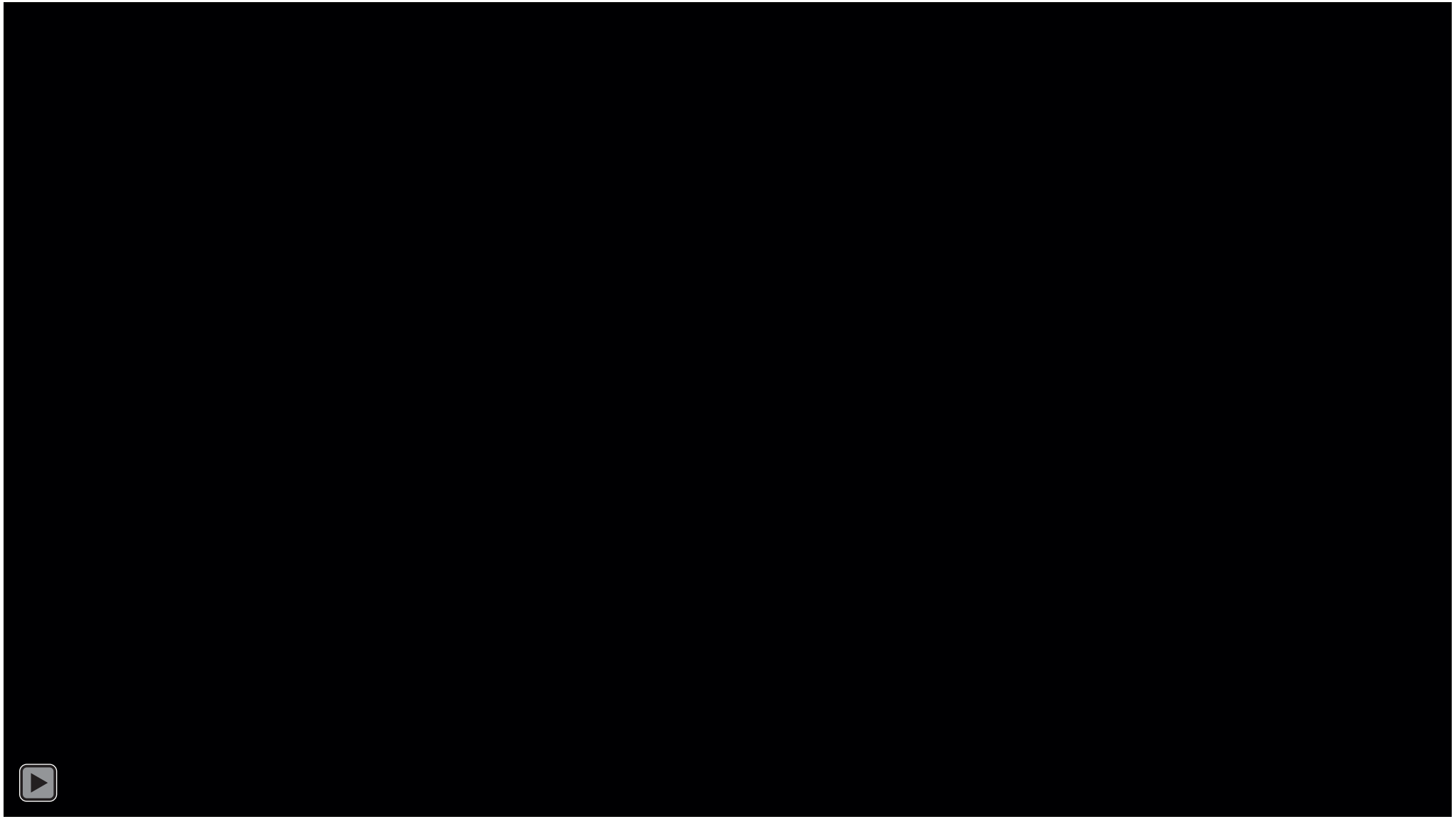




Ul-Hamid, A Beginners' Guide to Scanning Electron Microscopy



IMAGING LIFE FROM MOLECULES TO CELLS

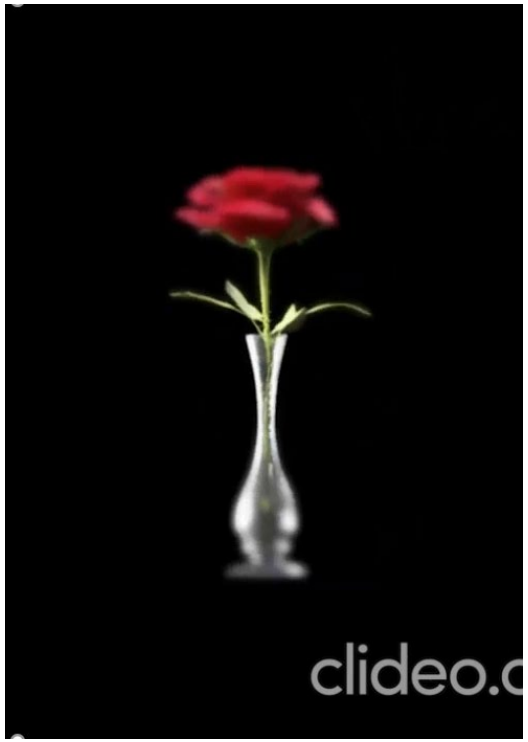


Canon.org website

Magnification

IMAGING LIFE FROM MOLECULES TO CELLS

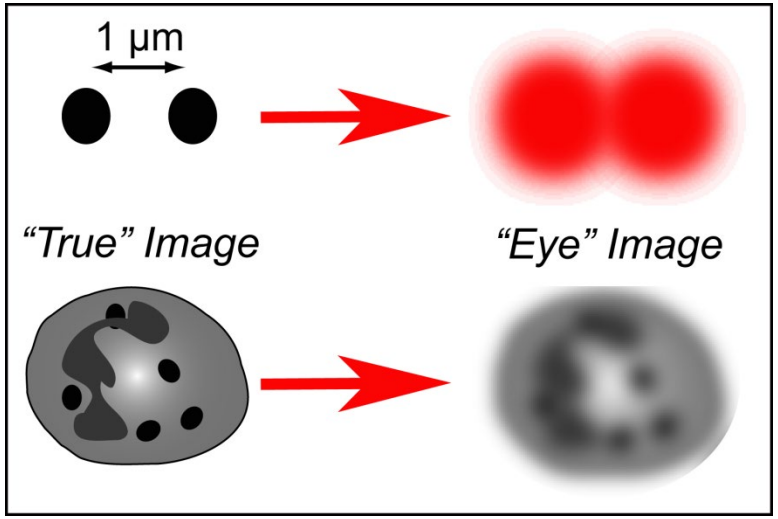
- How high can we go with the magnification?
- Is there a limit?
- What happen to the magnified image?



The Diffraction Limit

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

- High magnification does not translate into the ability to see infinitely small details. Instead, the resolution of light microscopy is limited because light is a wave and is subject to **diffraction**.
- The **diffraction** of light prevents exact convergence of the rays, causing a sharp point on the object to blur into **a finite-sized spot** in the image.



Ernst Karl Abbe

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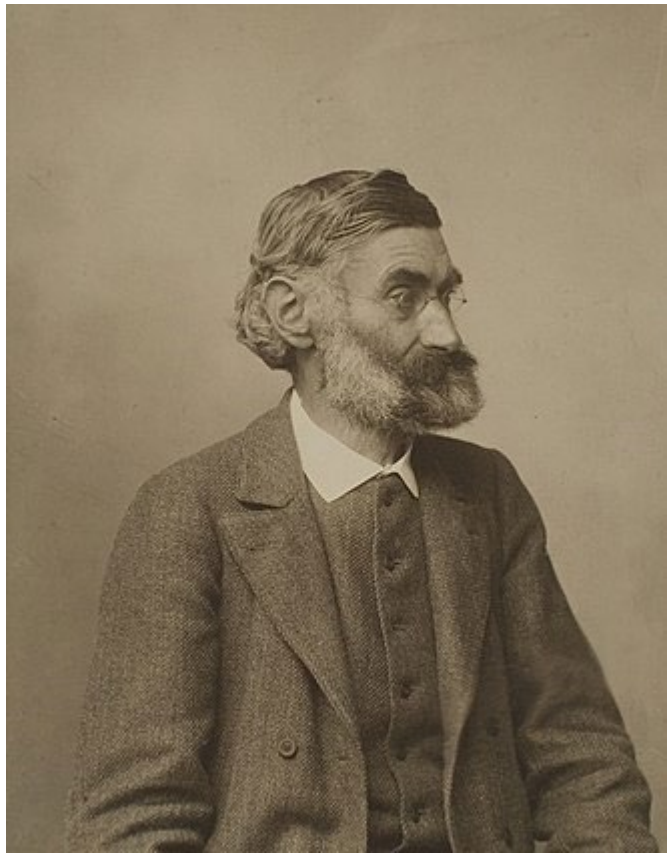

Ernst Karl Abbe approximated the diffraction limit of a microscope as $d = \frac{\lambda}{2\,n\,\sin\Theta}$

Abbe Diffraction Limit

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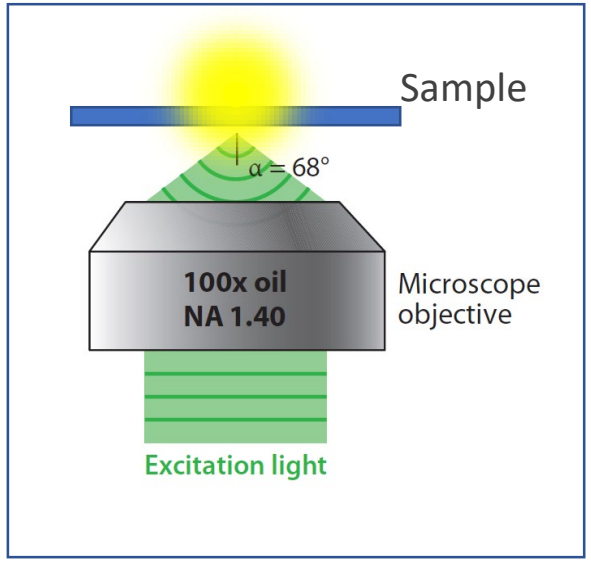
$$d = \frac{\lambda}{2n\sin\Theta}$$

NA

d - resolvable feature size
λ - wavelength
n - index of refraction of the medium being imaged in
Θ - half-angle of maximum cone of light of the objective
NA - objective numerical aperture



In light microscopy the diffraction limit is approximately half the wavelength ~300nm

Numerical Aperture (NA)

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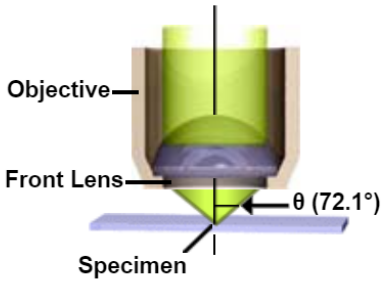
$$NA = n \sin\theta$$

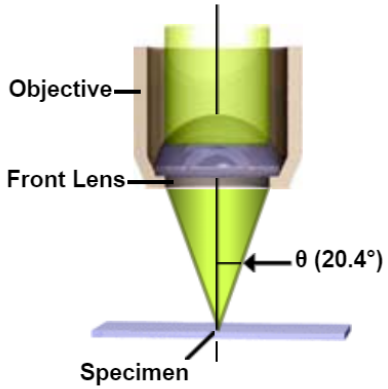


NA = n sin(θ)
0.95 = 1.0 sin 72.1°

NA = Numerical Aperture
n = Refractive Index
= 1.00 (Air)
θ = 1/2 Angular Aperture

Approximate Magnification: 100x

Numerical Aperture = 0.95

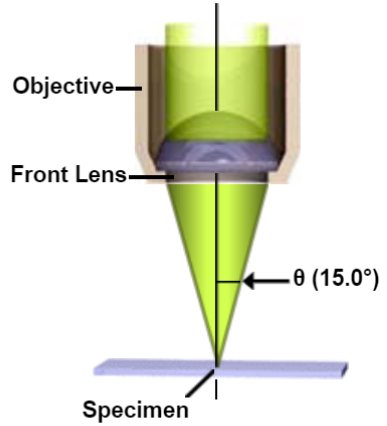


NA = n sin(θ)
0.34 = 1.0 sin 20.4°

NA = Numerical Aperture
n = Refractive Index
= 1.00 (Air)
θ = 1/2 Angular Aperture

Approximate Magnification: 20x

Numerical Aperture = 0.34



NA = n sin(θ)
0.25 = 1.0 sin 15.0°

NA = Numerical Aperture
n = Refractive Index
= 1.00 (Air)
θ = 1/2 Angular Aperture

Approximate Magnification: 10x

Numerical Aperture = 0.25

Higher NA

↓

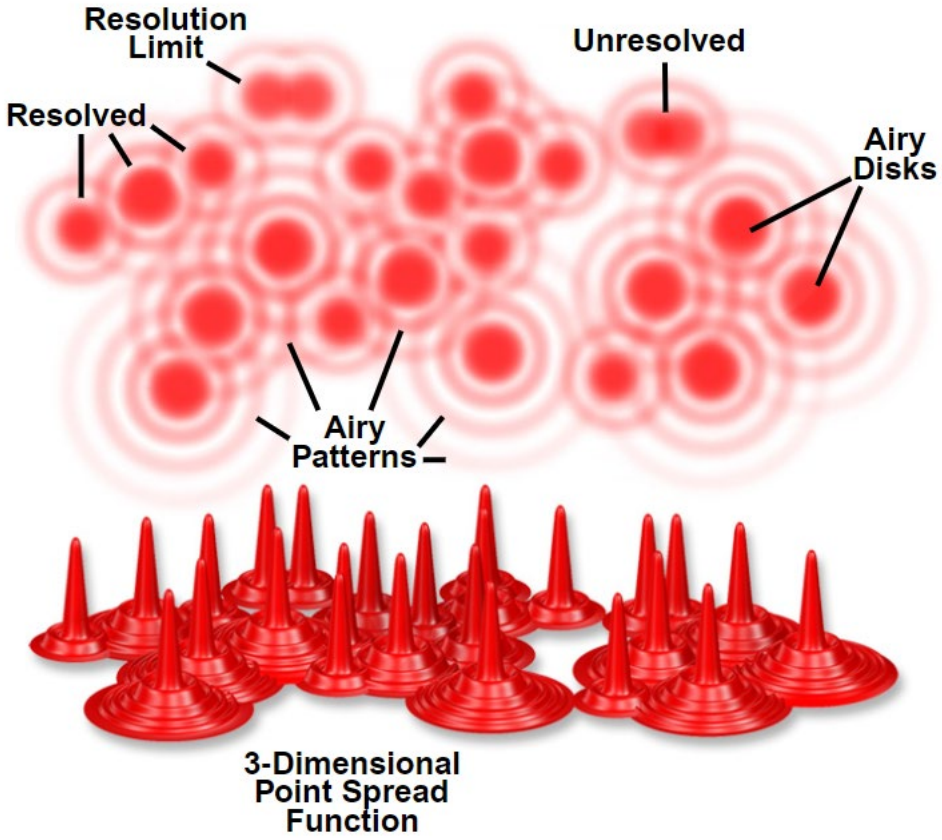
Gathering light over larger sets of angles

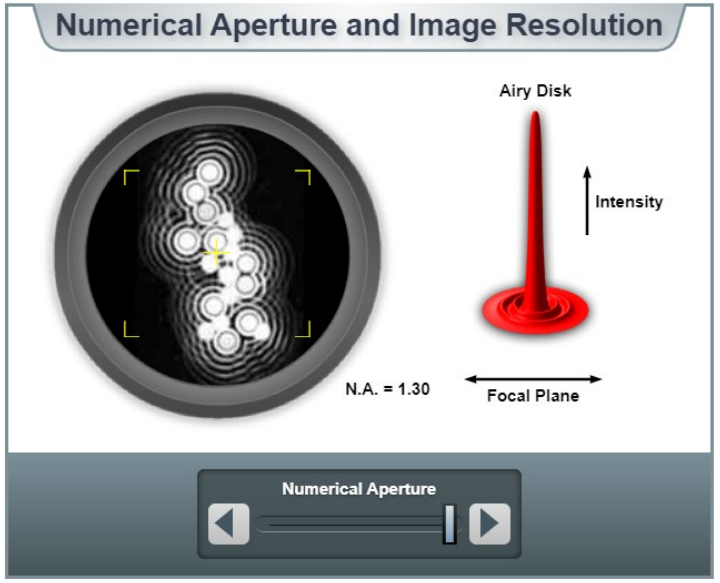
↓

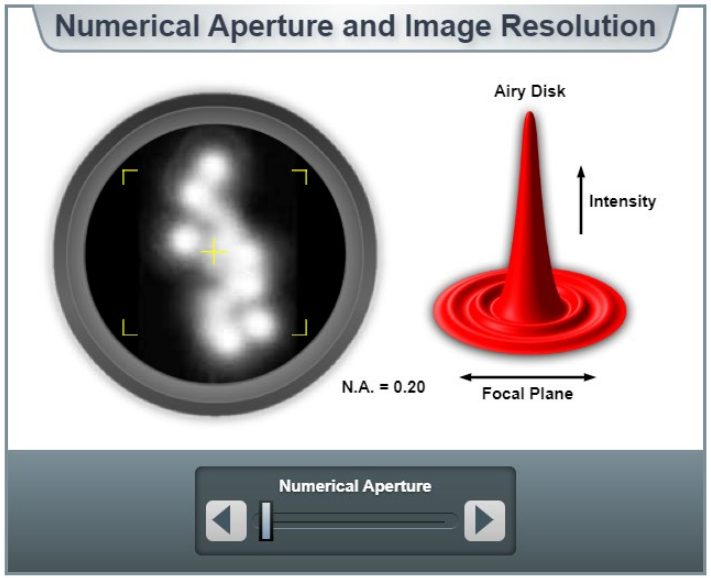
Higher resolution

https://micro.magnet.fsu.edu/primer/java/nuaperture/index.html

Point Spread Function

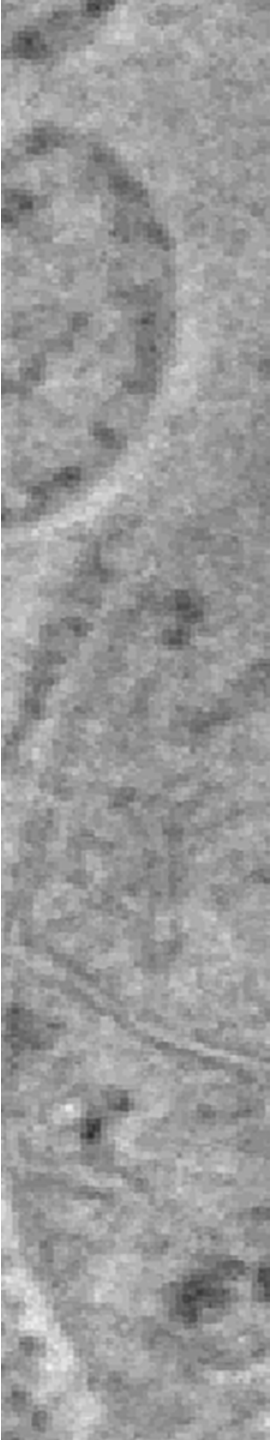






Three-dimensional representation of the diffraction pattern near the intermediate image plane is known as the **point spread function**

https://www.microscopyu.com/microscopy-basics/resolution



Resolution and not Magnification

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How can we improve the resolution?

- Use shorter wavelength – electron microscopes
- Break the diffraction limit – super resolution microscopy

Fluorescence Phenomena

- “Fluorescence”: named by George Gabriel Stokes (1852) after the mineral fluorite which lights up when illuminated with UV.
- He realized that the exciting light wavelength will always be shorter than the emitted light wavelength.
- The Stokes shift, which describes this light conversion, is named in Stokes's honor.

Fluorite

George Gabriel Stokes (1918-1903)

Wikipedia

What is Fluorescence?

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Tonic water (quinine)

Shining light on some molecules, excitation light, results in light emission at a longer wavelength.

Stokes Shift

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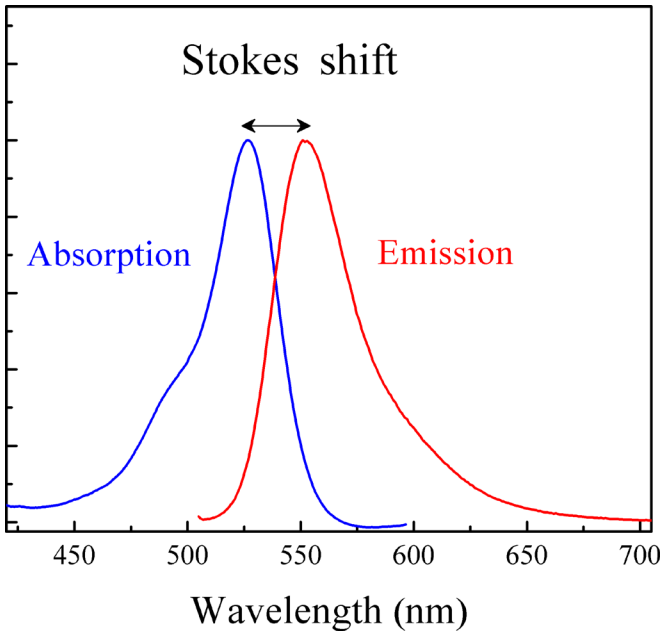
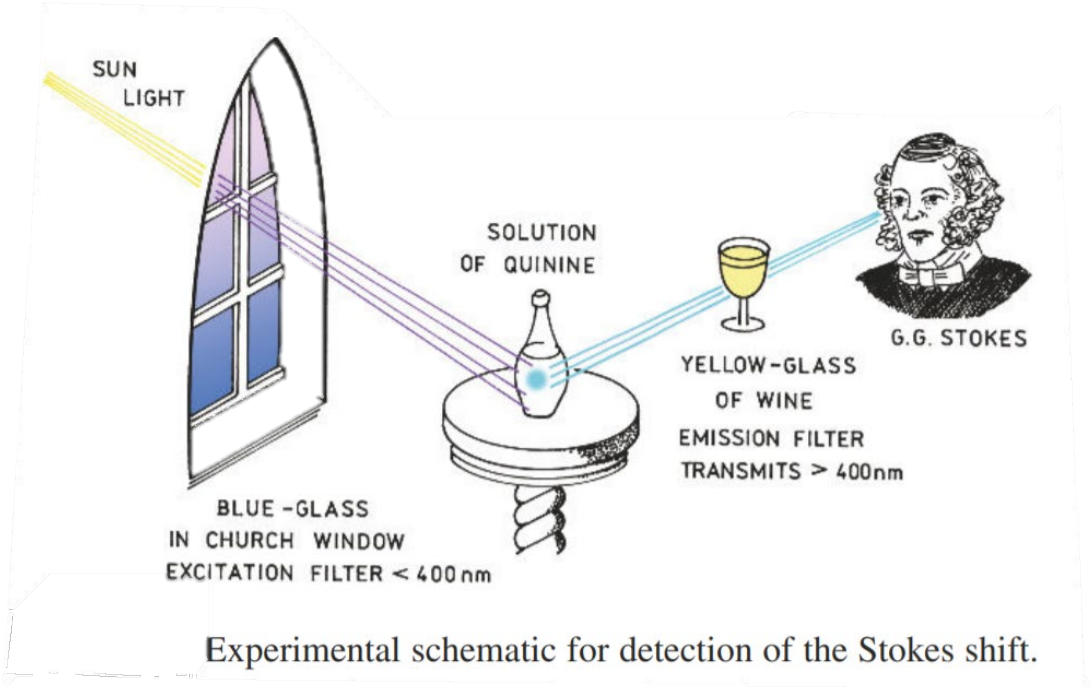
The energy of the emission is typically less than that of absorption.
Fluorescence typically occurs at lower energies or longer wavelengths.

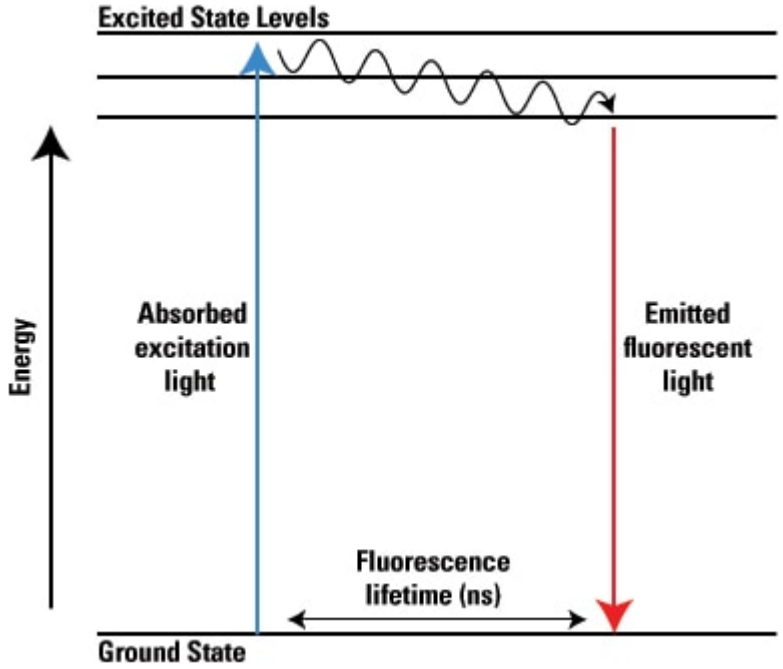




Experimental schematic for detection of the Stokes shift.

Principles of Fluorescence Spectroscopy, Joseph R. Lakowicz

Jablonski Diagram

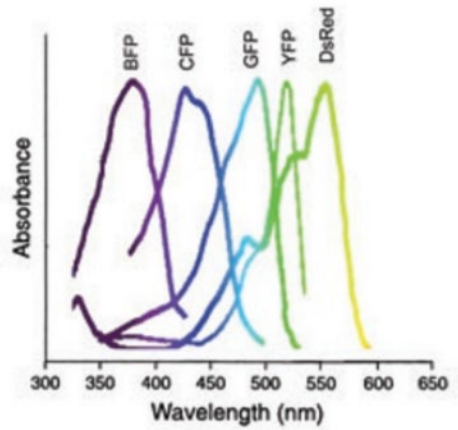


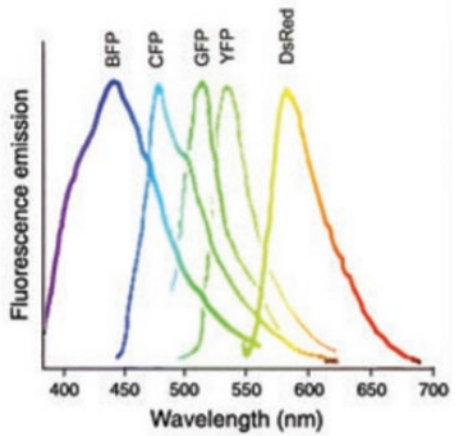
Fluorescein

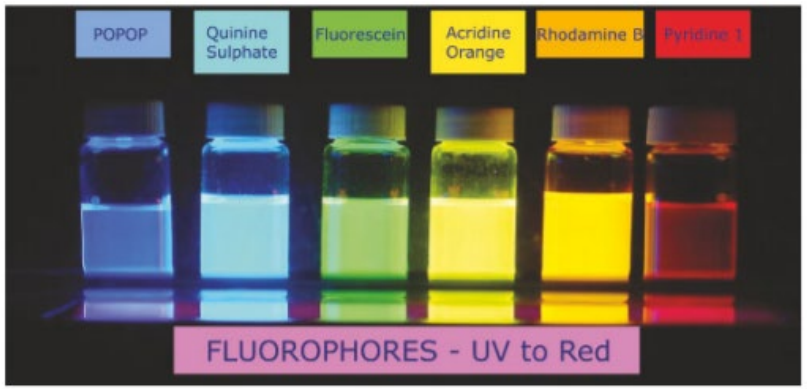
Shining light on some molecules, excitation light, results in light emission at a longer wavelength.

IMAGING LIFE FROM MOLECULES TO CELLS

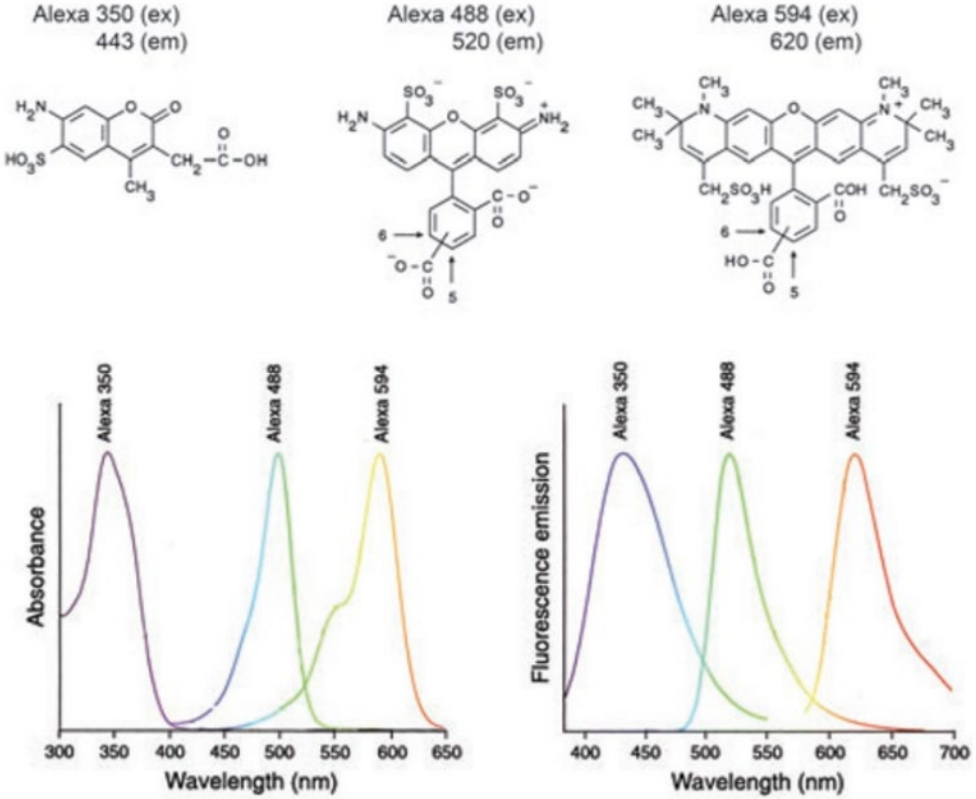
Fluorescent proteins







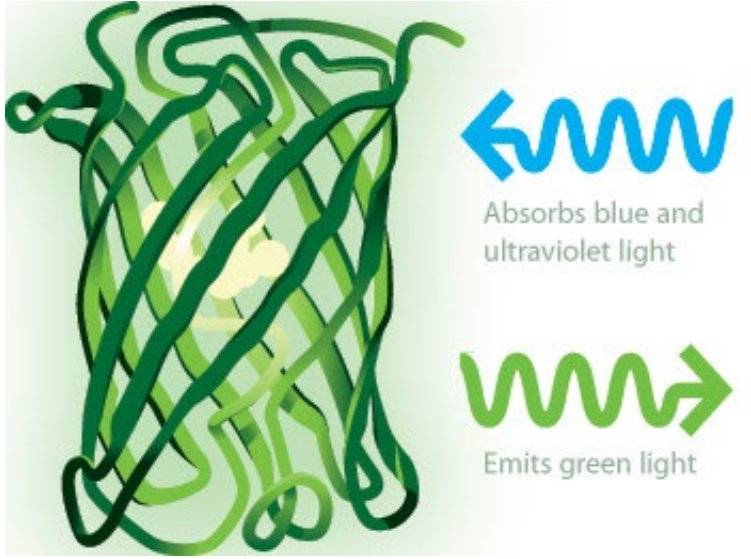
Fluorescent organic molecules



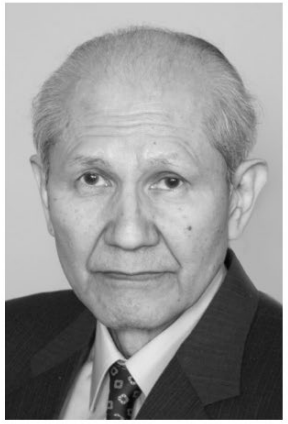
Structural basis of fluorophores is conjugated double bonds acting as ‘antenna’

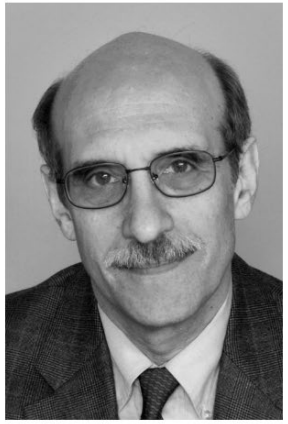
NATURE METHODS, VOL.2 NO.12, DECEMBER 2005, Principles of Fluorescence Spectroscopy, Joseph R. Lakowicz

Green Fluorescent Protein (GFP)



The Nobel Prize in Chemistry 2008


© The Nobel Foundation. Photo: U. Montan
Osamu Shimomura
Prize share: 1/3


© The Nobel Foundation. Photo: U. Montan
Martin Chalfie
Prize share: 1/3


© The Nobel Foundation. Photo: U. Montan
Roger Y. Tsien
Prize share: 1/3

Osamu Shimomura first isolated GFP from the jellyfish Aequorea victoria, and discovered that it glowed bright green under ultraviolet light.
Martin Chalfie demonstrated the value of GFP genetic tag for biological phenomena
Roger Y. Tsien contributed to our general understanding of how GFP fluoresces and extended the color palette

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

https://www.nobelprize.org

Fluorescence Microscopy

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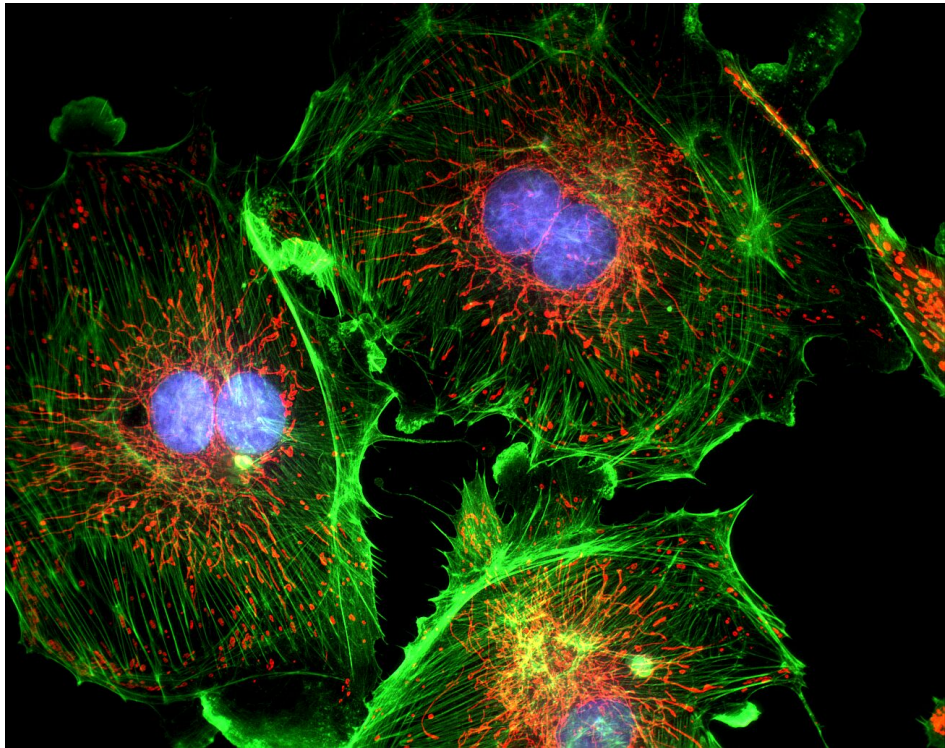
Why Fluorescence?

- High specificity
  -Fluorescent proteins (GFP…)
  -Antibodies conjugated to fluorescent molecules
- High contrast
  - Bright signal on dark background
- Quantitative
- Live cell imaging – dynamics
- Natural imaging conditions



Filters

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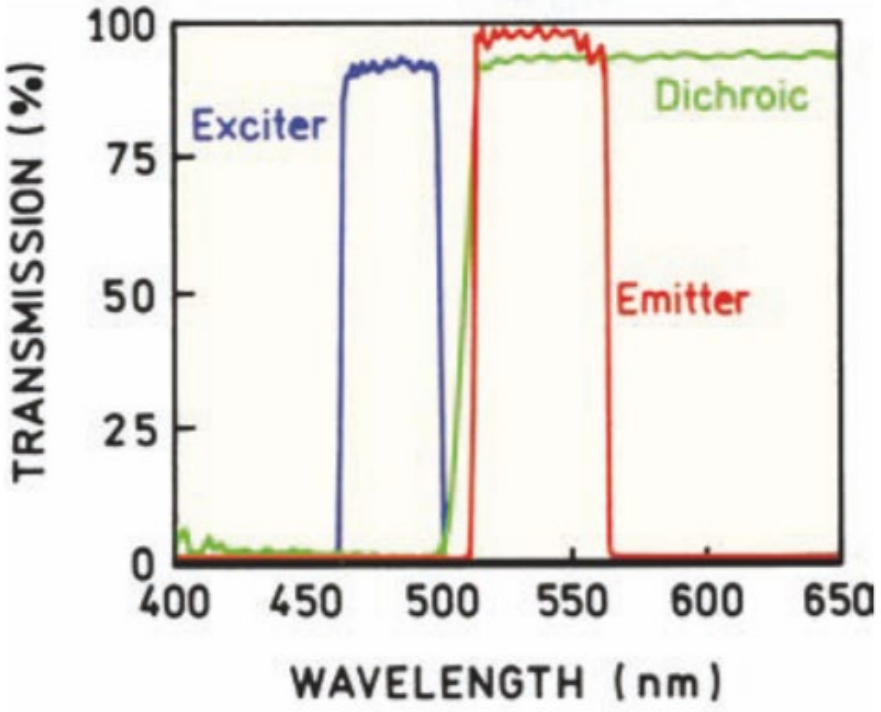
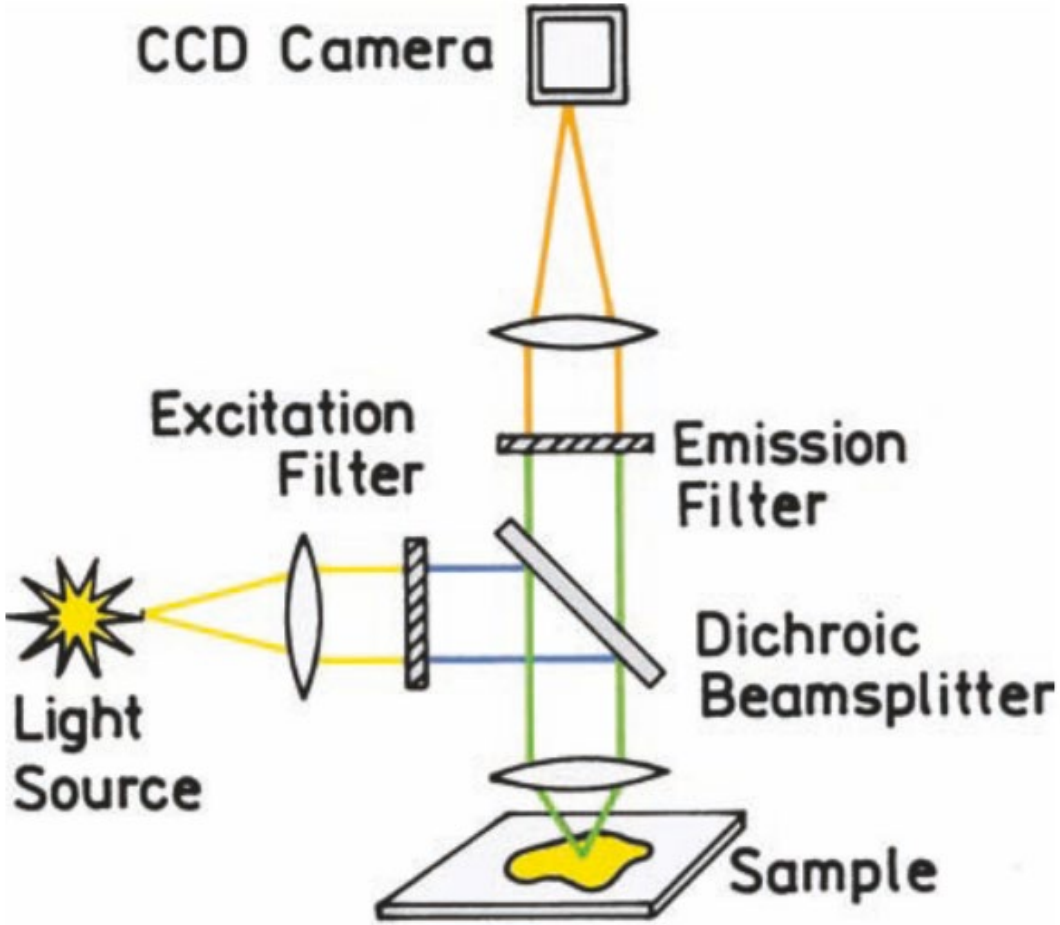
Epifluorescence filter set for Cy5





Principles of Fluorescence Spectroscopy, Joseph R. Lakowicz

Super Resolution Microscopy

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The Nobel Prize in Chemistry 2014
Eric Betzig, Stefan W. Hell, William E. Moerner

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The Nobel Prize in Chemistry 2014

Photo: Matt Staley/HHMI
Eric Betzig
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© Bernd Schuller, Max-Planck-Institut
Stefan W. Hell
Prize share: 1/3

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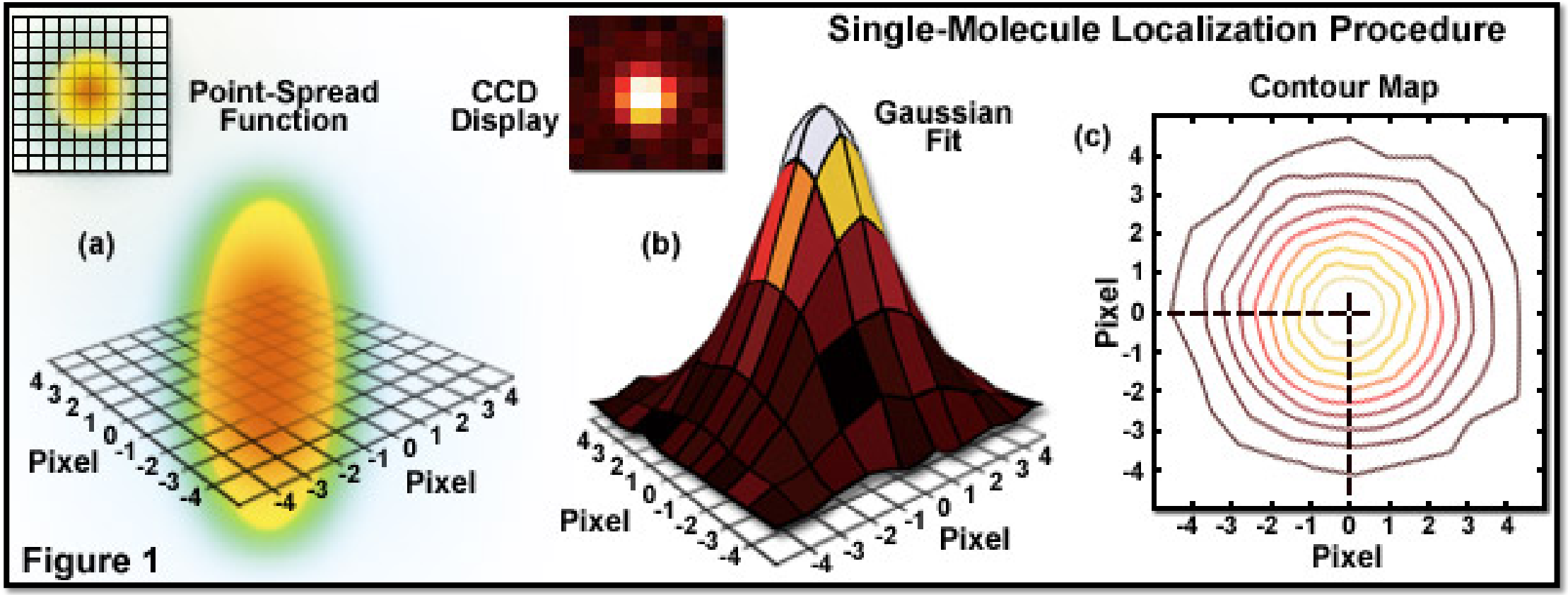
William E. Moerner
Prize share: 1/3

The Nobel Prize in Chemistry 2014 was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner *"for the development of super-resolved fluorescence microscopy"*.

Stochastic Optical Reconstruction Microscopy (STORM)

IMPaCT IMAGING LIFE FROM MOLECULES TO CELLS

Point Spread Function (PSF)

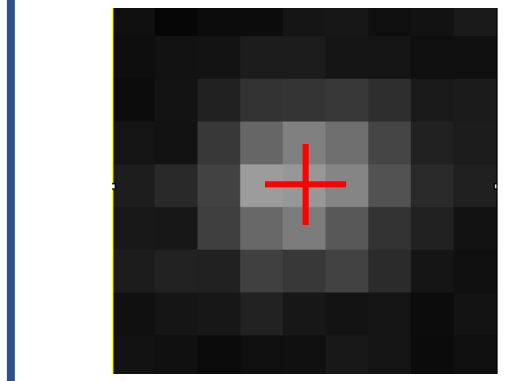


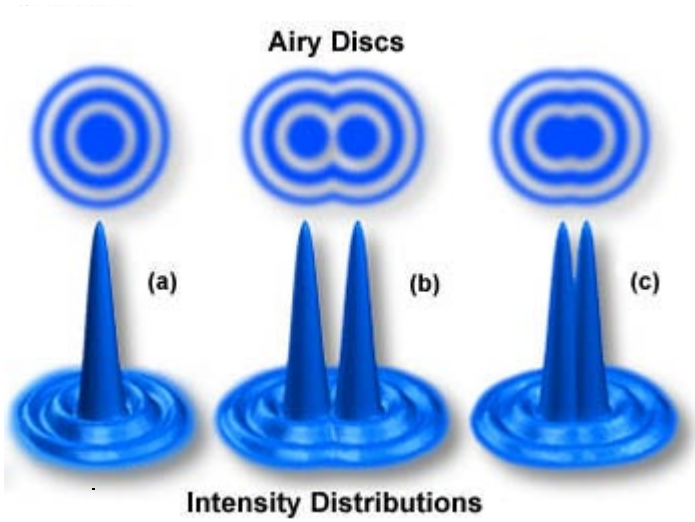
- PSF describes the response of an imaging system to a point source.
- Fitting the image using Gaussian function allows to determine the center of the spot with about an order of magnitude higher resolution.

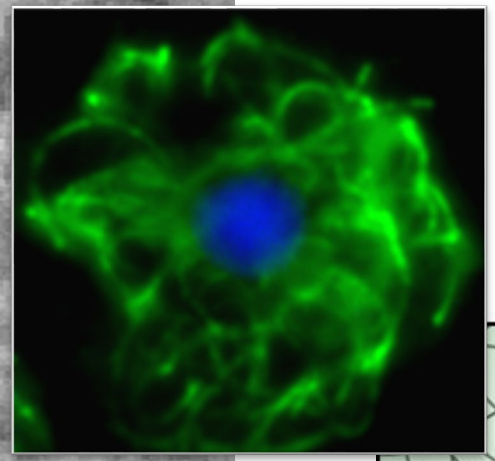
Zeiss website

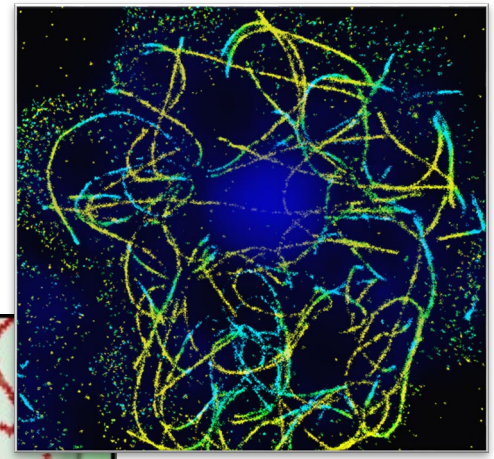
Stochastic Optical Reconstruction Microscopy (STORM)

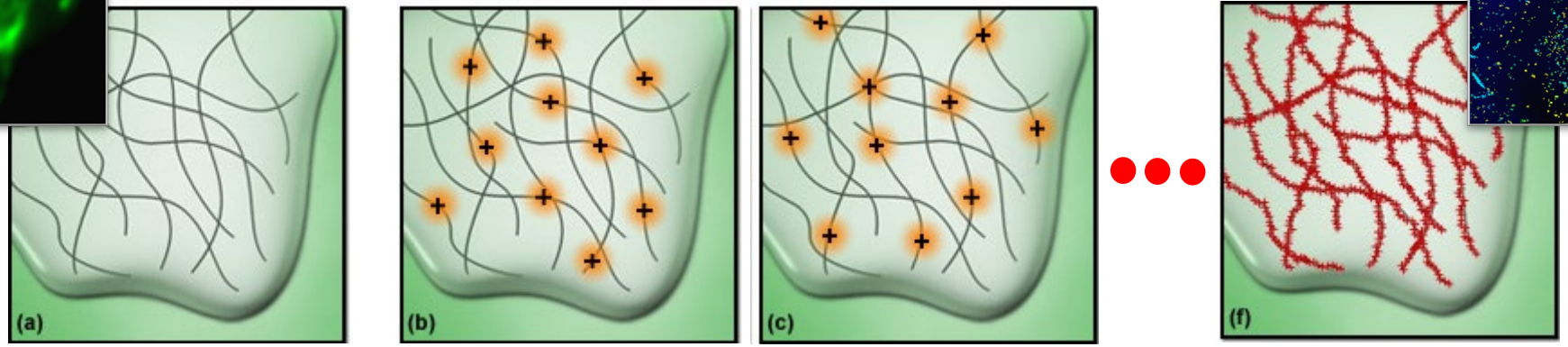
IMPACT IMAGING LIFE FROM MOLECULES TO CELLS









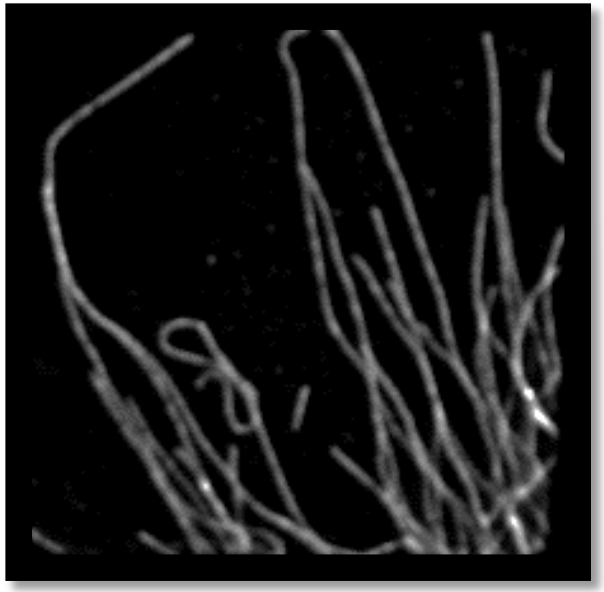


Separation of the molecules is done by switching them ON and OFF stochastically.

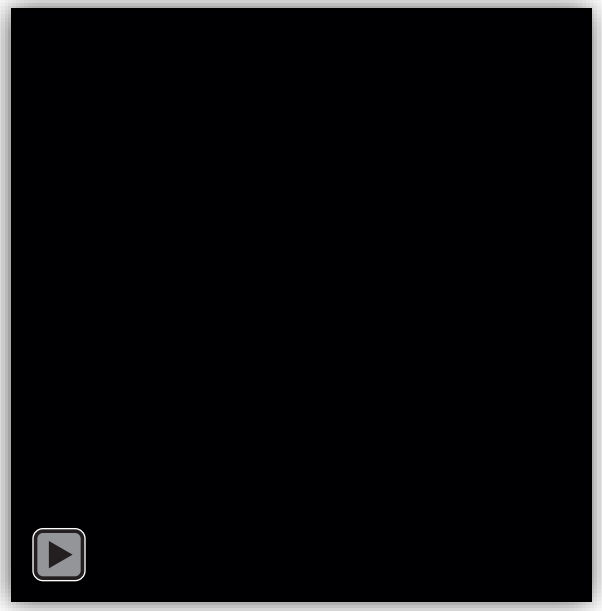
Stochastic Optical Reconstruction Microscopy (STORM)

IMPACT IMAGING LIFE FROM MOLECULES TO CELLS

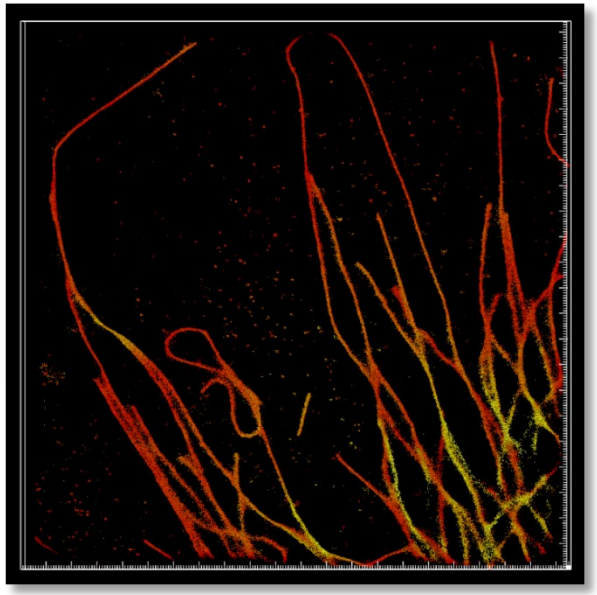
Conventional Fluorescence



Blinking



Super Resolution



Vutara 352 (Bruker)

- Lateral resolution of 20 nanometers
- Axial resolution of 50 nanometers
- Up to 5-micron imaging depth (with z-stack acquisition)
- Simultaneous 2-color imaging in super-resolution mode (up to 4 colors in wide-field mode)
- 3D particle tracking with ~10nm precision

3D STORM - microtubules

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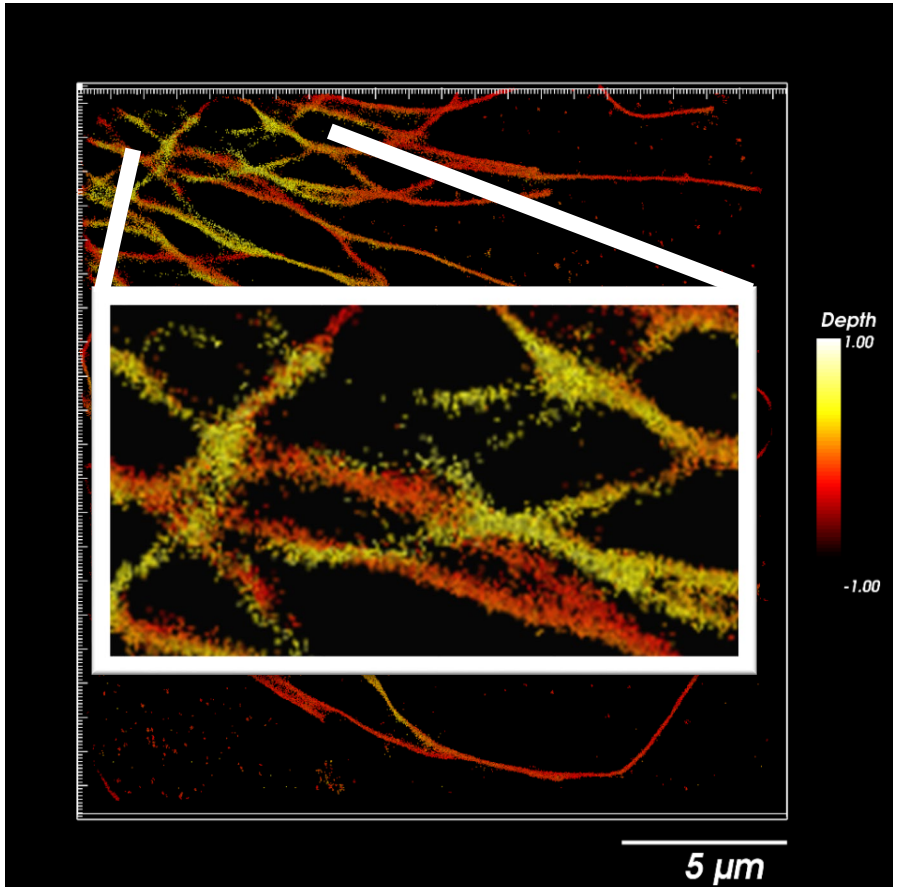
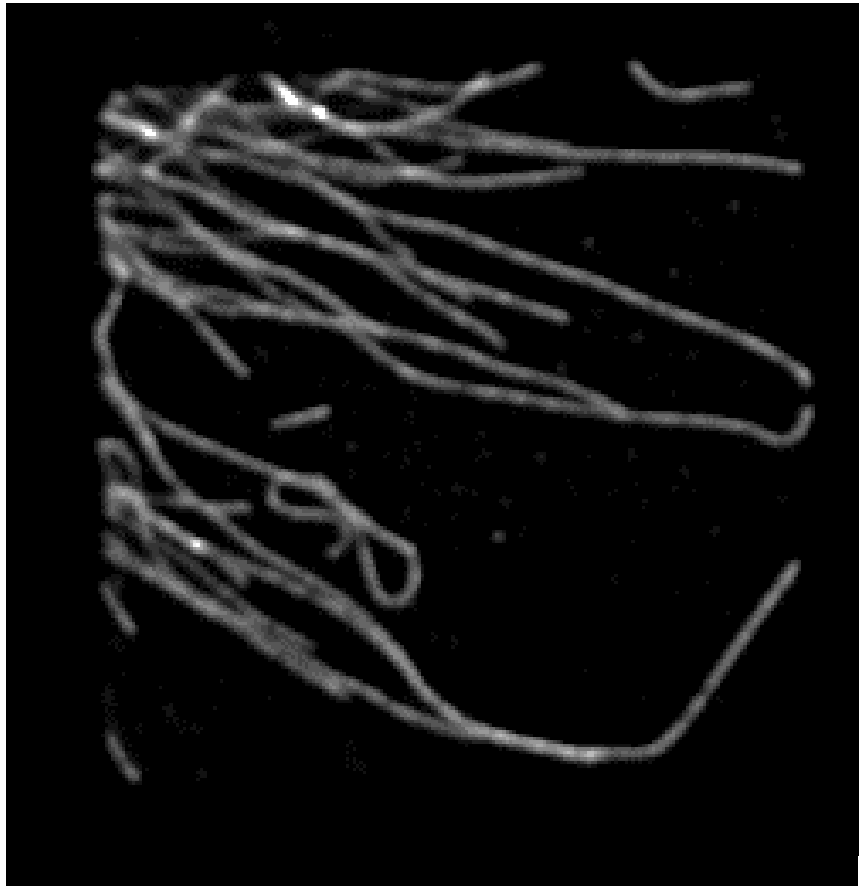
IMAGING LIFE
FROM MOLECULES
TO CELLS

Super resolution

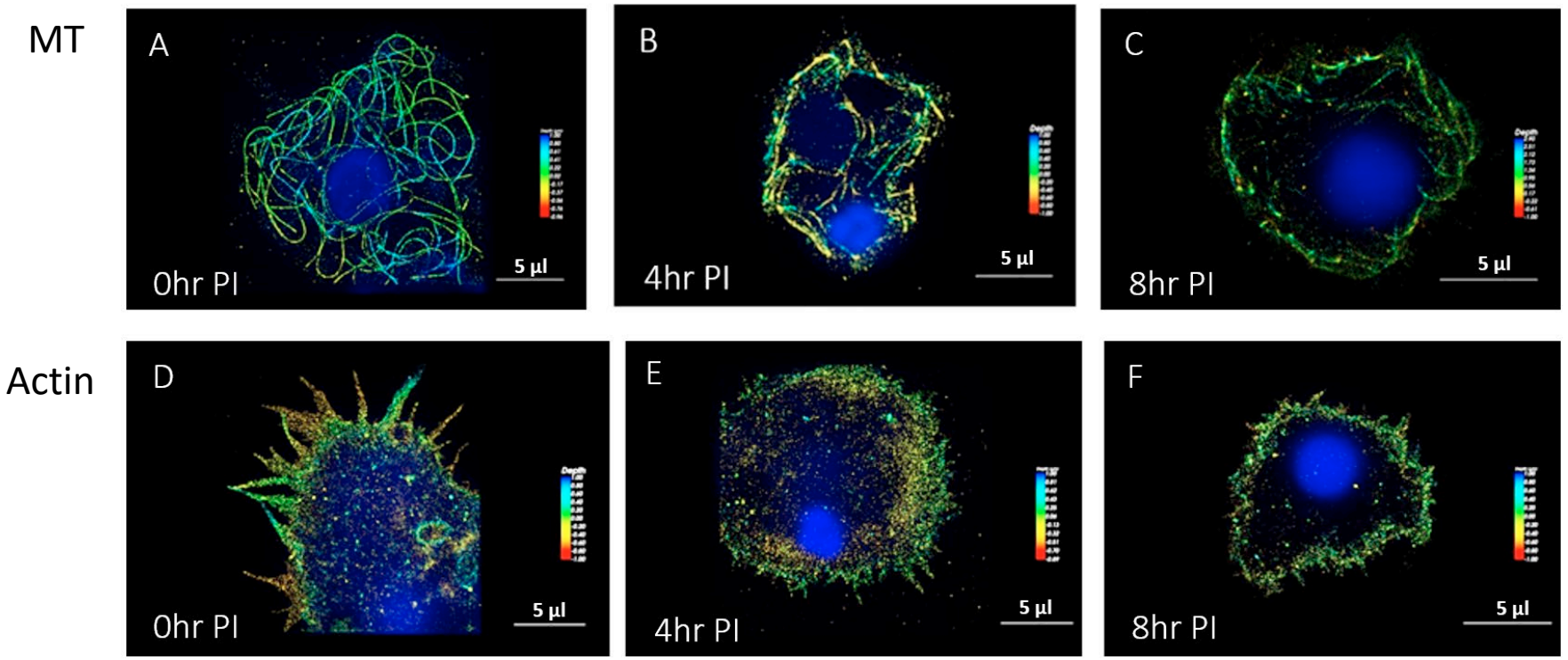


Conventional fluorescence



Morphological changes of the cytoskeleton in Mimivirus-infected cells

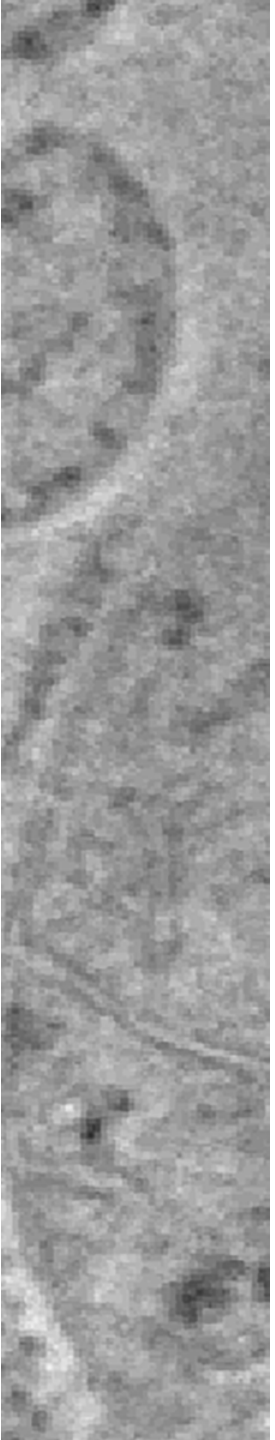
IMPaCT IMAGING LIFE FROM MOLECULES TO CELLS



Actin and microtubule filaments rearrangements throughout the infection cycle of Acanthamoeba at different time points post infection.

The cells were infected, followed by fixation and staining of microtubules using anti-alpha-tubulin antibodies (A,B,C) or staining of actin fibers using Phalloinin-647 (D,E,F), and DNA using DAPI (blue).

Prof. Avi Minsky and Liran Ben Yaacob



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Prof. Avi Minsky and Liran Ben Yaacob

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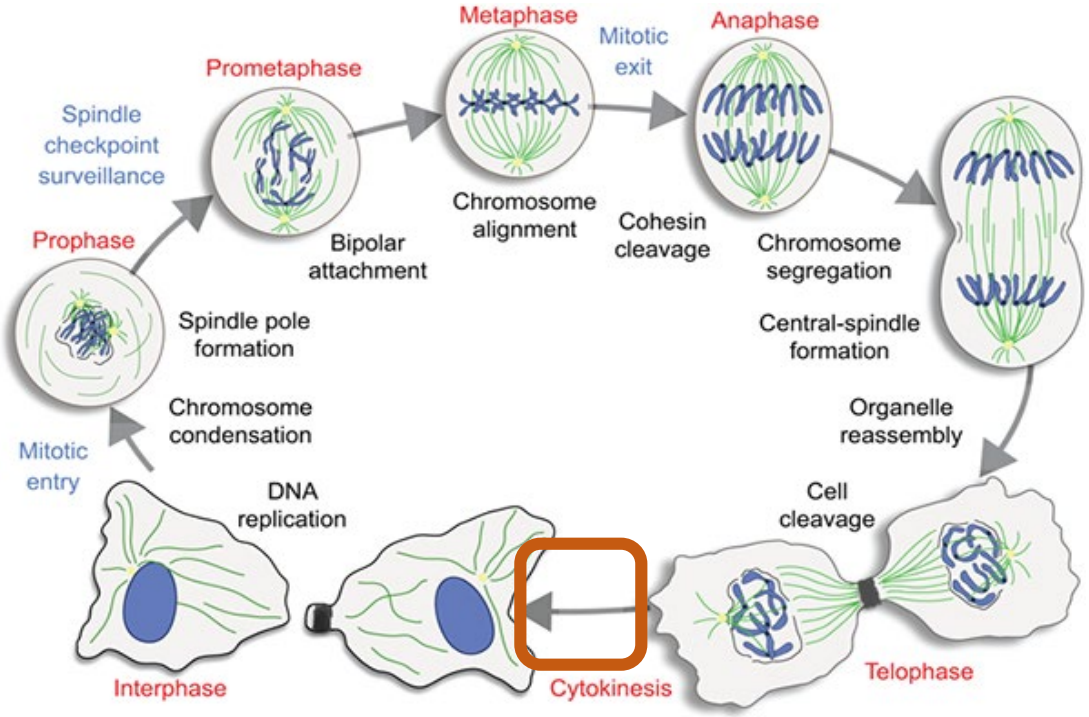
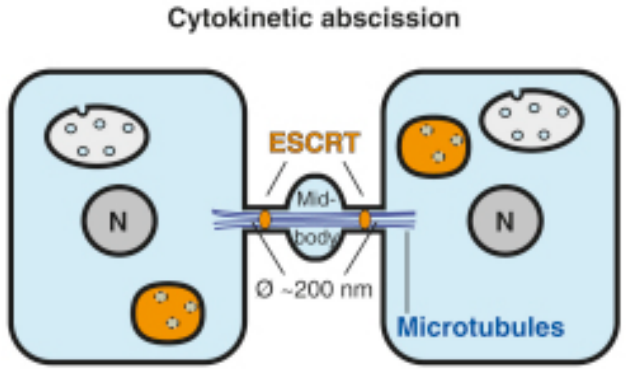
Cytokinetic abscission

ESCRT (Endosomal Sorting Complex Required for Transport) proteins play a role in biogenesis of multi vesicular bodies, HIV budding and cytokinesis.



ESCRT machinery involves in the final scission of the bridge connecting the two daughter cells



Oliver Schmidth, current Biology 2011

Dr. Natalie Elia and Inna Goliand, BGU

Spatial organization of the ESCRT-III protein (IST1) in the intercellular bridge of dividing cells at different stages of abscission

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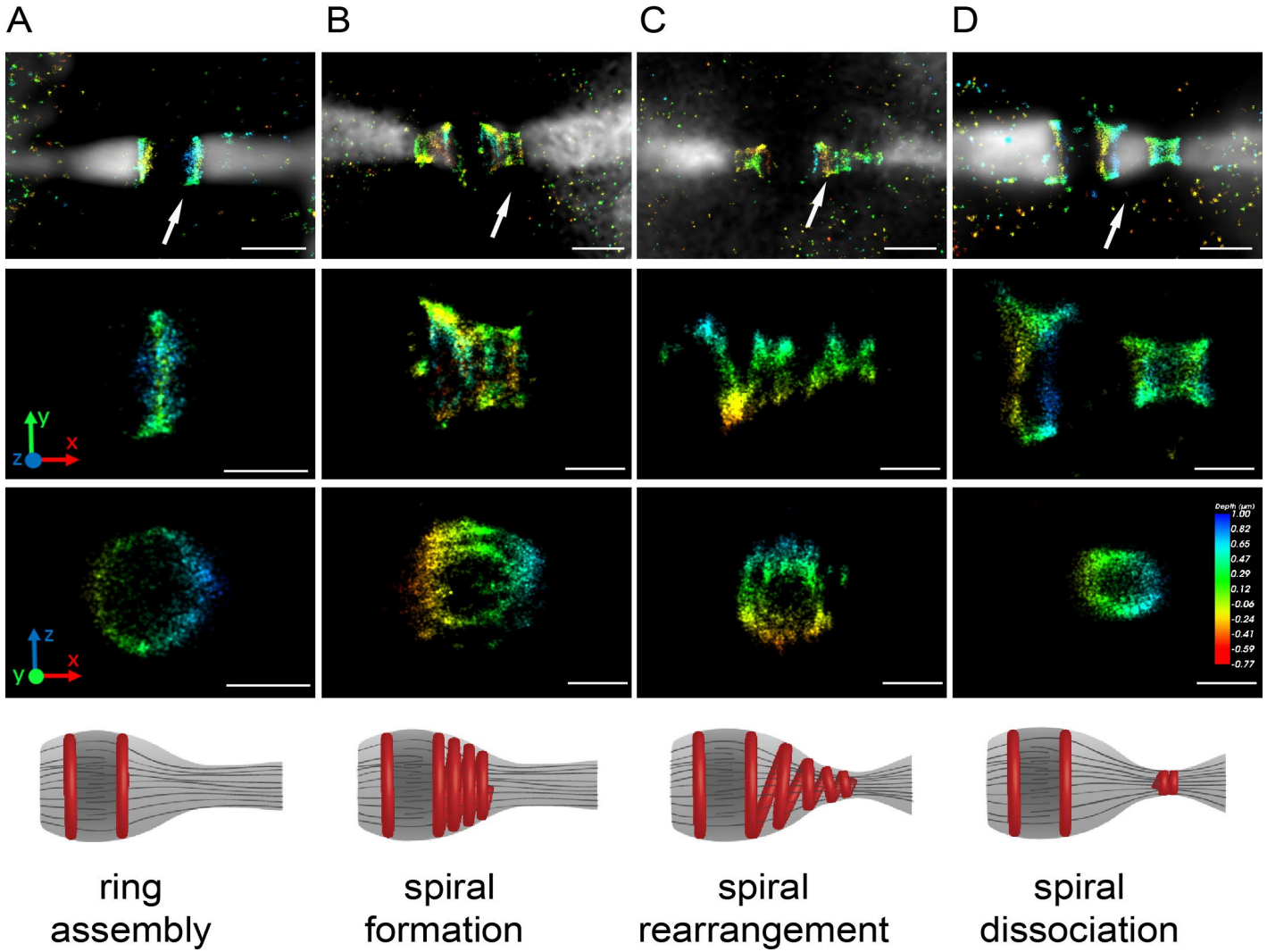
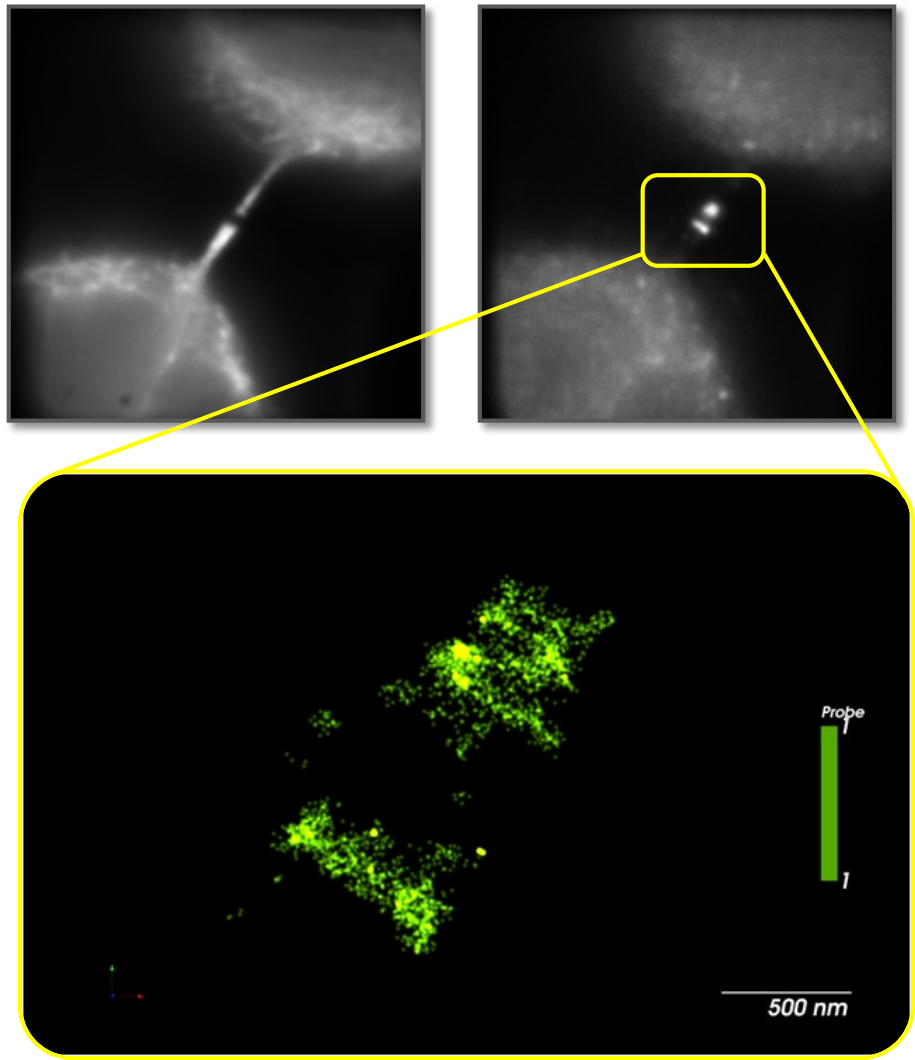


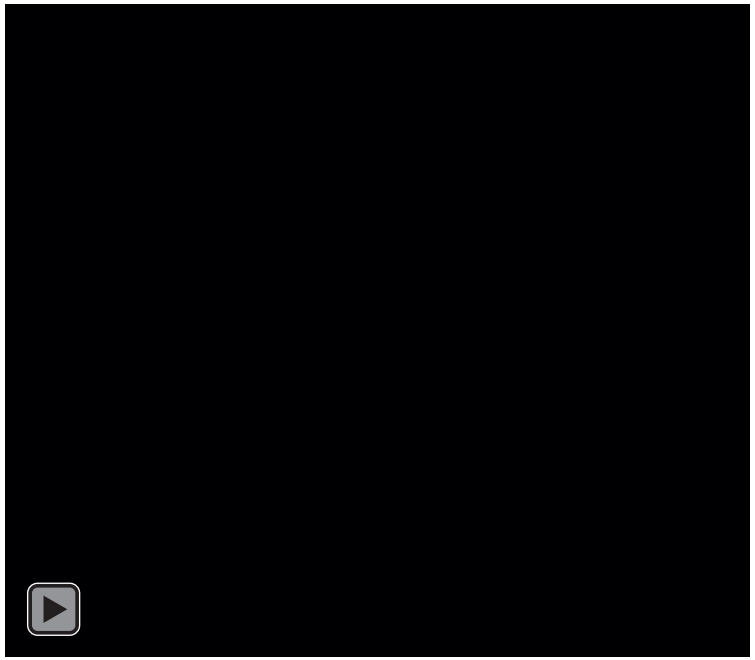
Dr. Natalie Elia and Inna Goliand, BGU

IMAGING LIFE FROM MOLECULES TO CELLS

Tubulin Alexa488

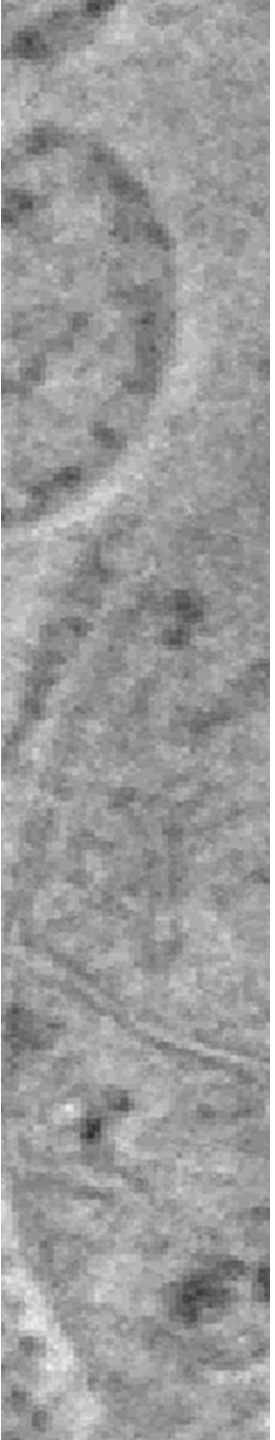
IST1 Alexa647





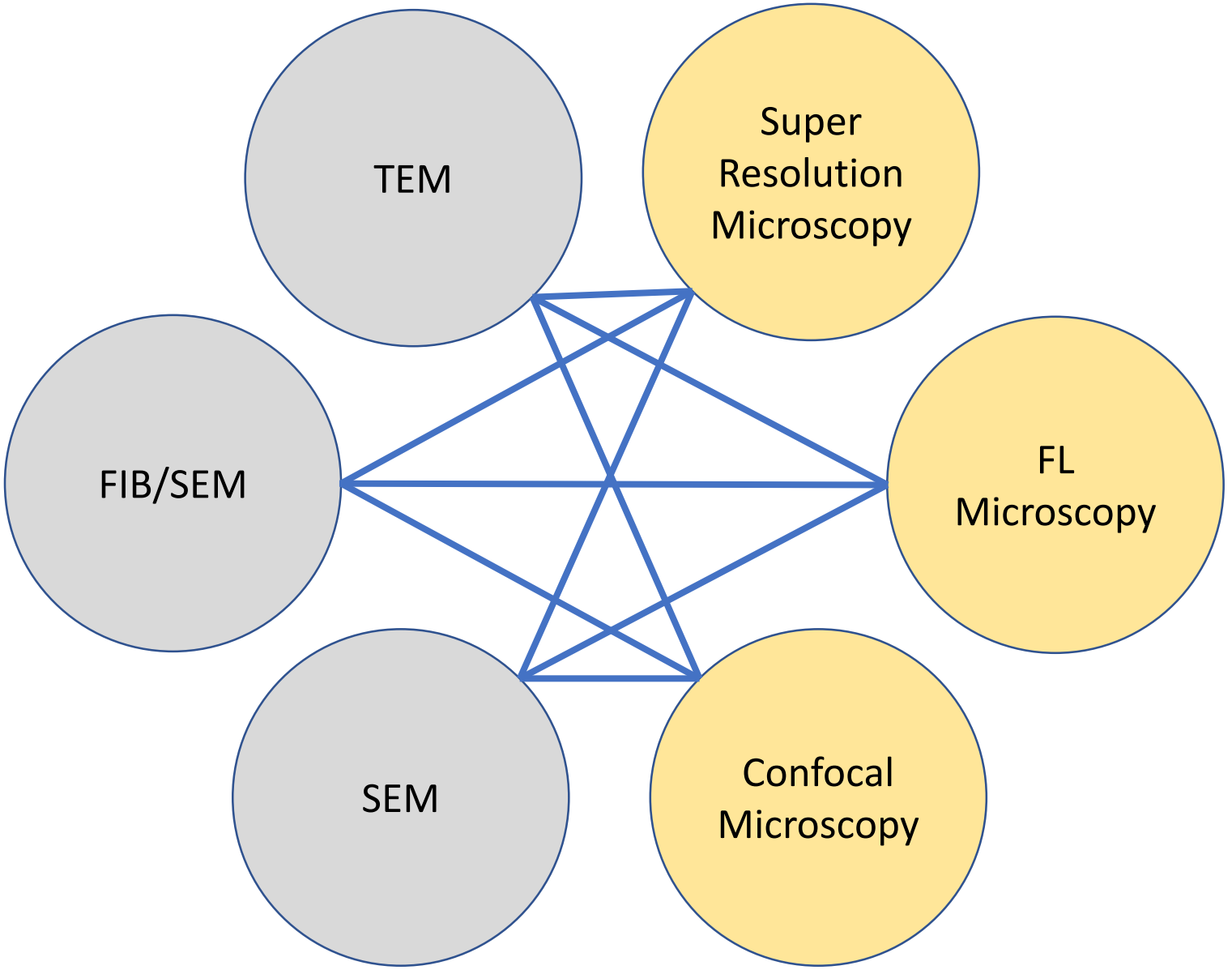
The first direct evidence that the ESCRT –III create helical filaments!!!

Dr. Natalie Elia and Inna Goliand, BGU



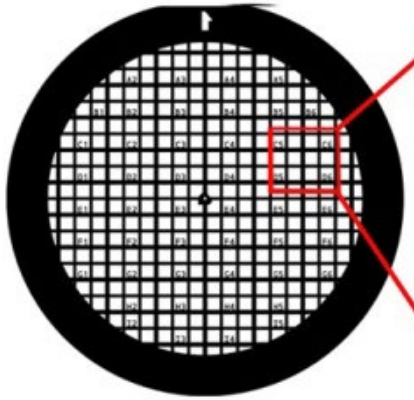
Correlative Light and Electron Microscopy (CLEM)

IMPACT IMAGING LIFE FROM MOLECULES TO CELLS

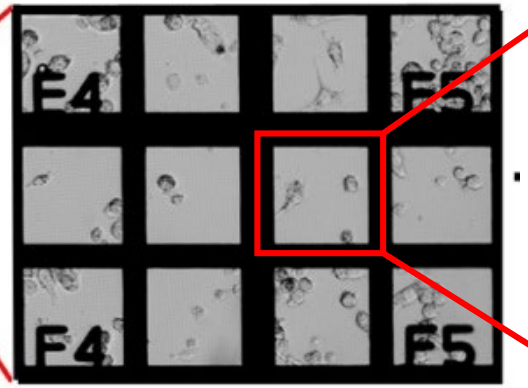


General CLEM workflow

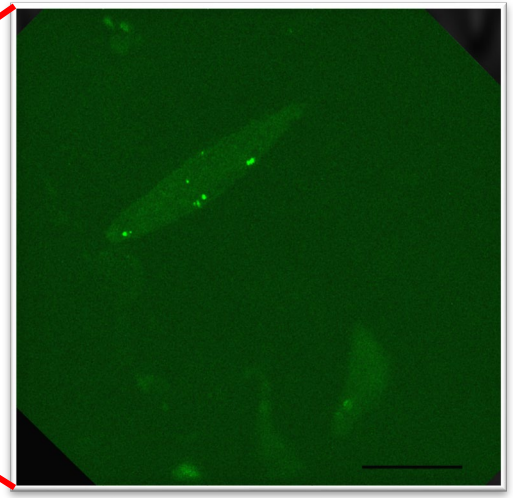
IMPACT IMAGING LIFE FROM MOLECULES TO CELLS



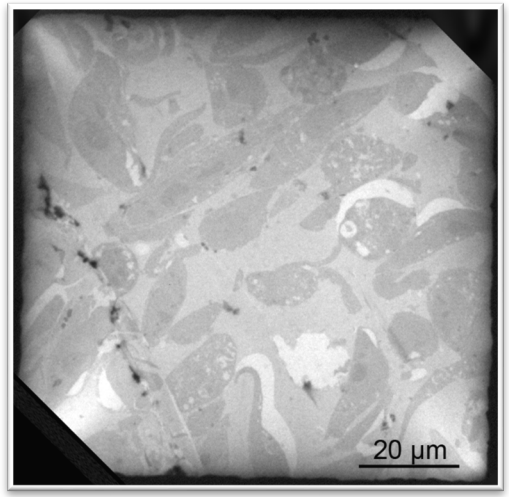
Sample on a grid



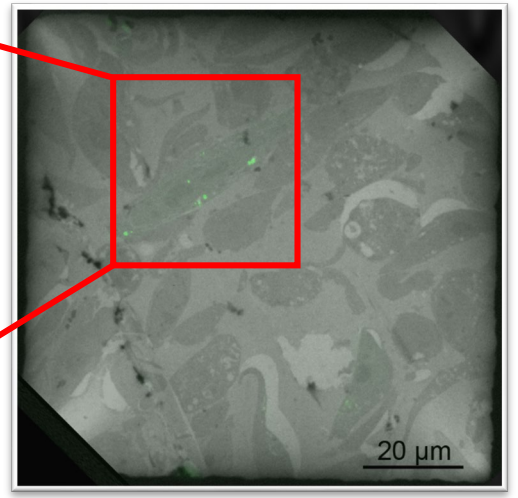
Mapping the grid



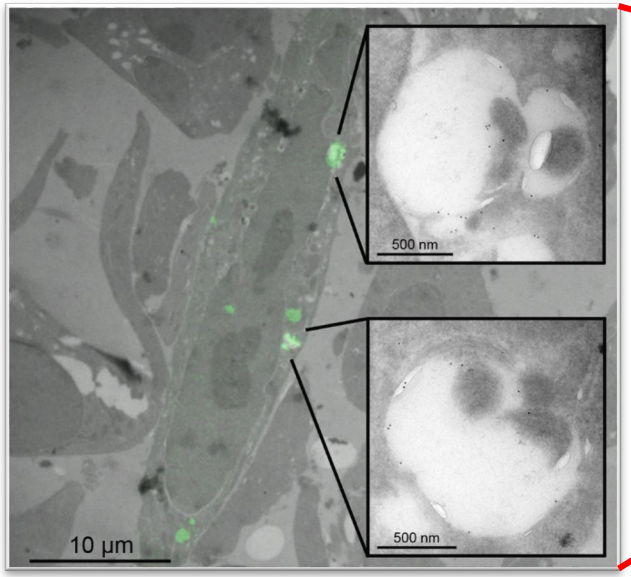
Focusing on a specific **FL** labelled cell



Locating the cell in the **TEM**

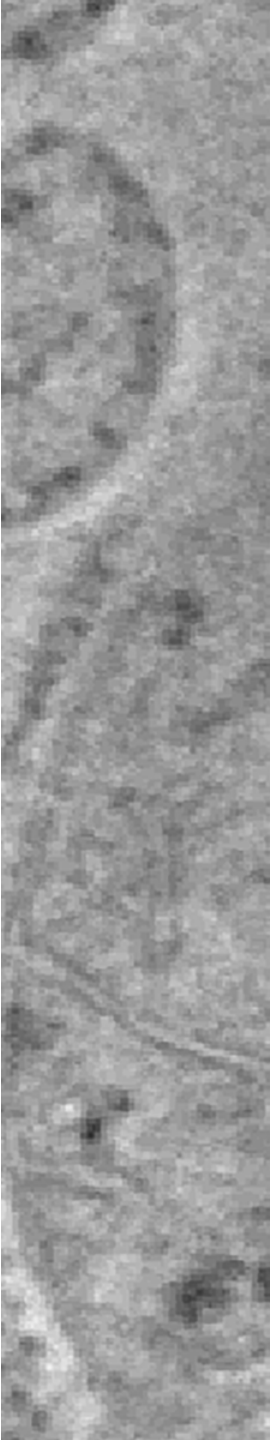


Overlay the **FL** and **TEM** images



Zoom in for high resolution and morphology

Dr. Smadar Zaidman



Correlative Light and Electron Microscopy (CLEM)

IMPaCT IMAGING LIFE FROM MOLECULES TO CELLS

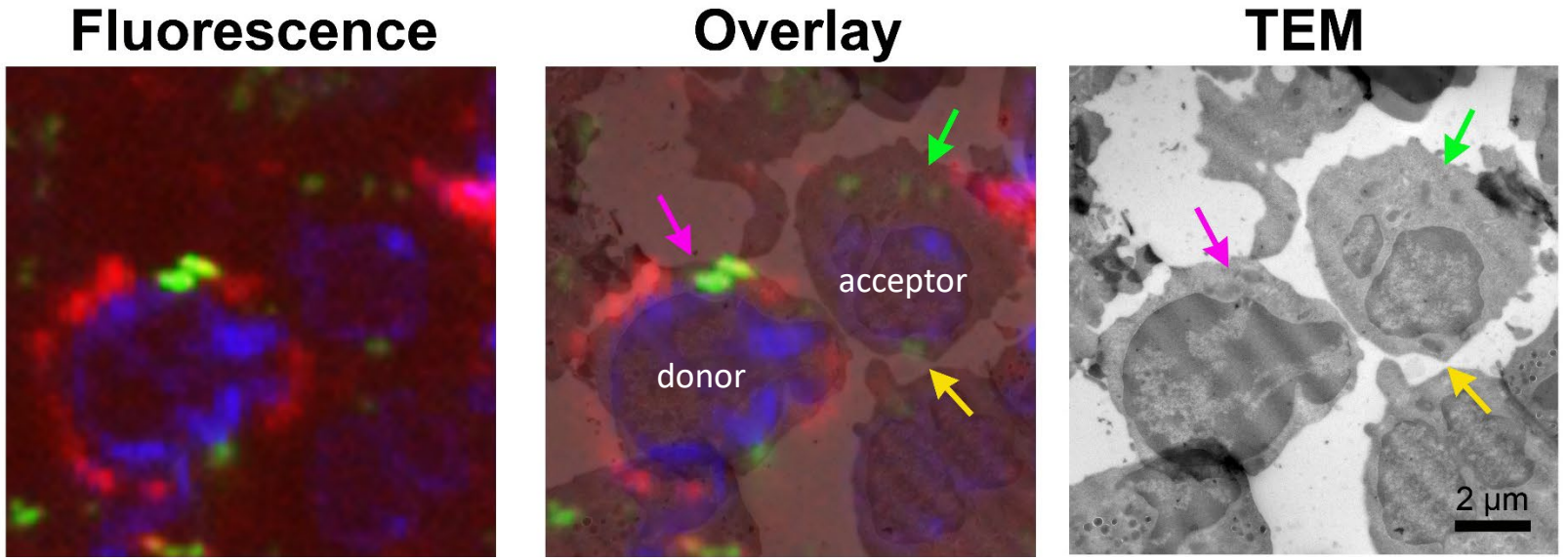
1. Taking advantage of both imaging techniques (FL and EM):
   - Specificity of fluorescent markers to identify or pre-select cellular targets
   - High resolution and morphology of EM

2. An efficient approach to the “needle in a haystack” challenge: targeting sparse events in a sample such as proteins, organelles and bacteria.

How to distinguish between sick and healthy cells?

IMPACT IMAGING LIFE FROM MOLECULES TO CELLS

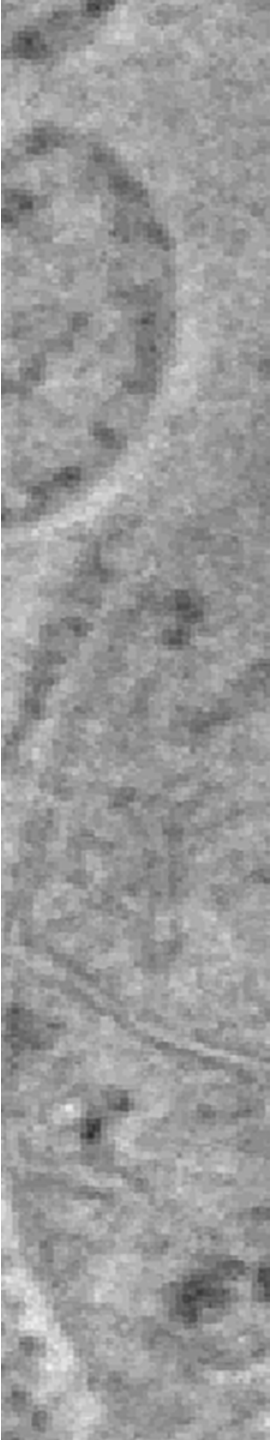
CD45 (donor)
mitochondria (Dendra2)
Nuclei (DAPI)



Donor (healthy) hematopoietic cells transfer functional mitochondria to the irradiated host (sick) bone marrow following total body irradiation.

Dr. Smadar Zaidman

Golan K, et al., Blood 2020



Correlative Light and Electron Microscopy (CLEM)

IMPaCT IMAGING LIFE FROM MOLECULES TO CELLS

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   - High resolution and morphology of EM

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How long would it take to find Waldo??

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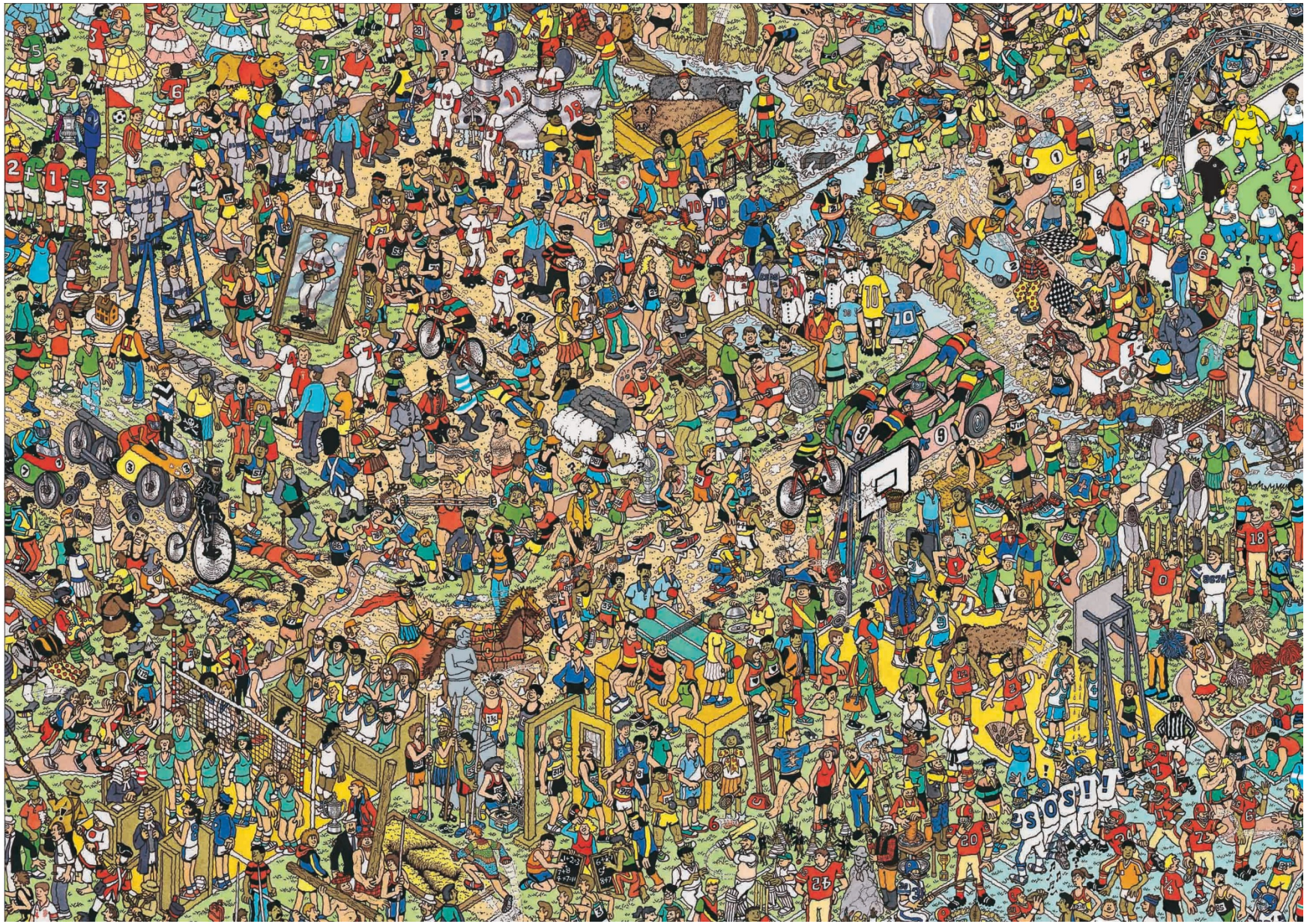
FROM MOLECULES
TO CELLS



Now…??

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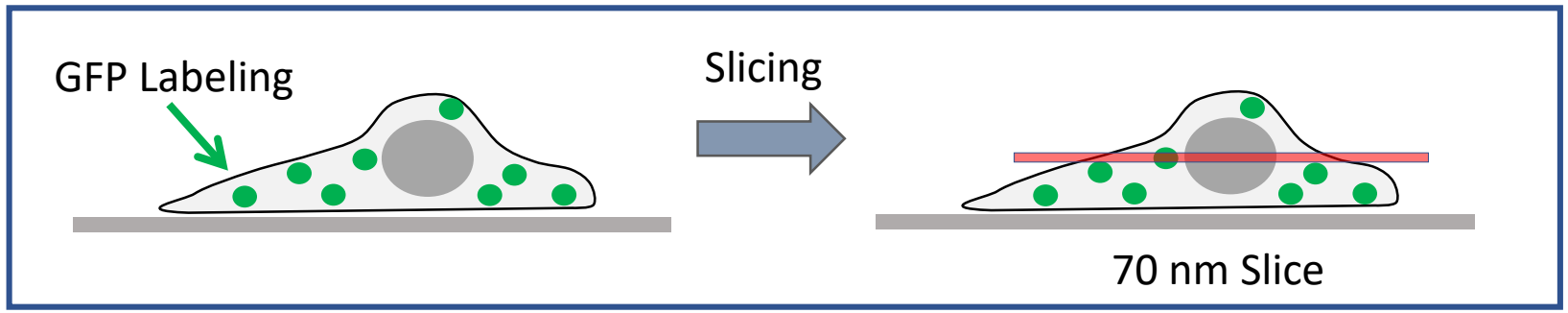
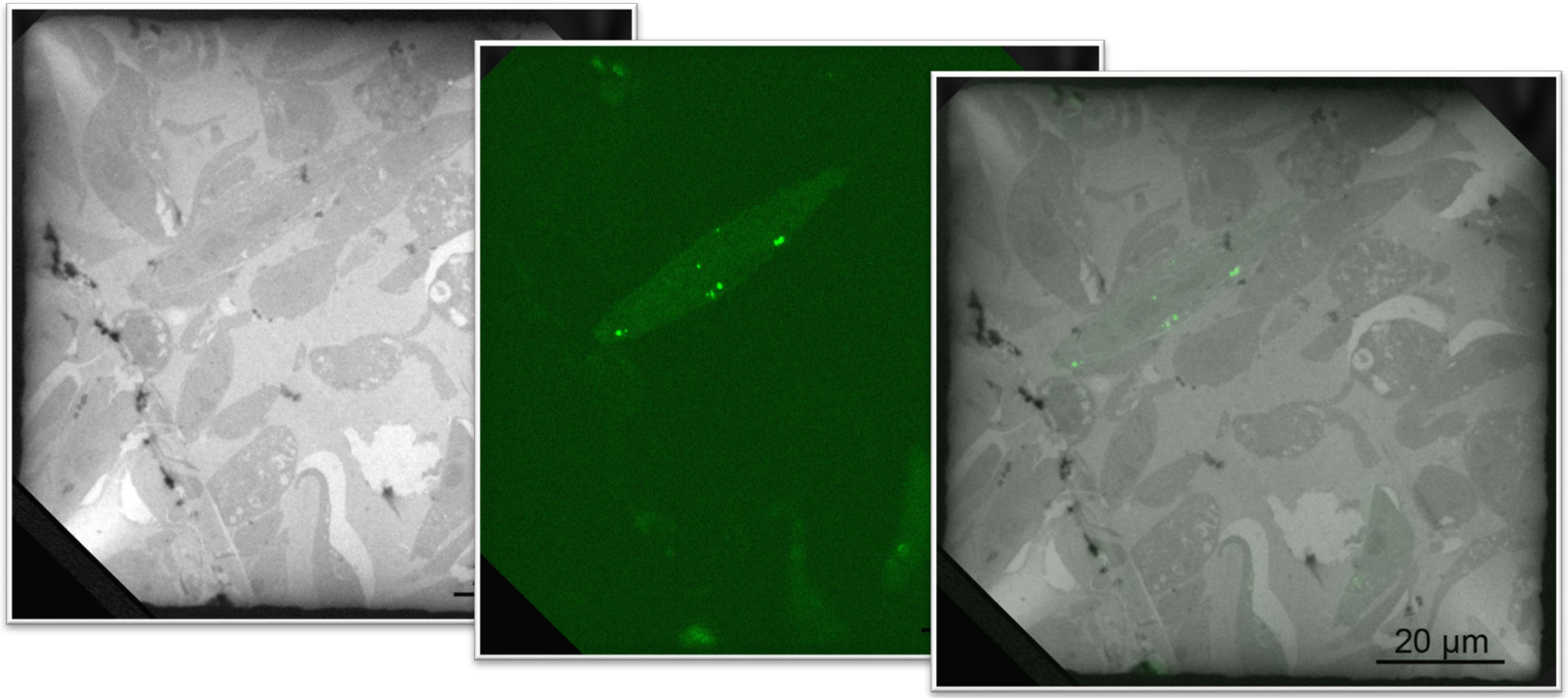
TO CELLS

Correlation of Fluorescence and TEM

(Tokuyasu technique)

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS





Correlative workflow

Sample fixation

↓

Sucrose embedding

↓

Cryo sectioning

↓

Light Microscopy

↓

Immuno-gold labeling

↓

TEM

Dr. Smadar Zaidman

Abada, A., S. Levin-Zaidman, Z. Porat, T. Dadosh, and Z. Elazar, PNAS 2017

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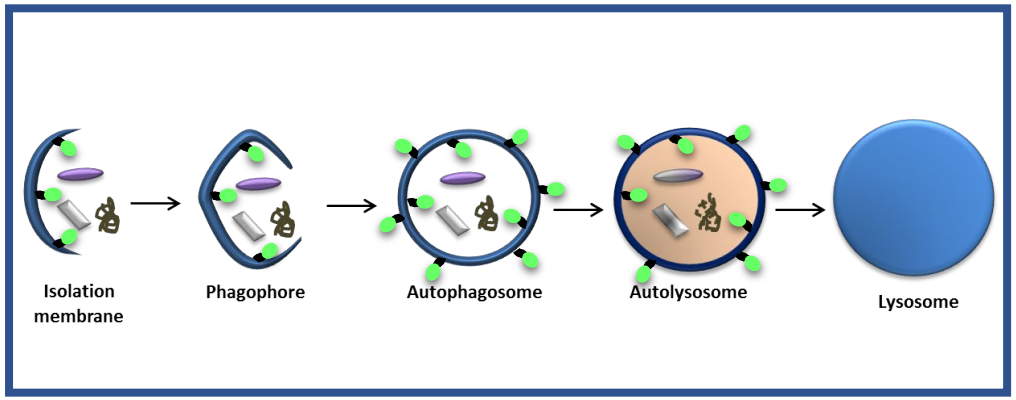
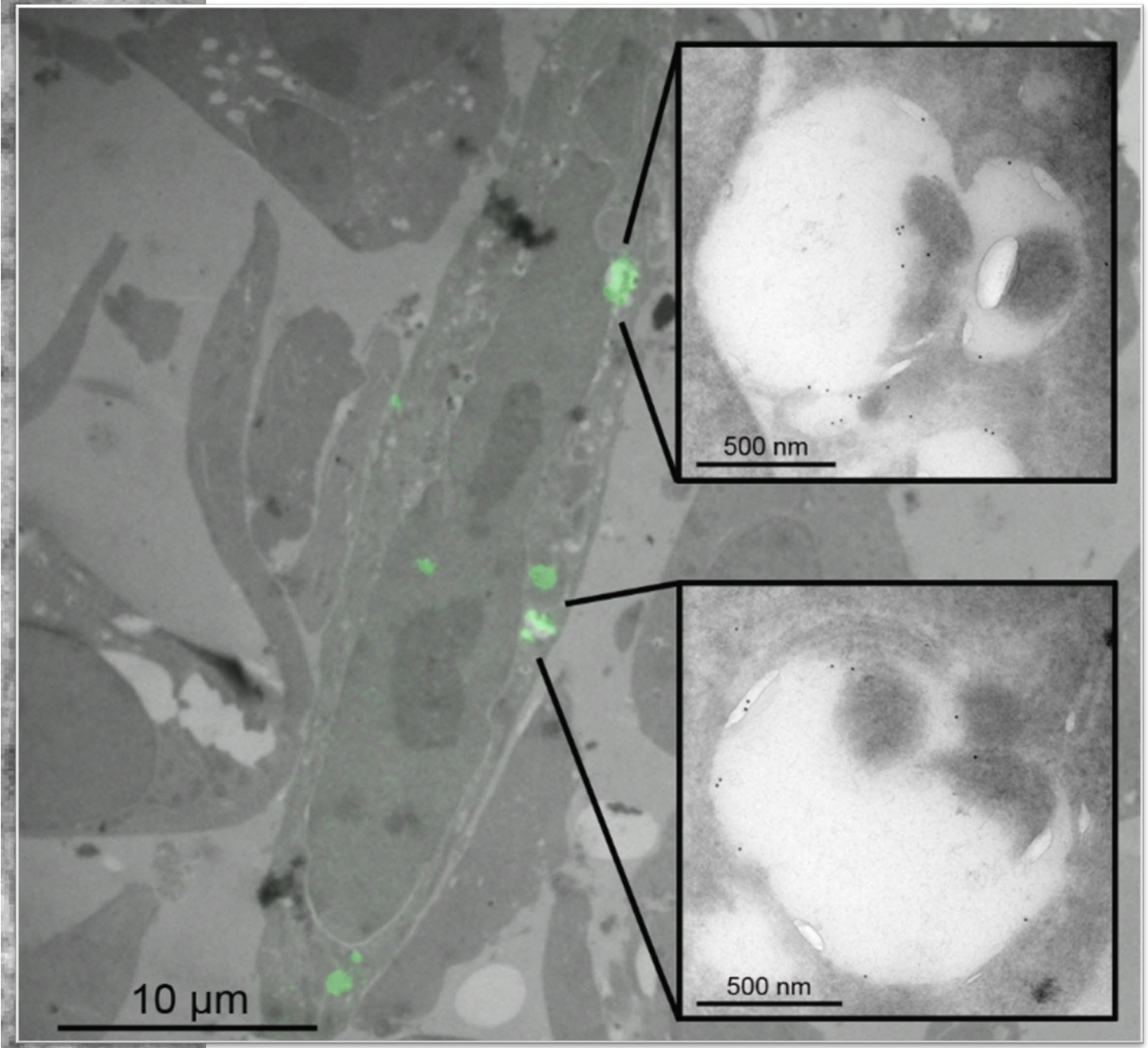
FROM MOLECULES
TO CELLS

Autophagosome Biogenesis





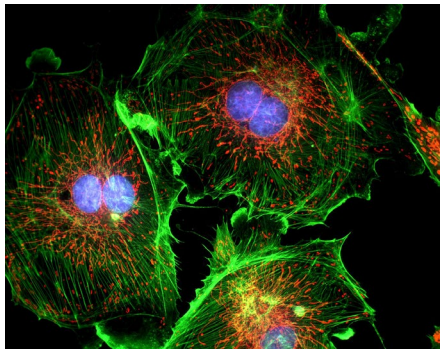
LC3-GFP-10nm Gold NPs

Abada, A., S. Levin-Zaidman, Z. Porat, T. Dadosh,and Z. Elazar, PNAS 2017

Challenges

Fluorescence labeling of the sample

- Genetically encoded fluorescent proteins (GFP, mCherry etc.)
- Organic fluorophores (bright and photo stable).

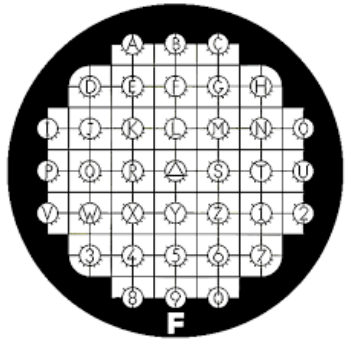


How to keep the fluorescence alive after treatment for EM?

- Fluorescence is quenched by dehydration, fixatives, heavy metals and resins. Hence, Protocols should be optimized and compromised – specific acrylic resins, no or very little osmium and UrAc…

Registration of images from both modalities

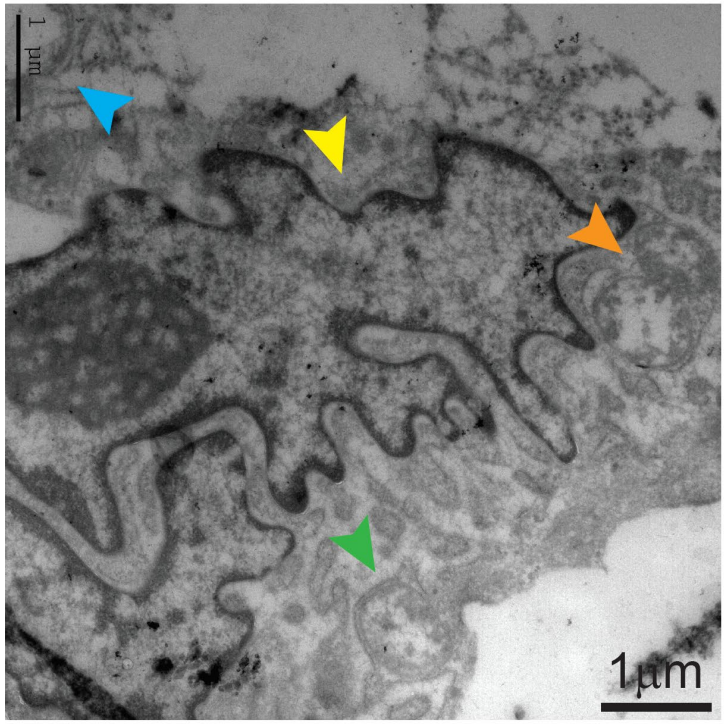
- For navigating and low resolution registration - marked substrate such as finder grid.
- For more precise registration - fiducial markers (FL beads, Nucleus labeling etc.).



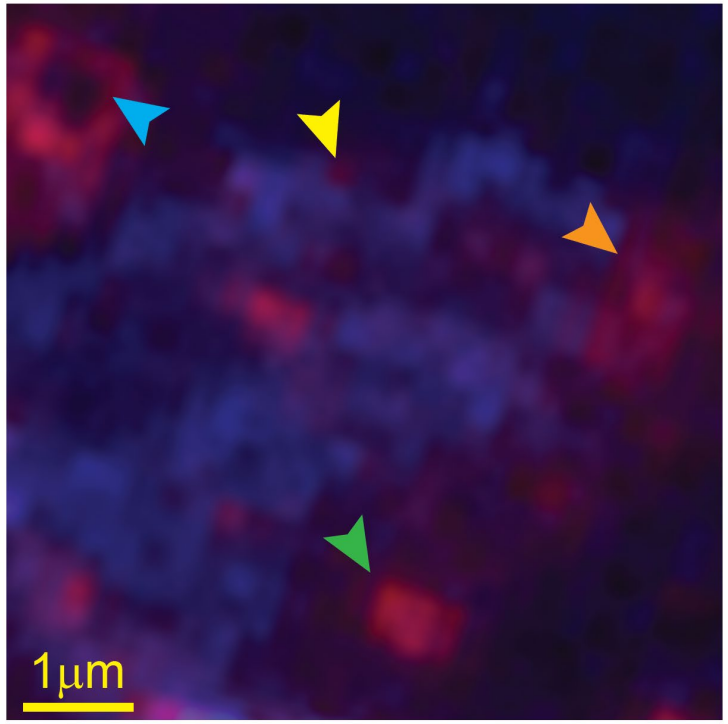
CLEM targets intra-cellular bacteria in human breast cancer

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

TEM

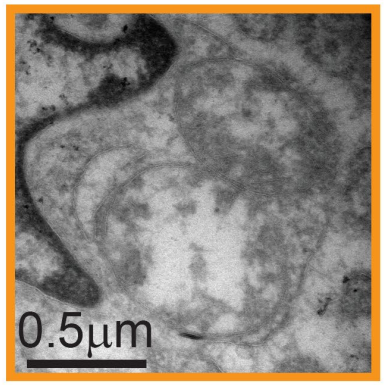


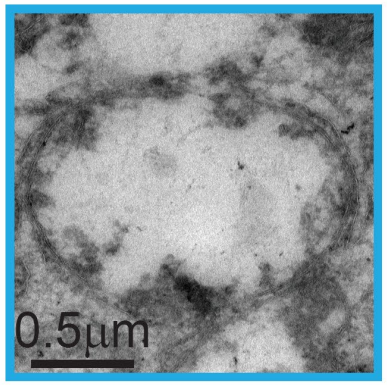
Fluorescence

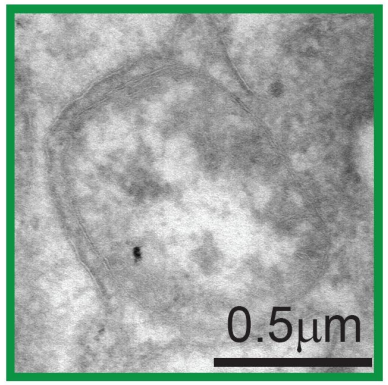


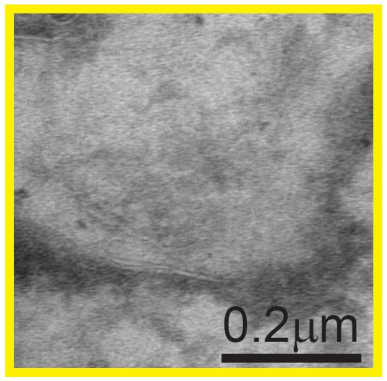
Identification of bacteria in human breast cancer tumor cells.

Red – Bacteria (Anti-LPS)
Blue – nucleus (DAPI)









Dr. Smadar Zaidman

Nejman D. et al., Vol. 368, Issue 6494, pp. 973-980, Science 2020

Correlative SEM and STORM

The cellulosome (Large multi-enzyme complex) in *Clostridium Clariflavum*

Correlative workflow

STORM Imaging (fixed sample)

↓

OsO4 post fixation

↓

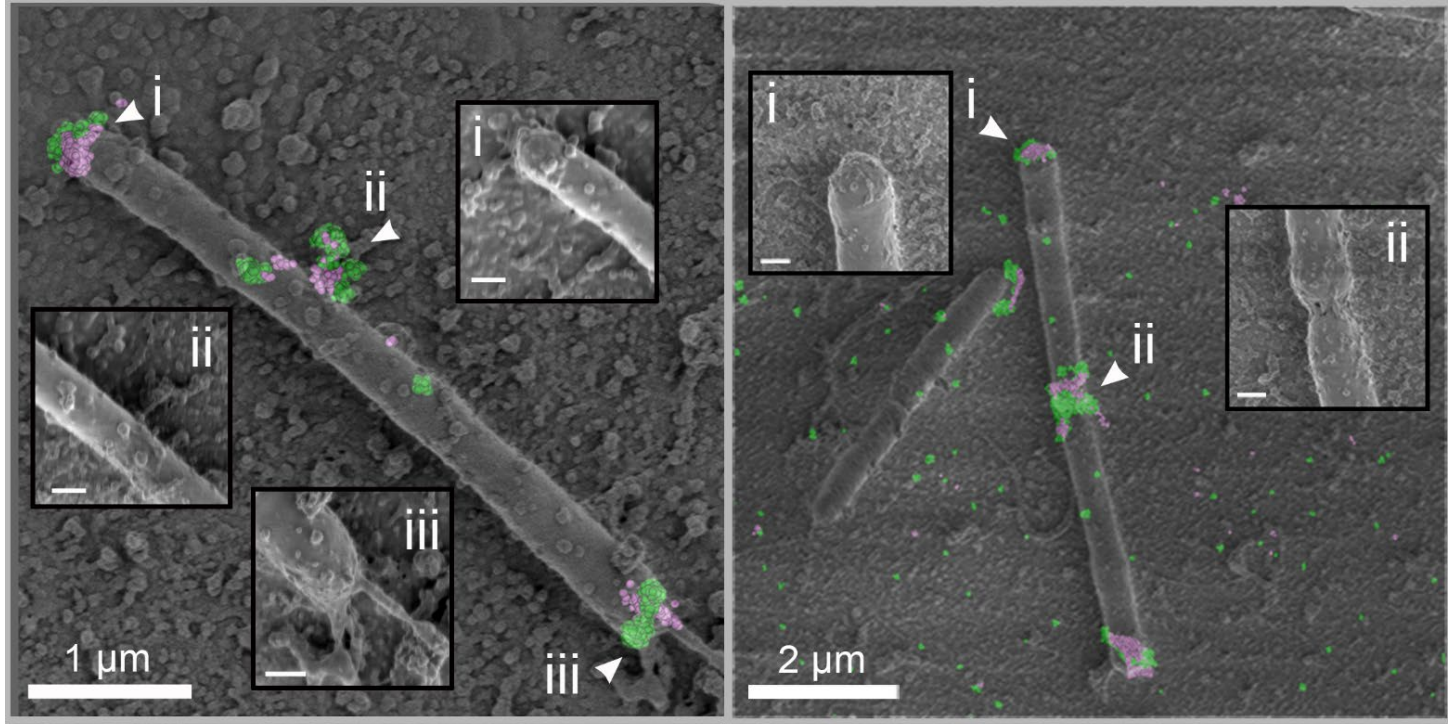
Ethanol dehydration

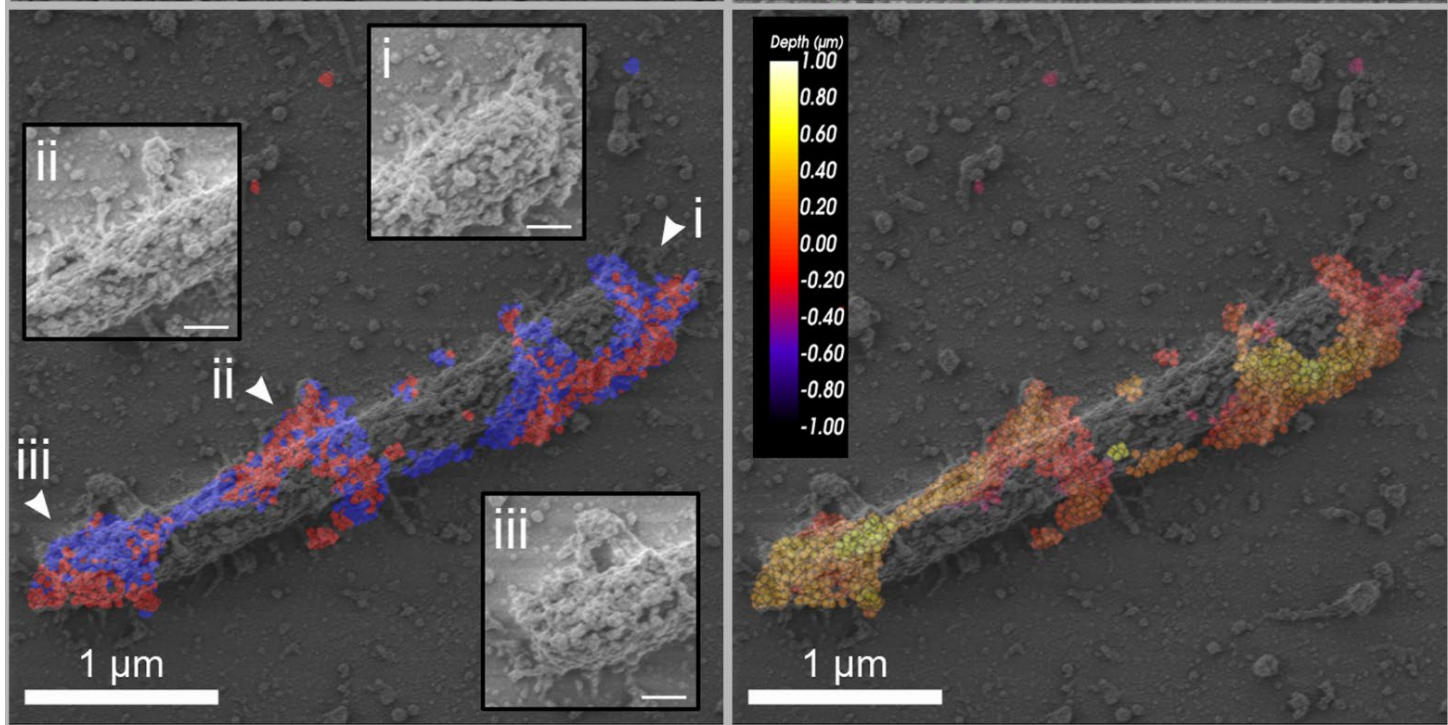
↓

Critical point drying

↓

SEM

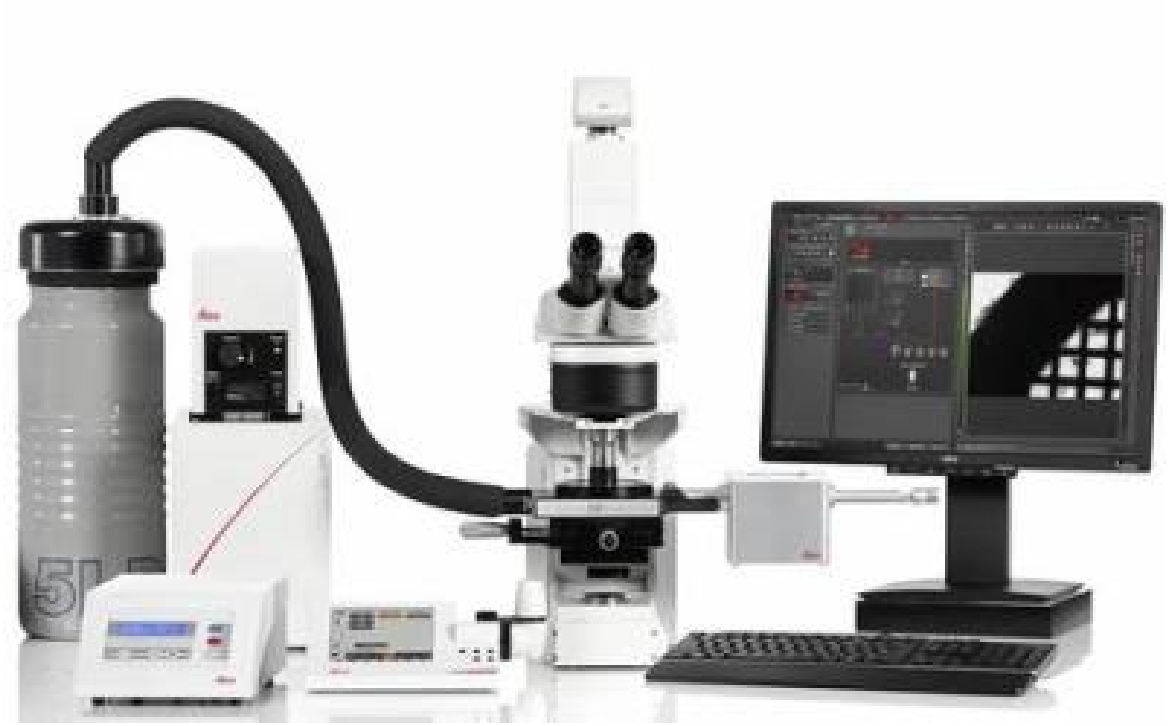




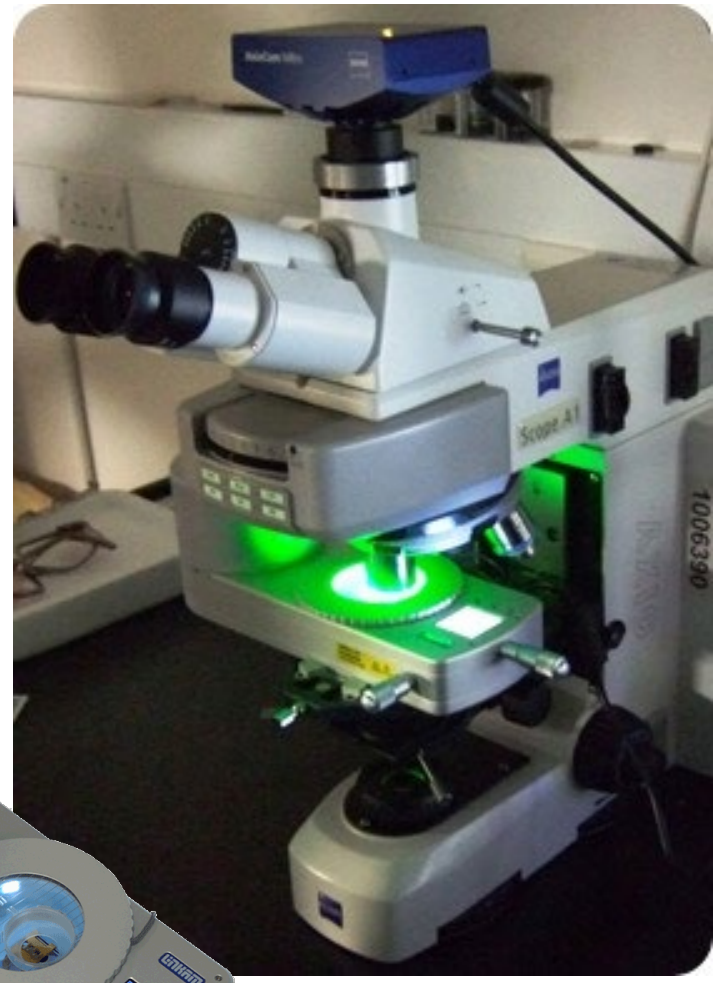
Artzi L., Dadosh T., Milrot E., Moraïs S., Levin-Zaidman S., Morag E., Bayer E., mBio 2018

Cryo-CLEM

IMPaCT IMAGING LIFE FROM MOLECULES TO CELLS



Cryo-CLEM Leica

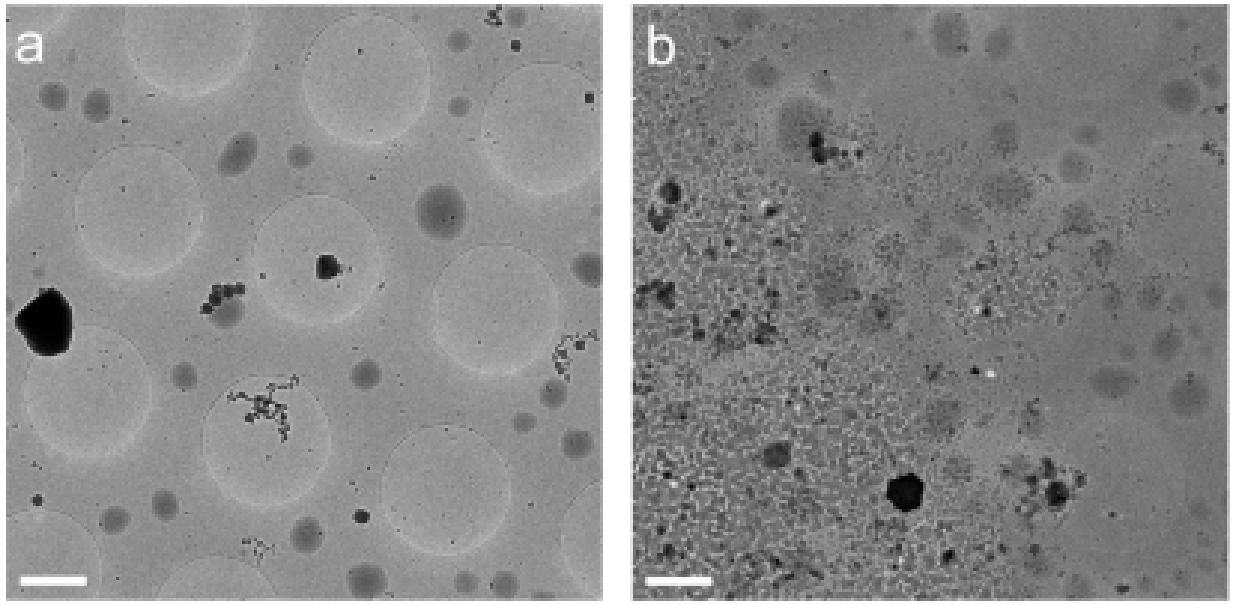


Cryo-CLEM Linkam

Stability
Laser damage
Long working distance objectives (lower NA)

Damage of vitreous water by laser illumination

IMPACT IMAGING LIFE FROM MOLECULES TO CELLS



550 W/cm2
30 min

650 W/cm2
5 min

Exposure to high intensity laser light can devitrify cryo samples.

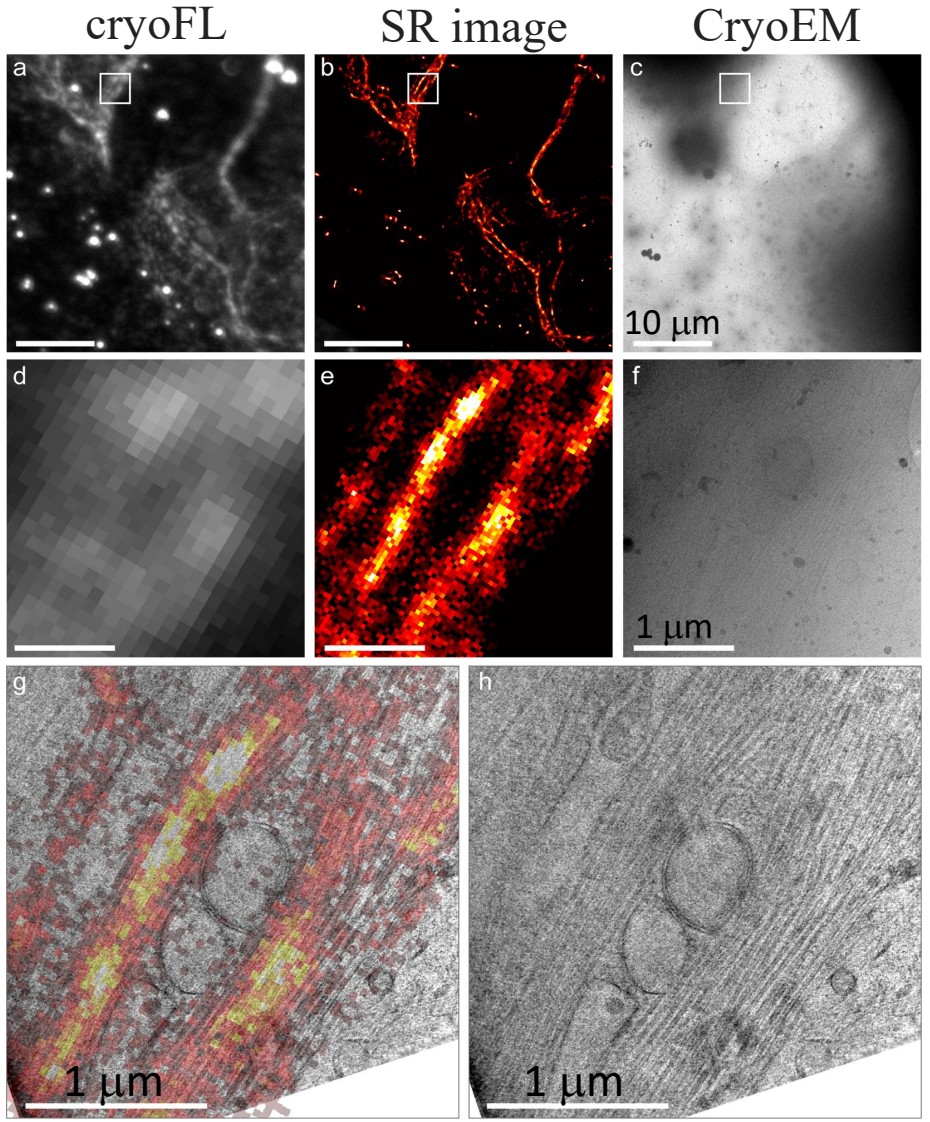
Devitrification is dominated by the laser intensity, and not the illumination time.

Tuijtel, M.W., Koster, A.J., Jakobs, S. *et al.* *Sci Rep* **9,** 1369 (2019).

CryoEM and Cryo Super Resolution

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

Human bone osteosarcoma epithelial (U2OS) cells transfected with plasmid encoding rsEGFP2 fused to microtubule-associated protein 2 (MAP2).



Overlay SR image over 18.6 nm tomographic thick slice

Tuijtel, M.W., Koster, A.J., Jakobs, S. *et al. Sci Rep* **9,** 1369 (2019).

מכון ויצמן למדע
WEIZMANN INSTITUTE OF SCIENCE

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

My colleagues at the Electron Microscopy Unit
Weizmann Institute of Science

Smadar Zaidman
Eyal Shimoni
Sharon Grayer Wolf
Katya Rechav
Nili Dezorella
Olga Brontvein
Anna Eden Kossoy
Nadav Elad
Lothar Houben
Ifat Kaplan Ashiri
Eelena Kartvelishvily
Sergey Kapishnikov
Orna Yeger

Thank you!

Michael Elbaum - Weizmann