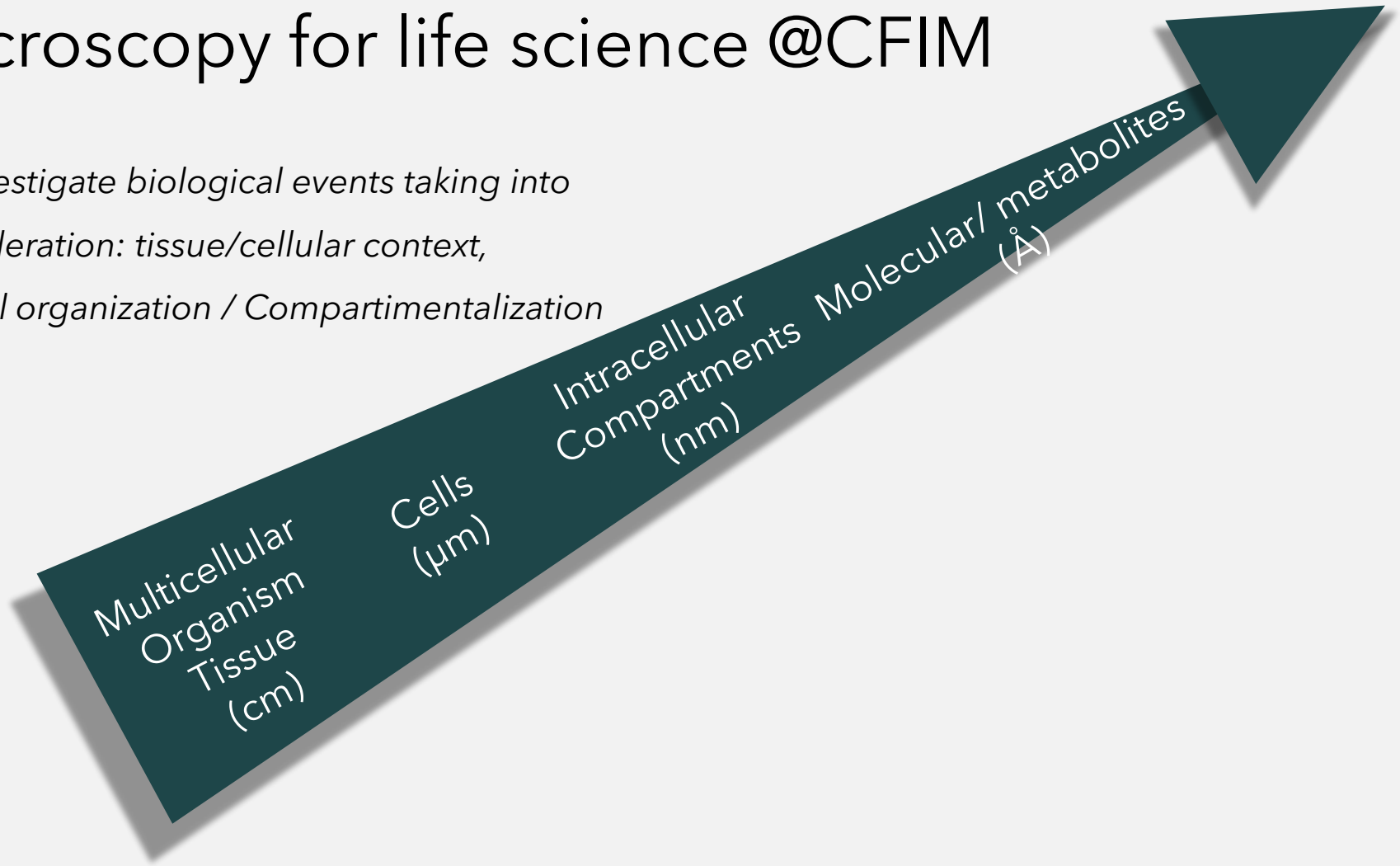
THE CORE FACILITY FOR INTEGRATED MICROSCOPY

Faculty of Health and Medical Sciences, University of Copenhagen

THE CORE FACILITY FOR INTEGRATED MICROSCOPY

Microscopy for life science @CFIM

To investigate biological events taking into consideration: tissue/cellular context, Spatial organization / Compartimentalization



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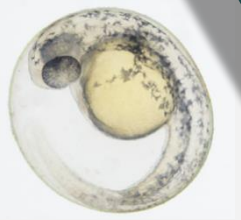
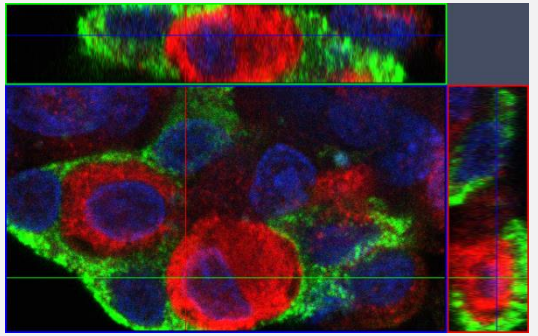


Multicellular Organism Tissue (cm)

Organism development / Cell migration / Tissue organisation / Pathology



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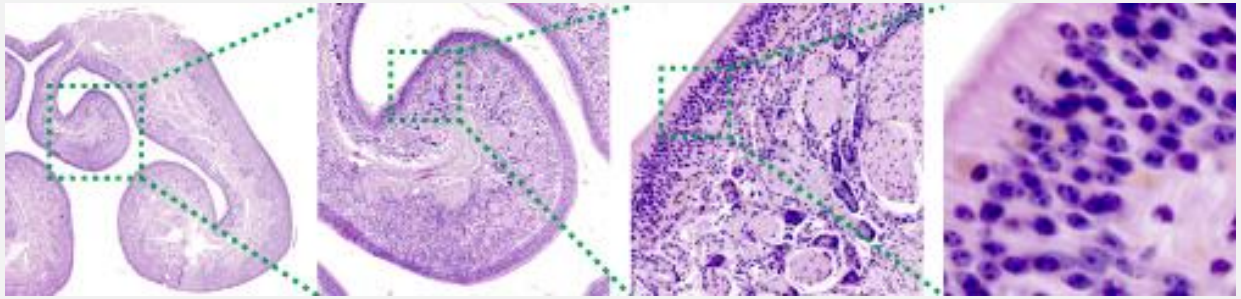
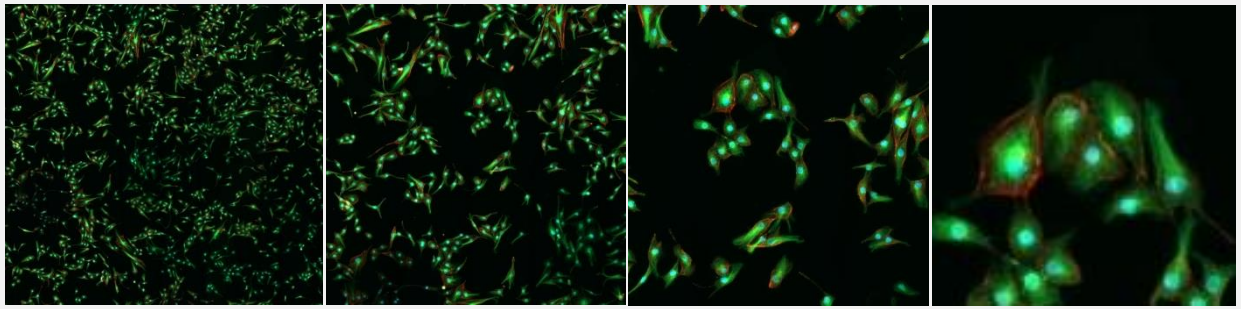
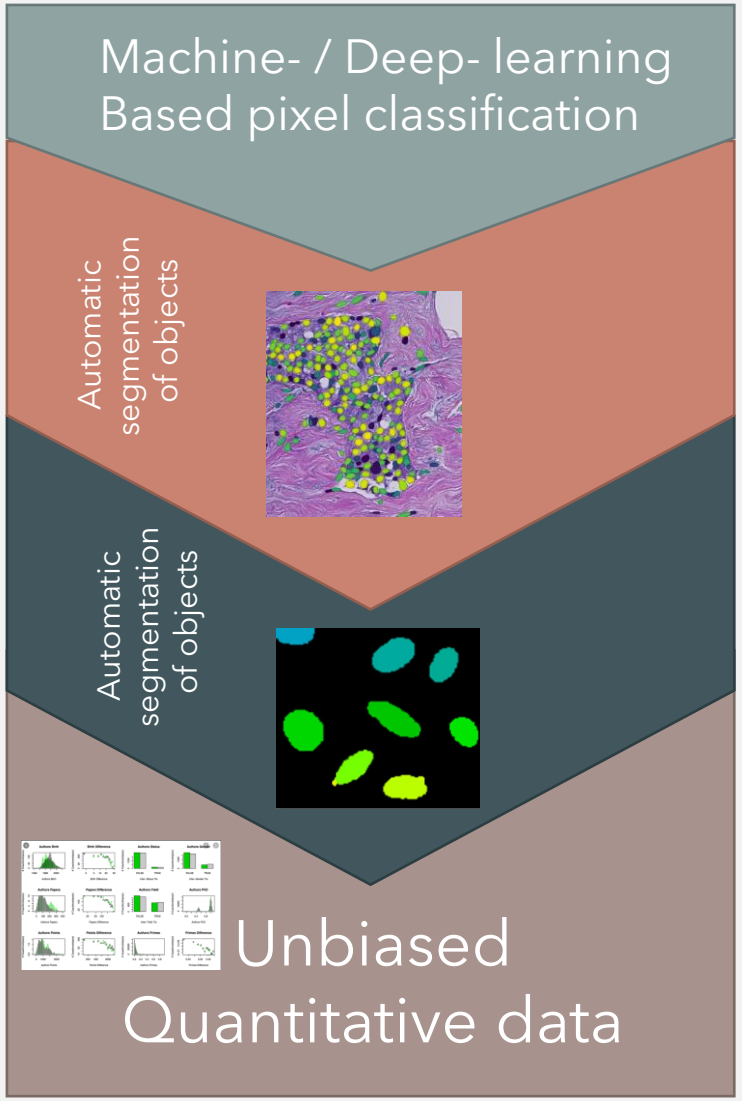
Digital Pathology





Large amounts of image-based data



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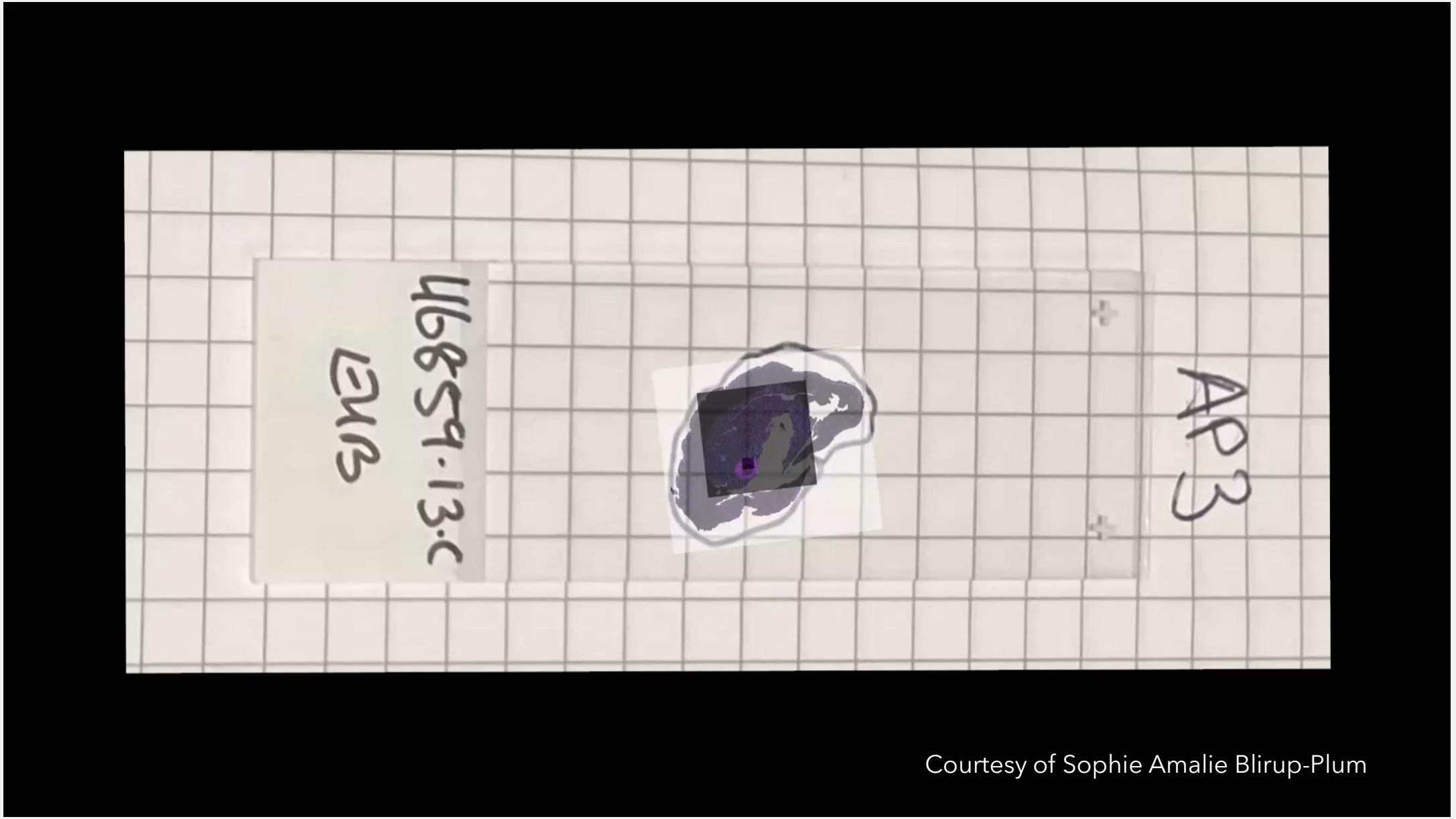

Courtesy of Sophie Amalie Blirup-Plum

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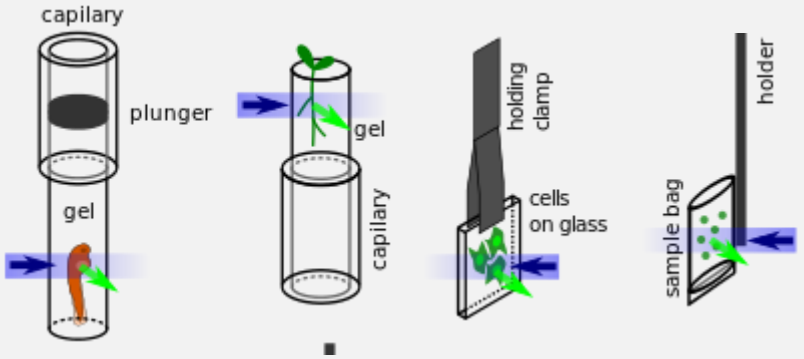
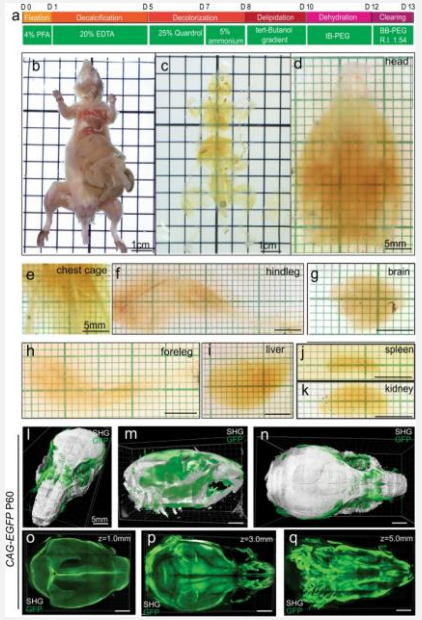
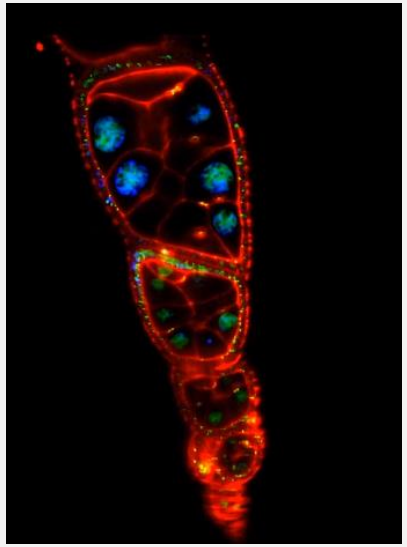
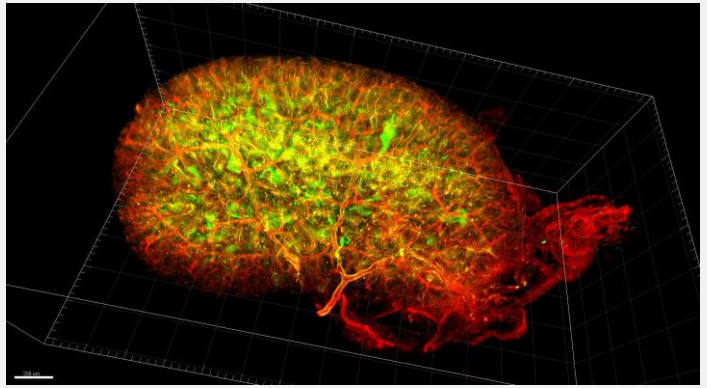
Selective Plane Illumination Microscopy
Light sheet Microscopy (live and cleared)

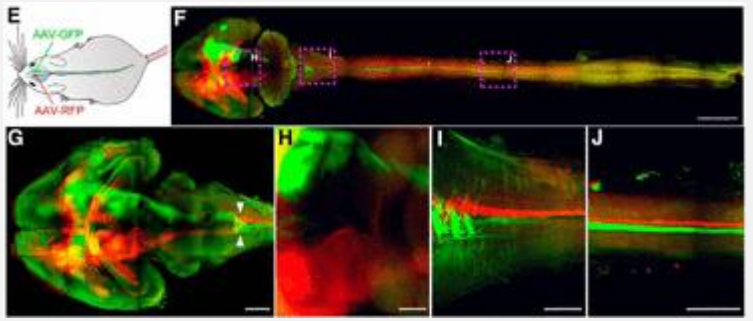
Relatively NEW From 2021











Mano et al 2018

NNF infrastructure
Bio DEEP

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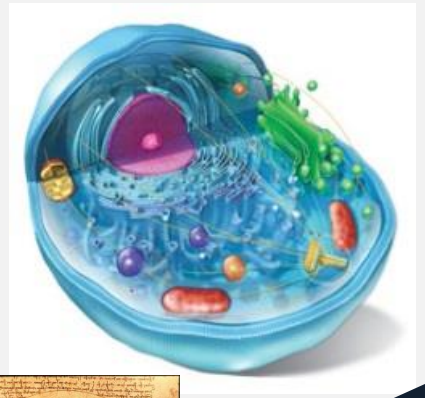
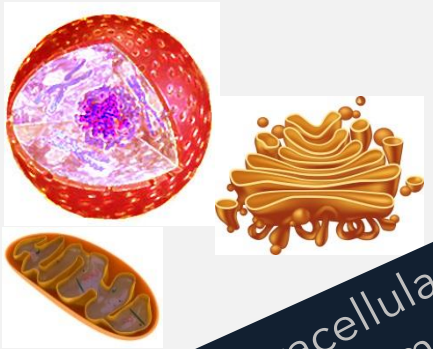
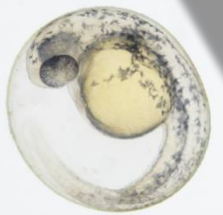


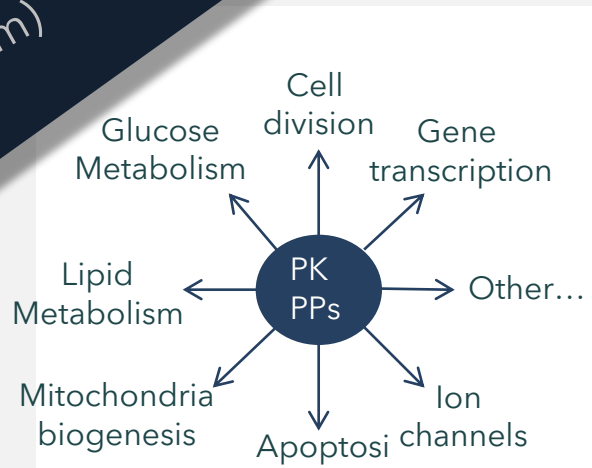


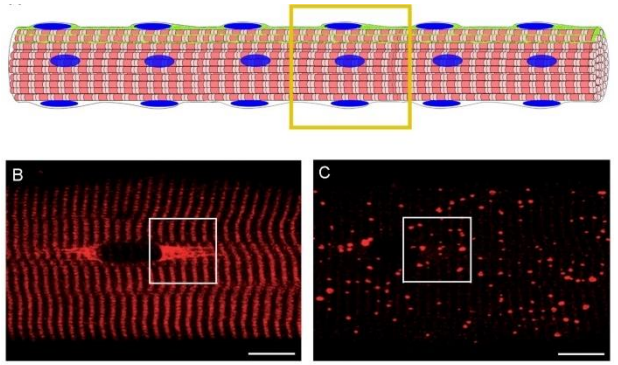


Multicellular Organism Tissue (cm)

Cells (μm)

Intracellular Compartments (nm)





Prats et al. JBC 2009

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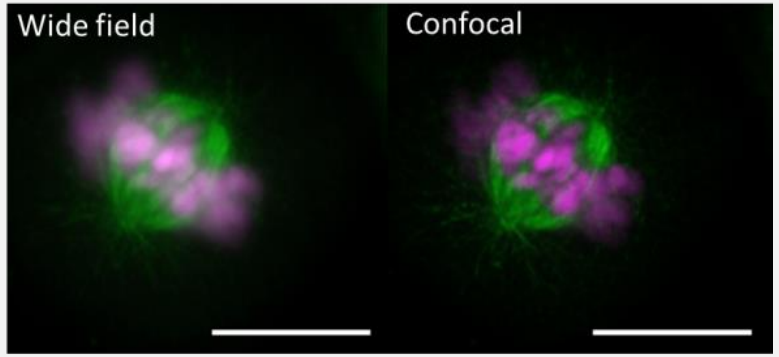
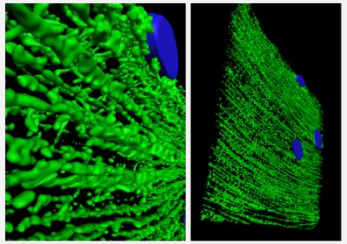
- Cell behaviour
- Dynamics of Intracellular processes
- 3D imaging of intracellular structures

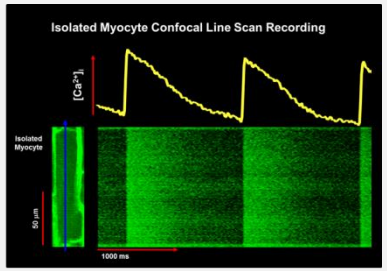
Widefield fluorescence Microscopy
Laser Scanning Microscopy



by Laure Plantard

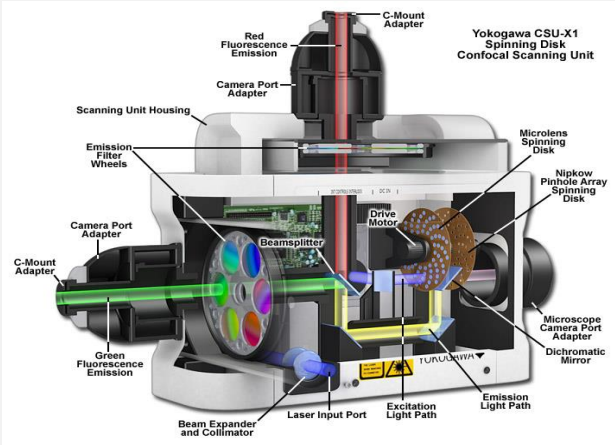


by Clara Prats



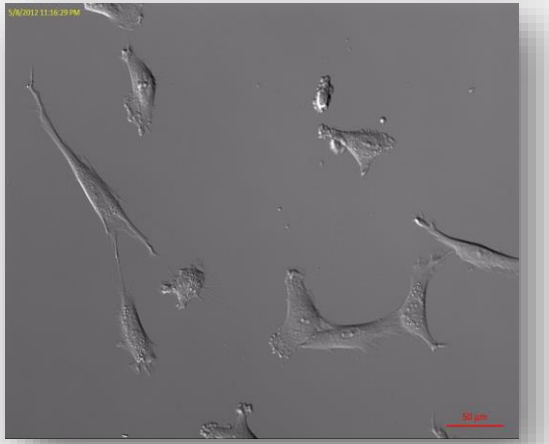
by Søren Grubb

Spinning disk



Very fast

Very Slow



Cell division (~ 9hours - app. 0.1 Hz) by Vibe Nylander

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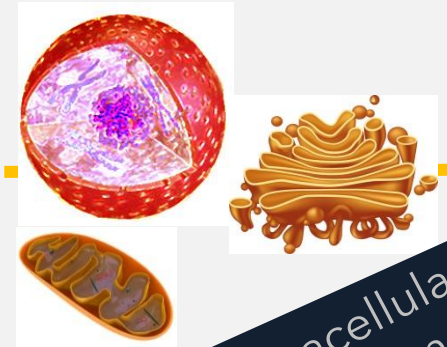
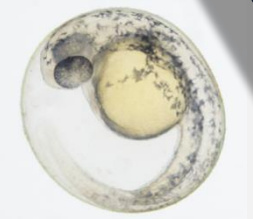




Multicellular Organism Tissue (cm)

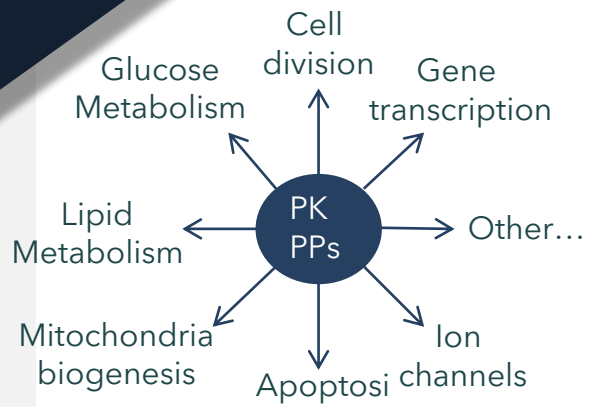
Cells (µm)

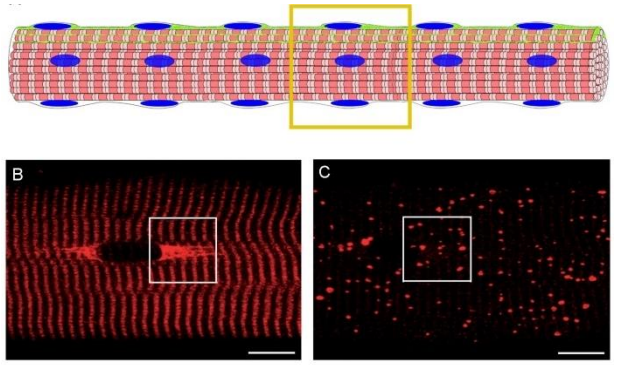
Intracellular Compartments (nm)

Light microscopy resolution limit

x,y ~ 275 nm and z ~ 500 nm

$$R = \frac{0.61\lambda}{\mathrm{NA}} \approx \frac{\lambda}{2\mathrm{NA}}$$





Prats et al. JBC 2009

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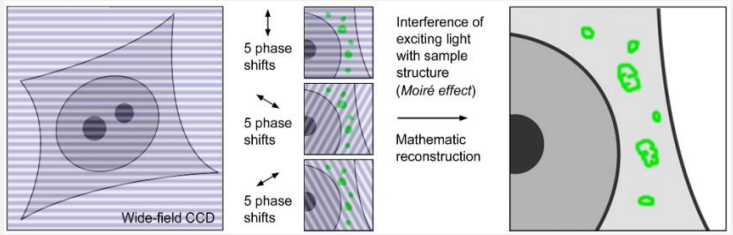
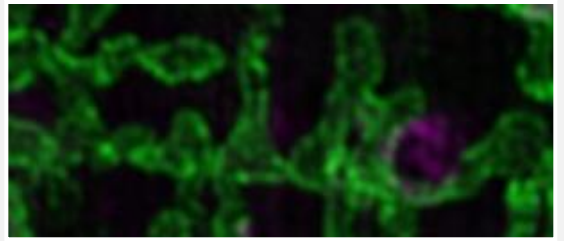
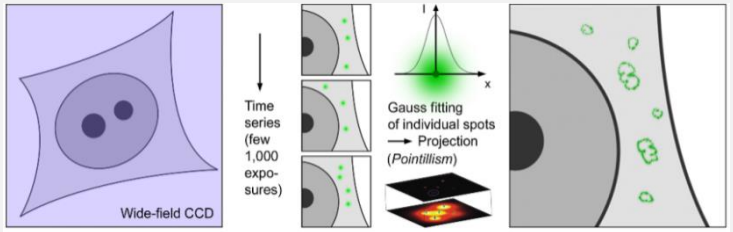
- Structured Illumination Microscopy

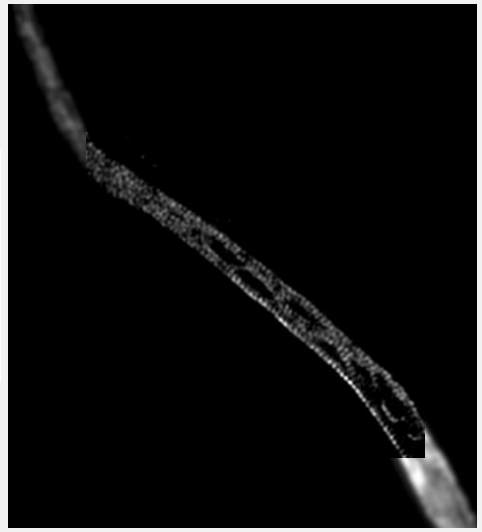




- Single Molecule Localization Techniques

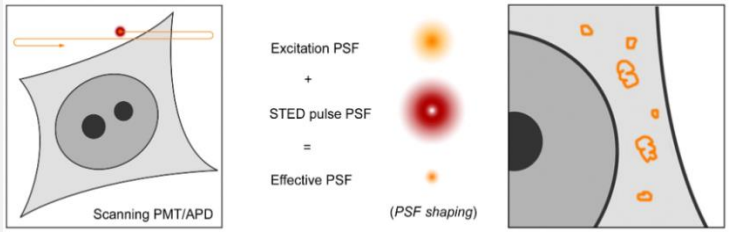
  Eric Betzig ♔
  Moerner ♔





- Stimulated Excitation Depletion - STED

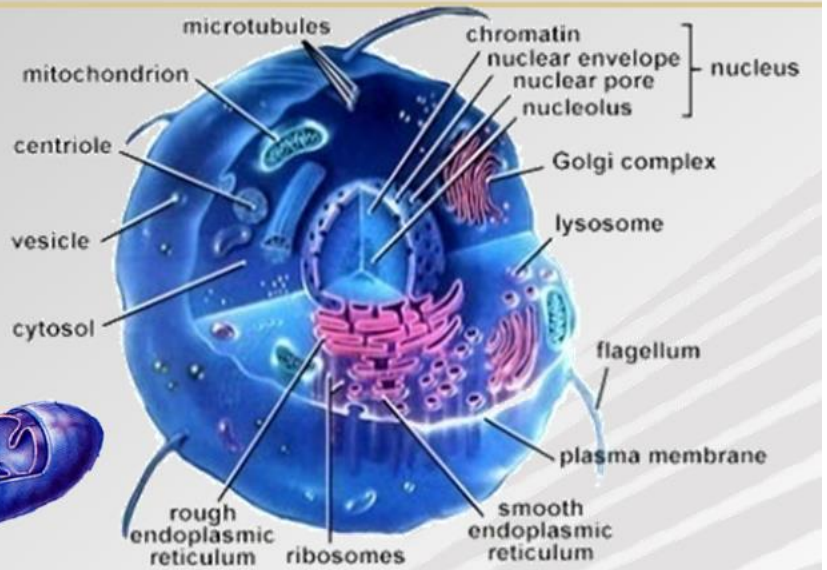
  Stefan Hell ♔

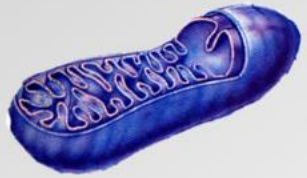


♔ Nobel prize in Chemistry 2014

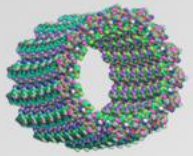
Microscopic Scale

What do you need?





Mitochondrion

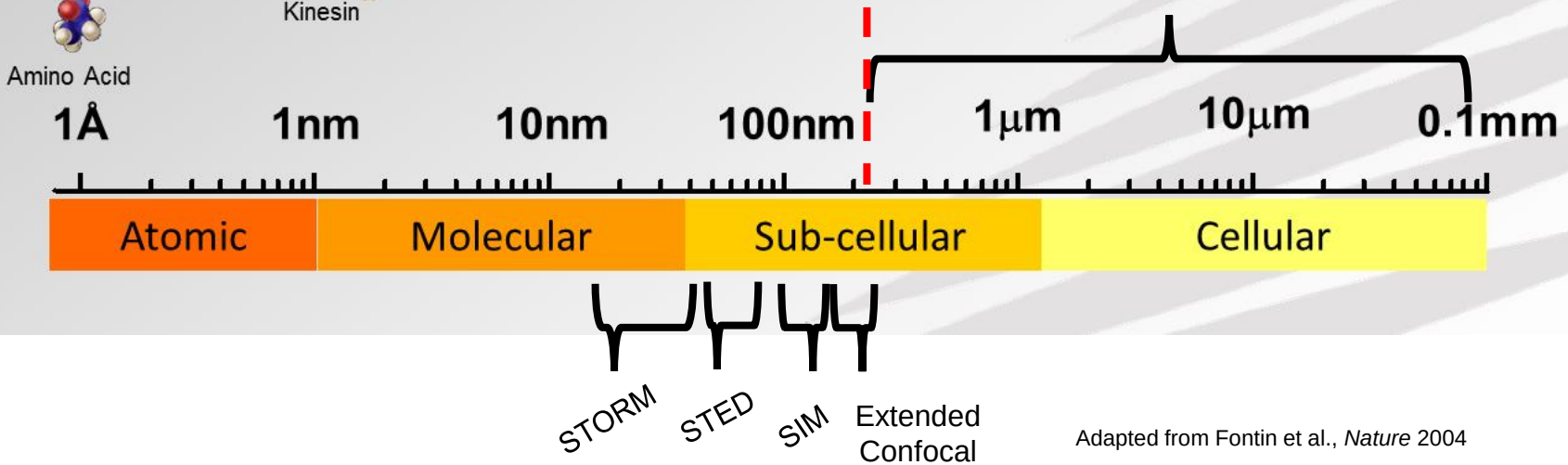


Microtubule

Kinesin

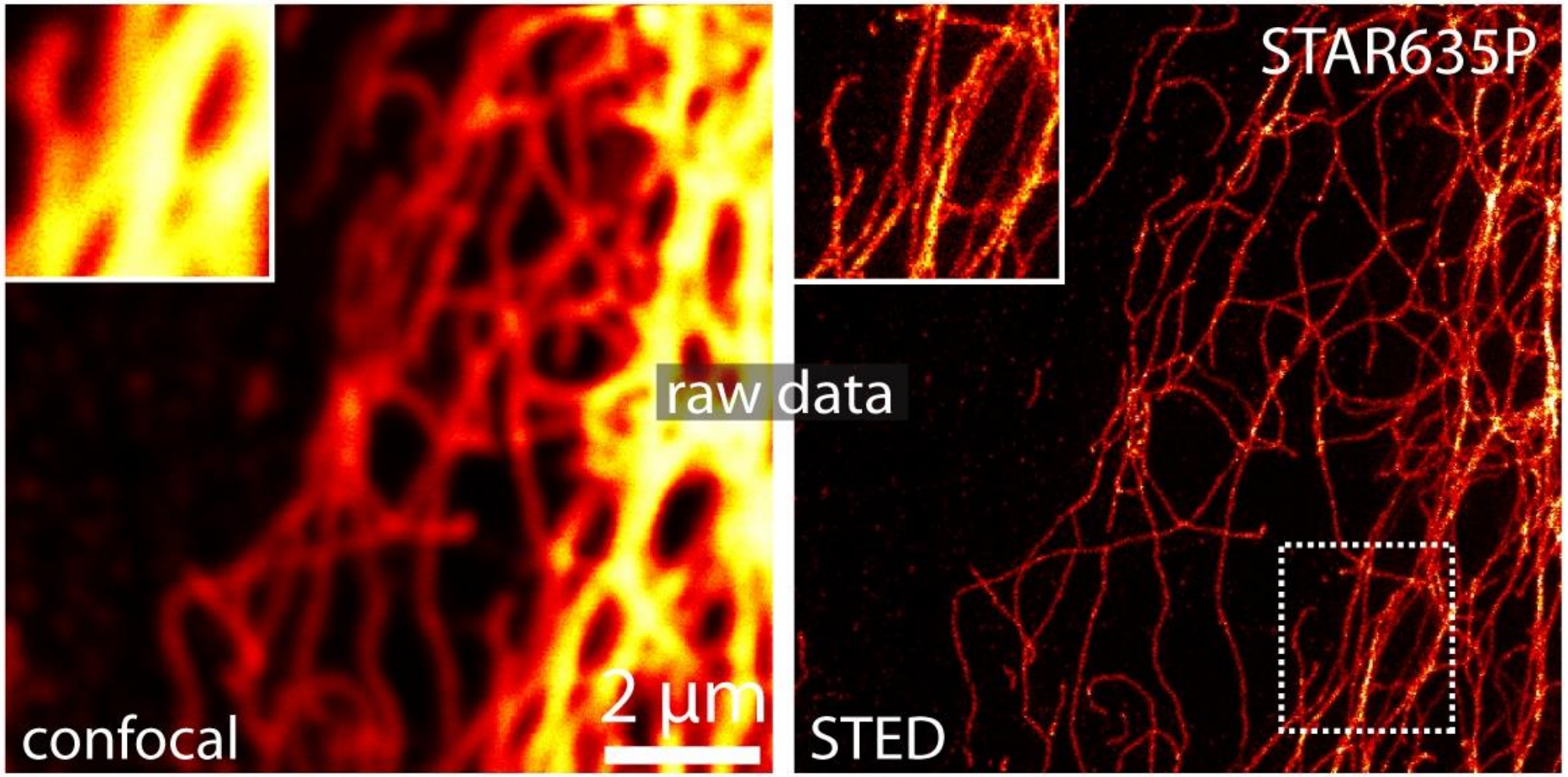
Amino Acid

Conventional light microscopy



Adapted from Fontin et al., *Nature* 2004

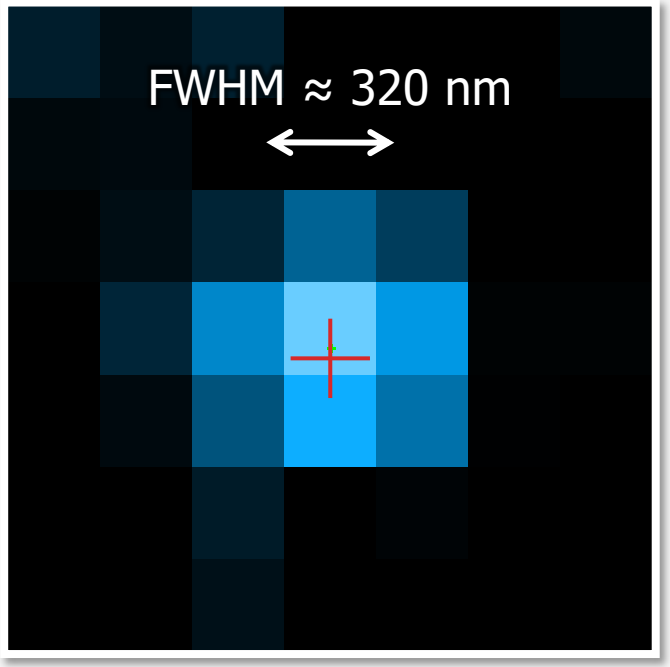
STED imaging example

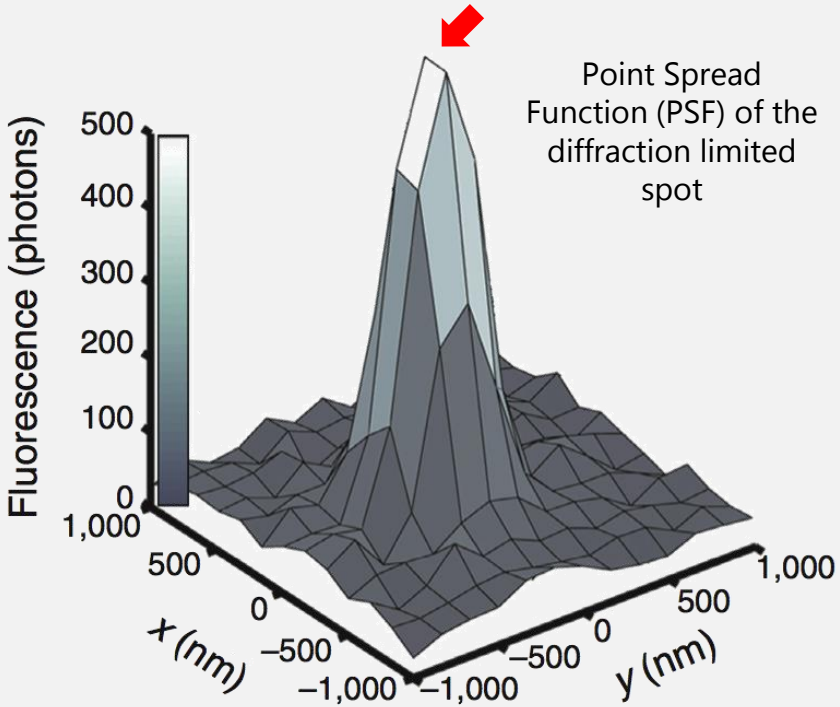


Single molecule localization microscopy

Single-Molecule Localization: Gaussian fitting

Diffraction limited blinks





Point Spread Function (PSF) of the diffraction limited spot

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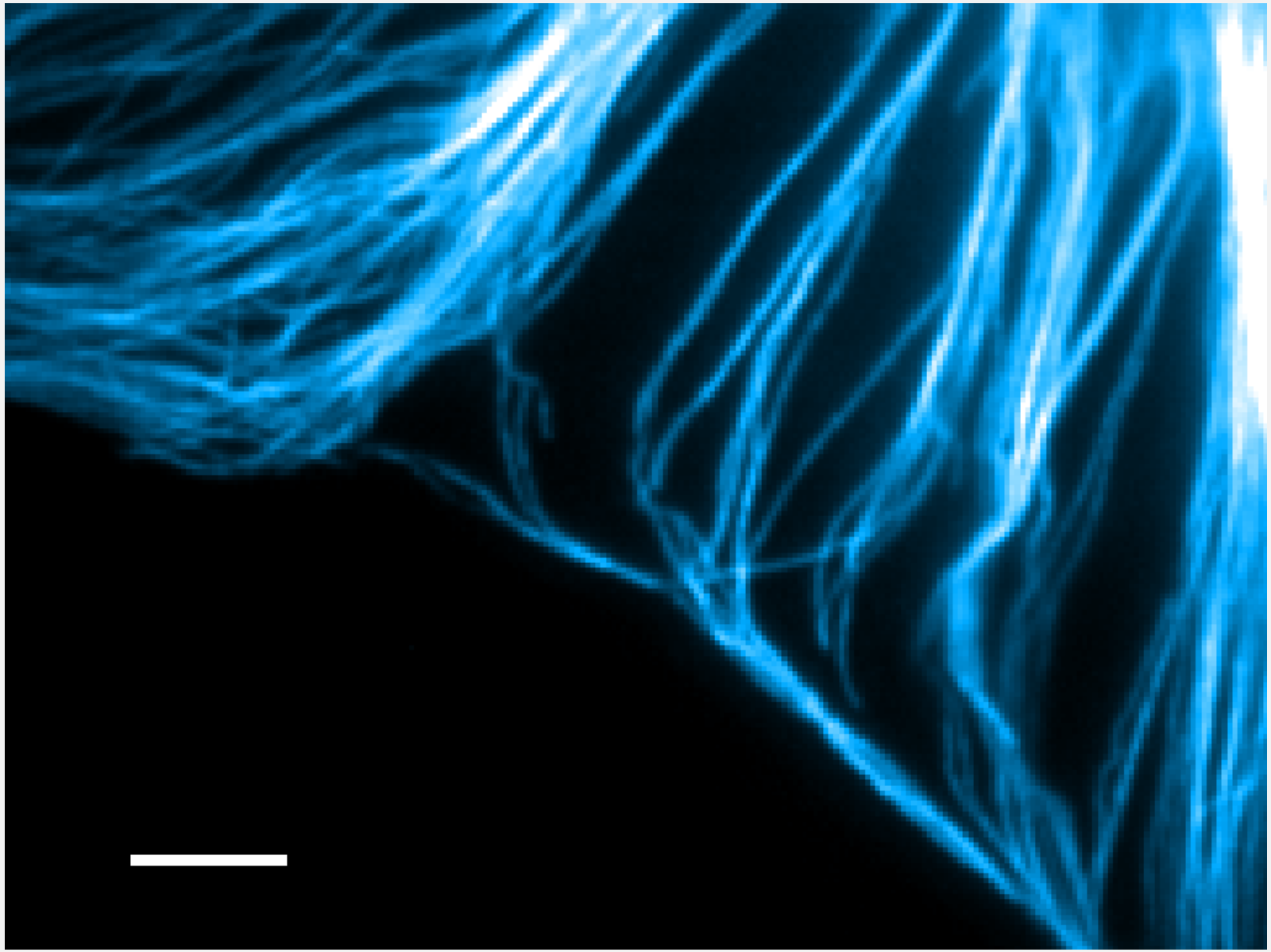

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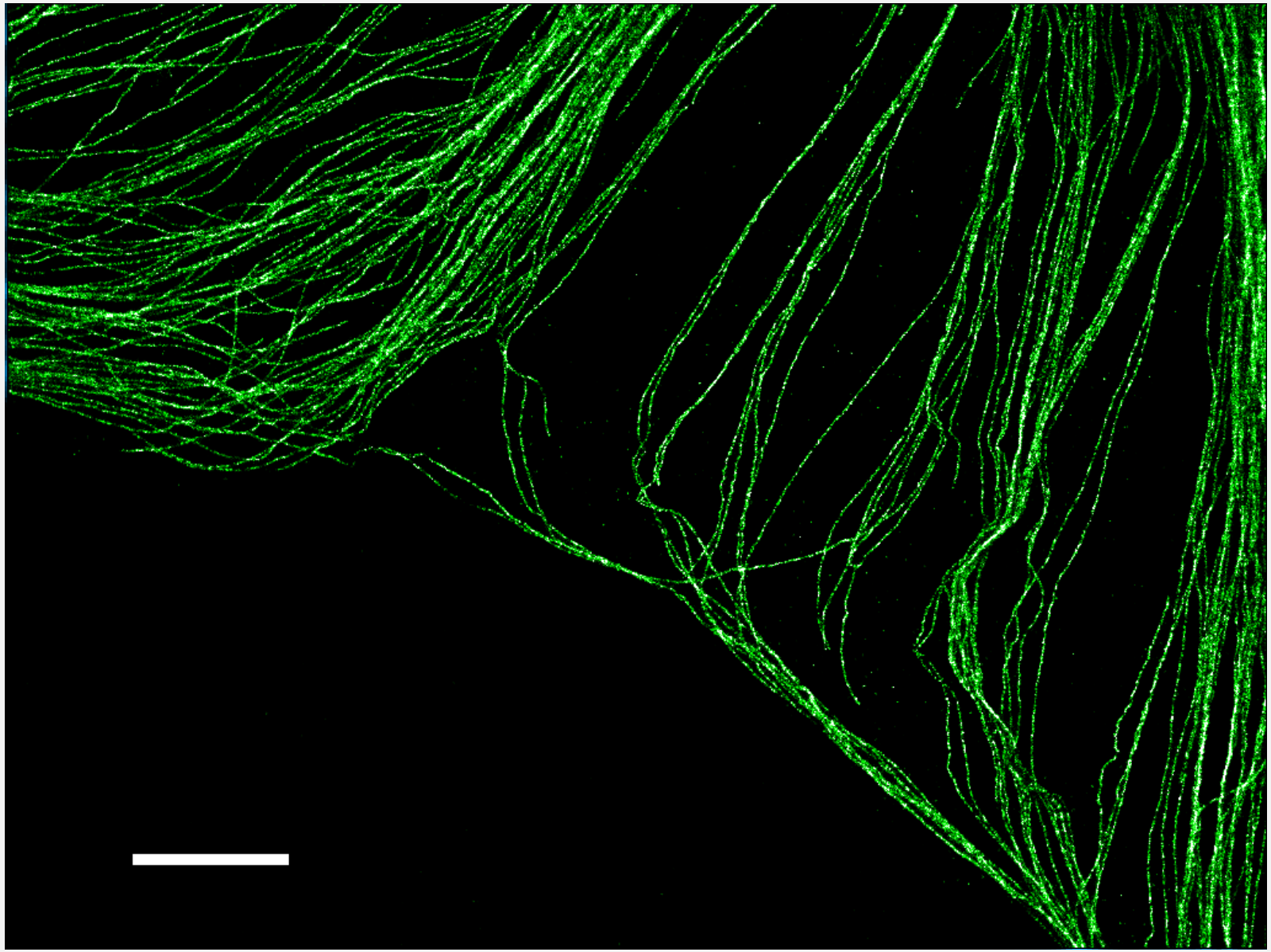
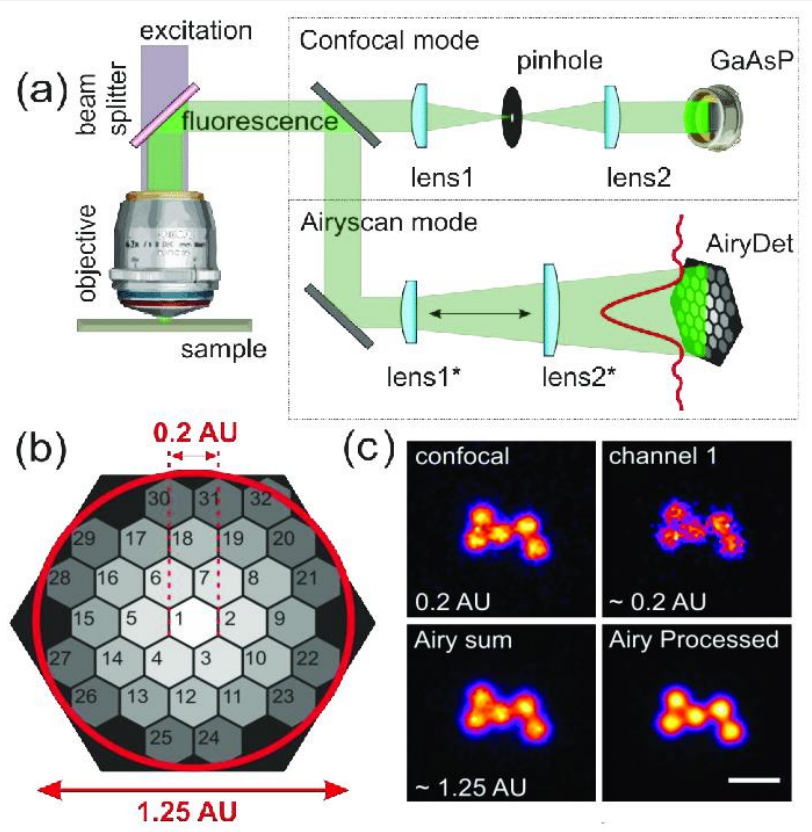
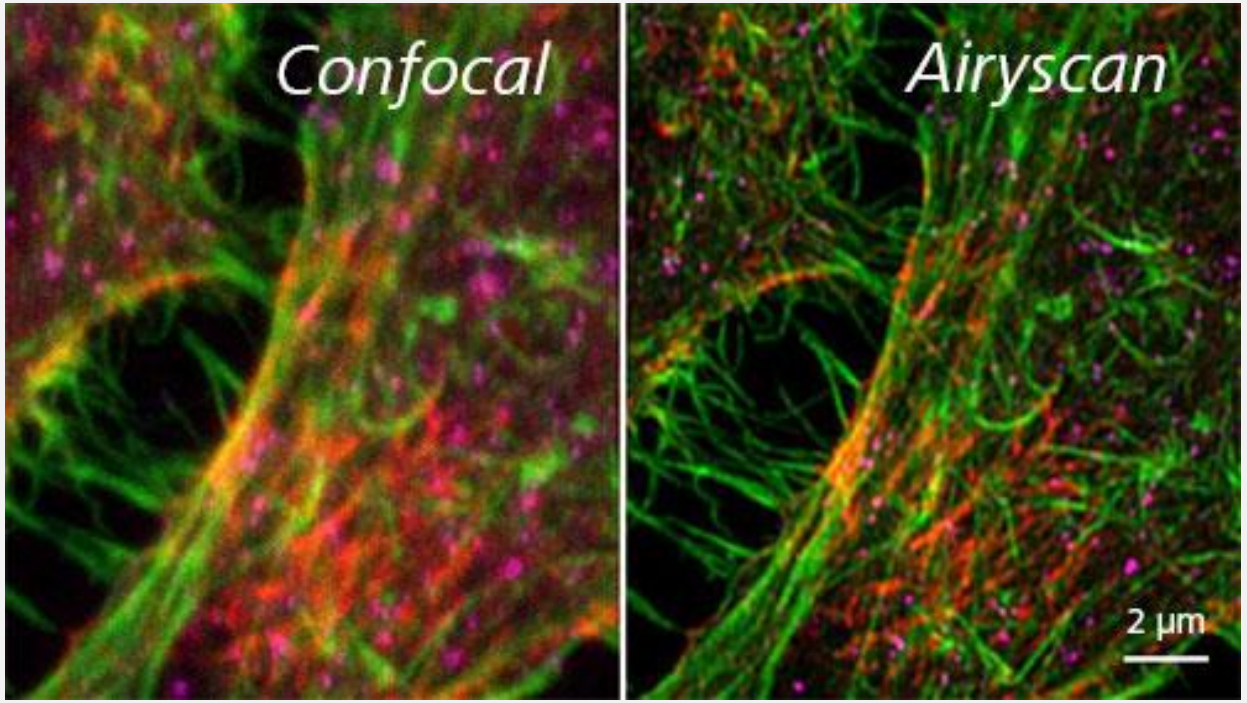


NEW

Airyscan microscopy (LSM900 and LSM980)



Korobchevskaya et al.



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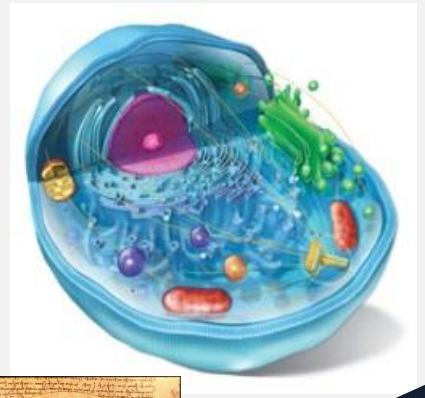
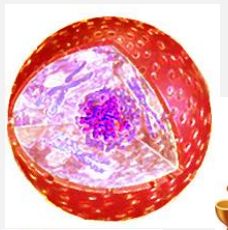
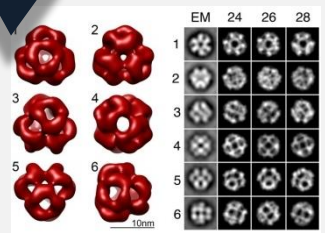
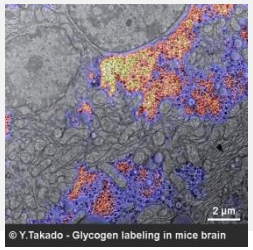
Electron Microscopy

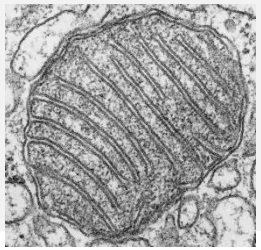
- \+ Ultrasctructure resolution
- \- Static









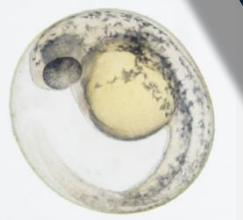


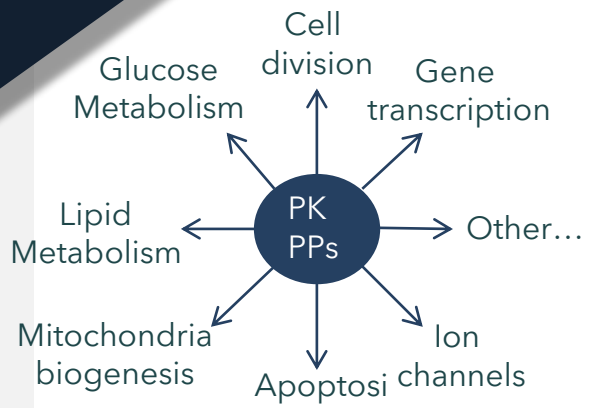
Multicellular Organism Tissue (cm)

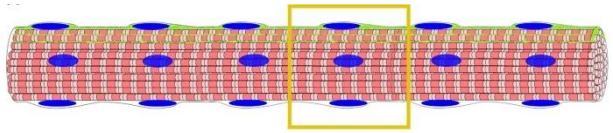
Cells (µm)

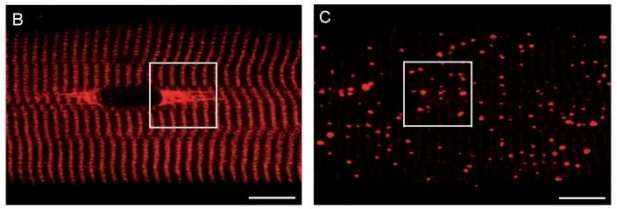
Intracellular Compartments (nm)

Molecular/ metabolites (Å)





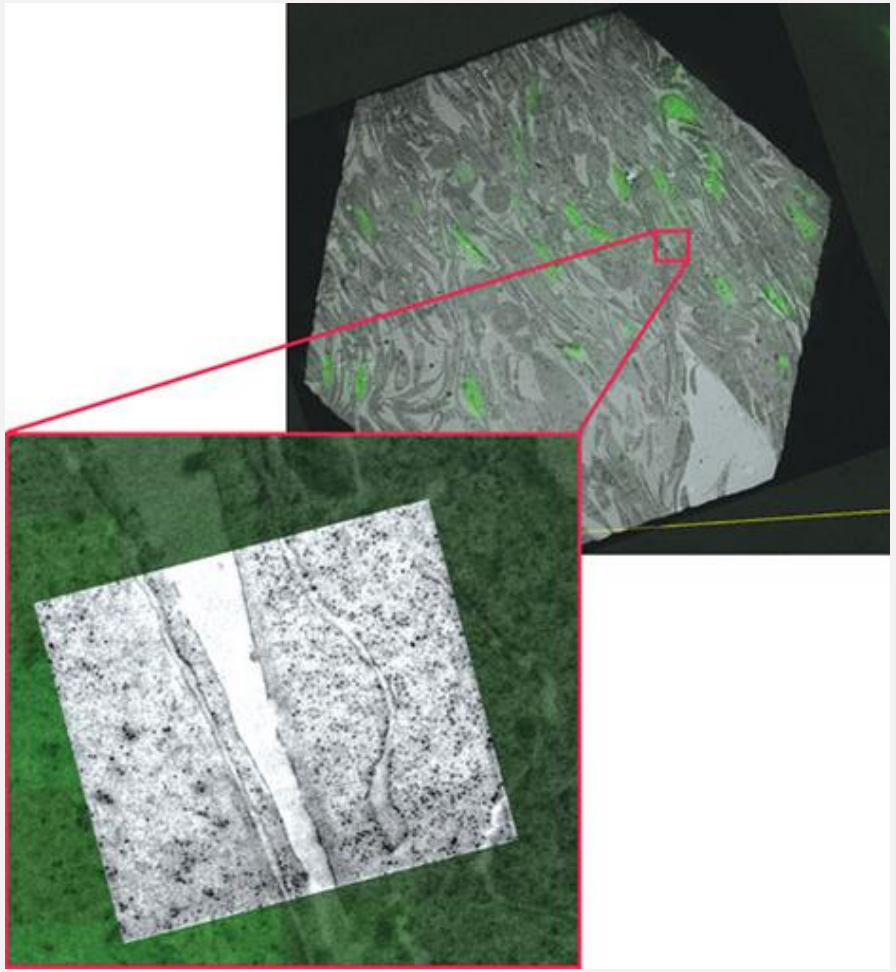




Prats et al. JBC 2009

THE CORE FACILITY FOR INTEGRATED MICROSCOPY

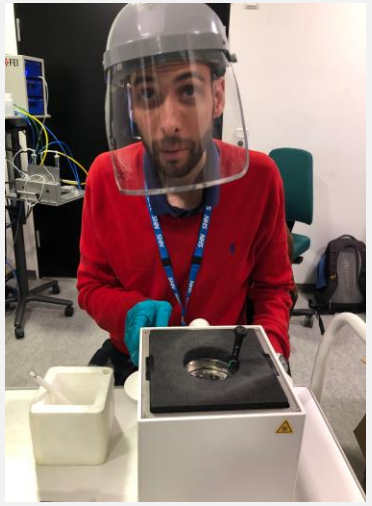
CLEM – Correlative Light and Electron Microscopy

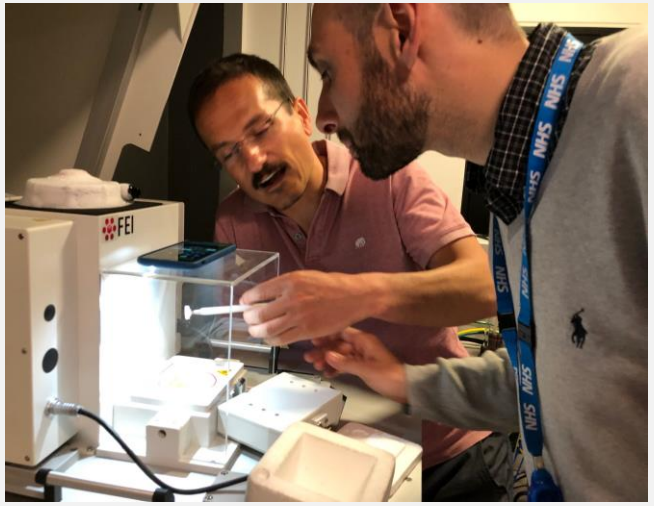


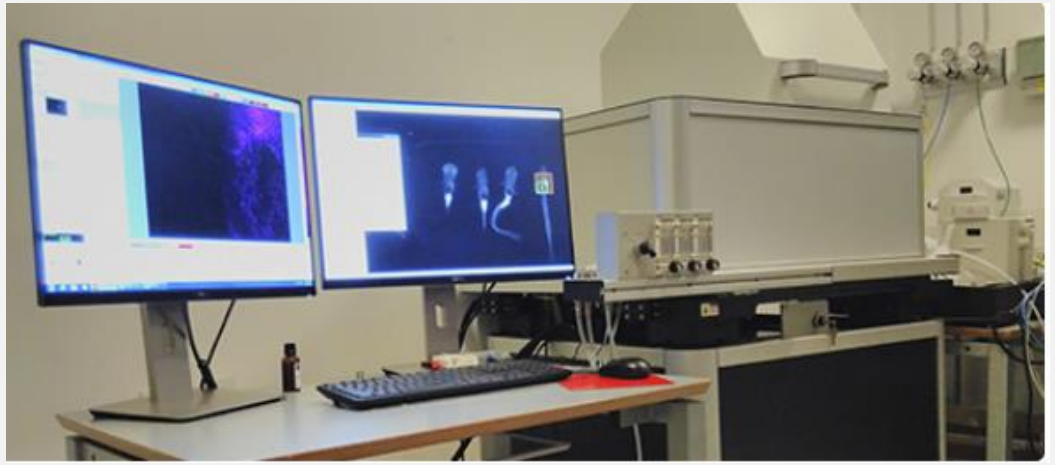
THE CORE FACILITY FOR INTEGRATED MICROSCOPY

FEI (ThermoFisher) CorrSight

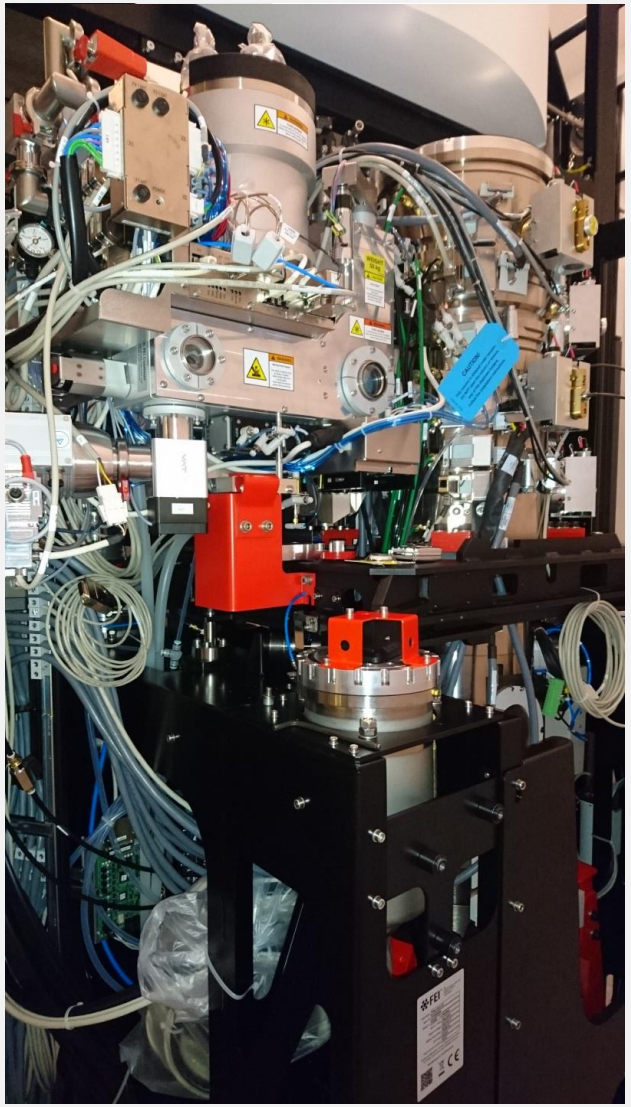
The CorrSight is a light microscope built for **live microscopy** as well as **correlative light and electron microscopy** experiments. It's run by the MAPS software, which is also present on some of CFIM electron microscopes, allowing registration between the images recorded in electron and light microscopy.

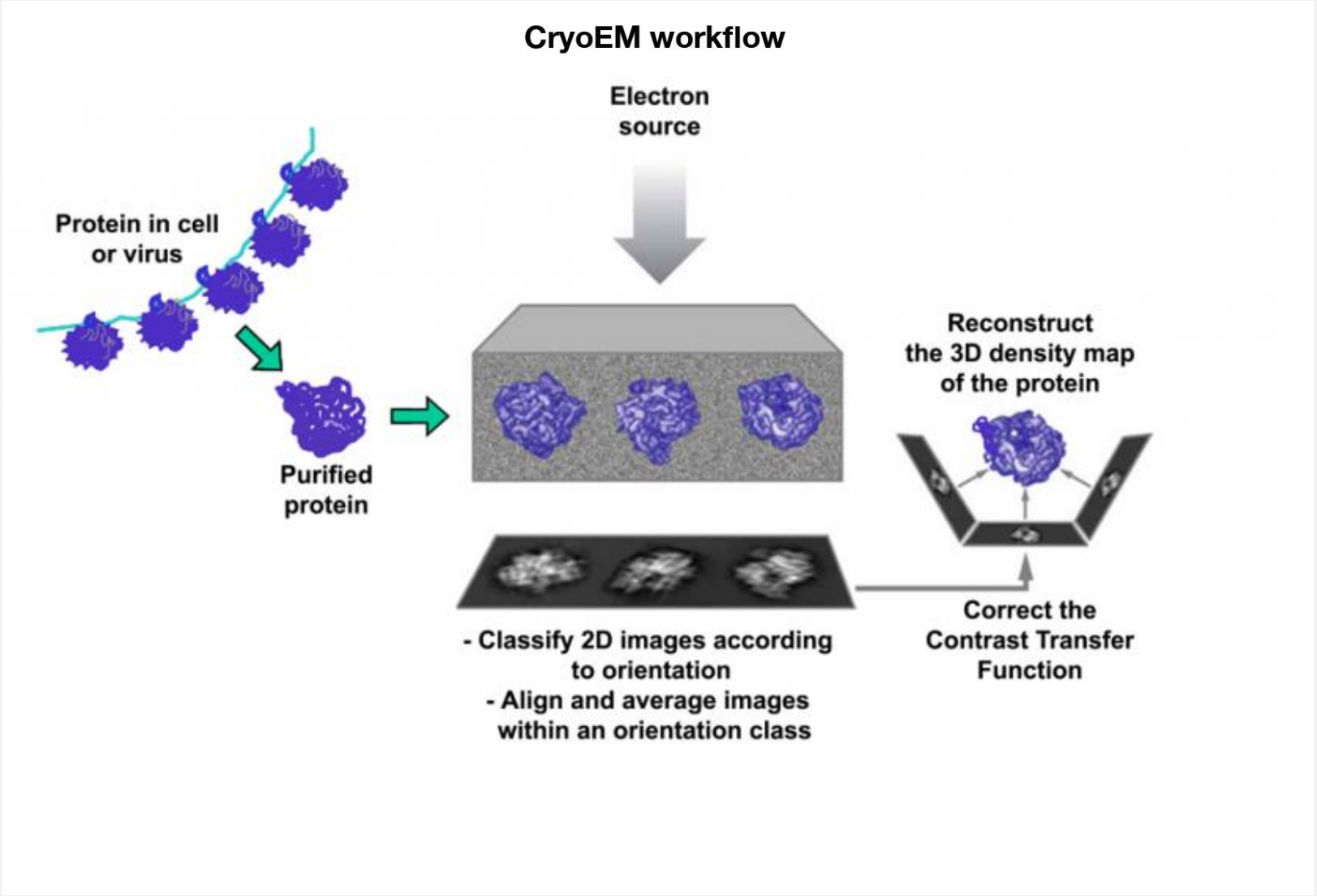






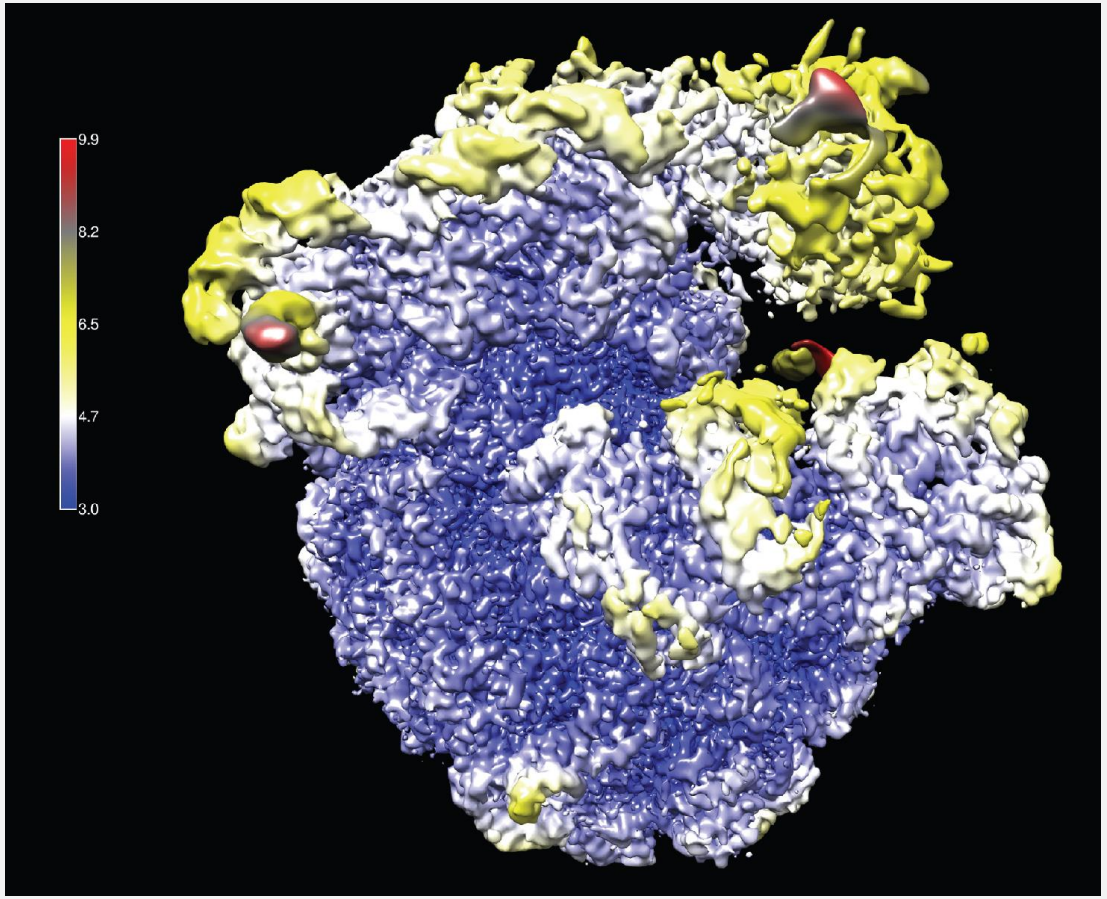
THE CORE FACILITY FOR INTEGRATED MICROSCOPY

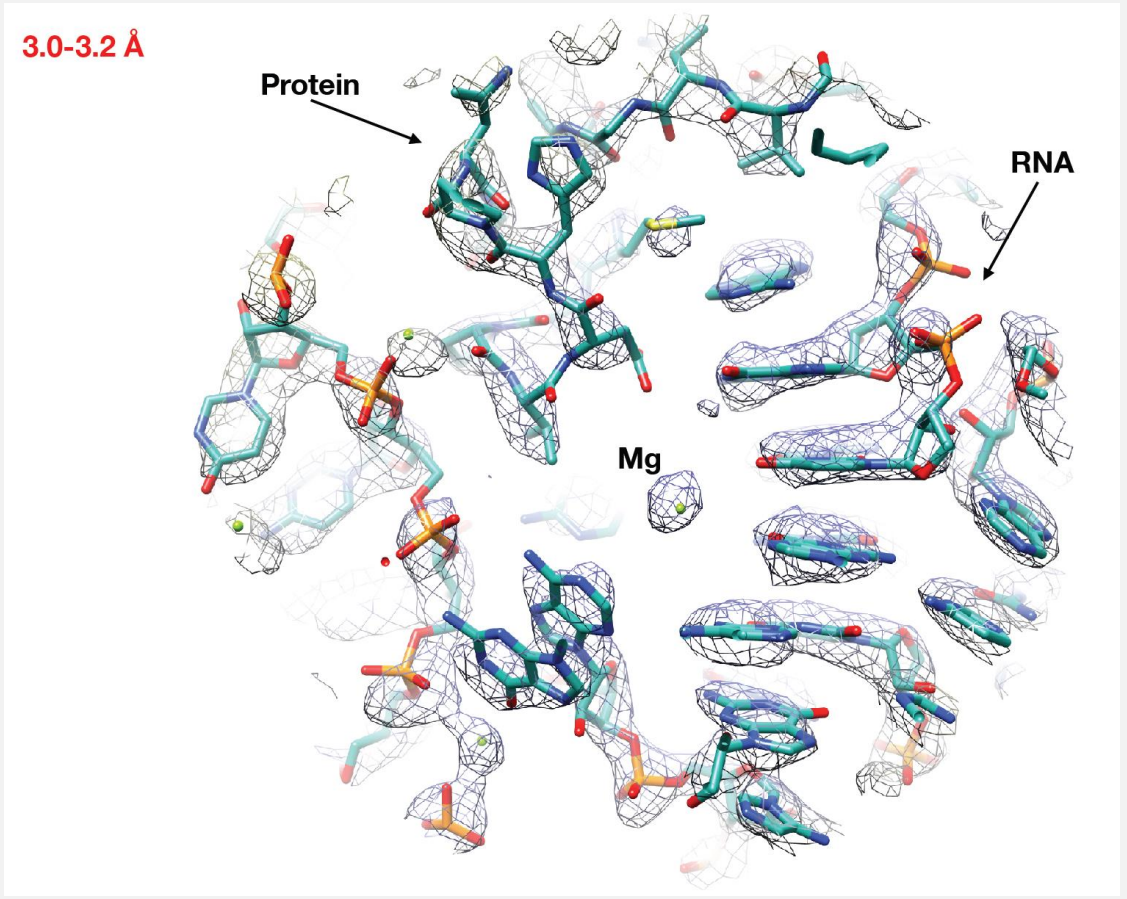




THE CORE FACILITY FOR INTEGRATED MICROSCOPY

70S structure



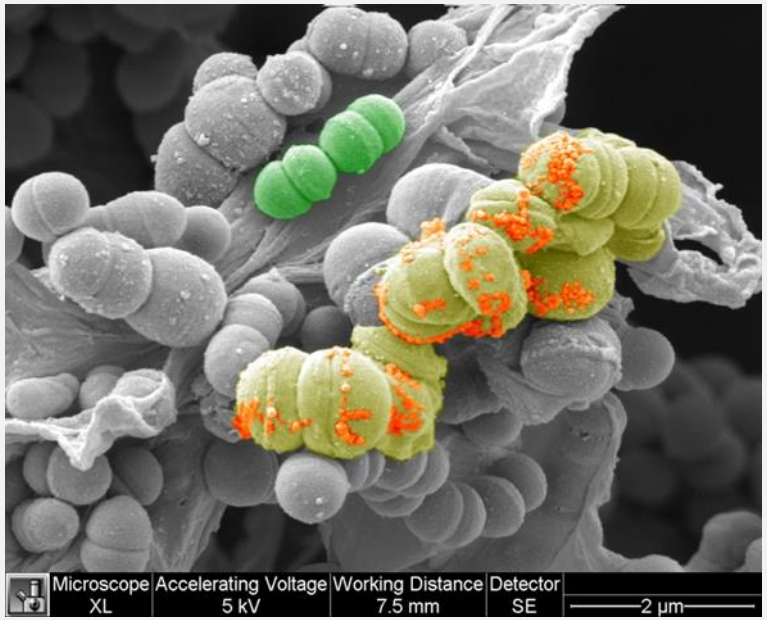


Courtesy of Pablo Mesa and Guillermo Montoya

THE CORE FACILITY FOR INTEGRATED MICROSCOPY

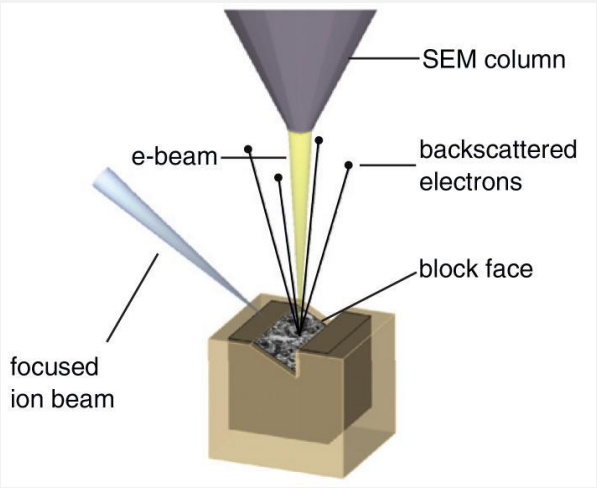
Scanning Electron Microscopy (SEM)

Topography

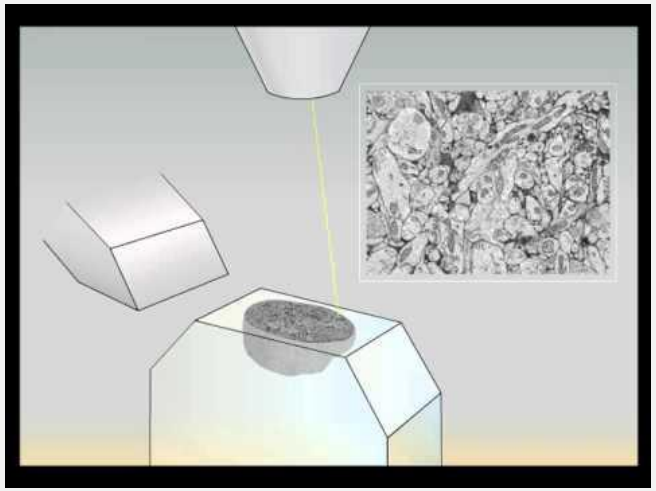


Serial Block face SEM

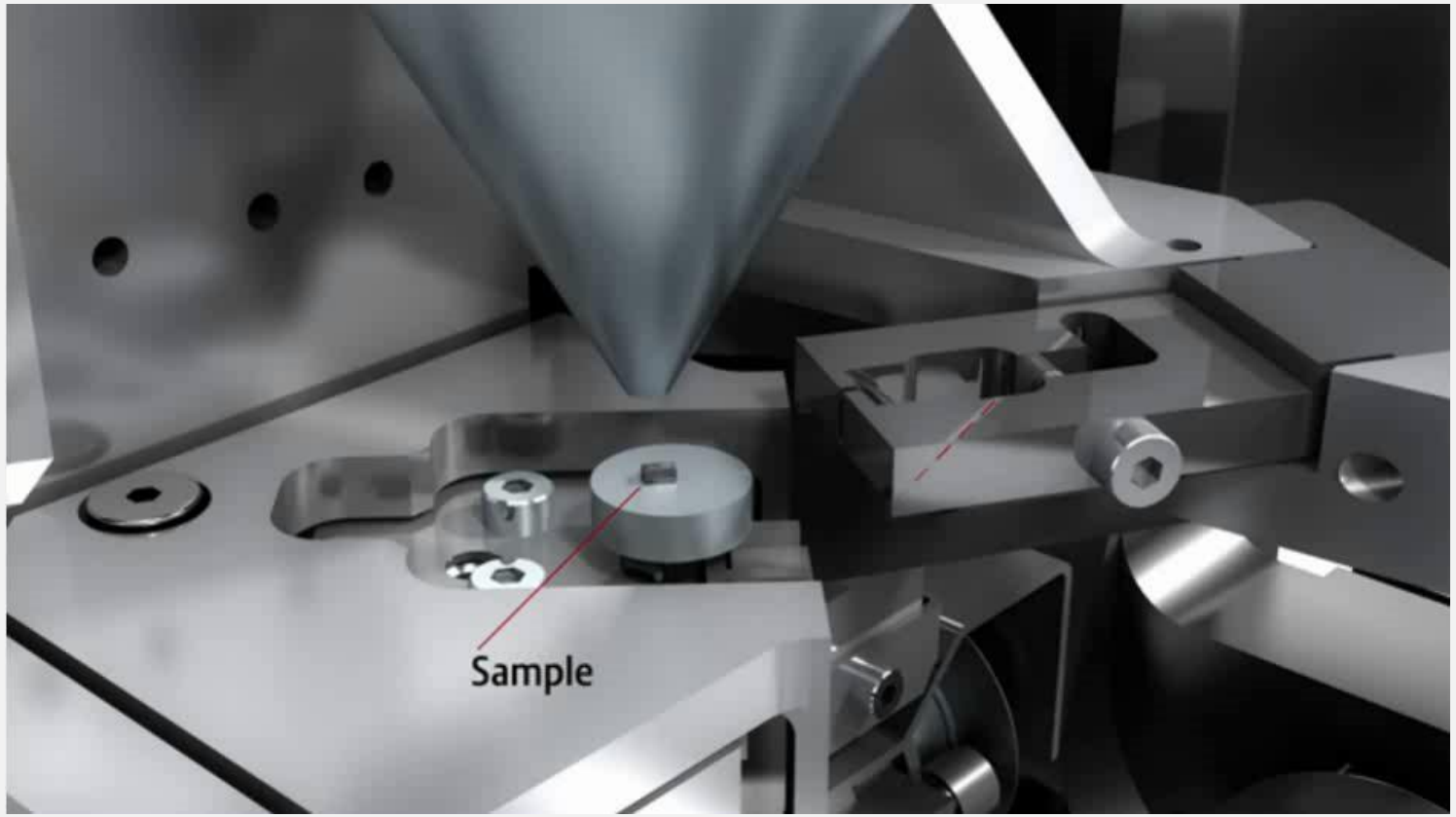
FIB/SEM



Serial block face

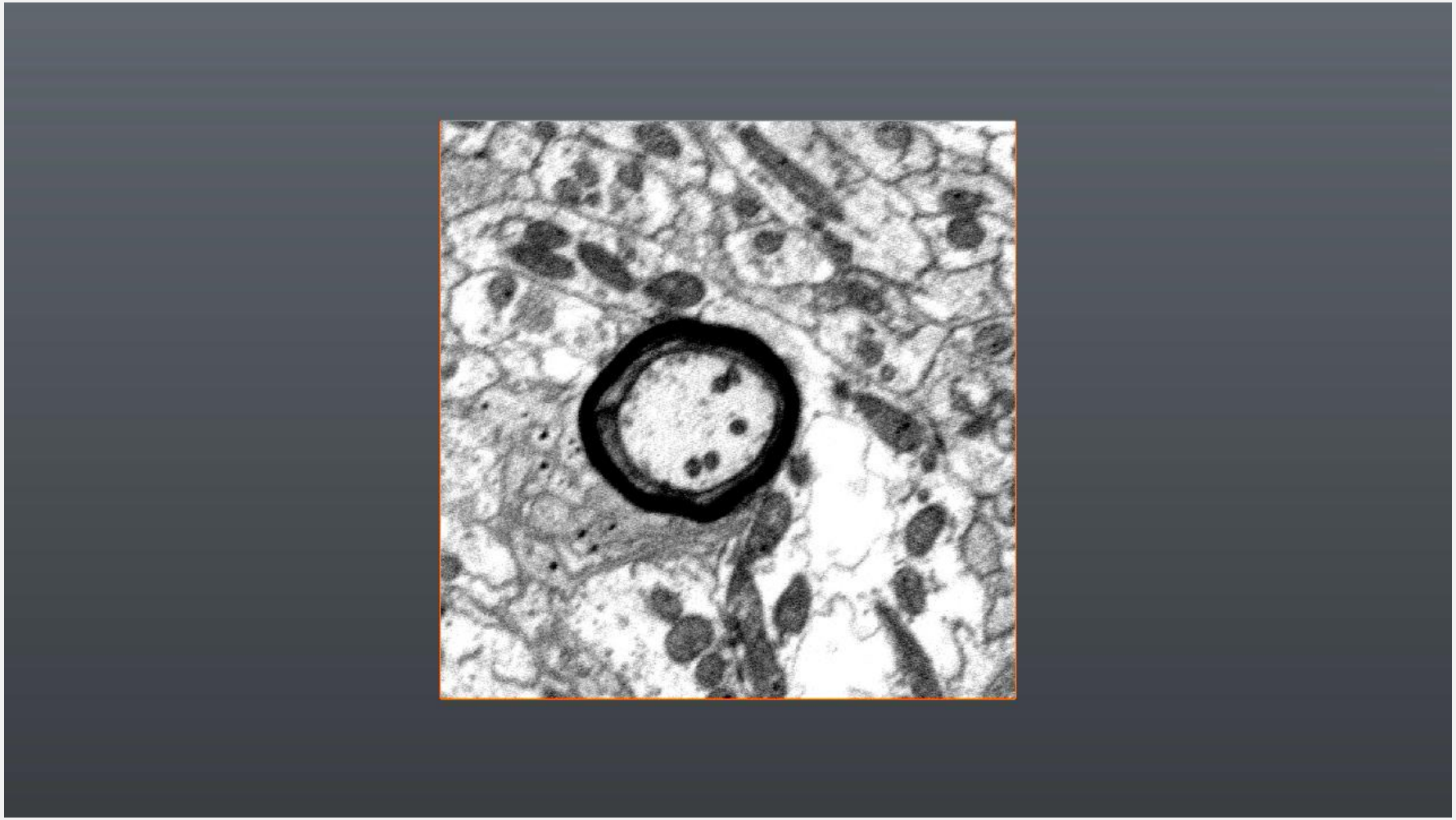


THE CORE FACILITY FOR INTEGRATED MICROSCOPY

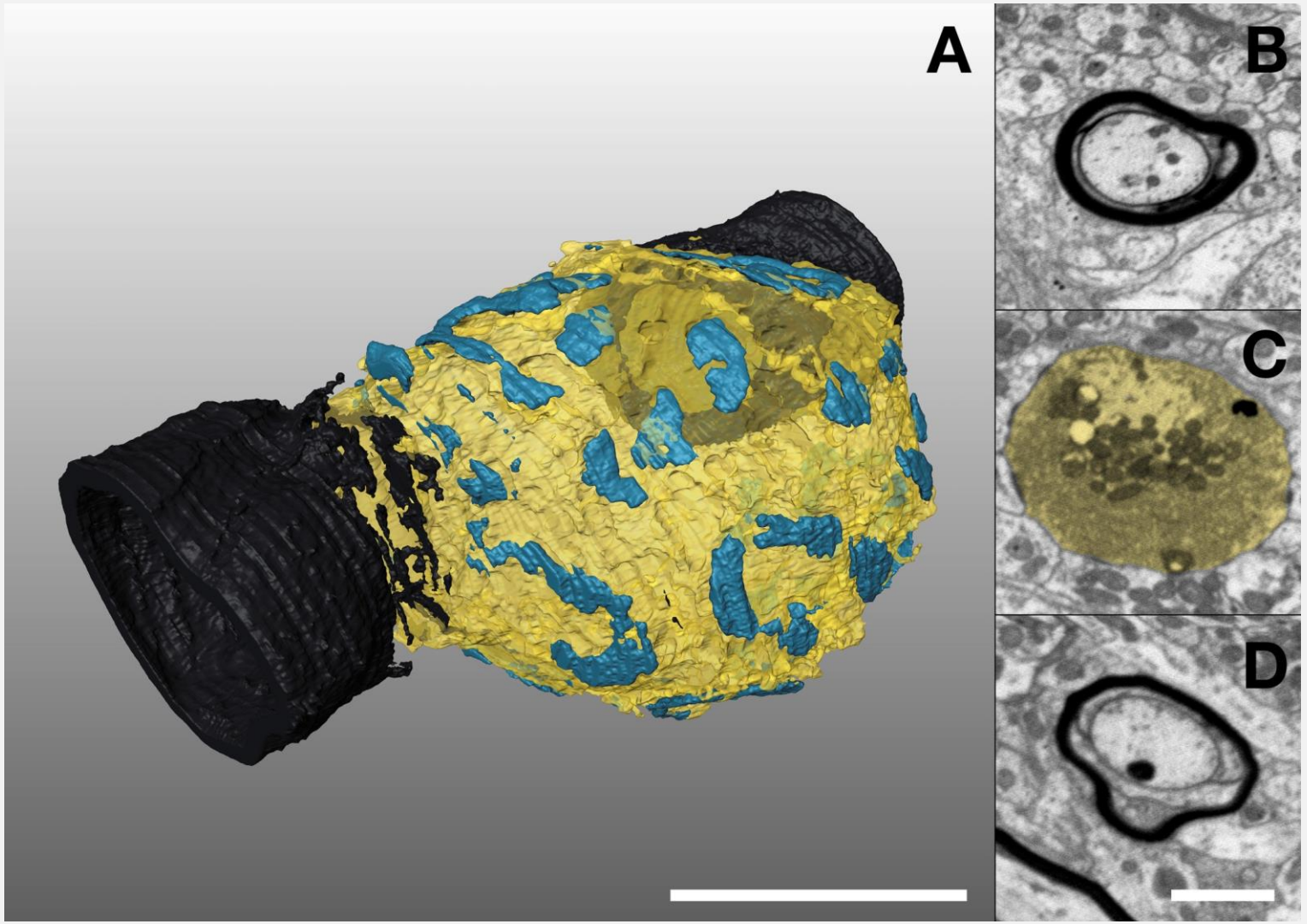


THE CORE FACILITY FOR INTEGRATED MICROSCOPY

FIB/SEM



THE CORE FACILITY FOR INTEGRATED MICROSCOPY



THE CORE FACILITY FOR INTEGRATED MICROSCOPY

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THE CORE FACILITY FOR INTEGRATED MICROSCOPY

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PhD, Image Analyst

Richard Denis Maxime De Mets
richard.demets@sund.ku.dk

Computers

AUGUST KÖHLER
CPU Score : 45826
GPU Score : 4352
RAM : 128GB
Zen 3.4, Fiji, QuPath, CellProfiler

FRITZ ZERNIKE
CPU Score : 15750
GPU Score : 4352
RAM : 96GB
Zen 3.4, Fiji, QuPath, CellProfiler

ERNST ABBE
CPU Score : 45826
GPU Score : 4352
RAM : 128GB
Zen 3.5, Fiji, QuPath, Amira 2019.2

ERNST RUSKA
CPU Score : 45826
GPU Score : 10496
RAM : 128GB
Zen 3.5, Fiji, QuPath, CellProfiler, R

MANFRED VON ARDENNE
CPU Score : 45826
GPU Score : 4352
RAM : 128GB
Zen 3.6, Fiji, QuPath, Amira 2019.2, CellProfiler

SANTIAGO RAMÓN Y CAJAL
CPU Score : 81343
GPU Score : 10496
RAM : 256 GB
Zen 3.5, Fiji, Huygens, Arivis, Imaris, Matlab, ...

ADA LOVELACE (SOON)
CPU Score :
GPU Score :
RAM :
Zen 3.5, Fiji, QuPath, CellProfiler, Amira 2019.2

Free software

CELLPOSE
CELLPROFILER
FIJI
ICY
ILASTIK
NAPARI
QUPATH
ZEROCOSTDL4MIC

Commercial software

AMIRA-AZIVO
ARIVIS
IMARIS
MATLAB
HUYGENS
ZEN

The Core Facility for Integrated Microscopy

Training and support

Since 2010 – around 2000 users
Currently – 319 unique active users in 2021

Education

Principles Light and confocal Microscopy - PhD course

The course is suitable not only for beginners in microscopy, but also for those who already use microscopy in their work and now want to extend their knowledge of basic principles and more specialised techniques.

The wide range of microscopes available at CFIM allows for a strong practical element, with time for each student to gain hands-on experience coupled with lectures given by renowned scientists.

The course is run in collaboration with The Royal Microscopical Society.

Content

The course in Light Microscopy consists in two modules:

Basic Principles of microscopy

- Optics of the microscope
- Diffraction, Resolution and Contrast

Fluorescence and Confocal Microscopy

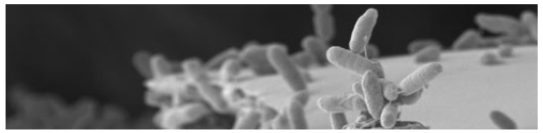
- Fluorescence microscopy (fluorophores, illumination…)
- Basic Confocal microscopy (Lasers, multi-dimensional Image Recording …)
- Advanced techniques in fluorescence microscopy (FRET, FLAP, FCS)

Next course 9th - 13th & 21st-27th January 2017 - to be confirmed

Preliminary programme

For further information please contact Ragnhild Mostert, rmostert@sund.ku.dk
Registration: search for Light microscopy in the Course catalogue

Electron Microscopy - PhD Course



The course is suitable not only for beginners in microscopy, but also for those who already use microscopy in their work and now want to extend their knowledge of basic principles and more specialised techniques.

The wide range of microscopes available at CFIM allows for a strong practical element, with time for each student to gain hands-on experience coupled with lectures given by renowned scientists.

The course is run in collaboration with The Royal Microscopical Society.

Course content

The course provides an essential grounding in the basic principles of electron microscopy, covering topics such as:

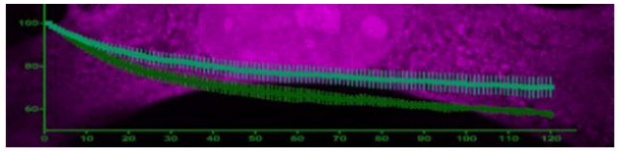
- Principles of Scanning and Transmitted Electron Microscopy
- Biological Specimen Preparation
- Advanced electron microscope techniques (immunogold labeling, electron tomography, and data analysis/visualization).

Next course 17th - 21st of October 2016

Last edition's Programme

For further information please contact Ragnhild Mostert, rmostert@sund.ku.dk
Registration: search for Electron microscopy in the Course catalogue

Image Processing - PhD Course



The course is suitable not only for beginners in image analysis with no experience, but also for those who want to extend their knowledge of basic principles and more specialised tools.

The course is mostly based on interactive lectures given by renowned scientists, where the students will perform themselves all processing and analysis tasks in parallel with the lecturers.

Course content

This course is an introduction to image processing (both light and electron microscopy images), analysis tools and basic Macro programming. The course will focus mainly on Fiji software but Cell profiler, Matlab, Amira, iMOD and iTEM will be demonstrated.

Main topics:

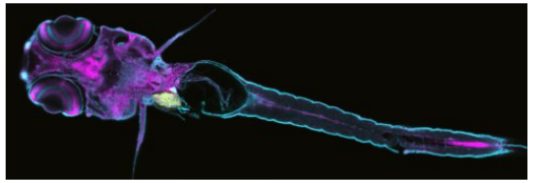
- what is a digital image? How and when to perform basic image processing tasks?
- Tools: segmentation, 2D and 3D measurements, tracking…
- Basic Macro programming for automated image analysis with Cell profiler and Fiji.

Next course: to be announced

Preliminary programme will be available soon.
Former edition's programme

For further information please contact Ragnhild Mostert, rmostert@sund.ku.dk
Registration: search for Image Processing in the Course catalogue

Light microscopy two-day crash course January 2022



January 2022 - 17th and 18th of January from 9.00 to 16.30.

This condensed course is aimed at everybody, who wants to understand the basic principles of optics and fluorescence in microscopy. Theoretical lectures are followed by practicals. The course will cover:

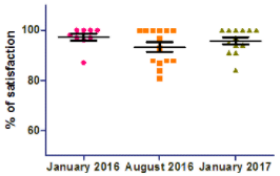
Basic Principles of microscopy

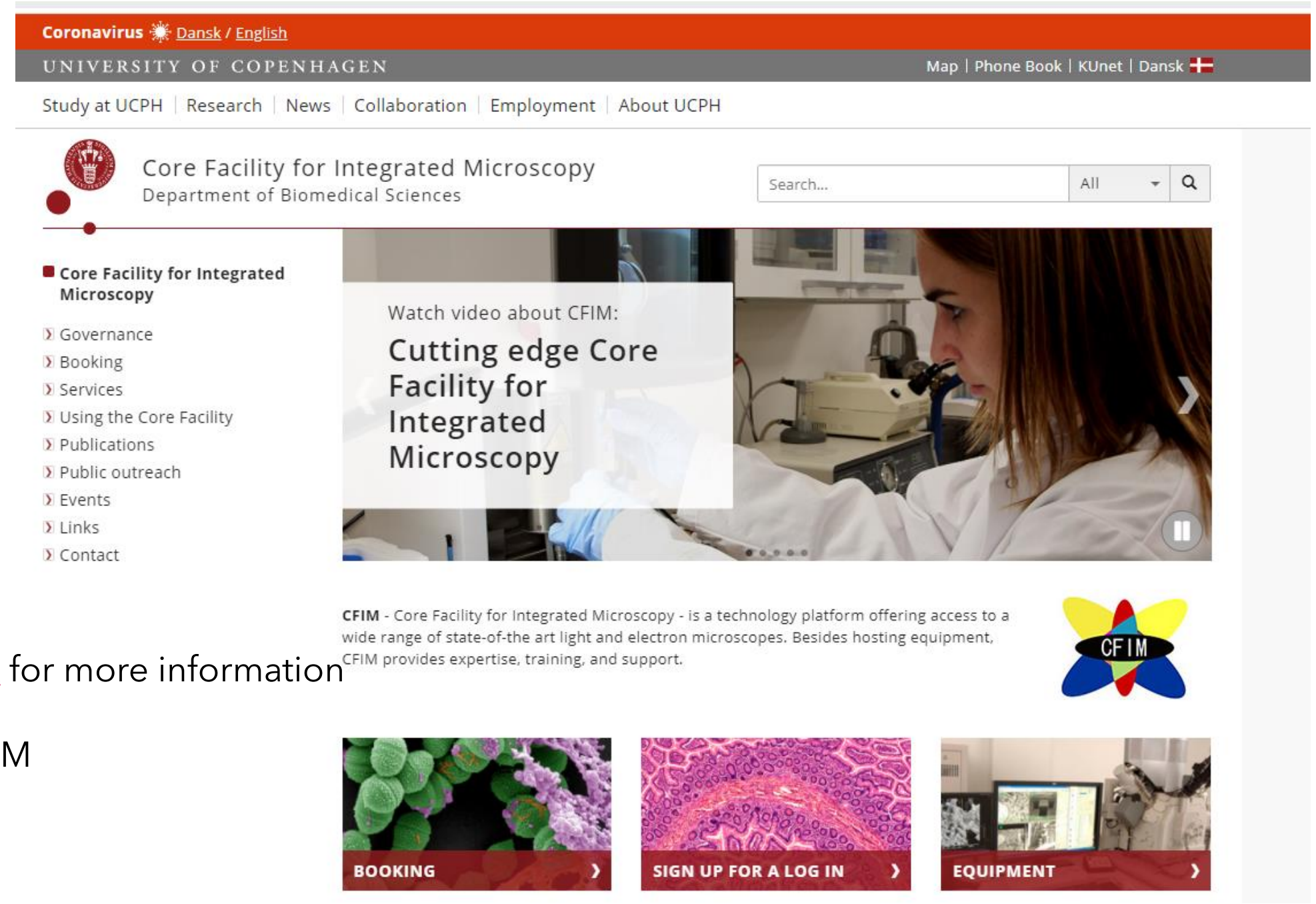
- Optics of the microscope
- Diffraction, Resolution and Contrast

Fluorescence and Confocal Microscopy

- Fluorescence microscopy (fluorophores, illumination…)
- Basic Confocal microscopy (lasers, multi-dimensional image acquisition, live imaging …)

Overall satisfaction of previous students

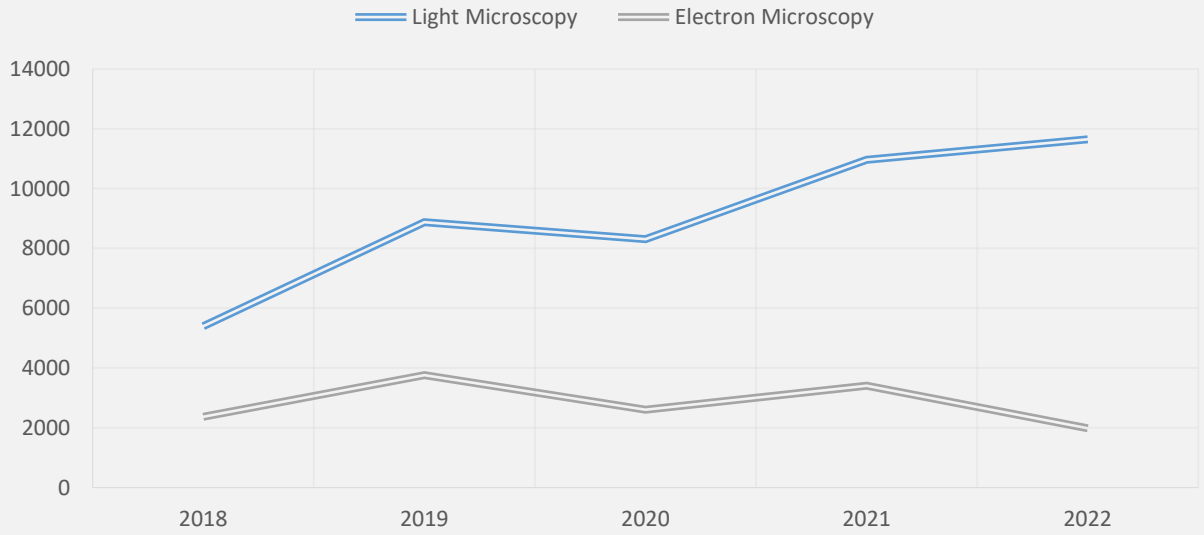




Visit www.cfim.ku.dk for more information or contact:
Klaus Qvortrup for EM
Clara Prats for LM

THE CORE FACILITY FOR INTEGRATED MICROSCOPY

USAGE (OPERATIONAL HOURS)



SUPPORT HOURS

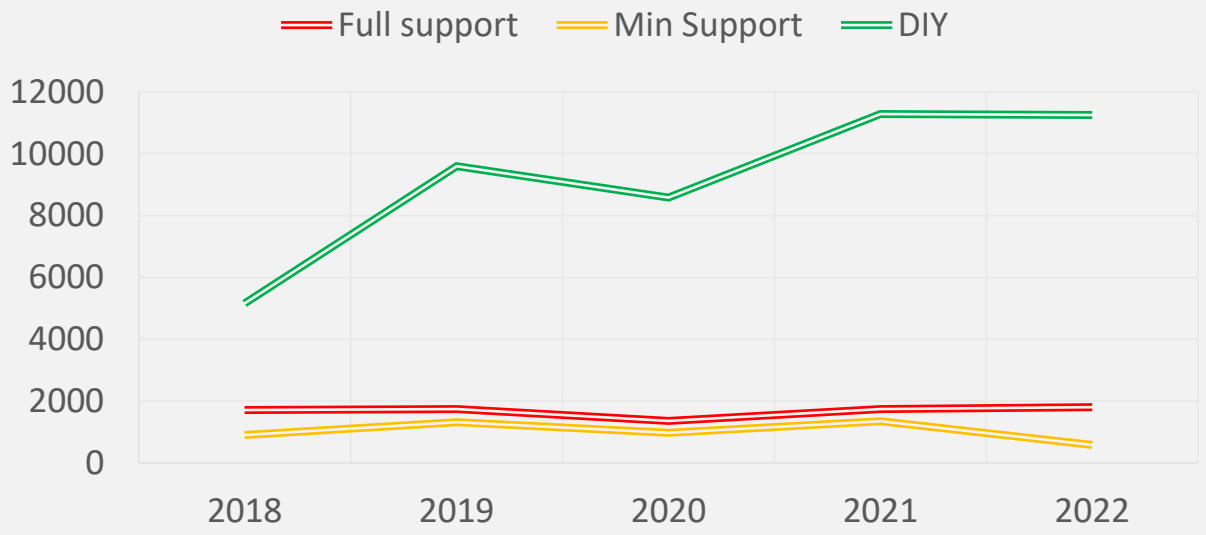


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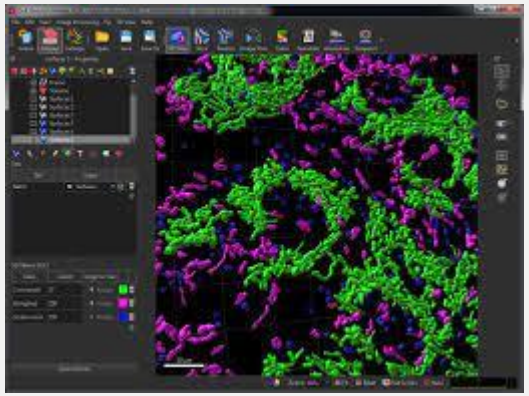
Image analysis

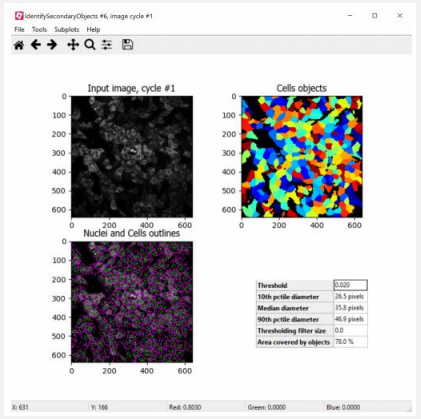
Richard De Mets

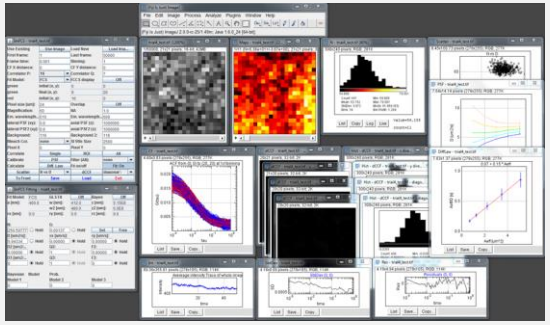
Bioimage Analyst at CFIM

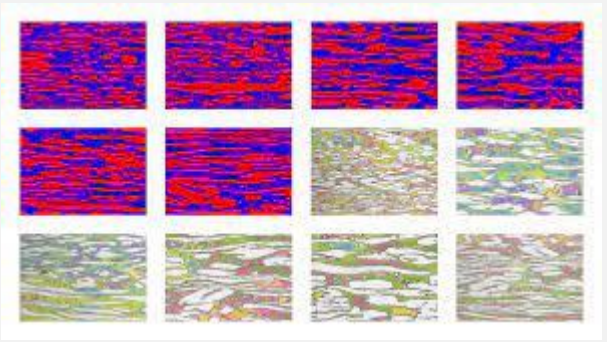
- PhD in BioPhysics
- MSc Imaging Robotics and Life Engineering



- Five powerfull workstations for booking
- Image analysis software packages (Incl. Commercial and open source)



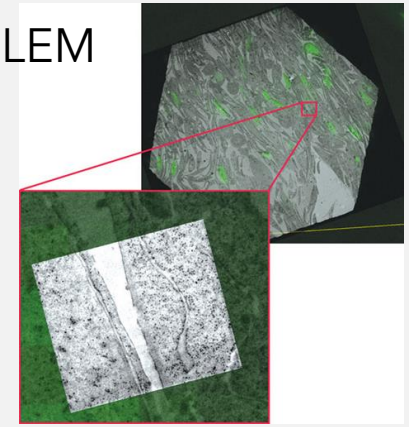


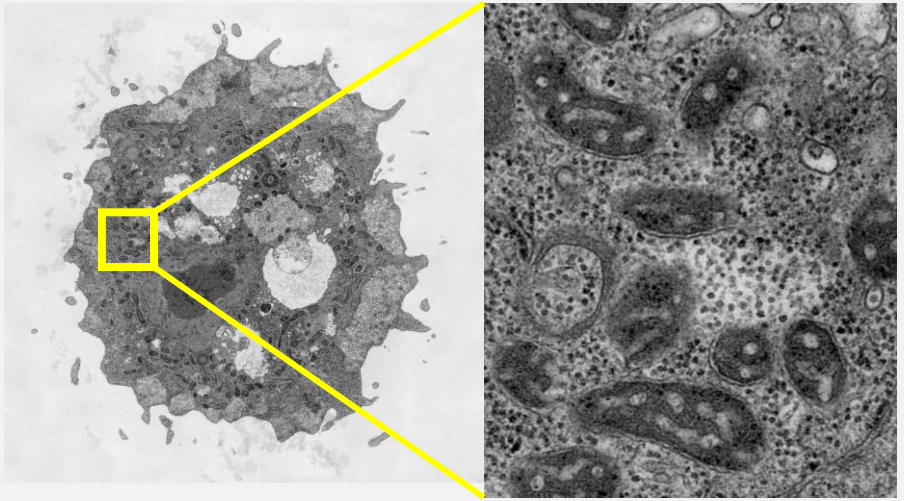


THE CORE FACILITY FOR INTEGRATED MICROSCOPY

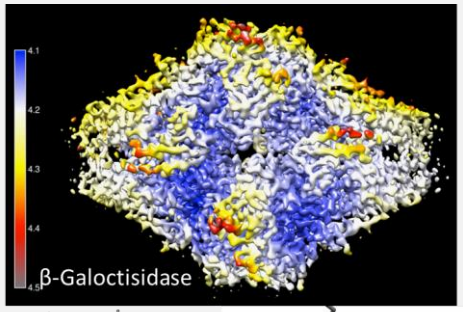
Transmission Electron Microscopy (TEM)

CLEM



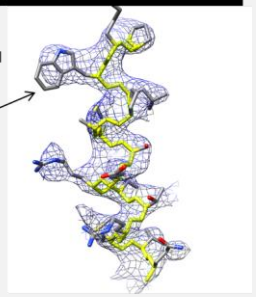


Cryo –TEM
NNF CPR

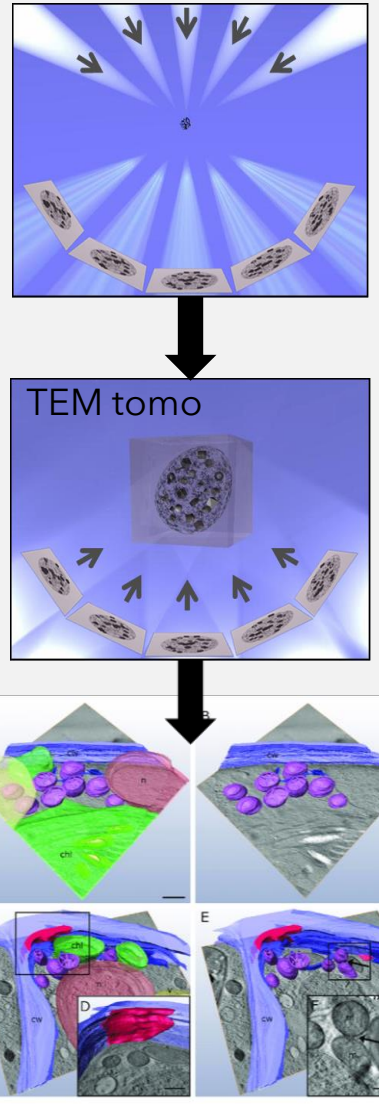


Resolution 4.2 Å
65,000 particles in total
26,000 particles for this model
by Pablo Mesa, CPR-SUND

lateral chains on the β-galactosidase



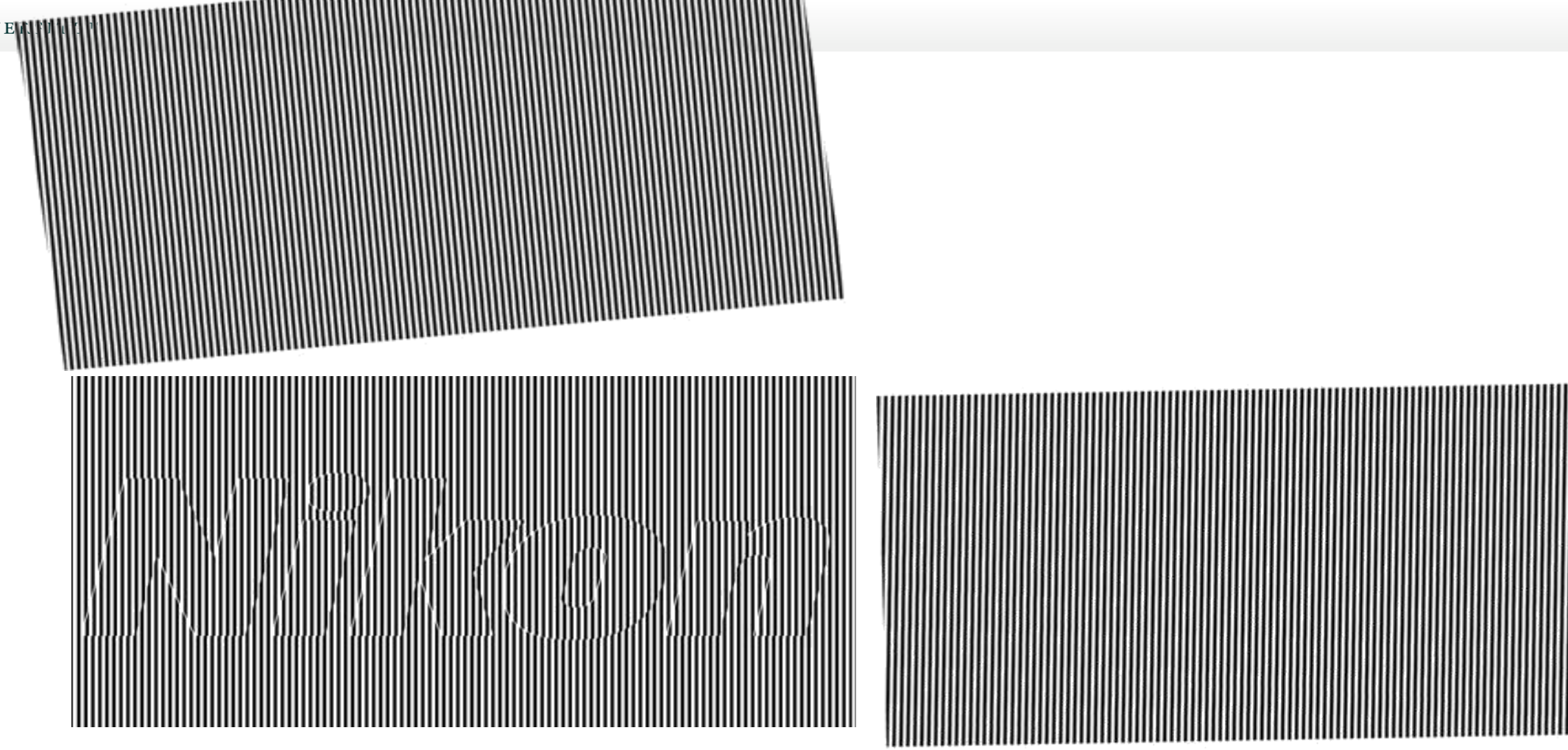
TEM Tomography



Improving resolution

		@ 488 nm xy	@ 488 nm z
1873 Abbe	Diffraction limit	250 nm	850 nm
1957 Minsky	Confocal	200 nm	500 nm
1994 Gustafsson	3D-SIM	100 nm	250 nm
2008 Zhuang	3D-STORM	20 nm	50 nm

Minimal object size (PSF)



- SR information is observable as a Moiré pattern created by the interaction of sub-resolution structures in the sample and the illumination pattern.
- The interference pattern is *coarser* then the sample and can be picked up by the optical system

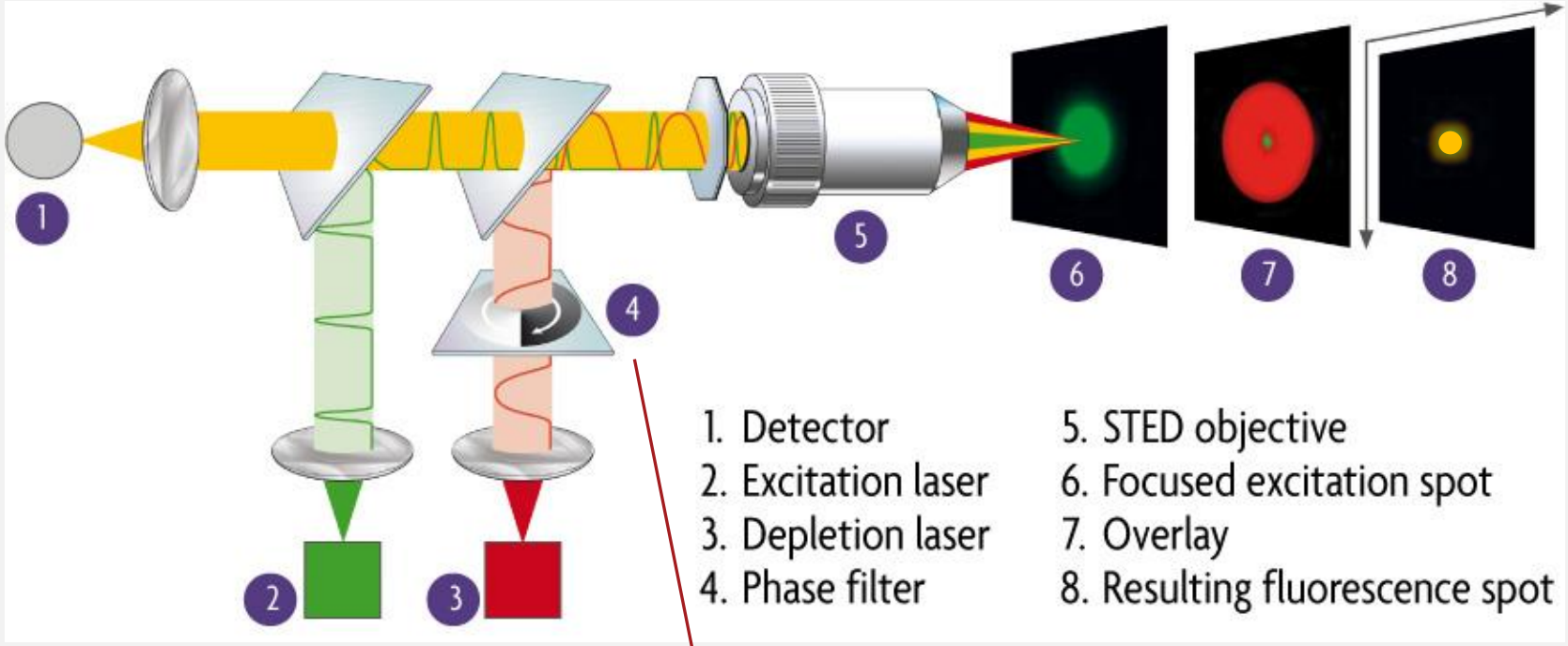
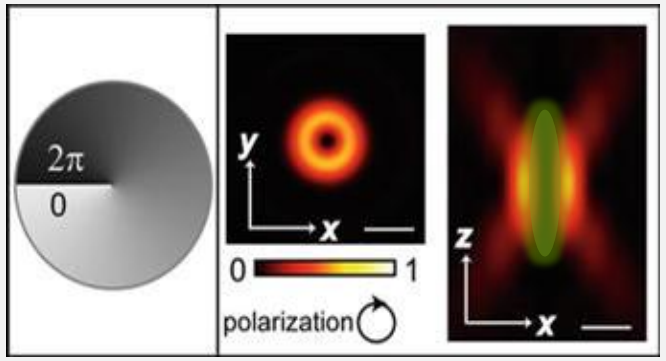


Image source: Leica

STimulated Emission Depletion