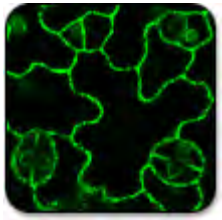
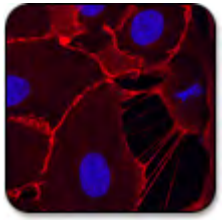


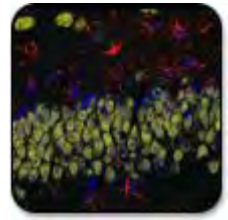




MIC

Molecular Imaging Center

Endy Spriet
Chief engineer





MIC Personnel/Associated people

Frits Thorsen
Professor
Platform Leader

Jaakko Saraste
Professor

Endy Spriet
Chief Engineer

Hege Avsnes Dale
Chief Engineer

Linda Sandven
Staff Engineer

Hans Olav Rolfsnes
Chief Engineer

Heidi Espedal
Senior Engineer

Associated:

Arvid Lundervold
Professor IBM

Emmet McCormack
Professor K2

Kai Gunter Brandt
Staff Engineer K1

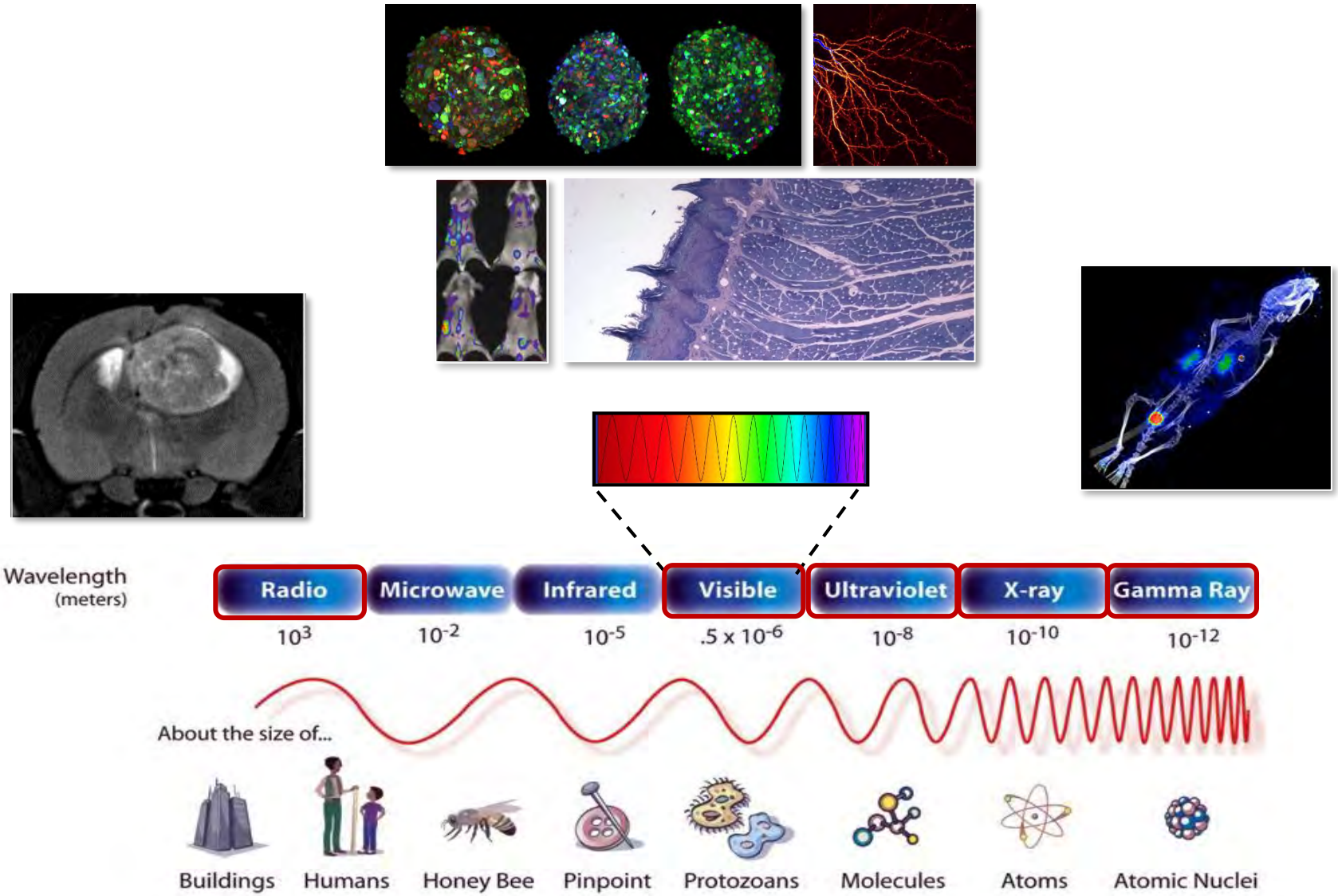
Jose Gomez
Staff Engineer K1

Administrative:

Elisabeth Hove

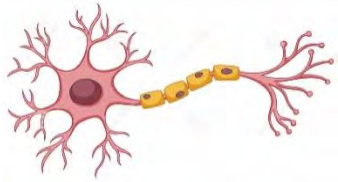
Janne Gotaas

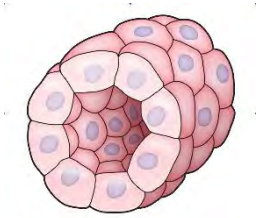
The electromagnetic spectrum for image creation

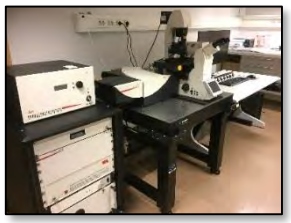


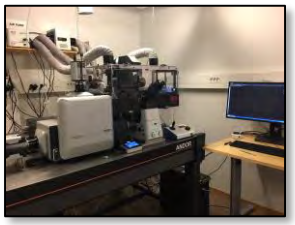
MIC
Molecular Imaging Center

Cell & Tissue imaging





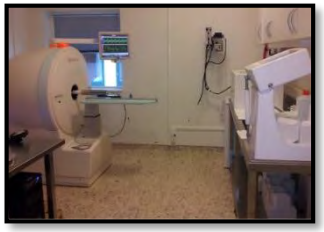




Small animal imaging

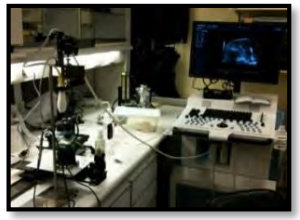
Instruments:

- Training
- Individual use
- Assisted use



Sample prep:

- SEM
- TEM
- Paraffin sectioning



Advice in:

- Imaging protocols
- Staining
- Analysis

Available Equipment

Electron microscopy

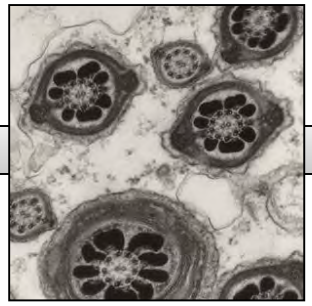
- Sample prep. lab
- Scanning EM
- Transmission EM

Fluorescence / confocal

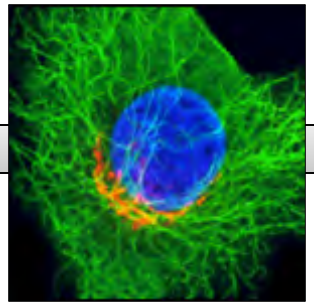
- Fluorescence microscopy
- Confocal microscopy
- Live cell confocal imaging
- Multiphoton microscopy
- High throughput imaging

Small animal imaging

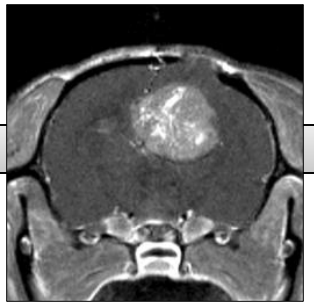
- Optical imaging
- MRI
- Flow cytometry
- (PET)



Nanometer



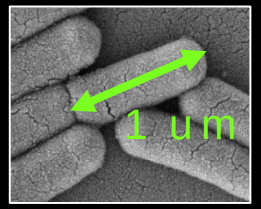
Micrometer

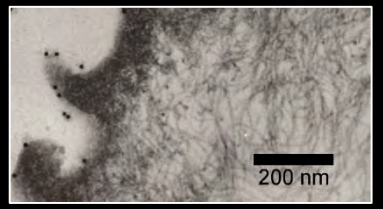


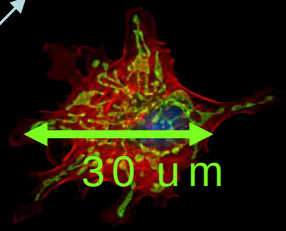
Sub mm

Resolution: The ability to see more details

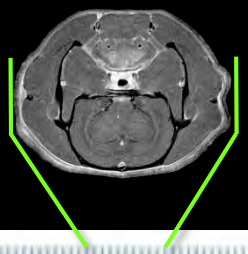
1 um = 1000 nm

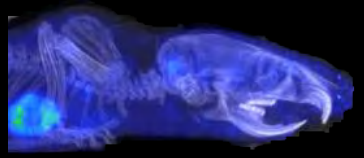






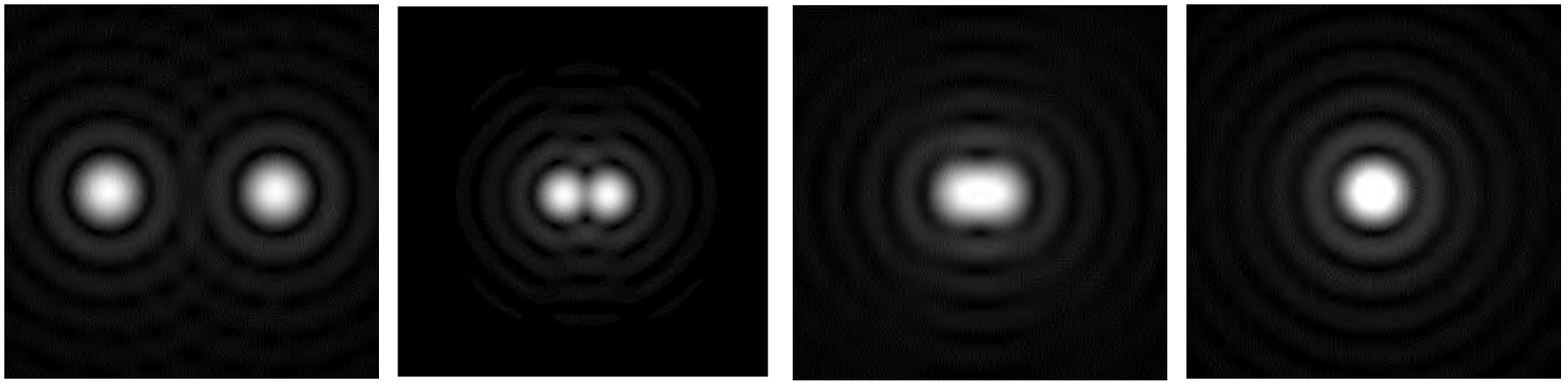
1 mm = 1000 um





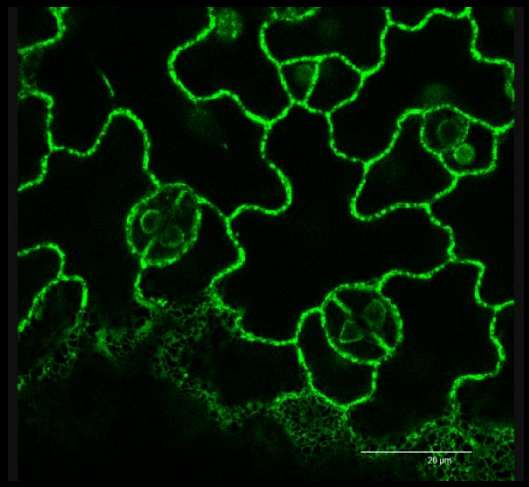
Oppløsning

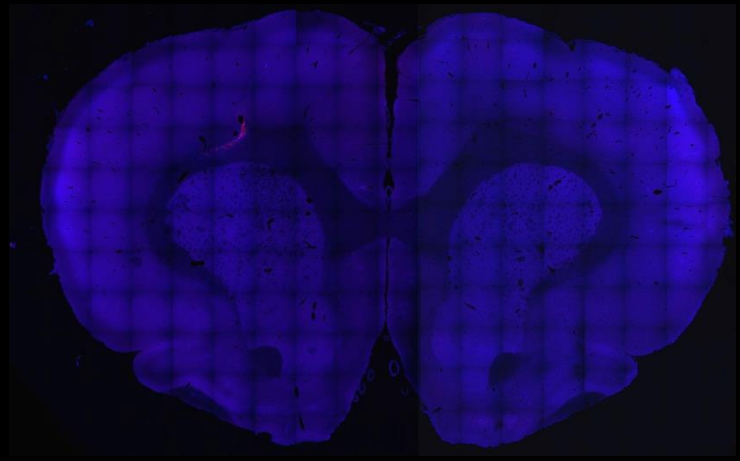
Evnen til å skille små ting fra hverandre.

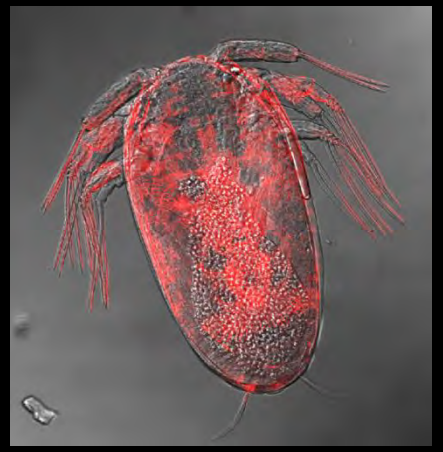


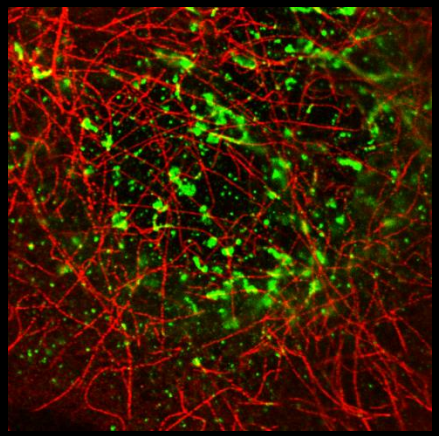
Konfokal mikroskopi: 200 µm

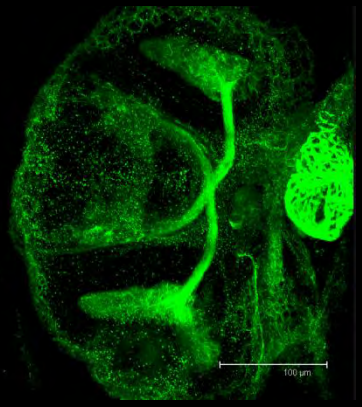
Elektron mikroskop: 10 nm

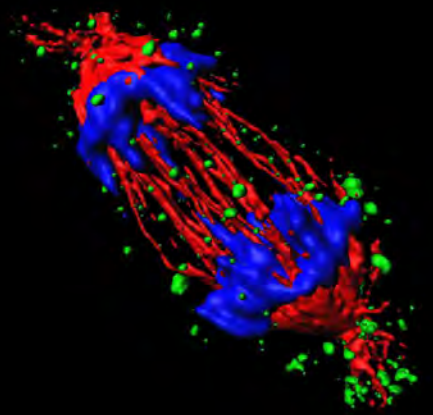

20 µm

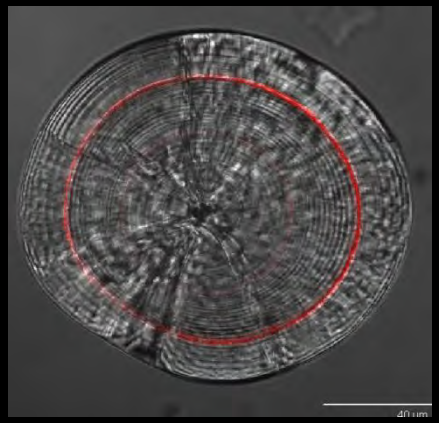


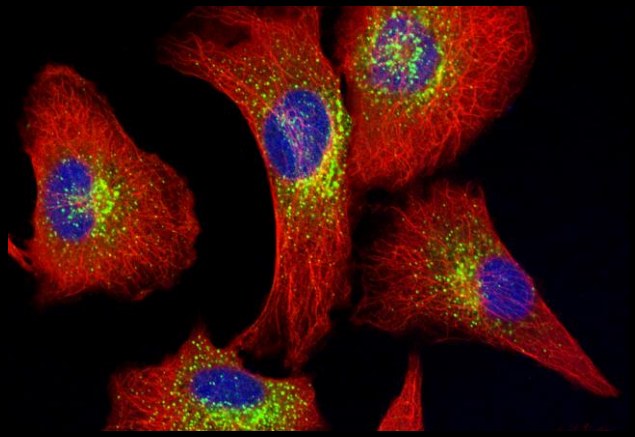


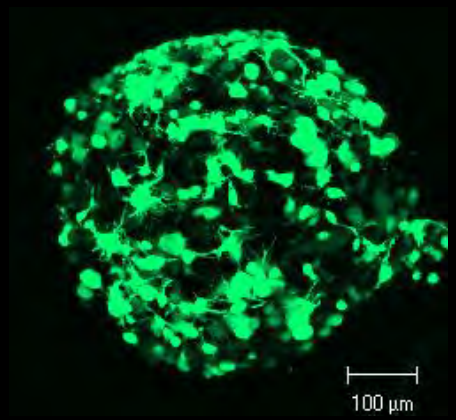



100 µm

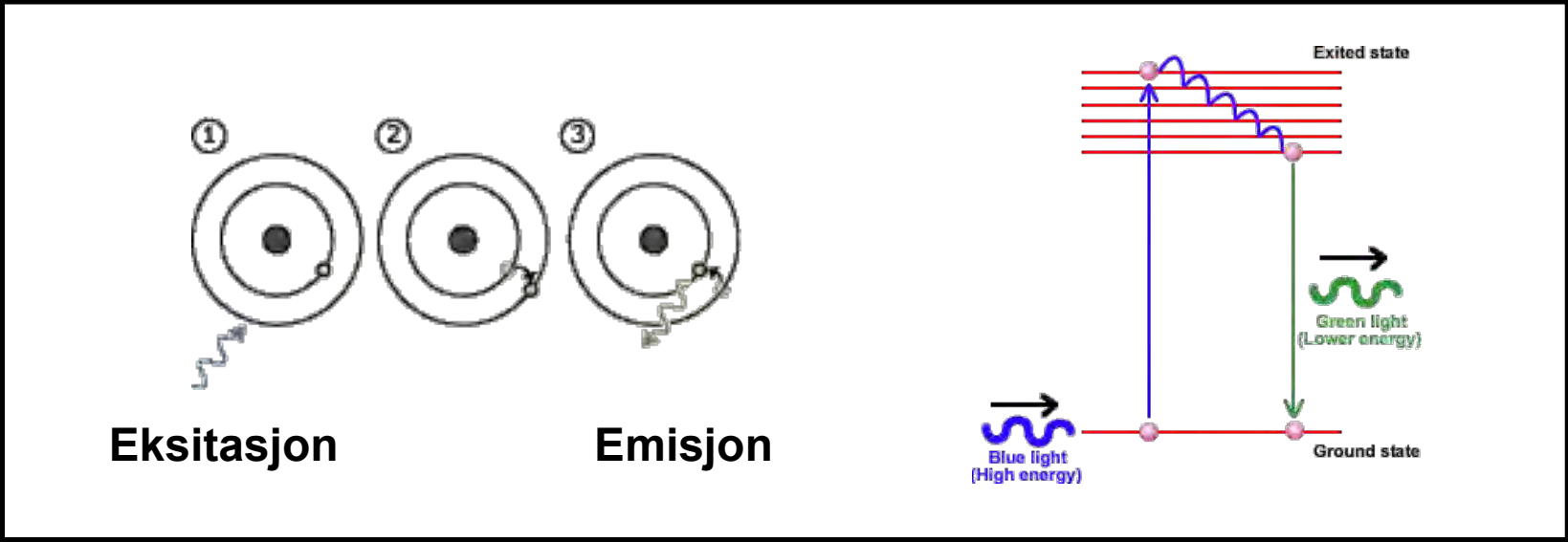



40 µm




100 µm

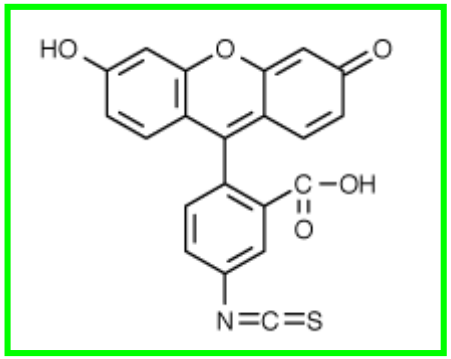
Fluorescens:
Hva skjer på atomnivå?



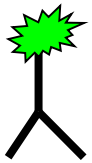
Eksitasjon: Energien fra et foton løfter et elektron til et høyere energinivå.

Emmisjon: Etter en runde rundt atomet faller elektronet tilbake til grunntilstand og gir fra seg et foton med lavere energi.

Metoder for farging med fluorescens



FITC

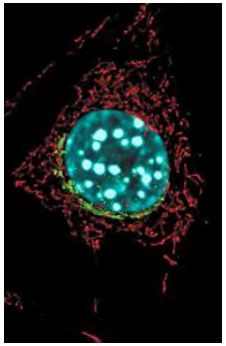


Sekundære antistoff med fluoriserende molekyl bundet gjenkjenner primært antidtoff

Primære antistoff gjenkjenner proteinet av interesse

GFP

Uttrykk av et fluoriserende protein sammen med proteinet av interesse.



Eksempel:
Kjernen: DAPI
Mitokondria: MitoTrackerRed
Golgi apparatet: BODIPY FL ceramide

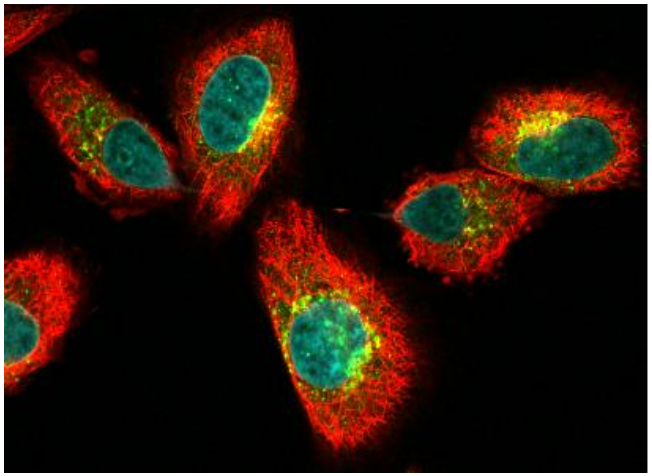
Organelle spesifikke fluoriserende probes

Tilsettes direkte til levende eller fikserte celler.

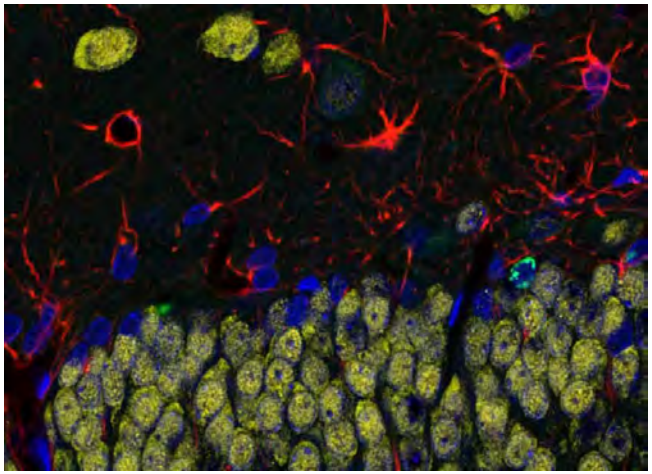
http://www.invitrogen.com/site/us/en/home/brands/Molecular-Probes.html

The most common samples

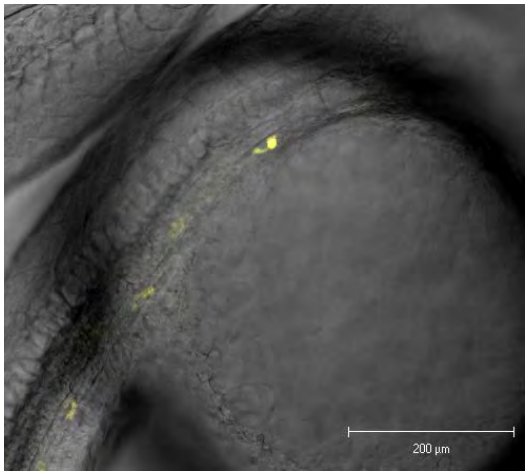
Cell cultures



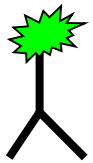
Tissue



Small animals



Staining of *fixed* samples:

1 

2 probes

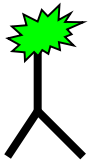
3 

Staining of *live* samples:

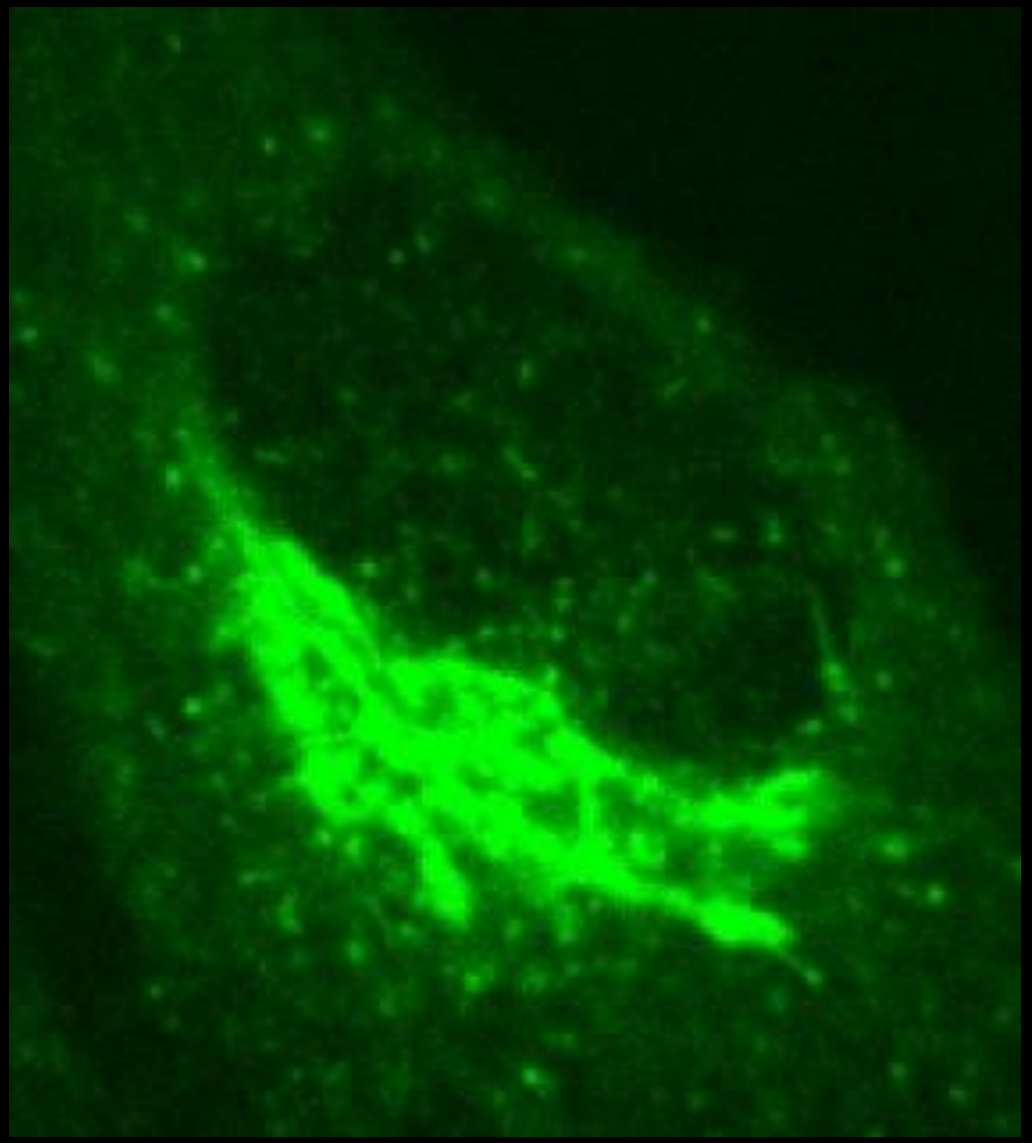
1 

2 probes

(if membrane permeable)

3 

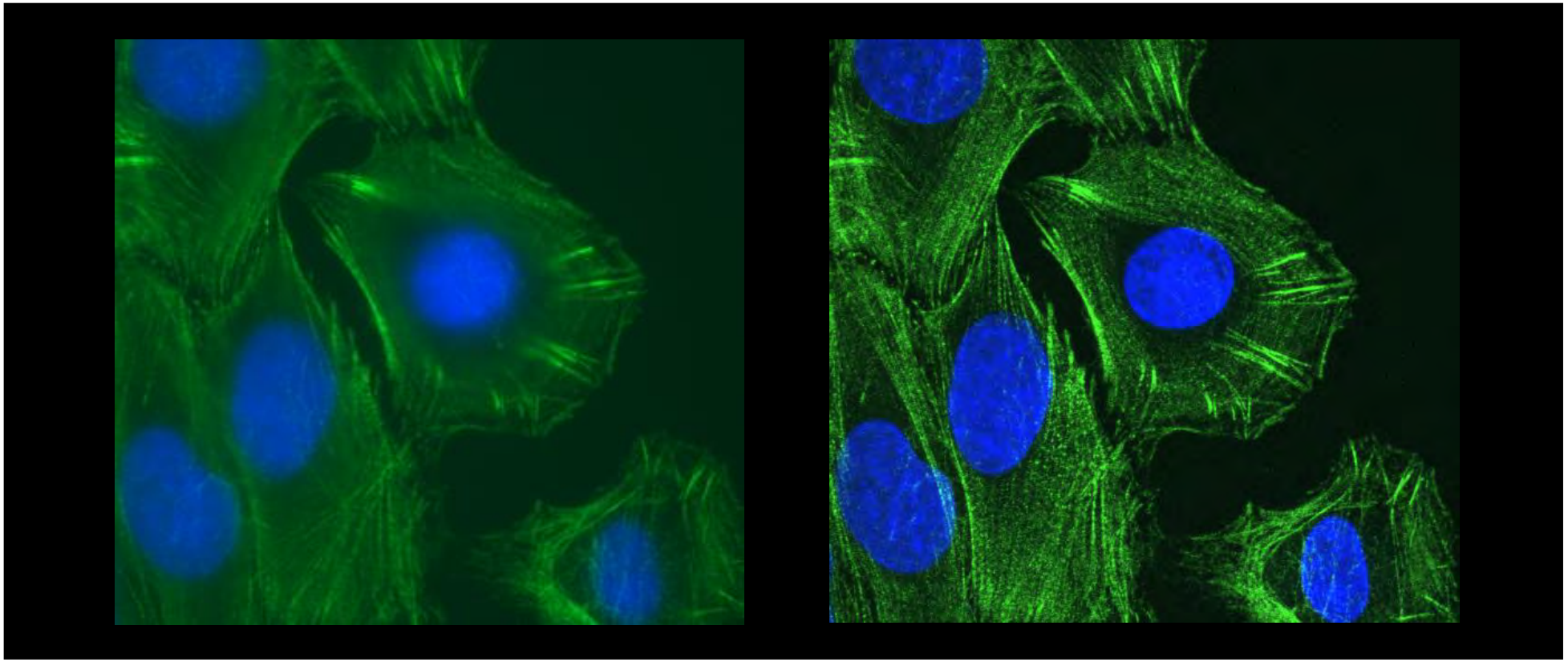
(if internalized or microinjected)



Timelapse of Rab1 tagged eGFP in NRK cells (spinning disk microscopy)

Courtesy of Michaël Marie

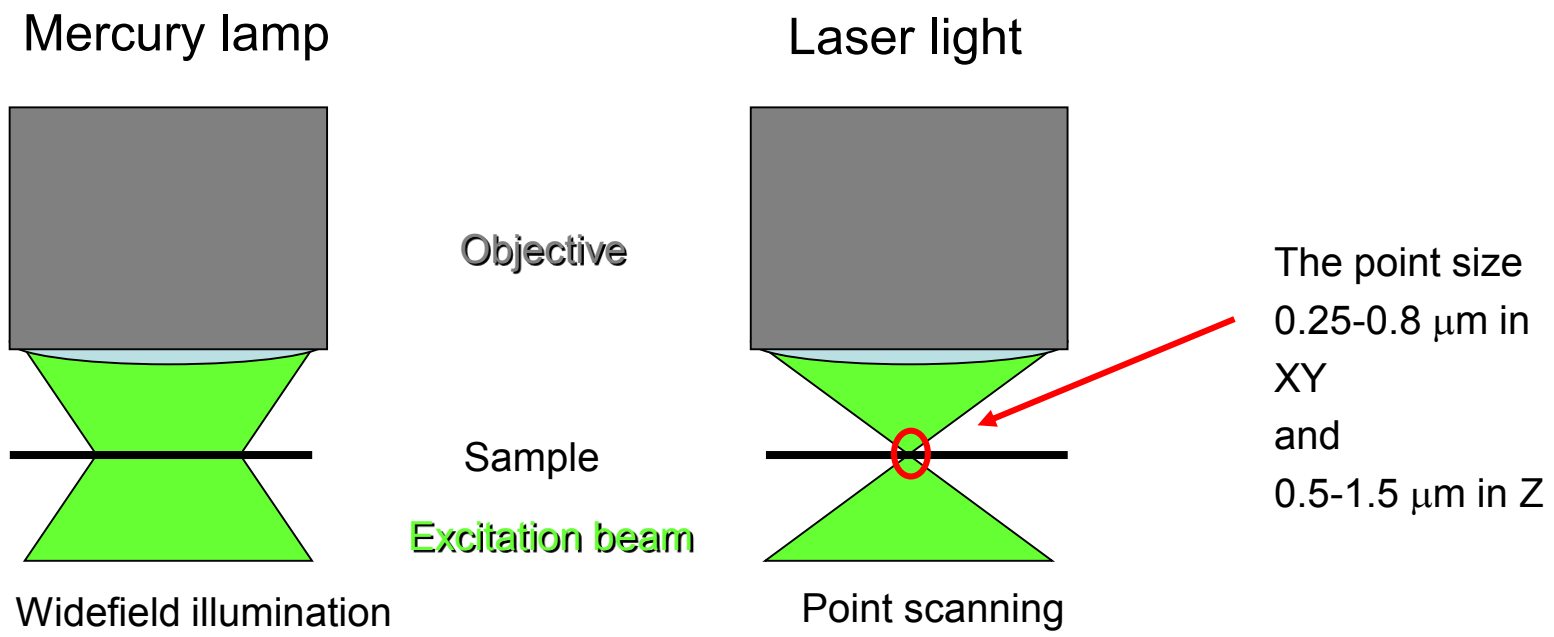
Conventional fluorescence *vs* Confocal microscopy



Conventional widefield fluorescence image

Confocal image (Z-projection)

Conventional fluorescence *vs* Confocal microscopy



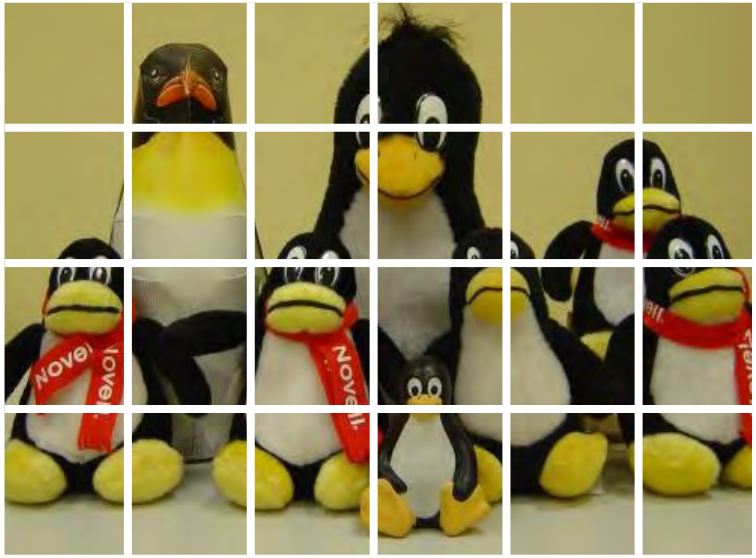
The whole field is illuminated
at the same time,
and the whole picture
is taken immediately.

The sample is illuminated point by point,
and the final image
is the information from all these
small points together.

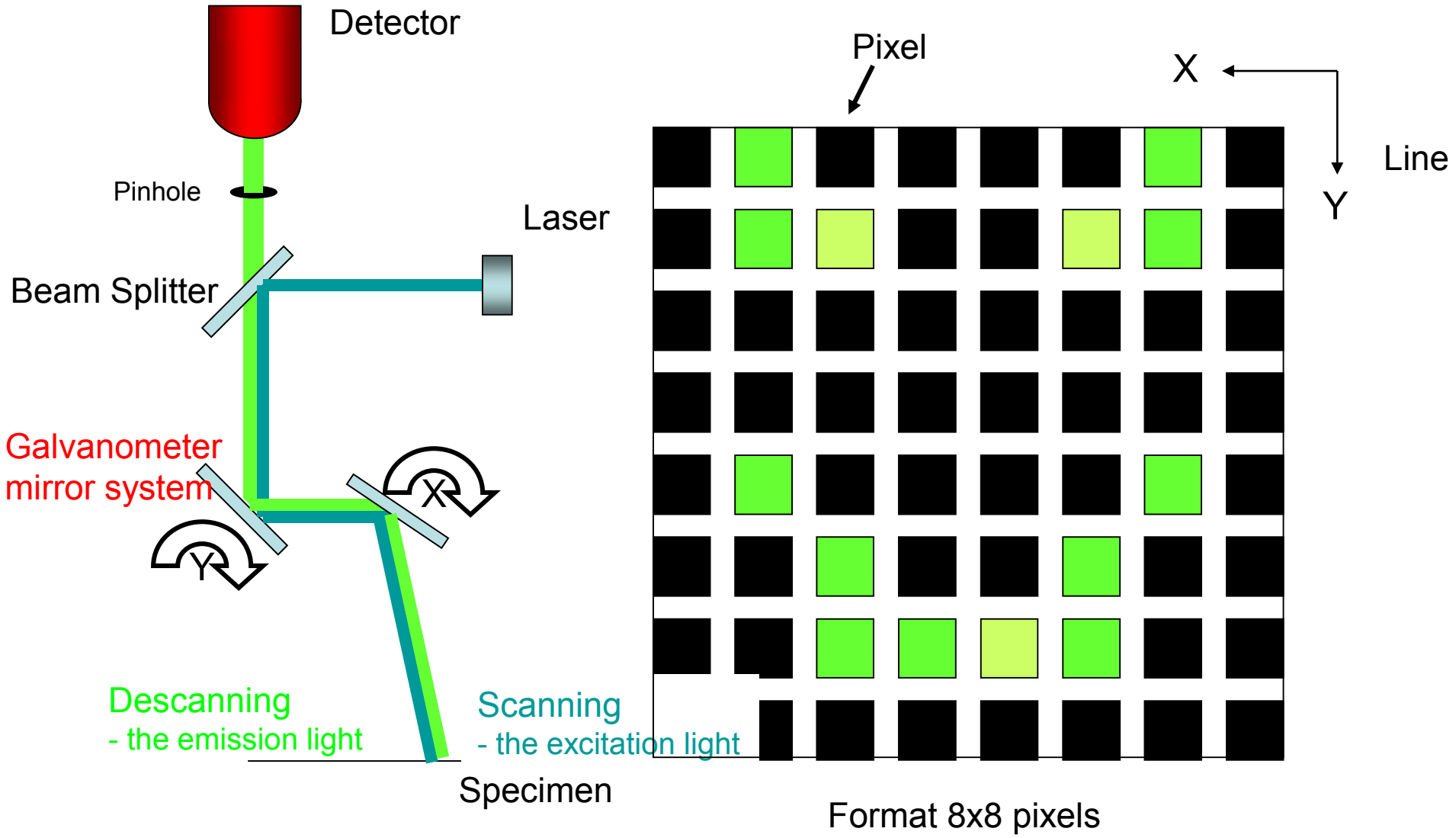
Conventional fluorescence *vs* Confocal microscopy

Instant image

Point by point

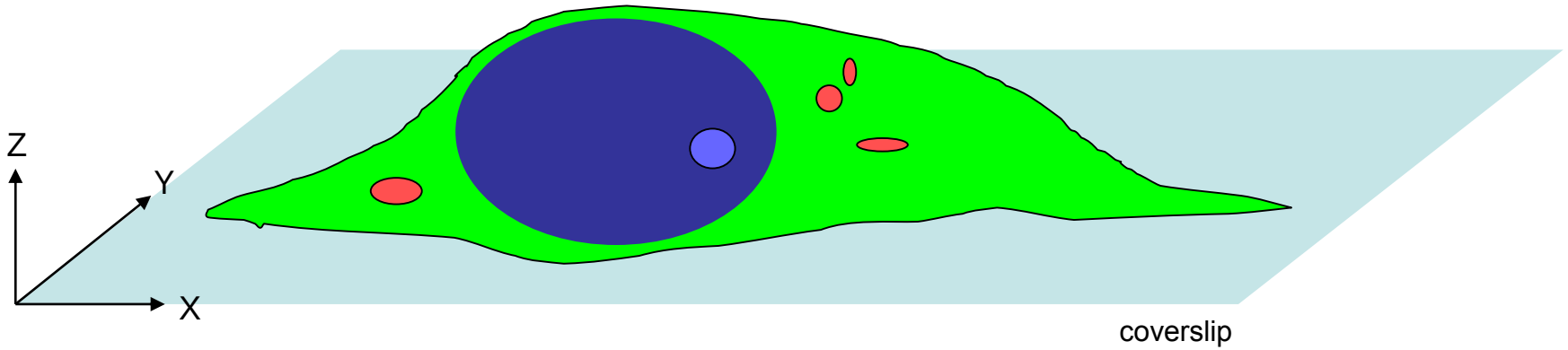


Point scanning



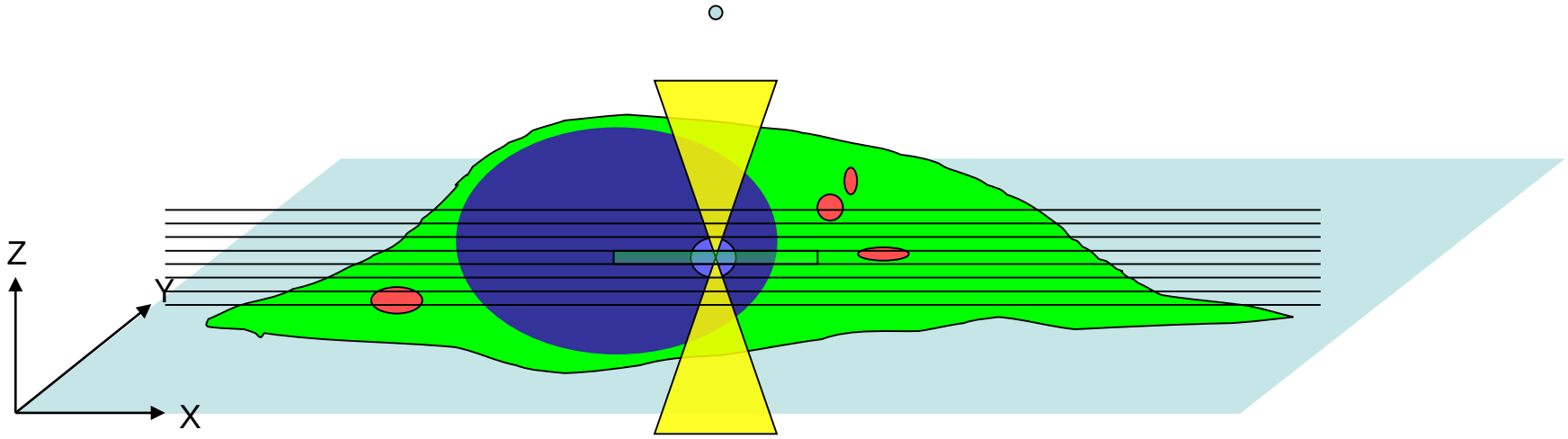
Optical slicing

A typical cell: thickness of approx. 10 µm

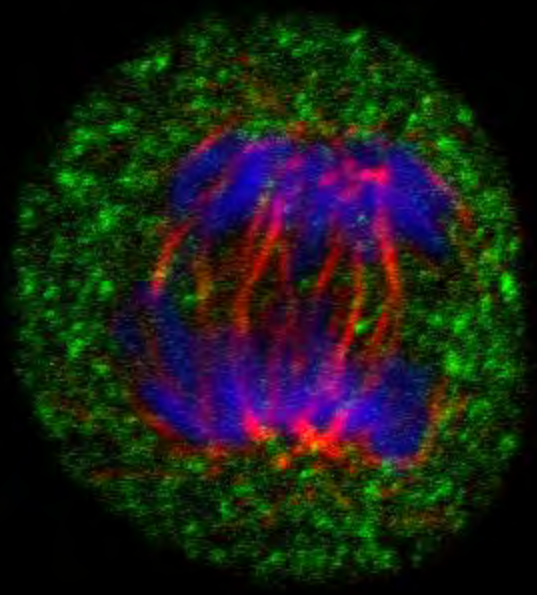


Optical slicing

Generally; the smaller the pinhole the thinner the slice

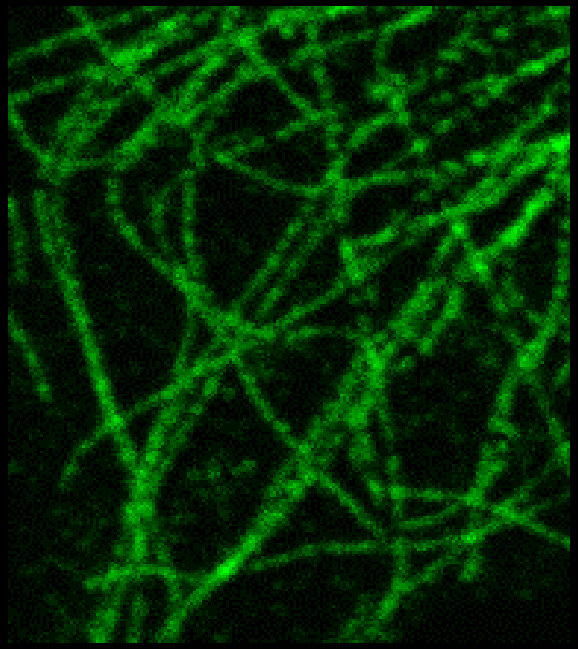


Celledeling; Blå kromosomer, rødt cytoskjelett, grønt protein i cytoplasma

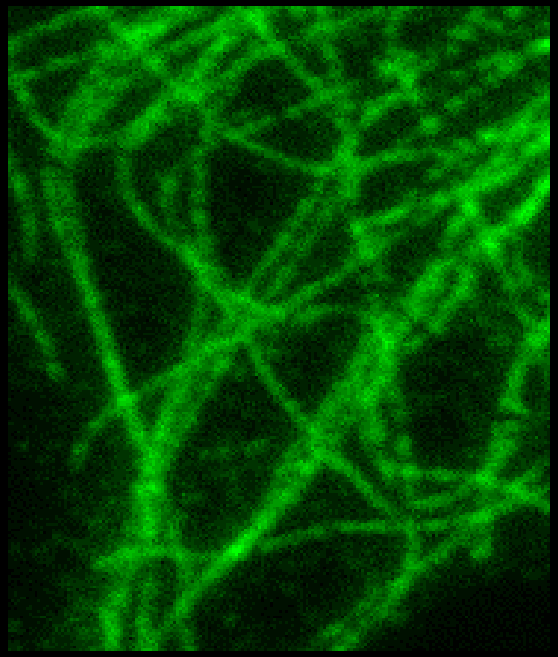


20 µm

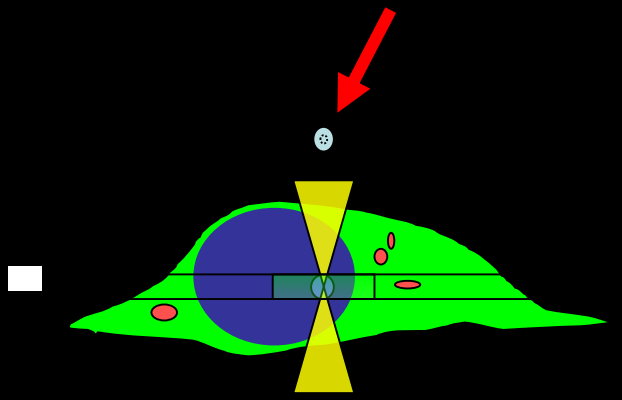
The smaller the pinhole, the better rejection of out of focus light, and the thinner section acquired.



Airy 0.8



Airy 5.0

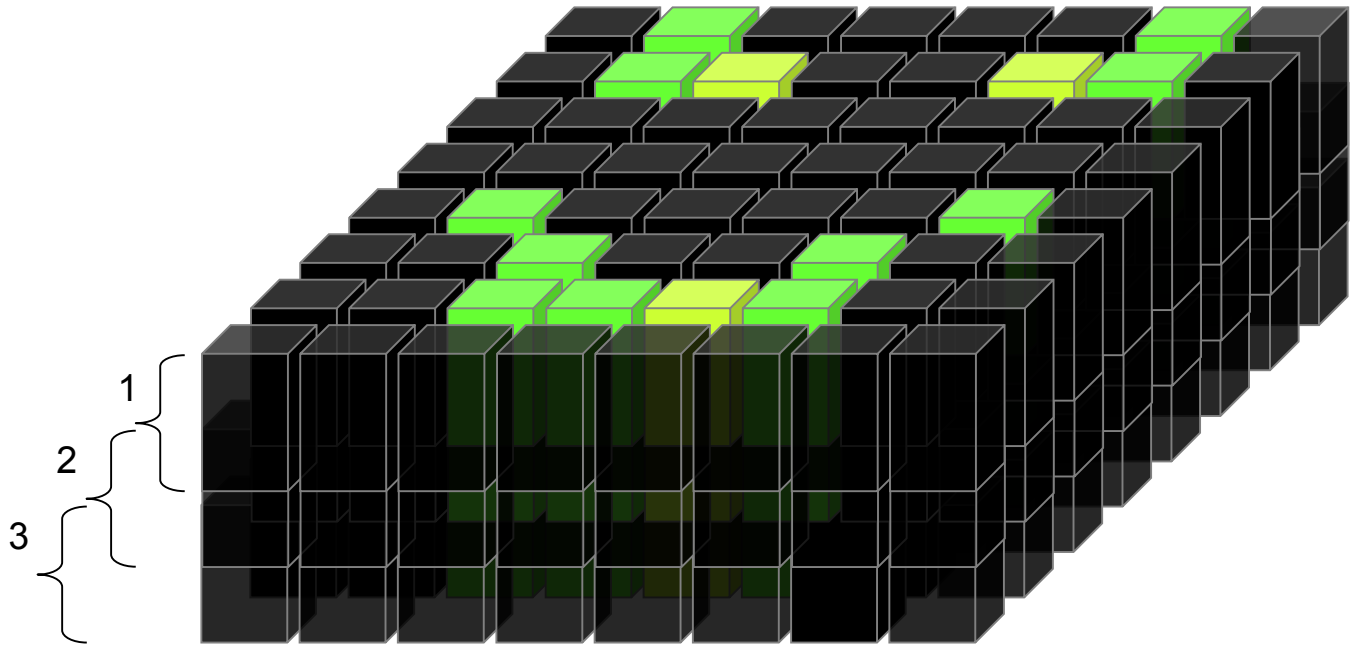


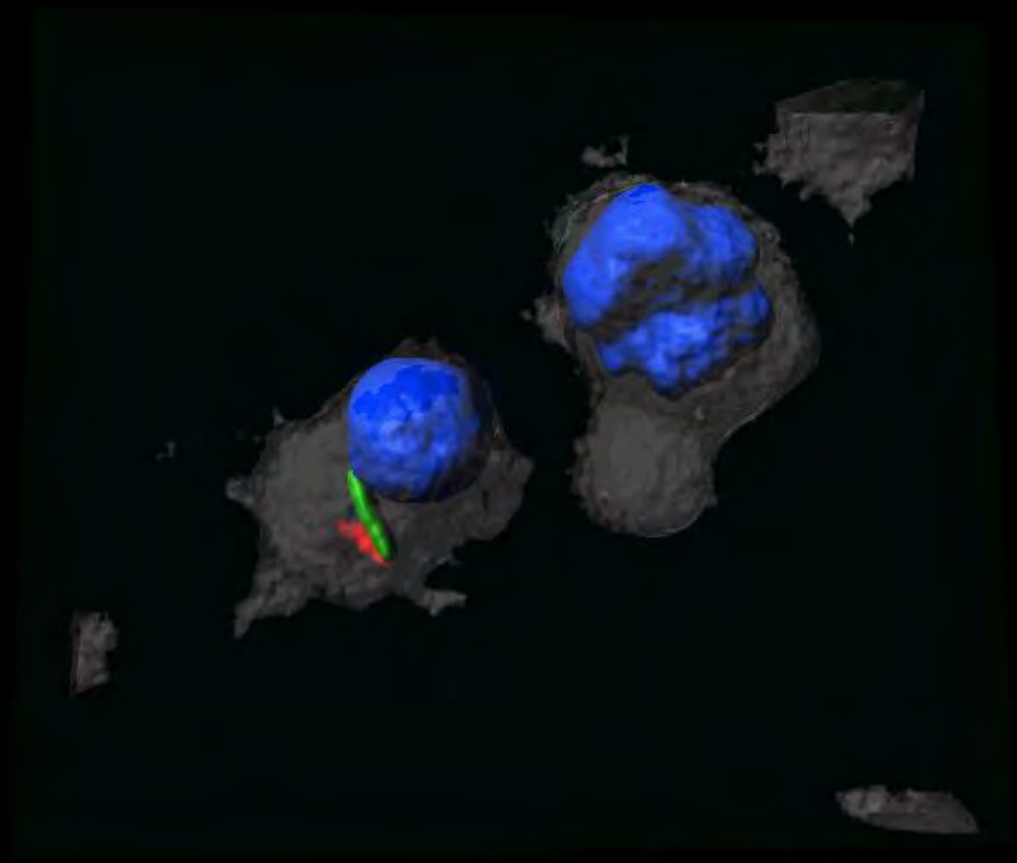
small pinhole	large pinhole
sharp image	loss of definition
dim image	bright image

Optimal image acquiring for 3-D reconstruction
“Nyquist criterion”

The optical slice has to overlap 50 % with the next

→ 2x over sampling in the Z-axis

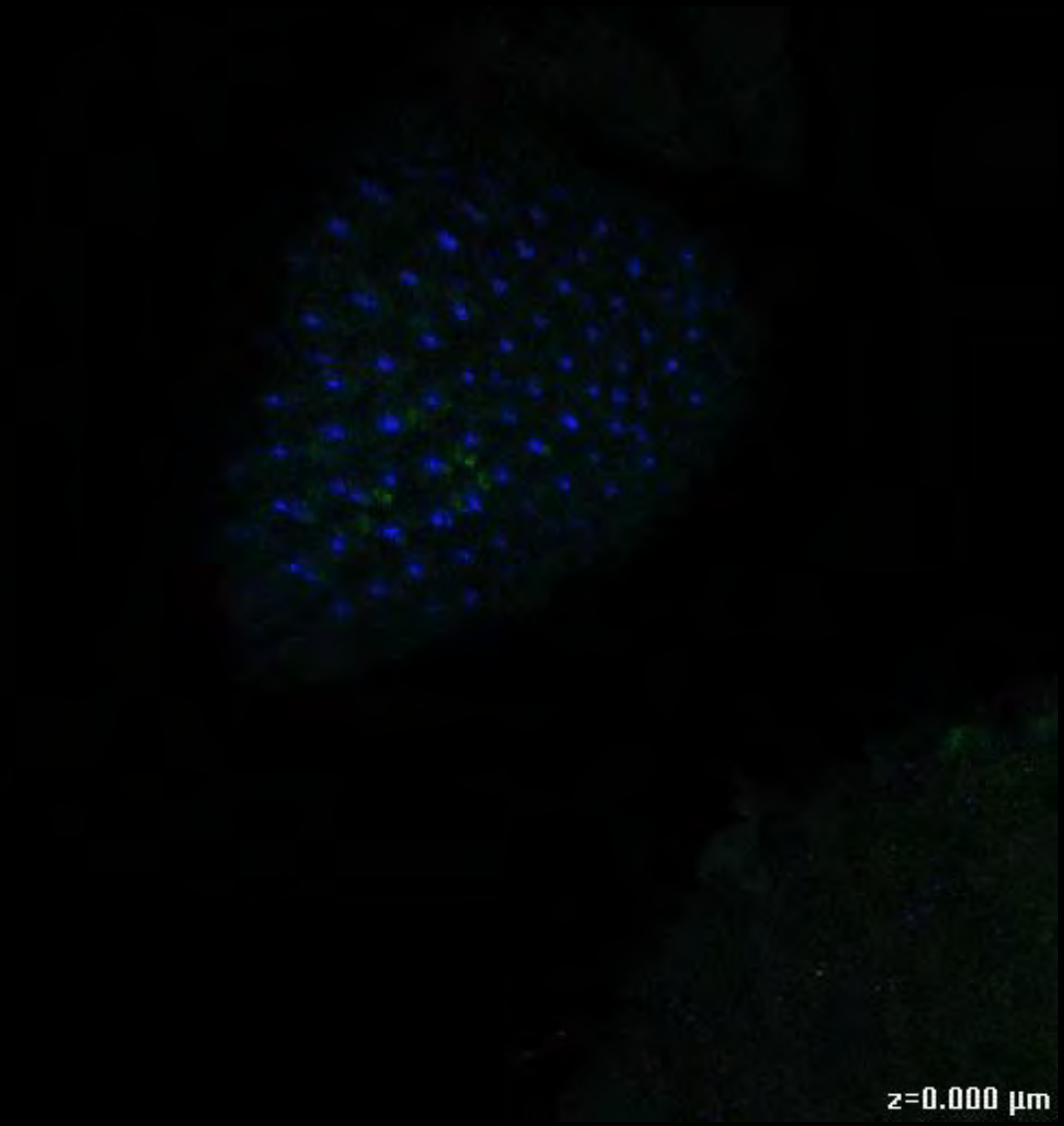




Rendered cell showing bacteria (green) penetrating and leaving deposit (red)

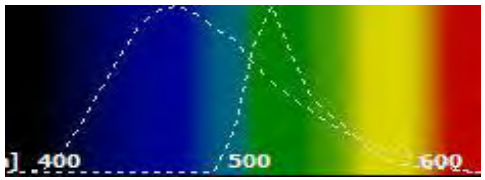
(confocal microscopy)

110 optiske snitt gjennom øyet på en bananflue



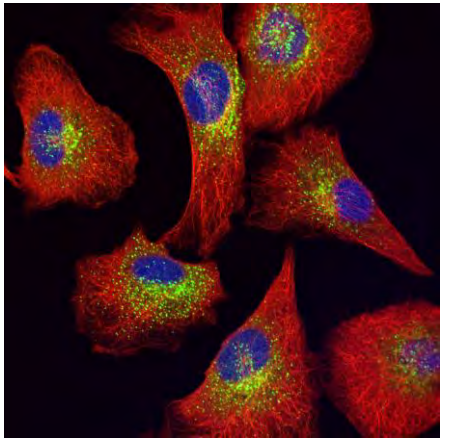
Parts involved in acquiring the best confocal image

Sample preparation, cover slip, mounting medium and fluorochrome

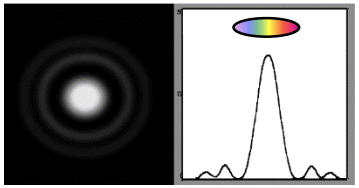


Cross excitation and cross emission

Laser choise/intensity

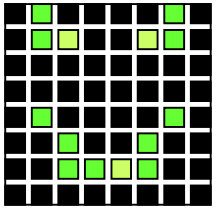


Objective, NA and immersion media



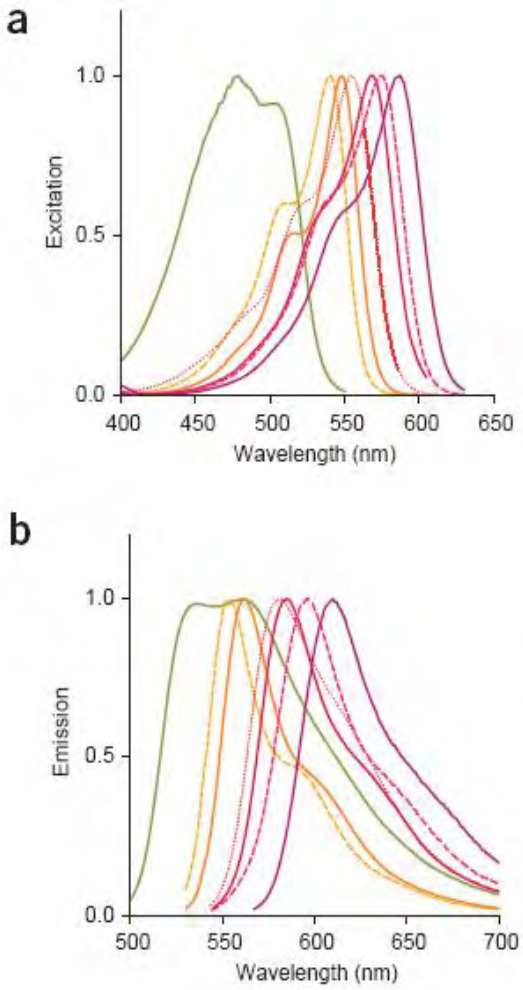
Pinhole size

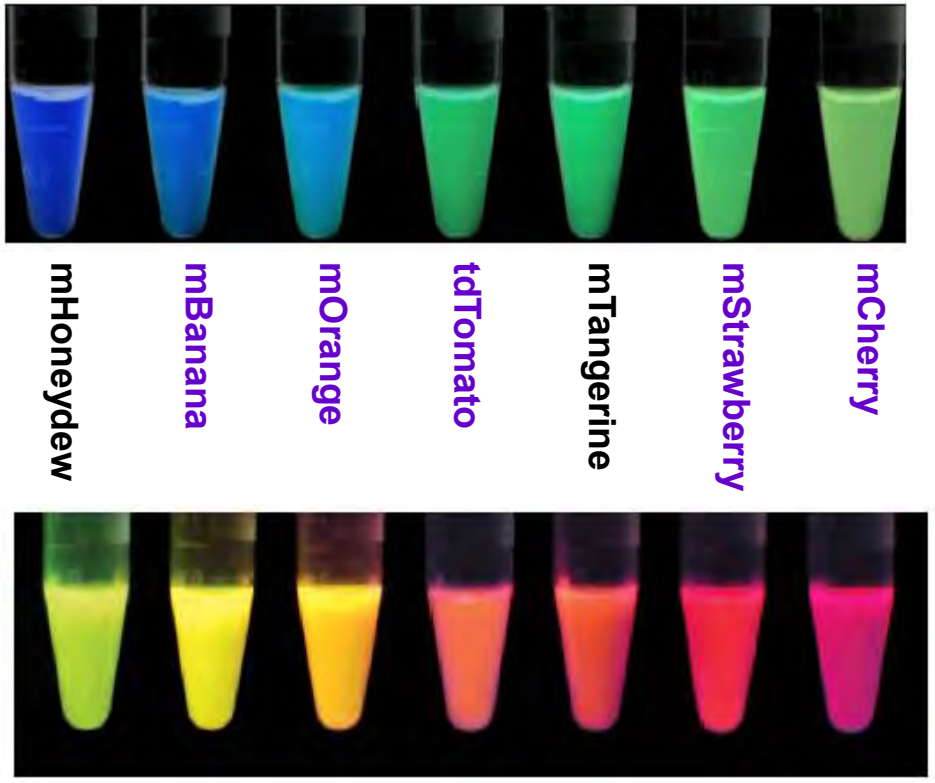
Detector settings (detection range and V)



Format and voxel size

More red shifted mutants generated by Campell, Tsien and coworkers (2004)





It's possible to request the different FP-vectors:

https://www.addgene.org/search/advanced/?q=

Select the correct objective for you sample (media, working distance)

Adjust the corection collar if applicable

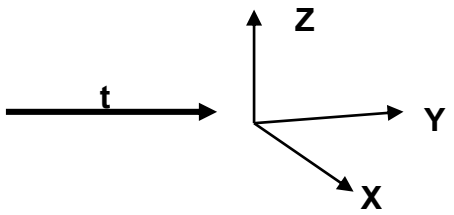
Mount samples in appropriate mounting media (cure?) with appropriate RI

Match the immersion oil to the objective

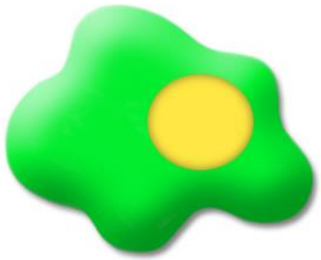
Use appropriate coverglasses or dish - #1.5 high precission

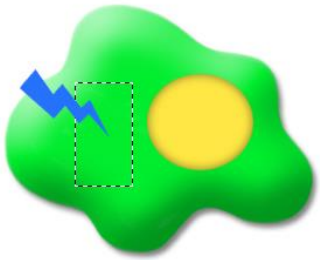
Common applications on living cells
> *following dynamics* <

3D over time = 4D

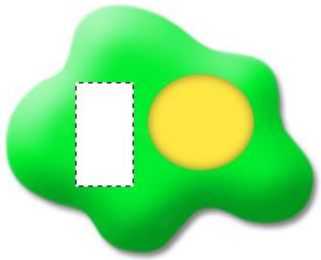


Fluorescence Recovery After Photobleaching (FRAP); measures association

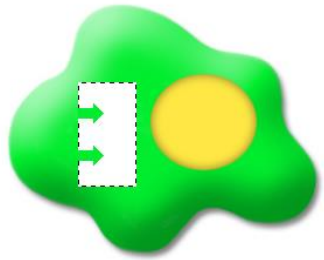




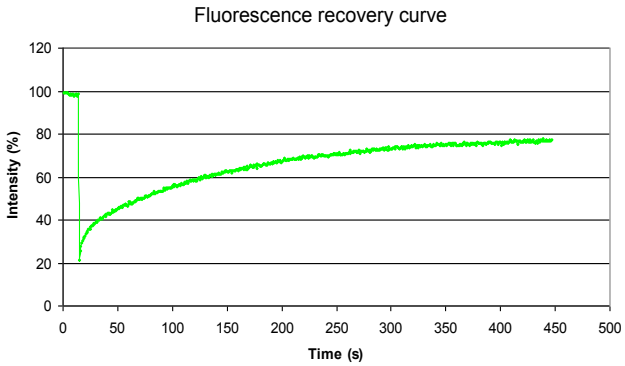
High-intensity laser pulse in Region Of Interest (ROI).



Fluorescent molecules inside ROI become irreversable non-fluorescent



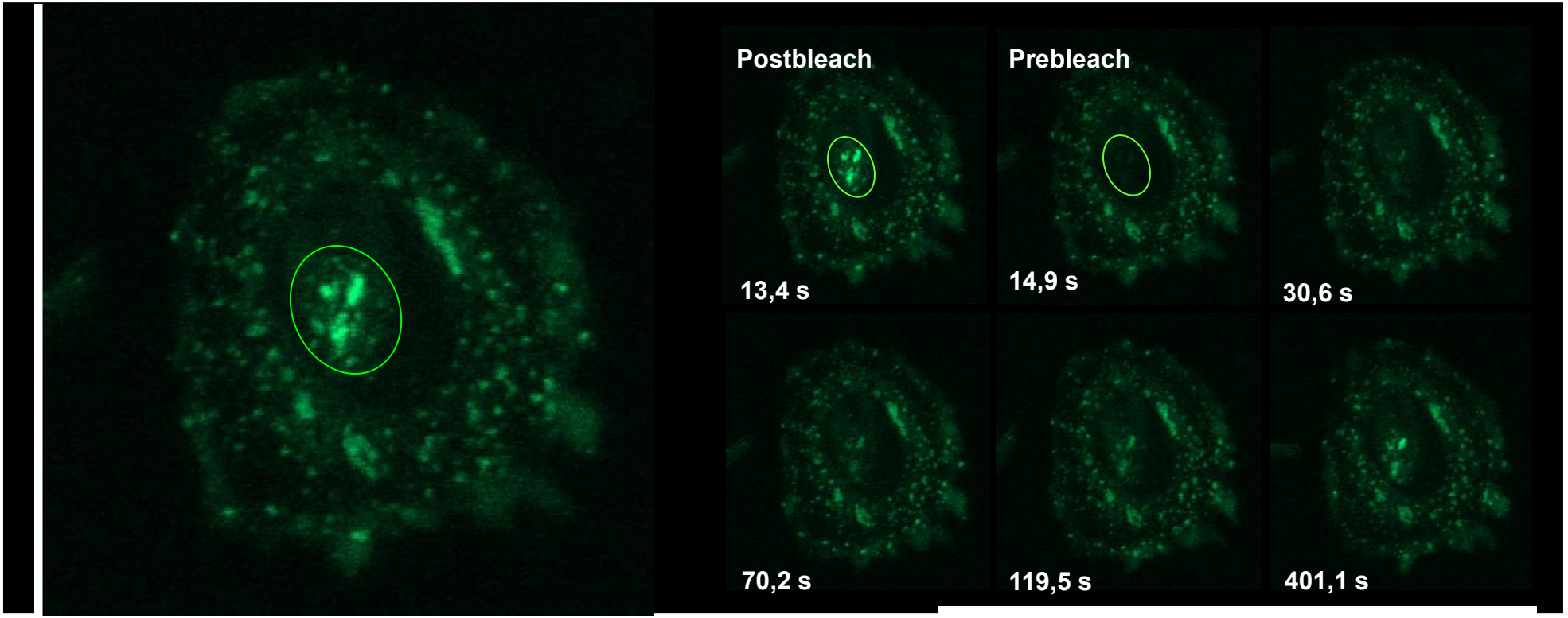
Unbleached molecules move into bleached ROI



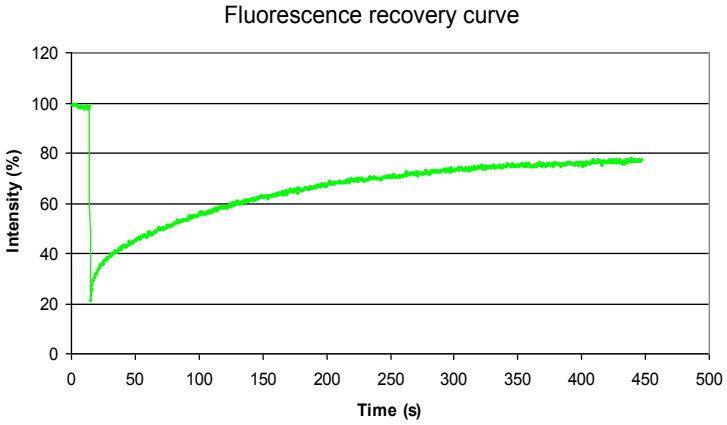
Photoactivation – opposite effect of FRAP; measures dissociation

Photoconversion – monitoring molecules in and out of ROI

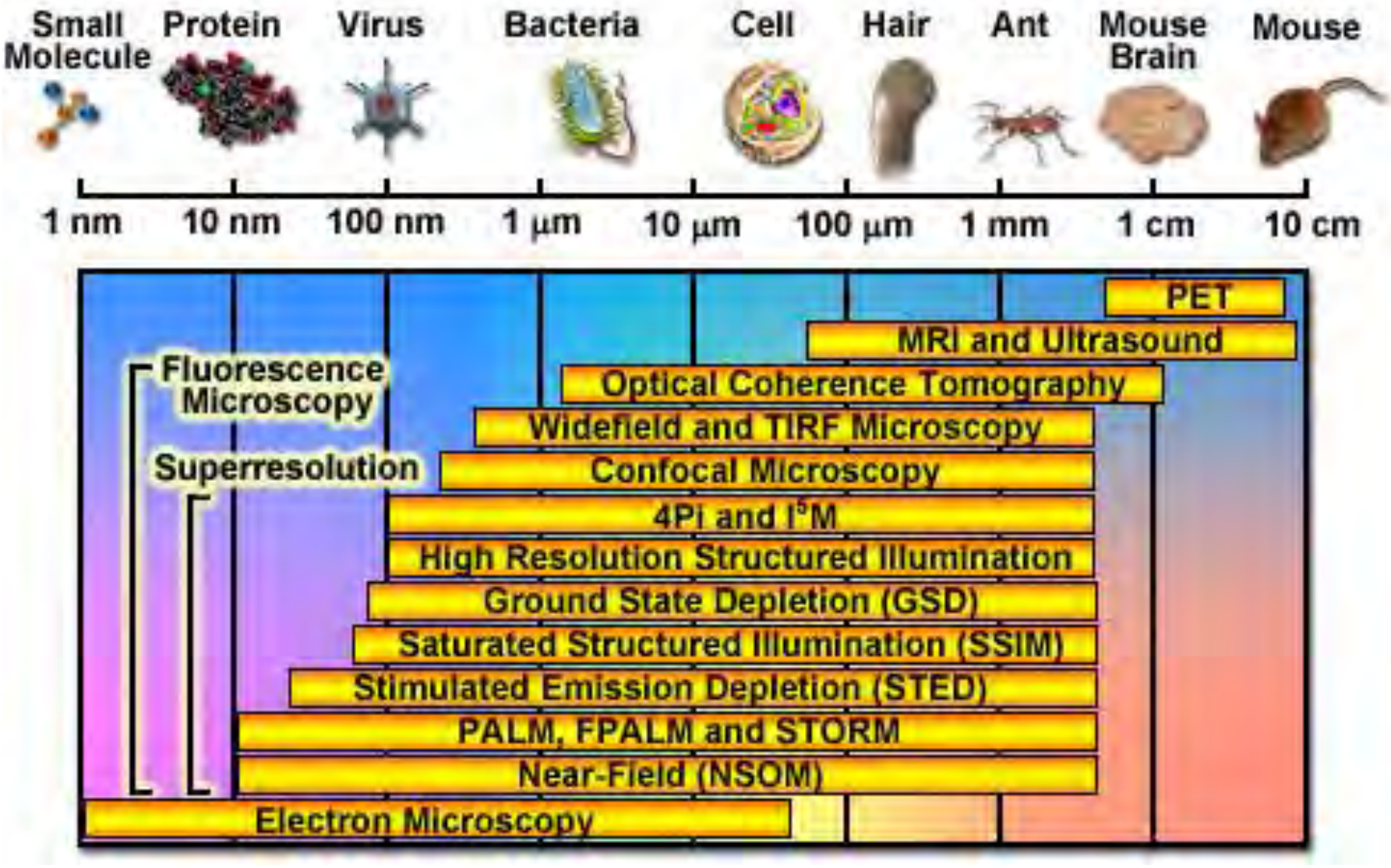
Fluorescence Recovery After Photobleaching (FRAP)



Scan speed: 245 ms pr. frame
Acquisition speed: 1 frame pr. 750 msec
Movie play speed: 16 frames pr. sec

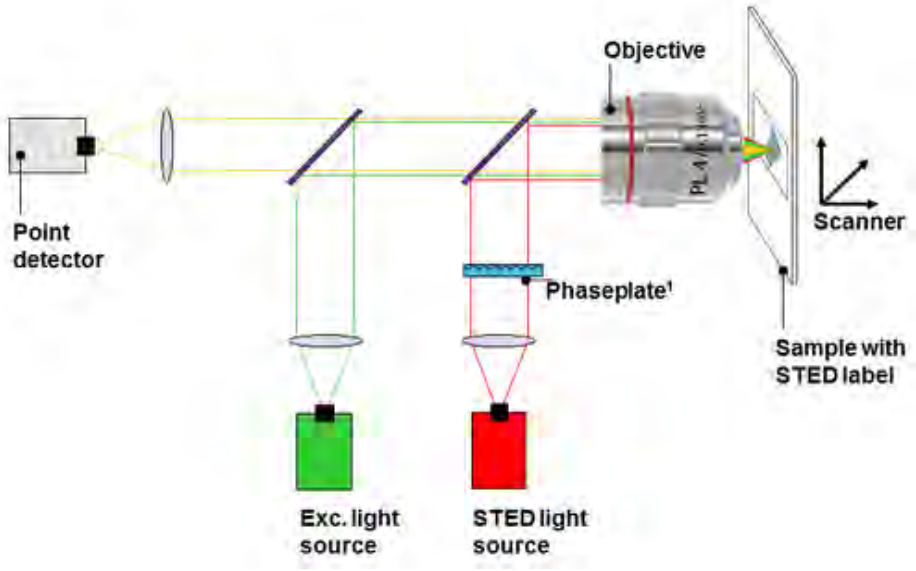


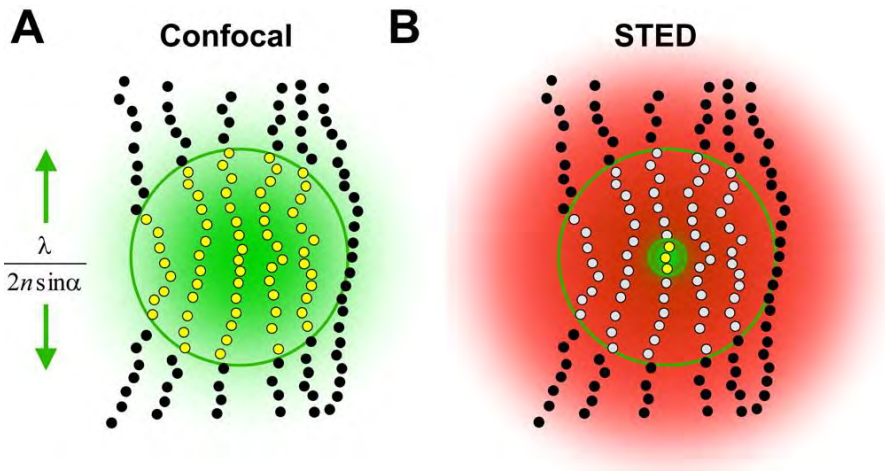
Spatial Resolution of Biological Imaging Techniques



The STED principle

STimulated Emission Depletion



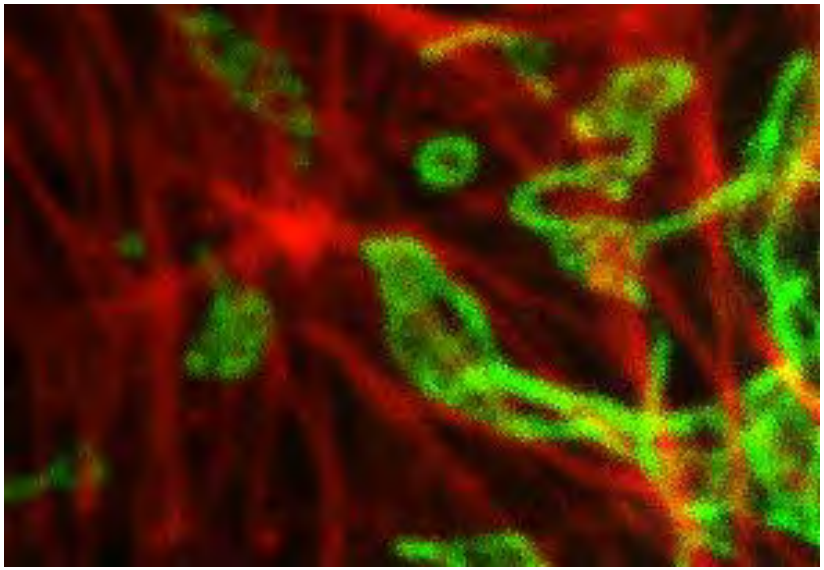


- Based on lasers scanning over the sample, resembling a confocal.
- Each excitation event is followed by a donut shaped pulse of depletion laser.
- The emission signal from the middle of the donut is detected point by point by detectors.

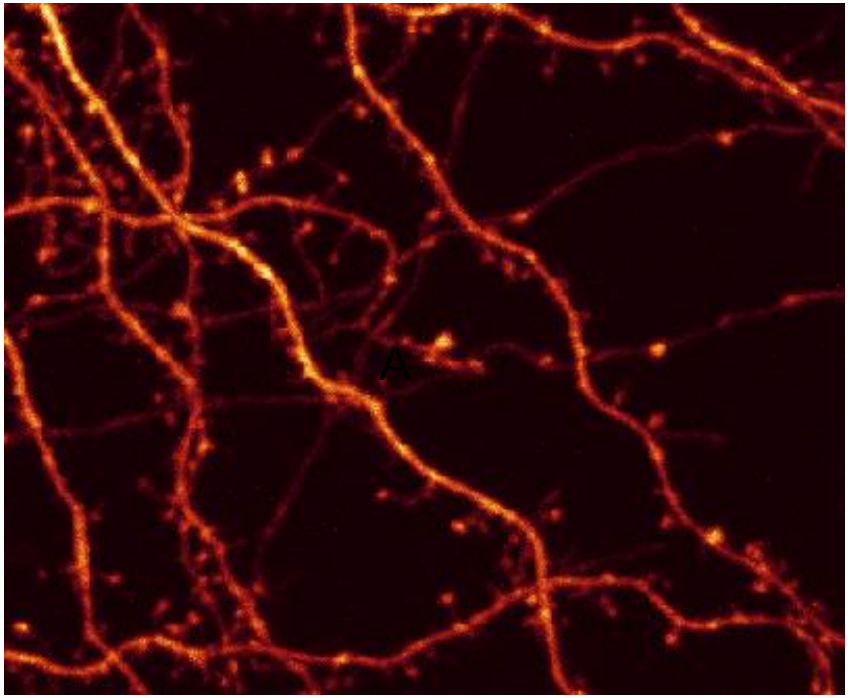
When do you need super-resolution?

In intra-cellular imaging

– when you need to see more and finer details than this:

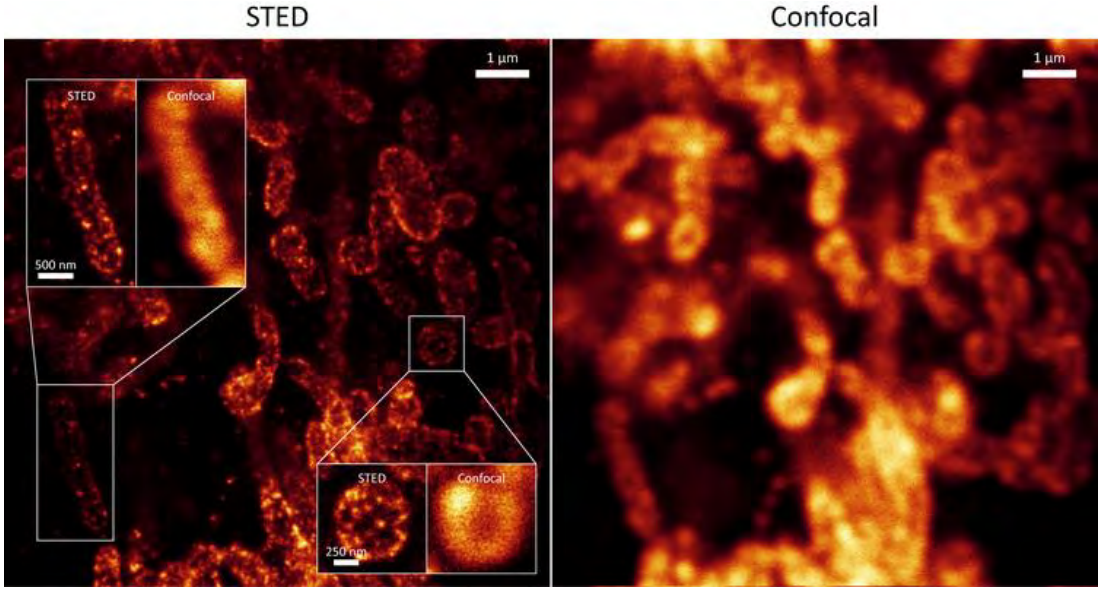


Actin (red) and mitocondria (green)

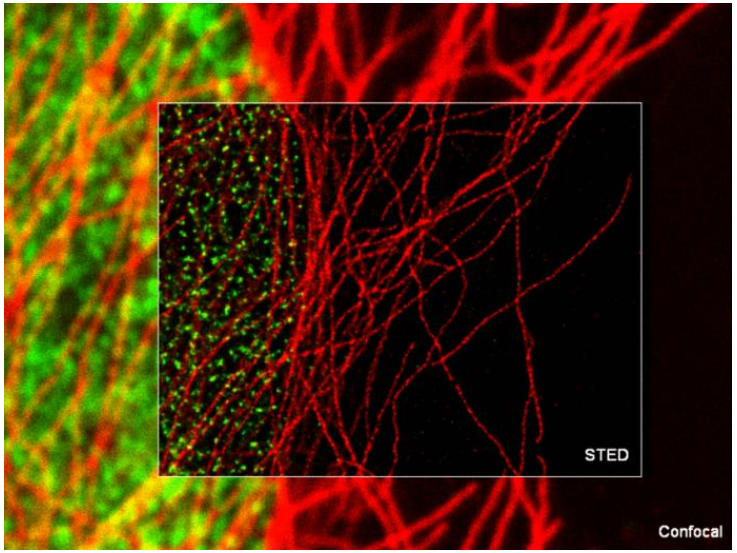


Neurons with spines

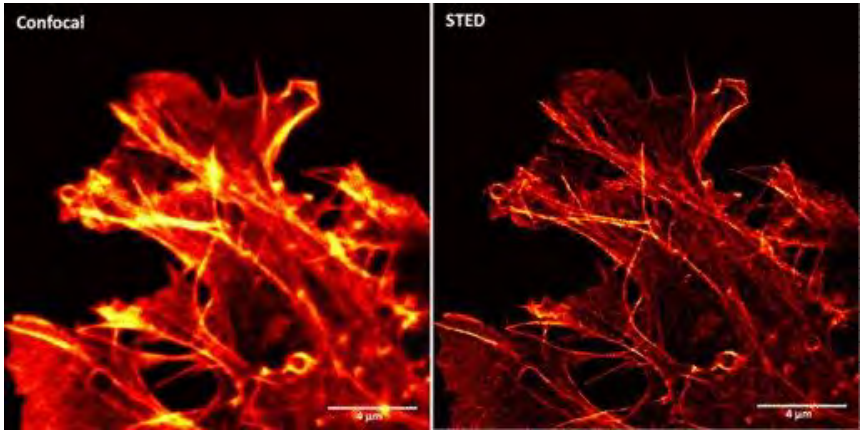
Comparison of confocal and STED images



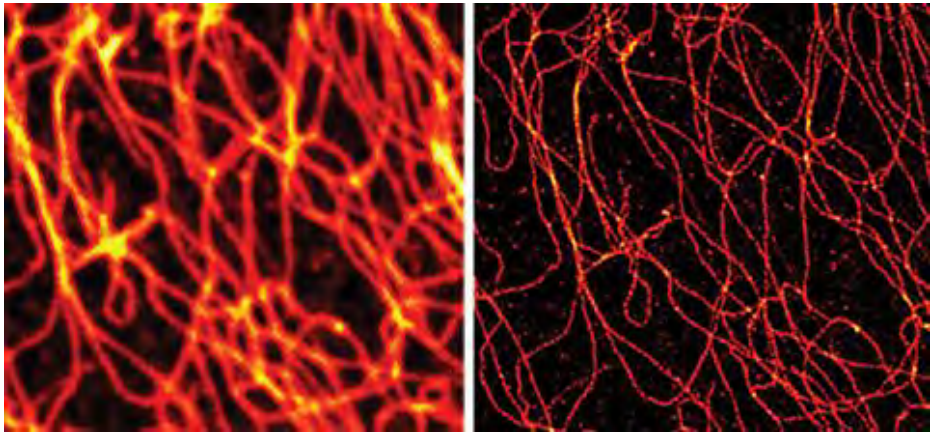
Mitochondria



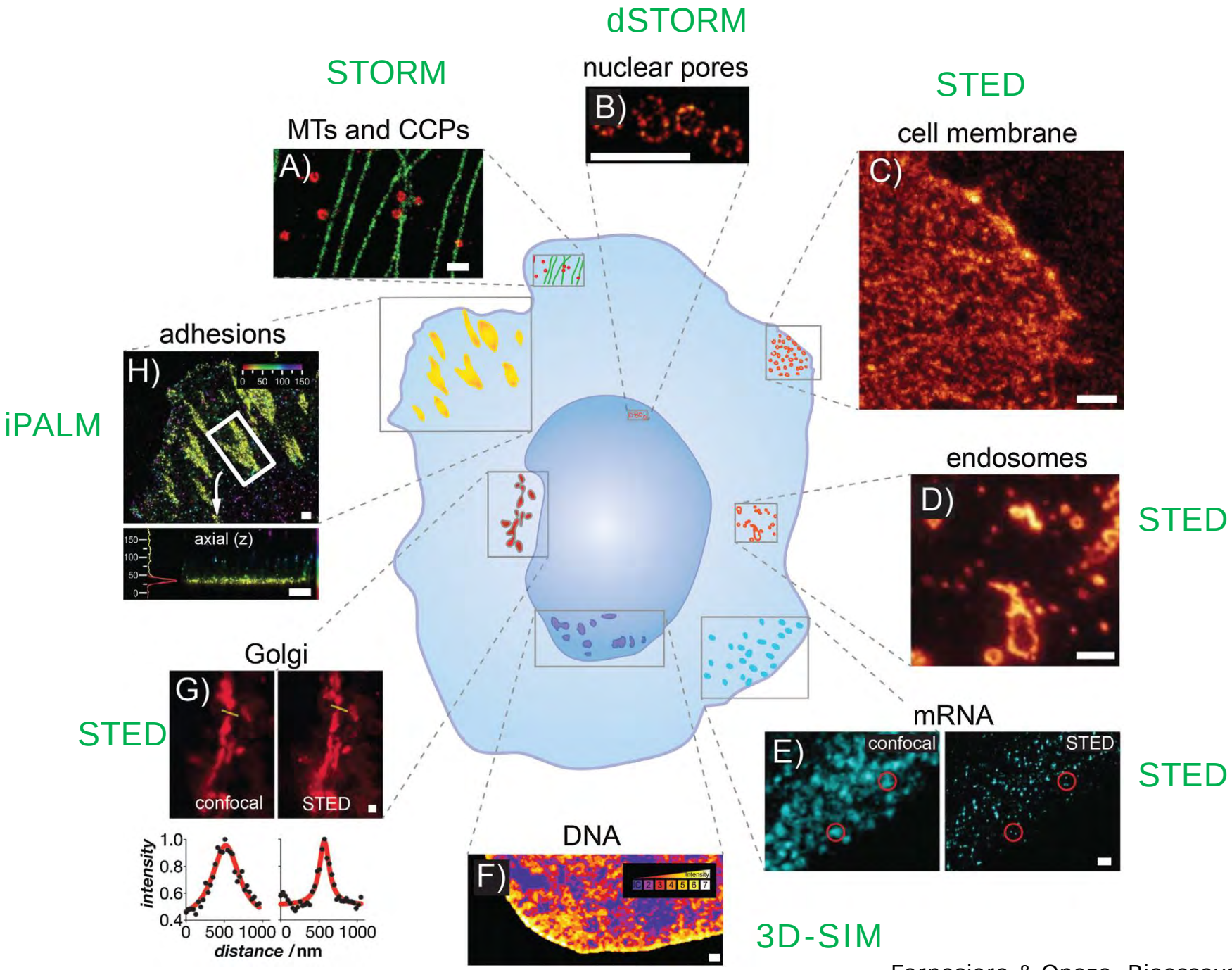
Tubulin



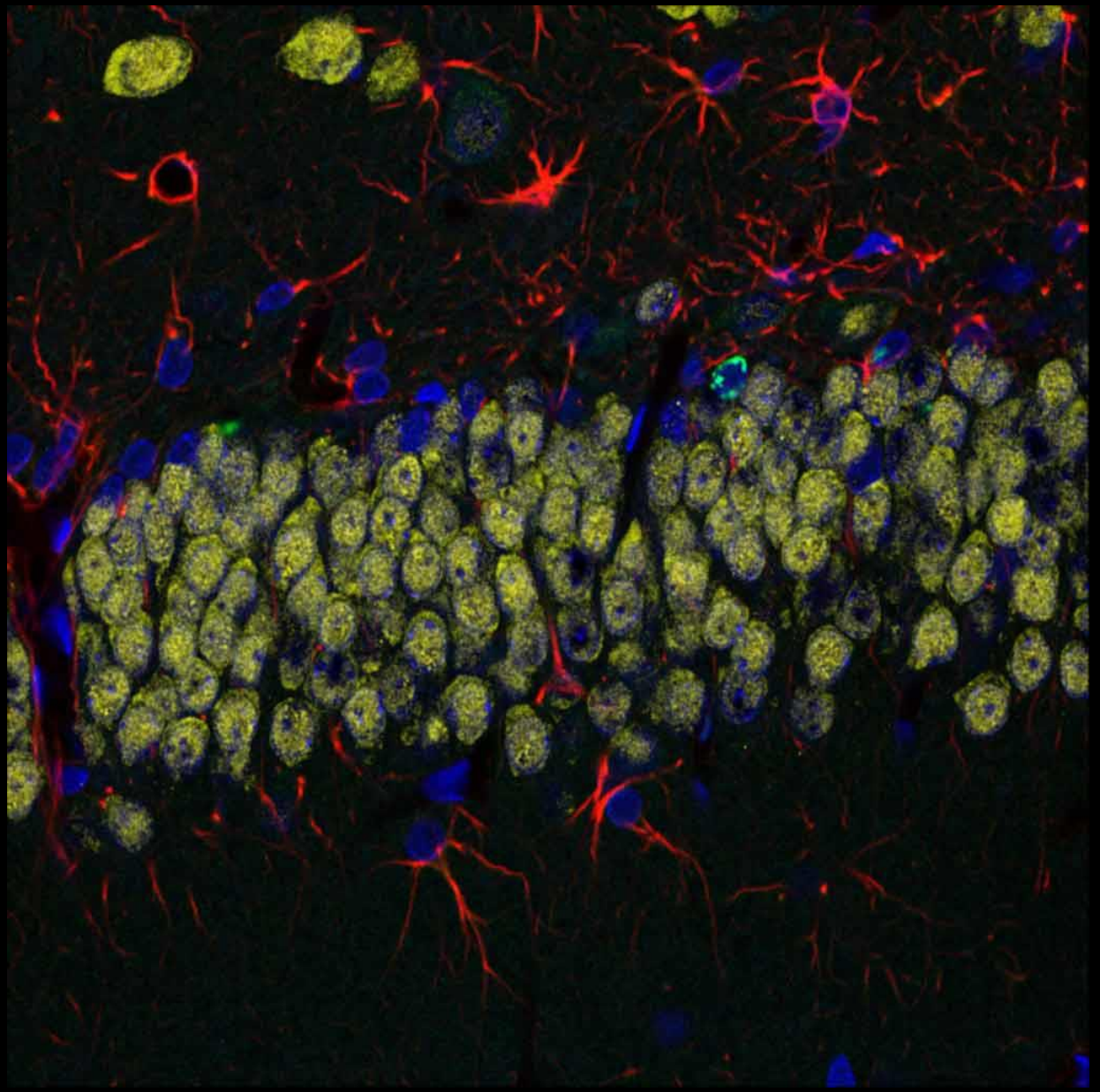
Actin



Vimentin

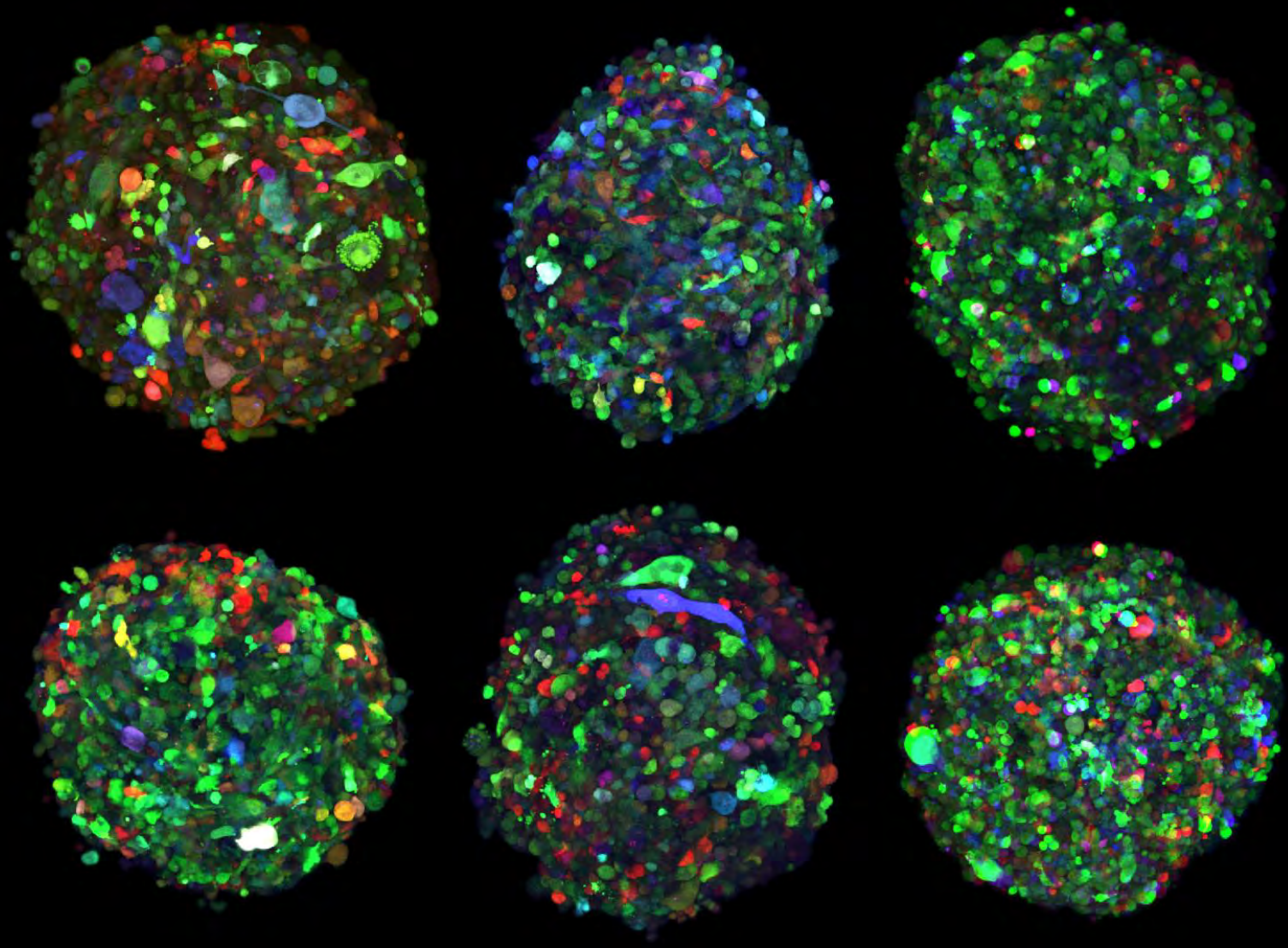


Fornasiero & Opazo, Bioassays 2015



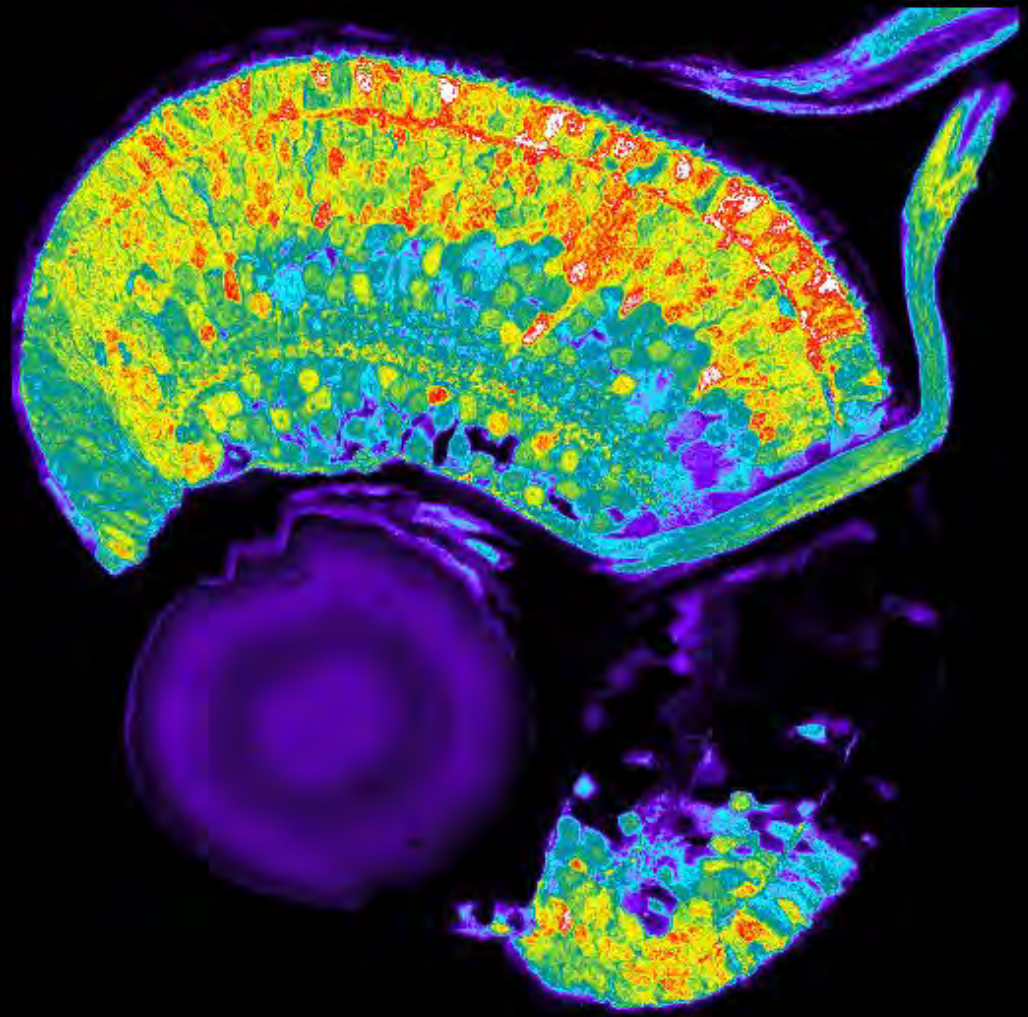
Utsnitt fra rottehjerne: Neuroner (røde) og gliaceller (gule) med cellekjerner i blått.

Sjoukje Kuipers, Biomed, UIB

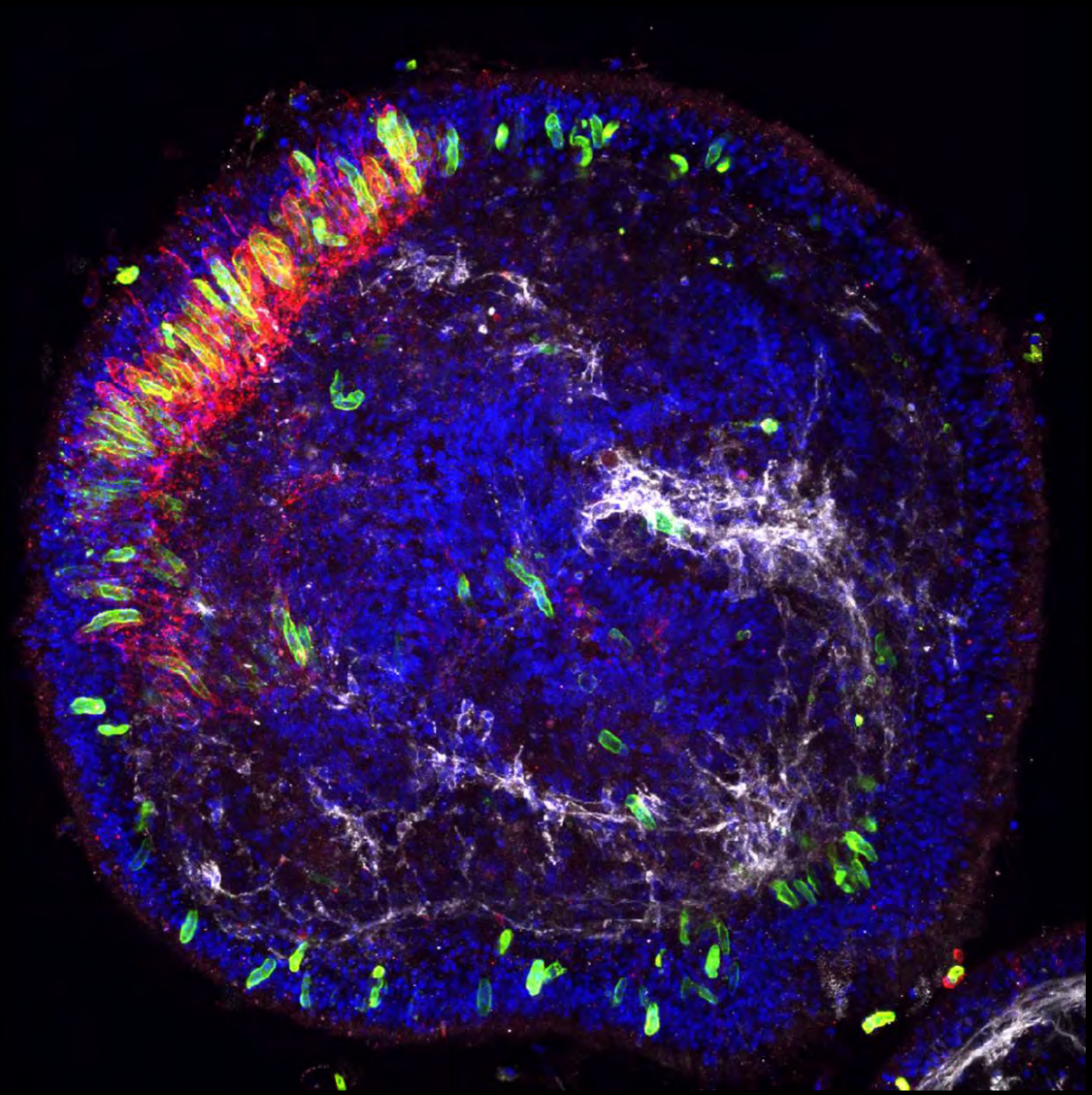


Spheroids of cancer cells expressing GFP, CFP and mCherry (confocal microscopy)

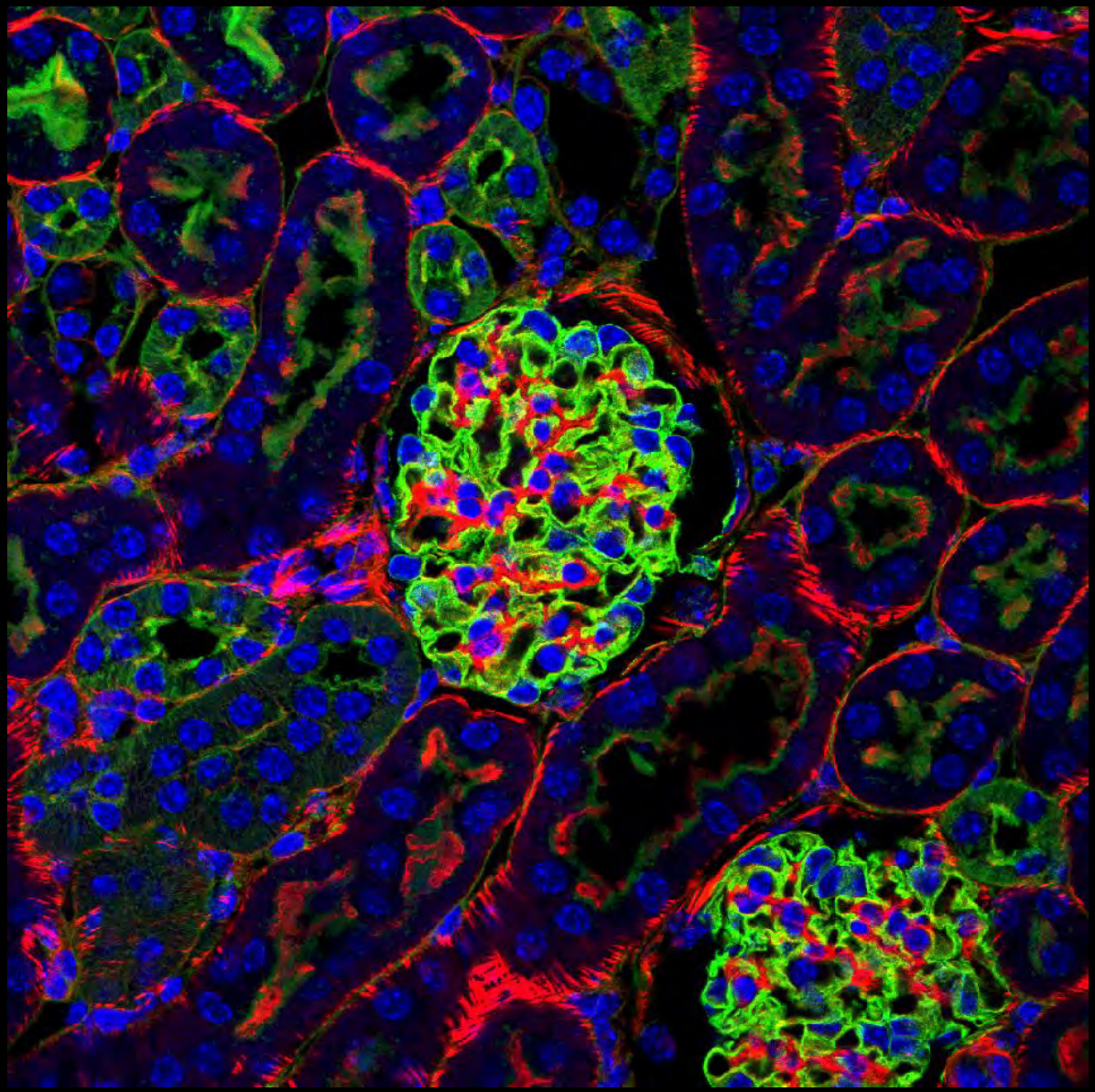
Courtesy of Lars Prestegaarden



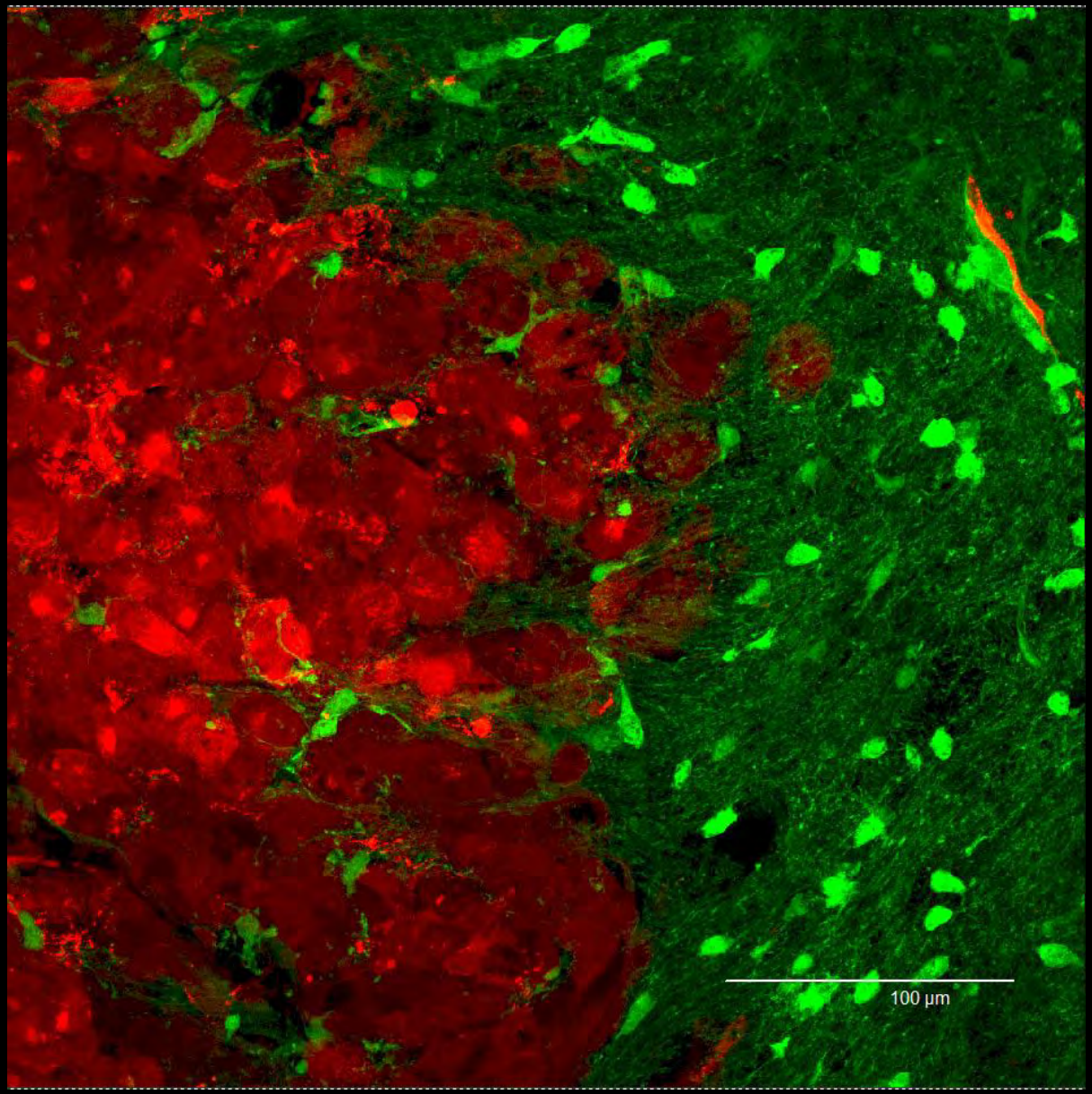
GFP positive cells from the retina of a zebrafish eye (confocal microscopy)



Nematostella embryo (confocal microscopy)



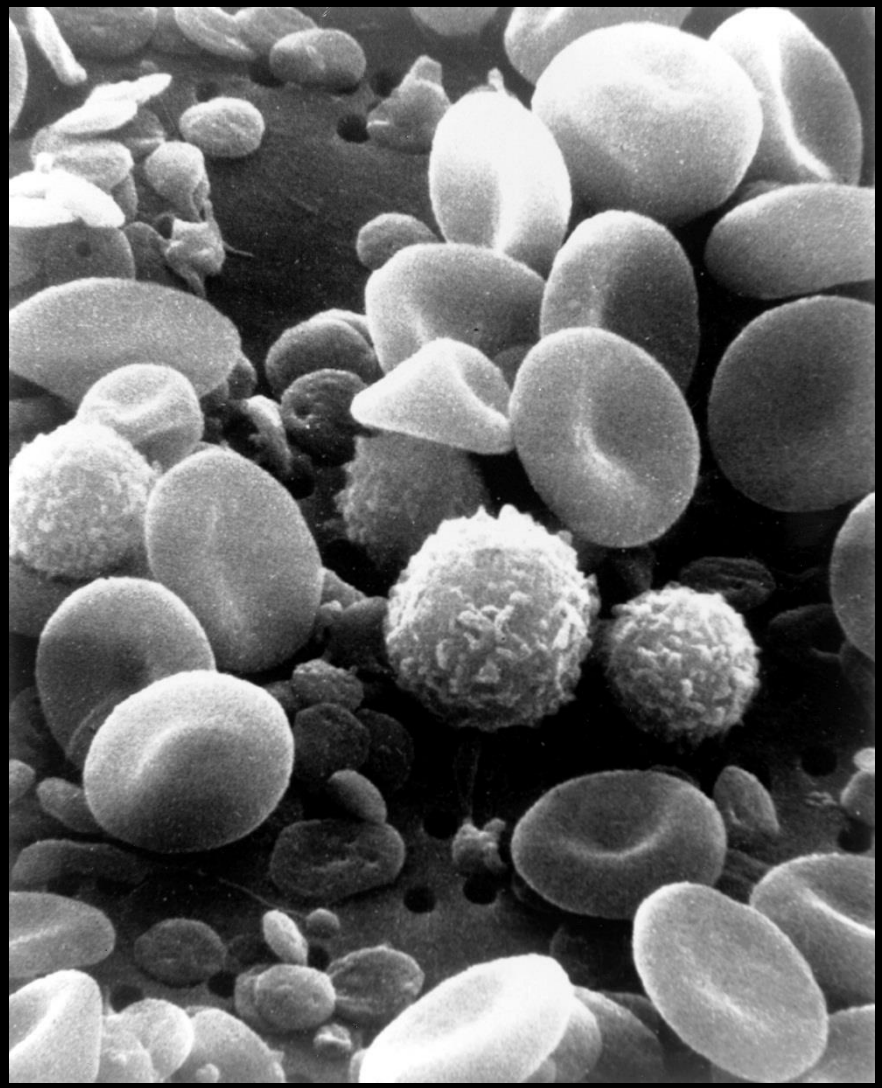
Kidney section – bowman capsule (confocal microscopy)

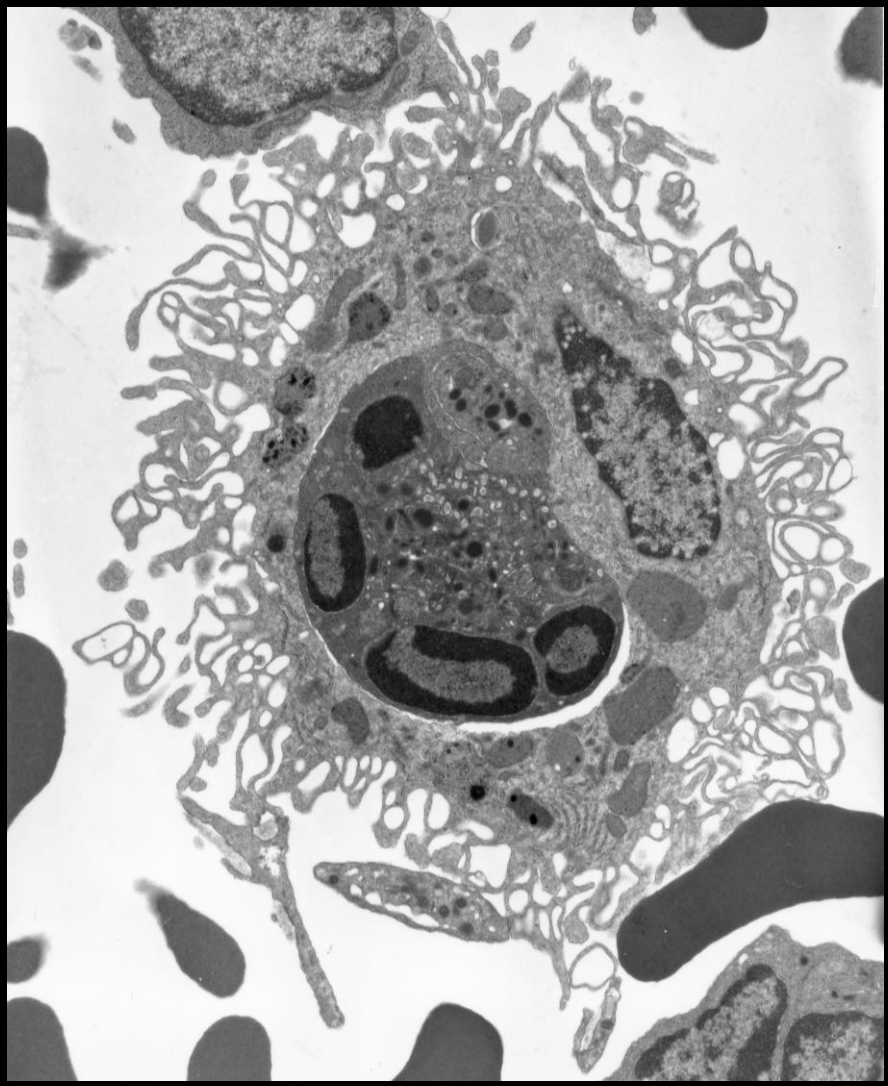


Mouse neural cells (green) and invasive DsRed human tumour cells

(Confocal microscopy)

Elektron mikroskopi

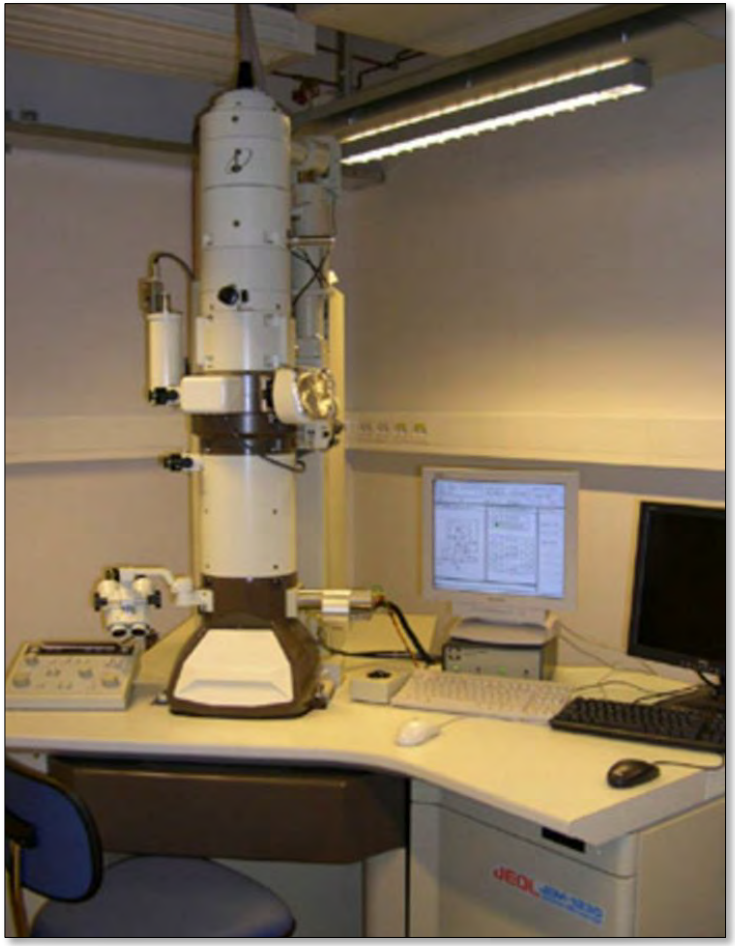




Elektron mikroskop ved MIC

Scanning EM

Transmission EM



Preparering av prøvene for transmisjons elektron mikroskopi

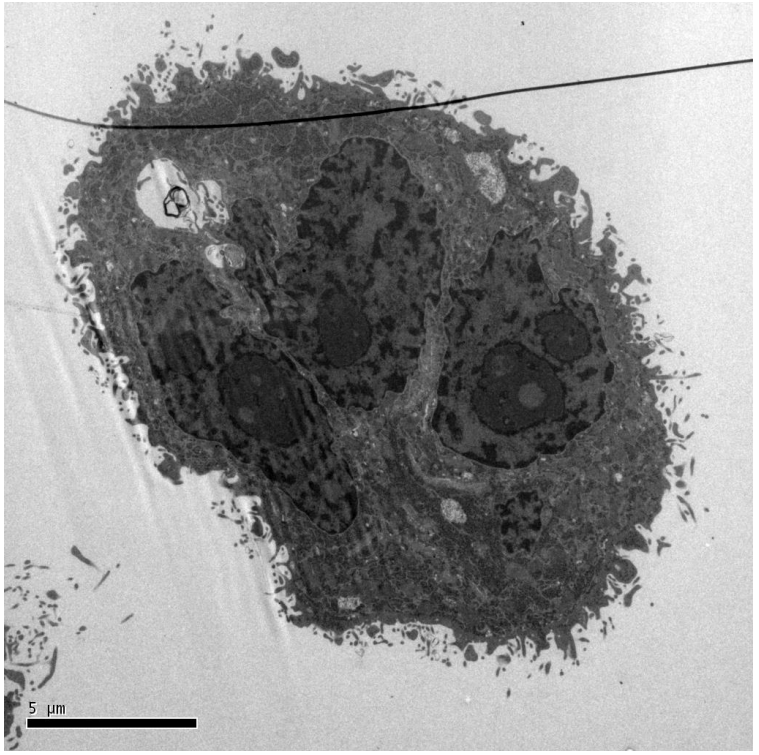
- **Fiksering: oppbevarer 3D struktur (postfiksering).**

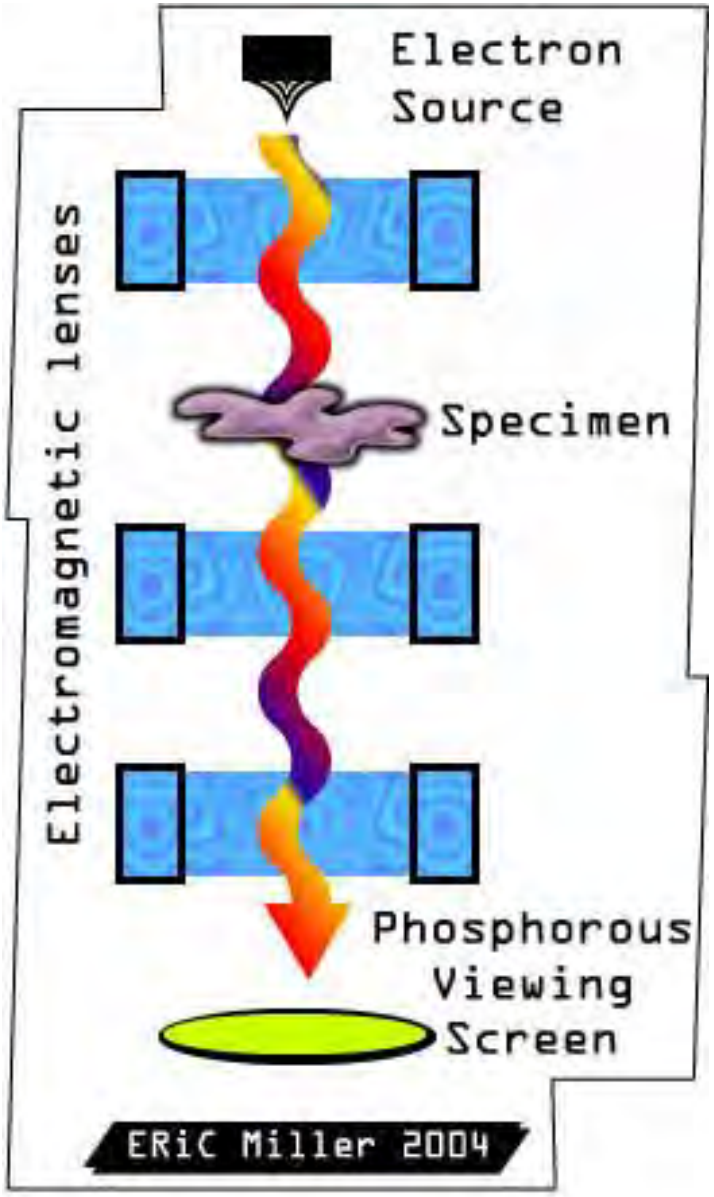
- **Dehydrering: fjerner alt vannet fra preparatet (vha alkohol).**

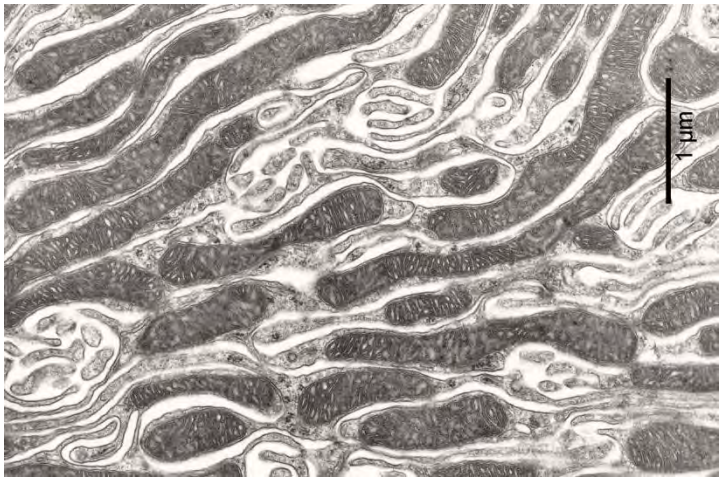
- **Innleiring i plast og polymerisering:**

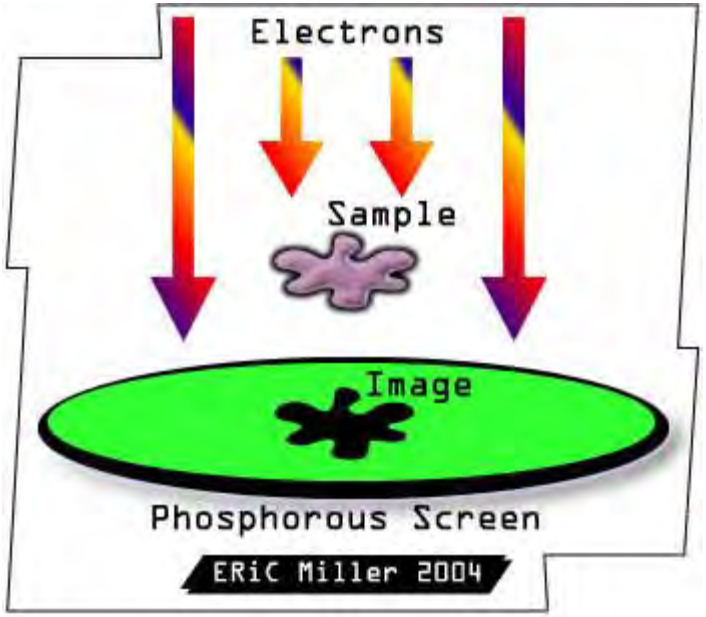
- **Snitting (45-60 nm) og montering på gridd:**

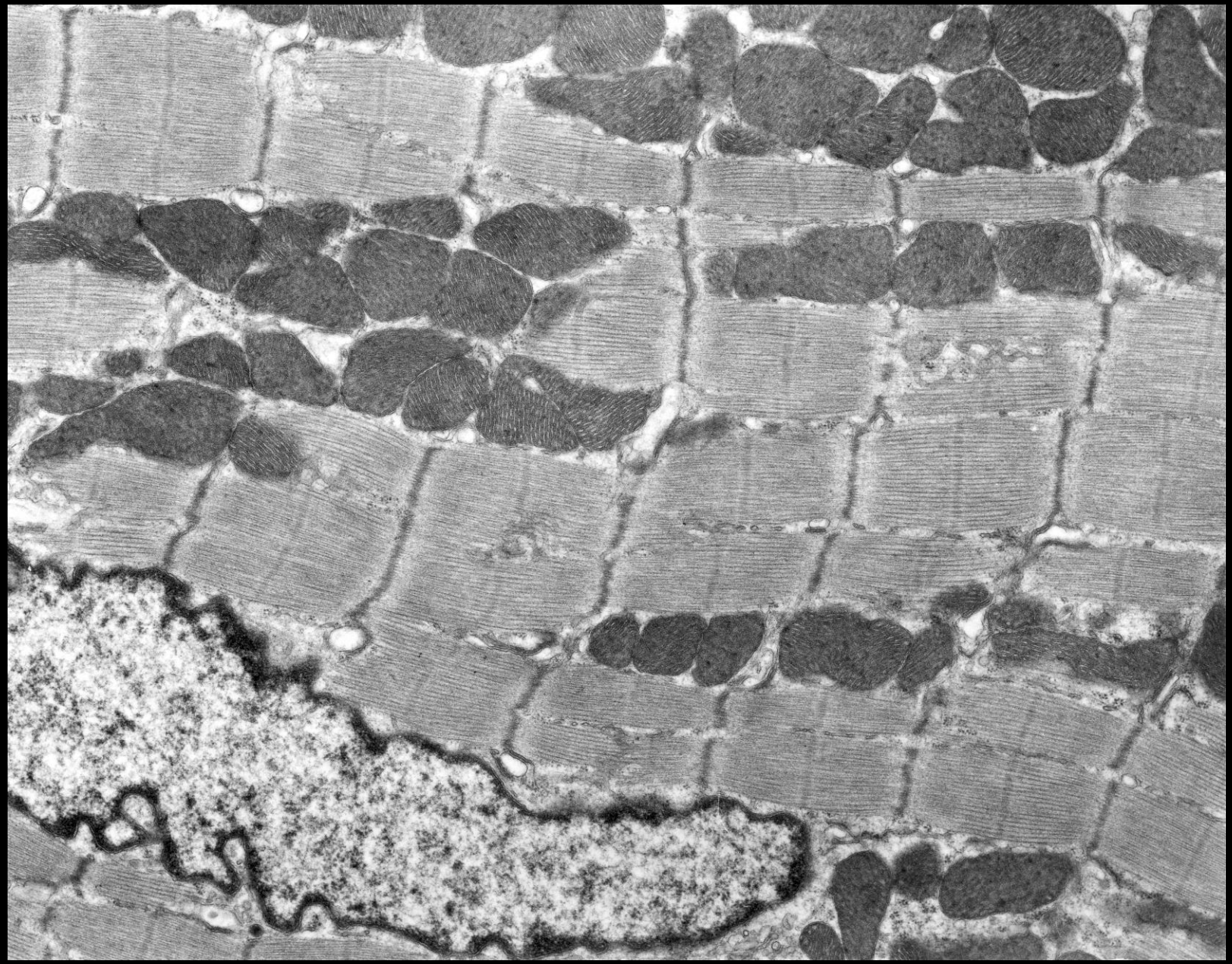
- **Farging: med uranylacetat og bly.**

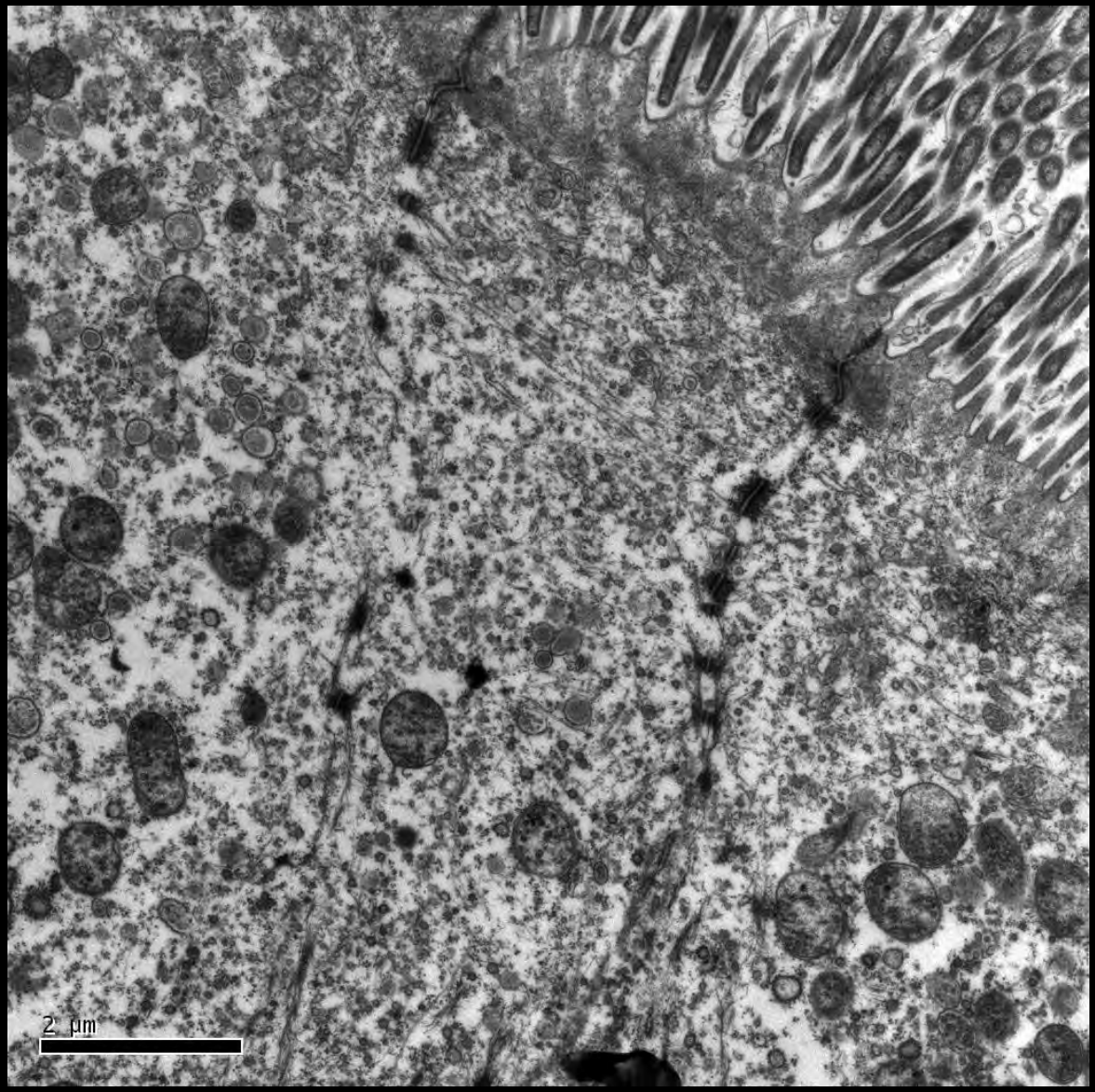



Electron Source
Electromagnetic lenses
Specimen
Phosphorous Viewing Screen
ERiC Miller 2004

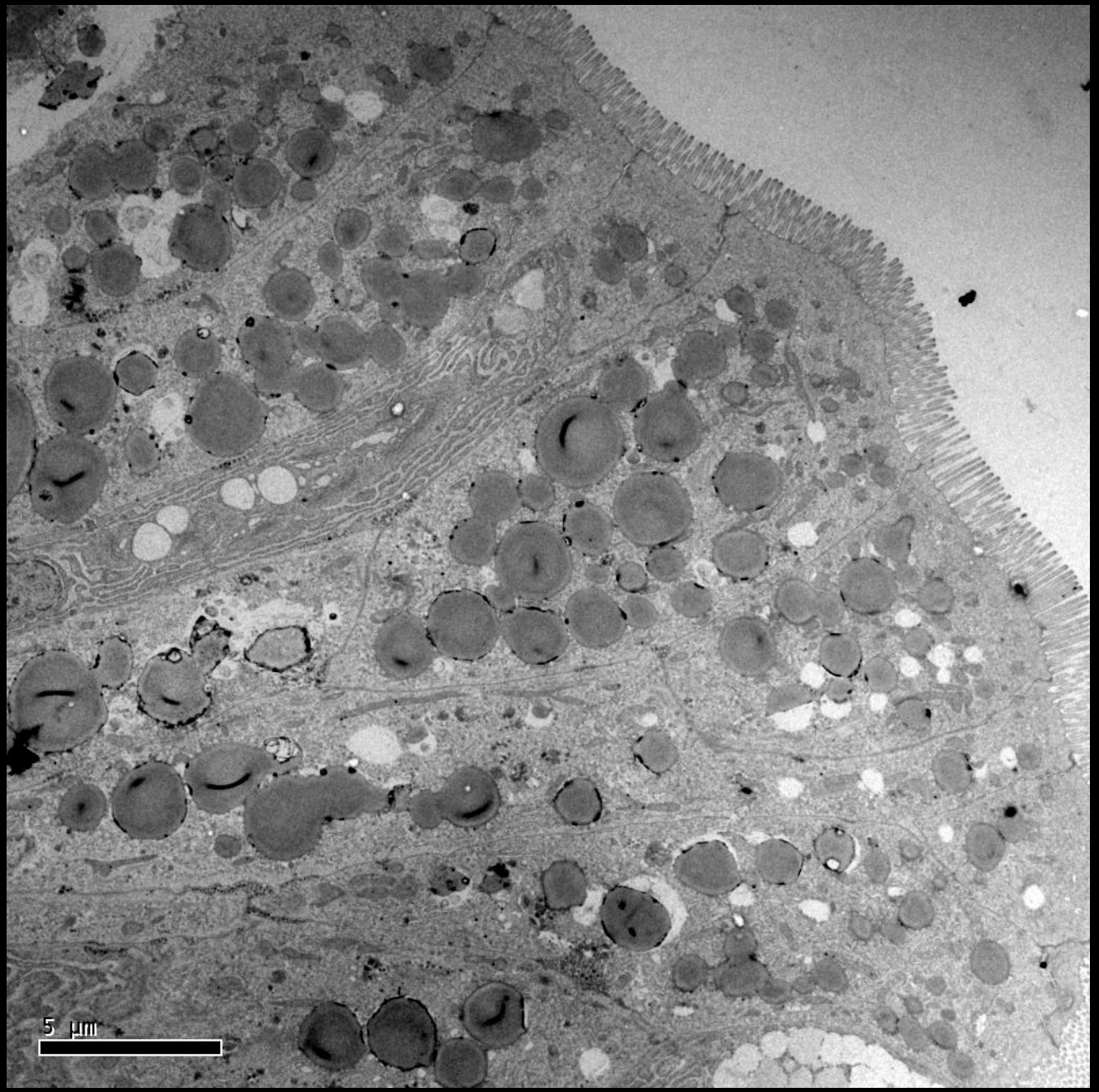

1 µm


Electrons
Sample
Image
Phosphorous Screen
ERiC Miller 2004



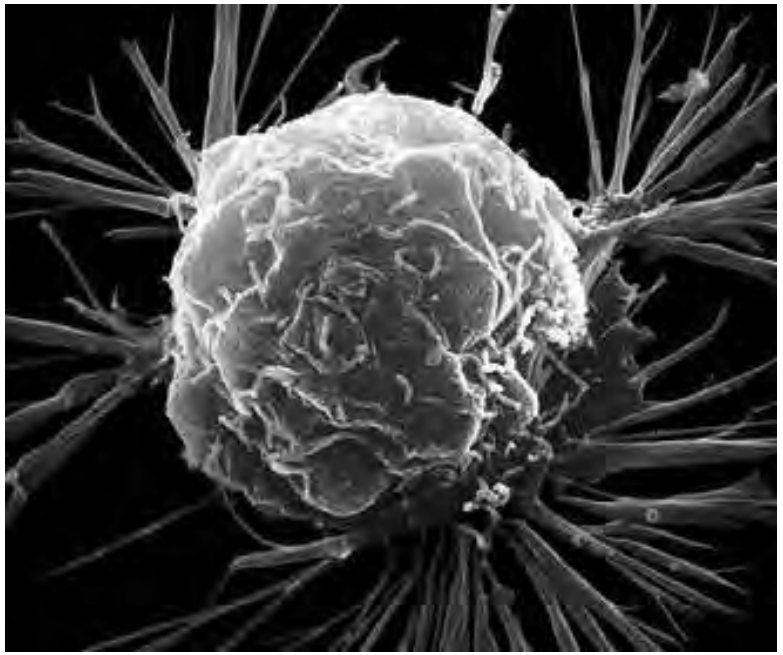


Desmosome and tight junctions in epithelial cells (transmission electron microscopy)


5 µm

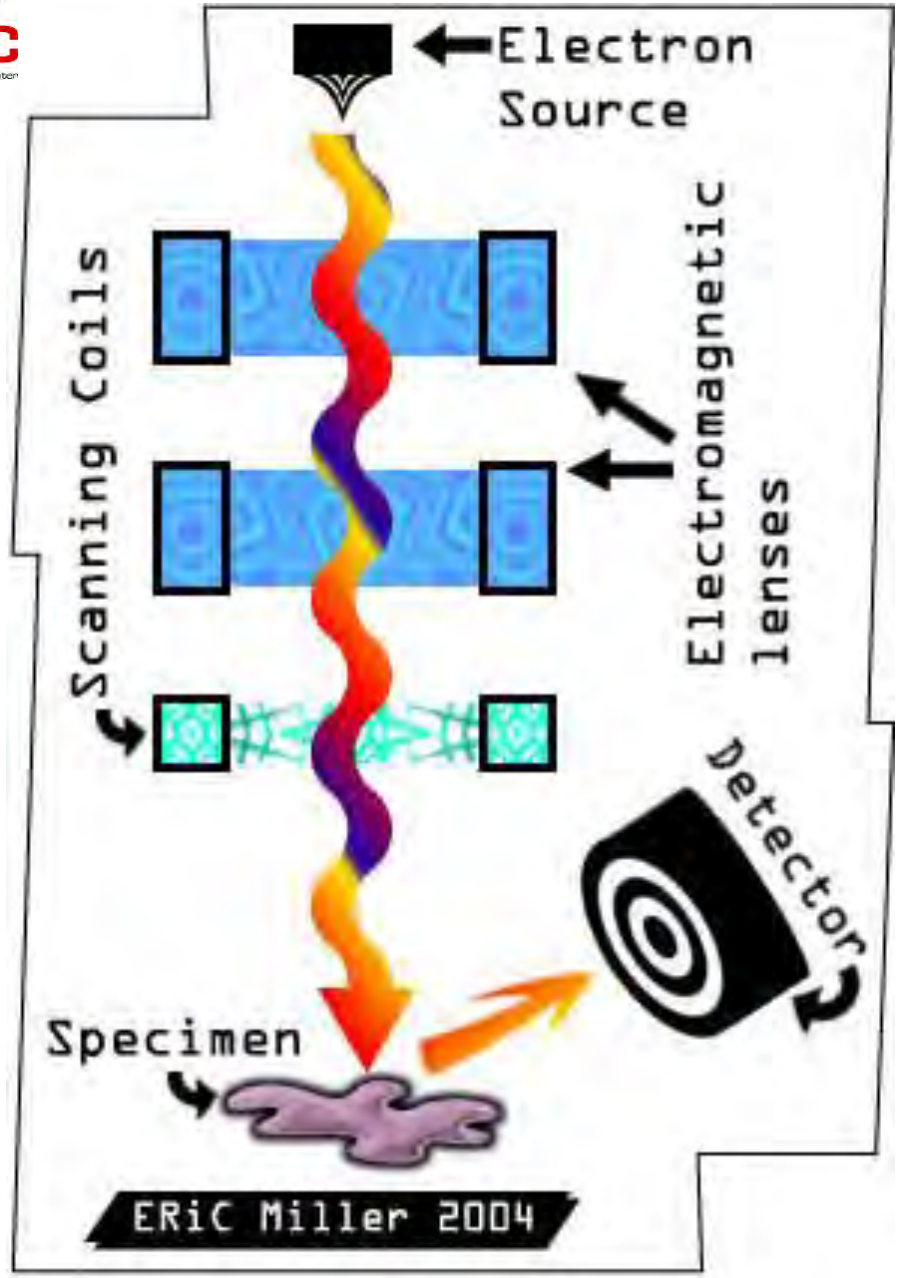
Preparering av prøvene for skanning elektron mikroskopi

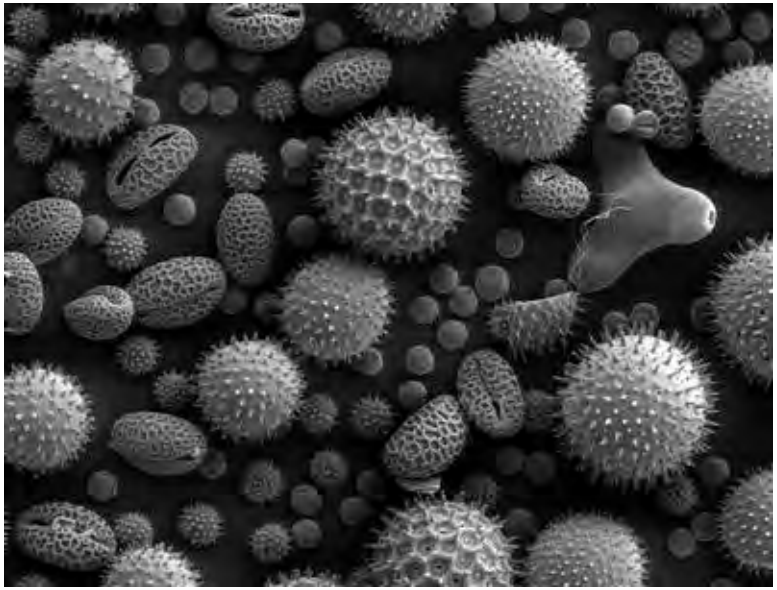
- **Fiksering: oppbevarer 3D struktur (postfiksering).**
- **Dehydrering: fjerner alt vannet fra preparatet (vha alkohol).**
- **Kritisk punkt tørking: fjerner alle vannrester vha vakum.**
- **"Coating": overflaten på preparatet blir dekket av et tynt lag metall (palladium/gull).**

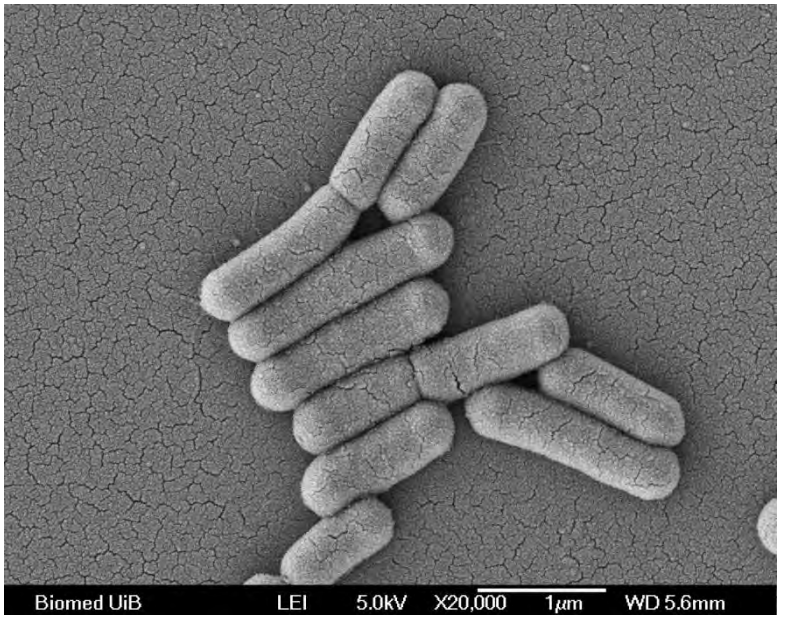


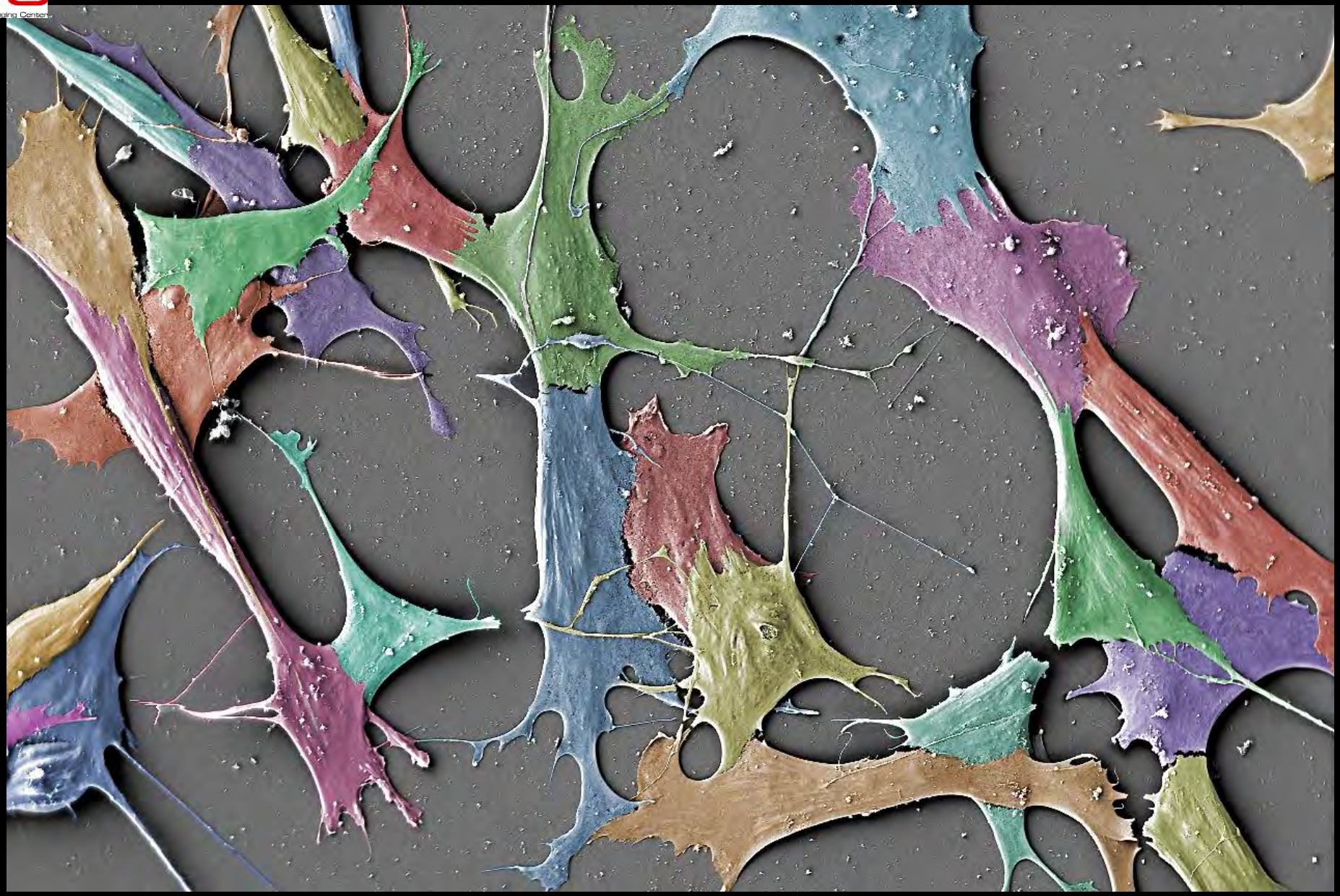
Prøver slik som tenner, bein, stein, organismer med hardt ytre skjelett trenger ikke fiksering.

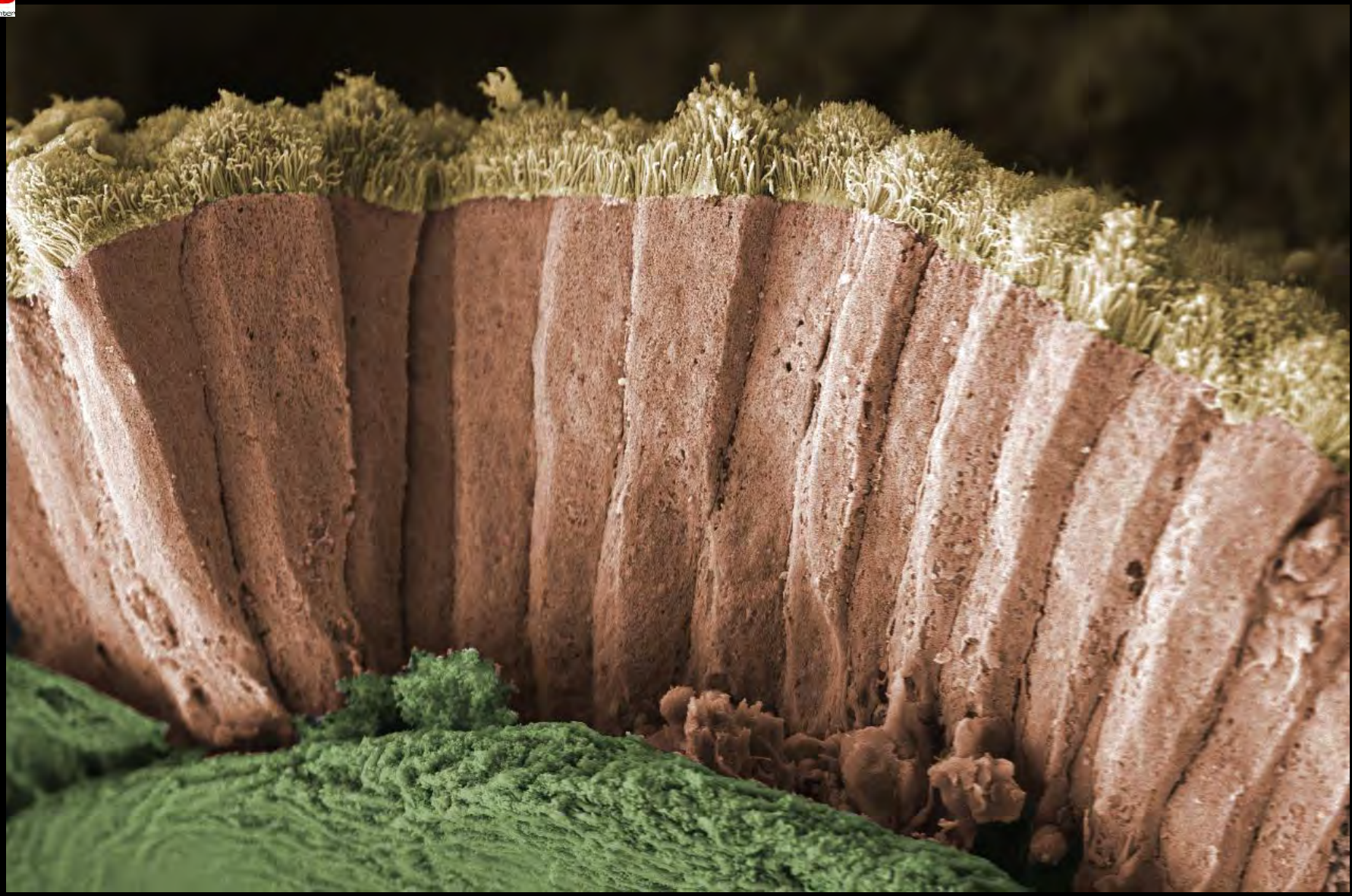
MIC
Molecular Imaging Center

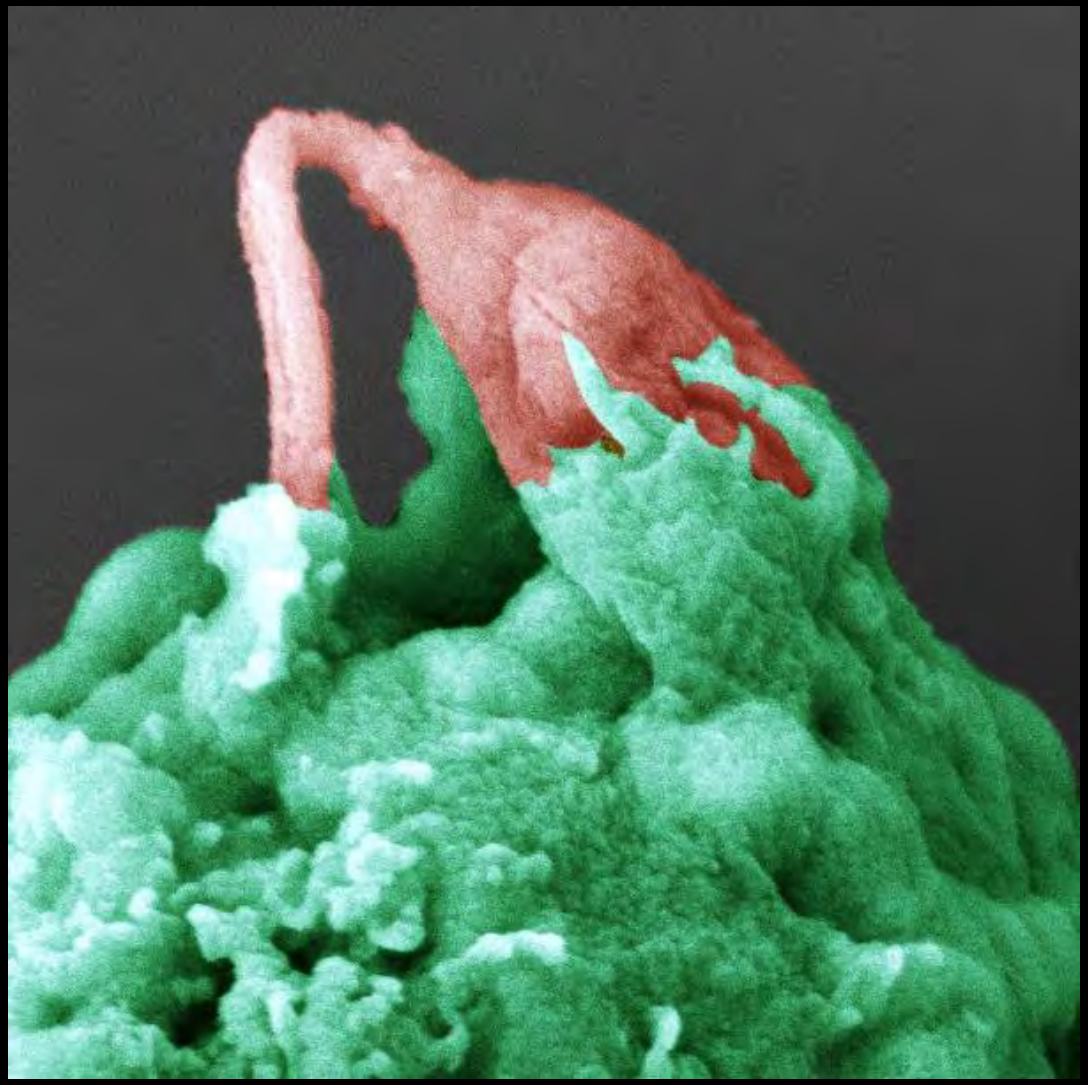








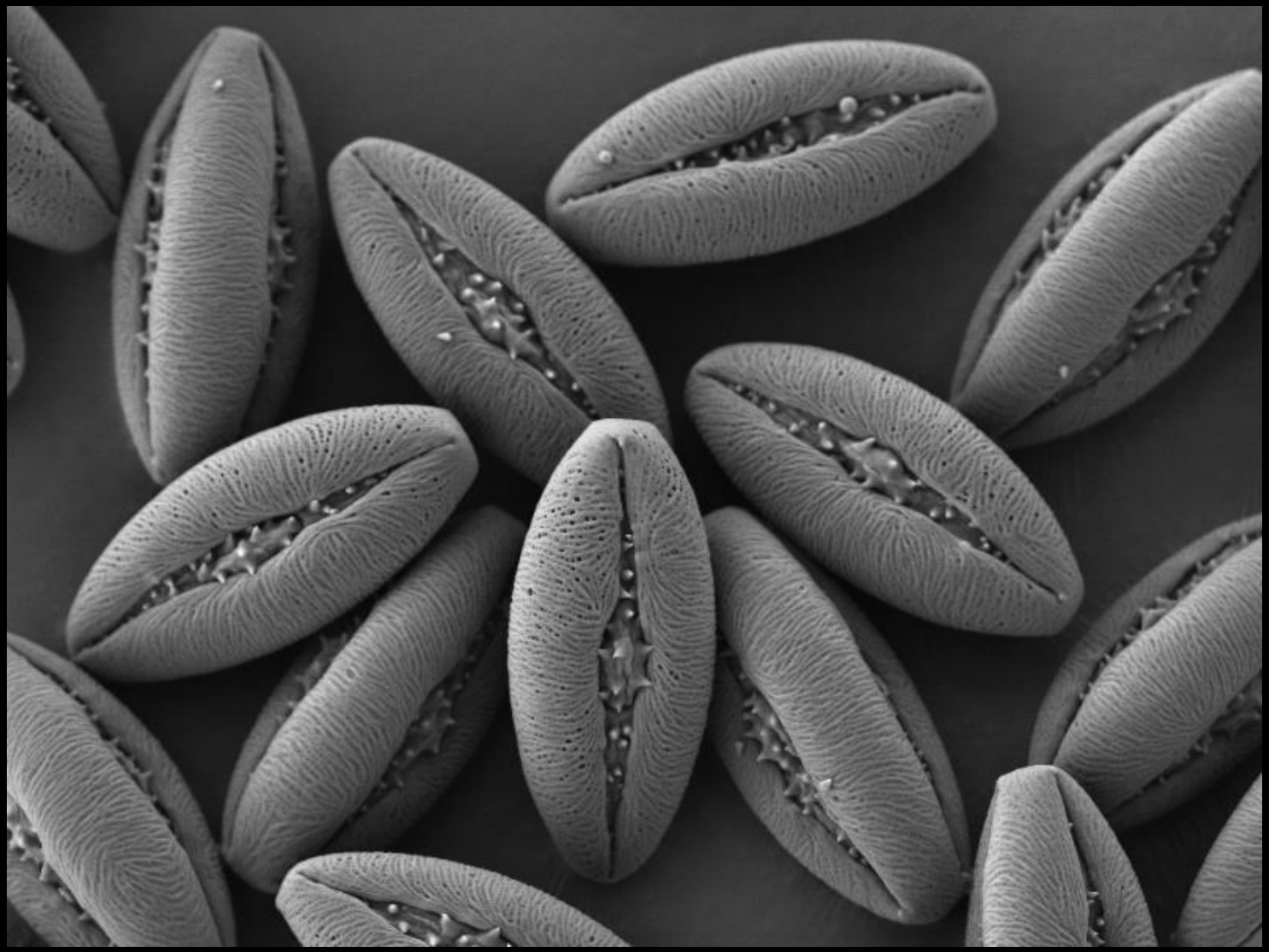




Leishmania parasite being phagocytosed by white blood cells

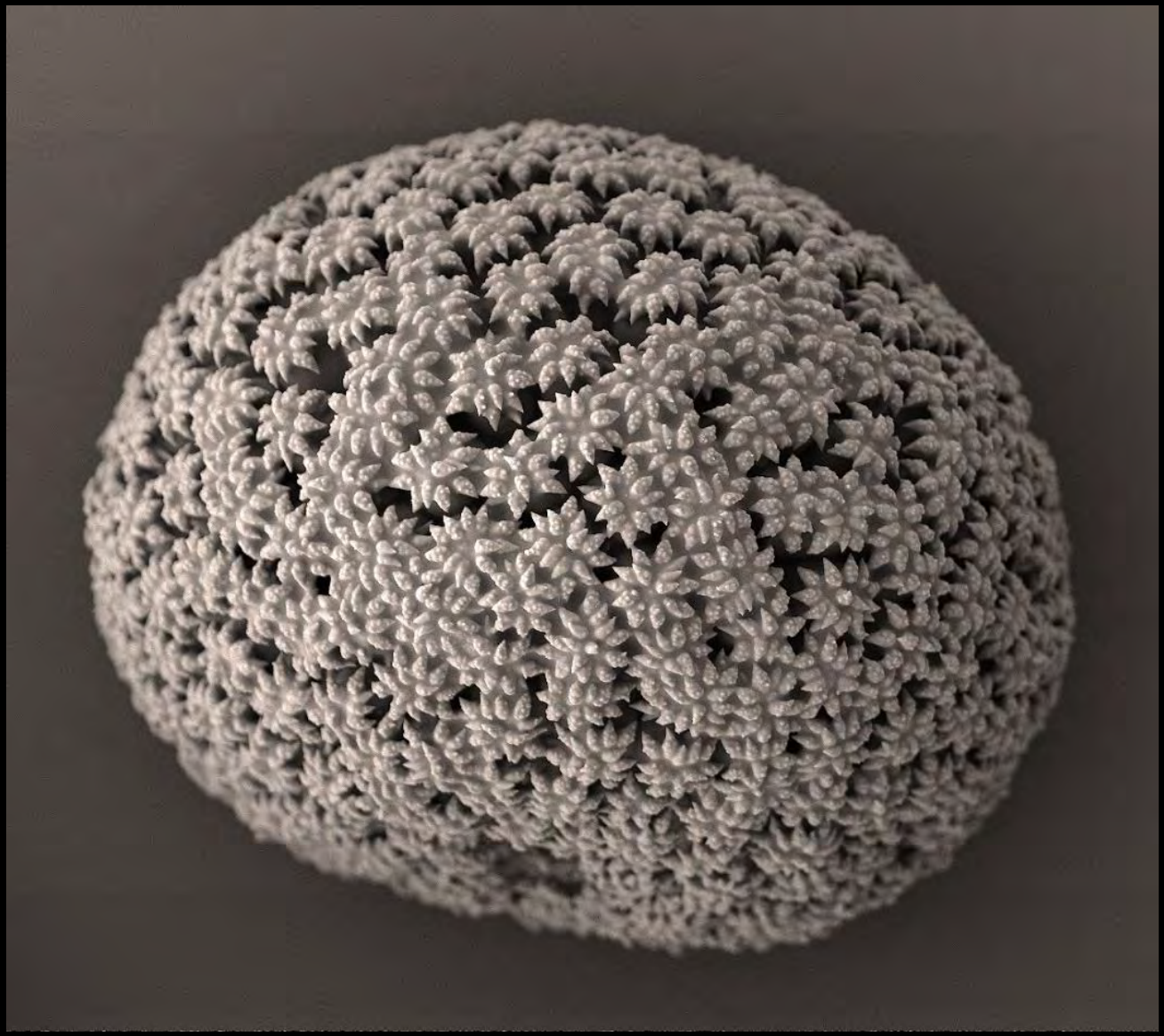
(scanning electron microscopy)

Courtesy of K Rebbestad



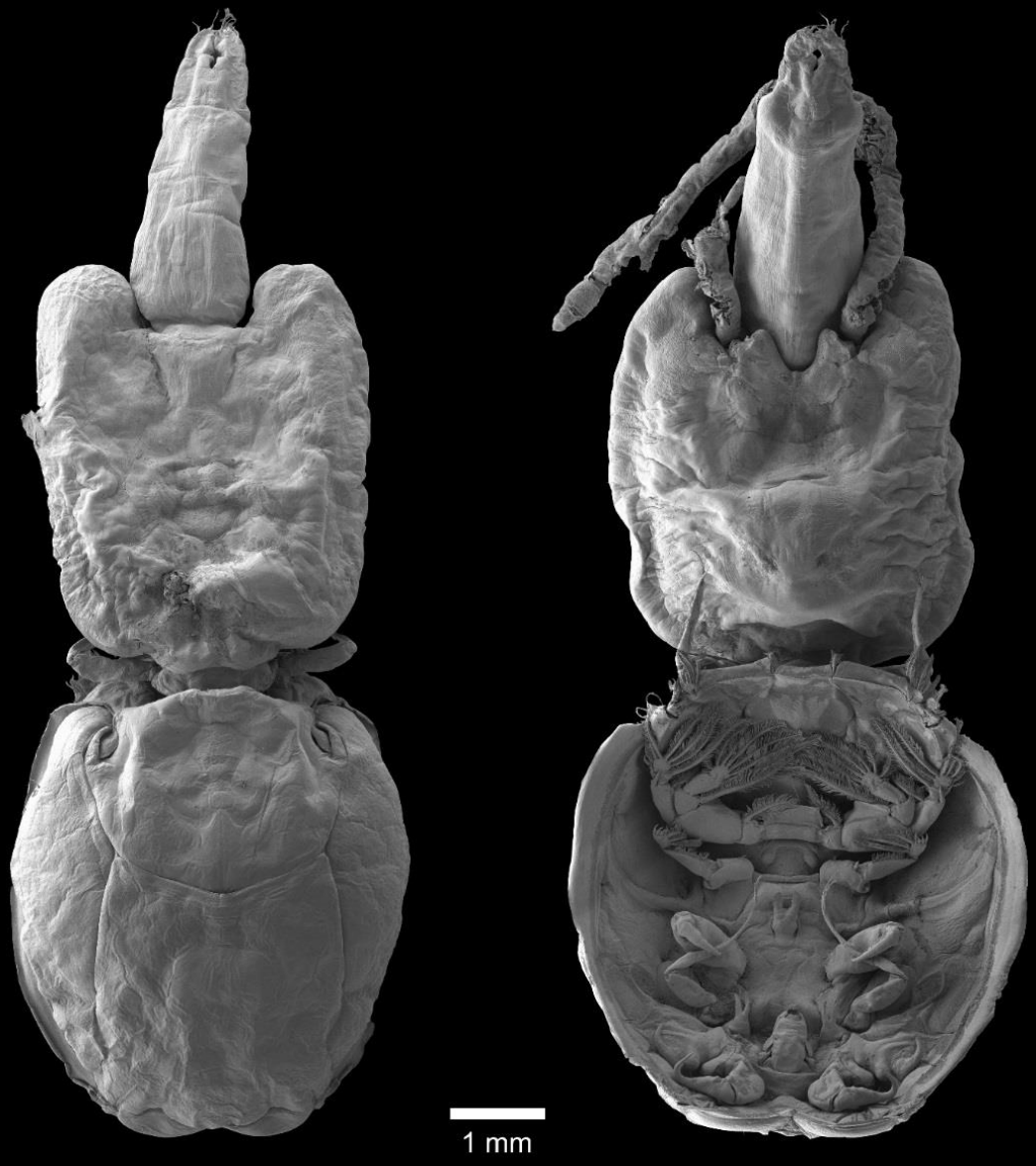
Pollen from Chestnut (scanning electron microscopy)

Courtesy of Caroline Schmid

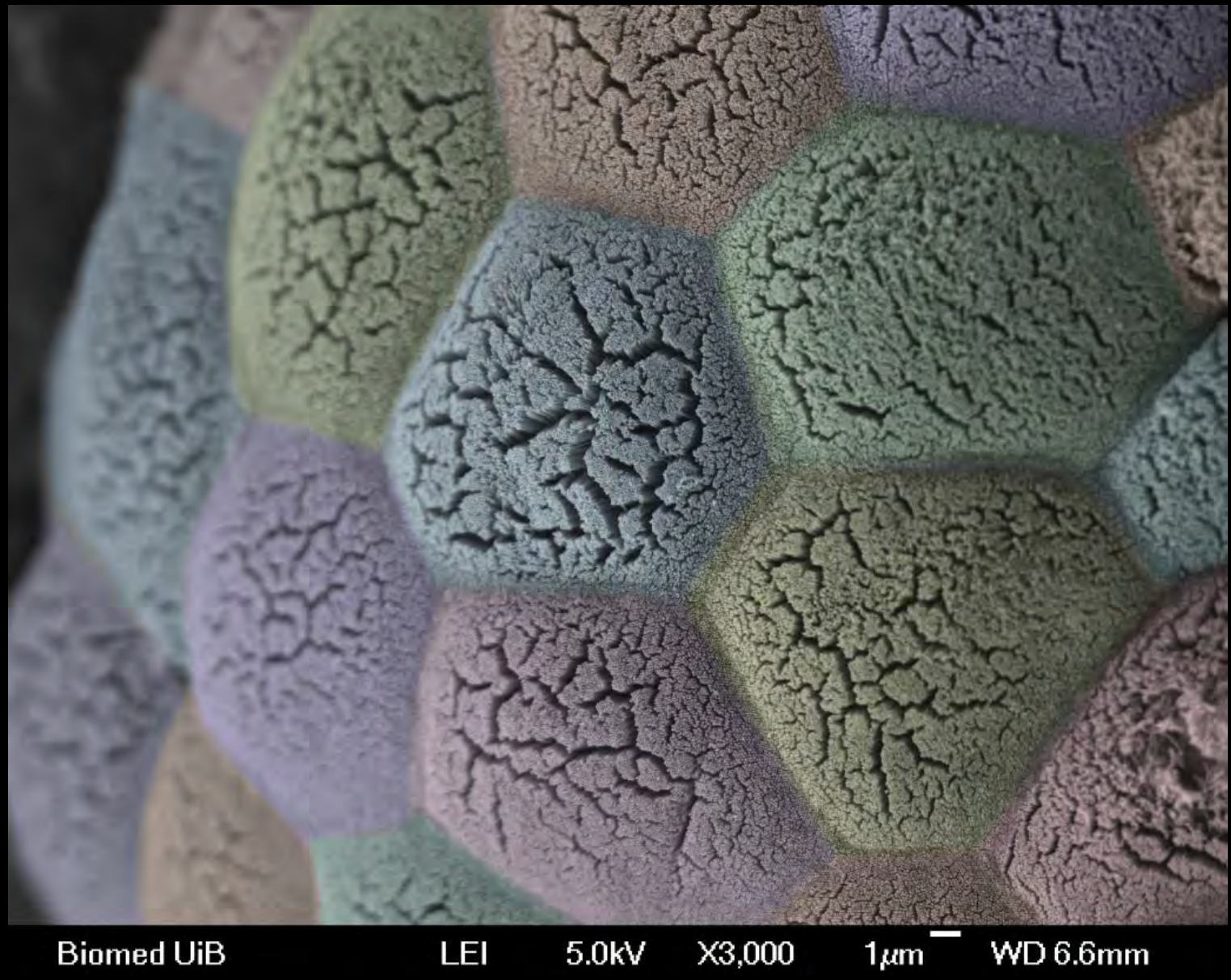


Coccolithosphore: a unicellular, eukaryotic phytoplankton algae

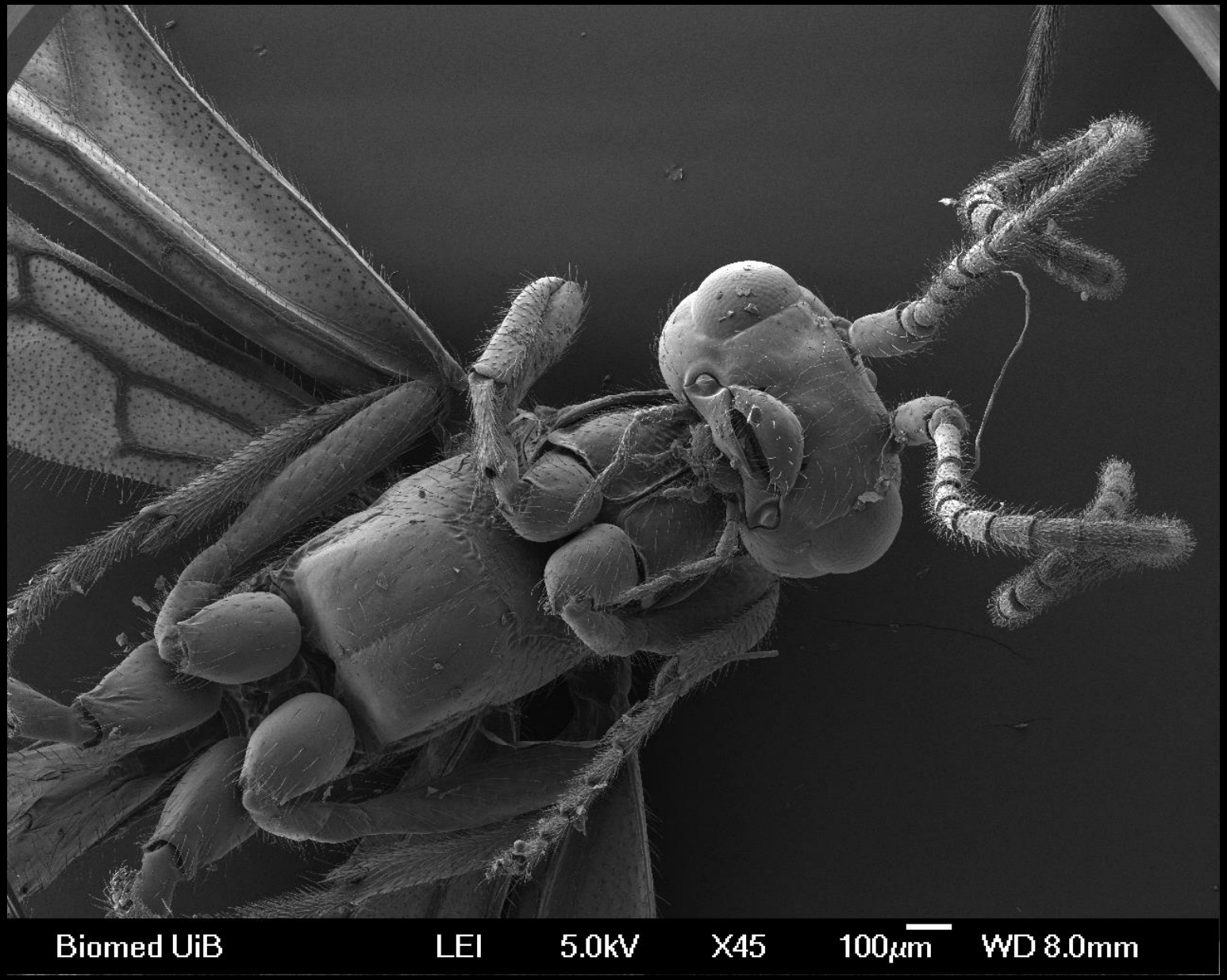
(scanning electron microscopy)

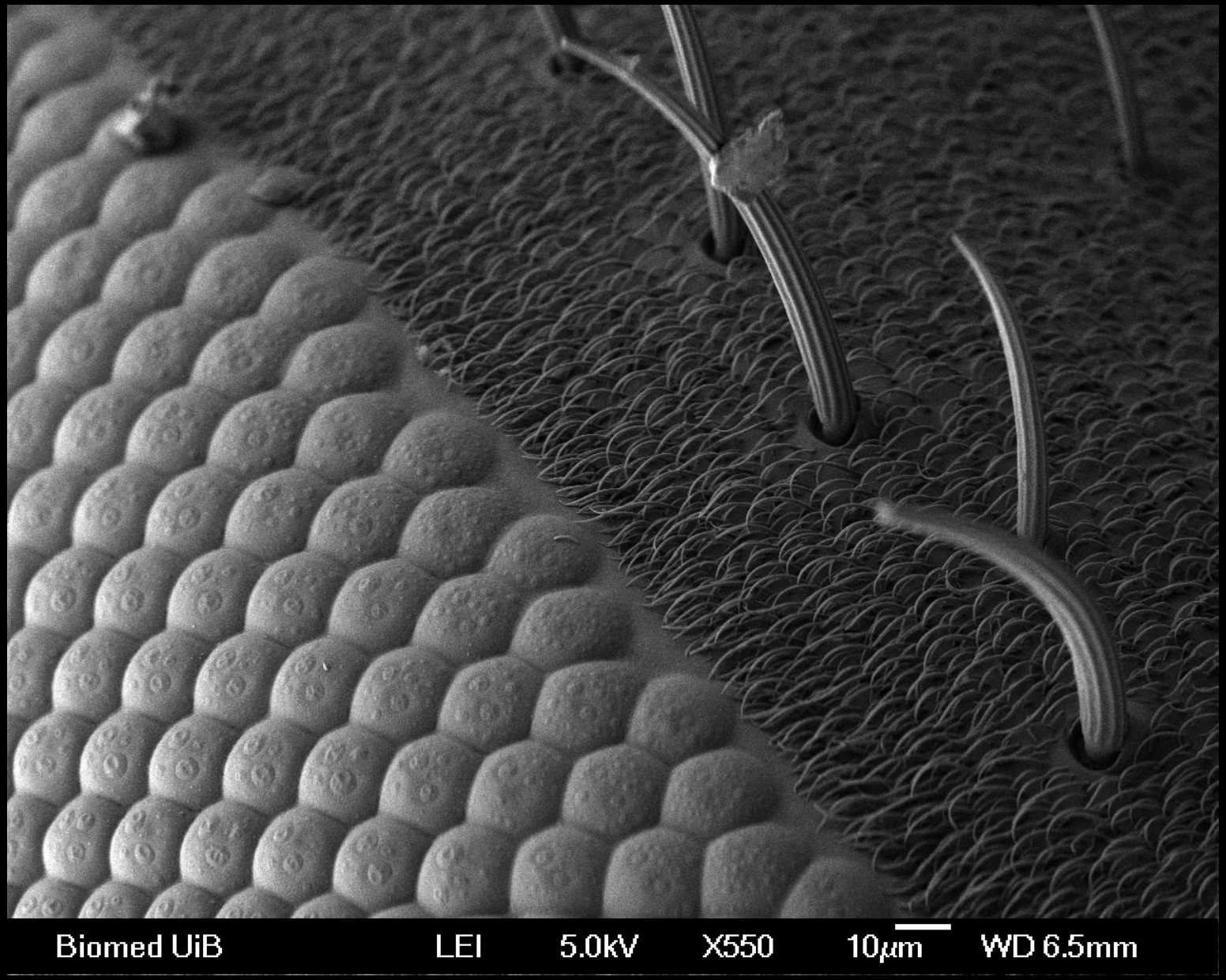


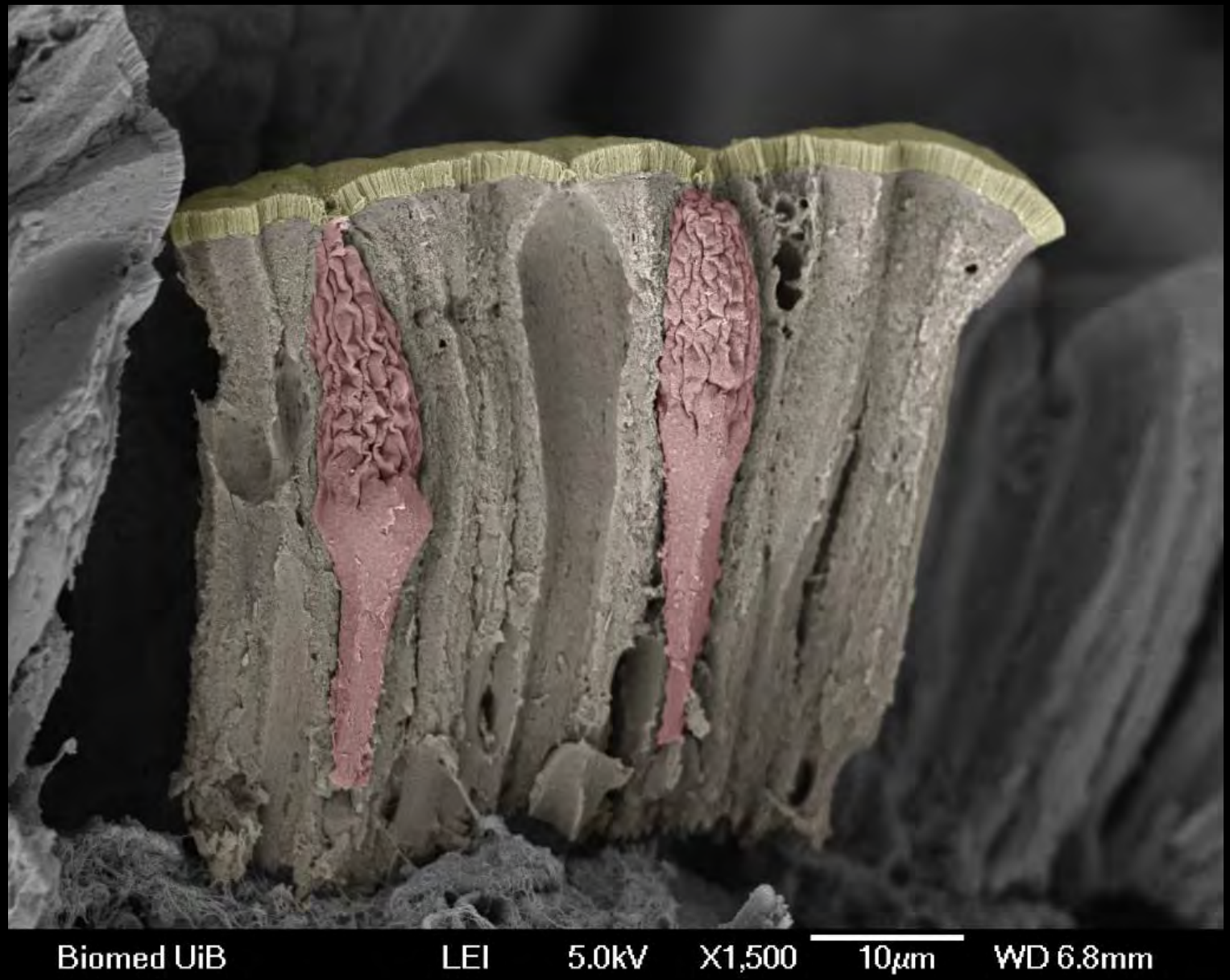
Salmon lice (scanning electron microscopy)



Ciliated intestinge cells (electron microscopy)

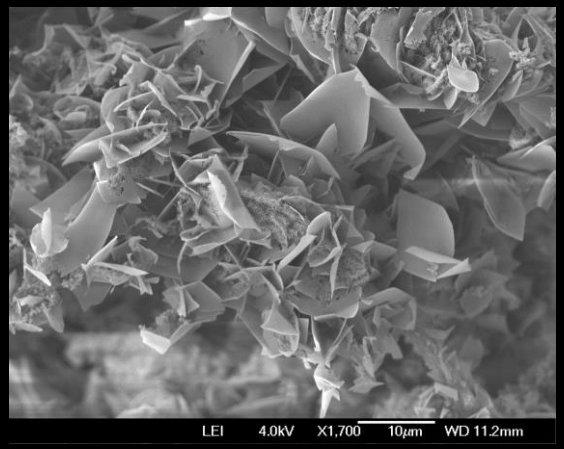

Biomed UiB
LEI
5.0kV
X45
100µm
WD 8.0mm


Biomed UiB
LEI
5.0kV
X550
10µm
WD 6.5mm



Intestine and goblet cells (pink) from a fish intestinge (scanning electron microscopy)

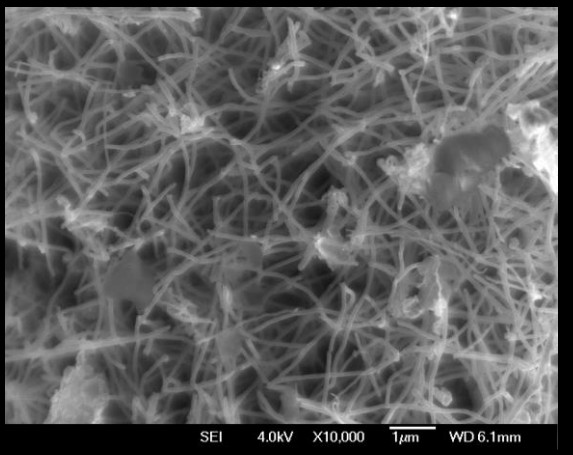
Images of non-biomedical material



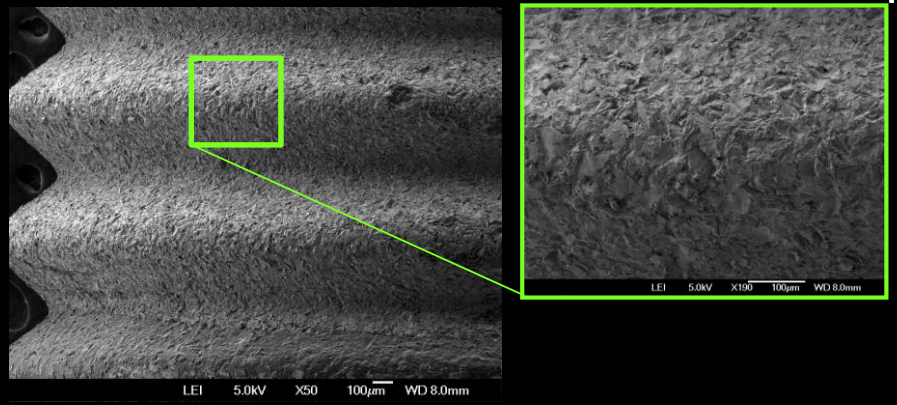
Salt crystals

Scanning electron micrographs (SEM)

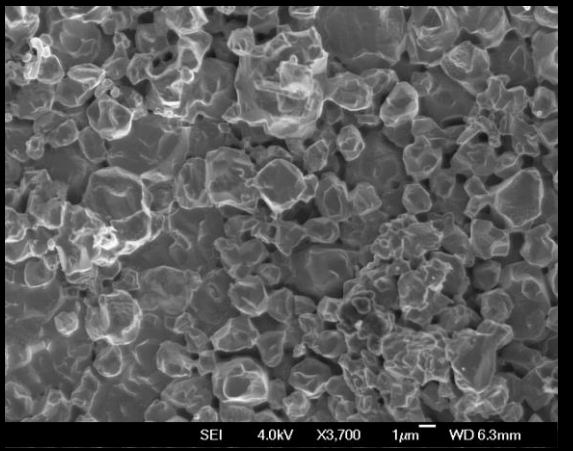
Dehydrated & metal coated samples.



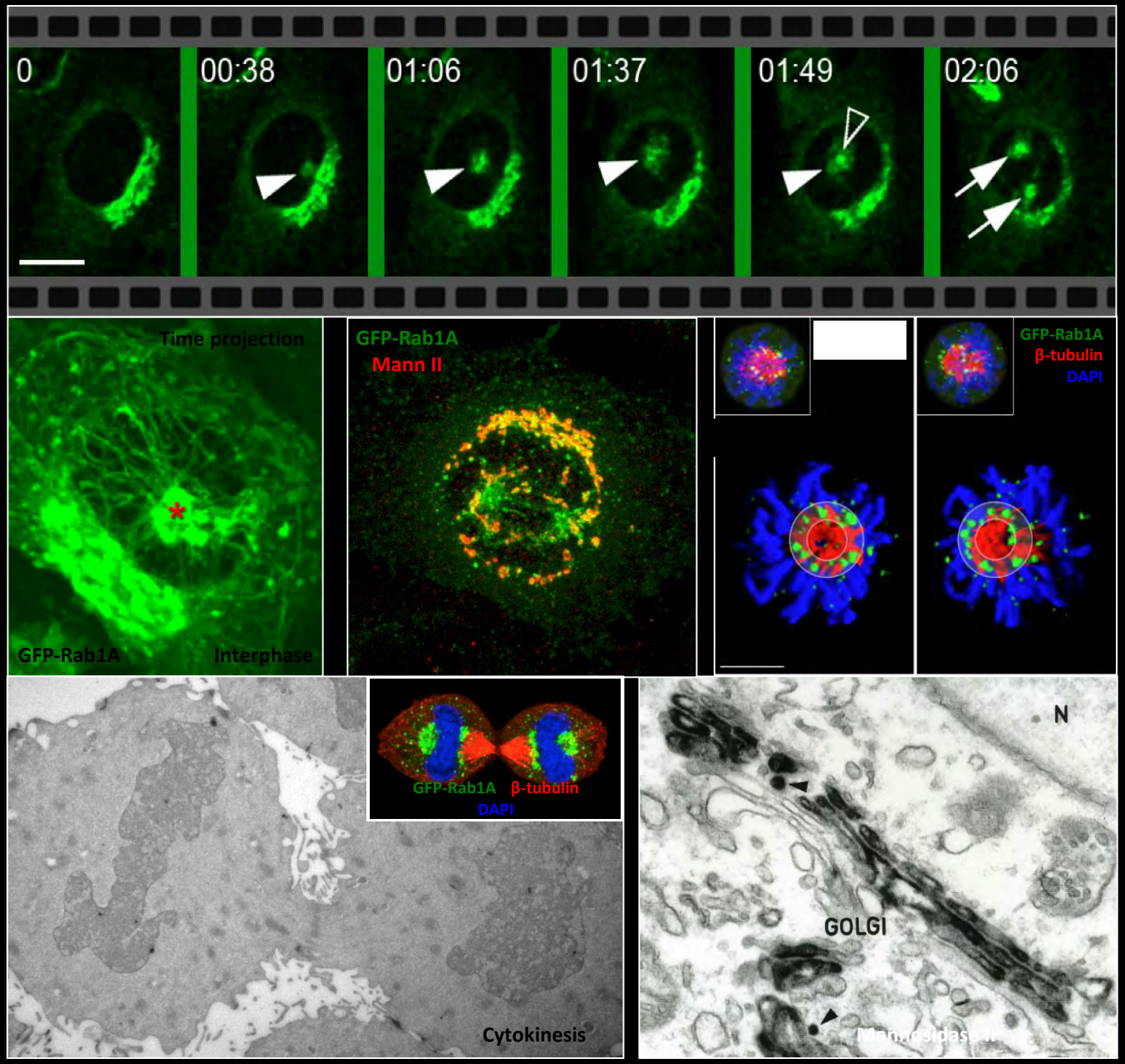
Carbon structures



Small surgical screw

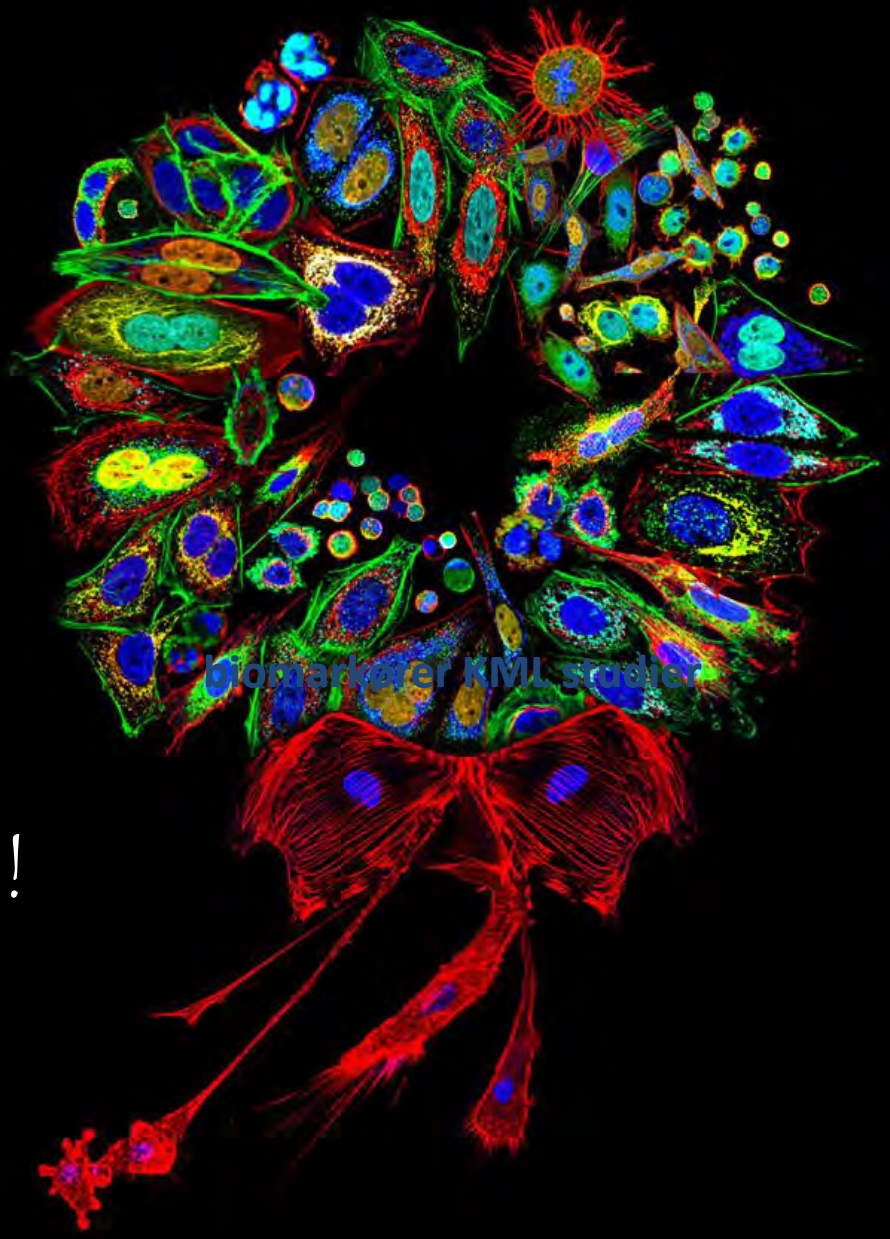


A story validated by different techniques



- Live cell imaging – GFP-marked protein
- Fixed samples – green and red fluorescent antibodies and blue fluorescent DNA probe
- Transmission electron microscopy – immunogold stain

Courtesy of Jaakko Saraste



Takk for meg!

Spørsmål?

Mammalian cell collage, Donna Stolz, 19th place in Nikon Small World competition 2011