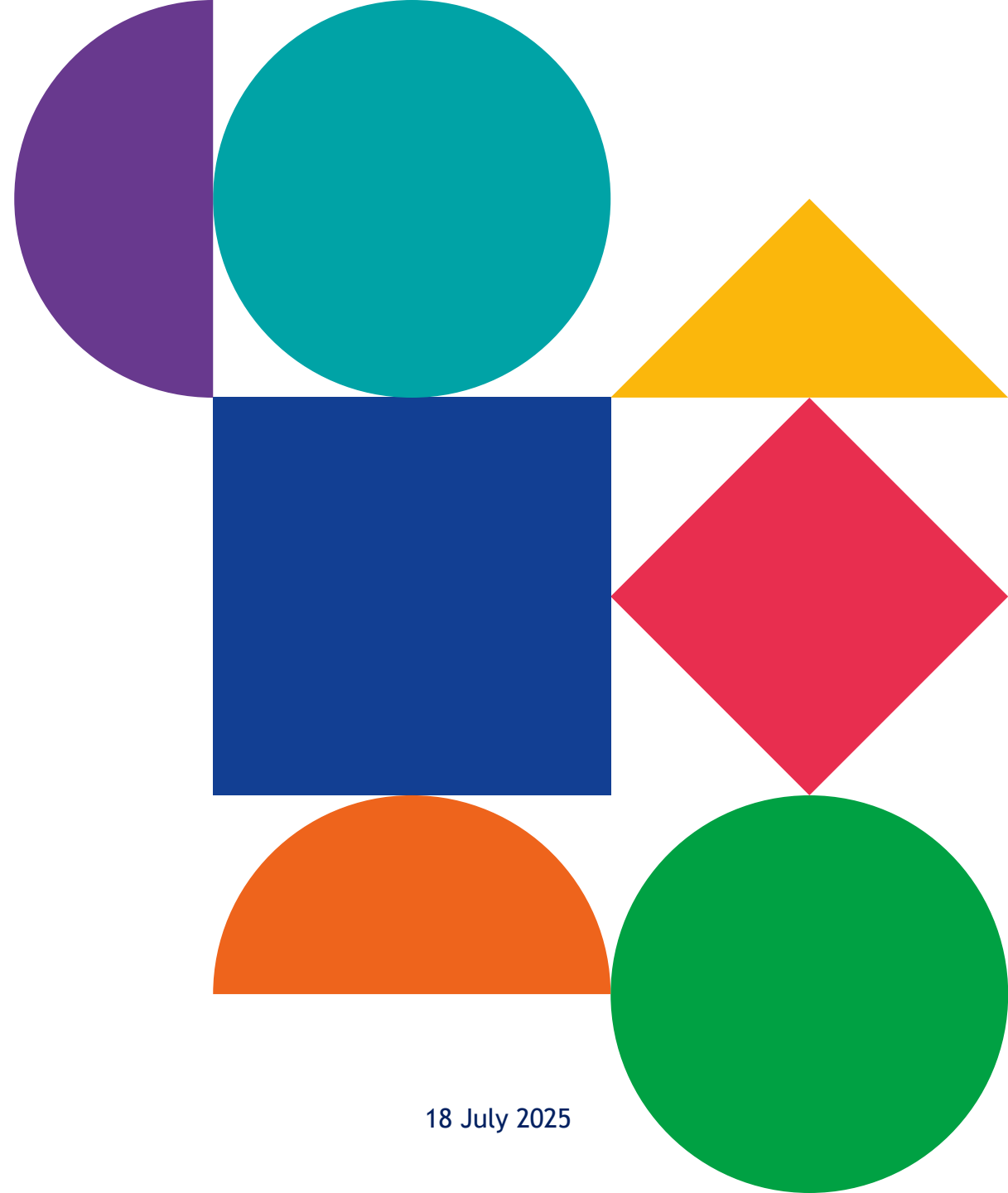


Artifacts: Friend or Foe?

Erin Tranfield, PhD

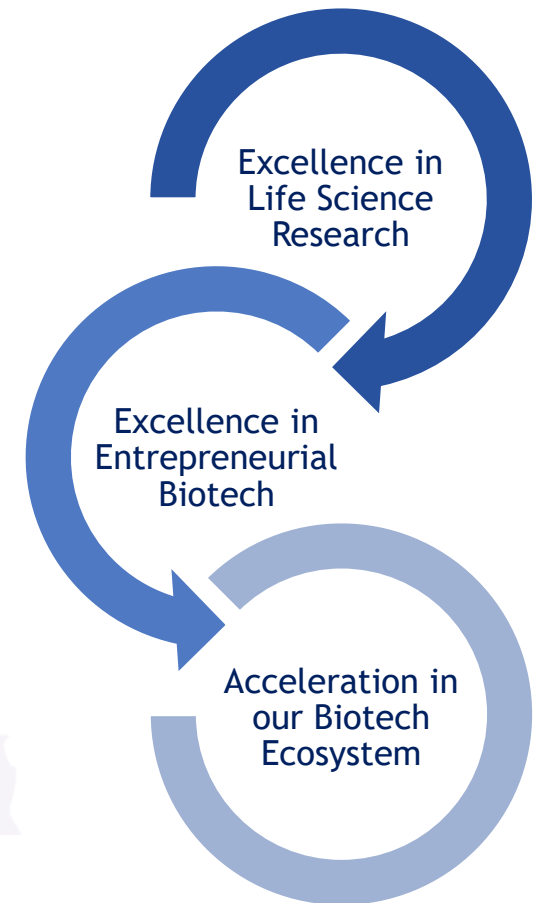
Head of BioImaging at VIB Ghent



18 July 2025

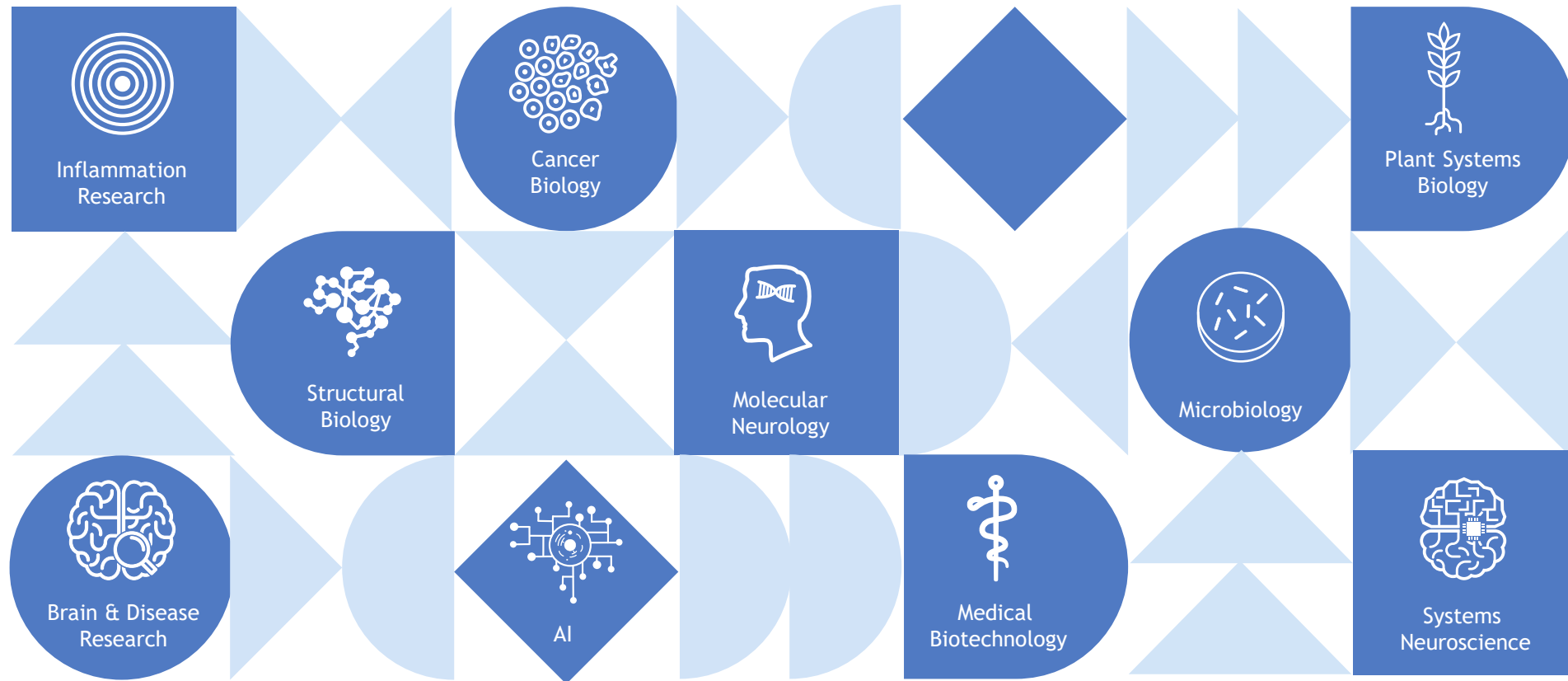
VIB, Flemish Institute for Biotechnology

A leading life sciences research institute in Belgium with an entrepreneurial spirit.



VIB Pioneering Life Sciences Research

10 focused research areas



VIB Technologies

Foundation for cutting-edge
life sciences research



Biolmaging Core in Ghent

Light Microscopy



Eef
Parthoens



Erin Tranfield

Electron Microscopy



Anneke
Kremer

Data Analysis



Jari
De Vuyst



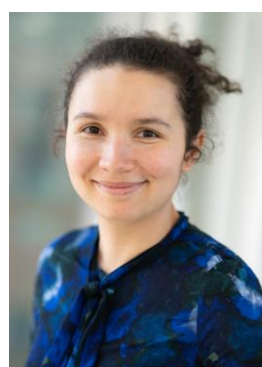
Jérémy
Verbeke



Amanda
Gonçalves



Benjamin
Pavie



Tatiana
Woller



Jan
Felix
(starting 09/25)



Michiel
De Bruyne



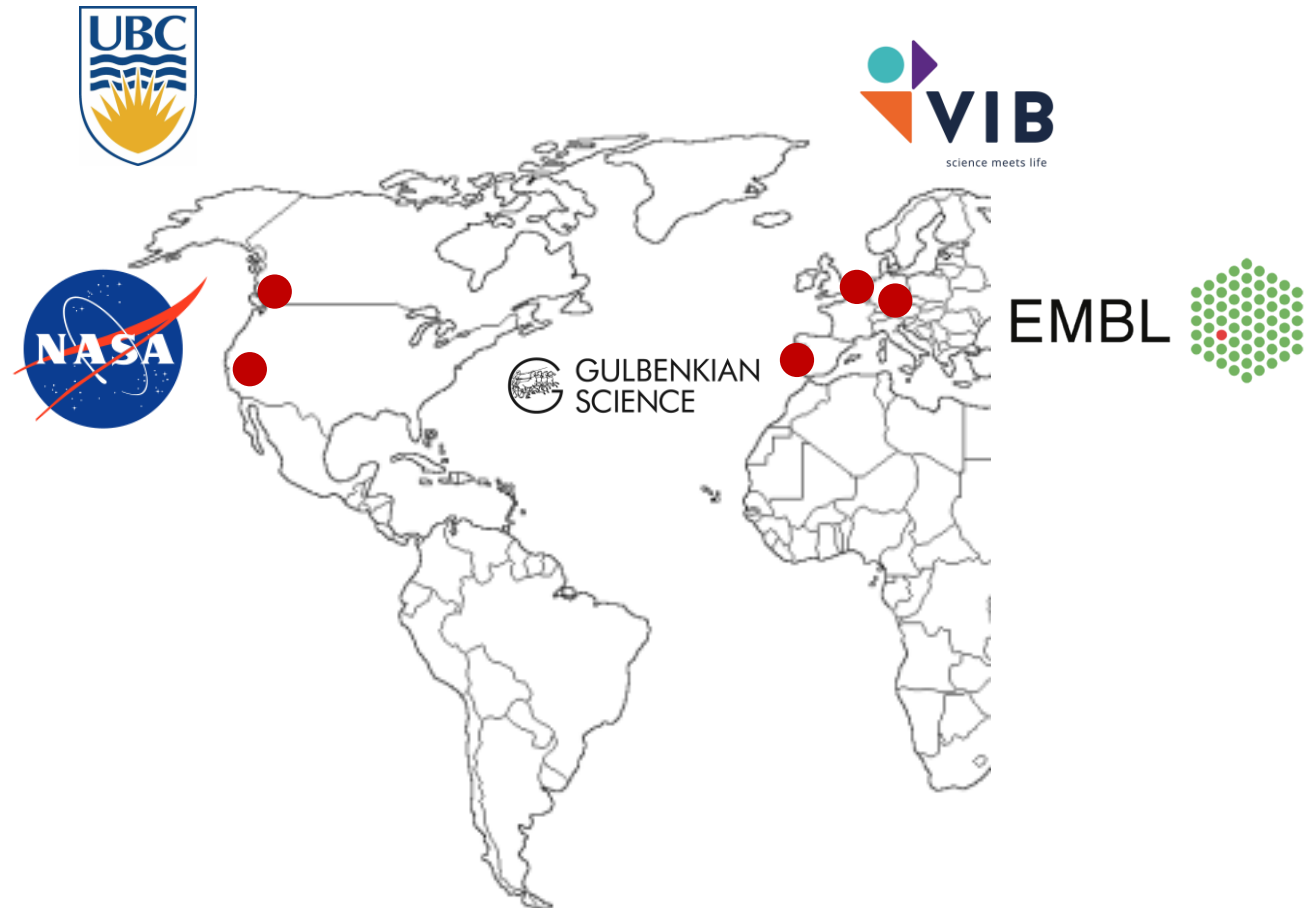
Femke
Baeke



Riet
De Rycke
(until 03/26)

Erin Tranfield

- 2002 - 2007: PhD in Pathology at University of British Columbia (Vancouver, Canada)
 - How air pollution alters the ultrastructure of the cardiovascular system
- 2007 - 2009: Post Doc at NASA Ames Research Center (San Francisco, USA)
 - How lunar dust impacts the lungs, eyes and skin of astronauts
- 2009 - 2013: Post Doc at European Molecular Biology Laboratory (Heidelberg, Germany)
 - Electron tomography on the Meiotic spindle
- 2013 - Feb 2024: Head of Electron Microscopy Facility, Instituto Gulbenkian de Ciência (Lisbon, Portugal)
 - 2017 Horse Riding Accident - entered the world of patients
- Mar 2024 - current: Head of Biolmaging Core - Ghent



Electron Microscopy in Pathology vs Fundamental Research

Pathology - patient must come first

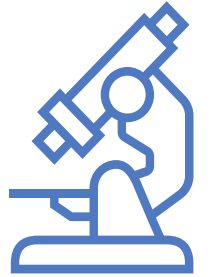
- Samples from human patient
 - Samples obtained by biopsy or autopsy
- Goal is diagnosis of a known disease
 - Samples rarely have controls, replicates
 - Sample preservation is complex due to patient needs, and less critical



Desired outcome is to diagnosis what is affecting the patient, not expanding the medical understanding of a disease. Samples may have artifacts.

Fundamental Research - sample fidelity first

- Samples from a research model
 - Cells, organoids, animal models
 - Human tissue is rare
- Goal is the study and characterization of a previously uncharacterized disease
 - Diseased samples are paired with controls
 - Replicates are done
 - Sample preservation is essential to avoid inaccurate characterizations.

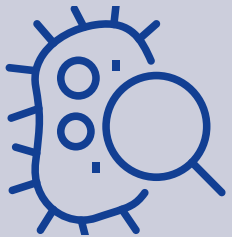


Desired outcome is to expand medical understanding of a disease. Samples should have as few artifacts as possible.

Definition of an artifact



An object made by a human being, typically one of cultural or historical interest.



Something observed in a scientific investigation or experiment that is not naturally present but occurs as a result of the preparation or investigation procedure.

In microscopy what can lead to artifacts?

Physical handling:

- Cutting
- Drying
- Mounting

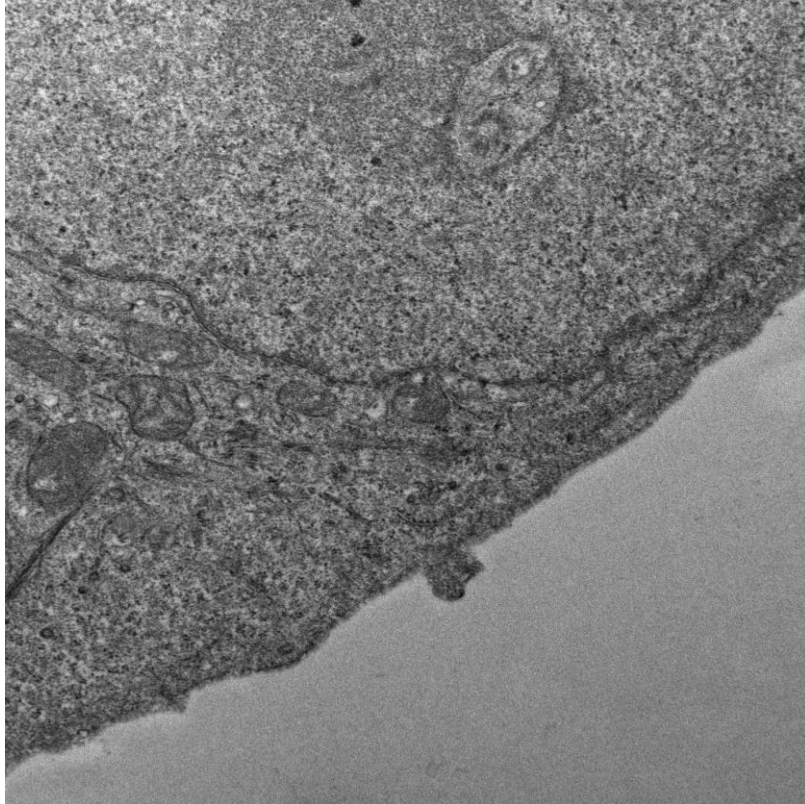


Chemistry choices:

- ⑩ Fixative
- ⑩ Vehicle / buffer
- ⑩ Time
- ⑩ Temperature

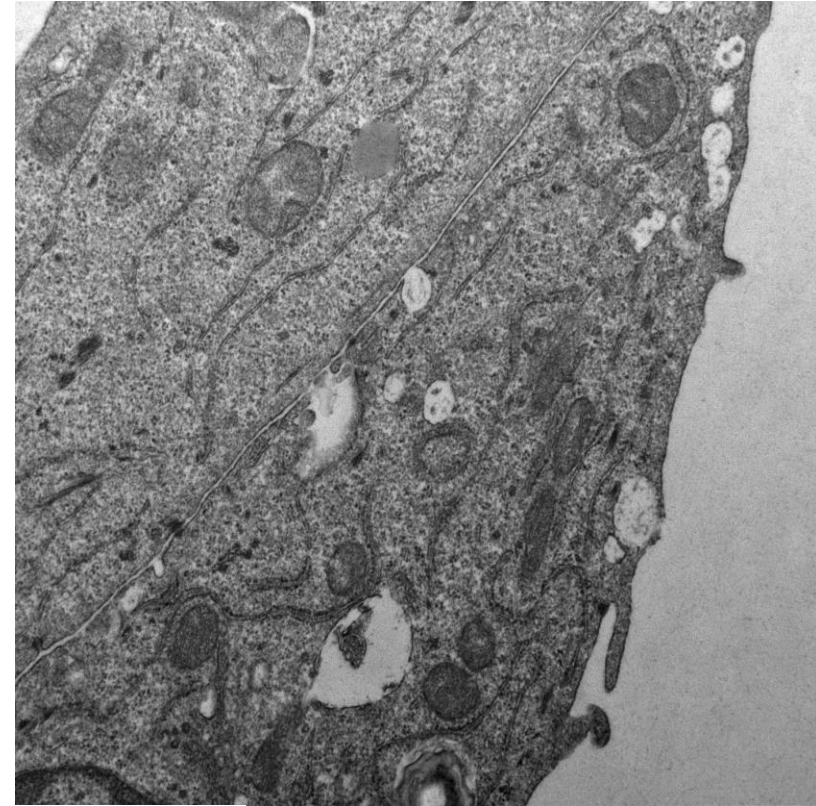


There is one technical difference - any guesses what it is?



500 nm
HV=100.0kV
Direct Mag: 5000x

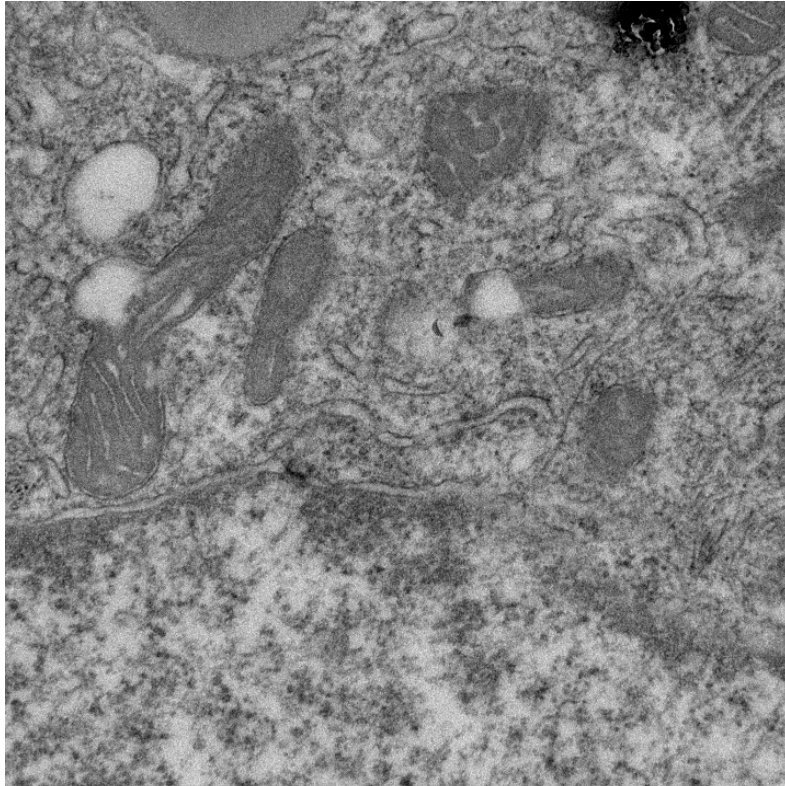
Osmium fixation at room temperature



500 nm
HV=100.0kV
Direct Mag: 5000x

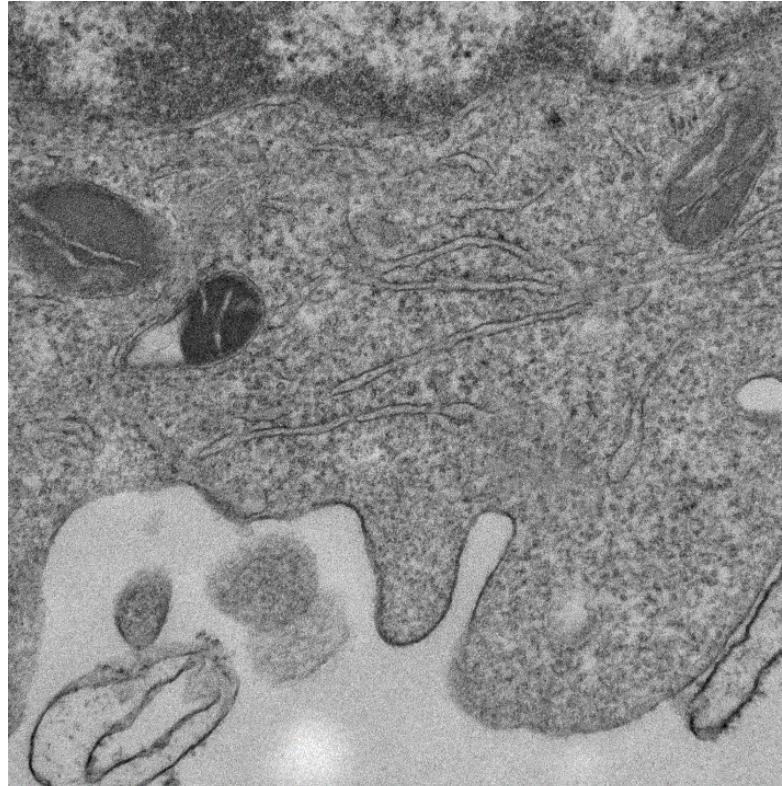
Osmium fixation on ice

There is one technical difference - any guesses what it is?



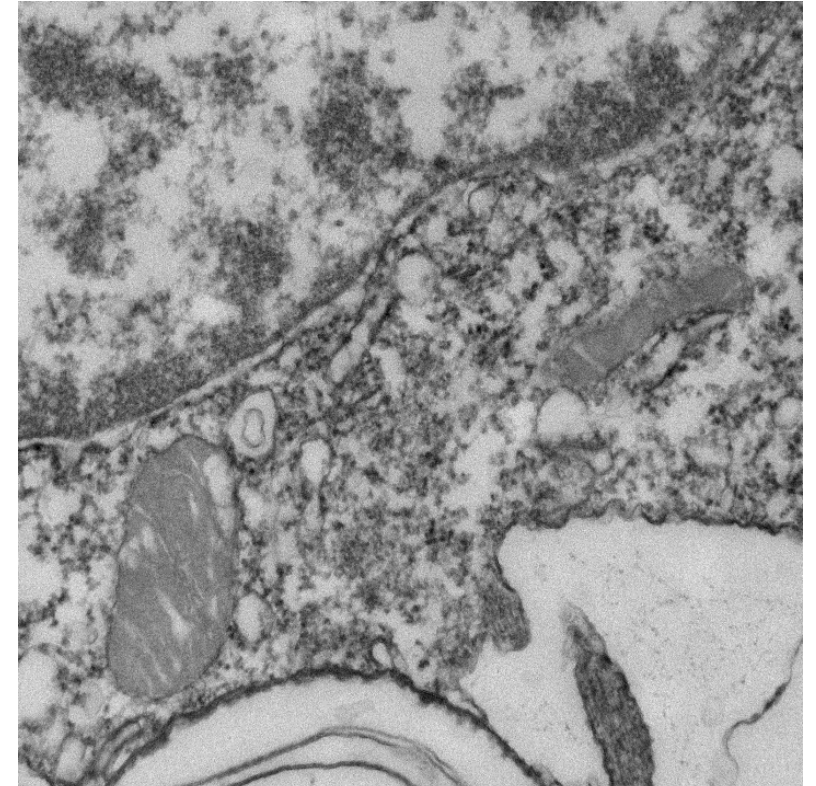
500 nm
HV=100.0kV
Direct Mag: 12000x

0.1M Cacodylate Buffer



500 nm
HV=100.0kV
Direct Mag: 12000x

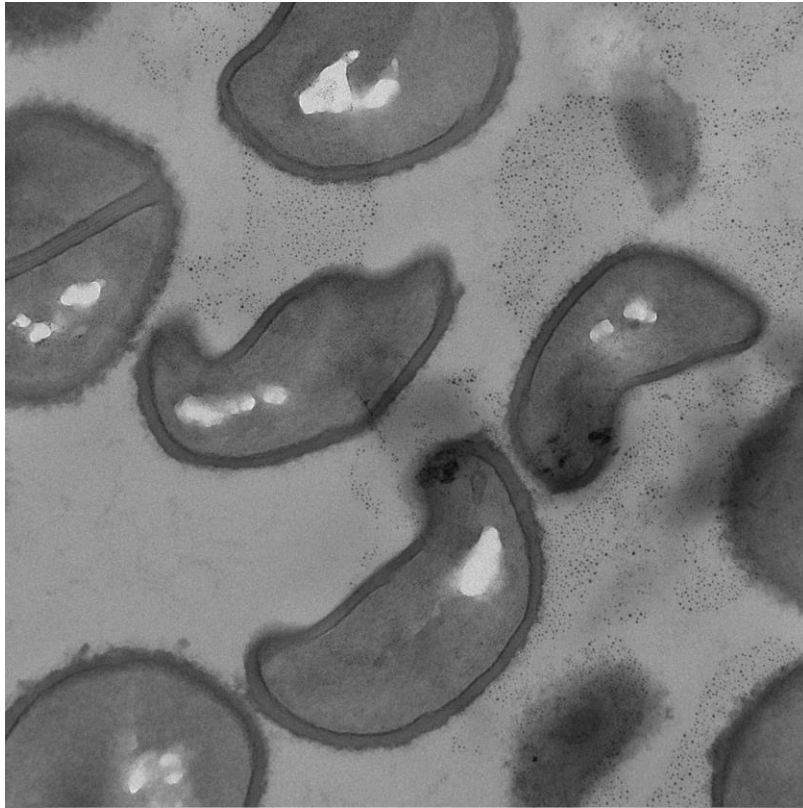
0.1M Phosphate Buffer



500 nm
HV=100.0kV
Direct Mag: 12000x

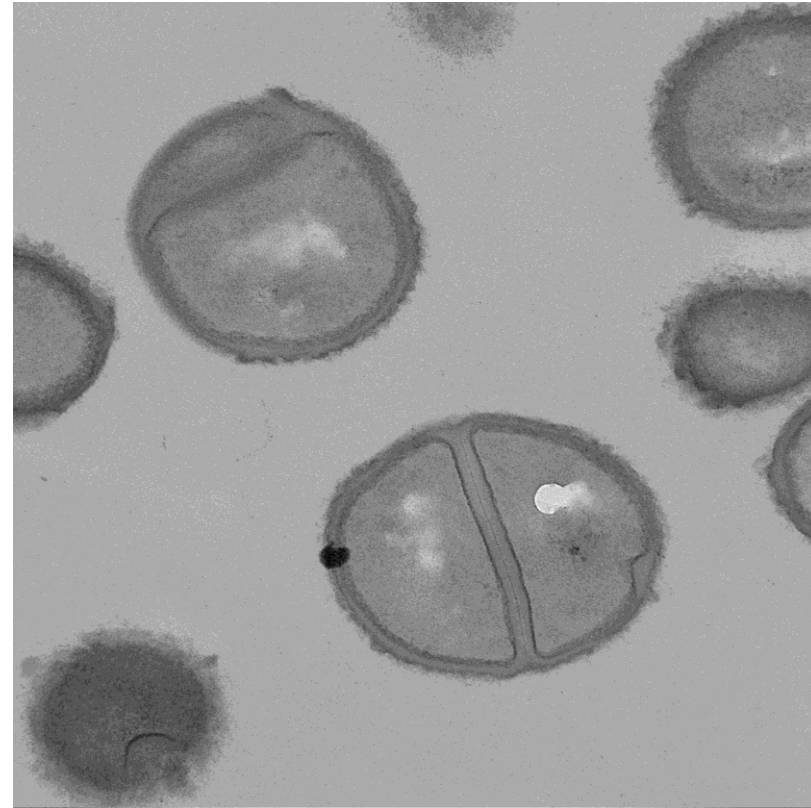
Water

There is one technical difference - any guesses what it is?



500 nm
HV=100.0kV
Direct Mag: 15000x

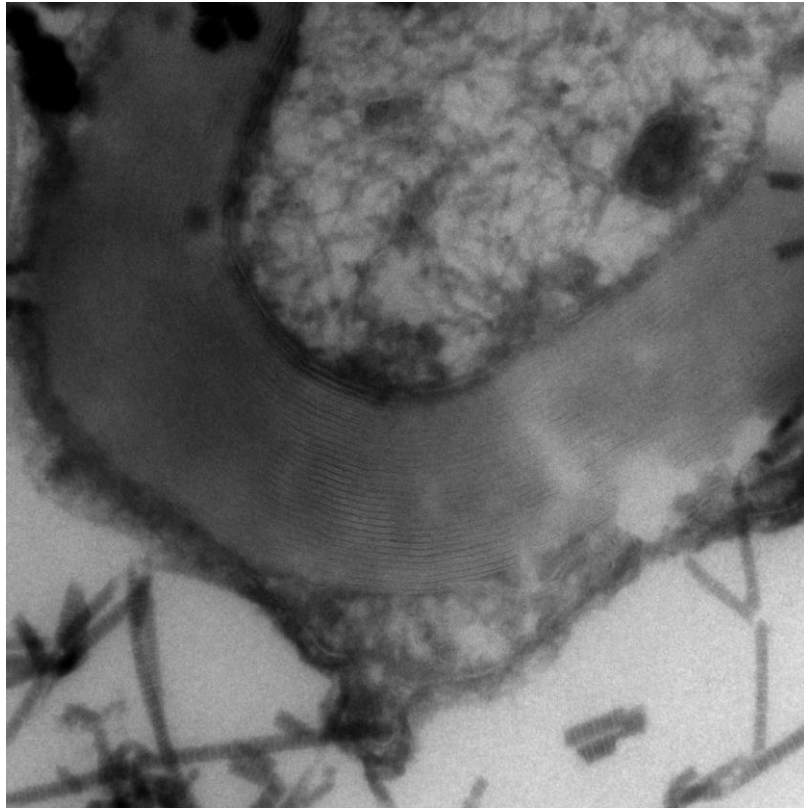
Dehydration using Ethanol



500 nm
HV=100.0kV
Direct Mag: 15000x

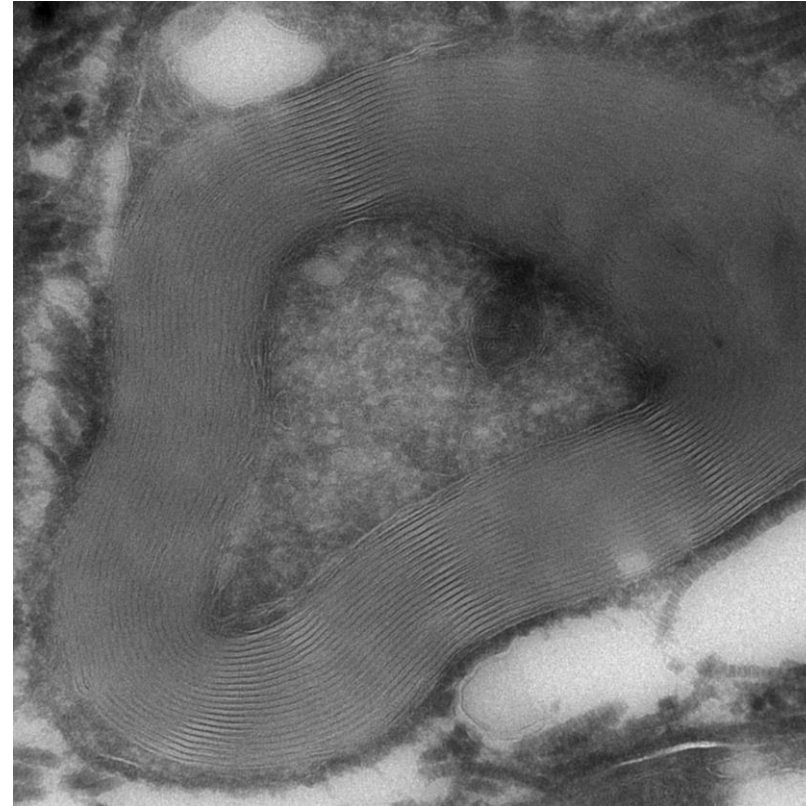
Dehydration using Acetone

There is one technical difference - any guesses what it is?



500 nm
HV=100.0kV
Direct Mag: 15000x

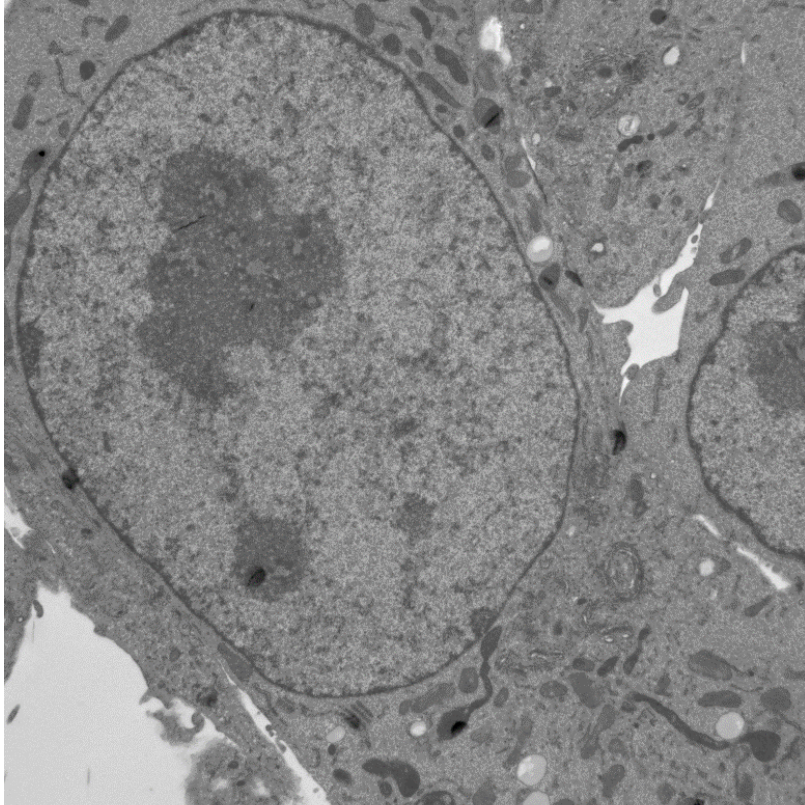
4% Formaldehyde only



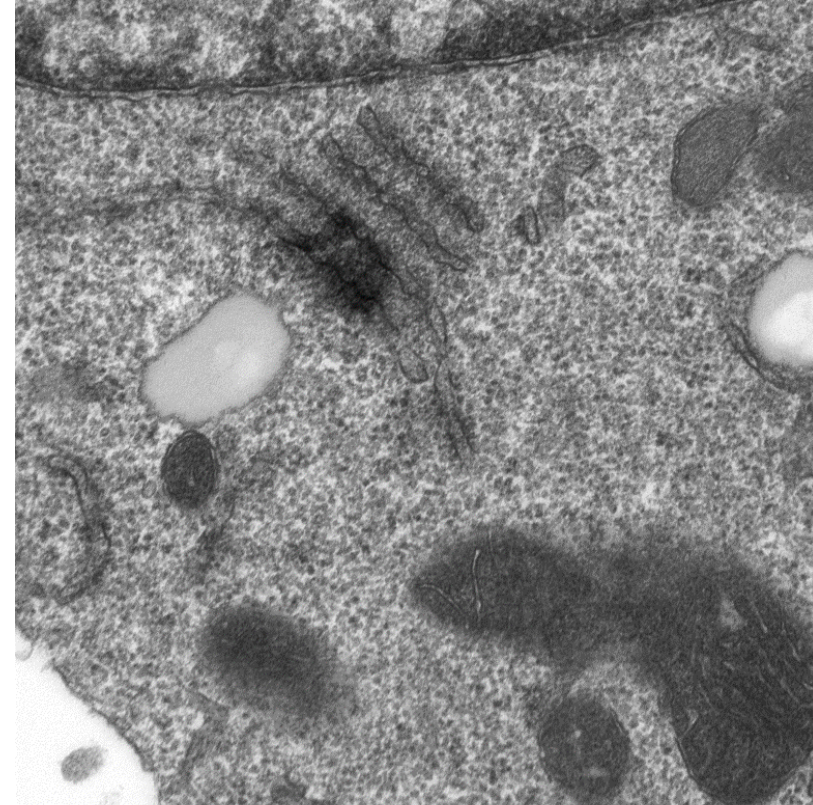
500 nm
HV=100.0kV
Direct Mag: 15000x

0.2% Glutaraldehyde and 2% Formaldehyde

Nice control cells

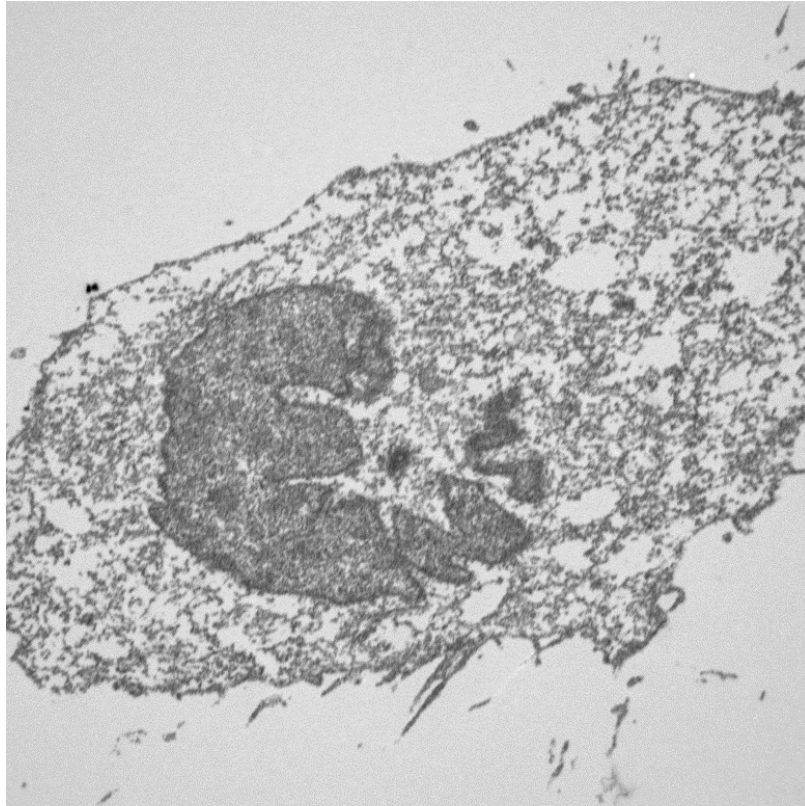


2 μm
HV=100.0kV
Direct Mag: 1500x

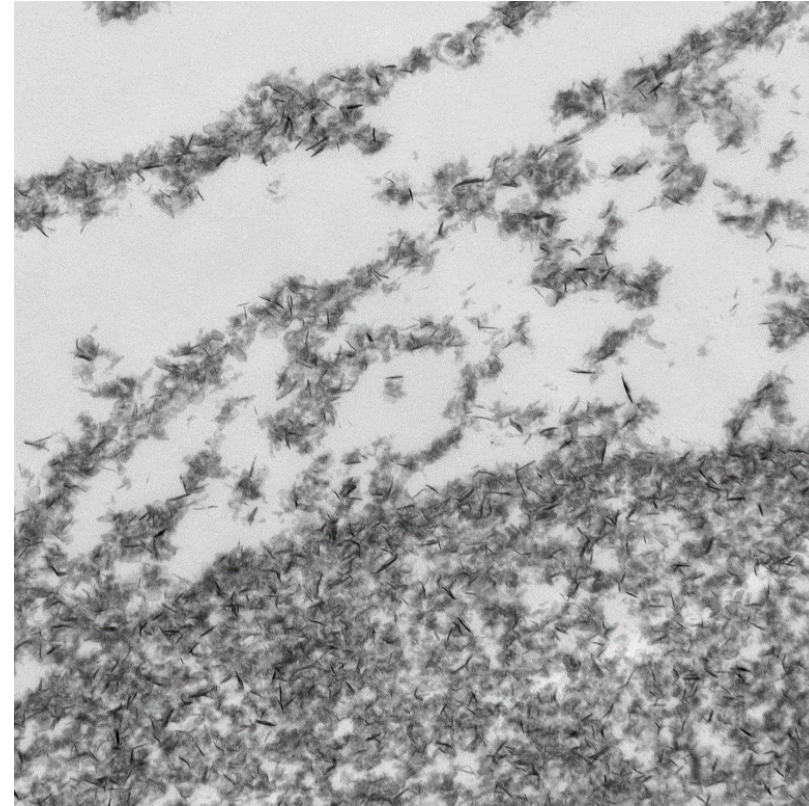


500 nm
HV=100.0kV
Direct Mag: 12000x

Oh dear...

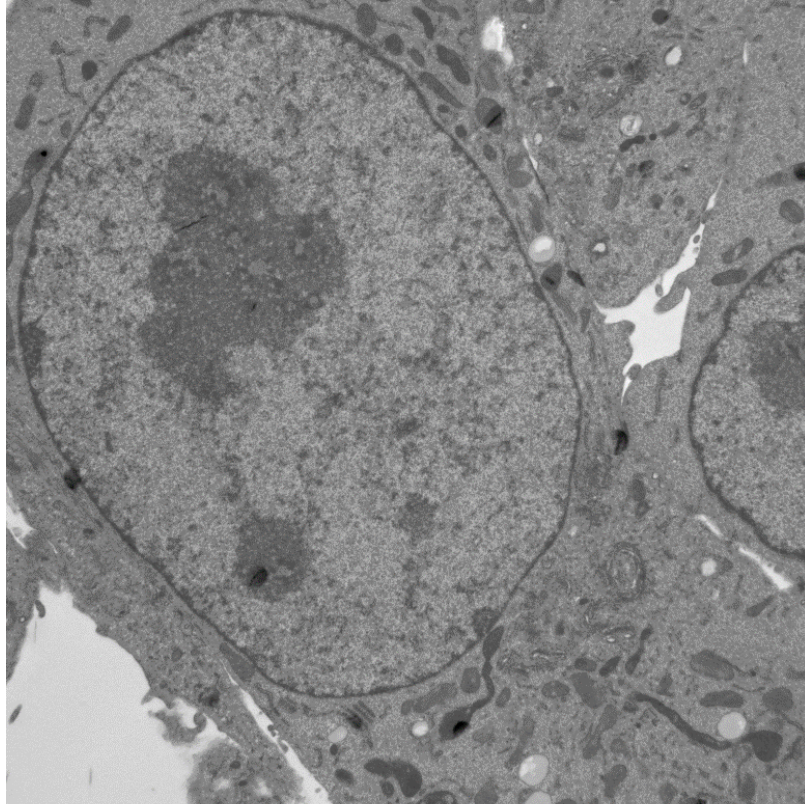


2 μ m
HV=100.0kV
Direct Mag: 1500x

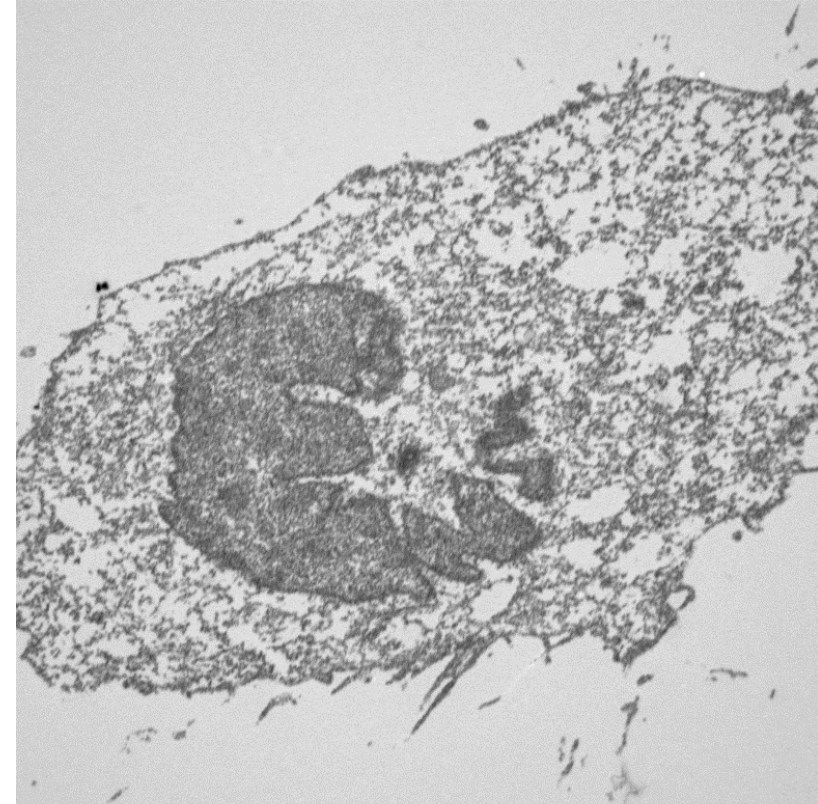


500 nm
HV=100.0kV
Direct Mag: 12000x

What is different?



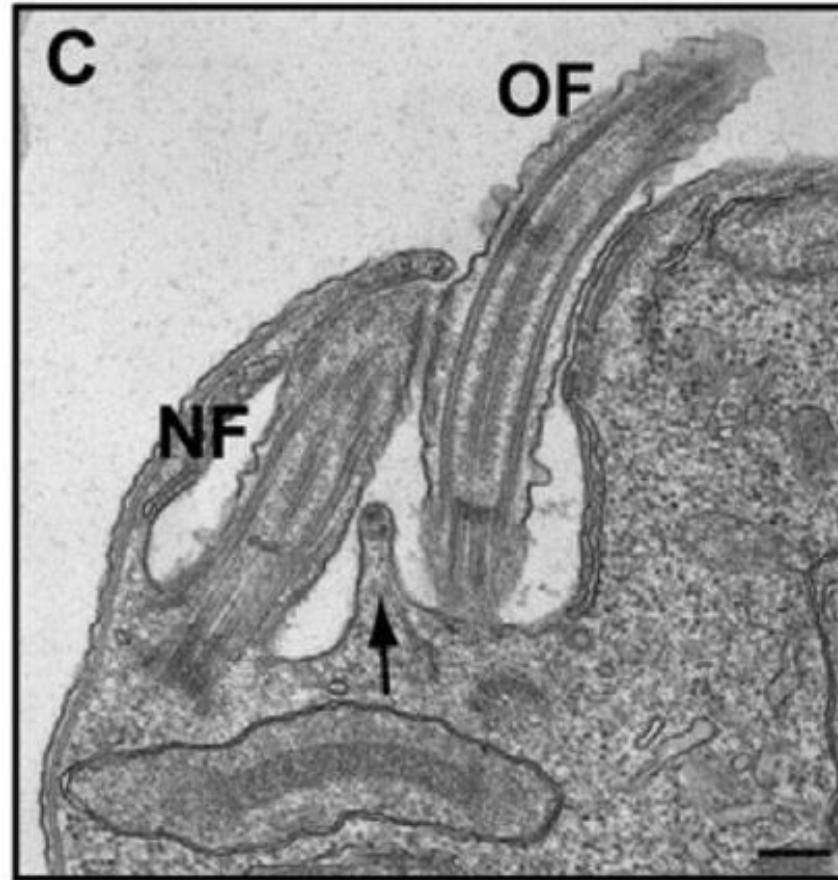
Control Cell



Cell following Immunofluorescence imaging
(0.1% Triton X-100 in PBS)

Artifacts can alter scientific interpretations (in the same research group)

Room Temperature Fixation



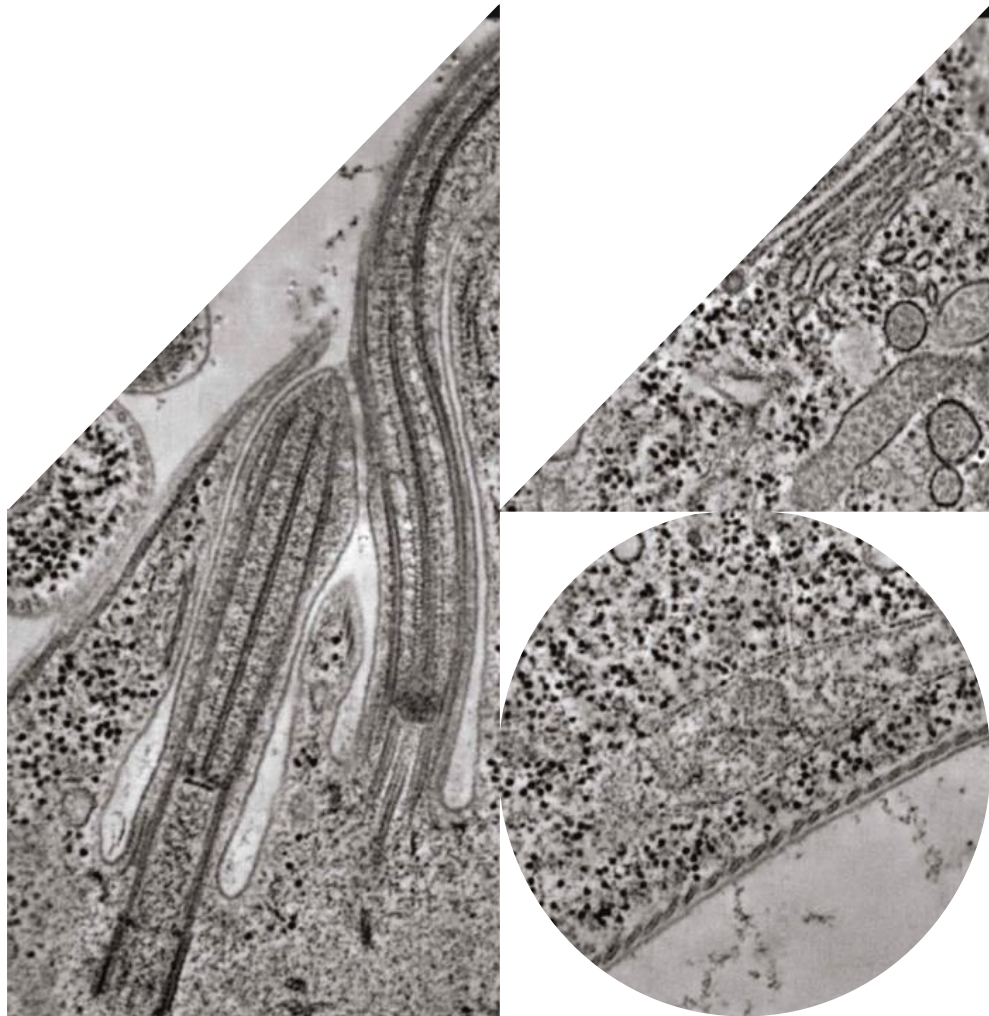
Trypanosoma brucei
OF = Original Flagellum
NF = New Flagellum

Lacomble, 2010, J Cell Sci

HPF/ AFS



Höög, 2014, eLIFE



Knowing your techniques and knowing your artifacts is essential to good science

But does preservation always need to be “perfect”?

In a world of limited budget and time can it be perfect?



Roksana
Pirzgalska

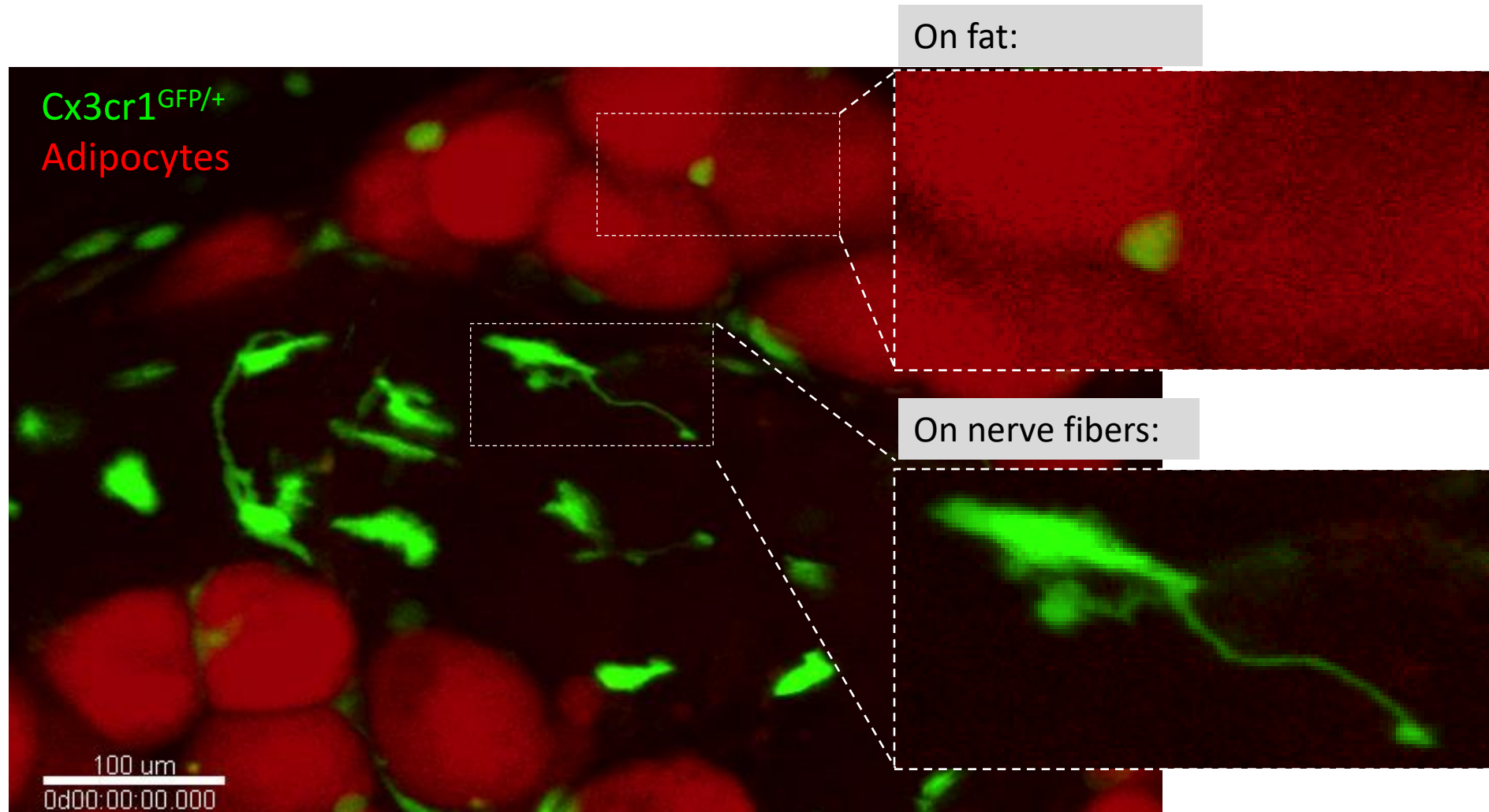


Ana
Domingos

Work done in collaboration with the Obesity group of
Ana Domingos at the Instituto Gulbenkian de Ciência,



The Electron Microscopy Facility Team and the Histopathology
Facility Team at the Instituto Gulbenkian de Ciência



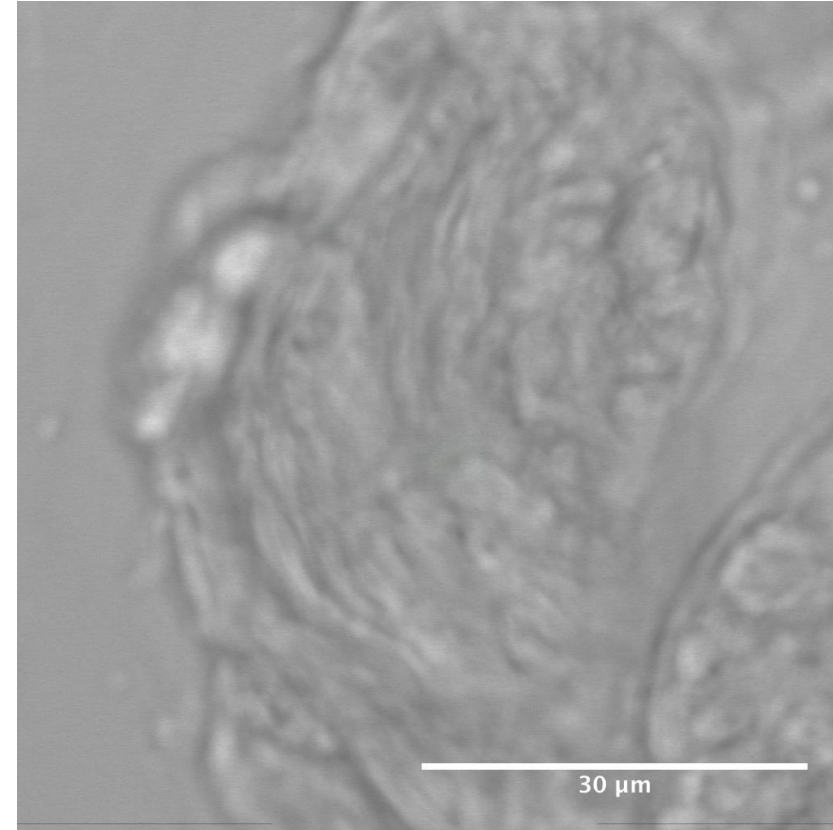
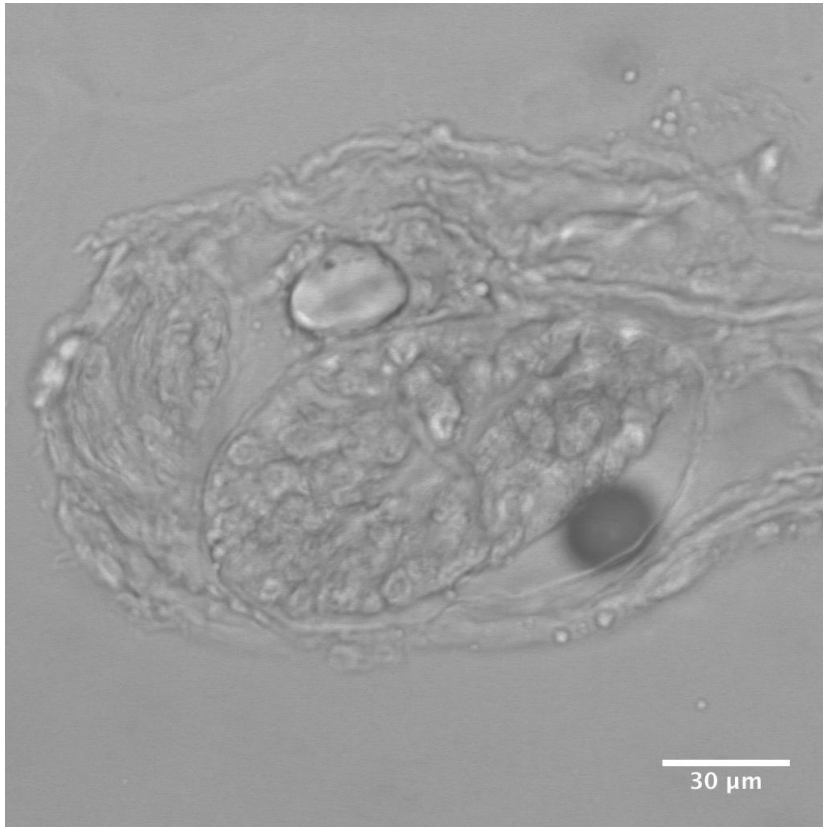
Perfusion with 2% formaldehyde, 0.2% glutaraldehyde in 0.1M phosphate buffer (pH 7.4).

Fibers were isolated and immersion fixed for 2 hours at room temperature in the same fixative

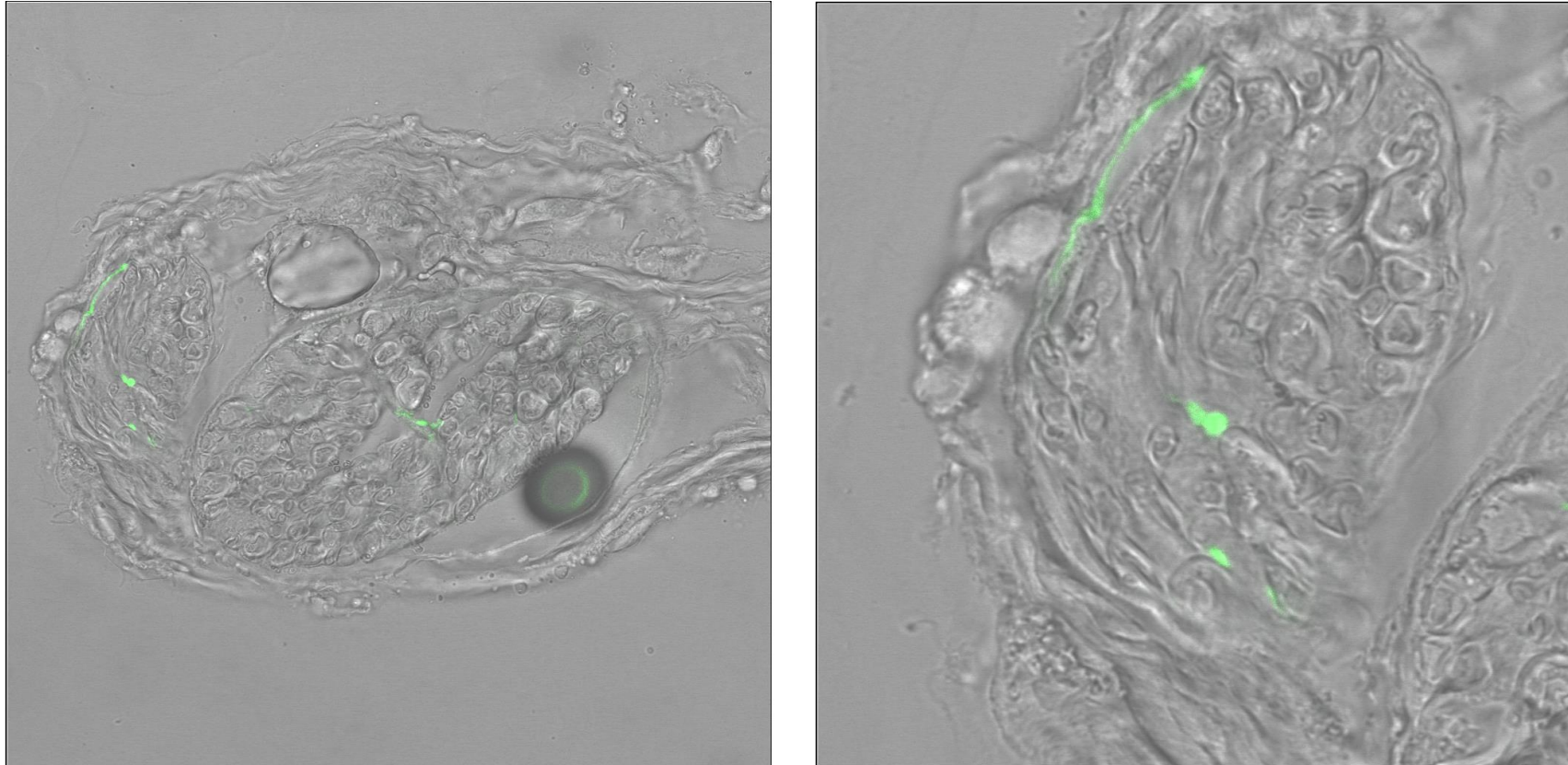


Fibers were embed in 2% agarose before cryoprotection in 30% sucrose ON at 4°C

Embed samples were placed in OCT and plunge frozen in liquid nitrogen. 10µm sections were cut on a cryostat and picked up on cover glass coated with 2% APTES in acetone



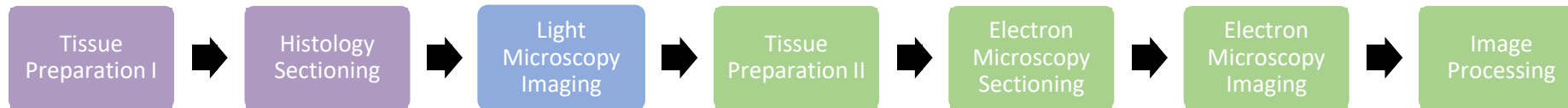
Light Microscopy Imaging in 10µm cryostat sections



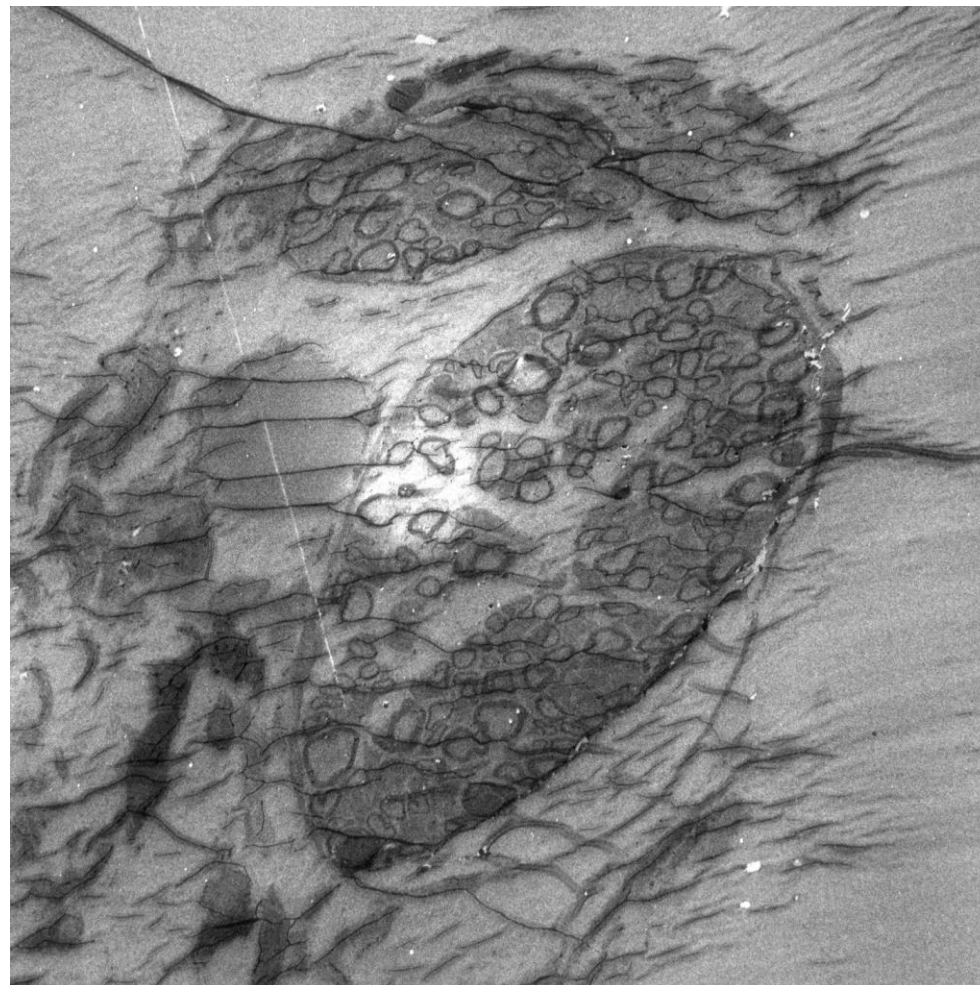
Light Microscopy Imaging in 10µm cryostat sections

With CLEM we could confirm the Macrophages in Adipose Tissue

samples washed 10 times with 0.1M PB
post-fixed in 1% reduced osmium tetroxide in PB for 30 minutes, on ice.
graded ethanol dehydration and embedded in Epon



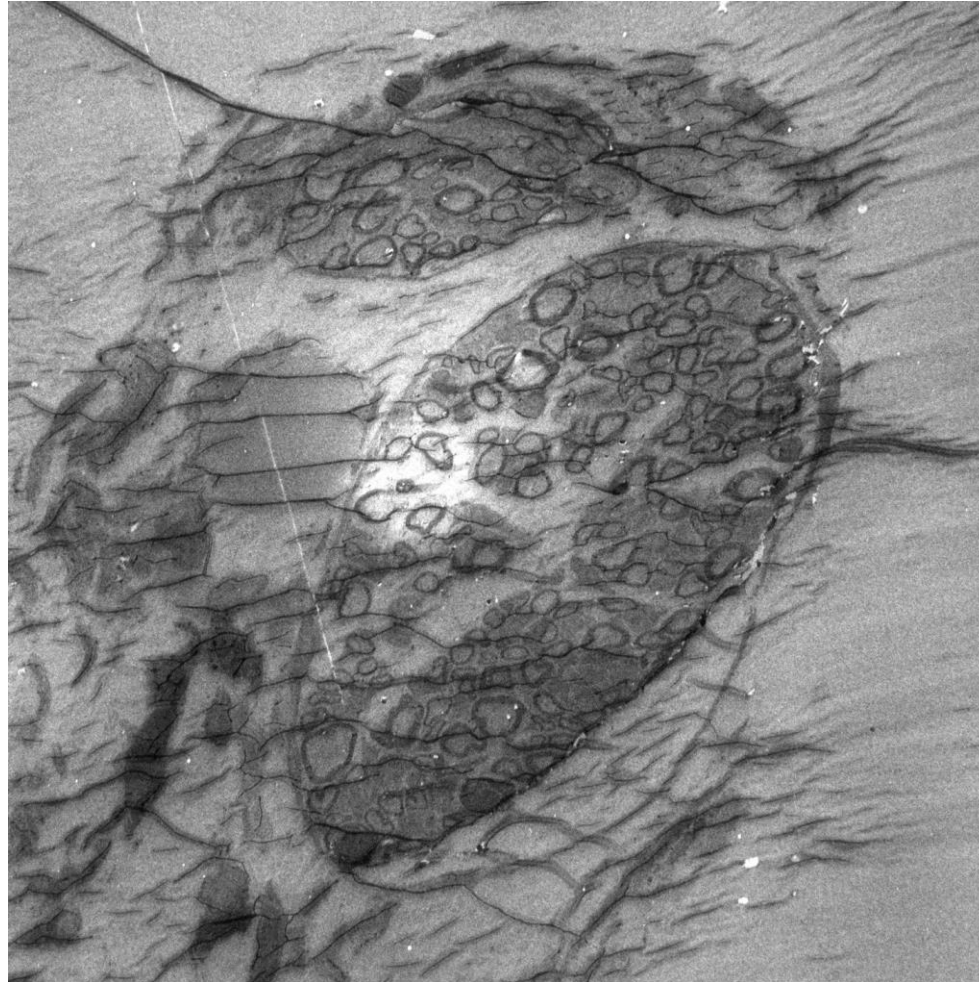
70nm serial sections were cut on an ultramicrotome.
Post – staining 1% uranyl acetate and lead citrate for 5 minutes each.



Trans23.block2647section18-001.tif
Cal: 0.075115 um/pix
14:14:25 24/02/17

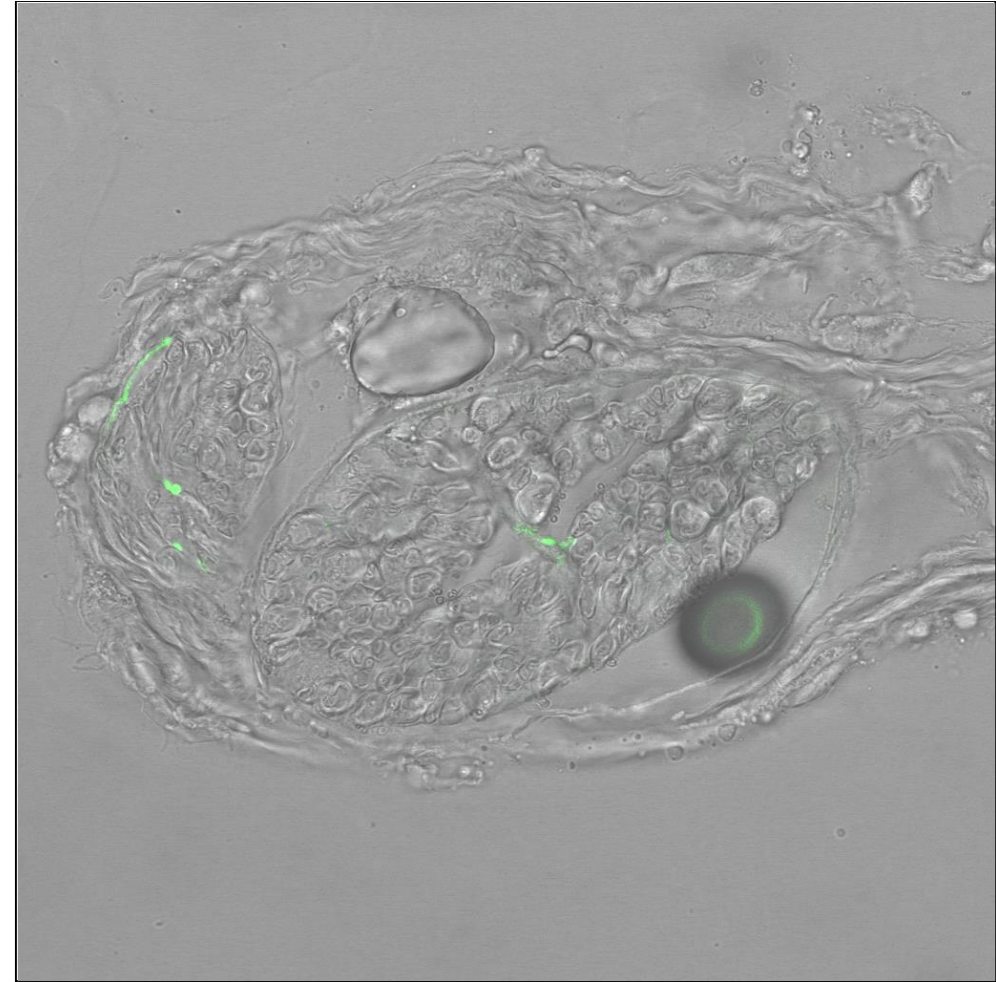
10 μ m
HV=100.0kV
Direct Mag: 200x

Science: Pirzgalska RM et al., Nat Med. 2017 Nov;23(11):1309-1318.
Method: Sousa AL, et al., Methods Cell Biol. 2021;162:13-37.

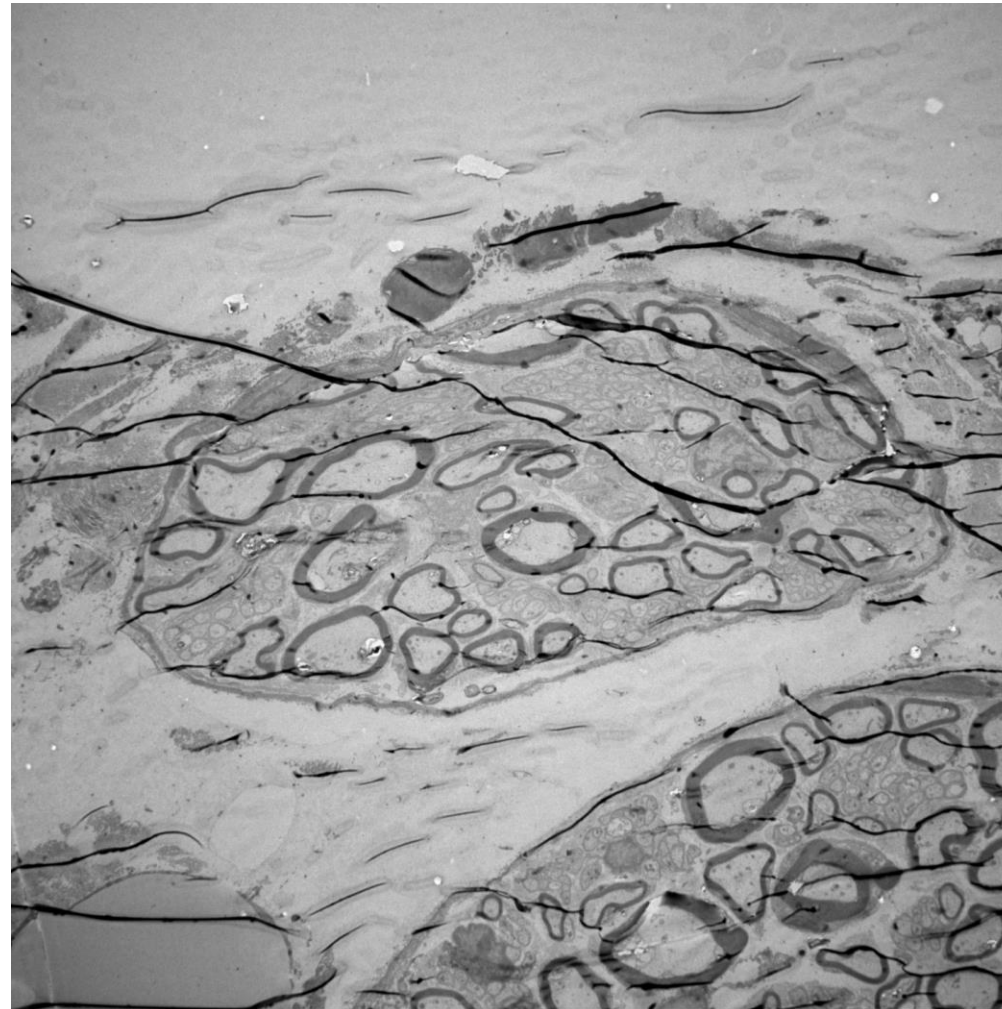


Trans23.block2647section18.001.tif
Cal: 0.075115 um/pix
14:14:25 24/02/17

10 μ m
HV=100.0kV
Direct Mag: 200x



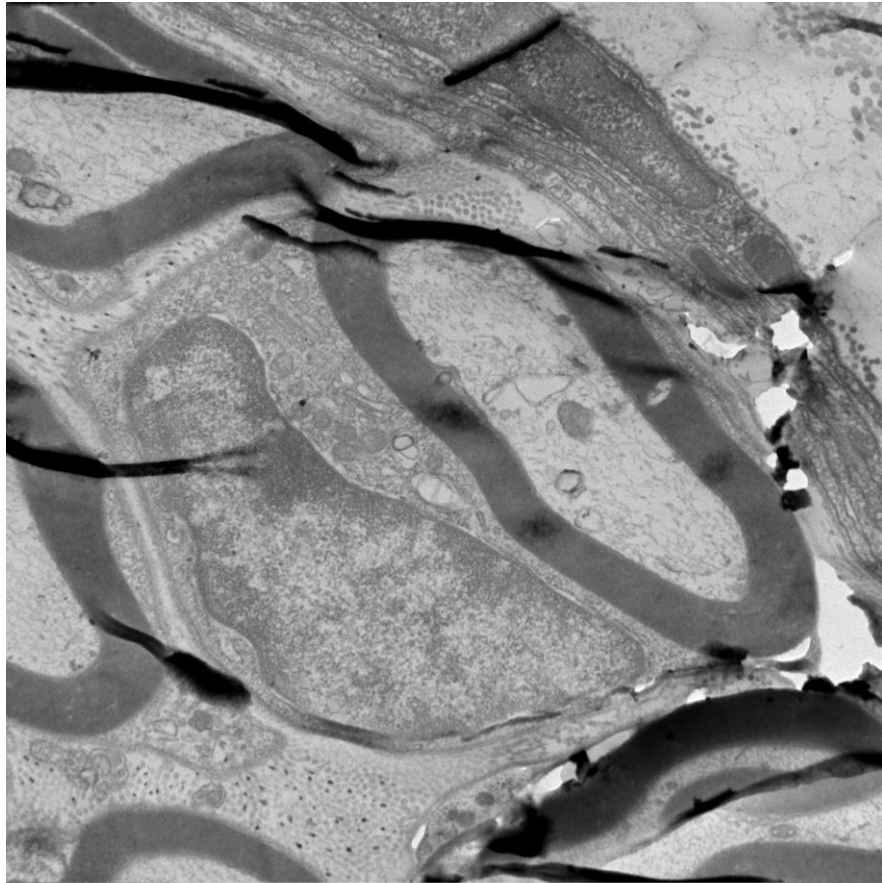
Science: Pirzgalska RM et al., Nat Med. 2017 Nov;23(11):1309-1318.
Method: Sousa AL, et al., Methods Cell Biol. 2021;162:13-37.



Trans23.block2647section18.002.tif
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14:21:36 24/02/17

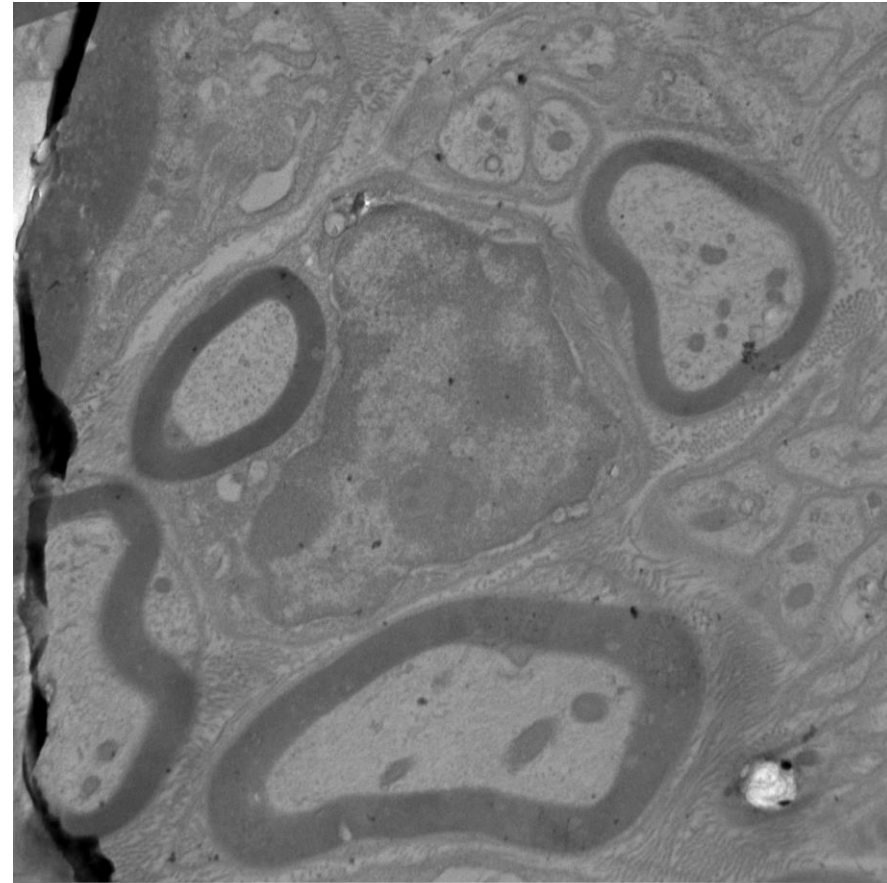
10 μ m
HV=100.0kV
Direct Mag: 500x

Science: Pirzgalska RM et al., Nat Med. 2017 Nov;23(11):1309-1318.
Method: Sousa AL, et al., Methods Cell Biol. 2021;162:13-37.



Trans23.block2647section18.005.tif
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14:27:07 24/02/17

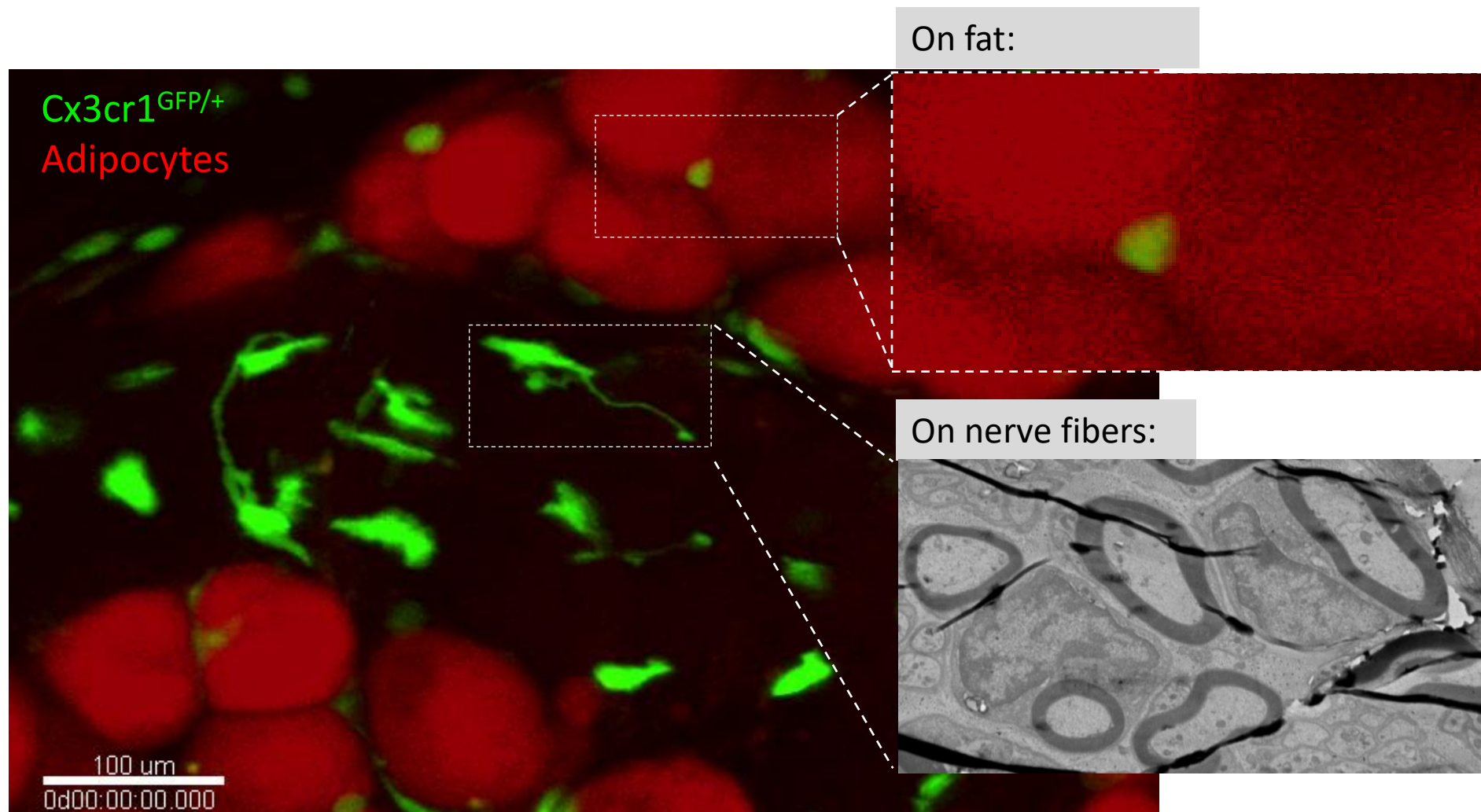
500 nm
HV=100.0kV
Direct Mag: 4000x



Trans23.block2647section36.002.tif
Cal: 0.005008 um/pix
14:37:20 24/02/17

2 μm
HV=100.0kV
Direct Mag: 3000x

We could confirm the Macrophages in Adipose Tissue



Knowing your techniques and knowing your artifacts is essential to good science

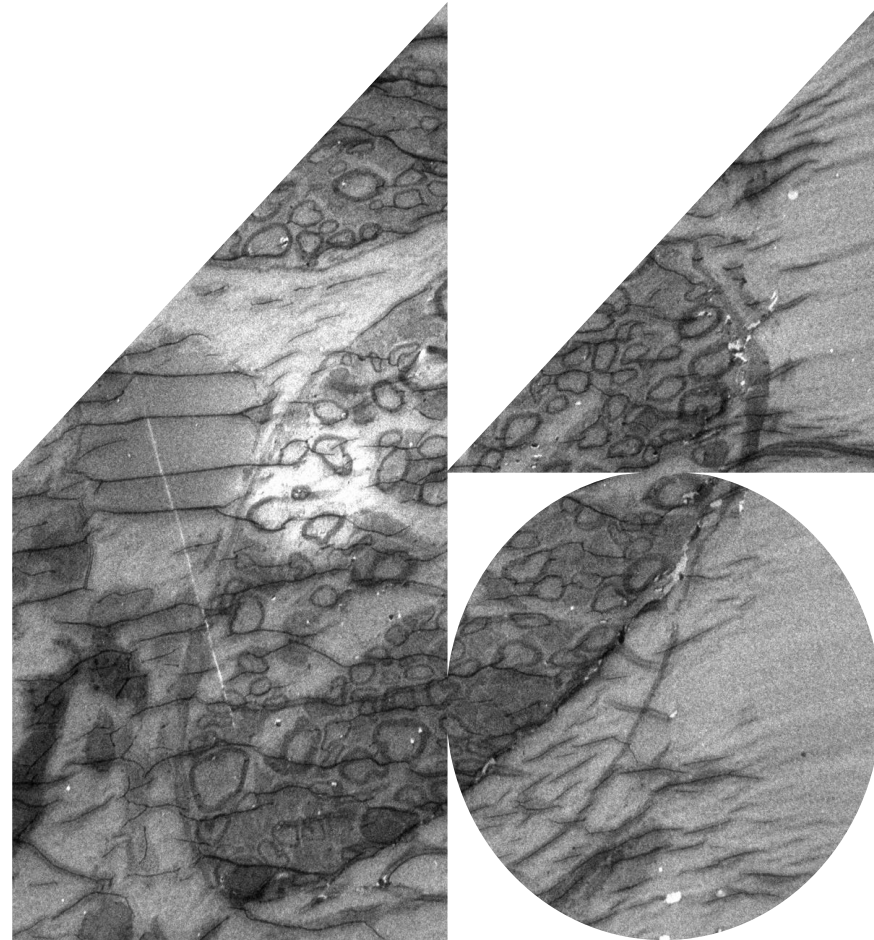
No, sample preservation does not always need to be perfect if you can confidently answer your biological question

It is important to know the purpose of the sample collection

- Pathology assessment?

- Research investigation?

- What is needed to answer the biological question?



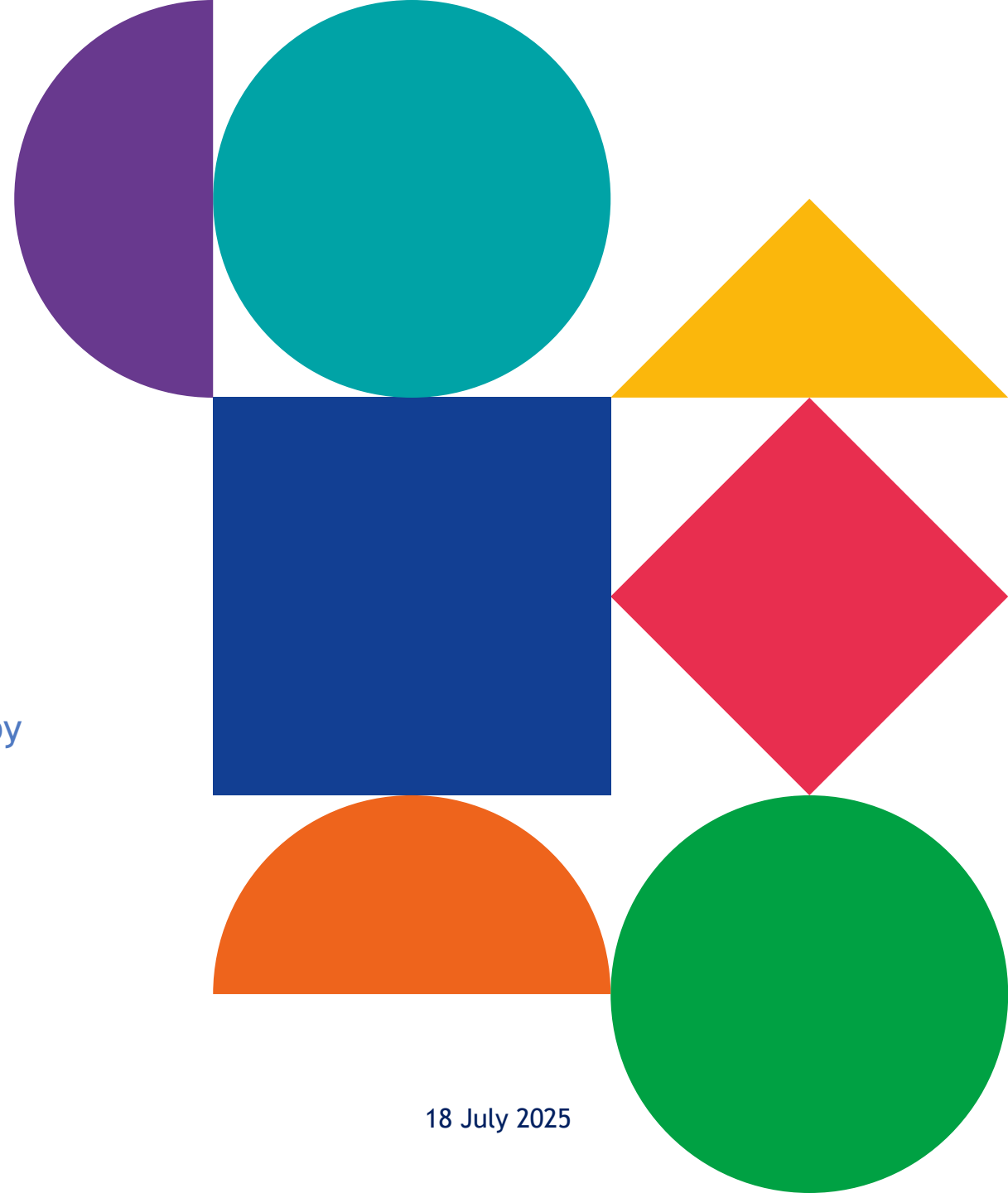
Artifacts: Friend or Foe?

There is no such thing as artifact free microscopy

An artifact is a problem if:

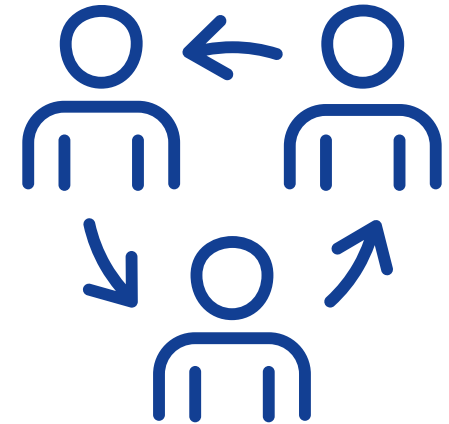
- It interferes with your ability to answer your biological question
- **You pretend it does not exist**

Sometimes an artifact in the sample may unintuitively help answer your question



The TechEM Seminars

- Started in 2020 during lockdown
- Goal: to share all technical nuances that are often left out of papers
- Format: online presentations, with lots of discussion, target is 60 minutes, typically at 11:00 CET/CEST on Thursday, 10-14 seminars per year.
- Topics range from detailed sample preparation protocols, to talks about imaging artifacts, to introductions to new topics (art in EM, material science EM etc)
- Speakers range from summer students to Nobel Laureates, everyone is encouraged to ask questions and welcome to present
- If you wish to join us, email me at TechEMseminars@vib.be and I can add you to the mailing list



The TechEM Seminars

If you wish to join us,
email me at
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and I can add you to the
mailing list.





Erin Tranfield

Erin.Tranfield@vib.be or TechEMseminars@vib.be

