CEITEC

Středoevropský technologický institut

BRNO | ČESKÁ REPUBLIKA

Electron microscopy

InnoCore project

Jiri Novacek

EVROPSKÁ UNIE
EVROPSKÝ FOND PRO REGIONÁLNÍ ROZVOJ
INVESTICE DO VAŠÍ BUDOUCNOSTI

2007–13
OP Výzkum a vývoj pro inovace

Sylabus

- **Lecture 1: Applications of electron microscopy in life-science research**
- **Lecture 2: Transmission electron microscope, cryo-electron microscopy, principles of image formation**
- **Lecture 3: Data alignment in 2D, techniques for 3D model determination in cryo-EM**

Sylabus

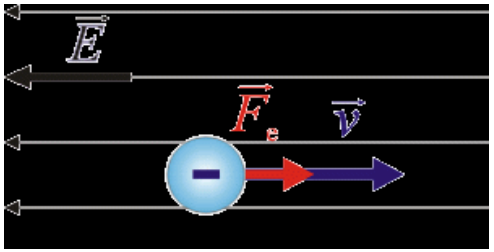
- **Lecture 1: Applications of electron microscopy in life-science research**
- **Lecture 2: Transmission electron microscope, cryo-electron microscopy, principles of image formation**
- **Lecture 3: Data alignment in 2D, techniques for 3D model determination in cryo-EM**

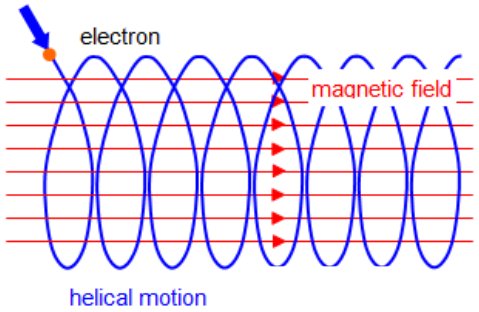
Electron

rest mass:	**9.109 e-31 kg**
charge:	**-1.61 e-19 C**
spin:	**1/2**

Electron in electric and magnetic field

$$\mathbf{F} = q(\mathbf{E} + \mathbf{v} \times \mathbf{B})$$ (Lorentz force)





Electron

Dual character of electron

$$\lambda = \frac{h}{p} = \frac{h}{mv}$$

$$\lambda_{\text{de Broglie}} = \frac{h}{p} = \frac{h \cdot c}{\sqrt{(e \cdot V_\text{a})^2 + 2 \cdot e \cdot V_\text{a} \cdot m_\text{e} \cdot c^2}}$$

Acceleration Voltage [kV]	Non-relativistic wavelength [pm]	Relativistic wavelength [pm]
2	27.35	27.32
20	8.65	8.57
100	3.87	3.69
200	2.73	2.50
300	2.23	1.96

Abbe diffraction limit

$$\Delta x \cong \frac{\lambda}{2n \sin \alpha}$$

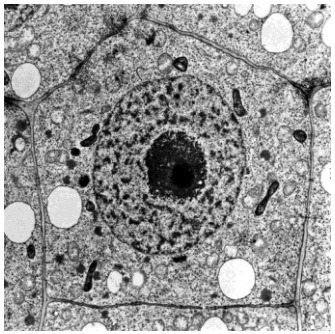
Scales in electron microscopy

1 mm

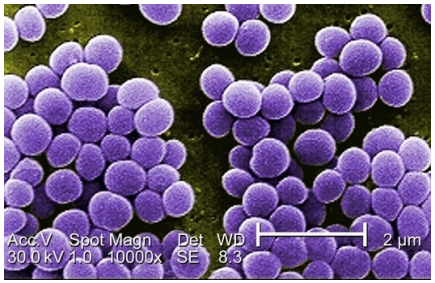
1 um

1 nm

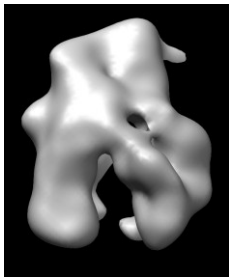
Tick (ESEM)



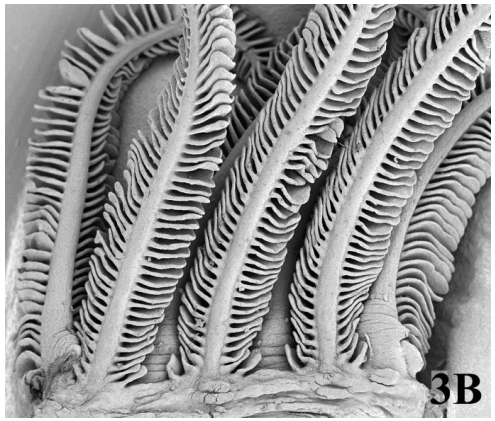
Plant cell (TEM)



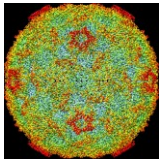
Bacteria (SEM)



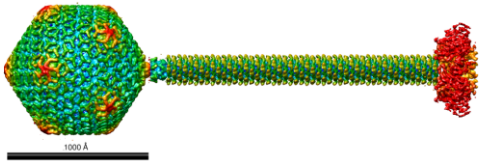
RNA polymerase (TEM)



Plant (SEM)



Virus (TEM)



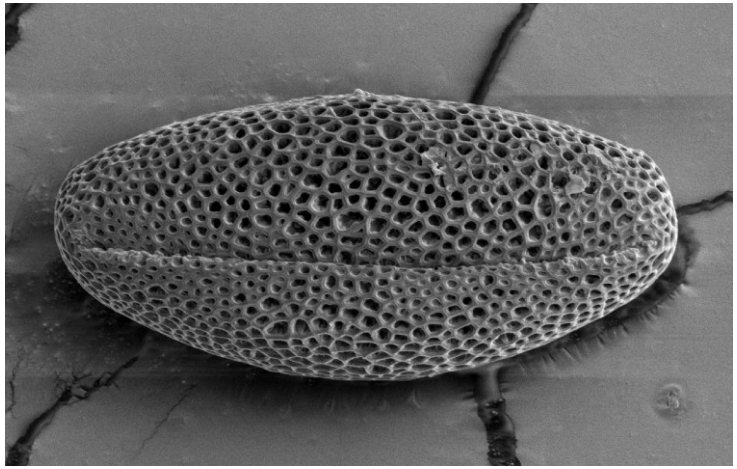
Bacteriophage (TEM)

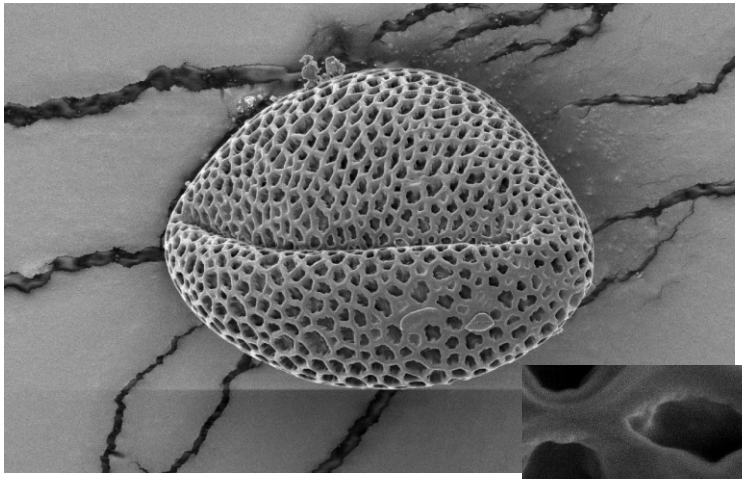
CEITEC

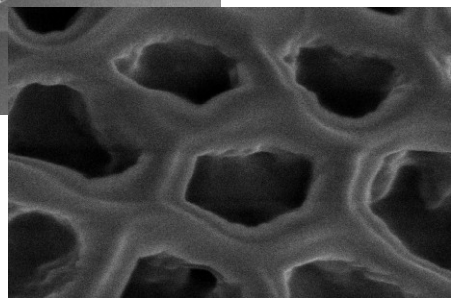
Applications in life-sciences

- SEM imaging
- Block face imaging
- Structural Biology cryo-EM
- Cellular cryo-EM techniques

SEM imaging





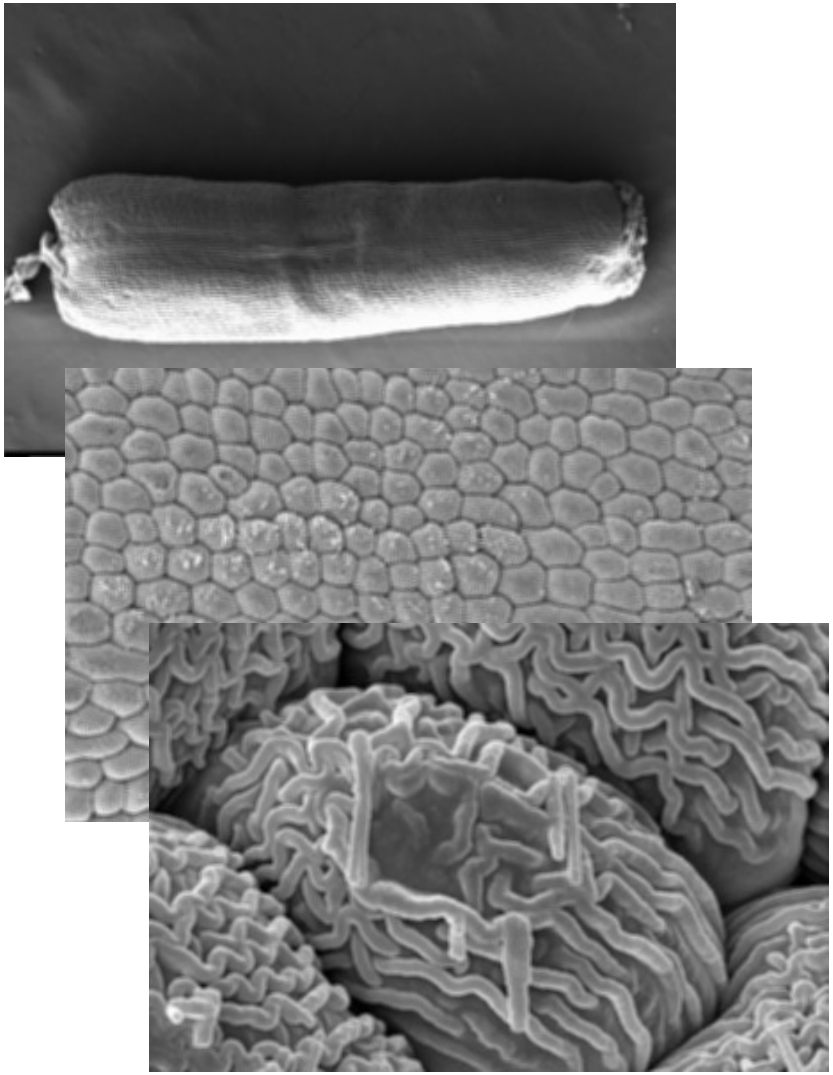


Pros:
- imaging of sample morphology at significant scale difference(1mm - 10nm)
- fast sample preparation

Cons:
- non-native (sample dehydrated)

Sample preparation:
- air drying
- metal sputtering (Pt, Au, Ir)

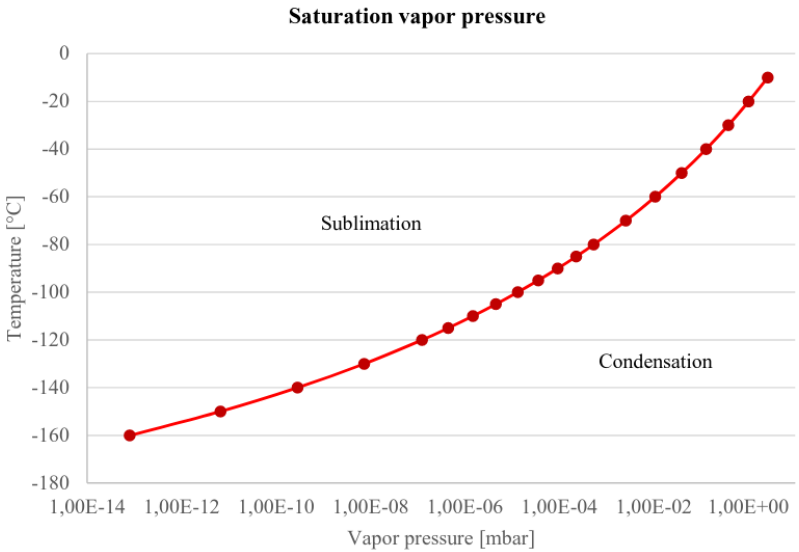
SEM imaging



Pros:
- imaging of sample morphology at significant scale difference(1mm - 10nm)
- fast sample preparation

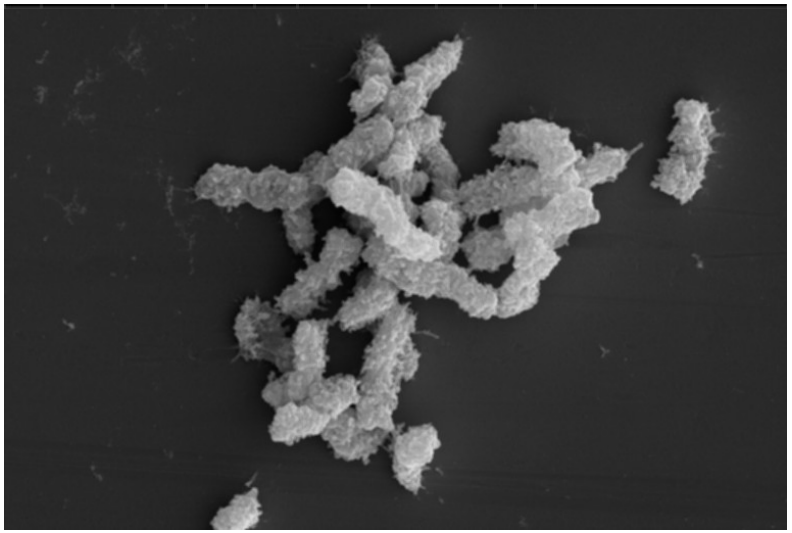
Cons:
- non-native (sample dehydrated)

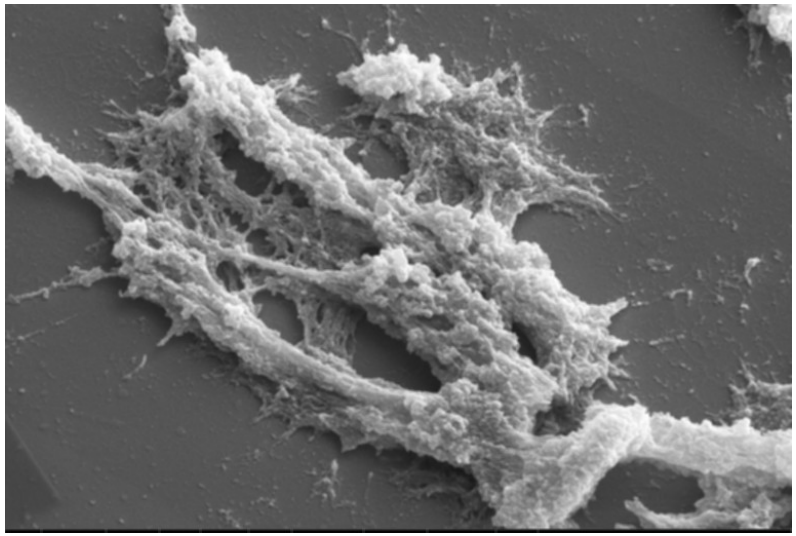
Sample preparation:
- freezing into LN2
- sublimation
- metal sputtering (Pt, Au, Ir)



CEITEC

SEM imaging

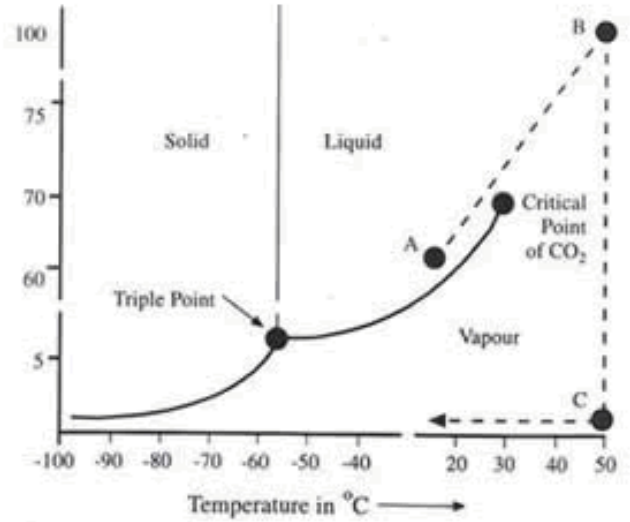




Pros:
- imaging of sample morphology at significant scale difference(1mm - 10nm)
- fast sample preparation

Cons:
- non-native (sample dehydrated)

Sample preparation:
- chemical fixation
- contrasting (Pt,U)
- dehydration (EtOH,aceton,HMDS)
- critical point drying
- metal sputtering (Pt, Au, Ir)



CEITEC

Block face imaging

Pros:
- 3D volume reconstruction at ultrastructural level of detail
- high signal to noise
- low dose sensitivity
- robust (easy sample handling)

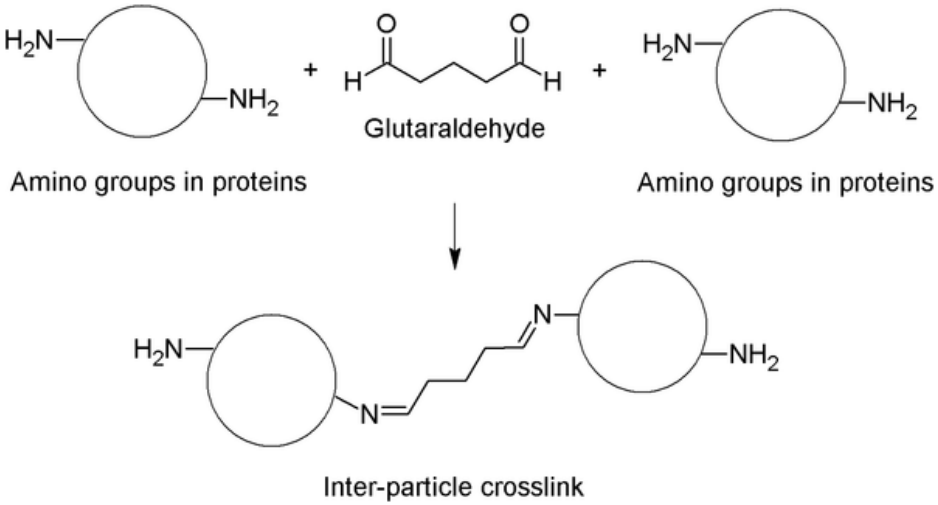
Cons:
- non-physiological conditions during sample prep
- artefacts (changes in cell structure, depression of proteins)
- extremely toxic chemicals (OsO_4)
- attainable level of detail limited

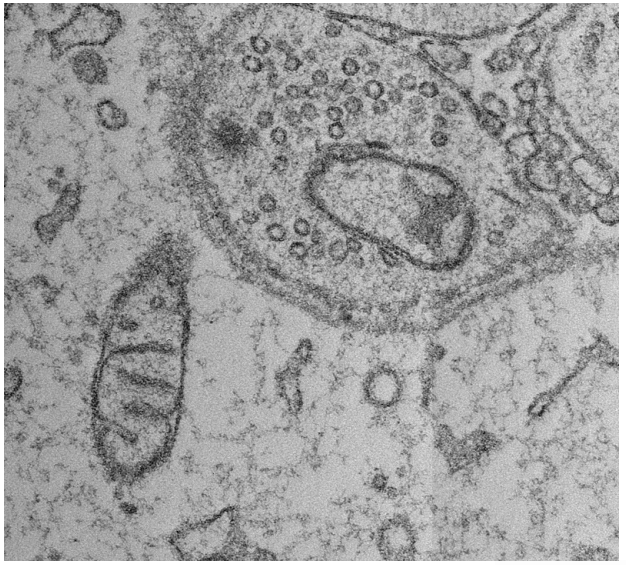
Block face imaging

Sample preparation 1:
- formaldehyde, glutaraldehyde
- chemical fixation - ~2% solution in water or buffer
- variable duration – 2-24 hours (sample thickness)
- contrasting (OsO4, UAc, Pb)

Pros:
- 3D volume reconstruction at ultrastructural level of detail
- high signal to noise
- low dose sensitivity
- robust sample preparation

Cons:
- non-physiological conditions during sample prep
- artefacts (changes in cell structure, depression of proteins)
- extremely toxic chemicals (OsO4)
- attainable level of detail limited





CEITEC

Block face imaging

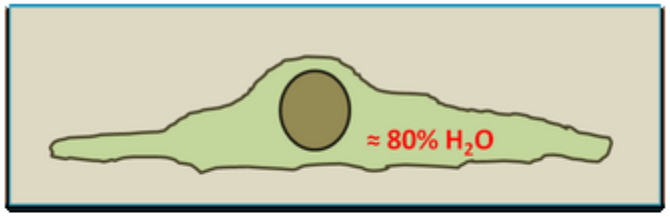
Sample preparation 2:

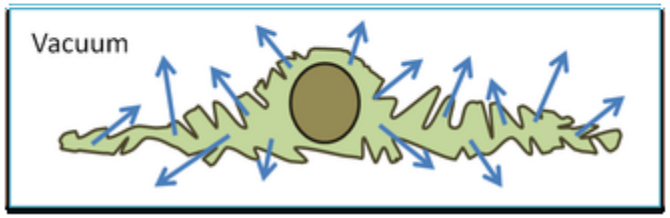
Dehydration – EtOH or aceton series
(30% for 15mins, 50% for 15min, 70% for 15mins, 90% for 15mins, 100% - 3x)
- shrinking of protein and lipids
- sample shrinking up to 40%
- formation of various artefacts

Resin embedding – resin infiltration (2:1 propylen oxide: resin for 1h, 1:1 for 1h, 1:2 for 1h, 100% resin overnight
- polymerazation 24-72h at 60-70C

Pros:
- 3D volume reconstruction at ultrastructural level of detail
- high signal to noise
- low dose sensitivity
- robust sample preparation

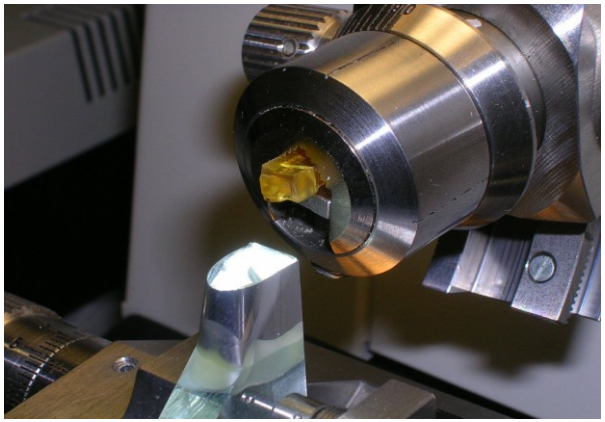
Cons:
- non-physiological conditions during sample prep
- artefacts (changes in cell structure, depression of proteins)
- extremely toxic chemicals (OsO4)
- attainable level of detail limited

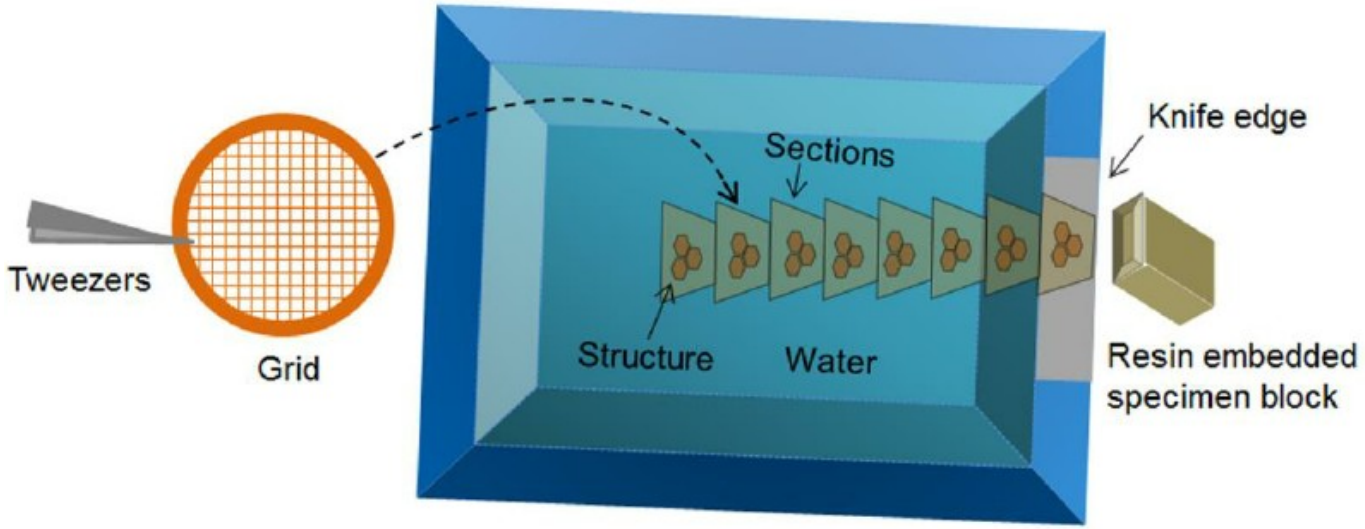




Block face imaging

Mechanical sectioning for TEM

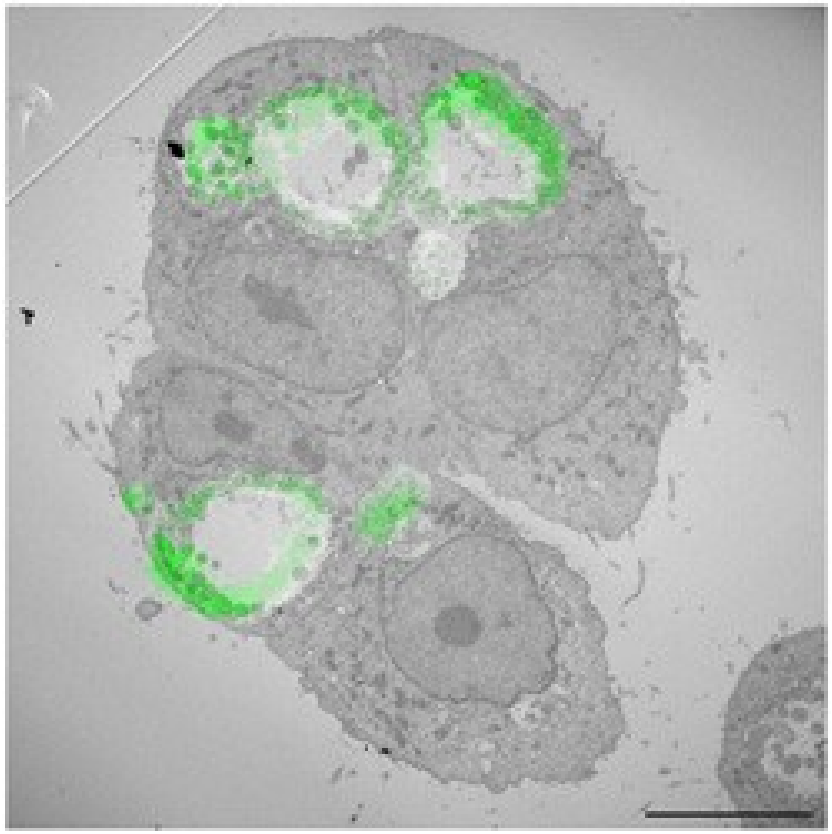




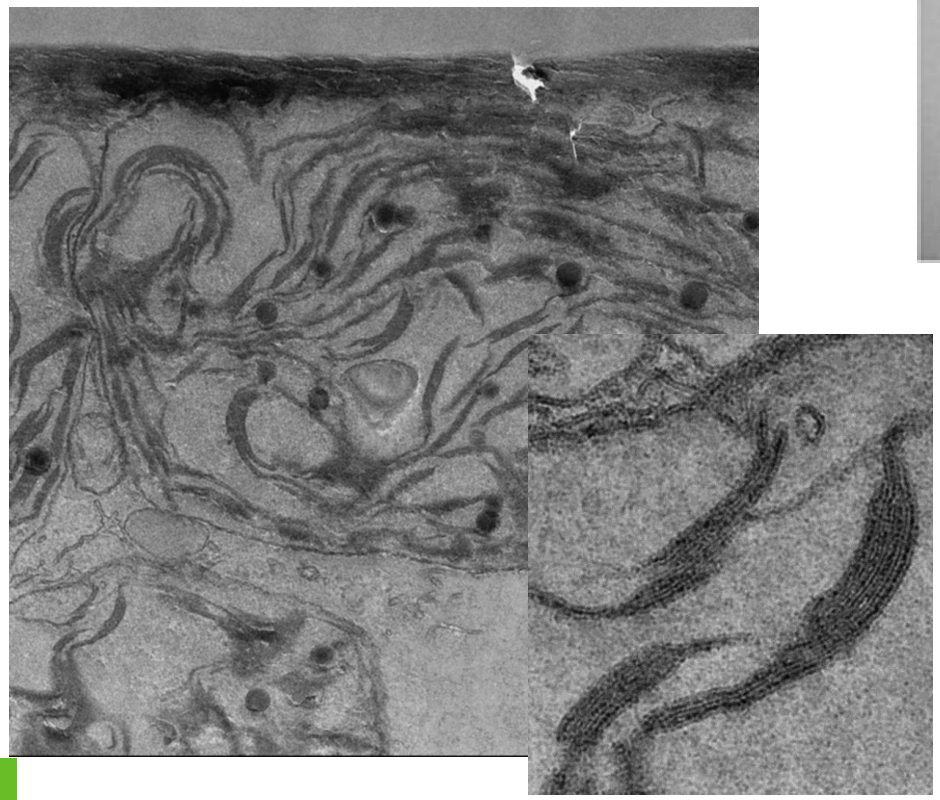
Block face imaging

Mechanical sectioning for TEM

- 50 – 70 nm thick sections
- high-resolution imaging in TEM (tomography)
- 3D volume reconstruction
- resolution limited by sample preparation
- staining with EM contrasting agents (nanoparticles) or fluorescent markers (CLEM) for targetting



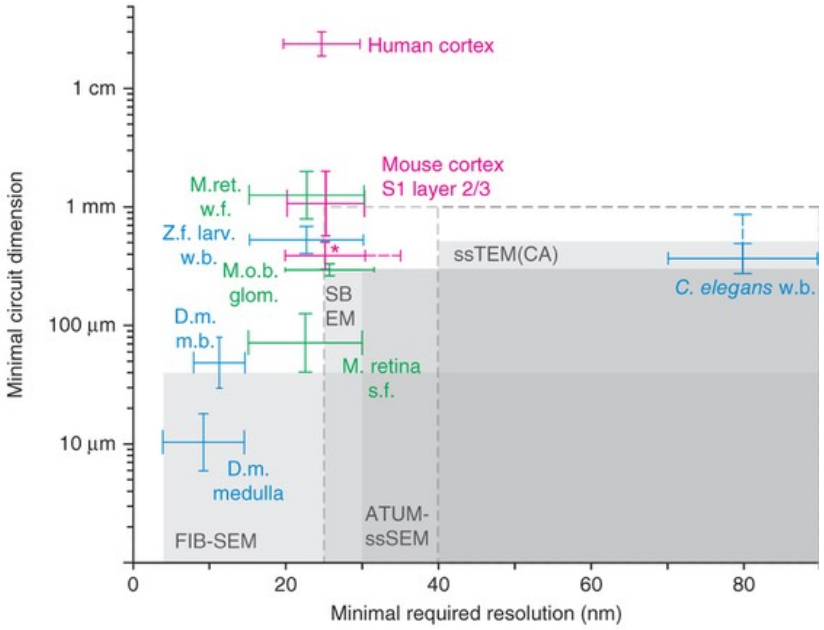
NIH el. mic. facility

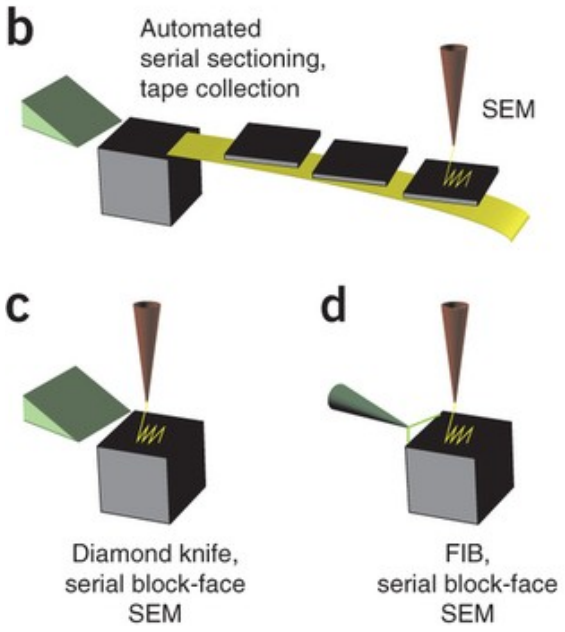


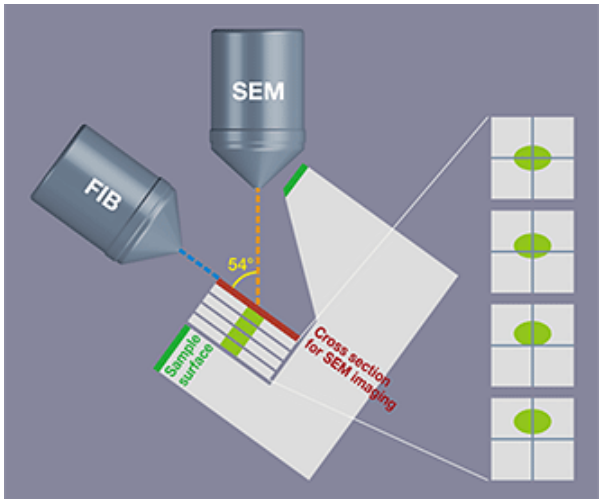
Block face imaging

Mechanical or FIB sectioning for SEM

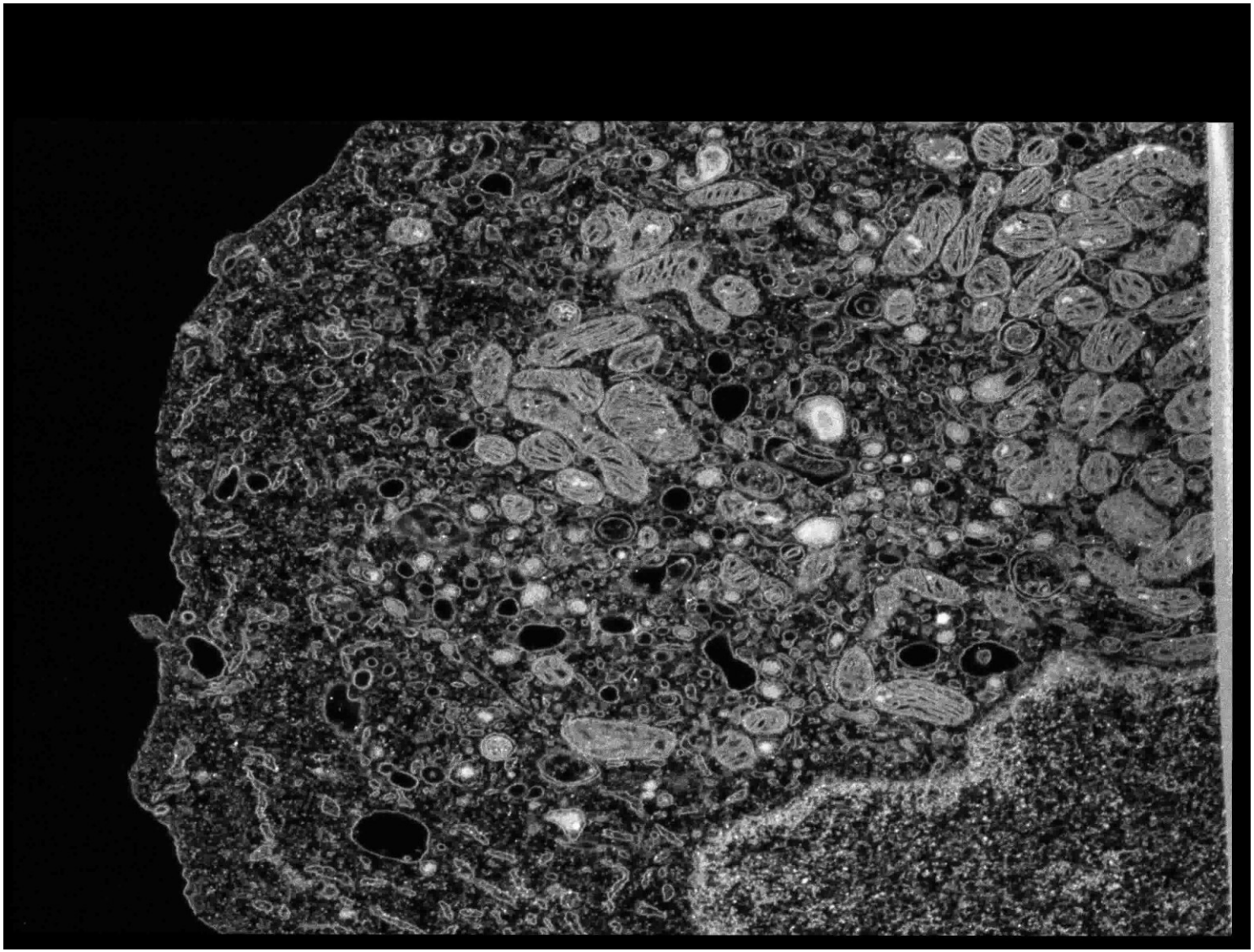
- detection of back scattered electrons
- mechanical sectioning either inside or outside SEM
- FIB sectioning (10nm)
- FIB-SEM tomography – correlative studies limited



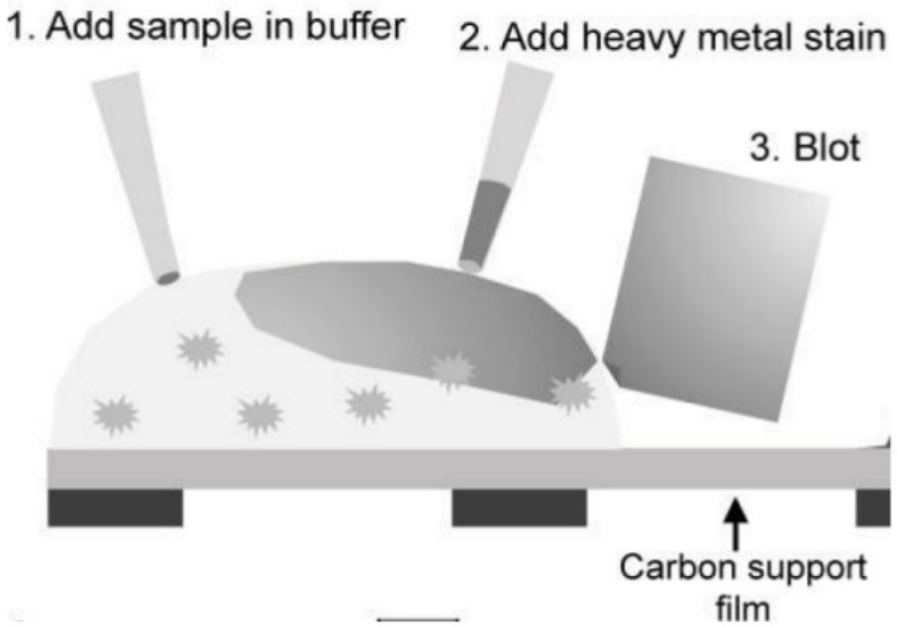


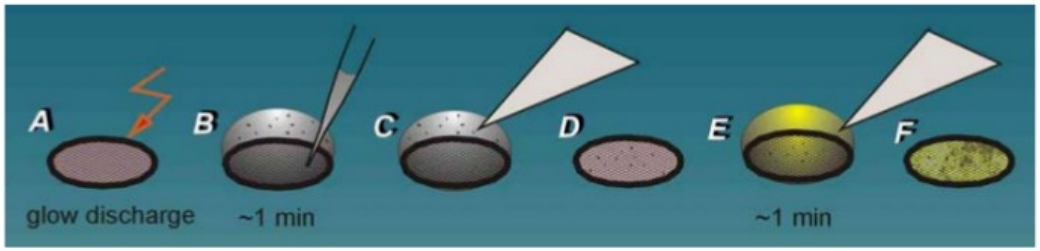


Block face imaging



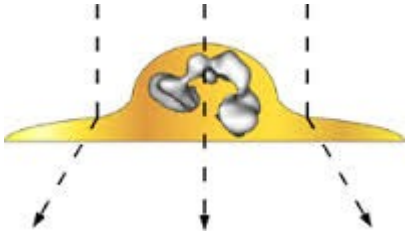
Heavy metal staining (negative staining)





Stains: uranyl acetate (pH=4)
uranyl formate (pH=4)
ammonium molybdenate (pH=7)
phosphorus thungstanate (pH=7)

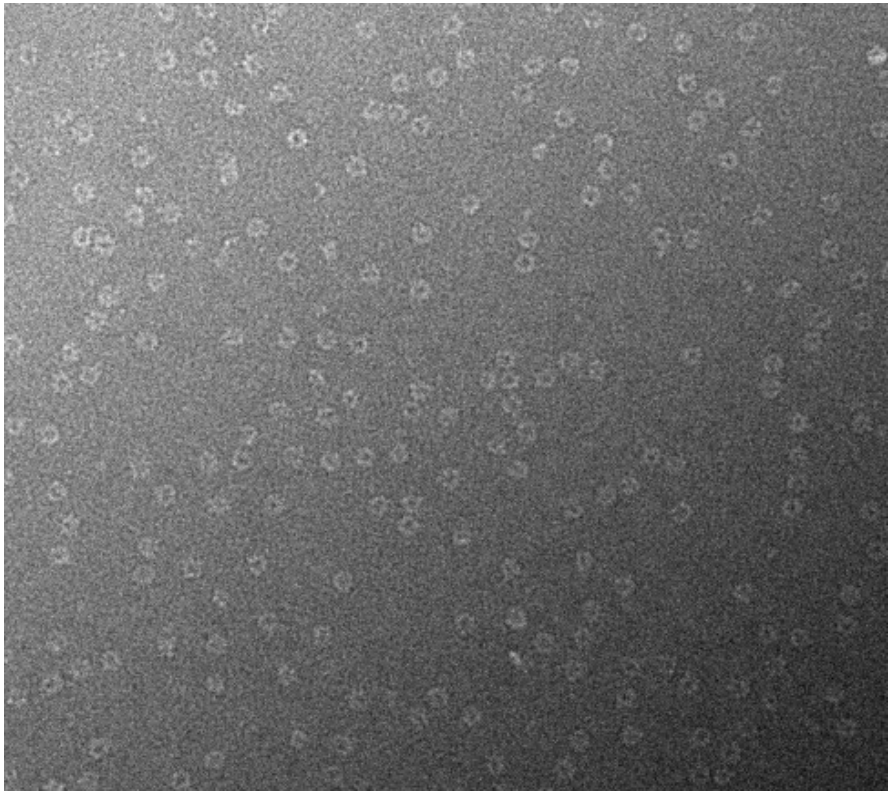
Heavy metal staining (negative staining)



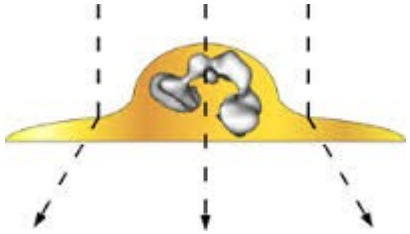
Pros:
- quick sample screening

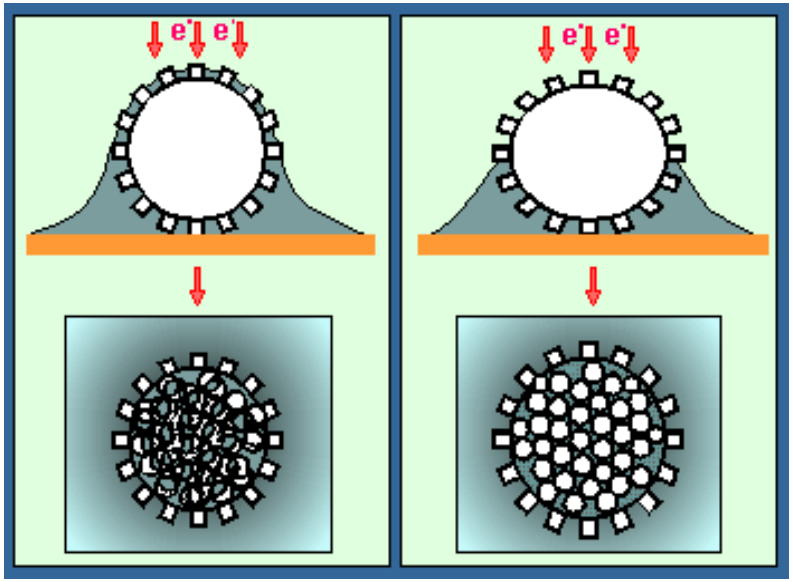
- high contrast

- less prone to beam damage



Heavy metal staining (negative staining)





Pros:
- quick sample screening

- high contrast

- less prone to beam damage

Cons:
- limited resolution (20A)

- flattening artefacts

- denaturation of proteins

Cryo-EM techniques

Plunge freezing:

- rapid immersion of buffered sample into cryogen (liquid ethane, ethane:propane mix)

- vitrification has to be fast 10e4-10e5 K/s

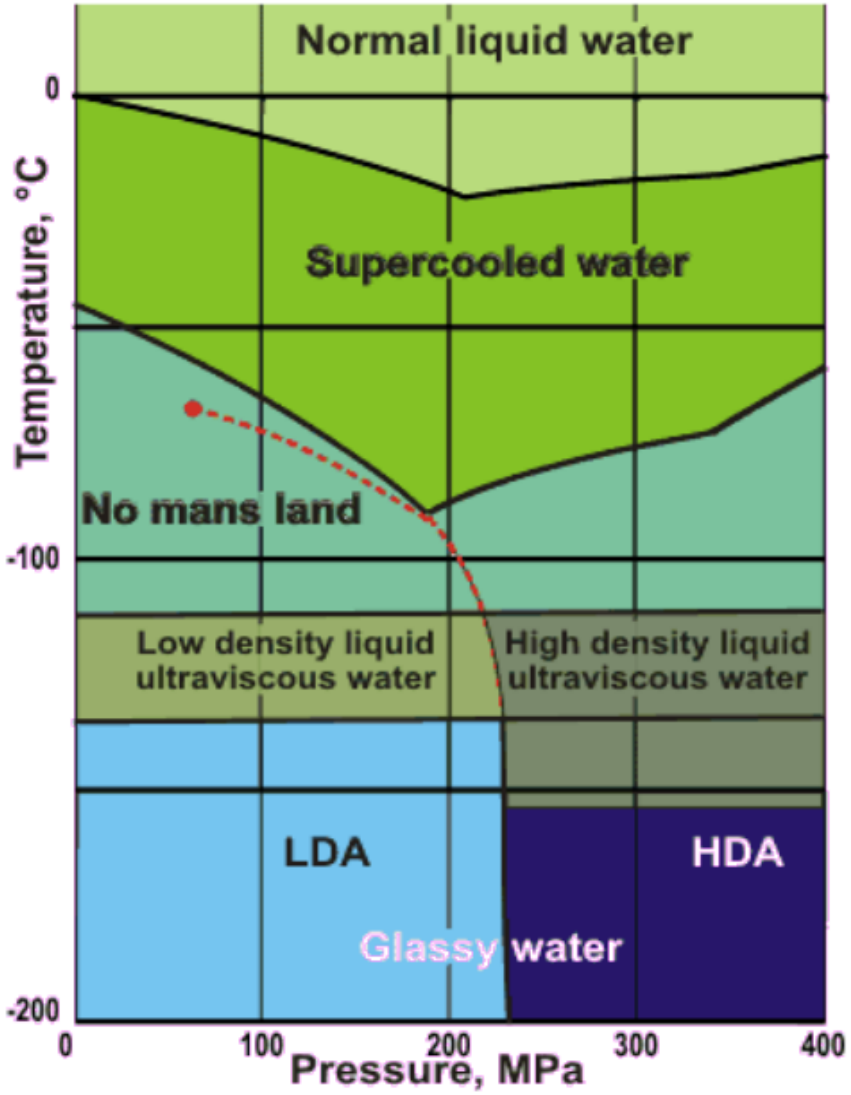
- available only for samples ~<10um thick

High pressure freezing

- sample thickness <200um

- freezing with liquid nitrogen

- 2000 bars, 20 ms



Cryo-EM techniques

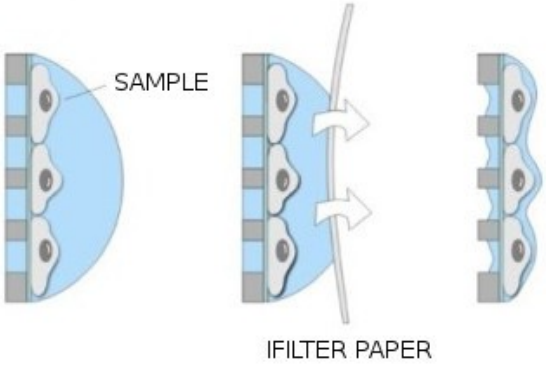
Plunge freezing

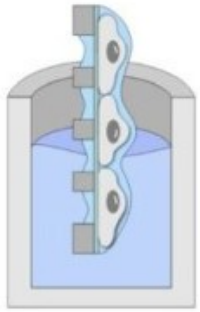
-
-

Cryogens	Melting point (°C)	Boiling point (°C)	Cooling rate (10^3 °C/s)	Relative cooling efficiency*
Ethane	-183	-89	-260 – -258	1.3
Liquid nitrogen	-210	-196	-272	0.1
Propane	-189	-42	-263 – -261	1.0
Freon 22	-160	-41	-267 – -265	0.7

3-4ul
mg/ml for purified protein complexes
OD~0.5 for bacteria

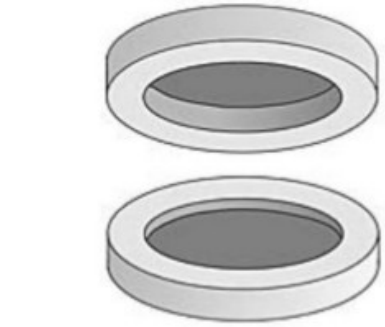
3mm diameter

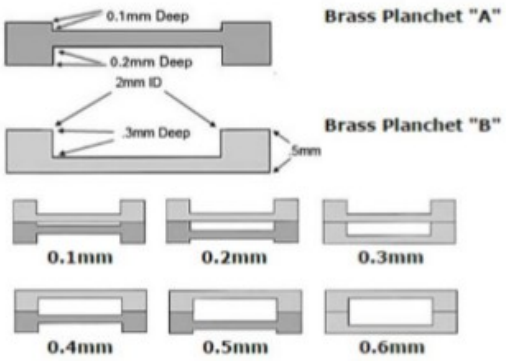




Cellular cryo-EM techniques

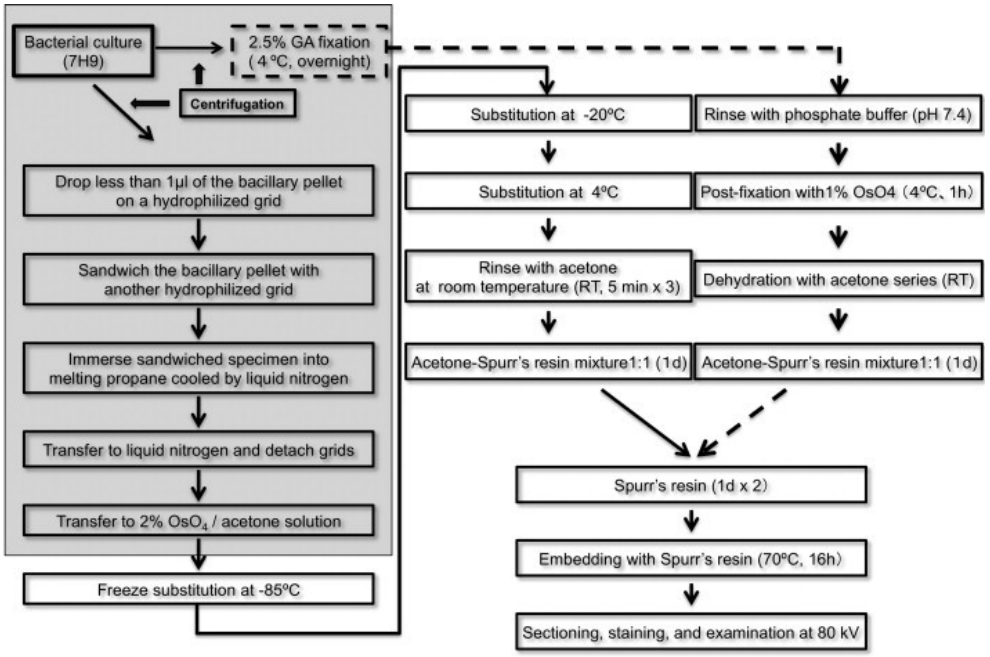
High pressure freezing, freeze substitution





www.leica-microsystems.com

Freeze substitution
- reduction of ultrastructure changes compared to dehydration at ambient temperature
- dehydration at temperatures <-70C (aceton typically -90C)
- fixatives are evenly distributed before cross-linking at ambient temperature
- resin embedding for ultramicrotomy at room temp.



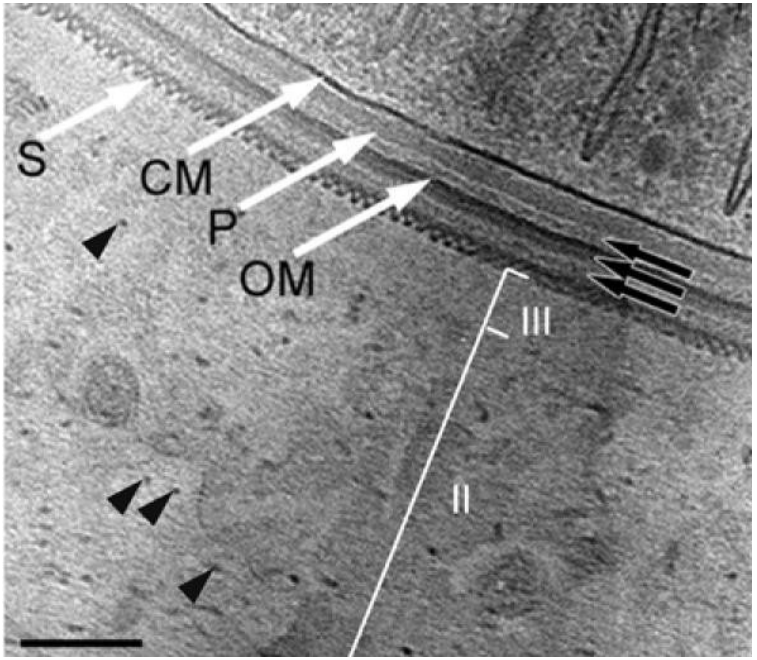
Yamada et al. JMM 2010

CEITEC

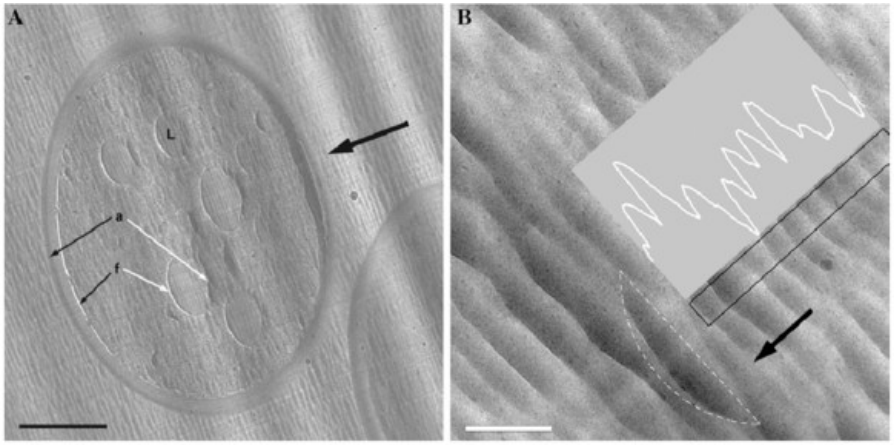
Cellular cryo-EM techniques

CEMOVIS – cryo-EM of vitrous sections

- - no chemical fixation, dehydration or contrasting
- low contrast
- preservation of the sample in near-native conditions
- mechanical sectioning by ultramicrotome at LN2 conditions
- sectioning artefacts



Al-Amoudi et al. EMBO J 2004

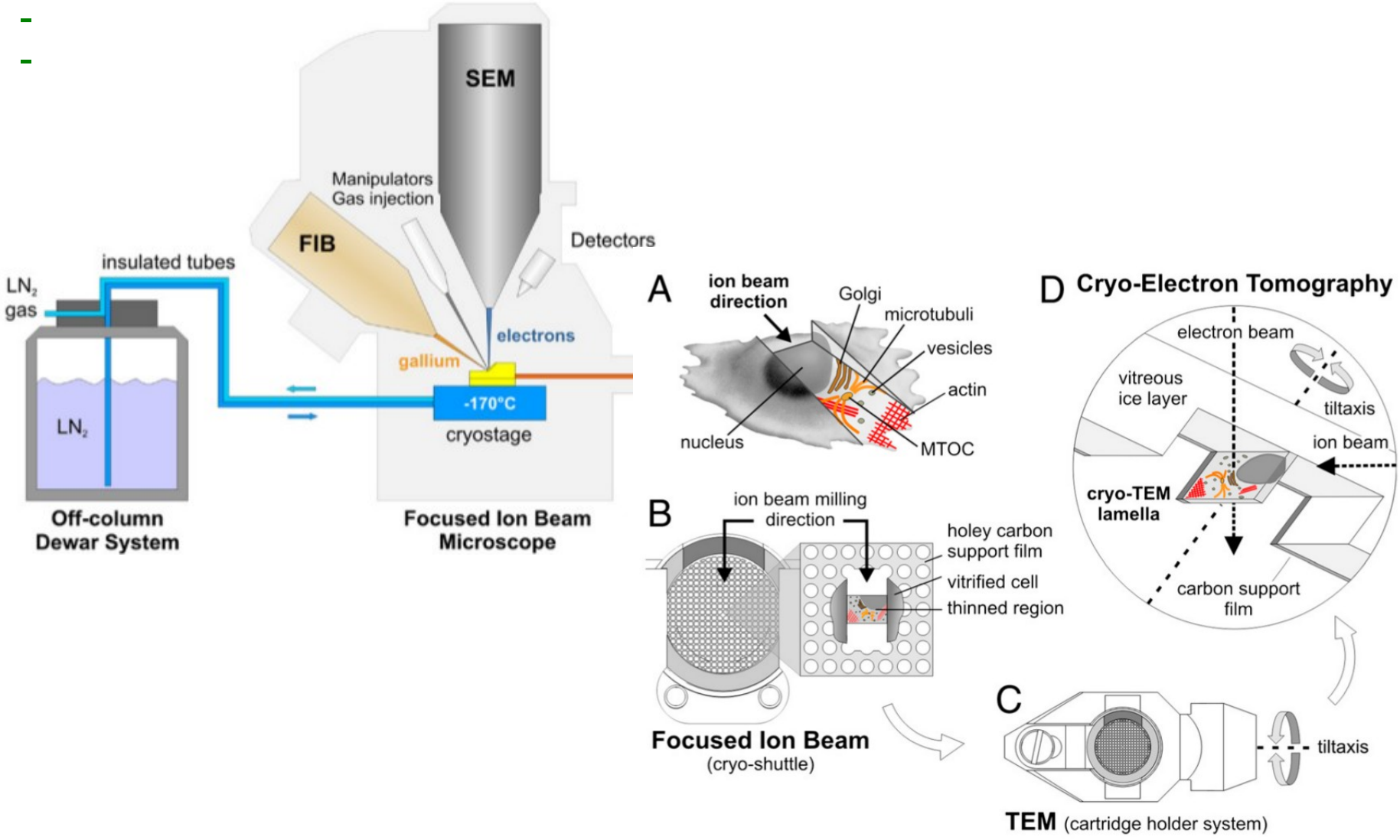


Al-Amoudi et al. JSB 2005

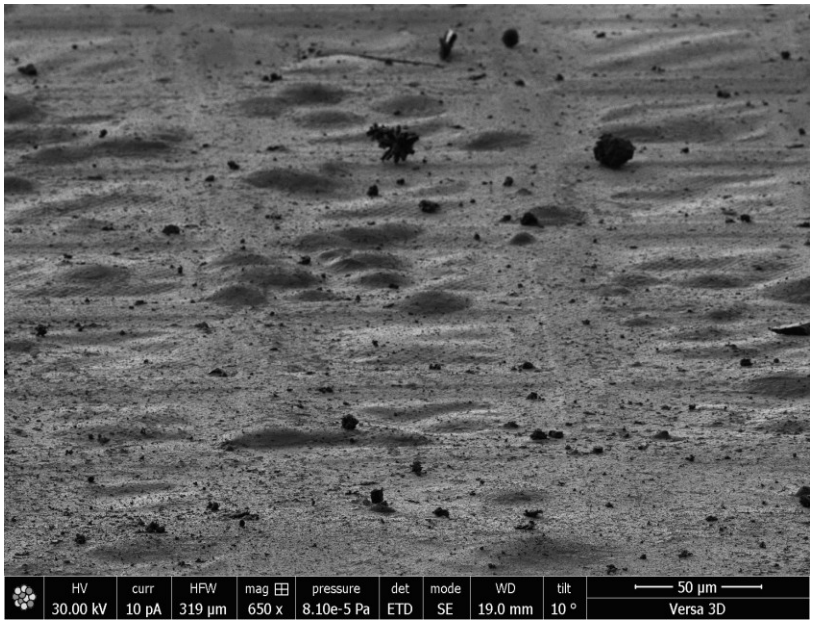
CEITEC

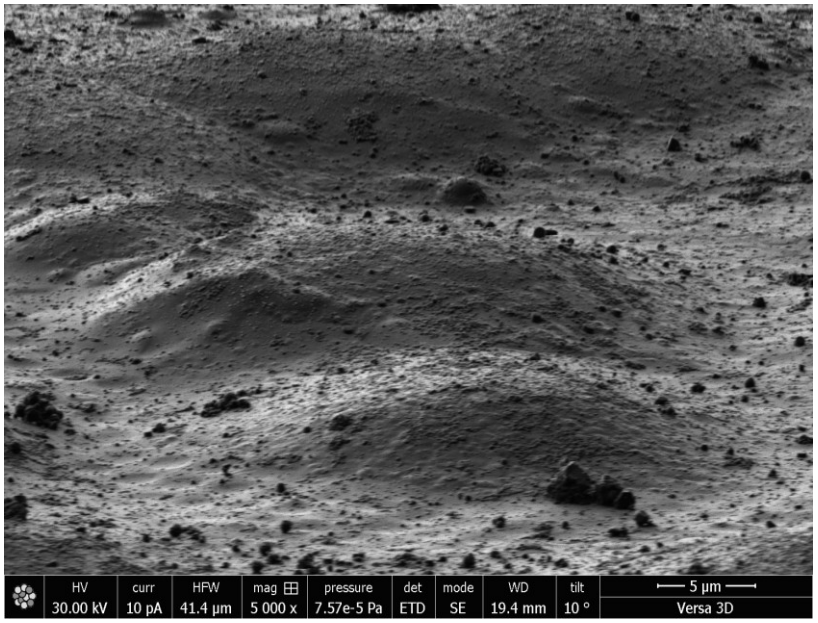
Cellular cryo-EM techniques

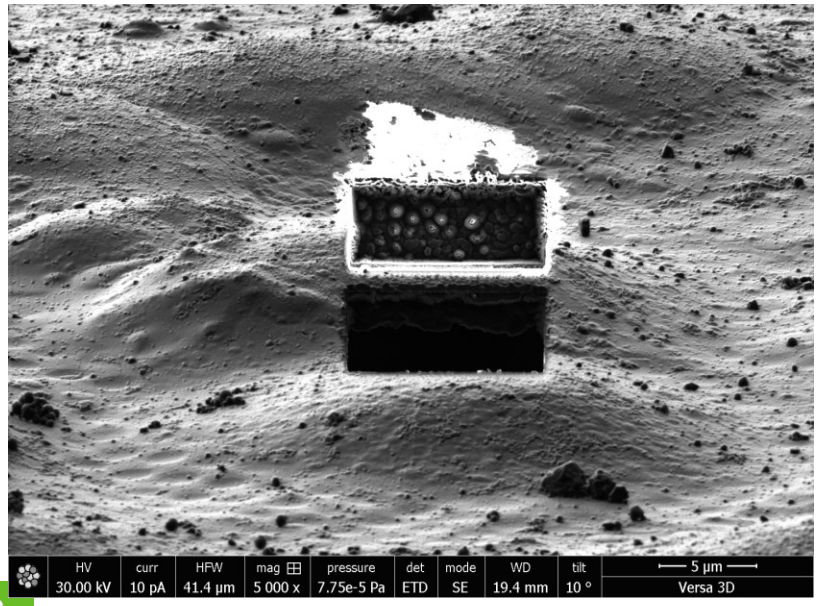
Focused ion beam milling of cellular lamellas

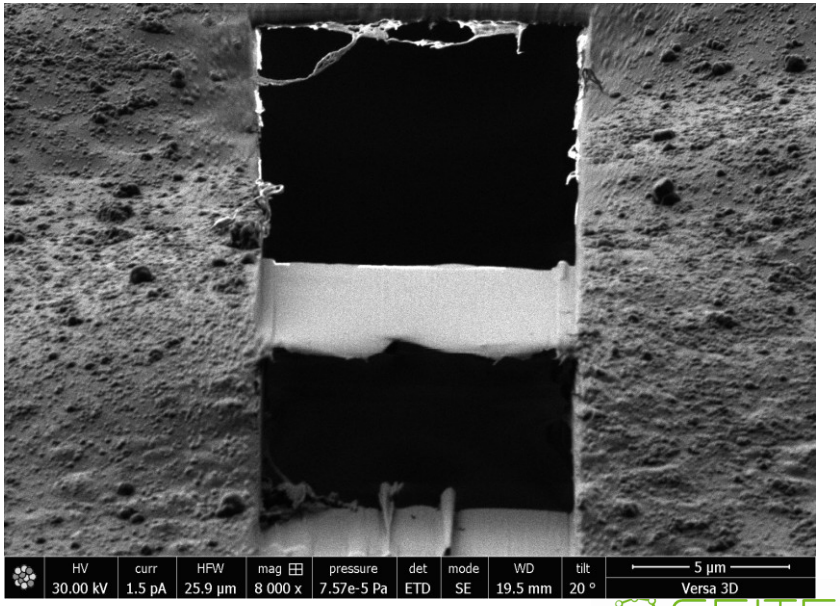


Cellular cryo-EM techniques



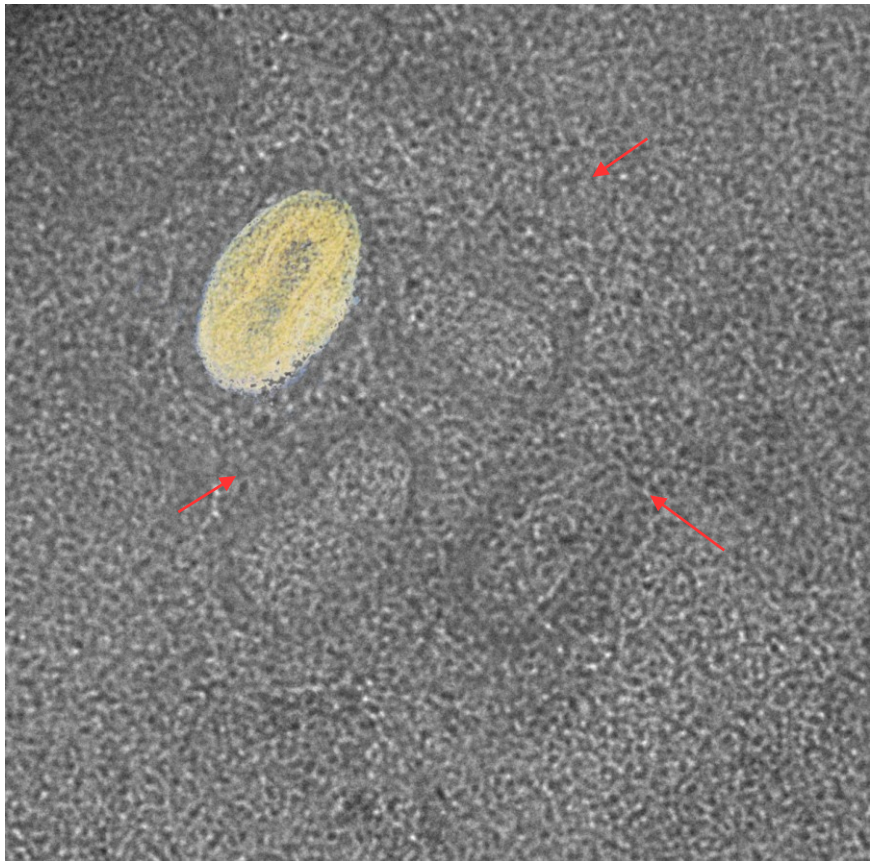






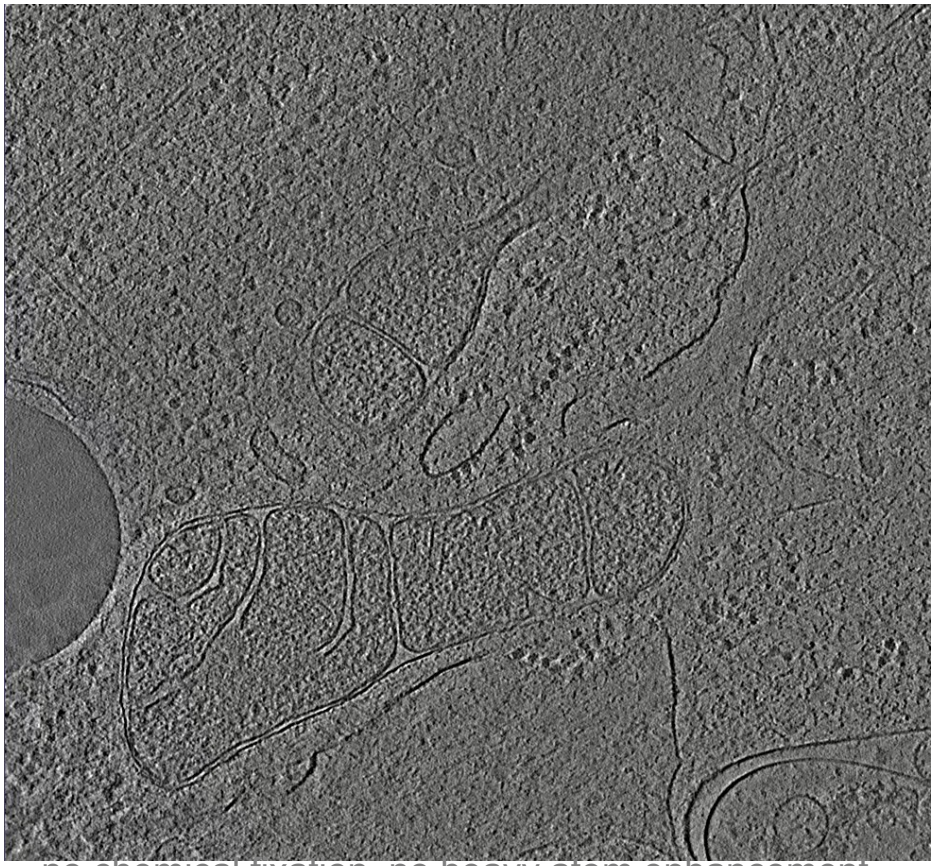
Cellular cryo-EM techniques

Vaccinia virus inside cell



Pavel Plevka group

HeLa cells



- no chemical fixation, no heavy atom enhancement

- true, near-native representation of the cellular interior

- low contrast

Thank you for attention

jiri.novacek@ceitec.muni.cz

CIISB
Czech Infrastructure for Integrative Structural Biology

instruct ERIC

iNEXT DISCOVERY