Cryo-Electron Microscopy in Structural Biology Research

Simone Mattei
EMBL Imaging Centre
Team Leader
EM Service and Technology Development

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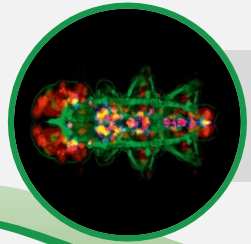
European Molecular Biology Laboratory (EMBL)

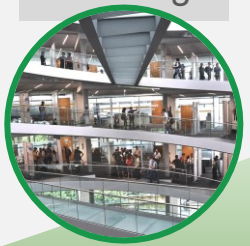
Europe's intergovernmental laboratory for life science research

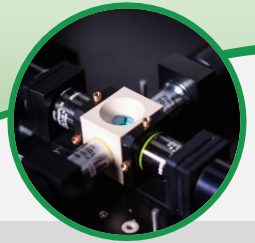


Excellent Research

Scientific Services

Advanced Training





Innovation and Translation

Integrating life sciences

1900 people	**96** nationalities	**27** member states	**729** publications	**82 million** daily web requests to EMBL-EBI data services	**6700** annual user visits to experimental services

2020 metrics shown

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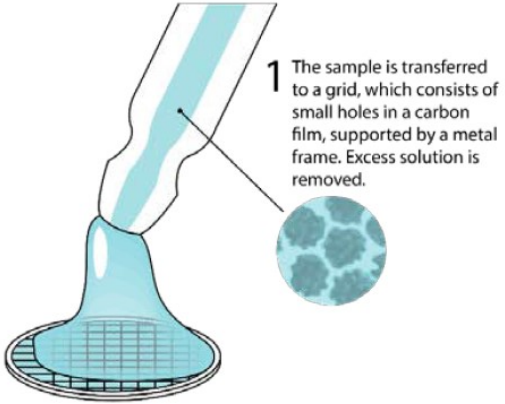
Cryo-Electron Microscopy



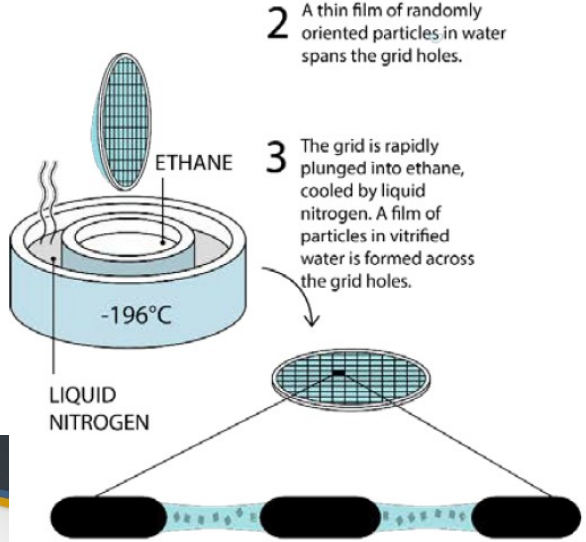
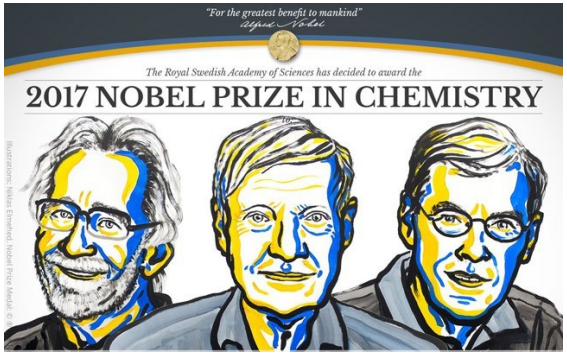
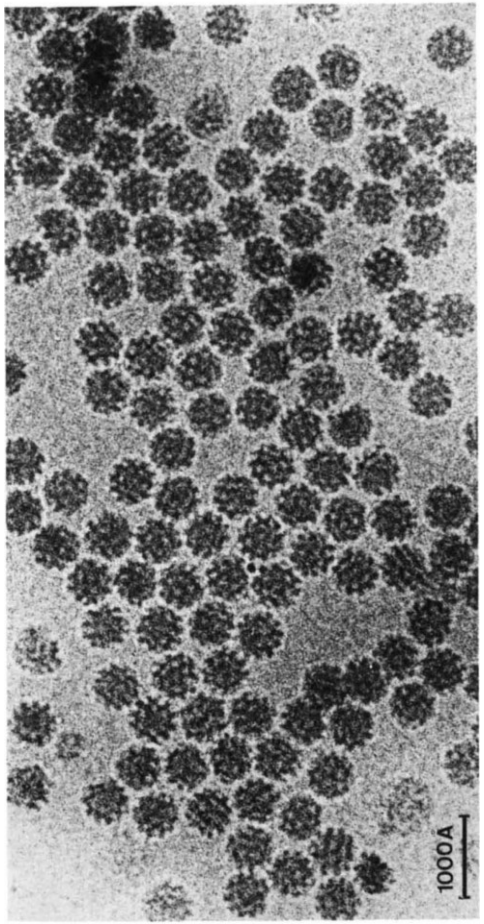


Illustration: © Johan Jarnestad - The Royal Swedish Academy of Sciences.





Adrian et al., 1984 Nature

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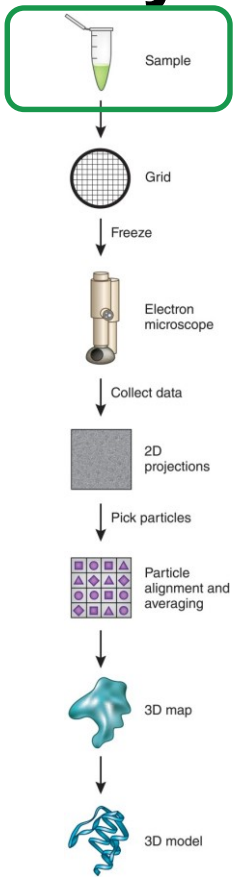
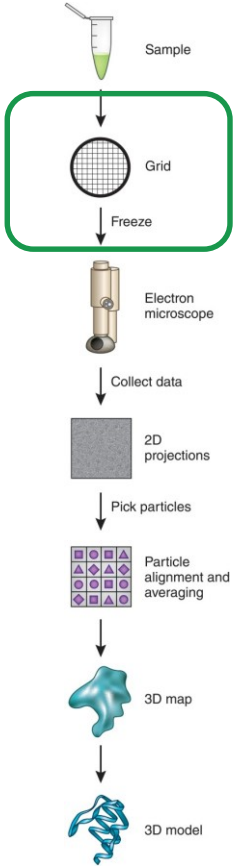
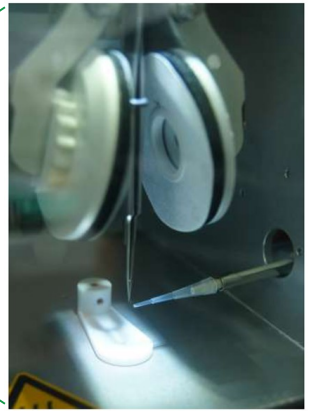
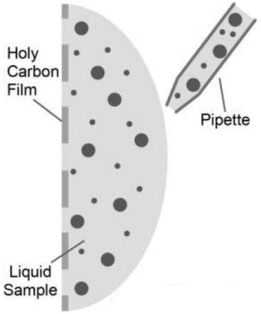
Cryo-EM workflow – first things first, biochemistry!

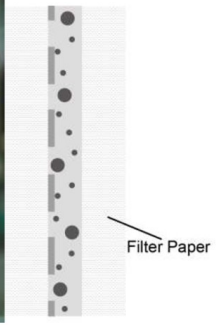


Cryo-EM workflow – Sample preparation

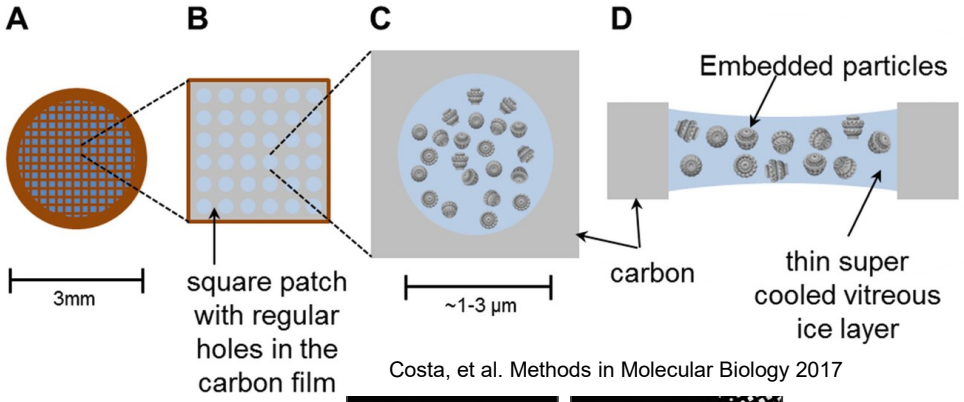




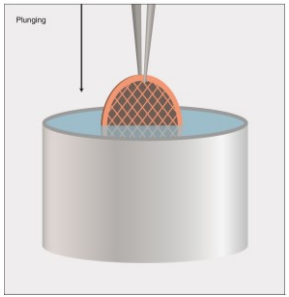




www.hic.ch.ntu.edu.tw

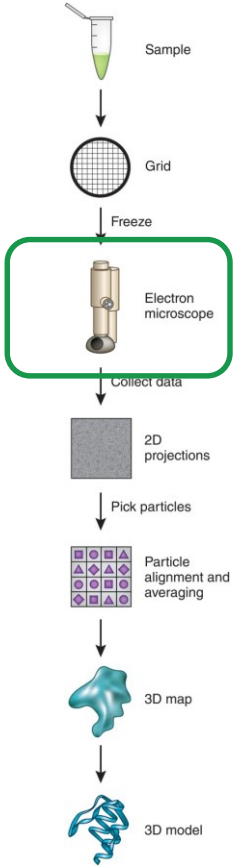


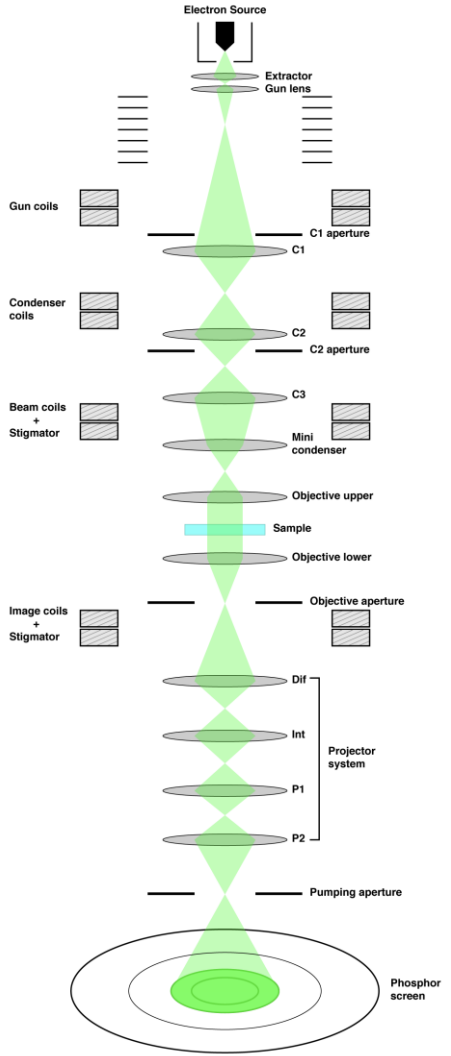
Costa, et al. Methods in Molecular Biology 2017



Weissenberger, et al. Nat Methods 2021

Cryo-EM – Image Acquisition





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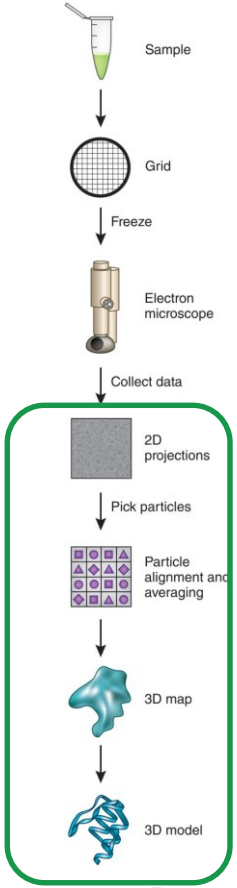
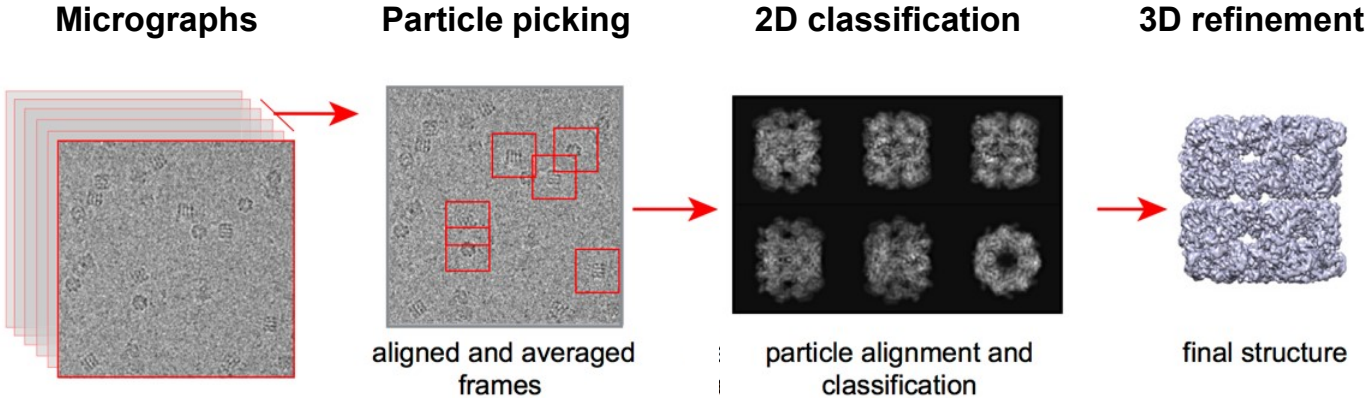
Cryo-EM – Image Processing





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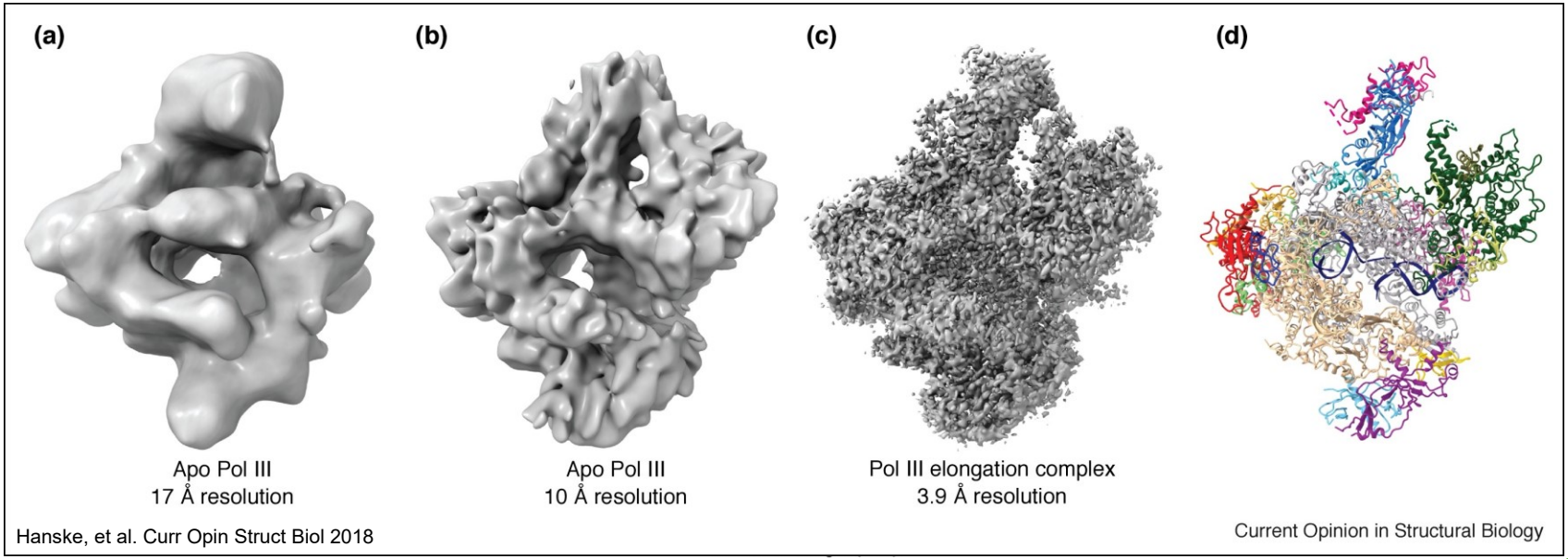
Cryo-EM and the “Resolution Revolution”

- Hardware (better transmission microscopes, direct electron detectors)
- Software (image processing capabilities and user friendliness)



EMBL Imaging Centre

Open access
to new imaging technologies

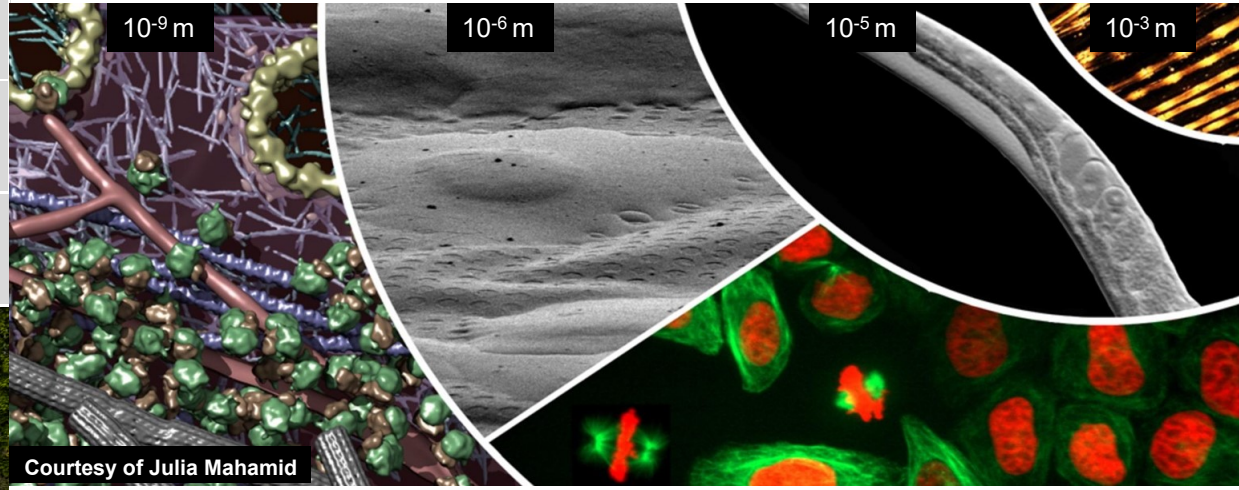
Tailored project support
by expert technical staff

Advanced training
of users and facility staff

Commercialisation
of new technologies and workflows

Imaging across the scales of biology

Molecules ▶ Molecular Machines ▶ Cells ▶ Tissues/Organisms



Structural analysis ◀ Functional characterization ◀ Understanding health & disease

Open call - Autumn 2021

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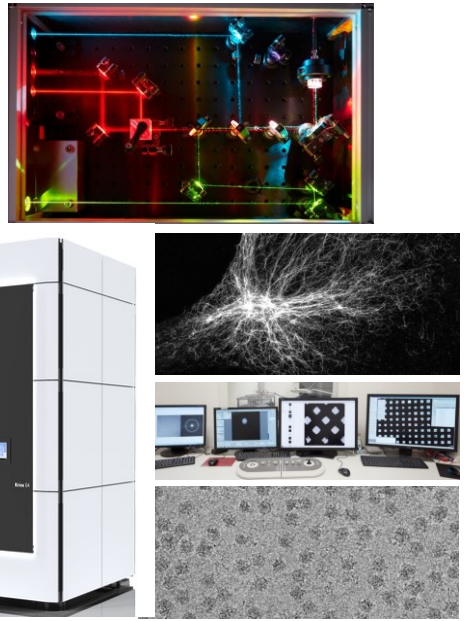
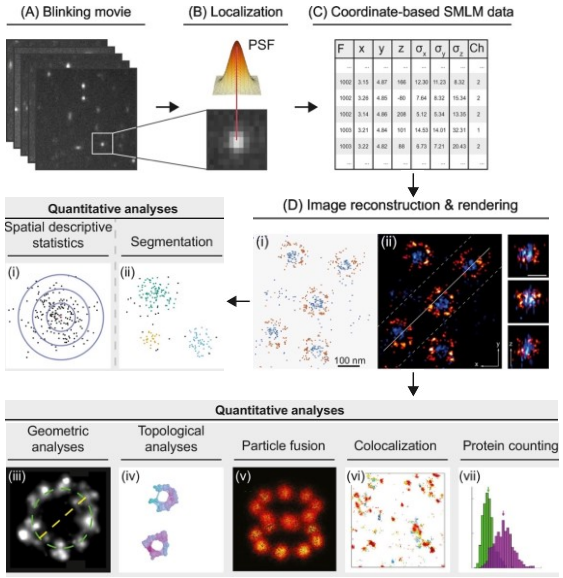
Tailored Project Support

Sample Preparation

Image Acquisition with High-Tech Instrumentation



Data Analysis



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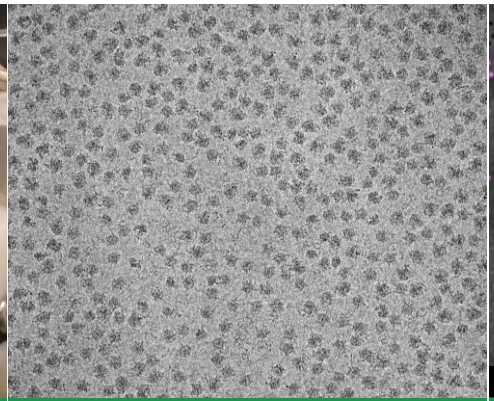
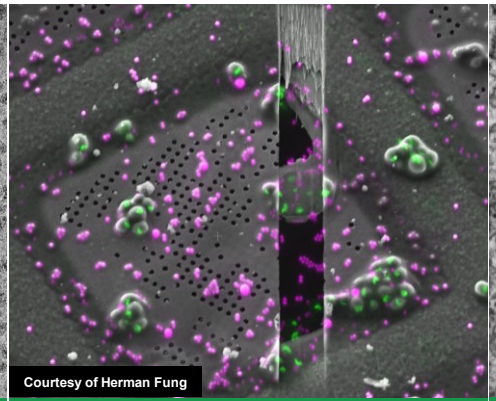
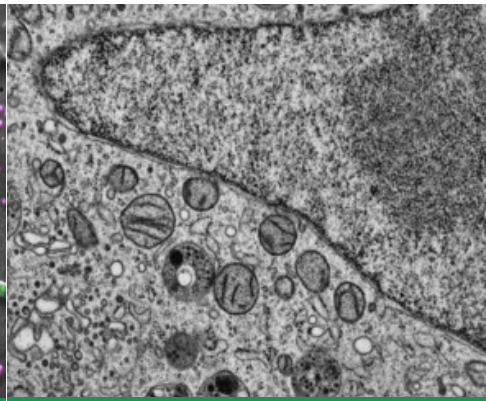
Tailored Support for cryo-EM and CLEM workflows

Sample Preparation	Image Acquisition with High-Tech Instrumentation		Data Analysis
			
Sample vitrification	**TEM data collection**	**Cellular tomography**	**Volume imaging**

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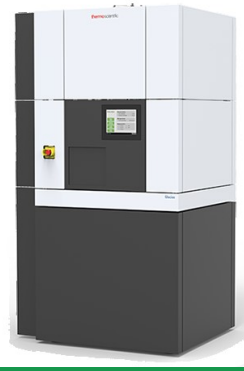
Instruments for EM and CLEM

Titan Krios G4

- Single particle analysis
- High-res tomography
- Cellular tomography



Glacios

- Sample screening
- Single particle analysis

Aquilos 2

- FIB-milling
- Lift out (coming soon)

Crossbeam 550

- FIB-milling
- volume imaging

LSM 900 Airyscan 2

- Cryo-Confocal LM

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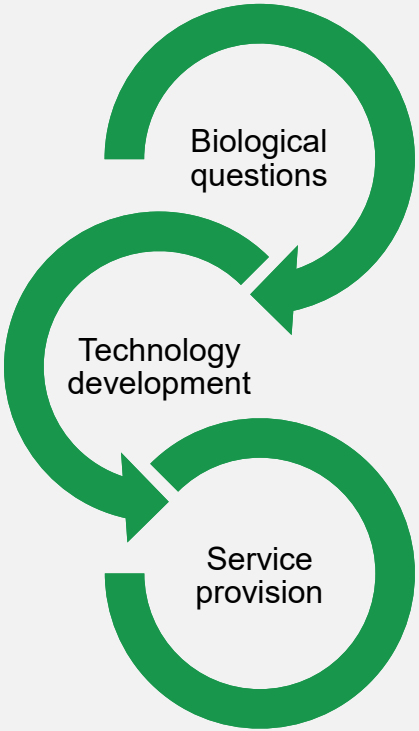
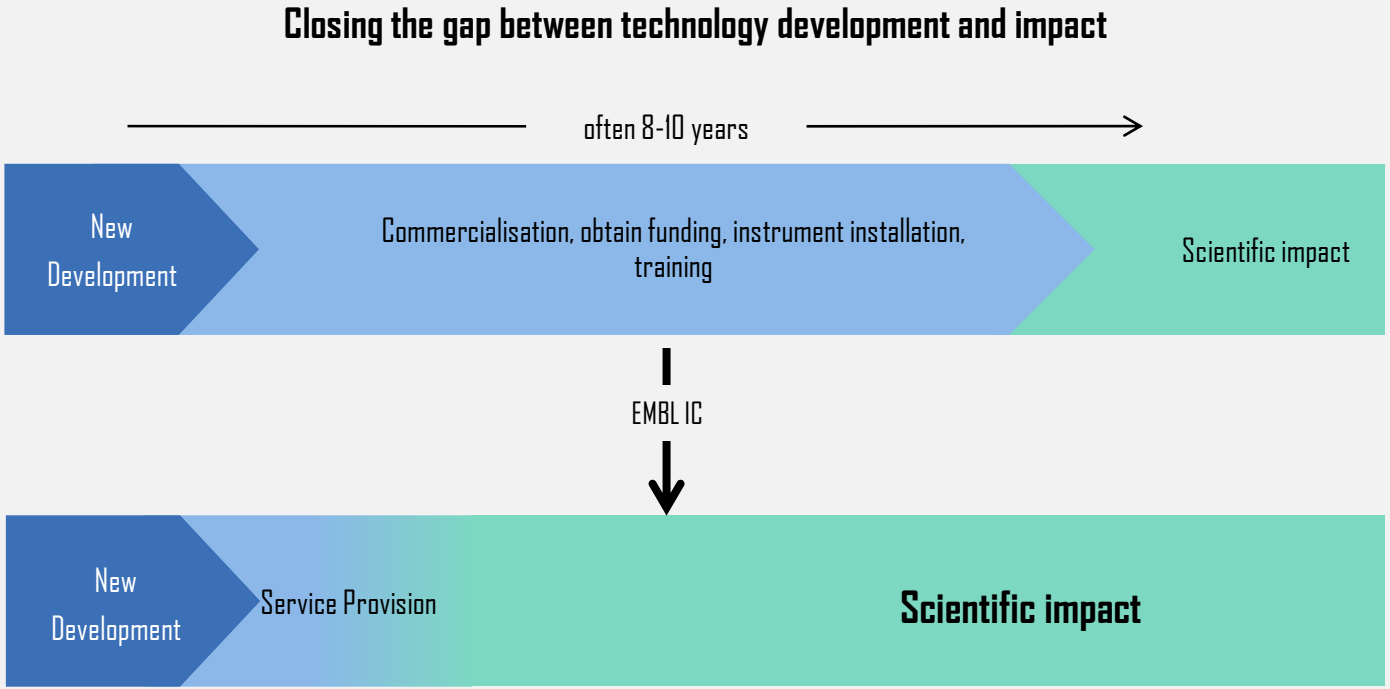
Technology development and Open Innovation





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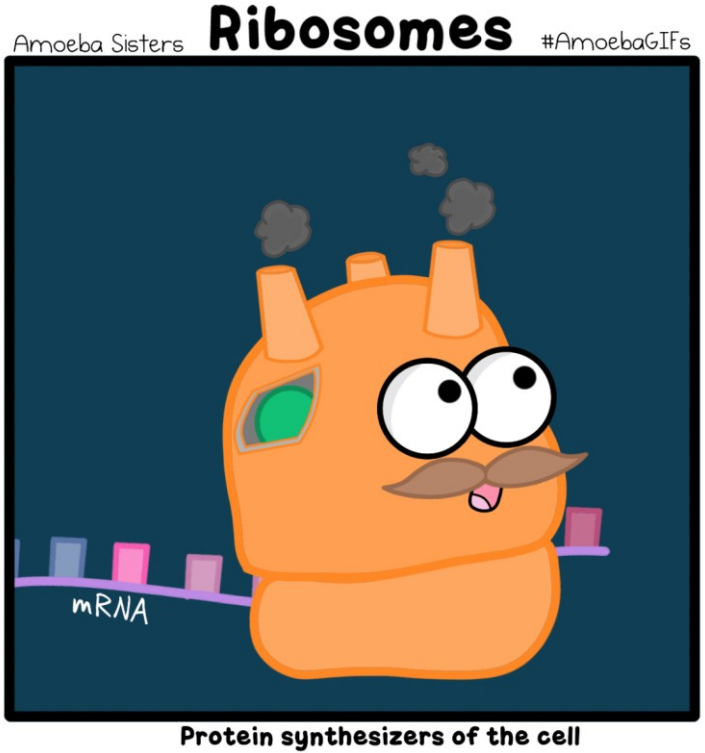
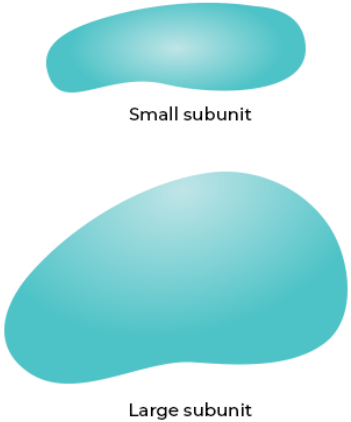
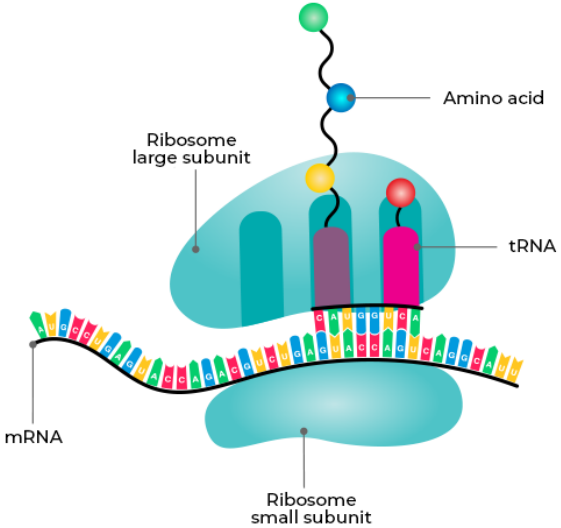
Protein translation in a cell – the ribosome







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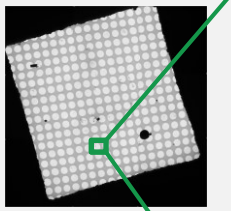
Cryo-EM services: from sample to structure

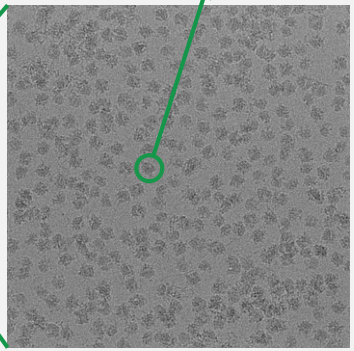
Sample preparation for cryo-EM by rapid plunge freezing

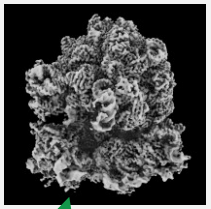
Sample screening to identify grids suitable for high-end data collection

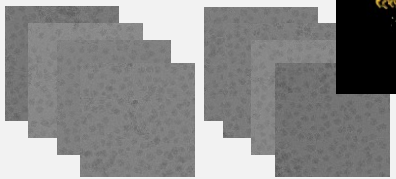
High-end data collection and data analysis

Purified 70S ribosomes









1.55 Å resolution

Fromm, et al. Nat Commun 2023

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Cryo-EM services: from sample to structure

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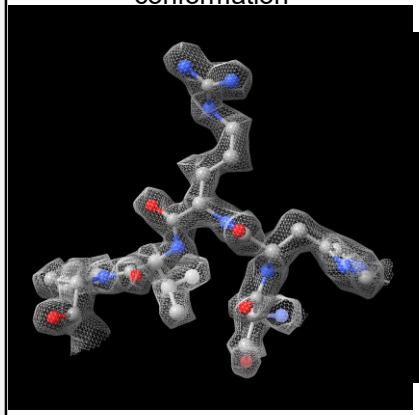
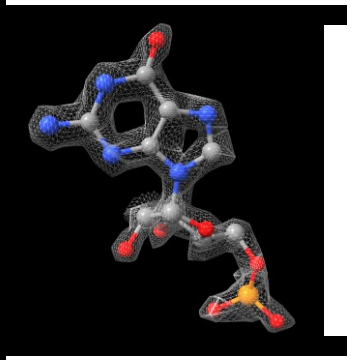
Sample screening to identify grids suitable for high-end data collection

High-end data collection and data analysis

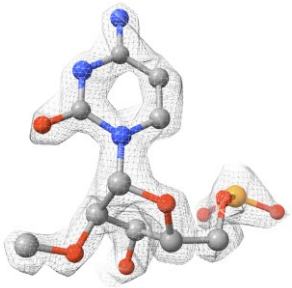
Side chain conformation



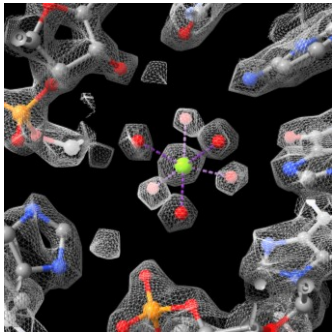
Nucleic base conformation

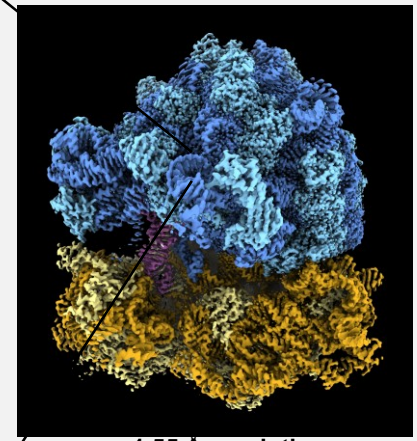


Chemical modification (2'-O-Methylcytidine)



Magnesium coordination by water molecules

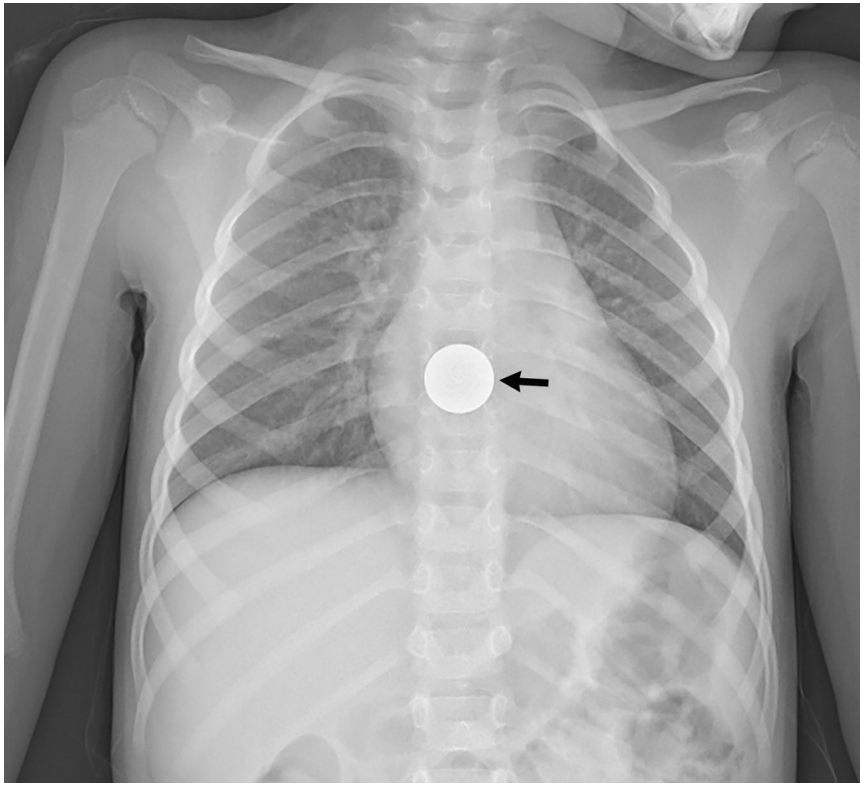




1.55 Å resolution

Fromm, et al. Nat Commun 2023

Tomography – the need of different views



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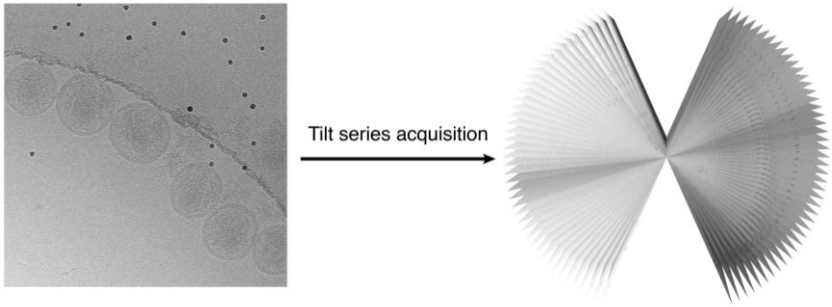
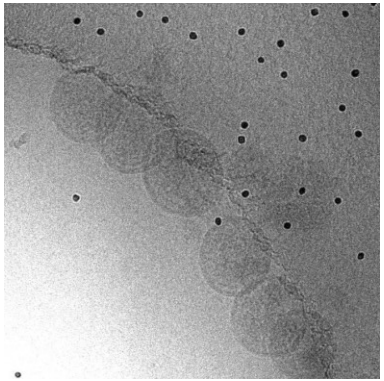
Tilt series acquisition



Tilt series



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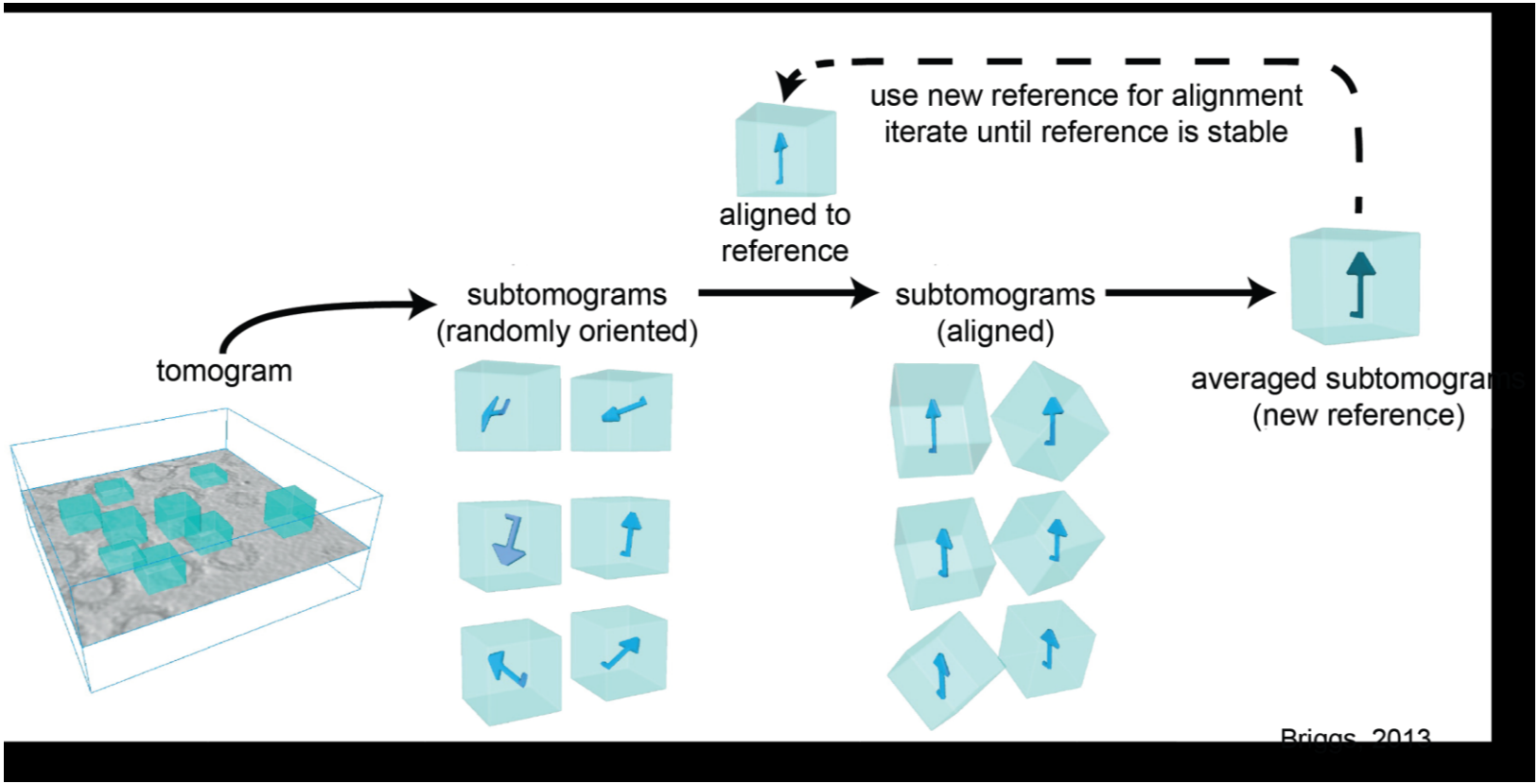
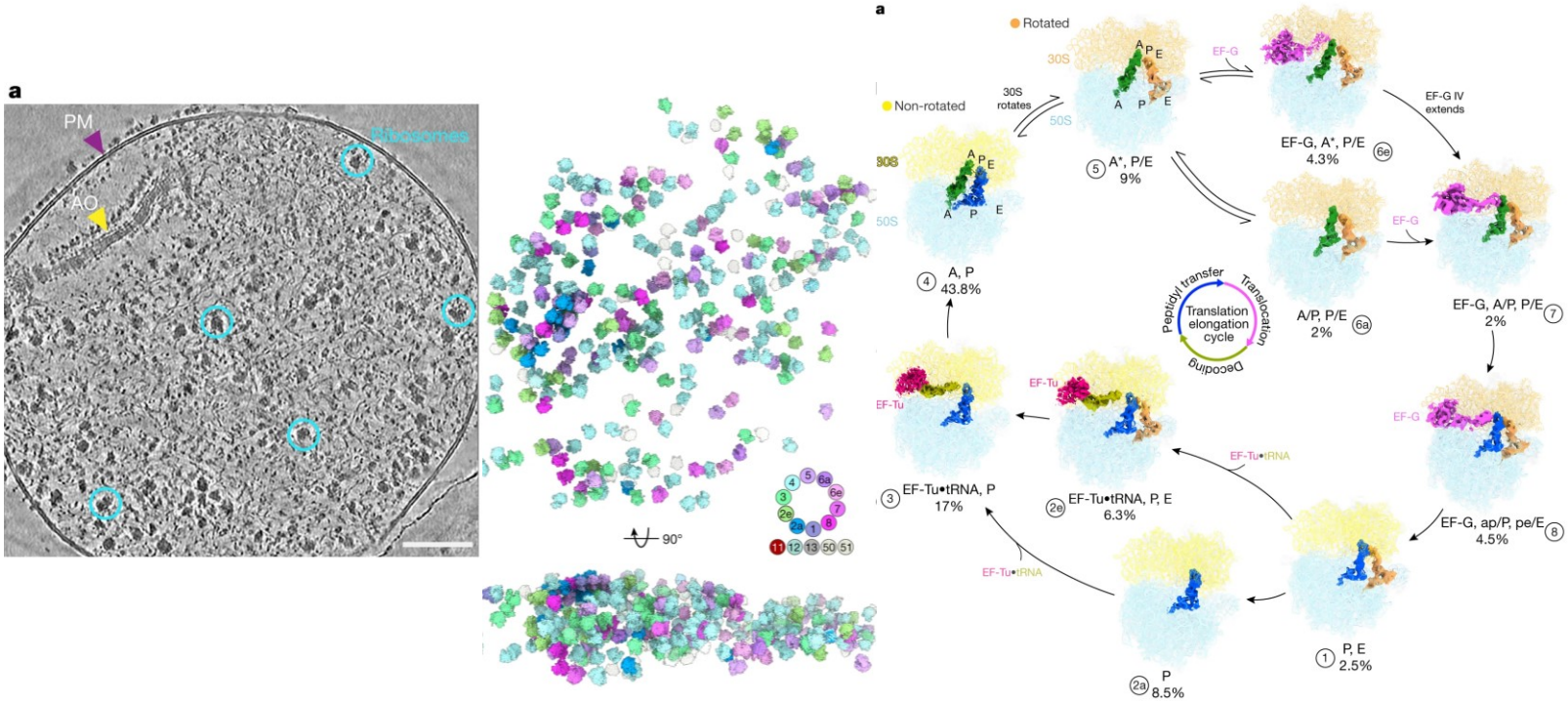
Subtomogram averaging



M. pneumoniae translation dynamics at atomic detail

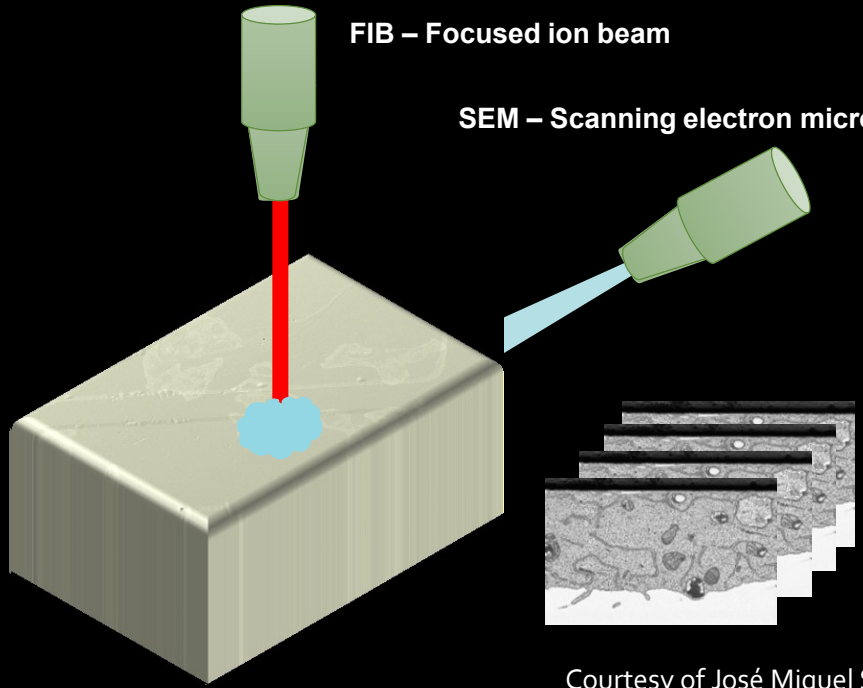


Xue, et al. Nature 2022

Focused Ion Beam – Scanning Electron Microscope

- x/y pixel size: 2-10 nm
- z slicing: 5-50 nm (tried up to 1 µm)
- Field of view: 10-30 µm²
- Imaging time/image: 1.5-2.5 min
- "Unattended" acquisition

  (1000-10.000 images)



Courtesy of José Miguel Serra Lleti and Anna Steyer

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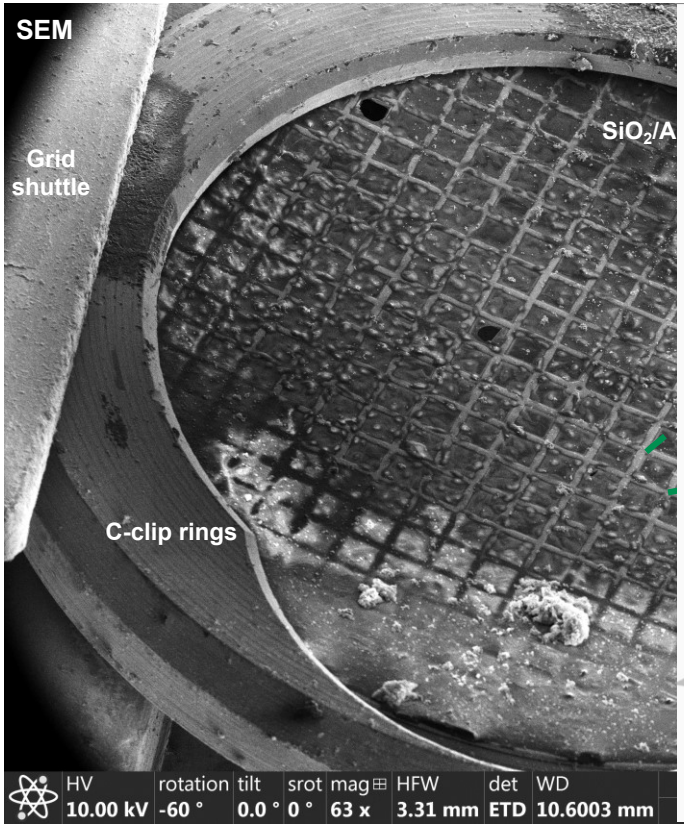
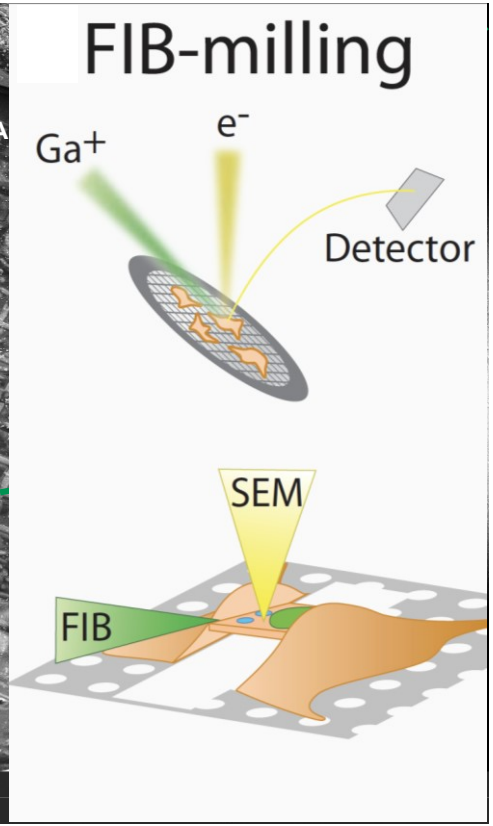
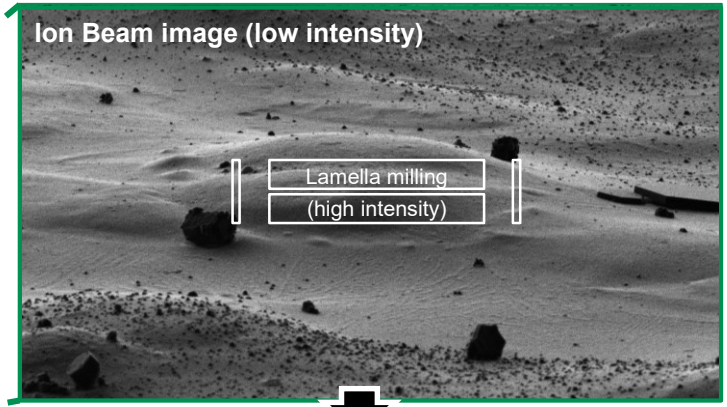
Spehner *et al.* 2020, *J Struct Biol*

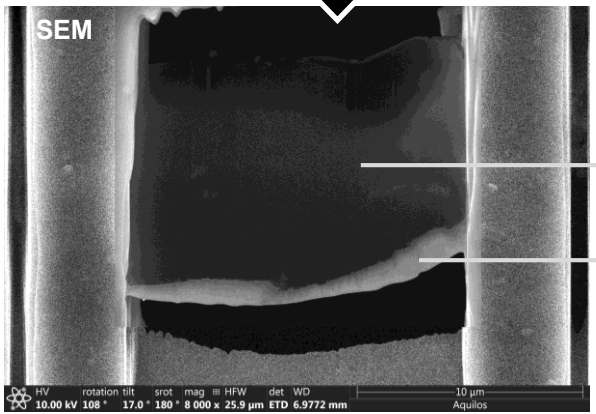
Courtesy of José Miguel Serra Lleti and Anna Steyer

Lamellae milling of vitreous HeLa cells









Blowing fork axis (during medium removal)

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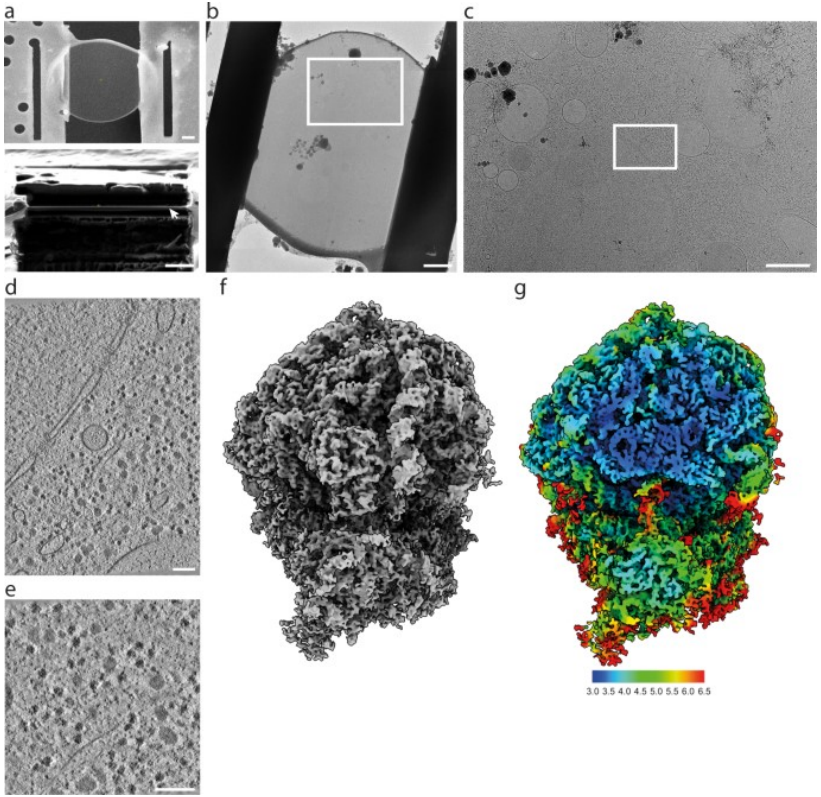
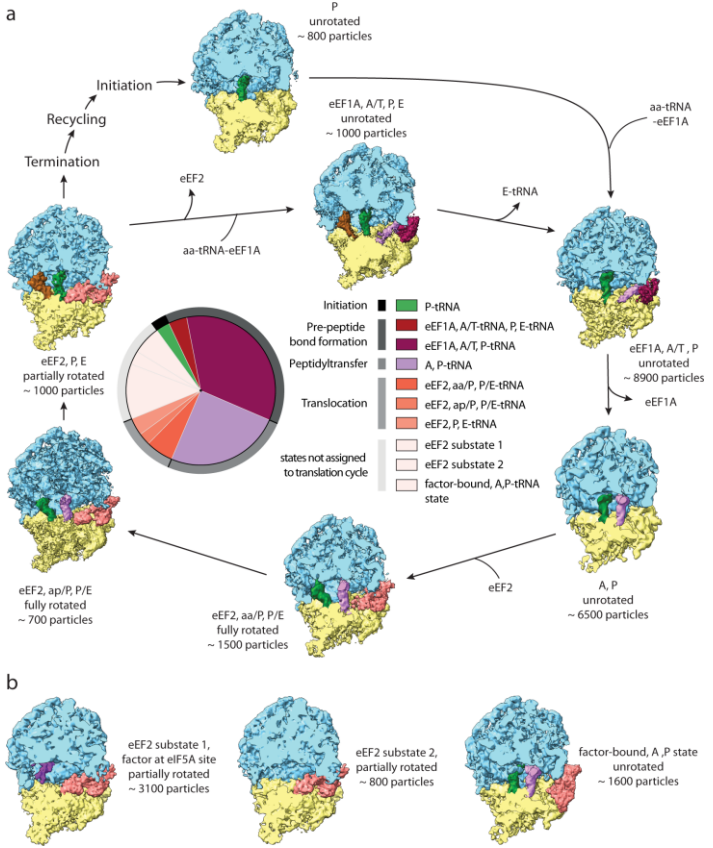
Eukaryotic ribosome translational states *in situ*





Hoffman, et al. Nature Communications 2023

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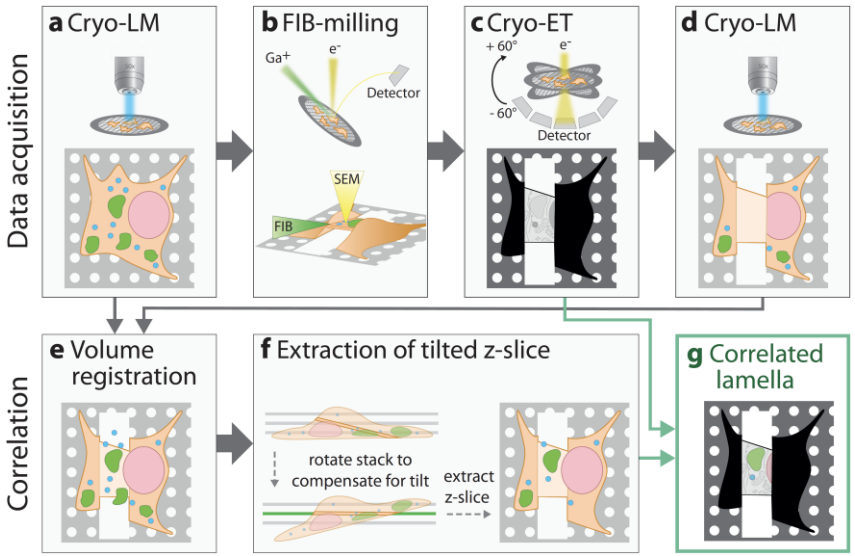
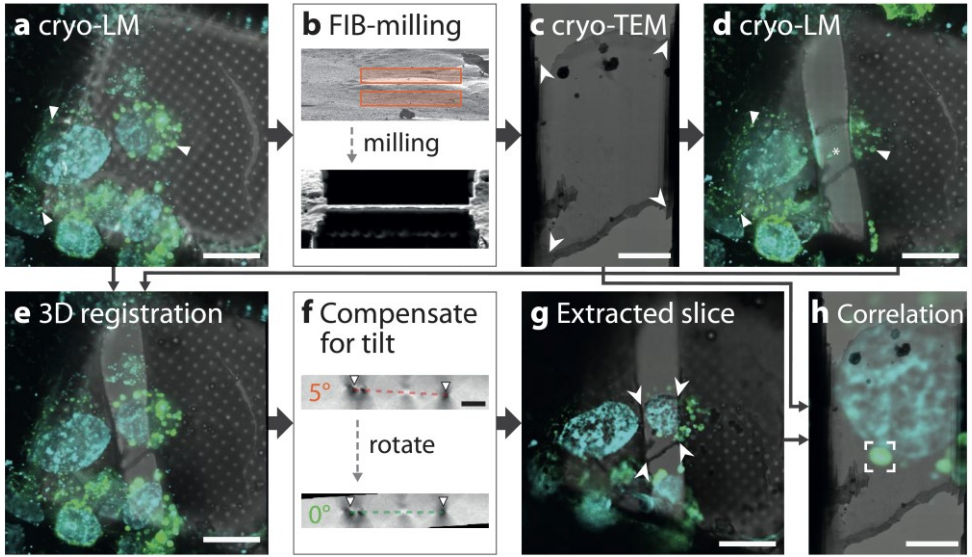
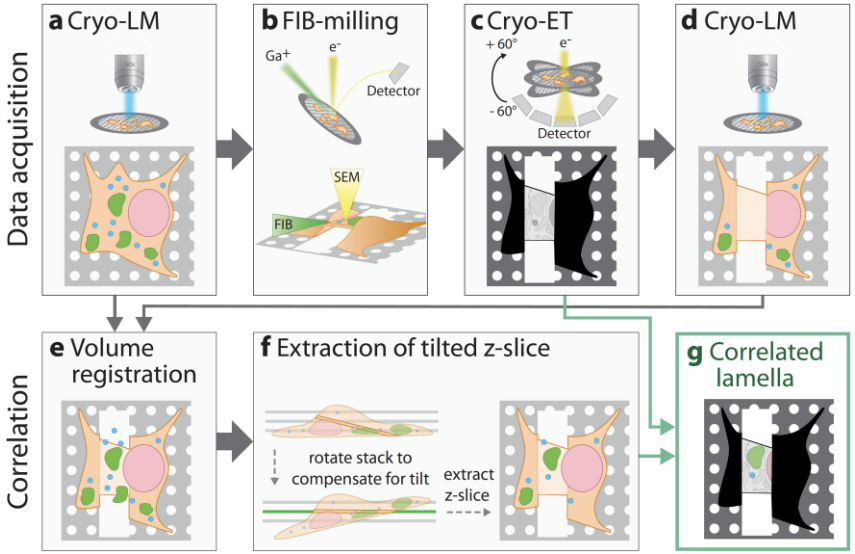
Cryo-CLEM & FIB-milling



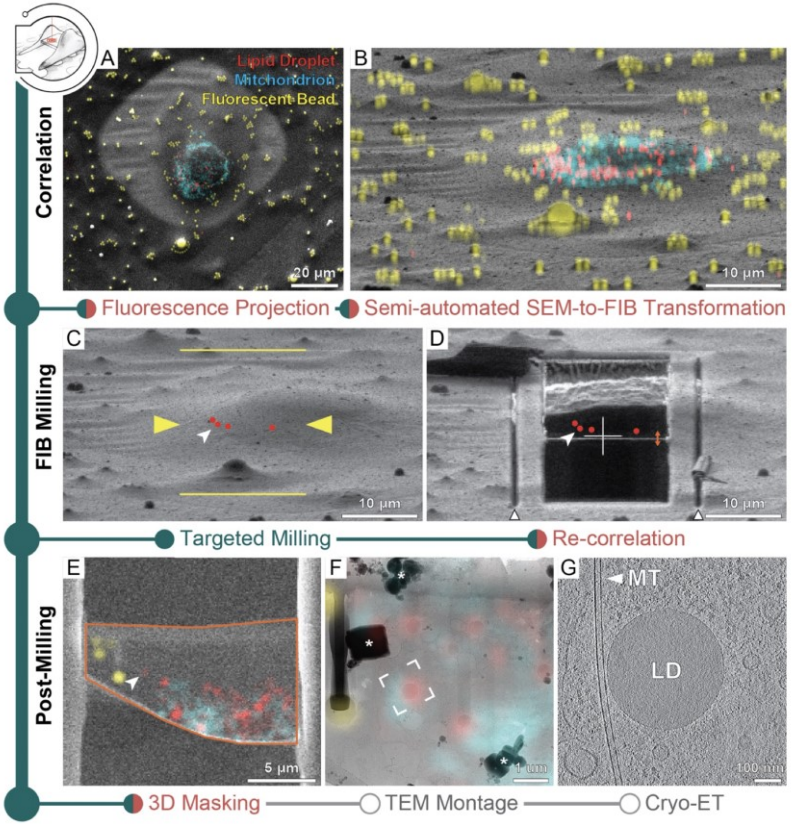


Klein, et al. Commun. Biol. 2021

Cryo-CLEM & FIB-milling



Klein, et al. Commun. Biol. 2021



Klumpe, et al., BioRxiv 2021

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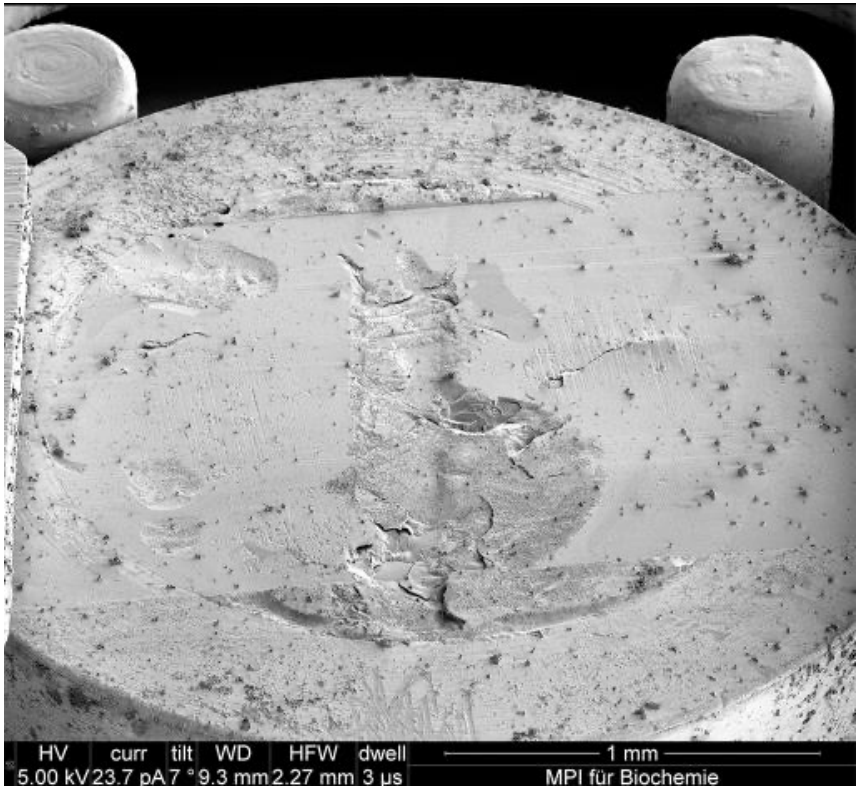
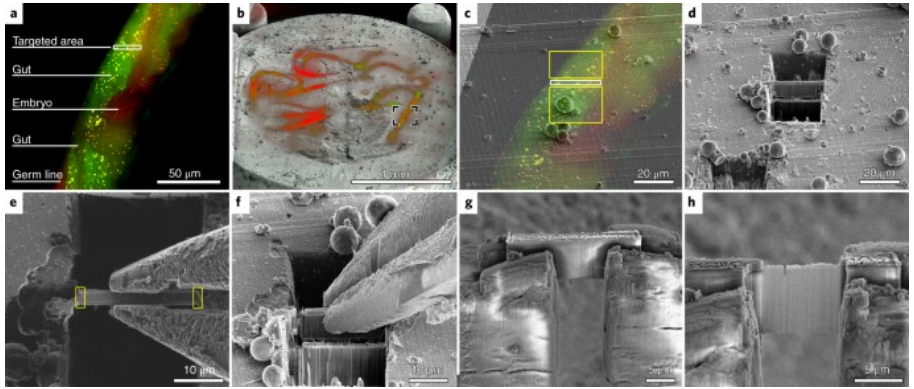
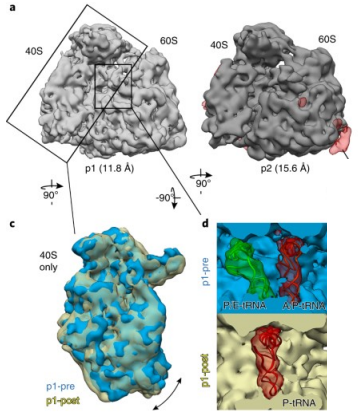
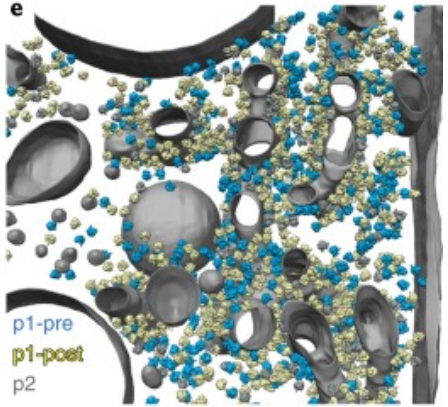
The Future – targeted cryo-lift out









Schaffer, et al. Nature Methods 2019

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HEIDELBERGCEMENT

EIPOD4

CLEM workflows – many steps, many problems…

Sample Preparation

EM GP2
- Plunge freezing

EM ICE
- High Pressure Freezing

Cryo confocal LM

Zeiss LSM900
- Linkam cryo-stage

Cryo FIB-SEM

Aquilos 2
- FIB-milling
- Integrated wide-field LM
- Micromanipulator for lift out

Cryo TEM

Titan Krios G4
- Single particle analysis
- Tomography

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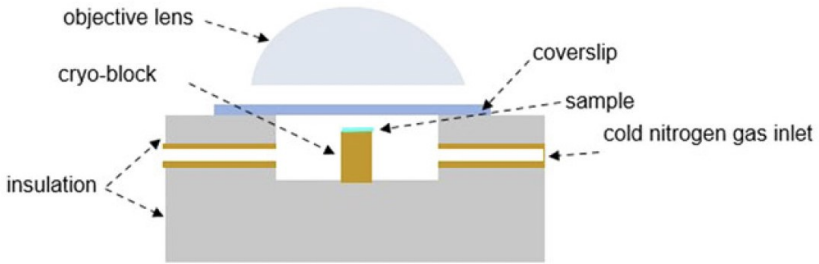
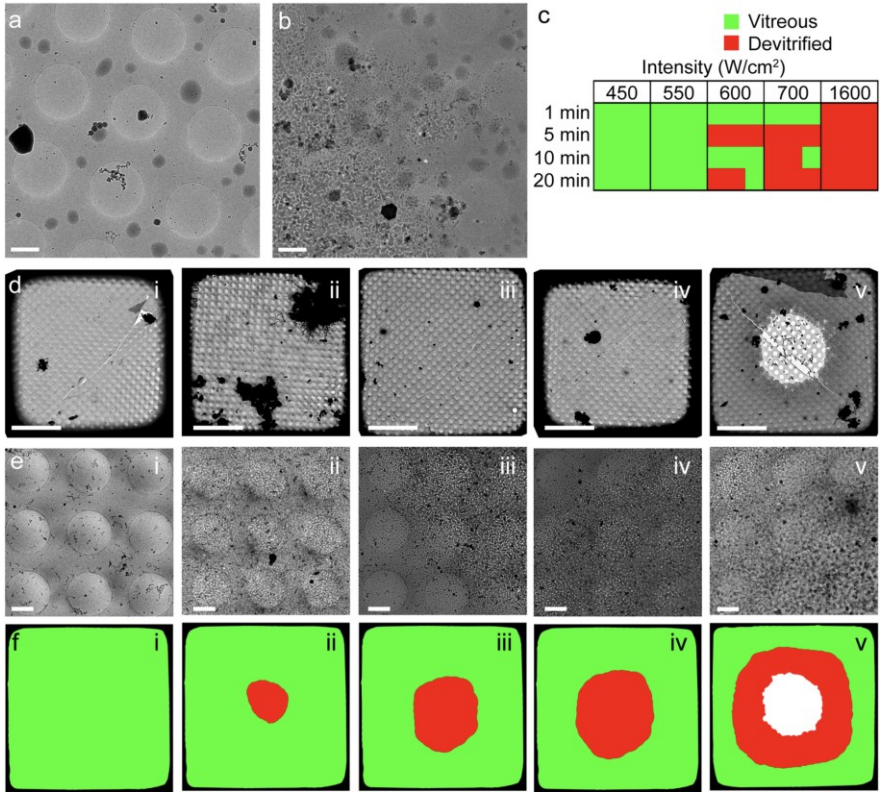
Doing cryo-LM without melting the sample

For very thin layers of ice, an intensity greater than ~550 W/cm^2 can heat the sample enough to cause the vitreous ice to become microcrystalline and compromise the quality of the cryo-EM images



DeRosier, Quart. Rev. Bioph. 2021



Tuijtel, et al. Sci. Rep. 2019

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Access Modes

Direct Access

- Evaluated by the external referees of the project evaluation committee
- Users contribute to the maintenance costs of the microscopes and consumables of their experiments

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