Vitrifying biological samples for cryo-imaging experiments

Gergely Papp

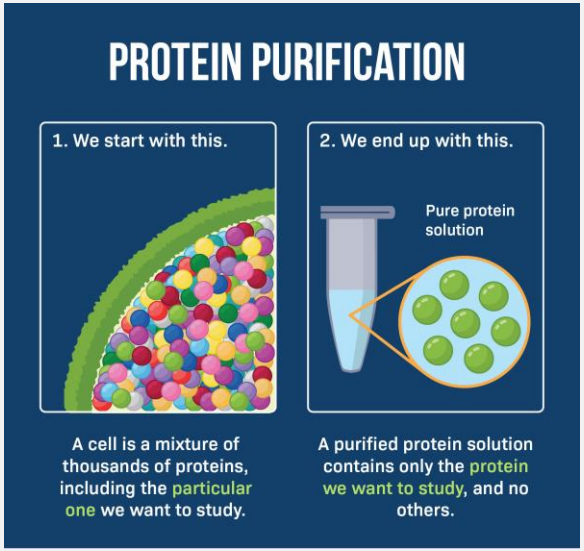
Team Leader

EIRO FORUM ESI – 14/5/2024

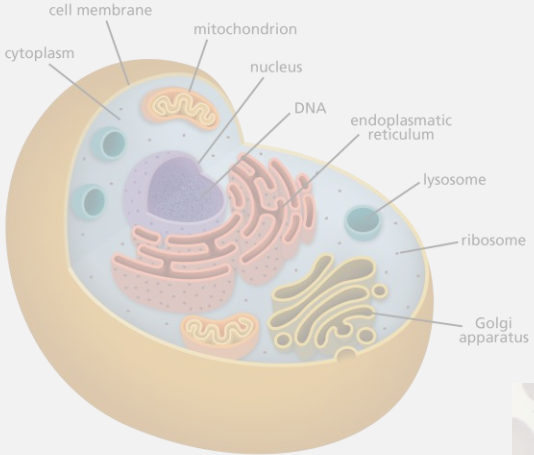
EMBL

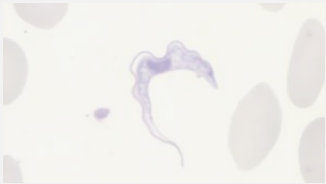
Which samples?

- Purified protein soltions (Single Particle Analysis)
  - Struture determination at atomic resolution



- Cells, monocellular organisms
  - In-situ study, proteins in their netural environment





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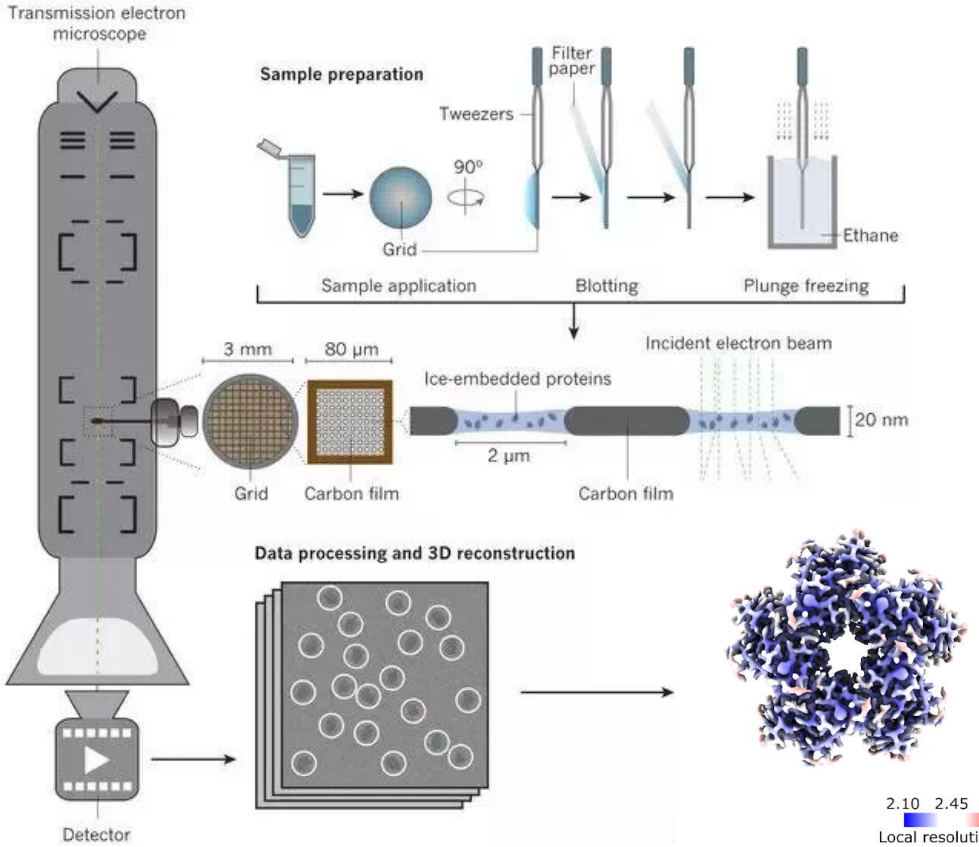
Cryo-EM principle



3D structure of the protein

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The traditional sample preparation method

One session at the Vitrobot

- **Poor reproducibility of thin ice film production**
  - Slow grid screening process
  - Multi-parametric optimizations
  - “No size fits all”

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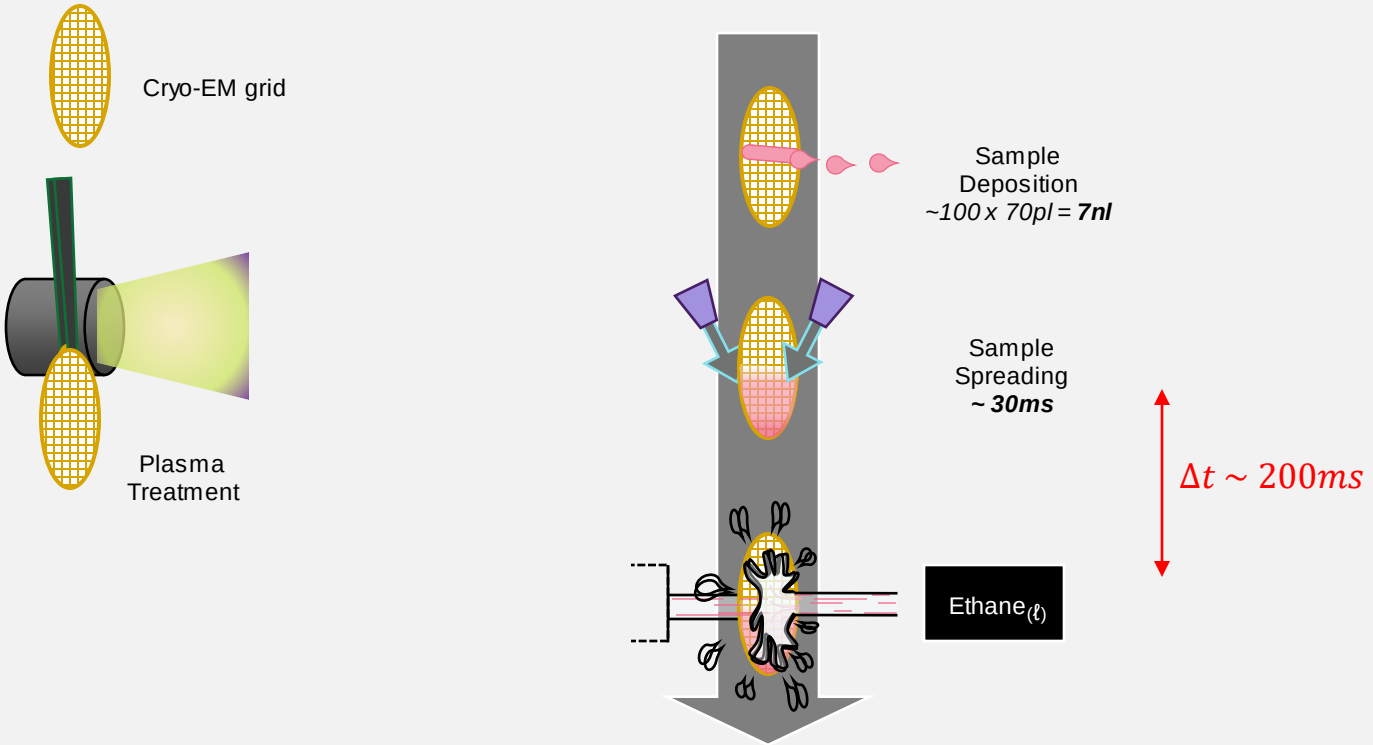
EasyGrid, automated Cryo-EM sample preparation

EMBL - GR

EMBL – HD
&
Imaging Centre

EMBL

Preparation process for SPA samples



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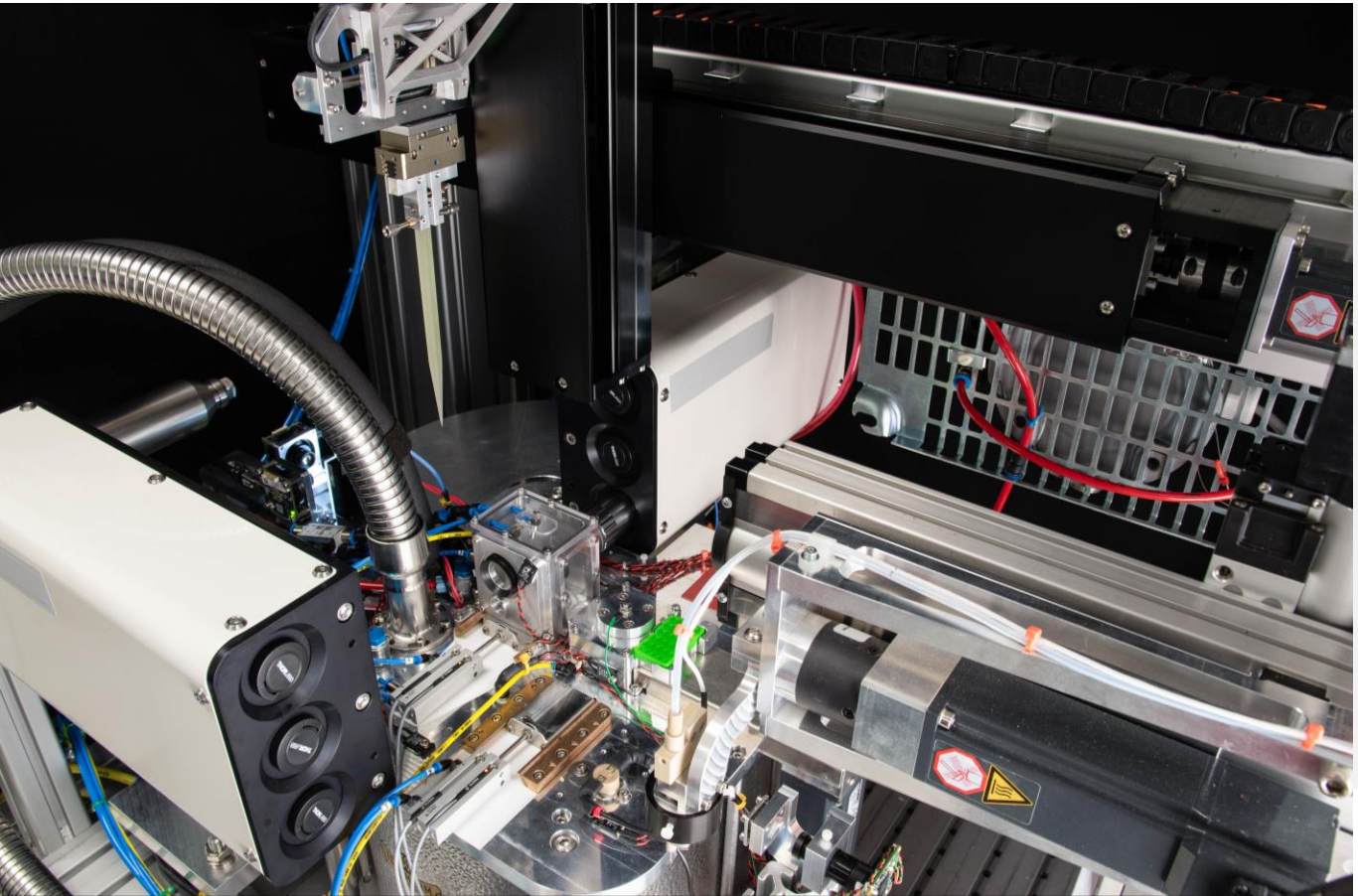
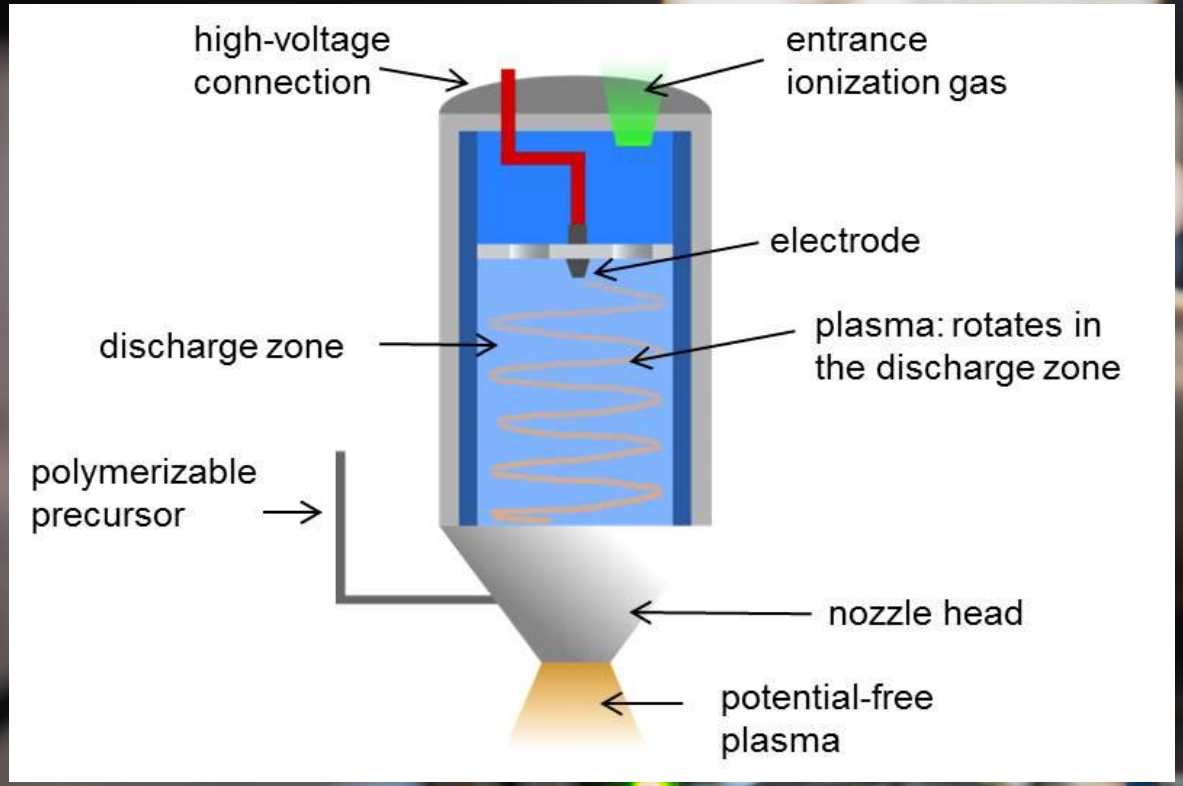
Components



- Atmospheric plasma treatment

EMBL

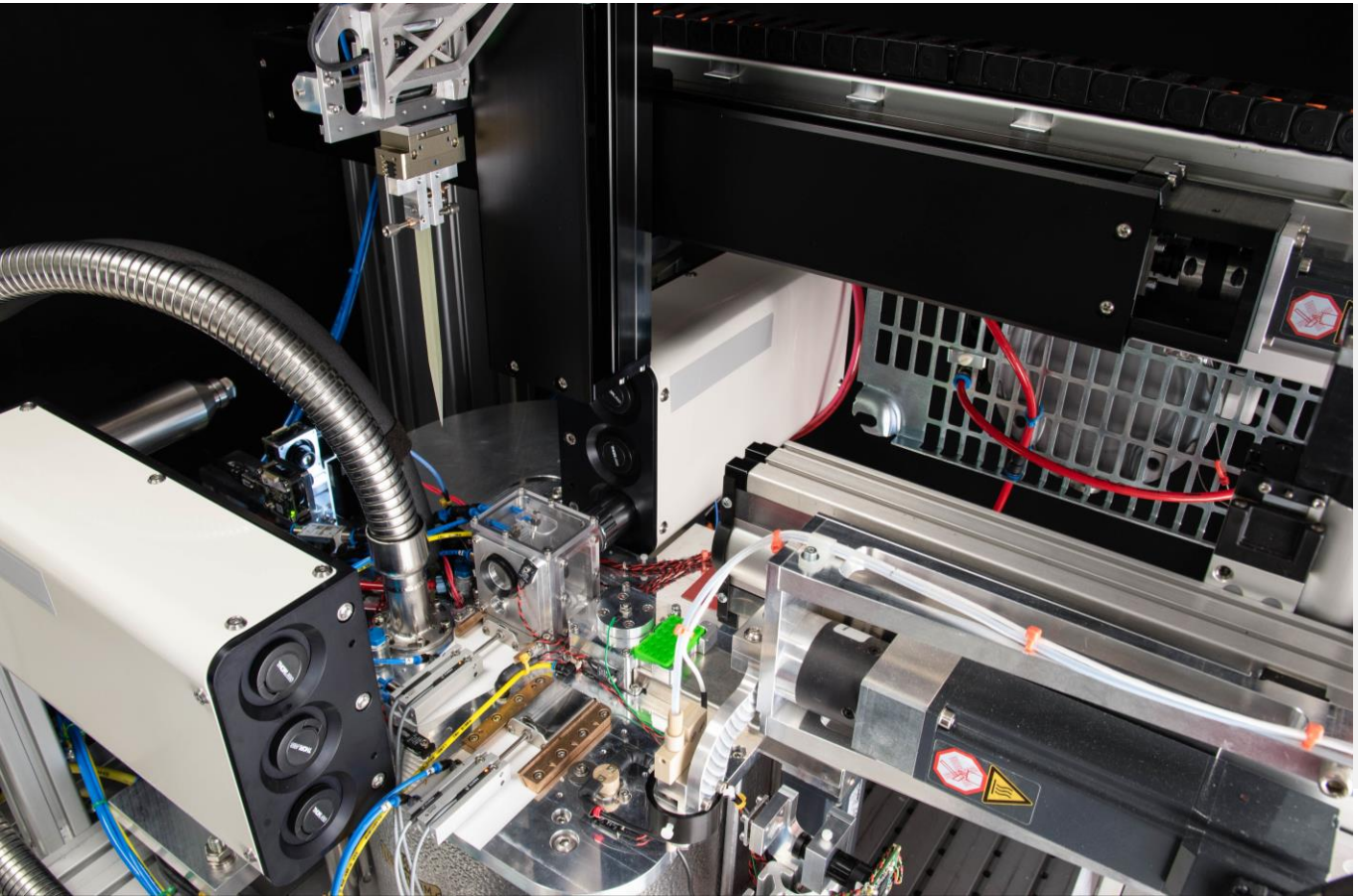
Components



- Atmospheric plasma treatment
- Advantage:
  - In-line hydrophilic treatment
  - Minimum time between treatment and sample deposition
  - Possibility of material deposition and use of other gases

EMBL

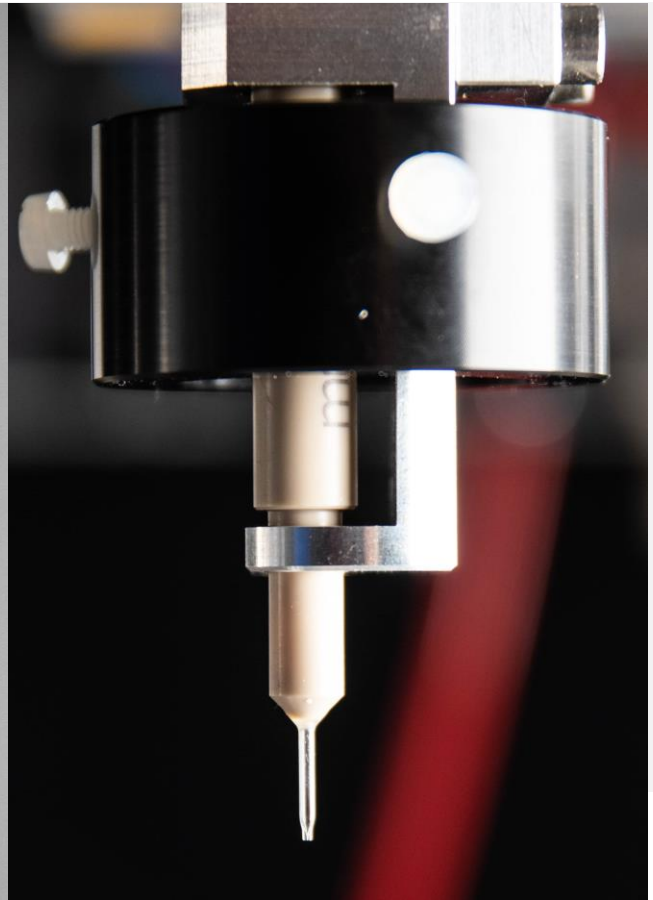
Components



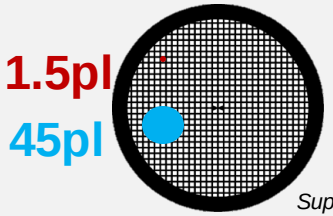
- Atmospheric plasma treatment
- Picoliter drop dispenser

EMBL

Components



- Atmospheric plasma treatment
- Picoliter drop dispenser
- Advantage:
  - Highly repeatable
  - Controlled deposition



Supposing 100nm sample thickness

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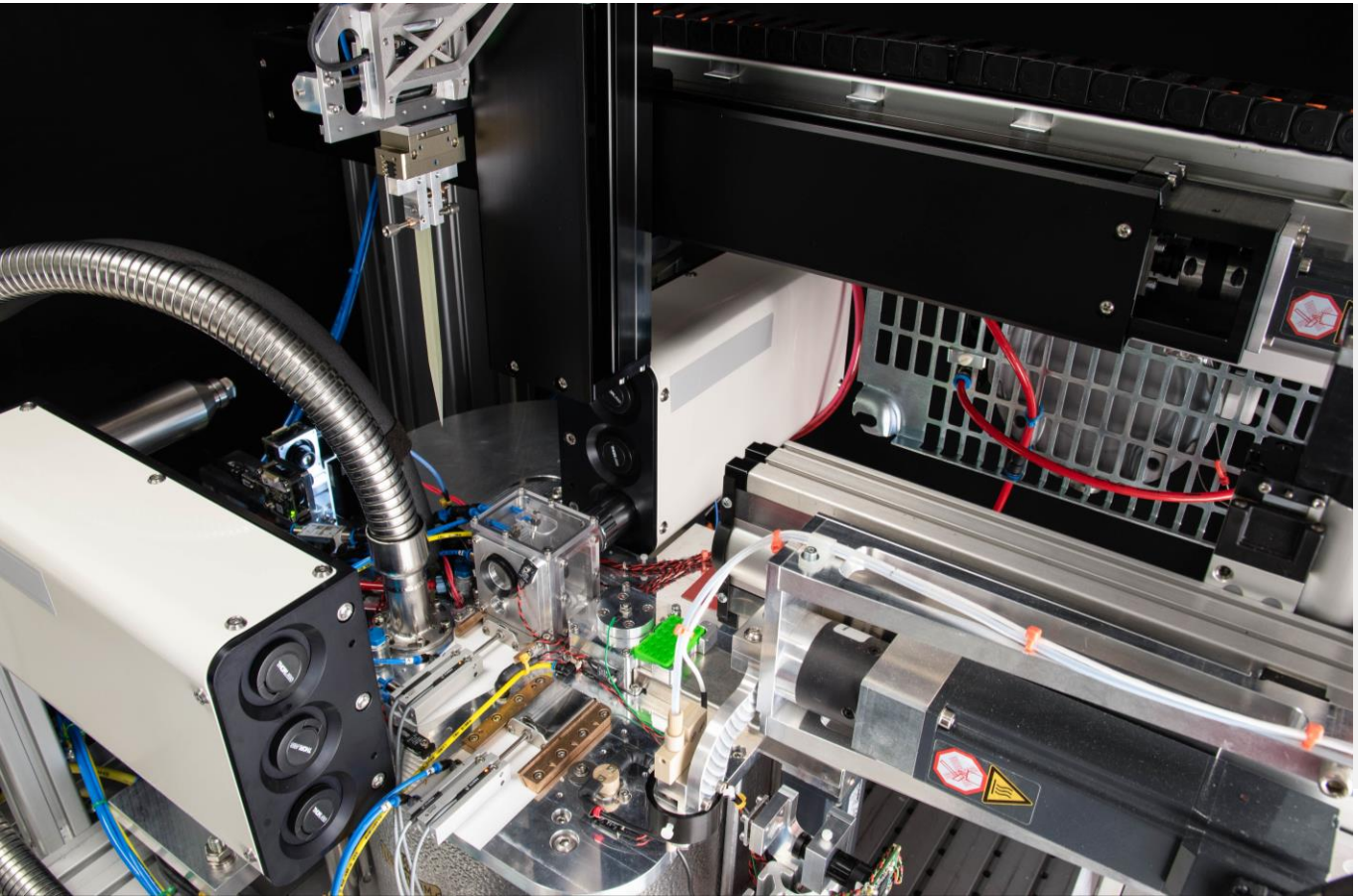
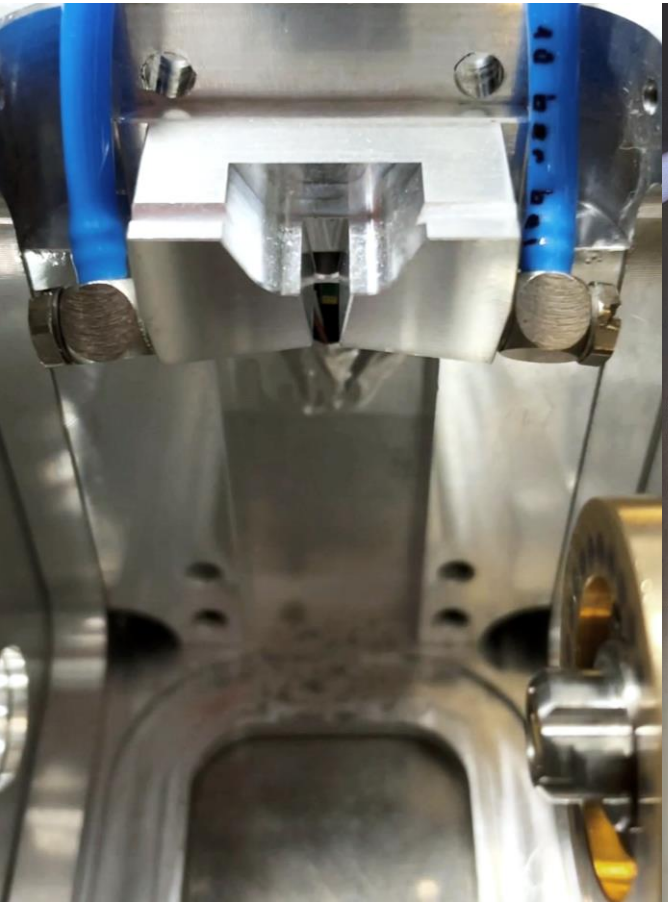
Components

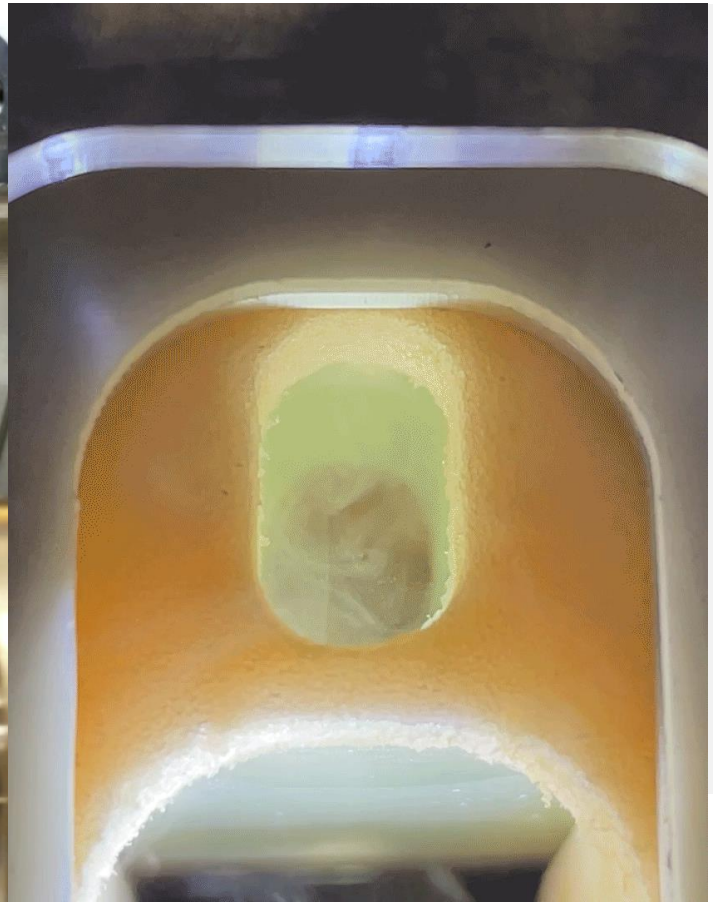


- Atmospheric plasma treatment
- Picoliter drop dispenser
- Chamber
  - Pressure wave generator
  - Humidity control
  - Ethane jet for vitrification

EMBL

Components

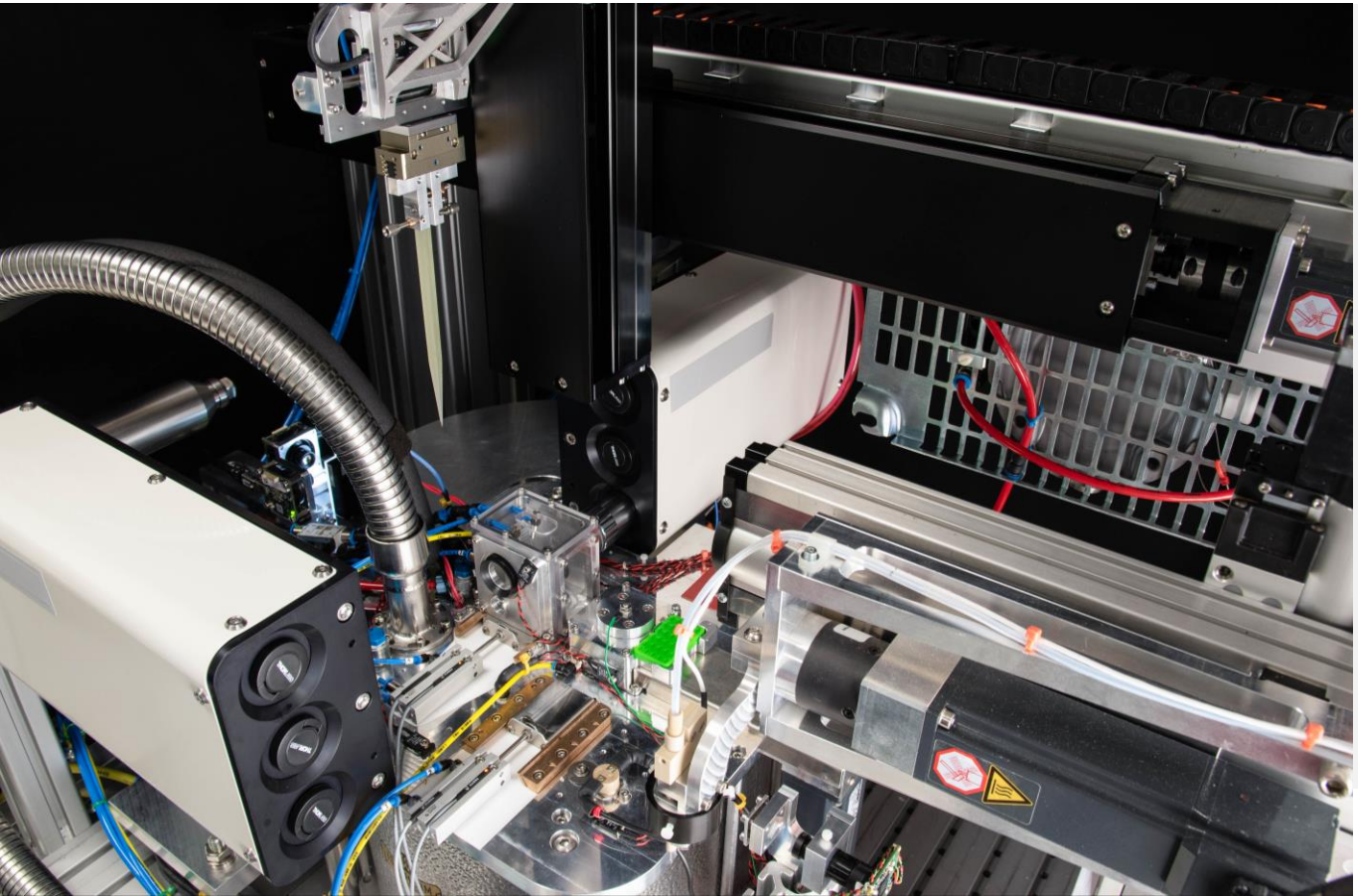




- Atmospheric plasma treatment
- Picoliter drop dispenser
- Chamber
  - Pressure wave generator
  - Humidity & temperature control
  - Ethane jet for vitrification
- Advantage:
  - Contactless spreading
  - Higher cooling rate

EMBL

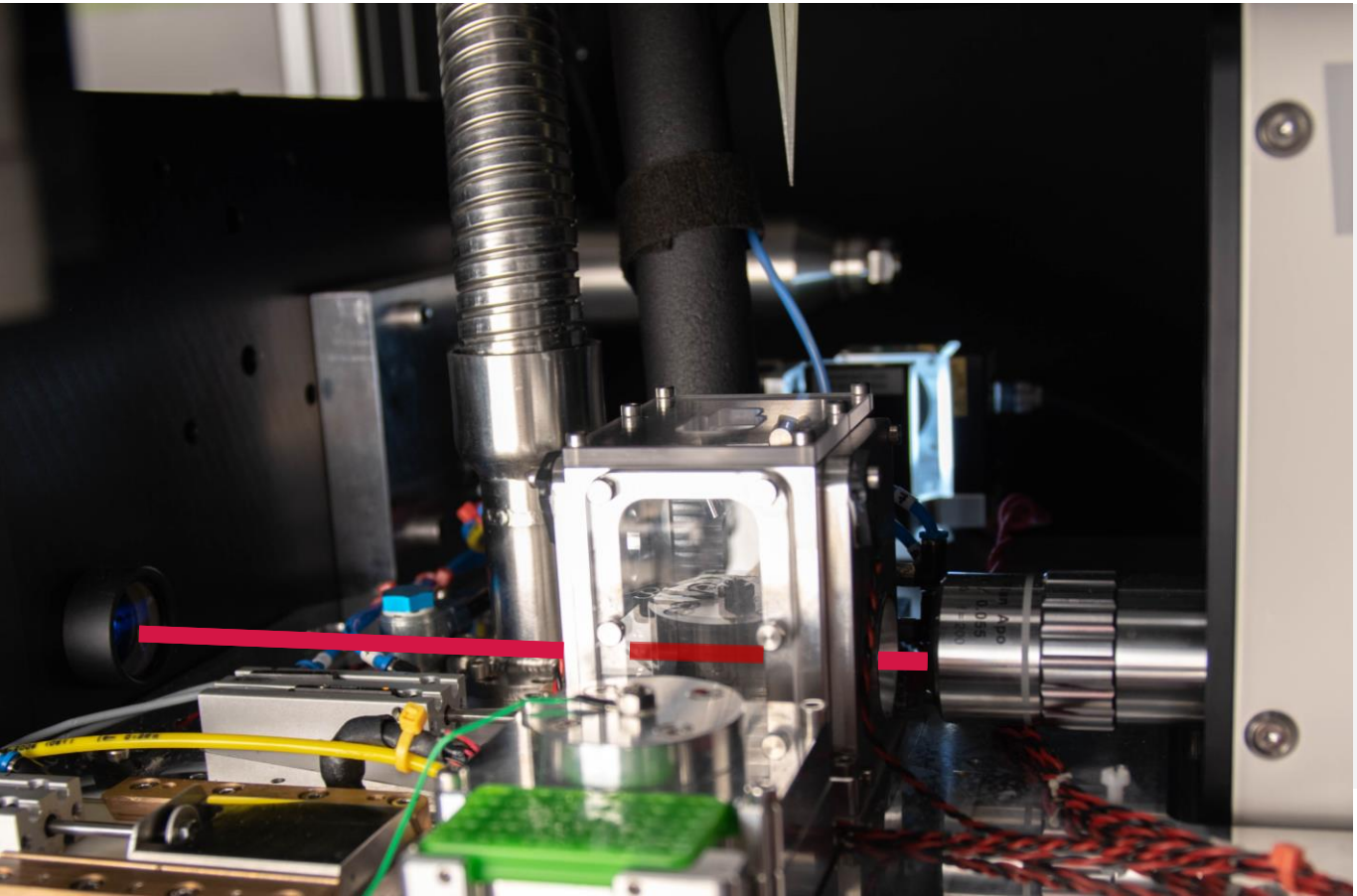
Components



- Atmospheric plasma treatment
- Picoliter drop dispenser
- Chamber
  - Air blades (spreading)
  - Humidity control
  - Ethane jet for vitrification
- Digital Holographic Microscope (DHM)

EMBL

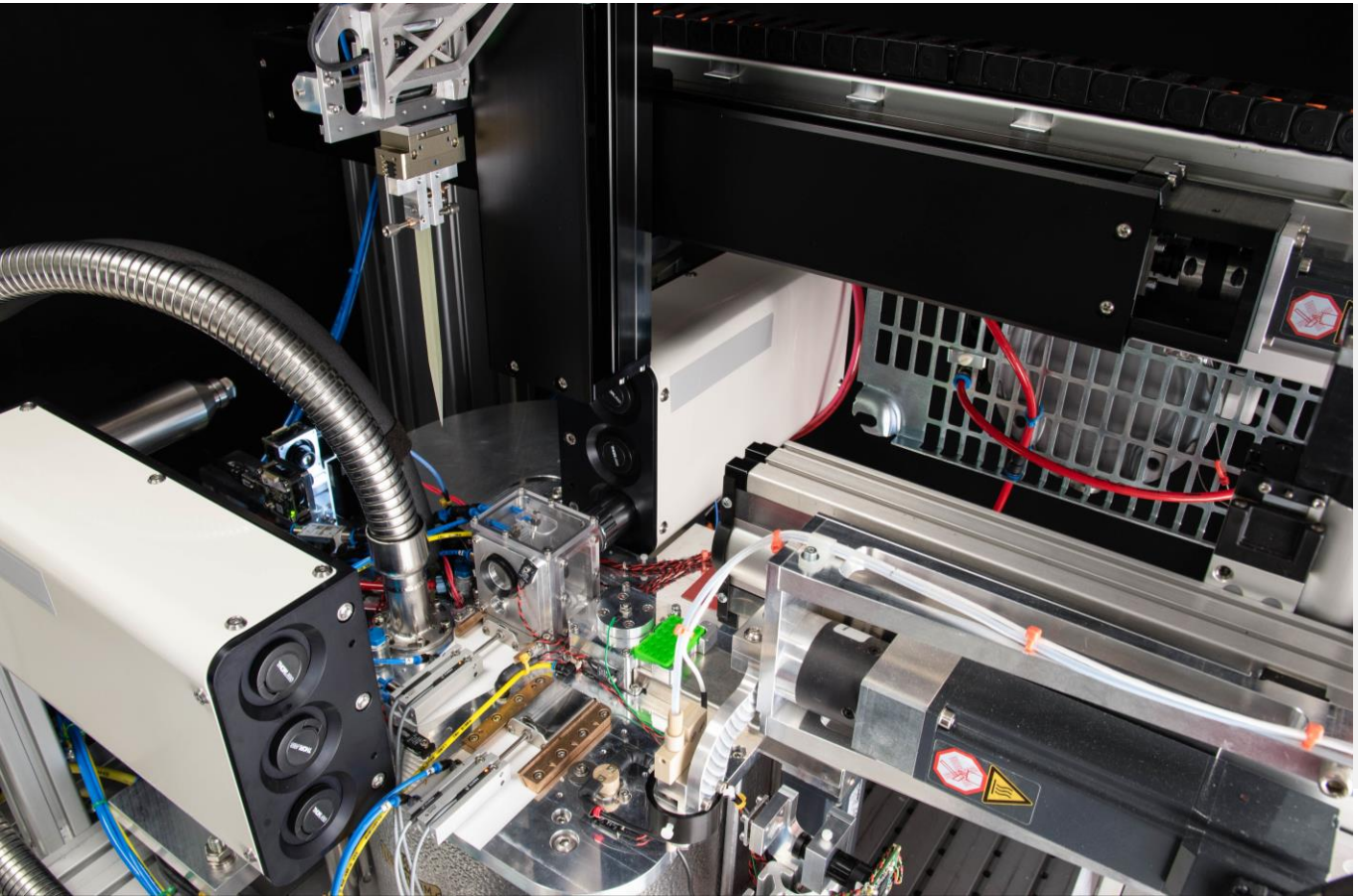
Components



- Atmospheric plasma treatment
- Picoliter drop dispenser
- Chamber
  - Air blades (spreading)
  - Humidity control
  - Ethane jet for vitrification
- Digital Holographic Microscope (DHM)
  - Control of the sample thickness

EMBL

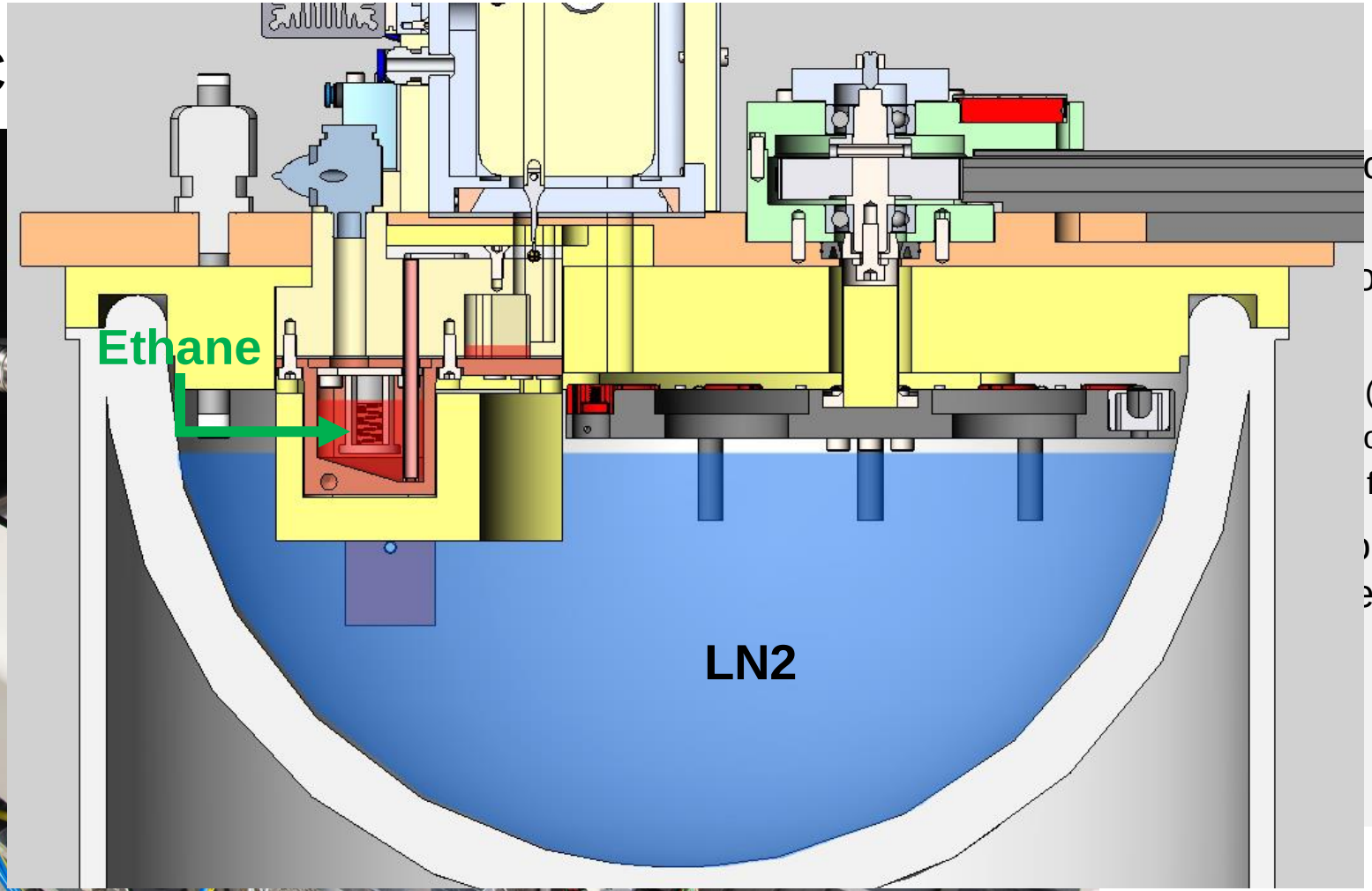
Components



- Atmospheric plasma treatment
- Picoliter drop dispenser
- Chamber
  - Air blades (spreading)
  - Humidity control
  - Ethane jet for vitrification
- Digital Holographic Microscope (DHM)
- Dewar
  - Storage in Liquid Nitrogen
  - Ethane reservoir

EMBL

C



c plasma

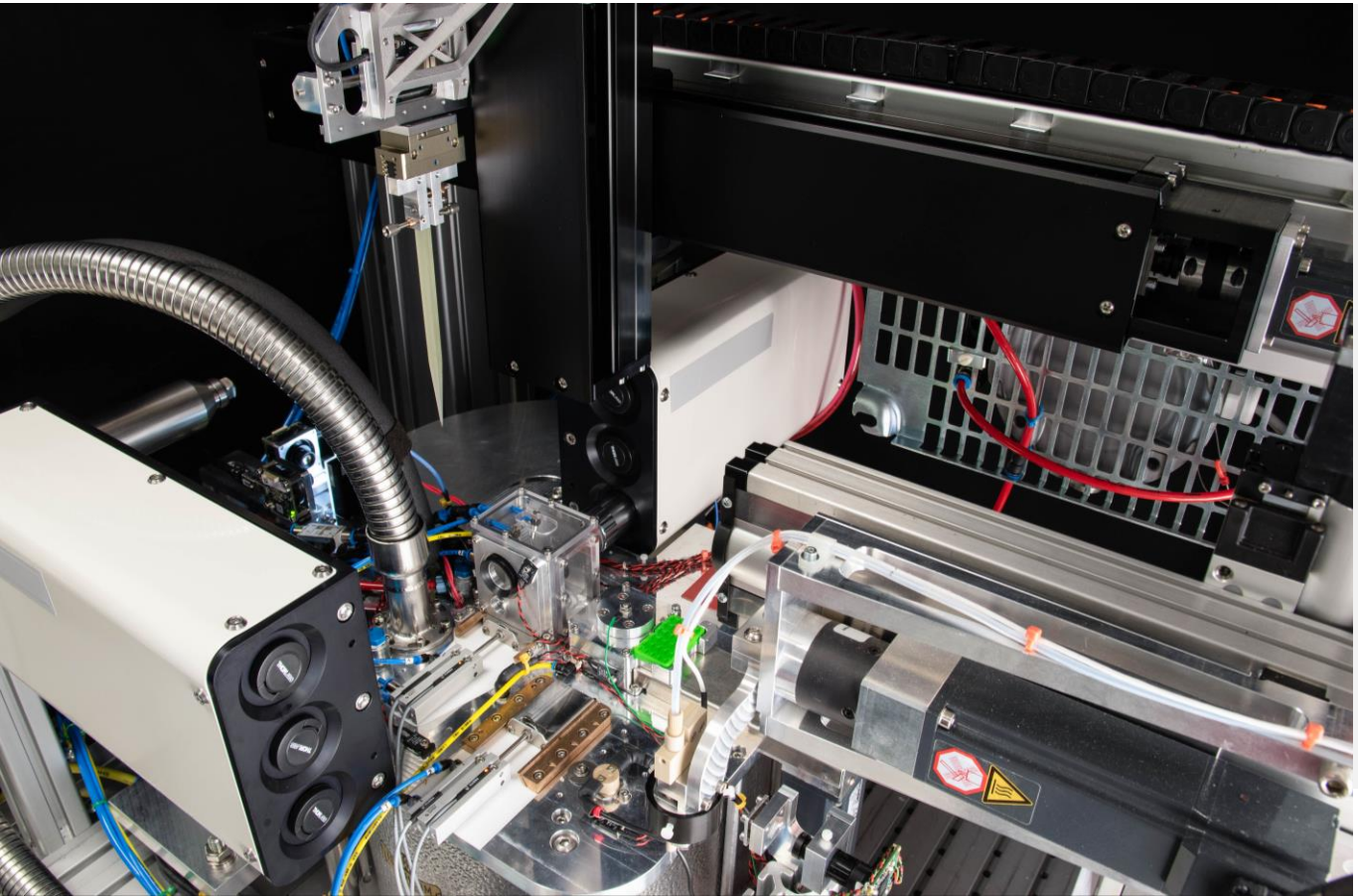
op dispenser

(spreading)
ontrol
for vitrification

ographic
e (DHM)

EMBL

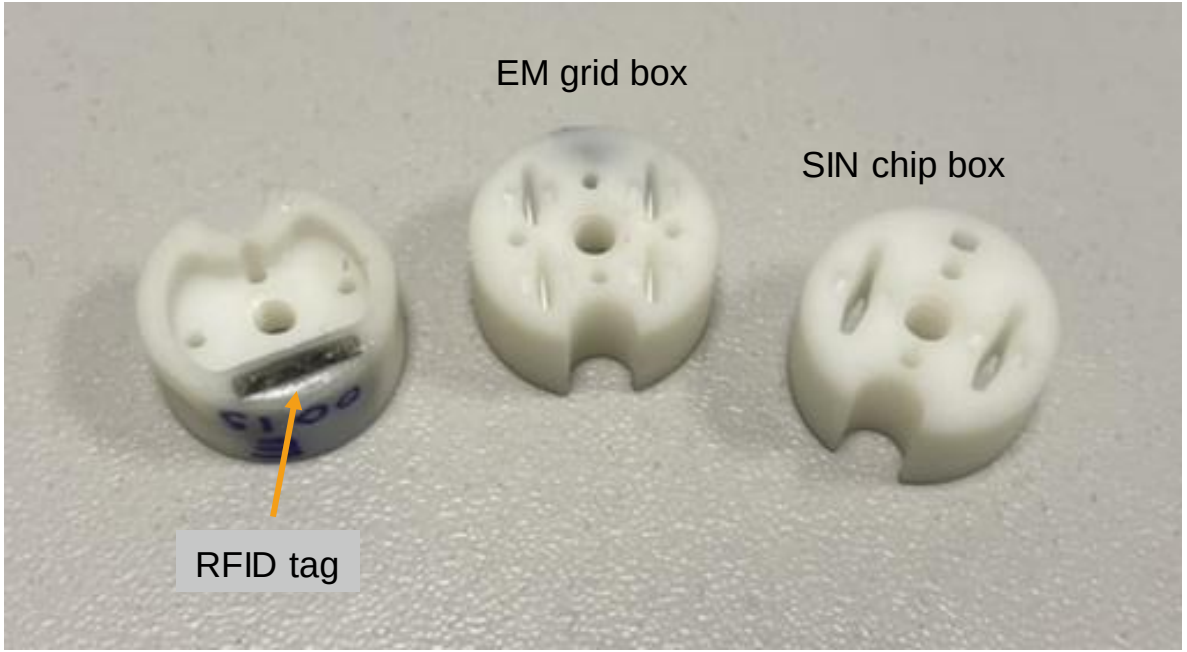
Components

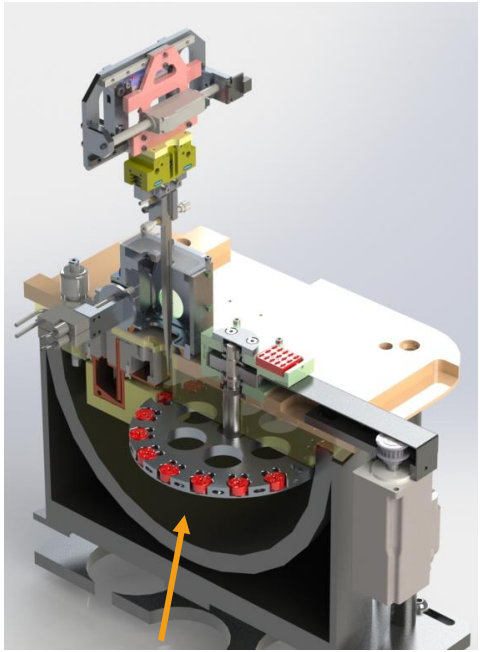


- Atmospheric plasma treatment
- Picoliter drop dispenser
- Chamber
  - Air blades (spreading)
  - Humidity control
  - Ethane jet for vitrification
- Digital Holographic Microscope (DHM)
- Dewar

EMBL

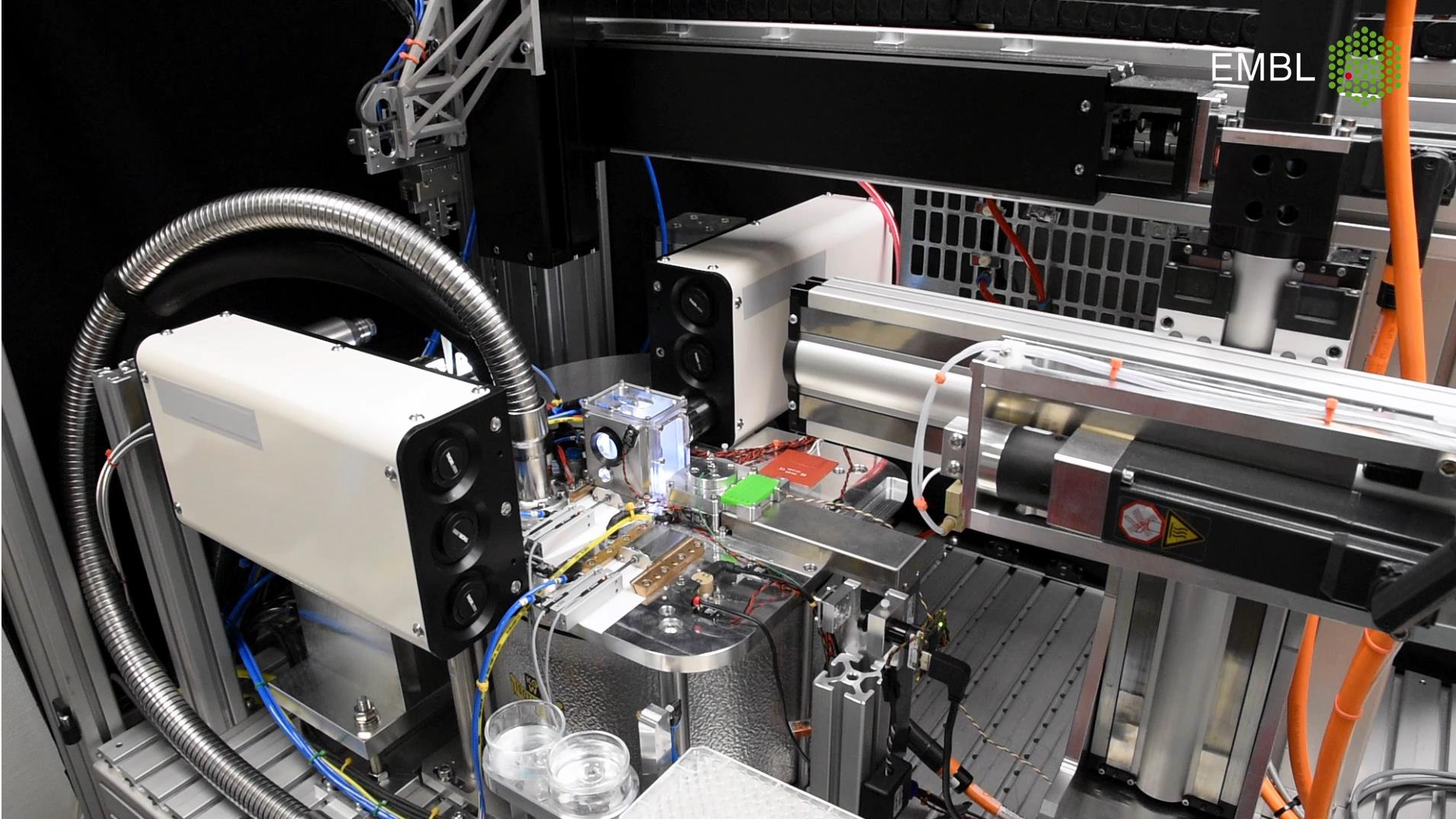
The EasyGrid machine – sample storage





Capacity : 10 boxes = 40 EM grids

EMBL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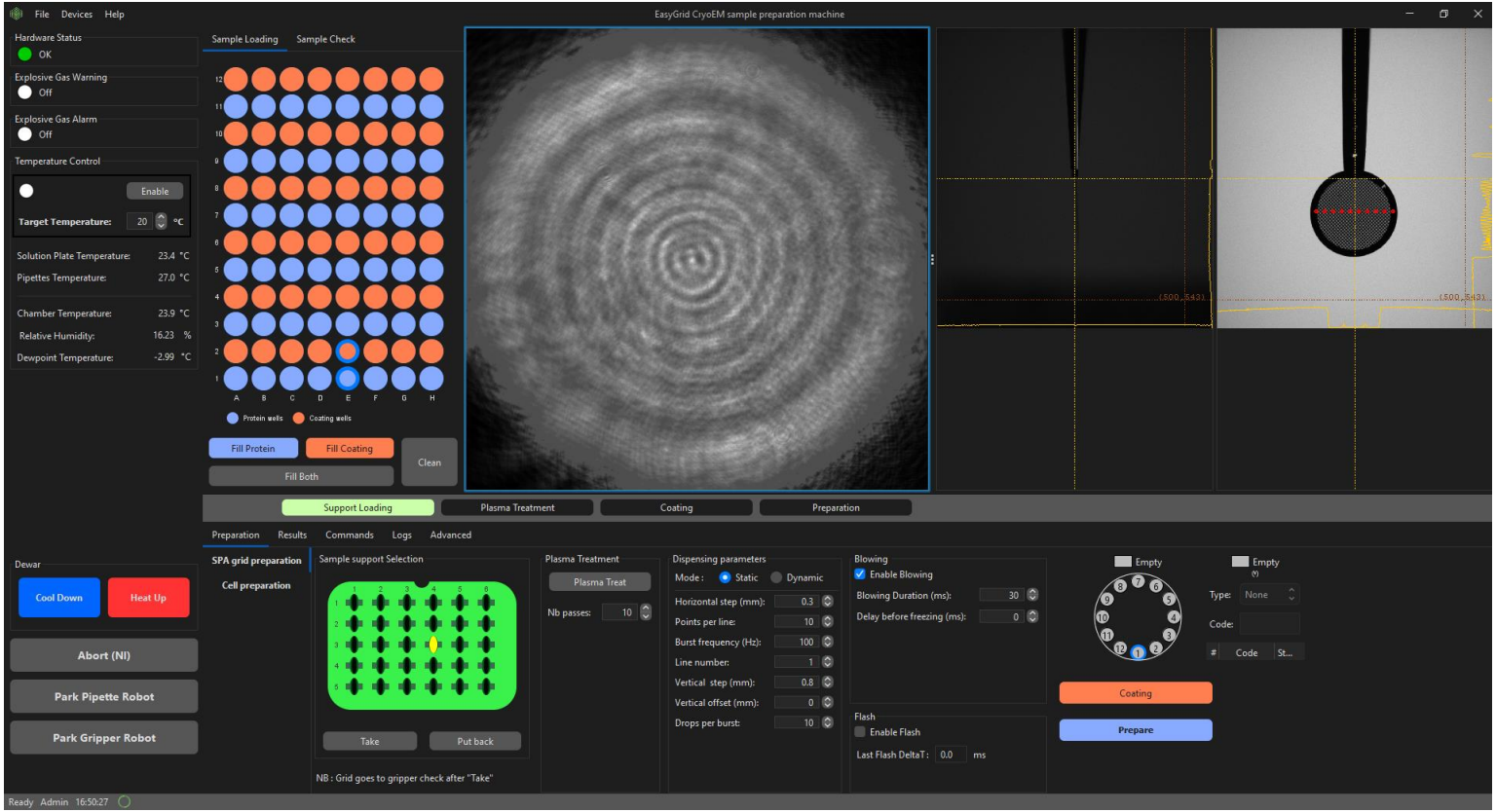
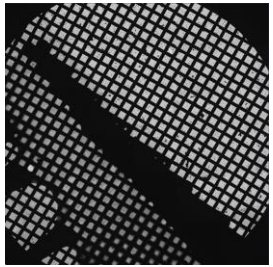
GUI

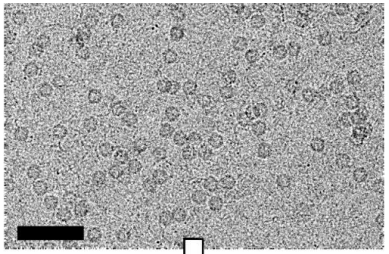

File
Devices
Help
EasyGrid CryoEM sample preparation machine
Hardware Status
OK
Explosive Gas Warning
Off
Explosive Gas Alarm
Off
Temperature Control
Enable
Target Temperature:
20
°C
Solution Plate Temperature:
23.4 °C
Pipettes Temperature:
27.0 °C
Chamber Temperature:
23.9 °C
Relative Humidity:
16.23 %
Dewpoint Temperature:
-2.99 °C
Sample Loading
Sample Check
Protein wells
Coating wells
Fill Protein
Fill Coating
Fill Both
Clean
Support Loading
Plasma Treatment
Coating
Preparation
Preparation
Results
Commands
Logs
Advanced
Dewar
Cool Down
Heat Up
Abort (NI)
Park Pipette Robot
Park Gripper Robot
SPA grid preparation
Cell preparation
Sample support Selection
Take
Put back
NB : Grid goes to gripper check after "Take"
Plasma Treatment
Plasma Treat
Nb passes:
10
Dispensing parameters
Mode :
Static
Dynamic
Horizontal step (mm):
0.3
Points per line:
10
Burst frequency (Hz):
100
Line number:
1
Vertical step (mm):
0.8
Vertical offset (mm):
0
Drops per burst:
10
Blowing
Enable Blowing
Blowing Duration (ms):
30
Delay before freezing (ms):
0
Flash
Enable Flash
Last Flash DeltaT :
0.0
ms
Empty
Empty
Type:
None
Code:
Code
Coating
Prepare
Ready
Admin
16:50:27

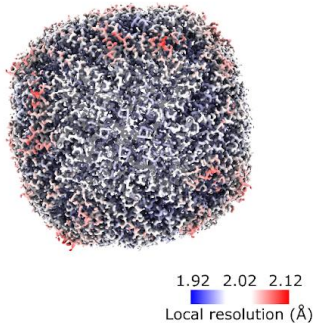
EMBL

Purified protein structures



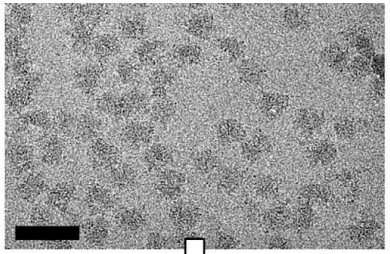
apoferritin

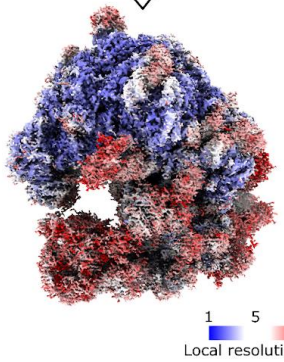




R_{avg} = 1.9Å

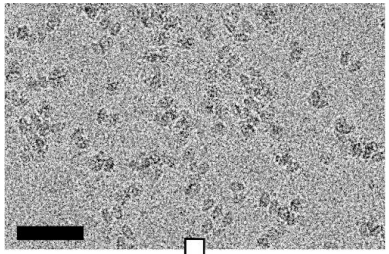
ribosome

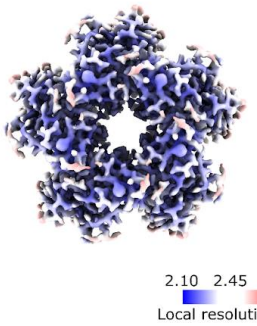




R_{avg} = 2.4Å

rhodopsin





R_{avg} = 2.3Å

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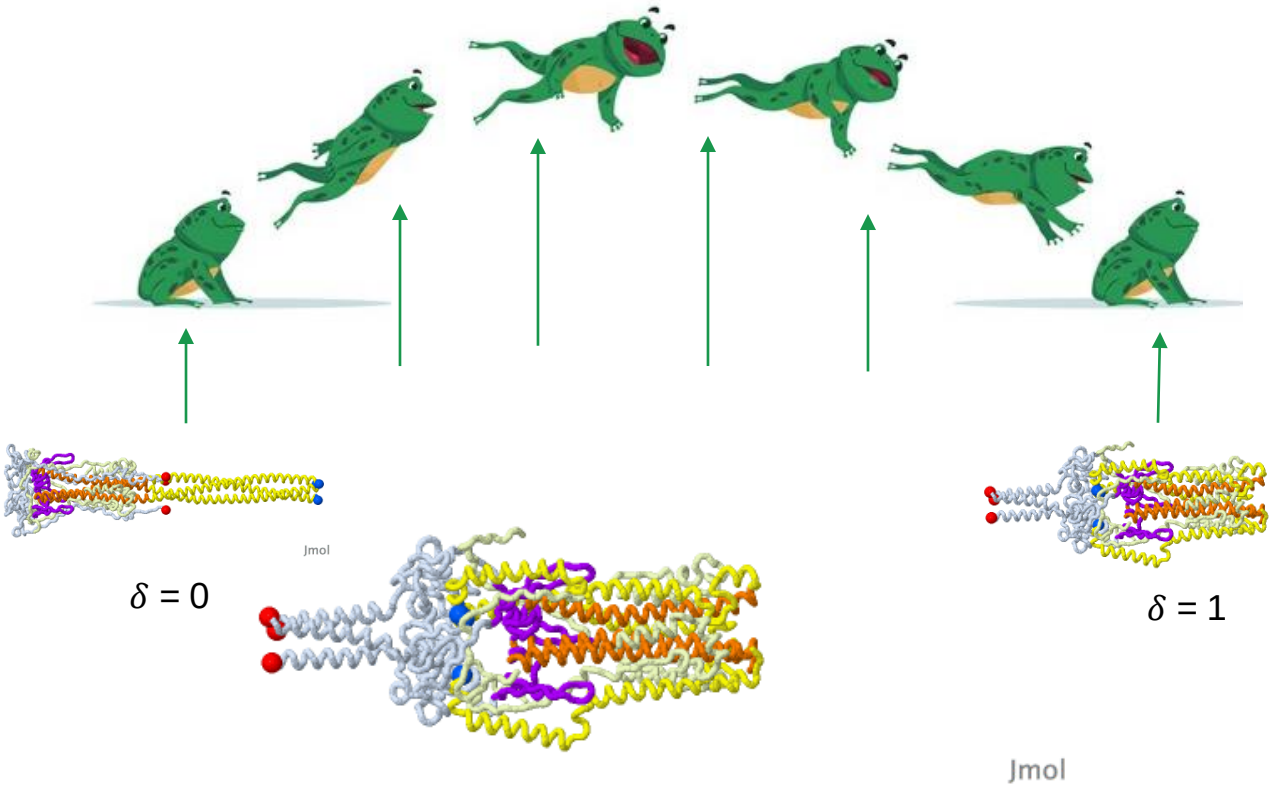
Time-Resolved experiments - Towards movies of biochemical reactions



adapted from protopedia.org

EMBL

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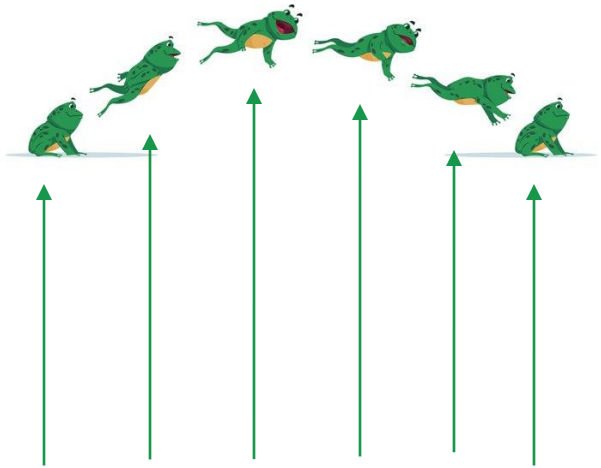


$\delta = 0$

$\delta = 1$

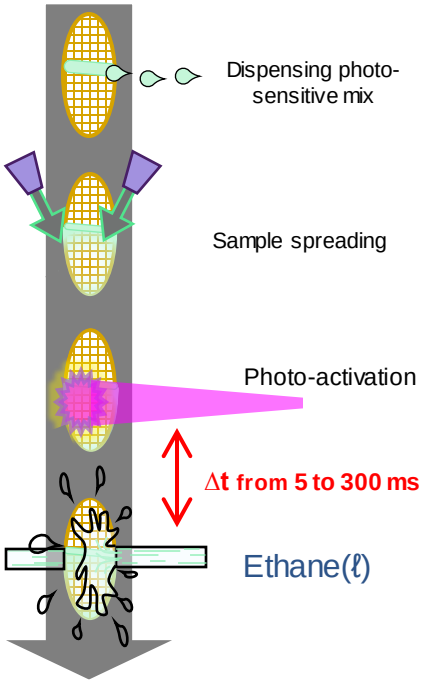
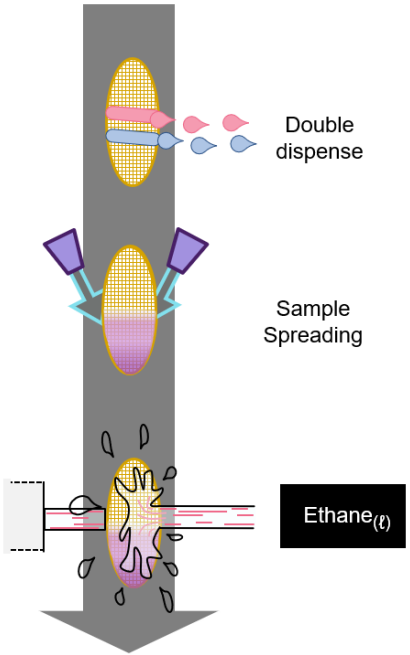


Photo-activation module



Mixing with double dispense

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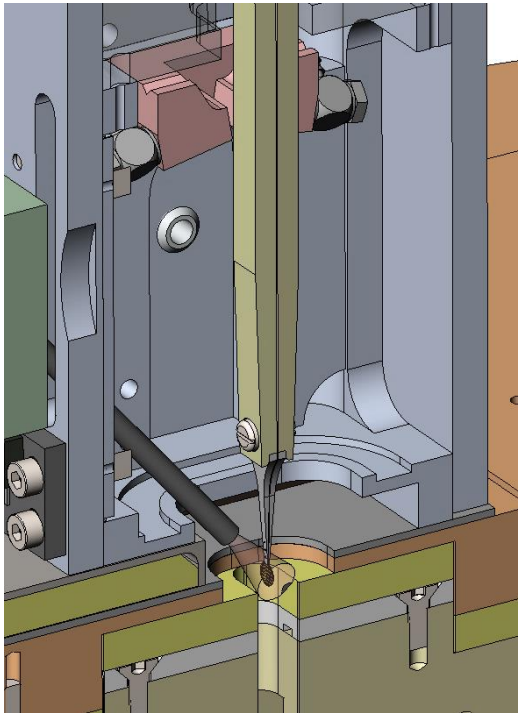
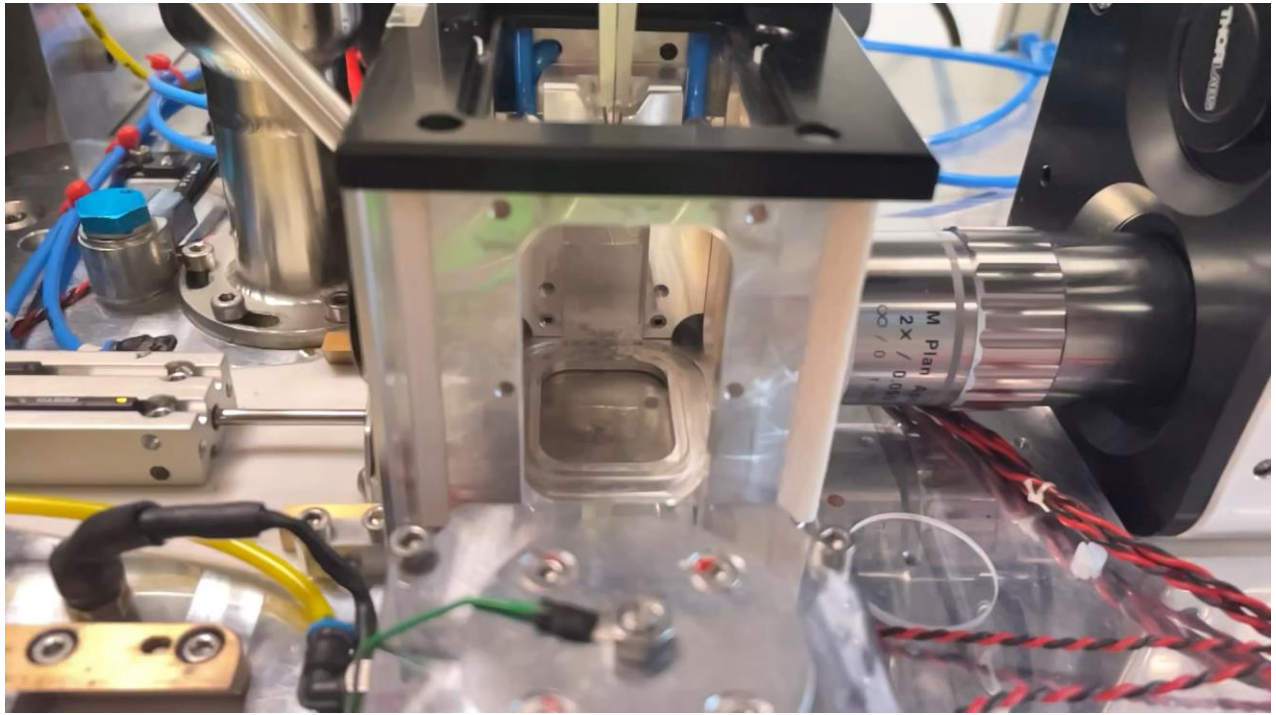
Towards time resolved studies (light triggerig)



Light triggering setup (CAD)



Light triggering video – **Slow motion**

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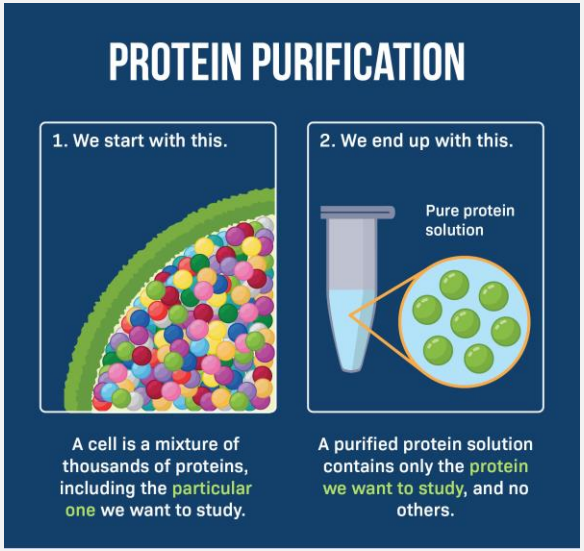
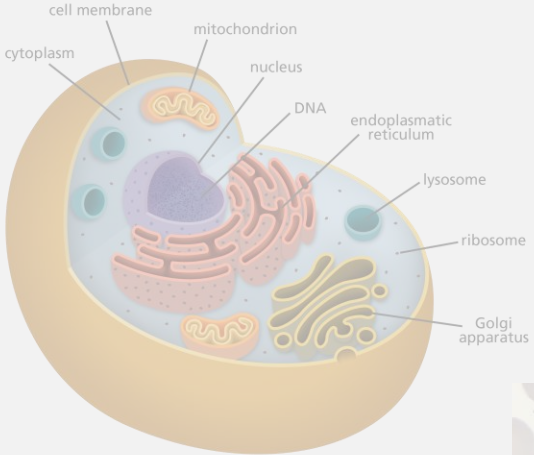
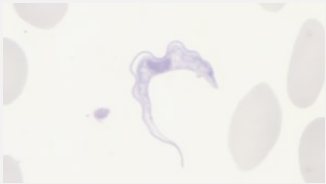
- Purified protein soltions (Single Particle Analysis)
  - Struture determination at atomic resolution



- Cells, monocellular organisms
  - In-situ study, proteins in their netural environment





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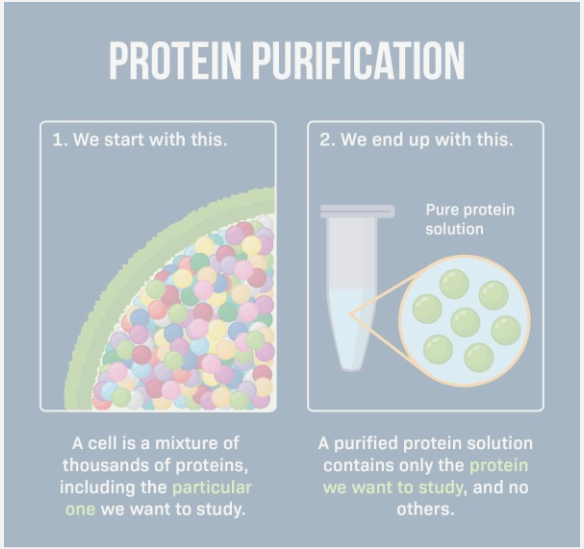

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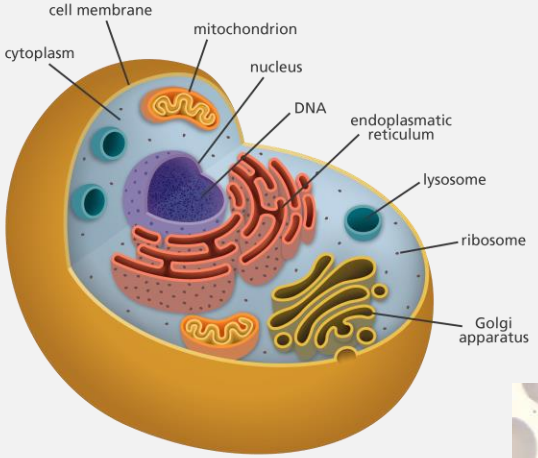

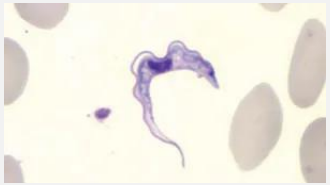

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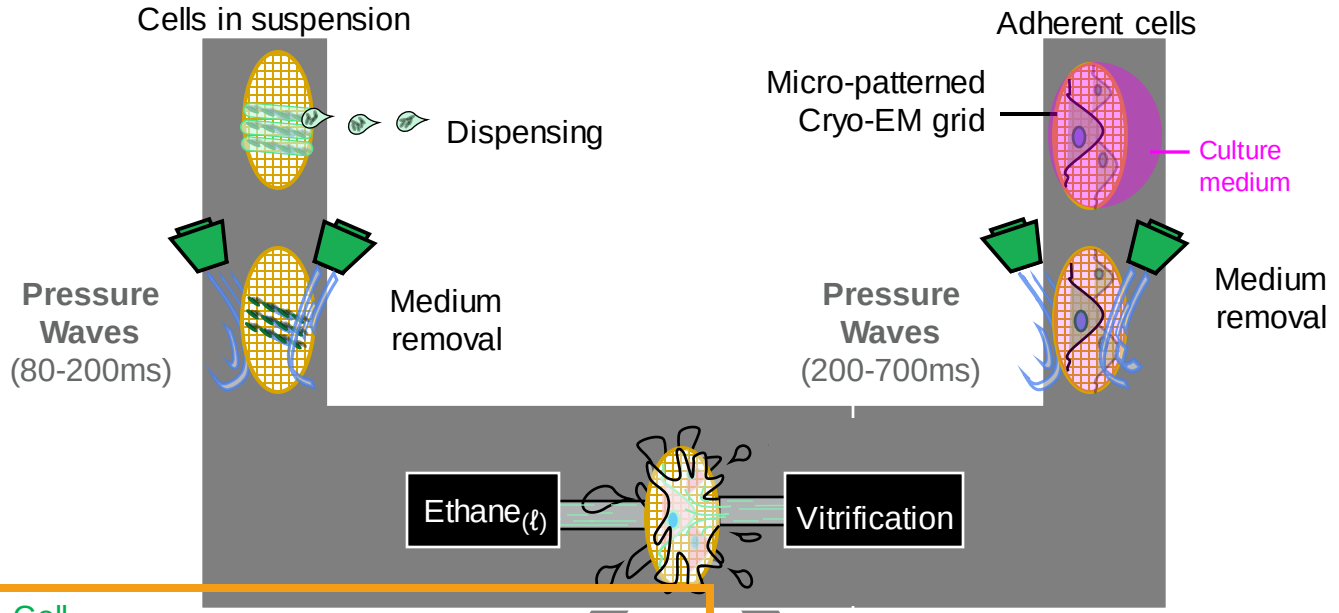
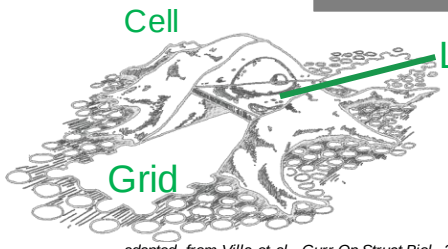
Cell vitrification for in-situ imaging





adapted from Villa et al., Curr.Op.Struct.Biol. 2013

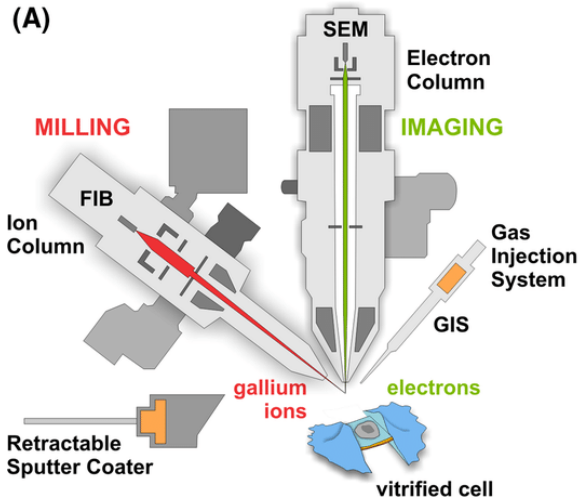
Focused Ion Beam (FIB)-milling

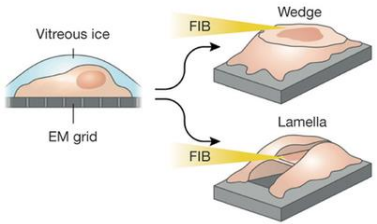
Cryo-ET

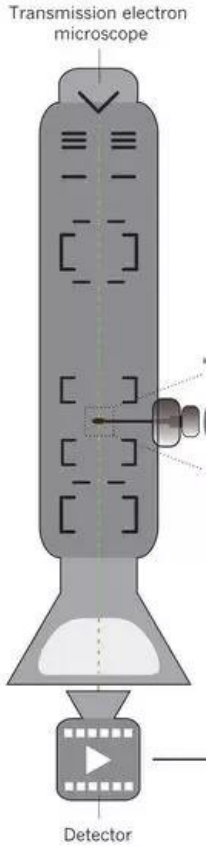
X-Ray imaging

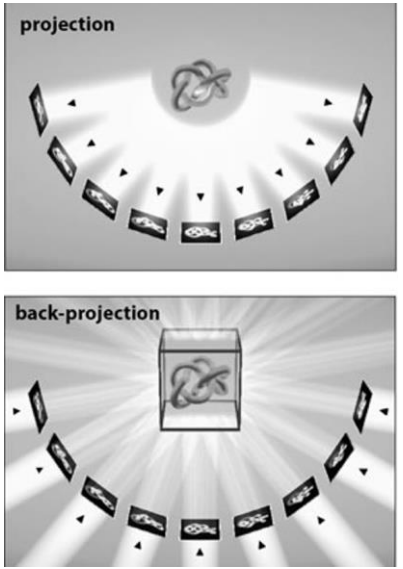
EMBL

Cryo-ET FIB-SEM and data collection





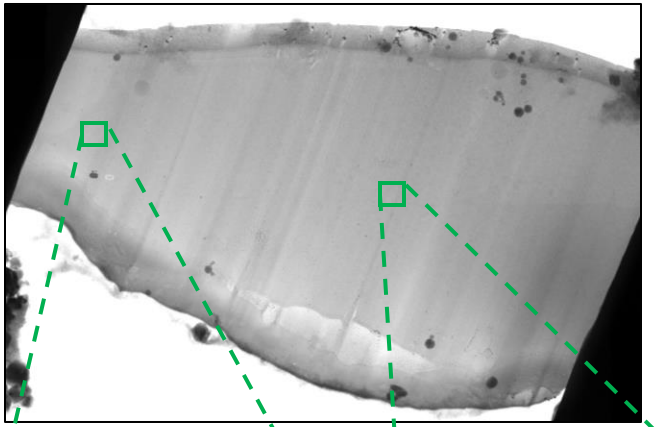




EMBL

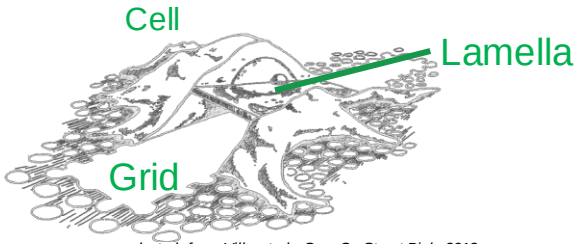
Cryo-Electron Tomography of HeLa cells

Lamella of *jet*-frozen HeLa cell (EasyGrid)

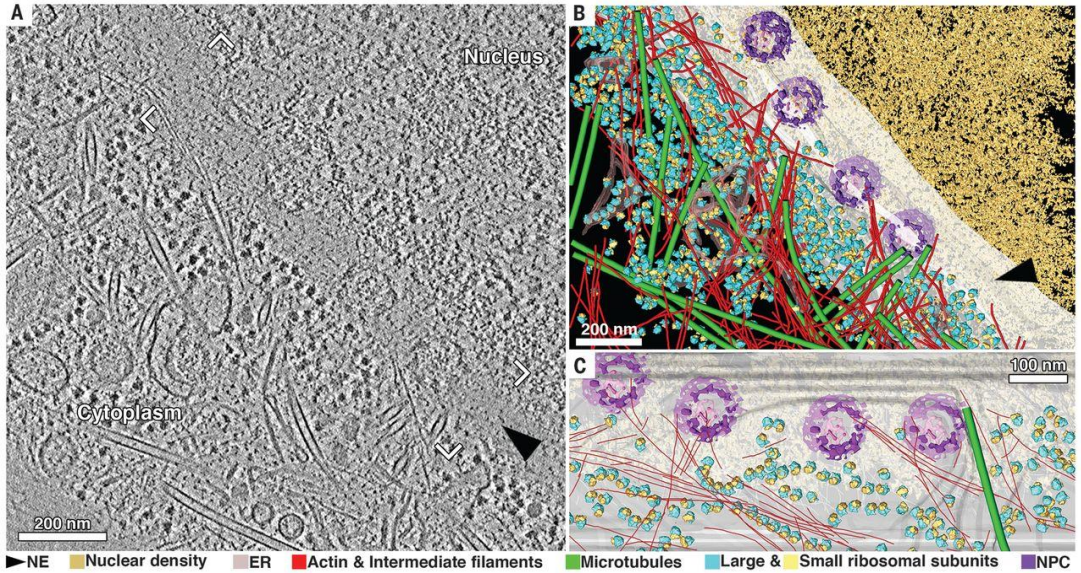


Cytosol

Nucleus



adapted from Villa et al., Curr.Op.Struct.Biol. 2013



Julia Mahamid *et al.*, Visualizing the molecular sociology at the HeLa cell nuclear periphery. *Science* **351**, 969-972 (2016). DOI: 10.1126/science.aad8857

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Acknowledgements

- Papp Team:
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  - Zhengyi Yang
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  - Steffen Klein
  - Anastasiia Babenko (alumna)
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  - Sylvain Bohic
- IBS
  - Guy Schoehn
  - Felix Weis
  - Martin Weik
  - Benoit Gallet

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Life Science ALLIANCE

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Thank you

Questions?

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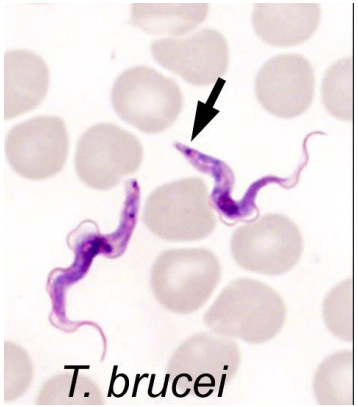
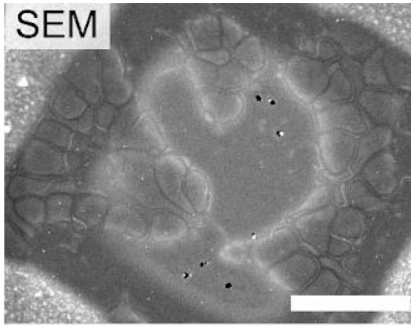
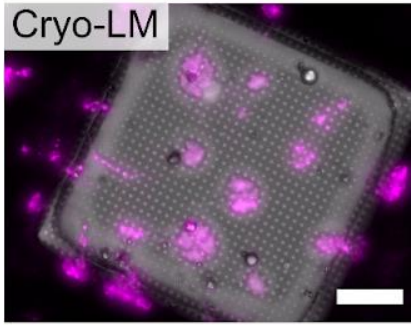
Preserving natural shape (Trypanosoma brucei)

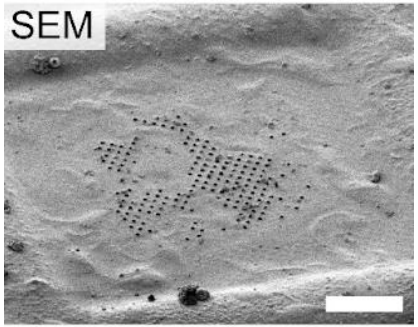


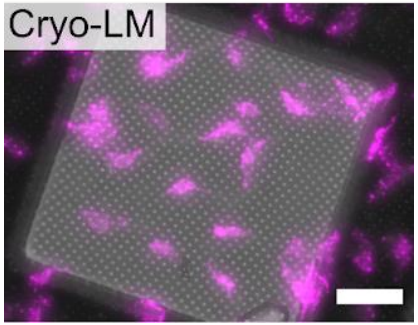
Leica GP2





EasyGrid





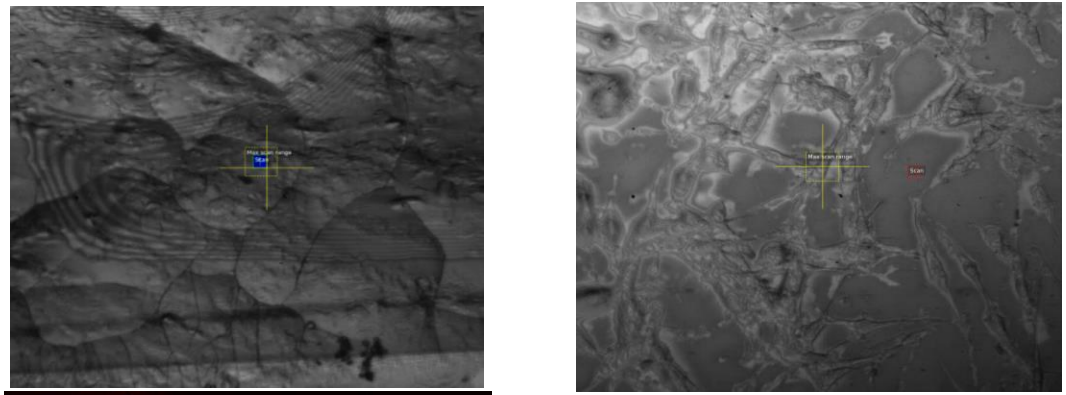
EMBL

X-ray nano imaging (ESRF ID16A)

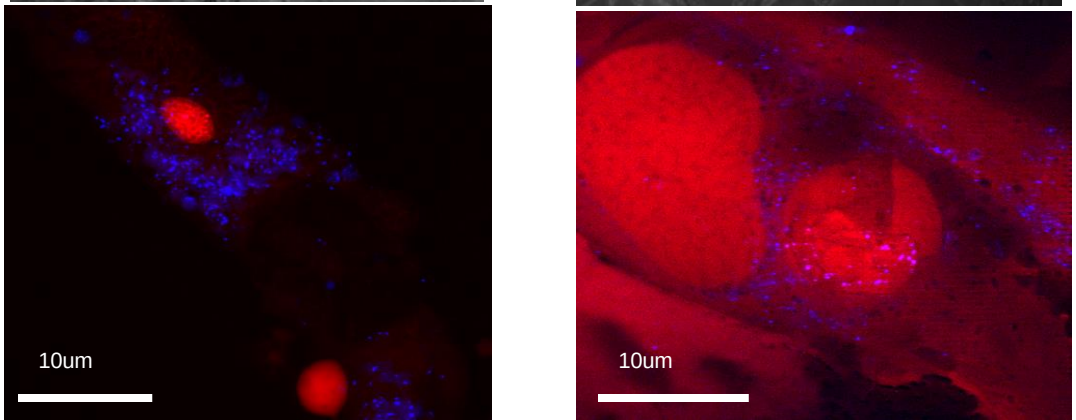
Toxoplasma Gondii infected HFF cells (24h post infection)

Leica cryo-plunger **EasyGrid**

Phase images



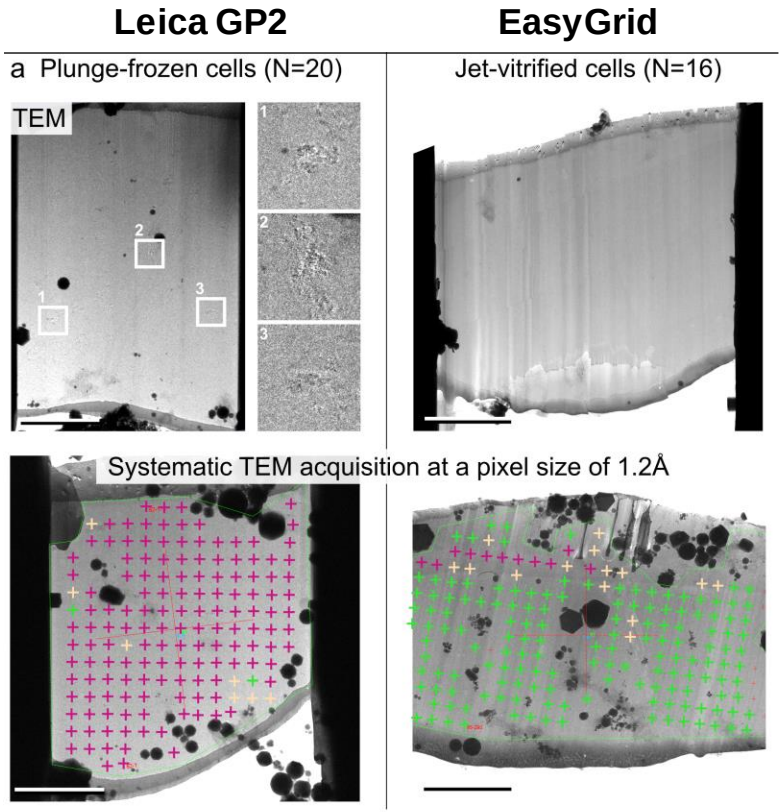
XRF image
K/*Fe*
distribution map
(area density ng/mm²)

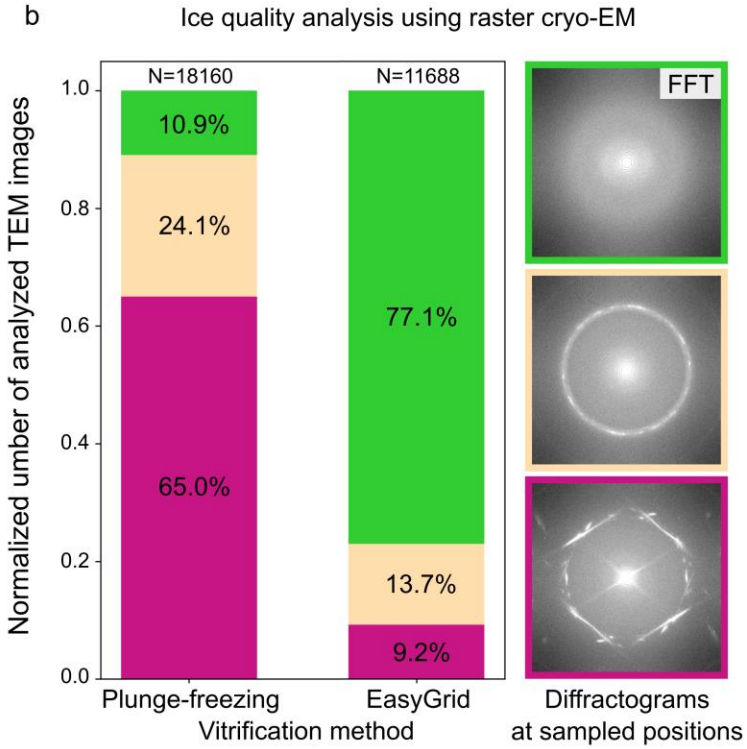


EMBL

Cryo-Electro Tomography principle

Improvement in ice quality (HeLa cells)

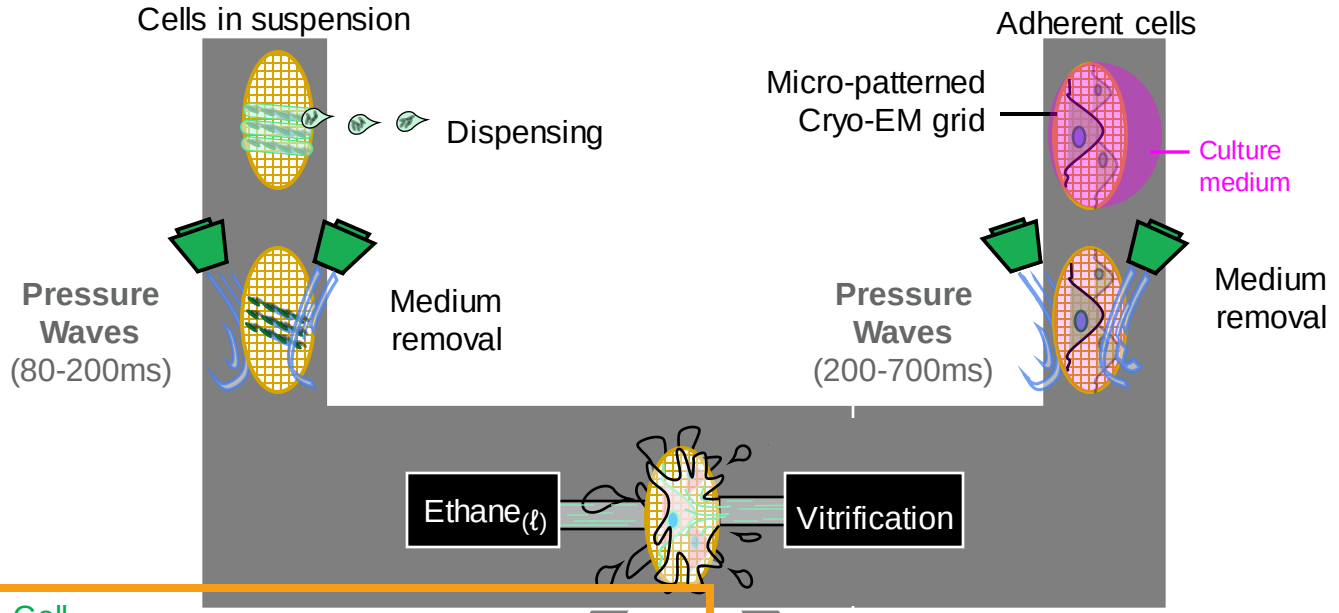




Vitreous Semi-crystalline Crystalline

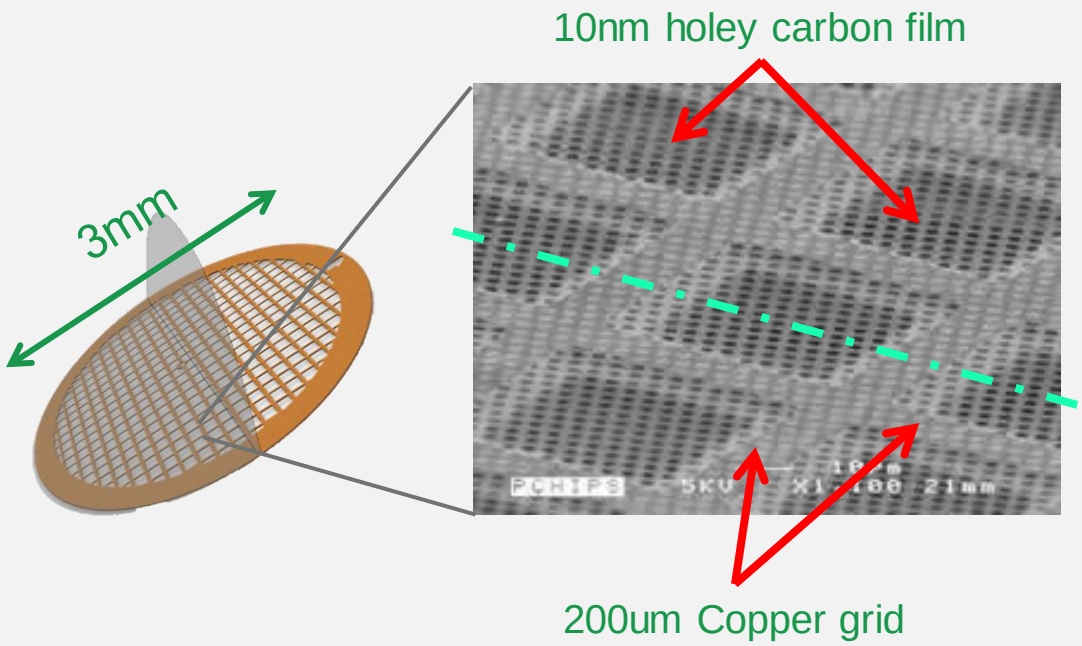
EMBL

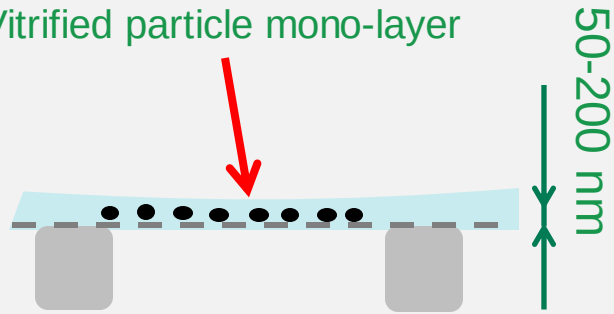
Cell vitrification for in-situ imaging



EMBL

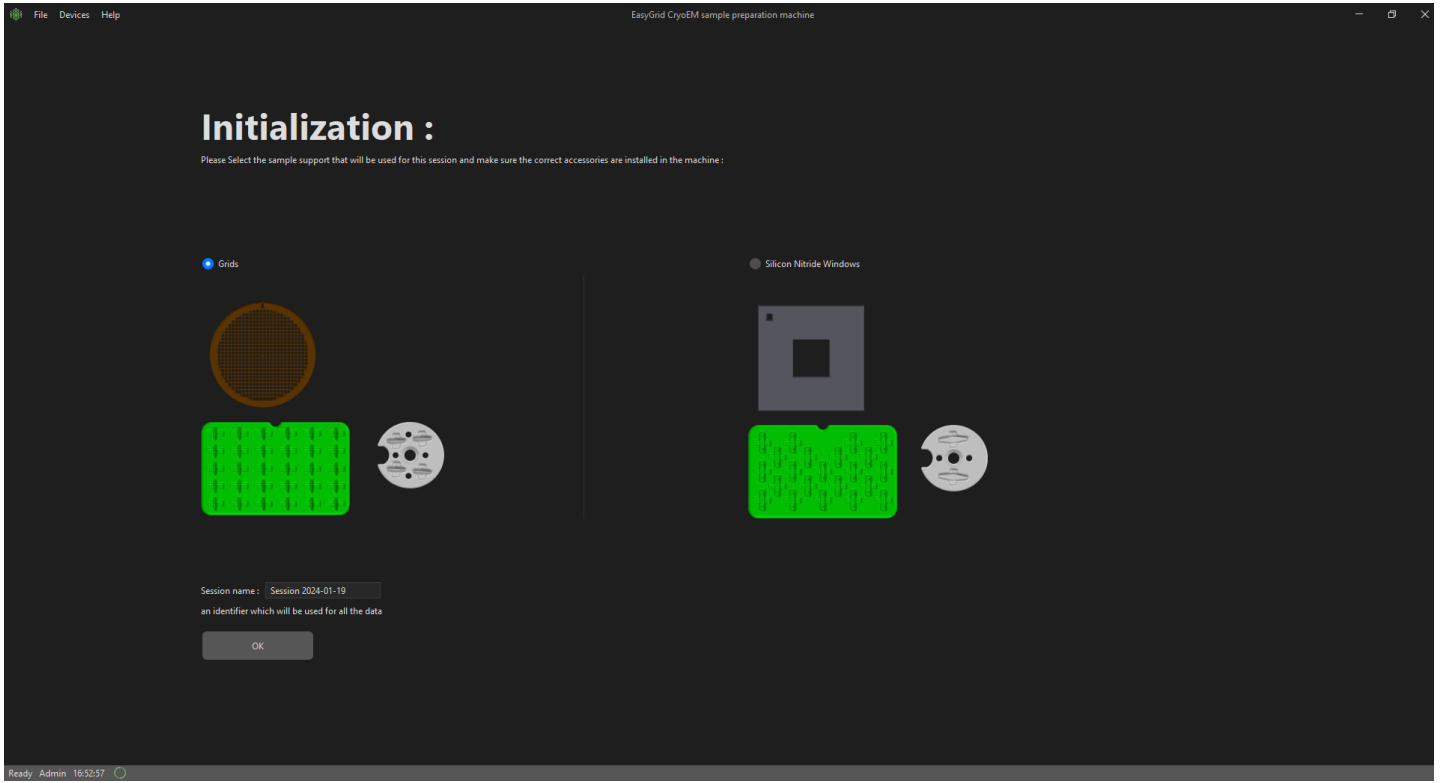
Ideal sample for Single Particle Analysis





Vitrification: rapid freezing of the sample to form amorphous ice and not crystalline.

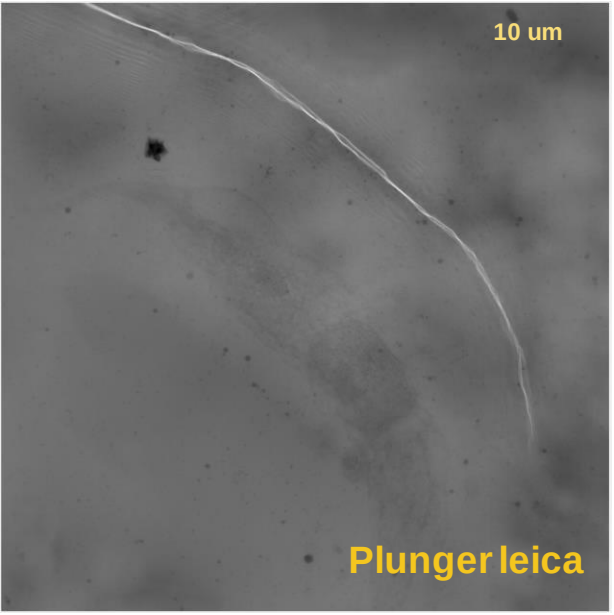
Rapid switch between sample supports

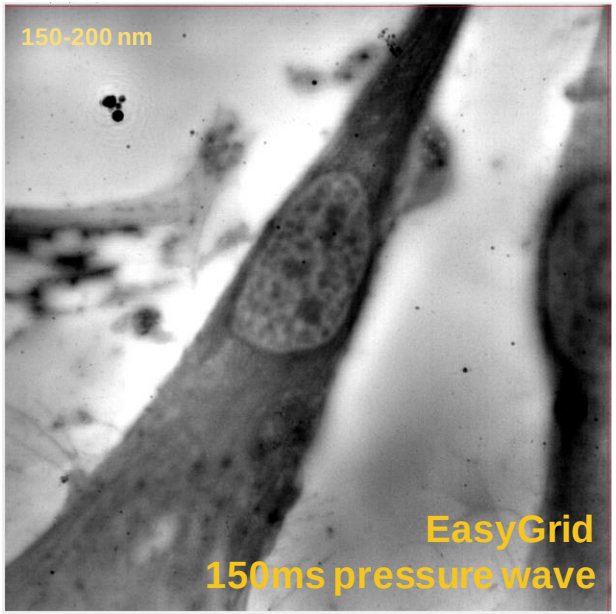


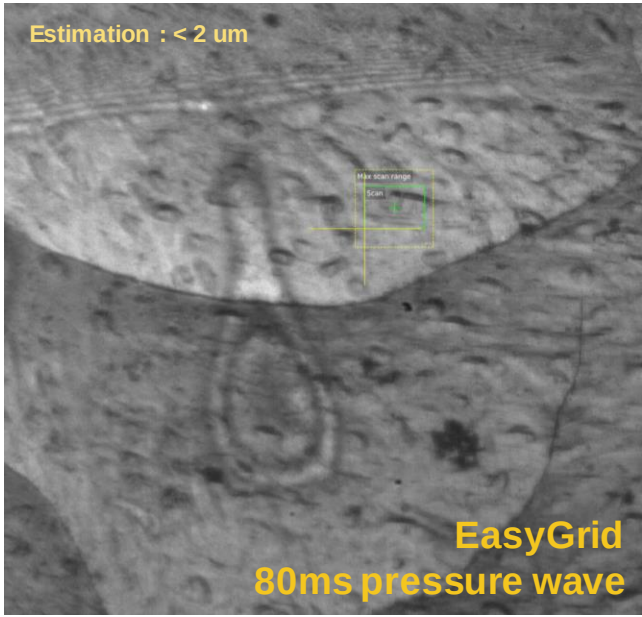
EMBL

X-ray nano imaging (ESRF ID16A)

2D Phase Reconstructions (25nm/pixel) - Toxoplasma Gondii infected HFF cells (24h post infection)



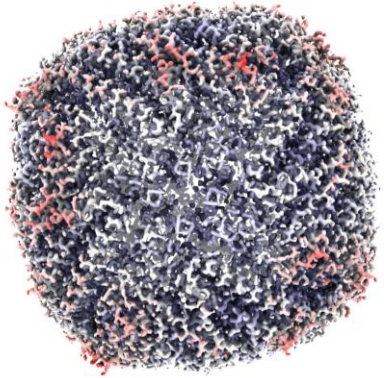




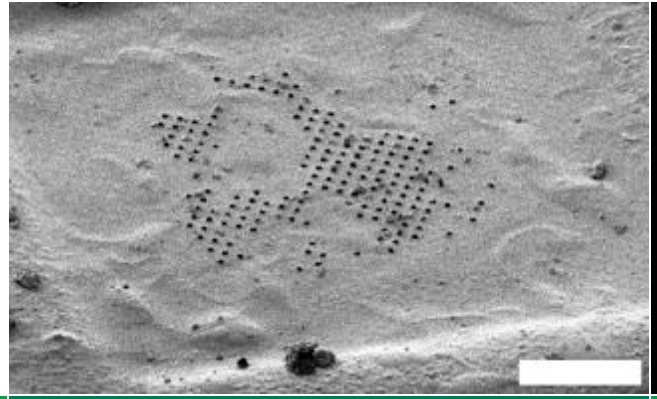
Ice layer thickness can be tuned

EMBL

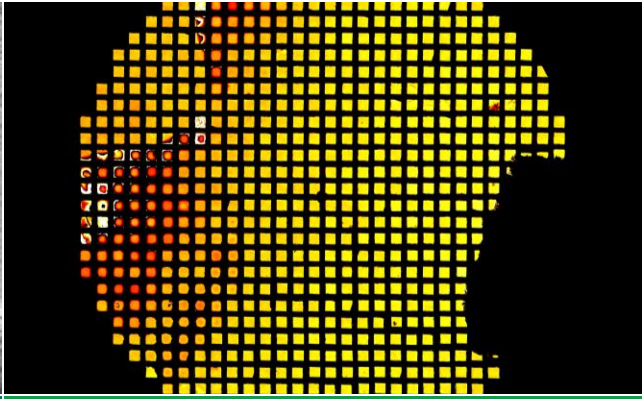
EasyGrid – use cases



Single Particle Analysis



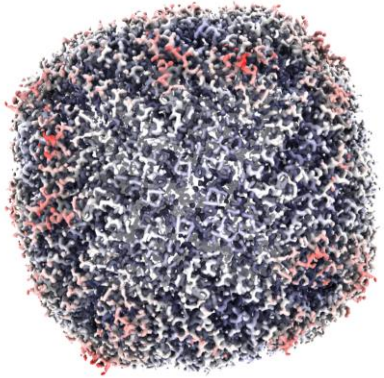
Cell vitrification



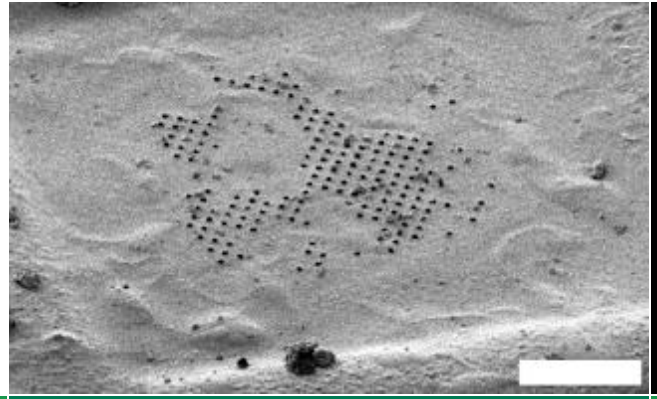
Quality control

EMBL

EasyGrid – use cases



Single Particle Analysis



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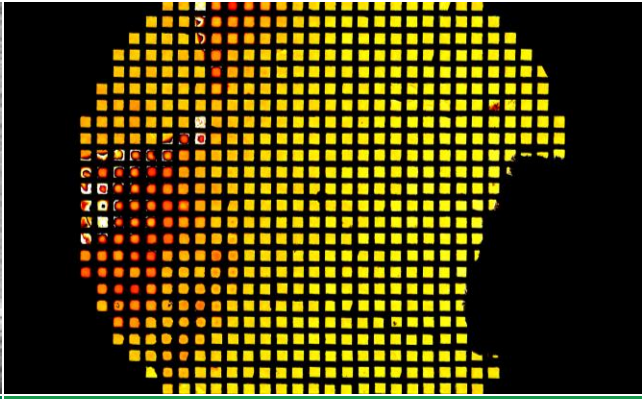


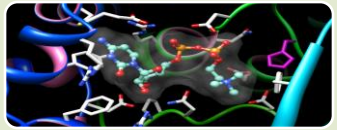
Quality control

EMBL

The traditional workflow for Cryo-EM SPA

Sample vitrification

Data-collection

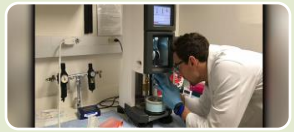
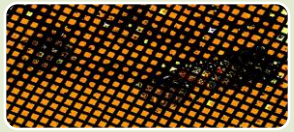


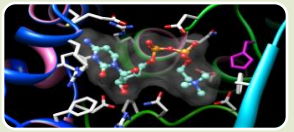
Structure

EasyGrid

EMBL

The EasyGrid workflow for Cryo-EM SPA

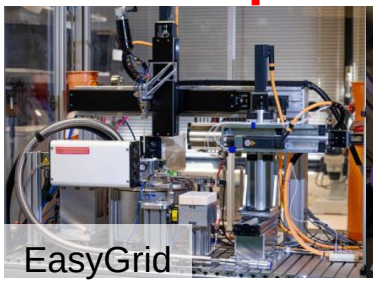


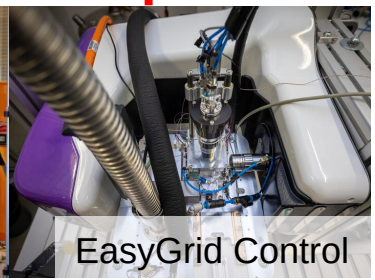
Sample vitrification

Quality control

Data-collection

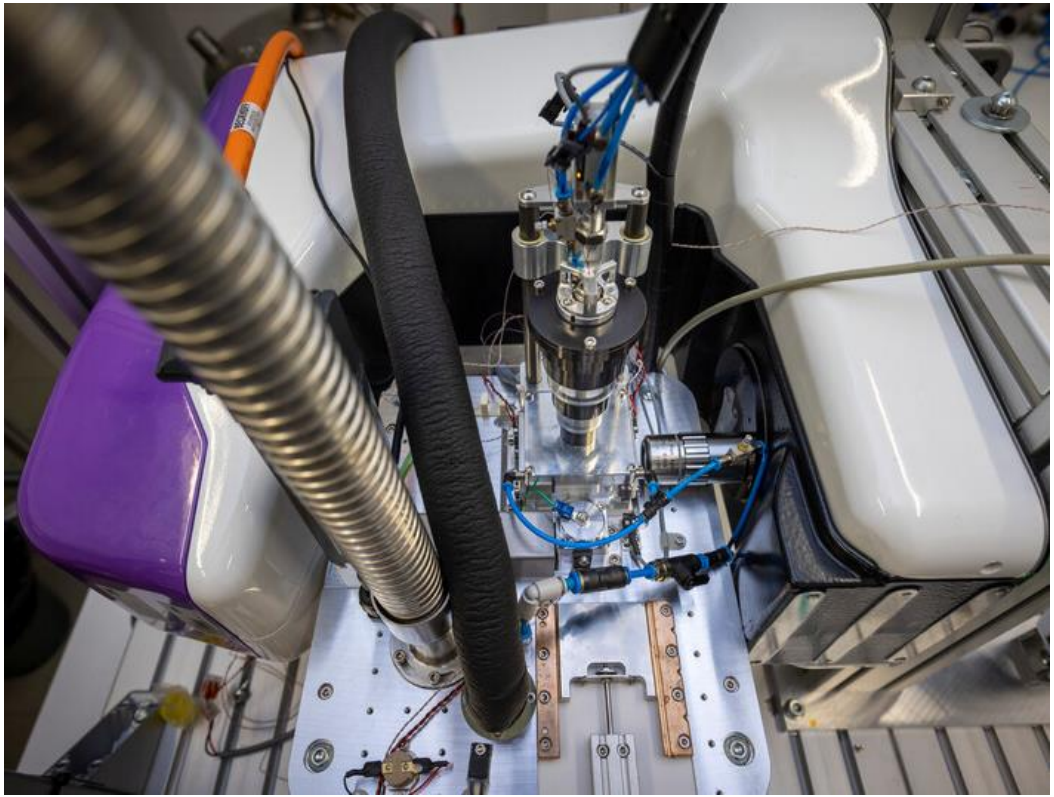
Structure


EasyGrid


EasyGrid Control

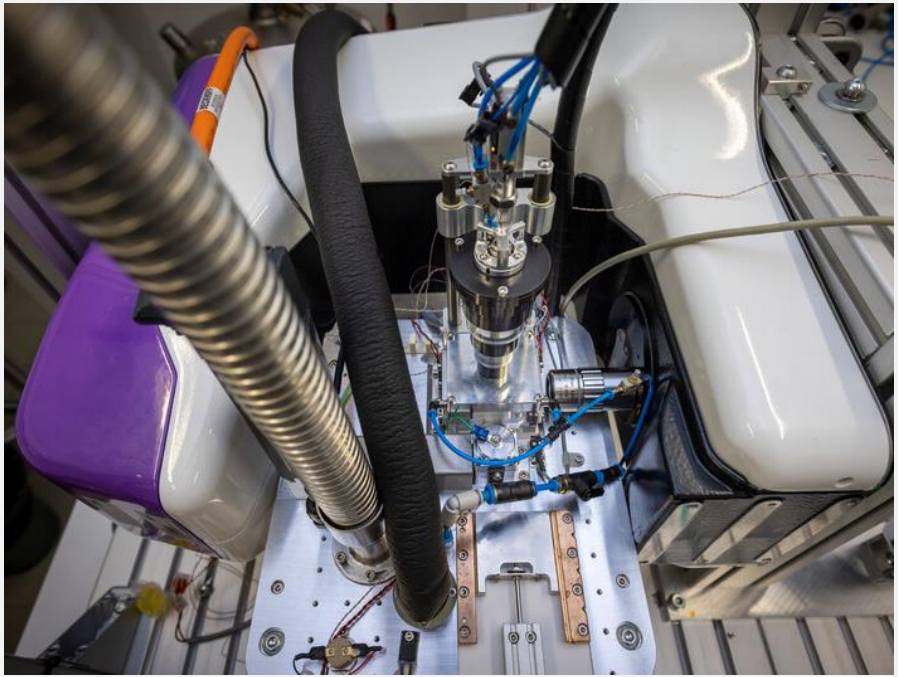
EMBL

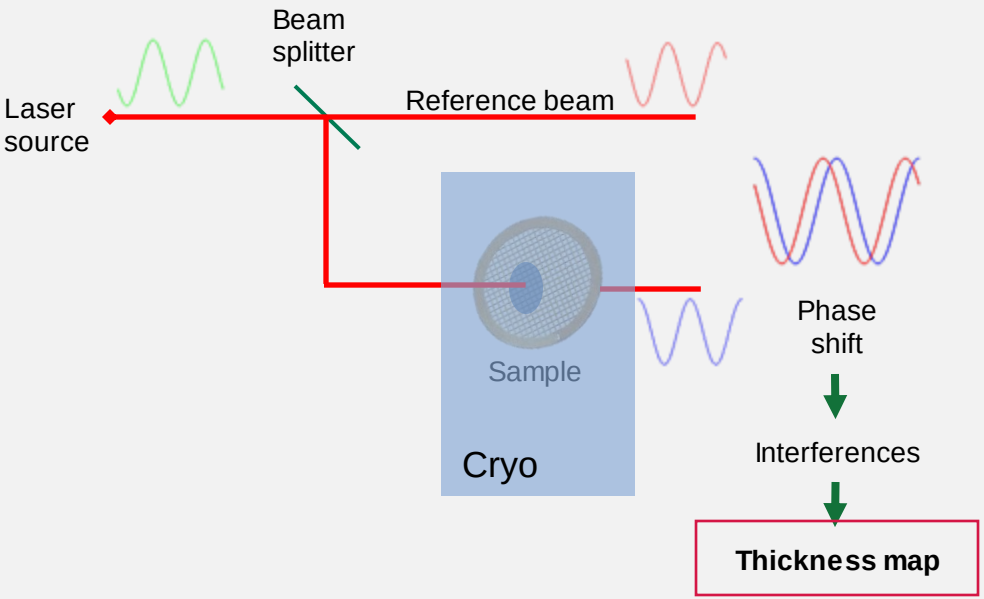
EasyGrid Control machine



EMBL

EasyGrid Control - principle



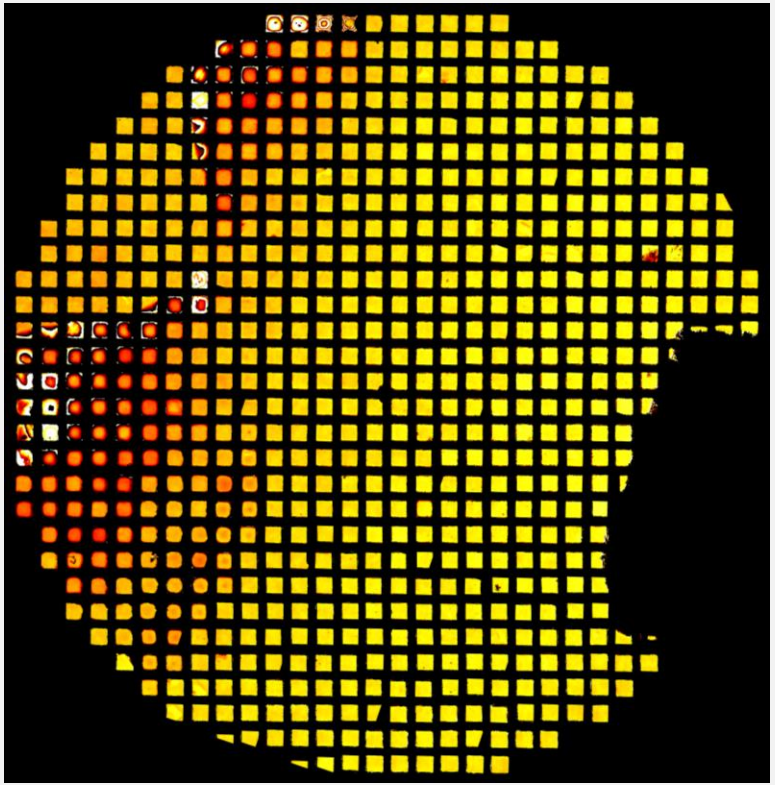


EMBL

EasyGrid Control cryo observation column

EMBL

SPA Grids – thickness map



EGC

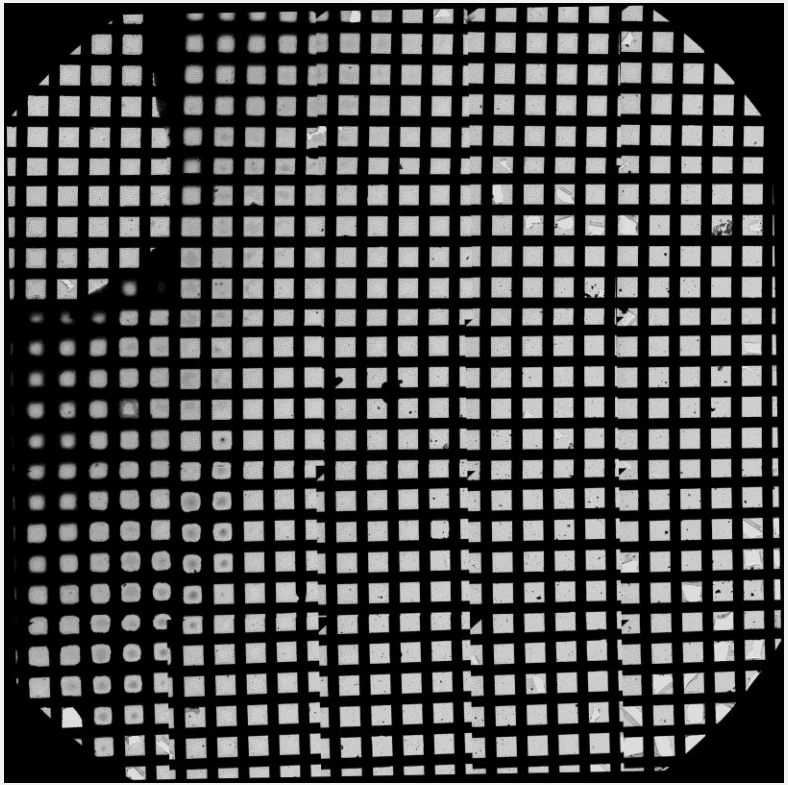
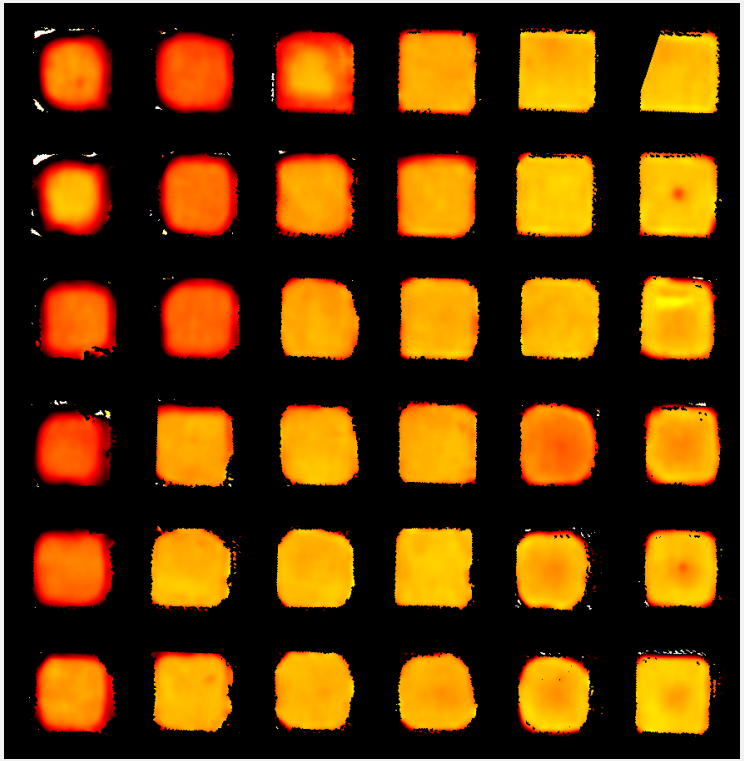


Image from Cryo-EM
Low resolution atlas

EMBL

SPA Grids – thickness map



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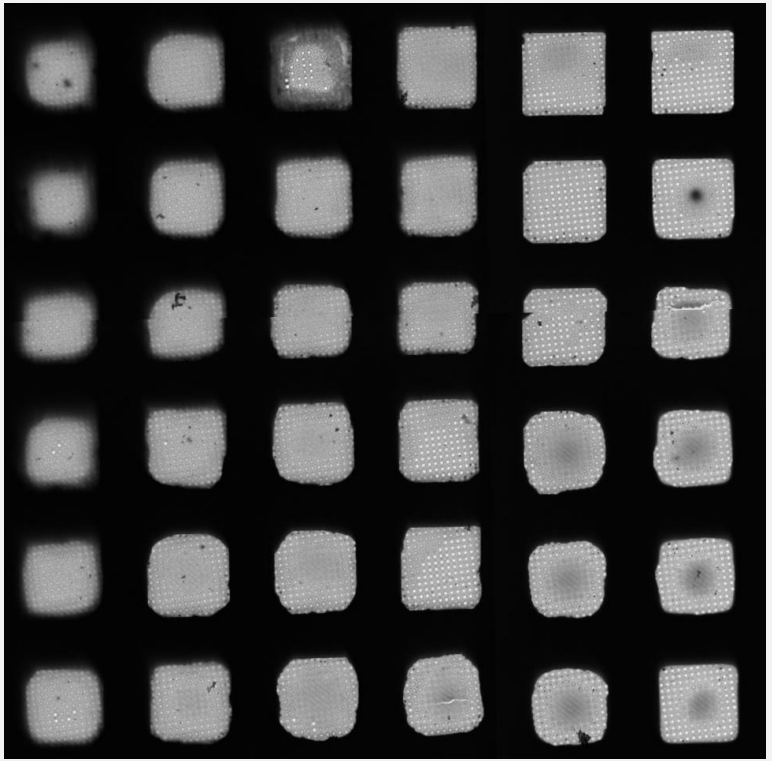
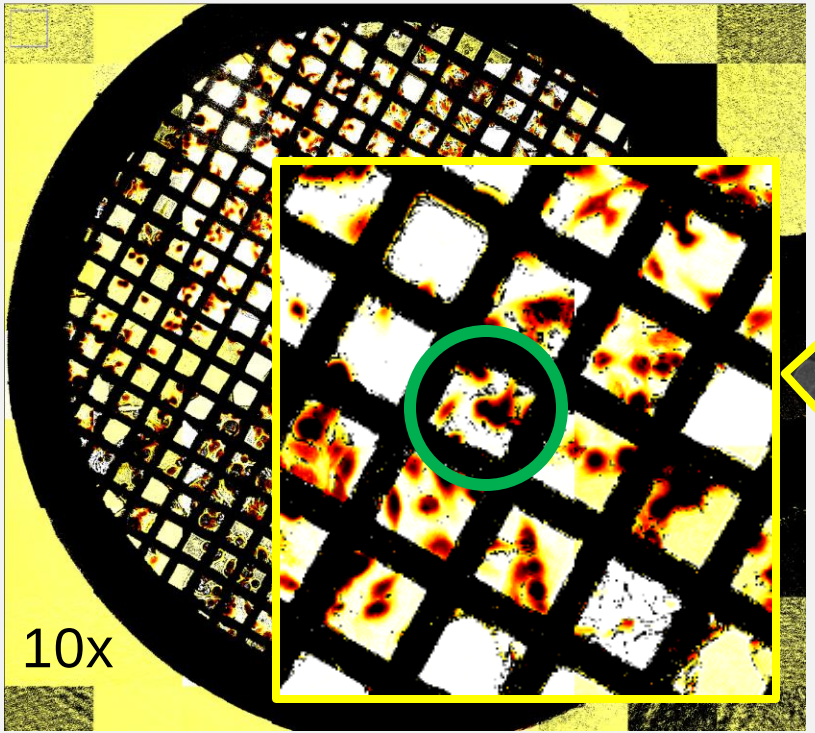


Glacios atlas

EMBL

Grids with vitrified cells

EGC (HeLa2-4_g1 : BF650ms)



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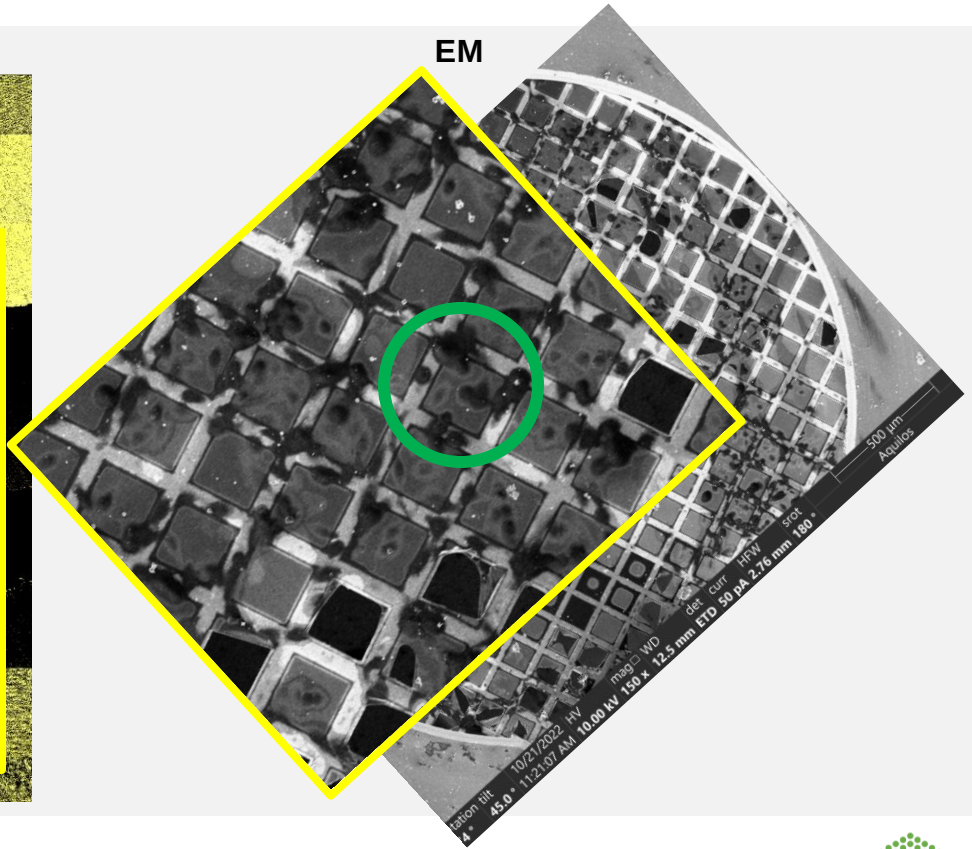

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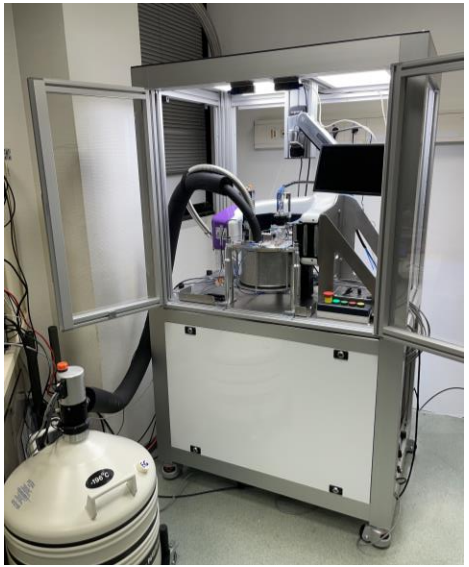
Optimizing sample preparation

EasyGrid



EasyGrid Control

FIB-SEM

Cryo-EM

X-ray nano imaging

EMBL

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  - Martin Weik
  - Benoit Gallet

iNEXT DISCOVERY

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Questions?

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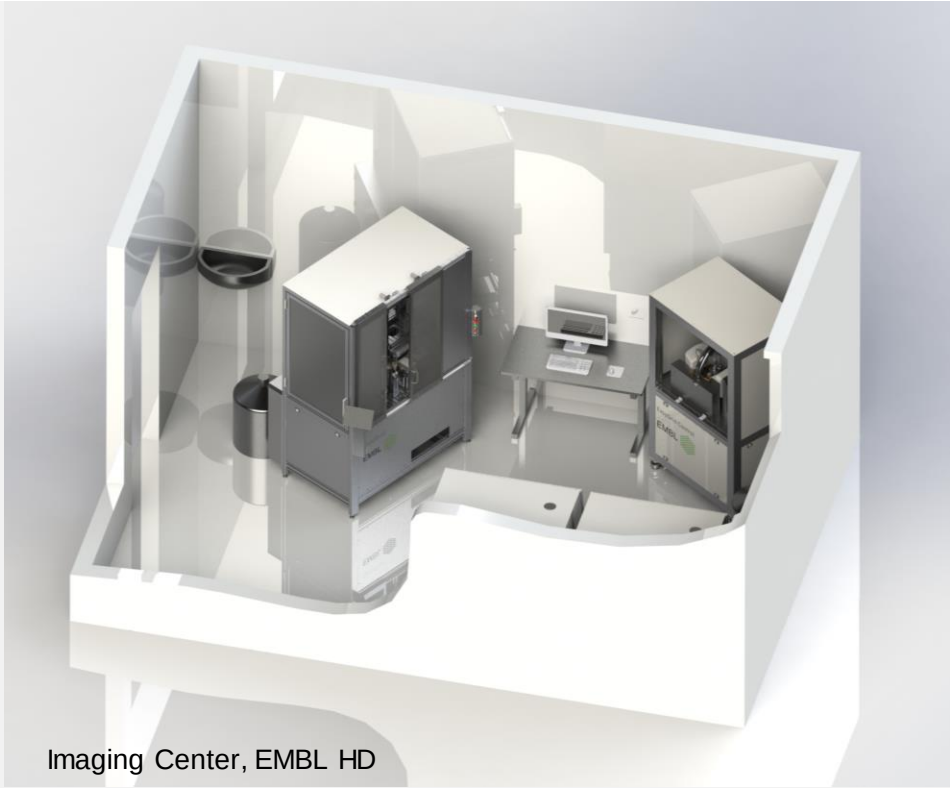
EasyGrid at EMBL-HD Imaging Center (2024)

EGC

EG2



Imaging Center, EMBL HD

EMBL

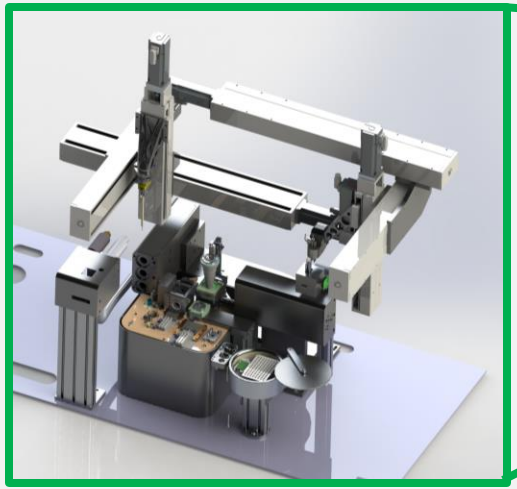
Next steps

EasyGrid 2 (EG2)
Sample preparation

EasyGrid Control (EGC)
Sample control

Installation to EMBL-HD Imaging Center

EasyGrid 3 (EG3)
Preparation & Control
Dedicated L2 lab @ EMBL-GR
Service provision - 2024



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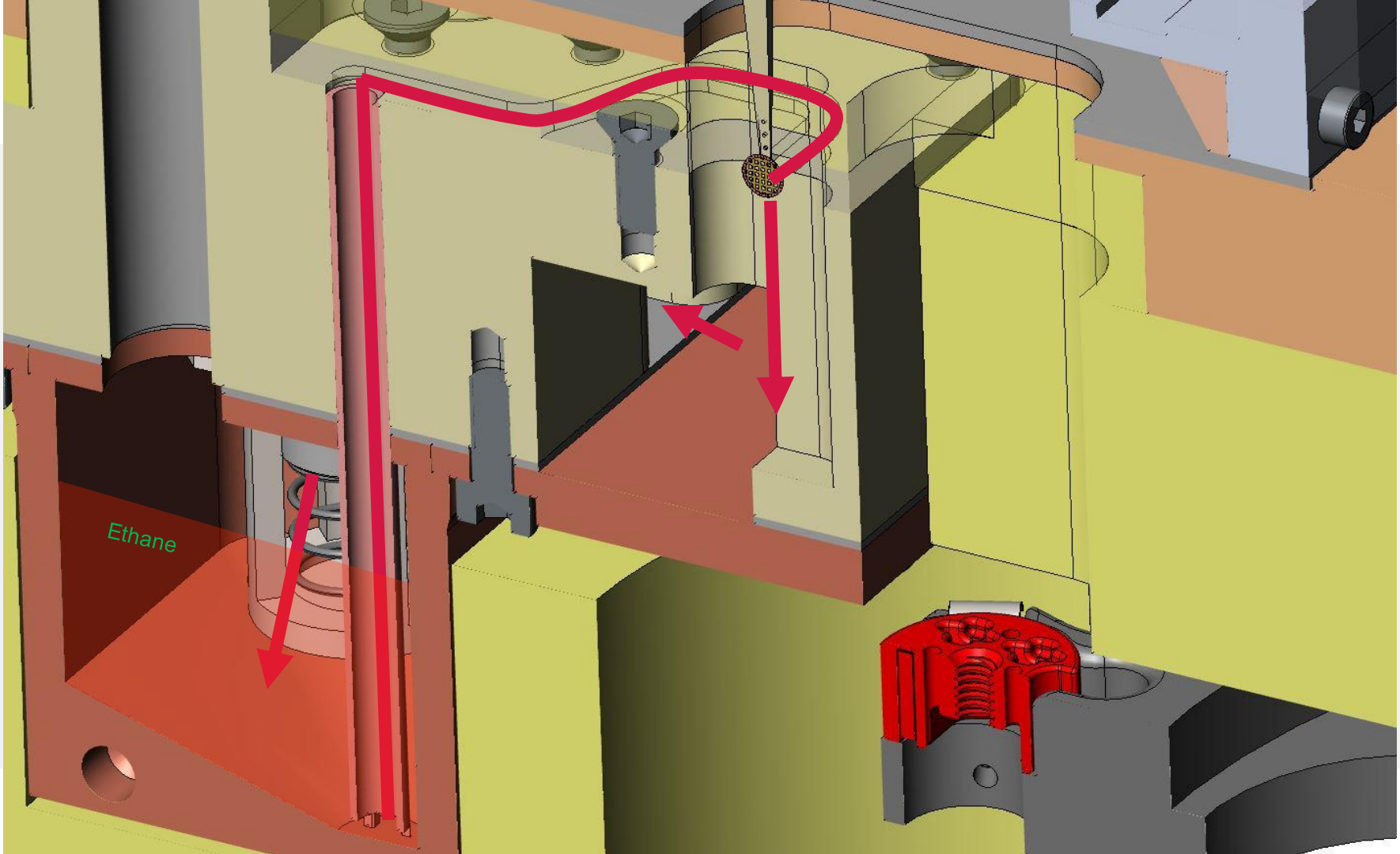
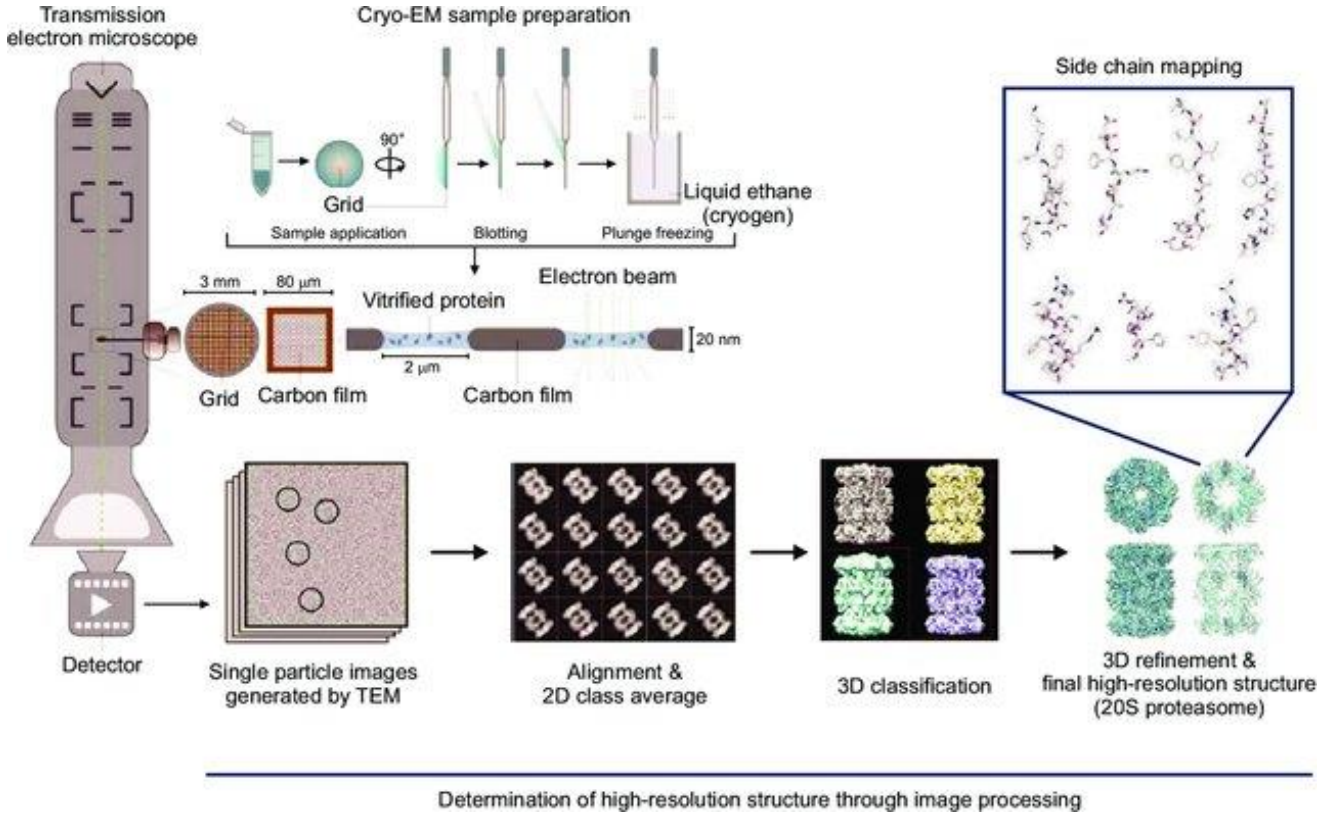

Ethane

Cryo-EM principle



EMBL