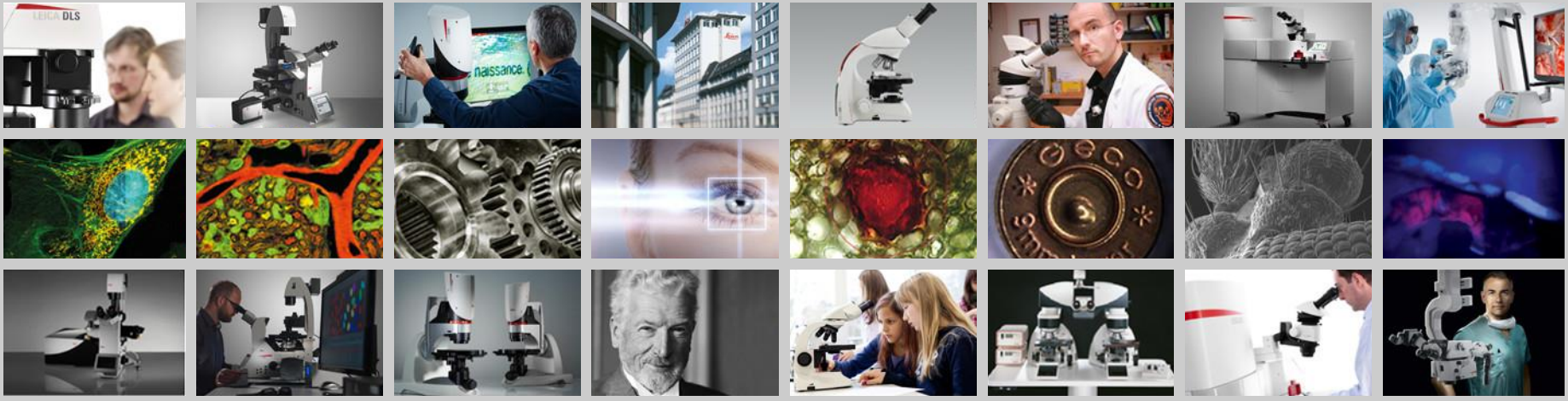
From Eye to Insight

Leica MICROSYSTEMS



The 1st Cryo Electron Microscopy and 3D Image Processing of Macromolecular Assemblies and Cellular Tomography (CEM3DIP) 2016

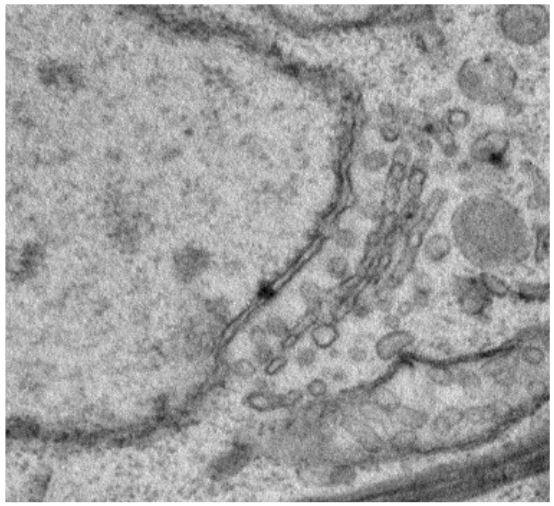
EM CRYO PREPARATION WORKFLOWS

Update on the Latest Instrumentation

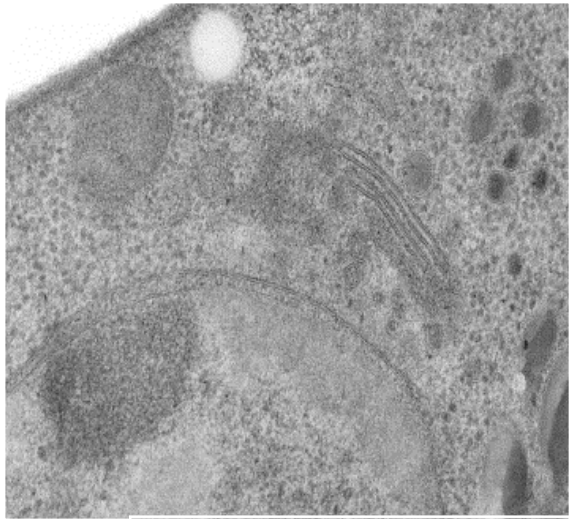
Science Lab

Why Cryo?

- Low Contrast in EM
- Critical Temperature -140° C
- Sensitive to Electron Beam
- Contamination (ice)

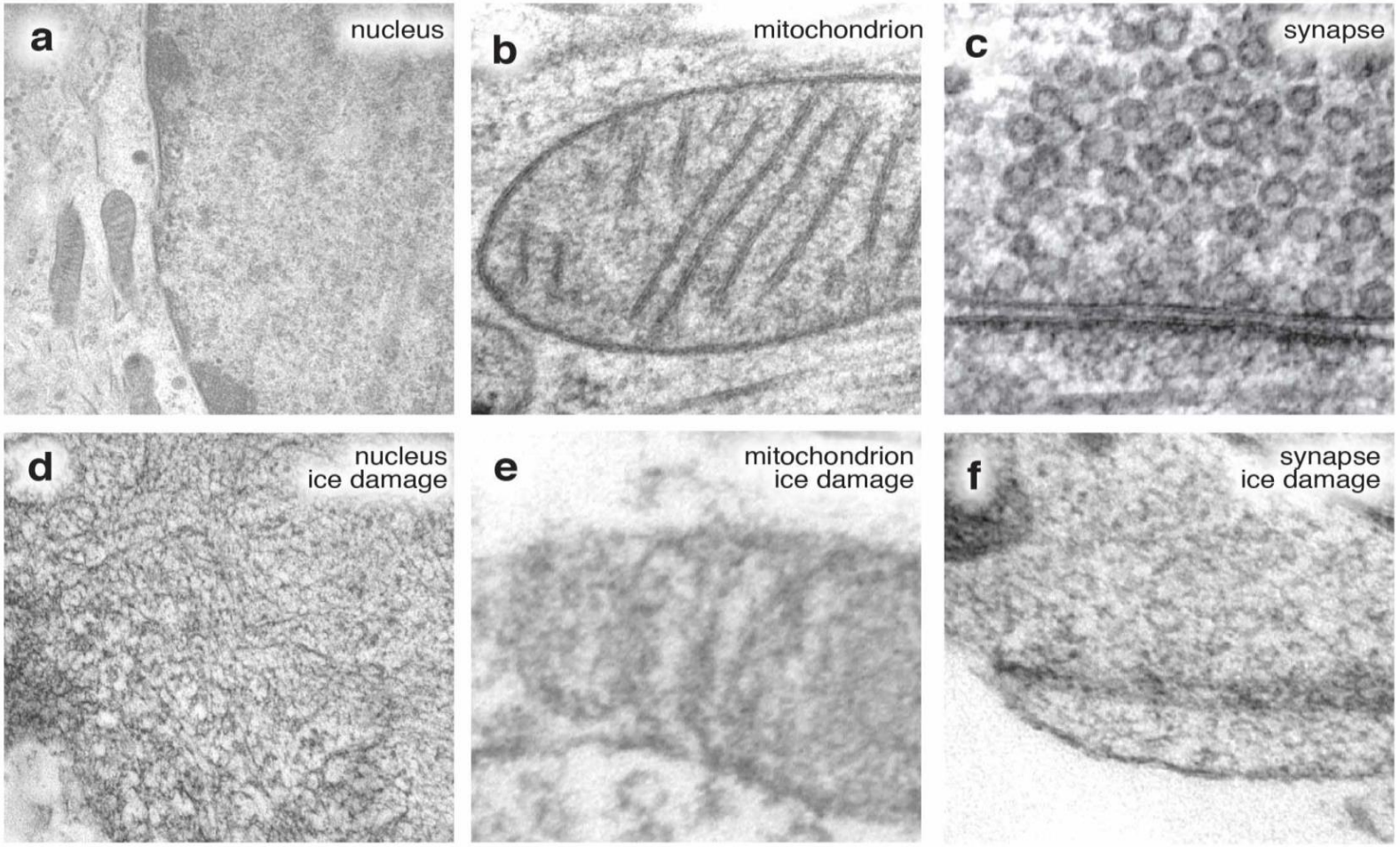


+ Low Artefact Formation During Fixation
+ Most Native Fixation and Analysis Method
+ Fastest Fixation
+ Frozen Hydrated Samples



Toxoplasma gondii

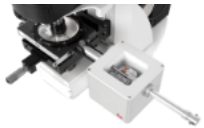
The “No-Ice Challenge”



Watanabe S[1], et al. Nature, 2013 Dec 12, 504 (7479):242-7 doi: 10.1038 nature 12809

Connectivity and Cryo-Preparation Workflows

1.



Cryo-CLEM

2.

Cryo Fixation by
High Pressure Freezing

Cryo-Transfer

4.

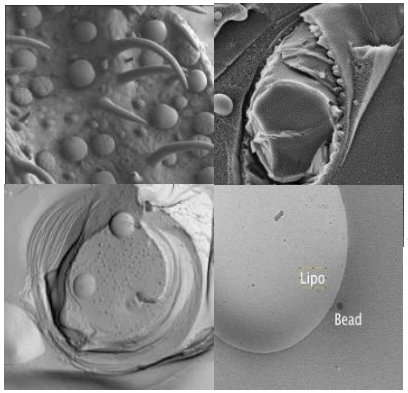
Cryo Ultramicrotomy

3. Freeze Fracture, Freeze Etching and Coating

5. Cryo Ion Milling

Cryo-Transfer

cryoEM



1. Correlative Light and Electron Microscopy

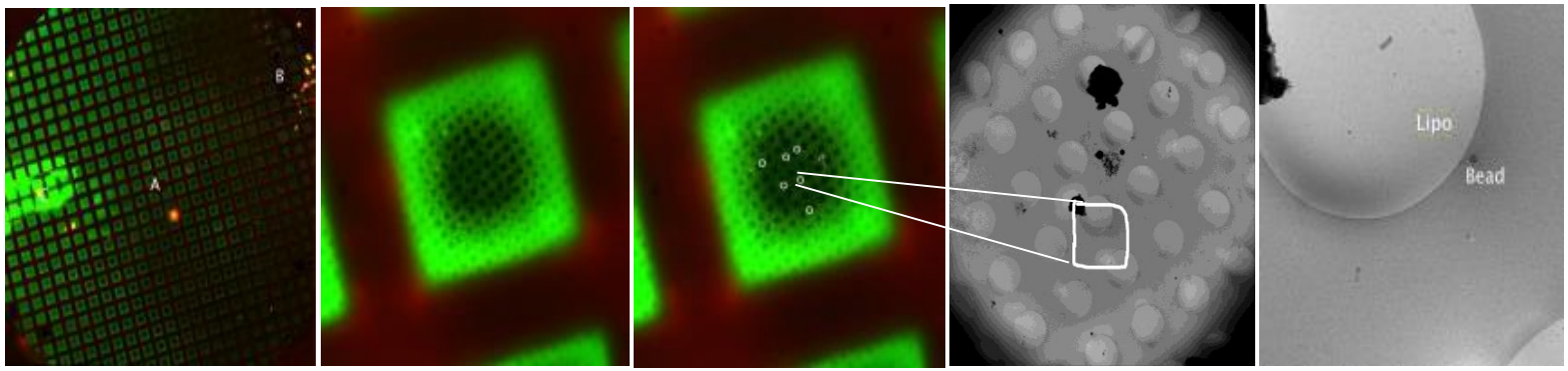
- Correlative Light and Electron Microscopy (CLEM) combines fluorescence light microscopy (FLM) and electron microscopy (EM) **imaging of the same sample**.

- A method which allows FLM **rapid screening** of large areas and fast determination of regions of interest in EM. This **reduces the user interaction time** on the EM significantly.

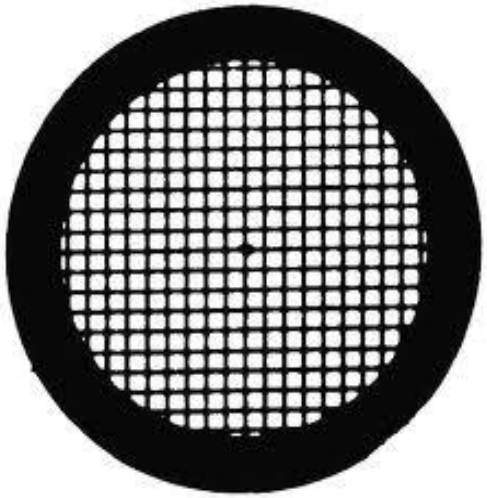
- Provides **deeper understanding** of analyzed sample by **overlaying complementary information** such as high resolution LM localization and EM ultrastructural context data.

Fluorescence Light Microscopy (FLM)

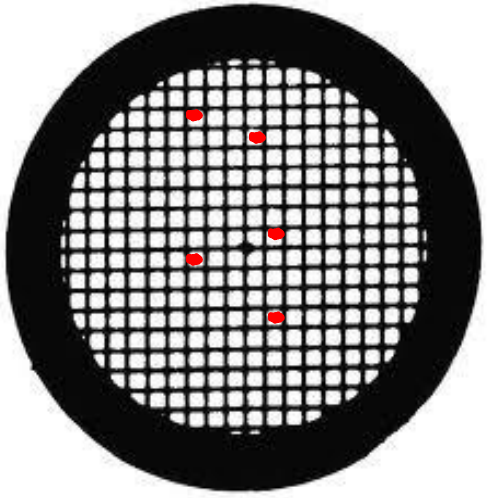
Transmission Electron microscopy (TEM)



CLEM Application – Rapid Screening

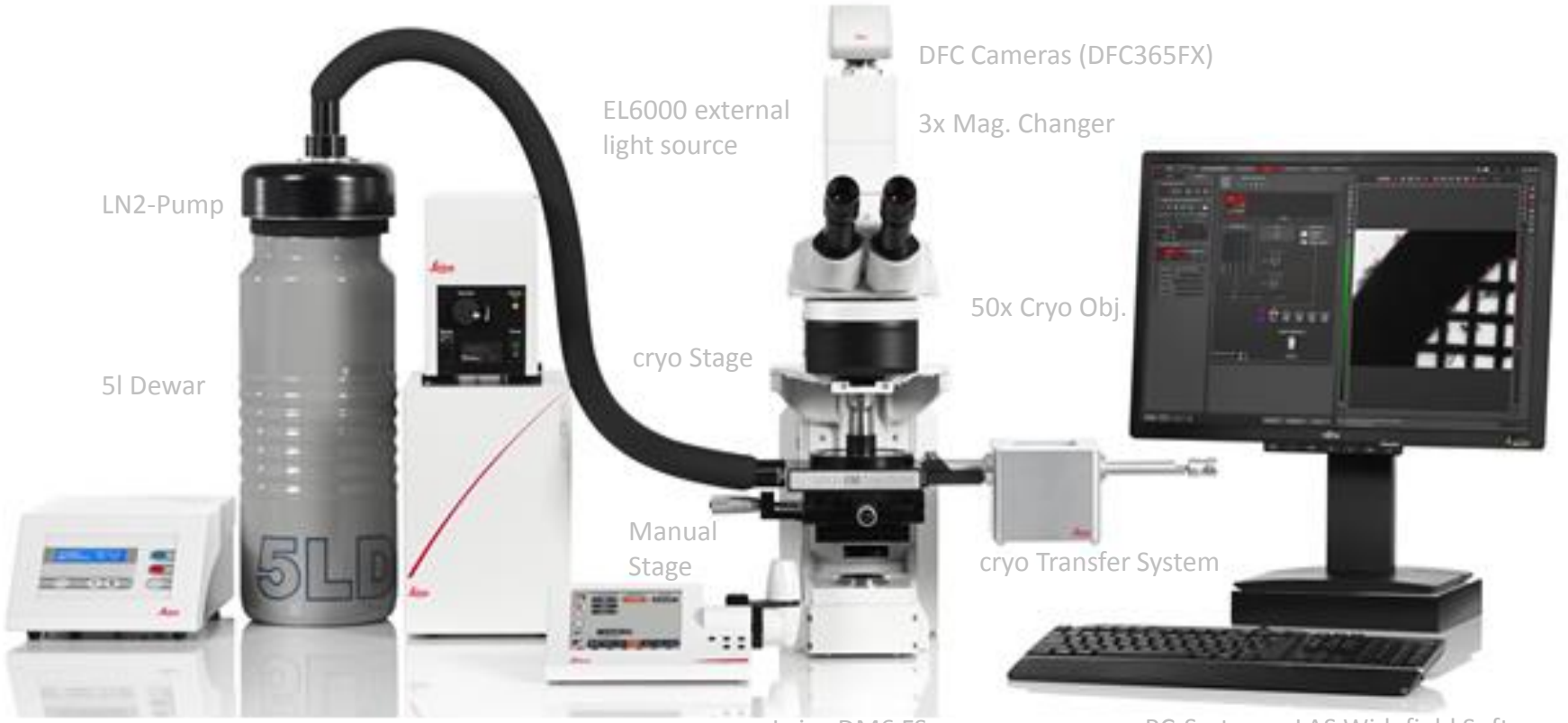


Without FLM prior the EM the user might have to check all grid squares to find adequate target structures



With FLM prior the EM the user knows which grid squares contains promising target structures

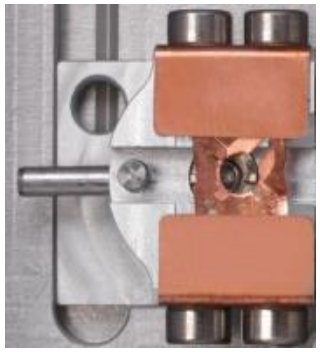
Cryo CLEM System



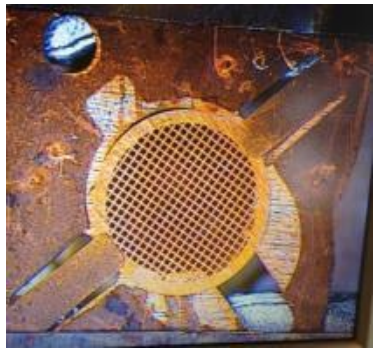
Cryo CLEM System

Plunge frozen
or
HPF/UM sample

Cryo transfer system
with cartridge loading station

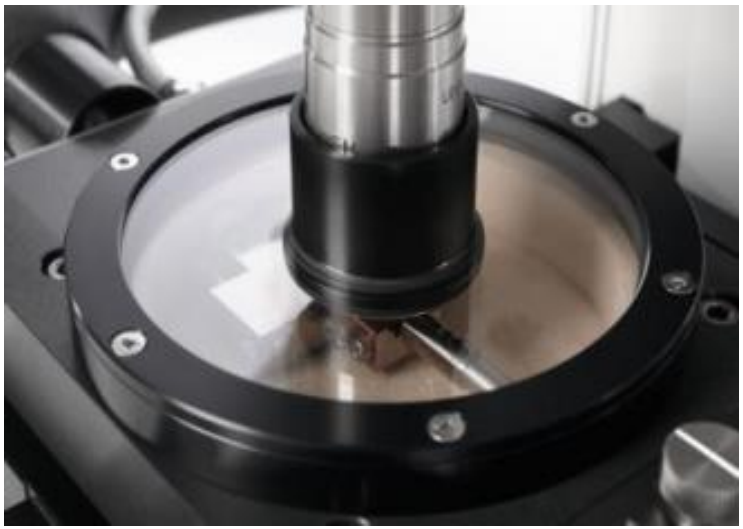


Cartridge loading station
with empty cartridge



Cartridge with 3mm
grid

Cryo transfer system docked to cryo stage



Leica cryo stage with cover and cryo
CLEM objective.
Temp. range –195 ° C to +60 ° C

Cryo CLEM System - Objective

Leica HCX PL APO 50x / 0,90 CLEM objective

- Apochromatically corrected
- Thermally decoupled lens
- NA 0.9
- Low working distance of 0.28 mm
- Localization of ~50nm

Workflow Solution

High Pressure Freezing,
Cryo-Ultramicrotomy

or

Grid Plunging

Cryo-Transfer
System

Leica Cryo Light Microscopy

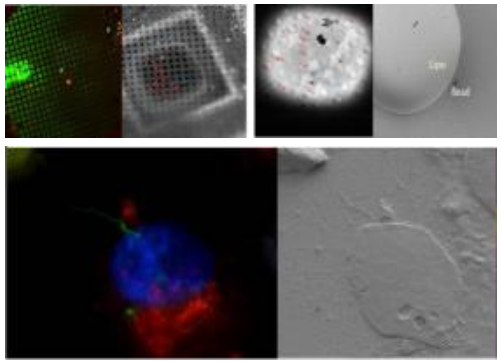
Cryo CLEM

Leica LAS X Widefield Images used for
correlation of LM marked structures in EM

Transfer to cryoTEM

Cryo-TEM

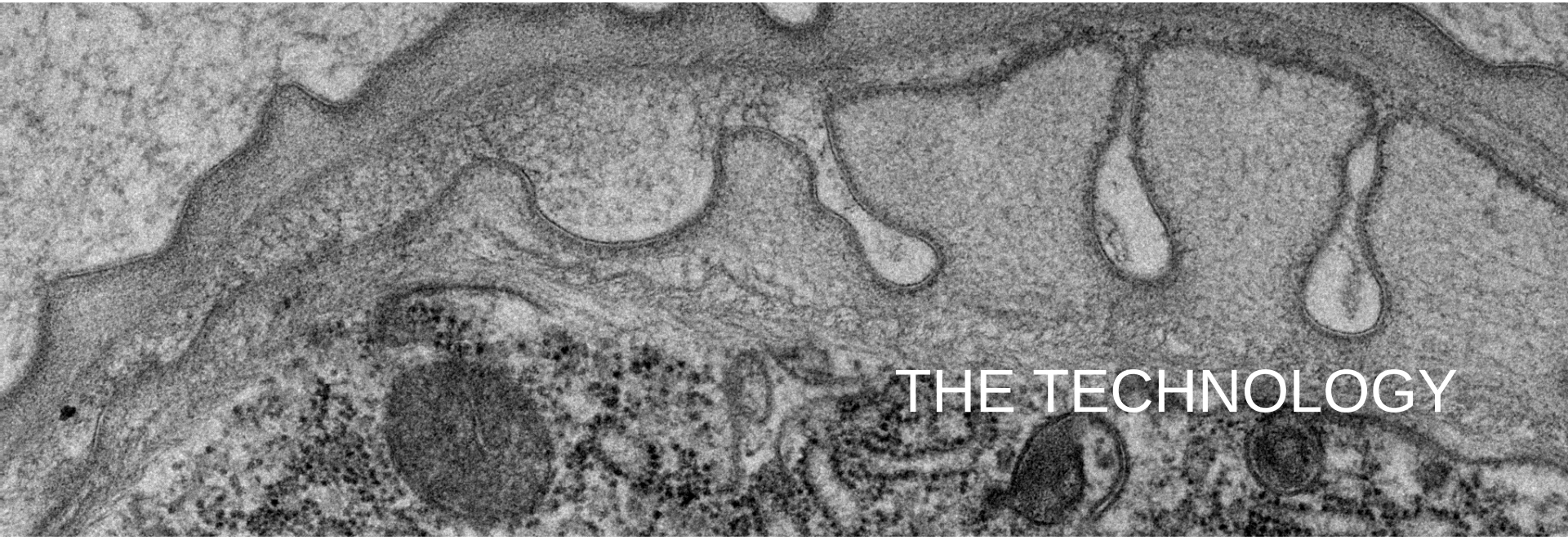
Image Analysis



2.

Leica
MICROSYSTEMS

A NEW ERA IN HIGH PRESSURE FREEZING



THE TECHNOLOGY

C. elegans, courtesy of Elly van Donselaar, Martin Harterink and Karin Vocking, Utrecht University,

A High Pressure Freezer arrests aqueous samples in their native state to deliver the best possible sample preservation.

LEICA EM ICE

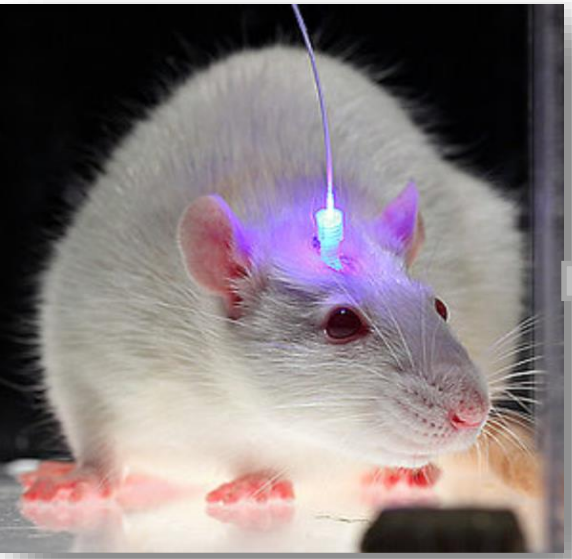
Leica EM ICE is a platform for game-changing discoveries.

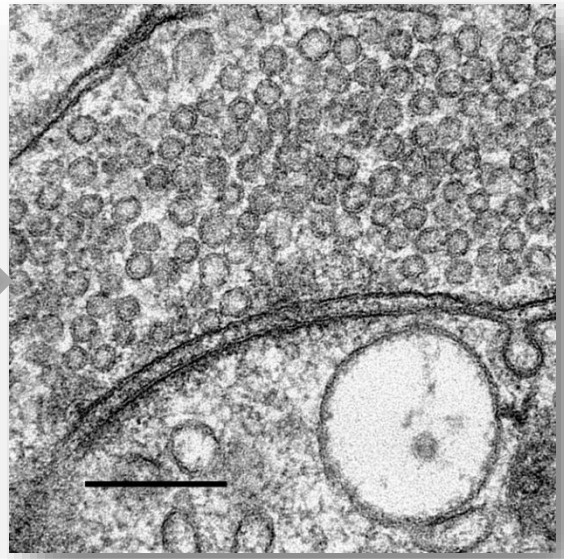
LEICA EM ICE LIGHT STIMULATION

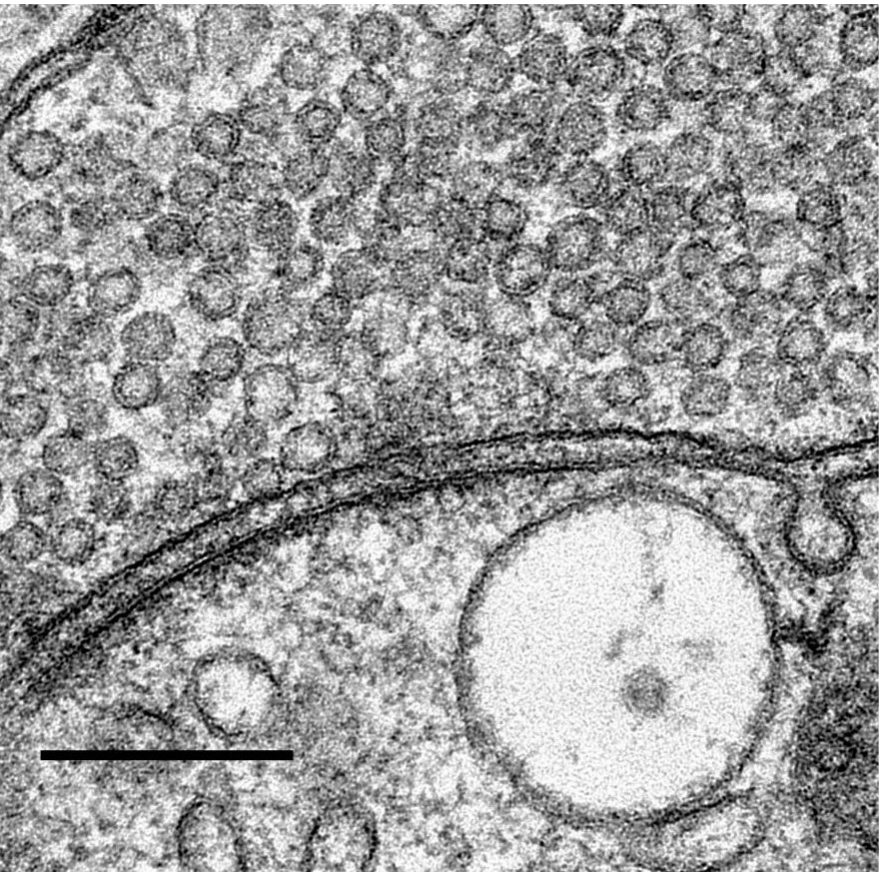
New possibilities for researchers in life science and industry

WHY LIGHT STIMULATION?

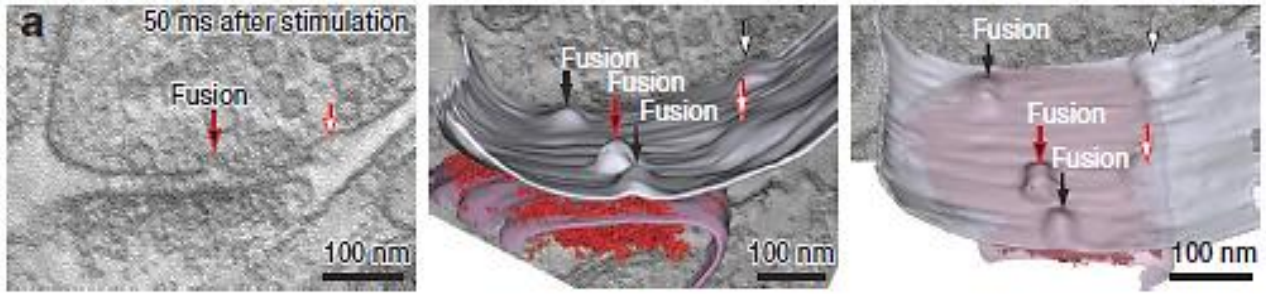
Utilizing light to understand the complex process of neurotransmission







The synchronization of light stimulation and high pressure freezing allows the visualization of highly dynamic process at a nanometer resolution and millisecond precision.



Nature. 2013 Dec 12; 504(7479):242-7

Variety of Light Spectra

- Five modules, five wavelengths automatically recognized by the instrument software

Program Your Experiment

- Precise correlation between the light impulse and the time of freezing

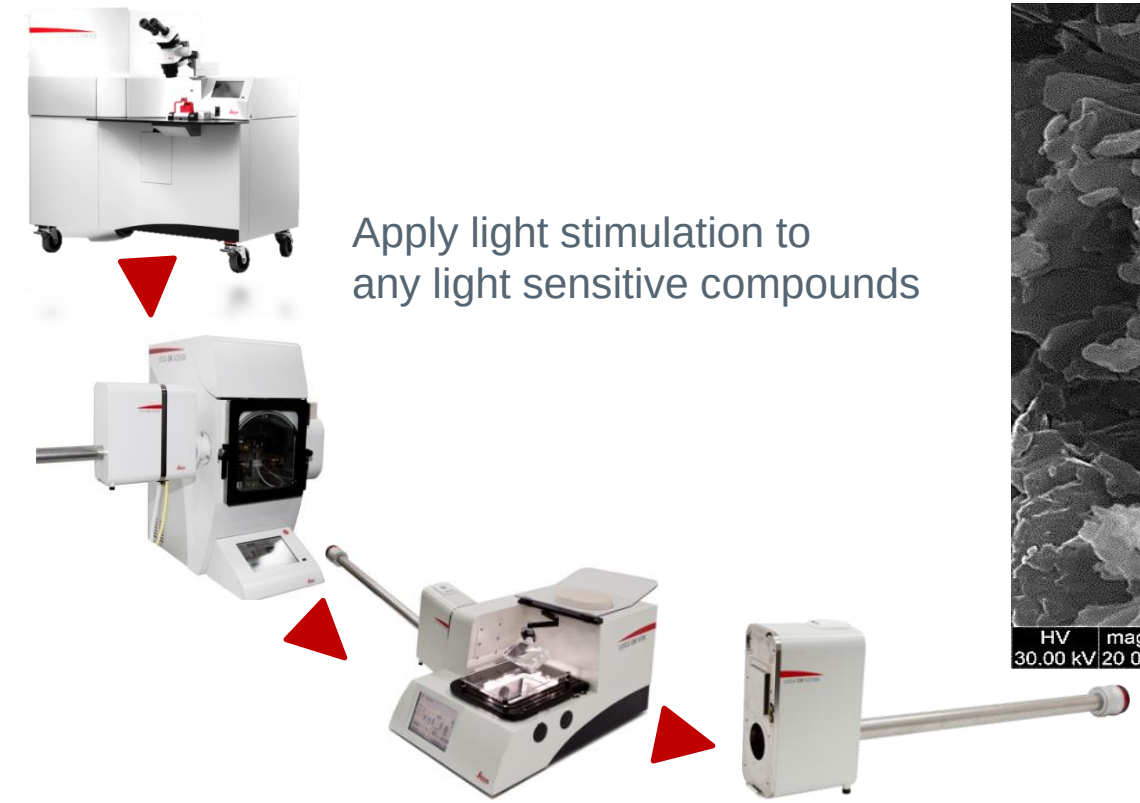
- One minute recovery time between freezing cycles
- One second from fresh-to-frozen
- Only 20 minutes cool-down time
- Only 30 L daily consumption of LN2


FOCUS ON YOUR SAMPLE
One move, fully automated loading
Closing the cover triggers the perfect alignment of cartridge assembly followed by the freezing process

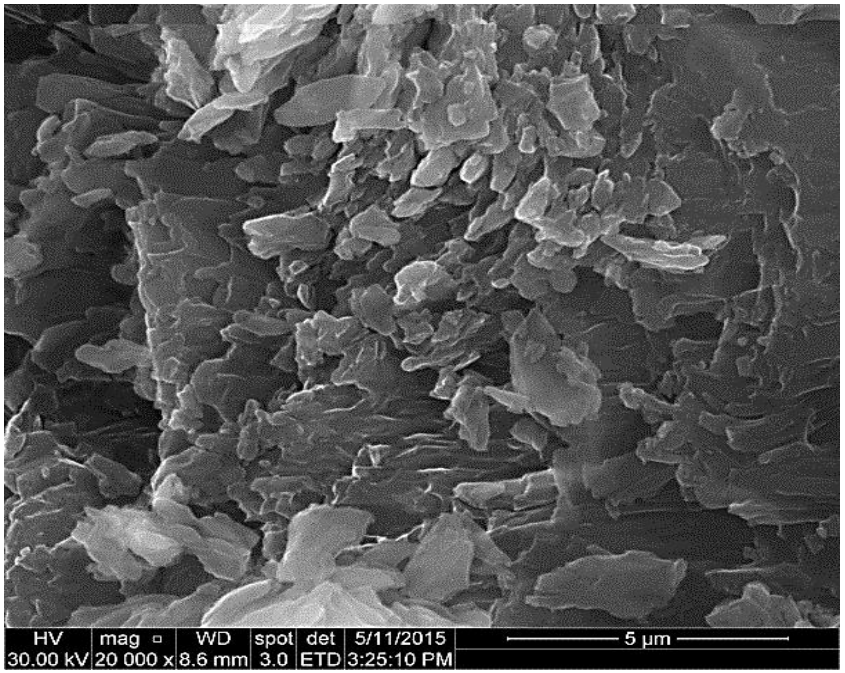
SAMPLE STORAGE DEWAR

- Three separate positions
- Nine consecutive freezing cycles
- Programmed rotation

DISCOVER NEW POSSIBILITIES



Apply light stimulation to
any light sensitive compounds



Sunscreen lotion frozen after millisecond UV light stimulation.

3. Cryo-preparation – Freeze Fracture

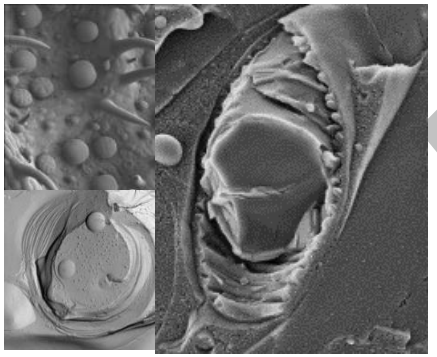
Cryo Fixation
High Pressure Freezing

Cryo-Transfer

Freeze Fracture, Freeze Etching and Coating

Cryo-Transfer

Cryo SEM



Leica
MICROSYSTEMS

A REVOLUTION IN FREEZE FRACTURE

READY TO OPERATE

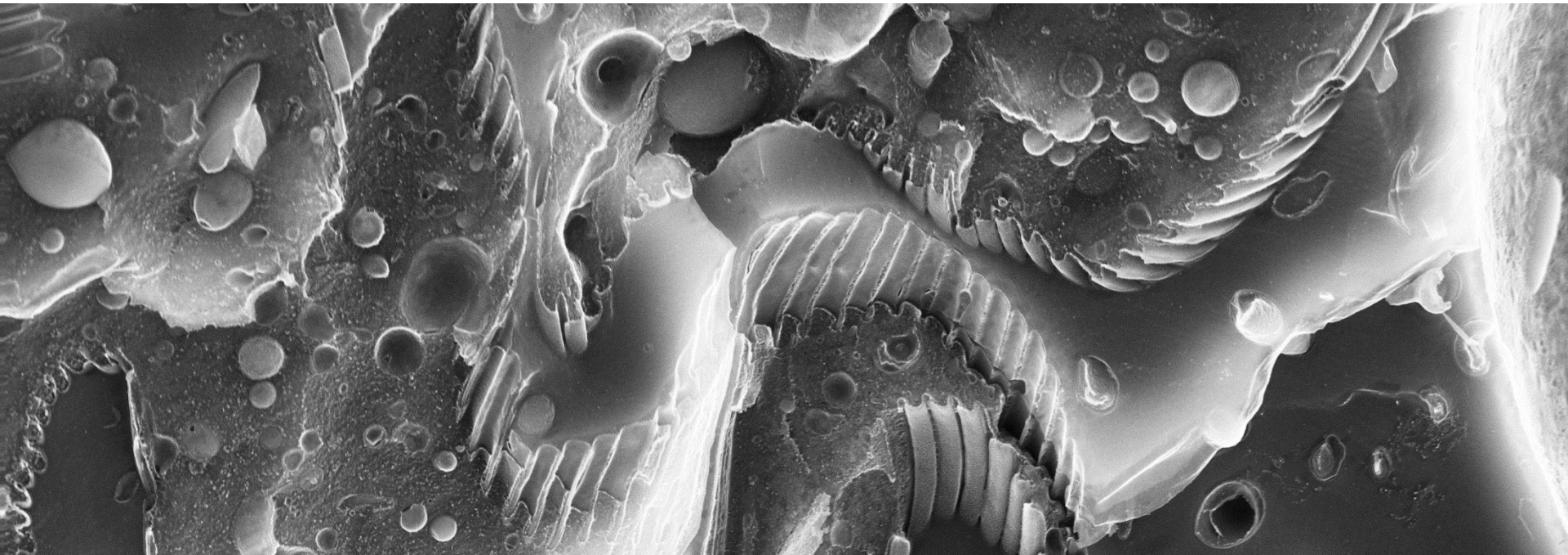
- Load locks for sample, microtome, e-beams
- New cooling, shielding, microtome, e-beams
- High vacuum 10-8mbar
- Connection to VCT500


PRECISE AND CLEAN CUTTING
Exchangeable and 3 axis movable knife

FREEZE FRACTURE

Freeze Fracture breaks frozen specimens to reveal internal structures

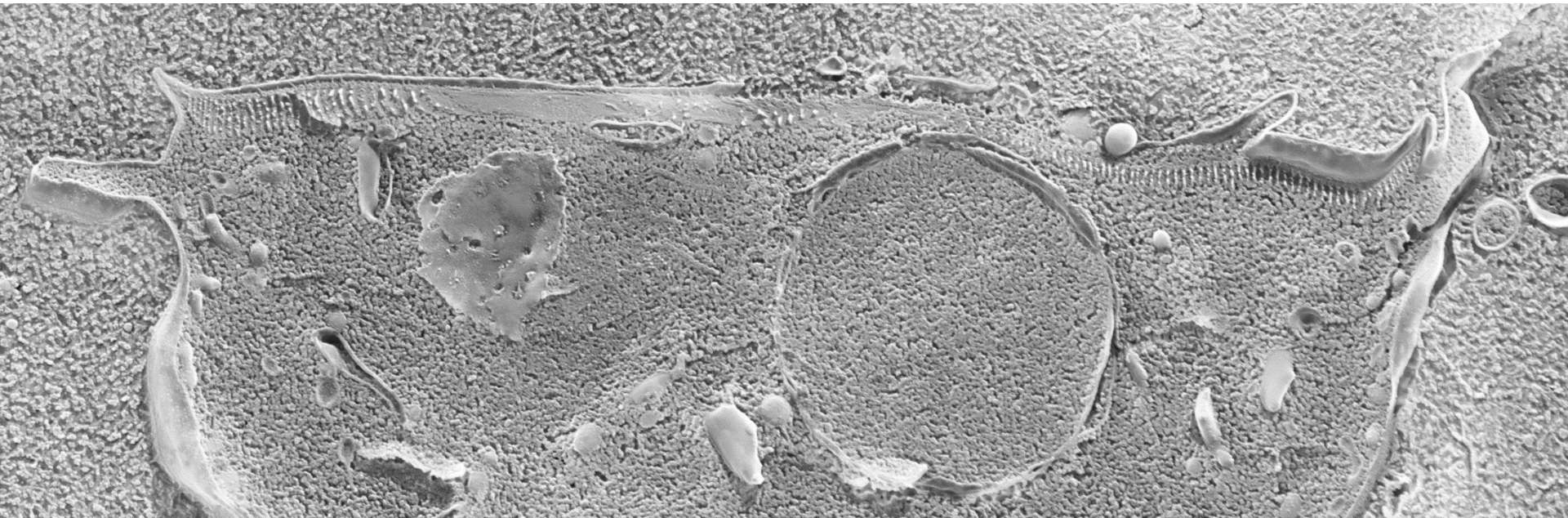
Euglina gracilis, courtesy of Andres Käch, Center for Microscopy and Image Analysis, University of Zurich, Switzerland



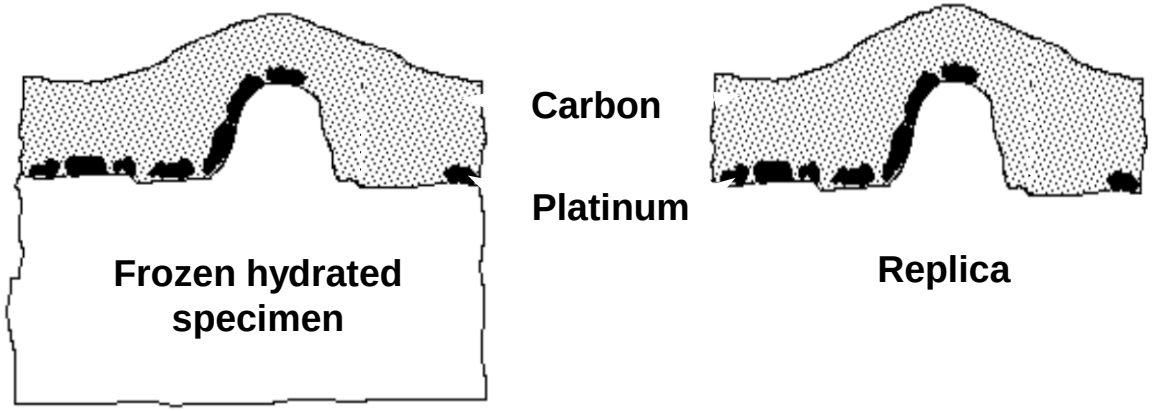
FREEZE ETCHING

Freeze Etching is the sublimation of surface ice under vacuum to reveal details of the fractured face that were originally hidden.

Giardia lamblia, courtesy of Andres Käch, Center for Microscopy and Image Analysis, University of Zurich, Switzerland



FREEZE FRACTURE & REPLICAS



cryo-SEM

Backscattered signal

TEM

Amplitude contrast

4. THE FUTURE IS CONNECTIVITY

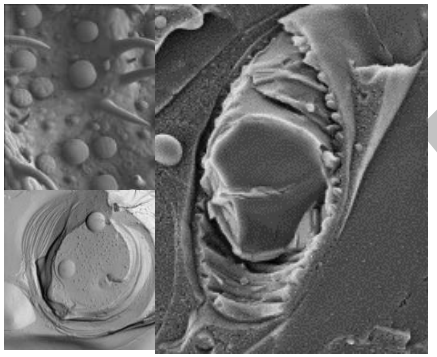
Cryo Fixation
High Pressure Freezing

Cryo-Transfer

Freeze Fracture, Freeze
Etching and Coating

Cryo-Transfer

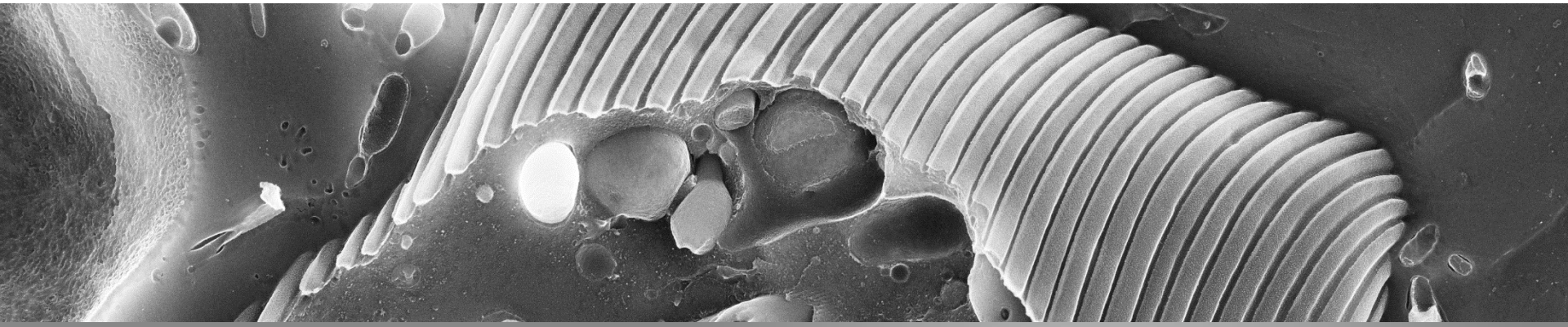
Cryo SEM

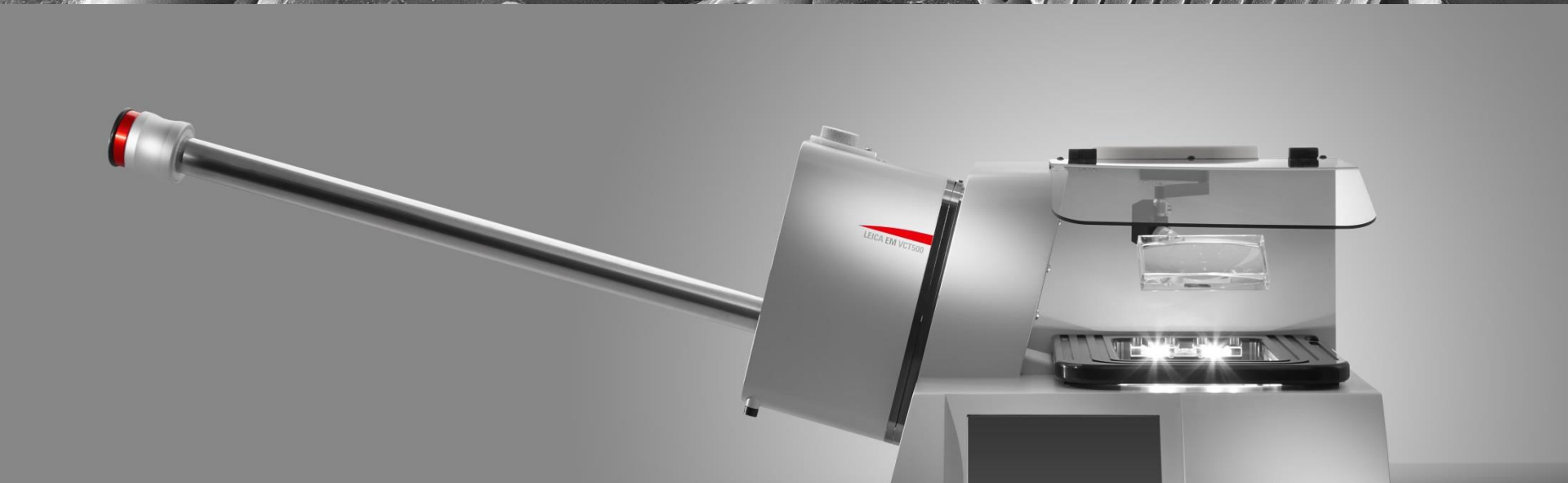


WHY VACUUM CRYO TRANSFER?

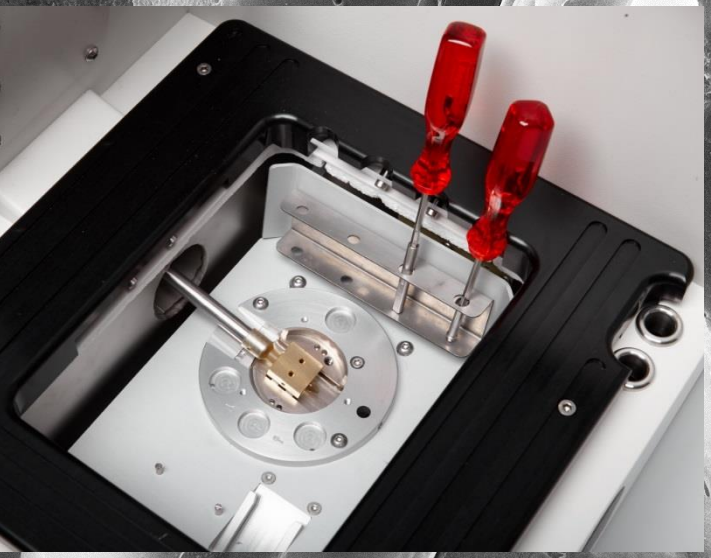
Contamination-free sample transfer between preparation and analysis systems.

Euglena gracilis, courtesy of Andres Käch, Center for Microscopy and Image Analysis, University of Zürich, Switzerland





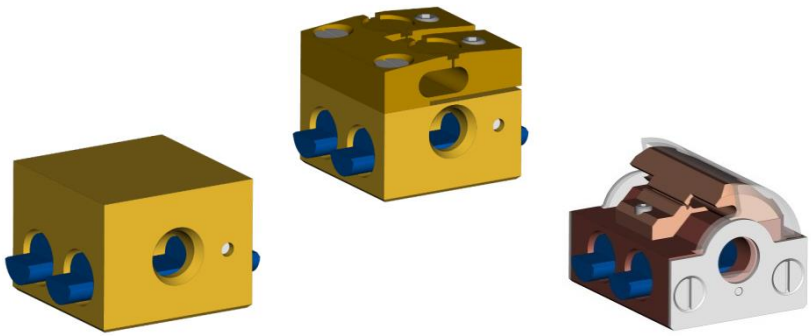
CONNECT WITH LEICA EM VCM



- All transfer under vacuum
- Contamination-free sample handling
- Improved connectivity

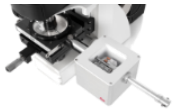
FLEXIBILITY TO CONNECT

- Glovebox
- SEM
- FIB
- Analysis systems, e.g. XPS
- Atomic probe
- Synchrotron
- ….



Connectivity for cryo-preparation

Cryo Fixation, e.g.
High Pressure Freezing

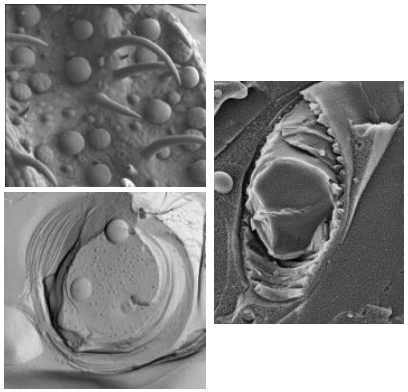


Cryo-CLEM

Cryo-Transfer

Freeze Fracture, Freeze
Etching and Coating

Cryo Ultramicrotomy

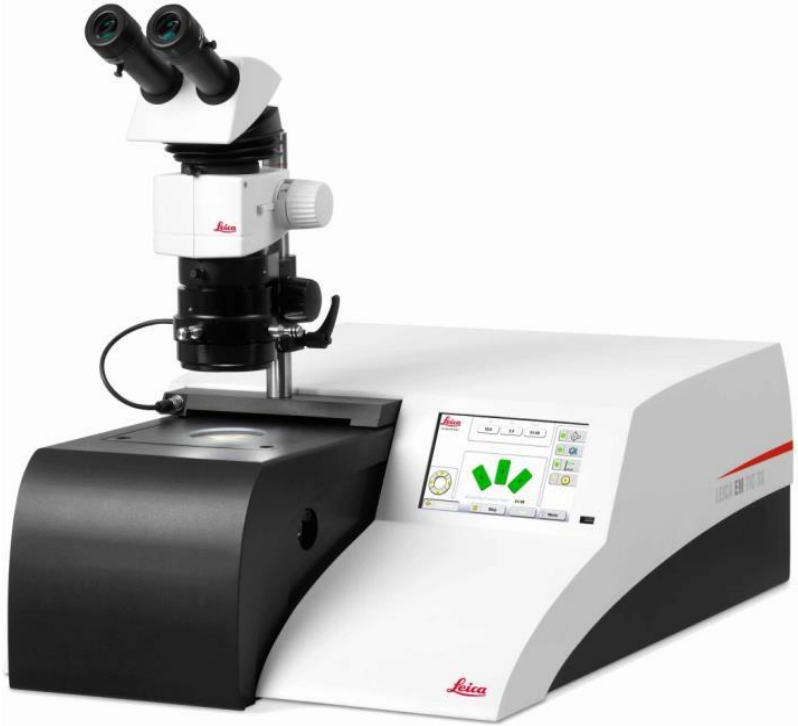


Cryo-Transfer

5. Cryo Ion Milling

5. Broad Ion Beam Milling Workflow

Sample preparation under controlled environmental conditions

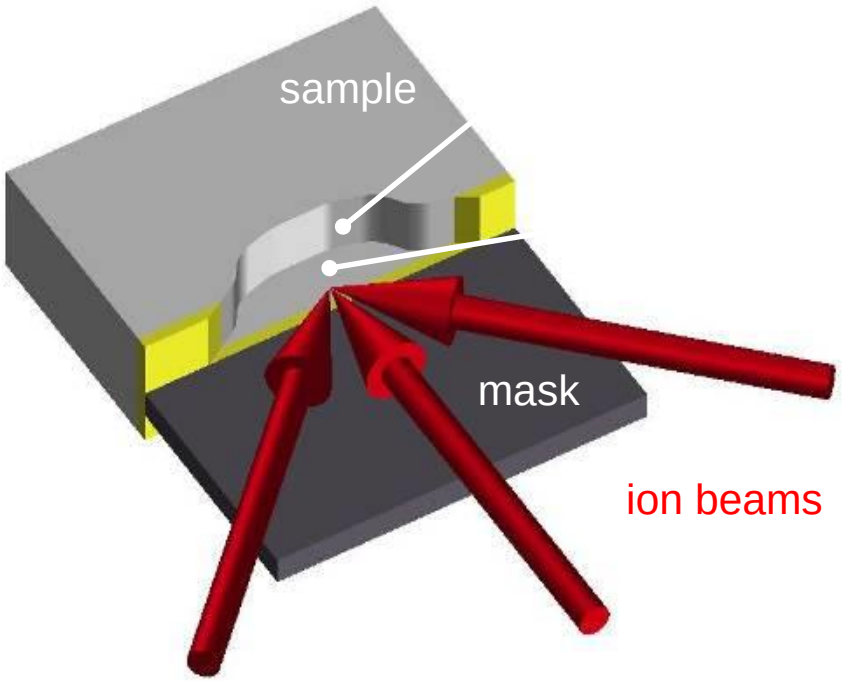


Ion beam slope cutting with Leica EM TIC 3X

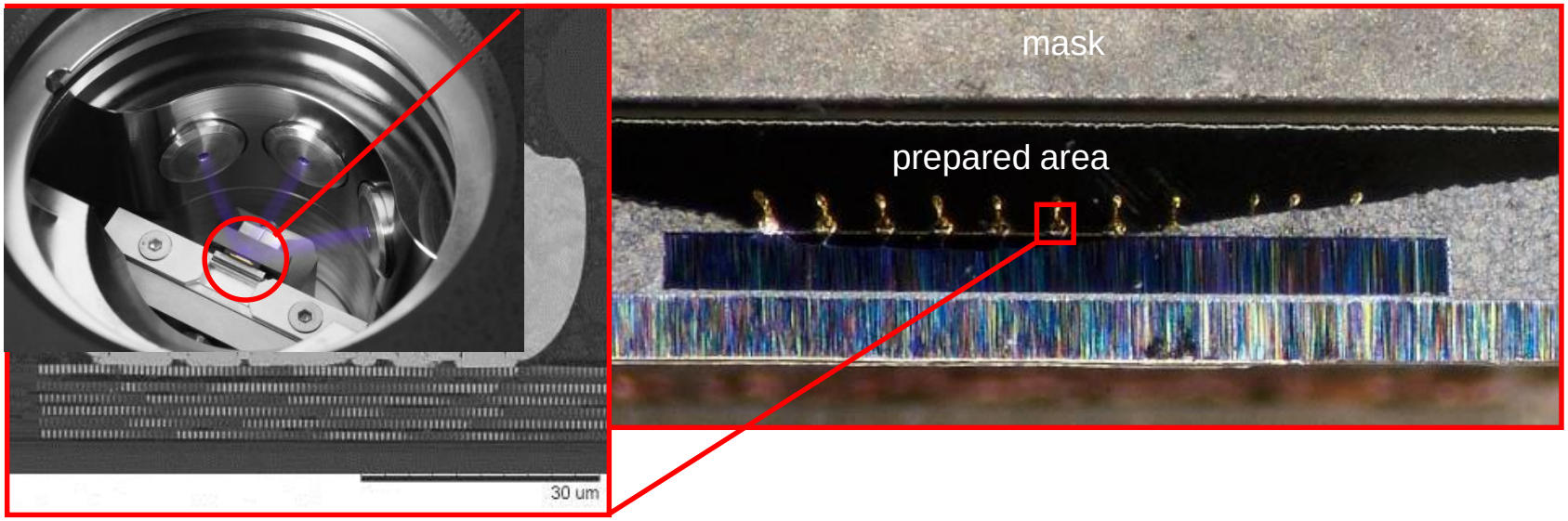
- Three ion beams hitting the sample from different directions (reduction of curtaining)
- Fixed sample (better heat transfer)

Features

- Cutting depth >1000µm
- Cutting width > 4000µm
- Cutting speed >150µm/h



Leica EM TIC 3X “Triple ion beam” slope cutting



What About Temperature, or Environmentally Sensitive Samples?

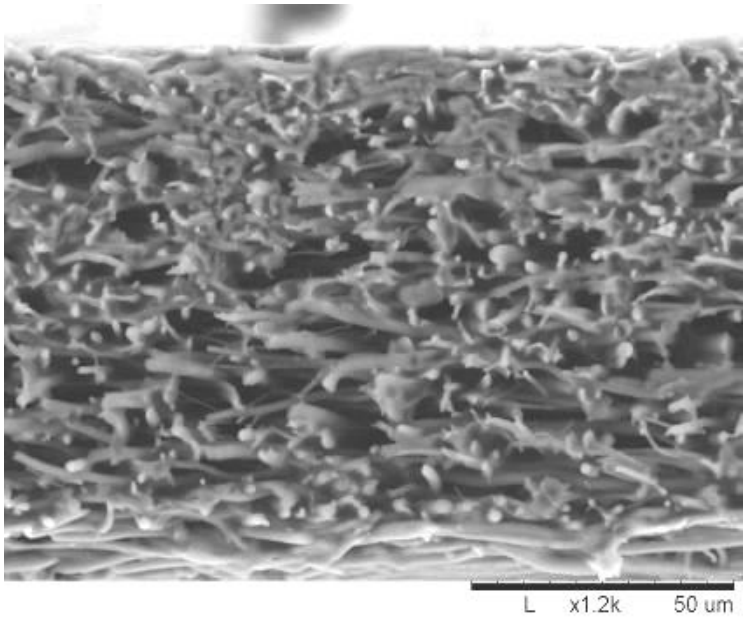
Heat-sensitive polymer fibres with water-soluble portion

Without cooling (~80° C)

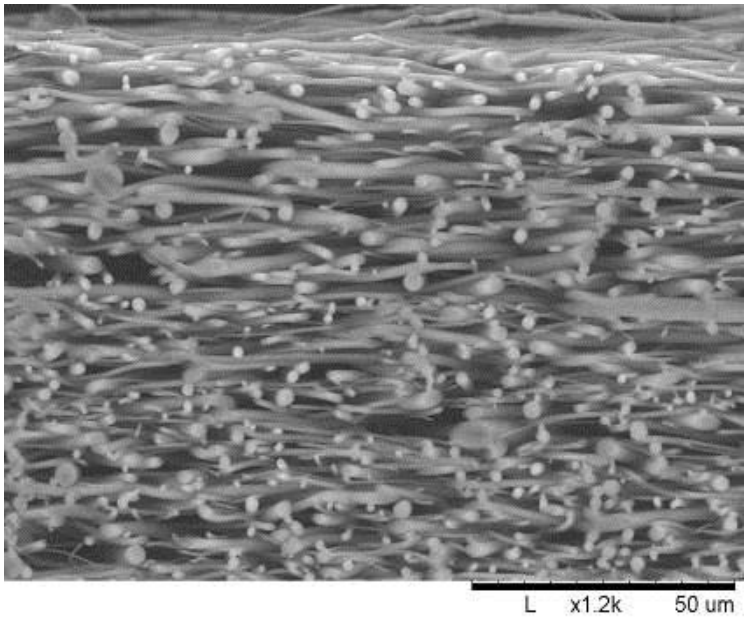
With cooling stage (-120° C)

Comparison

Heat-sensitive polymer fibres with water-soluble portion

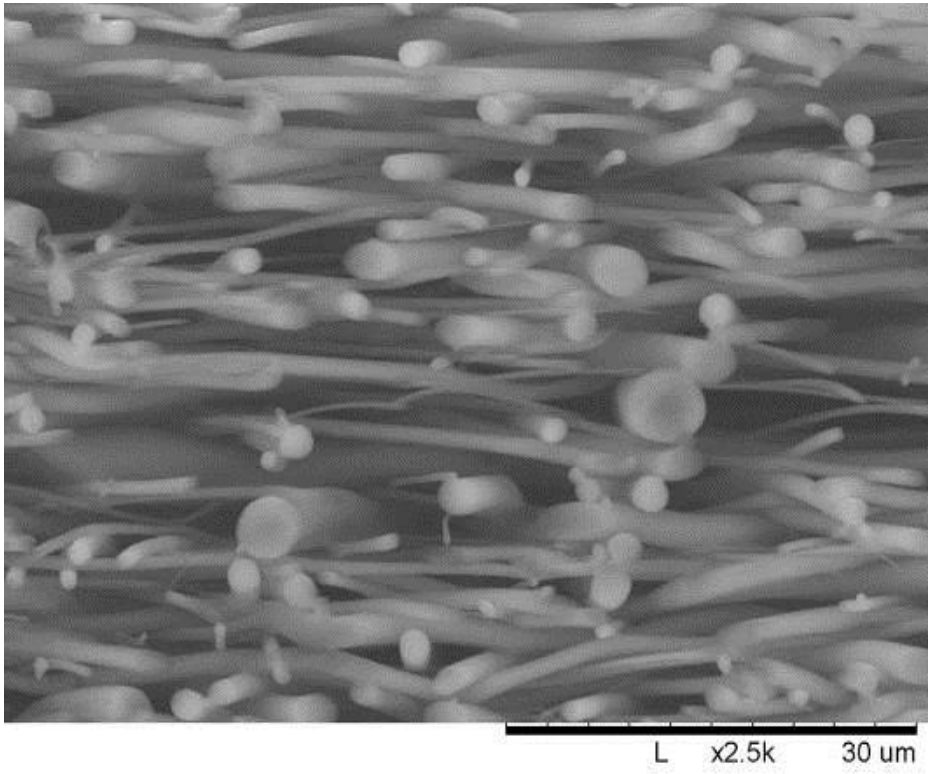


UC7 with FC7 -140° C



TIC 3X -120° C

Cooling stage result



Environmentally controlled workflow

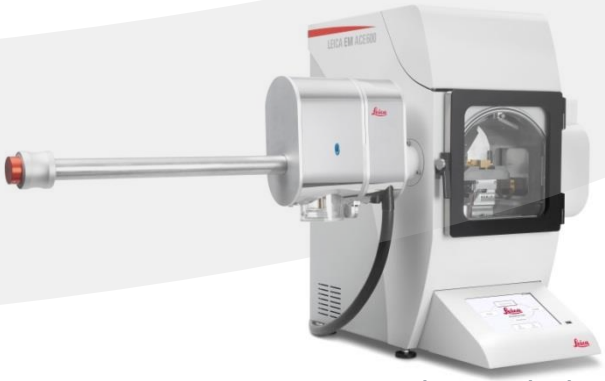
Sample Freezing

Cryo-loading station with **cryo-saw**
LN2 sample pre-preparation

EM TIC 3X with cryostage &
VCT docking station
(cryo-) BIB milling

(Cryo-)
SEM
FIB

VCT 100 shuttle
(cryo-) vacuum transfer

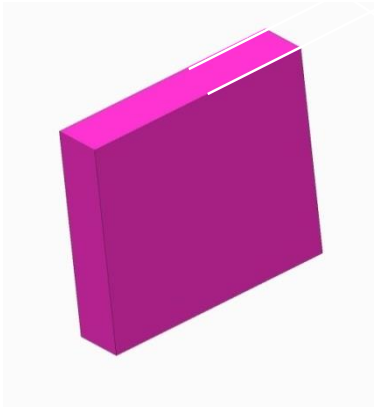


EM ACE 600 with VCT docking station
(cryo-) coating

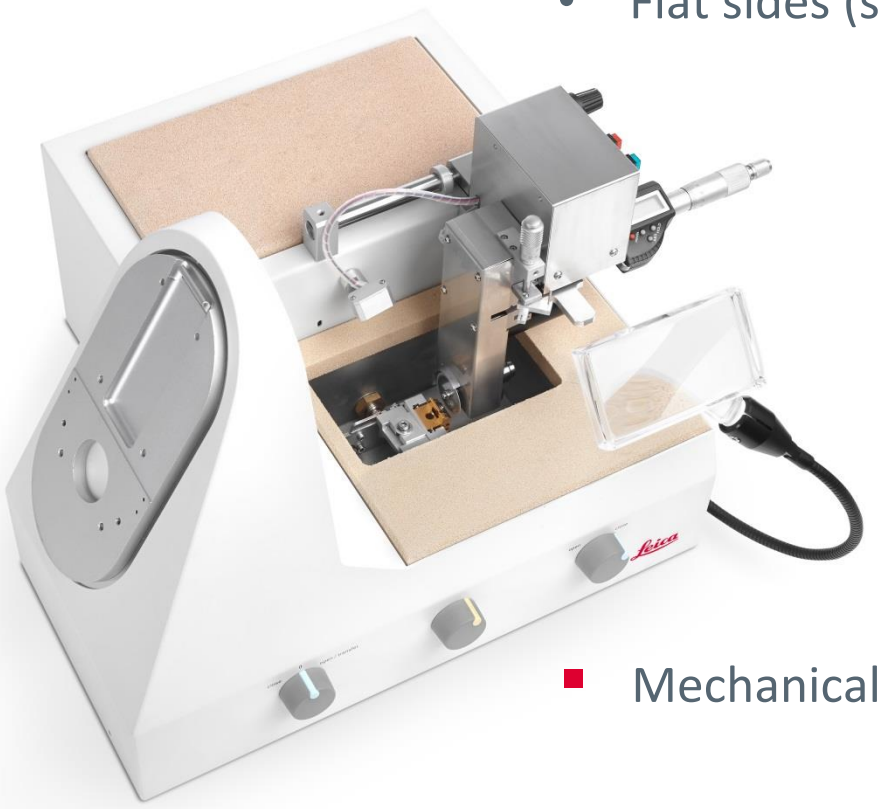
Cryo-saw and loading station

Sample size:

- Maximum 10x7x4(thick)mm
- Flat sides (saw-finish



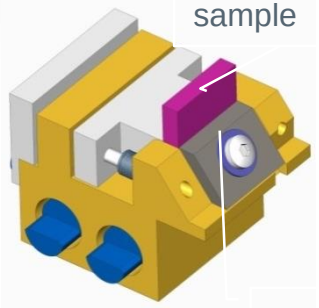
4mm



- Mechanical preparation under LN2 conditions

Preparation Process

- Freeze
- transfer to cryo-saw preparation station filled with LN2
- inserted in the VCT holder and pre-prepared with cryo-saw



VCT holder
(sample size max. 10x7x4mm)

Leica EM VCT500 connectivity

Cryogenic sample transfer to and from the broad ion beam etching system, TIC 3X and to (cryo)SEM

- After shaping to size, sample is placed in loading position
- VCT shuttle is attached and the Dewar filled with LN2
- Sample taken on board VCT shuttle, transferred and attached to the EM TIC 3X docking port
- Sample moved into position for ion beam slope cutting under cryo-conditions.

New environmentally controlled workflow

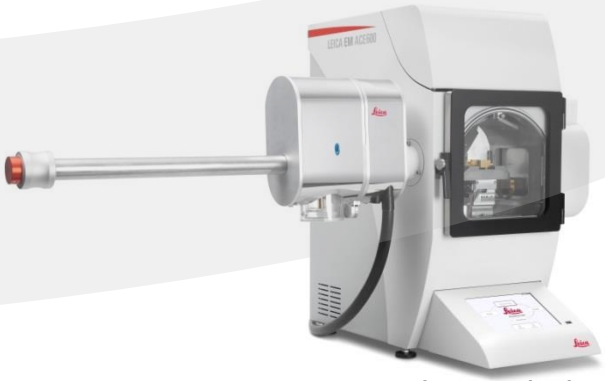
Sample
Freezing

Cryo-loading station with **cryo-saw**
LN2 sample pre-preparation

EM TIC 3X with cryostage &
VCT docking station
(cryo-) BIB milling

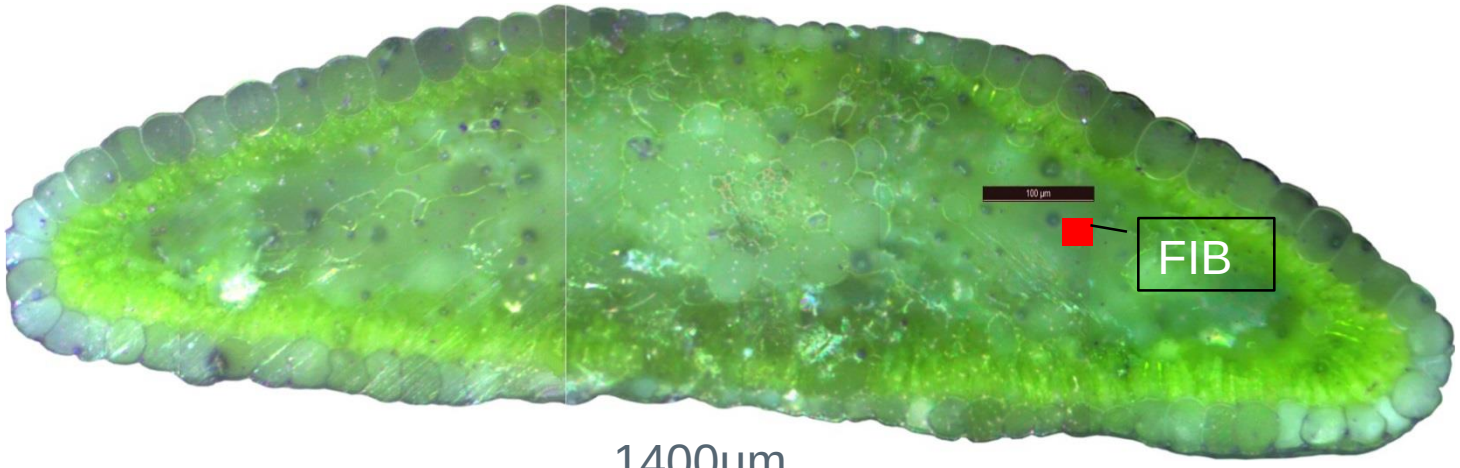
CryoSEM/FIB

VCT 100 shuttle
(cryo-) vacuum transfer



EM ACE 600 with VCT docking station
(cryo-) coating

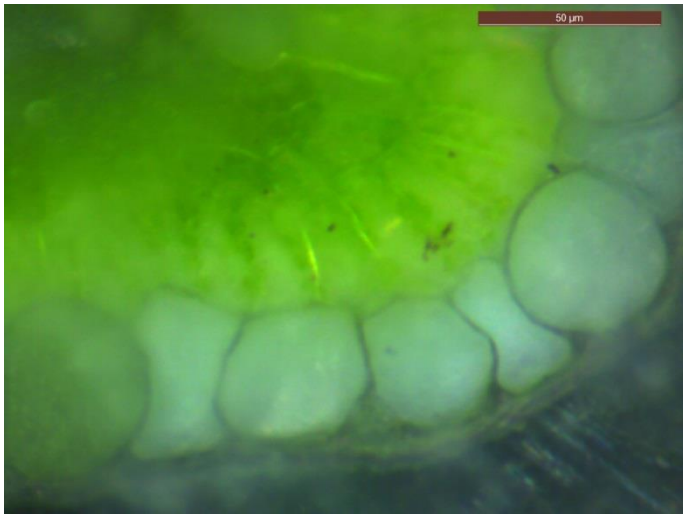
Applications



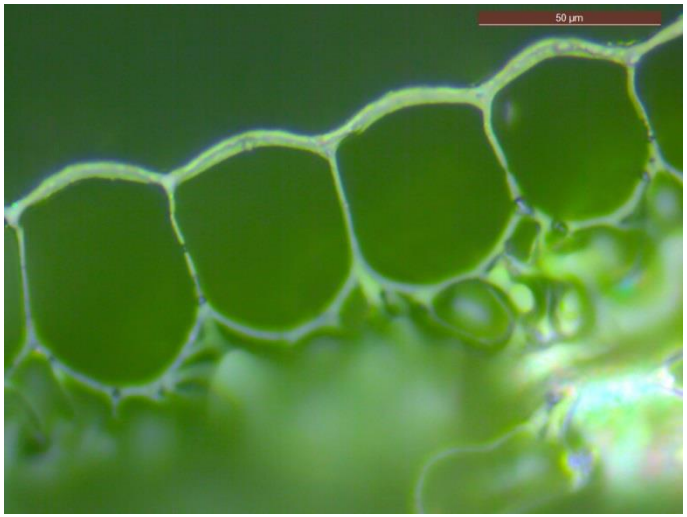
1400µm

Asparagus; plunged in LN2, pre-prepared with the Cryo-Saw-Loading station, Ion beam cross sectioned with the TIC 3X – VCT at -120° C and 6kV; transferred with the VCT100 and investigated in a "cryo-LM" 20x objective

Applications

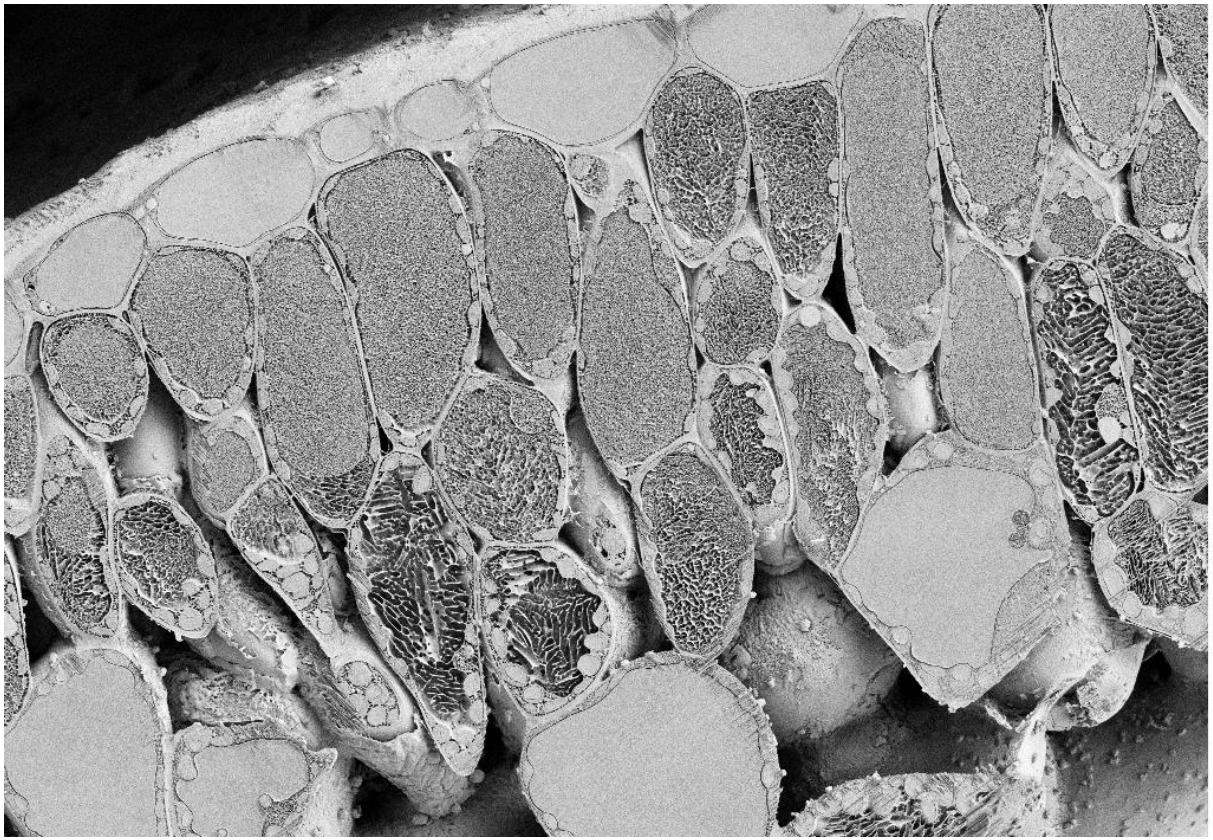


Asparagus; Cryo-TIC preparation; “Cryo-LM”



Asparagus; Cryo-TIC preparation; LM after the sample warmed up

Applications

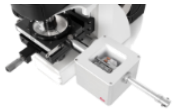


Taxus sp., plunged in LN2, pre-prepared with the Cryo-Saw-Loading station, ion beam cross sectioned with TIC 3X/VCT at -120C and 6kV; transferred with the VCT100 and investigated in a cryo-FIB SEM

Image courtesy of Harvard University

Connectivity for cryo-preparation

Cryo Fixation, e.g.
High Pressure Freezing

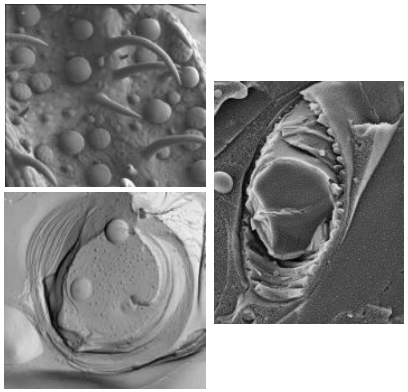


Cryo-CLEM

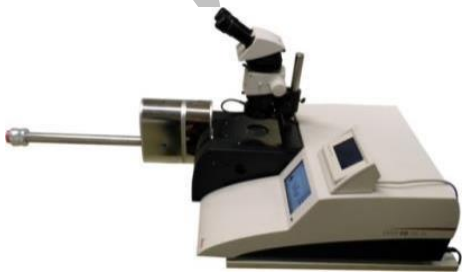
Cryo-Transfer

Freeze Fracture, Freeze
Etching and Coating

Cryo Ultramicrotomy



Cryo-Transfer



Cryo Ion Milling

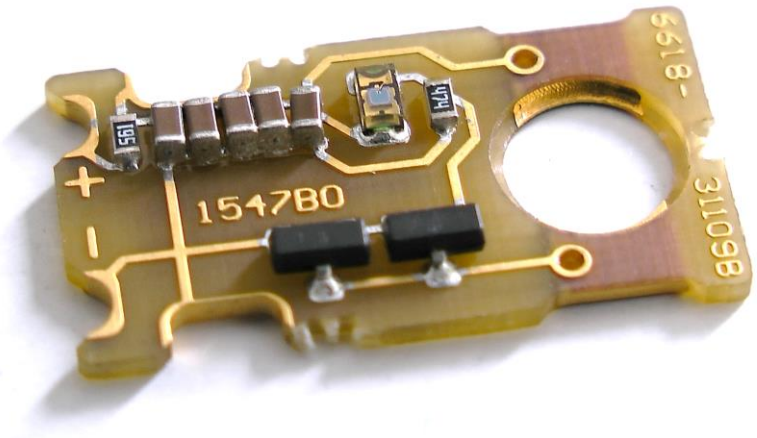
Future Developments

HPF with Electrical Stimulation

The Concept

- Middle plate includes a circuit board with capacitors to store the electrical energy

Middle plate with capacitors top-side view



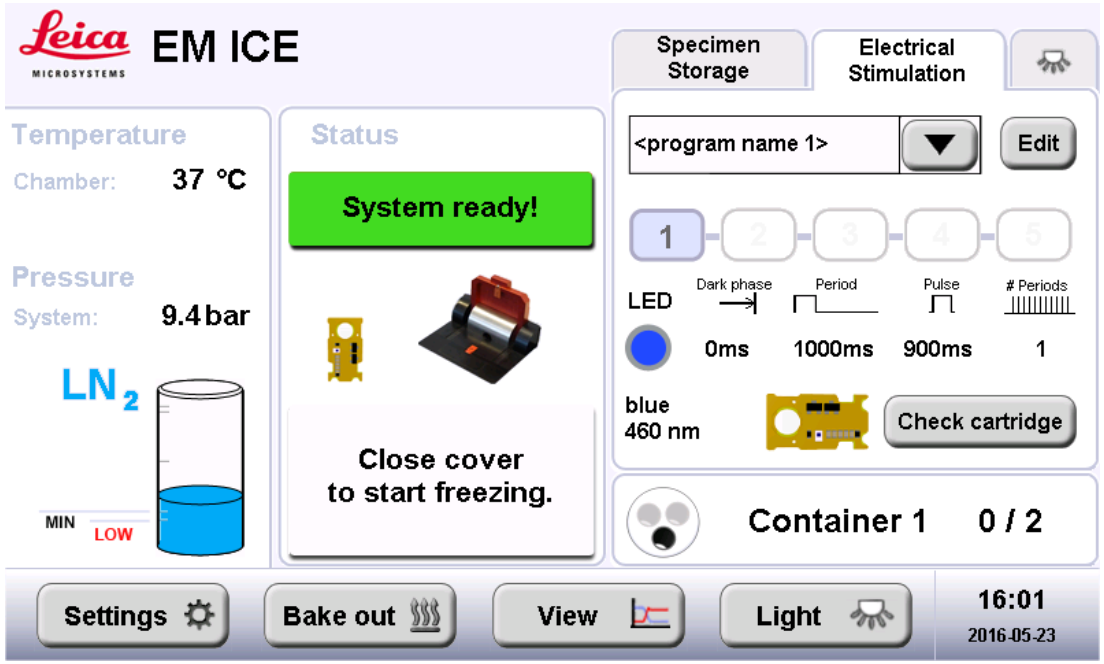
Middle plate back-side view

Spacer ring

Cover ring

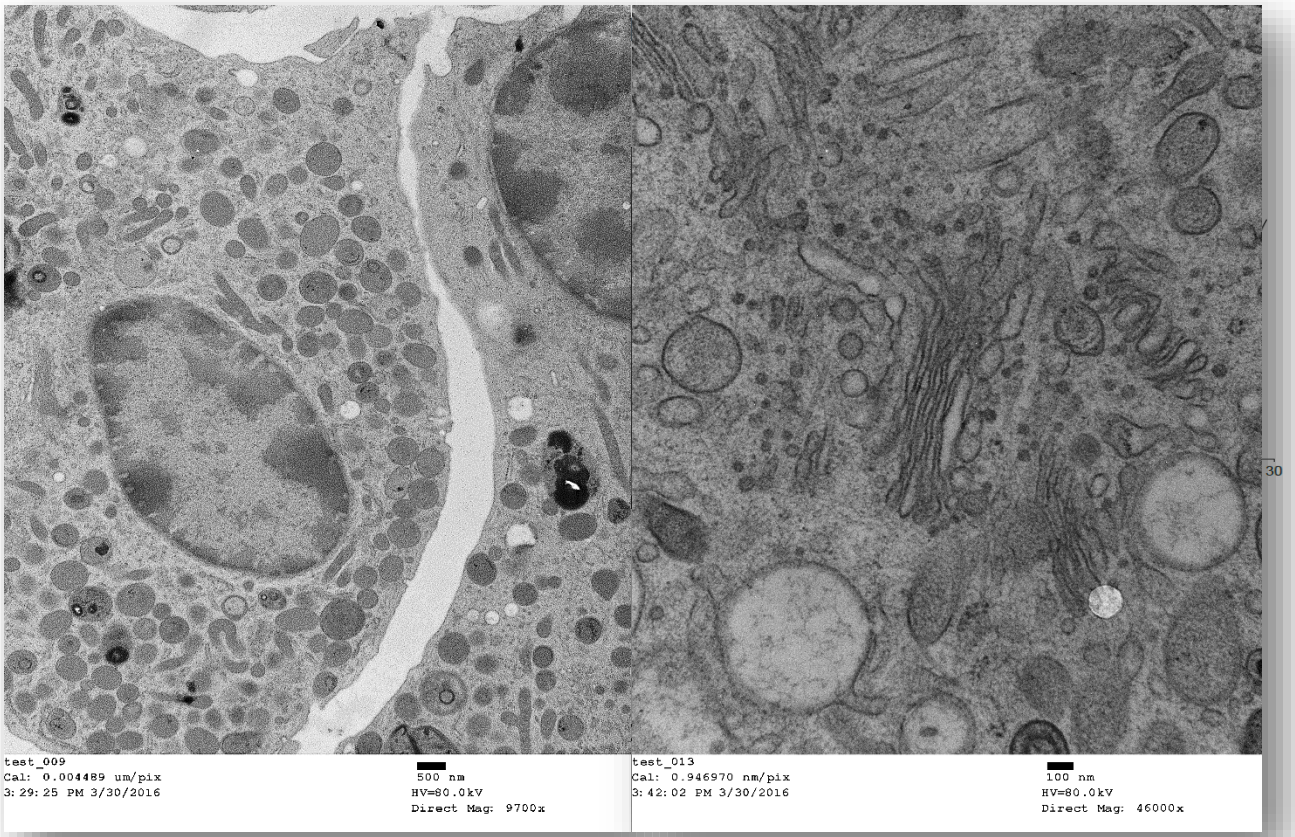
The Concept

- Blue LED module used as the trigger for releasing the power and having e-current delivered to the sample
- Software automatically recognising the ES mode. All settings saved in the log-file



Proof of Principle/Functional Model

Initial Sample Test



Future Developments

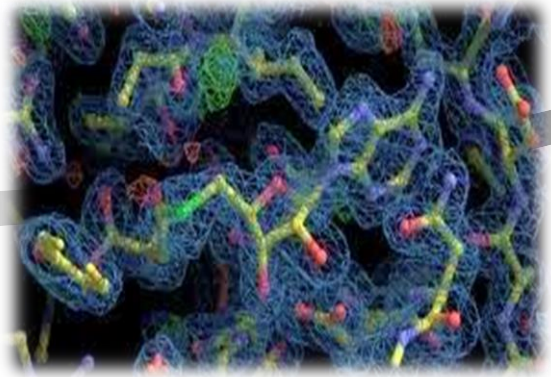
HPF for protein crystallography

Leica Protein Crystallography Solution

- Screening for a suitable cryoprotectant
- Disintegration and loss of crystals
- Time consuming

HPF

- Standardization
- Reliability
- High throughput



Drug discovery development

Thank You