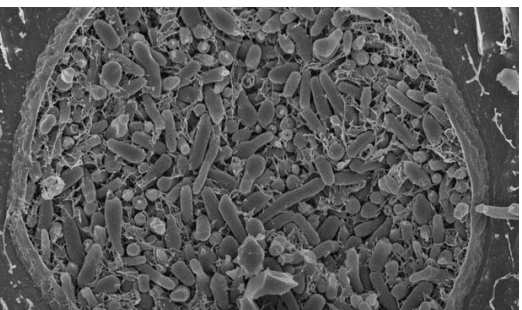
From Eye to Insight

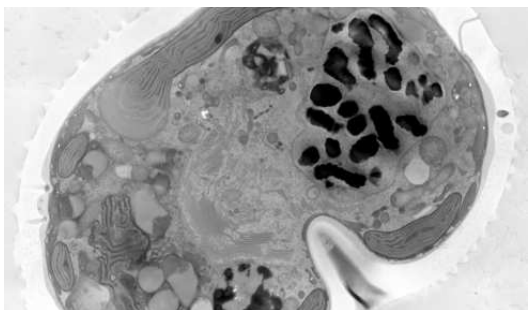
Leica
MICROSYSTEMS

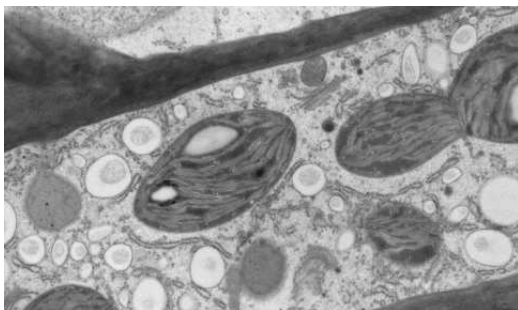
Sample Preparation at Leica Microsystems

WORKFLOW SOLUTIONS FOR LIFE SCIENCE RESEARCH

We focus on workflow solutions to provide a product range serving your needs in TEM, SEM, LM, and AFM investigations.







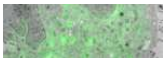
Sample Preparation Workflows for Life Science Research

Leica Microsystems offers an extensive portfolio of high-performance instruments for electron microscopy sample preparation. Designed to fit multiple sample preparation workflows, these solutions enable you to obtain top results.

In this publication, we present workflows for the most frequently used sample preparation methods. The solutions discussed are proven to bring reliable and reproducible results. If you have special requirements for your workflow or any questions regarding the topics shown here, our Leica experts will be happy to assist you at any time.

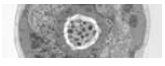
Cover images: top: Collembolen (source: Dr. Daniela Gruber, University of Vienna, Austria); bottom left: Cryo-SEM micrograph of a covalent hydrogel capsule with a continuous wall filled with *M. smegmatis*, Scale bar 5 µm. (Adapted from: Woodward-Rowe et al., Capsule Formation Mechanisms in Interfacially Initiated Macroporous Hydrogels to Tailor Microstructures for the Encapsulation of Living Bacteria, https://pubs.acs.org/doi/10.1021/acsapm.4c02458, https://creativecommons.org/licenses/by/4.0/); bottom middle: Micrographs of a dinoflagellate processed as described within the AppNote "How marine microorganism analysis can be improved with high-pressure freezing". Scale bar big image = 5 µm, scale bar smaller images = 1 µm (source: Dr. Yannick Schwab and Dr. Karel Lea Marie Mocaer, EMBL Heidelberg, Germany); bottom right: Ultrastructure of Arabidopsis thaliana primary root cells (source: Dr. Riet de Rycke, Department for Molecular Biomedical Research, 9000 Gent). Category image "Correlative Methodologies": In resin fluorescence overlay of a C1-GFP transfected HeLa cell (source: Dr. Christopher Peddie, The Francis Crick Institute, London, UK); Catergory image "Optogenetics & Electron-Physiology": Symmetric Synapse (source: Dr. Shuwen Chang, Charité Universitätsmedizin Berlin, Germany); Catergory image "Surface Analysis": Salivary gland (source: David McCarthy, UCL School of Pharmacy, London, UK); Catergory image "2D Tissue & Cellular Morphology": *C. elegans* cross section (source: Dr. Thomas Müller-Reichert, TU Dresden, Dresden, Germany); Catergory image "3D Tissue & Cellular Morphology": Trichomonas parasite in mouse gut, rOTO Embedding and serial sections for array tomography imaging (source: Isabelle Guerin-Bonne, Low Kay En, Electron Microscopy Unit, Yong loo Lin School of Medicine, National University of Singapore); Catergory image "Suspension & Macromolecules": Visualization of DNA Molecules (source: Michele Giannattasio, Istituto FIRC di Oncologia Molecolare (IFOM)-Electron Microscopy Facility (Single Molecules Visualisation), Milano, Italia, and Gisela Höflinger, Leica Mikrosysteme, Vienna, Austria); Image page 4: TauSense / TauContrast – lamella positions; 50x/0.90 dry cryo: 1312 x 1312 x 33 voxel; 146 x 146 x 11 µm: Nuclei - membrane - reflection (source: Dr. Jan De Bock, Leica Microsystems).

Table of Contents


Correlative Methodologies 06


Optogenetics & Electro-Physiology..... 09


Surface Analysis 10


2D Tissue & Cellular Morphology 11


3D Tissue & Cellular Morphology 13


Suspensions & Macromolecules 18

SAMPLE PREPARATION WITH LEICA MICROSYSTEMS – THE PORTFOLIO THAT GIVES YOU SUCCESS FOR YOUR APPLICATION

STELLARIS Cryo

STELLARIS Cryo is a confocal microscope system that helps you to target your area of interest for cryo-electron tomography (CryoET). STELLARIS Cryo gives you the precision to target reliably, while offering superior performance you can count on and sample safety for your experiments.



EM ICE

High pressure system for freezing aqueous samples delivers optimal sample preservation. Offers the highest flexibility to meet multiple application demands.



UC Enuity

Next generation ultramicrotome that saves you valuable time and resources through its automated setup functions, providing state-of-the-art sectioning quality. Experience the cutting egde of automation and precision with UC Enuity.



EM VCT500

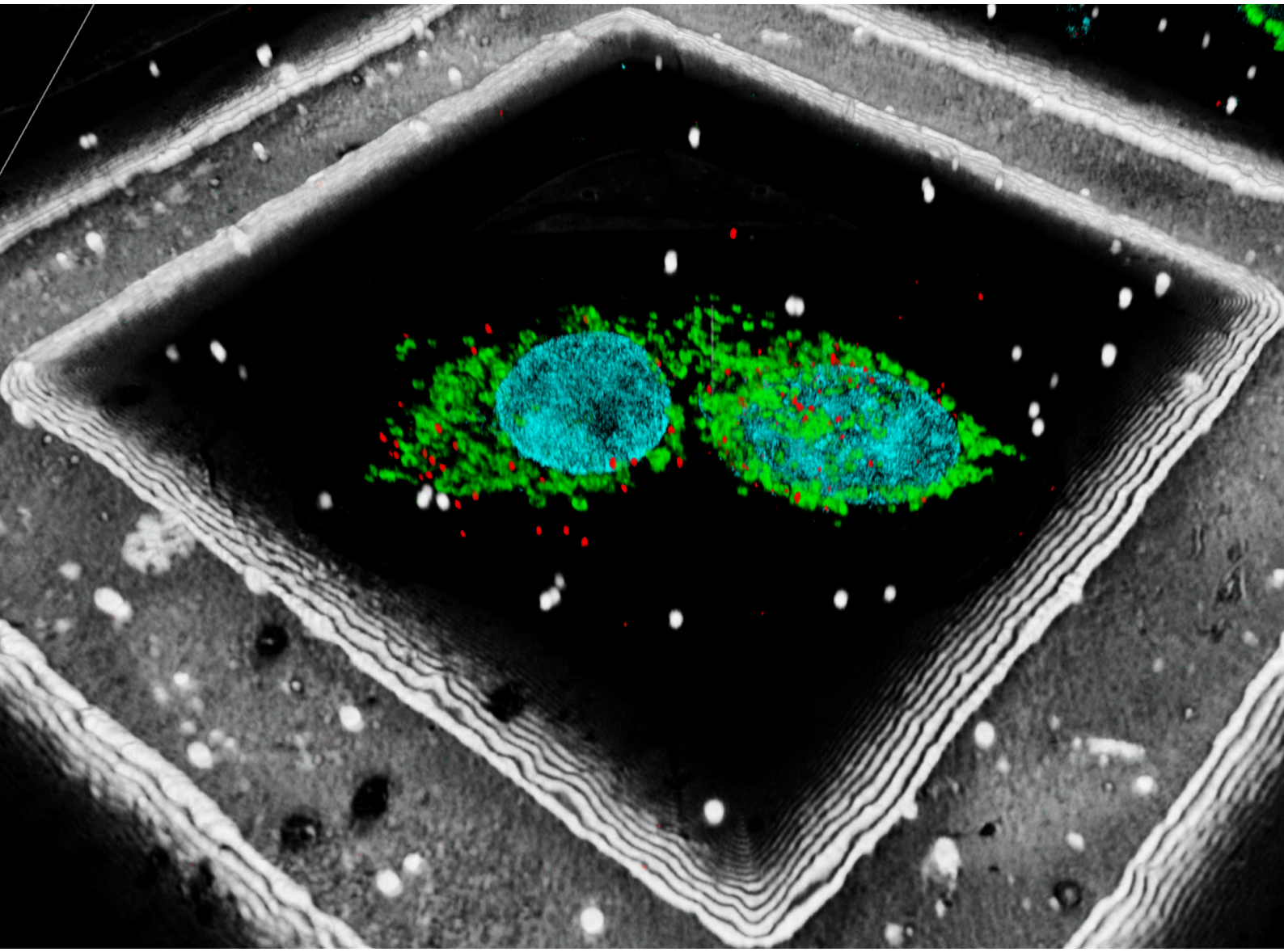
Versatile vacuum cryo transfer system for contamination-free transfer of specimens between different preparation and analysis instruments.



EM ACE600

Fully automated, versatile high vacuum coater producing very thin, fine-grained, conductive metal and carbon coatings. Up to two angled coating sources configurable. For high resolution analysis, preparation and freeze fracture technique.





CAPTION

Cryo Workflow

Optional step for better results

Trimming

Grid Plunging

Critical Point Drying

LM Investigation

Ultramicrotomy

Freeze Substitution

Micro CT

SEM Investigation

Sample Transfer

Coating

Slush Freezing

TEM Investigation

High Pressure Freezing

Freeze Fracture

Immuno Labelling

FIB

Light Stimulation

Tissue Processing

Chemical Fixing / Manual Processing

X-Ray

Electrical Stimulation

Staining / Contrasting

Dedicated Cryo FIB



EM AC20



EM CPD300



EM VCM with EM VCT500



UC Enuity



EM ACE200



STELLARIS Cryo



EM VCT500



UC Enuity - Fluorescence configuration



EM ACE600



EM GP2



EM AFS2



UC Enuity - Cryo configuration



EM RAPID

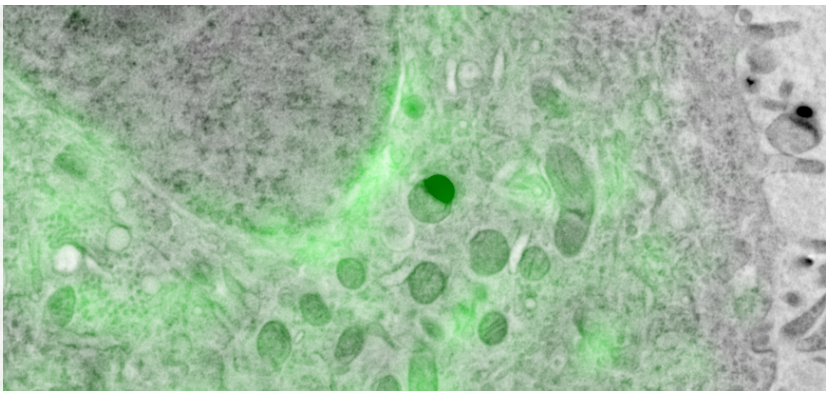


EM ICE



EM TP

CORRELATIVE METHODOLOGIES

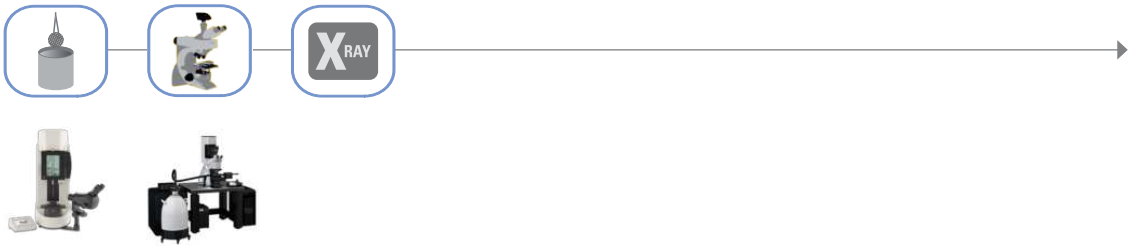


Cryo-CLEM

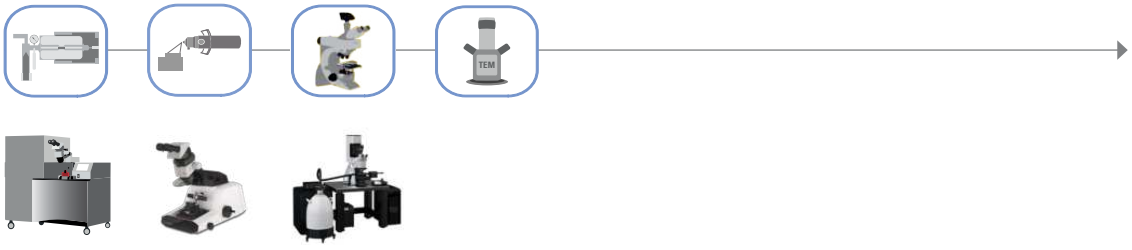
This workflow joins on-grid plunge freezing with cryo-fluorescence microscopy to target, relocate and overlay the structure of interest with the subjacent ultrastructure imaged with the cryo-TEM.

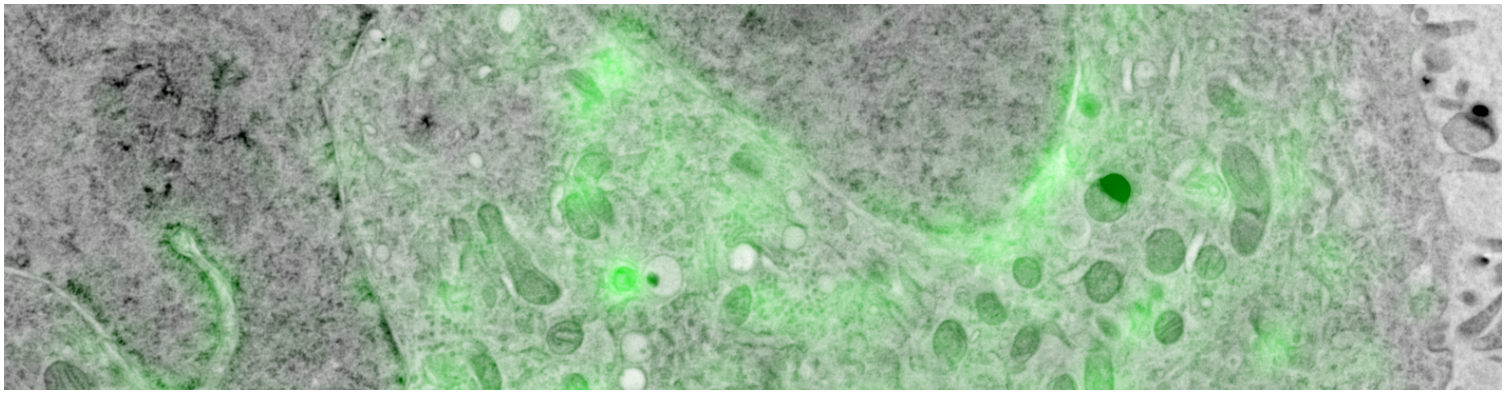


Workflow to target the cell of interest on the grid by cryo-fluorescence microscopy for subsequent cryo-soft-X-ray-tomography.



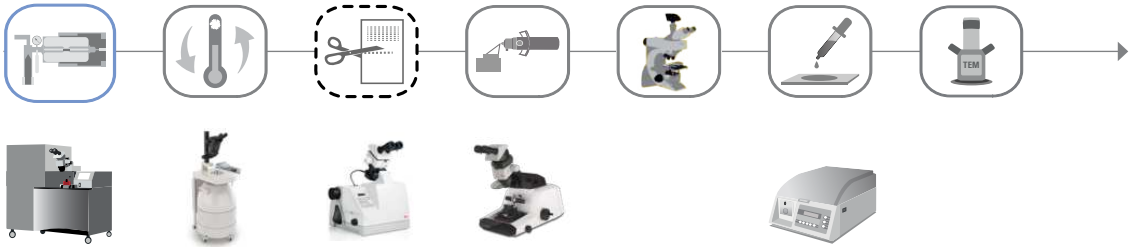
Combining CEMOVIS of high-pressure frozen samples with cryo-fluorescence microscopy allows to target the structure of interest within cryo sections. This is then superimposed with the underlying ultrastructure.





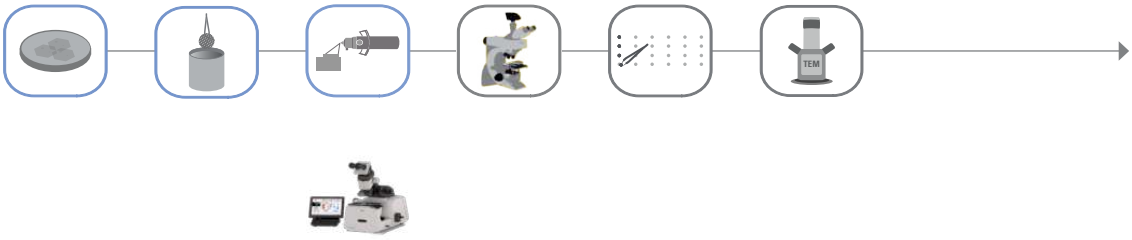
In resin CLEM

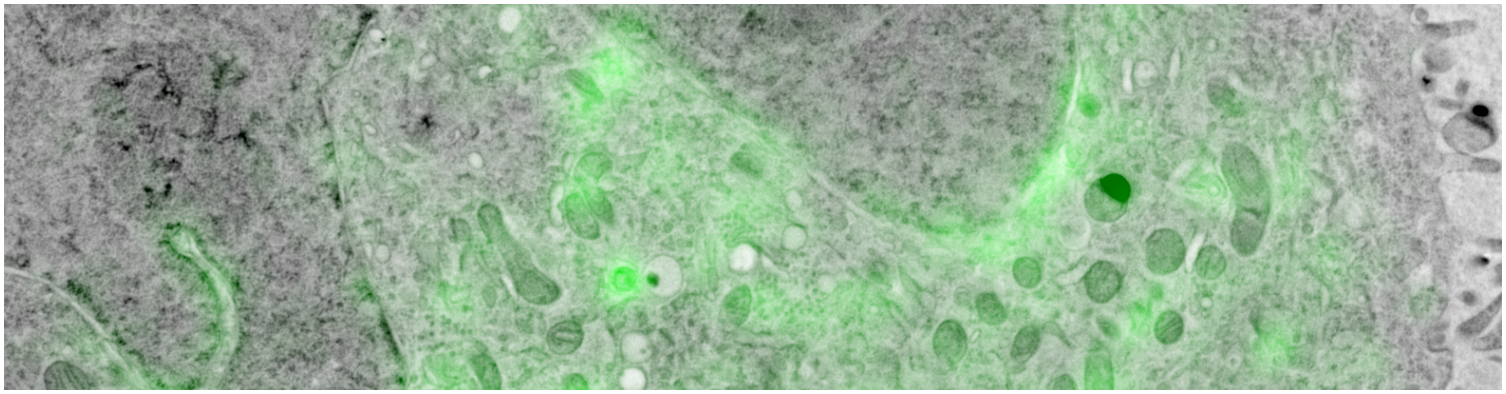
This workflow allows to correlate fluorescently labelled structures in different image modalities to combine the advantages of fluorescence microscopy with the superior ultrastructure of high pressure freezing in RT processing.



Tokuyasu CLEM

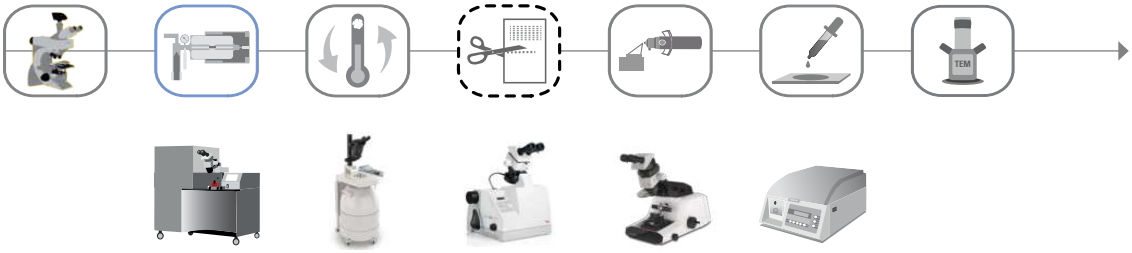
Introducing light microscopy into a classic Tokuyasu workflow is used to identify the region of interest on the section followed by a more precise labelling localisation at the ultrastructural level.





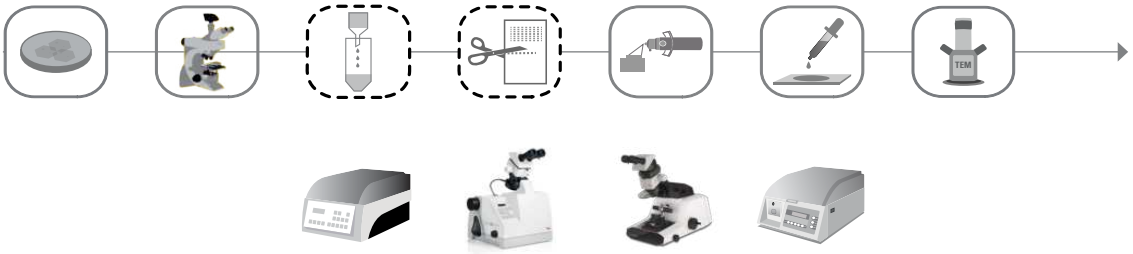
Live cell CLEM

Understand ultrastructural changes over time by combining live-cell imaging and instant fixation by high pressure freezing.

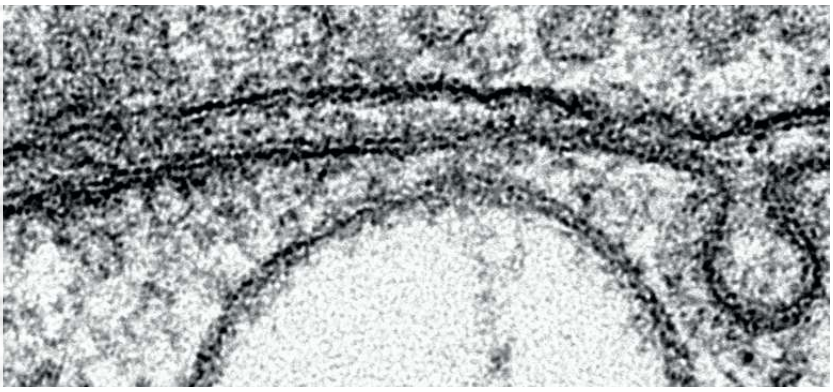


Post fixation epoxy CLEM

Identify the structure of interest in tissue samples by using a mild chemical fixation, followed by LM imaging.
Afterwards the samples will be further processed for electron microscopy.

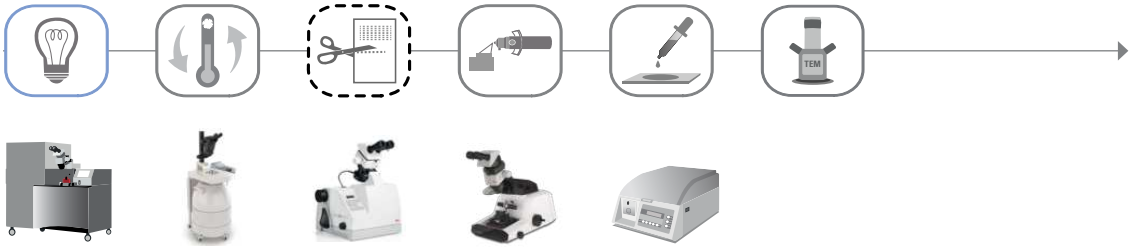


OPTOGENETICS & ELECTRO-PHYSIOLOGY



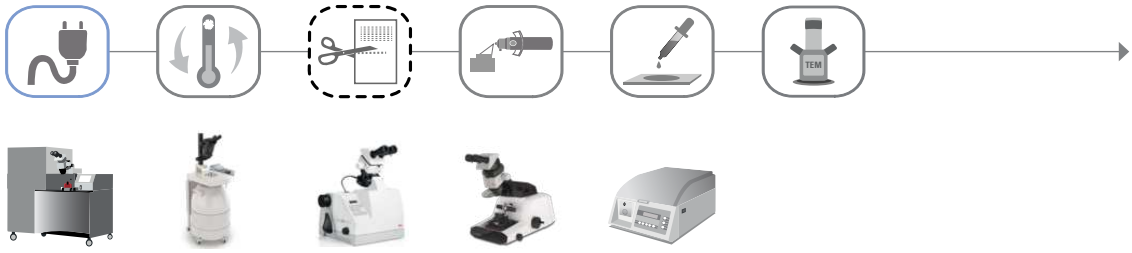
Light Stimulation

Using light stimulation in combination with high pressure freezing a moment in time can be frozen. Based on optogenetics, this workflow enables to trigger action potentials in excitable cells and arrest the process at any specific time.

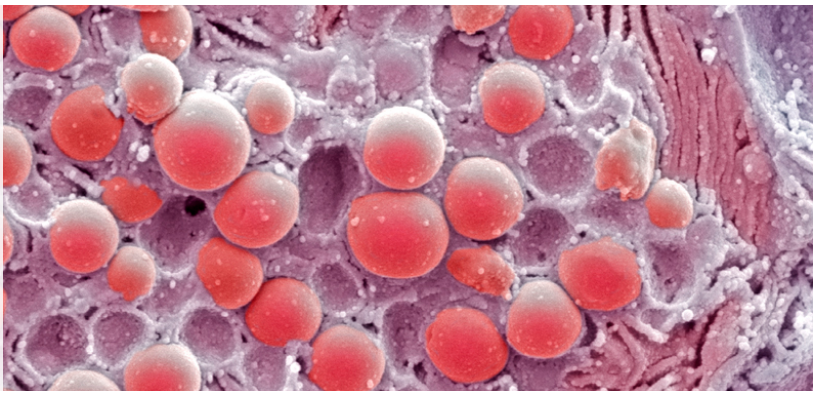


Electrical Stimulation

Coordinate electrical stimulation of neuronal tissue with rapid freezing to visualize membrane dynamics at millisecond precision.

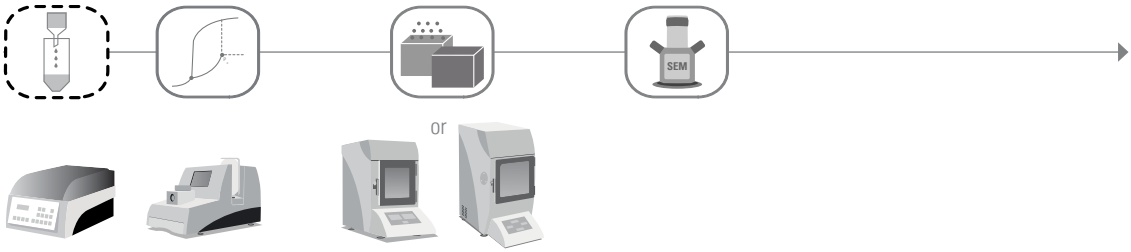


SURFACE ANALYSIS



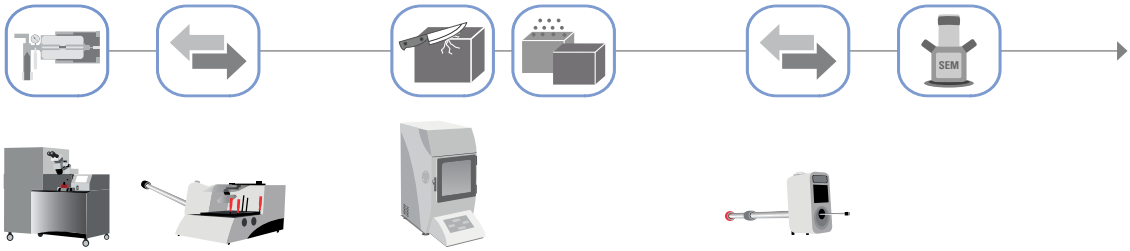
SEM

Investigate surface architecture of chemically fixed samples.

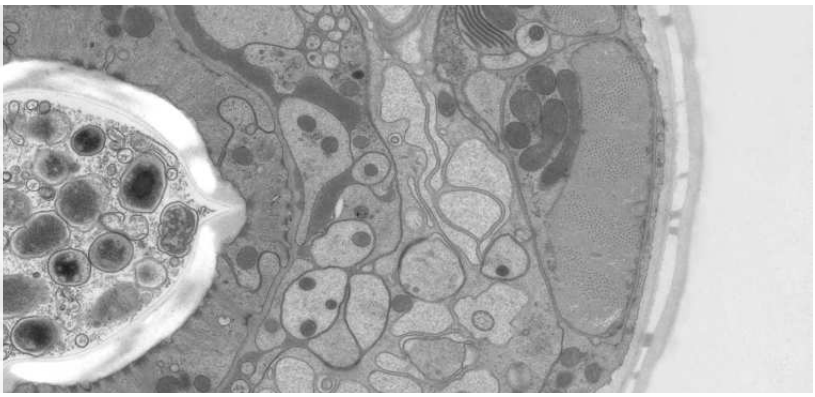


Freeze Fracturing & Cryo SEM

Cryo SEM imaging is a fast way to receive high-resolution images of internal structures or surfaces of biological and industrial samples.

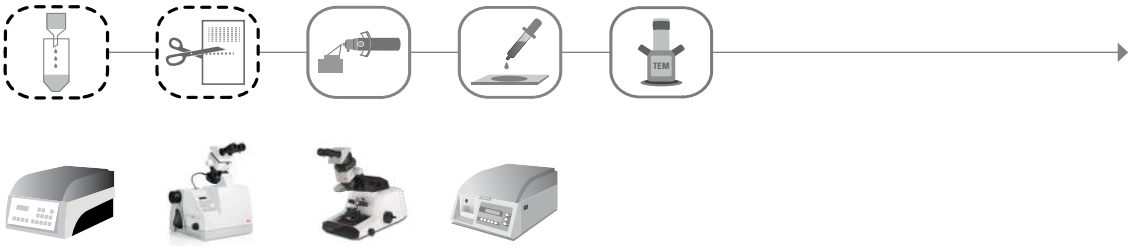


2D TISSUE & CELLULAR MORPHOLOGY



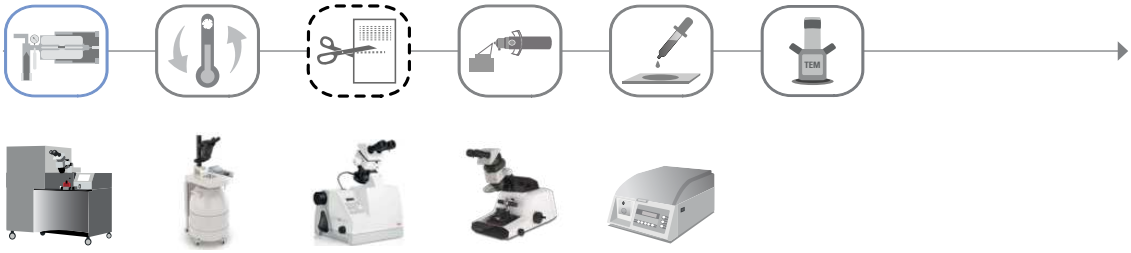
Room Temperature Processing

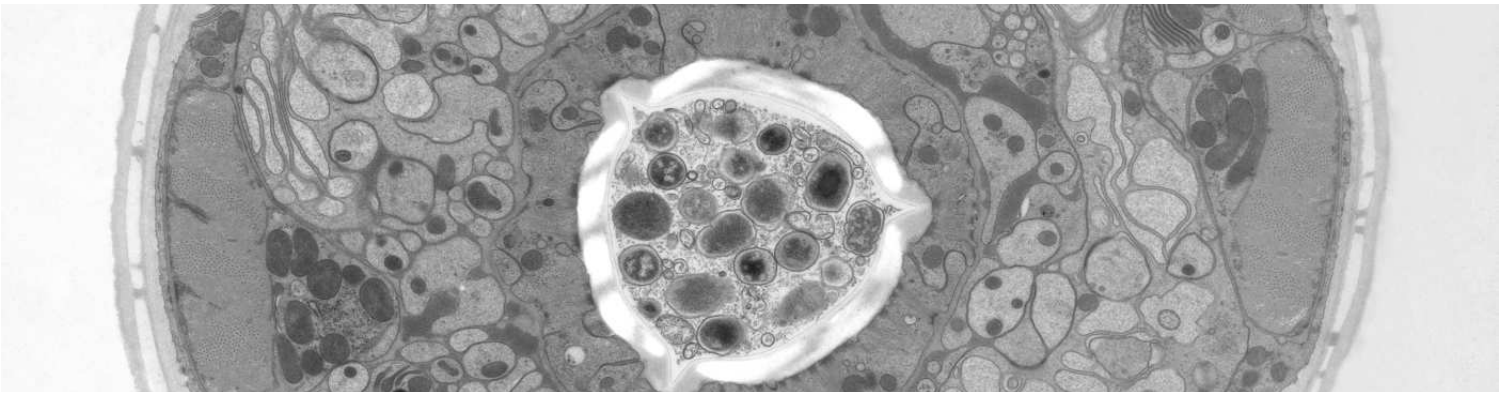
The classic technique used for ultrastructural analysis of biological materials. Sample preparation is done at room temperature.



Hybrid Processing

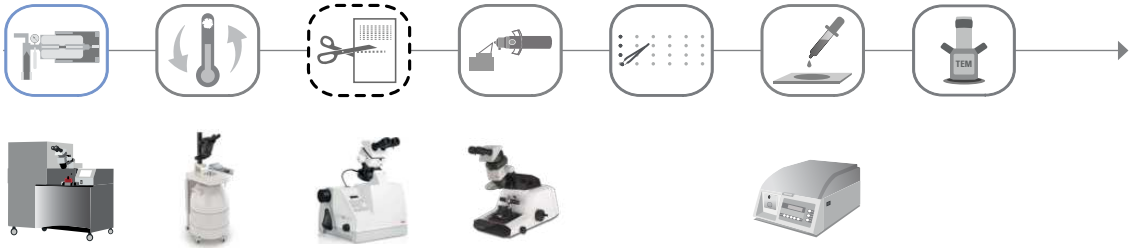
This standard workflow combines superior ultrastructural fixation by high pressure freezing with room temperature manipulation using freeze substitution and resin embedding.





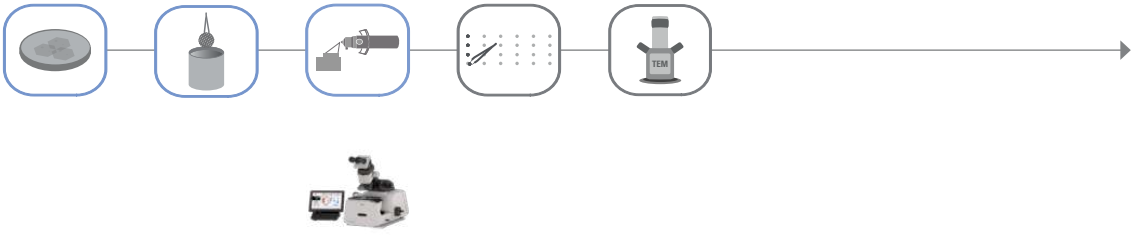
Immunolabelling

A different way to label the structure of interest on a nanometre scale is to use cryo fixation in combination with low temperature resin embedding to preserve the antigens for subsequent labelling.

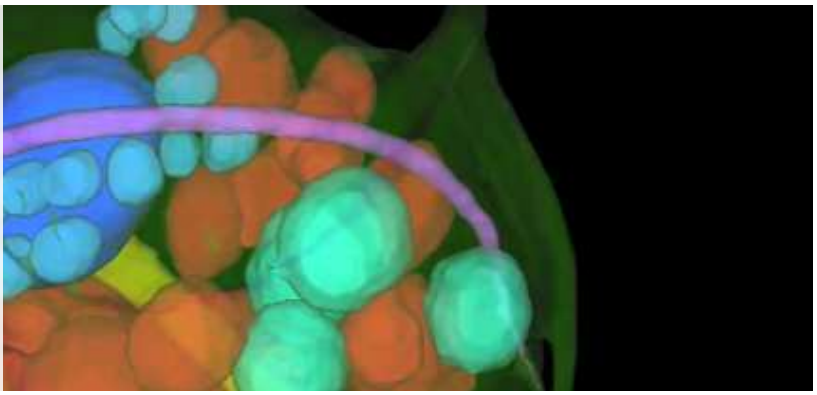


Tokuyasu Immunolabelling

A widely used method for preparation of biological material for immunolabelling by using chemical fixation, sucrose infiltration and plunge freezing. Because of the well-preserved antigens, this technique is a fast and safe way to acquire results on ultrastructure and immunolabelling.

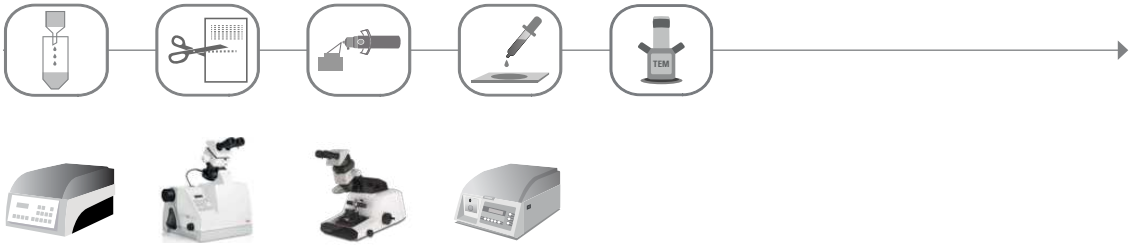


3D TISSUE & CELLULAR MORPHOLOGY

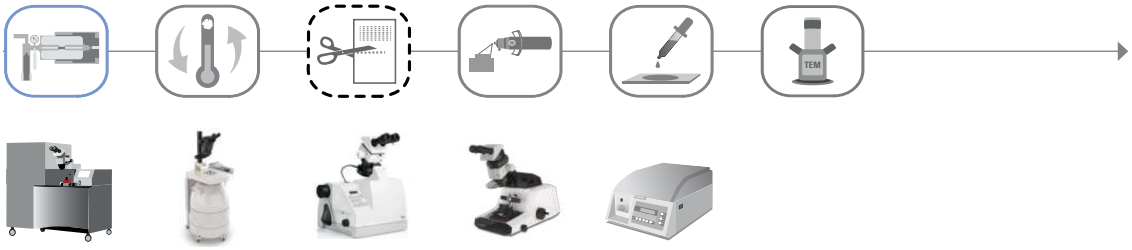


Section tomography 3D

3D electron tomography enables to study the organization and interaction of biological structures within three dimensions in a defined volume. In this workflow the samples are processed at RT, followed by serial sectioning onto TEM grids.



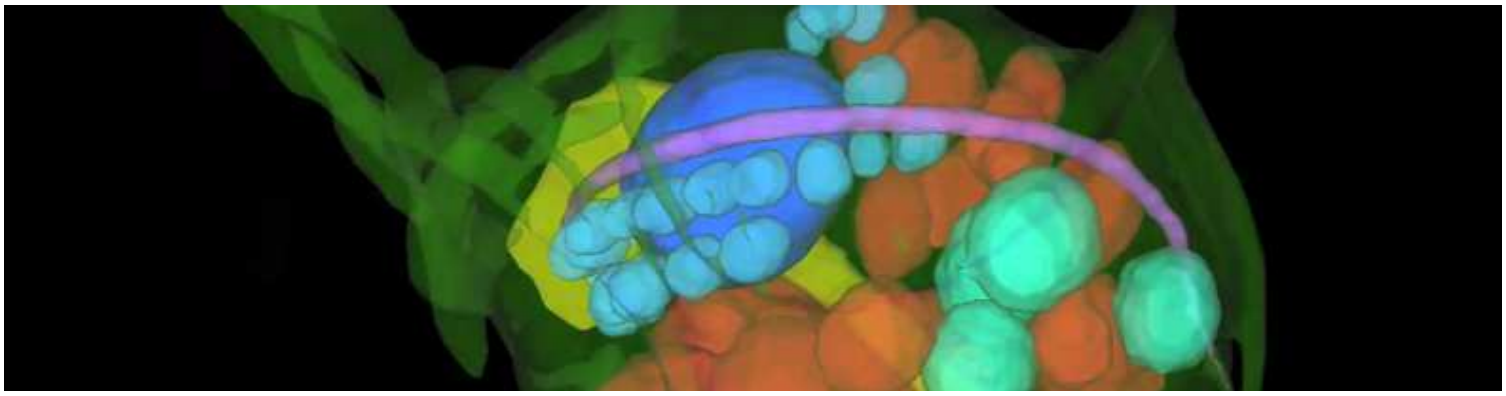
The same approach can be applied using high pressure freezing as a fixation method to increase structural preservation and minimize introduction of artefacts.



CEMOVIS 3D

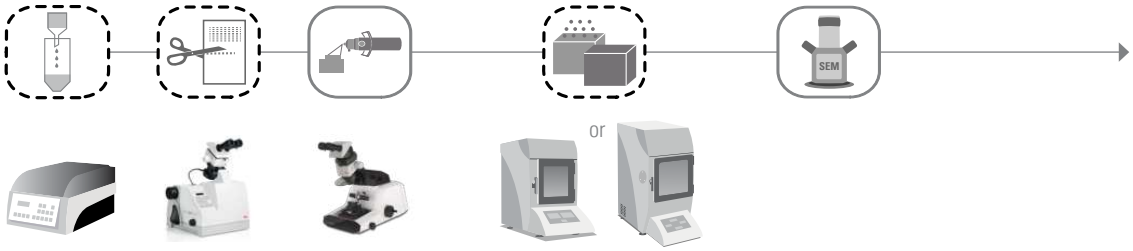
CEMOVIS enables not only the study of vitrified cells and tissue in 2D but also in 3D. The recently optimized double micromanipulator enables a better control of the cutting process. Combined with cryo electron tomography, it allows to study the supramolecular organization of cells.



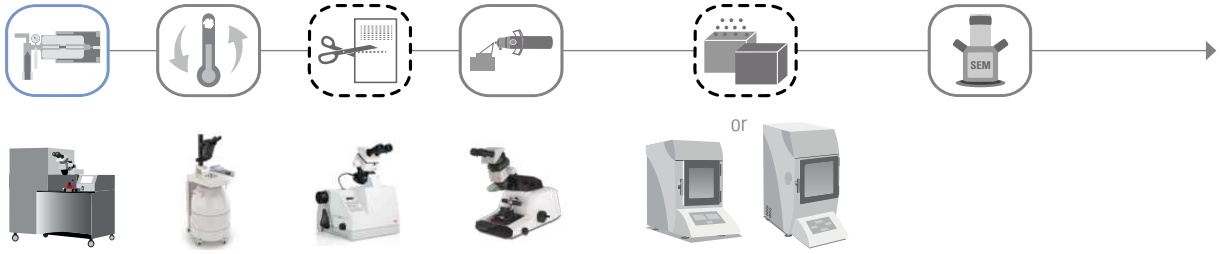


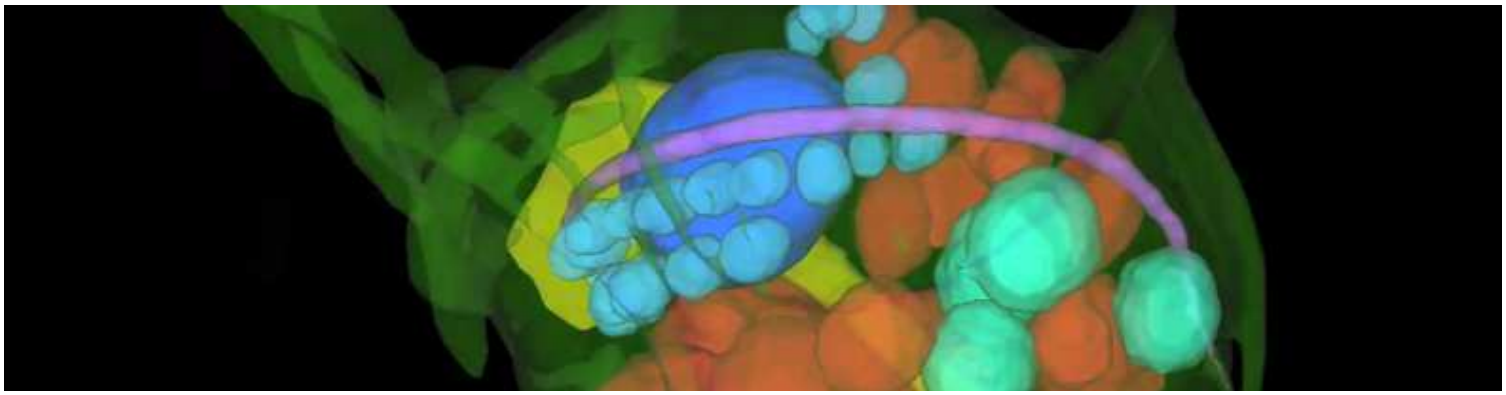
Serial Section SEM

Optimised dimensional structural analysis of biological material can be achieved with this technique. Thin sections are collected on silicon wafers to be imaged in a SEM for higher throughput.



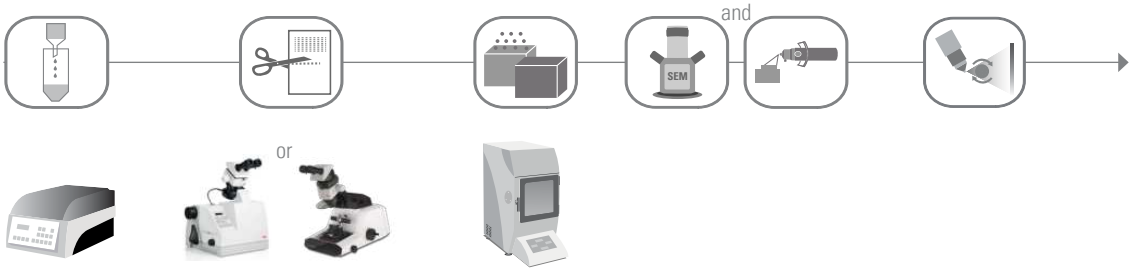
The same approach can be applied using high pressure freezing as a fixation method to increase structural preservation and minimize introduction of artefacts.



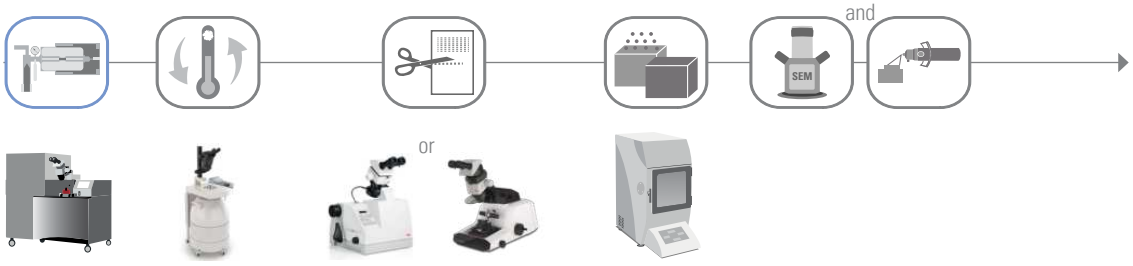


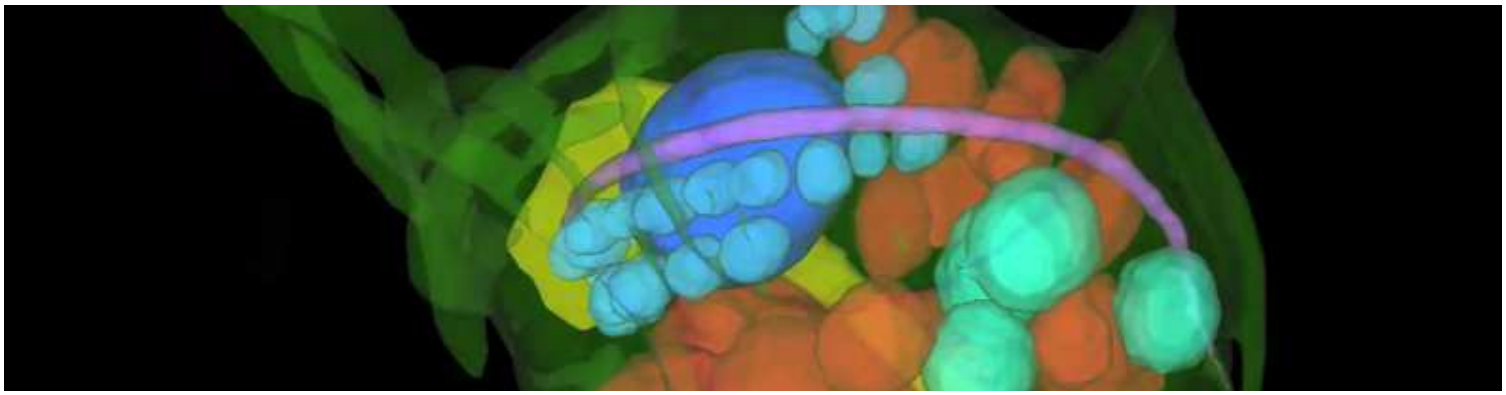
SEM Serial Blockface

Serial block face imaging is an integrated solution of ultrathin serial sectioning and simultaneous imaging inside a SEM. This workflow is suitable for chemically fixed samples to generate 3D datasets of big volumes. In case a µCT is used to identify a ROI within the sample volume, µCT based target trimming functionality in UC Enuity can be used.



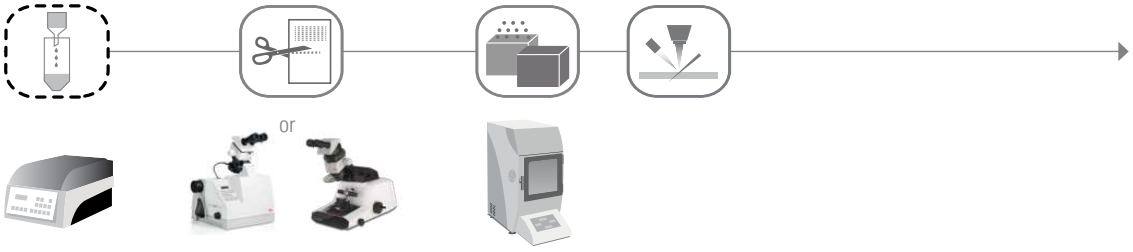
Similar approach can be applied using high pressure freezing as a fixation method to increase structural preservation and minimize introduction of artefacts.



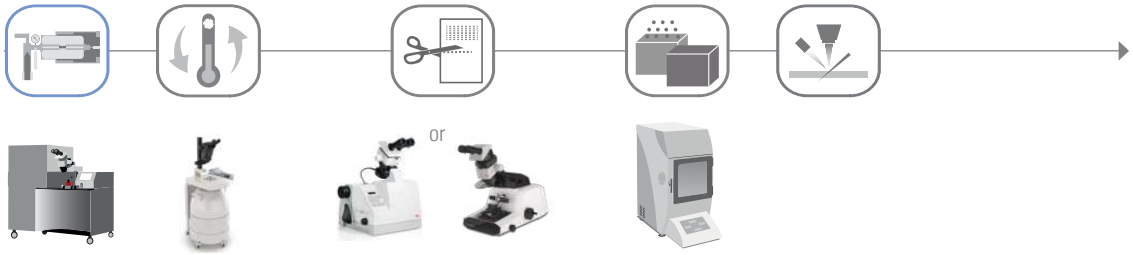


FIB SEM

In contrast to serial block face imaging, sample sectioning is achieved by using a focused ion beam. Samples are chemically fixed and processed at room temperature. The SEM images can be combined to generate 3D datasets. In case a µCT is used to identify a ROI within the sample volume, µCT based target trimming functionality in UC Enuity can be used.

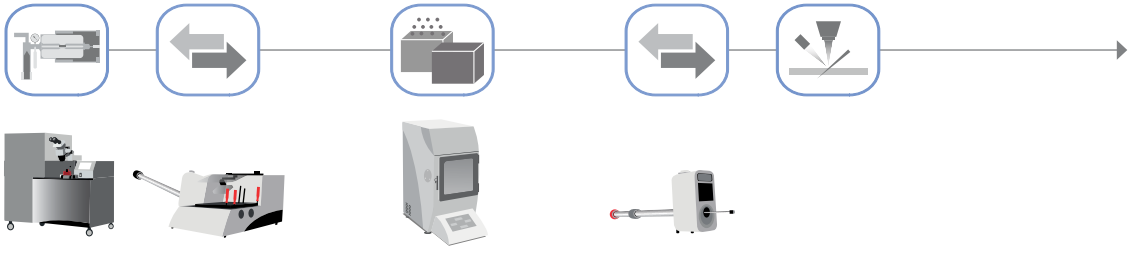


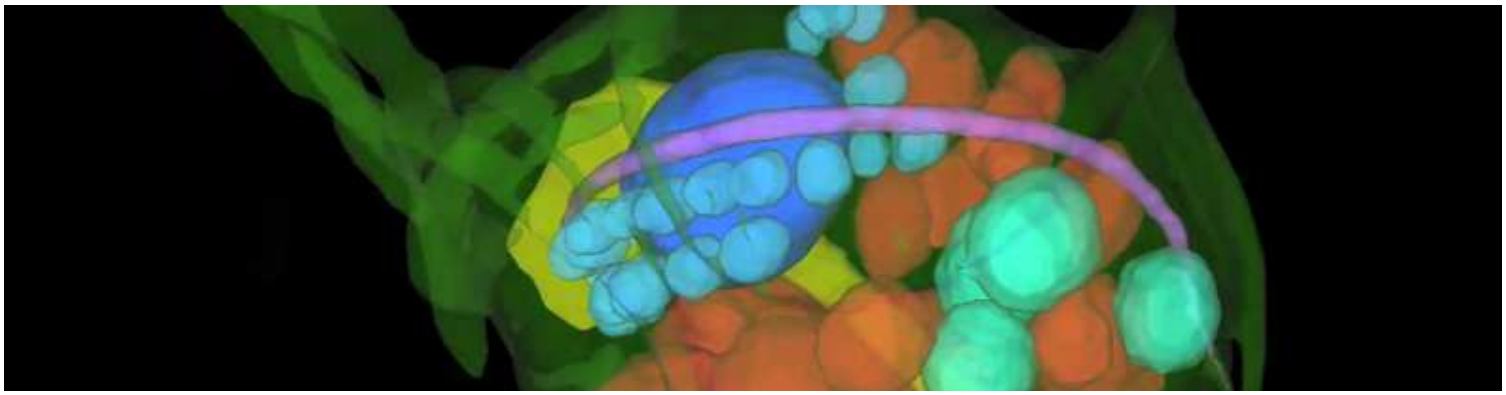
The same approach can be applied using high pressure freezing as a fixation method to increase structural preservation and minimize introduction of artefacts.



Cryo FIB SEM

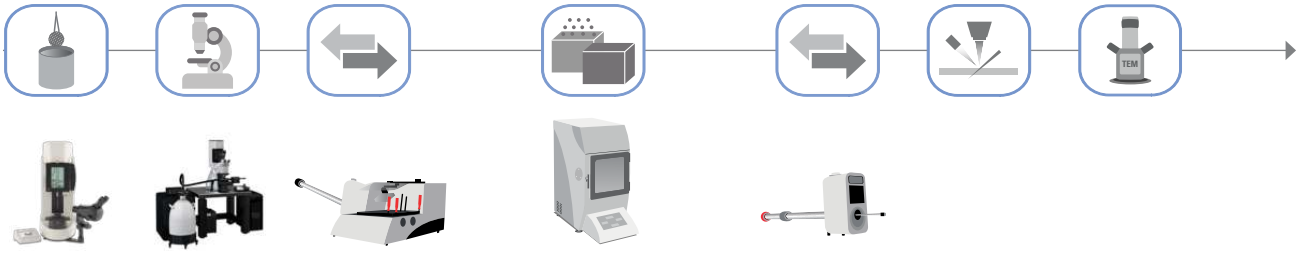
This method has the advantage to image under cryogenic conditions, not introducing any artefacts by FS or RT sample processing.



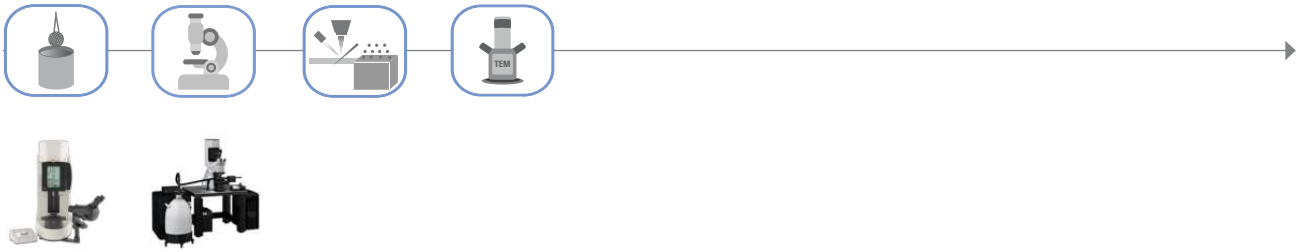


Cryo FIB on grid lamella

From vitrification up to sample transfer under cryogenic condition, this workflow guarantees samples free from contamination for cryo-TEM. An intermediate, optional cryo light microscopy step, helps identify regions of interest.

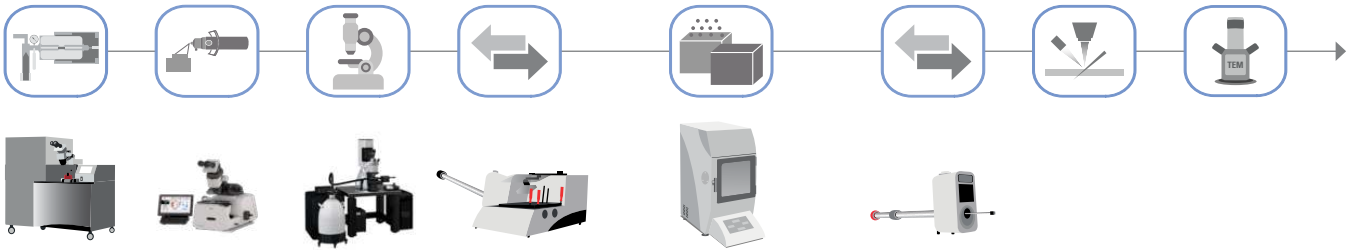


Using a dedicated cryo FIB to speed up sample retrieving and milling.

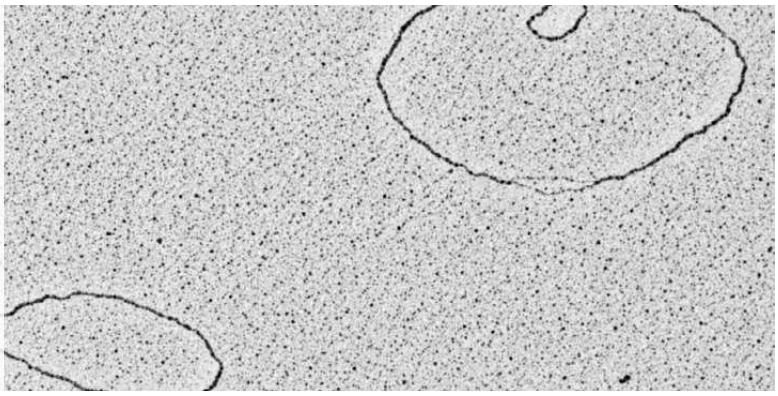


Cryo FIB lift out

Vitrified cells or tissue prepared by high pressure freezing can be prepared for Cryo TEM using a Cryo FIB to mill/produce a lamella which can be transferred onto a grid for Cryo TEM. In combination with an intermediate cryo-LM step, it facilitates identification of regions of interest easier and enables to prepare the lamella at a specific location within the sample.



SUSPENSIONS & MACROMOLECULES



Single particle analysis

A standard cryo-TEM workflow enables 2D and 3D studies of ultrastructure of proteins, bacteria, viruses and cells.



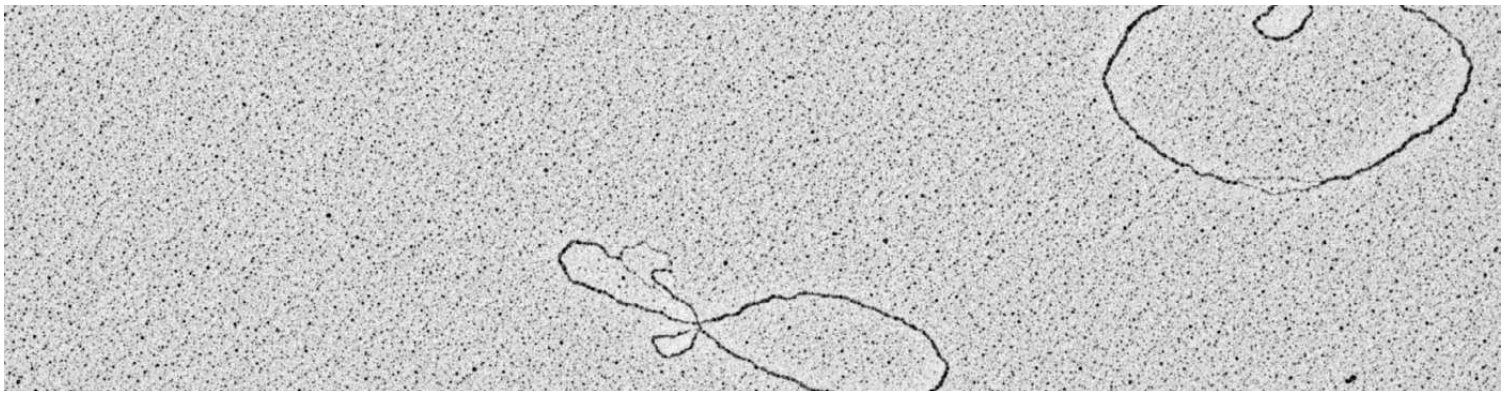


Low angle rotary shadowing

Coating at low angles enables structure resolution of small molecules like DNA or proteins.

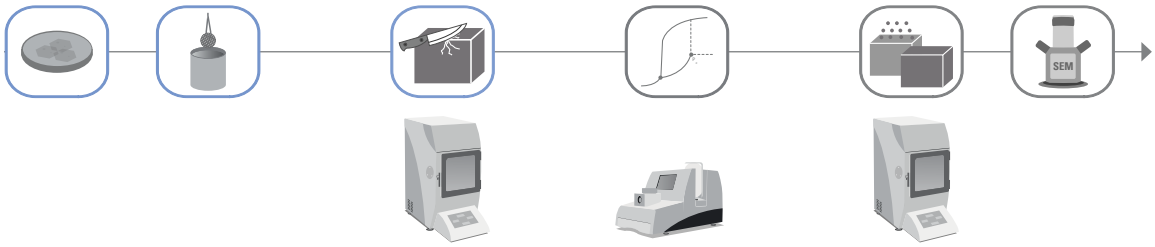






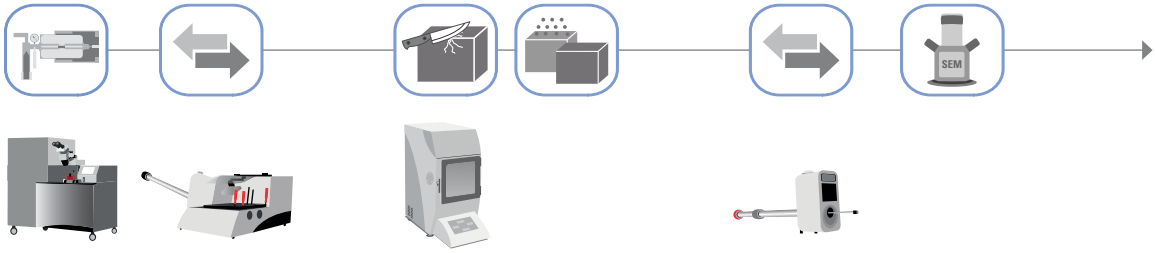
Freeze Fracturing & SEM

To analyse the interior structure of chemically fixed samples by SEM, they can be frozen and fractured as an intermediate step, then further processed by standard SEM sample preparation.



Freeze Fracturing & Cryo SEM

This method is an efficient technique to get high resolution images of internal structures of samples in suspensions without any introduction of artefacts by room temperature processing.



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