

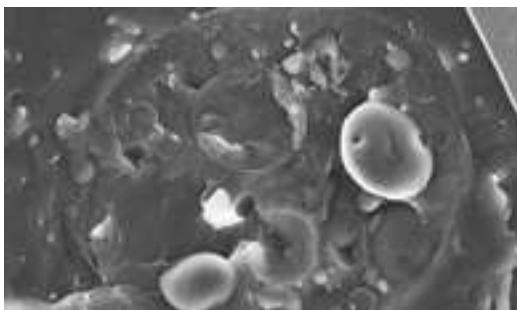
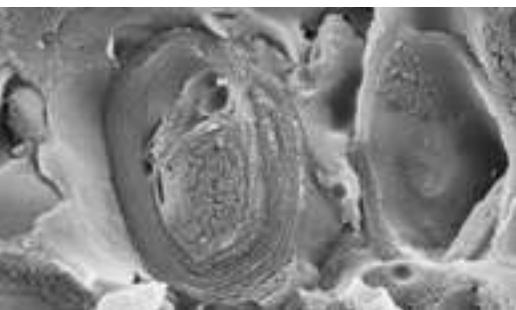
From Eye to Insight

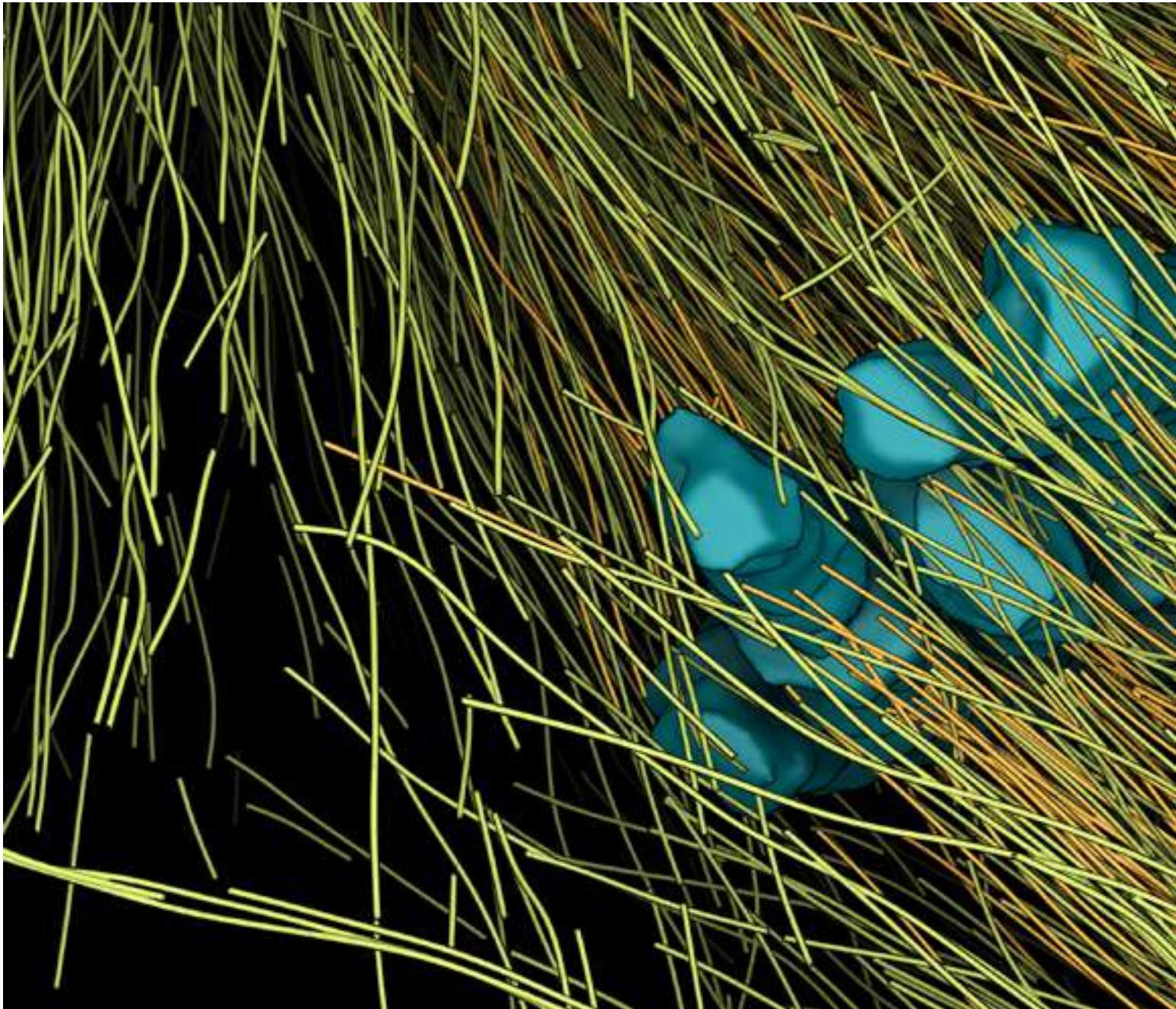


Leica Nano Technology

WORKFLOW SOLUTIONS FOR LIFE SCIENCE RESEARCH

Concentrating on workflow solutions, we provide a product range that is aligned to your needs in TEM, SEM, LM, and AFM investigations.





Great Results with the Workflow of your Choice

Do you need to pay attention to every detail – not just during image acquisition and analysis, but also sample preparation? Leica Microsystems offers you an extensive portfolio of high performance instruments for electron microscopy sample preparation. Designed to achieve effective sample preparation workflows, these solutions enable you to obtain top results.

In this publication, we have compiled and present in a clear way appropriate workflows for the most frequently required sample preparation methods. The solutions discussed are proven to bring about great results, both reliable and reproducible, within a short time. 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.

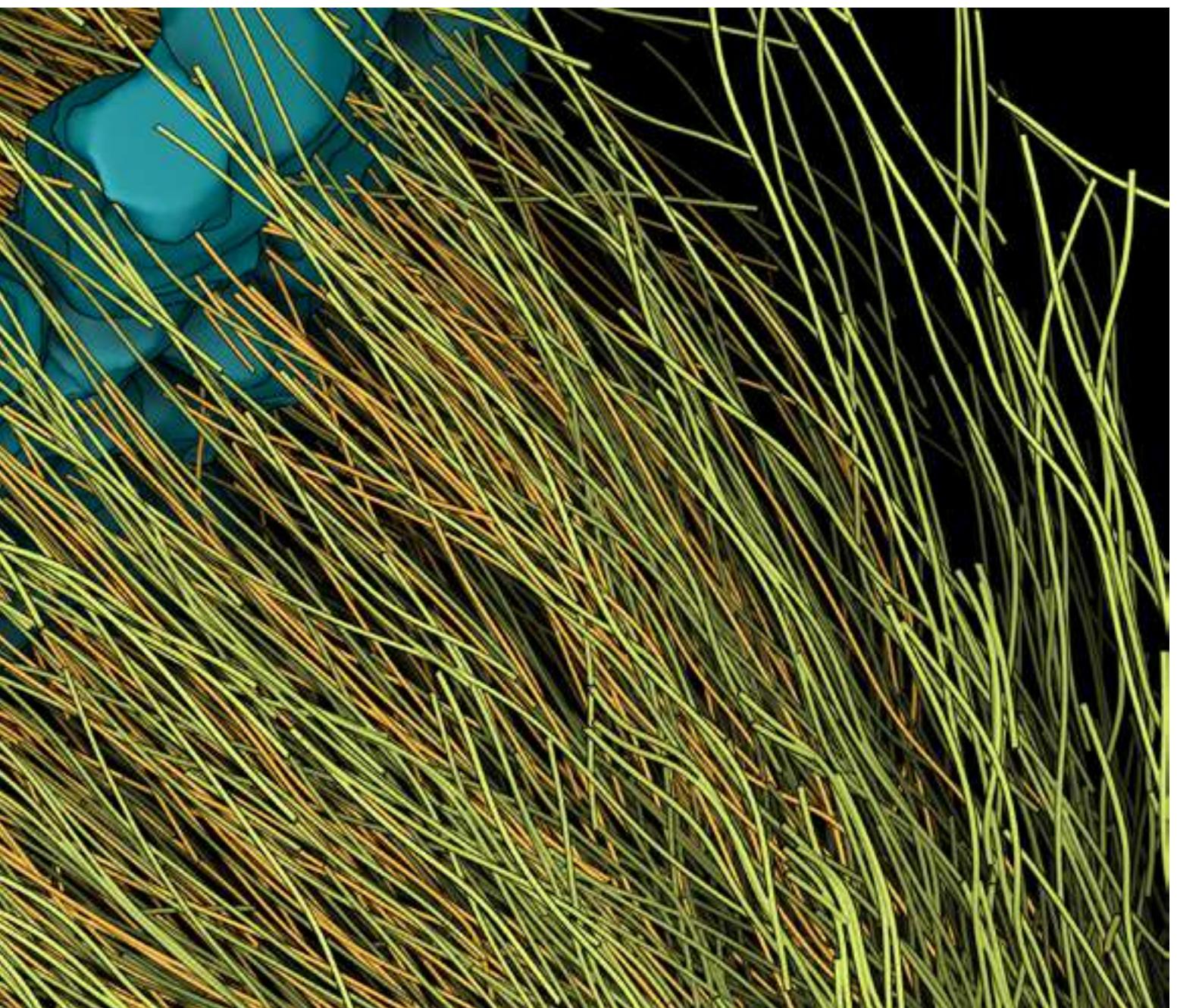


Table of Contents

	Correlative Methodologies	04		2D Tissue & Cellular Morphology	10
	Optogenetics & Electro-Physiology.....	07		3D Tissue & Cellular Morphology	12
	Surface Analysis	08		Suspensions & Macromolecules	18

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

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, required for FE-SEM and TEM applications.



EM Cryo CLEM

The system ensures contamination-free sample transfer and loading from cryo sample preparation instruments to a Leica fix stage light microscope. Maintains sample vitrified during cryo imaging.



EM ICE

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



See our complete portfolio, browse through our product websites and download all application notes from this brochure on
www.leica-microsystems.com/products/sample-preparation-for-electron-microscopy

Explore the knowledge portal on EM sample preparation on
www.leica-microsystems.com/science-lab
and get detailed information on Application Notes, Articles and Tutorials!



EM ACE900

High-end sample preparation system to perform freeze fracture, freeze etching, and e-beam coating to create replicas for TEM analysis and to image in the Cryo-SEM using the EM VCT500 connection.



EM UC7

Ultramicrotome for ultrathin sectioning of biological and industrial samples for TEM, SEM, AFM and LM examination. With the ARTOS 3D you can also simplify your preparation for Array Tomography.



EM VCT500

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



Cover images: top: Collembolen (source: Dr. Daniela Gruber, University of Vienna, Austria); bottom left: Cross-fractured axon showing myelin layers, (source: Dr. Andres Käch, Center for Microscopy and Image Analysis, University of Zurich, Switzerland); bottom middle: Euglena gracilis Klebs (source: Dr. Andres Käch, Center for Microscopy and Image Analysis, University of Zurich, Switzerland); bottom right: Ultrastructure of Arabidopsis thaliana primary root cells (source: Dr. Riet de Rycke, Department for Molecular Biomedical Research, 9000 Gent). Top image page 2: 3D reconstruction of the first mitotic spindle in a *C. elegans* embryo (Dr. Stefanie Redemann, University of Virginia, Charlottesville, USA); 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); Category image "Optogenetics & Electron-Physiology": Symmetric Synapse (source: Dr. Shuwen Chang, Charité Universitätsmedizin Berlin, Germany); Category image "Surface Analysis": Salivary gland (source: David McCarthy, UCL School of Pharmacy, London, UK); Category image "2D Tissue & Cellular Morphology": *C. elegans* cross section (source: Dr. Thomas Müller-Reichert, TU Dresden, Dresden, Germany); Category image "3D Tissue & Cellular Morphology": 3D reconstruction of an intercellular bridge in a *C. elegans* embryo (source: Dr. Julia König, TU Dresden, Dresden, Germany); Category 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 25: Reconstructed z-slice of an intercellular bridge of a sea urchin one-cell embryo (source: Dr. Julia König, TU Dresden, Dresden, Germany).

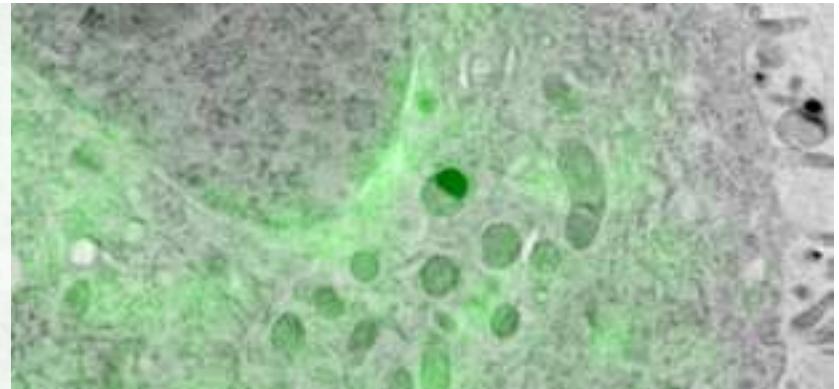


CAPTION

	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 <small>(discontinued end 2019)</small>		EM CPD300		EM TRIM2		EM VCM with EM VCT500
	EM ACE200		EM Cryo CLEM		EM UC7		
	EM ACE600		EM GP2		ARTOS 3D		
	EM ACE900		EM ICE		EM UC7 with EM FC7		
	EM AFS2		EM TP		EM VCT500		

CORRELATIVE METHODOLOGIES



Cryo CLEM

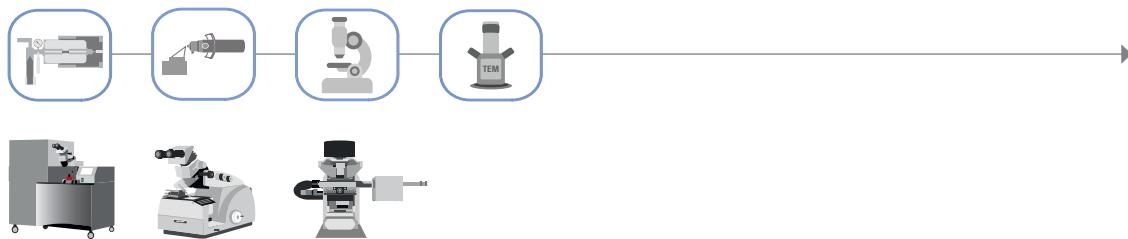
This workflow joins on-grid plunge freezing with cryo-fluorescence microscopy to target, relocate and overlay the structure of interest with the subjacent ultrastructure imaged with the cryo-TEM.

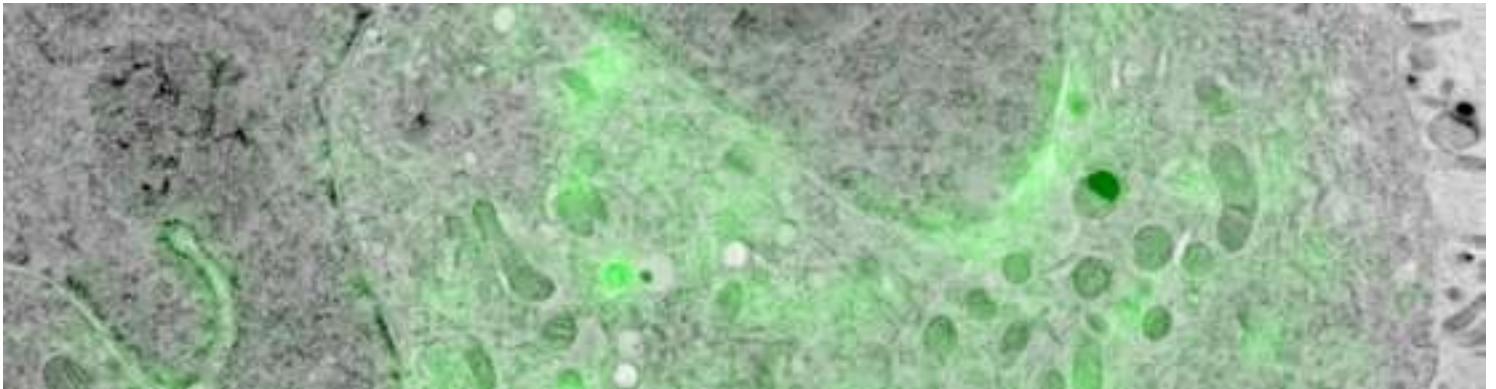


This is an ideal workflow to target the cell of interest on the grid by cryo-fluorescence microscopy for subsequent cryo-soft-X-ray-tomography.



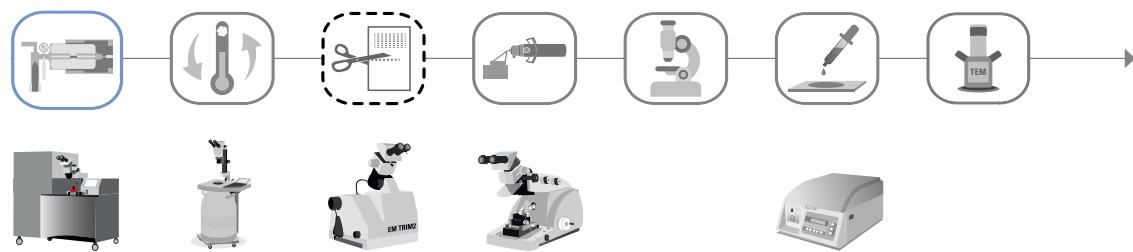
Combining CEMOVIS of high-pressure frozen samples with cryo-fluorescence microscopy allows to target the structure of interest within cryo sections. This is then superimposed with the underlying ultrastructure.



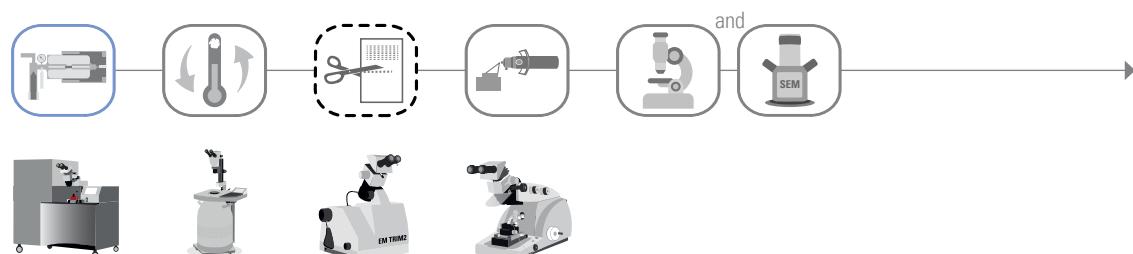


In resin CLEM

This workflow is optimised 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.

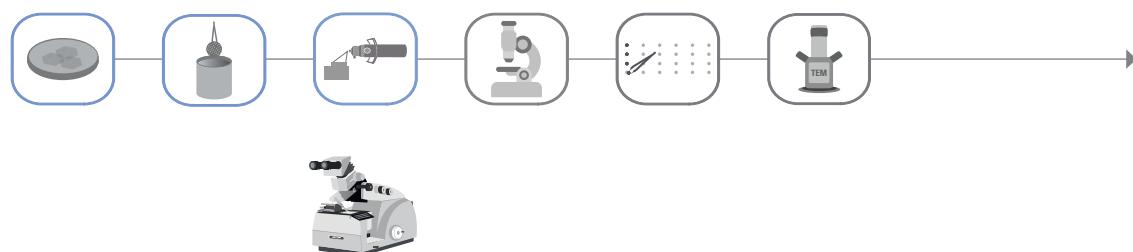


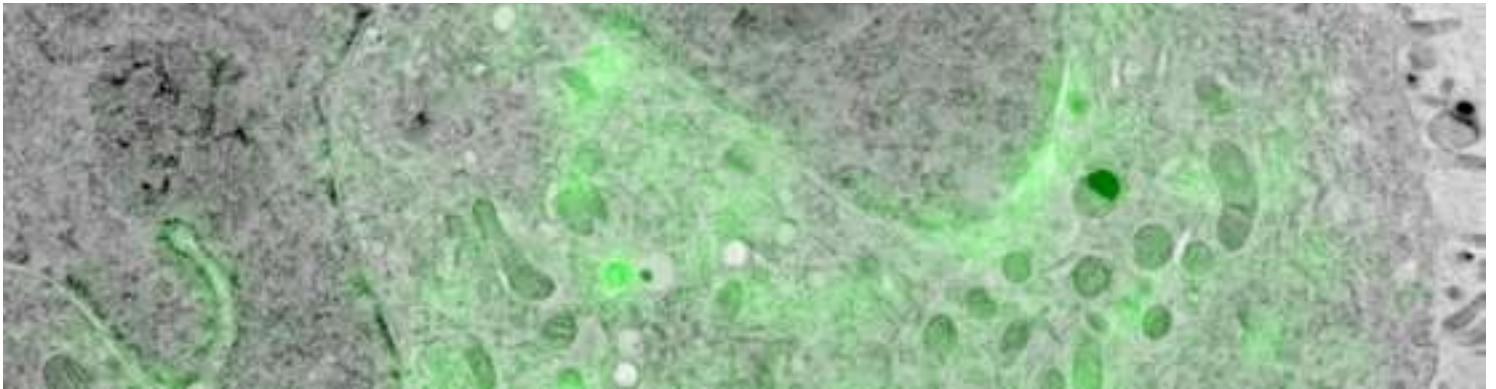
To achieve high precision correlation of the fluorescent and electron microscopic image, an integrated light and scanning electron microscope is utilized.



Tokuyasu CLEM

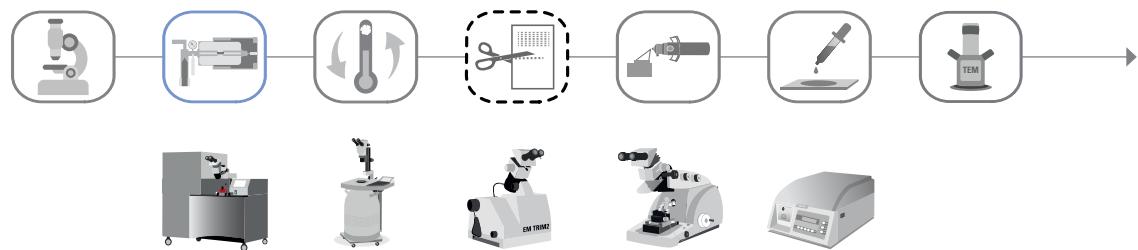
Introducing light microscopy into a classic Tokuyasu workflow is used to identify the region of interest on the section followed by a more precise labelling localisation at the ultrastructural level.





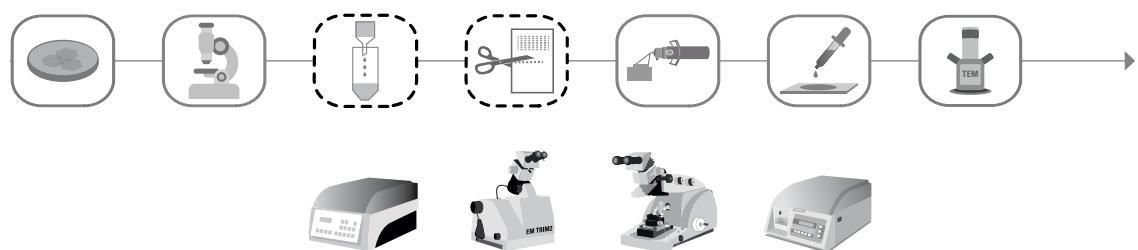
Live cell CLEM

This workflow aims to understand ultrastructural changes over time by combining live-cell imaging and instant fixation by high pressure freezing.

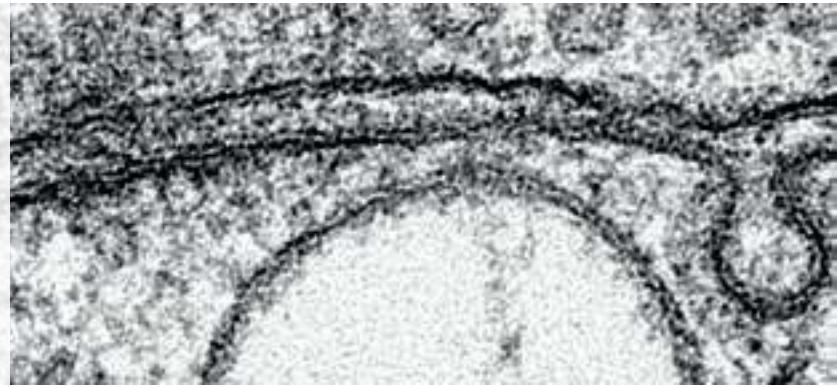


Post fixation epoxy CLEM

This is another opportunity to identify the structure of interest of tissue samples by using a mild chemical fixation, followed by LM imaging. Afterwards the samples will be further processed for electron microscopy.

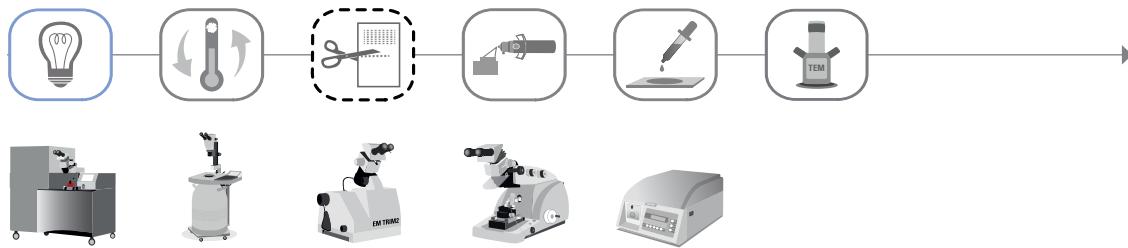


OPTOGENETICS & ELECTRO-PHYSIOLOGY



Light Stimulation

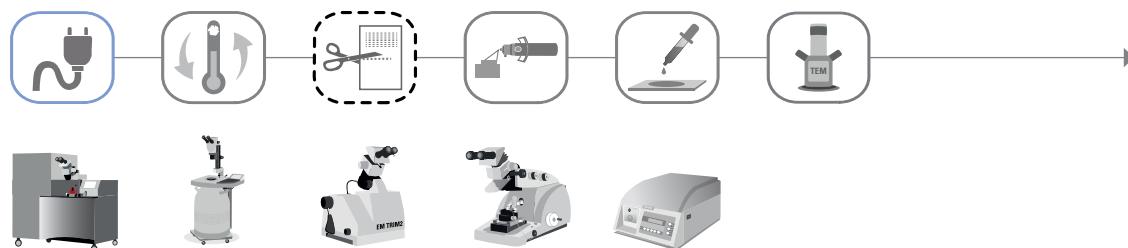
Using light stimulation in combination with high pressure freezing a moment in time can be frozen. Based on optogenetics, this workflow enables to trigger action potentials in excitable cells and arrest the process at any specific time.



Related Application Note downloadable from the following product website:
EM ICE: UV light stimulated sun screen lotion

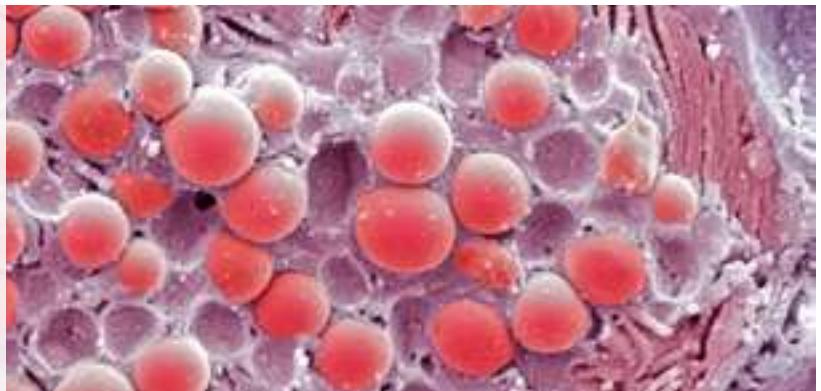
Electrical Stimulation

The workflow enables to coordinate electrical stimulation of neuronal tissue with rapid freezing to visualize membrane dynamics at millisecond precision.



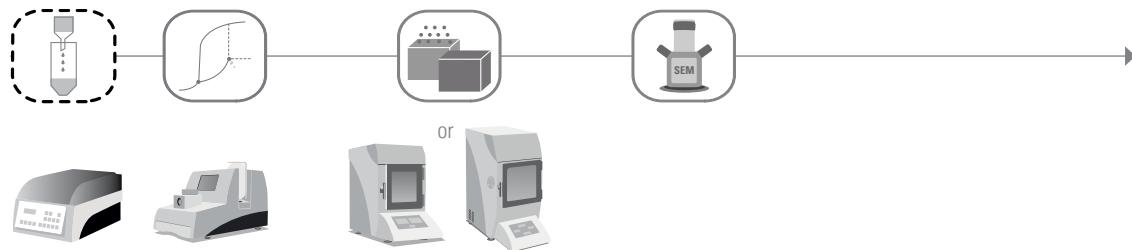
Related Application Notes downloadable from the following product website:
EM AFS2: Sample Preparation of Arabidopsis Thaliana Root Tips Cells
Cell envelope structure of a thermotolerant γ -proteobacterium Acidithiobacillus sp., strain HV2/2
Targeting of peroxisomal matrix proteins in the diatom Phaeodactylum tricornutum
Sample Preparation of Mouse heart
EM ICE: Symmetric Synapse
Visualization of membrane dynamics with millisecond temporal resolution

SURFACE ANALYSIS



SEM

This is the standard SEM workflow for biological samples to investigate surface architecture of chemically fixed samples.



Related Application Notes downloadable from the following product website:

EM CPD300: Bacteria Protocol

Black Mould Protocol

Clawed Frog Nuclear Envelope Protocol

Human Blood Cells Protocol

Micro-CT of Book Scorpion Musculature

Micro-CT of Insect Brain Protocol

Micro-CT of Insect Larva Protocol

Nematode *E. dianae* Protocol

Rice Anther Protocol

Rice Hull Protocol

Rice Root Protocol

Sludge Worm Protocol

Tobacco Leaf Protocol

Wall Cress Pod Protocol

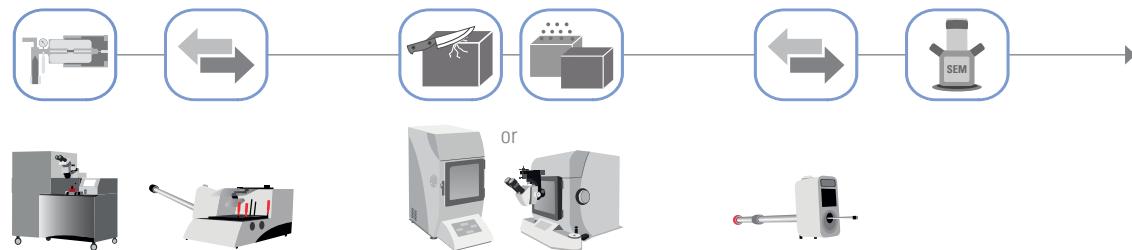
Wall Cress Stigma Protocol

Water Flea Protocol

Wrinkled Giant Hyssop Leaf Protocol

Freeze Fracturing & Cryo SEM

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



References on Science Lab:

Brief introduction to freeze fracture and etching

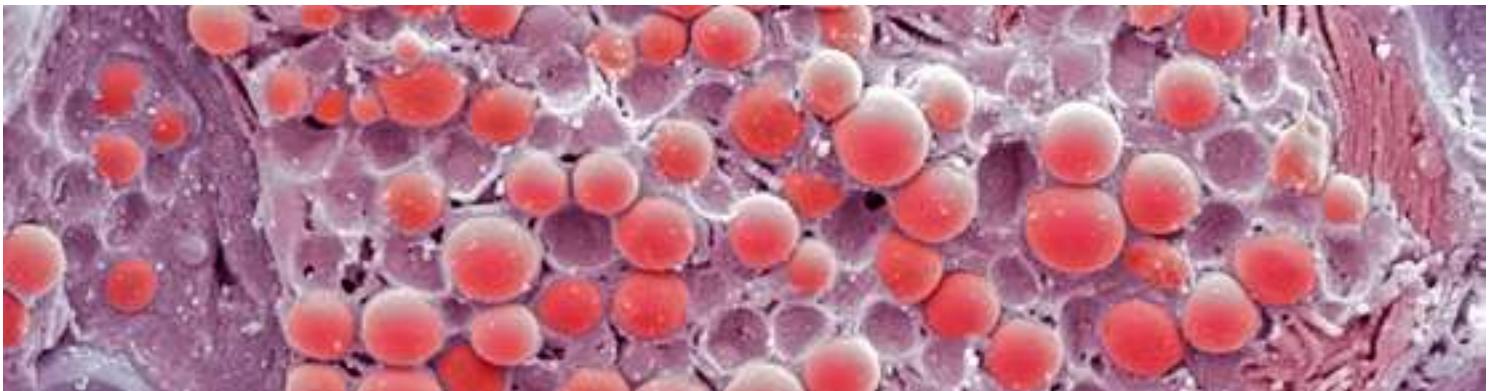
Giardia lamblia sample preparation for cryo sem

Drosophila larvae sample preparation for cryo sem

Cryo sem imaging of latex paint

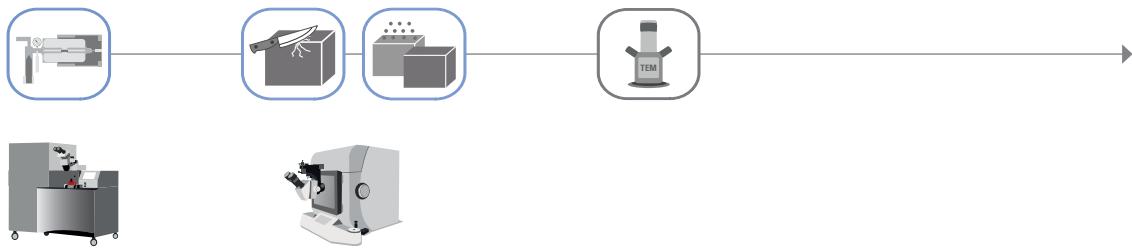
Related Application Note downloadable from the following product website:

EM ACE900: Cryo-SEM / Giardia lamblia



Freeze Fracturing & Replica

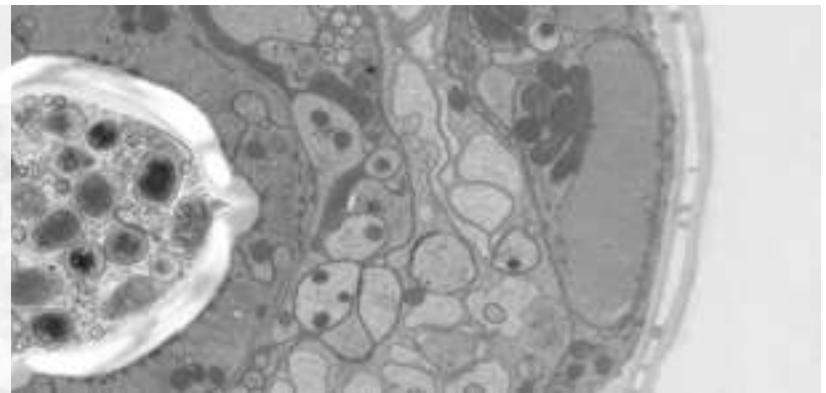
This is a classic freeze fracture workflow to receive a replica from the fractured surface of cryo fixed samples to get very high-resolution RT TEM images.



Related Application Note downloadable from the following product website:

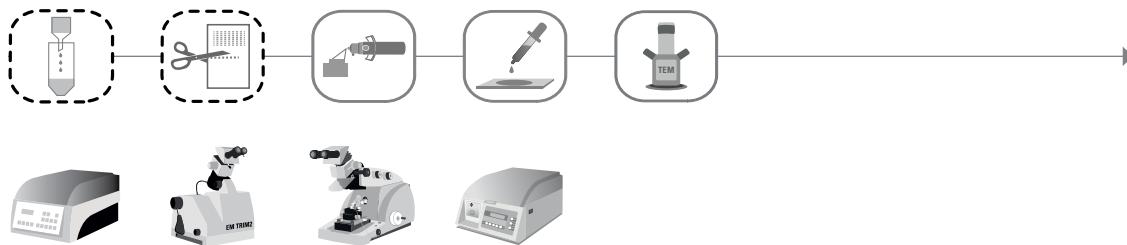
EM HPM100: Freeze-fracture replication of pyramidal cells

2D TISSUE & CELLULAR MORPHOLOGY



Room Temperature Processing

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

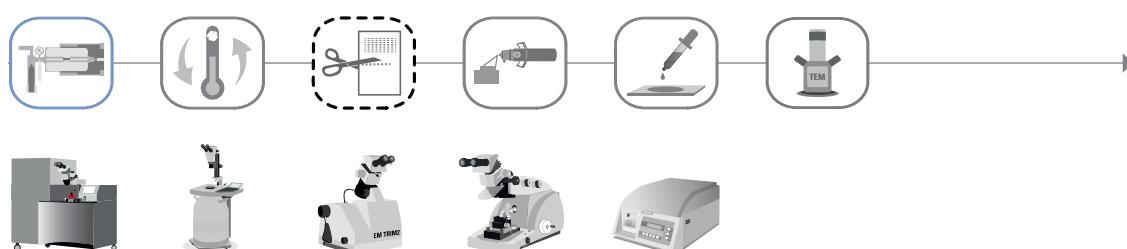


Related Application Note downloadable from the following product website:

EM TP: Embedding of murine fibroblasts grown on Aclar plastics for TEM

Hybrid Processing

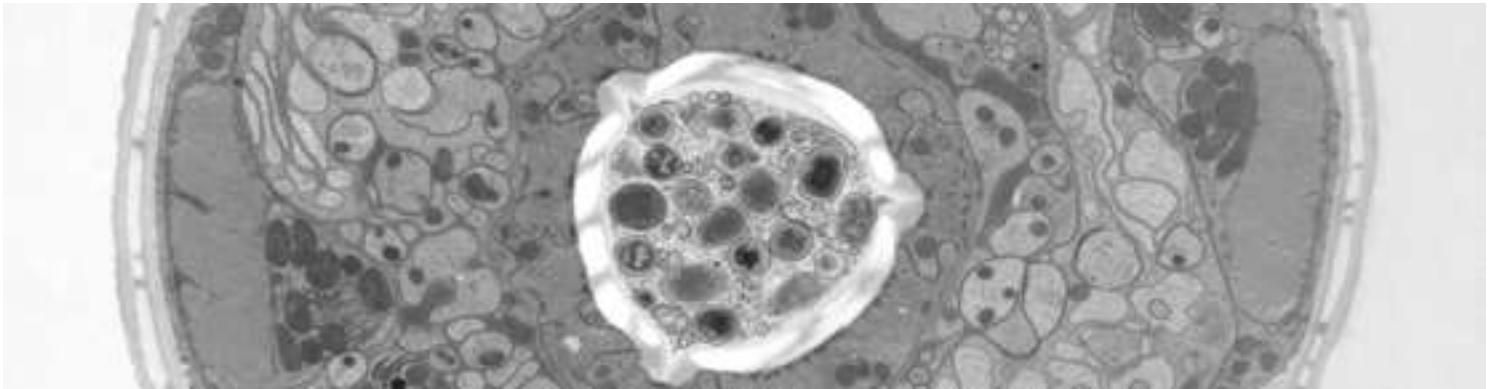
This standard workflow combines superior ultrastructural fixation by high pressure freezing with room temperature manipulation using freeze substitution and resin embedding.



Related Application Notes downloadable from the following product website:

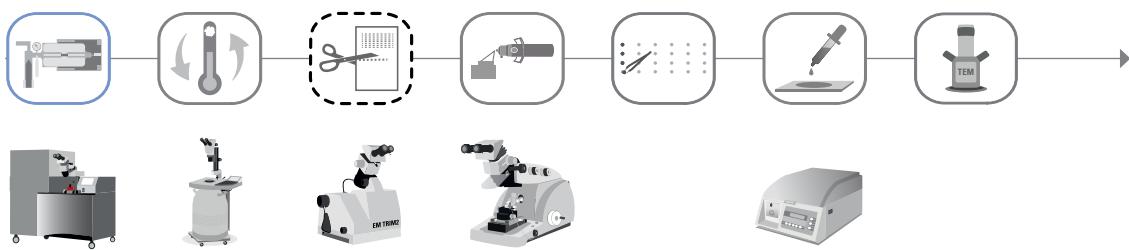
EM HPM100: Aureococcus (algae)
Hep-2 cells infected with Chlamydia pneumoniae
Maple (Acer saccharum) leaves
Maple (Acer saccharum) leaves
Mouse cerebellum
Poplar (Populus sp.) stem

Poplar (Populus sp.) stem
Rictus lutensis (protist)
Salmonella sp.
Structural study of Caenorhabditis elegans
Cultured Rat Hippocampal Neurons



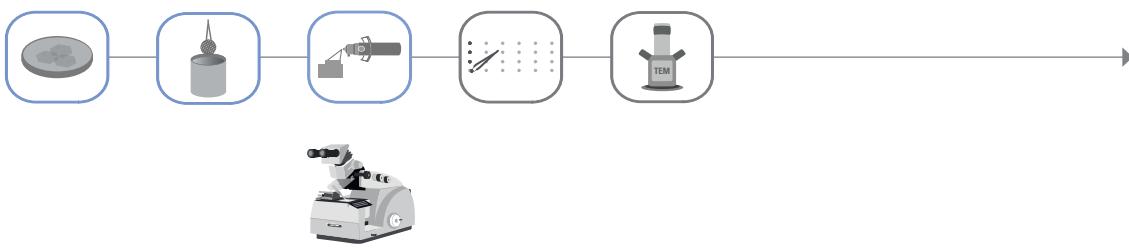
Immunolabelling

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

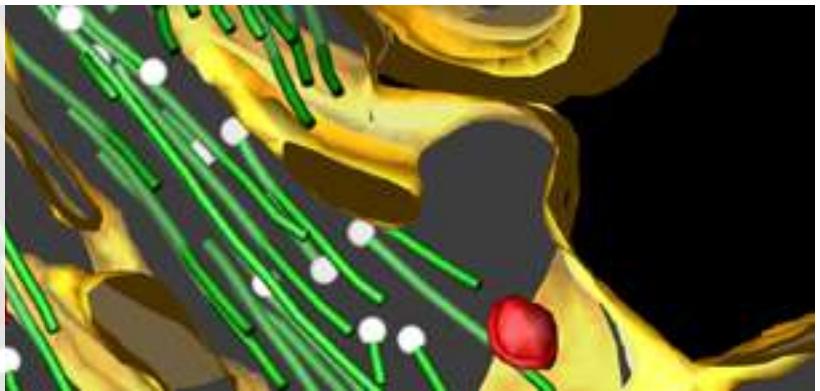
It is a widely used method for preparation of biological material for immunolabelling by using chemical fixation, sucrose infiltration and plunge freezing. Because of the well-preserved antigens, this technique is a fast and safe way to acquire results on ultrastructure and immunolabelling.



Reference

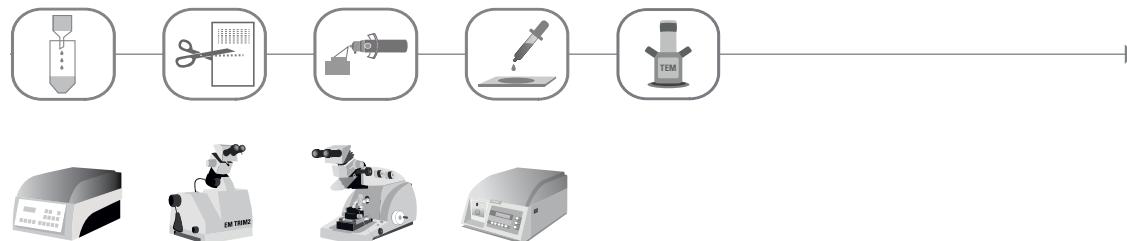
Basic course cryosectioning and Immuno-gold labeling of thawed frozen sections (Tokuyasu technique) at Cell Microscopy Core Utrecht, The Netherlands held by George Posthuma and others.
www.cellbiology-utrecht.nl/em-courses/basic-course-cryosectioning.html

3D TISSUE & CELLULAR MORPHOLOGY

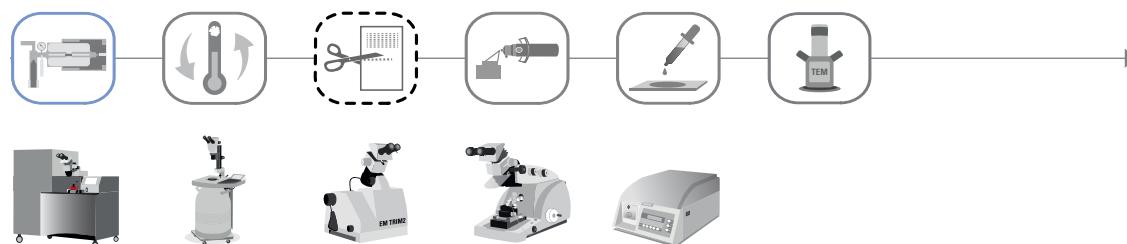


Section tomography 3D

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 semi-thick serial sectioning on 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.

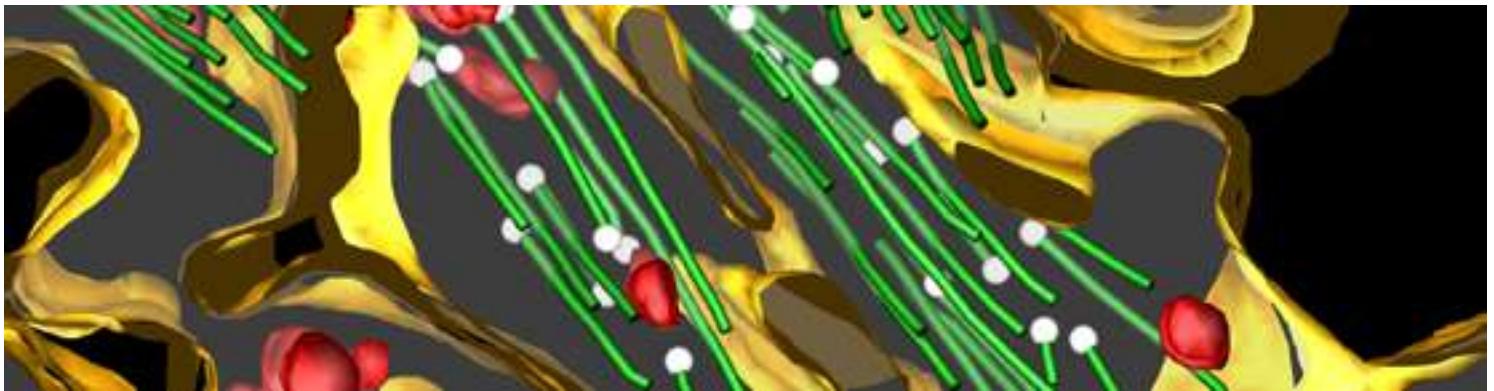


CEMOVIS 3D

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.

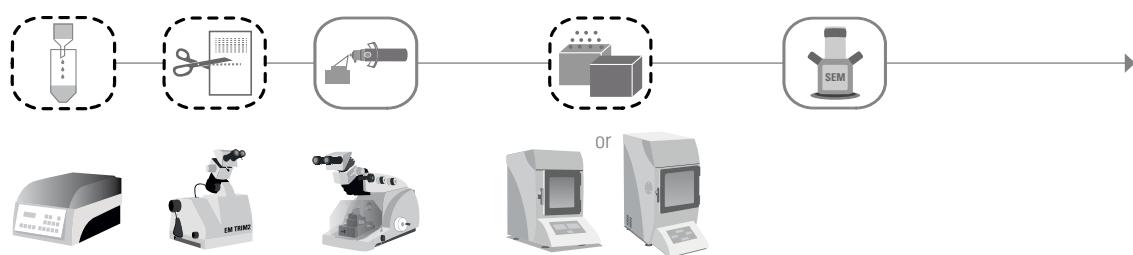


Related Application Note downloadable from the following product website:
EM HPM100: CEMOVIS of yeast

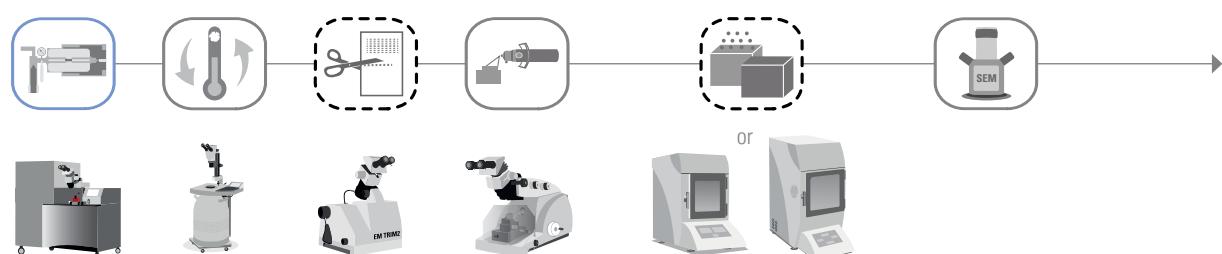


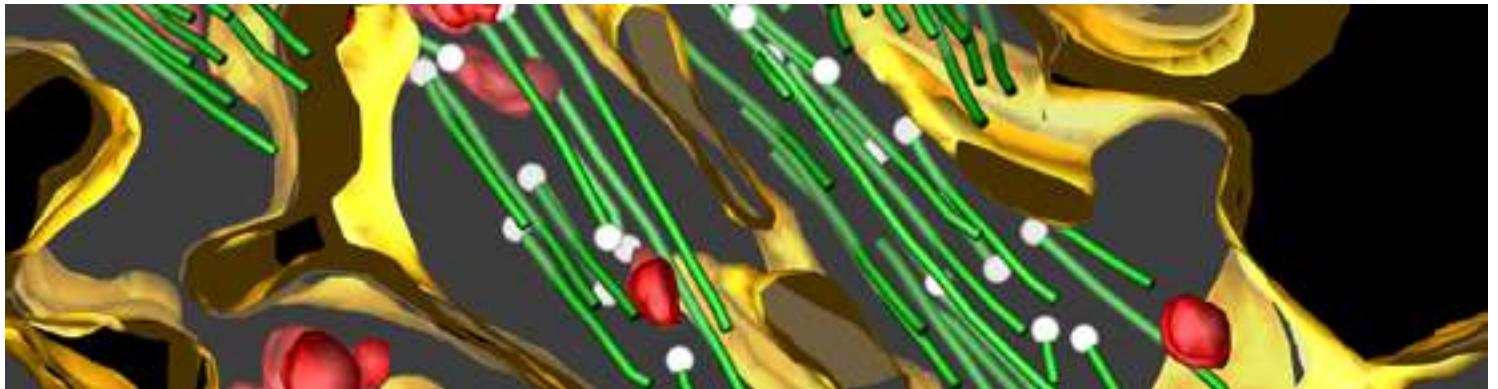
Serial Section SEM

Optimised dimensional structural analysis of biological material can be achieved with this technique. In comparison to the workflows above, thin sections are collected on silicon wafers to be imaged in a SEM for higher throughput.



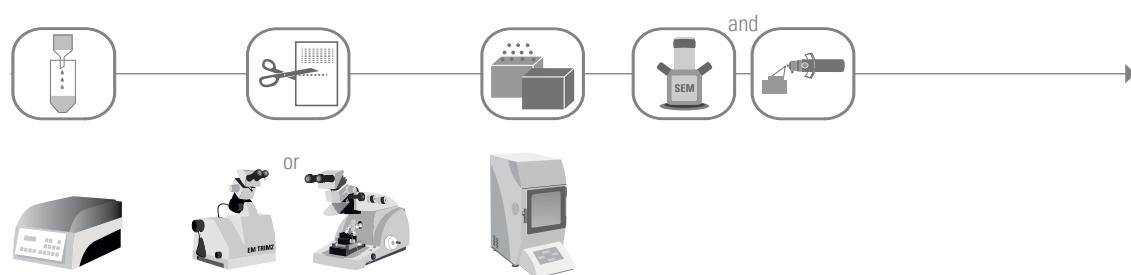
The same approach can be applied using high pressure freezing as a fixation method to increase structural preservation and minimize introduction of artefacts.



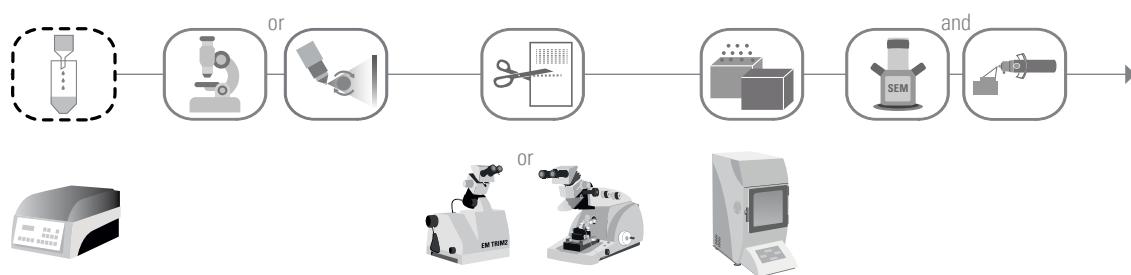


SEM Serial Blockface

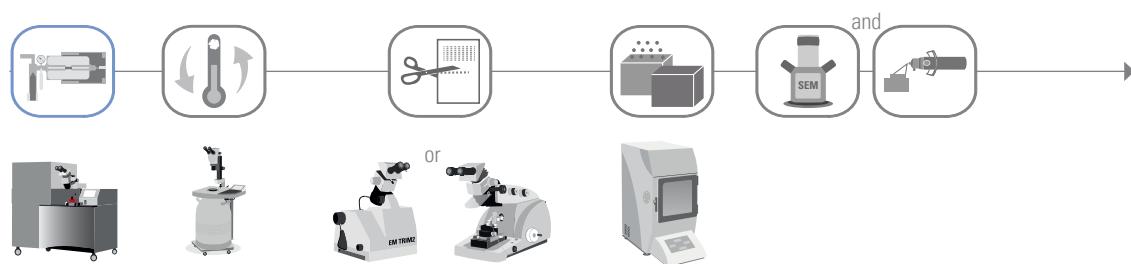
Serial block face imaging is an integrated solution of ultrathin serial sectioning and simultaneous imaging inside a SEM. This workflow is suitable for chemically fixed samples to generate 3D datasets of big volumes.



Same concept as above, but using a light microscope or a micro CT to target the region of interest within the volume for subsequent targeted trimming and serial block face SEM.



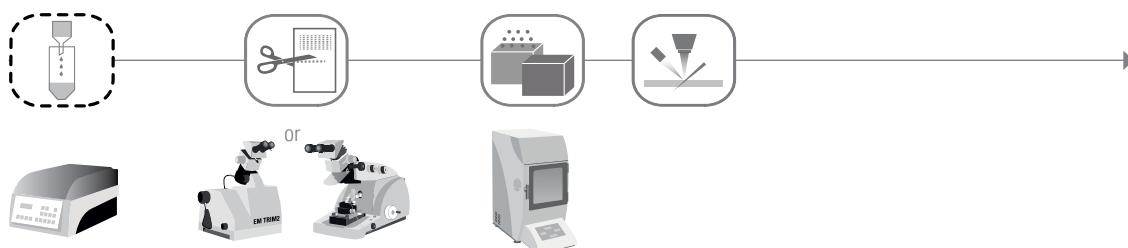
The same approach can be applied using high pressure freezing as a fixation method to increase structural preservation and minimize introduction of artefacts.





FIB SEM

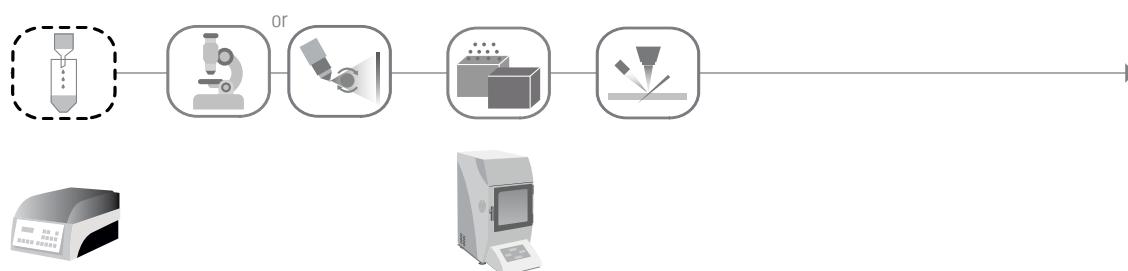
In contrast to serial block face imaging, the slicing of the samples is achieved by using a focused ion beam. Samples are also chemically fixed and processed at RT. The SEM images can be combined to generate 3D datasets.



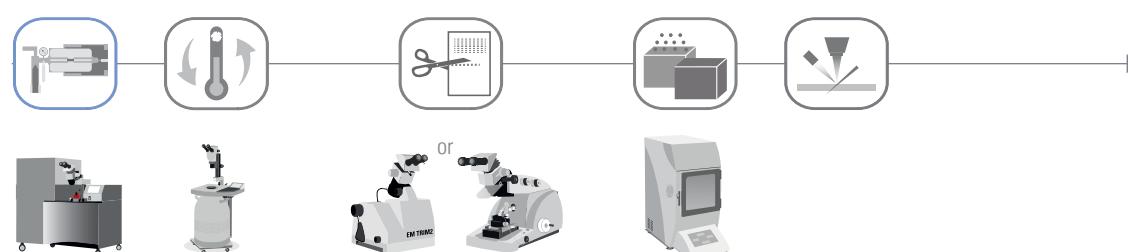
Related Application Note downloadable from the following product website:

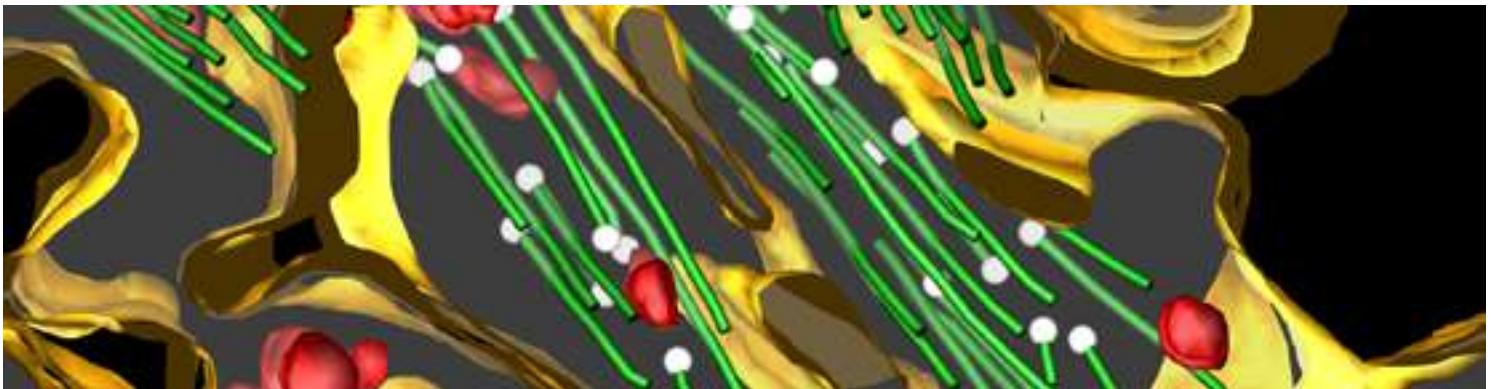
EM ACE600: Sample Protection prior to FIB processing

Same concept as above, but using a light microscope or a micro CT to target the region of interest within the volume for subsequent targeted trimming and FIB SEM.



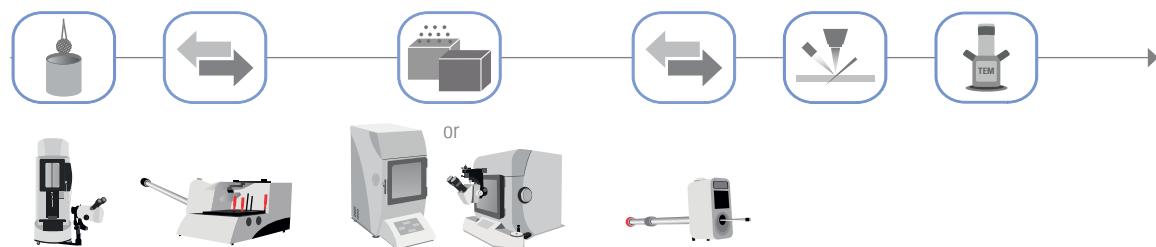
The same approach can be applied using high pressure freezing as a fixation method to increase structural preservation and minimize introduction of artefacts.



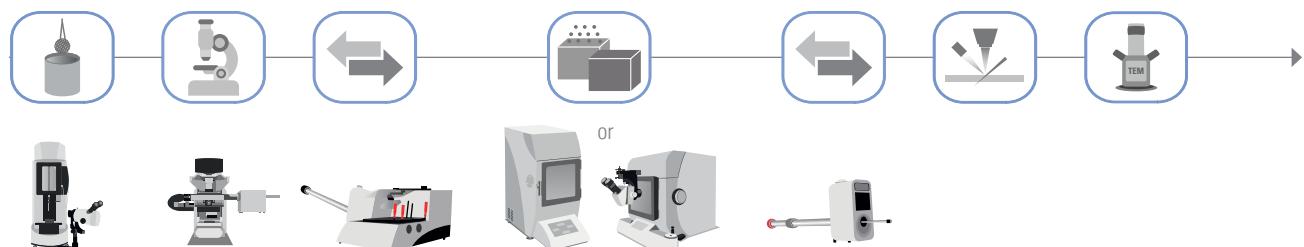


Cryo FIB on grid lamella

From vitrification up to sample transfer under cryogenic condition, this workflow guarantees samples free from contamination for cryo-TEM.

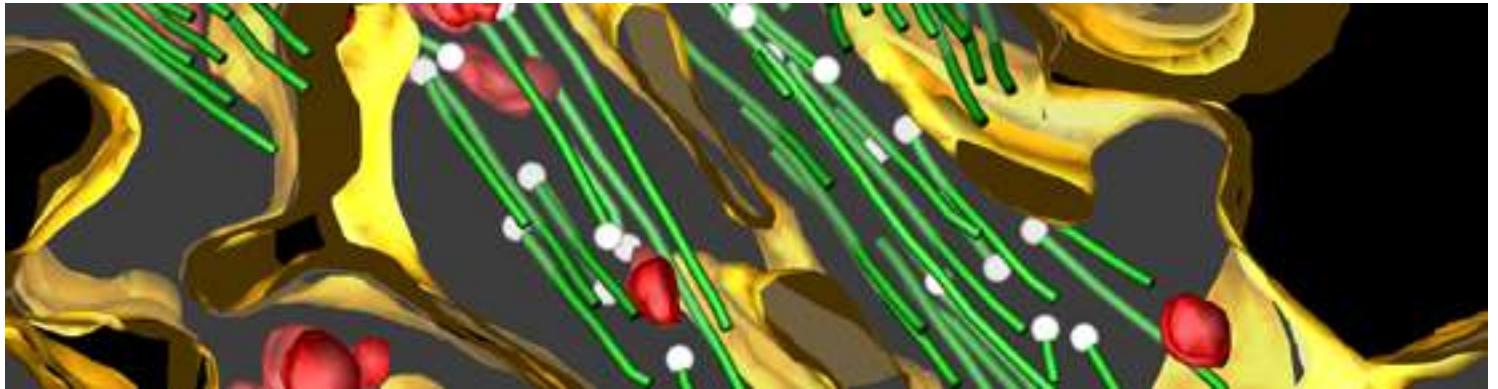


Combining the workflow above with an intermediate cryo-LM step, helps identify regions of interest easier and faster.



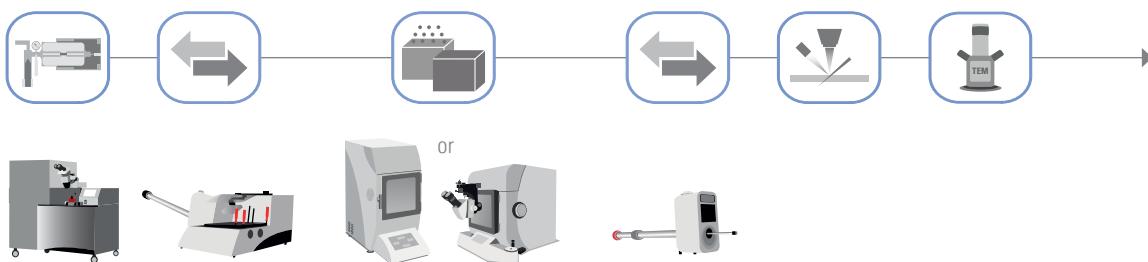
Using a dedicated cryo FIB to speed up sample retrieving and milling.



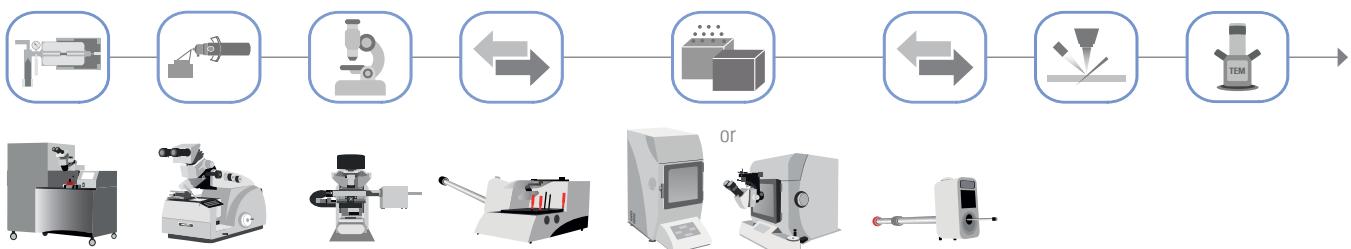


Cryo FIB lift out

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.

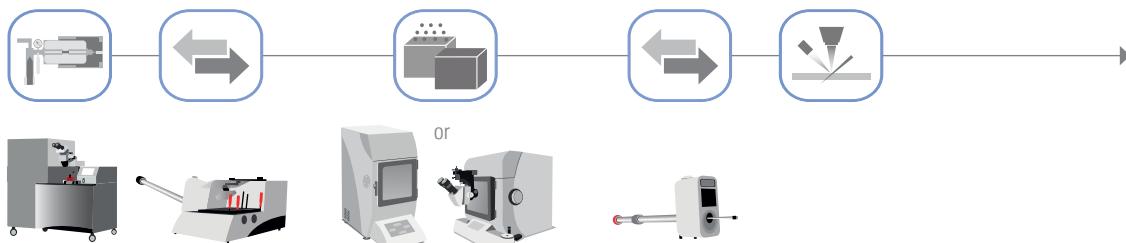


In combination with an intermediate cryo-LM step, it facilitates to identify regions of interest easier and enables to prepare the lamella at a specific location within the sample.

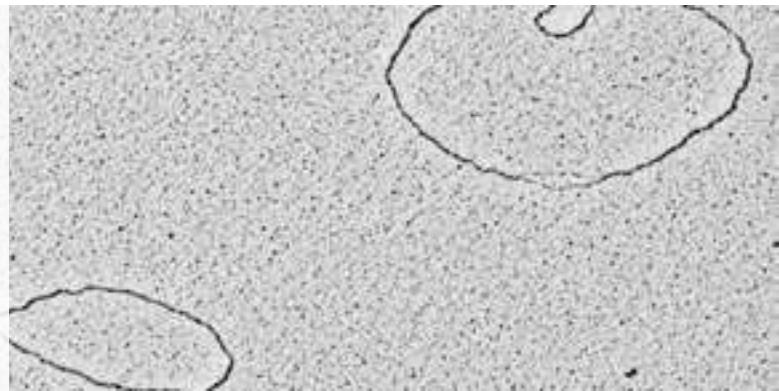


Cryo FIB SEM

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



SUSPENSIONS & MACROMOLECULES



Negative Staining

This is a classic TEM workflow to investigate the surface structure of small samples.



Single particle analysis

The standard cryo-TEM workflow enables studying the 2D and 3D ultrastructure of proteins, bacteria, virus and cells.



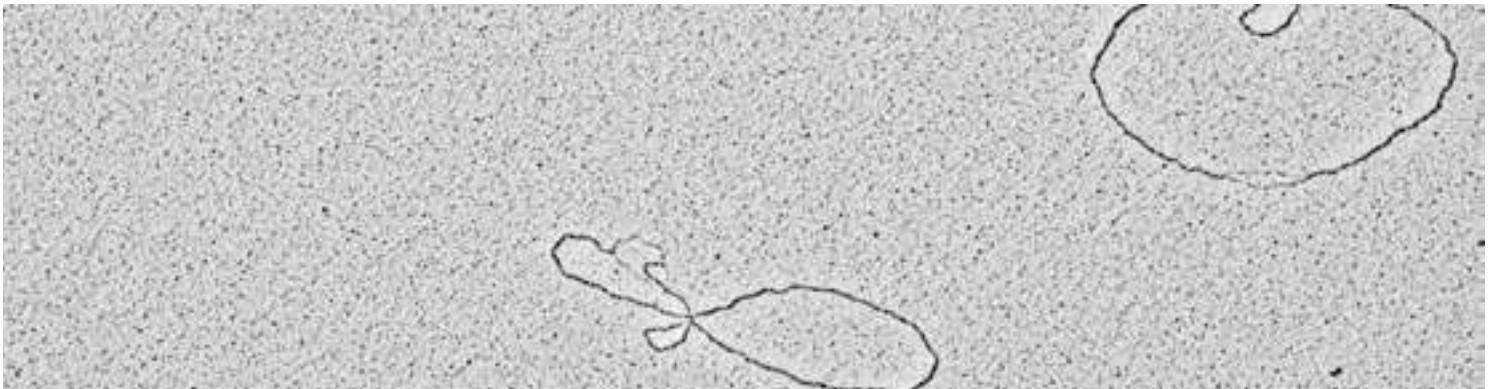
Related Application Notes downloadable from the following product website:

EM ACE600: Visualization of DNA Molecules
EM GP2: Plunge Freezing of Microtubules

Freeze Drying

The cryo fixated samples are kept at a sublimating temperature on the cryo stage in the vacuum system until all ice is removed and the samples are dry.





Low angle rotary shadowing

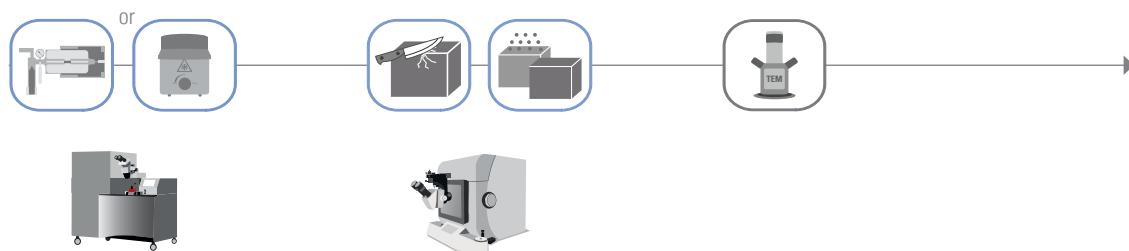
Coating in very low angles enables to resolve the structure of very small structures such as DNA or proteins.

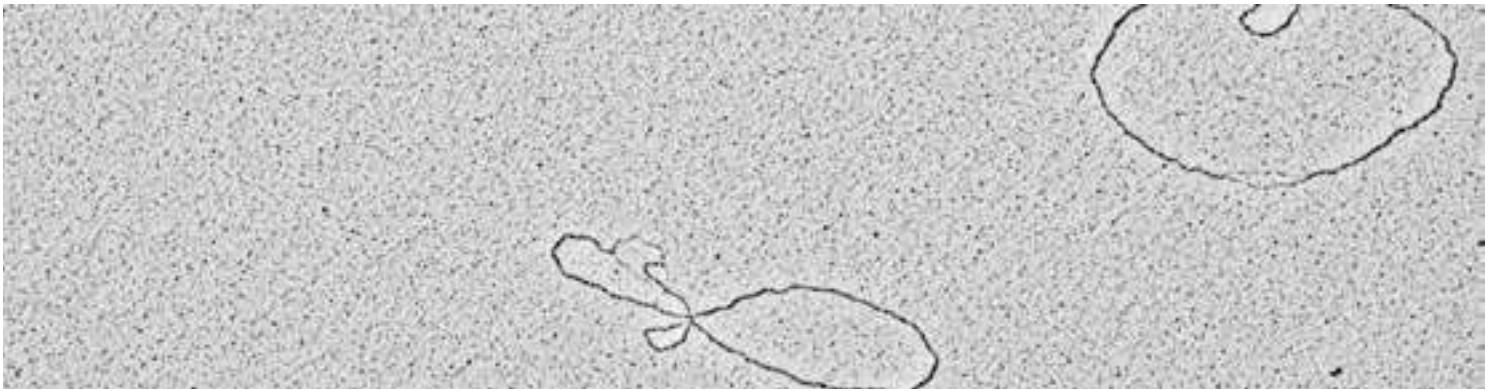


Related Application Note downloadable from the following product website:
EM ACE600: Visualization of DNA Molecules

Freeze Fracturing & Replica

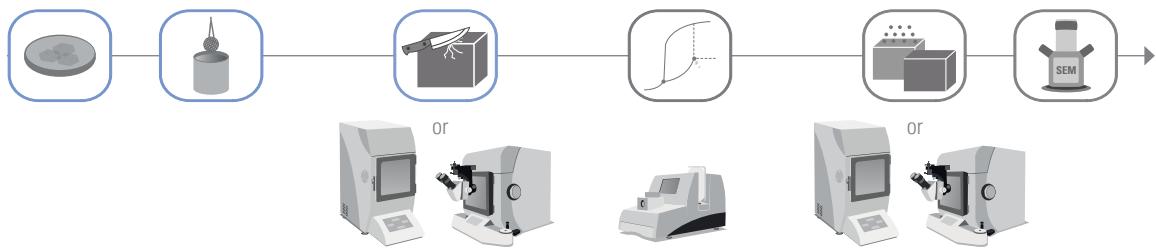
This is a classic freeze fracture workflow to receive a replica from the fractured surface to image in the TEM. Plunge freezing instead of high pressure freezing can be a quicker and easier way to fixate the samples.





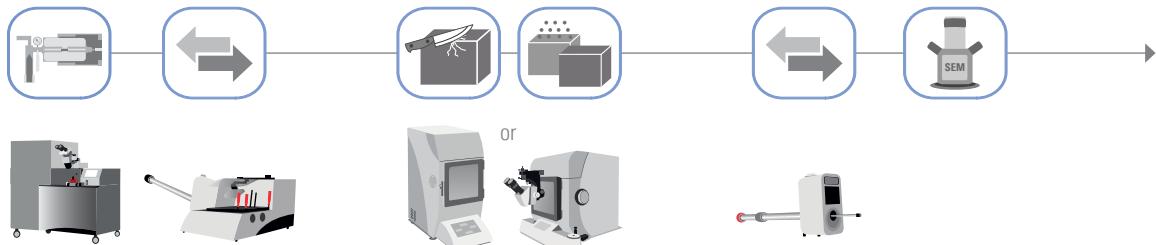
Freeze Fracturing & SEM

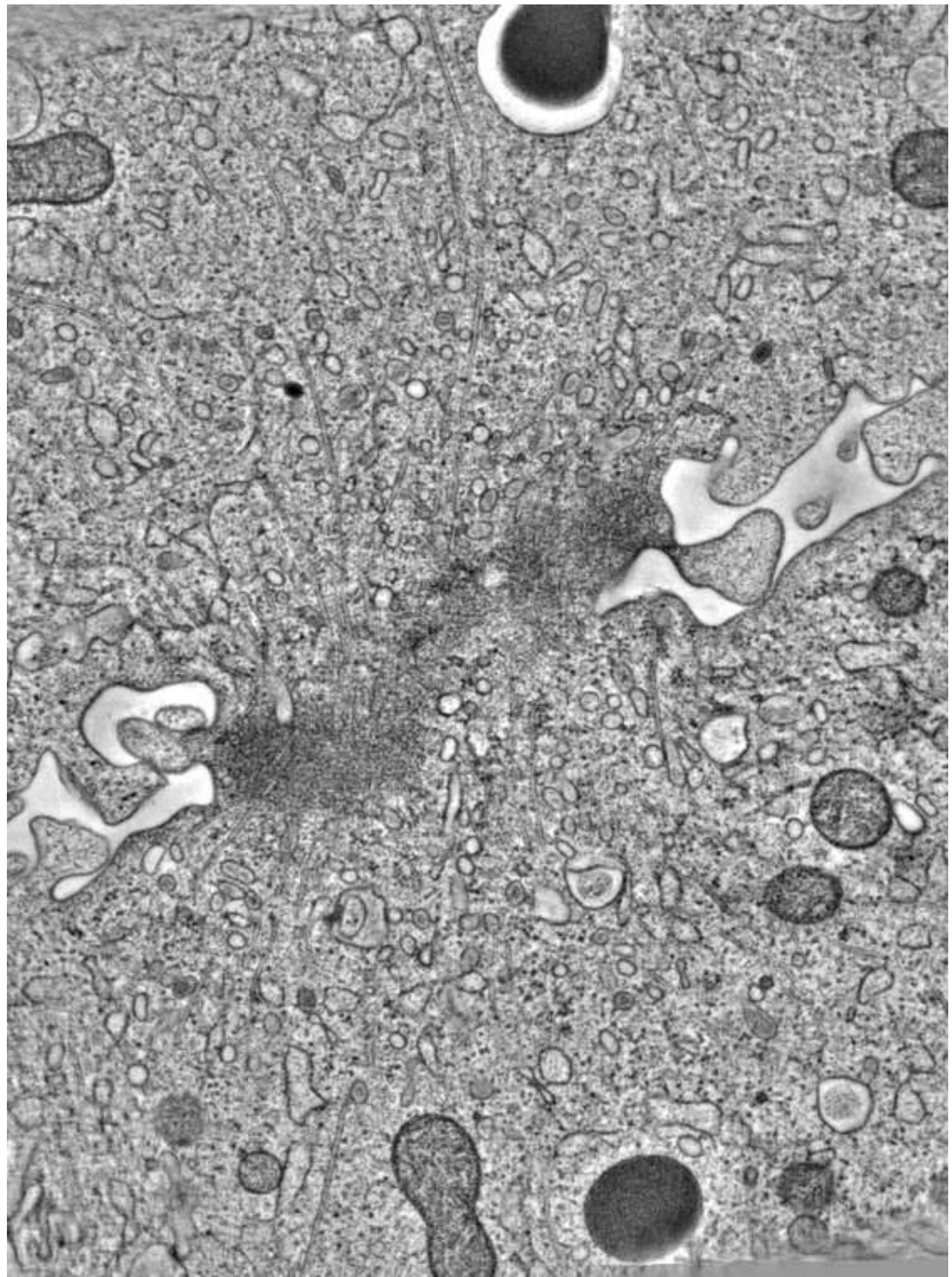
To analyse the interior structure by SEM of RT chemically fixed samples, they can be frozen and fractured as an intermediate step, then further processed by standard SEM sample preparation.

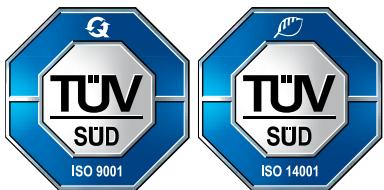


Freeze Fracturing & Cryo SEM

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







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