meteor

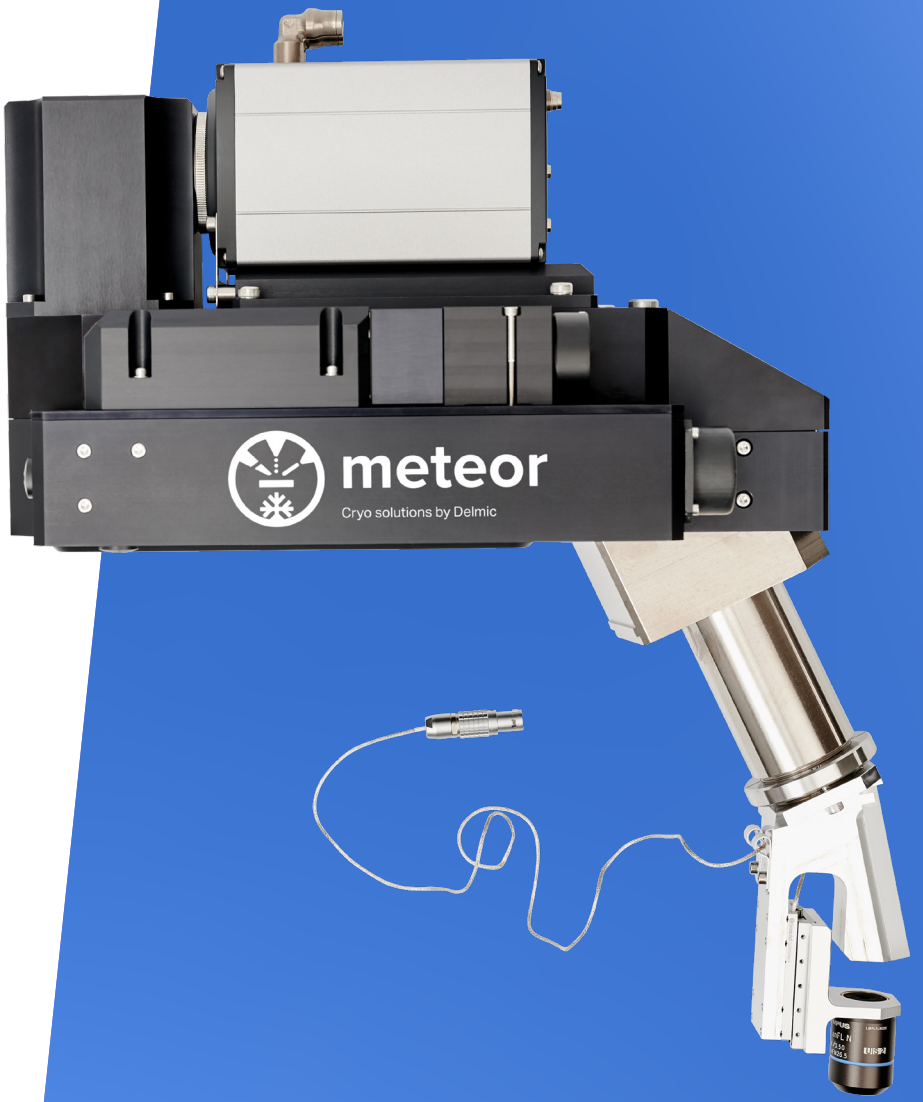
Cryo solutions by Delmic

Improve the cryo-ET workflow efficiency and streamline cryo-CLEM with in situ fluorescence imaging

delmic

Introducing **METEOR**

METEOR is an award-winning fluorescence light microscope (FLM) that can be directly integrated in selected cryo focused ion beam (FIB)/ scanning electron microscope (SEM) chambers. METEOR enables in situ cryo correlative light and electron microscopy (cryo-CLEM) and easily adapts to your workflow. Not only does it reduce the number of transfer steps between microscopes, protecting the fragile sample from unnecessary contamination, it will also allow you to image the region of interest (ROI) during and after milling. For the cryo-ET workflow this enables the verification of the ROI inclusion in the lamella. Not only does the FLM signal from the lamella confirm the target protein is being resolved by cryo-ET, it allows precious transmission electron microscopy (TEM) time to be better spent on useful samples only. In the 'mill and view' workflow, FLM data can be acquired in situ as more materials are milled away, improving functional and structural data correlation all through the volume of the biological material.



Key advantages of METEOR

Increase cryo-ET sample yield

Target your ROIs and correlate better and more effectively with the integrated FLM. Reduce transfer steps and thereby sample damage.

Optimise your CLEM workflow

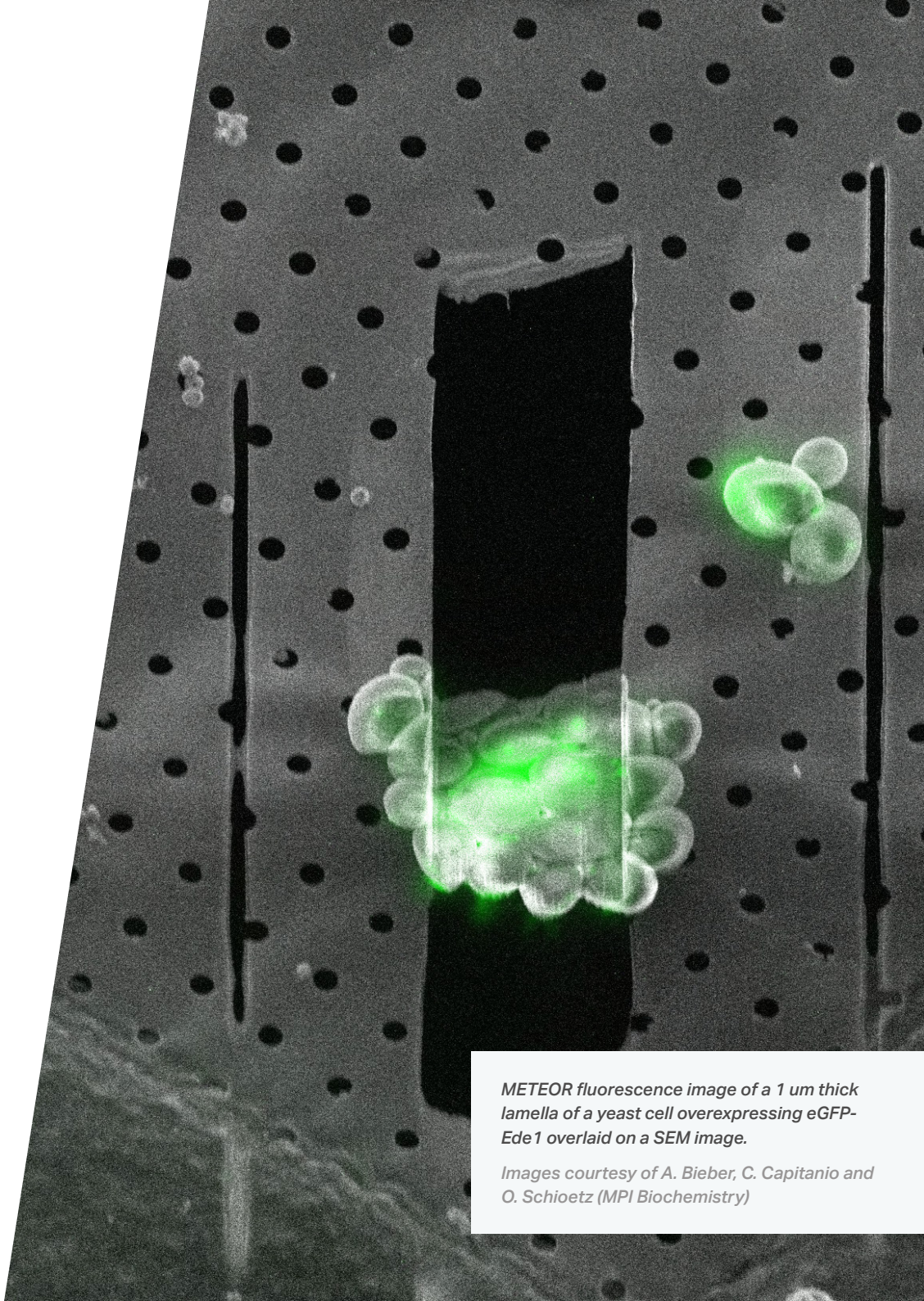
Collect FLM images more efficiently from the core of the sample or the lamella as materials are milled away.

Boost productivity

Produce high quality lamellae more easily. Obtain insight into your biological system through getting useful tomography data more quickly.

Improve cost efficiencies

Use your cryo TEM time more effectively on useful lamellae. Remove the need for a separate cryo FLM dedicated solely to ROI finding.



METEOR fluorescence image of a 1 um thick lamella of a yeast cell overexpressing eGFP-Ede1 overlaid on a SEM image.

Images courtesy of A. Bieber, C. Capitanio and O. Schioetz (MPI Biochemistry)

Current state of Cryo-ET and volume cryo-CLEM

Both cryo-ET and volume cryo-CLEM can uncover new insights in cell and molecular biology, virology, microbiology, immunology and drug development. The powerful insight obtained through cryo-ET helps to unravel biological structures in their near-native cellular environment at a sub-nanometre resolution. Not only is protein purification not required, the technique allows protein-protein and protein-drug interactions to be visualised. Thus, cryo-ET has the potential to offer a more effective and targeted drug design process and accelerate the drug development pipeline. With volume CLEM, functional and structural information can be obtained throughout the entire cell, providing a more holistic view. Changes in the organelles' morphology and their distribution can easily be linked back to, for example, the level and site of protein expression. Unfortunately, the speed of getting useful insight through cryo-ET and volume cryo-CLEM is hampered by their complex workflow. The current workflow, which involves imaging the sample beforehand with a separate cryo-FLM and then transferring it to a cryo-FIB, can be tedious and frustrating. The potential sample damage and contamination during sample transfer and difficulty inspecting the sample as material is milled away have a severe impact on the success in getting good data.

These pains and the simultaneous huge potential of cryo-ET and volume cryo-CLEM are what inspired us to develop solutions that would streamline the process and allow the researchers to reach their full potential.

What would you be able to achieve if you could get high quality data much more effectively?

Anna Bieber and Cristina Capitanio,
Max Planck Institute (MPI) of Biochemistry, Germany

We really wished for a way to check our lamellae for the target signal without having to do additional transfer steps, which would again increase the risk of contamination.

Katherine Lau,
Director of Life Sciences, Delmic

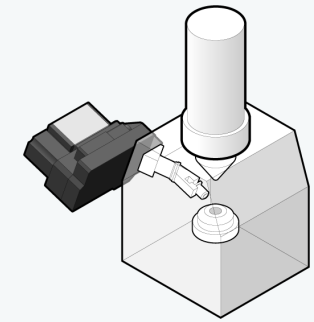
We want to allow researchers to improve sample yield and to get high-quality data more easily and make important scientific breakthroughs more quickly.

Workflow

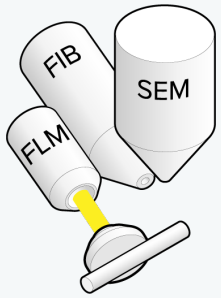
Fluorescence guided lamella preparation

METEOR is a flexible system that can be used in a variety of workflows including fluorescence guided lamella preparation and volume cryo-CLEM.

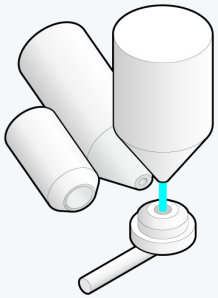
Using METEOR to guide lamella preparation, the user first loads a sample into the cryo-FIB/SEM equipped with METEOR. The microscope stage is then moved to the METEOR position to acquire an overview image and a highly precise z-stack, which enables the ROI to be located. The stage is then moved to the SEM position to capture a SEM image of the same ROI. Based on the image correlation the user determines where to generate a lamella and starts the milling. During and after milling the presence of the ROI in the lamella can be verified based on the fluorescence signals before transferring the sample to the cryo-TEM. Not only does the FLM signal from the lamella confirm the target protein is being resolved by cryo-ET, it allows precious transmission electron microscopy (TEM) time to be better spent on useful samples only.



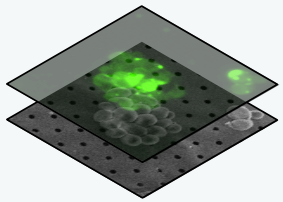
1. Load sample in cryo-FIB/SEM/FLM.



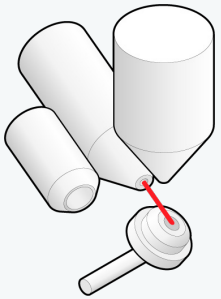
2. Move to FLM position and capture FLM image.



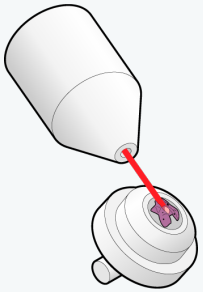
3. Move to SEM position and capture SEM image.



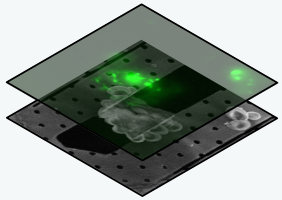
4. Image correlation.



5. Move to a region of interest (ROI) based on the FLM image.



6. FIB mill lamella.

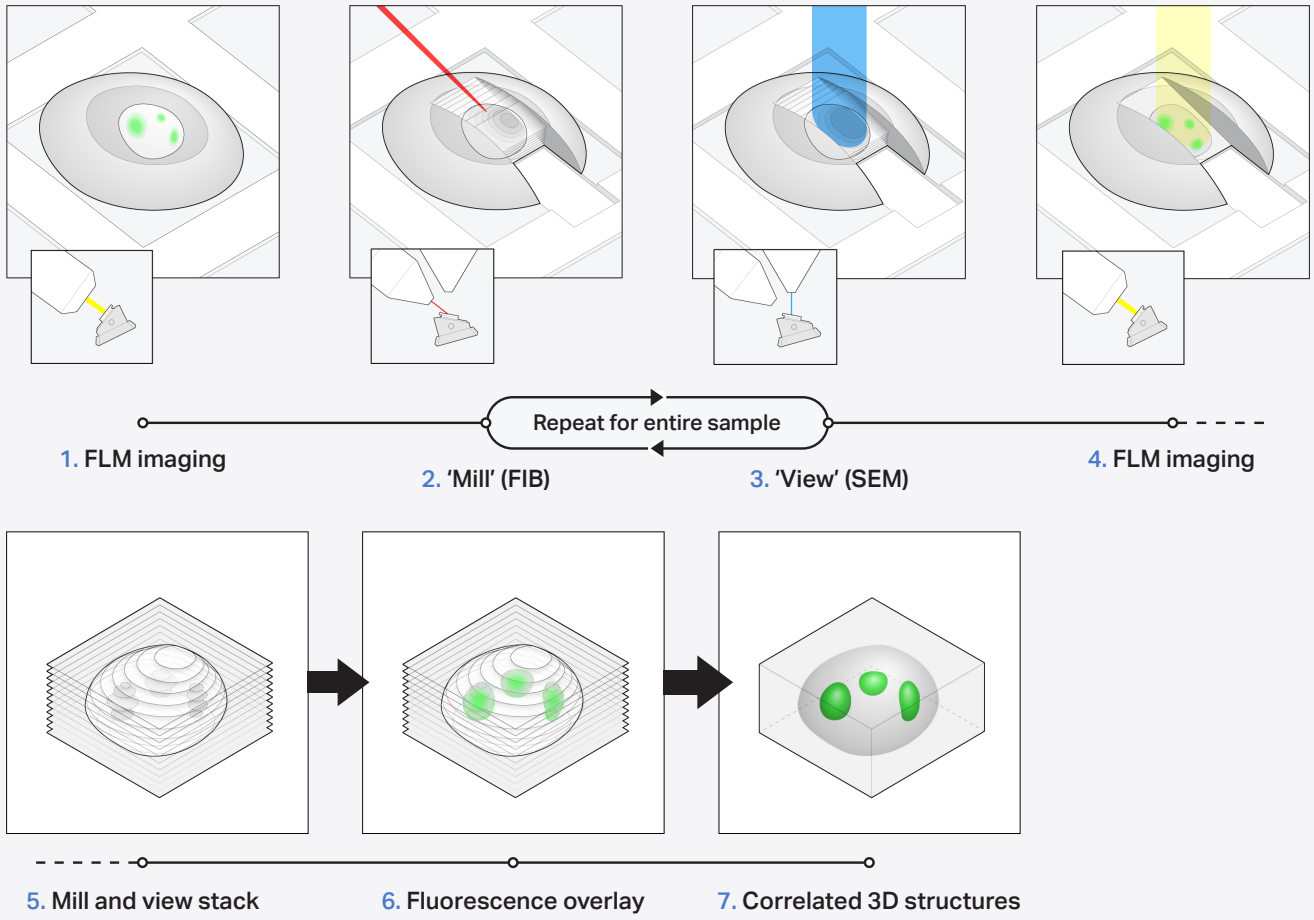


7. After milling: verify if fluorescence is still present.

Workflow

In situ volume CLEM through coupling with ‘mill and view’

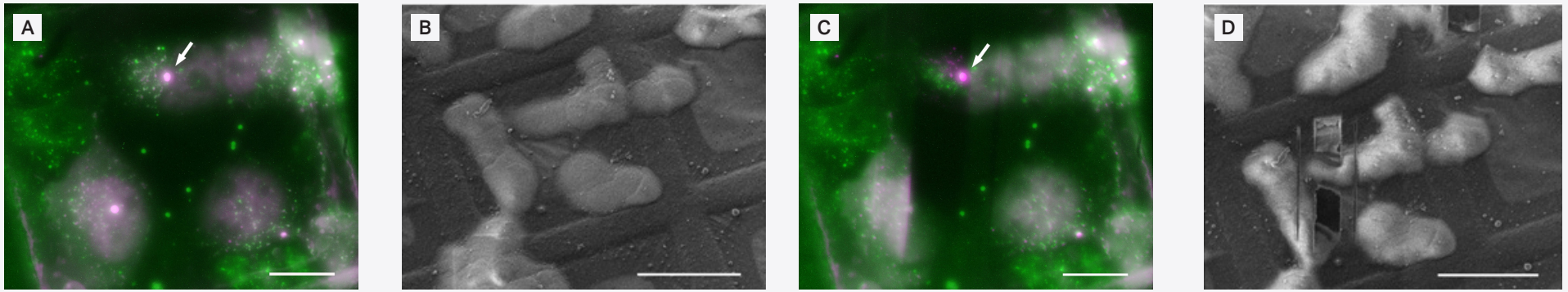
For volume cryo-CLEM the sample is also first imaged with METEOR to identify the ROI. Next, a high contrast image of the ROI is acquired with the SEM and subsequently the top layer of the sample is ablated with the FIB after which the freshly exposed surface is imaged by the SEM again. This ‘Mill and View’ workflow is repeated for the entire volume of the sample. By using METEOR the fluorescence of the ROI can be inspected as more material is milled away, improving functional and structural data correlation all through the volume of the biological material.



Optimize your cryo-ET research

Cryo-ET provides the unique opportunity to study cellular structures and molecules in the context of interaction. METEOR helps to increase the sample yield and significantly simplifies the sample preparation for cryo-ET allowing more researchers to use cryo-ET. METEOR has helped to streamline the cryo-ET workflow for targeted lamella milling of HeLa and yeast cells.

How would your sample yield change if you could guide lamella milling with a METEOR?



HeLa cells

HeLa cells serve as a model for complex biological systems such as mammalian cells and they have contributed to many medical breakthroughs. We used a HeLa cell line in which two proteins were tagged: a protein localizing on the mitochondria was tagged with eGFP (green) and the second protein of interest was tagged with mCherry (pink). The location where the two fluorescence signals colocalized was our ROI. In **Figure 1** we show electron beam and METEOR images before (**Figure 1A, B**) and during lamella milling (**Figure 1C,D**). The white arrows indicate the ROI, which can easily be visualized before milling and enables targeted lamella milling. The ROI can also be seen during milling confirming that the current milling strategy will result in a ROI-containing lamella.

*Figure 1 **SEM and FLM images of HeLa cells** taken before milling and at 1.5 µm lamella thickness **(A, C)** Maximum intensity projections of FLM Z-stacks (30 slices at 400 nm steps), Olympus LMPLFLN 50X/0.5 objective. Green: LED excitation at 484 nm and a 525 nm emission filter. Pink: LED excitation at 560 nm and a 607 nm emission filter. The targeted lamella is indicated by a white arrow. (scale bar: 20 µm) **(B, D)** Electron beam images (HV: 5 kV curr: 13 pA t: 1 µs detector: ETD) (scale bar: 60 µm).*

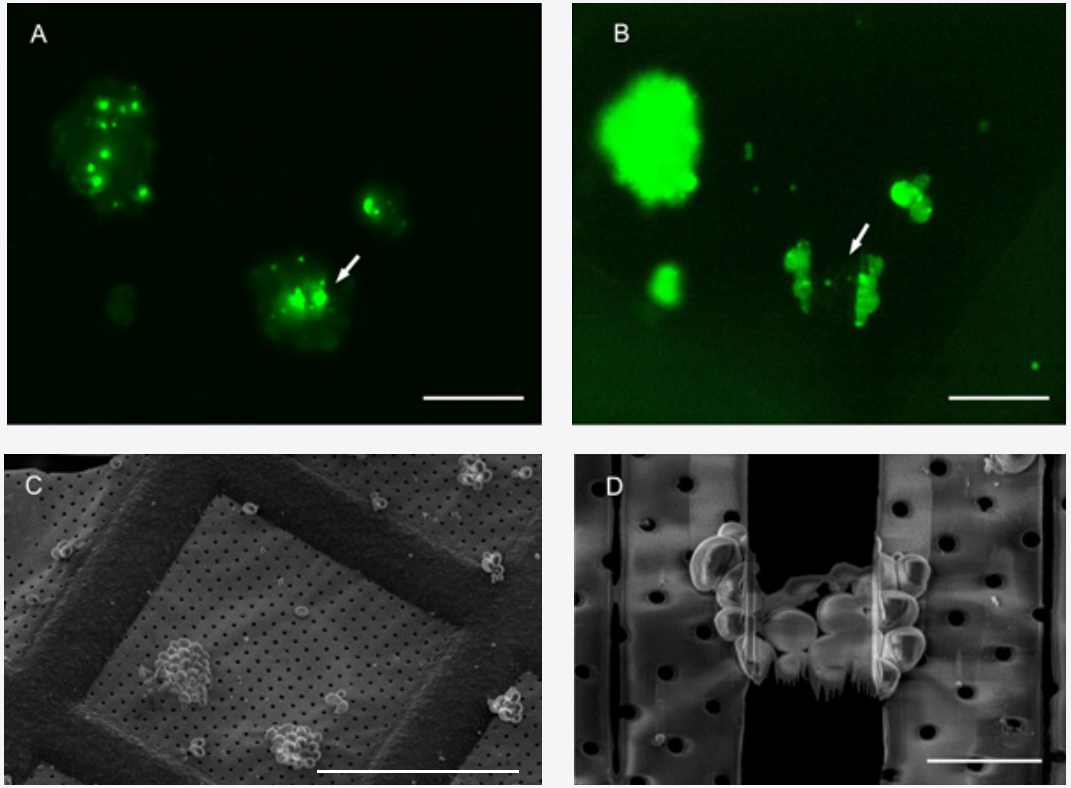


Figure 2 ***SEM and FLM images of yeast cells*** *taken before milling and at 150 nm lamella thickness* ***(A-B)*** *Maximum intensity projections of FLM Z-stacks (70 slices at 400 nm steps, objective: Olympus LMPLFLN 50X/0.5, LED excitation at 484 nm and a 525 nm emission filter). The targeted lamella is indicated by a white arrow.* ***(C-D)*** *Electron beam images (HV: 5 kV curr: 25 pA t: 1us det: ETD) Scale bar (A-B): 20 µm (C): 100 µm (D): 20 µm.*

Sample courtesy of Max Planck Institute of Biochemistry, Martinsried, Germany

Yeast cells

Yeast cells share many basic properties with mammalian cells and thus serve as an easy to work with model organism. We prepared targeted cryo-lamellae from yeast overexpressing Ede1 tagged with eGFP. Ede1 is a selective autophagy receptor and involved in the recruitment of proteins that form large condensates at the plasma membrane and within autophagic bodies. In **Figure 2** we show METEOR and electron beam images before (**Figure 2A, C**) and after milling a lamella (**Figure 2B, D**). The white arrows indicate that the ROI is present in the final lamella, confirming that the lamella is good enough to transfer to the cryo-TEM.

Customer story

Anna Bieber and Cristina Capitanio are PhD candidates in the groups of Prof. Dr. Wolfgang Baumeister, Prof. Dr. Brenda Schulman and Prof. Dr Juergen M. Plitzko respectively at the MPI of Biochemistry in Martinsried, Germany. They and their colleagues study cellular processes with in situ cryo-ET. In the process of planning, designing and performing experiments they are constantly thinking about and trying out ways to improve the existing workflow to make correlative cryo-FIB milling and cryo-ET more robust, more effective and easier to use.

Anna Bieber and Cristina Capitanio are currently focusing on autophagy, an important cellular waste disposal and recycling system. Using cryo-FLM, cryo-FIB milling and cryo-ET, they are characterizing all the steps that a cell needs to take to engulf cargo into so-called autophagosomes for degradation.

The big challenge Anna Bieber and Cristina Capitanio faced was confirming that specific cellular structure they were interested in was present after the cryo-FIB milling. It was only possible to confirm the presence of the ROI in a cryo-fluorescence microscope after milling – an error-prone and time-consuming procedure.

Delmic's METEOR allows monitoring the presence of the target signal during and after milling within the FIB chamber. By using METEOR, Anna Bieber and Cristina Capitanio were able to check for the presence and precise location of the target signal in lamellae without having to transfer the sample to a separate cryo-FLM or waste time on them in the cryo-TEM.

Anna Bieber and Cristina Capitanio,
Max Planck Institute (MPI) of Biochemistry, Germany

Anyone who wants to perform correlative cryo-FIB milling can benefit from an integrated FLM system. People with a well-defined and abundant target structure can increase their yield by using METEOR in combination with automation. Additionally, METEOR can bring improvements when targeting very rare and even novel structures.

ODEMIS user-friendly software suite

METEOR is fully controlled by the Delmic control PC with our software suite ODEMIS. This intuitive, open-source software allows you to control all the FLM settings as well as the FIB/SEM sample stage. It ensures image acquisition parameters are primed for high-quality fluorescence images. You can easily navigate the sample, while maintaining focus, and effortlessly switch between SEM, FIB, and FLM imaging modes. You can furthermore implement your own scripts in Python to automate routine processes, e.g. camera exposure time optimization.

Easy overlay

You can perform the image overlay by using fiducial markers or other available methods. Upon installation the system will be calibrated and the relative movement from FIB/SEM imaging to FLM imaging by METEOR will be determined. This calibration allows you to seamlessly switch from FLM to FIB/SEM imaging and back with the click of a button.

Communication with other systems

ODEMIS communicates with the FIB/SEM through an XTlib adapter. You can control the Z movements of the METEOR objective and the FIB/SEM stage movements through ODEMIS for easy sample navigation and a fluid cryo-FLM imaging workflow. All the non-FLM imaging actions such as SEM imaging, FIB milling and gas injection system (GIS) coating are controlled through the FIB/SEM control PC.

Easy-to-export image file formats for downstream analyses

METEOR images are saved in the OME-TIFF format that can be processed using various image processing software programs such as ImageJ (Fiji) and Huygens deconvolution from SVI. Correlation software such as the 3D correlation toolbox can also be used in combination with METEOR data.

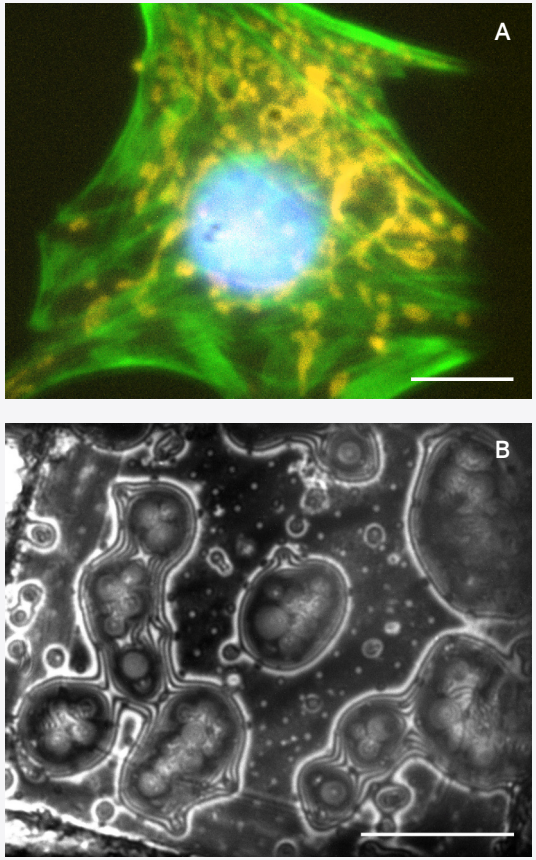


Figure 3 (A) Bovine pulmonary artery endothelial cells (BPAEC). Orange: mitochondria (MitoTracker® Red CMXRos), Green: F-actin (Alexa Fluor® 488 phalloidin), Blue: nucleus (blue-fluorescent DNA stain DAPI). Scale bar: 10 µm. (B) Reflection image (objective: Olympus LMPLFLN 50X/0.5, LED excitation at 484 nm). Scale bar: 30 µm .

Specifications

Compatible FIB/SEM systems

- Thermo Fisher Scientific Aquilos
- Thermo Fisher Scientific Helios
- Thermo Fisher Scientific Scios
- Zeiss Crossbeam 550
- Zeiss Crossbeam 550L

Optics

Objectives: the following options are available*

- Olympus Fluorite objective:
  - 20x (WD 3.1 mm, NA 0.45)
  - 50x (WD 1.0 mm, NA 0.80)
  - 100x (WD 1.0 mm, NA 0.90)
- Olympus Semi-Apochromat objective:
  - 50x (WD 10.6 mm, NA 0.50)

* Depending on shuttle and sample stage configuration the choice of objectives can be limited due to space restrictions.

Objectives stage:

- Travel range: 31 mm
- Minimal incremental motion < 50 nm

Lightsource:

Omicron LedHub fitted with 4 LED sources:

- 385 nm
- 470 nm
- 505 - 600 nm - bandpass filter
- 625 nm

Camera: the following options are available

- sCMOS camera - 6.5 µm pixel size (Andor Zyla 4.2)
- sCMOS camera - 6.5 µm pixel size (Andor Sona 4.2B-6)

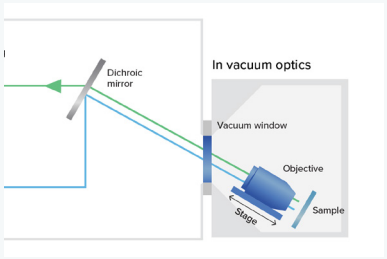
Filters:

Equipped with a filter wheel with four slots, several multi-band or single-band configurations are possible that can be optimized to the user's needs. Example configuration:

- 432/36 nm single-band bandpass filter
- 515/30 nm single-band bandpass filter
- 595/31 nm single-band bandpass filter
- 698/70 nm single-band bandpass filter

Imaging modes:

- Fluorescence imaging
- Reflection imaging



Technical Note

Curious to learn more about METEOR's light path and optical components used for in-vacuum imaging? Read the technical note.

READ NOW

Specifications

Dimensions

The METEOR system consists of 3 main parts. The electronics rack (placed in the microscope room), the body (mounted on the FIB/SEM) and the in chamber optics (mounted inside the FIB/SEM). The dimensions (H/W/D) are given below. Please note that depending on the exact configuration chosen these dimensions might change.

- Electronics rack (80 cm x 60 cm x 80 cm)
- METEOR body (30 cm x 30 cm x 20 cm)
- METEOR in-chamber (6 cm x 6 cm x 20 cm)

System Control

Comes with a control PC next to the FIB/SEM computer for acquiring your fluorescence images.

Odemis Integrated Software

ODEMIS, a user-friendly open-source acquisition software, is installed together with METEOR. It will ensure image acquisition parameters are primed for high quality fluorescence images. Users can furthermore implement their own scripts in Python to automate routine processes, e.g. camera exposure time optimisation.

Software functions:

- Adjust all relevant imaging parameters (LED power, camera exposure time and gain, excitation and emission filter)
- Tiling and stitching functionality to acquire large sample areas overview
- Control of the FIB/SEM stage to navigate the sample and find the region of interest
- Switch between the SEM and FLM positions to acquire new FLM images as materials are milled away
- Save sample and objective stage coordinates to quickly reinspect milled lamellae
- Switch between SEM and FLM positions to verify fluorescence signals in the lamella
- Z-stack acquisition
- Camera pixel binning option
- Multicolour imaging
- Odemis viewer to inspect the images
- Licence-free (free updates)

Meet Delmic Cryo Solutions

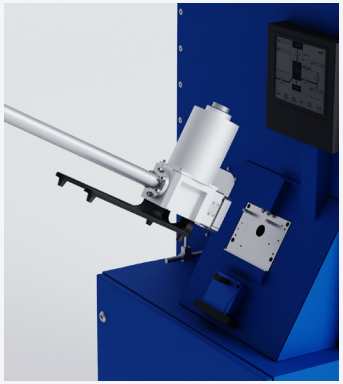
Our mission is to help you better understand how the building blocks of life work with cryo-ET

METEOR Reduce transfer steps and improve sample yield with integrated cryo-CLEM

- Capture FLM images and SEM images without sample transfer, directly move to the ROI after correlating the two images.
- Confirm the presence of ROI with FLM after milling
- Save time and work more efficiently by having both FLM and FIB in the same microscope

CERES Clean Station Prepare cryo-EM samples in the bespoke and highly effective Clean Station for complete protection against ice contamination

- Prepare your cryo-EM samples in an anhydrous enclosure (<1% humidity / 1 ppm water) with ease at every step.
- More effective, more comfortable, and environmentally friendly than a low humidity room.



CERES Vitri-Lock A high vacuum cryo transfer module that enables safe and ice contamination-free cryo sample transfer

- Transport your cryogenic sample between the CERES Clean Station and the cryo-FIB/SEM in high vacuum to minimize the presence of moisture during the transfer.
- Keep samples vitrified for up to 30 minutes thanks to the actively cooled chamber.

CERES Ice Shield Offers the ultimate protection against ice contamination during lamellae preparation

- Protect your cryo-sample from parasitic ice growth during lamella milling using our patented technology.
- Prepare more ice contamination-free lamellae using your cryo-FIB to obtain high quality tomograms and high throughput.

Interested?

For more information on this topic visit www.delmic.com

About

Delmic is a passionate high-tech company based in Delft, the Netherlands that develops powerful and user-friendly solutions for light and electron microscopy. Our systems are used by researchers and companies all over the world in fields ranging from life sciences, geology, material sciences to nanophotonics.

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Subscribe to our newsletter to stay up to date with our latest Cryo-ET developments

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