iFLM Correlative System

Integrated fluorescence light microscope for Thermo Scientific DualBeam Microscopes

The Thermo Scientific iFLM Correlative System is an integrated light microscope for cryo-correlative imaging inside the high-vacuum chamber of Thermo Scientific Cryo-FIB SEM microscopes.

Correlative fluorescence and electron imaging in one system

The Thermo Scientific™ iFLM™ (Integrative Fluorescence Light Microscope) Correlative System allows you to combine fluorescence imaging and ion milling within a single Thermo Scientific™ DualBeam™ Microscope. The iFLM System is an optional optical module for new instruments that can also be retrofitted into existing Thermo Scientific Cryo-FIB SEM microscopes (after compatibility check). The integrated light microscope enables identification, targeting, and validation of fluorescence signals in frozen-hydrated samples and can be used in conjunction with in situ cryo-lamella or cryo-lift-out workflows. It also supports the re-correlation of fluorescence data obtained using external light microscopes.

Extending the capabilities of the cryo-FIB

Thermo Scientific cryo-FIB systems lead the way for cryo-electron tomography lamellae production. Cryo-lamellae can be made from cells on EM grids as well as from high-pressure frozen (HPF) samples with the optional Thermo Scientific™ Easylift™ Cryo Lift-Out System. The possibility to inspect both sample types using the iFLM Correlative System extends the range of applications and offers increased throughput and specificity for cryo-tomography.

Inspect and verify fluorescent targets inside finished cryo-lamellae

A key benefit of the iFLM Correlative System is the ability to check the final lamellae for fluorescently labelled targets, ensuring they are located within the ~200–300 nm thin cryo-lamellae. This is particularly useful in combination with the included Thermo Scientific™ AutoTEM™ Cryo Software, which can produce several lamellae on a single EM-grid. The iFLM Correlative System can then inspect these lamellae and verify which contain the targets of interest. This facilitates and accelerates subsequent data acquisition in the cryo-TEM. Additionally, fluorescence images acquired in the cryo-FIB can be used to correlate data from an external cryo-light microscope.

Key features

Correlative targeting workflow in one system. The iFLM Correlative System allows you to select target sites for cryo-lamellae production and samples to be imaged directly within the high-vacuum chamber of the cryo-FIB without additional transfer steps from an external cryo-light microscope. This saves time and reduces the risk of sample contamination.

Fluorescence validation during and after milling. Correlation with the iFLM Correlative System allows the lamella milling process to be monitored step by step to ensure that the target is contained in the final lamella. This increases efficiency for TEM analysis.

Proprietary sample shuttles for a range of applications. Dedicated sample shuttles for AutoGrid and HPF carriers, which can be selected in the software, allow you to handle a broad range of specimens and workflows including cryo-lift-out and volume imaging.

Optimized optic design for targeting. Minimized chromatic aberrations for multichannel fluorescence acquisition using a custom-made tube lens plus UV and low-autofluorescence compatible vacuum window.

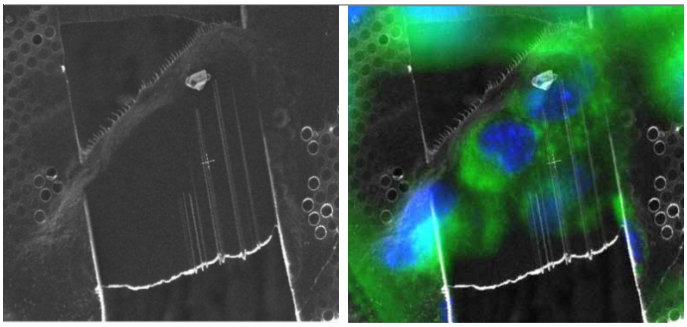

Figure 1. Top: Cryo-lamella prepared with the Thermo Scientific™ Aquilos™ 2 Cryo-FIB. Bottom: Overlay of SEM image with fluorescence image obtained with iFLM Correlative System.

Correlative software

Included Thermo Scientific Maps Software enables data correlation between the iFLM Correlative System and FIB-SEM. Data from the iFLM and other correlative light microscopy data can be imported for targeting and to identify features of interest. The iFLM Correlative System features its own software interface that controls the fluorescence microscope and allows the user to switch between electron and light microscope imaging positions. Data from the iFLM can be swiftly imported into Maps Software while retaining positional information from the grid.

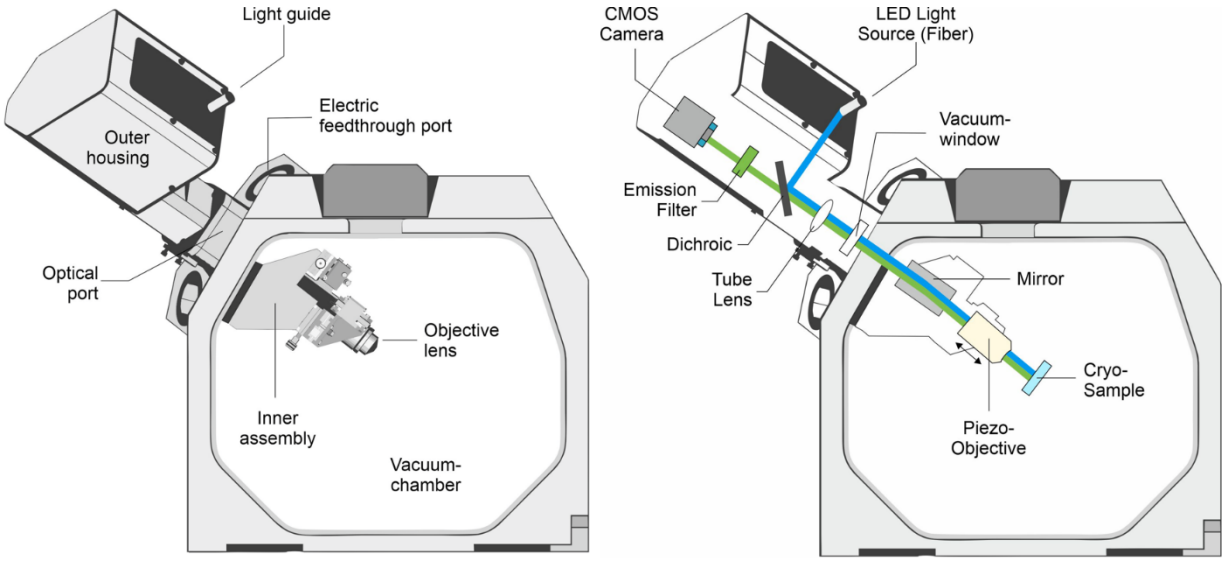

Figure 2. Left: Schematic of the iFLM Correlative System showing the outer housing connected via an optical port to the inner assembly inside the high-vacuum chamber. **Right:** iFLM Correlative System beam path.

Improved targeting workflow with Maps Software

An additional feature in Maps Software allows you to mark fluorescent targets and relocate them later in AutoTEM Cryo Software with the help of a reference point. First, as shown in Figure 4, the position of the focal plane between the reference point (X-Fiducial) and the target point of interest (POI) is determined in Maps Software. These coordinates can then be recalled in AutoTEM Cryo Software by ticking a checkbox. With the Crosshair feature, the reference point can be correlated within the ion milling image at any given ion incidence angle. This functionality enables more targeted preparation of the cryo-lamellae.

Cryo-Lift-Out workflow example

The iFLM Correlative System is particularly useful for identifying fluorescence within high-pressure frozen samples and for precisely localizing target sites. In contrast to the in-situ lamella preparation use case, the advantage in the cryo-lift-out case is that imaging can be performed perpendicular to the sample surface. Using the iFLM System (Figure 6), fluorescent sample sites can be precisely localized within the bulk sample and bulk lamellae containing the target can be excised. After lift-out and polishing to the desired thickness, the sample can be checked again to ensure that the fluorescent target is contained within the lamella.

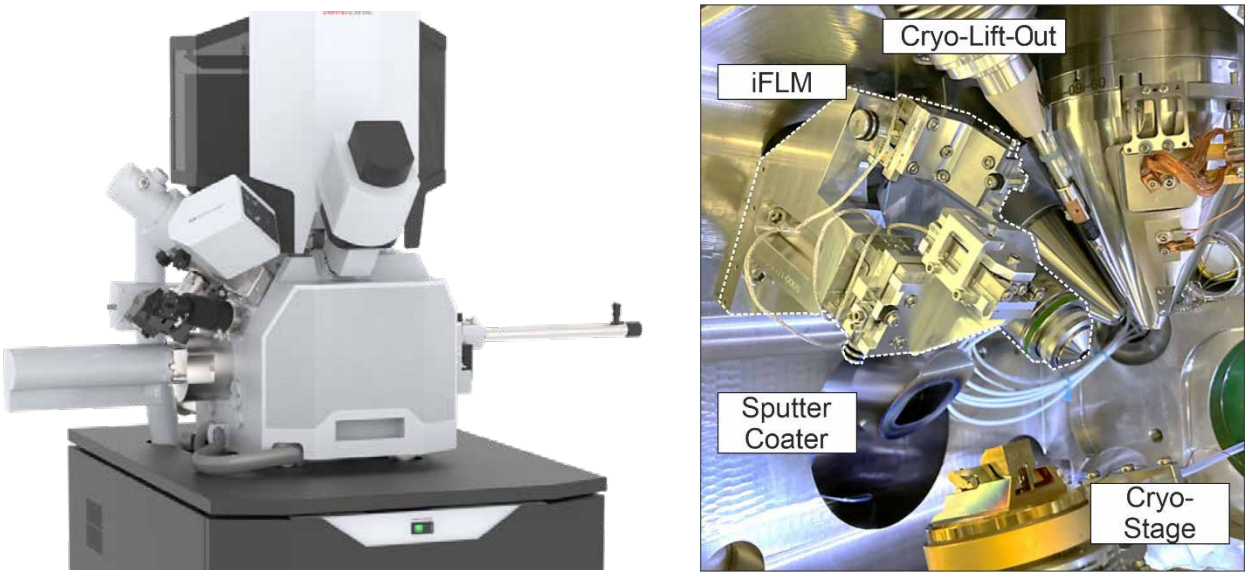

Figure 3. The iFLM Correlative System module on the Aquilos 2 Cryo-FIB and set up inside the high-vacuum chamber.

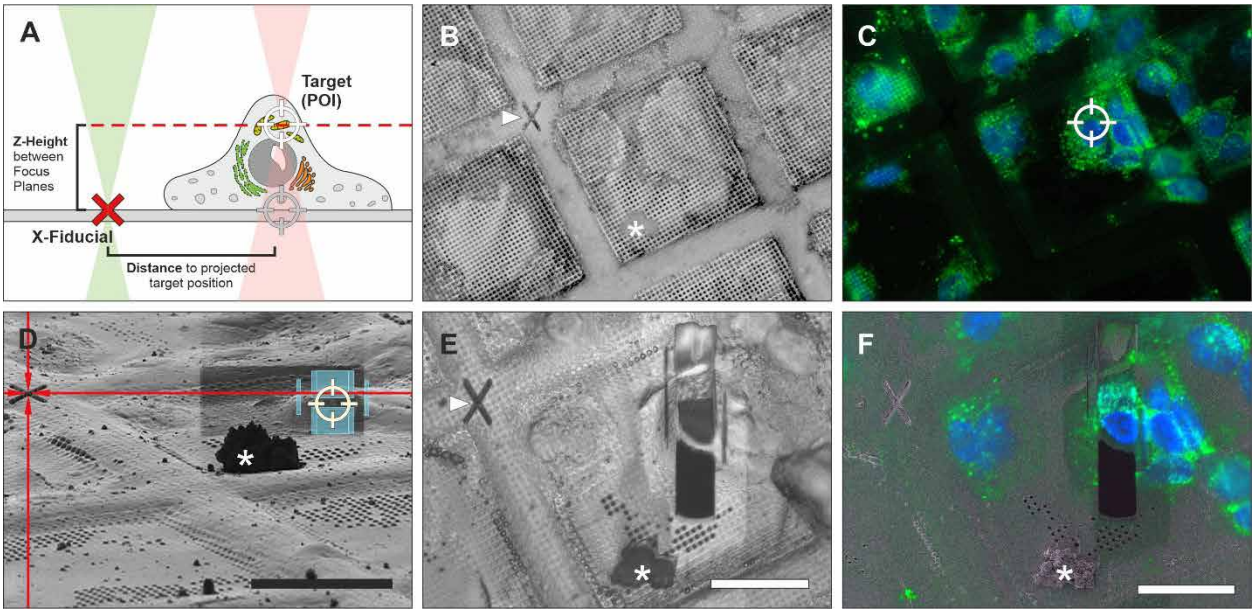

Figure 4. (A) Determination of the region of interest and accurate positioning of the milling box in an ion beam image using fiducial and z-focus information obtained by optical microscopy. (B) Reflection and (C) fluorescence images of fiducial region and target cell. (D) Positioning of the fiducial marker within the ion beam image in AutoTEM Cryo Software. (E) Corresponding reflection and (F) fluorescence images after lamella preparation exhibiting the milled lamella with fluorescent features. Scale bars: 40 µm; white arrowheads: X-fiducial; white crosshair: POI; asterisk: cluster of ice particles.

Customized sample shuttles for the cryo-FIB workflow

A good match between the objective of the integrated light microscope and the geometry of the specimen shuttle is very important for successful operation. Given that samples are inside an AutoGrid and held by the shuttle clamp, working distances that are too short (< 1 mm) must be avoided, otherwise the distance for focusing will be very small which may result in damage to the front lens of the objective. The iFLM System uses an objective lens with a 1.3 mm working distance. All available sample shuttles (Figure 7) are optimized for a pretilt angle of 35° and can accept AutoGrids, HPF planchettes, and sapphire discs, thus ensuring sample versatility. The pretilt angle of 35° degrees is optimal for imaging with both the fluorescence light microscope and SEM, as well as for setting the final shallow milling angle under the ion beam. Both the HPF and sapphire disc shuttles always have a second position to accommodate an AutoGrid that serves as the receiver grid for the cryo-lift-out lamellae and which is subsequently transferred to the cryo-TEM.

Example of targeting using the iFLM Correlative System

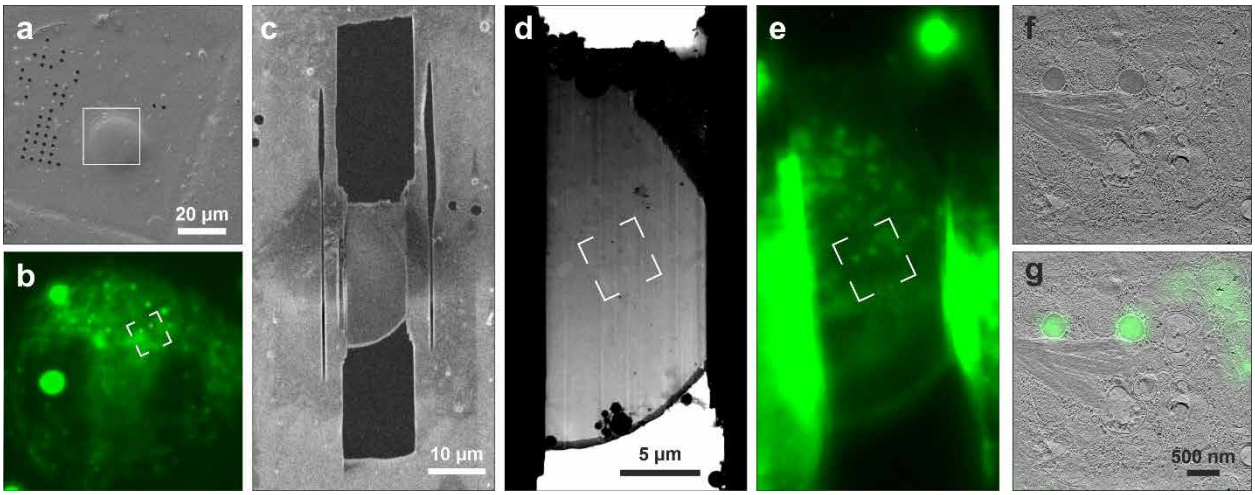

Figure 5. Targeting of lipid droplets (label: BODIPY; small green dots) from a widefield fluorescence image stack of a target HeLa cell. Correlation of fluorescence data from the final polished cryo-lamella and correlation to the cryo-TEM images. *Sample courtesy Dr. Jae Yang and Dr. Elizabeth Wright, Cryo-EM Research Center and the Midwest Center for Cryo-ET, Department of Biochemistry at the University of Wisconsin, Madison.* DOI: https://doi.org/10.1101/2023.07.11.548578

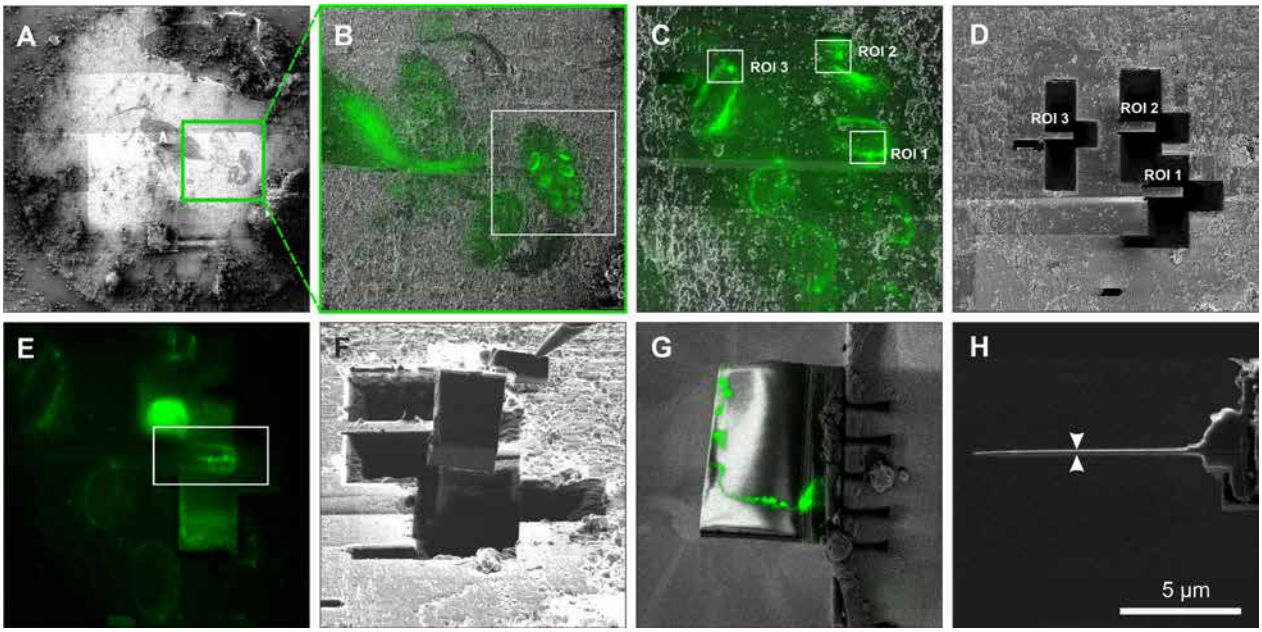

Figure 6. Utilizing the iFLM Correlative System for cryo-lift-out on a high-pressure frozen (HPF) sample. (A) SEM overview of a 3 mm planchette containing Drosophila oocytes expressing GFP-NUP358. (B) magnified region where fluorescence signal is overlaid with the SEM image. (C) Without the iFLM Correlative System, the GFP-labelled nuclear pores and the nuclear envelope would be impossible to discern in the SEM image. (D-F) Cryo-lift out sample preparation ensuring that the nuclear envelope is part of the bulk lamella that is lifted out. (G-H) Checking fluorescence after the bulk lamella has been thinned to TEM transparency. *Sample courtesy Sven Klumpe, Max Planck Institute for Biochemistry, Martinsried*

iFLM Correlative System	
System	Widefield optical microscope
Objective	20x Zeiss Epiplan-Apochromat, NA 0.7 (Piezo-driven)
Working distance	1.3 mm
Modes	Fluorescence and Reflection (motorized filter changer)
Filters	Semrock LED-DA/FI/TR/Cy5-B-000 (Quadband)
Camera	Basler ace 2 (2A4504-5gmPRO; Sony IMX541 CMOS sensor)
Tube lens	Corrected tube lens for minimized chromatic aberrations
Imaging FOV	500 x 500 µm (700 µm diagonal)
LED source	CoolLED, 4 channels (365 nm/450 nm/550 nm/635 nm)
Shuttles	Dedicated iFLM System shuttles (for example AutoGrid, HPF, Sapphire disc, Leica CLEM)
Warranty and training	1-year warranty, choice of service maintenance contracts, choice of training options
Compatibility	Fully compatible with Thermo Scientific DualBeam Microscopes (for example: Thermo Scientific™ Aquilos™ 2 Cryo-FIB and Hydra Bio™ Plasma-FIB)

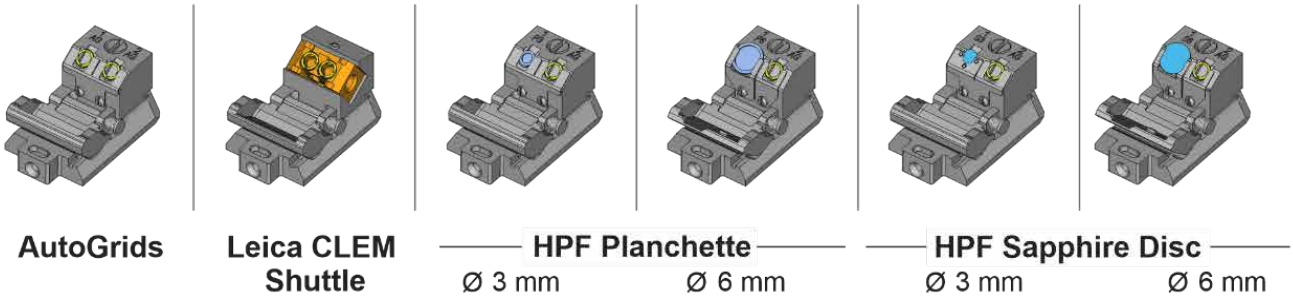

Figure 7. Overview of available shuttles for Thermo Scientific Cryo-FIB SEM microscopes. All shuttles have a 35° pretilt and are fully compatible with the iFLM Correlative System.

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