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CryoTomo Software Suite
Efficient acquisition and real-time analysis for cryo-electron tomography

The Thermo Scientific™ CryoTomo Software Suite facilitate access to cryo-electron tomography (cryo-ET), offering integrated software tools designed for this cutting-edge technique. This software solution enhances the efficiency and accuracy of cryo-ET, enabling researchers to gain deeper understanding and detailed visualization of cellular processes.

Building on user-friendly Thermo Scientific™ Tomography 5 Software, the CryoTomo Software Suite combines advanced components to streamline routine use and allows both experienced and new users to quickly start their tomography data acquisitions. With additional support for multigrid use cases, the suite is capable of motion correction while also integrating the functionality of Thermo Scientific™ Tomo Live™ Software for parameter-free, automatic, on-the-flytomogram reconstruction.

Tomo Live Software runs on the microscope's Data Management Platform server, which integrates with the Thermo Scientific™ CryoFlow™ Software portal. Together, all the functionalities allow CryoTomo Software Suite to enable fast, efficient and accurate cryo-electron tomography data acquisition and analysis.

Usability and interactivity
The CryoTomo Software Suite offers functions for all the steps of automated cryo-ET data collection. The user-friendly setup and imaging interface is organized in a logical sequence of tabs and buttons that guide you through the workflow. For example, in the Preparation tab, different "imaging presets" are defined for various standard tasks such as focusing, feature of interest tracking, and the acquisition of tilt series.

All imaging parameters for each task can be expressly set to avoid unnecessary exposure of the sensitive sample to the electron beam, including specific magnification, illumination conditions, and beam shift parameters. Other tabs offer the recording of low-magnification tile sets for entire grids (atlases), testing and calibrating autofunctions (Optimize Optics, focus, astigmatism, eucentricity, etc.), and the ability to set up and run acquisition sessions.

| Key Benefits |
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| Reduced learning curve with logical, step-by-step guided set up of tomographic data acquisition |
| Automatic cassette mapping of up to 12 grids for initial grid-quality assessment and lamella identification |
| Intuitive graphical set up of target areas for tomography, with task parallelization for optimized microscope usage |
| High throughput with multishot acquisition, where beam/image shift allows for several tomogram positions to be recorded per tilt with only one focus and tracking area |
| Automated tilt series acquisition for multisite, multishot, and multigrid batch tomography with high targeting precision |
| Full integration with Selectris Imaging Filters and Falcon Direct Electron Detectors, along with several image output formats, provides optimized image quality |
| Sample evaluation facilitated by parameter-free on-the-fly reconstruction and visualization of tomograms |
| Ability to guide future acquisitions by back tracing the recorded position for each tomogram |

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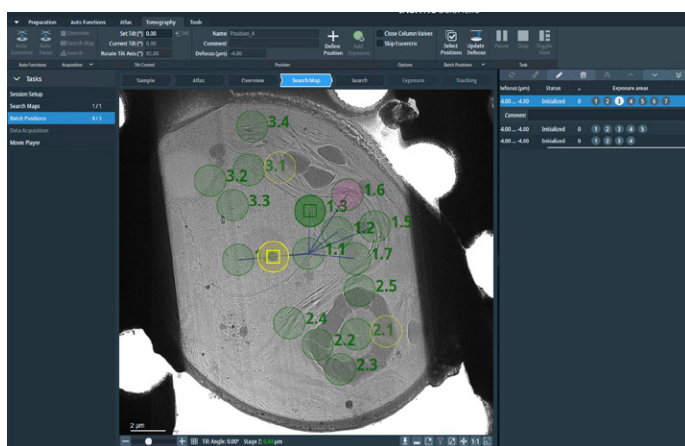
Figure 1. User interface for session setup in the CryoTomo Software Suite, featuring step-by-step settings organized into tabs and tasks. The central viewport displays image data and supports interactive navigation. Grid transitions and re-localization of targets are automatic, minimizing manual interventions and maximizing microscope use, thereby lowering costs.

Increased throughput with multisite, multishot, and multigrid batch tomography

The CryoTomo Software Suite features automatic cassette mapping of up to 12 grids stored in the cryo-transmission electron microscope (cryo-TEM) Autoloader system. For each grid, an atlas is recorded to assess grid quality and efficiently screen for objects of interest, such as cryo-lamella regions. A batch tomography mode ensures optimal instrument capacity by enabling continuous tomogram acquisition at multiple sites on one or more individual grids. The fully automated data acquisition can be started as soon as all settings are defined.

With a single mouse click, areas for tilt-series acquisition can now be added directly to a Search Map, a tile set of images at intermediate magnification. It is no longer necessary to record a search image before adding each tilt-series acquisition position. Additionally, multishot acquisition can be used to significantly enhance throughput. This feature uses beam/image shift to capture several tomogram positions with only one focus and tracking area per tilt, thereby streamlining the data acquisition process and increasing efficiency.

The batch tomography capabilities of the CryoTomo Software Suite also extend to multiple grids, allowing for unattended tomogram acquisition at numerous locations across selected grids.

Task parallelization for efficient usage of microscope time

Acquired images can be used to set up subsequent acquisitions while an automated acquisition is in progress. This allows you to perform set-up tasks in advance to avoid unnecessary wait times, shortening time to results and optimizing beam usage time. Once an atlas has been acquired, you can define sites for subsequent Search Map acquisition.

Additionally, positions for tilt-series imaging can be defined on recorded Search Maps while subsequent Search Maps are being acquired, or even during an ongoing tilt-series acquisition. The time saved by parallelization depends on the number of grids and the locations to be imaged, but can potentially save hours per session.
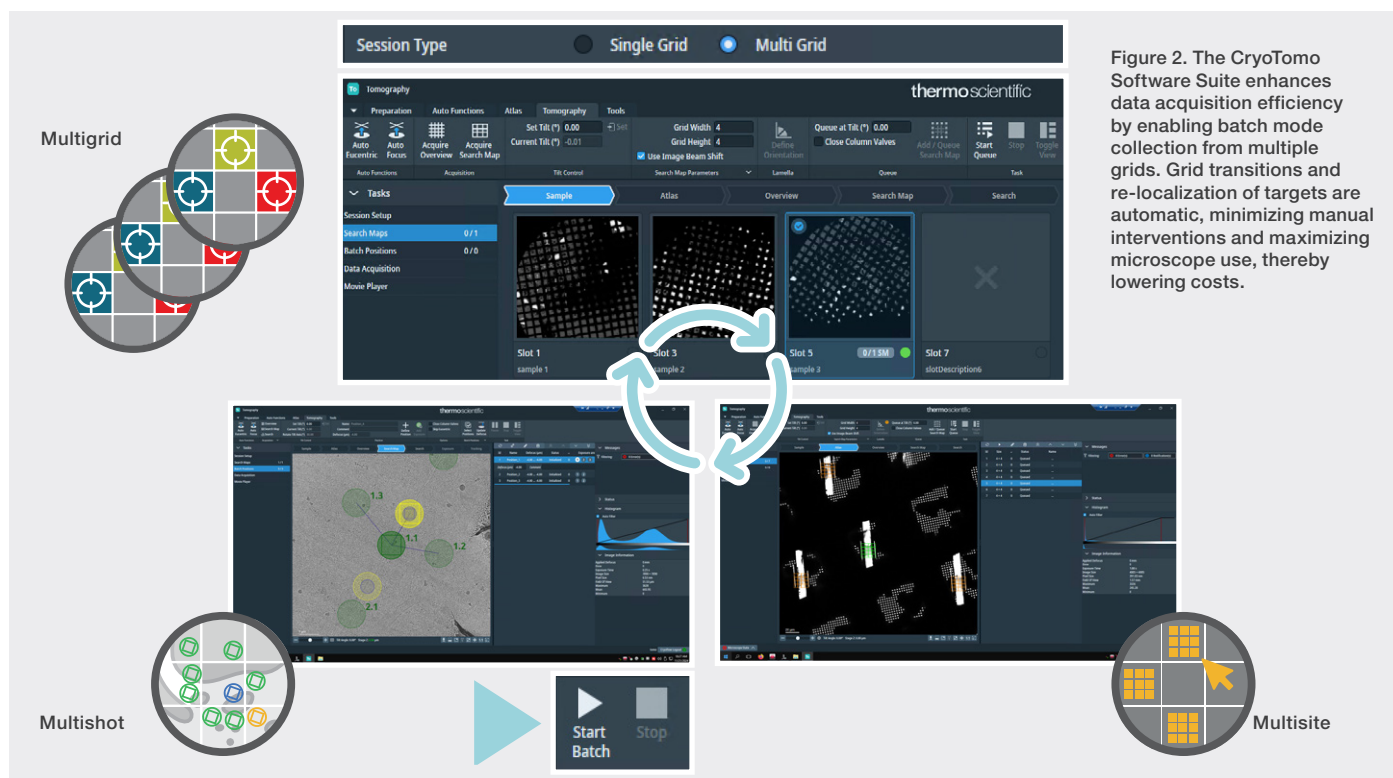

Figure 2. The CryoTomo Software Suite enhances data acquisition efficiency by enabling batch mode collection from multiple grids. Grid transitions and re-localization of targets are automatic, minimizing manual interventions and maximizing microscope use, thereby lowering costs.
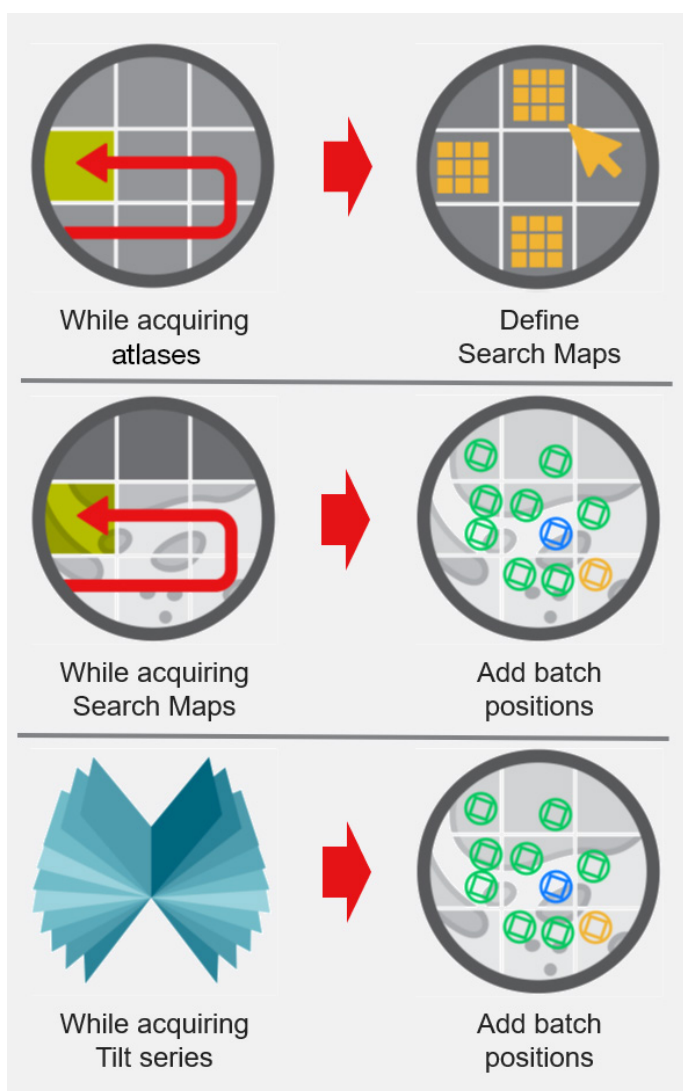
Figure 3. Task parallelization in the CryoTomo Software Suite. The definition of Search Maps and the addition of batch positions can be done without interfering with beam usage, increasing overall microscope efficiency.

Imaging with optimal electron dose distribution

Vitrified biological samples are susceptible to beam damage from electron radiation. To address this, a dose-symmetric acquisition scheme is available in the CryoTomo Software Suite, ensuring optimized electron dose distribution during data acquisition for high-resolution sub-tomogram averaging. During a tomographic tilt series, this scheme uniformly exposes the sample in the lower negative and positive angular tilt ranges, preserving high-resolution information. This collection scheme optimizes resolution (i.e., in the sub-nanometer range), providing considerably better results than continuous or bi-directional tilt schemes.

Enhancing tomogram contrast and resolution

Tomogram contrast and resolution can be significantly increased during data acquisition by limiting the range of allowed electron energies. Thermo Scientific™ Selectris™ and Selectris X Imaging Filters greatly improve the signal-to-noise ratio of tomography data while boosting contrast and resolution through zero-loss filtering, which removes inelastically scattered electrons. The suite also offers a selection of output formats, EER decoding (in 4k x 4k or 8k x 8k pixels), and on-the-fly motion correction for systems utilizing a Thermo Scientific™ Falcon™ 4i Direct Electron Detector. Using the full spatial and temporal resolution of the EER format increases image quality and enhances resolution in downstream sub-tomogram averaging pipelines.
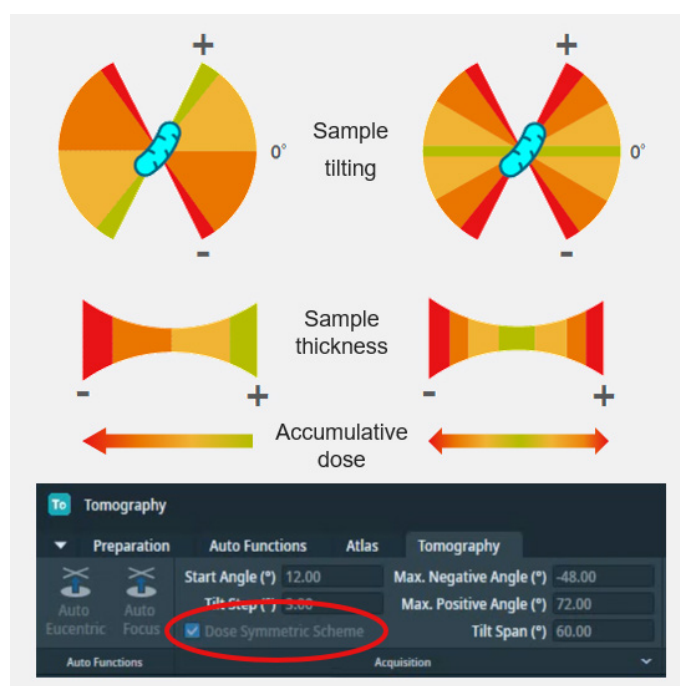

Figure 4. Electron dose distribution in a continuous (left) and dose-symmetric (right) tilt scheme. Initiating the exposure at high-tilt angles, and subsequently covering the entire tilt range, has a detrimental effect on the quality of thinner projections due to cumulative dose damage (green to red). Initiating the acquisition at zero tilt and then alternating between negative and positive tilts in a progressive, uniform manner, meanwhile, effectively retrieves the high-resolution information available at low tilt angles, where the apparent sample thickness is low.
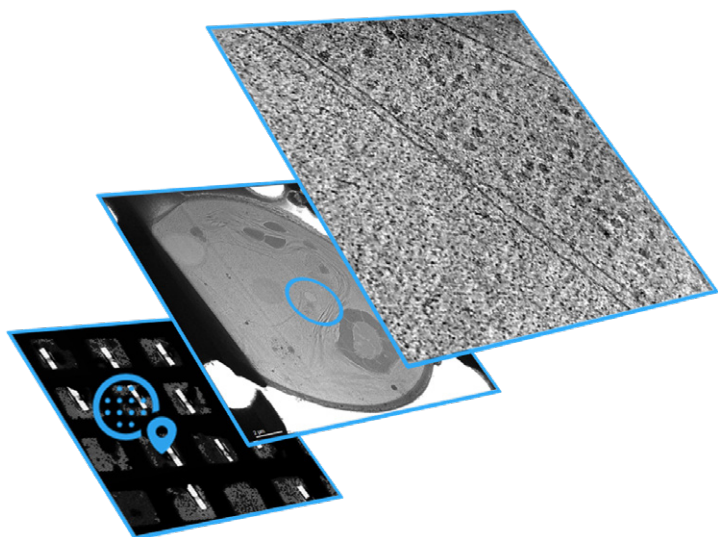
Figure 5. The CryoTomo Software Suite includes the Discovery Viewer, an interactive tool that allows you to track the positions of each tilt series in Search Maps and atlases.

The CryoTomo Software Suite works with:

- Cryo-TEMs equipped with Autoloaders, such as Thermo Scientific™ Krios™, Glacios™, and Talos Arctica™ Cryo-TEMs

- Other TEMs with room-temperature or cryo- side-entry holders, including Thermo Scientific™ Talos™ L120 and F200 TEMs

- Cameras: Falcon 3/4/4i Direct Electron Detectors and Thermo Scientific™ Ceta™ Cameras, as well as Gatan K2/K3/OneView Cameras

- Imaging filters: Selectris and Selectris X Imaging Filters as well as Gatan BioContinuum and BioQuantum

- Data formats: EER (Falcon 4/4i Detector only), MRC, TIF

The CryoTomo Software Suite is available on new microscopes or as a potential upgrade, depending on the system.

Automatic on-the-fly reconstruction

The CryoTomo Software Suite integrates a series of algorithms, previously offered individually as Tomo Live Software, to enable on-the-fly quality monitoring of generated data by performing real-time reconstruction of tilt series into 3D volumes. Tomo Live Software functionality can perform motion correction of fraction movies, fiducial-free tilt series alignment, and 3D reconstruction without user intervention during tilt series recording. The resulting reconstructed tomograms allow for immediate data quality evaluation, identification of high- and low-quality tilt series, and exporting of selected tilt series and reconstructed tomograms for further processing, archiving, and sharing. Tomo Live Software runs on the microscope's Data Management Platform server, and its user interface is integrated into the CryoFlow Software portal.

Tomo Live Software algorithms use patch tracking for tilt-series alignment of fiducial-free *in-vitro and in-situ* samples, as well as iterative reconstruction. By combining accurate and comprehensive metadata from cryo-TEMs with smart automatic parameter estimation, no user input is needed.

Reconstructions appear within five minutes after each tilt series is recorded. No copying, saving, or loading is needed to view the data. You can log in to the CryoFlow Software portal via a web browser to review data at your convenience, whether in or out of the lab.
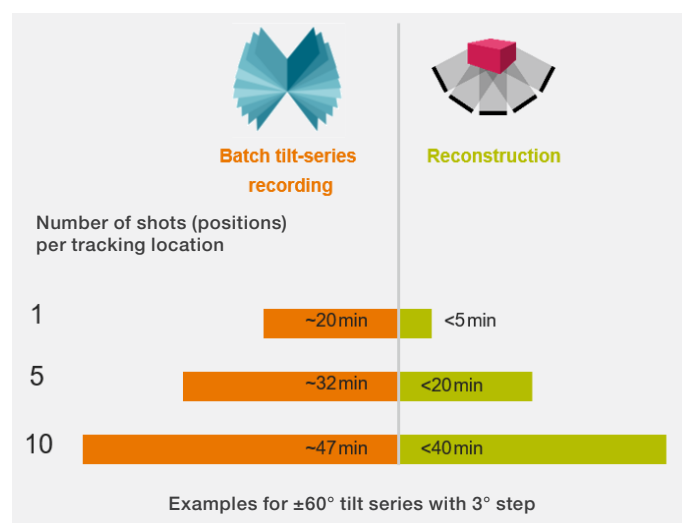

Figure 6. On-the-fly reconstruction in the CryoTomo Software Suite is achieved with multishot acquisition of up to ~10 positions (depending on settings). Live data processing is compatible with automated batch acquisition.
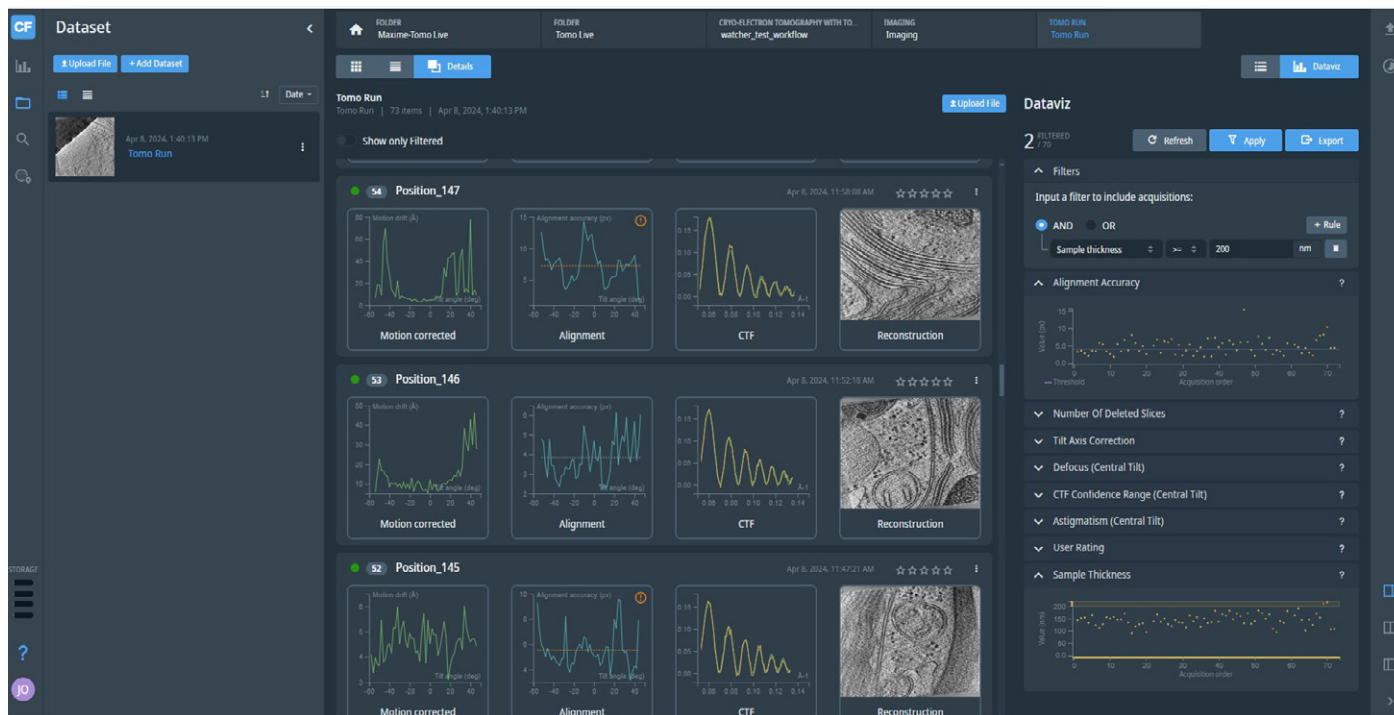
Figure 7. CryoFlow Software web portal showing reconstructed tomograms and associated metadata generated by Tomo Live Software algorithms.

Quality metrics and data curation

Cryo-ET sub-tomogram averaging requires the acquisition of tens or even hundreds of tomograms. Curation is crucial, as sub-optimal data increases processing time and reduces the resolution of averaged particles. The CryoTomo Software Suite displays quality metrics, derived during the alignment and reconstruction processes, alongside the reconstructed tomograms. These quality metrics can be used to interactively define rulesets for data export and include the average defocus of tilt series, alignment accuracy, number of projections removed during motion correction and alignment, sample thickness, devitrification score, and a subjective, user-defined quality rating (stars).

CryoFlow Software and the Discovery Viewer

CryoFlow Software is a data integration platform designed to optimize cryo-EM workflows. In tomography, it hosts the functionalities of the CryoTomo Software Suite, including Tomo Live Software algorithms for real-time image analysis and reporting, enabling you to make quick decisions regarding sample quality and imaging conditions. Additionally, CryoFlow Software features the Discovery Viewer, an add-on that helps precisely determine where specific tilt series were captured on a sample during the entire tomography workflow, including the atlas/grid, Search Map/lamella, and batch positions/tomogram. This can help you evaluate multigrid screening results and identify optimal areas and imaging conditions for subsequent data acquisition.
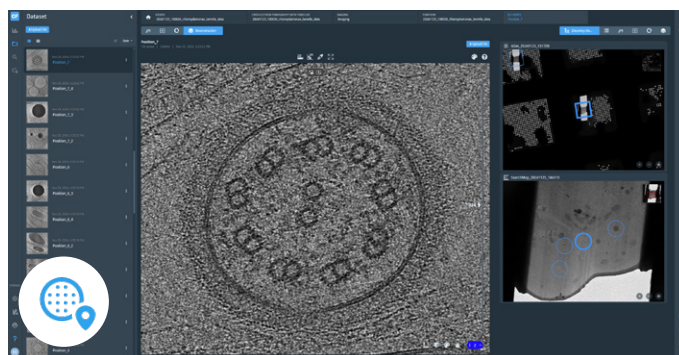

Figure 8. CryoFlow Software web portal, showing a reconstructed tomogram and associated positions in grid and lamella.

Learn more at **thermofisher.com/cryotomo-suite**

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