Tomography 5 and Tomo Live Software
User-friendly batch acquisition for and on-the-fly reconstruction for cryo-electron tomography

Cryo-electron tomography (cryo-ET) is a label-free cryogenic imaging technique that can generate 3D datasets of cellular organelles and protein complexes at Angstrom resolution. It is the highest resolution technique for imaging of a cell’s molecular machinery at physiological conditions. The in situ capabilities of cryo-tomography allow it to bridge the gap between single particle analysis cryo-electron microscopy (cryo-EM) and structural cell biology, linking molecular insights to cellular behavior.

Thermo Scientific™ Tomography 5 Software has a new, user-friendly interface to enable both experienced and new users to jump-start their tomography data acquisition. It was purposely designed to follow the same intuitive logic as Thermo Scientific EPU Software for single particle analysis. This eases the learning curve and ensures a seamless transition between the two software packages. Tomography 5 Software also connects with Thermo Scientific Tomo Live Software for automatic on-the-fly data reconstruction and review in any browser.

**Key Benefits**
- Automated 3D data acquisition for multi-site batch tomography
- Higher throughput through multi-shot acquisition, in which several tomogram positions are recorded with only one focus and tracking area
- Automatic cassette mapping of up to 12 grids for grid-quality assessment and lamella identification
- Reduced learning curve with step-by-step set-up of tomographic data acquisition, designed around the same intuitive logic as EPU Software
- Intuitive graphical setup of tomography areas and corresponding focus and tracking regions
- Fully integrated with Selectris Imaging Filters and Falcon Direct Electron Detectors

**Imaging with optimal electron dose distribution**
Vitrified biological samples are susceptible to beam damage by electron radiation. A dose-symmetric acquisition scheme has been added to Tomography 5 Software in order to ensure optimal cryo-tomography data acquisition and high-resolution sub-tomogram averaging. During a tomographic tilt series, the acquisition scheme exposes the sample in the lower-negative and positive angular tilt range uniformly, preserving high-resolution information. This collection scheme optimizes resolution (i.e., the sub-nanometer range) and provides considerably better results than continuous or bi-directional tilt schemes.
Integrated with the Selectris and Selectris X Imaging Filters
Tomogram contrast and resolution can be significantly increased during 3D data acquisition by limiting the range of allowed electron energies. The Thermo Scientific Selectris™ and Selectris X Imaging Filters greatly improve the signal-to-noise ratio (SNR) of tomography data while boosting contrast and resolution through zero-loss filtering (i.e., the removal of inelastically scattered electrons). The imaging filter is fully integrated with Tomography 5 Software.

Usability and interactivity
For a tomography experiment, different “imaging presets” are often defined for various standard tasks, such as focusing, tracking a feature of interest, and tilt series acquisition. In Tomography 5 Software, all imaging parameters are purposely set to avoid unnecessary exposure of the sensitive sample to the electron beam, with specific magnification, illumination conditions, image and beam shift parameters corresponding to each task. A modern, user-friendly interface allows step-by-step adjustments of imaging conditions, control of the Selectris Imaging Filter, setup of the area of interest, as well as tracking and focusing of adjacent regions.

Figure 3. Slice from a tomogram acquired with Tomography 5 Software and Selectris Imaging Filter capturing a cytoplasmic region within a Chlamydomonas cell. Data courtesy of S. Khavnekar, W. Wietrzynski, P. S. Erdmann, Max Planck Institute of Biochemistry, Martinsried, Germany.

Figure 4. Tomographic slice showing a cross-sectioned mitochondrion within a S. cerevisiae cell reconstructed with Tomo Live Software.

Figure 5. User interface of Tomography 5 Software. Step-by-step settings are made via tabs. User-friendly sections enable users to adjust parameters for each step, view status messages, and display image information. A central viewport allows for display of image data as well as interactive navigation and recording of tomogram areas. Tomography 5 Software is used to assign the acquisition region (green) as well as focus (blue) and tracking areas (yellow) along the tilt axis.
**Increased throughput with cassette mapping and batch tomography**

Tomography 5 Software features automatic cassette mapping of up to 12 grids stored in the cryo-transmission electron microscope (cryo-TEM) autoloader system. For each grid, a low-magnification map of the entire grid is recorded in order to assess grid quality and efficiently screen for cryo-lamella regions. A batch tomography mode ensures optimal instrument capacity by enabling serial tomogram acquisition on each grid. Tomography 5 Software allows you to set up tomogram acquisition at several regions of interest, which are automatically acquired. The fully automated data acquisition runs as soon as all settings are defined.

**Search Atlas**

Areas for tilt-series acquisition can now be added directly onto a montage of search images using the Search Atlas. It is no longer necessary to record a search image before each tilt series acquisition position is added — this can now be done with a single mouse click.

**Tomography 5 Software works with:**

- Cryo-TEMs equipped with autoloaders, such as the Thermo Scientific Krios™, Glacios™, and Talos Arctica™ Cryo-TEMs
- Other TEMs with room-temperature or cryo-side-entry holders: Thermo Scientific Talos™ L120 and Talos F200 TEMs
- Imaging filters: Thermo Scientific Selectris and Selectris X Imaging Filters as well as Gatan BioContinuum and BioQuantum
- Export data formats: MRC, EER (Falcon 4/4i Detector only), TIF
- Tomography 5 Software is available on new microscopes or can be upgraded (depending on system)
Tomo Live Software
Automatic on-the-fly reconstruction with data curation

Tomo Live Software enables on-the-fly quality monitoring of data generated with Tomography 5 Software by performing real-time reconstruction of tilt series into 3D volumes. It performs motion correction of fraction movies, fiducial-free tilt series alignment, and 3D reconstruction without user intervention during tilt series recording. These reconstructed tomograms allow for impromptu 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 the user interface is integrated in the Thermo Scientific Athena™ Software portal.

Automatic reconstruction
Tomo Live Software uses state-of-the-art algorithms to motion-correct movies from fractionating cameras, tilt-series alignment of fiducial-free lamellas with patch tracking, and iterative reconstruction. By combining 100% accurate and complete metadata from Thermo Scientific TEMs with smart automatic parameter estimation, Tomo Live Software is entirely automatic and does not require any user input.

Reconstructions appear within 5 minutes after each tilt series is recorded. No copying, saving, or loading is needed to view the data. Simply log in to the Athena Software portal in your web browser and review data at your leisure — in or out of the lab.

Curation
Cryo-ET with sub-tomogram averaging requires acquisition of tens or hundreds of tomograms. Curation is important, as sub-optimal data increase the data processing time and reduce the resolution of averaged particles. Tomo Live Software shows quality metrics, which are derived during the alignment and reconstruction process, next to the reconstructed tomograms. These quality metrics can be used to interactively define rulesets that are then applied during data export (e.g., the number of projections that had to be removed during alignment or the alignment accuracy).

Tomo Live Software
Motion correction
Tilt-series alignment
Back-projection
Web UI for data management

Segmentation
Structure determination by particle picking and averaging
Export
Curated tomograms can be exported to a network drive in the laboratory network. Both raw (ERR tilt series) and processed (motion corrected, aligned tilt series and reconstructed stacks) can be exported with full meta data as .mrc files.

Reconstructed tomograms can be used directly for segmentation of cellular architecture in Thermo Scientific Amira™ Software. The motion-corrected and aligned tilt series are in suitable formats for particle picking and sub-tomogram averaging workflows.

Tomo Live Software system requirements:
- Platform Software 2.7.0 (Glacios or Arctica Cryo-TEM), 3.7.0 (Krios Cryo-TEM)
- DMP Version 2.6 or higher
- Athena Data Management Platform version 1.16 or higher
- Tomography Software version 5.10 or higher