

CryoTomo Software Suite

Frequently asked questions

General questions

1: What are the hardware and system requirements for installing CryoTomo Software Suite?

A: CryoTomo Software Suite runs on a high-performance workstation or server installed and configured by a Thermo Fisher Scientific field service engineer (FSE) or digital solutions engineer (DSE) during the suite setup. The server is equipped with the required GPU acceleration, RAM, and fast storage.

2: Is CryoTomo Software Suite compatible with existing Thermo Scientific electron microscopes?

A: Yes. CryoTomo Software Suite is designed to integrate seamlessly with Thermo Scientific™ Cryo-TEMs, including the Krios™ G5 Cryo-TEM and Glacios™ 2 Cryo-TEM. To check if your system is supported, please consult your account manager.

3: How does CryoTomo Software Suite differ from Tomography 5 Software?

A: Tomography 5 Software is a component of the CryoTomo Software Suite responsible for tilt series acquisition. The suite's Tomo Live component extends the capabilities of Tomography 5 Software with novel capabilities, including different output formats in certain hardware configurations, a queueing system for multiple grids, and initial real-time processing of data (alignment and reconstruction of tilt series).

4: Is data generated by CryoTomo Software Suite compatible with third-party analysis tools?

A: The CryoTomo Software Suite supports multiple image output formats, including MRC, EER (specific to the Thermo Scientific Falcon 4/4i Detector), and TIF. For tilt-series alignment, it supports export of standard file formats (e.g., MRC, STAR) to enable downstream processing in third-party tools such as IMOD or RELION 4. Metadata and alignment files are structured to support seamless handoffs.

5: Can CryoTomo Software Suite be used by researchers who are new to tomography?

A: Absolutely. CryoTomo Software Suite is designed with intuitive, user-friendly interfaces and includes automated setup wizards and guided workflows, making it accessible for both expert users and tomography beginners.

6: What training resources are available for new users?

A: New users have access to guided onboarding, tutorials, and in-app tips within CryoTomo Software Suite. Thermo Fisher also offers remote and in-person training sessions, user guides, and workflow documentation to support a smooth learning curve.

7: Can CryoTomo Software Suite be run remotely or accessed via a network?

A: Yes. CryoTomo Software Suite can be configured for remote access through institutional networks using secure protocols (e.g., VPN). This allows you to monitor acquisitions or process data from outside the microscope room or off-site.

8: How many users can access CryoTomo Software Suite at once?

A: CryoTomo Software Suite supports multi-user environments and offers data review for up to 15 users. Volumetric visualization of reconstructed tomograms is limited to two simultaneous users.



9: How is my data managed and protected when using CryoTomo Software Suite?

A: CryoTomo Software Suite uses Thermo Scientific CryoFlow Software for data management. CryoFlow Software supports secure data storage and transfer through your institution's infrastructure. The initial data storage occurs on the dedicated server where CryoFlow Software resides. Data is protected using RAID architecture and can be backed up or exported to long-term storage with built-in functions for export and archiving. Institutions can restrict local access by using password-controlled logins and are responsible for backups.

10: How do I get the latest updates for CryoTomo Software Suite?

A: Thermo Fisher Scientific provides quarterly software updates for CryoTomo Software Suite. Users are notified via email and social media channels when a new update is available. User guides and release notes can be accessed at any time on the [Cryo-EM Software Updates](#) page.

Technical questions

11: Is CryoTomo Software Suite customizable for specific needs?

A: Yes. While CryoTomo Software Suite is designed for simplicity and automation, you can customize parameters such as tilt schemes and their angular distributions through the expert settings panel.

12: Can CryoTomo Software Suite handle batch processing of multiple datasets?

A: Yes. CryoTomo Software Suite includes tools for automated batch processing of tilt-series and tomograms. This feature allows you to queue multiple datasets for unattended reconstruction, which is helpful for high-throughput labs or overnight runs.

13: How does CryoTomo Software Suite support subtomogram averaging pipelines?

A: CryoTomo Software Suite provides alignment and reconstruction outputs that can be used as inputs for subtomogram averaging pipelines. It also includes visualization and filtering tools to help you discriminate high-quality tilt series adequate for subtomogram averaging in external platforms.

14: How does CryoTomo Software Suite handle drift correction and alignment of tilt-series?

A: CryoTomo Software Suite includes robust, automated drift correction and tilt-series alignment algorithms that correct for sample motion and stage inaccuracies. These steps are performed with minimal user input and optimized for consistency across large datasets. For technical detail, see [this research paper from IUCr](#).

15: Does CryoTomo Software Suite include visualization tools for tomogram inspection?

A: Yes. CryoTomo Software Suite features integrated 2D visualization tools for reviewing raw tilt-series, alignment quality, and reconstructed tomograms slices. You can explore slices, annotate regions of interest, and inspect particle distribution directly within the software.

16: How does CryoTomo Software Suite handle contrast transfer function (CTF) estimation in tomography?

A: CryoTomo Software Suite includes support for per-tilt CTF estimation. It automatically calculates defocus parameters from tilt-series data during the acquisition process. Additionally, an estimation of astigmatism is calculated for the zero-tilt projection.

17: Can CryoTomo Software Suite perform on-the-fly processing during data acquisition?

A: Yes. CryoTomo Software Suite is optimized for on-the-fly alignment and reconstruction, enabling immediate feedback on data quality and structural visibility that helps you adjust acquisition strategies in real time.

18: Does CryoTomo Software Suite support fiducial-based and fiducial-less alignment?

A: CryoTomo Software Suite only supports marker-less alignment for samples where fiducials are not present or usable. It uses robust feature detection algorithms to support alignment, which can also be successful for samples with fiducials under a good quantity and distribution of particles.

19: What is the typical processing time for reconstructing a tomogram in CryoTomo Software Suite?

A: Processing time varies by dataset size, GPU configuration, and reconstruction method. For example, a standard 20-tilt tomogram (4k x 4k) using SIRT on a high-end workstation can be reconstructed in about five to 10 minutes per tomogram.

20: Does CryoTomo Software Suite support 8k images?

A: Yes. CryoTomo Software Suite fully supports 8k image acquisition and processing, providing a larger field of view and greater sampling flexibility for cryo-ET workflows.

21: At what magnification or pixel size does it make sense to use 8k images instead of 4k?

A: For small pixel sizes (e.g., about 1 Å/pixel), 8k images offer no significant benefit given the current resolution limits of *in-situ* subtomogram averaging. However, 8k imaging can be advantageous under two criteria:

- A large field of view is needed (typically at greater than 2 Å/pixel)
- The sample contains macromolecular complexes well suited for subtomogram averaging at medium to high resolution (e.g., ribosomes, microtubules, actin).

If subtomogram averaging is not part of the workflow, using 8k images provides no added value regardless of magnification or pixel size.

22: Can I change the settings of motion correction?

A: No, the only selectable option is between 4k and 8k output size. Tomo Live Software was designed as a parameter-free pipeline to provide a user-friendly experience.

23: How can Tomo Live Software be “parameter-free?”

A: Through smart automatic estimation of parameters, auto-tuning of filtering and thresholds, and adaptive detection of bad images. Examples:

1. **Motion-correction**
 - The optimal number of fractions is determined from exposure time and electron dose
2. **Stack cleaning**
 - The threshold for excluding images with grid bars or large ice particles is automatically tuned
3. **Pair alignment**
 - Cross-correlation images are tuned with adaptive parameters
 - The threshold for excluding images with a low correlation score is determined from data
4. **Tilt –series positioning**
 - Feature tracking automatically detects and ignores outliers
5. **Patch tracking**
 - Patches are filtered with adaptive parameters
 - Bad patches are found and replaced
 - Images with too few matching patches are removed
 - “Theoretical trajectories” are created on basis of patch trajectories and known geometry

 Learn more at thermofisher.com/cryotomo-suite