

## CryoFlow Software

### Streamline your cryo-EM workflow with integrated data management

Thermo Scientific™ CryoFlow™ Software is a built-in data management solution for cryo-electron microscopy (cryo-EM) workflows. Whether you're routinely generating protein structures using single particle analysis (SPA) or analyzing cellular structures with cryo-electron tomography (cryo-ET), it serves as a single platform for handling data from Thermo Scientific electron microscopes.

CryoFlow Software's modular applications guide workflows and provide real-time reporting on cryo-EM images and their analysis. The interactive interface enables real-time monitoring and visualization of data quality during acquisition, including unsupervised sessions.

#### Seamless integration

Designed to work seamlessly with Thermo Scientific cryo-transmission electron microscopes (cryo-TEMs), CryoFlow Software is installed and configured on the Data Management Platform, or DMP. The DMP is a dedicated, powerful, and versatile

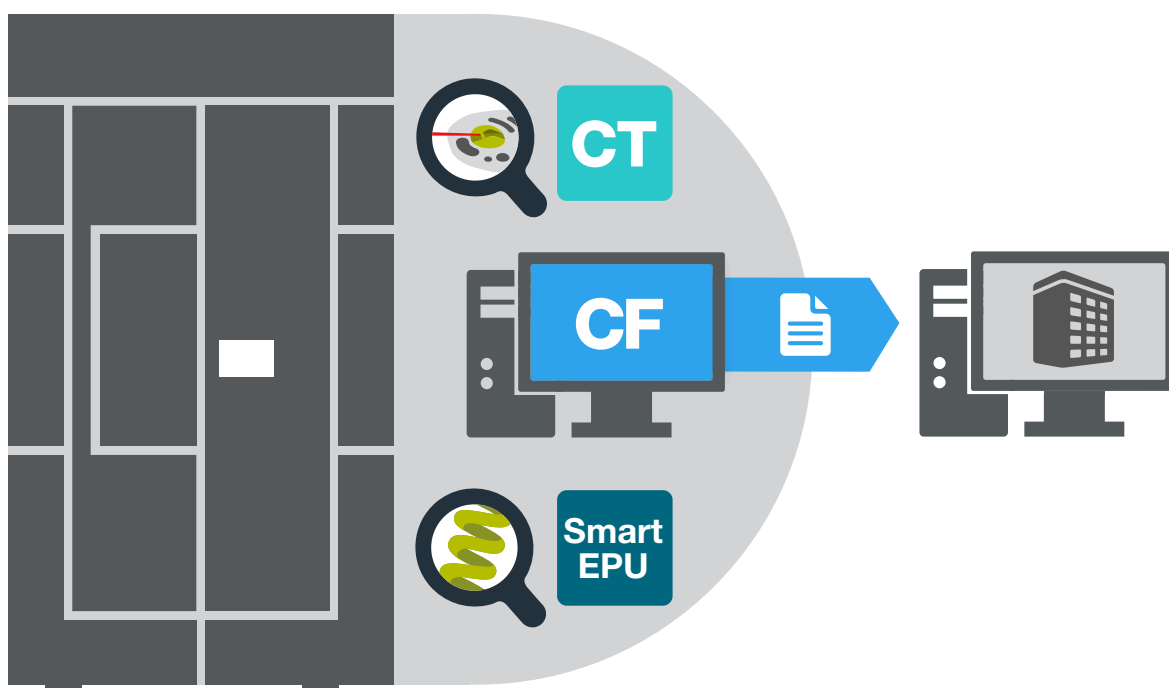


Figure 1. CryoFlow Software manages data for cryo-EM workflows. With modular organization, it provides a convenient single location to track experiments through individual steps in SPA and cryo-ET workflows.

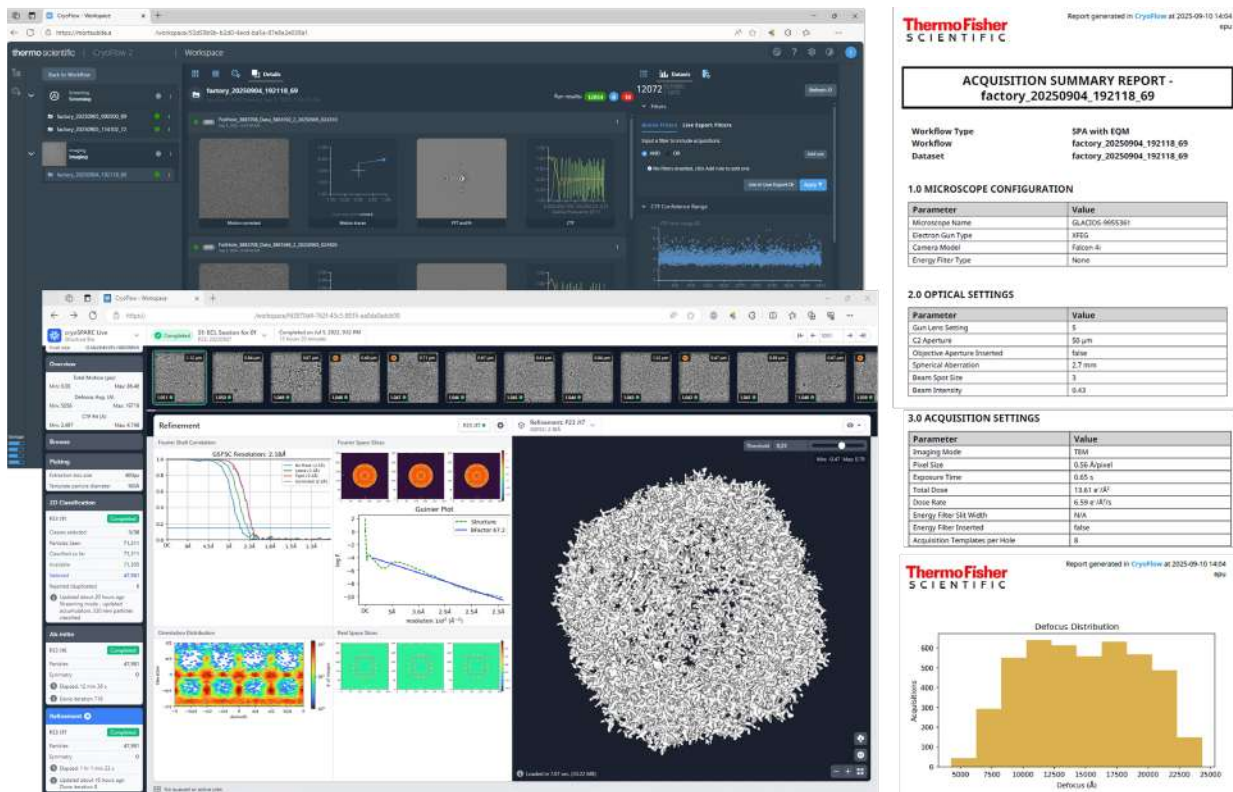


Figure 2. CryoFlow Software supports Smart EPU Software with Quality Monitor (top, left) and Embedded CryoSPARC Live (above, right). When collecting SPA data, image quality information is presented on the fly, allowing for fast modification of acquisition parameters if needed. After collection, a report can be generated with key information about microscope settings and collected data for future reference (right).

server that's delivered with Thermo Scientific™ Tundra™, Glacios™, and Krios™ Cryo-TEMs. It offers a 10 Gb network interface and configurable GPU computing power, providing outstanding performance that lets you quickly offload raw experiment data from the microscope, process images on the fly, and deliver real-time feedback to enhance data collection. This server is an integral part of the overall system, making data management more efficient and providing convenient access to CryoFlow Software through a web browser from any location within your network.

Once experiment data is on the DMP, CryoFlow Software displays the images and associated metadata, which you can annotate, share with collaborators, or export. All your data is securely stored, and built-in data administration tracks all experiments performed with the microscope, replacing unintegrated forms of administration like paper records. CryoFlow Software is password protected so that data from each acquisition session can be accessed by only the authorized operator and designated collaborators. Data and associated metadata are stored for as long as desired within the storage capacity of the DMP server.

CryoFlow Software can transfer raw and pre-processed data, in real-time or post-acquisition, to process clusters and storage systems without custom scripts. When exporting data, you can apply image-quality filters to limit exports to the best data. Metadata is stored in its own format and can be exported in a format commonly used by third-party image processing pipelines. Automated connectivity ensures that data is captured

correctly under the proper project and with all necessary data and metadata, eliminating the burden of clicking and checking.

CryoFlow Software also allows you to select data for further processing and generate PDF reports to summarize the results for each dataset, including microscope information, data characterization, and representative images. These reports provide offline access to critical records and support easy sharing with collaborators.

### Applications enabled by CryoFlow Software

CryoFlow Software supports Thermo Scientific Smart EPU Software and the Thermo Scientific CryoTomo Software Suite, serving as a bridge between data acquisition and data processing. These applications run on the DMP.

#### Smart EPU Software

Smart EPU Software is a self-monitoring data acquisition program for SPA. It includes:

- EPU 3, the latest version of the EPU acquisition program
- Two programs for live image evaluation, EPU Quality Monitor and Embedded CryoSPARC Live
- Smart Plugins, which are decision algorithms used when setting up data collection sessions or to automatically modify data acquisition parameters on the fly based on the reported image evaluation

CryoFlow Software bi-directionally communicates with all Smart EPU Software components to further automate SPA data collection.

By orchestrating data transfer and analysis, CryoFlow Software makes it possible to execute Smart EPU Software's AI- and DL-based plugins for automating critical routine tasks. For example, the pre-trained AI models can automatically select grid squares and foil holes that will deliver high-quality images, a task that traditionally required a high level of user expertise.

CryoFlow Software also launches Smart EPU Software's image evaluation programs. Immediately after starting data collection, EPU Quality Monitor can be used to perform real-time motion correction and contrast transfer function (CTF) estimation for each acquired movie. Micrograph thumbnails and the corresponding EPU Quality Monitor processing results are displayed within the CryoFlow Software user interface with continuously updated graphical summaries of the ongoing data collection. EPU Quality Monitor metrics enable you to manually adjust acquisition parameters in Smart EPU Software to optimize results early in the data collection process, helping you focus on collecting high-quality data.

CryoFlow Software tracks the origin of each high-resolution image collected in a Smart EPU Software session. This makes it possible to easily identify the foil hole, grid square, and grid where each image was collected and associate a given sample's data quality with the corresponding ice properties, which is crucial when setting the acquisition area for a large dataset based on a screening session.

As an alternative to EPU Quality Monitor, Embedded CryoSPARC Live offers a complete pipeline for real-time cryo-EM data analysis, including motion correction, CTF estimation, particle picking and extraction, 2D classification, ab-initio

3D reconstruction, and streaming 3D model refinement. You can start the program with CryoFlow Software for a direct association of imaging and image processing. When using Embedded CryoSPARC Live, you can make decisions based on measurements of image and sample quality to select adequate sample preparation conditions for high-resolution imaging, adjust parameters for optimal high-resolution data collection, or interrupt jobs when further sample optimization is still needed. CryoFlow Software's storage and export capabilities facilitate advanced data post-processing with CryoSPARC Software or other specialized processing pipelines.

Unsupervised monitoring of image quality is also possible via Smart Plugins. These algorithms can access results from both image evaluation programs and automatically adjust EPU Software data acquisition parameters in real time. For example, they can maintain image focus within a targeted range. CryoFlow Software plays a central role in the execution of these feedback loops by assuring proper data transfer.

To facilitate supervised monitoring, the Discovery Viewer is a valuable add-on to the CryoFlow Software portal. It helps determine the specific location on a sample (grid square and foil hole) where a particular image was captured. In the Discovery Viewer interface, you can easily navigate from atlas images to micrographs taken by EPU Software. It proves particularly useful in evaluating results from a multigrid screening session, helping you identify the necessary grid squares, foil holes, and defocus range required for subsequent data acquisition. This tool plays a crucial role in enhancing sample traceability and further automating workflows.

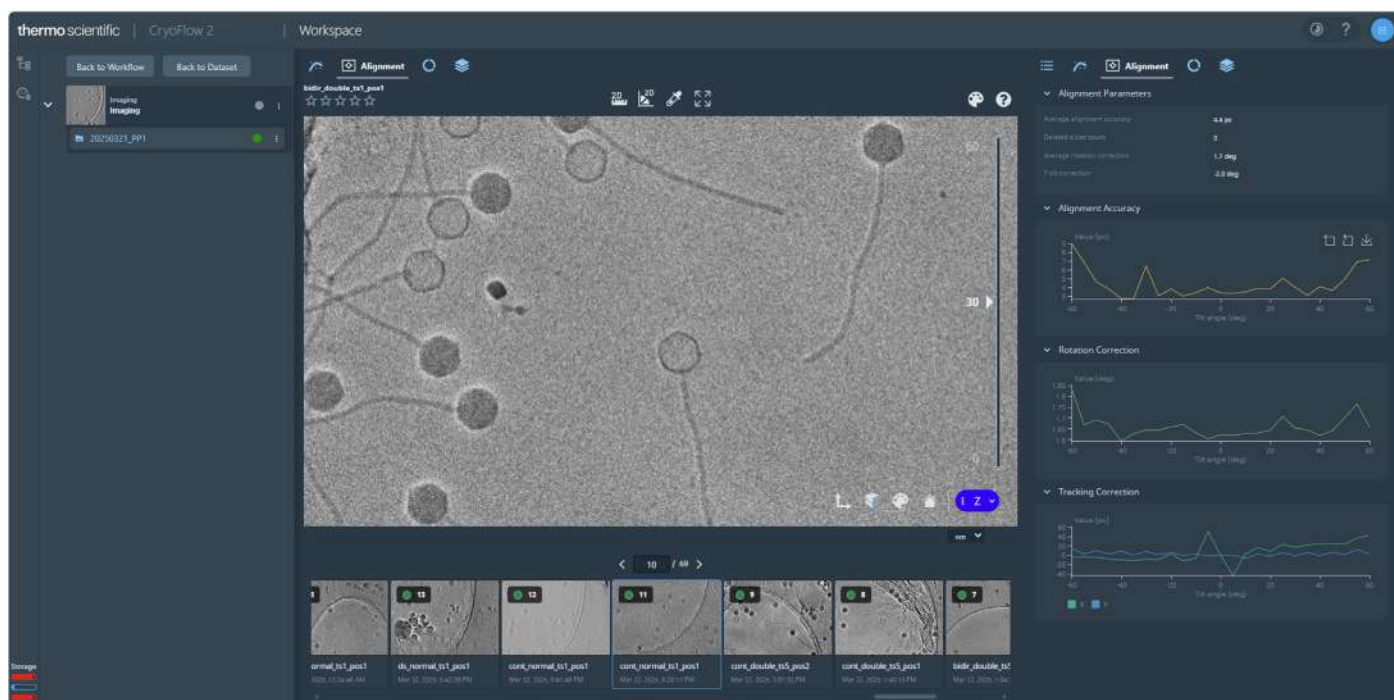


Figure 3. CryoTomo Software Suite results displayed in the CryoFlow Software user interface.

## CryoTomo Software Suite

The CryoTomo Software Suite is an integrated software solution for cryo-ET workflows, comprised of Tomography 5 Software, Tomo Live Software, and multigrid support capabilities. Tomo Live Software picks up tilt series acquired by Thermo Scientific Tomography 5 Software and offers on-the-fly data monitoring through real-time reconstruction of tilt series into 3D volumes. When recording tilt series, it automatically performs motion correction of fraction movies, fiducial-free tilt series alignment, and 3D reconstruction.

Tomo Live Software runs on the DMP, and its user interface is integrated into the CryoFlow Software user interface. CryoFlow Software's 2D and 3D data visualization capabilities help you evaluate cryo-ET data quality and identify high- and low-quality tilt series on the fly.

Data can also be filtered using quality metrics obtained during or after the alignment and reconstruction process. These metrics include the number of deleted slices that could not be aligned, the residual alignment error after iterative patch tracking alignment, and estimated sample thickness.

CryoFlow Software can export selected tilt series and reconstructed tomograms for further processing and sharing.

Equivalent to the capabilities of image tracking in the SPA workflow, the CryoTomo Software Suite and CryoFlow Software work together to trace the origin of each collected tilt series. You can easily identify the region of the grid (search map) and grid (atlas) where each tilt series was acquired.

## Hardware requirements

CryoFlow Software requires a dedicated server, the DMP, with network connectivity to a microscope and your institution's infrastructure.

## Data Management Platform (DMP)

The DMP is the standard storage server delivered with all Thermo Scientific cryo-TEMs and the primary storage location for data collected on these microscopes. When CryoFlow Software is installed, the Thermo Fisher Scientific field service engineer and digital solutions engineer will configure the DMP for your requested applications and connect it to your institution's network. If additional hardware components are needed, such as GPUs, RAM memory, or an NVMe SSD, they will be included in the corresponding Smart EPU Software or CryoTomo Software Suite offer and installed by the FSE. While it is technically possible for users outside of your institution's network to access the DMP, your IT department is responsible for providing controlled access and implementing regular security patches provided by Thermo Fisher Scientific.

Once installation is complete, the DMP evolves from being a standard storage server to an enhanced tool for temporarily hosting, processing, and exporting experimental data.

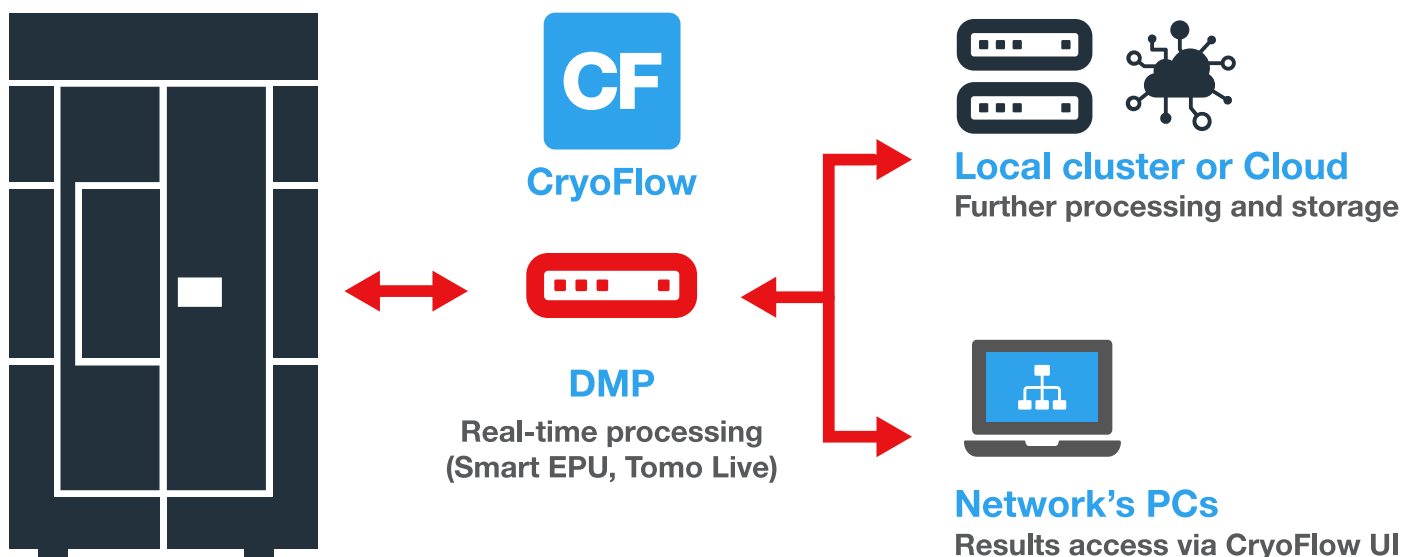


Figure 4. Connecting the DMP to your network makes it possible to offload data from the microscope and transfer it to local clusters.

## Connecting the DMP to your institution's infrastructure

The DMP is configured according to the boundaries of your institution's IT department and to the specific product being purchased. The DMP uses 10 Gb connections to both the Thermo Scientific instrument and your institution's network to transfer images and data quickly from the instrument or camera to the DMP and onward to your processing servers and long-term storage locations.

The following network connections should be in place between the microscope room and the room where the DMP is located:

- Three 1 Gb Ethernet network connections for communication between the microscope PC or support PC and the DMP
- Three 10 Gb OM3 fiber connections for data transfer from up to three cameras on the instrument to the DMP

The following items should be in place in the room where the server is to be located:

- 19-inch server rack to store the DMP and its associated switch
- Two 220 V power sources, preferably on different power circuits for redundancy in the event of a power outage (the server has two power supplies for redundancy)

The following network requirements should be in place between the DMP room and your institution's network or long-term data storage:

- One 1 Gb Ethernet network connection to make CryoFlow Software's user interface accessible within your institution's network; the connection requires:
  - Static IP address provided by your institution's IT group
  - Domain Name System (DNS) name to be applied to CryoFlow Software's user interface for it to be accessible via a web browser within the institution
  - DNS server information
- One 10 Gb network connection for exporting data from the DMP to your institution's long-term, on-premises storage and processing node or to a cloud storage and processing node
  - OM3 fiber/CAT6A or highest possible

Due to the sound and heat generated by the DMP, it is highly advised to locate it in a separate room close to the microscope.

## Digital solutions engineers deliver end-to-end service

Digital solutions engineers (DSEs) are part of Thermo Fisher Scientific's service organization. With backgrounds and experience in IT and network configuration, the DSE team will help you define how to best integrate CryoFlow Software and the DMP into your infrastructure. If you expect to receive a DMP or CryoFlow Software server, be prepared to discuss the products being delivered with your IT department and the DSE team, who can be contacted via email at [DSEsupport@thermofisher.com](mailto:DSEsupport@thermofisher.com).

Once the DMP is installed on site by Thermo Fisher's field service engineers, a DSE will remotely connect and install CryoFlow Software and the associated applications. All future software updates, including further integration with your institution's IT facilities, can also be scheduled with a remote DSE, saving time and additional maintenance for your IT department.

## Software capabilities

### Single microscope solution

Each microscope is delivered with its own DMP server hardware and with individual CryoFlow Software instances.

### Data storage

The DMP is not meant to serve as long-term storage. Its storage capacity is used as a buffer for bulk image data, allowing the camera to continuously offload data and ensure uninterrupted microscope operation. Bulk data must be transferred to another storage location using CryoFlow Software's export function. The DMP has a RAID 6 architecture to prevent data loss in case of disk failure. You have full control over deleting image data.

### Camera support

CryoFlow Software can support up to three cameras on a single microscope. When installed locally on a microscope, CryoFlow Software does not support cameras on other microscopes because of the high data rate connection requirements.

### Concurrent users

CryoFlow Software supports access for up to 15 concurrent users.

### Reference

Comet, M., et al. (2024) Tomo Live: an on-the-fly reconstruction pipeline to judge data quality for cryo-electron tomography workflows. *Acta Cryst.* (2024). D80, 247–25. doi: 10.1107/S2059798324001840

Learn more at [thermofisher.com/cryoflow](https://thermofisher.com/cryoflow)

**thermo** scientific