

# CryoFlow Software

## Integrative data management for cryo-EM workflow optimization

Thermo Scientific™ CryoFlow™ Software is a paperless data management solution for cryo-electron microscopy (cryo-EM). CryoFlow Software enables applications for the optimization of cryo-EM workflows by facilitating the routine generation of protein structures by single particle analysis (SPA) and cellular structural analysis using cryo-electron tomography (cryo-ET).

CryoFlow Software is designed to work seamlessly with Thermo Scientific cryo-transmission electron microscopes (cryo-TEMs). To ensure optimal performance, CryoFlow Software is installed and configured on an on-premises server, provided by Thermo Fisher Scientific, that is strategically positioned near the microscope at the host institution. This server acts as an integral part of the overall system, facilitating efficient data management. Once it is set up, you can conveniently access the CryoFlow Software through a web browser from any location within the host institution's network.

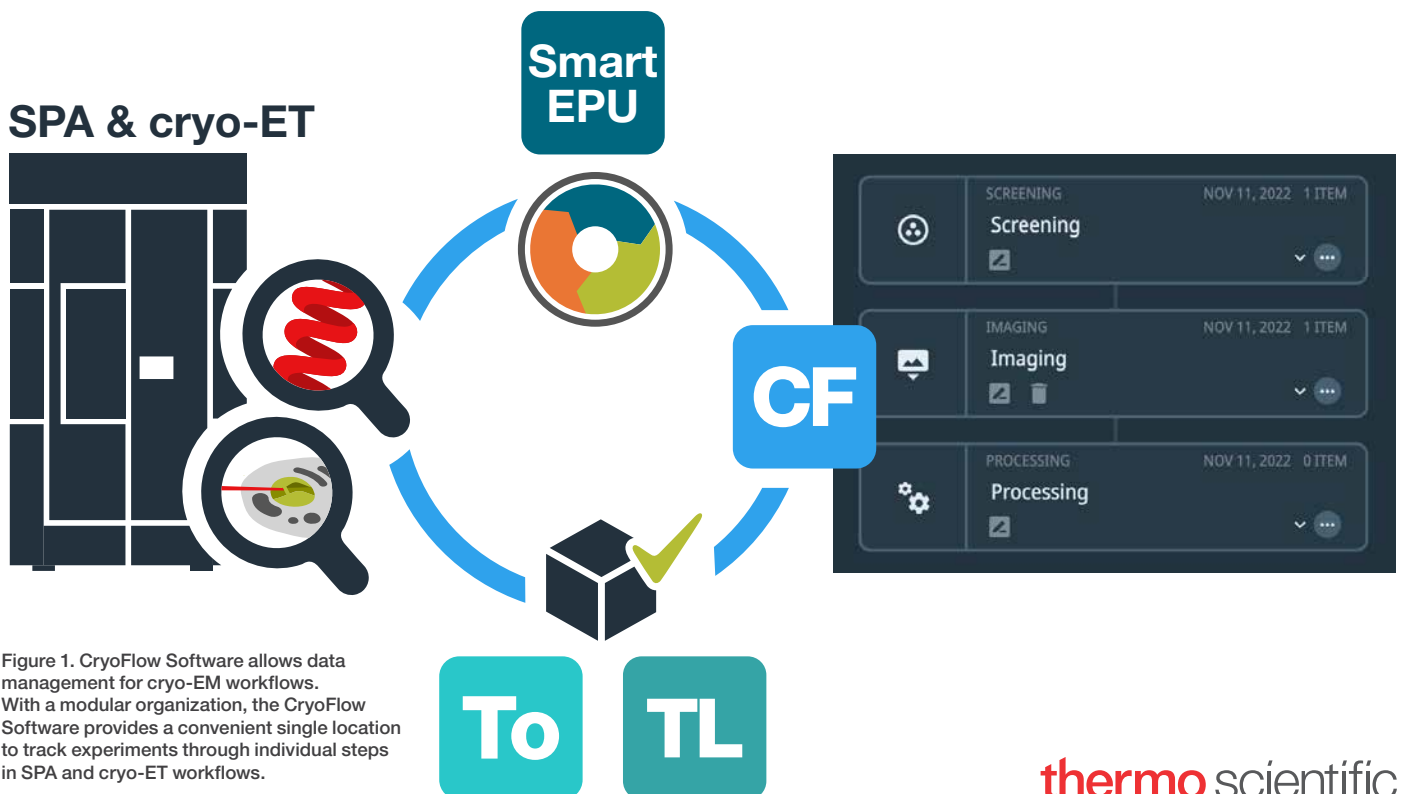


Figure 1. CryoFlow Software allows data management for cryo-EM workflows. With a modular organization, the CryoFlow Software provides a convenient single location to track experiments through individual steps in SPA and cryo-ET workflows.

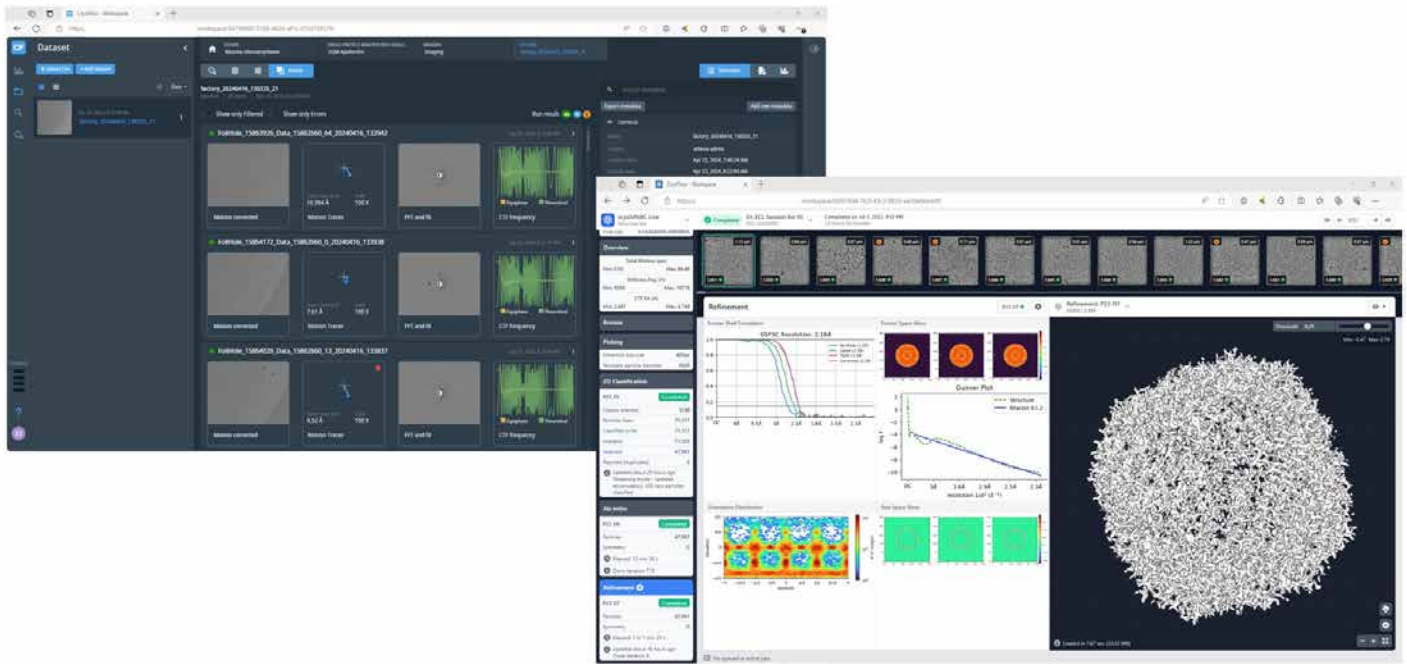


Figure 2. Smart EPU Software with Quality Monitor (background) or Embedded CryoSPARC Live (foreground) enabled by CryoFlow Software. During execution of SPA data collection, image quality information is presented on-the-fly, allowing fast modification of acquisition parameters if needed.

With the addition of modular applications, CryoFlow Software enables execution of guided workflows, as well as real-time reporting of cryo-EM images and their analysis. The CryoFlow Software user interface allows you to interact with the data as your acquisition is ongoing on the microscope. This permits supervised live monitoring of cryo-EM data acquisition or results in visualization for unsupervised sessions.

CryoFlow Software, as a cutting-edge data integration platform, is a valuable tool for cryo-TEM lab management because it offers a common platform for data handling associated to Thermo Scientific instrumentation.

Now, a suite of dedicated software applications enabled by CryoFlow Software operates in the interface between data acquisition and data processing during execution of cryo-EM workflows. These applications are Thermo Scientific Smart EPU Software with EPU Quality Monitor or with Embedded CryoSPARC Live™ Software, and Thermo Scientific Tomo Live Software.

CryoFlow Software applications run on dedicated hardware, a powerful and versatile server called Data Management Platform (DMP), currently delivered with the Thermo Scientific Tundra™, Glacios™, and Krios™ Cryo-TEMs. The optimal performance of CryoFlow Software-based applications is reliably assured by the DMP, with its 10 Gb network interface and configurable GPU computing power. The CryoFlow Software applications can take advantage of the fast offloading of raw experiment data from the microscope camera to the DMP and use their resources to process images on the fly for instant feedback to the microscope. The DMP guarantees the necessary speed to keep up with the data acquisition throughput, enabling real-time feedback to enhance data collection.

CryoFlow Software takes the experiment data on the DMP and presents all images and associated metadata to you, making them available for annotating, sharing with collaborators, or exporting, while meeting high security standards for data management. CryoFlow Software has built-in data administration that tracks all experiments performed with the microscope, replacing unintegrated (e.g., paper) forms of administration. Data and associated metadata are stored for as long as desired within the storage capabilities of the DMP server.

CryoFlow Software has a featured function for data transfer to processing clusters and storage systems. Metadata is stored by the CryoFlow Software in its own format and can be exported in a format commonly used by third-party image processing pipelines (STAR format). You do not need to write your own export scripts to transfer data. 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.

Once the DMP is installed on site by Thermo Fisher's Field Service Engineers (FSE), Thermo Fisher's Digital Solutions Engineers (DSE) remotely connect and install the CryoFlow Software and associated applications. All future software updates, including further integration with the institution's IT facilities, can also be undertaken by our remote DSE with the proper scheduling, saving time and additional maintenance by the IT department, as the whole product is offered as a turnkey solution.

### **Applications enabled by CryoFlow Software**

An increasing number of applications related to cryo-EM workflows are enabled by CryoFlow Software, including Smart EPU Software and Tomo Live.

#### **Smart EPU Software**

Smart EPU Software is a self-monitoring data acquisition program for SPA that includes EPU 3, the latest version of the EPU acquisition program; two alternate programs for live image evaluation, EPU Quality Monitor and Embedded CryoSPARC Live; and Smart Plugins, decision algorithms used during the setup of data collection sessions or for automatic modification of data acquisition parameters on-the-fly based on the reported image evaluation. CryoFlow Software bi-directionally communicates with all Smart EPU Software components to achieve higher automation of SPA data collection.

The orchestration of data transfer and data analysis provided by CryoFlow Software allows the execution of Smart EPU AI/DL-based plugins for the automation of critical routine tasks. Grid squares and foil hole selections for high-quality imaging, actions that traditionally require a good level of user expertise, are now automated thanks to the robust performance of pre-trained AI models.

The Smart EPU image evaluation programs are started via CryoFlow Software, which itself is accessible from the Smart EPU Software user interface. The login for CryoFlow Software is password protected so that data from each acquisition session can be accessed by only the authorized operator and designated collaborators.

Immediately after starting data collection, EPU Quality Monitor can be used to perform on-the-fly motion correction and contrast transfer function (CTF) estimation for each acquired movie. Micrograph thumbnails and the corresponding Quality

Monitor processing results are displayed within the CryoFlow Software user interface together with continuously updated graphical summaries for the ongoing data collection. Quality Monitor metrics allow you to manually adjust acquisition parameters in Smart EPU Software to optimize results early in the data collection process, increasing throughput of high-quality data. The CryoFlow Software user interface enables you to select data for further processing objectively and interactively.

The origin of each high-resolution image collected in a Smart EPU Software session is trackable in CryoFlow Software. You can easily identify the foil hole, grid square, and grid from where each image was taken. This facilitates the association between a given sample's data quality and the corresponding ice properties, which is crucial in the setting of a large dataset acquisition based on results of a screening session.

As an alternative to 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. The program is started via CryoFlow Software, allowing a straightforward association of imaging and image processing. When using Embedded CryoSPARC Live, you can then make decisions based on the image and sample quality measurements 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. The storage and export capabilities of CryoFlow Software 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, keeping the image focus within the targeted range. CryoFlow Software plays a central role in the execution of these feedback loops, assuring proper data transfer.

To facilitate supervised monitoring, the Discovery Viewer is a valuable add-on to the CryoFlow Software portal. It helps in determining 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 multigrad screening session, enabling you to identify the necessary grid squares, foil holes, and defocus range required for subsequent data acquisition. Moreover, this tool plays a crucial role in enhancing sample traceability and will become essential for achieving further automation.



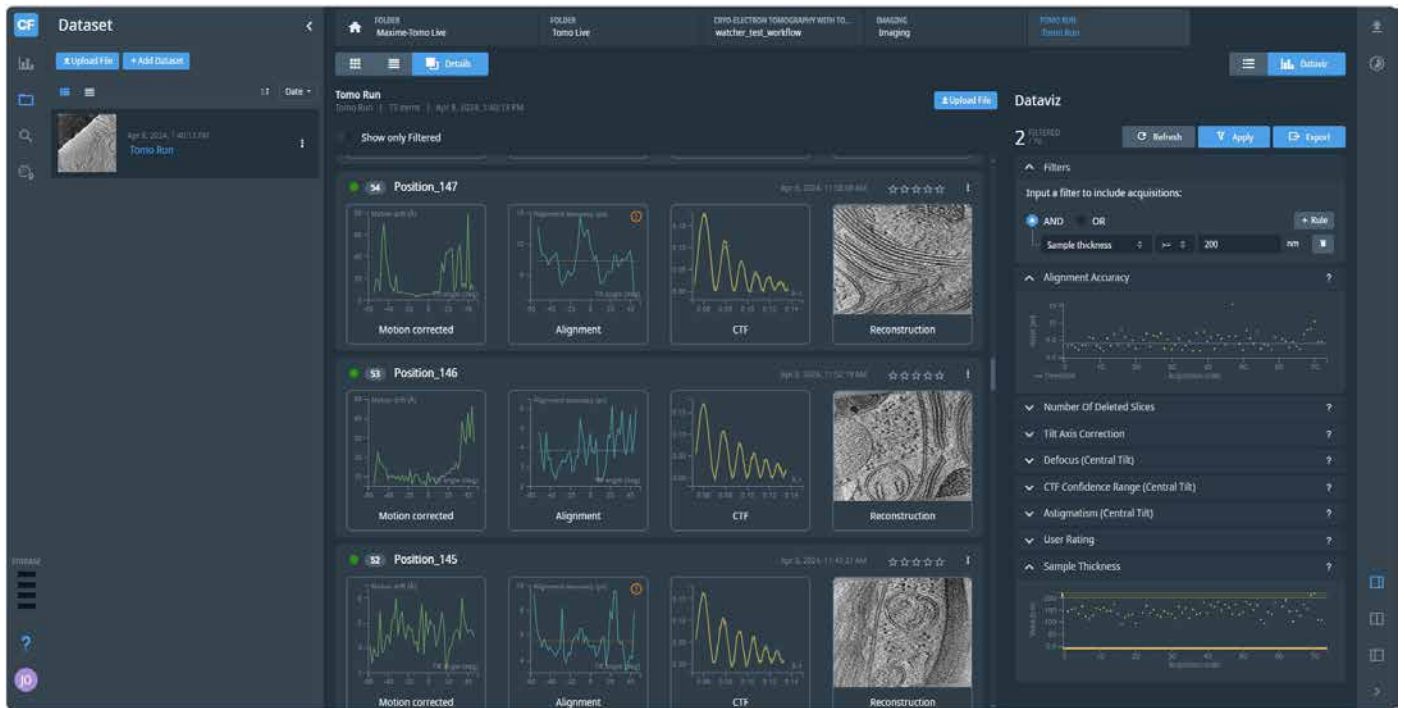


Figure 3. Tomo Live Software results displayed in the CryoFlow Software user interface.

## Tomo Live Software

CryoFlow Software-based Tomo Live Software picks up tilt series acquired by Thermo Scientific Tomography 5 Software. It offers on-the-fly data monitoring through real-time reconstruction of tilt series into 3D volumes. During recording of 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 the user interface is integrated in the CryoFlow Software user interface. The CryoFlow Software capabilities for 2D and 3D data visualization assist you in an impromptu cryo-ET data quality evaluation and identification of high- and low-quality tilt series.

Data can also be filtered using quality metrics obtained during or after the alignment and reconstruction process. Examples for such metrics are the number of deleted slices that could not be aligned, the residual alignment error after iterative patch-tracking-alignment, or estimated sample thickness. CryoFlow Software allows exporting of selected tilt series and reconstructed tomograms for further processing, archiving, and sharing.

Equivalent to the capabilities of image tracking in the SPA workflow, in tomography, it is possible to trace back the origin of each tilt-series collected in Tomography Software using CryoFlow Software. You can easily identify the region of the grid (search map) and grid (atlas) from where each tilt series was acquired.

## Hardware requirements

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

### Data Management Platform (DMP)

The DMP is the standard storage server being delivered with all Thermo Scientific cryo-TEMs. In its most basic form, the DMP is the primary landing point for data being collected on these microscopes. During the CryoFlow Software installation phase, the FSE and the DSE will configure the DMP according to the requested applications and connect it to the hosting institution's network. If additional hardware components are needed (GPUs, RAM memory, or NVMe SSD), they will be included in the corresponding Smart EPU Software or Tomo Live Software offer and installed by the FSE. Access to the DMP to users external to the hosting institution's network is technically possible, being the responsibility of the hosting institution's IT department to provide controlled access (i.e., via VPN) and to implement the installation of 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 temporary hosting, processing, and exporting of experimental data.

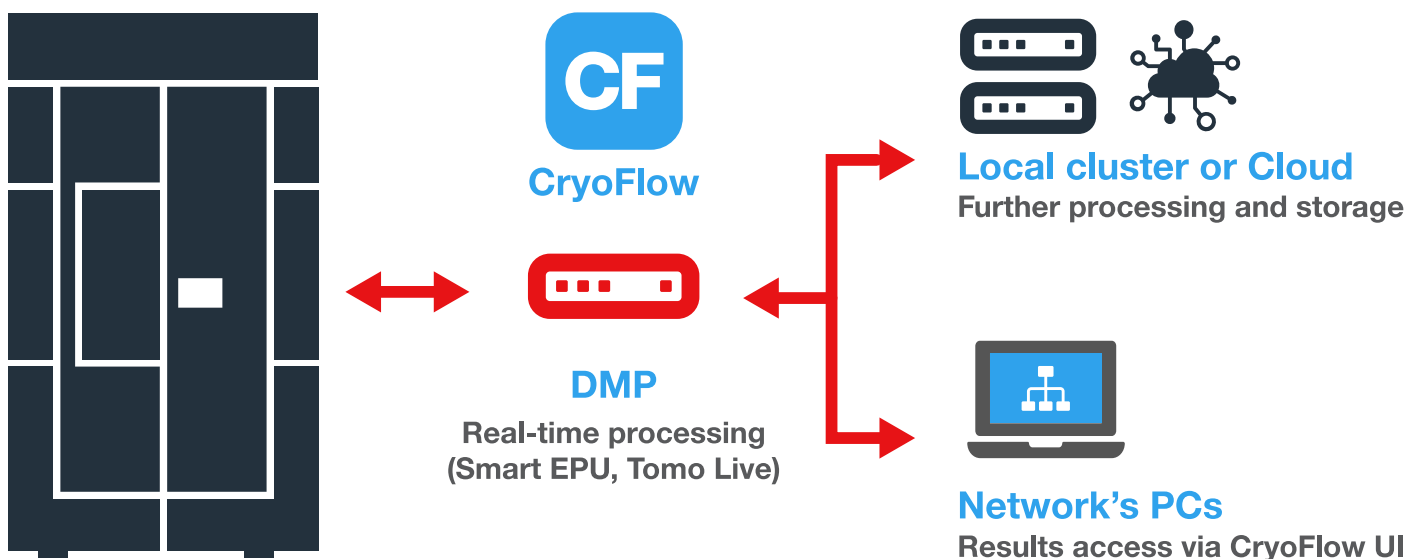


Figure 4. DMP and network connectivity. Offload data from the microscope and transfer to local clusters or cloud uses fast network connections.

### DMP connectivity to the hosting institution's infrastructure

Each DMP is configured according to the boundaries of the hosting institution's IT department and to the specific product being purchased. Connecting to both, the Thermo Scientific instrument and the institutions network, the DMP utilizes 10 Gb network connections to transfer images and data quickly from instrument/camera to the DMP and onwards to the customers processing servers and/or to long-term storage locations.

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

- 3 x 1 Gb ethernet network connections for communication between the Microscope PC/Support PC and DMP.
- 3 x 10 Gb OM3 fiber connections for data transfer for 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.
- 2 x 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 the hosting institution's network/long-term data storage:

- 1 x 1 Gb ethernet network connection. This will be the connection point for the CryoFlow Software user interface to be accessible within the hosting institution's network; therefore, it needs:
  - Static IP address provided by the host institution's IT group.
  - Domain Name System (DNS) name to be applied to the CryoFlow Software user interface for it to be accessible via web browser within the institution.
  - DNS server information.
- 1 x 10 Gb network connection. This network connection will allow for the data first stored on the DMP to be exported to the institution's long-term, on-premises storage/processing node or cloud storage/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 another room close to the microscope.

### Service delivery via the Digital Solutions Engineers

The Digital Solutions Engineers (DSEs) are an addition to the Service organization of Thermo Fisher Scientific. With backgrounds and experience in IT and network configuration, the DSE team will help you define how to best integrate the CryoFlow Software and the DMP into the infrastructure of the host institution. If you are expecting to receive a DMP/CryoFlow Software server, you should be prepared to discuss 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).

The DSEs will support the FSEs installation remotely and deliver the applications and features required remotely, allowing for future updates to be delivered promptly.

### Software capabilities

#### Single microscope solution

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

#### Long-term data storage

DMP is not meant as long-term storage; the storage capacity is used as a buffer for bulk image data to ensure continuous operation of the microscope (so the camera can always offload data). The DMP has a RAID 6 architecture to prevent data loss in case of disk failure. Transfer of bulk data is required and is made easy by the CryoFlow Software export function. Image data deletion is controlled by you to adjust to specific situations.

### Camera support

CryoFlow Software can support up to three cameras on the same microscope. A local instance of CryoFlow Software does not support cameras on a different microscope than that on which it is installed (because of 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](https://doi.org/10.1107/S2059798324001840)

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