

Smart EPU Software

A platform for automated screening and data acquisition in single particle analysis using cryo-EM

Thermo Scientific™ Smart EPU Software offers increased automation capabilities for high-quality data acquisition. It provides on-the-fly, AI-assisted decision-making algorithms to help beginners and offers a higher degree of flexibility for advanced users.

Single particle analysis using cryo-electron microscopy (cryo-EM) is a popular technique in structural biology used to solve high-resolution structures of proteins, macromolecules, and complexes. While recent improvements in instrumentation have made it more efficient to obtain large amounts of data, the quality of single particle analysis (SPA) data still largely depends on the expertise of the microscope operator. Smart EPU Software is an innovative platform that combines well-known features of previous EPU Software releases with novel, easy-to-use tools designed for further software automation:

- Easy microscope preparation for optimal imaging
- Straightforward data collection setup
- Optimal Autoloader usage for screening and data collection
- Fast selection of imaging areas and self-monitoring of imaging conditions for higher efficiency

Smart EPU Software frees the operator from tedious and repetitive tasks while enabling new users to adopt the cryo-EM workflow faster.

By encoding expert knowledge into neural networks, the Smart EPU software can automatically recognize and categorize areas in cryo-EM grids suitable for imaging, discarding those that would lead to suboptimal results. You no longer need to predefine the perfect setup for your acquisition. Instead, you can rely on the power of AI-driven algorithms to help refine your choices and selections on the fly.

The Smart EPU Software can include two optional components for real-time image processing: EPU Quality Monitor (EQM), a component that performs motion correction and contrast transfer function (CTF) determination to dynamically adjust data collection, or Embedded CryoSPARC Live™, a powerful image processing package that offers 2D and 3D information of the molecular content in the sample. Integrated into the EPU workflow, Smart EPU Software includes a unique set of algorithms that analyzes the results of EQM or Embedded CryoSPARC Live to power

Key Benefits

EPU 3 Software, combined with AI-powered image filtering, reduces the required level of user expertise and helps train new users across all compatible instruments.

On-the-fly image analysis, carried out by EQM or Embedded CryoSPARC Live and remote data access (via Thermo Scientific™ CryoFlow™ Software), enables fast and objective responses to image quality.

AI-enabled solutions provide instant feedback to steer data collection on the fly, eliminating the need for manual monitoring.

Fast setup time, queuing of multiple grids by EPU Multigrid, and real-time feedback loops from Smart Plugins enable more efficient use of microscope time and increased data quality.

Open API allows AI developments to be tailored to address specific questions in individual samples.

Continuous improvements are offered through frequent updates from our development team and professional maintenance is supported by our service organization and application specialists.

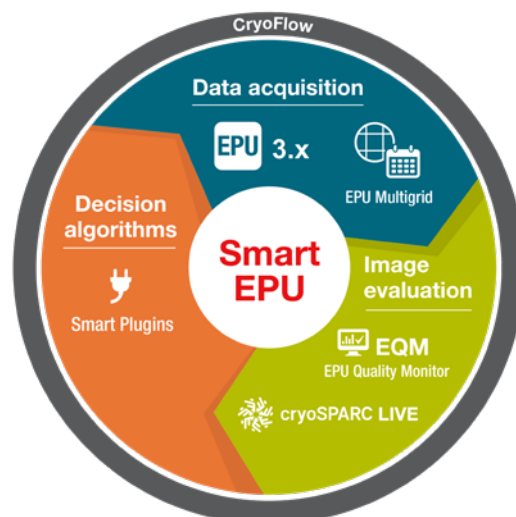


Figure 1. Smart EPU Software enables efficient, unattended acquisition of thousands of cryo-EM micrographs, calculating and evaluating image attributes on the fly.

online feedback loops and optimize ongoing acquisitions. These processing routines, called Smart Plugins, favor quality data and efficiency and further simplify setup.

Additionally, an open application programming interface (API) provides maximal flexibility for expert users by allowing AI algorithms to influence and automate a number of EPU acquisition strategies, thus addressing very specific needs.

EPU 3 and EPU Multigrid Software

EPU Software is a popular choice for the cryo-EM SPA workflow largely due to its robust performance and intuitive interface. Unexpected interruptions of automatic acquisitions have become rare events, allowing thousands of images to be collected in a few hours for structural determination of proteins at up to atomic resolution. EPU Software has continuously evolved for more than a decade, and its most recent version—EPU 3 Software—has been designed to further facilitate its use, saving you time and effort.

As a core component of the Smart EPU Software, EPU 3 Software contains functions for all the steps of automated SPA data collection. The interface is organized in a logical sequence of tabs and buttons that guide users through the workflow.

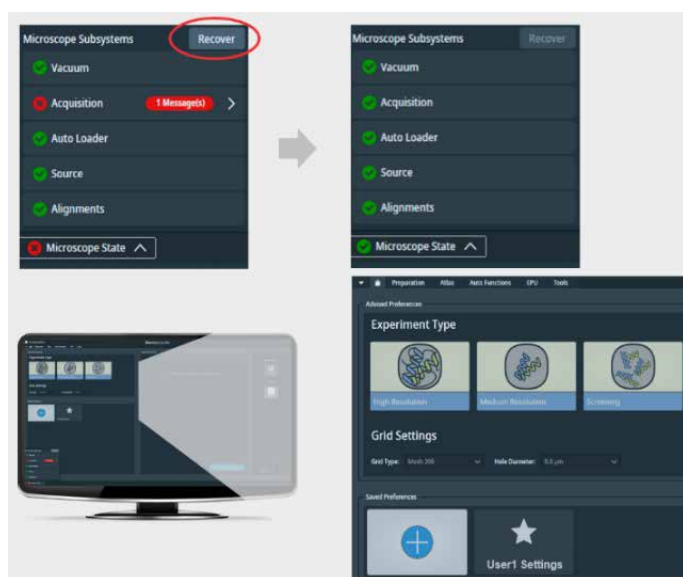


Figure 3 The Smart EPU Software user interface presents the main steps of an acquisition run in a logically organized sequence. Recently implemented features include a Traffic Lights panel and a Home Page to start sessions faster.

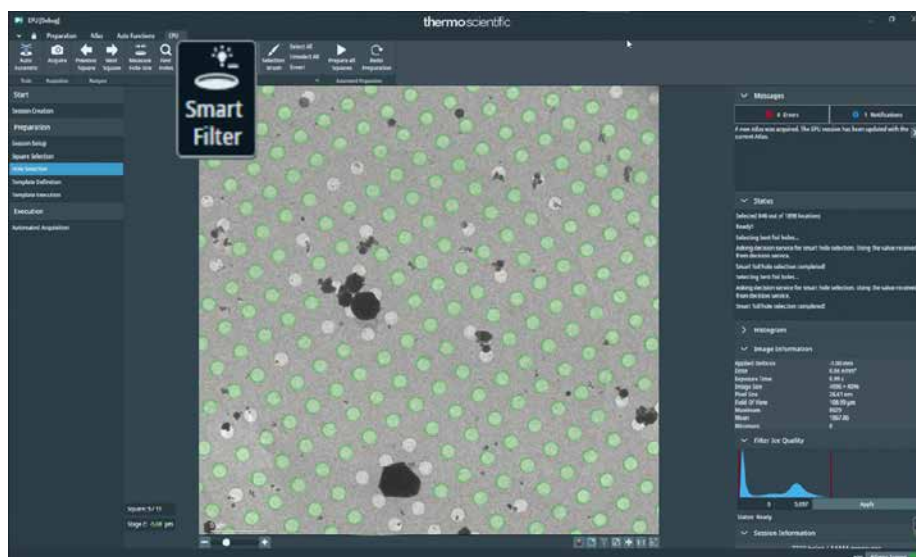


Figure 2. In Smart EPU Software, a smart filter facilitates curation of hole selections. Our AI-powered grid hole selector can distinguish holes suitable for data acquisition (highlighted in green) from those that are empty or in ice-contaminated areas. Smart EPU Software can automate many laborious tasks, significantly reducing the time and expertise needed to perform an experiment.

These tasks include:

1. Saving and loading all relevant preferences that define an acquisition on the Home Page
2. Preparing optical sets
3. Recording low-magnification grid atlases
4. Testing and calibrating autofunctions (focus, astigmatism, eucentricity, and Volta phase plate activation)
5. Setting up and running acquisition sessions

These tasks are divided into subtasks, such as hole selection, for which Smart EPU Software contains a relevant AI-element. The Smart Filter function, for instance, acts as an automated foil hole selector that picks hole populations with the highest probability to yield high-resolution structures. Smart Filters can be combined with the conventional Auto Filtering function, which selects holes based on relative thickness. After preparation of only one square, the same selection criteria can be applied automatically to other selected grid squares, excluding ice-contaminated holes or holes with ice that is too thick.

Another component of Smart EPU Software is EPU Multigrid, which maximizes efficiency in Autoloader-equipped microscopes by setting up a queue of automated acquisitions across multiple grids. EPU Multigrid enables long periods of unattended runs. Imaging conditions set for one grid are applied to many others in the queue, reducing the operator's work. It facilitates the logistics of sample screening and data acquisition of multiple grids, increasing the number of datasets you can generate in each working day, including unattended overnight and weekend runs. EPU Multigrid reduces idle beam times for an overall increase in microscope productivity.

EPU Quality Monitor (EQM) and Embedded CryoSPARC Live

Smart EPU software can be configured with two alternative integrated programs for on-the-fly monitoring of data quality: EPU Quality Monitor (EQM) Software or Embedded CryoSPARC Live.

EQM provides real-time information about image quality throughout the entire data collection process. Drift and optics-related information derived from motion correction and CTF-estimation are accessible for each image immediately after acquisition.

Embedded CryoSPARC Live offers 2D and 3D information derived from the data while collection is still ongoing, enabling both image and biological sample quality to be monitored in real time. Embedded CryoSPARC Live facilitates the adjustment of imaging and processing parameters at any time, increasing overall data quality while allowing the user to discriminate between successful and unsuccessful experiments objectively and rapidly. A first high-resolution reconstruction of the object under scrutiny can be achieved while still collecting data. In case of poor-quality samples, Embedded CryoSPARC Live avoids waste of resources (beam time and storage) while providing insights for further improvement of sample preparation, such as preferential orientations or low ligand binding stoichiometries.

In the Smart EPU platform, the information gathered from both programs can be used to adjust acquisition parameters not only manually (as has been the case previously) but also automatically with Smart Plugins. With EQM or Embedded CryoSPARC Live, acquisition parameters can be adjusted at any time, maximizing the potential to succeed in structure determination. Notably, the filtering capabilities of EQM can exclude poor-quality images from image processing, reducing cost and time.

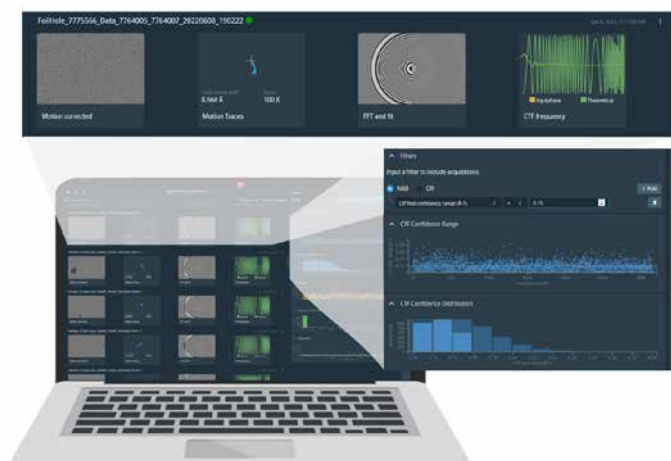


Figure 5. EPU Quality Monitor and CryoFlow Software allow EPU data to be accessed remotely for evaluation and sharing.

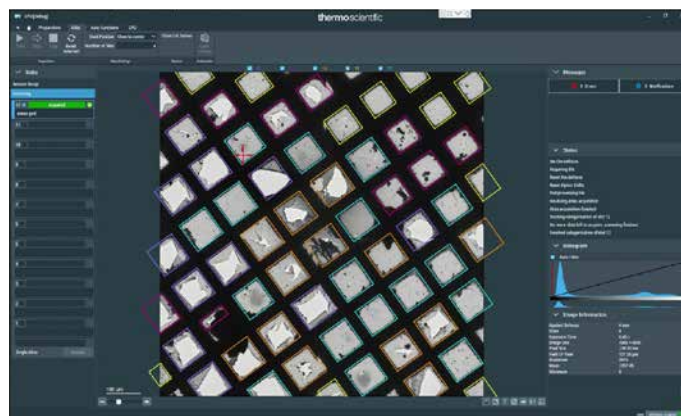


Figure 4. EPU 3 Software can automatically acquire atlases for the specimens in multiple slot positions and automatically classify grid squares to facilitate user selection.

Smart Plugins

Smart Plugins are a set of decision-making algorithms for improving the microscope efficiency. Smart EPU Software presents a set of innovative AI/ML plugins that automate steps that hitherto needed manual actions during the setup of EPU sessions. Four Smart Plugins take over the task of grid squares classification according to ice quality, foil holes identification, foil holes selection based on ice thickness and curation of initial selections to avoid highly contaminated foil holes. Neural networks have been trained to automatize these laborious and repetitive tasks with focus on sample screening, helping users to determine favorable conditions for further collection of large datasets.

In Smart EPU Software, the image evaluation from EQM or Embedded CryoSPARC Live is accessible to other Smart Plugins tightly integrated with EPU 3 Software in order to modify the course of a run. This opens a new era in electron microscopy data acquisition, making it possible to automate feedback loops based on quantitative parameters derived from images. Three of these feedback plugins are included with Smart EPU Software: Smart Focus for autofocus stabilization, Smart Stage Time to adjust stage waiting time based on drift motion, and Smart Grid Skip for skipping suboptimal grid squares based on the information limit. Additional Smart Plugins will become available through frequent updates.

Open API

An important feature of Smart EPU Software is an open Application Programming Interface (API) that allows advanced users to develop their own plugins for Smart EPU Software. These user-developed plugins can evaluate microscope outputs and influence Smart EPU Software according to specific needs. The open API facilitates innovation and the exchange of setups in the broader community.

CryoFlow Software

The tight integration of components within Smart EPU Software is enabled by the CryoFlow Software, which is a platform

for data exchange management, data analysis, and visual reporting. It assures the smooth, secure transfer of images and associated metadata between components.

CryoFlow Software plays a crucial role in the Smart EPU Software. It makes recorded images accessible to EQM and Embedded CryoSPARC Live, allows Smart Plugins to evaluate them, and transfers automated decisions to microscopes on the fly. CryoFlow Software presents all results in a comprehensive and visually attractive design using a portable graphical interface. A dedicated, interactive panel allows you to filter datasets based on these parameters. It also allows you to easily organize, view, and share SPA projects.

The CryoFlow Software platform relies on the imaging server delivered with most microscope configurations. It is accessible via a web interface on computers and mobile devices that have access to the local network. Privacy and permission settings define who can access individual experiments.

Smart EPU Software is ready to use after installation and is supported by our instrument service team, digital solutions engineers, and application specialists. We are committed to continuously improving the Smart EPU Software experience.

Smart EPU Software with EPU Quality Monitor is pre-installed on Thermo Scientific™ Tundra™ Cryo-TEMs and is available for Thermo Scientific™ Glacios™ and Krios™ Cryo-TEMs (Table 1). Smart EPU Software is also available as an upgrade to any existing platforms. It integrates the latest technological advances, such as the Thermo Scientific™ Falcon™ 4i Direct Electron Detector (including Electron Event Representation) and the Thermo Scientific™ Selectris™ Imaging Filter (as well as the Gatan BioContinuum/K3 Filter) to significantly accelerate automated data acquisition.

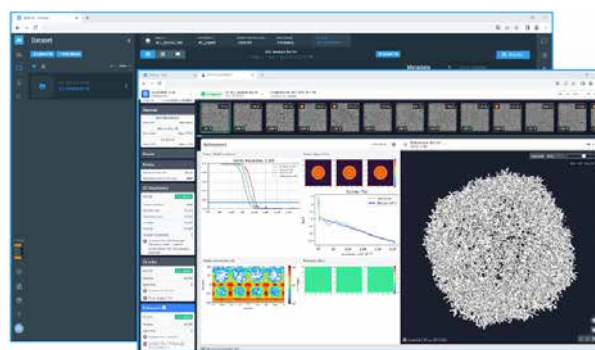


Figure 6: Embedded CryoSPARC Live readily started from CryoFlow Software allows on-the-fly EPU data processing, from basic SPA analysis steps through generation of 3D-structures.

Software solutions that work out of the box

Component	Capability	Description	Tundra Cryo-TEM	Glacios Cryo-TEM	Krios Cryo-TEM
EPU 3 Software	Data acquisition	Enables microscope automation	Included	Included	Included
EPU Multigrad	Scheduling of data acquisition	Set up unattended and/or multi-day data collection runs	N/A	Optional	Included
EPU Quality Monitor	Real-time data evaluation	Real-time motion and CTF correction	Included	Optional	Optional
Embedded CryoSPARC Live	Real-time data evaluation	Real-time motion, CTF correction, particle picking, and 2D and 3D particle averaging	Optional	Optional	Optional
Smart Plugins	Decision algorithms*	Automatically adjust microscope parameters using real-time data from EQM or Embedded CryoSPARC Live	Included	Optional	Optional
Open API	Decision algorithms	Enables tailored scripting to adjust microscope parameters	Included	Included	Included
CryoFlow Software	Data management	Facilitates workflow integration, reporting, and data sharing	Included	Included	Included

Table 1. Overview of Smart EPU Software components and availability with Thermo Scientific Cryo-TEMs. All Smart EPU components require Windows 10. No additional hardware needed for recent instruments. Consult with your sales account manager for retrofit requirements.

*All Available Smart Plugins (Smart Selection of Grid Squares, Smart Hole Finder, Smart Ice Thickness Prediction, Smart Filter, Smart Focus, Smart Stage Time, and Smart Skip Grids Squares) depend on the availability of a real-time data evaluation component.

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