Smart EPU Software
A platform for automated screening and data acquisition in cryo-EM single particle analysis

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 as well as a higher degree of flexibility for advanced users.

Single particle 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 a neural network, the Smart EPU Software can automatically recognize and discard areas in cryo-EM grids that would lead to inferior micrographs. You no longer need to predefine the perfect setup for your acquisition. Instead, you can rely on the power of AI-driven algorithms to refine your choices and selections on the fly.

The Smart EPU Software can include EPU Quality Monitor (EQM), a component that performs motion correction and contrast transfer function (CTF) determination to dynamically adjust data collection. Integrated into the EPU workflow, Smart EPU Software includes a unique set of algorithms that analyzes the results of EQM to power online feedback loops and optimize ongoing acquisitions. These processing routines, called Smart Plugins, favor quality data and efficiency and further simplify setup.

Key Benefits

<table>
<thead>
<tr>
<th>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.</th>
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<tr>
<td>On-the-fly image analysis, carried out by EQM and remote data access (via Athena Software), enables fast and objective responses to image quality.</td>
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<td>AI-enabled solutions provide instant feedback to steer data collection on the fly, eliminating the need for manual monitoring.</td>
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<td>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.</td>
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<td>Open API allows you to tailor AI developments to address specific questions in individual samples.</td>
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<td>Continuous improvements are offered through frequent updates from our development team and professional maintenance is supported by our service organization and application specialists.</td>
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</table>

Figure 1. Smart EPU Software enables efficient, unattended acquisition of thousands of cryo-EM micrographs, calculating and evaluating image attributes on the fly.
Additionally, an open application programming interface (API) provides maximal flexibility for expert users to implement custom decision algorithms and automation routines to power feedback loops, 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 determinations 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.

As shown in Figure 2, Smart EPU Software incorporates a smart filter that 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.

**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 accounts for 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.

As shown in 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.
EPU Quality Monitor (EQM)

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. In the Smart EPU Software, this information can be used to adjust acquisition parameters not just manually (as has been the case previously) but also automatically with Smart Plugins.

With EQM, you have the power to adjust acquisition parameters 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.

Smart Plugins

Smart Plugins are a set of decision-making algorithms for improving the microscope efficiency. In Smart EPU Software, the image evaluation from EQM is accessible to 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 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 with more complex algorithms 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.

Athena Software

The tight integration of components within Smart EPU Software is enabled by the Thermo Scientific™ Athena™ 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.

Athena Software plays a crucial role in the Smart EPU Software. It makes recorded images accessible to EQM, allows Smart Plugins to evaluate them, and transfers automated decisions to microscopes on the fly. Athena 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 Athena 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.
Software solutions that work out of the box

<table>
<thead>
<tr>
<th>Component</th>
<th>Capability</th>
<th>Description</th>
<th>Tundra Cryo-TEM</th>
<th>Glacios Cryo-TEM</th>
<th>Krios Cryo-TEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPU 3 Software</td>
<td>Data acquisition</td>
<td>Enables microscope automation</td>
<td>Included</td>
<td>Included</td>
<td>Included</td>
</tr>
<tr>
<td>EPU Multigrid</td>
<td>Scheduling of data acquisition</td>
<td>Set up unattended and/or multi-day data collection runs</td>
<td>N/A</td>
<td>Optional</td>
<td>Included</td>
</tr>
<tr>
<td>EPU Quality Monitor</td>
<td>Real-time data acquisition</td>
<td>Real-time motion and CTF correction</td>
<td>Included</td>
<td>Optional</td>
<td>Optional</td>
</tr>
<tr>
<td>Smart Plugins</td>
<td>Decision algorithms</td>
<td>Automatically adjust microscope parameters using real-time data from EQM</td>
<td>Included</td>
<td>Optional</td>
<td>Optional</td>
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</table>

Table 1. Overview of Smart EPU Software components and availability with Thermo Scientific Cryo-TEMs.