

# EPU 2 Software

## Sample Screening and Data Acquisition Software for Single Particle Analysis Workflow (cryo-EM SPA)

With improved ease of use and advanced automation, Thermo Scientific EPU 2 software ensures enhanced productivity for all users.

Single particle cryo-EM uses the particles extracted from thousands of images to reconstruct 3D structures of macromolecules. The yield of an acquisition session at the microscope depends on the time to set up the run and the speed of the acquisition itself.

Thermo Fisher Scientific™ EPU 2 software is designed to maximize SPA throughput. Its simplified user interface and advanced automation ensure that setting up a data acquisition session is faster and more accessible, allowing users to focus on their research and make efficient use of the microscope.

Before starting the data collection, EPU 2 software will guide users through four principle steps and their subtasks for setting up the run.

In the **Preparation** step, users define acquisition and optics settings for screening and high-resolution imaging.

The second step controls the **Auto Functions**, which allow users to verify proper microscope alignment for high-resolution data acquisition. The EPU 2 software user interface gives access to auto-eucentricity, auto-focus, auto-coma, and auto-stigmat.

For the third step, EPU 2 software acquires a grid overview (**Atlas**) to determine whether vitrified grids are of sufficient quality for an automated recording session. All 12 grids in the **Autoloader** cassette can be batch screened, and the software examines ice quality and groups grid squares into categories of similar quality automatically.

In the final step, users select grid squares and foil holes for acquisition and define the acquisition template for each foil hole. Grid square and foil hole selection can be performed manually or automatically by applying user-defined filter settings. Likewise, the software assists users in setting up the template acquisition applied to all selected foil holes once the user starts the **Automated Acquisition** run.

### Key benefits

Microscope-embedded SPA software solution for automated grid screening and data acquisition provides a unified user experience across all compatible instruments.

Improved ease of use and throughput via systematic user guidance, automated alignments, automation of tasks and support of the latest hardware developments.

Tightly integrates with EPU Quality Monitor and Data Management (powered by Athena) software, enabling remote data access and on-the-fly analysis of data quality.

Professionally maintained, updated regularly, and supported by our service organization and application specialists.

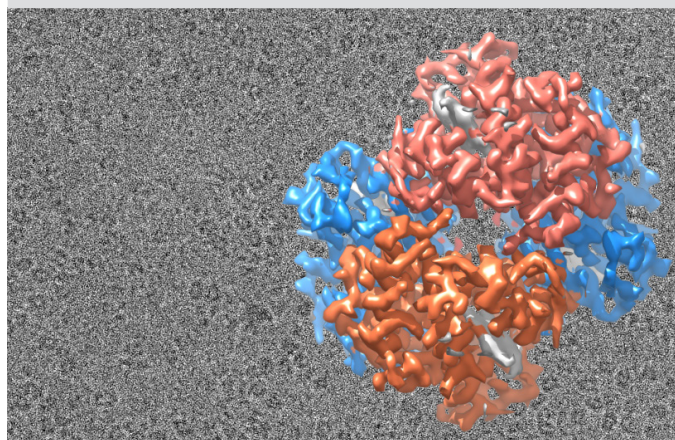


Figure 1. EPU 2 software enables the unattended acquisition of thousands of cryo-EM micrographs that are used to reconstruct protein representations, such as the hemoglobin shown here. The sample was acquired using the Selectris X Imaging Filter.

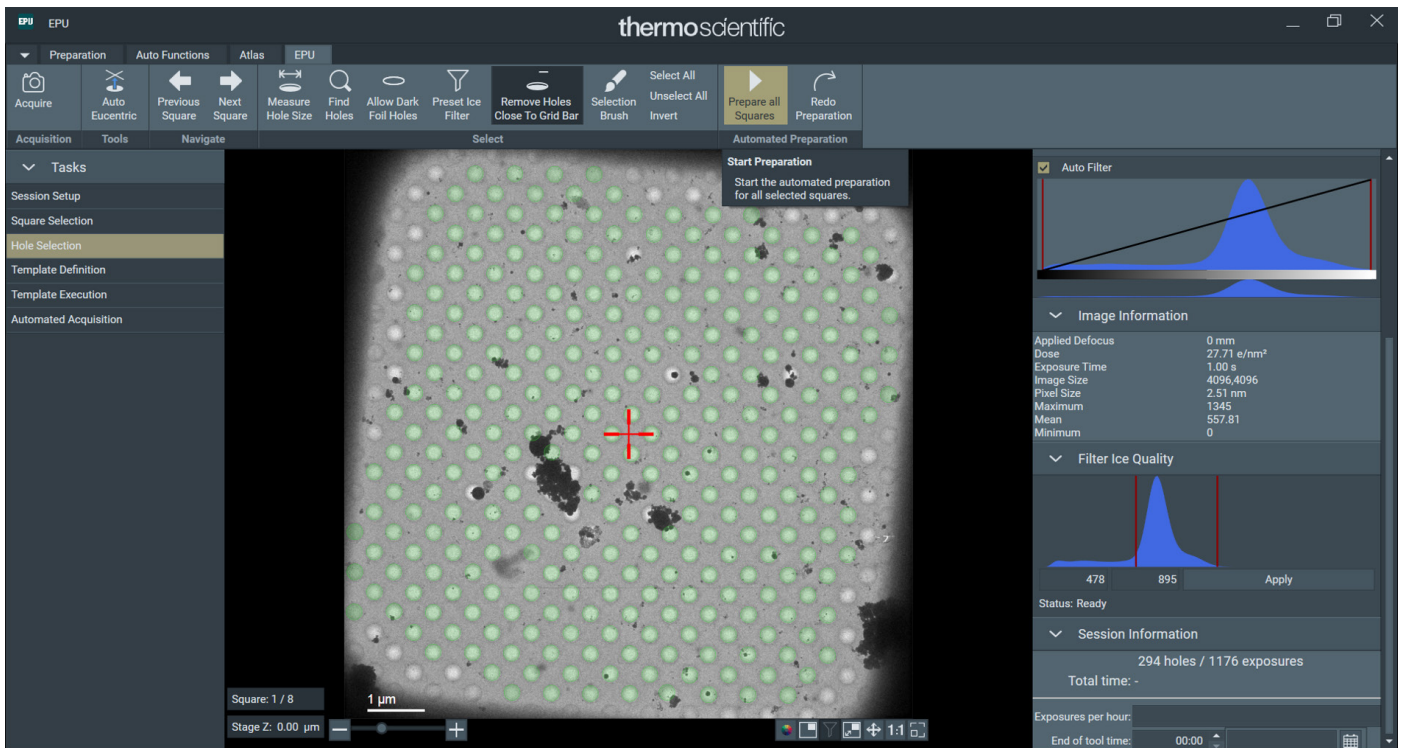


Figure 2: The EPU 2 user interface guides the user through all actions that are needed to prepare and execute an acquisition run. The main tabs for preparing the microscope, daily alignments, screening atlases and image acquisition are subdivided into tasks. EPU 2 software can automate many tasks and significantly reduce the time and expertise needed to perform an experiment.

To set up a repeat experiment faster and reduce the required user expertise, EPU 2 software allows saving and loading all relevant preferences defining an acquisition: presets and calibrations, session setup information, hole selection information, template information, and automated run options.

EPU 2 software supports semi-automated grid square preparation. Rather than setting up selected grid squares one by one, users now have to do so only for the first grid square. The software then performs auto-eucentric height, foil hole detection, and the removal of unsuitable foil holes automatically for all squares—requiring users only to inspect and potentially adjust its selection before starting the run.

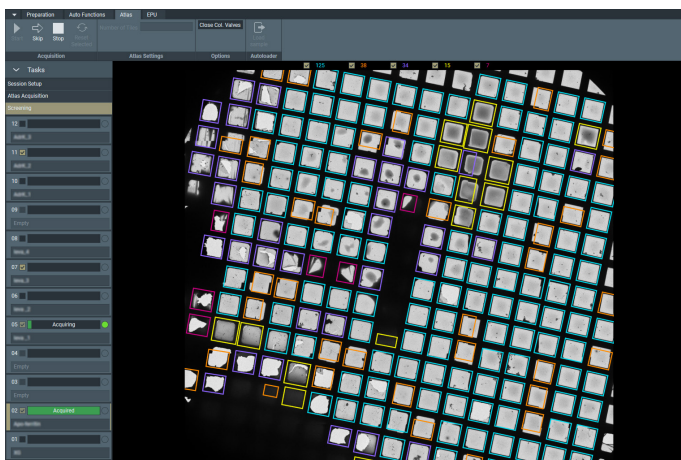


Figure 3: EPU 2 software can automatically acquire Atlases for the specimens in multiple slot positions and indicates the suitability of grid squares for high quality data acquisition.

EPU 2 software is pre-installed on all Thermo Scientific cryo-TEMs. It integrates the latest technological advances such as the Thermo Scientific Falcon™ 4 Detector (including Electron Event Representation), Thermo Scientific Selectris™ Imaging Filter as well as the Gatan BioContinuum/K3 Filter.

Combined with these direct electron detectors, EPU 2 software can accelerate automated data acquisition to more than 300 movies per hour. This **Faster Acquisition** mode processes foil holes in groups using image/beam shifts to reduce the number of stage shifts. It relies on aberration-free image shift (AFIS), a new optical mode that performs large beam shifts without off-axis coma and astigmatism, and greatly benefits from the presence of fringe-free imaging (FFI).

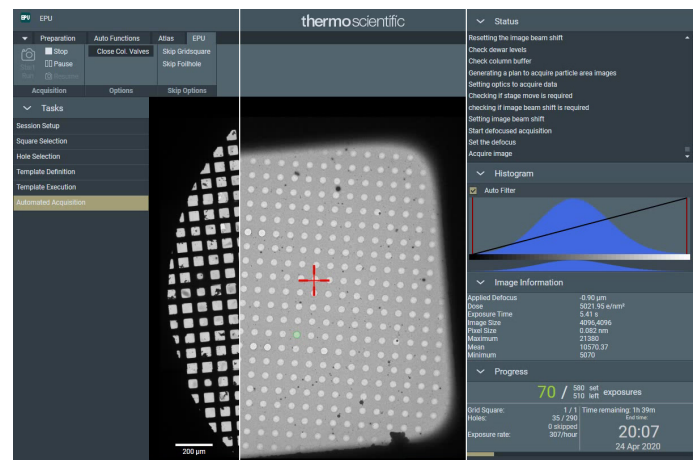


Figure 4: EPU 2 software's Faster Acquisition mode allows users to acquire >300 movies per hour.

## EPU Quality Monitor and Data Management software

EPU 2 software tightly integrates with EPU Data Management (powered by Athena) software and the optional EPU Quality Monitor software. The Thermo Fisher Scientific Athena platform and the server hardware used by the Falcon 4 and the Selectris Imaging Filter form the basis for this data management solution. It allows users to organize, view, and share single particle analysis projects easily.

In the session setup, users can select a predefined project as a streaming target for image data and metadata. Projects are organized hierarchically and can contain multiple experiments, workflows, and datasets. Users can add data from all steps of the single particle analysis workflow, including sample preparation, vitrification, and grid screening, to collect all relevant data in one place. Athena is accessible via a web interface on computers and tablets that have access to the local network. Privacy and permission settings define who can access individual experiments.

The optional EPU Quality Monitor software analyzes and visualizes EPU 2 data on-the-fly, enabling users to judge the image quality of running or completed acquisitions remotely. The results from motion correction and CTF estimation augment the information stored in the data management solution with statistics on defocus, astigmatism and astigmatism distribution, CTF confidence distribution, and when using the Volta Phase Plate, phase shift and phase shift distribution. The data visualization panel allows filtering datasets based on these parameters graphically.

The combination of EPU 2, EPU Quality Monitor and Data Management software helps to generate high-quality results quickly, with confidence, and with the same ease of use for which EPU is already known.

## Software solutions that work out of the box

The EPU 2, EPU Quality Monitor and Data Management software tools are ready to use from day one. They are set up and supported by our service and digital solutions engineers and our application specialists. We are committed to continuously improving the EPU experience.



Figure 5: EPU Quality Monitor and Data Management (powered by Athena) software allow EPU data to be accessed remotely.

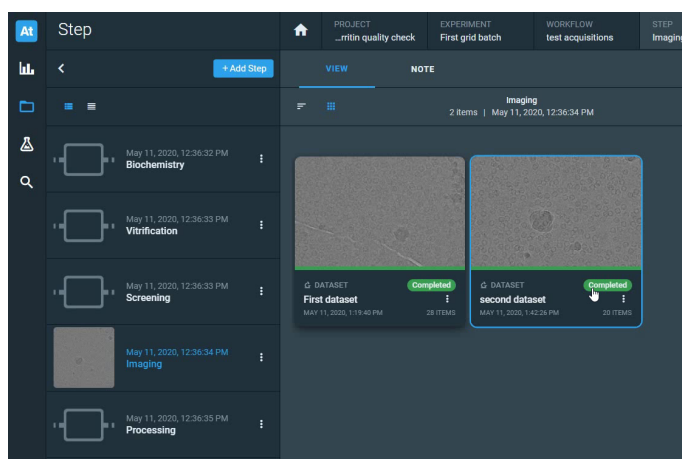


Figure 6: EPU Data Management (powered by Athena) software helps to organize, view, and share SPA data.

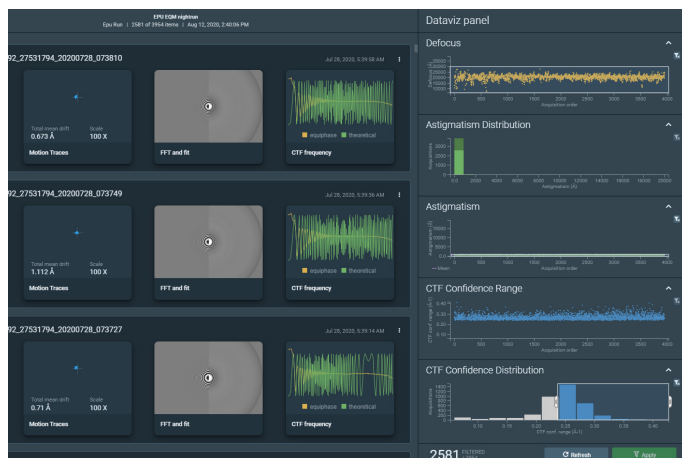


Figure 7: EPU Quality Monitor software analyzes and visualizes the quality of EPU data on-the-fly.

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