

# 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.

Discovering the 3D structure of a protein is key to understanding biological systems.

Biological research is increasingly focusing on unraveling interactive processes and pathways at the molecular level. For this, a high-resolution transmission electron microscopy (TEM) is indispensable. At the same time, being able to image macromolecular complexes in their native, hydrated state is of paramount importance.

Cryo-electron microscopy single particle analysis (cryo-EM SPA) has become an established technique for 3D image creation. A large number of vitrified complexes are imaged at low electron dose conditions, which ultimately – after conformational classification and particle averaging – results in a high-resolution 3D representation. Due to the low signal-to-noise ratio in the recorded images, obtaining high-resolution information is dependent on the averaging of large amounts of well-aligned 2D data. EPU automated screening and data acquisition has been developed to efficiently collect these large, high-quality datasets consisting of vitrified particles within a thin layer of amorphous ice.

This implies that the software should provide a solution for both identifying suitable areas for data collection (i.e. screening for the best sample areas) and for efficiently acquiring the large number of high-resolution images needed for subsequent 3D image reconstruction and processing.

The Thermo Scientific™ EPU 2 software facilitates these crucial steps. With sample screening functionalities and full control of the Autoloader, it provides efficient grid screening capabilities.

The advanced automation and simplified user interface of the EPU 2 software ensure that setting up the data acquisition scheme is faster and easier than ever before.

### Key benefits

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

Enhanced ease-of-use. Systematic user guidance combined with automated alignments allows for easy operation from one single interface.

Efficient screening capabilities. Unattended batch screening of up to 12 grids, creating overview atlases and categorizing the ice film quality for guided selection of grid squares.

Professional software. The software is professionally maintained by a service organization and supported by application specialists.

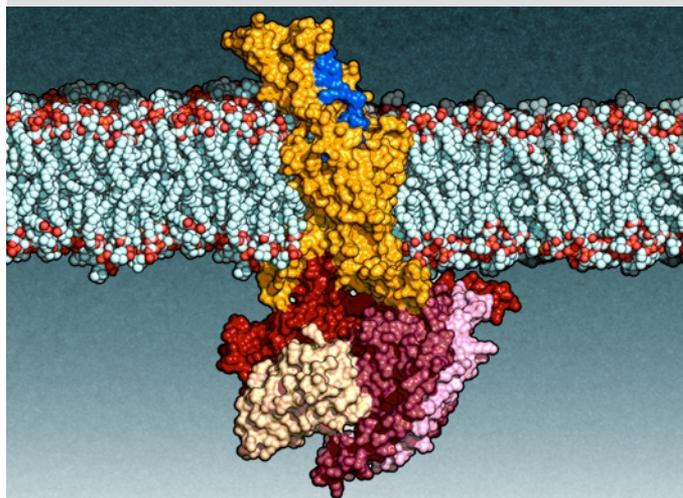


Figure 1. EPU software enables the unattended acquisition of thousands of cryo-EM micrographs that are used to reconstruct protein representations, such as the G protein-coupled receptor shown here. Courtesy Prof. Patrick Sexton and Dr. Alisa Glukhova at Monash Institute of Pharmaceutical Sciences, Monash University.

## Planning for Success

EPU 2 software enables the automated collection of numerous vitrified particles from pre-selected areas on a grid.

Prior to data collection, a few simple set-up steps are required, and the new EPU 2 user interface will guide you through the SPA experiment in a user-friendly manner.

1. All essential settings for data acquisition are located in the Preparation tab (Figure 2). During the Preparation step, the preset is defined for camera settings and optics presets are defined for all subsequent steps in the screening and data acquisition process.
2. The second step controls the Auto Functions, which allow you to verify that the microscope is properly aligned for high resolution data acquisition. Automated user alignments such as Auto-Eucentricity, Auto-Focus, Auto-Coma and Auto-Stigmatate can be executed via the EPU user interface (Figure 3).

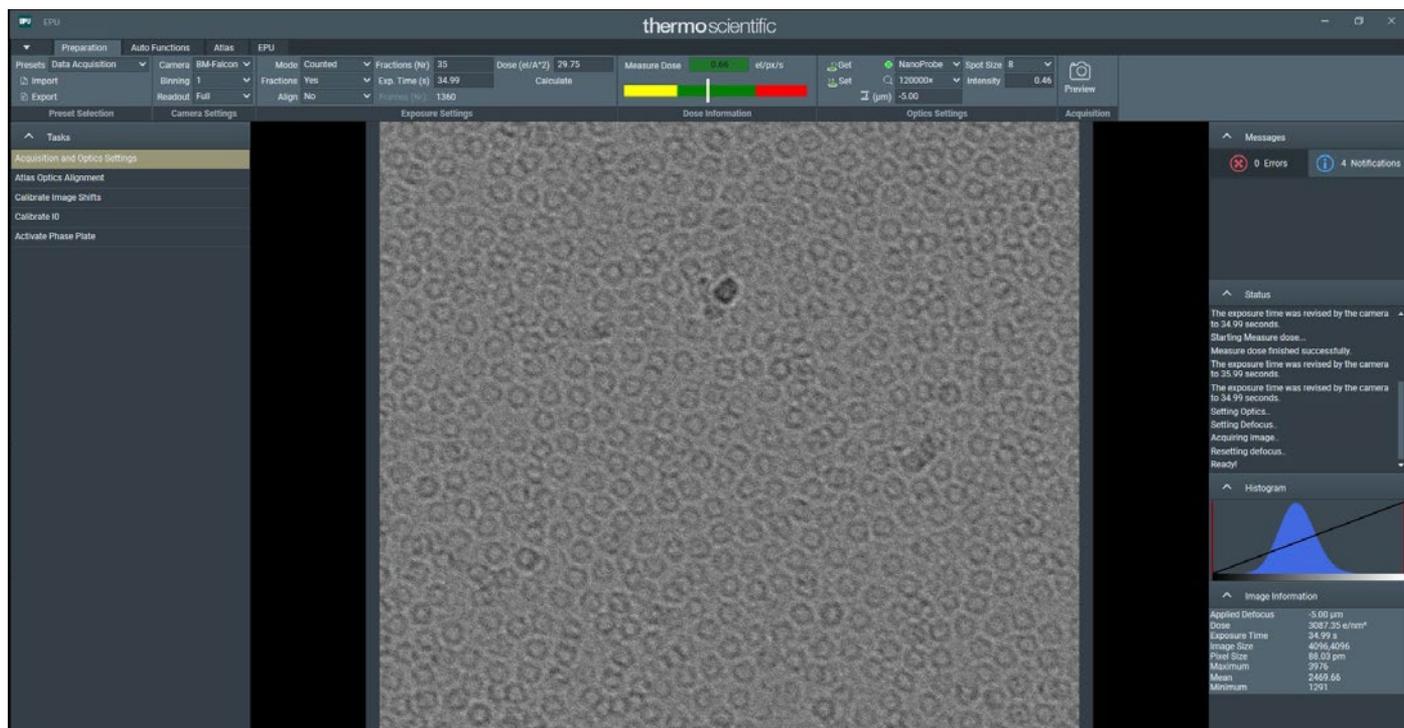


Figure 2: All camera and optical presets for the subsequent steps are defined in the Preparation tab

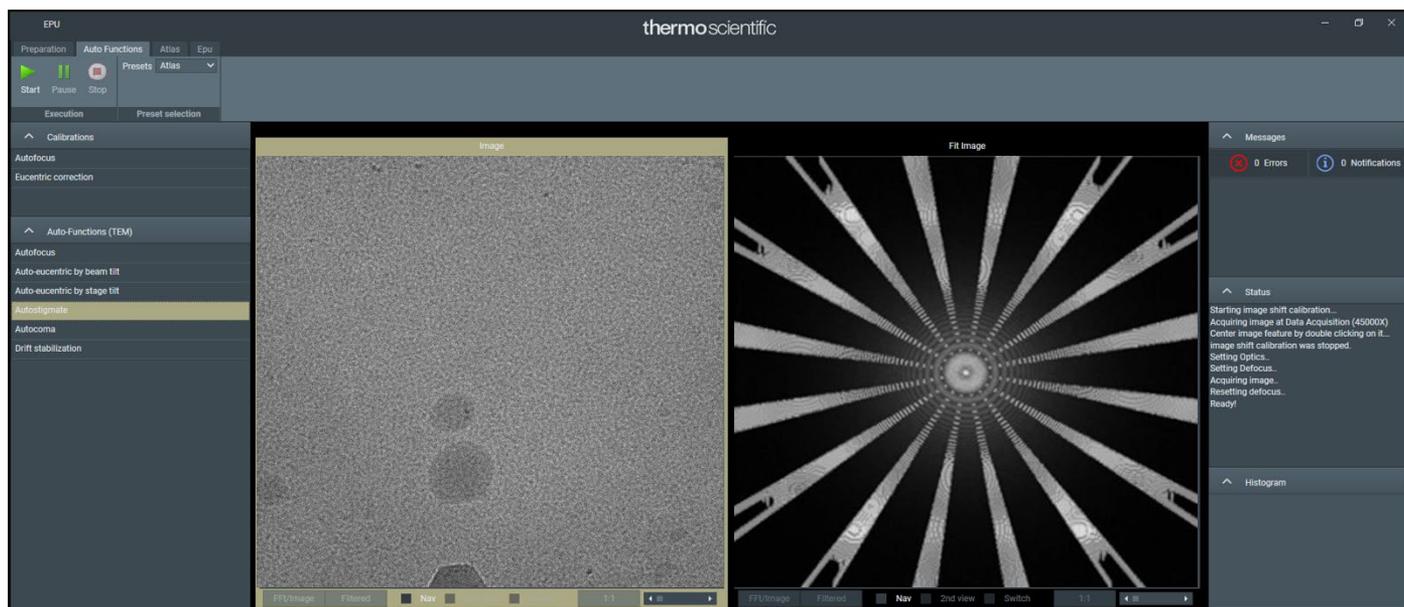


Figure 3: EPU 2 software automates all daily alignments needed for an experimental run

- In the Atlas step, users will be guided through grid screening functionalities.

To determine whether vitrified grids are of sufficient quality for an automated recording session, a grid survey is performed. For this purpose, an atlas of the entire grid is automatically recorded. In addition, with full control of the Autoloader, all 12 grids in the Autoloader cassette can be batch-screened.

Subsequently, the software examines the ice film's quality (presence and thickness) and automatically groups it into a number of categories defined by user input for the grid square (Figure 4).

- In the final EPU step the data collection is executed. The grid squares that meet the quality requirement can be selected for acquisition. This can be done either manually or automatically by applying user-defined filter settings.

Subsequently, a personalized, specific recording scheme or template can be set, visualizing where the acquisitions should be done in the selected holes. The acquisition scheme is graphically displayed (Figure 5). Upon pushing the Acquisition button, the process is initiated.

The experiment will now run unattended. High resolution images will be collected based on the defined template pattern at each selected grid square.

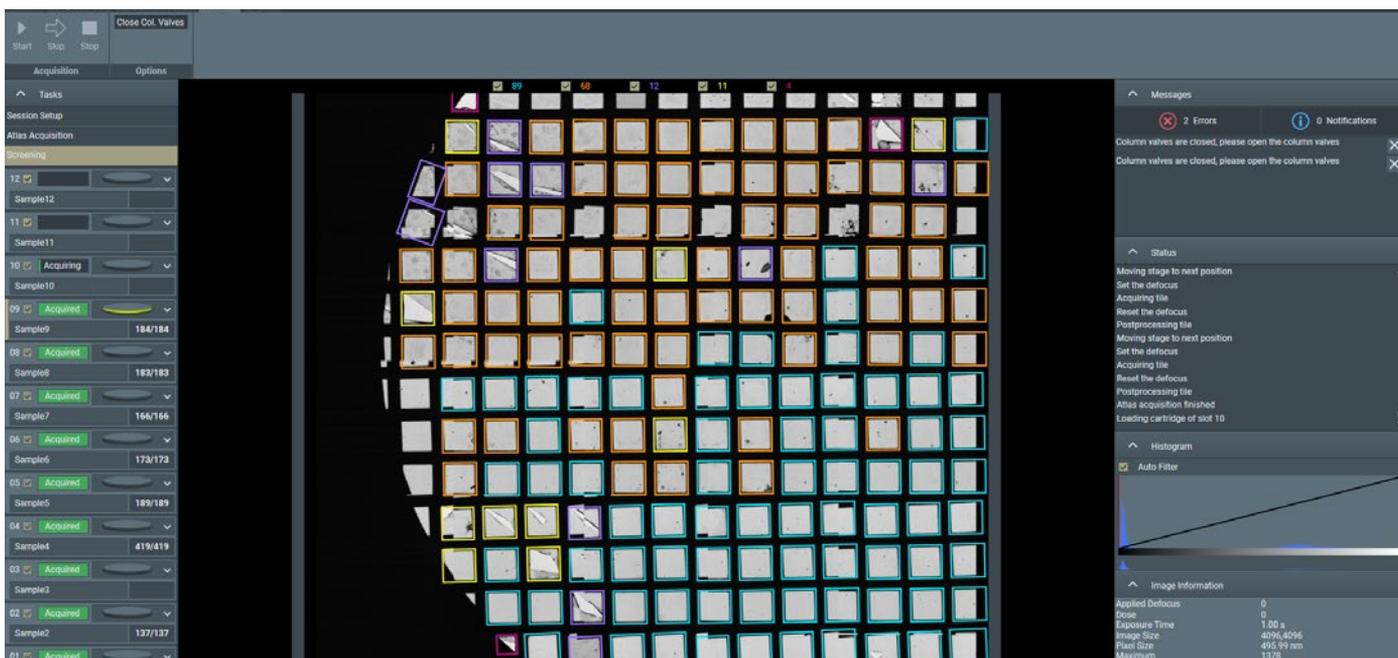


Figure 4: EPU 2 software has integrated Autoloader functionality and performs grid surveying for efficient screening.

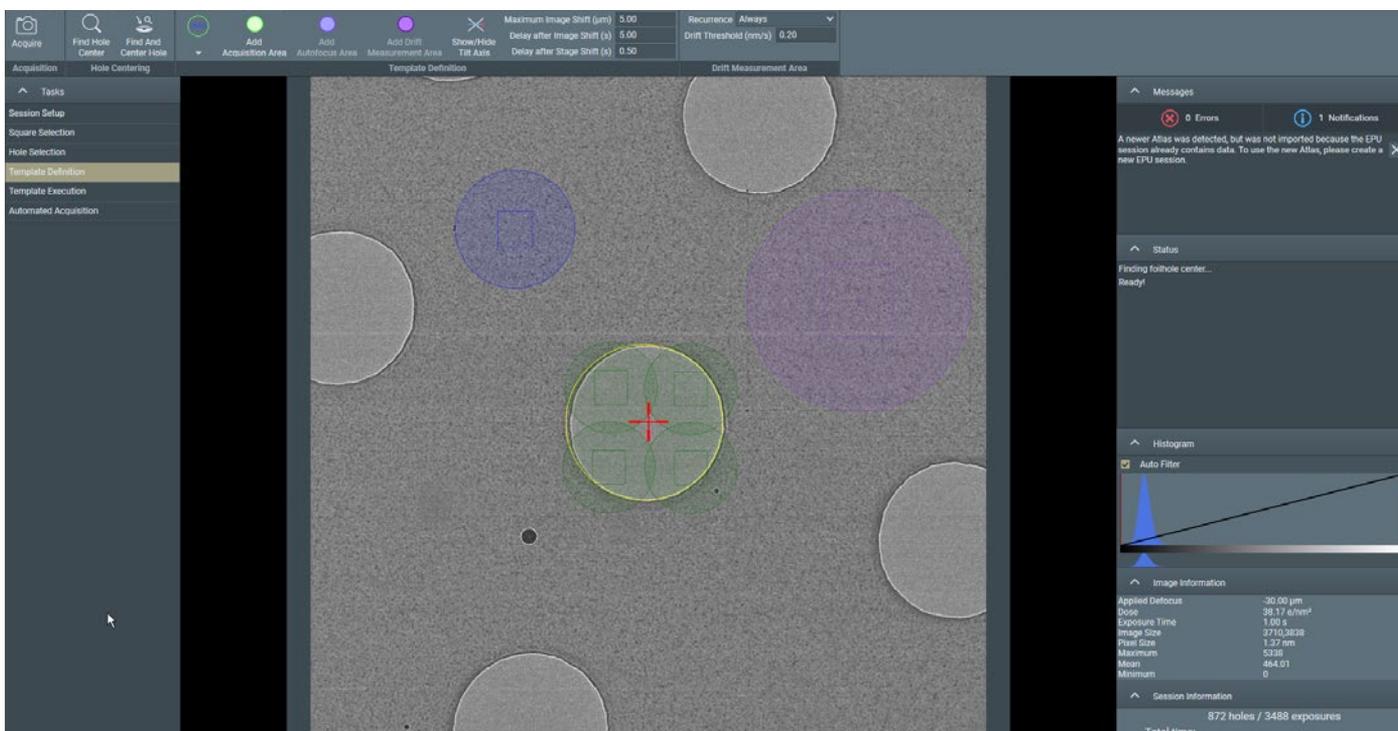


Figure 5: The acquisition scheme. Acquisition, drift, and focusing positions are graphically displayed for the user's convenience.

## **Professional support**

The EPU software is ready to use from day one. Being professional software, our service organization is committed to keep it running and up-to-date. With growing application support staff we are ready to support you for training.

## **Ensuring efficiency and ease-of-use**

Throughput of the entire cryo-EM SPA workflow is an important measure of success. Since collecting numerous images is a time-consuming process, it is essential that data collection software, such as EPU, contributes to its simplicity and ensures high throughput.

EPU 2 software also provides a solution for the automatic recording of single particles. Its new intuitive user interface, which includes automation features and screening capabilities, allows for straightforward planning of single particle experiments for all user expertise levels.

Find out more at [thermofisher.com/EM-Sales](https://thermofisher.com/EM-Sales)