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DATASHEET

EPU Software

Automated Data Acquisition Software for the Single Particle Analysis Workflow

3D imaging is key to understanding biological systems.

Fundamental research within the scope of structural biology, cell biology, and tissue biology is increasingly focusing on unraveling interactive biological processes and pathways at the molecular level. For this, 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.

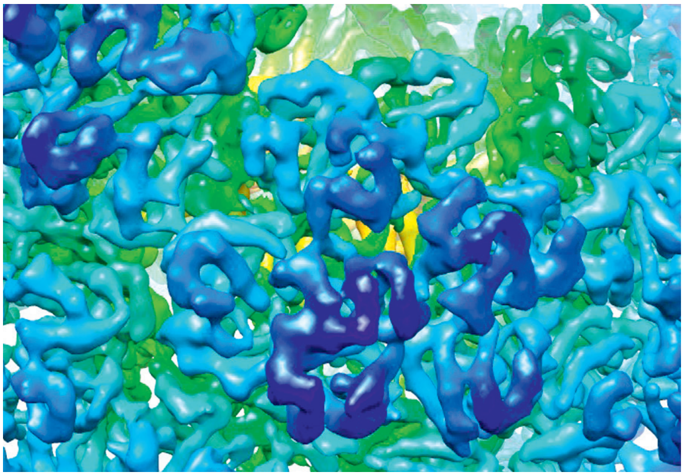
A crucial step for obtaining 3D structural information is the availability of application software that can acquire multiple 2D images of the specimen under investigation. During single particle analysis, a large number of vitrified, low-contrast complexes are imaged at low electron dose conditions, which ultimately—after conformational classification and particle averaging—results in a high-resolution 3D representation.

Cryo-electron microscopy (cryo-EM) for single particle analysis is approaching atomic resolution for biological samples. Since biological material is highly sensitive to electron radiation, an approach which utilizes an extremely low dose of electrons is used to keep specimen damage to a minimum. 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.

The efficient collection of a large number of images requires a high degree of automation. Thermo Scientific™ EPU Software was developed specifically to optimize the automated collection of single particle analysis datasets. These datasets consist of thousands of images containing up to hundreds of thousands of particles for further reconstruction. The actual reconstruction is performed by various open source software applications.

EPU Software will facilitate the process of optimal area selection – the first crucial step of the total single particle analysis workflow. It also facilitates high-throughput data collection from the microscope, which is a primary prerequisite for high resolution 3D imaging.

Key benefits
Microscope-embedded solution for single particle analysis data acquisition
Optimized for high-throughput particle collection
Recording schemes defined for commonly used grid types
Intuitive, graphic display of experimental set-up
Compatible with CCD cameras, and direct electron detectors
Designed for Krios™, Glacios™ and Talos™ TEM platforms



Lumbricus terrestris hemoglobin
Dr. Sacha De Carlo and Dr. Gert Oostergetel, NeCEN, The Netherlands.

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Planning for Success

EPU Software enables the automated collection of a large number of vitrified particles from preselected areas on a grid. Prior to data collection, a few simple set-up steps are required:

- Determination of the grid quality. To judge whether a vitrified grid is a proper candidate for an automated recording session, a grid survey is performed. For this purpose, an atlas is automatically recorded of the entire grid. Potential grid squares for investigation should contain thin, electron-translucent ice-filled holes and be devoid of broken carbon (Figure 1).
- Upon selection of the grid squares that meet the quality requirements, individual foil holes containing amorphous, vitreous ice of the proper thickness can be selected (at higher magnification). This can be done either manually or automatically by applying user definable filter settings (Figure 2).
- Subsequently, a specific recording scheme or template can be defined, visualizing at which locations in the selected holes the acquisitions should be done. The acquisition scheme is graphically displayed for the user's convenience (Figure 3).
- Pressing the Start Run button will initiate data acquisition on the foil holes that were defined in the location selection of the setup. The experiment will now run unattended. The stage will move to the center of the first selected square. All detected holes are now checked via the ice thickness filters mentioned above. Only those holes that meet the criteria will be used in the experiment. The stage will move to the center hole and start executing the template pattern as defined previously. The stage will visit all holes in an outward spiraling way. The complete procedure will be repeated from the start for each selected grid square.

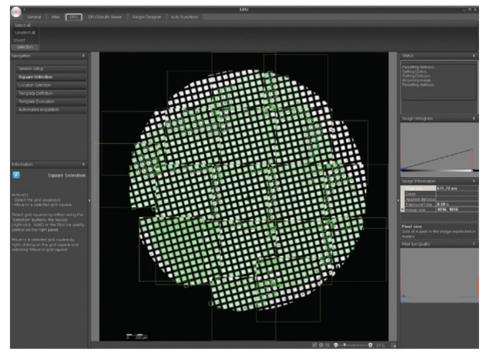

Figure 1:
The square selection pane displays the same atlas overview image, but with all detected grid squares selected by default (green).

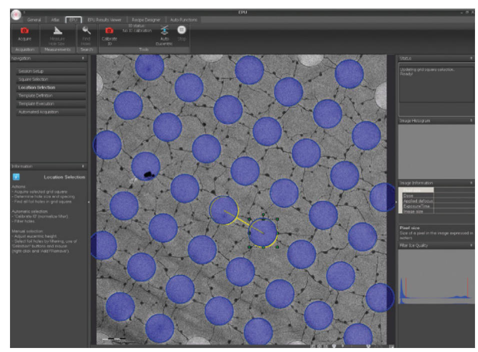

Figure 2:
Selection of the holes inside the grid squares.

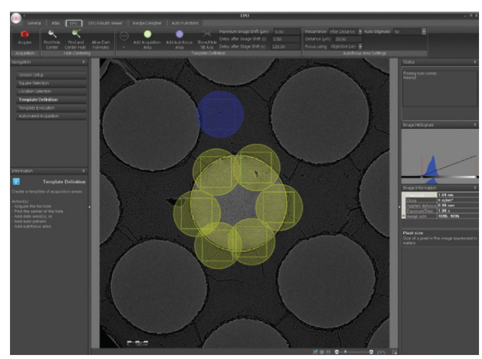

Figure 3:
Targeting the template for recording of the selected holes.

Single Particle Analysis Workflow

With the introduction of EPU, we have set another milestone in optimizing the workflow for single particle analysis. Combined with existing solutions available for sample preparation (Thermo Scientific Vitrobot™) and 3D imaging (Thermo Scientific Krios, Thermo Scientific Glacios, Thermo Scientific Talos Arctica, Thermo Scientific Falcon™ 3EC), EPU Software provides a solution for the automatic recording of single particles. Its intuitive user interface allows for straightforward planning of single particle experiments and thus contributes to the scientific challenges and endeavors of the structural biology community.

Find out more at **thermofisher.com/EM-Sales**

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