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doi:10.1017/S143192761901417X
Microsc. Microanal. 25 (Suppl 2), 2019

High-End Data Collection for Single-Particle Cryo-EM

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Single-particle cryogenic transmission electron microscopy (cryo-EM) can be used to elucidate the 3D structure of macromolecular complexes. The sample is embedded in a thin layer of vitreous ice and maintained at liquid nitrogen temperature. It is then imaged directly in the microscope and a 3D reconstruction may be calculated from projections of individual macromolecular complexes by determining the orientations of the projections. For many years cryo-EM was limited to low-resolution, but recent advances have made it a method of choice for the determination of high-resolution structures, termed as "The Resolution Revolution" [1]. Sub-3 Å resolution structures are now published every week and sub-2 Å resolution structures are also reported [2-4]. There are several contributors to this progress. The first one is the development of a new generation of electron detectors recording images with unprecedented quality. The second one is the availability of highly stable and fully automated electron microscopes allowing long unattended operation and automated data collection. The last one is the simultaneous development of improved image processing procedures. The synergy between all these breakthroughs led to cryo-EM structures with unrivalled final resolution.

These developments deeply modified the way of collecting cryo-EM data. Currently, after only a few hours of setup, data collection can run in a fully unattended and automated way for several days, producing dataset of 10,000 micrographs or more. Such an amount of data led to new challenges in term of data handling, storage and processing and new tools were required to tackle these questions. This tutorial will present how high-end data collection is performed at the EMBL Heidelberg cryo-EM platform, insisting on some of the key steps of the workflow: (a) Expectations and needs in term of sample quality; how to optimize the sample for cryo-EM (buffer screening, cross-linking…); how to overcome grid freezing problems (surface treatment, support films…). (b) Setting up a high-throughput and fully automated data collection using SerialEM [5]. (c) "On the fly" pre-processing and monitoring of the data during acquisition using WARP [6], allowing rapid inspection and validation of the running acquisition. (d) Alternative collection strategies for samples with very low concentration and uneven spreading on the grid [7] or exhibiting preferential orientations [8].

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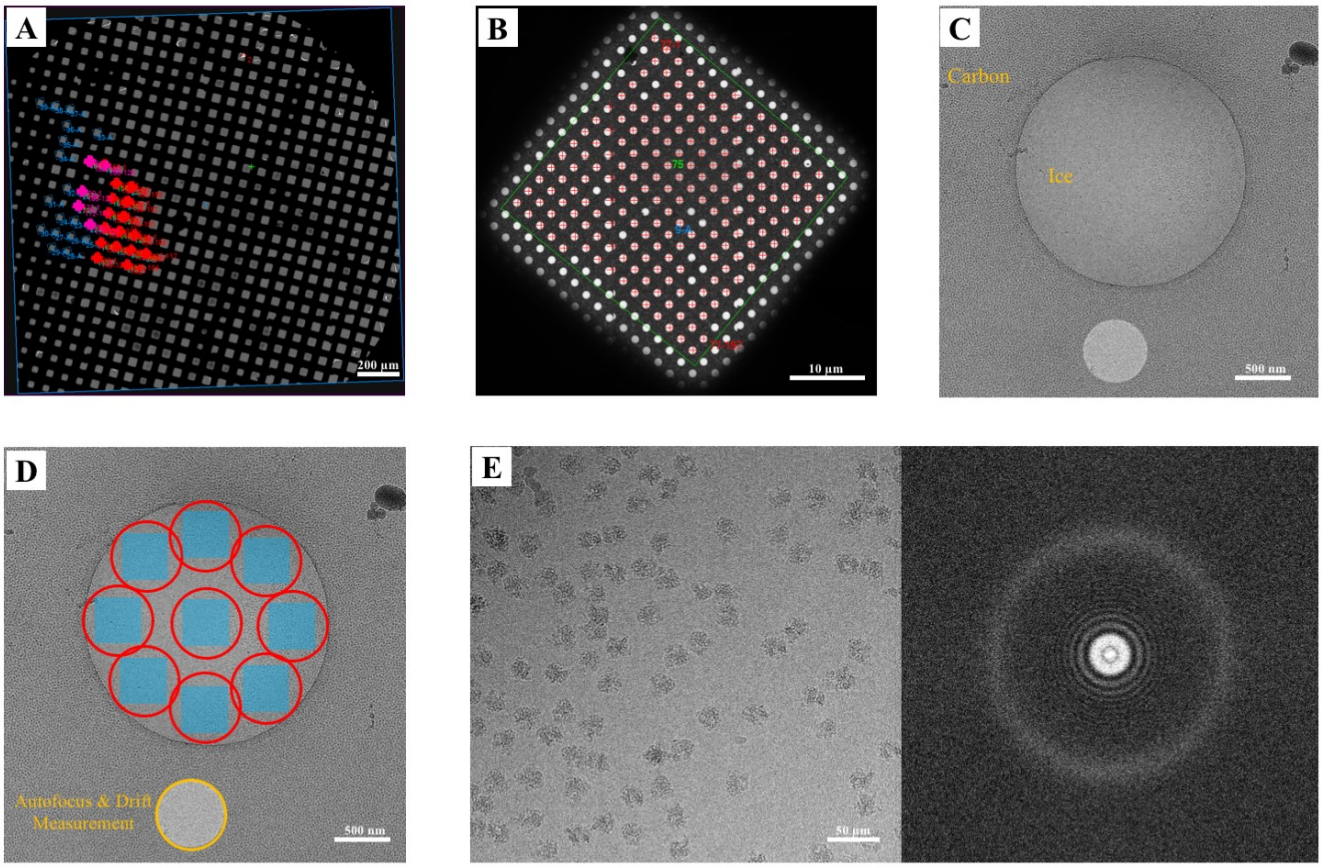

Figure 1. Single particle automated acquisition workflow in SerialEM. (A) Grid map generated at low magnification, used to select the squares of interest. (B) Square map generated at the lowest SA magnification, used to select the holes for data collection. (C) Hole image generated at intermediary magnification (x15,000); the hole will be subsequently centered. (D) Acquisition pattern; the size of the beam at high magnification (x130,000) corresponds to the red circle, the area covered by the camera corresponds to the blue square, the area used for autofocus function and drift measurement is on the carbon next to the hole. (E) Representative micrograph (1.04 Å/pixel) of *Escherichia coli* 70S ribosome with its corresponding power spectrum.

https://doi.org/10.1017/S143192761901417X Published online by Cambridge University Press