

# Stream processing of CryoElectron Microscopy images at the acquisition site

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## Abstract

Cryo-Electron Microscopy (CryoEM) has established as one of the key techniques in Structural Biology. The throughput of electron microscopes for cryo-samples of biological macromolecules is steadily increasing in image size and rate. This large increase in acquisition speed must be accompanied by a tight control on the quality of the acquisition so that the amount unusable data is minimized. This is accomplished by an online analysis of the images being acquired. The two most typical monitored parameters are the average frame drift over time and the microscope defocus as proxies of the stability of the acquisition and correction of the optical setup. In this abstract, we show that the quality of the sample itself can also be monitored by automatically finding particles in the micrographs, classifying them in two-dimensions, and assigning in real-time the newly acquired particles to the found classes. This quality may change over time depending on the ice thickness and the quality of the region of the grid being analyzed. If the quality is low, then the acquisition may be shifted to some other region or the grid changed.

In this poster, we introduce the use of Scipion for this purpose. Scipion is a workflow engine especially designed for image processing in CryoEM. We have carefully analyzed the bottlenecks affecting our software performance and optimized it so that the image analysis can be performed in real-time. The executed workflows are highly configurable and every facility can design its own image analysis pipeline including the most popular image analysis packages (motioncorr2, relion, cistem, cryoSPARC, Xmipp, gctf, gautomatch, sphire, etc.). Additionally, Scipion and Xmipp bring quality control protocols that warns the microscope operator if the acquisition goes out of specifications. Finally, HTML reports of the acquisition status can be generated and, if desired, made accessible from outside the facility, so that the user can have automatic feedback on his/her acquisition in real-time.