CRYO ARM™ series microscopes for cryo-EM in structural analysis of proteins and viruses

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Introduction

Cryo-EM has seen an enormous increase in capabilities and potential in recent years owing to a number of technological advances, e.g. direct detector devices and improved scope automation. JEOL released two electron cryo-microscopes in 2017 specifically designed for automated and unattended, continuous operation at 200 and 300 kV, the CRYO ARM™ series. A recent update on both type of CRYO ARMs has the potential of increasing the throughput well beyond the current limit of 20,000 images/day, namely north of 50,000 images/day as well as extending the resolution to nearly true atomic resolution, i.e. 1.2Å.

Minimum Fringe Illumination and Aberration Free Image Shift

In order to increase the throughput to current levels two techniques have been critically important. One is the reduction of Fresnel fringes from the CL apertures when matching the beam diameter to the field of view of the camera. JEOL has introduced Köhler illumination on the CRYO ARM series microscopes to achieve this (Fig. 1). For the CRYO ARM™ 200 this results in Minimum Fringe Illumination, whereas on the CRYO ARM™ 300 this is implemented as Zero-Fringe Illumination. The second technique is the implementation of the Aberration Free Image Shift, where a narrow parallel beam gets steered inside the hole of a Quantifoil specimen using beam shift and image shift. This approach can be extended to cover multiple adjacent holes with an optional coma correction. (Fig. 1).

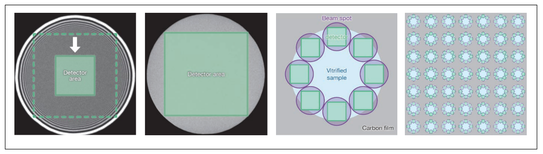

Fig. 1: Minimum (or Zero) Fringe Illumination implemented on the JEOL CRYO ARM™ series electron cryo-microscopes. Using Köhler illumination the Fresnel fringes arising from the CL apertures (left) can minimized allowing the beam diameter to be matched to the field of view of the detector without sacrificing usable area (middle left). Using beam shift and image shift with an optional correction for coma, the narrow, parallel beam can be steered inside a Quantifoil hole to create an intra-hole multi-record (middle right). Extending this to adjacent creates the inter-hole multi-record, here depicted as a 7x7x8 pattern (right).

Long-term stability

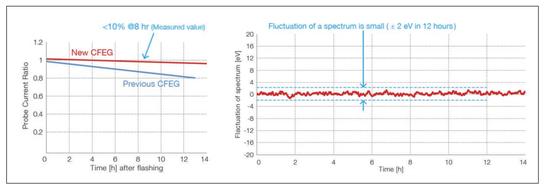

Fig. 2: Improvement of the CFEG reveals minimal requirements for flashing the tip (left). The Omega-style energy filter now shows minimal drift of the Zero Loss peak (right).

To enable long running imaging sessions two other areas were improved, i.e. stability of the Cold Field Emission gun (CFEG) and the Omega-style in-column energy filter. The CFEG requires periodic flashing to reset the emission to 100%. The second-generation CFEG can now operate for an entire day without requiring this (Fig. 2). If flashing is needed, remote control software will interrupt the imaging run, flash the tip and continue, a process which takes < 1 min. No adjustment of coma is required. The stability of the energy filter has also been dramatically improved. Both these improvements not only dramatically increase run times for imaging, but also minimize the amount of time users need to spend with the microscope resulting in better throughput. Finally, tuning of the Omega filter is now done automatically.

Interoperability

In order to optimize inter-operability between JEOL and other brands of cryo microscopes it is imperative to be able to transfer frozen-hydrated grids between microscopes. From fig. 3 we can glean that reverse transfers work. Minimal ice contamination is present on the sample after the reverse and subsequent second cryo-transfer.

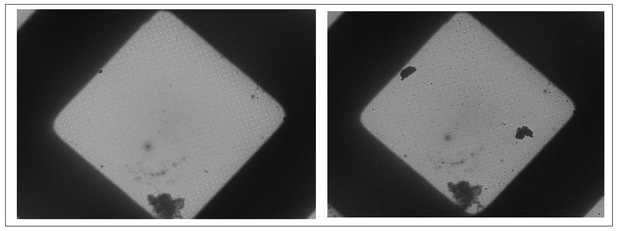

Fig. 3: Results after first cryo-transfer (left) and after a reverse and subsequent cryo-transfer into a CRYO ARM 300 II (right). Minimal ice contamination accumulates during the second transfer.

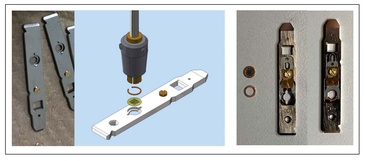

Fig. 4: Standard cartridges for the CRYO ARM™ series microscopes (left) and the AG-compatible cartridges (right).

Figure 4 shows the main cartridges used in the CRYO ARM™ series electron cryo-microscopes for imaging in SPA workflows and single-axis tomography. For complete inter-operability with non-JEOL brand cryo-microscopes JEOL have developed a cartridge that is compatible with AG-clipped grids (Fig. 4). Whereas the standard cartridge uses a c-clip to secure the grid, the AG-cartridge employs a sliding cover.

Figure 5 shows results obtained from apo-ferritin using an AG-compatible cartridge after only 30 minutes of data collection. The grid square image does not show significantly larger amounts of ice-contamination. The 3D map was obtained using only 150 micrographs and from the FSC curve can be seen to have a resolution of 2Å.

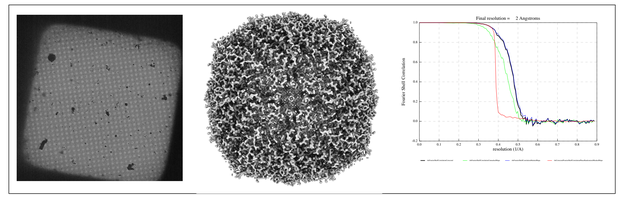

Fig. 5: Results from apo-ferritin sample in an AG-compatible cartridge.

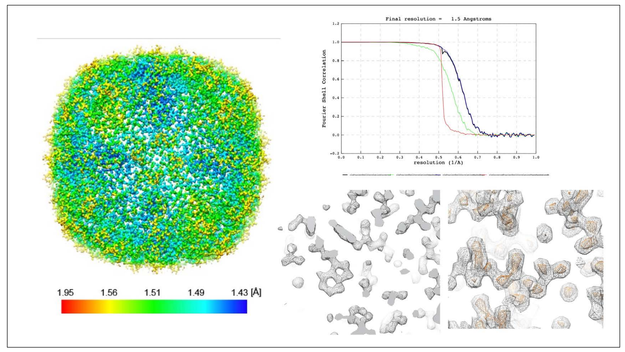

Fig. 6: Results from apo-ferritin obtained to determine the amount of data/time needed to break 1.5Å. The results were obtained after only 30 minutes.

Throughput challenge I

Figure 6 shows results from an experiment attempted to determine how much data is needed to break 1.5Å resolution using apo-ferritin mounted in a standard cartridge. On the CRYO ARM™ 300 II this took only a half hour of data collection. In fact, the core of the particle shows a resolution of better than 1.5Å strongly suggesting that a well-behaved sample can yield usable data in about an hour. Whereas elsewhere it has been suggested that screening ought to be done using a dedicated tool, these results suggest that not only can screening be done effectively on a CRYO ARM™ 300 II - note that sample transfer and retrieval does **NOT** affect already loaded samples - but these results also give a clear indication how well behaved the sample is and thus a good indication how far the data can be pushed.

Throughput challenge II

Figure 7 shows results from pushing the data collection and processing as far as it would go within reasonable time frames. A total of 7500 micrographs were collected from which 500,000 particles were selected. Processing yielded a 1.29Å close to the limits discussed by Yip et al. (Nature 587 (2020) 157. The CFEG clearly gives data that is extremely close to be officially called atomic resolution, but in a standard package in for a price considerably less than the other-brand cryo-microscopes.

Final note: all the data collection is done using serialEM employing a multi-record scheme with a 5x5x4 pattern imaged on a K3 camera

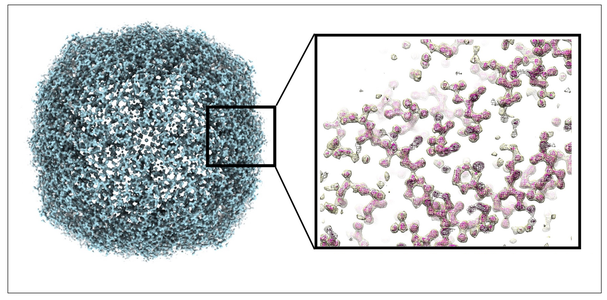

Fig. 7: Results from apo-ferritin sample. Final map is at 1.29Å resolution.

Teaser

Ask us about our CRYO ARM™ 200 II electron cryo-microscope. Also, ask us about our uptime guarantee.

1. **The JEOL CRYO ARM™ series electron cryo-microscopes enable researchers to get fast results**
2. **The JEOL CRYO ARM™ series electron cryo-microscopes allow researchers to get nearly atomic resolution data**
3. **The JEOL CRYO ARM™ series enjoys impressively robust usage times of more than a year with single interruption**