High resolution Micro-Electron Diffraction of protein crystals with Falcon 4(i)

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Over the past century, X-ray crystallography has revealed the structures of thousands of macromolecules. Despite these great advances, the requirement for large, well-ordered crystals remains a bottleneck for this technique. Growing protein crystals is difficult, requires a lot of time and effort and is sometimes even not possible. However, quite often plenty of microcrystals and nanocrystals are produced during crystallization trials. Such crystals are regarded as useless for conventional X-ray diffraction experiments. Recently, microcrystal electron diffraction (MicroED) combined crystallography and cryo-electron microscopy (cryo-EM) into a method that can be used for high-resolution structure determination. Since electrons interact strongly with matter and are less damaging per scattering event, electron diffraction can produce high-resolution data from crystals that are an order of magnitude smaller. Compared to the traditional synchrotron and X-ray free electron laser, the microED method is much easier to access and has similar throughput. During rotation of the 3D nanocrystal in a high-energy electron beam the diffracted signal is recorded on the electron detector in a same manner as done for X-ray crystallography.

Direct electron detectors and energy filters have shown to improve the data quality and the resolution significantly with single particle analysis and cryo-electron tomography. This is because of the improved camera DQE and the removal of inelastically scattered electrons. Here we will show the use of Falcon 4(i) to achieve electron diffraction at atomic resolution using proteins and small molecule crystals. Surprisingly, Falcon 4 can still be used in counting mode, despite the high electron flux in the diffraction spots.