Optimized Electron Diffraction Detection and Data Acquisition in Diffraction Tomography: A Complete Solution by Gatan

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Abstract : Continuous electron diffraction tomography, also known as microcrystal electron diffraction (MicroED) or three-dimensional electron diffraction (3DED), is a powerful technique, which in combination with cryo-electron microscopy (cryo-ED), can provide atomic-scale 3D information about the crystal structure and composition of different classes of crystalline materials such as proteins, peptides, and small molecules. Unlike the well-established X-ray crystallography method, 3DED does not require large single crystals and can collect accurate electron diffraction data from crystals as small as 50 – 100 nm. This is a critical advantage as growing larger crystals, as required by X-ray crystallography methods, is often very difficult, time-consuming, and expensive. In most cases, specimens studied via 3DED method are electron beam sensitive, which means there is a limitation on the maximum amount of electron dose one can use to collect the required data for a high-resolution structure determination. Therefore, collecting data using a conventional scintillator-based fiber coupled camera brings additional challenges. This is because of the inherent noise introduced during the electron-to-photon conversion in the scintillator and transfer of light via the fibers to the sensor, which results in a poor signal-to-noise ratio and requires a relatively higher and commonly specimen-damaging electron dose rates, especially for protein crystals. As in other cryo-EM techniques, damage to the specimen can be mitigated if a direct detection camera is used which provides a high signal-to-noise ratio at low electron doses. In this work, we have used two classes of such detectors from Gatan, namely the K3® camera (a monolithic active pixel sensor) and Stela™ (that utilizes DECTRIS hybrid-pixel technology), to address this problem. The K3 is an electron counting detector optimized for low-dose applications (like structural biology cryo-EM), and Stela is also a counting electron detector but optimized for diffraction applications with high speed and high dynamic range. Lastly, data collection workflows, including crystal screening, microscope optics setup (for imaging and diffraction), stage height adjustment at each crystal position, and tomogram acquisition, can be one of the other challenges of the 3DED technique. Traditionally this has been all done manually or in a partly automated fashion using open-source software and scripting, requiring long hours on the microscope (extra cost) and extensive user interaction with the system. We have recently introduced Latitude® D in DigitalMicrograph® software, which is compatible with all pre- and post-energy-filter Gatan cameras and enables 3DED data acquisition in an automated and optimized fashion. Higher quality 3DED data enables structure determination with higher confidence, while automated workflows allow these to be completed considerably faster than before. Using multiple examples, this work will demonstrate how to direct detection electron counting cameras enhance 3DED results (3 to better than 1 Angstrom) for protein and small molecule structure determination. We will also show how Latitude D software facilitates collecting such data in an integrated and fully automated user interface.

Keywords : continuous electron diffraction tomography, direct detection, diffraction, Latitude D, Digitalmicrograph, proteins, small molecules
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