Continuous Electron Diffraction Tomography with Gatan Electron Counting Cameras and Latitude® D

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Continuous electron diffraction tomography, also referred to as Microcrystal electron diffraction (MicroED) or Three-dimensional electron diffraction, is a powerful technique used for determining high-resolution structures of chemical compounds and biological macromolecules by placing the specimen crystal in a transmission electron microscope (TEM) and continuously tilting the stage while collecting diffraction patterns [1,2]. These diffraction patterns from the microcrystals are then used to reconstruct a 3D electron density map of the crystal structure. The microED technique has been used to solve structures of various proteins [2,3], small molecules [4], zeolites [5], metal-organic frameworks [6], and covalent organic frameworks [7], demonstrating the versatility and effectiveness of microED in characterizing complex molecular structures in different scientific domains. Unlike X-ray crystallography, which uses high-intensity X-rays and requires large, well-ordered crystals, microED can be performed with a low-dose electron beam on crystals just a few nanometers in size [2]. This makes microED valuable for studying small crystals that are susceptible to radiation-induced damage. The susceptibility of the specimen to beam-induced damage constrains the amount of electron dose that can be used to gather data with microED, thereby increasing the difficulty in obtaining high-resolution structures. This also poses a limitation on using scintillator-based fiber optic cameras to acquire high-quality data due to noise generated during the conversion of electrons to photons in the scintillator, the transfer of light through the fibers to the sensor, and the frame readout. Increasing the electron dose on the specimen can partially address this problem by improving the signal-to-noise ratio (SNR). However, this increases beam-induced damage to the specimen, significantly affecting the data quality. Therefore, researchers must carefully balance the need for high-quality data with the need to minimize damage to the sample when using the microED technique. Direct detection electron counting cameras, such as Gatan K3®, Stela™ [8], Alpine™, and Metro™, are more advantageous than scintillator-based fiber optic cameras for microED due to their ability to count individual electrons, resulting in significantly improved SNR and detective quantum efficiency at all spatial frequencies. Electron counting eliminates signal read noise and variability caused by electron scattering, ensuring accurate measurement of diffraction spot intensities, especially at high-resolution frequencies with lower amplitudes. This work will demonstrate the superior diffraction capabilities of various Gatan electron counting direct detectors and provide a guide for effectively acquiring high-quality electron diffraction data using these cameras. MicroED data collection involves various steps, including repetitive switching between imaging and diffraction mode in TEM optics, screening specimen crystals, adjusting the mechanical eucentric height, and capturing diffraction data while tilting the stage at an optimal speed. This process has traditionally been performed manually or with semi- automated scripts and tools, which can lead to inefficiencies and errors in data collection. In recent years, several software platforms have been employed to improve automation during microED data collection. Here we will present Latitude® D as a turnkey solution that offers automation and optimization for microED data collection, resulting in greater efficiency with Gatan cameras.

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