

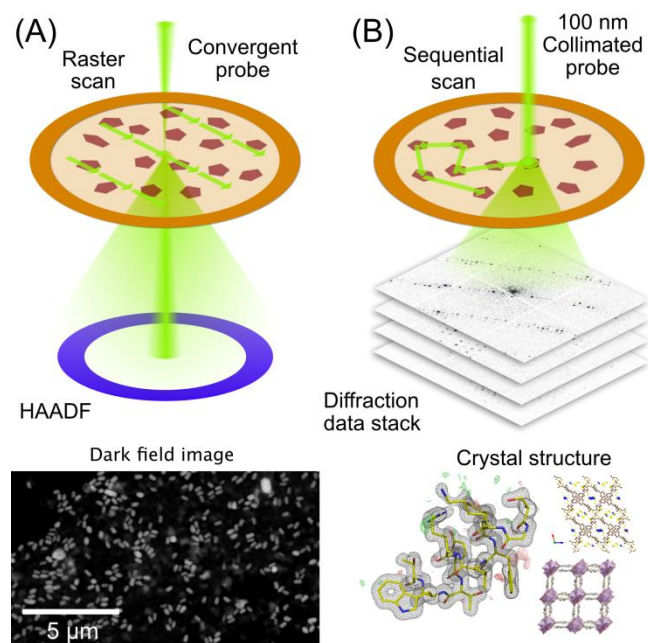
## Serial Electron Crystallography: New Developments for Data Collection and Analysis

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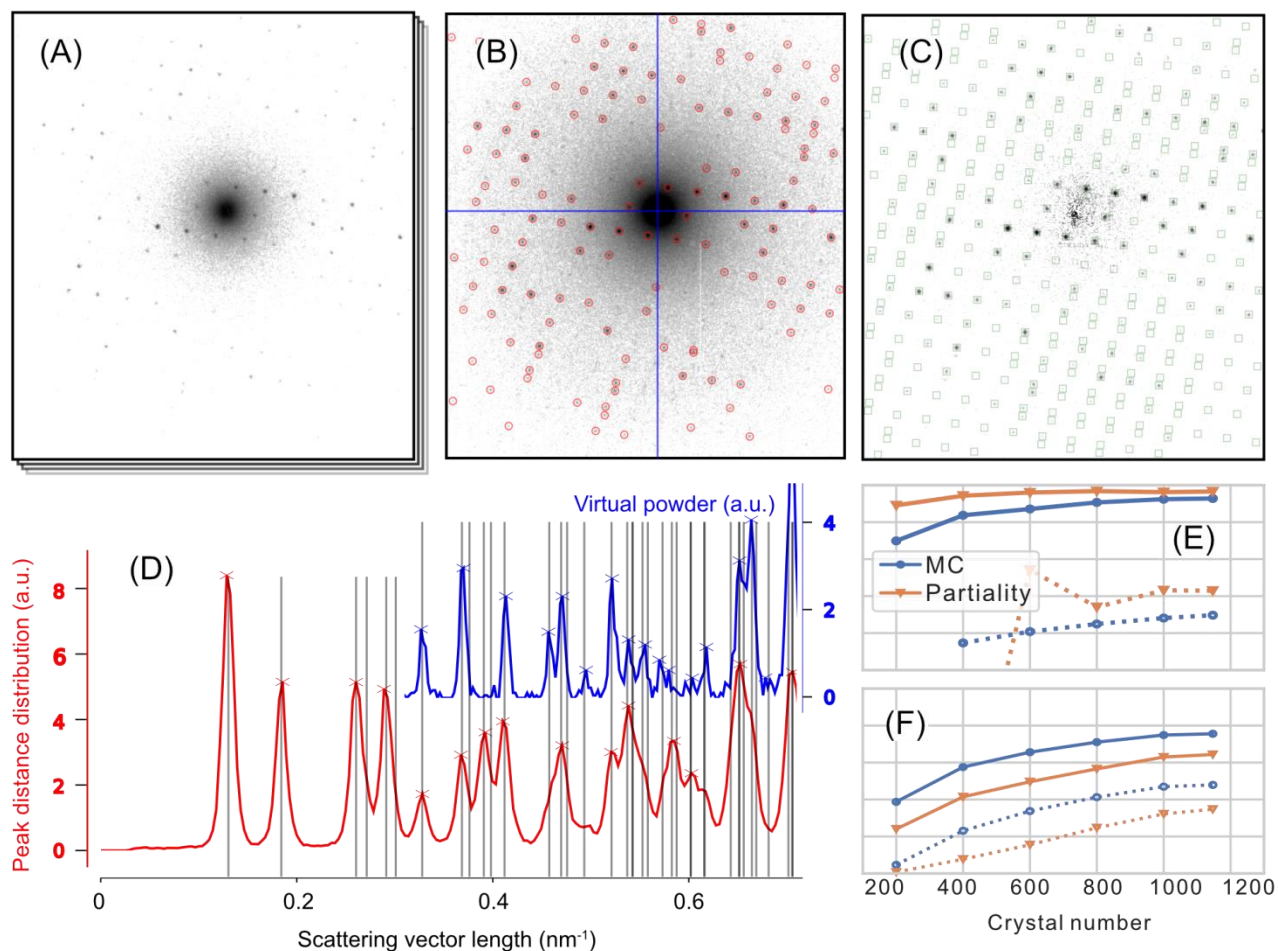
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Serial electron diffraction (SerialED) (Smeets, Zou and Wan, 2018; Bücker *et al.*, 2020) is an emerging three-dimensional electron diffraction (3D-ED) method (Gemmi *et al.*, 2019; Nannenga and Gonen, 2019), where data from a large ensemble of macromolecular or small-molecule nano-crystals is merged into a high-resolution structure solution. Like in serial X-ray crystallography at synchrotrons and X-ray free-electron lasers (Mehrabi *et al.*, 2020), SerialED is typically performed in the limit of rapidly acquired single or dose-fractionated diffraction shots per diffracting volume, minimizing deleterious effects of radiation damage. However, the flexibility of a scanning TEM (S/TEM) enables a much broader range of data collection strategies, blurring the lines between serial crystallography (SX), scanning electron nano-diffraction (SEND), and massively automated rotation electron diffraction (RED). Furthermore, the flexibility of SerialED along with the large volume and unique nature of the obtained diffraction data, that is, purely two-dimensional reciprocal-space slices of unknown orientation, call for new approaches for data processing (Jiang *et al.*, 2009; Smeets and Wan, 2017; Bücker, Hogan-Lamarre and Miller, 2020; Gevorkov *et al.*, 2020).

We present results from a range of biological and organic samples such as in-situ grown proteins, covalent and metal-organic frameworks (COFs/MOFs), highlighting the excellent performance of SerialED in obtaining structures of highly radiation sensitive crystals, as well as robustness against contaminated and inhomogeneous specimens. Also, considerations regarding the optimal data collection and analysis strategies for a given sample, as well as various methods of determining unknown unit cells from two-dimensional snapshot data will be discussed. The recently developed software package *diffractions* (Bücker, Hogan-Lamarre and Miller, 2020), is introduced, which allows for easy pre-processing and data reduction of many-terabytes SerialED data sets, and wraps *CrystFEL* (White *et al.*, 2012) for the tasks of crystal orientation determination and merging of partially excited reflections. Finally, an outlook on ongoing projects towards time-resolved SerialED, and serial/scanning ED on filamentous samples, is given.



**Figure 1.** Scheme of a SerialED experiment. (A) A sample region containing nano-crystals is screened in STEM low-mag real-space imaging. (B) Diffraction patterns from each crystal are acquired in rapid succession by steering the beam to each crystal. From the resulting patterns, the crystal structure is reconstructed.



**Figure 2.** Steps of SerialED analysis. (A) Schematic stack of dose-fractionated frames comprising a single diffraction pattern snapshot. (B) Aggregated pattern, i.e., partial sum over frames in (A), with found peaks and pattern center annotated. (C) Background-corrected pattern after indexing, with Bragg reflection positions predicted from the found crystal unit cell and pose annotated. (A–C) are shown on the same logarithmic scale. (D) Histogram of radial peak positions (blue) and pair-wise peak distances (red) aggregated over many tetragonal lysozyme nano-crystals. The data allows to refine the unit cell parameters by fitting predicted d-spacings (grey lines). (E) Validation metrics CC1/2 and completeness for a protein-crystal dataset increase as a function of merged crystal numbers. A clear difference is found between merging strategies with (orange) or without (blue) taking into account partiality.

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