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Unifying 3D electron diffraction and serial electron diffraction into a high-resolution, high-accuracy and high-throughput structural analysis technique

Xiaodong Zou

Department of Materials and Environmental Chemistry, Stockholm University, United States

Knowing the 3D atomic structure of a material or molecule is crucial for understanding its properties and functions. X-ray crystallography is the main technique for atomic structure determination of all crystalline compounds, with > 1 100 000 structures in the Cambridge Structure Database and > 170.000 entries in the Protein Data Bank. One major limitation of X-ray crystallography is the demand of large crystals (> 5 μm^3). Electron crystallography can be used for studying nano- and micrometer-sized crystals that are too small to be studied by X-ray diffraction. A major breakthrough has been the development of 3D electron diffraction (3D ED) techniques.[1-3] which has revolutionized structural analysis of nano- and micron-sized crystals.[4] A unique advantage of 3D ED is that sub-Ångström data resolution (0.5 -1.0 Å) can be easily achieved on a standard TEM. The resolution depends mainly on the sample or beam damage, not the microscope. 3D ED has been applied for crystal structure determination of a wide range of materials and molecules, from inorganic materials, organic compounds to macromolecules. [4]

A common feature of 3D electron diffraction (3D ED) is that a series of ED patterns are collected at different angles while the crystal is tilted around an arbitrary goniometer axis. A 3D reciprocal lattice is reconstructed from the 3D ED data, from which the unit cell parameters and space group can be determined (Figure 1a). The intensities of diffraction spots are extracted and used for determination of atomic positions, in a similar way as for X-ray diffraction. Standard X-ray crystallography software can be used for data processing (e.g. XDS, Dials), structure solution and refinement (e.g. SHELX, Jana).

My group developed protocol and software *RED* for collection of step-wise rotation electron diffraction (RED) data, by combining beam tilt and goniometer tilt. [5] We later developed software *Instamatic* for high-throughput collection of continuous rotation electron diffraction (cRED) data, by which it is also possible to track the movement of the crystal during tilting, using defocused diffraction patterns and applying a set of lens changes.[6] A DigitalMicrograph script *InsteaDMatic* was also developed for automated cRED data acquisition[7], which can be used on any TEM with a Gatan detector. Today, a complete 3D ED dataset can be obtained in less than a minute. By applying 3D ED, my group has determined more than 200 crystal structures, ranging from inorganic materials,[8] organic compounds[9] to protein crystals[10] (Figure 2). We show *ab initio* structure determination from 3D ED data is robust. Despite dynamical effects, atomic positions determined from cRED data are very accurate, on average within 0.02-0.10 Å when compared to those determined by X-ray diffraction[11].

To automate data collection and data analysis, we developed serial electron diffraction (SerialED) [12] by taking ED patterns (snapshots) of individual particles. ED patterns from more than > 3500 particles can be collected automatically in less than one hour on a standard TEM (Figure 1b). We also combined data collection by SerialED with cRED and developed SerialRED to perform fully automated 3D ED data collection, including automated crystal screening, crystal tracking, data collection[13]. A typical data collection screens up to 500 crystals per hour, and cRED data are collected from suitable crystals. We developed a data processing pipeline to process the SerialRED datasets, and implemented hierarchical cluster analysis to group and identify the different phases present in the sample and to find the best matching datasets to be merged for subsequent structural analysis. SerialED and SerialRED provide new

possibilities to obtain high resolution data from very beam sensitive crystals. The large number of datasets from different particles makes it possible for phase analysis, and for detection of minor phases in the sample.

In conclusion, 3D ED is a promising technique for high-resolution, high-accuracy and high-throughput structural analysis, complementary to X-ray diffraction and cryo-EM.

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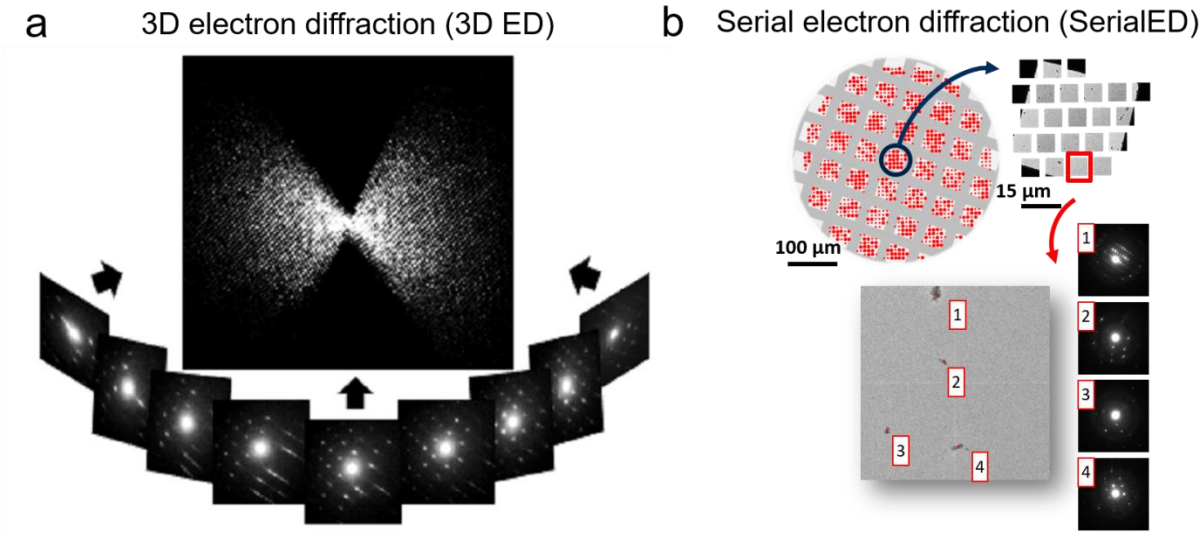

Figure 1. Illustration of (a) 3D electron diffraction (3D ED) and (b) Serial electron diffraction (SerialED).

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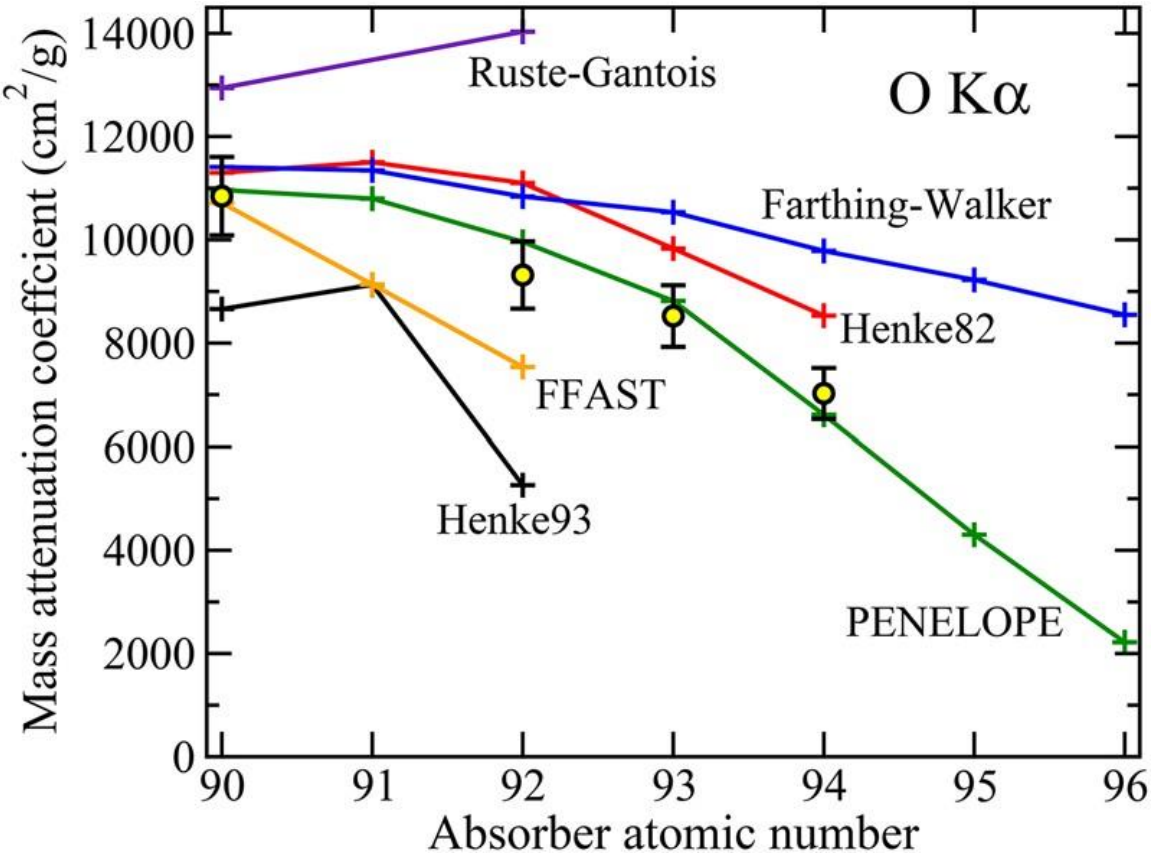

Figure 2. Selected examples of structures determined by 3D electron diffraction in my group.

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