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Acquisition and Analysis of Serial Electron Diffraction Data for Structure Determination

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Crystallography has transformed science by giving us the means to visualize matter at an atomic level thus providing vital insights on structure-function relationships. This technique exploits a beam of X-rays or electrons to strike a crystal generating diffraction patterns that are computationally processed and refined to determine the crystal structure [1,2]. Although numerous technologies have been established to accomplish this goal, generating well-ordered and high-diffracting crystals is challenging. Therefore, we have developed a method to generate the crystal nuclei required to give better quality crystals. This strategy holds the potential as a high throughput method for electron diffraction requiring minimal sample and can be explored to generate much smaller nanocrystals compatible with electron crystallography and applied for serial electron diffraction (SerialED) analysis. SerialED is an emerging structural determination tool that sequentially hits thousands of randomly oriented nanocrystals with electrons. SerialED significantly minimizes the radiation damage compared to microED in which crystals are rotated during the data collection [3].

In this work, SerialED has been implemented in a scanning transmission electron microscope (STEM). A darkfield STEM image of the sample is obtained using a focused electron probe for crystal mapping (Figure 1-A). The free lens control is then used for adjusting the beam condition to get a nearly parallel nanoprobe for diffraction (Figure 1-B,C). Data collection has been done on a Hitachi HT7700 TEM using the Azorus package developed by Hitachi High-Tech Canada Inc. and Python scripting. The crystal mapping is performed by a plugin custom developed by the author in Azorus using the *diffractem* package [4]. The crystals are selected automatically by segmenting the image to locate the center of each segment. The software then moves the electron probe to each crystal coordinate sequentially and captures the diffraction pattern on the camera. By this method, hundreds of diffraction patterns can be collected in a short amount of time with minimal electron beam damage on the sample.

As a proof of principle, we present SerialED data of guanine nanocrystals and the indexed results which is shown in Figure 2. Through crystallographic data processing the crystal structure can then be resolved. The prospects of applying these methods to various samples and solving the structure from the acquired data will also be discussed.

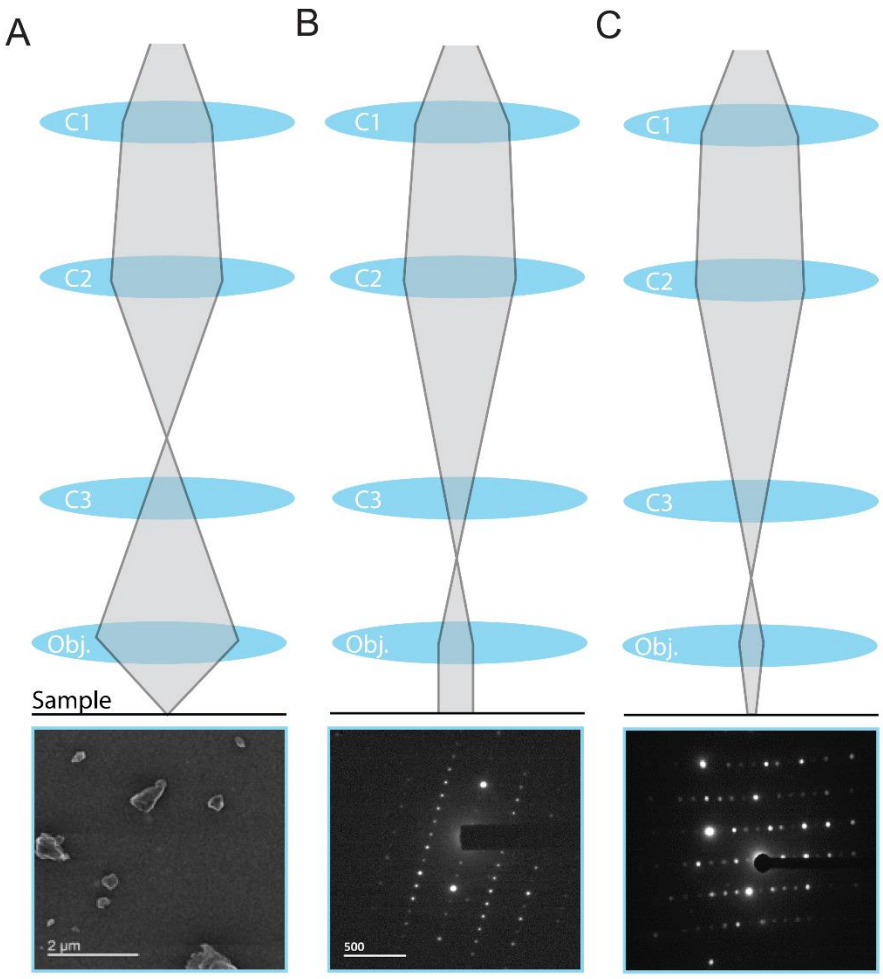

Figure 1. The electron beam probe in the STEM mode can be set to different configurations by adjusting the condenser lenses (C1, C2, C3). **(A)** Focused beam suitable for high resolution STEM imaging. **(B)** Collimated beam suitable for diffraction of large structures, like proteins. **(C)** Semi-collimated beam suitable for nano-diffraction of small molecules.

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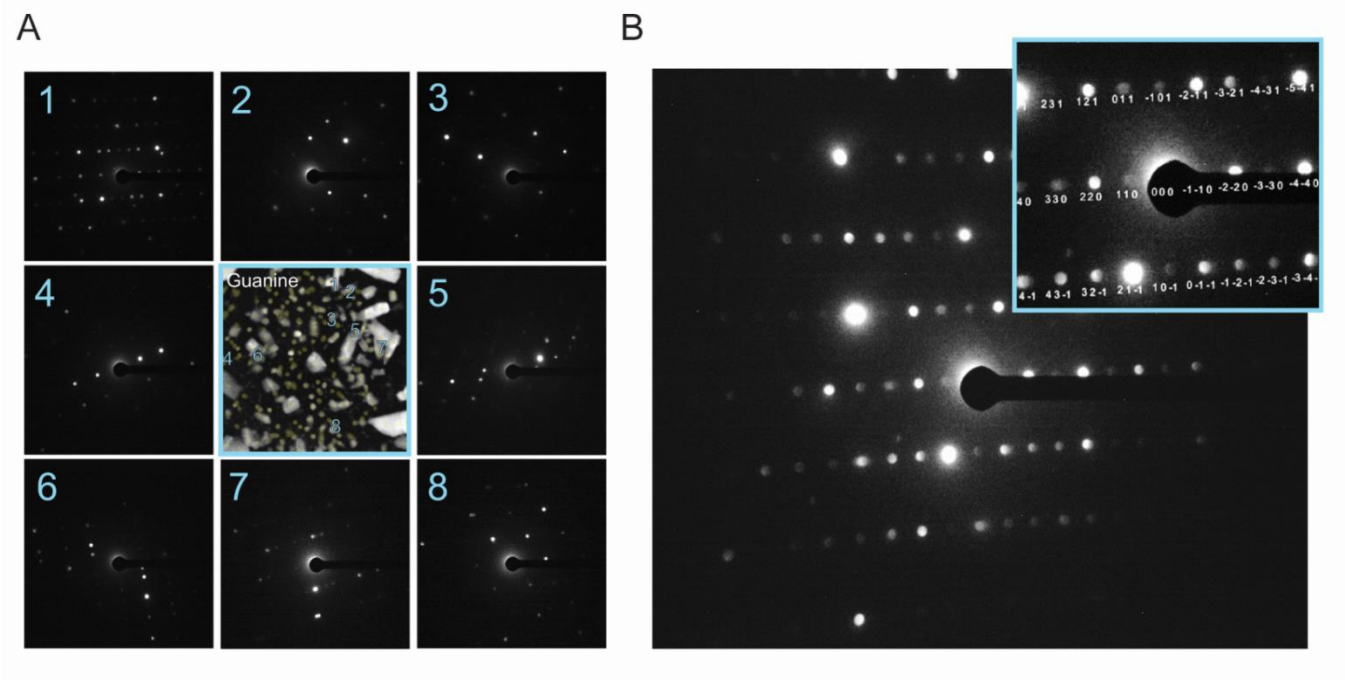

Figure 2. (A) Serial electron diffraction (SerialED) of guanine nanocrystals. Subfigures 1-8 show a series of diffraction images selected from 276 patterns obtained from the STEM image section of the Guanine sample. SerialED diffractograms were obtained on a Hitachi HT7700 at the Centre for Nanostructure Imaging (CNI) at the University of Toronto. **(B)** Indexed guanine diffraction image from SerialED data. The result of the indexing of one of the diffraction patterns obtained shows the [1 -1 1] zone axis of the guanine crystal. Indexing was carried out using the CrysTBox software [5, 6].

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