Progress Towards More Accurate and Precise MicroED Measurements

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MicroED is a technique that can generate *ab initio* structures from nanoscale crystals (for example, proteins 14-240 kDa in mass, molecules even smaller) [1, 2]. Diffraction patterns acquired under parallel illumination conditions while continuously rotating the goniometer produce movies that can be processed using established X-ray data reduction programs [4] used to generate structural solutions.

Comparisons of X-ray to electron diffraction data must account for significant discrepancies in the wavelengths of the incident quanta (which result in a substantial change in the radii of the corresponding Ewald spheres) and changes in atomic form factor (due to the different, but related, cross sections experience by X-rays and electrons). These differences add to constricted sampling of the reciprocal lattice due to experimental geometry to limit the precision to which the basis and lattice can be determined. Without an internal calibration for each recorded dataset, lattice parameters may be imprecise below to the level of 5-10%.

Comparisons of X-ray and electron diffraction data must account for several intrinsic differences between the two techniques. TEM electron wavelengths generate Ewald spheres with substantially larger radii than their X-ray counterparts. Furthermore, electrons' inherent charge and mass produce different atomic scattering amplitudes relative to form factors for uncharged, massless X-ray photons (linked by the Mott-Bethe formula). In addition, the limited tilt range of the TEM restricts sampling of reciprocal space. Overall, differences in both fundamental physical properties and experimental geometries combine to limit the precision to which the basis and lattice constants can be determined. Without an internal calibration standard for each recorded dataset, unit cell parameters may be imprecise at the level of 5-10%.

We will discuss how a graphene oxide support structure can be used to increase the precision of the XDS input parameters to better than 1% at the time of acquisition (the calibration standard and the crystal of interest are sampled simultaneously). This allows for a precise determination of (a) the camera length used in the experiment and (b) the location of the centre of the diffraction pattern. Although the camera length and the electron wavelength cannot be determined independently from this type of standard, we can use precise values of fundamental constants to determine the electron wavelength to the accuracy given by the TEM manufacturer's high-tension specification (typically around 1%). Other parameters, such as the oscillation range (a value denoting the thickness of each slice through reciprocal space, expressed in degrees), cannot be determined directly from the goniometer due to mechanical difficulties in measuring the exact angle at which a given diffraction pattern was acquired during continuous rotation (thanks to delays between shutter activation and camera readout, etc.). Here we use statistical analysis of the observed R-factor to determine the oscillation range at the 0.92±0.01°, or ~1% level, see inset of Figure 1.

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Progress in accurate and precise 3D ED measurements is not only useful in identification of new biochemical compounds, but also in determination of small molecule crystals too small to be analyzed by X-ray methods. In addition, the compatibility of 3D ED workflows with nearly all TEMs, from low-level instruments to state-of-the-art platforms, enables wide access to high speed *ab initio* ED crystallography. [5]
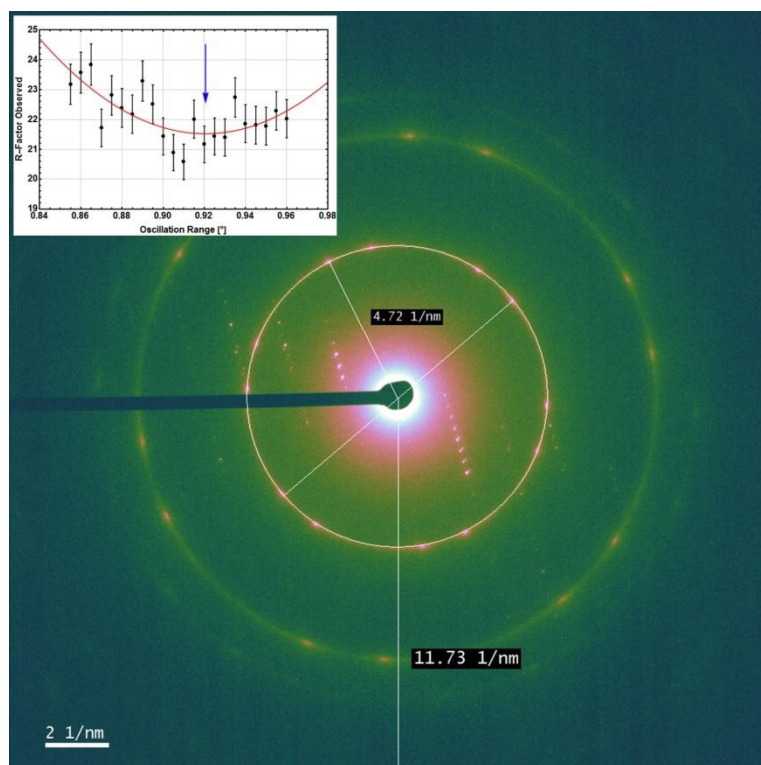

Figure 1. A SAED of Jacobsen's ligand acquired on a TFS Talos F200C, inset a determination of the oscillation range using error-minimization techniques. A ~1 um sized crystal of N,N′-bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexanediamine (546.8 Da in mass) on top of several graphene oxide flakes producing both Friedel pairs and Debye–Scherrer rings. Friedel pairs can be used for precise beam center determination, whereas both Friedel pairs and Debye-Scherrer rings provide an internal calibration standard. The inset shows statistical analysis of the oscillation range which results in the least-square determination of the lowest R-factor. The blue arrow indicates the minimum.

References:

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