

Seeing Is Believing: Visualizing Submicron Crystals as A first Step Towards Direct Protein Sample Preparation for Microed Experiments

Miranda L Lynch¹, Elizabeth Snell¹, Sarah EJ Bowman¹

¹Hauptman-Woodward Medical Research Institute

mlynch@hwi.buffalo.edu

Microcrystal electron diffraction (microED) has experienced incredible growth as a powerful technique for structure determination, and has seen increased use for structures of small molecules and peptides, and for an expanding range of protein targets. The growth of application of microED has spawned a concomitant expansion of methodologies enabling use of this structural technique. A salient feature of microED for protein structure determination is the requirement for generation of ultrasmall crystalline protein samples, typically in the submicron size range. Given this requirement, a host of methods for generating optimally sized crystals for microED have emerged, spanning techniques for processing macrocrystals into the appropriate size range (via crushing, vortexing, FIB milling, etc) to direct growth of submicron-sized crystals. Both of these methods for sample preparation present unique challenges. Protein macrocrystal processing is subject to the same primary bottleneck affecting conventional X-ray crystallography -- that of generating large, diffraction-quality crystal samples. When used for microED, these crystals also need to be sufficiently robust to maintain their lattice after manipulation. There is evidence that many protein samples that fail to produce large crystals needed for conventional macromolecular X-ray diffraction can in fact produce small, seemingly 'unusable' crystals. The separate approach to develop techniques that attempt to detect and handle those samples has been more limited. The first step to making those small, 'unusable' samples available for microED is to reliably detect and characterize the crystals. These submicron particles are not amenable to the standard brightfield imaging methods typically employed in screening for protein crystallization. In this work, we describe our techniques for using nonlinear optical microscopy and multiphoton tools for submicron crystal detection, with a focus on SHG (second harmonic generation). We discuss how use of SHG can inform on sample properties (such as crystal sizes and homogeneity of the sample) as well as crystal localization within the drop. We also present our novel analysis methods for image processing. We make use of complex wavelet transforms for image fusion of signals from SHG and UV-TPEF (a multiphoton method) microscopies, to combine the unique features of each imaging modality for informing on both sample crystallinity and whether crystals are protein containing. We also use our complex wavelet transforms for image enhancement and denoising the signals, to maximize the visualization power of these imaging modalities. The dual-tree complex wavelet transforms we employ for image processing provide nearly shift-invariant processing of the image signals, and have substantial improvement in directional selectivity over standard discrete wavelet transforms typically used in image analysis. Given the good performance of these complex transforms for handling photon shot noise in images (which forms a large portion of the noise in photon-counting detectors such as used in SHG signal generation), they provide a major advantage for our goal of not just seeing at the submicron scale, but also localizing signals within the sample volume. We discuss how the localization properties that arise out of our specific analysis tools can lead towards better sample handling and possibly contribute to in situ sample preparation.