Peak Intensity Analysis for Serial Femtosecond Crystallography Experiments at the Compact X-ray Light Source

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This research introduces innovative Bragg peak integration methods to advance peak intensity analysis in X-ray crystallography, crucial for accurate structure determination. The Compact X-ray light source (CXLS) will produce femtosecond duration X-ray pulses, allowing the collection of "diffraction before destruction" data. However, the flux will be limited compared to traditional full-scale X-ray free electron laser facilities, warranting the development of unique data analysis tools to fully exploit the unique capabilities of these sources for macromolecular crystallography. By improving the differentiation between signal and noise, coupled with a novel data collection scheme, our approach will enable experiments at the compact X-ray light source at Arizona State University.

Experimentally, out method enables two essential serial crystallography benefits to be maintained despite the limited flux; namely, radiation damage free data collection by exposing the crystal to a single femtosecond X-ray pulse and minimizing the crystal size (e.g. required for accurate time-resolution during pump-probe experiments, containing protein production costs etc.). Enabled by our unique time-over-threshold integration Dectris Eiger detector, after the initial single X-ray pulse low-signal, low fluence image has been collected a second diffraction pattern of the same crystal will be collected for which multiple X-ray pulses will be integrated until sufficient signal is obtained for robust indexing. The indexing solution from the second high dose, high damage diffraction pattern can be then applied to the initial signal-shot diffraction pattern, and a damage-free dataset assembled.

At the heart of our data analysis methodology are two critical components: the *PeakThresholdProcessor* for identifying peaks via threshold analysis, and *ArrayRegion* for managing and extracting data regions of interest. These tools enable precise peak region extraction and interactive exploration, leading to a 3D scatter plot visualization of peak spatial distribution and intensity.

This work unfolded in three progressive phases, each building upon the last to introduce more sophisticated data processing techniques: (1) The first phase, Background Subtraction (*h5_background_subtraction_10_2_23.py*), establishes the foundation with core Python functions and classes for managing HDF5 files and threshold processing. (2) The second, Stream Background Subtraction (*h5_stream_background_subtraction_10_2_23.py*), adapts the initial methodology to handle stream files, (indexing results as produces by the software package CrystFEL[1]), focusing on dictionary creation and intensity array reconstruction for enhanced peak and surrounding value calculation. (3) Finally, the Overwrite Background Subtraction, (*h5_stream_background_subtraction_10_2_23.py*) processes stream files at *high* and *low* incident flux values, by reading the indexing solution from the high flux, high dose image but integrating the Bragg intensities from the low-dose radiation damage free image.

Our innovative approach not only advances the precision of peak intensity measurements but also highlights the importance of continuous methodological evolution in crystallography. By combining customized processing classes and interactive analysis functions, this research marks a significant leap forward in data analysis accuracy, with broad potential for scientific application.

Keywords: Crystallography, Data Analysis, CXFEL, CXLS, Water Background Subtraction, Peak Intensity, CrystFEL, HDF5, Python Programming.

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