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Spectral X-Ray Diffraction using a 6 Megapixel Photon Counting Array Detector

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Abstract

Pixel-array array detectors allow single-photon counting to be performed on a massively parallel scale, with several million counting circuits and detectors in the array. Because the number of photoelectrons produced at the detector surface depends on the photon energy, these detectors offer the possibility of spectral imaging. In this work, a statistical model of the instrument response is used to calibrate the detector on a per-pixel basis. In turn, the calibrated sensor was used to perform separation of dual-energy diffraction measurements into two monochromatic images. Targeting applications include multi-wavelength diffraction to aid in protein structure determination and X-ray diffraction imaging.

Introduction

X-ray imaging underpins technologies ranging from security to health to protein structure determination.¹⁻⁵ In each case, energy-resolved X-ray imaging can offer significant additional benefits beyond detection of fluence alone. In tomography and X-ray imaging, the attenuation cross-sections of materials (e.g., bone, metals, liquids, etc.) depend on X-ray wavelength, such that the spectral pattern of attenuation provides additional composition-specific contrast inaccessible from the measured intensity alone.^{3, 6} This additional information can in turn inform models for tomographic reconstruction by explicitly including effects from beam hardening, for example, in which the spectral content of the detected X-ray beam differs from that of the source due to wavelength-specific attenuation cross-sections.

In macromolecular X-ray diffraction, the benefits are even more pronounced. Structure determination from measurement of the diffraction pattern is complicated by uncertainties in the phase.^{7, 8} The intensity of each diffraction spot is recorded, but the Fourier transform relationships connecting diffraction to structure are only invertible if the phase of each diffraction spot can be known or inferred. Inference of phase is currently most routinely performed by comparison of diffraction patterns close to and far from absorption edges of particular atoms in the lattice.^{8, 9} In these multiple-wavelength anomalous dispersion (MAD) measurements, the phase-shift of the diffraction peaks close to resonance alters the interference conditions and affects in turn the pattern of diffracted intensity. This subtle

change provides additional on phase to assist in inversion of the diffraction pattern to recover the underlying macromolecular structure.

While ubiquitously used, current approaches for MAD measurements are typically plagued by artifacts from X-ray damage and $1/f$ noise. Macromolecular crystals exhibit dose-dependent losses in X-ray diffraction, with the highest resolution features decaying at the highest rates.^{10, 11} MAD measurements performed sequentially with different exposures at each wavelength are therefore subject to associated complications in data analysis.

We present here demonstration of an approach working toward single-shot spectral X-ray imaging by combining statistical methods with a physics-based model for the response in a photon-counting array detector. Pixel-array detectors are capable of performing photon counting in each pixel on a massively parallel scale (e.g., 6 million parallel photon counting circuits per detector array for fluences $>10^6$ counts per second per pixel).^{12, 13} The initial effort demonstrated herein targets dual-wavelength analysis, consistent with the requirements of MAD measurements used in macromolecular X-ray diffraction.

Model for the Instrument Response

The general strategy used for spectral imaging is based on measuring the count rate as a function of a threshold value set on a comparator on a per-pixel basis to perform energy-dispersive spectral detection. A physical model for this process is illustrated in Figure 1, in which an initial current plume generated from X-ray absorption is ultimately recorded by a digital counter. The number of counts at each pixel are then read to generate an image. The number of photoelectrons produced in the initial current plume and the peak height of the corresponding voltage transient are dependent on X-ray energy. As such, the measured counts as a function of the threshold voltage can allow determination of X-ray energy.

In practice, two key interactions complicate precise recovery of the X-ray energy by this approach. First, the number of photoelectrons produced by X-ray photon absorption is inherently a random variable, the variance of which sets a limit on the precision of energy discrimination. Second, X-rays absorbed at the junctions between adjacent pixels result in charge sharing between them. As a result, the probability density function (pdf) for the anticipated voltage upon X-ray absorption is a nontrivial function that includes both whole and fractional counts.

Given the difficulties in generating a reliable analytical expression for the anticipated pdf, fitting of the data from each pixel was instead performed by comparison of the measured results with a normalized pdf generated numerically by Monte Carlo modeling of the predicted instrument response as a function of the free parameters used in the fits. The Monte Carlo simulations were performed using a sufficient number of predictions to ensure that the measurement noise was much greater than the Poisson noise introduced in the fitting through the finite number of simulations.

Experimental Methods

All data were acquired at beamline 17-ID, IMCA-CAT, at Argonne National Laboratory. Diffuse scattering of vitreous ice was measured with a Dectris Pilatus-6M single threshold detector at several detector comparator threshold levels (V_{th} in Figure 1). The detector had a pixel size of $172 \times 172 \mu\text{m}^2$ with a silicon active area thickness of $320 \mu\text{m}$. Five second exposure times were taken at each detector threshold for both 13.5 keV and 15 keV incident X-ray energies in a standard lattice (153 ns between X-ray pulses). Absolute detector voltage thresholds at each pixel were automatically calibrated through Pilatus' automatic internal voltage trim system to maintain threshold accuracy. A low gain input amplifier setting was used for all measurements. The resulting internal voltage threshold levels are denoted here as equivalent thresholds in units of keV, which describes the equivalent X-ray energy that would deposit this mean level of voltage. The 13.5 keV incident energy measurements were serially taken with equivalent threshold energies from 7.5 keV to 21.0 keV in steps of 0.5 keV with a detector distance of 0.700 m. The 15 keV incident energy measurements were serially taken on a later day with a new ice sample with equivalent threshold energies from 7.5 keV to 20.9 keV in steps of 0.2 keV with a detector distance of 1.000 m. In all cases, the incident photon flux was kept low enough to ensure a low probability of pulse pileup affecting counting results.

All data analysis was performed in MATLAB with custom software. Data files were read using the MATLAB macros package for cSAXS (Paul Scherrer Institute). ImageJ was also used to view data files using a plugin (CBF reader plugin, written by JLM).

Results and Discussion

A series of diffuse X-ray scattering images were acquired at two different X-ray energies and summed to produce the image stack shown in the top of Figure 2. Prior to analysis, every pixel in the stack at each wavelength was independently fit to three parameters: the mean and standard deviation of the one-photon peak height distribution and the spatial spread of the current plume related to fractional photon counting. From these parameters, the set of summed images could be recovered by treating the composite as a linear combination of the two pure components.

From inspection of the comparison with the ground truth results in Figure 2, overall the pure component images are recovered with reasonable accuracy. One notable exception is the bright location in the bottom right portion of 13.5 keV image, which results in a weak but nonzero ghost in the recovered 15 keV image. This artifact is attributed to the multiplex disadvantage in Poisson distributed measurements, in which the sum of a large and small number produces noise dominated by the large number but distributed over both upon separation. As a result, the noise in the smaller number is significantly higher than would be expected by Poisson statistics alone.

The measurements shown in Figure 2 demonstrate proof-of-principle for strategies that can perform single-shot spectral imaging. Substantial contributions from $1/f$ noise in both the X-ray source and the detector array can be dramatically reduced by patterning the threshold

values within a given array on a pixel-by-pixel basis. Even in diffraction measurements producing sharp reflections, the diffracted spots still extend over many pixels. Consequently, a pattern as simple as a checkerboard with two different threshold values could allow recovery of spectral-selective images without substantial loss in intrinsic image information content. More elaborate patterns could be envisioned depending on the number of desired spectral channels and/or spectral parameters to be determined from the images, and the nature of the images themselves. However, those efforts are beyond the scope of the current proof-of-concept demonstration.

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References

- [1]. Frojdh C, Norlin B, Frojdh E. Spectral X-ray imaging with single photon processing detectors. *Journal of Instrumentation*. 2013;8.
- [2]. Roessl E, Proksa R. K-edge imaging in x-ray computed tomography using multi-bin photon counting detectors. *Physics in Medicine and Biology*. 2007; 52(15):4679–4696. [PubMed: 17634657]
- [3]. Boone JM, Shaber GS, Tecotzky M. Dual-Energy Mammography - A Detector Analysis. *Medical Physics*. 1990; 17(4):665–675. [PubMed: 2215412]
- [4]. Srajer V, Teng TY, Ursby T, Pradervand C, Ren Z, Adachi S, Schildkamp W, Bourgeois D, Wulff M, Moffat K. Photolysis of the carbon monoxide complex of myoglobin: Nanosecond time-resolved crystallography. *Science*. 1996; 274(5293):1726–1729. [PubMed: 8939867]
- [5]. Schlichting I, Almo SC, Rapp G, Wilson K, Petratos K, Lentfer A, Wittinghofer A, Kabsch W, Pai EF, Petsko GA, Goody RS. Time-Resolved X-Ray Crystallographic Study of the Conformational Change in HA-Ras P21 Protein on GTP Hydrolysis. *Nature*. 1990; 345(6273):309–315. [PubMed: 2111463]
- [6]. Butler APH, Anderson NG, Tipples R, Cook N, Watts R, Meyer J, Bell AJ, Melzer TR, Butler PH. Bio-medical X-ray imaging with spectroscopic pixel detectors. *Nuclear Instruments & Methods in Physics Research Section a-Accelerators Spectrometers Detectors and Associated Equipment*. 2008; 591(1):141–146.
- [7]. Clifton, IJ.; Duke, EMH.; Wakatsuki, S.; Ren, Z. Evaluation of Laue diffraction patterns. 1997.
- [8]. Pahlér A, Smith JL, Hendrickson WA. A probability representation for phase information from multiwavelength anomalous dispersion. *Acta Crystallographica Section A*. 1990; 46(7):537–540.
- [9]. Guss J, Merritt E, Phizackerley R, Hedman B, Murata M, Hodgson K, Freeman H. Phase determination by multiple-wavelength x-ray diffraction: crystal structure of a basic “blue” copper protein from cucumbers. *Science*. 1988; 241(4867):806–811. [PubMed: 3406739]
- [10]. Holton J. A beginner’s guide to radiation damage. *Journal of Synchrotron Radiation*. 2009; 16(2): 133–142. [PubMed: 19240325]
- [11]. Massover W. Radiation damage to protein specimens from electron beam imaging and diffraction: a mini-review of anti-damage approaches, with special reference to synchrotron X-ray crystallography. *Journal of Synchrotron Radiation*. 2007; 14(1):116–127. [PubMed: 17211078]

- [12]. Muir RD, Pogradichney NR, Muir JL, Sullivan SZ, Battaile KP, Mulichak AM, Toth SJ, Keefe LJ, Simpson GJ. Linear fitting of multi-threshold counting data with a pixel-array detector for spectral X-ray imaging. *Journal of Synchrotron Radiation*. 2014; 21(5):1180–1187. [PubMed: 25178010]
- [13]. Henrich B, Bergamaschi A, Broennimann C, Dinapoli R, Eikenberry EF, Johnson I, Kobas M, Kraft P, Mozzanica A, Schmitt B. PILATUS: A single photon counting pixel detector for X-ray applications. *Nuclear Instruments & Methods in Physics Research Section a-Accelerators Spectrometers Detectors and Associated Equipment*. 2009; 607(1):247–249.

Figure 1.

(Adapted from Ref. 12) In each pixel in the Pilatus detector array, absorption of an X-ray photon results in a charge plume that is amplified in a current to voltage converter and introduced to a comparator. For voltage transients exceeding the comparator threshold, a counter is incremented and stored locally until readout of the array. Modeling the parameters affecting the peak voltage introduced to the comparator allows quantitative spectral information to be extracted from multithreshold data.

Figure 2.

(adapted from Ref. 12) Sum of two-color measured images obtained for multiple comparator threshold values (top) and the recovered pure component images (middle). The ground truth results are also shown (bottom).