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Cost-effective EMCCD-based detector for time-resolved biological SAXS applications

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Abstract

Third generation synchrotron sources such as the Advanced Photon Source at Argonne National Laboratory, Argonne, IL, are outstanding tools for X-ray diffraction and scattering studies of non-crystalline biological materials. However, these studies are hindered by the lack of adequate detectors that can provide multiple frames of detailed structural information on the required millisecond time scale at the extremely high count rates available at the APS. RMD is developing a cost effective detector for time-resolved small angle X-ray scattering, using a cooled, 512×512 pixel electron multiplying CCD (EMCCD). This paper describes the detector design, its efficacy for time-resolved SAXS studies, and its imaging performance with frame rates of 30 to 500 fps.

Keywords

Synchrotron detector; EMCCD; SAXS; time resolved SAXS; Advanced Photon Source

1. Introduction

The availability of small angle X-ray scattering (SAXS) instruments on third generation synchrotron sources has been invaluable for static and time-resolved studies of various non-crystalline biological systems [1]. Time-resolved X-ray diffraction (XRD) of muscle has historically been a driving force in synchrotron instrumentation development [2]. Other applications of time-resolved XRD, such as scattering from macromolecules in solution [3], kinetics of protein folding [3]–[5], study of phase transitions in model membrane systems [6], etc., have a significant biotechnological relevance.

To make effective use of these high performance SAXS instruments, detectors must be able to operate at high global count rates ($\sim 10^7$ – 10^8 photons/s) and have high sensitivity (ability to detect single photons), high spatial resolution (50 μ m–100 μ m), large active area (>10cm×10cm), and a read out with millisecond-scale time resolution. Unfortunately, detectors that fulfill all of these requirements simultaneously are not currently available.

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Here we present the results of preliminary tests of a custom-designed EMCCD-based camera system that promises to fulfill the demanding needs of SAXS applications. This system consists of an EMCCD coupled to a phosphor via a fiber optic (FO) taper. Prospects for new larger active area, high time resolution detectors based on this concept are discussed.

2. Electron multiplying CCD's

New EMCCDs manufactured by e2v Technologies [7] work like conventional CCDs, except that they provide internal gain via an avalanche mechanism. Thus, an imaging system based on the EMCCD does not require the use of intensification stages, even though it allows detection of extremely low light levels at high frame rates [7],[8]. As shown in Fig. 1, the EMCCD consists of an imaging area of active pixels and an additional area of optically shielded pixels as a frame transfer array. During conventional operation, the detector integrates the image for a specified time, and then shifts it out of the image zone and into the frame transfer array at a rate of $0.8 \mu\text{s}$ per row. Thus the entire 512 rows are shifted out in $410 \mu\text{s}$. For Andor's iXon DV887 back illuminated EMCCD with 512×512 pixels used in our design, the entire pixel data is read out in $\sim 31 \text{ ms}$ for a maximum speed of $\sim 32 \text{ fps}$. Higher frame rates of up to 526 fps can be achieved by sacrificing pixel resolution by binning or reducing the imaging area.

3. EMCCD as a fast and sensitive X-ray detector

The dynamic range of an EMCCD-based detector in terms of the detectable number of 12 keV X-ray photons depends on the internal gain setting and the number of light photons per 12 keV X-ray provided by the phosphor and transmitted by the fiber optic system. For an internal gain of 1–3, the dynamic range is $(250,000 e^- \text{ full well}) / (30 e^- \text{ per X-ray with a 6:1 taper})$, or ~ 8500 12 keV X-rays. For higher frame rates of 500 fps , the read noise is $\sim 50 e^-$ and a gain of 50 is needed to effectively eliminate the effect of read noise. Thus, a pixel capacity of $850,000/50$ or $17000 e^-$ is useful at 500 fps operation. This corresponds to ~ 570 12 keV X-ray photons/pixel/ 2 ms frame, which is significantly higher than the expected X-ray photon flux per pixel from a strong diffraction feature in 2 ms .

One of the most attractive features of an EMCCD-based detector is that the internal gain is software controllable and can be changed in real time during an experiment, thereby providing the flexibility of simultaneously controlling the sensitivity and dynamic range. Pixel binning may be used to allow higher speeds by sacrificing resolution. For time-resolved studies of fibrous material such as muscle, or in SAXS studies, high resolution is critical in one dimension, while it may be relaxed in the other dimension.

4. Methods

4.1. Test configuration

The test instrument consisted of a customized Andor iXon DV887 back illuminated EMCCD sensor with 512×512 pixels. With its $16 \mu\text{m}$ pixels, this camera has an effective active imaging area of only $8.2 \text{ mm} \times 8.2 \text{ mm}$. Fiber optic tapers with 3:1 and 6:1 demagnification ratios were coupled to the chip using an optical grease and specially designed mechanical fixtures to increase the active area. The phosphor used was a commercially available $\text{Gd}_2\text{O}_2\text{S}(\text{Tb})$ screen (Kodak Min R 2000). The EMCCD chip is cooled to -35°C with thermoelectric cooling. Under these conditions, the dark noise is $< 1 e^-/\text{pixel}/\text{s}$.

4.2. Scintillator selection

For a high speed imaging application like time-resolved SAXS, it is important to have a scintillator with fast decay time. For the preliminary SAXS experiments, we used commercially

available Kodak Min R 2000 screens. Although this scintillator was able to demonstrate the practicality of using an EMCCD detector for time-resolved SAXS application, a scintillator with higher light output and minimal afterglow is required to exploit the potential of the EMCCD-based SAXS detector. Towards that end, we have fabricated screens of vapor deposited CsI:Tl,Eu [9]. To demonstrate the extent to which the afterglow suppression can improve imaging performance under high-speed conditions, we integrated a 200 μm thick CsI:Tl,Eu film into the EMCCD camera, and acquired X-ray images of a test phantom. For comparison we also evaluated a standard 200 μm thick microcolumnar CsI:Tl screen produced by RMD. A 3:1 fiberoptic taper was used for the experiment.

For these tests the X-ray source was set to 50 kVp and 10 mA. The EMCCD camera was used to acquire images 30 ms in duration, spaced 1 ms apart. The object was exposed to X-rays only during the first integration period, with the subsequent frames acquiring the residual image provided by the afterglow. The resulting data for a conventional CsI:Tl film and the new CsI:Tl,Eu film are shown in Fig. 2.

As can be seen from these figures, with conventional CsI:Tl a residual image is visible even after 10 frames (normally beyond the sensitivity of commercial CCDs), whereas CsI:Tl codoped with Eu shows virtually no residual image in any of the subsequent frames. These data show that the afterglow suppression directly translates into dramatically improved dynamic imaging.

4.3. Experiments for solution SAXS and muscle diffraction

The experiments for SAXS applications were performed using the standard SAXS instrument on the BioCAT beamline 18-ID at the APS at Argonne National Laboratory using a camera length of 1.82 m and 0.103 nm X-rays.

Experiments for macromolecular solution scattering were conducted using a sample of 20 mg/ml NtrC1C protein (courtesy T. Nixon, Penn State University) in buffer solution. Individual time frames were summed and compared to static patterns of the same total exposure time taken with an existing high sensitivity 4k $\times$ 7k (12 μm pixel) SAXS detector developed at Brandeis University [10] to compare signal/noise characteristics. The EMCCD camera has a pixel size of 16 $\mu\text{m}\times$ 16 μm (corresponding to effective pixel sizes of 48 μm and 96 μm for 3:1 and 6:1 FO tapers, respectively) and the Brandeis CCD detector was used in 4 $\times$ 4 binning mode (48 $\mu\text{m}\times$ 48 μm pixels). Scattering of the protein solution and scattering of the buffer were measured using the same configurations at room temperature ($\sim 23^\circ\text{C}$). The samples were filled in a quartz capillary of diameter 1.5mm and kept flowing with a Hamilton syringe pump (Microlab 540C) to reduce X-ray damage to the protein. Background scattering of the buffer was subtracted from the scattering of the protein solution in the process of data reduction, and the data were averaged azimuthally. The reduced scattering intensity is presented as the number of photon-generated counts per binned pixel versus scattering vector Q , where $Q=4\pi/\lambda \sin(\theta/2)$ with θ being the scattering angle.

Diffraction patterns were also taken from fixed, embedded muscle tissue samples provided by M.K. Reedy, Duke University. Time-resolved experiments were simulated by taking sequences of patterns from muscle samples at various pixel binning and speeds ranging from 30 fps to 143 fps, at gain settings of 150 and 255. For both the fiber and solution experiments dark images were subtracted from the signals. No attempt was made to correct for inhomogeneities in detector response or spatial distortions.

5. Results and Discussion

Fig. 3(a) shows a comparison of scattering data from 20 mg/ml ADP NtrC1C protein taken with the prototype system for gains of 150 and 255, and a 6:1 taper, to data taken with the Brandeis detector [10] using 4×4 binning with an exposure time of 600 ms. At a gain of 255 the EMCCD data (no binning) gave a signal 1.8 times larger than that of the Brandeis detector. With a 6:1 taper, the effective pixel size was 96μm in the test system vs. 48μm with the Brandeis detector. This means that at an ADU level, the test system was 0.44 times as sensitive as the Brandeis system.

Fig. 3(b) shows data from similar measurements taken with the test system using the 3:1 taper and no binning. In this case the signal was 2.5 times that of the Brandeis detector, as the pixel sizes were the same. In the Brandeis detector there are 10 ADU's per 12 keV X-ray photon, with about 3 ADU's of read noise [10]. This implies that for the test system there are ~25 ADU's and 4.4 ADU's per 12 keV X-ray photon for the 3:1 and 6:1 tapers respectively. The estimated read noise in the case of the EMCCD detector is <1 ADU per pixel per read on either the 150 or 255 gain settings. Thus, the SNR is at least 25:1 to 4.4:1 for 3:1 and 6:1 tapers, respectively.

Fig. 4(a) shows a portion of the fiber diffraction pattern from the embedded muscle specimen taken in 30 ms, using the high sensitivity Brandeis CCD detector. Fig. 4(b) shows a single 33 ms frame taken using the test system with no binning, at a gain of 255 with a 3:1 taper. The Z-scale was adjusted to account for the factor of 2.5 enhanced sensitivity between the test system and the Brandeis detector. Fig. 4(c) shows an 11 ms frame with 4×4 binning, also with a gain of 255 using the 3:1 taper. Consistent with the SAXS data, the EMCCD data frames are noisier and have somewhat less spatial resolution than the data taken with the Brandeis detector, but give interpretable data even with the 11 ms exposure and 4×4 binning. Streaking appears from over-loaded pixels due to the lack of anti-blooming in this device. Streaking can be avoided by selectively attenuating the strong features of a pattern.

6. Perspectives

Our tests show that the sensitivity of the test system with the 3:1 FO taper even in this preliminary, non-optimized form is close to what we need from a useful detector for SAXS applications. The 92fps frame rate is high enough for capturing protein structural changes in stopped flow experiments, and the data quality at this frame rate with a gain setting of 255 is good enough for this purpose. The transient conformation of the protein structure can be deduced from model fitting the scattering profile of individual frames, thus continuously capturing individual scattering frames at high frequency offers a great way to follow the process of structural changes. Similarly, useful X-ray diffraction data from muscle can be obtained in similar time scales. The ability to do asymmetric binning in the EMCCD can increase speed while retaining spatial resolution in the direction it is needed. It is also possible to increase speed by only examining a small region of interest.

In order to match the SNR characteristics of the high speed EMCCD detector to the state of the art Brandeis detector (a slow detector with several seconds per read cycle), a phosphor with higher light output is needed. In the experiments reported here, a standard, commercial Gd₂O₂S(Tb) phosphor screen was used. However, the newly developed CsI:Tl,Eu scintillator, with its extremely low after-glow, holds great promise for high-speed imaging applications such as time-resolved SAXS. It is anticipated that achieving a 2 to 3 fold improvement in light output over the commercial screens should be feasible with existing technologies and would allow the fast framing detector to have comparable SNR characteristics as the Brandeis detector while permitting high frame rates needed for the time resolved studies.

The 512×512, 16μm pixel sensor we used is too small to be the basis for a practical device for X-ray detection for SAXS applications. Even with a 6:1 taper, the ~50mm×50mm active area provides too limited a θ range to be useful for most applications for weakly scattering systems such as macromolecules in solution and muscle applications. It could, however, be a very useful and economical detection scheme for stronger diffracting systems such as model membrane and synthetic polymer systems. However, EMCCD chips with higher pixel resolution and larger pixel size are being developed (e.g., 1600×1600, 16μm pixels), that can be read out at 10 MHz, allowing ~4fps imaging at full resolution. The possibility exists to modify these chips to implement four outputs, each reading at 40 MHz, which would allow full frame readout at 64fps, with 1024 fps readout achievable with 4×4 binning. Using such a chip with an active area of 25.6 mm, FO tapers of 3:1 to 6:1 would give a very useful range of detector active areas (78mm×78mm – 156mm×156mm). This detector would represent a relatively economical and simple but high performance device that could accommodate a wide variety of time-resolved SAXS applications at third generation synchrotron sources.

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Fig. 1.
Schematic of EMCCD sensor developed by e2v [7].

Fig. 2.

Comparison of dynamic imaging with a conventional columnar CsI:Tl screen and the newly developed CsI:Tl,Eu screen. Each frame lasts 30 ms and is separated from the next by 1 ms. The X-ray pulse occurs during the first frame only, but the image in CsI:Tl persists even after ten frames. The corresponding data for CsI:Tl,Eu shows hardly any residual image even in the very next frame, demonstrating the practical utility of the new material.

Fig. 3. Comparison of experimental data using the EMCCD-based SAXS detector and the current state-of-the-art detector. Solid lines denote the response of the Brandeis detector.

Fig. 4. Partial fiber diffraction pattern. (a) Brandeis detector 30 ms, (b) EMCCD 33ms 1×1 binning, (c) EMCCD 11 ms 4×4 binning gain=255.