Digital Detectors for Electron Microscopy

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Abstract

Film has traditionally been used for recording images in transmission electron microscopes but there is an essential need for computer-interfaced electronic detectors. Cooled-CCD detectors, developed over the past few years, though not ideal, are increasingly used as the preferred detection system in a number of applications. We describe briefly the design of CCD-based detectors, along with their main properties, which have been used in electron crystallography. A newer detector design with a much bigger sensitive area, incorporating a 2 by 2 tiled array of CCDs with tapered fibre optics will overcome some of the limitations of existing CCD detectors. We also describe some preliminary results for 8 keV imaging, from (direct detection) silicon hybrid pixel detectors, which offer advantages over CCDs in terms of better spatial resolution, faster readout with minimal noise.

Keywords: Digital electron microscopy, CCD detectors, hybrid pixel detectors, electron crystallography

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1. Introduction

Electron microscopy plays a major role in the solution and understanding of the structure of biological molecules and macro-molecular complexes, especially those which are difficult to crystallise, to atomic or near-atomic resolution. In some favourable cases if the protein is available in the form of a two-dimensional crystal, electron crystallography can be applied to obtain structural details to high resolution (2.5 Å⁻¹). Due largely to recent progress in the technical quality of electron microscopes and better algorithms for data analysis, electron tomography on single particles, e.g. viruses, etc, can also produce near-atomic resolution. Finally, with higher energy electron microscopes, there is also interest in using electron tomography on thicker structures of interest to cell and neurobiologists, though the results are available only to a lower resolution.

As the amount of data being generated in electron microscopy is quite large, there is a clear need for an efficient and easy to use detector system, which can also be automated. CCD based detectors, which fall in this category, are becoming more popular, for certain types of microscopy. The main reasons for the popularity of CCDs are that before results become available, film needs to be developed and densitometered, a slow and tedious process. CCD detectors, on the other hand, normally operate under computer control, making results immediately available for on-line viewing and analysis, which allows a more efficient use of the specimen and user time [1-4]. Speeding up and automating data collection, which is only feasible with electronic detectors, is also important for high-throughput structure determination. A major experimental achievement, illustrating the use of CCD detectors has been in the analysis of the structural intermediates in the photo-cycle of bacteriorhodopsin, a membrane protein which acts as a light-driven proton pump. The complete investigation combined diverse techniques, including site-directed mutagenesis and cryo-electron crystallography [5,6].
2. Background to CCD Detectors

As mentioned earlier, the most common form of electronic detectors used in electron microscopy are based on cooled CCDs (for a recent review on biological applications of CCDs, see [4]). CCD detectors usually operate on an 'indirect' detection method, using a phosphor as the first element in the detector, which converts the primary electron energy into visible radiation. The phosphor is usually coated as a thin layer on a fibre optics face-plate, which de-magnifies the light image on to the CCD, where it is integrated and stored. To illustrate the general principles of CCD detectors, Fig.1 shows a schematic of a detector system designed, built and used at the MRC laboratory [2].

![Fig.1. Schematic diagram of a CCD detector with tapered fibre optics, installed on a 120 keV electron microscope. The phosphor (P43) is deposited as a thin layer on the front face of the fibre optics assembly. The complete detector and some electronics are housed in vacuum to avoid any material in the electron path [2].](image-url)
Fig. 2. A typical diffraction pattern from a two dimensional crystal of bacteriorhodopsin with the radial background subtracted. A backstop is required in the centre of the pattern to stop the direct beam from striking the phosphor. Diffraction spots are visible out to \( \sim 2 \ \text{Å}^{-1} \) resolution.

One of the main uses of the detector was in acquiring electron diffraction patterns from \textit{bacteriorhodopsin}, kept at liquid nitrogen temperatures, and an example of such a pattern is shown in Fig.2 [3].
The main drawback of CCD detectors using phosphor-fibre optics assembly is that the spatial resolution is relatively poor compared to film, caused largely by multiple light scattering within the phosphor and the fibre optics. This effect results in the long tails of the point spread function, generated by recording the image of an extremely fine beam of electrons on the detector [2]. The main consequence of the poor resolution is that the incident electron is not recorded in a single pixel; instead, the signal gets shared between a number of adjacent pixels, resulting in a loss of resolution. Although recording of electron diffraction patterns is not affected by the poor resolution, it does have an adverse effect on high-resolution imaging due to the cut-off in the modulation transfer function at higher spatial frequencies. According to a recent evaluation of this signal-sharing problem [4], the size of an ‘independent’ pixel can be considered to be ~100 µm in the phosphor plane. This value is the full width at 10% maximum in the point spread function, which is defined as an independent pixel. It is not, unfortunately, possible to reduce the size of the independent pixel without altering the basic design of the detector. However, it is possible to increase the total number of independent pixels in a CCD detector by having four times as many pixels (i.e. 4 CCDs) and a larger de-magnification in the fibre optics [7]. The new detector has a much larger sensitive area (140 by 130 mm), consisting of a 2 by 2 array of CCDs (model: 55-30 from Marconi), similar to the ones used in single-CCD detectors. Each CCD has 1242 by 1152 pixels, resulting in a total of 5.7 million pixels. The fibre optics, consisting of four sections for the four CCDs, has a demagnification of 2.5:1, giving a pixel size of 56 µm square in the phosphor plane (~100 µm dead spaces in the fibre optics joints). A schematic of the detector is shown in Fig.3 where, for clarity, only half the system is shown.
3. Direct detection in hybrid silicon detectors

A different approach to electron imaging which could potentially yield superior spatial resolution is to use a direct rather than indirect detection method. Instead of using a phosphor for converting the incident electron energy, it relies on generating the signal from electron-hole pairs in a suitable semiconductor. The signal needs to be much higher than noise levels in the detection electronics to be recorded with high efficiency. Direct detection in silicon, the most common semiconductor material used, is certainly feasible, as sufficient energy is deposited in a layer of 100-300 µm. Another important requirement
is to be able to detect electrons with very good spatial resolution, a property inherent in hybrid pixel detectors.

The operation of pixel detectors is different from CCDs in that an incident electron, which deposits energy above a pre-defined threshold, increments a digital counter associated with the pixel instead of accumulating charge in a pixel well [8]. The (digital) image is built up from the accumulation of counts due to individual electrons in counters, which are equivalent to the charge accumulated in the CCD pixel wells. The readout of the digital image from pixel detectors is also similar in principle to readout from CCDs; data from individual pixels is shifted into an external register to be read and stored in a RAM. It should be possible to read out pixel detectors at faster speeds due to the purely digital nature of the readout. Pixel detectors, unlike CCDs, do not have the equivalent of dark current or readout noise despite operating at room temperatures; the absence of readout noise allows a ‘noise-less’ summation of a number of images for improved statistics. Since the detectors are intrinsically digital, the dynamic range of the detector is determined by the size of the storage memory rather than the size of the pixel well capacity, as for CCDs. The problems associated with X-ray induced noise signals in the electron microscope should be less troublesome as they can only register as a single count [4]. According to Monte Carlo simulations for trajectories of 120 keV electrons impinging on silicon, the expected point spread function should be narrower by 2-3 times over the measured PSF in CCD detectors. The improved resolution should allow diffraction data to be recorded to a higher resolution than is possible with CCD systems, or allow high-resolution imaging to be carried out. It may also be possible to improve the resolution by using a higher density and Z material, e.g. GaAs or Cd(Zn)Te as the detector, and these material are under investigation by the Medipix Collaboration [9]. The detectors and the electronics need to be radiation hard and these issues are being actively worked on by micro-electronics
designers in particle physics since much higher radiation levels will also be encountered in the vicinity of colliding beams, where similar detectors will have to be placed in future. We are in the process of evaluating a hybrid silicon pixel detector (Medipix-1) developed by the original Medipix collaboration [8], for electron and X-ray imaging. The Medipix-1 assembly consists of an array of 64 by 64 pixels, each pixel being 170 µm square. The detector chip is bump-bonded to a custom designed readout chip consisting of a amplifier, discriminator and 15-bit counter. In future we hope to use the more recent version of the assembly, viz. the Medipix-2 assemblies, which are currently being designed by the ‘enlarged’ Medipix collaboration, in 0.25 µm CMOS, with 256 by 256, 55 µm square pixels.

3.1 Response of Medipix-1 to 8 keV radiation

A general assessment of how well hybrid pixel detectors could match the needs of electron microscopy was made recently [12]. Two of those issues are addressed below. Firstly, is the sensitivity of silicon detector assemblies adequate for recording 120 keV (and higher energies) electrons and secondly, is the spatial resolution improved compared to previous systems. Our primary goal is to record electrons used in electron microscopy, which range from 120-300 keV. Due to easier access to low-energy X-ray sources, the preliminary tests have been carried out using 8 keV radiation from an Am$^{241}$ (+ copper target) source, prior to tests in the microscope. The low energy tests at 8 keV show that, even with a very small fraction of energy deposited by an energetic electron, they would be recorded with high efficiency. Although Medipix-1 electronics was originally designed for medical applications using X-rays with energy >20 keV it has been found that, with care in setting individual pixel thresholds, it is possible to record X-ray photons with energies as low as 5.4 keV [10]. A feature of the readout electronics is a 3-bit DAC, which applies an offset to the global threshold voltage and is used to even out the variations in the optimum
threshold settings between different pixels [11]. The experimentally determined threshold adjustment values are stored as a mask file, which is applied as a ‘correction’ voltage to modify the global threshold value, when obtaining data. It is also possible, using a simpler procedure, to mask out any ‘noisy’ pixels in the assembly [11].

Due to lack of perfection in the Medipix-1 assembly used for the measurements, there were some noisy pixels, seen clearly in Fig.5, which were not disabled in the mask generation. The response of the silicon assembly for 8 keV can be assessed by performing a threshold voltage scan with 8 keV radiation incident on a fully depleted silicon detector (detector bias: 100 volts). The results in Fig.4 show a plot of the accumulated counts in the selected region of the assembly, containing 400 (~10% of the total) pixels, as a function of the threshold voltage setting. Noise counts dominate below 0.97 Volt and there is a plateau starting at ~0.975 volt corresponding to the start of the 8 keV peak counts. There is a small 'contamination' of 60 keV radiation from the Americium decay, mixed with the 8 keV from the copper target, but as the detection efficiency for this radiation is very low in 300 µm of silicon, it can be ignored. The results are very encouraging for using the medipix-1 assembly for recording 8 keV radiation and since the energy deposited by 120 keV electron will probably exceed 8 keV by a factor of 5, there should not be a problem in obtaining an adequate signal.

3.2 Resolution at 8 keV

An estimate of the expected resolution in the medipix-1 assembly can be obtained from the image shown in Fig.5. The image was generated by covering the face of the detector with a metal plate containing 16 holes (450 µm diameter) on a semi-regular lattice; there are also two smaller holes with a diameter of 250 µm. The image shows the larger 16 holes clearly, but the smaller holes are more difficult to pick out. The intensities for one of the smaller holes are printed below suggesting that the cross-coupling of signal into
Fig. 4. Response of the Medipix-1 assembly (the silicon detector was fully depleted with a 100 volts bias) to 8 keV radiation as a function of threshold voltage.

adjacent pixels is minimal and is roughly what would be expected from geometrical (parallax) effects.
**Fig.5** Image of a mask (metal plate with holes drilled - see text) on medipix-1 using 8 keV radiation. The raster of intensities, printed below, is from points in the 250 µm hole region:

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5. References

(1) A.R.Faruqi, H.N.Andrews and R.Henderson

(2) A.R.Faruqi and H.N.Andrews,

(3) A.R.Faruqi, R.Henderson and S.Subramaniam

(4) A.R.Faruqi and Sriram Subramaniam
Quarterly Rev. of Biophys. 33, 1, (2000)

(5) S.Subramaniam, M.Lindahl, P.Bullough, A.R.Faruqi, J.Tittor,
D.Oeterhelt, L.Brown, J.Lanyi and R.Henderson

(6) S.Subramaniam, A.R.Faruqi, D.Oesterhelt and R.Henderson

Nucl. Instr. and Meth. To be published (2001)

(8) C.Schwarz, M.Campbell, R.Goeppert, E.H.M.Heijne, J.Ludwig,
G.Meddeler, B.Mikulec, E.Pernigotti, M.Rogalla, K.Runge,
A.Soeldner-Rembold, K.M.Smith, W.Snoeys, and J.Watt

(9) http://medipix.web.cern.ch/MEDIPIX/

(10) A.Fornaini, D.Calvet and J.Visschers
Submitted to Nucl. Instr. and Meth. Aug.2000

(12) A. R. Faruqi, Nucl. Instr. and Meth. To be published (2001)