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Characterization of a Direct Detection Device Imaging Camera for Transmission Electron Microscopy

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Abstract

The complete characterization of a novel Direct Detection Device (DDD) camera for transmission electron microscopy is reported, for the first time at primary electron energies of 120 keV and 200 keV. Unlike a standard Charge Coupled Device (CCD) camera, this device does not require a scintillator. The DDD transfers signal up to 65 lines/mm providing the basis for a high-performance platform for a new generation of wide field-of-view high-resolution cameras. An image of a thin section of virus particles is presented to illustrate the substantially improved performance of this sensor over current indirectly coupled CCD cameras.

1. Introduction

A key advance in transmission electron microscopy (TEM) is the development of new imaging detectors that combine large field-of-view and fast inline digital data acquisition with high modulation transfer function (MTF) and detective quantum efficiency (DQE). However, current imaging devices, most notably film and indirect scintillator coupled charge-coupled-device (SCCD) cameras, have intrinsic properties that constrain high-resolution, high-sensitivity TEM imaging. Conventional photographic emulsions yield wide field-of-view images but require extensive post processing and digitization that prevents immediate feedback and high throughput. SCCD cameras avoid these problems, but have reduced field-of-view, relatively poor resolution and efficiency, and narrow dynamic range when considered against the requirements for imaging, diffraction and spectroscopy in the TEM [1]. These limitations are directly related to the indirect detection that uses a scintillator screen to convert fast beam electrons into photons, fiber optics or a lens coupling to project these photons onto the sensor, and a CCD to convert these photons into electrical signals that are finally digitised. As an alternative we note that an ambitious post specimen deceleration mount has been constructed [2] which enables optimization of the spreading in the scintillator to the detector pixel size.

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A promising alternative to these conventional systems are camera systems that are exposed directly to the incident high energy electron beam, thus avoiding the use of a scintillator[3,4,5,6,7,8,9]. This paper presents work with a Direct Detection Device (DDD) based on a new CMOS sensor. The DDD is based around a radiation-hardened monolithic active-pixel sensor that retains the linearity and immediate digitization of a SCCD, but has substantially faster digital readout. Furthermore, direct exposure to incident electron beam significantly improves the signal-to-noise ratio in comparison to a SCCD. Previous feasibility studies [3,10] have demonstrated the operation of this sensor, including radiation tolerance. However a complete quantitative characterization of sensor performance has not yet been reported. This letter presents the characterization of a prototype DDD used to directly capture 120 keV and 200 keV electron images. A typical image of a lightly stained, epoxy embedded biological specimen is also included to illustrate the practical advantages of this type of sensor.

2. Experimental Method

The DDD used in these studies has a pixel pitch of 5 microns on a 1024 by 1024 pixel array giving a factor of 4 increase in imaging area and an increase in the number of readout ADCs from 4 to 16 compared to earlier prototypes [3]. The sensitive epitaxial layer is approximately 8 microns. We also note that no additional process has been added to increase the radiation hardness beyond increasing the readout speed and reducing the scale of the fabrication technology. The DDD was mounted in a modified film drawer of a JEOL JEM 2000EX TEM for the measurements using a 200 keV electron beam. The 120 keV beam measurements were carried out on an FEI Spirit TEM with the DDD mounted in a custom housing beneath the viewing chamber. In both cases the sensor was cooled to 258 K to minimize radiation damage and decrease dark current noise. Incident electron beam dose was measured using a Faraday plate adjacent to the sensor. The DDD has a noise RMS of 2.95 ADC and an ideal linearity up to 2,380 ADC in a single readout cycle with a conversion factor of 3.6 ADC to 1 mV. Taking into account loss of dynamic range due to fixed pattern noise and the remnant noise floor of 9.33 ADC after dark field correction in a sum of 10 frames a dynamic range of 68 dB is computed for a readout speed of 1.25 MHz and an output frame rate of 1 Hz, a frame rate comparable to a slow-scan SCCD. The dynamic range of the DDD is thus similar to the typical values of 72 dB for slow-scan SCCDs operating under similar conditions [1], largely due to its increased readout speed.

Three datasets were recorded at a frame rate of 5 Hz to measure the MTF and DQE at 120 keV and 200 keV. Stochastic images of the projected shadow of an opaque metallic mask with an edge inclined with respect to pixel rows to enable effective oversampling of the experimental data were captured to calculate the MTF [11]. Scattering of electrons at the mask edge was monitored in the resulting images and could be eliminated using a mask with a sharp edge. Random images of uniform electron flux across the detector were recorded to provide suitable white noise data required for detector DQE and pixel gain values. A third dataset in the absence of an electron beam was also recorded to obtain pixel dark-noise values. For each dataset, 20 images were recorded, with a total image dose of just under 100 electrons/pixel. This low number of incident electrons was chosen to illustrate one of the main applications of an imaging detector with a high signal to noise ratio in the imaging of radiation sensitive specimens.

Edge shadow and white noise datasets were corrected first for variations in pixel gain and dark-noise levels across the detector using the average of the white noise images and null datasets, respectively. The average profile of the edge shadow was subsequently obtained from the corrected dataset using an interpolation factor of 8 points/pixel and correcting for any possible curvature of the opaque mask. The MTF was then calculated from this using

fixed step differentiation followed by an absolute Fourier transform. The average Noise Power Spectrum (NPS) was obtained from the average of power spectra calculated from each image in the white-noise dataset. Finally, the DQE was calculated using the modulation transfer, noise spectrum and dose and correcting for bandwidth limitation [11,12].

As a demonstration image, Flock House virus particles in *Drosophila* cells were used for TEM. Flock House virus infected *Drosophila* cells were prepared using high pressure freezing and freeze substitution, embedding in resin and mechanical sectioning to 90 nm [13]. Sections were lightly stained with Sato's triple lead solution to enhance contrast [14]. The final image was composed by summing 1,000 frames acquired at an exposure of 106 msec/frame, producing a total average signal of 2,000 incident electrons/pixel and was also corrected for pixel gain variation and dark noise.

3. Results and Discussion

The calculated MTF is shown in Figure 1. The MTF is attenuated at high spatial frequencies due to strong scattering of primary beam electrons in the sensor as is typical for imaging detectors used in TEM [15]. The characteristic charge sharing between pixels in CMOS sensors may also cause attenuation at frequencies close to the Nyquist limit. Increasing the electron energy from 120 keV to 200 keV, decreases the MTF at low spatial frequencies and increases it at high spatial frequencies. This is explained by a decreased fraction of beam electrons that pass through the sensitive layer and back scatter from the substrate to produce signal far from the initial point of impact.

A kink is observed in the MTFs at 200 keV and 5 lines/mm, and at 120 keV and 20 lines/mm, which are considered to indicate that MTF at each electron energy is a sum of two contributions. The first contribution, which dominates at high frequencies, is attributed to the signal produced by the high energy electrons as they bombard the detector and penetrate the epitaxial layer. The second contribution to MTF, dominant at low frequencies, is attributed to the energy deposited by electrons backscattered from the substrate into the epitaxial layer. Thus the kink in the MTF curve is considered to represent the point of transition between the two contributions to the MTF, with the lower spatial frequency at 200 keV illustrating that the corresponding back-scattered electrons have larger lateral displacement than those at 120 keV. It is also proposed that the shape of this kink provides information on the relative strength of these two contributions at this spatial frequency, showing that the backscattered signal is much stronger at 200 keV than at 120 keV.

A typical SCCD transfers modulation up to 50 lines/mm but by comparison the DDD can transfer modulation up to 100 lines/mm and the MTF of the DDD for 200 keV electrons is 30% at 25 lines/mm, compared with typical values of 10-20% for a SCCD at the same frequency [16,17]. Moreover modulation transfer is 5% beyond 65 lines/mm, dropping to 1% at the 100 lines/mm limit.

The Normalised NPS is shown in Figure 2. This shows that noise is added at low frequencies and attenuated at high frequencies, with transition points at 13 lines/mm and 16 lines/mm for electrons with energies of 120 keV and 200 keV respectively. Images with 200keV electrons are noisier than those recorded at 120 keV, especially at low frequencies. However this increase in noise cannot be attributed to the DDD since the double-sampled readout of signal from each pixel has a noise level significantly lower than the signal from single electrons [3]. Therefore noise is attributed to the strong probabilistic nature of 120 keV and 200 keV electron energy deposition in the sensor, which cannot be avoided in any imaging camera [15].

Figure 3 shows the resultant DQE for the DDD. The presence of low-frequency noise decreases the low-frequency DQE to 52% at 120 keV and to 36% at 200 keV. In addition, the strong attenuation of the MTF reduces this further to 4% for 120 keV and 13% for 200 keV at 25 lines/mm. The DQE drops below 5% at 22 lines/mm at 120 keV and at 44 lines/mm, at 200 keV, to a limiting value of 0.1% at the maximum 100 lines/mm. By comparison with reported values of conventional SCCDs, the DQE of DDD is much higher at high electron energies, reaching 17% at 200 keV for 16.7 lines/mm compared with 5% at 300 keV at the same spatial frequency [18]. However, a higher DQE value has been reported for a conventional SCCD operating at lower electron energy, 15% at 120 keV and 16.7 lines/mm [16], compared with 8% obtained here for the same energy and spatial frequency. Given that the MTF and NPS of the DDD are constrained only by lateral scattering of high energy incident electrons, we conclude that the DQE of the DDD is limited by electron scattering in bulk Silicon. The DDD is therefore indicative of the ultimate performance that can be obtained with directly-exposed standard CMOS sensors operating as flux-integrating imaging cameras.

To illustrate the sensitivity of the DDD, an image of a Flock House virus array in the cytoplasm of a cell is shown in Figure 4 at 5,600X magnification (0.89 nm/pixel). The virus particles are approximately 30 nm in diameter and the gold fiducial markers on the image are 6-10 nm in diameter. Due to partial staining of the complete virus coat protein, the diameter of the virus particles in the image often appears smaller than 30 nm. The life cycle of Flock House virus in an infected cell is of interest because much of the specific replication using cellular machinery is poorly characterized. A later stage in the virus life cycle is the formation of para-crystalline arrays and this image shows the highly ordered nature of the Flock House virus particles in these arrays [19].

4. Conclusions

The MTF, NPS and DQE of a new Direct Detection Device operated as a direct imaging sensor have been measured for images recorded using 120 keV and 200 keV electrons. The MTF and NPS are reduced at high spatial frequencies and noise is added at low spatial frequencies as is typical for images formed using electrons within this energy range. These characteristics produce a somewhat limited DQE, in spite of the high sensitivity of the DDD which is capable of resolving signals produced by single electrons. However, within these limitations, the detector transfers modulation to frequencies significantly higher than that of SCCD (up to 65 lines/mm at a 5% limit) demonstrating that the DDD provides a platform for much wider field-of-view, high-resolution imaging device for TEM with fast inline digital acquisition with immediate applications to low dose imaging and improved quantification of exit wave reconstruction approaches. These features have been initially illustrated with an example image of an array of Flock House virus particles that shows strong contrast at less than 0.89 nm/pixel at a magnification of only 5,600X.

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Figure 1.

Modulation transfer functions for images recorded with 120 keV and 200 keV electrons, compared to those of commercial cameras. Scatter points represent data reported by manufacturers for conventional SCCD cameras, with electron energy in brackets (where available). Cameras US400, SC1000, SC200 and SC200D are manufactured by Gatan [16]; cameras F224HD and F816 are manufactured by TVIPS [17].

Figure 2. Noise power spectra for images recorded with 120 keV and 200 keV electrons. Noise power spectra of commercial cameras are not available from the literature and therefore cannot be included here.

Figure 3.

Detective quantum efficiencies for images recorded with 120 keV and 200 keV electrons, compared with those of commercial cameras [16,18]. Scatter points illustrate performance of commercial CCD cameras, with electron energy in brackets, Cameras US400 and 794MSC are manufactured by Gatan [16]. Detective quantum efficiency for some of the commercial cameras referenced in Figure 1 are not reported in the literature and cannot be included here.

Figure 4. TEM image of an array of Flock House virus particles in the cytoplasm of an infected cell recorded using the DDD at 120 keV and at a magnification of 0.89 nm/pixel. Scale bar is 100 nm. The arrow on the left shows a gold fiducial marker and the arrow on the right indicates a Flock House virus particle.