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Evaluation of Medipix2 detector for recording electron diffraction data in low dose conditions

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ABSTRACT: The drive for elucidation of important macromolecular structures to high resolution in their 3D native or near-native state places continuously higher demands on the quality of the experimental data. For instance, recording of diffraction patterns good enough for structural studies from cryo-preserved bio-macromolecules at low dose conditions remains challenging and highly desirable. The emergence of hybrid pixel detectors opens up new possibilities for direct electron detection and superior detector performance. Here, we report on the characteristics of the Medipix2 detector in diffraction studies, with a special focus on the reliability of the intensities acquired in very low dose conditions. Diffraction data recorded on a Medipix2 detector were assessed in refinement analysis. R-factors lower than 10% were obtained from data recorded at electron dose of 0.05 el/Å^2. The reproducibility of the data was also shown to be high, given the correlation coefficient of the intensities being higher than 0.9970. The contrast that could be achieved at very low dose conditions was at least an order of magnitude better than that of image plates, based on a direct comparison.

KEYWORDS: Pixelated detectors and associated VLSI electronics; Radiation damage evaluation methods; Photon detectors for UV, visible and IR photons (vacuum); Neutron diffraction detectors

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1 Introduction

Electron cryo-microscopy has gained increasing popularity in structural biology over the past years. In diffraction mode, studies of frozen two-dimensional crystals using a standard cryo-electron microscope have been essential for solving structures of membrane proteins. One of the main limitations in studying biological molecules by electron microscopy is currently imposed by radiation damage. To partially remedy this problem, samples are cooled down to liquid nitrogen temperature. More sensitive detectors would further improve the situation. Imaging plates and CCD cameras are two powerful detectors used nowadays in electron microscopy for data recording. Photographic film, in spite of some disadvantages, is also widely employed. Direct electron detectors are an upcoming new development. A fundamental and often unappreciated problem in EM is that most of the energy of the electrons is converted into photons inside the microscope. These photons are produced when the electrons are being generated, focused by electromagnetic lenses, or interact with the sample, the walls of the EM or with the electron detector at the far end of the microscope. Although the energy of these photons is high (hard UV and X-ray), their energy is lower than that of imaging electrons. Nothing prevents these randomly scattered photons from depositing their energy alongside the imaging electrons on the electron detector. This inevitably means that conventional electron detectors have inflated signal-to-noise ratios, which often obscure essential details. This problem severely impacts studies that necessitate a limited electron dose, like time resolved studies or life science research. If there would be a way to detect *only* the signal (electrons), whilst *disregarding* the noise (photons), this would represent a quantum leap in electron microscopy, as this would allow studies with unprecedented contrast.

Quantum area detectors locally measure the energy of incoming quanta and can be programmed to count only events within a predetermined energy window. Llopart and Faruqi [1, 2] showed that the Medipix detector can also be used for electron detection in TEM. Here, based on structural diffraction studies performed in very low electron dose, we show that the use of Medipix for TEM can result in a vastly improved contrast: the same signal-to-noise ratio could be obtained at a dose that was an order of magnitude lower, when compared to imaging plates, the current best detector

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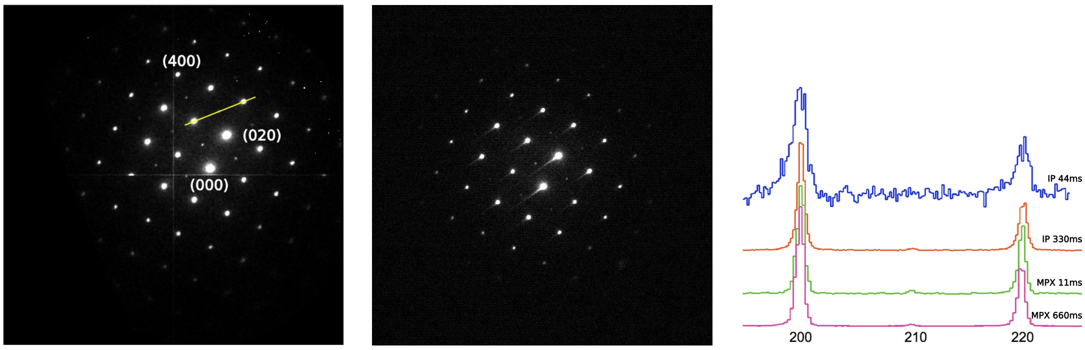

(a) (b) (c)

Figure 1. Electron diffraction pattern of the $SrTiO_3$ [001] zone recorded on Medipix2 using 330ms exposure time (a), IP using 330ms exposure time (b) and line scans of (200), (210) and (220) reflections indicated in figure 1a by a yellow line recorded with the Medipix2 using 11ms and 330ms exposure times and with IP using 44ms and 660ms exposure times.

for this application. We assessed whether the intensities recorded on Medipix in very low dose conditions are reliable and can be used for structural analysis. A special focus is set on the reproducibility of the data recorded in the conditions described. The performance of the detector for structural diffraction analysis is characterized in a direct comparison with imaging plates.

2 Medipix2 detector

Medipix2 is a hybrid pixel electron detector able to count individual events.

The dynamical range of the detector is limited by the level of detected background radiation, which can be substantially reduced using appropriate threshold setting.

Detailed characterization of the imaging performance of the detector was done by Faruqi [2] who showed that the optimal energy at which Medipix2 should be employed is 120keV. At higher energies a significant charge spreading to adjacent pixels occurs which leads to a deterioration of the image obtained. The radiation resistance of the detector was also characterized. It was reported that at a dose of 3.0Mrad per hour, no substantial damage was observed up to 200keV.

3 Experimental

To obtain a sample that was radiation hard and thin enough, a crystal of $SrTiO_3$ was crushed in a mortar. The sample was suspended in ethanol and a droplet of a few micro-litres was pipetted on an EM grid and let to evaporate leading to the formation of sub-micron crystals suitable for EM studies.

Electron diffraction experiments were performed on a CM200 microscope equipped with a field emission gun and operated at 200keV, using an electron dose of 5.2 el/Å^2/s. A thin crystal of $SrTiO_3$ was selected and oriented in [001] zone. Diffraction data from the same part of the crystal were collected on the Medipix2 detector and imaging plates. The Medipix2 detector was installed on the off-axis port of the microscope. DITABIS imaging plates were loaded on the same microscope for the purpose of the experiment. This allowed experiments to be performed under the

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same illumination conditions. The plates were read with a Micro DITABIS scanner with 24 bits dynamic range. Two data channels were employed to view the data, each with a 16 bit dynamic range. The second data channel was used for increasing the dynamic range and measures the data with a 30 fold higher gain. The two images were then combined into one.

4 Results and discussion

Imaging plates have a large dynamic range which makes them often the preferred recording medium for diffraction experiments especially if quantitative analysis is aimed in the study. Therefore plates were chosen for the purpose of the experiment. For the comparative study a crystal of $SrTiO_3$ was selected. This has atoms only on special positions, hence does not require a positional refinement, and the material is very stable in the electron beam, hence one does not have to take into account a change in the diffraction pattern due to radiation damage. [001] diffraction patterns from exactly the same area of the crystal were recorded on DITABIS image plates and the Medipix2 detector at the same microscopy settings with different exposure times. In this specific orientation of a $SrTiO_3$ crystal reflections with strong as well as very weak intensities are present on the pattern. A diffraction pattern recorded with Medipix2 and line-scans through the same three reflections in diffraction patterns recorded with image plate (44 and 660 ms) and Medipix2 (11 ms and 440 ms) are given in figure 1. The presence of the weak h+k=2n+1 reflections on the [001] diffraction patterns was shown to be fairly independent of the sample thickness within the range of thicknesses studied. For each exposure time a number of patterns (up to 20) were recorded on both detectors. The data were further analyzed with the programs GREED and MSLS [3] from the package ELSTRU [4].

First data reduction on the diffraction reflections was performed with GREED. The integrated intensity of each reflection was determined by enclosing the reflection spot with a circular mask. By correcting using a background calculated from the edges of the enclosing mask, one assumes that the background underneath the peak behaves linearly. For refinement, only those reflections were used that had intensity higher than their standard deviation as measured by the inferred background. Two parameters were refined for all datasets: crystal misorientation and thickness. A thickness search was performed in the range between of 5.0Å and 500Å with a step size of 5.0Å. The maximum resolution used in the refinement process was set to 0.7Å. Data were collected with different exposure times ranging from 1s to 5ms for the Medipix detector and from 1s to 22ms for imaging plates. In the case of imaging plates the exposure time was limited by the software of the microscope. In all cases a thickness of 31(1) nm and very similar misorientations were refined. The results from the refinement are presented in table 2.

The Medipix detector allowed collection of intensities reliable for structural analysis at exposure time of 11ms and even lower. In total 20 datasets collected at 11ms were analyzed and the R-values obtained from the refinement process were all found to be in the range between 7.9 and 10.1%. The reproducibility of the data was also studied based on the correlation coefficients of the intensities. The analysis was performed with the program COMPAR from the package ELSTRU. For this purpose, integrated data (output from the program GREED) were fed into COMPAR. Correlation coefficients (intensity by intensity) of the different datasets collected at 11ms were calculated. The intensities were found to correlate very highly with coefficients ranging between 0.9970 and 0.9988 (see table 1). High correlation coefficients of the intensities were also found

Table 1. Refinement results of data recorded with Medipix2 detector. Successful refinements could be done with all datasets, as shown by the R-factor (preferred R-value below 10%). Correlation coefficients show high reproducibility of the data recorded with Medipix2 even at very low exposures.

Exposure time (ms)	Number of datasets	R-Value (%)	Correlation coefficient (reference is 1 set of 11 ms)
2	3	-	0.9420-0.9751
4	3	-	0.9677-0.9846
7	3	-	0.9877-0.9925
9	3	-	0.9893-0.9967
11	20	7.9-10.1	0.9970-0.9988
22	1	11.5	0.9977
440	1	8.0	0.9979
660	1	7.7	0.9974
880	1	8.4	0.9989

Table 2. Refinement results recorded on DITABIS image plates. The reliability and reproducibility of the electron diffraction data recorded on image plates are strongly dependent on the exposure used.

Exposure time (ms)	Number of datasets	R-Value (%)	Correlation coefficient (reference is 1 set of 1000 ms)
22	1	44.7	0.8015
44	2	24.4-28.7	0.9356-0.9520
66	3	10.5-15.0	0.9454-0.9664
88	3	8.8-10.0	0.9797-0.9861
111	3	5.9-10.3	0.9748-0.9761
154	3	6.1-8.3	0.9809-0.9951
220	3	7.2-9.2	0.9876-0.9908
330	3	6.1-8.5	0.9902-0.9941

between datasets taken with different exposure times (see table 1). Diffraction data collected with the Medipix detector within the same series of experiments using an exposure time of 5ms was also subjected to refinement analysis. In this case the automatic peak search failed probably due to the weak signal. Therefore diffraction reflections were selected manually. A relatively high R-value of 18% was obtained from the refinement process using this dataset (an R-factor below 10% is usually preferred when studying small molecules). Line scans performed on the (210) reflection (see figure 1) of datasets collected at 1s, 88ms, 66ms, 44ms, 22ms, 11ms and 5ms showed that up to 11ms the reflection could clearly be resolved. At 5ms exposure time there were only enough statistics to resolve the strong h+k=2n reflections.

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In a different series of experiments aimed at investigating the reproducibility of the intensities collected on the Medipix detector at electron dose even lower than 0.05 el/Å^2, diffraction data were collected from $SrTiO_3$ in the same [100] orientation at 11ms , 9ms , 7ms, 4ms and 2ms using an electron dose of 5.2 el/Å^2/s as in the previous study. The results are presented in table 1. A dataset of 11ms was used as a reference for calculating the correlation coefficients. Up to 7 ms exposure time (0.0364 el/Å^2) the diffraction intensities were found to correlate highly (see table 1).

In the case of imaging plates the data were analysed in the same manner as discussed for the Medipix detector (table 2). In this case an R-factor around 10% could be obtained at exposures of 66ms-88ms. However, the correlation coefficients were lower in this case. The diffraction data collected on plates were characterized in general by lower reproducibility at the conditions studied. Stable results with an R-factor below 10% and a correlation coefficient between 0.9876 and 0.9908 were calculated from datasets taken at exposure at least of 154 ms.

5 Conclusions

By employing a silicon pixelated quantum detector (Medipix2) it was possible to collect reliable intensities in a reproducible way at very low electron dose. The improved efficiency of the Medipix2 detector may be attributed largely to removing photon noise. The detector can be mounted on the off-axis of the microscope, which is an added advantage, since it makes it compatible also with other recording media such as CCD cameras or negatives if those are needed on the same machine. The dynamic range of the detector is in practice limited only by the read-out system, which makes the detector perfectly suited for structural diffraction studies. With the development of a faster read-out system, a further significant improvement of the data collection can be achieved allowing more data (or a stronger signal) to be recorded for the same amount of time. However, even with the current set-up, Medipix2 was shown to have superior performance.

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