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Potential impact of silicon pixel detectors on structural biology

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ABSTRACT

X-ray crystallography and cryo-electron microscopy play key roles in high-resolution biological structure determination. It is important to minimise the radiation dose during imaging as all biological specimens are susceptible to radiation damage. This requires the use of the most efficient detectors possible to collect data. A detector with good spatial resolution is needed to capture high-resolution features in the image. Detection efficiency is also important; efficiency and resolution can be combined in a single property, defined as the detective quantum efficiency (DQE), and particularly DQE as a function of spatial frequency, which is very useful in comparing properties of different detectors. This review deals mainly with two types of pixellated silicon devices, viz. monolithic active pixel sensor (MAPS) and hybrid detectors (Medipix). We discuss the performance of both these detectors for electron microscopy and hybrid pixel detectors for X-ray crystallography, emphasizing the potential advantages they bring to data collection.

1. Introduction

Detectors have played a vital role over many years in helping unravel a large number of biological structures with X-ray or electron sources. As there is a huge range of structures, in terms of size and complexity that need to be tackled there are correspondingly a variety of physical techniques that can be used. For instance, if a specimen is available in a crystalline form (ordered in three-dimensional (3D) or two-dimensional (2D) crystals) it is possible to use X-ray or electron crystallographic methods. Structural determination can be arbitrarily divided into 'low' resolution (2–10 nm), 'medium' resolution (0.5–2 nm) or 'high' resolution (0.15–0.3 nm). Low-resolution data can be obtained with small-angle X-ray scattering (SAXS) or electron microscopy (EM) of negatively stained specimen. To attain somewhat higher resolution it is necessary to use electron cryo-microscopy (cryo-EM) at liquid nitrogen temperatures or X-ray crystallography. The highest resolution has yet to be achieved with cryo-EM of single particles (but 0.38 nm has already been reported [1], which could be considered as near atomic). So only X-ray crystallography is able to get to these limits in a routine fashion.

The main part of this review concerns highlights of detector applications in cryo-EM with a brief section on X-ray crystallography. These applications serve to remind us of what has already been achieved in the past along with the shortcomings of previous detector systems, which need to be addressed with newer technology. The latter forms the major subject of this paper in subsequent sections.

2. Some highlights of detector applications

Cryo-EM is particularly useful when good 3D crystals are not available, which could be used for X-ray crystallography. In some slightly favourable cases it is possible to obtain 2D crystals to which electron crystallographic techniques can be applied and obtain near-atomic resolution [2]. It is possible to get excellent diffraction patterns from purple membrane (containing a 2D array of a transmembrane protein, bacteriorhodopsin—bR), shown in the report [3] and in Fig. 2. In the absence of 2D or 3D crystals, it is possible to obtain structural information from imaging of isolated molecules [1]—a technique commonly known as single-particle imaging. Fairly sophisticated software is required to align and average a large number of individual particles to attain high resolution. Finally, electron tomography allows one to study the structure of large macromolecular complexes and even larger structures such as mitochondria within whole cells [4]. A large number of images are needed at different tilt angles as the specimen is gradually rotated on a goniometer. Due to the large integrated dose during imaging, the role of electronic detectors with a high detective quantum efficiency (DQE) is crucial in minimising radiation damage.

2.1. Study of rapid molecular changes

A key feature of biological systems is that, in order to function, they frequently exhibit some form of dynamical behaviour. A comprehensive understanding of such systems can be obtained by studying the different stages of the dynamics, i.e. by recording time-resolved data—occasionally at sub-millisecond time scales. We have taken just two examples in this review from a wide range

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of experiments to illustrate the diverse use of detectors—one based on time-resolved X-ray diffraction from live muscle [5] and the other based on an electron crystallographic study of the bacteriorhodopsin photocycle [6].

2.1.1. Time-resolved muscle diffraction

The regular arrangement of filamentous proteins into thick (mainly myosin) and thin filaments (mainly actin) are implicated in force generation in vertebrate muscle. Due to the periodic nature of the protein structure within the filaments, it is possible to obtain strikingly informative small-angle X-ray diffraction patterns from living muscle and correlate them with images obtained in an electron microscope. During force generation a series of projections, known as cross-bridges from the myosin filaments, make contact with the adjacent actin filament. The cross-bridges then undergo a structural change on a sub-millisecond time scale, fuelled by ATP hydrolysis, resulting in a relative movement of ~10 nm between the two filaments. The amount of force generated by one cross-bridge is miniscule, around only a few pico-newtons, but due to the large number of cross-bridges acting in parallel, a significant amount of force can be generated over the whole muscle. In some of the earliest experiments using time-resolved techniques, Huxley et al. [7] and Huxley and Faruqi [8] followed the time course of the primary reflection arising from the myosin cross-bridges with a spacing of ~14.3 nm on a millisecond time scale. By imposing small but rapid length changes on a contracting muscle in less than 1 ms it was observed that the intensity of the 14.3 nm reflection decreased with the same time course as the drop in the generated force, as shown in Fig. 1, providing an excellent confirmation of the tilting cross-bridge theory. Similar experiments with much improved time resolution, 0.1 ms, have confirmed the earlier findings [9]. In all these experiments, gas-filled multiwire proportional chambers were used to record the diffraction patterns. Possessing high DQE with zero noise, the main detector-related effort was required in increasing the count rate capability to have significant counts in the very short time intervals being studied; a summary of the earlier detector work is given in a review [8].

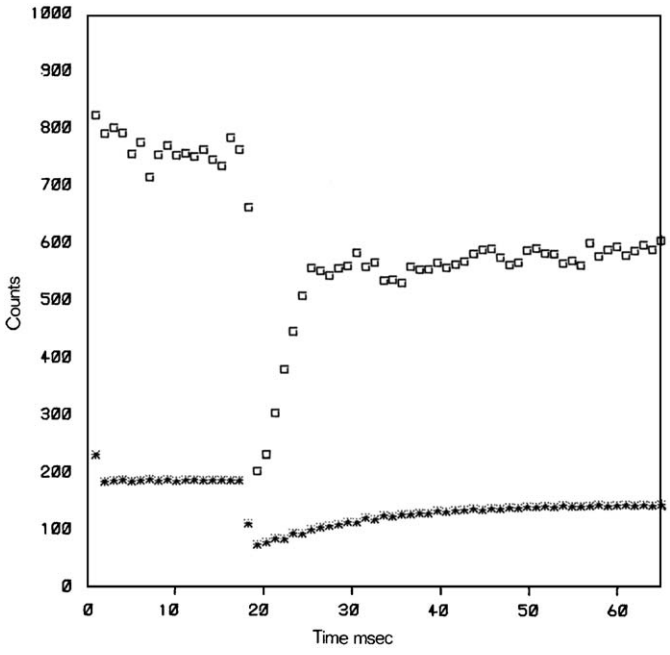

Fig. 1. Time course of the 14.3 nm meridional reflection (shown as boxes), arising from the periodic arrangement of myosin cross-bridges, at a resolution of 1 ms [7]. The force generated by the muscle is shown in the lower trace as asterisks. When the muscle is allowed to shorten suddenly during an isometric (constant length) contraction to a new shorter length, the intensity of the 14.3 nm reflection decreases in synchrony with the force.

Fig. 2. Electron diffraction pattern from two-dimensional crystals of bacteriorhodopsin recorded on a CCD camera [3] at 120 keV.

2.1.2. Conformational changes in bacteriorhodopsin

The study of conformational changes in bacteriorhodopsin, the main constituent of purple membrane, was carried out by recording diffraction from 2D crystals in a CCD detector (). The role of bR is to act as a light-driven proton pump, transferring an H^+ across the cell membrane. The creation of a proton gradient across the membrane results indirectly in the production of ATP; bR thus acts as a transducer converting light (solar) energy into chemical energy within the cell. Conformational changes in bR were studied by Subramaniam et al. [10] by trapping intermediate states in the bR photocycle. Since many of the intermediate states have extremely short lifetimes, the authors used genetically modified molecules in which the intermediate states have a longer lifetime, which were studied by freezing in vitreous ice, but are structurally similar to the wild type. Due to difficulties in obtaining 3D crystals of bR (but not 2D crystals), this work was done using electron crystallography [2]. This type of experiment would have been very difficult with films due to the large number of diffraction patterns needed. A large quantity of electron crystallographic data was acquired on CCD detectors [11,12].

3. Detectors: evolution of direct detectors

Despite the similarity in detector requirements for X-rays and electrons, there are also many differences. These are partly due to the very different energy range of interest for the two radiations: X-rays are typically 10–20 keV whereas electrons are of much higher energies ranging between 100 and 300 keV.

Since electrons are easily scattered or absorbed in matter the detectors need to operate within microscope vacuum. The present generation of electronic detectors for both techniques is based mainly on indirect detection. The incoming radiation is converted into light in a phosphor/scintillator, which is imaged by a cooled,