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Area detectors in structural biology

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Abstract

An overview of area detectors in structural biology is presented. Development of these detectors is one of the main reasons for the exponential rise in the number of structure determinations of large biological complexes. The different techniques used for structure determination put different demands on area detectors. The techniques used in structural biology, X-ray and electron diffraction and electron imaging are discussed and the requirements for a good detector are highlighted. Furthermore, an overview is given of the current state of the art of high-resolution area detectors.

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1. Introduction

It is not much more than 15 years ago that most data in X-ray crystallography and electron microscopy structure determinations of proteins and other large biological molecules were still collected on photographic film. The exponential rise in the number of structures determined since then [1] must largely be attributed to the emergence of powerful, high-resolution area detectors and ever more intense X-ray sources. Two fundamentally different principles are used in high-resolution studies of macromolecules: diffraction and direct high-resolution imaging (Fig. 1). Casual comparison of the optics of diffraction and high-resolution imaging indicates that each approach places different demands on a detector. Here a brief overview is given of these demands.

1.1. X-ray diffraction

In protein X-ray crystallography, X-rays with a wavelength between 6.2 and 25 keV (0.05 and 0.2 nm) are used [2]. As there are no effective lenses for such radiation, data are collected in diffraction mode. Diffraction experiments of crystalline material generate images that are substantially

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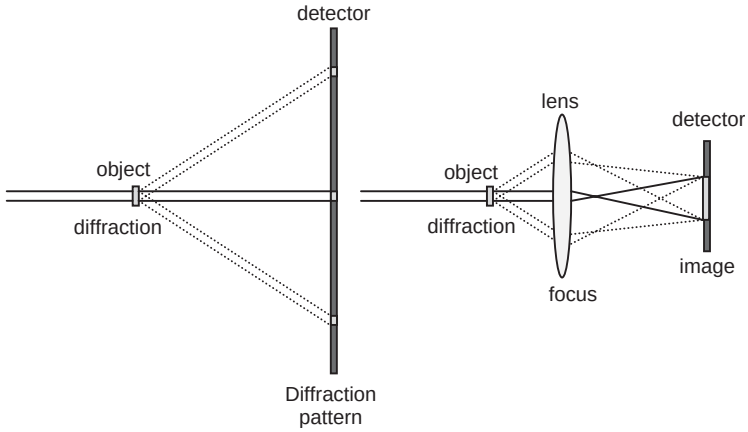
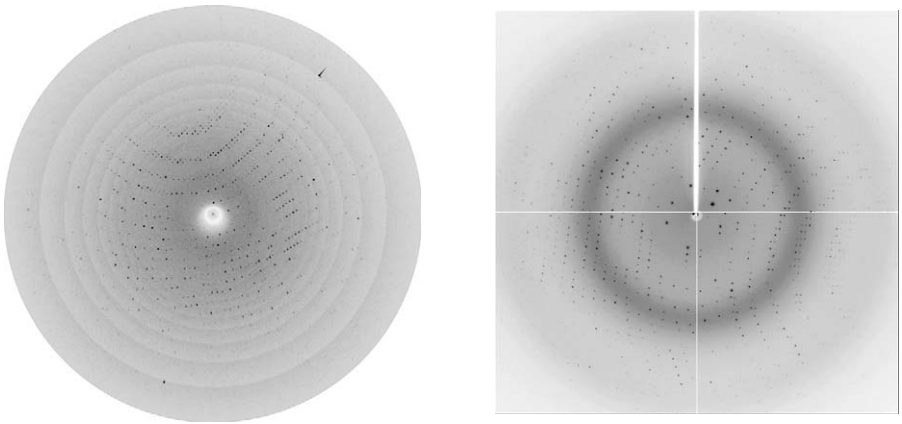


Fig. 1. A comparison between the optical principle of diffraction (left) and a high-resolution imaging (right) experiment. In diffraction, the resolution of a Bragg spot on the detector is therefore determined by its distance from the direct beam (usually located in the center of the detector). The resolution of diffraction data is determined by the maximum angle of diffraction and *not* by the intrinsic resolution of the detector, which is determined by its point spread function. In a direct imaging experiment, the point-spread function of the detector *does* determine the resolution of the data.



(a) (b)

Fig. 2. (a) Diffraction pattern of a lysozyme crystal recorded on a MAR345 image plate detector upon exposure on a home source 5 kW rotating anode X-ray generator. The geometry of the experiment is as in Fig. 1 (left). Though weak, diffraction spots can be seen to the edge of the detector, corresponding to a resolution of about 1 Å. (b) Diffraction pattern of a protease inhibitor crystal recorded on a tiled CCD detector, consisting of four CCDs, collected on ID29 at the ESRF in Grenoble. Resolution at the edge is about 1.6 Å. The white shadow is caused by the beam stop, which blocks out the direct beam. Bragg diffraction spots can be seen above a fairly isotropic background due to inelastic scattering, lattice defects, random or correlated motions of the molecules in the crystal and scattering by air or the support medium of the crystal.

different from imaging experiments. In diffraction images, the high-resolution information is determined by the angle of diffraction of the Bragg spots and the experiment aims to determine with the greatest accuracy the intensity of each of these diffraction spots (Fig. 2). Inevitably, X-ray diffraction also generates diffuse background scatter, resulting from inelastic scattering, solvent scatter-

ing, lattice defects, random or correlated motions of the molecules in the crystal and scattering by air or in the support medium of the crystal. In the absence of absorption by air, the intensity per area of this background falls off quadratically with the distance from the sample, whereas the intensity of the Bragg peak remains constant in the case of a well-focused beam and a crystal with low mosaicity. In order to measure the intensity of the discrete diffraction spots as accurately as possible, the background can therefore be reduced by using a large detector that is placed as far as possible from the crystal, ensuring that the high angle, high resolution data can are collected close to the edge of the detector. Profile fitting of Bragg spots to the two- or three-dimensional shape of the (local) average diffraction spot is used to enhance the accuracy of the intensity measurement [3]. These profile-fitting techniques require a Bragg spot to extend across multiple independent detector pixels.

On the basis of these considerations, the specifications of a good detector for X-ray crystallography can be formulated:

- High dynamic, linear range (intensities of Bragg spots vary over many orders of magnitude);
- high sensitivity, as measured for instance by the DQE[1] (high resolution spots may have an intensity similar to the standard deviation of the background, and still be useful for structure refinement);
- large surface area (to reduce the background);
- fairly fine sampling, typically 2000×2000 pixels;
- fast readout times (exposures at synchrotrons can be as short as 0.1 s);
- a point-spread function that is not too bad (1/500th the total width of the detector is acceptable).

On low intensity sources, image plate scanners are currently the most widely used detectors due to their large size, high sensitivity and high dynamic range. On high intensity synchrotron sources, CCD cameras are more popular in view of their substantially faster readout times.

[1] DQE = Detective quantum efficiency, defined as: $(\text{Signal}_{\text{out}}/\text{Noise}_{\text{out}})^2/(\text{Signal}_{\text{in}}/\text{Noise}_{\text{in}})^2$.

1.2. Electron diffraction

Electron diffraction of frozen two-dimensional crystals using a standard cryo-electron microscope has been essential for solving structures of membrane proteins (e.g., [4]). The same considerations apply for the detector as in X-ray crystallography and in addition the detector should operate well inside the high vacuum of the microscope. The Bragg spots of electrons are focused on the detector, so the size of the diffraction spots can be tuned to a certain extent to the point-spread function of the detector. A CCD detector is mostly used for measuring electron diffraction, although image plates are also used for specific applications where a higher dynamic range is required.

1.3. Electron imaging

In high-resolution electron microscopy imaging, lenses generate a real space projection of the sample on the detector. This means that the high-resolution and low-resolution information are recorded locally, which places different demands on the detector compared to the recording of diffraction patterns. In principle, it is possible to visualize atomic details with a modern electron microscope, but radiation damage rules out the collection of sufficiently strong images of macromolecular biological complexes at high resolution. To partially remedy this problem, samples are cooled down to liquid nitrogen temperature. Nevertheless, high-resolution recordings of biological complexes are by necessity underexposed. The poor signal-to-noise ratio of such weak images can be enhanced by averaging over many particles. In non-crystalline samples, individual particles need to be aligned and averaged computationally in three dimensions [5]. State-of-the-art cryo-electron microscopy allows the determination of the structures of large protein complexes to a resolution of 0.6–0.7 nm (Fig. 3). The resolution of such structure determination is not limited by the wavelength of the electrons (which can be down to 0.025 Å) or by the microscope (which can achieve a line resolution of 1.7 Å), but by the order in the sample and by the quality of alignment and

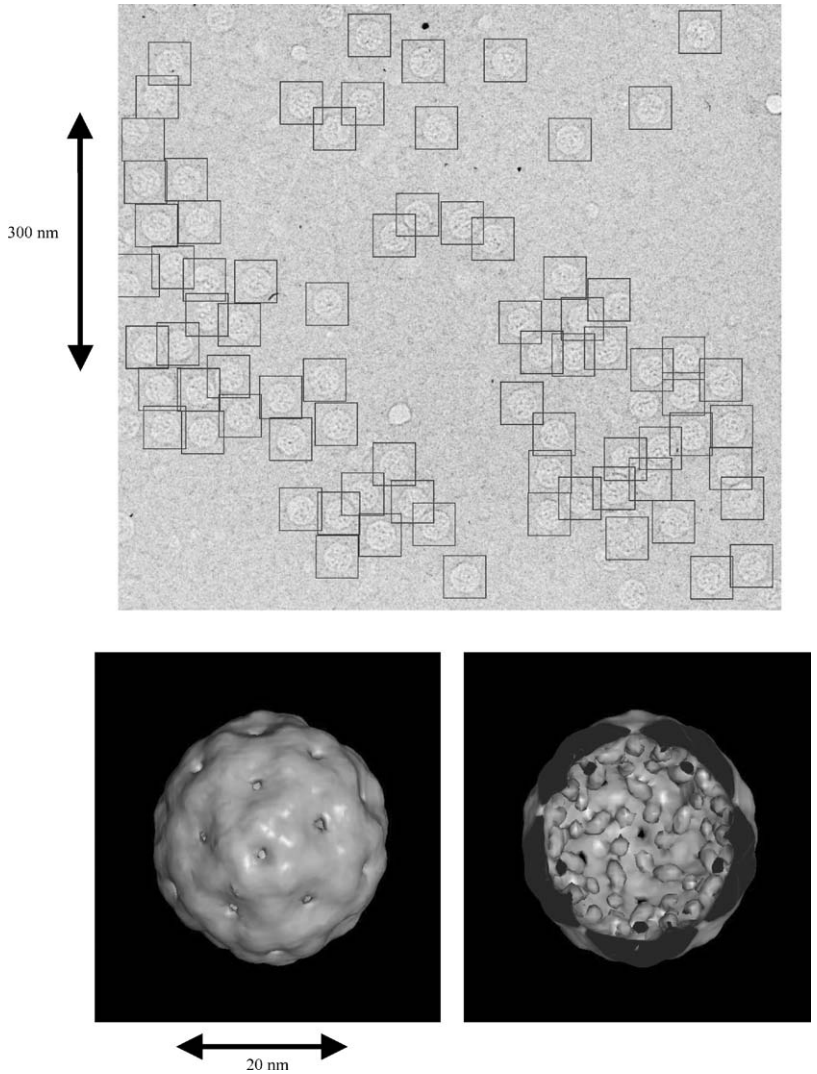


Fig. 3. Top: electron micrograph of a high-resolution cryo-EM image of bacteriophage MS2. Individual virus particles are boxed. Bottom: a high-resolution three-dimensional reconstruction of bacteriophage MS2 (left: the intact particle; right: a view into the core of the virus), calculated by aligning and averaging 2600 boxed particles and imposing the known icosahedral symmetry constraints.

averaging procedures. Detectors for electron microscopy need the following characteristics:

- High sensitivity (since exposure time is limited by beam damage to the sample, the total count-per-pixel will be low);
- a very sharp point-spread function, with a width that ideally should not be much more than a single pixel;
- low intrinsic background noise (since the signal tends to be low);
- large surface area of at least 2000×2000 pixels;
- fairly fast readout times (an exposure typically takes about 1 s, but finding an area to image usually takes much longer in current practice—although this is likely to change with the introduction of automated data collection);

- a high dynamic range is not essential (contrast is very low, so the intensity of the signal does not vary much over the detector);

The most popular collection system for electron microscopy still is photographic film, because of its superb resolution. Disadvantages of film are obvious, hence more and more microscopes are equipped with a CCD camera, even though these devices do not achieve the spatial resolution of film.

2. Detectors

Table 1 summarizes the most common types of detector that have been used for imaging and diffraction in structural biology. In view of current developments, we only discuss three types of detector.

2.1. Image plate detectors

Image plate detectors that scan X-ray diffraction patterns on-line have been on the market since about 1992. The main manufacturers are MAR Research and Rigaku. They are based on a storage phosphor, in which X-ray photons excite electrons to higher energy levels, where they can remain trapped in color centers. The stored energy can be released by illuminating the color centers with red light, whereupon they emit the stored energy as blue light. The amount of blue light emitted is proportional to the number of X-ray photons that hit the color center and can be measured with a photo-multiplier. In practice, first the image plate is exposed to X-rays, then the beam is switched off and the plate is scanned. Several geometries for scanning image plates with red lasers are employed, and one of the most versatile ones is the spiral scan, where a red laser beam is translated across a rotating image plate.

A major advantage of image plate scanners is their very low intrinsic noise (although they do collect signals from cosmic rays) and their high dynamic range, which is linear up to at least 10^5 X-ray photons per $0.02\,mm^2$. Also their large size is beneficial (for example the MAR 345 detector has a disk-shaped image plate with a diameter of 345 mm). They would be the ideal detector for X-rays if it were not for the relatively long readout time, which is in the order of 50 kpixel per second, so a typical scan takes about 1 min or more.

Storage phosphors can also be excited by electrons, and image plates are used in electron diffraction as off-line detectors. The mechanics required for scanning an image plate cannot easily be built into an electron microscope and hence the image plates are treated in the same fashion as photographic plates prior to scanning. For electron diffraction their main advantage over photographic plates is their linearity and high dynamic range. The width of their point spread function disqualifies image plates as a detector for direct imaging by electron microscopy.

2.2. CCD cameras

Since X-rays and especially electrons are too damaging to CCD chips, CCD cameras use a phosphor as an intermediate step in detection. Furthermore, X-ray absorption in the thin active

Table 1
Overview of dectectors used in high-resolution structural biology

	X-ray diffraction	Electron diffraction	Electron imaging
Chemical detection: photography	Obsolete	Obsolete	State of the art
Multi-wire	Obsolete	Never used	Irrelevant
Television camera: FAST	Obsolete	Never used	Irrelevant
Image plate	State of the art	Used sometimes	Not used
CCD	State of the art	State of the art	Up and coming
Pixel detectors	Near future	?????	?????

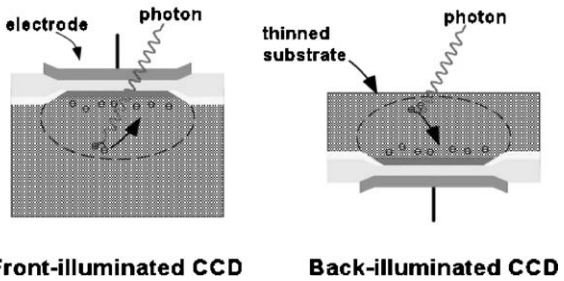


Fig. 4. Front-illuminated CCDs have a lower quantum efficiency than back-illuminated CCDs as in the former a portion of the photons are absorbed by the detection electronics.

layer of a CCD is relatively low. X-rays or electrons hit a phosphor layer, and the excited secondary photons are transmitted through a fiber optic that can be tapered to the CCD chip. The visible light generated by the phosphor hits a photoactive substrate and the electrons that are generated by this process are detected by read-out electronics. Recently, so-called 'back-illuminated' CCDs have been introduced, (e.g., the Fairchild 486), where the secondary photons do not have to pass the read-out electronics before hitting the photoactive substrate (Fig. 4). These CCD detectors have a higher quantum efficiency since more photons hit the photoactive substrate.

The advantages of cooled CCD detectors are their fast readout time, their low noise and their reasonable spatial resolution. However, they also have some disadvantages: they are limited in size (the largest CCD chips are currently about $3600\,mm^2$) and their dynamic range is limited. The small size of CCD chips requires demagnifying fiber optics, which can lead to a loss of signal and an increase in the width of the point-spread function. Recently, Bruker–Nonius introduced a commercial lens-based system that seems to be a viable and attractive alternative to fiber optics. Nevertheless, in view of their fast readout time, CCD detectors are becoming the detector of choice for intense X-ray beams. Furthermore, they have replaced film and are an excellent alternative for image plates in electron diffraction studies. Finally, very large CCD detectors are replacing film in electron microscopy, even though, due to the requirement of a phosphor, their spatial resolution relative to their size is worse than that of photographic film.

2.3. Solid state detectors

The most recent development in area detectors for structural biology is the direct-detection pixel-based solid state detector. In these detectors the X-rays or electrons directly hit a semi-conductor, creating free electrons and holes that are detected by electronics. Because the conversion by a phosphor into visible light is skipped, a higher spatial resolution and quantum efficiency can be anticipated. Read-out electronics can be designed to have a low noise, a high dynamic range and a fast readout. Several systems are currently being developed and tested. Without being exhaustive, we briefly discuss two of them.

The MARResearch solid state area detector uses an amorphous selenium semiconductor, and free electrons are detected by a thin film transistor array which is bonded to the scintillator. This solid state, direct conversion detector has a size of $430 \times 358\,mm^2$, and contains about 7.8 Mpixels with a surface of $140 \times 140\,\mu m^2$ each. Like CCD detectors, the electronics of each pixel accumulate the signal in a storage capacitor. Reading out the detector requires little time, (in the order of about 1 s), but is sufficiently long that the X-ray exposure has to be interrupted for readout. This detector has already been tested on demanding samples, including virus crystals (Fig. 5).

X-ray pixel detectors built in hybrid design consist of a radiation sensor, a reverse biased pn-diode (usually silicon) with a segmented contact structure on the p-side, and a readout ASIC-chip, with the same pixel structure. Sensor and read-out chips are connected via tiny conducting indium balls, called bump-bonds. X-rays are detected in the sensor and the resulting signal is amplified in the pixel chip. Due to the direct detection of the X-rays with a silicon sensor, the point spread function of such detectors is excellent. The DQE is given by the absorption of the X-rays by the silicon which is 75% at 12 keV for a 0.3 mm thick sensor. One of the most advanced systems is the three module-array of the PILATUS detector.

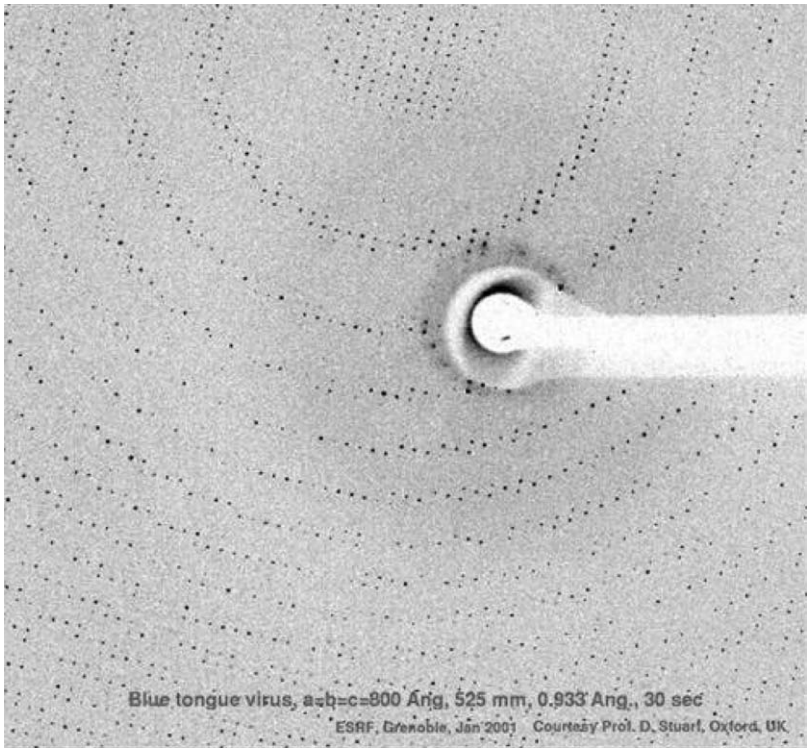


Fig. 5. A zoomed view of a diffraction pattern collected by a MARResearch direct conversion area detector of a Blue Tongue virus crystal (cubic space group, $800 \times 800 \times 800\,\text{Å}^3$ unit cell), in a geometry comparable to Fig. 1 (left). Despite the close spacing of the diffraction spots, a very good separation is achieved through the combination of the large size (which also reduces background) and the low point spread of the detector.

This detector is $35.3 \times 238.7\,\text{mm}^2$ and has a readout time of 6 ms. The principle and the specifications of the full detector are described in Ref. [6].

A desired mode of data collection in macromolecular crystallography is fine-ϕ-slicing, preferred because it improves the signal to background ratio of the Bragg reflections. In this mode the crystal is rotated by a fraction of its angular acceptance (0.02°–0.2°) during each frame, leading to data sets of as many as 9000 frames for a full 180° rotation. Clearly, such an experiment is very time consuming with a CCD detector. Therefore a very fast framing detector is required, so that the crystal can be rotated *continuously* in the beam without opening and closing the shutter for each frame. For such an experiment, an electronically gateable detector is needed, because of the absence of a mechanical shutter. Since the crystal is rotated without any shutter operation, the read-out time of the detector needs to be as fast as possible, in order to reduce the dead-time during the rotation of the crystal. And, most important, the detector should not add any additional noise to the data, when being read out. Pixel Detectors as e.g., the PILATUS detector are specifically designed to take data in fine-ϕ-slicing mode (Fig. 6).

Currently, direct conversion, solid state pixel detectors are only used for detecting X-rays in structural biology. However, there is no intrinsic reason why they could not be used for detecting electrons, despite some technical hurdles. Such a detector would be a viable alternative to CCD or image plate for electron diffraction studies. Currently, the pixel size of the direct conversion

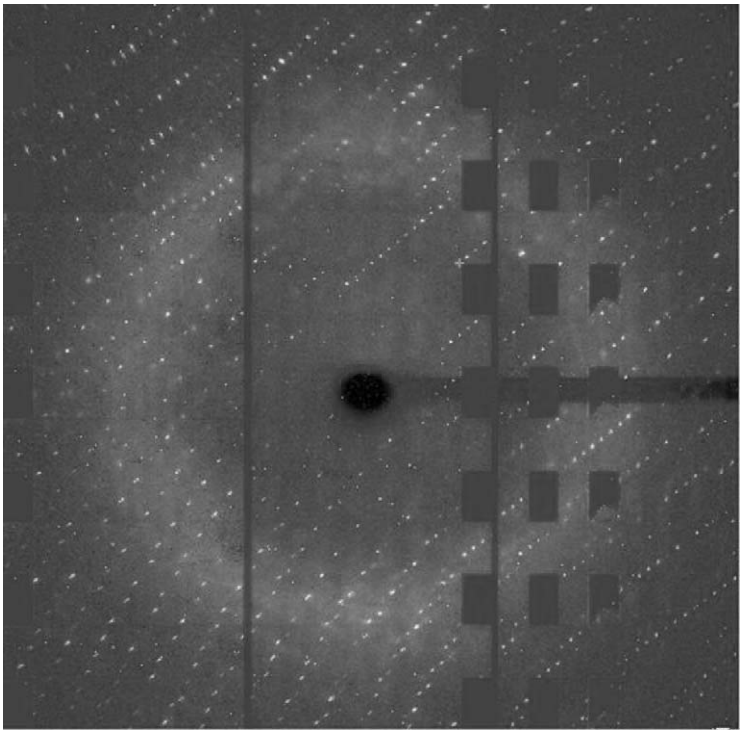


Fig. 6. A diffraction pattern (after flat field correction) of a lysozyme collected by the PILATUS solid state area detector developed by Dr. Broennimann at SLS in the standard geometry of data collection (Fig. 1, left). The crystal was rotated for 1° and simultaneously exposed for 2 s with 12 keV X-rays. Due to the still limited size of the detector, it was translated and the pattern is a composite of the data collected at 7 detector positions. Three of the 48 chips of this detector were unresponsive at the time of the experiment, explaining the missing patches. The detector has three modules, spaced 2.38 mm apart.

detectors is still too large for electron imaging. However, if these detectors could be made large enough to compensate for the relatively large pixel size (which may be reduced to about $40 \times 40\,\mu m^2$ with current technology), they would become an attractive alternative due to their fast readout, high quantum efficiency and low point spread.

3. Conclusions

Development of high-resolution area detectors has contributed significantly to the increase in the number of structure determinations of proteins and other large biological complexes. Image plates and CCD detectors are now widely used in X-ray and electron diffraction. In high-resolution electron imaging, however, photographic film is still the preferred instrument of detection due to its superb resolution. CCD cameras are now slowly becoming available for this technique. Further development of these and other detectors may, especially when combined with automation of the data collection, dramatically improve the throughput of structure determinations using electron microscopy.

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