Taking a look at the calibration of a CCD detector with a fiber-optic taper

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Received 5 October 2015

Accepted 8 January 2016

Edited by V. T. Forsyth, Institut Laue–Langevin, France, and Keele University, UK

Keywords: CCD detectors; fiber-optic tapers; flat-field calibration; data scaling; anomalous signal.

Supporting information: this article has supporting information at journals.iucr.org/j

At the Structural Biology Center beamline 19BM, located at the Advanced Photon Source, the operational characteristics of the equipment are routinely checked to ensure they are in proper working order. After performing a partial flat-field calibration for the ADSC Quantum 210r CCD detector, it was confirmed that the detector operates within specifications. However, as a secondary check it was decided to scan a single reflection across one-half of a detector module to validate the accuracy of the calibration. The intensities from this single reflection varied by more than 30% from the module center to the corner of the module. Redistribution of light within bent fibers of the fiber-optic taper was identified to be a source of this variation. The degree to which the diffraction intensities are corrected to account for characteristics of the fiber-optic tapers depends primarily upon the experimental strategy of data collection, approximations made by the data processing software during scaling, and crystal symmetry.

1. Introduction

Protein crystallography data have been collected successfully for more than 20 years using charge-coupled device (CCD) detectors. Many groups have been involved in developing CCD detectors (Strauss *et al.*, 1990; Stanton *et al.*, 1993; Phillips *et al.*, 1993; Thiel *et al.*, 1995; Tate *et al.*, 1995; Pokrić *et al.*, 2002), including a very successful program located at Argonne National Laboratory, which created several CCD detectors with fiber-optic tapers that were used for many years at the Structural Biology Center, sector 19, at the Advanced Photon Source (APS) (Westbrook & Naday, 1997; Naday *et al.*, 1998, 1999). These detectors were eventually replaced by commercial detectors from Area Detector Systems Corporation (ADSC) (12550 Stowe Drive, Poway, California 92064, USA). A comprehensive review of the history of CCD detector development, particularly as it relates to detectors with fiber-optic tapers, has been given by Gruner *et al.* (2002).

In examining several of these papers the reader is given the distinct impression that the detector manufacturers are undertaking the sole responsibility to calibrate the detector in all aspects, such as non-uniformities caused by irregularities in the phosphor, light losses due to physical changes to the fiber diameters created during taper construction and light losses that occur within the taper itself as the bend of the fibers increases with higher demagnification. Calibration files are always provided with the detector, so it is natural to assume that the intensities from the corrected images, *i.e.* images corrected for all known systematic effects, will be on a uniform scale and vary by, at most, a few per cent.

After more than 10 years of operation, the last Argonne designed detector was replaced at beamline 19BM by an ADSC Quantum 210r detector in 2009. It has produced high-quality data ever since it was installed and continues to do so today. As a routine precaution we periodically check the integrity of all beamline equipment, including the detector, to ensure it is operating at peak levels. Most of the detector checks involve taking routine data sets on a crystalline sample of lysozyme. However, since the detector was more than 5 years old, it was decided to look more closely at the detector performance. In this instance, a modified flat-field calibration was performed, followed by mapping out the intensity profile of a test reflection as it was moved across one-half of a detector module. The goal was to validate detector operations and, if necessary, detect any problems if they existed.

1.1. Detector construction

A description of an ADSC Quantum 315r detector has been provided elsewhere (Holton *et al.*, 2012). Since the ADSC Quantum 210r detector is built from a smaller number of the same type of modules, most of the details are the same. For clarity, some of these details will be repeated here.

The ADSC Quantum 210r detector is a 2×2 tiled array of four sub-detectors. Each sub-detector is composed of a

thermoelectrically cooled CCD chip hard epoxy bonded to the narrow end of a fiber-optic taper. The taper (Incom, Charlton, MA, USA) has a nominal 3.7:1 demagnification ratio. The X-ray-sensitive phosphor is a 20–40 μm layer of powdered $\text{Gd}_2\text{O}_2\text{S:Tb}$ (particle size less than 5 μm) sandwiched between the end of the taper and a thin aluminized black plastic front window that also serves as a vacuum barrier. The absorption of X-rays in the phosphor generates fluorescent photons, which are emitted in random directions, so they can be (i) transmitted into the optical fibers of the taper, (ii) scattered in the phosphor and transmitted into the taper, or (iii) lost. The point-spread function resulting from this process may have some local variations due to phosphor layer granularity, but otherwise should be uniform across the detector (Holton *et al.*, 2012).

An individual taper contains millions of individual fibers with the diameter on the exit site being much smaller than the pixel size of the CCD chip. Each fiber consists of a core glass of high index of refraction, surrounded by a lower index glass cladding, which serves to contain light by total internal reflection. When light reflects within a fiber at an angle steeper than the total internal reflection angle, it will leak into the cladding. To maintain high contrast, a stray light absorber (generally referred to as EMA, for extramural absorption) is added to the fiber-optic tapers between or around individual fibers (Roper Scientific Inc., 3660 Quakerbridge Road, Trenton, NJ 08619, USA, technical note, <http://www.photometrics.com/resources/technotes/pdfs/fiberoptics.pdf>). Single fibers (10 μm) are stacked into a set of canes of hexagonal cross section and these canes are fused into a large round bundle. The fiber-optic bundle is heated and pulled into an hourglass shape, then cut in two to form two individual tapers. The axial fibers will remain straight and tapered after pulling, while peripheral fibers will be both bent and tapered. To bond to a square CCD chip and to allow for placement into a square array, both ends of the taper are machined into a square shape (Holton *et al.*, 2012). Fig. 1(a) shows a finished fiber-optic taper inside a mounting fixture without the bonded CCD chip. Figs. S1(a) and S1(b) in the supporting information show a view of the narrow end of the fiber-optic taper along with a view of the hexagonal pattern of the bundled fibers.

In ADSC Quantum 210r and 315r detectors, each taper is machined down from a starting bundle (ratio 3.7:1) to approximately $105 \times 105 \text{ mm}$ at the wide end, narrowing down to $25 \times 25 \text{ mm}$ at the narrow end, resulting in a wide-to-narrow end ratio of 4.2:1. Differences from the nominal value of demagnification (3.7:1) are due to image distortions, which are corrected in the manufacturer software with the geometric transformation of CCD output data. However, the geometric transformation corrects only for distortion of the image and not variability of the point-spread function of the detector. Fig. 1(b) shows room light transmission through the fiber-optic taper to illustrate one of the consequences of the optic fibers' bending. As the bending increases, the incidence angle of the light changes within the fiber and light transmission stops when the angle exceeds the critical angle. Therefore, fibers with a higher bending radius closer to the edges of the module

Figure 1
(a) Photograph of a machined fiber-optic taper inside mounting hardware without the bonded CCD chip. Yellow tape surrounding the taper is for the purposes of assembly and has no impact on operations. (b) Overhead light transmission through the taper. Dark areas are locations in the taper where the critical angle of internal reflection has been exceeded.

lose more light, which results in a darker, roughly radially symmetric pattern about the center of the module.

Light transmission will depend primarily on the physical properties of the taper, such as the refractive index of the glass, the core/cladding ratio of the individual fibers, the effective numerical aperture and taper ratio, and the fusing conditions used in making the fiber-optic bundle (Gruner *et al.*, 2002). The effective numerical aperture of a single tapered fiber is proportional to the magnification ratio and describes the taper's overall transmission abilities. Any light entering beyond the acceptance angle escapes through the fiber walls while traveling down the taper and it should be absorbed by the EMA. Transmission will also depend on the angular distribution of the incident light and its wavelength. Of particular importance is the change in magnification from center to edge as the bend and size of the fibers change with location in the taper (Gruner *et al.*, 2002).

It is not possible to calculate the exact light output from the phosphor and transmission factor through the taper for a given location on the detector owing to the variables involved, such as variations in phosphor thickness, fiber dislocations, distortions that occur during taper construction, changes in taper radius *etc.* This can only be done accurately through direct measurements. This is why the calibration of each detector is so important.

1.1.1. Detector performance with lysozyme. To determine a measure of detector performance prior to any calibration checks, a series of experiments were conducted to look at the overall R_{merge} of crystalline hen egg-white lysozyme as a function of beam attenuation. R_{merge} was chosen as a guide simply to establish a performance baseline. Since all data sets cover the same rotation range and were collected at the same rotation rate, this comparison should be informative. Typically,

the detector is performing well if any data set from a high-quality, well diffracting crystal gives a linear R_{merge} under 3% overall when processed to a resolution of 1.8 Å.

Low-mosaic, beamline calibration lysozyme crystals are grown using the micro-seeding procedure described in detail in the supporting information. The particular crystal used in this study was obtained from 0.100 M NaOAc (pH 4.75), 4% (w/v) NaCl, 27% (v/v) ethylene glycol. Tetragonal hen egg-white lysozyme crystallizes in space group $P4_32_12$, which in this case yielded cell parameters of $a = b = 78.87$, $c = 36.84$ Å upon cryocooling.

Each data set consisted of 90 binned images of 1° oscillation width, collected at 12.66 keV (just above the nominal Se absorption edge), with each frame collected at a rate of 1° per second. The typical photon flux at 19BM is 1.6×10^{11} photon s^{-1} at 100 mA ring current. The overall redundancy was 6.8 for each resolution shell and the data were processed to 1.8 Å for the purposes of comparison. The crystal mosaicity ranged between 0.21 and 0.25°, which indicates a high-quality crystal that lacks macroscopic disorder. The first data set was unattenuated followed by increasing attenuation up to data set 19, which has an attenuation factor of 415. Data set number 20 (attenuation factor 7.5) was the last in the reported series and is the only data set taken out of the increasing attenuation order. Each data set was collected for the exact same time per frame over the identical oscillation range. All data were processed using *HKL3000* (Minor *et al.*, 2006; Otwinowski *et al.*, 2003; Otwinowski & Minor, 1997). Results are plotted in Fig. 2 with selected attenuation values shown in parentheses on the plot.

As the attenuation factor goes from high to low, statistics improve, but only to a certain point. The mean $I/\sigma(I)$ for merged observations plateaus at about 72, near an attenuation factor of 4.5 (linear $R_{\text{merge}} = 0.019$), corresponding to an average (I) equal to 720 in arbitrary units, but constant for all data sets. This is the point where the number of saturated reflections starts to noticeably increase.

Very little improvement (*i.e.* a decrease in linear R_{merge}) is achieved past this point. Even with a fourfold increase in average (I) for the unattenuated beam the linear R_{merge} only drops to 0.018. This means that we have reached the minimum value obtainable with this detector, given the experimental conditions, crystal quality, detector calibration and data processing procedures used (Diederichs, 2010; Borek *et al.*, 2003).

While radiation damage certainly occurred during the experiment, it was not sufficient to change the expected results from data set 7 (attenuation factor 8.63) and data set 20 (attenuation factor 7.5), which had average (I) values equal to 386 and 435, respectively. In addition, the mosaicity was reported to be the same for the first data set (attenuation factor 1.0) and the last data set collected on the crystal. Finally, the scaling B -factor increase for the first data set acquired without attenuation was only 1 Å², which is equivalent to a dose of 1 MGy (Borek *et al.*, 2007, 2010, 2013; Kmetko *et al.*, 2006) while 20–40 MGy is considered a limiting factor (Owen *et al.*, 2006). All other data sets owing to the attenuation used

Figure 2

Data sets of crystalline lysozyme collected as a function of attenuation. Selected attenuation values are shown in parentheses. The highest attenuation factor (415) corresponds to the lowest average (I) (open circles) and lowest mean $I/\sigma(I)$ (solid points). As the attenuation factor decreases, the linear R_{merge} decreases proportionately to the increase in statistics. When the average (I) reaches ~ 720 for the data set, mean $I/\sigma(I)$ plateaus near a value of 72. Beyond this point, very little change occurs in linear R_{merge} , regardless of the increase in average (I).

had a cumulative dose that was lower than 1 MGy. Therefore, we concluded that radiation damage did not significantly influence these results.

Our results confirm that the total error in measurements of diffraction intensities comprises the counting statistics contribution (Poissonian) and the residual systematic errors. Increased exposure reduces the Poissonian error of intensity integration to the point where multiplicative systematic errors begin to dominate (Diederichs, 2010). Having observed that significant systematic errors exist, we will look for evidence of these detector-specific errors during further detector verification tests.

2. Flat-field calibration

A limited calibration to validate manufacturer specification was performed, with one-half of the top-left CCD module, as viewed facing the detector. This module is unobstructed by the beamstop shadow, allowing a clear path to a metal-foil-generated fluorescence source.

Using a 7 μm -thick copper foil placed at the sample location, the detector was positioned at a distance of 305 mm with the beamline energy set to 12.66 keV. This was close to the minimal accessible foil-to-detector distance before interference from copper powder diffraction lines becomes an issue. Ten fluorescence images were collected, each for 90 s. Using an ion chamber, a measure of the foil attenuation was obtained. After adjusting the direct beam to account for this attenuation factor, a series of ten air scatter measurements were recorded.

Both the fluorescence images and air scatter images were averaged to produce a single image of each type. Pixel locations were chosen that covered half of the module from outer edge to center. In total, seven lines composed of 23 points each

Figure 3

Copper fluorescence flat-field validation. The standard deviation from the average intensity for all data is 1.1%, indicating the results are a close match to the ADSC Quantum 210r detector calibration. Final ADUs per datum point for these measurements averaged $\sim 17\,000$. Locations of the points displayed for this plot match those recorded for the Si(311) experiment shown in Fig. 4(a).

were created and labeled as a percentage of the distance across the module. For example, the 50% line is a vertical line positioned halfway across the module in the horizontal direction. These are the same locations that will be used when performing the Si(311) reflection measurements that will be discussed later. After subtracting for background air scatter, correcting for a $1/r^2$ fall-off in intensity from the source to the detector, and making a small absorption adjustment for path length differences across the module face, final intensities were obtained. Path length absorption corrections were computed using cross sections from the McMaster tables (McMaster *et al.*, 1969). For plotting purposes the data were arbitrarily normalized to the center position of the module. Results are plotted in Fig. 3.

For the flat-field calibration to agree with the manufacturer's calibration, all points should be identical across the module. At the extreme locations, the partial flat-field calibration differs only by $\pm 2\%$. The standard deviation from the average value, using all data, is 1.1%. This is particularly gratifying since the manufacturer calibrates over a relatively broad energy range, roughly 12 ± 2 keV using a molybdenum X-ray tube operated at 20 mA and 15 kV (C. Nielsen, Area Detector Systems Corporation, private communication), while our measurements were conducted at the copper fluorescence energy of 8.05 keV.

In this section we confirmed that the manufacturer's flood-field correction performs well even after years of detector use. It was decided to complement this measurement using diffraction data as the calibrating source.

3. Silicon single-crystal reflection measurements

A $20 \times 9 \times 0.2$ mm-thick silicon single-crystal slab was mounted on the goniometer at room temperature. The beamline energy was set to 12.66 keV. Under normal conditions the horizontal beam at 19BM has a demagnification ratio of 6:1 (9:1 vertical). The beam size at the monochromator is 16×2.5 mm (H $\times$ V), with a minimum measured horizontal focus (full width at half-maximum, FWHM) at the sample position of 0.045 mm. For this experiment the monochromator horizontal focus was expanded to produce a wide, flat beam at the sample position. This limited the horizontal beam divergence and produced a consistent beam shape at the detector face when the detector was moved over a range of 150 mm during the experiment. The horizontal slits were reduced to 0.15 mm to define the horizontal beam width and the vertical slits were arbitrarily opened to 2.5 mm to ensure vertical beam movement would not impact the measurement; the vertical beam focus (FWHM) of 0.028 mm at the sample position was left undisturbed. The crystal was positioned to intercept the incident beam completely at all times. The beam intensity was fully tuned but, owing to the horizontal defocusing, only a factor of 7 attenuation was required to reduce the maximum pixel levels (called analog to digital units, ADUs) in any diffraction spot to $\sim 31\,000$ using a 2×2 binned image. Each binned pixel is 102.4×102.4 μm .

After examining the orientation of the silicon crystal, the Si(311) reflection was chosen as the calibrating source. The reflection position was varied horizontally by moving the detector downstream at discrete intervals. The total distance traversed by the detector was 150 mm. Because the detector is attached to an adjustable vertical stage, vertical spot location could be controlled by moving the detector up and down. In

(a)

(b)

Figure 4
(a) Overlay of Si(311) reflection positions measured across the module. Each reflection position was recorded five times. Partial reflections on the far left edge of the overlay are an artifact of the measurement process and did not interfere with the integration of individual reflections. (b) CCD detector integrated reflection profile from a reflection near the center of the module as viewed with the *HKL3000* display program. Each binned pixel is $102.4 \times 102.4 \mu\text{m}$.

combination, seven lines were generated across half of a CCD module. A total of 23 points were measured for each line; each point in each line was measured five separate times. A continuous intensity monitor (Alkire *et al.*, 2000) downstream of the slits was used to monitor incident beam intensity during all measurements. Raw integrated intensities for each reflection were initially obtained using the *HKL3000* display program under high-zoom conditions (Minor *et al.*, 2006) with an integration radius of 0.36 mm.

Corrections to the integrated intensities occurred in only two ways. First, a correction was made for changes in beam intensity that occurred as a result of tune drift and ring current decay as determined by the in-line intensity monitor. Beam intensity corrections for individual measurements ranged from 1 to 3.5%. Second, a correction factor was made to account for air absorption with detector distance and path length changes across the detector face. Air absorption distance corrections ranged from 1 to 4.7%, with up to an additional 1–3.4% (computed correction) for path length variations. Air absorption corrections with detector distance were based on direct beam measurements made with an ion chamber. All correction factors and data set statistics were compiled to arrive at the error bars for each measurement. The position of each measured reflection (Fig. 4a) is shown as an overlay compiled from individual measurements. An example of one of the integrated reflection profiles is shown in Fig. 4(b). Average integrated intensities as a function of position from the Si(311) experiment are shown in Fig. 5. For the purposes of normalization, 100% corresponds to the integrated intensity recorded in the center of the module.

Unlike in the flat-field correction experiment, reflection intensities vary dramatically. For the 50% line, the intensity varies a modest 5% between the middle and outer ends of the line. However, as the reflection moves away from the module center, the intensities steadily decrease. At the farthest

Figure 5
Si(311) reflection intensities measured at different points across the fiber-optic module after applying corrections for changes in the incident beam intensity, air absorption and path length differences. Labels (in %) indicate the vertical line location as a percentage of distance in the horizontal direction across the module face.

corners, the intensities fall off by more than 30%. Each reflection position should produce identical integrated intensities because each location represents the same reflection acquired and integrated using the same spot size.

Unlike proteins, silicon does not suffer radiation damage under these photon flux levels, even at room temperature. The

integrated intensities are quite high for these measurements, ranging from a high of $\sim 241\,000$ ADUs near the module center to a low of $\sim 158\,000$ ADUs in the corner. This is about the same intensity as the strongest reflections for the data set with an average (I) of 400 in the lysozyme experiment. Furthermore, the precision of each measurement is also very

high. The average standard deviation for each set of five measurements (*i.e.* each position of the reflection in each line) is 0.44%, three times lower than the lowest per shell linear R factor for the lysozyme data.

We repeated the integration of Si(311) reflections with *Denzo* (Otwinowski & Minor, 1997) using the summed intensity integration approach and the above corrections and changing the radius of integration from 0.4 mm to 2.0 mm (Fig. 6). For the purposes of normalization, 100% now corresponds to full intensity as measured with a 2.0 mm radius. The profile of integration 0.4 mm (~ 4 pixels) is roughly equivalent to the one used in the first integration procedure described earlier. Standard data processing protocols in macromolecular crystallography use an integration radius close to this value as a default. We observe that for an integration radius of 2.0 mm the unevenness of the integrated peaks' intensity is minimal and consistent with the flat-field calibration. Shorter radii of integration result in a gradual loss of total integrated intensity and an increased variability of response across the detector surface, with 1.5 mm corresponding to the loss of 3% of the total intensity in the center of the CCD module and 5% in the module corner, 1.0 mm corresponding to the loss of 5% of the total intensity in the center of the CCD module and 12% in the module corner, 0.75 mm corresponding to the loss of 6% in the center of the module and 17% in the corner of the module, 0.5 mm corresponding to the loss of 9% in the center and 26% in the corner, and 0.4 mm corresponding to the loss of 12% in the center and 31% in the corner.

The dependence of the variability of the integrated intensity on the diffraction peak integration radius indicates that the fiber-optic taper is the source of the problem. Our results predict complex interactions between diffrac-

Figure 6

Integration of Si(311) reflections with *Denzo* as a function of position on the detector surface using the summed intensity integration approach and changing the radius of integration over the range of (a) 0.4, (b) 0.5, (c) 0.75, (d) 1.0, (e) 1.5 and (f) 2.0 mm; data are normalized to 100% using the 2.0 mm integration radius and averaged intensity of 12 spots close to the center of the module. The dependence of the variability of the integrated intensity on the diffraction peak integration radius indicates that the fiber-optic taper is the source of the intensity loss.

tion spot sizes, the detector point-spread function and the area of spot integration. In practice, none of the integration programs adjusts the integration area by taking the variable point-spread function of the detector, described above, into account. The main factor defining the integration area is the diffraction spot size, and therefore the diffraction patterns with small spot sizes will be affected differently by the variability of the detector point-spread function compared to the diffraction patterns consisting of large spots.

3.1. Linearity check

As a final check on detector performance, a linearity check was performed as a function of attenuation. To establish if the detector responds linearly with changing intensity, two reflections were recorded on a single image and the ratio of their integrated intensities computed with the fixed radius of integration (0.36 mm). This measurement was repeated using various attenuation levels to see if the ratios changed with attenuation. Each measurement was repeated six times for each attenuation setting. For this experiment, the beamline energy was set to 12.66 keV, and the rocking curve was detuned to 10% of peak intensity to avoid radiation hardening with increasing attenuation. The same silicon single crystal used for the Si(311) reflection measurements was mounted at room temperature. Two reflections were oriented so as to strike the detector at the module center and at the corner as shown in Fig. S2. Capturing these reflections at the designated positions on the detector required adjusting the position of the detector vertically as well as in distance from its typical setting. Each image was collected over a 12° range for 12 s in order to capture the two reflections on a single image. Images were integrated in a similar manner to before and the ratios of intensities were computed. The peak ADUs per pixel per

reflection (corner/center) were $\sim 61\,000/32\,000$. The average integrated intensities (corner/center) started at $\sim 932\,000/541\,000$ ADUs and finished at 2855/1617 ADUs, for an attenuation range of 1:330. A plot of the computed ratios is shown in Fig. 7.

Within the experimental error, the ratios remain the same, regardless of the change in integrated reflection intensity. This means the detector is responding linearly with intensity, even when the reflections are positioned near the minimum and maximum bending radius of the fiber-optic taper.

4. The fiber-optic taper performance tests

Under the experimental conditions in this study, the diffracted beam size at the detector was approximately 1.5 (binned) pixels wide by 0.3 (binned) pixels high. As can be seen in Fig. 4(b), the tails of the point-spread function broaden this diffracted beam out significantly. When a flat-field calibration is performed, light output is generated from the impact of X-rays hitting adjacent points on the phosphor. Each impact point generates its own light output field proportionate to the X-ray intensity incident upon it. Because the detector point-spread function widens the light output from each position, the overall effect is to merge output from many regions into a more global distribution. A flat-field calibration, therefore, yields a smoothed-out intensity distribution over a wide area, which is insensitive to variability of the point-spread function of each diffraction reflection.

One way to see this smoothing feature directly is to use the same Cu flood-field conditions and place a flat steel plate vertically near the edge of a module. For this experiment, a commercial steel ruler of dimensions $300 \times 25.2 \times 1.2$ mm (L $\times$ W $\times$ H) was placed vertically so one edge was 4.5 mm from the centerline of the detector as shown in Fig. S3. The steel ruler is secured to the detector frame front surface, placing it about 5 mm from the black plastic cover window. Five flood-field images were collected for 100 s each and averaged, followed by five air scatter images, which were also averaged. The air scatter image was subtracted from the flood-field image to produce the final image used for profiling. The storage ring was operating in reduced horizontal beam, non-top-up mode for these measurements. However, the beam decay was less than 1% during the measurements so it was not necessary to correct for changes in the ring current.

Three different vertical heights along the ruler's edge closest to the centerline are presented, corresponding to 4, 10 and 52.5 mm down from the top, plus one profile from the outboard edge of the ruler at the same 52.5 mm height. The outboard location is horizontally displaced 30 mm from the centerline of the detector. Profile labels indicate the distance from the detector centerline, followed by the vertical height of the measured profile. All horizontal profiles have been plotted so their edges coincide and so that their intensities go from high to low in the same direction.

Air scatter background has been subtracted from the flood-field but no corrections for intensity losses in the flood-field due to distance have been made. This is to ensure that the

Figure 7

Ratio of corner/center integrated reflections as a function of attenuation, covering a range of 1:330. Within the experimental error, no change in the ratio is observed, indicating the detector is responding linearly with intensity, even when the reflections are positioned near the points in the fiber-optic taper with minimum (center) and maximum (corner) bend in the fibers.

intensities beneath the ruler are represented accurately. Figs. 8(a) and 8(b) show the magnified top and bottom portions of the intensity profiles. All edges have been aligned so that zero (mm) corresponds to half peak intensity.

Fig. 8(a) shows intensity losses starting to occur pre-edge at different distances away from an edge depending upon where the profiles were recorded. Where the intensity drops start to occur depends on the fiber-optic's characteristics. The ruler's outboard edge location (30 mm from the detector centerline) is in an area of the taper with the least amount of fiber bend,

(a)

(b)

Figure 8

Horizontal intensity profiles recorded during a Cu flood-field measurement, with part of the detector obscured by a steel ruler positioned vertically, parallel with the detector centerline. Profile labels designate the distance from the ruler's edge to the detector centerline, followed by the vertical distance down from the top of the module to the profile position. For clarity, all profiles have been positioned so their edges and intensities change relative to a common edge point. (a) Intensity losses pre-edge, *i.e.* the rounded portions of each curve near the ruler's edge, are due to the lack of contributing photons from adjacent pixels in the fiber-optic taper covered by the steel ruler. (b) Intensity gains post-edge, *i.e.* where light should not be present, result from the point-spread function increase with fiber bend and any stray light passing through the EMA absorber. As shown in both (a) and (b), light losses/gains increase as the fiber-bending radii increase.

relative to other areas covered by the ruler. Intensity losses (pre-edge), due to lack of light contributing from areas covered by the ruler, start to occur about 0.3 mm from the edge. On the inboard side, where fiber bend is higher, intensity losses begin roughly 0.6, 0.9 and 0.9 mm from the edge, corresponding to the 52.5, 10 and 4 mm vertical locations, respectively.

Fig. 8(b) shows the areas beneath the ruler where X-rays will not penetrate and so no intensity should be present. Intensity in this area results from the tails of the point-spread function, as well as any light losses that would be incurred by light passing beyond the critical angle of the fiber and continuing on outside the originating fiber and into a different region of the taper. Again, the distribution of light depends upon where in the taper the profile is measured.

At the 4 mm vertical position near the top of the module, light beneath the ruler is highest, even though the flood-field peak intensity (pre-edge) is lower than all other profiles. At the 1% intensity level, the tails of the 4 mm profile extend out to 1.5 mm. Judged against the ruler's outboard edge profile at 1%, this is an increase of more than 0.5 mm. From the plot one can see that as the profiles move closer to the center of the fiber-optic taper, *i.e.* less fiber bend, they become sharper, the same as observed pre-edge.

5. Software corrections during data merging

The problem with the performance of fiber optics was noticed by developers of software for data processing in macromolecular crystallography. Their approaches rely on analyzing integrated intensities under the symmetry constraint to establish the unevenness of detector response.

First, the corner correction in *XDS* was introduced in 2006 to account for this problem using a two-dimensional empirical function calculated on a grid selected automatically so that its areas contain a sufficiently high number of reflections for data analysis (Kabsch, 2010). Marian Szebenyi identified the need to apply the corner correction for the Q-270 detector at the CHESS F1 beamline. This correction is applied as a multiplicative, position-dependent factor after data integration but prior to scaling and merging. Arvai *et al.* (2007) presented a method to construct and apply a correction related to the corner effect in multi-module CCD detectors. *AIMLESS*, starting from version 0.2.1, also corrected integrated diffraction intensities for this corner effect (Evans & Murshudov, 2013). In this case, the correction uses a scaled and shifted radial erfc function [<http://www.ccp4.ac.uk/dist/html/aimless.html>, appendix 2(d)]. This choice was inspired by truncated two-dimensional Gaussian integrals, which describe how a variable Gaussian spot shape is affected by keeping an integration area constant. The correction fits nine parameters per detector module and restraints are imposed for the values of three parameters to be similar between modules while seven parameters are restrained to 0.

Scalepack takes into account both the crystal absorption surface and all sources of the potential detector non-uniformity response, including the fiber-optic leakage described

here. This is done by applying exponential modeling (Otwinski *et al.*, 2003), in which the base functions for the detector correction are two-dimensional Chebyshev polynomials of n th order. The order is determined by the detector properties. Using data presented in this paper, seventh-order

polynomials were used to model detector signal-loss properties for each of the 2×2 ADSC Quantum 210r CCD detector modules. For comparison, 15th-order polynomials were used to model a 3×3 module MAR Mosaic 225 CCD detector at sector 22ID at the APS (Rayonix LLC, 1880 Oak Avenue, Evanston, IL, 60201, USA) as shown in Fig. 9.

In the lysozyme experiment described earlier, the *Scalepack* correction improved the estimates of a very weak native anomalous signal (expected to be 0.56% at 12.66 keV) sufficiently to identify and refine the heavy-atom substructure with *SHELXD/E* (Sheldrick, 2008) and build a model with *ARP/wARP* (Lamzin & Wilson, 1993; Perrakis *et al.*, 1997) run within *HKL3000*. Without the correction, 10 000 cycles of *SHELXD* could not find the heavy-atom substructure. We used a single data set (90° of oscillation, attenuation factor of 4.5) for this test. Although the structure could also be solved from the data set acquired with attenuation factor of 1 upon applying the correction, it was more difficult because of the presence of overloaded reflections.

6. Discussion

Accurately calibrating a CCD detector with a phosphor at or below the 1% level is difficult for many reasons, particularly since these detectors are subject to intensity variations in all components of the optical chain, plus changing characteristics with angle and energy (Barna *et al.*, 1999). Because the point-spread function is not completely uniform across the detector face, deconvoluting it out completely would require a pixel-by-pixel validation with a source that is smaller than the point-spread function. This is considered too tedious, so it is not performed in practice (Gruner *et al.*, 2002). However, there is legitimate concern that the intensity recorded in a small number of pixels will not be corrected as accurately as a larger spot (Tate *et al.*, 1995). Nevertheless, the detector manufacturers use the flood-field method and accept the limitations on accuracy (Gruner *et al.*, 2002).

Our experiments revealed that the flood-field calibration is insufficient to account for variability of the point-spread function resulting from bending in the fiber-optic taper.

We performed a set of tests to characterize this phenomenon for one of the modules of the ADSC Quantum 210r detector at beamline 19BM at the APS. The tests with the Si(311) reflection and ruler revealed that the spatial variability of the point-spread function has a significant impact on the CCD detector's performance where sharp features (diffraction reflections) are concerned. The integration area for the diffraction peaks has to be restricted in data processing in macromolecular crystallography owing to the presence of neighboring peaks, so the very remote tails of the peaks are not integrated. Increasing the integration area to correct for the problem described here would require simultaneous deconvolution of profiles for different diffraction peaks, which would result in increased noise of their estimated intensities. Therefore, such an approach is not feasible. However, the variability of the point-spread function can be corrected by data processing software with standard approaches that are

Figure 9

The loss of signal due to missing long tails of reflections during integration as determined by scaling corrections in *Scalepack* and mapped on the surface of the detector. (a) 2×2 modules of the ADSC Quantum 210r detector at beamline 19BM at the APS and (b) 3×3 modules for the MAR Mosaic 225 detector at sector 22ID at the APS.

used in the scaling of diffraction intensities and which rely on comparing symmetrically equivalent reflections.

Using the Si(311) reflection data presented here, an empirical correction factor modeling these local effects was derived for one-half of the module. To accomplish this, a line was drawn from the center of the module to one of the corners. Intensities along this line, interpolated from Fig. 5, which used a 0.36 mm integration radius, were fitted to a polynomial. A graph of the intensities and the polynomial fit parameters are shown in Fig. S4. Data from Fig. 5 were corrected using this intensity-loss model and the results are presented in Fig. 10.

Using only the radial fall-off correction term, the data are now within a range of $\pm 5\%$ at the outermost edges, with a standard deviation from the average of $\pm 1.3\%$. Based on this correction model and a 0.36 mm integration radius, 55% of an ADSC Quantum 210r CCD module with a fiber-optic taper is within what could be termed the central core of the module, the area where fiber bending is minimal and the intensity losses are within 5% of that measured at the module center. In turn, the remaining 45% suffers from intensity losses in excess of 5–30%. Because there are four modules in the detector, these intensity-loss regions cover reflections over a wide range of resolution levels.

Similar results have been achieved with *Scalepack* (Fig. 9), which applied the correction for detector sensitivity based on exponential modeling with two-dimensional Chebyshev polynomials as base functions. The loss-of-integrated-signal distribution map shows that, although the overall assumption about intensity decreasing radially owing to fiber bending holds, there are many module-specific variations for the ADSC Quantum 210r detector at 19BM.

The bending in the taper depends not only on the taper ratio, but also on the pulling speed during taper expansion. Therefore, reducing the taper ratio does not eliminate the need to correct for the taper effects. As shown in Fig. 9, the

Figure 10

Si(311) reflection integrated intensities measured at different points across the fiber-optic module, using a 0.36 mm integration radius, after applying the polynomial-fit modelled corrections. The standard deviation from the average for all data is now $\pm 1.3\%$, compared to variations in excess of 30% prior to the fit corrections.

loss-of-signal map for the MAR Mosaic 225 CCD detector at sector 22ID, which has taper ratios of 2.7:1, is of similar amplitude but with quite different patterns from that created for the ADSC Quantum 210r with taper ratios of 3.7:1. These differences result from a different number of modules. Problems associated with such loss of signal are potentially more severe with 3×3 multi-modular detectors. With a four-module detector the high-loss edge of one module is covered by the beamstop assembly. With a symmetric nine-module detector, such as the MAR Mosaic 225 or ADSC Quantum 315r, the beamstop assembly and high Lorentz correction areas are all located along a line in the center of the detector, effectively removing a significant portion of the least distorted and best calibrated areas of the center modules.

The impact of this inadequacy in detector performance will be very significant for detection of weak anomalous signals and for charge-density studies, particularly when no attempt is made to correct for this effect during scaling. Additionally, the impact on estimates of anomalous signals will strongly depend on the crystal orientation and beam position. In some orientations this problem will affect the Friedel pairs by the same amount, so the anomalous differences will not change very much. The impact on structure solution with molecular replacement or on molecular refinement is much smaller, which can explain why this effect was not detected earlier.

The ADSC Quantum 210r is performing exceptionally well in our experiments, which allowed us to determine an additional source of systematic errors. Although the current, generalized *Scalepack* implementation of the software correction for this effect performs well for all tested multi-module CCD detectors built with reducing fiber-optic tapers, it shows module-specific variations specific to the particular detector. Therefore, the future approach will focus on detector-specific corrections that will require detector-specific calibration experiments.

7. Conclusion

An Area Detector Systems Corporation Quantum 210r detector was put through a series of tests to check its calibration and performance characteristics. A partial flat-field testing showed a uniform response consistent with the manufacturer's calibration across the face of the module tested. However, experiments testing the same surface with a diffraction peak from a silicon single crystal revealed a position-specific fall-off of intensity as high as 30% as one moved from the center of the module to its corner. The subsequent experiments and data analysis revealed that the variability of the point-spread function within the detector is responsible for this effect. The flat-field calibration is insensitive to the variability of point-spread functions, so corrections for this problem are not supplied by the detector manufacturer. Similarly, integration over a circle with a 2 mm radius is not a practical solution. Instead, scaling software corrects for the problem by comparing symmetrically equivalent reflections and modeling observed variability (Kabsch, 2010; Otwinowski *et al.*, 2003; Otwinowski & Minor, 1997; Evans & Murshudov,

2013). However, there are inherent problems in such a phenomenological approach: (i) potentially very strong correlation of sensitivity corrections with absorption modeling, (ii) in typical fixed-distance experiments, lack of sufficient restraints during scaling, which causes suboptimal estimates, and (iii) profile shape changes that contribute as well to these estimates, so that the results vary between different crystals. Therefore, higher-quality, integration parameter and energy-specific calibrations should be performed for each detector to enable detector-specific software corrections, which could lead to estimates of diffraction data produced by CCD detectors that are closer to quantum statistical limits.

Acknowledgements

The authors would like to thank Kay Diederichs for very helpful discussions in the preparation of this paper. Argonne National Laboratory's work was supported by the US Department of Energy, Office of Biological and Environmental Research under contract DE-AC02-06CH11357. The work of ZO and DB was supported by NIH grant Nos. R01GM053163 and R01GM117080.

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