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Synchrotron X-ray crystallography techniques: time-resolved aspects of data collection

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Synchrotron X-radiation (SR) is intense, polychromatic and collimated. These properties are exploited routinely now to measure data at high resolution from proteins or from large unit cells (e.g. viruses), particularly using a monochromatized short wavelength beam. The time needed to measure protein crystal data-sets (in rotation geometry) can be quick (hours or minutes) compared with laboratory sources (weeks or days). Even so, more rapid data collection is of interest for time-resolved macromolecular crystallography. White beam: stationary crystal (Laue) geometry at SR sources offers shorter exposure times (seconds and less with film). Laue data-sets can be sensitive to subtle structural differences. Technical challenges still presented by SR Laue patterns include the energy overlap of low-resolution data and the spatial overlap of spots, both of which affect the completeness of data-sets. Some energy deconvolution is already possible by the use of multiple films. The spatial overlap problem can be alleviated by the use of three-dimensional detectors, such as a 'toast-rack' of plates. Monitoring of a crystalline process, via the Laue pattern, requires time slicing detector systems (e.g. based on CCDs) to be developed.

1. Introduction

Time-resolved crystallographic studies of macromolecules and small molecules undergoing reactions and perturbations is an area of intensive research and development at synchrotron sources. Applications of time-resolved techniques in structural molecular biology have been started.

Synchrotron monochromatic studies have been made of carbon monoxide binding to myoglobin (Bartunik *et al.* 1981) and of catalysis in crystals of the enzyme phosphorylase *b* (Hajdu *et al.* 1987*a*). These studies used the (so-called) rotation method. The time for the measurement of a complete protein data set on a focused wiggler beam instrument (Helliwell *et al.* 1986) can be as short as 30 min (Hajdu *et al.* 1987*a*). The Weissenberg (1924) monochromatic geometry can also be exploited for time-resolved work (a revival of this method at SR sources is due to Sakabe (1983)).

The synchrotron Laue method offers significant advantages for time-resolved work, complementary to synchrotron monochromatic methods of recording X-ray data, because exposure times are short (seconds and less) and the sample is held stationary during the exposure (Moffat *et al.* 1984; Helliwell 1984, 1985; Hajdu *et al.* 1987*b*). Historical objections to the Laue method, particularly the multiplicity problem, have been found not to be as limiting as once thought (Cruickshank *et al.* 1987, 1991). In particular the total proportion of RLPs on single rays is actually greater than 83% for all $\lambda_{max}/\lambda_{min}$. Unfortunately there is a zero probability of

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recording those RLPS on single rays which have d spacings greater than $2d_{min}$ and are stimulated by wavelengths longer than $2\lambda_{min}$ (up to λ_{max}). Hence, the completeness of Laue data sets (derived from singles alone) is poor between $2d_{min} < d < \infty$, although it can be high for $d_{min} < d < 2d_{min}$. In establishing the credentials of the method one can quote, for example, the results of Liljas *et al.* (Lindahl *et al.* 1992 and this volume) who achieved a completeness from Laue data-sets, measured from carbonic anhydrase with bound HSO_3^-, of 67% overall, with 81% for $d_{min} < d < 2d_{min}$ and 16% for $2d_{min} < d < \infty$. These data were able to discriminate successfully between tetrahedral and square bipyrimidal configurations around the essential zinc atom. Hence, the Laue method can distinguish between subtle structural differences such as occur in catalytic mechanisms of proteins, at least up to a molecular weight such as this (29000 Da).

A time-resolved enzyme crystal structure study using the synchrotron Laue method has also now been performed successfully. This was a study of the ras p21 protein (Pai *et al.* 1989) during GTP hydrolysis following laser reaction initiation of caged GTP (Schlichting *et al.* 1989, 1990). The synchrotron facilities at EMBL in Hamburg were used. Another successful application of the synchrotron Laue method, in a time-resolved study of trypsin, is reported by Sweet in this volume.

The completeness of Laue data between $2d_{min} < d < \infty$ can be improved by the application of an existing method of energy deconvolution. Zurek *et al.* (1985) presented a method for the unscrambling of harmonic reflection intensities from spots on Laue patterns based on the use of multiple films and the different rates of attenuation of the component reflections in a spot, each reflection being stimulated by a different wavelength. Further details of this method of energy deconvolution and results for the protein pea lectin (MW 50000 Da) were presented in Helliwell *et al.* (1989*b*). The use of solely the low-resolution component of energy deconvoluted doubles from an amylase crystal successfully revealed the position of a Hg atom (Helliwell *et al.* 1989*a*); the molecular weight of the amylase is 45000 Da. This method of energy deconvolution is now being applied in other studies (see Duke *et al.*, this volume). Another way of attacking the problem is being examined based on direct methods (Hao Quan, J. W. Campbell and M. M. Harding, unpublished results).

Improvements in instrumentation for Laue diffraction are being made. New detectors are being developed. A 'toast-rack' three-dimensional (3D) detector (Helliwell 1991; Cruickshank *et al.* 1991) has been designed and constructed so as to alleviate the spatial overlap problem. Also charge coupled device (CCD) detectors are being developed for time slicing and reaction monitoring (Allinson *et al.* 1989, 1992*a*, *b*). Faster time resolutions per exposure (milliseconds) are being realized for protein crystals by use of focused white SR beams, e.g. as at Station 9.5 of the SRS (Brammer *et al.* 1988; Helliwell 1991; Allinson *et al.* 1992*a*). The next generation of sources such as the European Synchrotron Radiation Facility (ESRF) should allow exposure times in the microsecond range (Wulff 1991).

This paper does not attempt to give a review of the synchrotron monochromatic and Laue techniques and their use for time-resolved macromolecular crystallography; for this the reader is referred to chapters 6, 7 and 10.4 of Helliwell (1992). Instead this paper examines the time-resolved aspects of the data collection experiment which deserves a detailed consideration. The need to measure as many reflection intensities as possible in the shortest overall time requires (*a*) short exposures, (*b*) the rapid clearing of the detector media/image, (*c*) the minimum

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Table 1. *Time dependent aspects of the X-ray crystallography experiment*

required operation	monochromatic methods: rotation photography	monochromatic methods: Weissenberg with image plate	Laue photographic method
align the crystal to a specific orientation	optional	yes	optional
rotate the crystal during exposure	yes	yes	no
(minimum exposure time)	(1 s deg^{-1} ?)	(10 s 10 deg^{-1} ?)	(120 ps)
translate the detector during exposure	no	yes	optional
rotate the crystal between exposures	no	no	yes
(approximate minimum number of exposures per 90° coverage)	(45)	(9)	(6)
change the film/plate or refresh detector image in general (for cubic cases)	yes (yes)	yes (possibly)	yes (no)

number of crystal settings and (*d*) the maximum number of processable spots per crystal setting. The need to find the most opportune time-slice to measure the main data-set requires a frequent sampling of the diffraction pattern; but limitations of current electronic area detector technology means that only parts of a pattern can be sampled at high temporal frequency. The most opportune time slice can be defined not only as when a reaction intermediate builds up but also when mosaic spread has not increased to an unacceptable level. Finally, a basic time dependent feature of macromolecular crystallography is radiation and thermal damage to the sample. Time slicing, diagnostic CCD detectors offer exceptional performance in establishing as precisely as possible the behaviour of a given sample in the beam.

2. An analysis of the time elapsed in crystallographic data collection

To measure the unique set of reflections for a given crystal sample a variety of ways of capturing the data can be used. These are the rotation and Weissenberg (monochromatic) methods as well as the Laue (white beam) method (table 1). In the rotation method the sample is rotated with a constant angular velocity in a monochromatic X-ray beam and the diffraction spots are captured on an area detector (film or image plate or electronic area detector). The rotation angular range, $\Delta\phi_{max}$, per 'photograph' is ultimately limited by the need to prevent spots overlapping. Hence, $\Delta\phi_{max} \approx d_{min}/a$, where d_{min} is the resolution limit and a is the cell parameter. $\Delta\phi_{max}$ varies with ϕ, the angular setting of the crystal, particularly so if the cell parameters are anisotropic. Successive images are recorded at contiguous angular intervals. For details see Arndt & Wonacott (1977).

The Weissenberg method is similar to the rotation method but the detector is translated during the exposure to avoid overlap of spots. Hence, a significantly greater rotation range can be used per image. In the screenless Weissenberg method (i.e. the variant used at synchrotron sources) the total range of rotation per image is *ca.* 10° (less for viruses). The sample is aligned prior to data collection. The successive images are recorded at contiguous angular intervals.

In the Laue method the sample is held stationary during the exposure. The Laue method is a polychromatic technique whereby reflections are integrated over wavelength and not angle. The region of reciprocal space covered (or the number of reflections stimulated) in an exposure depends on the total width of the

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illuminating bandpass (λ_{max}–λ_{min}). For comparison with the rotation methods one can define an equivalent angular range, $\Delta\phi_{equiv} = (\theta_{max} - \theta_c) = [\arcsin(\lambda_{max}/2d_{min}) - \arcsin(\lambda_{min}/2d_{min})]$. For values of λ_{min} of 0.45 Å† and λ_{max} of 2.0 Å, a useful range of wavelengths, and a d_{min} of 2.5 Å, $\Delta\phi_{equiv} = (23.6–5.2)° = 18.4°$. During processing the spots retained are those stimulated in the λ range, 0.5–1.6 Å, and for which $\Delta\phi_{equiv} = (18.7–5.7)° = 13°$. Successive Laue exposures involve re-orienting the crystal by approximately $\Delta\phi_{equiv}$.

The time dependent aspects can be compared by parameterizing the whole experiment as follows:

t_1, the time to make an exposure;

t_2, the time to change the film cassette or toast-rack box or clear the electronic detector image per exposure (alternatively, it is the time needed to reset the crystal angle between exposures, because these operations can be done in parallel, and are done with the shutter closed, we take whichever is the longer time);

N, the total number of exposures required to make a complete or near complete or 'as complete as possible' data-set.

Hence the total time, T, to measure a data set is given by

$$T = [Nt_1 + (N-1)\,t_2]. \tag{1}$$

How can T be minimized? This obviously involves separately minimizing t_1, t_2 and N.

To minimize t_1, the time to make an exposure, in the monochromatic method involves rapid rotation of the crystal at a constant angular speed; to reach a constant angular speed requires acceleration and deceleration of the crystal at the start and end of the rotation range for a given exposure. Because protein crystals are mounted wet in a capillary and are subject to slippage this will limit the angular speed. Clearly t_1 can be minimized most satisfactorily using the Laue method where the crystal is stationary and where most effective use is made of the polychromatic SR spectrum.

To minimize N, the number of exposures, requires a large $\Delta\phi$ range per exposure. $\Delta\phi$ is largest for the Weissenberg and the Laue methods. High symmetry also reduces N. The highest symmetry case is cubic. An angular sweep of 15°–20° is sufficient, with a cubic case, to reach a near complete data set (i.e. N becomes 1 and the total time T is then simply t_1).

To minimize t_2 involves as rapidly as possible changing a film cassette or toast-rack box or refreshing the electronic detector memory and, in the Laue case, rotating the crystal to its new angular setting for the new exposure. For example, the carousel on the Enraf–Nonius camera carries eight cassettes and to rotate the new cassette into position takes about 10 s. To reset the crystal angular setting through 15° takes a similar length of time. To refill a carousel with eight new cassettes requires the hutch interlocks to be broken and re-established, a procedure taking *ca.* 5 min. A crystal may also need translating to a fresh position or changing altogether. The $(N-1)\,t_2$ term in equation (1) can have the most impact on the time resolution of a given experiment. With image plates each image requires approximately 90 s to be digitised and the plate 'cleaned'. Several typical schemes of data collection are compared in table 2.

The minimum time realized in these typical examples are 10 min for the Weissenberg monochromatic case with a cubic crystal or for the Laue method 30 ms. However, once more than one exposure is required the time to change plates begins

† 1 Å = 10^{-10} m = 10^{-1} nm.

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Table 2. *Approximate measuring times for a protein crystal data-set*
(The number in brackets is the number of exposures to make a data set for a given method.)

method	X-ray source	orthorhombic	cubic
rotation IP	rotating anode	24–100 h (45)	5–20 h (10)
rotation photo	SR	2.5 h (45)	30 min (10)
rotation IP	SR	1.5 h (45)	20 min (10)
FAST	SR	2 h (900)	30 min (200)
Weissenberg	SR	1 h (9)	10 min (2)
Laue (unfocused)	SRS	1 min (6)	1 s (1)
Laue (focused)	SRS	1 min (6)	30 ms (1)
Laue (focused)	ESRF	1 min (6)	30 μs (1)

Notes. 1. A *ca.* 90° coverage of reciprocal space is assumed for orthorhombic and *ca.* 20° for cubic. 2. In the rotation photographic method a 2° rotation per exposure is assumed. At the synchrotron a typical protein crystal exposure time per 2° is *ca.* 150 s with film (2 Å data). The time to move a new film cassette into position is *ca.* 10 s and the time needed to search a hutch and refill the carousel with 8 cassettes is about 300 s. Hence, the time for a photographic data-set for the orthorhombic case is not less than $[45 \times 150 + 44 \times 10 + 5 \times 300] = 8690$ s (i.e. 2.41 h). 3. In the rotation image plate (IP) method the exposure time would reduce to say 30 s per 2° due to the extra sensitivity of the IP. Each image requires 90 s to be read and cleared. Hence, the time for a rotation IP set would be $[45 \times 30 + 44 \times 90] = 5310$ s (i.e. 1.48 h). 4. For the FAST an angular rotation of 0.1° per image is used. A typical exposure time at the synchrotron per image is *ca.* 3 s and the time to read and clear the image is *ca.* 5 s. Hence, the time to get an orthorhombic data set onto disc is $[900 \times 3 + 899 \times 5] = 7195$ s (i.e. 2 h). This assumes that one setting of the detector is adequate. 5. In the Weissenberg mode (with IP) an angular rotation range of $\approx 10°$ is used with an exposure time of *ca.* 150 s. The time to change the IP in the camera is perhaps 5 min. Hence, the time for an orthorhombic data-set is $[9 \times 150 + 8 \times 300] = 3750$ s (i.e. 1 h). In this mode a cubic data-set can be measured in $[2 \times 150 + 1 \times 300] = 600$ s (10 min); the large angular range of the method and the high symmetry of the crystal combine here to reduce nicely the time needed to measure a data-set. 6. In the broad band pass Laue photographic method six exposures, 15° apart, each of 1 s exposure time (unfocused SRS) would be needed. Also, a further 10 s to change each cassette on a carousel would be needed while simultaneously re-orienting the crystal. Overall the time required would be $[6 \times 1 + 5 \times 10] = 56$ s to measure a data-set. For the cubic case in this mode, one exposure would be adequate. Hence, as the exposure time decreases (realised with focused SRS station 9.5, or projected performance for focused ESRF multipole wiggler) it is the single exposure, cubic crystal case which shows the dramatic benefit of the short exposure times of the Laue method.)

to dominate the time required. Amemiya *et al.* (1989) have described a rapid image plate changer which gets a new IP into position and stationary ready for a new exposure in 50 ms. The short exposure of 30 ms for a protein crystal broad-band Laue photograph was realized at the SRS in Daresbury at station 9.5. To summarize, the time needed to measure a data-set with monochromatic methods can be quick (hours or minutes with SR compared with weeks or days with laboratory sources) but to reach the faster time resolutions the synchrotron Laue method is clearly preferable.

3. Analysis of Laue data and the wavelength normalization

The analysis of Laue diffraction photographs is described fully elsewhere. From the SERC Daresbury Laboratory a software suite for processing Laue photographs has been distributed worldwide for several years and has been used successfully at remote sites (see, for example, Schlichting *et al.* 1990). A description of the software

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suite and results that can be obtained is detailed in Helliwell *et al.* (1989*b*). The software development itself was a collaboration between computer specialists and experimentalists (Machin 1987). Recently the integration stage of the processing has been improved by using a method based on overlapping peak deconvolution as used originally in neutron crystallography and fibre diffraction. Shrive *et al.* (1990) give very nice results in deconvoluting spatially overlapping Laue spots. Further developments of this approach are in progress (Wakatsuki *et al.* 1992). A suite of analysis programs has also been developed at Cornell (Temple & Moffat 1987; Bilderback *et al.* 1988).

The measured intensity of a Laue reflection (whether a single or an energy deconvoluted multiple (Zurek *et al.* 1985)) needs normalization for a variety of wavelength dependent factors. The recorded Laue intensity (strictly, an integrated power) is given for the reflection $\boldsymbol{h}$ by (Campbell *et al.* 1986 and references cited therein)

$$I_{\mathrm{L}}(\boldsymbol{h}) = \left[\frac{e^2}{mc^2}\right]^2 \frac{\mathrm{d}I}{\mathrm{d}\lambda}\lambda^4 \frac{1}{2\sin^2\theta}\frac{V_x}{V_0^2}PAD|F(\boldsymbol{h})|^2. \tag{2}$$

Here, $\mathrm{d}I/\mathrm{d}\lambda$ denotes the spectral intensity distribution of the incident X-ray beam; V_x is the volume of sample illuminated; V_0 is the sample unit cell volume; θ is the Bragg angle for the reflection $\boldsymbol{h}$; P is the polarization factor; A is an absorption correction for the sample in its capillary and D is a detector sensitivity and obliquity factor. Quantities such as P, A and D vary with any or all of λ, θ and x, the position of the diffracted beam on the detector; the spectral intensity distribution is in general not precisely known in advance; and the detector may suffer from spatial distortion and non-uniformity.

Generally, the use of symmetry equivalent reflections recorded at separate wavelengths has allowed a wavelength normalization curve (λ-curve) to be calculated for a protein crystal (Campbell *et al.* 1986; Helliwell *et al.* 1989*a*, *b*). D. W. J. Cruickshank (personal communication and this volume) has pointed out that this method is similar to one used by Ewald (1914).

In the case of monitoring a sample of known structure via the Laue pattern it is possible to use the *fractional* differences whereby the wavelength dependent corrections cancel. This difference ratio method can be used with time-resolved or perturbation measurements. A simplification, in principle, of the time-resolved Laue experiment over its static counterpart now appears: only fractional changes in intensities are needed (Bilderback *et al.* 1984; Hajdu *et al.* 1987*b*)

$$(|F(\boldsymbol{h},t)| - |F(\boldsymbol{h},0)|) = |F(\boldsymbol{h},0)|\Delta F_{\mathrm{L}}(\boldsymbol{h},\lambda,t)/F_{\mathrm{L}}(\boldsymbol{h},\lambda,0). \tag{3}$$

4. Novel detector schemes

Improvements to the Laue method can clearly be made particularly with respect to the detector schemes used.

(*a*) *The 3D, toast-rack, arrangement of films*

A novel toast-rack arrangement of films, each spaced some distance apart, is now being used (figure 1). This scheme increases the number of processable spots on a Laue photograph by reducing the number lost due to spatial overlap while increasing the angular aperture subtended at the sample by the front film (Helliwell 1991;

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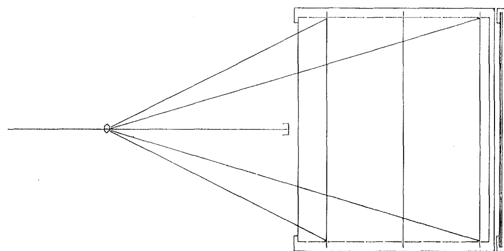

Figure 1. Schematic layout of the toast-rack (3D) film arrangement.

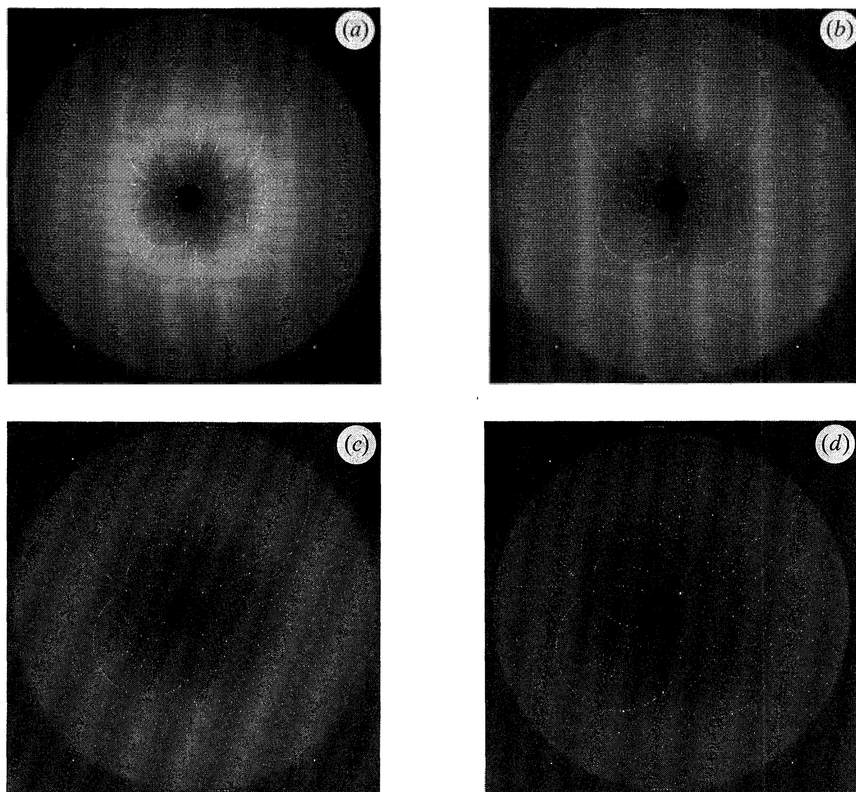

Figure 2. Four films exposed in a toast-rack set respectively at distances of (*a*) 115.5, (*b*) 155.5, (*c*) 195.5 and (*d*) 221.0 mm from a crystal of the protein concanavalin-A.

Cruickshank *et al.* 1991). A similar arrangement is also feasible with image plates or storage phosphors.

In the 'toast-rack', the short wavelength spots, although overlapped on the front film, will penetrate to the rear films where they will often be separated. The longer-wavelength singles, provided they do not lie on unacceptably high-density arcs will be recorded on the front film separated from neighbouring spots. It is possible to increase the number of measurable RLPs as compared with the standard multiple film pack.

Figure 2 shows four exposures from a protein crystal (concanavalin-A), using a relatively narrow bandpass of 0.45–1.0 Å with crystal to film distances of 115.5,

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Figure 3

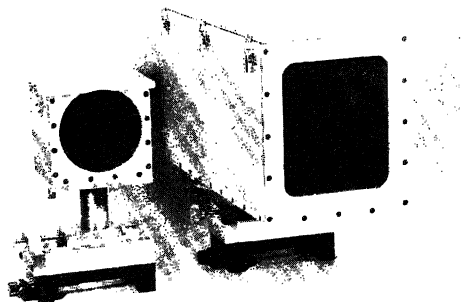

Figure 4

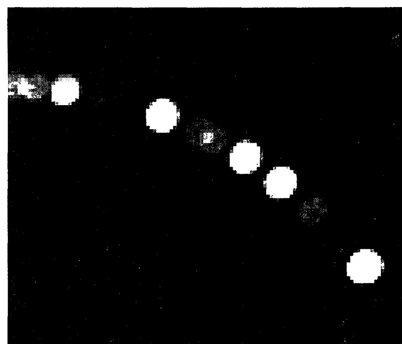

Figure 3. A large toast-rack, aimed at virus work, set side by side with the smaller one (referred to in figure 2) aimed at protein work. The aperture of the small toast-rack is 120 mm.

Figure 4. Laue pattern recorded on a CCD in direct detection mode from a cubic crystal of concanavalin-A on station 9.5 of the Daresbury SRS. The spots are 200 μm across, each detector pixel is 22×22 μm^2 and the spots are essentially 10×10 pixels each.

155.5, 195.5 and 221.0 mm; exposure time 5 s in each case. The purpose of this particular study was to realize high resolution (2.0 Å) with rapid exposures. To get to high resolution in Laue geometry required a truncation of the band pass.

Processing of the photographs in figure 2, along with the exposures at other orientations, has been completed and full details will be published elsewhere (Weisgerber & Helliwell, in preparation). The data quality is as good as data recorded with standard cassettes; $R_{\text{merge}}(I) = 9.4\,\%$ in Agrovata for 13871 unique reflections to 1.95 Å resolution derived from 65673 measured, spatially resolvable, singlets.

The benefits of the toast-rack increase as the bandpass increases (i.e. greater than 50 % more spots can be processed with bandpasses of 0.3–2.6 Å) and as the unit cell increases. Although the toast-rack shown in figure 1 is suitable for protein crystal Laue work (i.e. unit cells of 100 Å), large unit cells (greater than 300 Å) can benefit from a bigger toast-rack (figure 3). The big toast-rack is longer and takes larger films (240 mm × 180 mm). In a simulation harnessing the toast-rack concept, Cruickshank (personal communication) detailed a case involving a (300 Å)^3 unit cell, $d_{\text{min}} = 3.0$ Å, $\lambda_{\text{min}} = 0.5$ Å, $\lambda_{\text{max}} = 1.5$ Å, a plate radius of 100 mm, a spot-to-spot resolution criterion of 0.2 mm. With these parameters $\theta_{\text{max}} = 14.5°$ and $\theta_{\text{c}} = 4.8°$. There are 260000 accessible RLPs of which 230000 are singles (using Cruickshank *et al.* 1987). One plate placed at 270 mm yields 80000 spatially resolvable singlets. Three plates placed at 180 mm, 270 mm, 460 mm increases the number to 140000.

(b) CCDs *for on-line reaction monitoring*

In a time-resolved study a reaction has to be initiated in a crystal and then an interesting instant of the ensuing time course has to be identified. Reaction monitoring can be via real time monitoring of the Laue diffraction pattern itself. As a diagnostic, only a small part of the Laue pattern is required involving perhaps 50 spots. This number is sufficient to allow changes in the integrated spot intensities with time to be estimated and so to pinpoint the build up of reaction intermediates in the crystal. The method is complementary to the use of a microspectrophotometer (Hadfield & Hajdu 1990). Additionally, CCDs can also monitor changes in crystal mosaicity as a function of time.

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A CCD of size 17 mm × 26 mm, one of the larger ones available, can actually accommodate a stack of time slices. Such a scheme involves masking off a large area of the CCD from the diffraction pattern. Allinson *et al.* (1992*b*) document a design of such a CCD system. This new design, a MkII, builds on experience gained with smaller CCDs (MkI versions) where the whole area of a device is illuminated with part of a Laue pattern (Allinson *et al.* 1989). (An application of an up to date version of the MkI is given in the next section.)

The approach used involves direct detection by the silicon. This allows a fine point spread factor to be realized and avoids the long decay time processes (milliseconds) that are associated with phosphor coupled systems. The disadvantage of direct detection is a poorer absorption efficiency. However, exposure times in the 100 ms range are still feasible by direct detection, with an outstanding spatial resolution capability (figure 4).

The harnessing of CCDs is an active area of development by several other groups around the world (see, for example, Strauss *et al.* (1987) and also Clarke (personal communication), Gruner (personal communication) and Amemiya (personal communication).

5. A time-resolved study of radiation damage to a protein crystal

A significant advantage of synchrotron radiation is to reduce radiation damage to the sample. For example, the use of a short wavelength of approximately 0.9 Å increases the amount of data measured from one crystal. This effect, due to reduced absorption of the beam, has been harnessed in many studies particularly of viruses (see, for example, Acharya *et al.* 1989; Liddington *et al.* 1991). Further improvements might well accrue from use of even shorter wavelengths in reducing absorption of the beam by the sample (Helliwell 1992).

On line electronic detectors for reaction monitoring in the crystal, also offer much more precise diagnostic information than hitherto on the time course of radiation damage. Figure 5 shows a step in such studies. It depicts the Laue diffraction images recorded on a CCD, of the MkI type, from a protein crystal as a function of time. Seven positions (within the whole Laue pattern) were used in order to record an increased area of the Laue pattern with this CCD. The same overall area was then re-sampled five times in total. The total time involved in moving the detector around (and which included the reading of each image which was done in parallel) was 190 s. The X-ray shutter was closed in these time periods. The total sample irradiation or exposure time was 4.2 s for the mosaic of 35 images. The steady increase, from left to right, of the crystal sample mosaic spread is clearly visible as an increase in the size of spots.

6. Future synchrotron developments such as the ESRF

Large circumference synchrotrons allow for the insertion of multipole wiggler and undulator devices. The ESRF is a high energy, 6 GeV, low emittance machine of high brilliance in the X-ray range. Exposure times for Laue diffraction patterns, from protein crystals recorded with a variety of wavelength bandpass characteristics, will be in the range of millisecond to sub-nanosecond. The harnessing of these capabilities presents problems and opportunities. For the faster time-resolved experiments the Laue method based on a stationary crystal will be essential. The use of the broad

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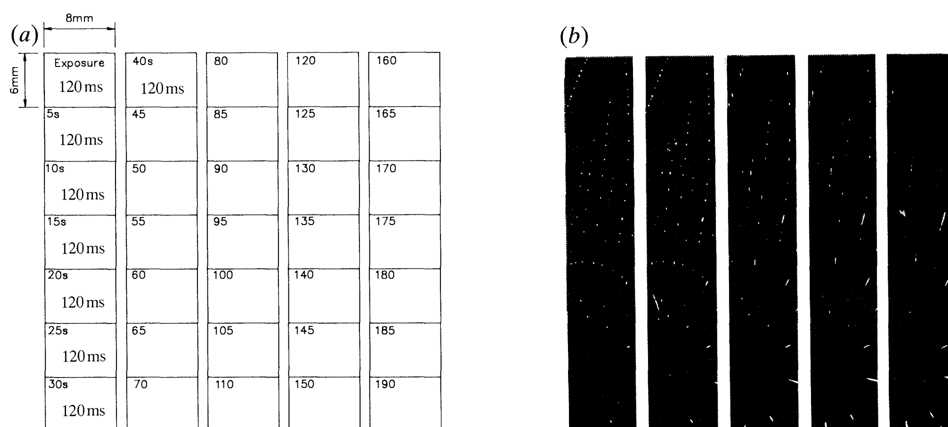

Figure 5. Time dependent study of radiation damage to a crystal of concanavalin-A, I222 form. (*a*) A schematic of the experiment illustrating how the CCD was exposed for 120 ms to the Laue pattern. The X-ray shutter was then closed and the CCD moved to a contiguous position, which took 5 s. The shutter was then re-opened for 120 ms, etc. A total of seven positions of the device make up one vertical stripe of the Laue pattern. To move the CCD from the bottom to the top position took 10 s. The number in the top left-hand corner of each exposed frame is the elapsed time, excluding the exposure time, which is relatively very small. (*b*) The complete set of exposed patterns. The steady increase of the crystal sample mosaic spread is visible as an increase in the spot sizes from left to right. (Experimental conditions: SRS 2 GeV, 272 mA; station 9.5; $\lambda_{min} = 0.5$ Å, $\lambda_{max} = 2$ Å; crystal to detector distance 90 mm. Detector CCD from English Electric Valve, size 8.1 mm × 6 mm, 375 × 275 pixels, each of area 22 μm × 22 μm, 30 μm deep depletion, direct detection. Crystal concanavalin-A, space group I222, $a = 88.7$, $b = 86.5$, $c = 62.5$ Å; dimensions of sample approximately 1 mm × 0.6 mm × 0.3 mm, X-ray beam (collimated) cross section 200 μm diameter.)

bandpass from a multipole wiggler will yield the largest number of Laue spots in the shortest exposure time. In the analysis of such multipole wiggler Laue patterns the wavelength normalisation analysis should be straightforward because of the smooth emitted SR spectrum from such a device. An undulator spectrum consisting of a series of narrow bandpass 'spikes' will be useful for some cases whereby more rapid Laue exposures are possible but yielding far fewer spots per exposure. The wavelength normalisation is more difficult than for the multipole wiggler case. Wulff (1991) describes an ESRF multipole wiggler Laue instrument for time-resolved macromolecular crystallography due to come on-line in 1994.

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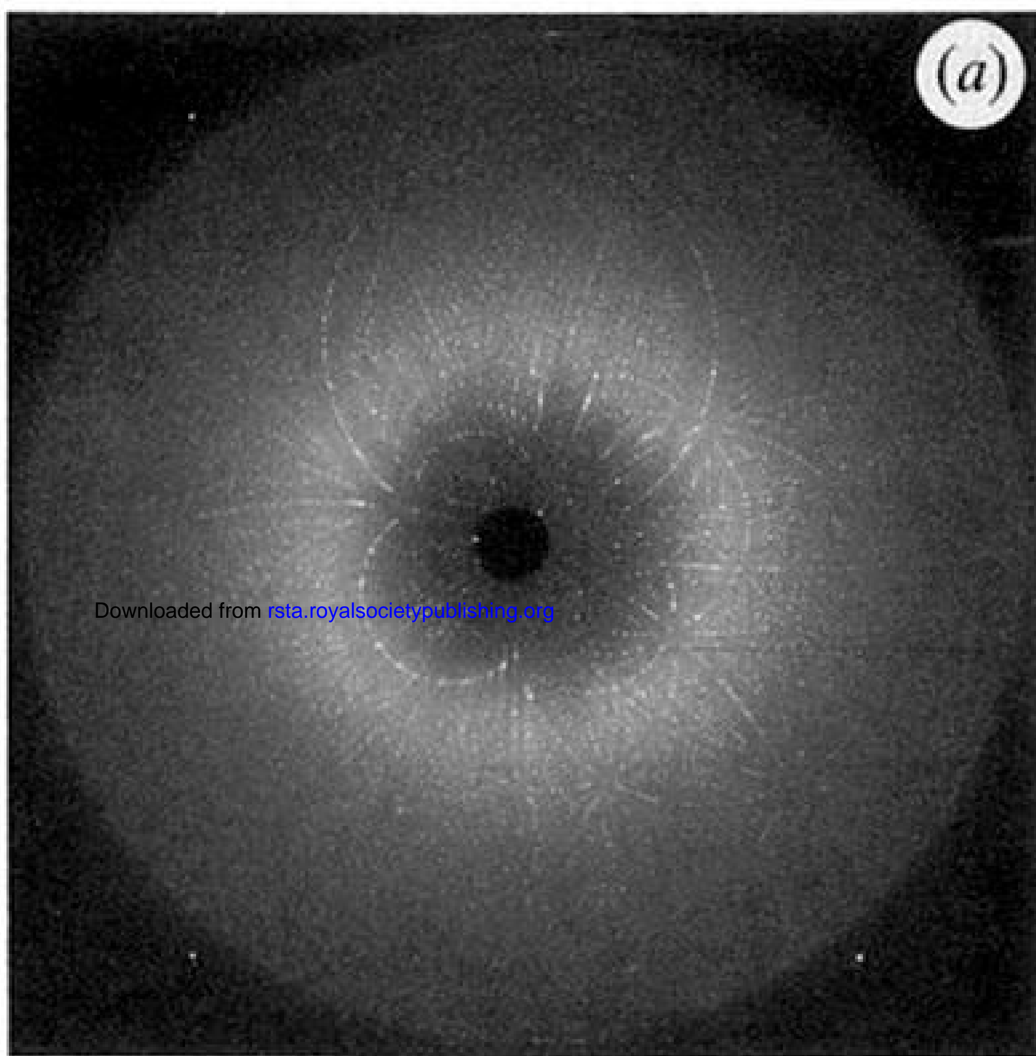

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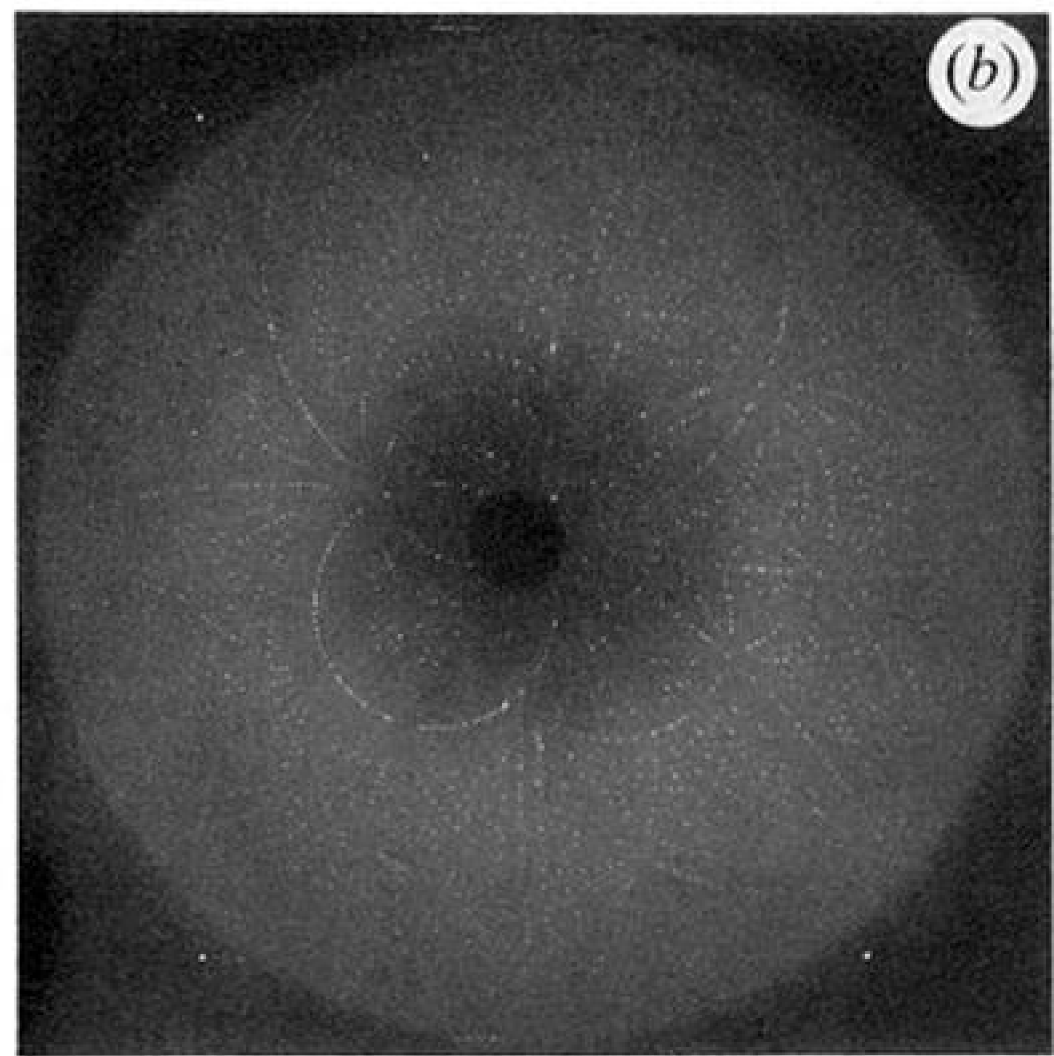

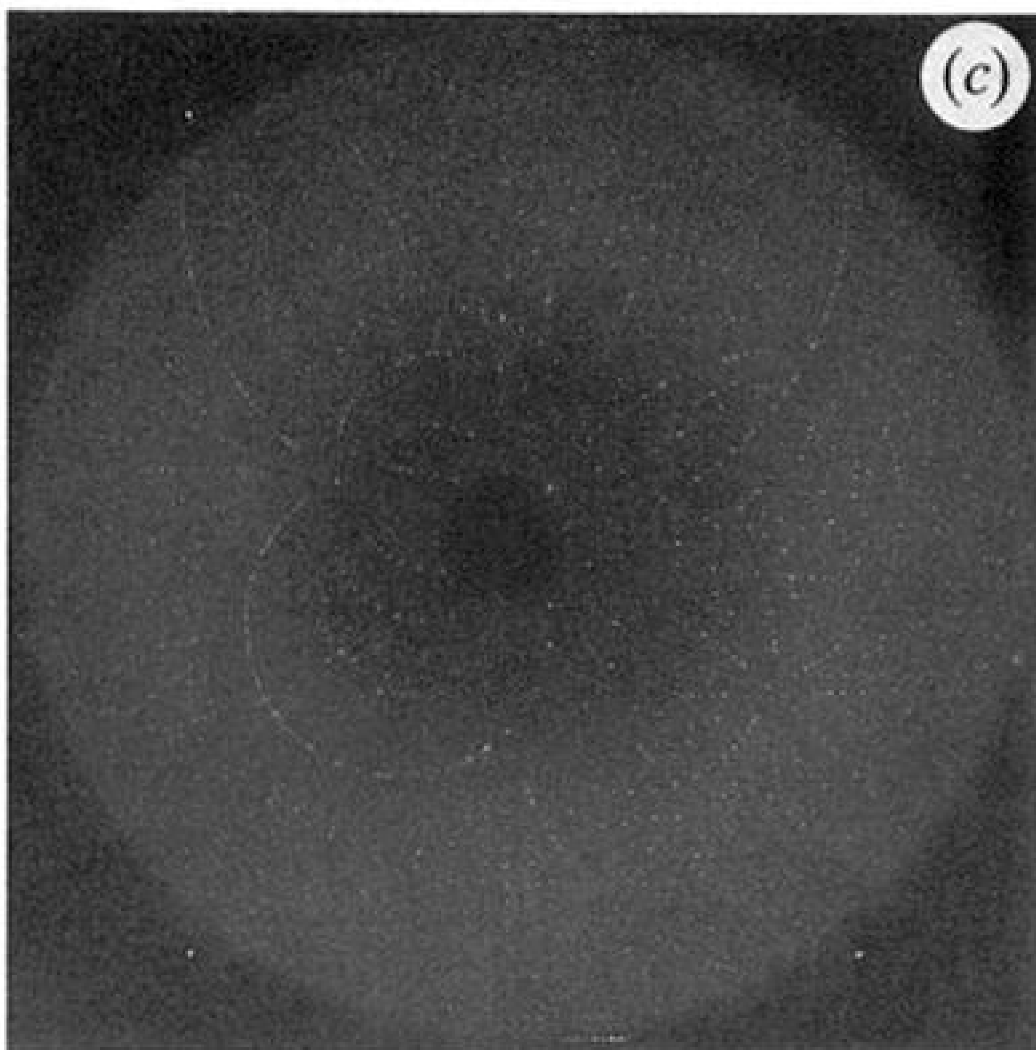

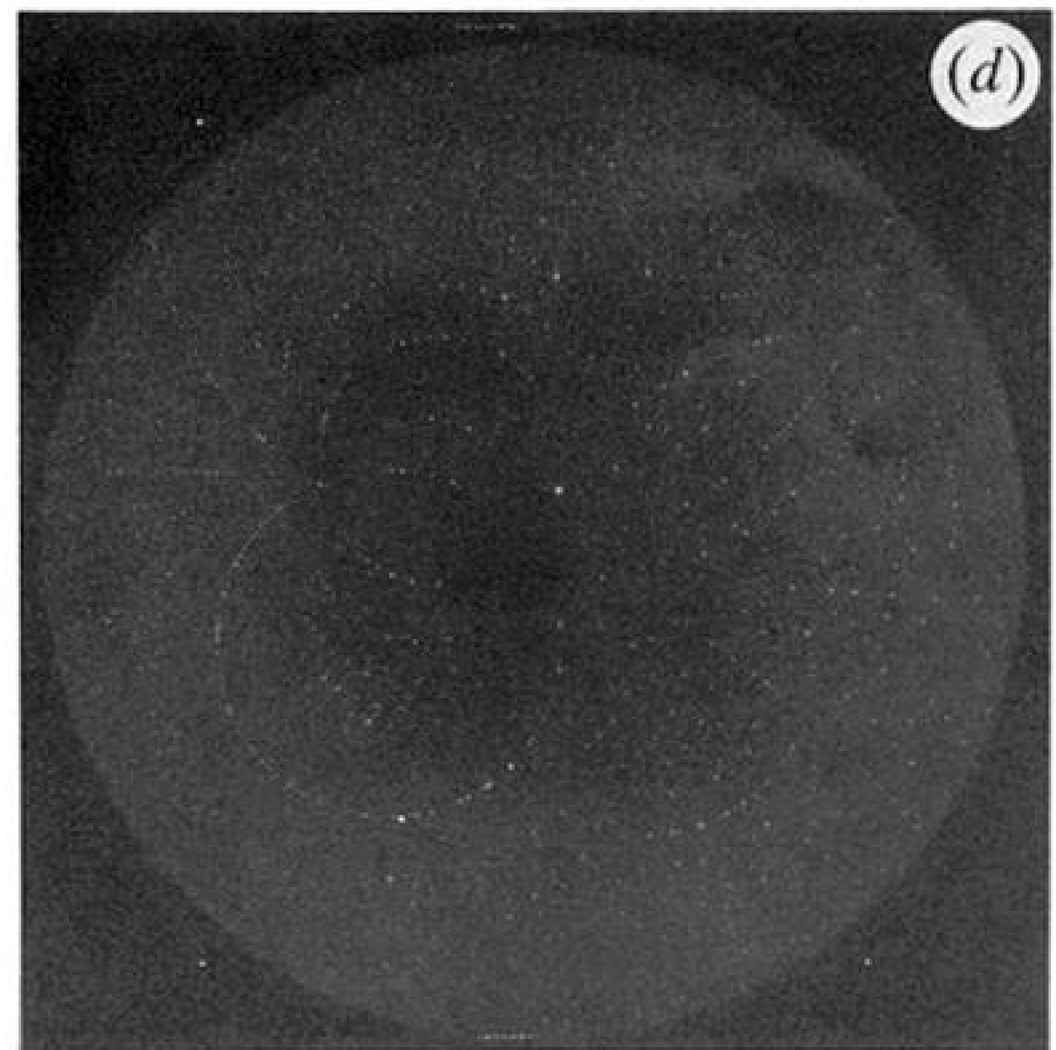

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igure 2. Four films exposed in a toast-rack set respectively at distances of (*a*) 115.5, (*b*) 155.5, (*c*) 195.5 and (*d*) 221.0 mm from a crystal of the protein concanavalin-A.

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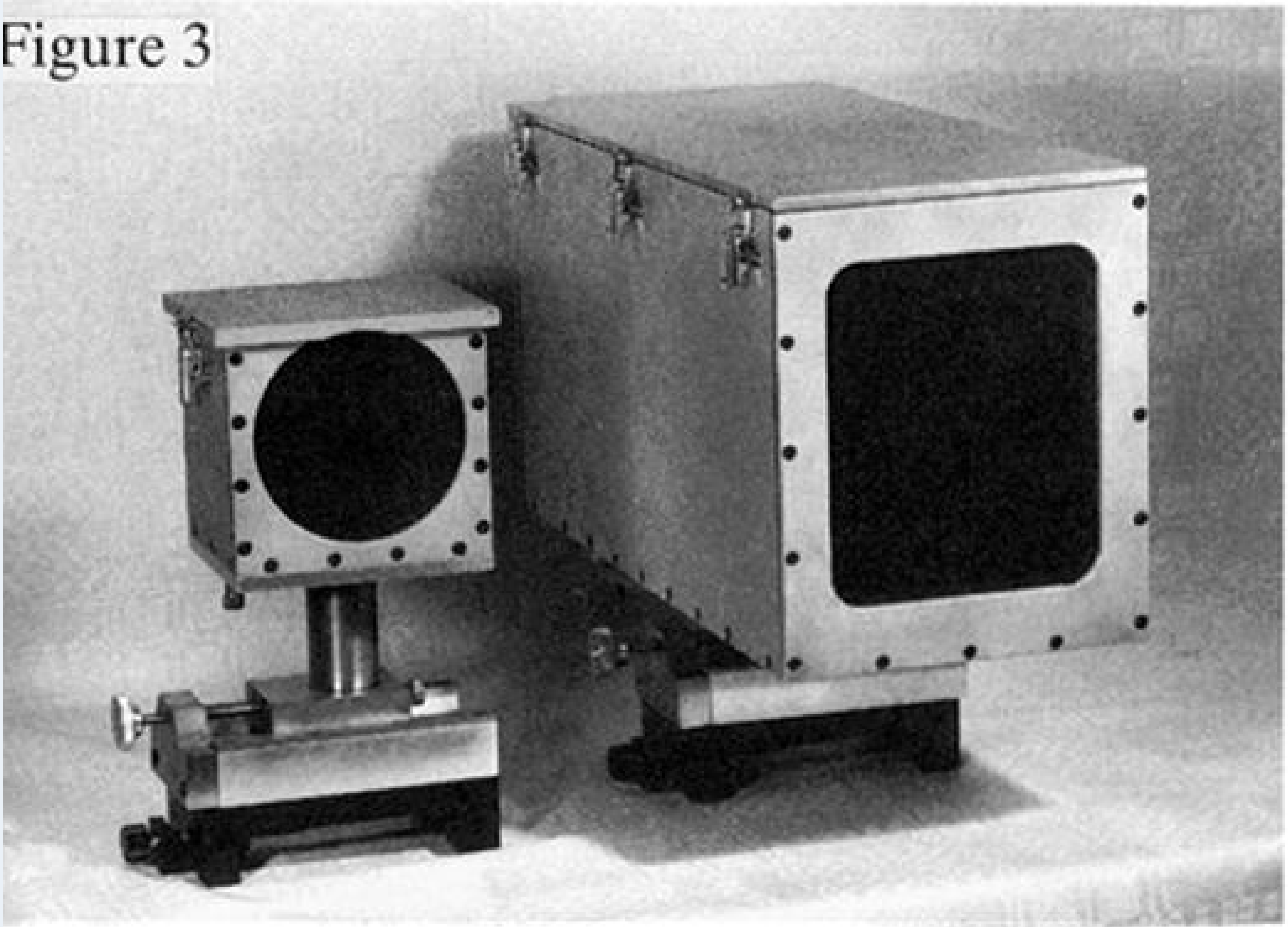

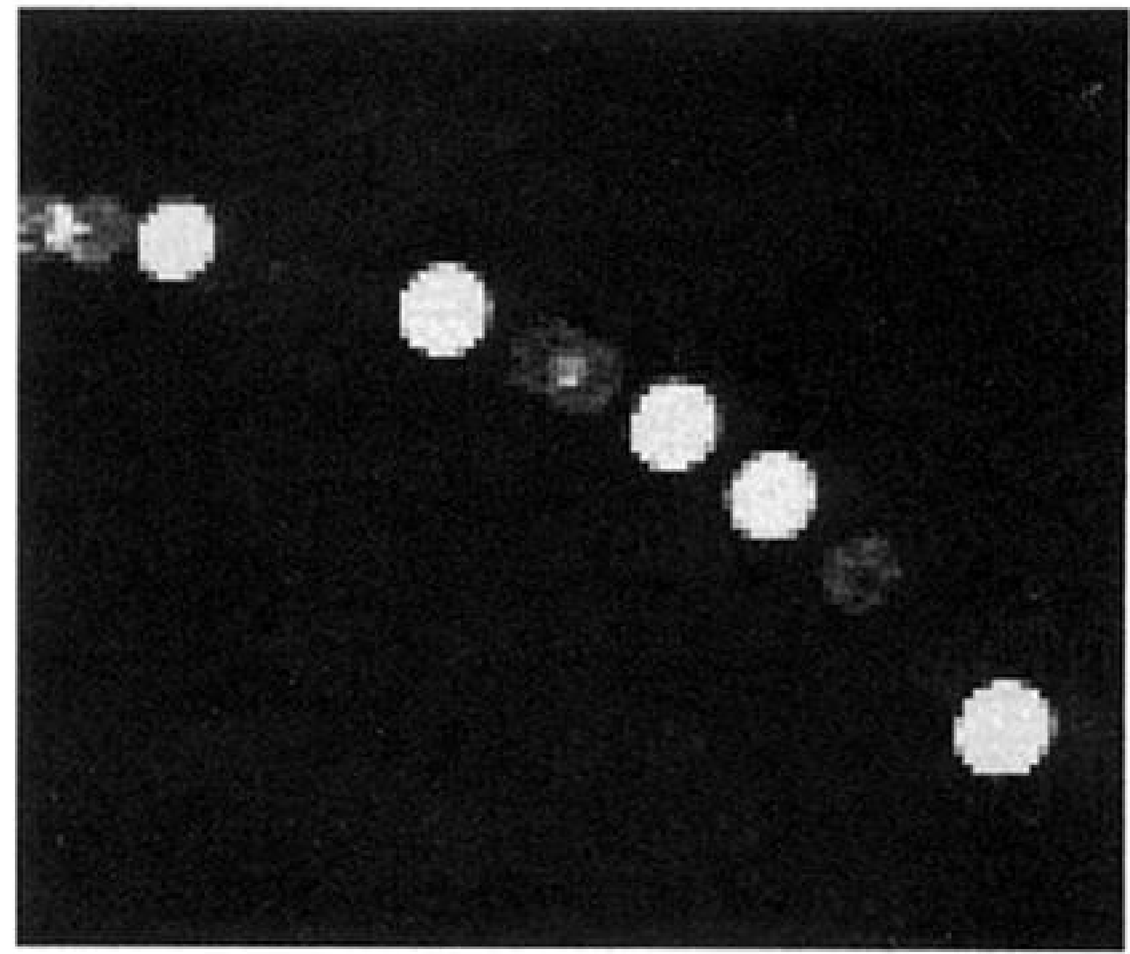

igure 3. A large toast-rack, aimed at virus work, set side by side with the smaller one (referred o in figure 2) aimed at protein work. The aperture of the small toast-rack is 120 mm.

igure 4. Laue pattern recorded on a CCD in direct detection mode from a cubic crystal of oncanavalin-A on station 9.5 of the Daresbury SRS. The spots are 200 μm across, each detector ixel is 22×22 μm^2 and the spots are essentially 10×10 pixels each.

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