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Micro-crystallography comes of age

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Abstract

The latest revolution in macromolecular crystallography was incited by the development of dedicated, user friendly, micro-crystallography beamlines. Brilliant X-ray beams of diameter 20 microns or less, now available at most synchrotron sources, enable structure determination from samples that previously were inaccessible. Relative to traditional crystallography, crystals with one or more small dimensions have diffraction patterns with vastly improved signal-to-noise when recorded with an appropriately matched beam size. Structures can be solved from isolated, well diffracting regions within inhomogeneous samples. This review summarizes the technological requirements and approaches to producing micro-beams and how they continue to change the practice of crystallography.

Introduction

Macromolecular crystallography is being transformed by the rapidly growing use of micro-crystallography. Micro-crystallography, coupled to novel data collection instruments and strategies, was developed to analyze crystals smaller than a few tens of microns (μm) to address many challenging and scientifically important problems. A highly brilliant X-ray beam of equivalent size is optimal for measurement of diffraction data from such micro-crystals [1]. Compared to conventional macromolecular crystallography experimental stations [2], micro-crystallography beamlines target samples smaller than 20 μm and beam sizes in the range of 1 – 20 μm [3]. Such micro-beams enhance the diffraction signals from micro-crystals by reducing background scattering from solvent and the sample support, thus providing high signal-to-noise in diffraction images [4]. Micro-beams can also address a variety of sample imperfections by illuminating only a well diffracting region within an imperfect crystal or a single-crystal region of a multi-crystal sample. Because many biological macromolecules and complexes yield small or clustered or inhomogeneous crystals, early successes at micro-crystallography beamlines spurred a huge demand for beam time and additional micro-crystallography capacity.

Small and highly brilliant X-ray beams suitable for micro-crystallography are available at third-generation synchrotron radiation facilities due to their low-emittance electron beams (<10 nm rad) and coupled insertion devices. Modern undulators produce X-ray beams with brilliance on order of 10^{20} photon/ $\text{mm}^2/\text{mrad}^2/0.1\%$ BW (yielding 10^{12} photon/sec or more

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in a 20- μm beam at the sample position) in the energy range of 5–35 keV (wavelength 2.5–0.35 Å). The advent of brilliant undulator radiation coincided with the widespread use of cryo-protection for protein crystals, enabling use of the new brilliant X-ray beams for conventional macromolecular crystallography (beams of 50 μm or larger). Together these advances set the stage for micro-crystallography. The early successes in micro-crystallography were from pioneering experiments at ESRF beamline ID13 [5,6,7,8], where macromolecular crystallography shared time with a variety of other experiments requiring a micro-focus beam. The ID13 micro-beam was critical for several structure determinations before the turn of the century (reviewed in [7]), and led others to develop a micro-beam capability on beamlines dedicated to macromolecular crystallography [9,10,11,12,13,14,15].

Several high-profile successes [16,17,18,19,20,21] published since the last micro-crystallography review in this journal [22] have opened up exciting areas of research where previously no tractable crystals existed. Micro-crystallography was key to solving all crystal structures of G-protein-coupled receptors (GPCR) reported in the past five years. Taken together, these structures are a major breakthrough in our understanding of how receptors respond to stimulation at the cell surface [17,19,20,23,24] and how receptor stimulation signals a cascade of events leading to an altered physiological state within the cell [21]. GPCRs and many other membrane proteins form notoriously small, weakly diffracting, radiation-sensitive crystals that can yield high-resolution structures in a micro-beam while being intractable to large-beam crystallography. Virus polyhedra are naturally occurring micro-crystals that enclose infectious insect viruses. Micro-crystallography provided high-resolution structures that explain the stability of the polyhedra under extreme environmental conditions except those favorable to infection [18,25,26]. Micro-crystallography has also provided fundamental insights into the structural basis for the high stability of the amyloid fibrils and toxic oligomers associated with many devastating diseases [16,27].

These successes along with the greater availability of micro-beams led to a rapidly growing demand that currently exceeds the capacity of the world's operating micro-crystallography beamlines. Here we summarize the critical features of micro-crystallography beamlines and the change in experimental practice that they are driving in structural biology.

Technologies for micro-crystallography: It's all about stability

The essential technologies that enable micro-crystallography are a high-stability and low-divergence beam from the storage ring, X-ray optics to create a micro-beam of high brilliance and stability, and a sample environment of high stability and precision including the diffractometer and sample-visualization system. Stability of the beam intensity and the relative alignment of the beam and crystal are paramount in micro-crystallography. Typical exposure times for diffraction images at modern crystallography beamlines range from a few seconds to a few tens of milliseconds with a fast-framing detector. Therefore, fluctuations on time scales of 0.01–200 Hz can result in decreased signal-to-noise through loss of incident intensity and therefore diffracted intensity and/or increased background resulting from beam motion. These fluctuations can originate from instabilities in the particle beam, front end, beamline optics and endstation equipment. The greatest success in achieving high-quality data from micro-crystals comes from a holistic approach to the beamline that considers everything from the particle beam along the X-ray beam path to the detector.

Factors that affect particle beam stability include day and night temperature changes in the storage ring tunnel, the tides and X-ray thermal load on components. For example, a 1° change in temperature causes an 11- μm expansion of a 1-m steel rod. One important development that improved beam stability is the “Top-Up” mode of operation [28,29] that maintains a constant beam current, thereby stabilizing the temperature of the tunnel and all

support structures in the front end. The incorporation of high-resolution X-ray beam-position monitors in positional feedback systems at some facilities has also improved beam stability.

On the beamline, environmental factors such as temperature and vibration contribute to beam instability. Most crystallography beamlines employ either a channel-cut or a double-crystal monochromator (DCM), which must provide a stable output beam under varying thermal load conditions. Channel-cut monochromators are less sensitive to vibrations and thermal load variation than are DCMs; however, energy changes require realignment of the entire beamline downstream of the monochromator. Loss of parallelism of the two crystals of a DCM, caused by thermal transients, can result in a slow drift of beam position and intensity at the sample position. Without correction, the beam position can drift by several microns over a few hours, which is a serious problem for beam and sample sizes less than 10 μm . The constant heat load on the optics in “Top-Up” mode significantly reduces the thermal drift. Vibrations of monochromator crystals cause high-frequency instability, which usually can be overcome by appropriate mechanical design, especially vibration dampening and vibration control of the circulating coolant [10,30]. Long-term instability can usually be reduced by the use of a fine positional feedback loop slower than 10 Hz [31,32,33,12]. Simple, heavy, stiff mechanisms of all optical components and granite support tables can provide great stability to environmental temperature changes and vibrations.

As the beam and crystal size are diminished, sample mounting, manipulation and visualization become more difficult, and the instrumentation must maintain a micron-sized crystal within a beam of similar dimensions for the duration of the experiment. The widely accepted target for the precision and stability of the interception of the beam and the crystal is one-tenth of the crystal or beam size, whichever is smaller. A decade ago, most macromolecular crystallography beamlines were designed for beam and crystal sizes of order 100 μm , requiring 10- μm stability, which was readily achieved with good mechanical design. Reduction of the crystal and focal spot size to 10 μm or even 1 μm presents increasing technical challenges. The requisite 1- μm stability for a 10- μm beam can be achieved by robust mechanical engineering to minimize vibrations and thermal fluctuations in the beamline optics and experimental enclosure [10,12,34,35]. Similarly, a 1- μm beam or crystal requires stability of 100 nm, and another order-of-magnitude improvement in stability and precision, including maintaining the endstation environment at a constant temperature to within $\sim 0.1^\circ\text{C}$ [13].

In addition, the visualization axis must coincide with the X-ray beam and must be aligned to the sample with similar accuracy and precision [6,13,36]. A high-performance microscope with sub-micron resolution (near the diffraction limit for visible light) is needed to precisely position samples and adjust the beam focus at the sample position. A microscope that is co-axial with the X-ray beam minimizes parallax errors [8], and is also useful for beam position monitoring with a high-resolution scintillation target mounted at the sample position.

‘Sub-micron’ precision goniometry is extremely challenging for instruments with a horizontal rotation axis due to gravity effects on the mechanisms for crystal rotation and translation, and currently is not possible for a multi-axis goniometer with, for example, kappa geometry. The peak-to-peak sphere-of-confusion (SOC) at the beamlines profiled below ranges from 3.0 μm to 0.4 μm (Figure 3). Air-bearing goniometers under development promise even smaller SOC values. Instruments with a vertical rotation axis avoid gravity effects, and should permit use of multi-axis kappa geometry.

Robotic sample handling improves stability by reducing temperature fluctuations from frequent openings of the enclosure door and inadvertent bumps of the goniometer and beam-

delivery hardware by experimenters. Additionally, sample automounting saves time as large numbers of crystals generally must be screened in order to find a high-quality crystal or to identify regions of a crystal or field of crystals with suitable diffraction [37,38,39]. Robotic systems for sample handling have become standard equipment at most crystallography beamlines, and a variety of micro-crystallography-compatible sample changers have been developed [40,41,42,43,44,45].

Sample cryo-cooling can contribute to temperature gradients in the experimental hutch. Careful venting of cold stream gas is a requirement for all micro-crystallography experiments.

Approaches to making a small beam

Approaches of four types have been implemented to achieve a small beam at the sample position (Figure 1). The choice for any beamline depends on the effective emittance of the source (product of the horizontal size and divergence), and the tradeoff between intensity and convergence in the beam. Micro-crystallography has been enabled by a steady emittance decrease at most synchrotrons, so that all four approaches can provide a micro-beam with a focal convergence in the range 0.1–1.5 mrad, as needed for typical unit cell dimensions and mosaicity of macromolecule crystals.

All four approaches to a small beam (Figure 1) are in use at successful beamlines. Here we profile one example of each approach. Table 1 is a summary of operating micro-crystallography beamlines and those in various stages of development.

The four profiled beamlines have several features in common. During the design and commissioning phases, considerable attention was given to ensuring a high degree of beam positional and intensity stability. The first optical component on all the beamlines is a high-stability monochromator where the number of motorized motions was reduced and turbulence in the circulating liquid nitrogen coolant was minimized to avoid exciting vibrations. The focusing optics are mounted on granite plinths to minimize vibrations. Accelerometers were used to detect vibrations and guide mechanical design improvements. The endstations incorporate high resolution, on-axis microscopes to visualize and optically center crystals measuring only a few microns on edge. Each of the endstations is maintained at a constant temperature within 1°C or less. Exhaust from the sample cryo-cooler is transported away from the sample environment, and heat sources in the experimental enclosure are kept to a minimum.

APS beamline 23-ID-D, for both traditional and micro-crystallography, is an example of the “aperture” approach (Figure 1a), which uses focusing optics to condense the beam followed by a beam-defining aperture near the sample position (Figure 2). To meet the stringent requirements of a micro-crystallography beamline, the DCM has undergone continuous modification [46]. A pair of bendable “bimorph” mirrors arranged in the Kirkpatrick-Baez (KB) geometry image the undulator source to $25 \times 65 \mu\text{m}^2$ at the sample position. The long mirrors (1050 mm horizontal and 600 mm vertical) collect the full central cone from the undulator. A quad-mini-beam collimator has user-selectable 5, 10 and 20 μm diameter beam-defining apertures for micro-crystallography and a 300 μm scatter guard aperture for traditional crystallography (Figure 2b) [11,47]. The beam-defining aperture defines a smaller, more Gaussian beam at the expense of flux, although it preserves the central flux density of the beam. The minimum achievable beam size is limited to $\sim 5 \mu\text{m}$ by the availability of small apertures and the beam convergence angle. Alignment of the beam and the rotation axis is stable for ~ 24 hours. Beam movements on the aperture are observed as intensity drift or fluctuations [34], which can be corrected by an automatic feedback system. The RMS intensity fluctuation is 1–2% for the 5- μm beam. A key element to the beamline

success is the rapidly (seconds) changeable beam size, and software to fully exploit the small beam sizes [37].

ESRF beamline ID23-2, dedicated to micro-crystallography, is an example of the “direct-focus” approach (Figure 1b), which relies on highly demagnifying optics to focus the beam directly to the desired size at the sample position. At ID23-2, the monochromator incorporates a single, side-bounce crystal that defines the fixed operating energy [12]. A pair of short (300 mm) mechanically bent, mirrors arranged in the KB geometry focus the beam to a standard focal size of $7.5 \times 7.5 \mu\text{m}^2$, but it can be slightly smaller. Here flux is lost because the focusing optics intercept only part of the undulator beam. A thermostatic enclosure around the mirror system is key to minimizing beam drift at the sample to less than $5 \mu\text{m}$ in 24 hours. Advantages of the direct-focus approach compared to the aperture approach are the shorter, *i.e.* less expensive, mirror optics; a shorter lever arm from the optic to the sample, which reduces sensitivity to slope error, mirror drift or vibrations; and smaller intensity fluctuations. However, to change the beam size, the mirror focus must be shifted downstream, which is a slow process (tens of minutes).

SPRING-8 beamline BL32XU, dedicated to crystals of dimension $10 \mu\text{m}$ or smaller, is an example of the “divergence-limited source” approach (Figure 1c) where highly demagnifying optics image a source point defined by slits in the divergent undulator beam. BL32XU (Figure 3) has the world's first $1\text{-}\mu\text{m}$ beam dedicated to macromolecular crystallography [13]. Following a high-precision DCM, an aperture of variable size creates the divergence-limited source, from which the beam is focused onto the sample using fixed-curvature, elliptical mirrors. To achieve a sub-micron focus, the mirror surfaces were fabricated to atomic-scale accuracy by Elastic Emission Machining (EEM) [48,49]. By changing the aperture size, the beam size can be varied rapidly (seconds) up to $10 \mu\text{m}$ with a minimum of $0.9 \times 0.9 \mu\text{m}^2$ (Figure 3a). A recently developed micro-diffractometer with an air-bearing rotation axis and linear XY positioners has sub-micron precision of rotation, *i.e.* a sphere-of-confusion (SOC) of $<0.4 \mu\text{m}$ (Figure 3b,c). The endstation is enclosed in a chamber within the experimental enclosure where the temperature is controlled to 0.1°C to maintain a high degree of positional stability. The first diffraction image with $d_{\text{min}} \sim 2 \text{ \AA}$ was collected from an illuminated volume of $1 \times 1 \times 2 \mu\text{m}^3$ in a $5 \times 5 \times 2 \mu\text{m}^3$ protein crystal (K. Hirata, personal communication).

DLS beamline I24 supports traditional and micro-crystallography, and is an example of using upstream optics to create a “secondary-source” (Figure 1d), which is then imaged to the sample position by a second set of optics [9]. A DCM selects the energy, and bimorph mirrors arranged in the KB geometry create an image of the undulator source several meters upstream of the sample position. Slits at this position define a spatially clean secondary source. A second set of KB mirrors creates an image of the secondary source at the sample position. By imaging a rigidly supported secondary source point, this approach provides beam stability at the sample position by decoupling from fluctuations in the source (position and angle) and in the upstream optics. The secondary source slits and second set of KB mirrors can be translated along the beamline to vary the focal demagnification, resulting in variable beam size at the sample position with constant intensity, effectively an “X-ray zoom”. When fully commissioned, beam size changes will take several minutes by zooming, and, at the expense of flux, more rapidly by varying the slit size. A key feature of this beamline is the combination of shutterless data collection and sample rastering, which significantly reduces the time to map out the quality of a large crystal or to locate microcrystals [38].

The aperture, divergence-limited-source and secondary-source approaches have the operational convenience of rapid (seconds) selection of the beam size by varying a slit or

aperture size, but at the cost of variable flux. In contrast, the direct-focus approach and the secondary-source zoom require a slower refocusing of the mirrors (several minutes), but can provide a constant integrated flux over various beam sizes and aspect ratios. A major advantage of the secondary-source zoom approach is the combination of a constant integrated flux and a focus maintained at the sample position, thereby minimizing any delirious effects from structure in the beam. As designed, the demagnification ratio of the beamlines increases from roughly 10:1 for 23-ID-D to 20:1 for ID23-2 to 30:1 for BL32XU. The higher demagnification ratios can lead to higher flux density at the focal position and also increased divergence, depending on what portion of the undulator central cone is accepted. The demagnification ratio on I24 is the product of the ratio for the first and second stages of focusing and varies from about 10:1 to 20:1.

Practice of micro-crystallography

The trend towards micro-crystallography is driving a fundamental change in the practice of macromolecular crystallography. Because single-crystal regions of samples no longer must have length many tens of microns, far more samples are screened relative to data sets collected, far more data sets are constructed from multiple crystals or from multiple regions of one crystal, and far more crystals now produce useful data that formerly were relegated to the dustbin. The shift in acceptable sample quality drives the rapidly growing demand for beam time at micro-crystallography stations. Where it is robust, user-friendly and available routinely, micro-crystallography has become the new normal.

What aspects of the nebulous quality of “user friendliness” are key to the success of micro-crystallography beamlines? Advances on the beamlines, together with improvements in the emittance properties and stability of storage-ring particle beams, deliver X-ray beams of high flux and low divergence to the sample. To be user friendly for micro-crystallography, a beamline must have beam and sample positional stability that is robust enough for users to rapidly tailor the beam size to the sample without need for beam realignment. If micro-crystallography is to be the new normal, beam tailoring without realignment must also include energy changes. Equally important are software tools for users to search quickly within the sample for the best diffracting regions, and to record diffraction images from all useful parts of the sample [39,37,38]. Compared to large-beam crystallography, fewer unit cells of the crystal are illuminated in micro-crystallography, a greater X-ray flux is delivered to a smaller sample volume, and radiation damage per illuminated volume is greater in each diffraction image. However, since a smaller crystal volume is damaged per diffraction image, software tools that spread X-ray exposure through the sample during data collection can effectively mitigate radiation damage. Schemes for “vector” or “helical” data collection have been quite successful [12,37].

Successful micro-crystallography beamlines are driving new technologies in sample handling, crystallogenesis and data collection. Two developments have especially high impact and may lead to further fundamental changes in practice. Meso-phase crystallization of membrane proteins has matured after many years of incubation [50,51,52] and resulted in spectacular success with several important membrane-protein crystal structures. Crystals grown in the lipidic cubic phase are generally small and are often difficult to see visually due to opacity of the lipid at the temperatures of sample cryo-protection (100 K). For these samples, software for a raster or grid search is essential to find well diffracting regions within a field of small crystals. Another exciting development is the ability to record diffraction data from crystals in their growth chamber, known as *in situ* diffraction [53,54]. This can be of enormous importance in screening crystallization conditions to distinguish crystals from non-crystalline solids as well as macromolecule crystals from salt or lipid crystals. However, it also promises to segue into a routine method for data collection where

single (or a few) diffraction images are recorded from each crystal. The first structures solved with *in situ* data were published this year [55]. *In situ* diffraction is necessarily done at the temperature of crystal growth, *i.e.* samples are not cryo-protected. Recent exciting evidence suggests that damage from radiation-generated free radicals can be “outrun” by use of fast-readout detectors [56,57].

A fundamental change on the horizon is a return to the practice of multi-crystal data collection, which was standard before crystal cryo-protection techniques were developed. Multi-crystal data sets are needed to achieve statistically significant diffracted intensity when extreme radiation damage results from large doses to micro-volumes [17,52] or to enhance a weak phasing signal [58]. New software tools for data processing, and especially for assembly of complete data sets from a large number of partial data sets, are under development in several labs and will become increasingly important as micro-crystallography and *in situ* diffraction become more widespread and as more focused beams of even greater flux are developed.

Radiation damage

Radiation damage is a major consideration in micro-crystallography because many fewer unit cells are illuminated than in large-beam crystallography, and consequently a greater X-ray dose (deposited energy per unit of crystal mass) is needed to record data to the diffraction limit of the crystal. In practical terms, radiation damage prevents collection of full data from one micro-crystal or from one site on a large crystal. Fundamental studies of radiation damage may address this problem in part. At the energies typically used for macromolecular crystallography (6–20 keV) and for the low Z elements that make up the bulk of the sample, the main mode of interaction is the absorption of an X-ray photon resulting in the emission of a high energy (keV) photoelectron and low energy (~100 eV) Auger electrons. Primary damage occurs when these electrons scatter around in the crystal until they are absorbed after a distance of a 0.1 μm for Auger electrons and 1–10 μm for photoelectrons. If the beam or the crystal is sufficiently small, the photoelectron can escape the illuminated volume, reducing damage within that volume. A series of calculations predicted the magnitude of this effect [59], which has been experimentally demonstrated with a 1- μm beam at 18.5 keV [60,61]. Although the damage was reduced for a given dose, the loss of diffraction from the smaller diffracting volume was greater than the loss in radiation sensitivity. Smaller beams and/or higher X-ray energies must be studied to determine whether photoelectron escape has a practical application in macromolecular crystallography. Alternatively, at low energies (<6 keV) the increase absorption in the sample results in increased damage, and radiation damage may be reduced by using microcrystals [62]. The fundamental studies of radiation damage within and beyond the beam footprint showed that damage to a protein crystal from 18.5-keV X-rays extended no more than 4 μm beyond the center of a 1- μm beam [60]. This result is of immediate practical use in micro-crystallography as the basis for estimating the size of a damaged zone or the minimum crystal translation required to expose an undamaged region of a crystal. In addition, the common practice of matching the beam size to the crystal size to reduce background has an advantage for microcrystals where the photoelectron can leave the crystal, resulting in reduced radiation damage.

Damage-free crystallography with an X-ray free electron laser

Initial feasibility experiments using the X-ray free-electron laser (XFEL) at the Linac Coherent Light Source (LCLS) hold the promise of damage-free crystallography. These experiments showed that a single X-ray pulse of several tens to 100 femtoseconds duration can produce usable diffraction data from protein crystals containing as few as 10^3 unit cells

[63,64,65,66]. The technique is named “diffract before destroy” because the short X-ray pulse is diffracted before it destroys the crystal. To realize the promise of damage-free crystallography, several challenges related to the X-ray source, sample handling [67] and data processing must be addressed. Data processing is complicated by the lack of any fully recorded Bragg reflections in the “still” diffraction images, and by the lack of multiple images from each crystal [68,69]. A vast number of crystals is required to estimate the full intensity of each reflection from a set of observations of unknown partiality. Additionally, the beam properties are variable as the lasing process produces X-ray pulses with considerably greater variation in intensity (5–12% RMSD), energy (~8 eV RMSD), and band width (~50 eV FWHM) [70], compared to beams from conventional synchrotron sources (less than 1% RMSD, 0.1 eV RMSD and 0.5 eV FWHM, respectively). Nevertheless the success of initial experiments holds great promise to eventually deliver damage-free crystal structures, especially with the increased capacity brought by new XFEL sources coming on line in Japan (SACLA) and Europe (XFEL) and in planning in Switzerland (SwissFEL) and Korea (PAL XFEL), as reviewed elsewhere in this journal [71].

The future

The rapid technical progress and a growing number of spectacular scientific achievements in the last five years demonstrate that micro-crystallography is indeed the “new normal” in macromolecular crystallography. Nevertheless several challenges remain. Beamlines of the requisite stability for 5- μ m diameter or smaller X-ray beams are difficult to develop, and especially challenging for retrofitting on older beamlines built to a different standard. Small samples and beams need powerful, rapid, automated methods to locate and center crystals in the beam. Automation is also key to monitoring and maintaining beamline robustness and stability, and to keeping clumsy human hands away from endstation hardware. Radiation damage is a perpetual challenge regardless of crystal cryo-protection. Fast-readout detectors may effectively extend the lifetime of room-temperature crystals by outrunning damage caused by diffusing free radicals. Through improved signal-to-noise, fast detectors also push the envelope toward even smaller and more weakly diffracting crystals. Smaller samples, hotter beams and more *in situ* experiments return us to an era of multi-crystal data sets, but in this era multi-crystal, ultra-high-redundancy data can be taken very quickly. There is a great need for sophisticated software to sift through many partial data sets from many crystals and to assemble complete merged data sets of the highest redundancy, taken from the most isomorphous subset of crystals, in their least damaged state. The need is especially acute for data taken at room temperature in *in situ* experiments. The challenges are substantial but not insurmountable and the future is bright – we will see more biology on more difficult systems with hotter, smaller beams that are delivered with great precision and controlled by sophisticated tools.

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Highlights

- X-ray micro-beams permit structure determination from small or imperfect crystals
- Robust micro-beams require source, optics and sample support to be extremely stable
- Four different approaches to making a 1–20-micron beam have been implemented
- A growing demand has led to ~20 beamlines for micro-crystallography worldwide
- Micro-beams enable data collection from LCP-grown crystals and crystals *in situ*

Figure 1. Approaches to making a small beam for micro-crystallography

(a) Aperture approach. The full central cone from the undulator source is focused by the mirrors and the beam size is defined with an aperture near the sample, typically 30 mm upstream. (b) Direct focus approach. Short mirrors near the sample capture a portion of the central cone from the undulator source and focus the beam directly to the sample position. (c) Divergence-limited source approach. A portion of the undulator central cone is selected by slits, which are imaged at the sample position by micro-focusing optics near the sample. (d) Secondary source approach. Upstream mirrors focus the full central cone of the undulator onto a set of slits. A second set of optics near the sample images the slits to the sample position. The beam direction is right to left. Focusing optics are depicted as blue ellipses, the source and focal points as red dots, and apertures and slits as black bars.

Figure 2. Micro-crystallography endstation at APS beamline 23ID-B

(a) Sample environment. The sample is mounted on an air-bearing goniometer with 1- μm peak-to-peak SOC [34]. The mini-beam quad collimator is positioned downstream of the high-resolution microscope that views the sample along the beam axis. The active beam stop is mounted from the right. A fluorescence detector is mounted in the upper left. The sample cryo-cooler, usually mounted in the upper right, is removed in this photograph. **(b)** Mini-beam quad collimator. In this schematic, the beam vector passes through the scatter guard aperture. Apertures in the other beam paths define 5-, 10- and 20- μm diameter beams. **(c)** Active beamstop. The beamstop is shown before (left) and after (right) assembly. X-rays impinge on the beamstop and eject photoelectrons, which yield a current that is linearly proportional to the incident intensity [34].

Figure 3. SPRing-8 BL32XU micro-focus beamline

(a) Focused beam profile at the sample position. The horizontal (left) and the vertical (right) beam profiles are from a wire scan. The FWHM of the focused beam is $0.9\ \mu\text{m}$ in both directions. (b) High-precision air-bearing Kohzu QKSU-1 goniometer, shown in a schematic drawing (top) and photograph (bottom). The QKSU-0 goniometer is installed on beamlines BL17 and BL01 at the Photon Factory, and beamlines BL41XU and BL32XU at SPRing-8. (c) Plot of the QKSU-1 SOC through 360° rotation. The displacement of a precise ball on the horizontally mounted goniometer was measured with a capacitive sensor. (Drawing and inspection data courtesy of Kohzu Precision Co., Ltd.)

Table 1

Micro-crystallography beamlines

Facility & beamline	Target beam size ^a	Energy range	Approach
Operating beamlines^b			
APS 23ID-B [11]	5, 10, 20 μm	3.5–20 keV	Aperture
APS 23ID-D [11]	5, 10, 20 μm	5–20 keV	Aperture
APS 17ID-B [47]	10, 20 μm	6–20 keV	Aperture
APS 19ID [72]	5, 10, 20 μm	6–17 keV	Aperture
APS 24ID-E	5–20 μm	12.66 keV	Aperture
APS 31ID	20 μm	9–13.8 keV	Aperture
Australia MX2	10 μm	5.5–28 keV	Aperture
CHESS A1 [73]	<20 μm	12.68 keV	Direct focus
CHESS F1 [73]	<20 μm	13.50 keV	Direct focus
CHESS F2 [73]	<20 μm	7–16 keV	Direct focus
Diamond I02	20 μm	5–25 keV	Aperture
Diamond I03	20 μm	5–25 keV	Aperture
Diamond I04	2×8 μm^2	13.1, 7.15 keV	Aperture
Diamond I24 [9]	7–10 μm	6.5–18 keV	Secondary source
ESRF ID13 EHII [6]	1 μm	5–17 keV	Direct focus
ESRF ID23-2 [12]	10 μm	14.2 keV	Direct focus
ESRF ID29 [15]	10, 20 μm	6–20 keV	Aperture
Photon Factory BL-17A [10]	20 μm	5.9–13.8 keV	Aperture
Photon Factory BL-1A	10 μm	2.7–3.0 keV	Aperture
SPring-8 BL32XU [13]	1–10 μm	8.5–20 keV	Divergence-limited source
SPring-8 BL41XU [14]	10 μm	6.5–35 keV	Aperture
SLS X06SA	(15)×5 μm^2	5.7–17.5 keV	(Aperture) direct focus
SSRL 12-2	7, 10, 20 μm	6.7–17.2 keV	Aperture
Beamlines in process			Status
ALBA BL13	300×7 μm^2	5–21 keV	Commissioning
Diamond I02	20, 10 μm	7–17 keV	Commissioning
Diamond I03	20, 10 μm	7–17 keV	Commissioning
Diamond I04	20, 10 μm	7–17 keV	Commissioning
PETRA III MX1	5, 10 μm ; 28×13 μm^2	5–17 keV	Commissioning
PETRA III MX2	4×1 μm^2	7–35 keV	Commissioning
APS 23ID-D [74]	1–20 μm	6–35 keV	Construction
NSRRC PX	1–50 μm	5.7–20 keV	Construction
SOLEIL PX2	20 μm^2	5–15 keV	Construction
SSRF NFPS	10×5 μm^2	5–18 keV	Construction
MAX IV BioMAX	20 μm	5–25 keV	Design

Facility & beamline	Target beam size ^a	Energy range	Approach
NSLS II FMX [75]	1–100 μm	5–20 keV	Design
NSLS II AMX [75]	5–300 μm	5–25 keV	Design
NSLS II NYX	5–50 μm	3.5–17.5 keV	Design

^a All dimensions are FWHM. If two dimensions are listed, the first is the horizontal dimension and the second is the vertical (HxV). Several beamlines have selectable beam sizes, designated by comma-separated discrete sizes or by a size range.

^b Beamlines with beams of dimension 20 μm or smaller; some also produce larger beams. For beamlines without a citation, the information was obtained from <http://biosync.sbkb.org/index.jsp> or from the beamline web site.