Introduction to single crystal X-ray analysis

III. Obtaining quality data from a microcrystal

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1. Introduction

Due to the recent development of fundamental technologies of X-ray generators and detectors, such as X-ray focusing optics and area detectors, the size of the measurable crystals by a laboratory source has been dramatically reduced. In these days, a crystal of a few microns can sometimes be measured. However, obtaining quality diffraction data from such a small crystal, namely a microcrystal, requires special attention that is not necessary for a regular sized crystal. In particular, the handling of a microcrystal becomes more troublesome as the size of the crystal becomes smaller. The retrospective glass fiber and glue seems to be a better method than today's conventional loop type mounts for a microcrystal. In this article, techniques and tools to measure diffraction data out of a microcrystal are introduced.

2. Measurement of a microcrystal

Regardless of the size of the crystal, the acquisition of quality diffraction data is the key to the successful structure analysis especially when the crystal has an additional complexity such as twining or severe disorders. Diffraction data precision is proportional to the magnitude of intensity because the counting statistics improve as the intensity increase. Therefore, one should make efforts to maximize the integrated intensity that is represented by the following equation

$$I = \frac{I_0\left(\frac{e^2}{mc^2}\right)^2 \lambda^3 v (Lp) A F^2}{V^2 \Omega}$$

where I is the integrated intensity of a diffraction spot, I_0 is the intensity of incident X-rays, e is the charge of an electron, m is the mass of electron, c is the speed of light, λ is the wavelength of X-rays, v is the volume of the crystal, Lp is the Lorentz and polarization factor, A is the transmittance, F is the structure factor, V is the volume of the unit cell and Ω is the scan speed that is equivalent to the exposure time for area detectors.

The equation above indicates that there are four parameters that can be manipulated to increase the intensity during the diffraction experiment: I_0, v, F and Ω.

The first parameter I_0, the intensity of the incident X-rays, is usually determined by selecting the instrument. One should be aware that it is brilliance that is more important than the flux especially for a microcrystal. The combination of a high brilliance X-ray generator and a multilayer focusing optics is optimal to achieve this requirement. Table 1 summarizes the comparison of brilliance of the focal point on the surface of the X-ray generators. Table 2 depicts the relative intensities delivered from the various combinations of an X-ray generator and optics. The relative intensities in Table 2 were calculated based on intensities recorded behind a 0.1 mm pinhole placed at the sample position.

The conventional HF type optics has a beam diameter of 200 μm at or near the sample position and the latest optics, VHF, has a 100 μm beam size. For Cu radiation, the VHF optics focus X-rays 2.5 times as intensely as the HF optics does when it is combined with the micro focus rotating anode X-ray generator, the RA-Micro7. The combination of the FR-E$^+$ and VHF optics delivers X-rays 5 times as intense as that of the RA-Micro7 and HF optics. Similar trends can be observed for Mo radiation. It is important to select a system equipped with not only a high brilliance X-ray source and optics but also a detector with a high sensitivity to maximize the efficiency. Figure 1 shows the combination of RA-Micro7, VariMax and Saturn 724$^+$ HG, a typical example of an optimal system best suited for measuring microcrystal diffraction.

The second parameter is the volume of the crystal. It is well known that the larger the crystal volume, the stronger the diffraction intensity. Since this article is about measuring microcrystals, there is no point in referring to this parameter.

The third parameter is the structure factor. The amplitude of the structure factor can be maximized by employing a low temperature measurement setup. At a low temperature, the thermal vibration of atoms is

Table 1. Comparison of brilliance among X-ray generators.

X-ray generator	ultraX18	RA-Micro7	FR-E$^+$
Power (kW)	5.40	1.20	2.47
Focal size	0.3 mm	ϕ70 μm	ϕ70 μm
Brilliance (kW/mm^2)	6.0	31.2	56.3
Relative brilliance	1.0	5.20	9.38

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Table 2. Combination of multilayer optics and X-ray generator.

X-ray generator	Power (kW)	Optics (VariMax)	Beam size (mm)	Relative intensity Cu	Relative intensity Mo
RA-Micro7	1.20	HF	0.21	1.00	1.00
		VHF	0.10	2.50	2.35
FR-E$^+$	2.47	HF	0.21	2.13	2.06
		VHF	0.10	5.00	4.84

Fig. 1. Overview of the VariMax with Saturn system.

reduced and this results in an increase of the amplitude of structure factors.

The last parameter is the scan speed, Ω. For area detectors, it is equivalent to the exposure time spent for each diffraction image. To increase the diffraction intensity, longer exposure time should be employed. However, every detector has its own specific characteristics based on the components used to create the detector and one should aware that there always is a limitation in the exposure time. Beyond the specific limitation, the signal to noise ratio deteriorates. One should make efforts to select the best exposure time in relation to the intensity of the source and the noise of the detector.

3. Tools to assist mounting a microcrystal

When the size of the crystal is as small as a few microns, the procedure of mounting the crystal under a microscope becomes extremely troublesome. In this chapter, tools to mitigate the difficulty are described.

The primary tool to mount a microcrystal is a high quality microscope. The microscope should have a sufficient magnification ratio that allows delicate manipulations. A standard inverted or upright microscope usually has a sufficient magnification ratio and may be able to be used to mount a crystal. However, those microscopes are often costly and the usage will be limited to small crystals. On the other hand, a stereo microscope with a zoom mechanism gives us maximum flexibility.

The necessary magnification ratio can be achieved by equipping an appropriate objective lens and eyepieces. For example, we used to use a standard SMZ1000

Fig. 2. A Nikon SMZ1500 stereo microscope.

microscope (Nikon) at our application laboratory, but it did not have a sufficient magnification ratio and did not enable us to mount a microcrystal. The SMZ1500 model (Nikon) shown in Fig. 2 has a zoom ratio of ×15, from 0.75 to 11.25. The additional outstanding feature of the SMZ1500 is the ability to adopt multiple objective lenses. Therefore, we obtained a system equipped with a revolver integrating two objective lenses having 1.6 and 1.0 magnification ratio. The 1.6 lens provides the highest magnification ratio of ×540 with a drawback of narrow working space between the stage and the bottom of the lens. This problem can be avoided by switching to the 1.0 lens when mounting regular sized crystals. Additional eyepieces with a different magnification ratios (such as ×20 and ×30) in addition to the standard ×10 eyepieces will be a good asset that gives us maximum flexibility to change the magnification ratio readily according to the size of the crystal. Each type of eyepiece should have a built in micrometer to measure the size of the crystals easily.

Fig. 3. A Narishige PC-10 glass puller.

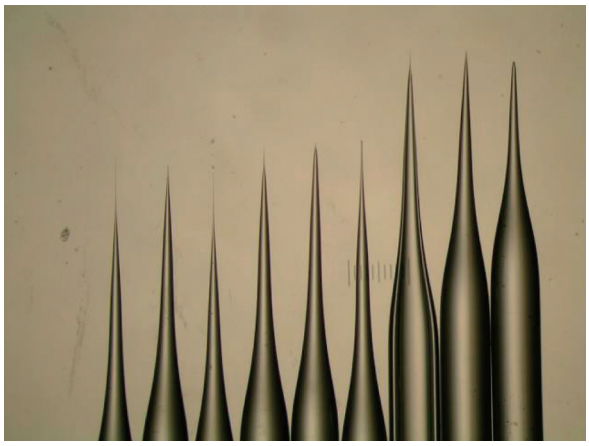

Fig. 4. Glass fiber created by the PC-10.

Usually a small crystal is stuck to the CryoLoop or MicroMount but a microcrystal is better to be placed to the tip of a very fine glass fiber with a small amount of oil. This is because a microcrystal often requires longer exposure time as it is mentioned before, and thin glass fibers produce less background than the organic polymer used in loops does. The thickness of the glass fiber can be reduced by heating and pulling it over a frame, but creating an extra fine fiber compatible with the size of the microcrystal is not an easy task. Figure 3 indicates a glass fiber puller, PC-10 (Narishige). It was originally developed for creating needles for the microinjection experiments. The system is capable of fine-tuning the thickness of the glass fiber (Fig. 4).

When mounting a microcrystal under the microscope, hand shakiness becomes a significant problem. We thought that a simple XYZ stage controlled by micrometers probably would be useful. Figure 5 shows is an example of such manipulator. The stage is fixed to the base of the microscope so that the relative position is kept constant. The XYZ manipulator allows a precise and subtle movement that cannot be possible with bare

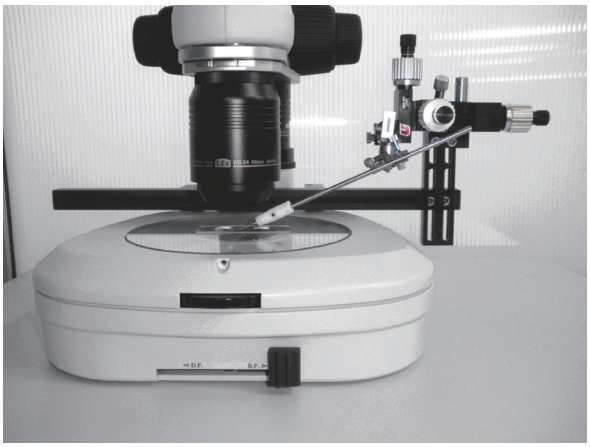

Fig. 5. An XYZ stage controlled by micrometers (Narishige Co., Ltd.) attached to a stereo microscope.

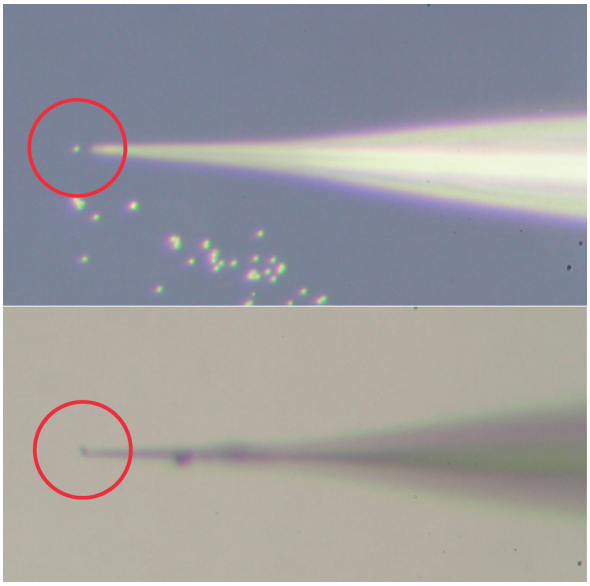

Fig. 6. A 3μm crystal before (upper) and after (lower) the mounting using the XYZ stage and the fine glass fiber created by the PC-10.

hands. We found that we can mount a 3 µm crystal easily by using this system (Fig. 6).

4. Summary

To obtain quality data out of a microcrystal, the intensity of the incident beam is critical. One should use a high brilliant X-ray source such as a combination of the micro-focus rotating anode X-ray generator and the multilayer focusing optics. The system should have a sensitive and low noise detector compatible with the X-ray source. The crystal should be mounted in a way to minimize the background because longer exposure time is often necessary for a very small crystal. At our application laboratories, we use an extra fine glass fiber. Selecting appropriate tools for mounting the crystal dramatically ease the difficulty of handling a microcrystal.