Alignment Protocol for Effective use of Hard X-ray Quad collimator for micro-crystallography

S. Xu, V. Nagarajan, R. Sanishvili and R. F. Fischetti

GM/CA-CAT, Biosciences Division, Argonne National Laboratory, Argonne, IL 60439, USA

ABSTRACT

In October 2009, a quad, mini-beam collimator was implemented at GM/CA CAT that allowed users to select between a 5, 10, or 20 micron mini-beam or a 300 micron scatter guard for macromolecular crystallography [1]. Initial alignment of each pinhole to the optical axis of each path through the mini-beam collimator is performed under an optical microscope using an alignment jig. Next, the pre-aligned collimator and its kinematic mount are moved to the beamline and attached to a pair of high precision translation stages attached to an on-axis-visualization system for viewing the protein crystal under investigation [1], [2]. The collimator is aligned to the beam axis by two angular and two translational motions. The pitch and yaw adjustments are typically only done during initial installation, and therefore are not motorized. The horizontal and vertical positions are adjusted remotely with high precision translational stages. Final alignment of the collimator is achieved using several endstation components, namely, a YAG crystal at the sample position to visualize the mini-beam, a CCD detector to record an X-ray background image, and a PIN diode to record the mini-beam intensity. The alignment protocol and its opto-mechanical instrumentation design will be discussed in detail.

Keywords: Quad collimator, kinematic mounting, high resolution translation stages, visual alignment, Alignment protocol

1. INTRODUCTION

Figure 1. Close up of the Quad Collimating system installed on the OAV. Two high resolution micro translation stages are combined to form an XY system for the collimators alignment. The travel range is 15 mm for both horizontal and vertical. The design resolution is 0.007 μm and the repeatability is 0.1 μm. The whole system stability is +/- 0.1 μm.
The Advanced Photon Source (APS) at Argonne National Laboratory (ANL) is a national user facility for synchrotron radiation research. GM/CA-CAT operates canted-undulator beamlines, 23ID-B and 23ID-D, at the APS. Both beamlines are rapidly tunable for Multiple Anomalous Diffraction (MAD) and are equipped with ALS-style sample automounters. Since 2007, we have provided micro-diffraction capabilities at both beamlines through versatile collimator systems and on-axis sample visualization (OAV) (Figure 1). Recently we developed a robust, monolithic quad-collimator containing user-selectable beam sizes of 5 μm, 10 μm, 20 μm or “full beam” (~25 μm (V) x ~75 μm (H) FWHM)[3]. The Quad Collimator assembly mounted in the experimental station on top of the On-Axis-Visualizer (OAV, has provided an enhanced tool for protein crystallographers and has encouraged users to bring crystals that are only a few microns in size. Software tools that raster to locate nearly invisible sample crystals or well diffracting regions in larger heterogeneous crystals are routinely and reliably used due to the development of the multi-collimator system. An effortless one-button automated switching between different sizes of collimators has removed barriers to scientists. Searching for samples with micron-sized collimators is now taken for granted. All of this is done while preserving all the benefits of the individual micron beam collimators—specifically background reduction and spatial resolution [4]. Precise alignment and proper orientation of the Quad collimator is critical for the efficient use of this apparatus.

2. QUAD COLLIMATOR SYSTEM

The Quad Collimator System [5] is an economical, compact, high precision multi-collimator (i.e. 5 -, 10 -, 20 -, 300-um apertures) system used for reducing the size of incident x-ray beam to that of the sample or smaller. The collimating system consists of three essential components: a uni-body quad collimator, a magnetically indexed kinematic mount and a precision motion system (Figure 2). Further descriptions of each component are provided below. It can be placed on beamlines or home X-ray sources and therefore can be installed on any existing x-ray system generally available.

Figure 2. GMCA’s X-Ray Uni-Body Quad Collimator system. It consists of Quad Collimators, kinematic mounting system and high resolution X-Z micro-translation stages.
2.1. Quad Collimator

The Quad Collimator system has evolved through actual testing of prototype models used by protein crystallographers. This refinement of the system has led to the current model, which provides three discrete mini-beam choices of the incident beam (5, 10, and 20 \( \mu \text{m} \)). A fourth scatterguard serves as background reduction for larger beam sizes that are restricted by focusing mirrors and continuously variable slits that are present upstream of the collimator assembly. The choices for the collimator apertures are customizable, depending on the application. Figure 3 is a semi-transparent, three-dimensional view of the quad collimator with its essential components. All four of the collimators are incorporated into a compact uni-body construction. The compact footprint was necessary to minimize any obstruction to the visualization of the sample. Other advantages of the uni-body construction are an increased durability relative to previous prototypes and a reduced exit aperture that further decreased background scatter.

2.2 Kinematic Mounting

A key design specification was to provide easy access for removal and replacement of the collimator from its mount. This is to accommodate changing experimental conditions, as well as maintenance/customization of the surrounding instrumentation. The collimators are then required to be replaced within sub-micron tolerances. A kinematic mounting system has been designed to reach these specifications (Figure 2 and Figure 5). The positional reproducibility of the mini-beam collimator on the kinematic mount was measured by optical metrology. The RMS deviation from the mean position was 0.24 \( \mu \text{m} \) in both the X- and Z-directions for 34 repeated manual mount and dismount operations. The stability of the assembled mount was monitored in the X-direction once per minute over a period of 20 minutes. The RMS deviation from the mean X-position was 0.06 microns. The stability in the Z-direction was not measured, but is expected to be smaller than the X-direction. [2]. A kinematic mounting system is designed for collimator vertical mounting. The two precision screw sets are installed on the holder plate. These screws feature tight tolerance and no excess grease.

2.3 High resolution X-Z micro-translation stages.

Two PI-M-111 high resolution micro translation stages are combined to form an xz system (Figure 2) for quick change of different aperture diameters (5, 10, 20 and 300 microns) according to sample size and also for collimator alignment.
The base plates of the kinematic mounts are attached to commercially available high-resolution micro translation stages providing horizontal and vertical translation. These stages have a uni-directional repeatability of < 0.1 \( \mu m \). Thus one can store the coordinates of each of the mini-beam collimators and the scatter guard allowing users to recall these coordinates to select a particular beam size. Measurements of the motion of the system over time show that it is stable to +/- 0.1 \( \mu m \). High precision screws that are part of the kinematic mount provide angular adjustment of the collimator assembly during initial alignment.

3. ON-AXIS VISUALIZATION

The collimating system follows the OAV (On-Axis Visualization) system. The On-Axis-Visualization system provides a parallax-free image of the direct beam at the sample position. This is accomplished by mounting a front-surface mirror at an angle of 45 degrees to reflect the image of the beam from a YAG crystal [6] at the sample position down to a 16:1 zoom optics and digital color CCD camera. Both the mirror and the objective lens nearest the sample have a 1.2 mm hole to allow passage of the x-ray beam. The on-beam-axis video microscope and YAG crystal are used to visually align the beam to the sample position. The collimator apparatus is located between the lens and the sample. It employs a pinhole coupled with scatter guards. A high degree of beam positional and intensity stability is achieved, in part, because the focused beam overfills the aperture.

4. ALIGNMENT PROTOCOL

Initial alignment of each pinhole to the optical axis of each path through the mini-beam collimator is performed under an optical microscope using an alignment jig. Next, the pre-aligned collimator and its kinematic mount are moved to the beamline and attached to a pair of high precision translation stages mounted on OAV system for viewing the protein crystal under investigation. The collimator is aligned to the beam axis by two angular and two translational motions. The pitch and yaw adjustments are typically only done during initial installation, and therefore are not motorized. The horizontal and vertical positions are adjusted remotely with high precision translational stages. Final alignment of the
collimator is achieved using several endstation components, namely, a YAG crystal at the sample position to visualize the mini-beam, a CCD detector to record an X-ray background image, and a PIN diode to record the mini-beam intensity. The alignment protocol and its opto-mechanical instrumentation design will be discussed in detail.

4.1 Pre-alignment in the laboratory by jig and microscope

The Device for pre-alignment was designed for the collimators. It enables the precise positioning of the shielding guard to the collimator body. Using the two adjustment screws the collimator yaw and pitch can be aligned. The optical axes of the four path channels are adjusted to be parallel and reside in the same plane using the microscope before gluing the pinhole cap. The quad collimator is mounted on XY stages under the OLYMPUS SZX12 microscope. When move the quad collimator to experiment station and mounting on XY stages which on the OAV.

4.2 Alignment of the Quad collimator with X-rays

Figure 5. Left is an alignment jig with kinematic mounting set. Right is a precision-alignment setting under the OLYMPUS SZX12 microscope.

The photograph shows the relationship of the quad collimator positioning and the path of the x-rays. A YAG crystal is mounted on the sample goniometer at the sample position. The photograph also shows the relative position of a highly sensitive photo voltaic diode (PIN diode) along the x-ray path and downstream of the YAG crystal.

Figure 6. Mini-beam Quad collimator mounted in the experimental station of beamline 23ID-D. The photograph shows the relationship of the quad collimator positioning and the path of the x-rays. A YAG crystal is mounted on the sample goniometer at the sample position. The photograph also shows the relative position of a highly sensitive photo voltaic diode (PIN diode) along the x-ray path and downstream of the YAG crystal.
The pre-aligned Quad collimator is positioned on the beamline by another kinematic base mounted on top of the on-axis visualization housing (Fig 1). The alignment of the focused x-ray beam to interact the horizontal rotation axis of the goniometer using a six step procedure is described elsewhere [1]. Alignment of the Quad collimator into the path of the x-ray beam is a multi-step process that involves the use of YAG crystal, PIN diode and a CCD detector.

4.2.1 Optical alignment with YAG

A YAG crystal is mounted on the goniometer at sample position as shown in Fig. 6. Initially the 300 micron pinhole is positioned in front of the hole in the lens of the on-axis visualization with the help of the translational stages. If this initial positioning of the quad collimator is parallel to the x-ray beam then a mere adjustment of the translation stages will result in the scintillation of x-rays on the YAG crystal (Fig. 7a). The scintillation can then be centered on the cross hairs (Fig 7b) by making adjustments to the position of the translation stages by a few microns. The 300 micron pin hole is then precisely centered by noting down the horizontal positions of the “half cut beam” in the right hand side of the cross hair and then the left hand side of the cross hair (Fig. 8). If the difference in the position of the horizontal stage between the two half cut beam positions is 300 microns then the pin hole is precisely centered in the horizontal direction. A similar procedure is followed to precisely center the vertical position of the pin hole with half cut beam above and below the cross hairs (Fig 8). After recording the position of the 300 micron pin hole translational stages are moved to the relative positions of the other pin holes and their scintillations centered on the cross hairs of the on-axis visualization (Fig 4).

Figure 7. Scintillation of the x-ray beam on YAG crystal mounted at goniometer sample position. (a) Scintillation off center (b) Scintillation centered on the cross hairs
4.2.2 Alignment with PIN Diode

Figure 9. The photograph shows the LED display of the voltage measured at the photo voltaic diode (PIN diode) placed downstream of the quad collimator along the path of the x-ray beam. The changing orientation and translation of the collimator results in increased voltage measured.

In general the kinematic base mounted on top of the on-axis visualization housing is not perfectly perpendicular to the x-ray beam, therefore the quad collimator is not parallel to the direction of the x-ray beam. Hence the initial positioning of the 300 micron collimator in front of the hole in the OAV lens does not yield scintillation on the YAG crystal. In certain other cases, the x-ray beam through the 300 micron pin hole is clearly seen on the YAG crystal but the collimator will need adjustment in the pitch and yaw for the other smaller size pin holes. In either situation hardly any x-rays is going through the pin hole as most of the x-rays are being blocked by the collimator block resulting in no scintillation visible on the YAG. This requires the utilization of the highly sensitive photo voltaic diode (PIN diode) and an adjustment in the pitch, yaw and translation of the quad collimator. The relative position of the PIN diode to the collimator and x-ray beam path is shown in Fig 6. Initially the gain of the photo voltaic diode is set very high to measure the relatively small voltage generated on the diode being produced by x-rays (Fig 9). Then the orientation and translation of the collimator are progressively changed resulting in an increase in the voltage recorded. As the orientation of the collimator comes closer to being parallel to the x-ray beam the scintillation on the YAG crystal appears stronger and eventually can be precisely centered on the cross hairs. The pin holes are aligned parallel to the beam and to the center of the cross hairs by an iterative process involving the YAG crystal and PIN diode.

4.2.3 Alignment with CCD detector

Once the pinholes have been centered, background scatter patterns are recorded downstream of the Quad collimator along the path of the x-ray beam on a MARmosaic 300 CCD detector (Now Rayonix) in order to assess the accuracy of the orientation of the collimator. Even minor misalignment of the quad collimator is clearly manifested in the quality of the background scatter images on the CCD detector. Slightly off center pin hole of the quad collimator produces rings and shadows in the background image pattern on the CCD detector as shown in Fig 10 (a) & (b), while a well centered pin hole of the quad collimator produces an image that has the lowest background with no rings or shadows Fig 10c.
Figure 10. Diffraction pattern recorded on the MAR mosaic 300 CCD detector. The shadow stretching from the central left end to the middle of the background pattern is due to a beam stop arm and the beam stop. The beam stop captures the highly intense direct x-ray beam to prevent damage to the detector. Images (a) and (b) are some of the typical background patterns obtained when the pin hole of the collimator is not precisely centered in the x-ray beam or the quad collimator body is not properly aligned along the direction of the x-ray beam. Image (c) is the background pattern of a well centered pin hole and a well aligned collimator. The continually varying background seen on the image is caused due to x-rays scattered by air.

5. SUMMARY

Uni-body Quad collimator system is a simple yet elegant and highly efficient system that automates the process of switching between various sizes X-ray beams with the click of a button. Precise alignment and successful integration of the Quad collimator into the beamline control and data acquisition software at GM/CA-CAT has helped structural biologists to conduct uncompromised experiments on their most important and often rare sample crystals. The number of groups self-reporting the use of Quad collimator is greater than 80% [7]. Our paper describing the mini-beam apparatus was published in early 2009 [1] and has attracted considerable attention. We have assisted in the construction and implementation of the Quad collimator at various beamlines at the APS (IMCA CAT, LRL CAT, sector 3). Several beamlines have placed orders for the construction of the apparatus.

6. ACKNOWLEDGEMENT

GM/CA CAT is funded with Federal funds from the National Cancer Institute (Y1-CO-1020) and the National Institute of General Medical Science (Y1-GM-1104). Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under contract Number DE-AC02-06CH11357.

7. REFERENCES

7. 2010 R&D 100 Award Entry – hard X-ray Quad collimator.