I24 Endstation upgrade – overview and engineering design

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Abstract **-** Beamline I24 at Diamond Light Source is a tuneable microfocus macromolecular crystallography beamline that has been in user operation since 2009. Upcoming upgrades to the beamline will result in a reduction of the beamsize from 8 x 8 µm to 3 x 4 µm. This together with the recent installation of a Pilatus-3 6M capable of data collection at 100 Hz necessitates the need for an upgrade to the current endstation. Furthermore, there is a growing demand from users to reduce the current downtime when switching between *in situ* and cryo-crystallography experiments. A unique two-goniometer design of the new endstation will facilitate this. This paper covers the design of the new endstation, preliminary measurements of the sphere of confusion of the vertical goniometer subassembly and the resolving power of the new on-axis viewing system.

Keywords**:** Diamond Light Source, Macromolecular Crystallography, Endstation, Goniometer, On-axis viewing system, Sphere of confusion

1. Introduction

A macromolecular crystallography beamline is expected to accurately record diffraction intensities from protein or virus crystals provided by the user, with the ultimate aim of determining the atomic structure of the protein or virus under study. Although many aspects of the experiment to obtain such data are considered routine, with many parts of the procedure automated, a significant fraction of samples diffract poorly. Such samples require particular attention regarding the details of the instrument design if useful information is to be obtained. Beamline I24 specializes in the acquisition of data from small and weakly diffracting samples that often result when particularly challenging biological targets are crystallised.

The original beamline design of I24 includes a double demagnification of the X-ray source via two pairs of Kirkpatrick-Baez (KB) mirrors, yielding a 8 x 8 µm beam at the sample and a horizontal goniometer (Evans et al., 2006). Planned improvements to the KB optics will reduce the beam-at-sample size to approximately 3 x 4 µm leaving the existing endstation instrumentation insufficient in terms of precision and repeatability to support this. Additionally the use of a Pilatus3 6M detector on the beamline capable of data collection at up to 100Hz means that the existing endstation is not able to fully exploit the speed of the detector when performing sample scans due to the performance of the positioning stages (Aishima et al., 2010). Furthermore, the existing endstation design requires significant manual intervention to enable the collection of data *in situ* (Axford et al., 2012). These are all motivating factors for the delivery of a new endstation design.

2. Overview of the Endstation

One of the unique features of the new endstation is the incorporation of two goniometers with very little change to the existing space envelope. The new vertical high precision kappa goniometer having a sphere of confusion of less than 1 µm is dedicated to cryo-crystallography experiments using a standard SPINE pin. Figure 1 shows the general assembly of the new endstation.

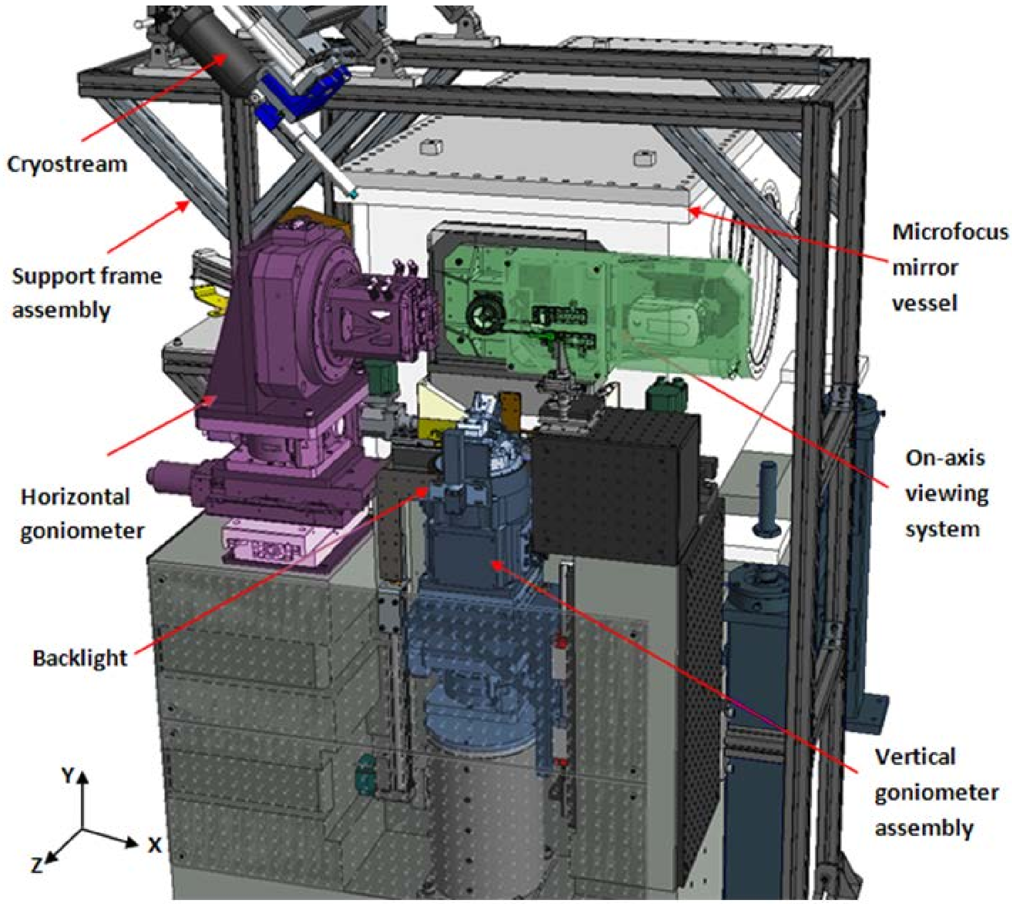

Figure 1 - 3D model of the new endstation showing the horizontal goniometer (purple), vertical goniometer (grey) and the on-axis viewing system (green) assemblies

The redesigned horizontal goniometer assembly is used for *in situ* experiments with crystallization microplates. Automated switching between cryo-crystallography and *in situ* experiments is possible reducing the downtime from 1 hour to less than a minute. The existing on-axis viewing system is replaced by a new compact system with improved sample viewing. It is also capable of laser irradiation of samples for photo-excitation. The existing beamstop is replaced by a micro-machined tungsten beamstop mounted on a high precision multi-axis stage minimising the shadow cast on the detector. To reduce possible problems from vibrations: both goniometers, the on-axis viewing system and microfocus mirrors are mounted on the same granite block to minimise relative motions. The redesigned granite block is grouted to the experimental hall floor.

3. Existing endstation

Figure 2 shows the general assembly of the existing endstation. A large part of the space below the sample position is occupied by the on-axis viewing system. Also, experiments are conducted with the Pilatus detector positioned as close as 195mm from the sample position. The lack of sufficient space to install a new vertical goniometer and the average image quality of the existing on-axis viewing system were the key drivers which influenced the redesign of the on-axis viewing system. In addition, due to the configuration of the existing viewing system, a time consuming procedure had to be followed to manually align the optical axis with the X-ray beam axis in the pitch and yaw directions.

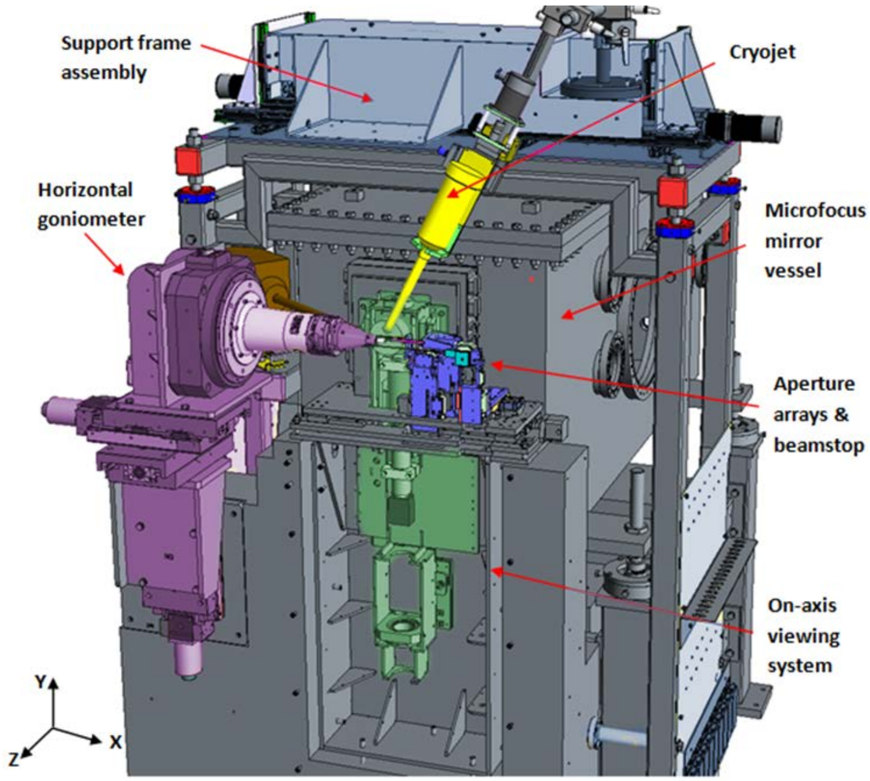

Figure 2 - 3D model of the existing endstation showing the horizontal goniometer (purple) and the on-axis viewing system (green) assemblies

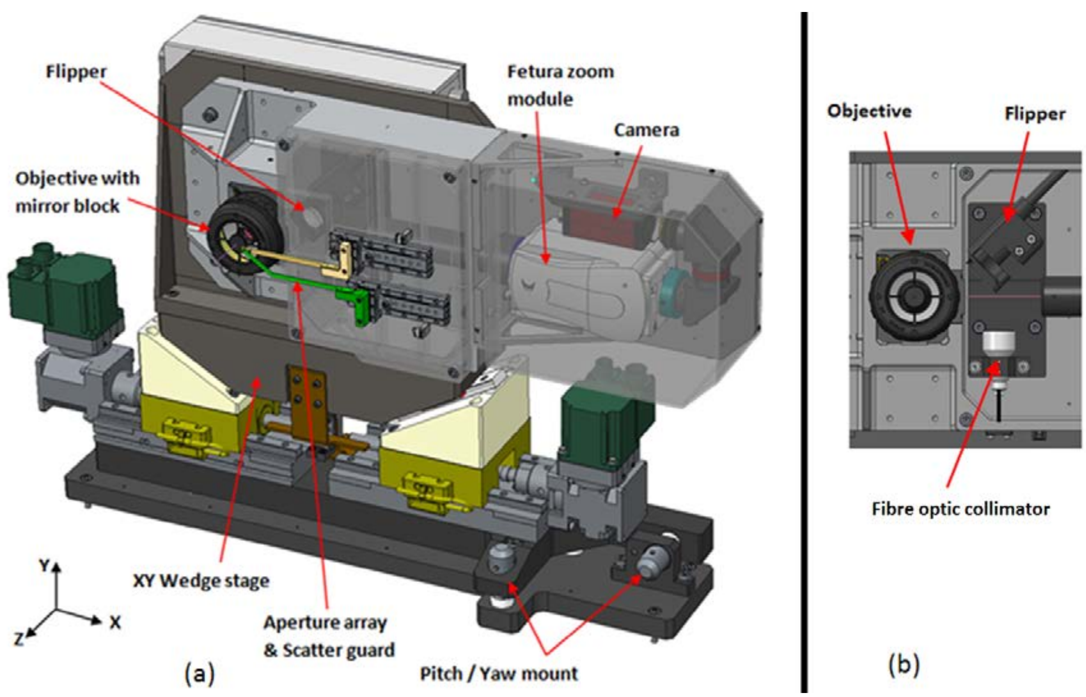

Figure 3 (a) 3D model of the new on-axis viewing system (b) Partial view showing the flipper and collimator

4. On-axis viewing system

The design of the new on-axis viewing system is shown in Figure 3**.** The objective lens assembly is mounted on an aluminium alloy block which has a mirror mounted at 45˚. Both the objective and the 45˚ mirror have a 1.6mm diameter hole which allows the X-ray beam to pass through. The Fetura programmable zoom lens system coupled with a relay lens is aligned to the 45˚ mirror. The image is received by a GigE camera attached to the Fetura zoom module through two 45˚ mirror blocks. A Newport 8893KM motorised flipper with a mirror is mounted at a 45˚ angle between the objective mirror block and the relay lens as shown in Figure 3(b). A Thorlabs F810SMA-543 fibre optic collimator mounted at 45˚ to the flipper is coupled to a laser source which can be used to illuminate samples.

4.1 Viewing system optical resolution tests

The new on-axis viewing system was initially tested with the existing objective, a custom design by Davin Optronics, using a USAF 1951 high resolution target (Web-1) mounted on a tip-tilt mirror mount and fixed onto a XYZ micro-positioning stage. The target was backlit with a CCS LFL-3212-SW2 flat panel diffused LED light. Images were captured for a zoom range between 1 and 12.5. The tests were repeated with a Newport 15x reflective microscope objective and a Mitutoyo 10x Plan APO objective lens.

4.2 Test results

Figure 4 shows the images taken by the Davin, Newport and Mitutoyo objectives. Of the three objectives under test, the Davin produced the lowest quality image with a non-parfocal image requiring some refocusing between zoom levels and some variation in focus across the image. 1 µm is just resolvable with this objective. The image quality with the Newport objective is improved over the Davin it is closer to parfocal and the focus is consistent across the image. It is able to resolve target lines of 0.7 µm however the working distance of 24mm, compared to 35.6mm of the Davin, makes *in situ* experiments extremely challenging. The Davin objective is therefore preferred. The image quality with the Mitutoyo objective is the best of the three. It appears perfectly parfocal with a flat focus and a resolution beyond 0.7 µm. However, this lens does not have a hole for the X-ray beam to pass through and it is physically longer than the other two lenses. Currently an objective with identical specifications to the Mitutoyo is being sourced from Qioptiq with a 1.6mm diameter hole in the centre. Minor modifications to the mirror housing and vertical goniometer mount will accommodate this objective and allow switching between the three lenses if required.

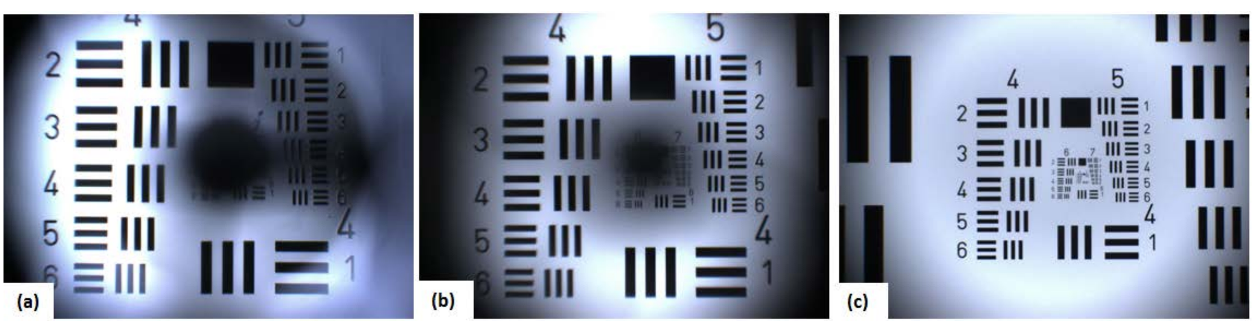

Figure 4 - Image at x1 zoom (a) Davin objective (b) Newport objective (c) Mitutoyo objective (width of line in Group 4 Element 2 is 27.86µm)

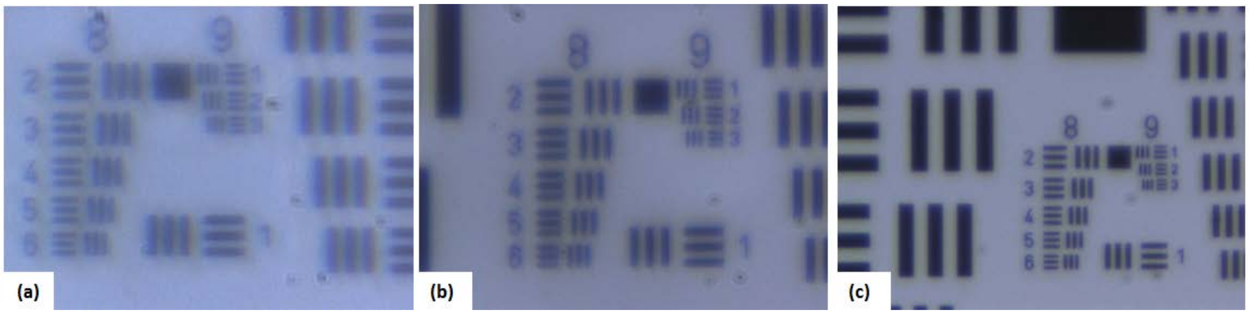

Figure 5 - Image at x12.5 zoom (a) Davin objective (b) Newport objective (c) Mitutoyo objective (width of line in Group 9 Element 3 is 0.78µm)

5. Vertical Goniometer assembly

One of the main requirements of the vertical goniometer was to achieve a sphere of confusion of less than one micron. In addition, the entire subassembly had to be retracted to provide a clear space for screening crystallization plates using the horizontal goniometer assembly. Hence, it was vital to ensure the entire setup was supported on a positioning stage with a very high stiffness and sufficient travel range.

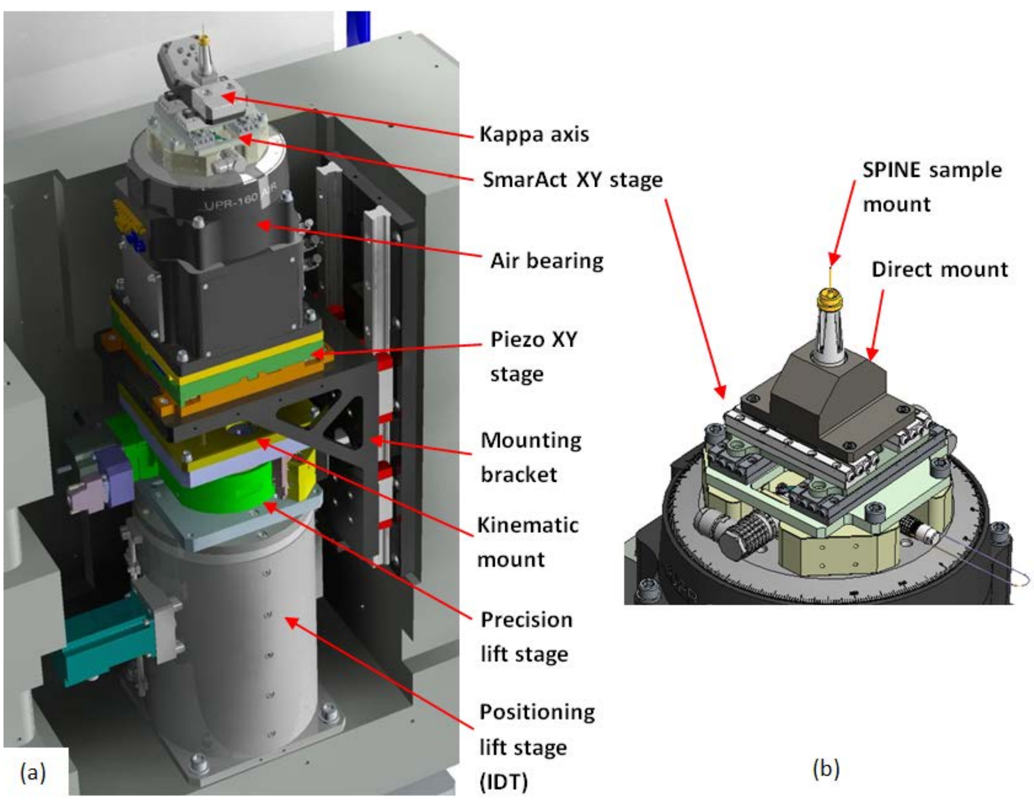

Figure 6(a) Vertical goniometer assembly with kappa axis (b) Air bearing with a fixed sample mount on the centering stage

A linear precision lift stage supplied by Instrument Design Technology (IDT) was selected since it had a high stiffness 450 nm/N in the lift or vertical direction however, the lateral stiffness of the stage was comparatively poor. In order to address this, the goniometer setup is supported on a bracket coupled to a set of preloaded high precision rails supplied by Schneeberger AG to overcome the lack of lateral stiffness in the IDT lift stage. An additional high precision lift stage supplied by Huber GmbH mounted on the IDT lift stage is used to manipulate the sample in the vertical direction. The set of lift stages is coupled to the goniometer mounting bracket via a kinematic mount. The entire setup is mounted on a synthetic granite base.

The main rotation axis is a PI Micos UPR-160 air bearing mounted on a custom design piezo XY stage supplied by PM Bearings to allow alignment of the rotation axis with the beam. Sample centering is achieved through a set of SLC-2475 and SLC-2490 piezo stages supplied by SmarAct GmbH. The kappa axis is a SmarAct SR-4513-S rotary stage. The phi axis is SmarAct SR-2013-S rotary stage.

5.1 Sphere of confusion measurements via an optical microscope

Preliminary measurements of the sphere of confusion of the vertical goniometer were performed using an optical viewing system comprising; Mitutoyo objective, an Infinity lens tube and Allied Vision Mako CCD camera all mounted on an XYZ micro-positioning stage. These initial tests were performed in a room with no temperature control. The vertical goniometer stack consisted of the PI Micos air bearing with the SmarAct centering stages. The air bearing was mounted on the PM bearing XY piezo stage through a bracket which houses a slipring. This was used to view an ultra-sharp tungsten pin mounted as a SPINE sample on the vertical goniometer stack. The pin was rotated in 10 degree increments and slices were taken from the image at the tip of the pin. A Gaussian function was fitted to each line-profile using the software package DAWN (Figure 7) and the fitted centre was used to record the deviation of the tip during rotation.

5.2 Test results

The sphere of confusion measured via this method was ~800nm peak-to-peak. This represents a significant improvement over the previous horizontal goniometer which displayed as much as 6 μm deviation peak-to-peak measured via the same method using an identical tungsten pin (Figure 8).

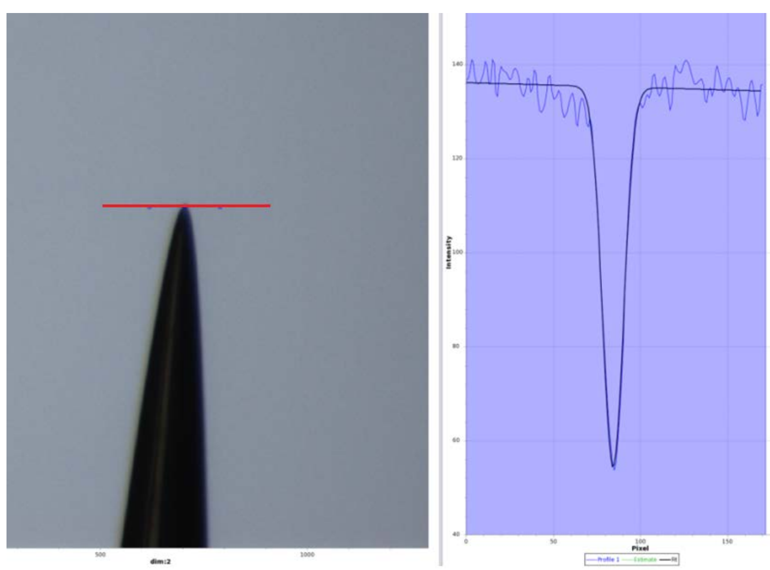

Figure 7 – Image of pin (image left); Gaussian function fitted to profile (image right)

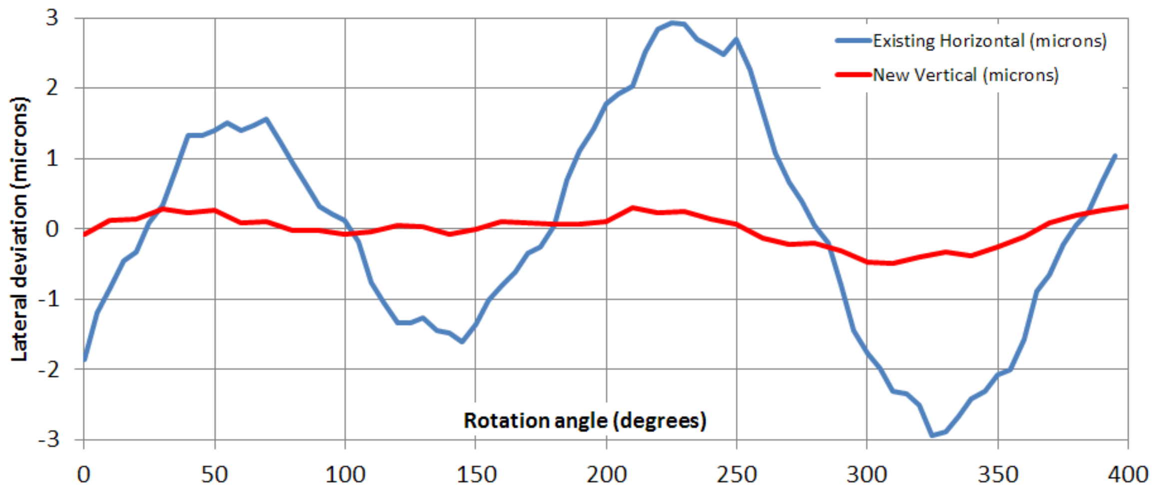

Figure 8 – Sphere of confusion: New vertical UPR-160 vs the existing horizontal ABRS-250MP goniometers.

6. Conclusion

The new I24 beamline endstation upgrade project evolves the beamline to the changing needs of the user community and has addressed a number of key limitations found in the existing endstation. Construction is nearing completion and full commissioning will commence soon prior to a resumption of user operation. Initial tests have already confirmed a significant improvement in both the goniometer sphere of confusion and the viewing system optical resolution. A test program has begun to accurately measure the sphere of confusion of the vertical goniometer using a Keyence high accuracy optical micrometer. It is anticipated that the dual goniometer set-up with new fast coordinated motion will greatly improve the beamline's ability to handle some of the most challenging cases in the field of macro-molecular crystallography.

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