Published in final edited form as:

Synchrotron Radiat News. 2018 ; 31(5): 3–8. doi:10.1080/08940886.2018.1506233.

Microscopy Instrumentation and Nanopositioning at NSLS-II: Current Status and Future Directions

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The development of ultra-brilliant synchrotron facilities and recent advances in the fabrication of efficient nano-focusing optics have stimulated rapid development and applications of the focused beams used to elucidate nanoscale phenomena in many areas of science and technology [1–5]. Various nano-focusing optics, including Kirkpatrick-Baez (KB) and elliptical mirrors, Fresnel zone plates (ZP), compound refractive and kinoform lenses, and multilayer Laue lenses (MLL), are routinely used to achieve nano-focusing. The latest experiments utilizing KB mirrors have demonstrated focal spot size of 13 nm [6] with the ZPs and MLLs being able to focus hard X-rays to 10 nm and below [6–9]. When the focused beam size is reduced to below 100 nm, there is a need to develop a dedicated system tailored for a specific application, which satisfies stringent mechanical, vibrational, and thermal characteristics to successfully enable nano-scale imaging. Multiple degrees of motion, including their positioning accuracy, resonance frequencies, and amount of heat being dissipated, have to be thoroughly considered in order to efficiently perform nano-focusing experiments.

Within the Imaging and Microscopy program at the National Synchrotron Light Source II (NSLS-II), a Department of Energy (DOE) Office of Science user facility located at Brookhaven National Laboratory, scanning and full-field imaging with direct spatial resolution down to 10 nm are available for the general user community. Most of the microscopes are developed in-house through a joint effort between the beamlines staff, the engineering group, and the nano-positioning team aimed to assist in design and construction of the cutting-edge microscopy instrumentation. Developed systems utilize state-of-the-art nano-positioning solutions with some of them being adopted beyond the Imaging and Microscopy program at NSLS-II. The few examples presented here represent microscopy and nano-positioning systems developed at NSLS-II in recent years.

MLL- and ZP-based scanning microscope at the Hard X-ray Nanoprobe (HXN) beamline

MLL optics were proposed as a new and efficient approach to achieve nano-focusing of hard X-rays and avoid the well-known “aspect ratio” problem of the ZPs, limiting their efficiency in the hard X-ray regime [10]. An individual MLL optic can be considered as a liner ZP, which requires angular adjustment to satisfy the Bragg condition and provide line focus. A pair of MLLs positioned orthogonally with respect to each other is required to achieve a point focus. In total, eight degrees of motion (five translational and three rotational) are

needed to perform a full alignment (Figure 1a). The typical focal distance of an MLL optic at 12 keV photon energy, 10 nm focus, and an aperture of 50 μm does not exceed 5 mm. The distance from an Order Sorting Aperture (OSA) to a sample is typically on the order of 1 mm or less. The orthogonality of two lenses plays a critical role and affects the ultimate focusing performance. Angular misalignment introduces an additional cross-phase term in the wave front causing blurring of the focus [11]. For a 10-nm-point focus, the orthogonality between two lenses should be better than 0.01° . For tomography experiments, a complicated sample stack needs to be positioned in close proximity to the OSA. When all motions are considered, an MLL microscope developed for 10 nm imaging resolution requires 20 or more linear and angular motions, with many of them providing single-nm precision.

In contrast to the MLL microscope and the technical challenges associated with its alignment, a ZP microscope is less demanding in terms of motions and working distances (Figure 1b). The working distance is greater than 5 mm (for a ZP with 30 nm outermost zone width and 10 keV photon energy). Based on the differences in alignment requirements and working distances, we have designed and constructed two independent modules (MLL and ZP) residing within the same vacuum chamber. Two modules are staggered and can be brought into the X-ray beam, depending on the application. The MLL module is used for ~ 10 nm spatial resolution routine imaging experiments and the ZP module is used for high-throughput ~ 30 nm and above resolution imaging.

Figures 2a–2c show the HXN microscope with two modules installed inside the vacuum chamber. The microscope allows users to perform scanning fluorescence (both 2D and 3D), differential phase and absorption contrast, ptychography and diffraction measurements. Both MLL and ZP modules rely on the piezo-driven actuators (Attocube, Smaract, PI, etc.) and utilize fiber optic interferometry for characterization and position read-back. Piezo actuators were selected due to their compact form factors, high-resonance frequencies, and reduced heat dissipation when compared to stepper, DC, or servo motors.

Prior to final design and construction, a number of prototype instruments have been built and evaluated in terms of resolution, stability, and drifts [12–16]. Critical microscope components were characterized in the nano-positioning laboratory of NSLS-II. Figure 2d shows the MLL module installed inside the vacuum chamber. Figures 3a and 3b demonstrate the sub-nm steps performed with a sample scanner of the MLL module and the corresponding resonance frequencies of the sample stack. Fundamental resonance frequencies well above 100 Hz of a fully loaded sample module efficiently diminish the influence of the background vibrations on the MLL module performance. Figure 3c demonstrates fluorescence images of a test pattern acquired at the HXN beamline at 12 keV photon energy [17–19]. The diameter of lithographically deposited platinum (Pt) circles is 80 nm and the line width is 20 nm. Figure 2e represents the ZP module with all key components labeled. Piezo-driven stick-slip actuators are utilized for both sample and ZP manipulations. Up to four different ZPs can be installed in the ZP carriage and positioned along the beam, depending on the photon energy and the ZP working distance. A parallel kinematic device on top of a rotary stage allows fine angular adjustments to be performed for both tomography and diffraction measurements. The MLL/ZP scanning microscope was commissioned in 2015 and is available to the general user community.

Transmission X-ray microscope at the Full-Field X-ray Imaging beamline

Precise knowledge of structural information plays a critical role in many areas of materials science and engineering. An ability to quantitatively image complex structural dynamics in 3D using non-distractive methods is important for *in-situ* studies and characterization. Full-field transmission X-ray microscopy (TXM) is a powerful tool well suited for fast 3D tomographic imaging. A number of TXM microscopes have been built and commissioned in recent years [20, 21]. At NSLS-II, a new TXM system has been designed, constructed, and recently commissioned at the Full-Field X-ray Imaging (FXI) beamline. A schematic of the microscope is shown in Figure 4a. Panels 4b and 4c demonstrate the actual microscope installed in the experimental hutch. As in the case of the HXN instrument, piezo-based actuators were employed for manipulation of the critical components; i.e., capillary condenser, ZP, pinhole, and the sample. The capillary condenser is positioned by a combination of linear and angular motors allowing adjustments with respect to the incoming beam. The sample stage is comprised of an XYZ piezo actuator residing on top of an air-bearing rotary stage. Samples of up to 0.5 kg can be loaded onto a kinematic sample mount. The unique feature of the sample stage pertains to the presence of a slip-ring as an integral part of the rotary stage. The integrated slip-ring allows for infinite rotation (fly-scanning data acquisition) and, at the same time, does not compromise quality of rotation (both synchronous and asynchronous rotational errors at the sample location do not exceed 120 nm). During fly-scanning data acquisition, complete tomograms can be acquired in one minute. The 30 nm outermost-zone-width ZP optic is manipulated by a set of XYZ stick-slip piezo-actuators.

Figures 4d and 4e show 3D reconstruction and a zoomed-in view of a tomogram of a Fe-Cu alloy. The pixel resolution for the measurement equals 30 nm. By upgrading the ZP optic, higher spatial resolution measurements will be attainable.

Ultra-high-speed piezo-based goniometer system for raster scanning serial micro-crystallography

Macromolecular crystallography is a powerful technique employing X-ray diffraction to obtain 3D structures of biological macromolecules at atomic resolution. The storage ring evolution to ever lower emittances has enabled the construction of microfocus beamlines [25, 26] to obtain structures from ever smaller crystals [27], typically encountered in the case of difficult to crystallize but key structure targets such as membrane proteins. The Frontier Microfocusing Macromolecular Crystallography (FMX) beamline [28, 29] exploits the low emittance of the NSLS-II storage ring to deliver a beam with a variable focus down to 1×1.5 (V×H) μm^2 and with a flux of 3×10^{12} ph/s at a photon energy of 12.7 keV. For a macromolecular crystallography (MX) experiment, this translates into a dose rate that surpasses microfocus MX beamlines worldwide by up to two orders of magnitude. The associated crystal lifetimes on the order of a few tens of ms, together with the micron beam size, place demanding boundary conditions on the goniometer systems used to position and orient the crystals in the beam.

A key application combining the advantages of high dose rate and small beam size is serial crystallography. By collecting and merging partial datasets from hundreds to thousands of microcrystals, structures can be obtained from tiny crystals that were previously considered too small for data collection. In a raster scanning serial crystallography approach [30, 31], new crystals are brought into the beam on a loop or thin membrane carrying the crystals. Raster scanning at the full flux of the FMX beamline requires scanning speeds of tens of linepairs/s over scanning ranges of hundreds of microns. Classical MX goniometer systems lack both the speed and the stiffness to perform at such specifications. In addition, typical sample holder sizes in MX experiments require travel ranges in the mm range, too large for single piezo positioners. The new FastForward goniometer system at FMX (Figure 5) combines an XYZ piezo cube (customized PI NanoCube) for fine positioning with a 200 μm travel range with a pair of piezo walk stages (PI NEXLINE) with a 4 mm range for coarse alignment (Figure 5b). To fit the tight confines of the FMX experimental station (Figure 5a), the piezo cube is tilted by 45° with respect to the laboratory coordinate system.

In contrast to a typical nano-positioning scanning setup in imaging applications, an MX experiment uses an infinite rotation axis, thus all electrical contacts have to be guided through a slip ring connection in the air-bearing rotation table (Nelson Air SP150). To minimize the effect of noise introduced to the encoder signals of the piezo system, the analog encoder signals are digitized on the sample side of the slip ring, with a resulting system repeatability of 25 nm for the fine and 100 nm for the coarse stages.

The runout of the complete goniometer system upon a full rotation is 1 μm peak to peak, with an asynchronous error of 150 nm. By using a calibration correction, we plan to reduce the goniometer runout to the size of the asynchronous error in a future controls system update.

Recent experiments at FMX established the beamline's ability to greatly reduce data collection times for raster scanning serial crystallography [32]. Complete datasets of $\sim 5\text{-}\mu\text{m}$ -sized proteinase K crystals were collected using the full flux of the beamline, and at detector frame rates from 100 to 750 Hz, with equally high data quality for each detector speed. The total shutter-open time at 750 Hz was under 20 s for the complete dataset. By employing nano-positioning, techniques originally employed in the imaging field, the new goniometer system enables data collection times for raster scanning serial crystallography in the sub-minutes time scale and at a millisecond time resolution. With its generic design, the system is also appealing for other X-ray methods that require fast rotations; for example, fluorescence computed tomography applications.

Outlook

At NSLS-II, the microscopy instrumentation and nano-positioning program aims to develop state-of-the-art instrumentation to support a broad range of scientific studies. For MLL nano-focusing optics, a concept of bonding two individual lenses together, yielding a monolithic 2D focusing element, has been explored [22, 23]. A novel Micro Electro Mechanical Systems (MEMS) approach has been considered and applied for alignment and manipulation of individual lenses. Various piezo-driven, interferometrically controlled

actuators have been developed to enhance performance of the next-generation MLL microscopes [24]. For future applications, the synergy between instrumentation available in the X-ray, electron, and scanning probe microscopy communities is envisioned to strengthen and benefit each of the scientific fields.

Acknowledgments

Funding

This work was performed using resources of the National Synchrotron Light Source II, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Brookhaven National Laboratory under Contract No. DE-SC0012704. The Life Science Biomedical Technology Research resource is primarily supported by the National Institute of Health, National Institute of General Medical Sciences (NIGMS) through a Biomedical Technology Research Resource P41 grant (P41GM111244), and by the DOE Office of Biological and Environmental Research (KP1605010).

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Figure 1:

Two types of nano-focusing optics implemented in the HXN microscope: (a) MLLs; (b) ZP optics. For MLLs, two linear optics have to be aligned orthogonally with respect to each other to achieve point focusing. The degrees of motion required to perform optics alignment and manipulation are shown.

Figure 2:

(a) Photograph of the MLL-based microscope installed in the experimental hutch of the HXN beamline; 1–granite support and the microscope vacuum chamber; 2–granite support and the fluorescence detector; (b) CAD model of the MLL/ZP modules installed inside the vacuum chamber; (c) inside view of the vacuum chamber from the downstream side, where MLL and ZP modules are staggered; (d) photograph of the MLL module; 1–the vertical MLL assembly; 2–horizontal MLL assembly; 3–OSA motion stack; 4–sample stage; 5–fiber optics interferometers; (e) photograph of the ZP module; 1–sample stage; 2–ZP carriage; 3–OSA assembly; 4–fiber optic interferometers.

Figure 3:

(a) 0.5 nm steps recorded by the interferometer during evaluation of the MLL module in the nano-positioning laboratory; solid line represents the trajectory of motion; (b) FFT spectrum of the sample stage in the y-direction; measurements were taken at the HXN beamline after the installation of the MLL module inside the vacuum chamber; fundamental resonance frequencies are well above 100 Hz; (c) XRF image of a Pt test pattern (L-edge, 12 keV photon energy, exposure time 0.2 s, 5 nm per pixel); diameter of the rings is 80 nm, line width 20 nm, and the height is 200 nm.

Figure 4:

(a) Schematic of TXM; (b) photograph of the TXM system installed in the experimental hut; 1–TXM microscope; 2–detector setup; (c) photograph of the TXM sample area; 1–sample stage assembly; 2–zone plate assembly; 3–pinhole support; 4–capillary condenser manipulator; (d) 3D reconstruction of an iron-copper (Fe-Cu) sample, pixel size 30 nm; (e) closer look at one of the tomograms.

Figure 5:

(a) The FastForward MX goniometer system installed in the FMX experimental station. (b) Schematic of the piezo scanner. P1, P2, and P3 are the motion directions of three shear piezo actuators of the NanoCube scanner. Yc and Zc are the motions of the NEXLINE coarse stages. Both fine and coarse stages are mounted on top of an air-bearing rotational stage, Ω . (c) Scanning trajectory measured in the x-direction at 40 Hz scanning frequency (blue solid line). The red dashed line demonstrates the selected trajectory. (d) Raster scan of 5- to 10- μm -sized proteinase K crystals loaded on a MiTeGen loop. The raster scan was performed as a fly-scan with 2 μm translation and 2.5 ms exposure time per frame (i.e., scanning frequency of 10 linepairs/s). The heatmap encodes the diffraction pattern spot count. The scan area is shown as a red-dashed rectangle in the inset.