1058
doi:10.1017/S1431927613007289
Microsc. Microanal. 19 (Suppl 2), 2013

Integrated Nonlinear Optical Microscope for Crystal Centering on a Synchrotron X-ray Beamline

Justin A. Newman[1], Scott J. Toth[1], Christopher M. Dettmar[1], Michael Becker[2], Robert F. Fischetti[2], and Garth J. Simpson[1]

1. Purdue University, Department of Chemistry, West Lafayette, IN, USA
2. GM/CA@APS, Advanced Photon Source, Argonne National Laboratory, Argonne, IL, USA

Synchrotron X-ray sources have become an invaluable tool for protein structure determination. Due to the high photon flux, energy tunability, and increasing drive towards tighter focusing, high resolution structures of proteins have been solved from increasingly smaller protein crystals and even subdomains within a single crystal using synchrotron X-ray beams[1]. However, to achieve these high resolution diffraction patterns, the crystal needs to be reliably centered in the X-ray beam. As the demand for high throughput structure determination grows, so too does the need for a rapid and reliable crystal centering method.

There are a variety of centering techniques commonly in use on synchrotron beamlines, including bright field image analysis and ultraviolet fluorescence (UVF) microscopy[2, 3], however as the field moves toward solving structures from smaller crystals, the detection limit of these techniques is being surpassed. Currently, the most reliable method for crystal centering involves performing a raster of the sample with the X-ray beam. From the resulting raster image the crystal is positioned so that the location in the raster image displaying the strongest Bragg-like diffraction is exposed to the X-ray beam[4]. However, this method can be time consuming (>2 s per raster cell) and can cause damage to the crystal from prolonged exposure and subsequent absorption of X-ray photons[5].

Recently, nonlinear optical (NLO) microscopy techniques, such as second harmonic generation (SHG) and two-photon excited ultraviolet fluorescence (TPE-UVF) have emerged as methods for rapid and non-destructive protein crystal detection[6, 7]. SHG, the frequency doubling of light, is a process selective to non-centrosymmetric crystal classes and is forbidden for highly symmetric crystals and disordered media. Due to the inherent chirality of proteins, an estimated 84% of protein crystals are able to be detected with existing SHG microscopy instrumentation[8]. TPE-UVF is a complimentary method to SHG that relies on the inherent fluorescence of aromatic amino acids within proteins, with no dependence on crystallinity. TPE-UVF is particularly useful in distinguishing protein crystals from SHG active salts that can form from the protein crystallization solutions, as well as in cases where the symmetry of a protein crystal generates no (or weak) SHG signal.

A NLO microscope was designed with both SHG and TPE-UVF imaging capabilities and was integrated with an existing synchrotron X-ray diffraction (XRD) beamline. A variety of samples were investigated including phenylalanine hydroxylase from *Chromobacterium violaceum* (cPAH), *Trichinella spiralis* deubiquitinating enzyme (TsUCH37), human κ-opioid receptor complex (kOR-T4L) grown in lipidic cubic phase (LCP), intimin grown in LCP, and α-cellulose. Good agreement was observed between the NLO images and the XRD raster images including the crystals grown in LCP. The turbid LCP media can make centering by bright field imaging nearly impossible. However, SHG images of intimin grown in LCP were able to accurately locate the protein crystal in the loop with excellent agreement with the XRD raster image (Figure 1).

NLO microscopy was found to be a fast and reliable method for centering protein crystals on the synchrotron beamline. Good agreement was achieved from both the NLO images and the XRD raster scans. The NLO instrument was able to obtain higher resolution images (2 μm spatial resolution) with faster image acquisition times (<10 s) compared to XRD raster scans (5 μm spatial resolution and 3-60 min image acquisition times). Once fully developed, NLO microscopy may serve as the primary method for crystal centering, thus significantly increasing the throughput of synchrotron beamlines and eliminating damage caused by exposure to X-rays during the raster scan[9].

References:

[1] Moukhametzianov, R., et al., Acta Crystallogr. Sect. D: Biol. Crystallogr., **64** (2008), p. 158-166.
[2] Jain, A. and V. Stojanoff, J. Synchrotron Rad., **14** (2007), p. 355-360.
[3] Vernede, X., et al., Acta Crystallogr. Sect. D: Biol. Crystallogr., **62** (2006), p. 253-261.
[4] Song, J., et al., J. Synchrotron Rad., **14** (2007), p. 191-195.
[5] Aishima, J., et al., Acta Crystallogr. Sect. D: Biol. Crystallogr., **66** (2010), p. 1032-1035.
[6] Kissick, D.J., et al., Analytical Chemistry, **82** (2010), p. 491-497.
[7] Madden, J.T., et al., Acta Crystallogr. Sect. D: Biol. Crystallogr., **67** (2011), p. 839-846.
[8] Haupert, L.M., et al., Acta Crystallogr. Sect. D: Biol. Crystallogr., **68** (2012), p. 1513-1521.
[9] Instrumentation development was supported in part by the Center for Direct Catalytic Conversion of Biomass to Biofuels (C3Bio), an Energy Frontier Research Center funded by the US Department of Energy, Office of Science, Office of Basic Energy Sciences, Award No. DE-SC0000997, and by the NIH-NIGMS through the R01GM-103401. GM/CA @ APS has been funded in whole or in part with federal funds from the National Cancer Institute (Y1-CO-1020) and the National Institute of General Medical Sciences (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 No. DE-AC02-06CH11357.

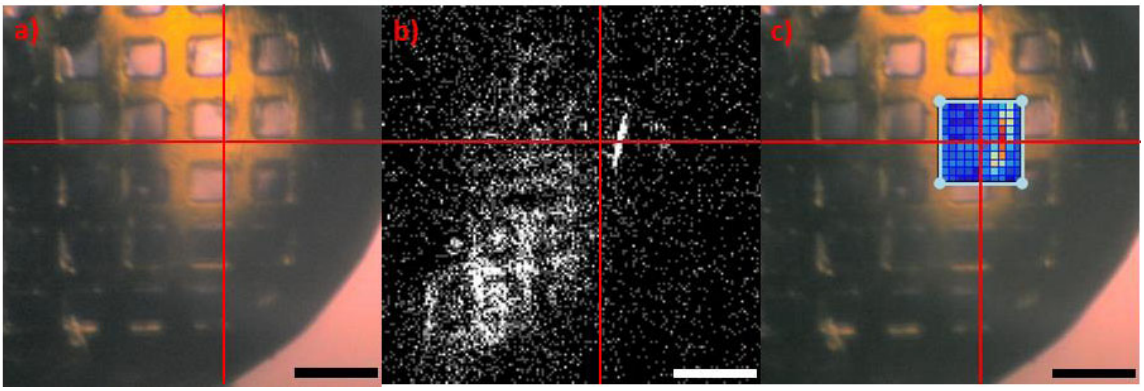

Figure 1. a) Bright field for an intimin protein crystal grown in LCP with corresponding **b)** trans-SHG, and **c)** X-ray raster summary overlay showing corrected Bragg-like reflection counts. Scale bars are 50 μm. Crosshairs were added to **(a)** and **(b)** to assist in orienting the field of view with respect to the diffraction raster images.

https://doi.org/10.1017/S1431927613007289 Published online by Cambridge University Press