Automated crystal shaping to facilitate native SAD phasing.

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Deep UV laser ablation is a technique to improve diffraction data quality from cryocooled macromolecular crystals by removing non-crystalline portion of the sample in the cryoloop or fabricating the crystal into symmetrical shape such as sphere [1]. It has been shown particularly effective for native single-wavelength anomalous dispersion (SAD) phasing in macromolecular crystallography (MX), where very accurate data is required to detect week anomalous signals from light atoms [2]. The systematic errors due to the beam absorption by the sample itself are reduced which is problematic in performing diffraction experiment using long wavelength X-ray. The application is straightforward, however, time consuming with human intervention, and even difficult in case the crystal is hard to identify by visual inspection, such as membrane protein crystals in lipidic cubic phase.

We are developing an unattended system to shape large number of samples based on the 3D map constructed by visual images or by X-ray raster images. In the case of using X-ray raster images, the map is automatically created from the raster images collected at several orientations at a synchrotron MX beamline in a standard, unattended manner. The map is then transferred to the laser shaping machine, allowing to visualize the envelope of the crystal on the mounted, ready-to-shape sample. By specifying the type of processing, e.g., 'removing non-crystalline region' or 'fabricating into inscribed sphere', the system automatically defines the orbit of the laser and repeats processing until the sample converges to the target shape. It is quite useful in preparing large number of shaped samples, typically in native SAD phasing experiment where high-redundant diffraction data is required.

[1] Kitano H. et al. (2005). *Jpn. J. Appl. Phys.* **44**, No. 2, 54-56.

[2] Basu S. et al. (2019). *IUCrJ*, **6,** 373-386.

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