s1.m2.o3 **Detection of Protein Crystals Frozen in Loop-Shape Holders.** Victor S. Lamzin & Sudhir Babu Pothineni, *European Molecular Biology Laboratory, c/o DESY, Notkestrasse 85, 22603 Hamburg, Germany*. *E-mail: victor@embl-hamburg.de*

**Keywords: Protein Crystallography; Crystal Centring; Image Analysis**

With an increasing use of modern robotics equipment, macromolecular crystallography is now aiming at a full-fledged software and hardware pipeline from protein samples to their atomic models. The fully automated data collection at synchrotron beamline is a key component of such a pipeline. Since a crystal, typically flash-frozen in a loop, is mounted onto a beamline goniostat, one of the important tasks is to automatically centre the crystal with respect to the beam. This has already been addressed by, e.g. Karain *et al.* (2002) and Philippe *et al.*, (2004) with the use of optical and X-ray (scattering and fluorescence) techniques, edge detection as well as sophisticated image processing. In both approaches the loop holding a crystal is first looked for. Here we present a computational approach for automatic detection of a crystal in a loop using image-processing techniques with an emphasis on the use of algorithms related to crystal structure determination. No prior knowledge about the size, location and orientation of the loop or the crystal is needed. The centre of the crystal can be accurately detected with either visible or ultra violet illuminating light and with the presence of background noise. Two complementary algorithms have been designed: MoRCI (Molecular Replacement in Crystal Imaging) and FuCHi (Fuzzy Categorisation of image Histogram). The first algorithm resembles a 2-dimensional analogue of molecular replacement in crystallography. In the current implementation a circular object (rolling disk) with variable radius is matched to the image so that the rotational component vanishes. The translation function becomes equivalent to an evaluation of a local variance that is efficiently implemented in reciprocal space (e.g. Terwilliger, 1999). The latter algorithm uses the fuzziness of the image gray levels that is achieved through an activation function. For images that contain light reflections and occlusions it has a higher performance. Application of the detection algorithms for crystals with different morphology will be discussed and the implementation of the software for high-throughput data collection will be presented.

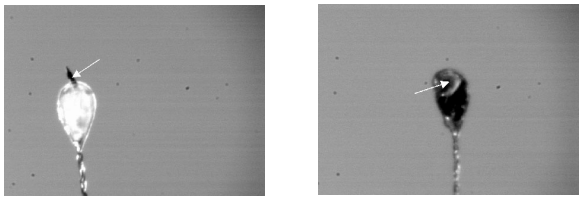

Figure: Examples of crystal detection in the loops

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[3] Terwilliger, T.C. (1999) $\sigma^2_R$, a reciprocal-space measure of the quality of macromolecular electron-density maps. *Acta Cryst.* **D55**, 1174-1178.

s1.m2.o4 **Searching for Polymorphs: a Novel Automated Parallel Crystallisation and X-Ray Powder Diffraction System.** Andrea Johnston,[a] Alastair Florence,[a] Norman Shankland,[b] and Sarah L. Price.[c] [a]*University of Strathclyde, Department of Pharmaceutical Sciences, 27 Taylor Street, Glasgow G4 0NR, UK;* [b]*CrystallografX Ltd., Queen Street, Glasgow, UK;* [c]*Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ, UK. E-mail: andrea.johnston@strath.ac.uk*

**Keywords: Parallel Crystallisation; Polymorph Screening; X-Ray Powder Diffraction**

A combined robotic parallel crystallisation and X-ray powder diffraction analysis system has been developed to allow systematic polymorph searching utilising a wide range of crystallisation conditions. The screening facility is part of a multi-centred collaborative programme entitled "Control and Prediction of the Organic Solid-State" funded under the auspices of the UK Research Councils Basic Technology Programme. This mid-throughput (*ca*. 25 samples per day) is built around a crystallisation platform which provides accurate control of several parameters critical to the crystallisation of organic solids from solution, namely: solvent identity; temperature (isothermal and controlled cooling within the 150 to -10°C); agitation (0 - 1400 rpm) and rate of evaporation. Furthermore, the degree of supersaturation in each crystallisation can be controlled through the automated dispensing of liquid and solid into each crystallisation vessel. Following the preparation of solutions, an in-line filtration process removes excess solid and prevents seeding of the final crystallisation solution. Recrystallisation is then controlled either by solvent evaporation, controlled cooling or the addition of anti-solvents. The recrystallised samples are typically polycrystalline and are readily evaluated using a multi-sample X-ray powder diffractometer equipped with foil transmission geometry, primary monochromated CuKα1 radiation and a linear PSD [1]. Data showed good angular resolution (*FWHM* as small as *ca*. 0.06°) and lattice parameters were easily obtained using the indexing program DICVOL-91. This instrument is therefore highly effective where there is a requirement to analyse 20 – 30 recrystallised samples per day, with an emphasis on obtaining the high-quality data that are important in pattern recognition and imperative in indexing. The application of this approach to polymorph screening will be demonstrated with examples including carbamazepine and theophylline.

[1] Alastair J. Florence, Bruno Baumgartner, Chris Weston, Norman Shankland, Alan R. Kennedy, Kenneth Shankland, and William I. F. David, *Int. J. Pharm.*, **92**, 1930, 2003.