

Automated crystal mounting and processing through laser photoablation. New opportunities for integrated macromolecular crystallography pipelines.

Jose. A. Marquez

*European Molecular Biology Laboratory, Grenoble Outstation,
marquez@embl.fr*

The introduction of fast pixel array detectors is opening new opportunities and increasing the capacity of modern synchrotron facilities for macromolecular crystallography. However, the preparation of crystals for diffraction experiments still requires manual, elaborate manipulations that can result in sample loss. In collaboration with the EMBL instrumentation team we have developed a novel approach called CrystalDirect that enables fully automated crystal mounting and cryo-cooling¹⁻³. This new technology is based on the use of a thin film as crystallization support from which crystals can be recovered by excising the film around the sample with a laser beam and attaching it to a data collection pin. By eliminating manual procedures, crystal mounting becomes a more reliable and controlled operation that does not depend on the skills of the scientist. Moreover, this approach offers an unprecedented level of control during sample processing opening a number of new possibilities. For example, a modification of the CrystalDirect protocol makes it possible to deliver small molecules and other chemicals to crystals by diffusion, providing an alternative to manual crystal soaking experiments. The CrystalDirect approach can contribute to closing the automation gap between crystallization and X-ray data collection thereby contributing to the advancement of challenging projects in structural biology that require the analysis of large numbers of crystals, like those targeting multi-protein complexes, membrane proteins or those involving large scale compound and fragment screening in the context of drug design campaigns. The experience from the use of this system at the High Throughput Crystallization Facility of the EMBL Grenoble outstation as well as the new opportunities enabled by the integration of crystallization and X-ray data collection into continuous, fully automated workflows will be discussed.

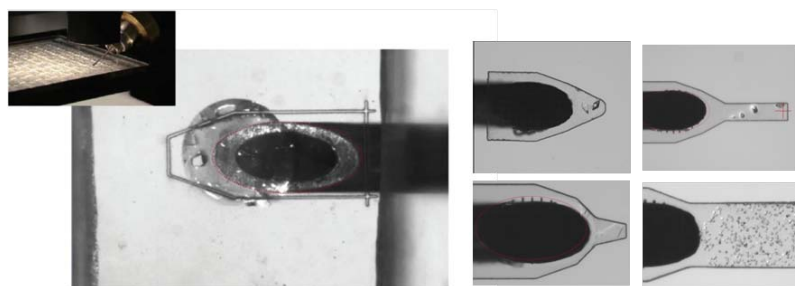


Figure 1. The left panel illustrates the principle of the CrystalDirect technology. The right panels show examples of crystals automatically mounted and cryo-cooled.

1. F. Cipriani, M. Rower, C. Landret, U. Zander, F. Felisaz, and J. A. Marquez, *Acta Cryst. D68* (2012), 1393-9.
2. J. A. Marquez, and F. Cipriani, *Methods Mol Biol*, 1091 (2014), 197-203.
3. U. Zander, G. Hoffmann, I. Cornaciu, et al., *Acta Cryst. D72* (2016), doi:10.1107/S2059798316000954.