

# Detection and Crystallographic Analysis of Crystals grown in the Crystal Former via the PX Scanner.

Tadeusz Skarzynski<sup>1</sup> and Morten O. Sommer<sup>2</sup>

## ABSTRACT:

**PURPOSE:** The ability to rapidly discern between protein and salt crystals and between the qualities of protein crystals *in situ* contributes tremendously to the increased efficiency of the structural biology pipeline. The PX Scanner (Oxford Diffraction) permits *in situ* visualization and diffraction analysis of crystals grown in SBS-format crystallization plates. In this study we evaluate the compatibility of the PX Scanner with Crystal Formers staged in SBS-format holders.

**METHODOLOGY:** Crystals of chicken egg white lysozyme were grown in the microchannels of the Crystal Former under known crystallization conditions for the protein. The Crystal Former channels were then imaged optically with the PX Scanner and the diffraction quality of the crystals analyzed *in situ*.

**RESULTS:** Protein crystals grown in the Crystal Former microchannels were readily identified using the optical microscope of the PX Scanner. Furthermore, the crystal quality could be directly assessed on X-ray exposure with minimal background from the Crystal Former.

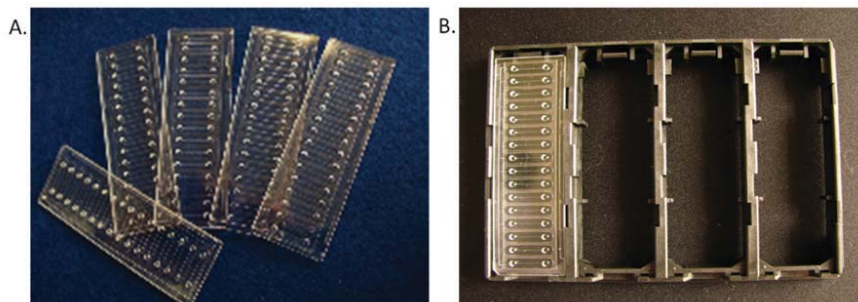
**CONCLUSIONS:** Crystals grown in the Crystal Former can be identified and their diffraction quality assessed with the PX Scanner. The compatibility of the Crystal Former with the PX Scanner thus creates a pipeline that significantly increases the efficiency of crystallization and crystallographic assessment.

**INTRODUCTION:** In the past decade, the push for rapid protein structure determination has been driven by large, collaborative structural genomics initiatives. These consortia have not only contributed a large volume of structural information to the public domain, but have radically shortened the timeline for structure determination. This has been accomplished through the introduction of technolo-

gies, including the development of robotics and the miniaturization of protein purification and crystallization, which have fundamentally influenced the paradigm of X-ray crystallography.

In both high-throughput and traditional academic environments, the parallelization of protein purification and crystallization have dramatically increased the demand to expedite crystal optimization, data collection and structure determination. This reduced timeline necessitates the ability to score numerous potential crystallization conditions for crystal quality and maximal resolution. Typically such a process would involve the identification of crystallization conditions in nanoliter-scale experiments, followed by microliter-scale crystal optimization using vapor-diffusion and manual crystal harvesting for diffraction analysis. This pipeline has been tedious and fraught with multiple points at which crystal quality may be significantly reduced. Structural genomics has thus inspired the application of high-throughput capillary crystallization and *in situ* diffraction of the resultant protein crystals.

The Crystal Former is a commercially available, microfluidic device that has been designed to permit gently diffusive mixing of the protein and crystallization solution inside a rigid microchannel (Figure 1). Owing to the favorable, convection-free environment, proteins crystals of high quality can be rapidly grown and utilized directly for structure determination. In addition to the ease with which the Crystal Former may be manually utilized, the device has been engineered for compatibility with a variety of robotic crystallization and crystal imaging systems typically employed in both academic and industrial structural biology laboratories. Here, we further expand the application range of the Crystal Former through a demonstration of its compatibility with the PX Scanner from Oxford Diffraction, thus permitting rapid *in situ* visual and crystallographic inspection of crystals grown in the microchannels.



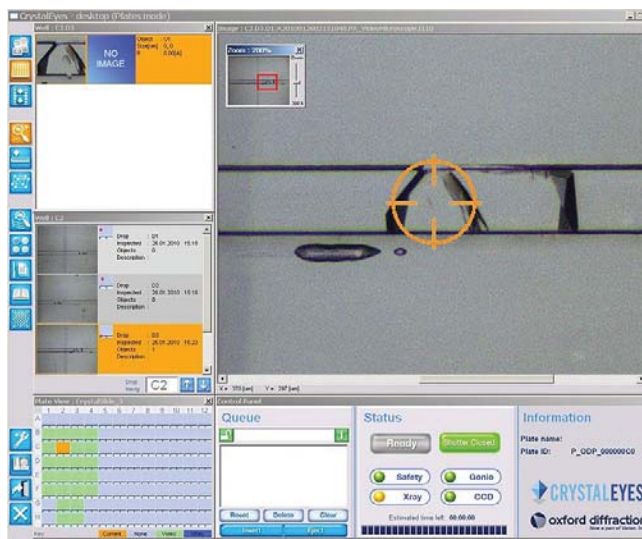
**Figure 1 | The Crystal Former microfluidic device.** (A) The Crystal Former comprises 16 microfluidic channels that are independent of one another, thus permitting harvesting of crystals for traditional mount and crystallographic analysis. (B) Staging of the Crystal Former chips with an SBS-format holder (SH-1 from Microlytic) renders the chips compatible with modern robotic technologies for crystallization, crystal imaging and *in situ* diffraction analysis.

<sup>1</sup>Oxford Diffraction, 2000 Kraft Drive, Suite 1103, Blacksburg, VA 24060 and <sup>2</sup>Microlytic North America Inc., 300 Trade Center, Suite 3650, Woburn, MA 01801, USA. Correspondence should be directed to MOAS: ms@microlytic.com

**METHODS:** Lysozyme was purchased from Jena Bioscience a stock solution of protein prepared with a concentration of 100 mg mL<sup>-1</sup> in 0.1 M sodium acetate, pH 4.8. Crystals were grown in the 16-channel Crystal Formers by equilibrating 0.5 uL of lysozyme against 0.5uL of 10% (w/v) NaCl, 0.1M sodium acetate pH 4.8. Samples were incubated at room temperature prior to inspection and diffraction analysis. The Crystal Former was then mounted into the SBS-format adaptor and loaded directly onto the PX Scanner™. Crystals were visually located using the standard optical video microscope and CRYSTALEYES interface. The diffraction quality was immediately assessed *in situ* at room temperature through the CRYSTALEYES interface of the PX scanner. For each image, 0.5° oscillations were collected with 10 second exposures. The PX Scanner comprises an x-y-omega goniometer, a series of digital microscopes, a high intensity Nova™ Copper X-ray source and a 165mm CCD detector.

**RESULTS:** *Identification of crystals grown in the Crystal Former using the optical video microscope*

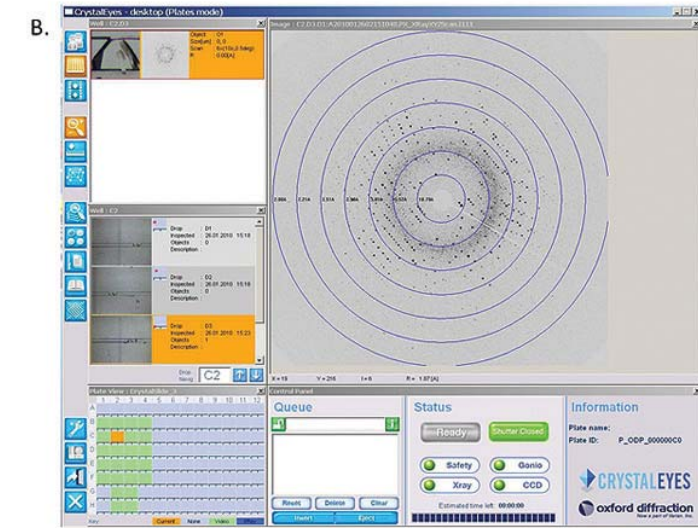
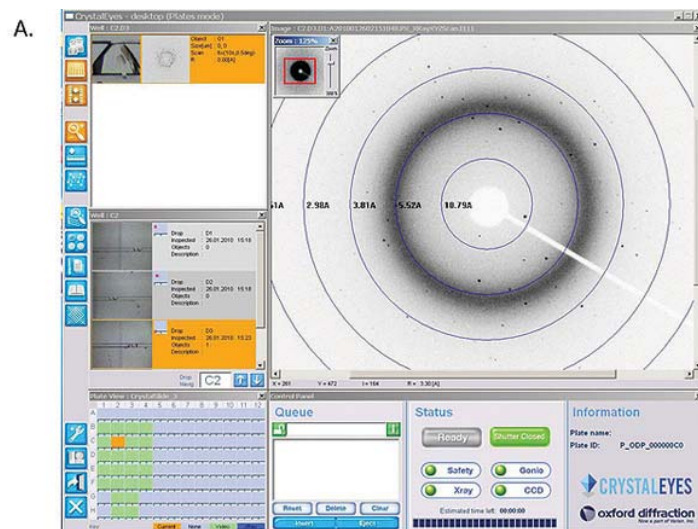
Once staged within the SBS-format holder, the Crystal Former chip was readily accommodated by the PX Scanner. Images were taken along the length of each capillary and were catalogued as separate “drop” samples for a given well. Crystals were readily identifiable by manual inspection of the images. Furthermore, the low background and birefringence of the Crystal Former permitted clear visualization of the crystal edges, even at low magnification (Figure 2).



**Figure 2 | Imaging of the Crystal Former with the PX Scanner optical microscope.** The individual microfluidic channels of the Crystal Former were readily imaged using the PX Scanner from Oxford Diffraction and the individual lysozyme crystals were easily identifiable.

*In situ diffraction testing of single crystals grown in the Crystal Former*

The *in situ* diffraction of a single lysozyme crystal was initially assessed with a single, 10 second exposure for 0.5° of oscillation. Diffraction spots were readily visible at resolutions better than 2.98Å (Figure 3A). For a single image, some scatter from the Crystal Former itself was apparent, as indicated by the presence of the shadow centered at 5.52 Å. This background, however, did not interfere with inspection of the diffraction pattern and was significantly reduced on generation of a composite diffraction image comprising 6 images, each of 0.5° oscillation and 10sec exposure (Figure 3B). Analysis of the composite image indicated a maximum resolution of 2.21 – 2.0Å for the same lysozyme crystal.



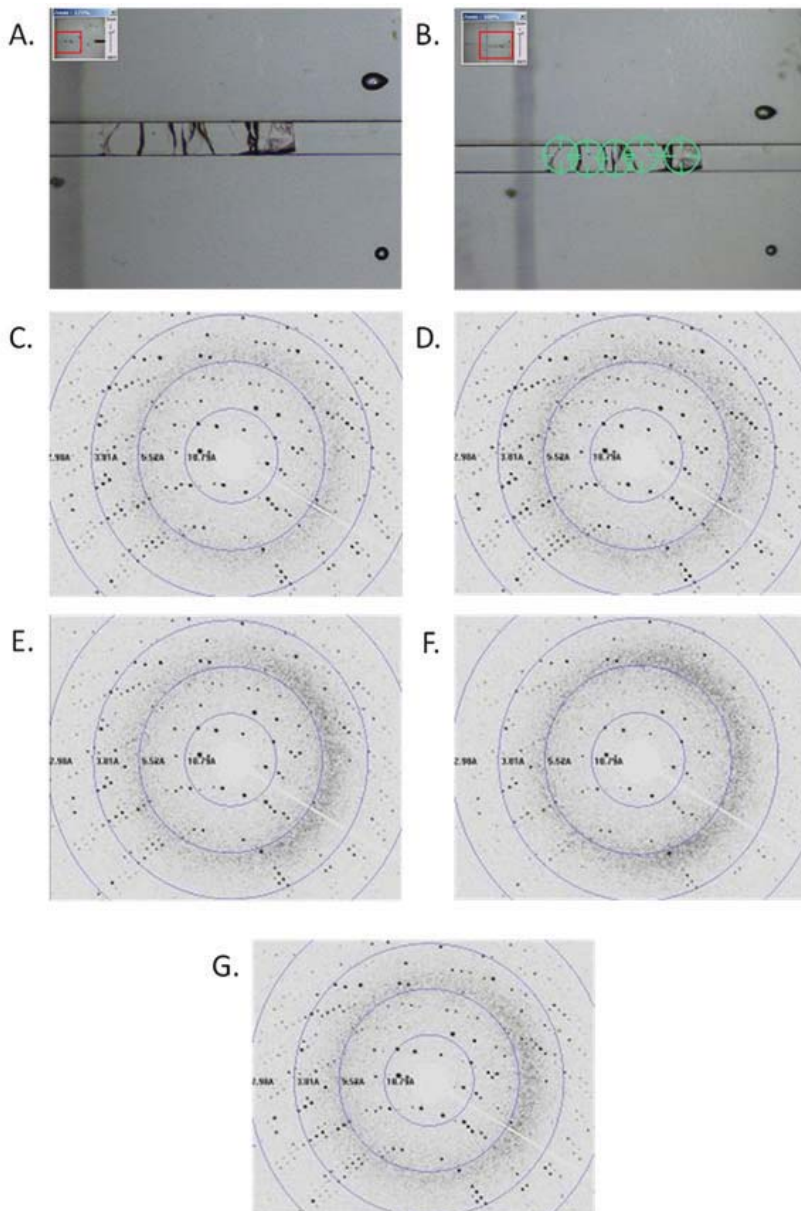
**Figure 3 | *In situ* diffraction testing of a single lysozyme crystal.** (A) A single lysozyme crystal grown within one of the microfluidic channels of the Crystal Former was tested *in situ* for diffraction quality via a single exposure of 10 seconds for a 0.5° oscillation. (B) The composite diffraction image of the same lysozyme crystal comprising 6 x 0.5° oscillations indicated a maximum resolution of almost 2.0 Å with excellent spot quality.

*In situ* diffraction screening using translation of a defective crystal grown in the Crystal Former

The growth of single, large and high-quality crystals from initial crystallization screening is a relatively rare occurrence. More frequently, initial crystals may appear inter-grown, physically twinned with severe cracks and other defects. As the manual mounting and diffraction analysis of crystals is a time consuming process, many of these early crystals are deemed unsuitable for diffraction testing following only visual inspection. The presence of visual defects, however, does not necessitate that the crystals be discarded. As diffraction screening with the PX scanner is a rapid and robust test for *in situ* crystal quality, we also tested the diffraction quality of a lysozyme crystal that appeared to possess numerous cracks and/or growth domains (Figure 4A). Five regions of the crystal were selected for diffraction analysis and a com-

posite image of  $6 \times 0.5^\circ$  oscillations (10 seconds per exposure) was generated for each translation. The results indicated that not only was the diffraction quality consistently good along the length of the crystal, but that the entire crystal clearly possessed a single growth domain (Figure 4B). Thus, the apparently intergrown cluster was in fact a single crystal of excellent diffraction quality, despite the visually obvious defects.

**CONCLUSIONS:** The resolution of the optical microscope and the intensity of the X-ray source of the PX scanner are well suited for the visual and crystallographic analysis of crystals grown in the Crystal Former. Furthermore, the beam focus and translational precision of the  $x$ - $z$ - $\omega$  goniometer of the PX Scanner readily permits the sampling of diffraction quality of for individual crystals, or regions of crystals, distributed along the microchannel of the Crystal Former.



**Figure 4 | *In situ* diffraction analysis while translating along a visually defective crystal.** (A) Initial visual inspection of a lysozyme crystal grown in the Crystal Former indicated the presence of serious defects and/or the intergrowth of multiple crystals. (B) Five translations were selected for diffraction analysis along the length of the defective crystal. (C-G) Composite diffraction images collected for translations along the apparently defective crystal actually demonstrated it to be a single crystal of good quality, despite poor scoring from visual inspection alone.