

# Visualization of macromolecular crystals using the Rock Imager from Formulatrix

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## ABSTRACT:

**PURPOSE:** The development of high throughput methodologies for protein crystallization has necessitated the automation of crystal detection and imaging. It is thus essential that new formats for protein crystallization be compatible with available imaging systems. The Crystal Former (Microlytic Inc.) is a novel crystallization device that combines unique surface chemistry with efficient sample mixing to facilitate protein crystal growth. The Crystal Former is available in two formats that are SBS compatible. In this study, we demonstrate the integration of both Crystal Former formats with the Rock Imager (Formulatrix, Inc.).

**METHODOLOGY:** Both formats of the Crystal Former were staged for imaging in the Rock Imager. Each microchannel was imaged as halves and a composite “whole-channel” image generated through the user interface.

**RESULTS:** Both the 16- and 96-channel Crystal Formers were fully compatible with the standard options of the Rock Imager. Individual microchannels were readily imaged using the visible light path and CCD camera without modifications to the sample stages or visible light path.

**CONCLUSIONS:** The Crystal Formers comprise a crystallization system that is highly flexible with regard to experimental format while retaining the necessary features for full compatibility with currently available robotics, such as the Rock Imager.

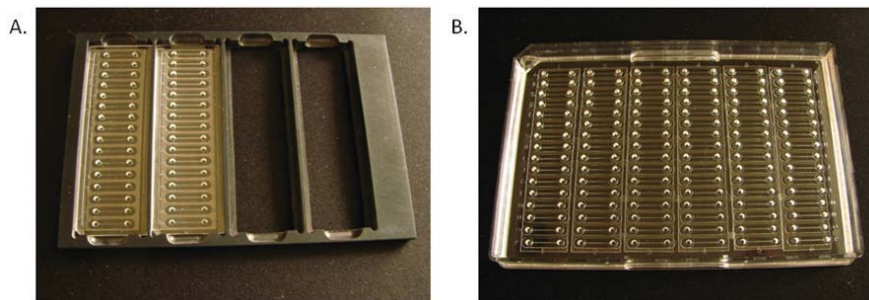
**INTRODUCTION:** The determination of three dimensional macromolecular structures via X-ray crystallography requires the production of relatively large, single crystals. Additionally, the desire for diffraction data of high quality and maximal resolution necessitates that the many growth domains that comprise a single crystal are well ordered. Despite the rapid advances in many aspects of protein crystallography in recent years, researchers still lack the ability to predict *ab initio* conditions that favor the crystallization of macromolecules.

For any given protein sample, several hundred crystallization trials must be thus explored in an appropriate manner for inspection, crystal optimization and crystal harvesting.

Much of the recent growth of Protein Data Bank from a crystallographic perspective can be attributed to the development of automated crystallization robots that are both affordable and compact. These systems have proved beneficial not only in terms of efficiency, but also in the reduction of sample volumes needed for crystallization trials. The miniaturization of crystallization drops has, therefore, opened the avenues of crystallization to previously intractable proteins for which expression levels were severely limiting in manual crystallization trials.

The dramatically increased throughput observed from automated crystallization platforms has thus necessitated the integration of automatic imaging systems capable of programmed plate inspection and interpretation of initial results. This has been especially relevant in industrial and structural genomics environments where thousands of crystallization trials are initiated on a daily basis. In this regard, several robotic imaging systems have been introduced to the crystallographic community in order to expedite the examination and evaluation of crystal trials. The rising popularity of such systems also places some restrictions on the development of new crystallization products such that novel strategies may be readily integrated with current robotics.

The Rock Imager from Formulatrix, Inc (Waltham, MA, USA) provides a robust, automated system for imaging protein crystallization trials. It permits the storage of up to 1000 microplates in either SBS or microbatch plate formats with tight temperature control in the imaging and incubation chambers, vibration insulation, user-defined imaging schedules, drop location algorithms and automated focus and exposure times. Given the popularity of this system in both industrial and large academic laboratories, we sought to integrate the both the 16- and 96-channel Crystal Former formats (Microlytic, Inc., Woburn, MA, USA) with the Rock Imager (Figure 1).



**Figure 1 | The Crystal Former Formats.** (A) The 16-channel Crystal Former is readily staged in the SBS-format holder (Product SH-2, Microlytic) for compatibility with the Rock Imager (Formulatrix). (B) The 96-channel Crystal Former (CF-HT, Microlytic) is directly compatible with the visible light path and CCD camera of the Rock Imager.

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**METHODS:** *Staging of Crystal Formers for imaging with the Rock Imager*

Both the original and Scale-up formats of the 16-channel Crystal formers are compatible with the SBS-format holder (Catalogue # SH-2, Microlytic Inc, Woburn, MA). Up to four Crystal Formers are placed into the holder and the assembly inserted into a storage slot of the Rock Imager. The 96-channel Crystal Former is directly compatible with the standard visible light optical path and CCD camera of the Rock Imager and does not require a plate adaptor.

*Image Capture*

The microfluidic channels are imaged in halves with the final whole-channel view represented as a composite view. The individual images are well aligned via the central overlap of images. Automated imaging of the Crystal Formers is easily configured through the user interface.

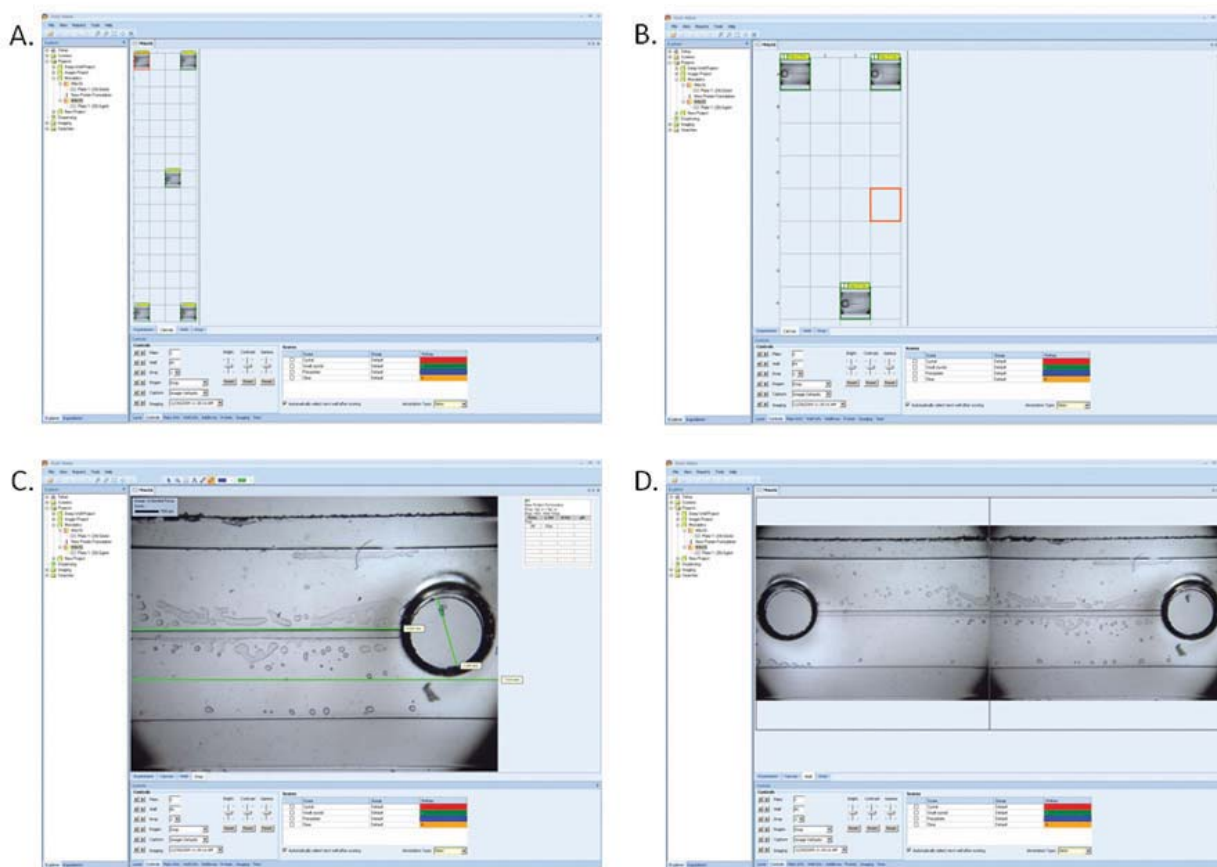
*Detection of Lysozyme Crystals*

A lysozyme kit was obtained from Hampton Research (HR-108) and 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

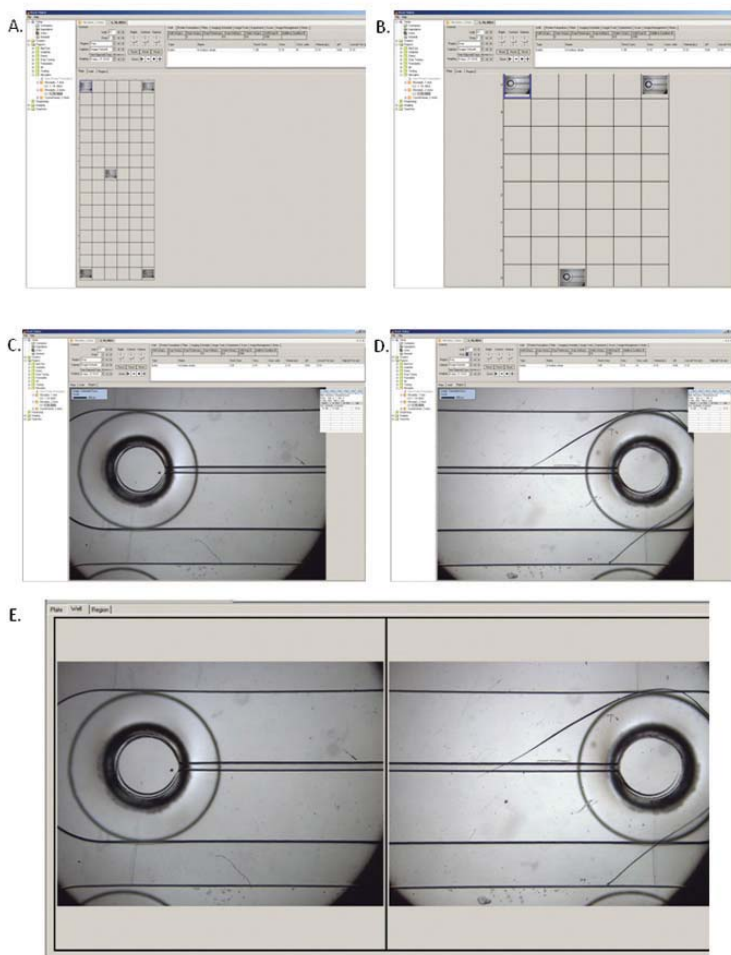
16-channel Crystal Formers by equilibrating 0.5 μL of lysozyme against 0.5μL of 10% (w/v) NaCl, 0.1M sodium acetate pH 4.8. Samples were incubated at room temperature prior to inspection.

**RESULTS:** *Imaging of the 16-channel Crystal Formers*

Once staged in the SBS-format holders, the individual channels of the Crystal Former are readily imaged with the Rock Imager. The Rock Imager is equipped with a 5-megapixel 2/3" color CCD camera and a Kohler light source with motorized condenser. The low birefringence and scatter if the Crystal Former permits clear visualization at high resolution along the length of the microchannel. Within the user interface (Figure 2 A and B), four individual chips are represented as a 16 x 4 array. Each microchannel is imaged as two “drops” for which each image captures one half of the microfluidic channel (Figure 2C). Precise alignment of the individual images is facilitated by the duplicate imaging of approximately 0.6 mm which creates a region of overlap in the final composite image (Figure 2C). Once the individual images are aligned and merged, a view of an entire channel is created allowing visualization of the individual sample wells, as well as the channel itself (Figure 2D).



**Figure 2 | 16-Channel Crystal Former Imaging with the Rock Imager.** (A) The user interface indicating the scheduled imaging of 4 individual Crystal Formers in the SH-2 holder. (B) A magnified view of the user interface. (C) Imaging of a single “drop” that equates to one half of the crystal former channel in the SH-2 holder. (D) The whole-channel view for a single microchannel in a 16-channel Crystal Former.



**Figure 3 | Imaging of the 96-channel Crystal Former with the Rock Imager.** (A) An overview of the user interface with the 96-channel Crystal Former defined. (B) A magnified overview of the user interface. (C-D) Individual “drop” images, each comprise one-half of a single microchannel. (E) The whole channel view for a single microchannel in the 96-channel Crystal Former.

### *Imaging of the 96-channel Crystal Former*

The 96-channel Crystal Former is directly compatible with the visible light path and standard CCD camera of the Rock Imager (Figure 3). Each microchannel of the 96-channel format is defined as a single sample, constituting a 16 x 6 array in the user interface, and the microchannels recorded in halves (Figure 3 A and B). As with the 16-channel Crystal Formers, imaging overlaps at the center of the channels (Figure 3 C and D) allow for the generation of a composite image comprising the entire microchannel and inlet wells (Figure 3E).

### *Detection of Lysozyme Crystals*

Protein crystals were readily detected in the Crystal Former channels using the standard imaging scheme of the Rock Imager. The crystals, present near the middle of the microchannel, were visible on both images for a given microchannel (Figure 4A). Furthermore, the analysis software permits the definition of a region of interest (Figure 4B) and the magnification of this region for closer inspection (Figure 4C).

**CONCLUSIONS:** The Rock Imager from Formulatrix is a popular device for the storage and automated imaging of protein crystallization experiments. The system is compatible with many crystallization plate formats and has been previously utilized in the imaging and scoring of crystals grown from vapor diffusion and microbatch trials. Here, we have demonstrated the compatibility of the Rock Imager with both the 16- and 96-channel Crystal Formers.



**Figure 4 | Detection and imaging of lysozyme crystals in the 16-channel Crystal Former.** (A) Lysozyme crystals located near the center of the microchannel were imaged in both of the individual photographs for a single microchannel. (B) The crystals were highlighted as a region of interest and (C) the image magnified for closer inspection.