

How to use Counter Diffusion CrystalSlide™

This brief document describes the use of CrystalSlide™ for Counter Diffusion Crystallography at the Accelerated Technologies Center for Gene to 3D Structure (ATCG3D) microfluidics group.



Figure 1: The image shows four CrystalSlides™ in a slide holder.

Background

Crystallisation in confined geometries using the counter diffusion concentration screening is a key tool in the search for diffraction quality crystals. It permits low protein consumption, easy handling and storage, automated imaging and *in situ* X-ray screening and collection, removing crystal manipulation from the process. CrystalSlide™ takes advantage of the gradient over solution concentration inherent in the counter diffusion method automatically fine screening a single initial condition.

CrystalSlide™ was designed with input from the ATCG3D microfluidics group and has been tested over the past months. The slides match the dimensions of a standard microscope slide, are very flat for imaging, have numbered channels and the materials characteristics are optimised for *in situ* imaging and diffraction data collection.* The geometry of the inlet ports enables loading of the slides with standard pipette tips (Greiner Bio-One crystal tips) and automated systems.

Each counter diffusion experiment takes 600 nl of protein to ensure liquid for a protein and precipitant interface during the addition of the precipitant. Air bubbles in the channels can be an issue but are avoided with careful setup.



Figure 2: Top down image of CrystalSlide™ with 12 straight numbered channels



Figure 3: Profile of CrystalSlide™. The inlet wells are raised for improved interface with pipette tips.

* All data and images (except Fig. 1 - 3) refer to the CrystalSlide™ prototype with 10 instead of 12 channels.

Channel Dimension



Figure 4: The channel width is 100 µm (ImagePro 6.0).

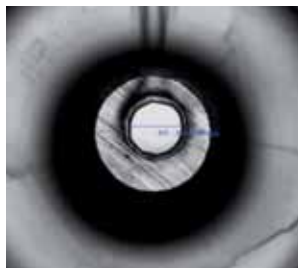


Figure 5: The well at channel height has a diameter close to 400 µm (ImagePro 6.0).

Each straight channel is 2 cm long, 100 µm wide and 100 µm in height.

Loading of the slides

Nothing beyond a 10 µl pipette and 0.5 - 20 µl Greiner Bio-One crystal tips, capillary wax and nail polish are needed to load protein and precipitant solutions in this counter diffusion crystallisation environment.



Figure 6: All tools necessary to set up a counter diffusion experiment in CrystalSlide™.

Product	Supplier	Cat.-No.
CrystalSlide™	Greiner Bio-One GmbH	444 820
Crystal Tips	Greiner Bio-One GmbH	765 290, 765 280, 765 271
Slide holder	Greiner Bio-One GmbH	on request

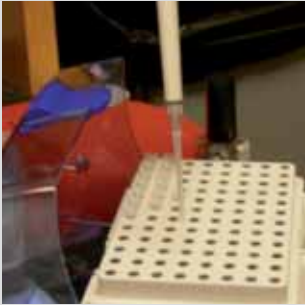
Table 1: Plastic consumables necessary to set up CrystalSlide™

Procedure

The inlet ports were designed to fit 0.5 - 20 μ l Greiner Bio-One crystal tips (Cat.-No. 765 290, 765 280, 765 271)

Loading Protein:

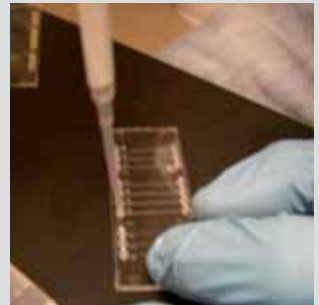
- 1) Aspirate 0.60 μ l protein solution into the pipette tip.



- 2) Insert the pipette tip into the inlet and press all the way down on the pipette button.

This sends protein solution down the channel and into the opposite well. The protein should be seen arriving in the opposite well.

Once comfortable with this step, several protein channels can be loaded before loading precipitant solutions.



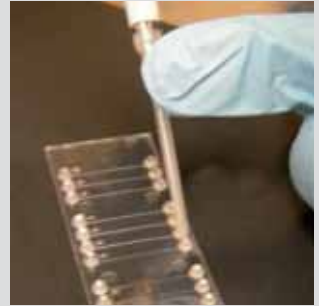
Loading Precipitant Solution:

- 1) Aspirate 0.45 μ l of precipitant solution into a new pipette tip.



2) Insert the pipette tip partially into the well on an angle so the tip is touching the wall near the protein at the bottom of the well.

The tip should be as close to the protein in the well as possible but not all the way in where it makes a seal.

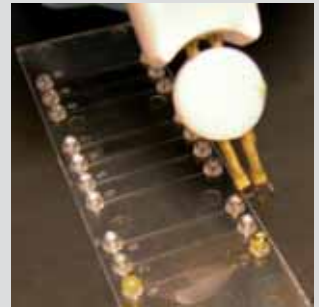


3) Pipette the 0.45 μ l of precipitant solution on top of the protein solution in the well slowly.

The contact of protein solution to precipitant solution is crucial. An air bubble between the protein solution and the precipitant solution will inhibit proper equilibration.

4) Seal both wells with capillary wax using the wax pen and capillary wax purchased from Hampton Research.

Melt some wax with the wax pen, let go of the wax pen button, collect a small amount of wax on the tip of the wax pen and while holding the tip of the wax pen over the well to be sealed press the wax pen button again to melt the wax and apply it to the well.



5) Cover the wax seal with nail polish on and around the wax of all inlets to complete the seal.

Make sure not to cover the channel with capillary wax or nail polish.



Imaging

The CrystalSlide™ can be imaged under any conventional microscope or many automated imaging systems. For example, the Formulatrix RockImager, manufactured to store and image crystallisation plates with standard microplate footprint, like the 96 Well Crystal-Quick™ low profile crystallisation plate seen in **figure 7**, can be easily programmed to completely image each counter diffusion channel when inserted into a slide holder with standard microplate footprint. The slide holder with 4 CrystalSlides™ ready for storage and imaging is demonstrated in **figure 8**.

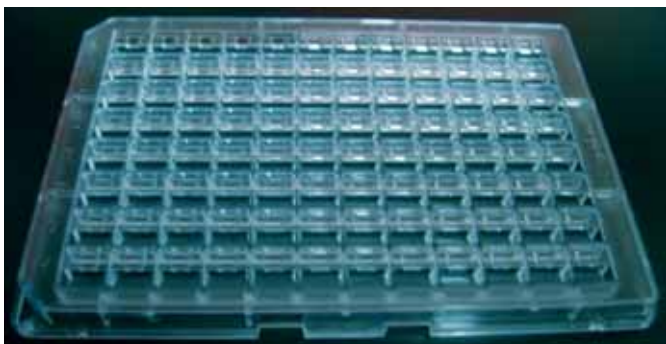


Figure 7: 96 Well Crystal-Quick™ low profile crystallisation plate.

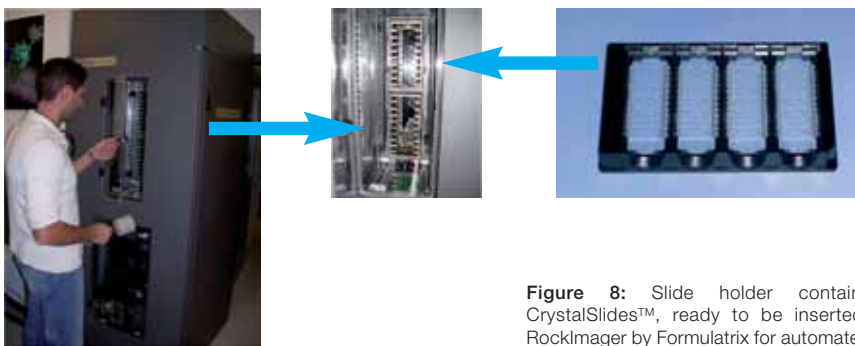


Figure 8: Slide holder containing four CrystalSlides™, ready to be inserted into the RockImager by Formulatrix for automated imaging.

Once the template for the four CrystalSlides™ and the slide holder is set, polarised and bright field images at the predetermined magnification are taken scheduled at the preference of the experimenter. Regions of interest can be selected for higher magnification imaging during the next imaging sequence. The below montage demonstrates the template for imaging the 40 channels (four cards) that fit in the frame along with a higher magnified region of interest in one channel.

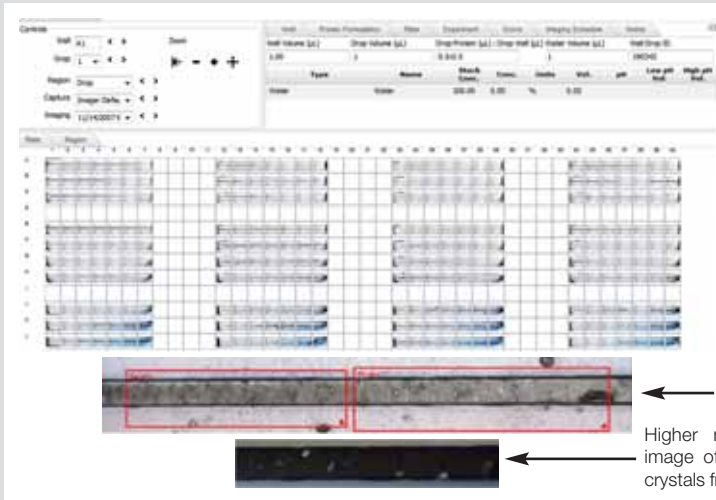


Figure 9: Well layout of CrystalSlide™ after imaging. Each block is a 3x magnification bright field and polarised image that can be viewed individually and scored. For higher magnification, regions of interest are drawn and imaged during the scheduled time.

← Bright Field image - H26 well
 ← Higher magnification polarised light image of possible Membrane Protein crystals from region 2 of H26 well image

The images from the RockImager can also be presented in a way to give the overview of the whole channel at each imaging event.

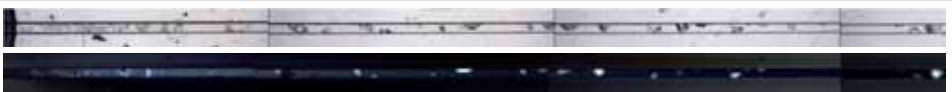


Figure 10: Stitched RockImager bright field (top) and polarised (bottom) images of thaumatin crystals in a CrystalSlide™ channel from “CapViewer” developed in The Kuhn Lab at The Scripps Research Institute.

CrystalSlides™ are UV-transmissive, as well, allowing ultra violet light activated fluorescence of protein crystals to be identified as seen below in the image of the same channel seen above under bright field and polarised light.



Figure 11: UV-fluorescence image of protein crystals in CrystalSlide™.

Mounting channels for X-ray analysis

Room temperature mounting for crystal screening:

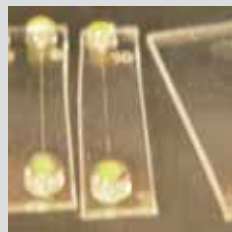
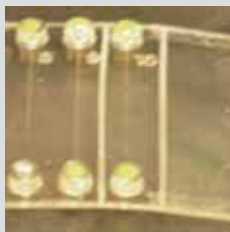
- 1) Locate the crystal to be screened.
- 2) With a marker, mark next to the channel on both sides of the crystal for future reference.

Some beam line bright field optics are not very useful in finding crystals in the channels so locating the crystal on the beam line is easier when a mark is used.

In line beam optics are the most complementary to the plastic, fixed geometry crystallisation channels.

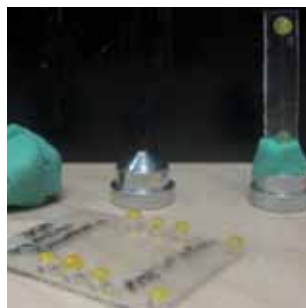


- 3) Cut the channel from the card (1, 2, 3, and 4 channel groups have been cut out and shot with success in certain orientations).



To collect the best data with the largest phi angle cut out a single channel and attach it lengthwise to the end of a magnetic base with modeling putty. (Colder than room temperature data collection can be achieved by directing a cryo stream toward the sample on the beam line.)

Figure 12: Modeling putty, a magnetic base and the resulting assembly (in the rear), four channels from a CrystalSlide™ (in the front)



In situ X-ray analysis

Because of the material selected, *in situ* X-ray analysis is routine.

Performance of the channels in an X-ray beam:

The plastic X-ray transmissive material allows *in situ* X-ray screening in a micro-focus beam (PXScanner from Oxford Diffraction) and synchrotron beam like in the images below (APS GM/CA CAT ID-D).

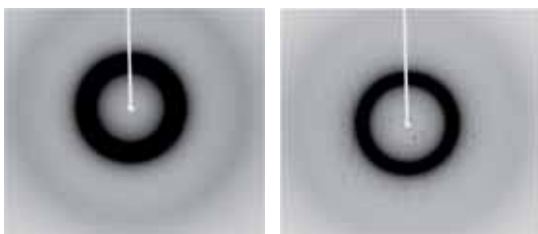


Figure 13: Single channel cut from CrystalSlide™ and mounted in front of an X-ray beam (APS GM/ CA CAT ID-D) for *in situ* X-ray screening.



Figure 14: (top left): Image of a channel cut from CrystalSlide™ flat in front of an X ray beam.

(top right): High magnification image of a lysozyme crystal in front of an X-ray beam perpendicular to the card.

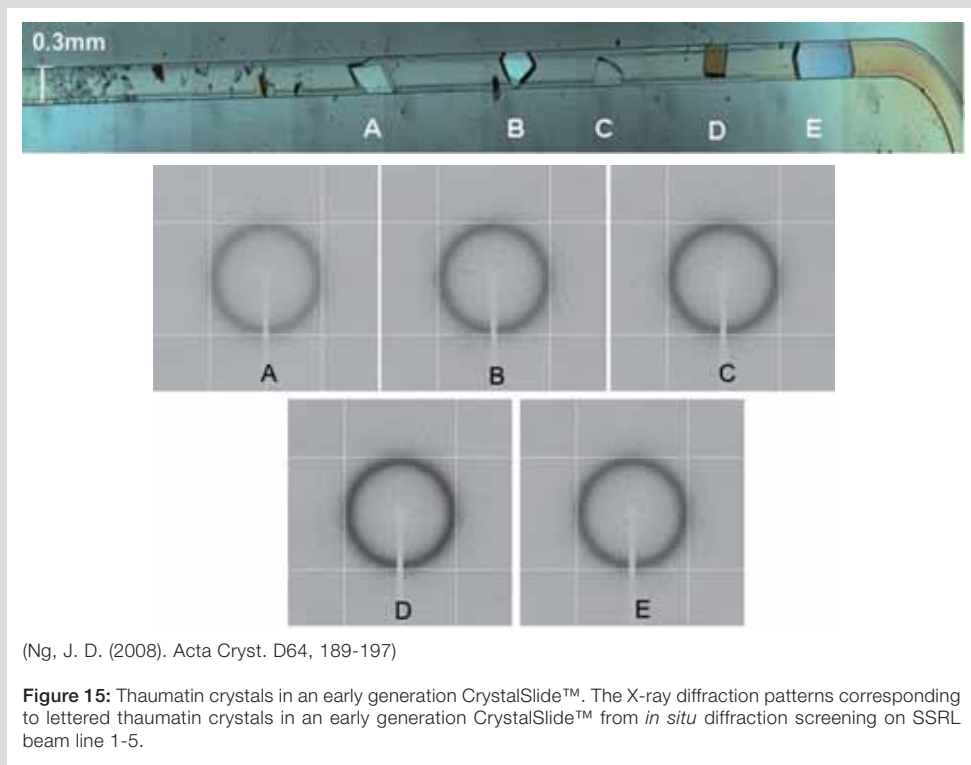


(bottom left): Image 32 from an X ray diffraction data collection on the lysozyme crystal shown in the top right. The slide started flat and spots become increasingly apparent as it gets closer to directly facing the channel as seen through a microscope.

(bottom right): Image 80 from the same lysozyme crystal when closer to a direct X-ray beam shot.

Lysozyme diffraction images, taken at the Advanced Photon Source GM/CA CAT ID-D beam line, with 10x attenuation and 1 degree oscillation at room temperature were indexed successfully using Mosfilm with resolution spots visible around 1.8\AA on the last image.

This data is consistent with earlier generation testing of earlier generations of CrystalSlide™ as seen below in data from Ng, J. D. (2008). Acta Cryst. D64, 189-197.



(Ng, J. D. (2008). Acta Cryst. D64, 189-197)

Figure 15: Thaumatin crystals in an early generation CrystalSlide™. The X-ray diffraction patterns corresponding to lettered thaumatin crystals in an early generation CrystalSlide™ from *in situ* diffraction screening on SSRL beam line 1-5.

Thaumatococcus

Space group	P4 ₁ 2 ₁ 2
Unit cell parameters	a=b=58.51Å c=151.24Å
Resolution range (Å)	25.0 to 2.0
No. of observations	144768
No. of unique reflections	18409
Completeness (%)	
Overall	98.8
Lowest shell	96.7
Highest shell	98.5
R_{merge} Σ(I-⟨I⟩)/Σ⟨I⟩ (%)	
Overall	9.5
Lowest shell	3.9
Highest shell	34.1
⟨I/σ(I)⟩	
Overall	18.42
Lowest shell	37.9
Highest shell	5.0
Lowest shell range	25.00Å to 4.30Å
Highest shell range	2.07Å to 2.00Å

(Ng, J. D. (2008). Acta Cryst. D64, 189-197)

Table 2: Statistics from *in situ* X-ray diffraction data collected on SSRL beam line 1-5 on thaumatococcus crystals in an early generation CrystalSlide™.

Results

ATCG3D has tested the CrystalSlides™ with a number of model proteins (thaumatococcus, lysozyme and insulin) along with a number of soluble and membrane proteins achieving crystallisation with well behaved samples. The slides had excellent performance with low protein consumption, easy handling and storage, automated imaging and *in situ* X-ray screening and collection. Although harvesting and freezing crystals grown in the slides has proven a challenge, the idea behind the slide was to provide a platform for successful crystallisation screening, and “touch free” X-ray analysis and this affordable, plastic slide is very suitable for that application.

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