Science Concept

For

Real-Time Protein Crystal Growth (RTPCG-1)

January 15, 2018

Investigator

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Sponsors

NASA: SLPSRA, ISS-PO

CASIS

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Experiment Phases	Hardware 1	Hardware 2	Hardware n	Responsible organization	Science Participation
Phase-1, Demonstrate that real time operational procedures and testing are feasible on orbit. Specifically, "*The study involves experimental evaluation and optimization of a crew procedure comparing two different flight approved vapor diffusion hardware systems (Mitogen 96-well plates and Protein Crystallization Apparatus for Microgravity (PCAM).*"	96 Well Plate	PCAM	TBD	SLPSRA	Consultants, transitioning to a Science Definition Team* (SDT).
Phase -2, Test reference proteins. Specifically, "*The initial evaluation will proceed to phase-2 provided a viable procedure (using either or both hardware systems) is developed. Phase-2 involves testing the phase-1 experimental protocol using a readily available, purified protein; hen egg-white lysozyme*	96 Well Plate	PCAM	TBD	SLPSRA/CASIS	SDT*
Phase 3, True Scientific Research. Specifically, the scope would be, *"Phase-3 will involve NASA/CASIS request for proposals (RFP) involving high value proteins that, in a 1g environment, yield crystals of inadequate quality for X-ray and/or neutron crystallographic structural analysis or to support drug discovery (this may involve co-crystallization studies with small molecules or peptides)."*	96 Well Plate	PCAM	TBD	CASIS	SDT**

Table 1. Experimental Approach and Collaboration

* Open Science for Phases 1 and 2 – This would be achieved by using the NPJ – Microgravity Blog. NASA would post a detailed description of RTPCG for comment by the PCG science community. This would be an iterative process. A core, existing Science Definition Team would incorporate as many of the blog ideas as feasible into the experiment.

** Open Science for Phase 3 – A Science Definition team would be selected through the CASIS RFP. The participants from the NPJ – microgravity blog would be well prepared to compete.

Real-time Protein Crystal Growth (RTPCG) Summary

Nearly all laboratory experiments performed on Earth require more than one try. Rarely does an experiment work exactly as expected the first time. In the past, iterations of experiments on the same flight have not been very practical for Protein Crystal Growth in microgravity. However, it has been noted that proteins which have been flown numerous times are more likely to produce higher quality crystals than those flown only once, indicating that such iterations could greatly improve possibilities for success. Recent experiments in DNA analysis on the ISS have shown that hand-pipetting small volumes of liquid in a microgravity environment can be accomplished, paving the way for setting up iterative crystal growth experiments. A bonus for this type of experiment is that more pharmaceutical corporations may be enticed to use microgravity for growing higher quality crystals, given that they won't have to wait months or even years to retry their experiments.

Several conference calls have been conducted to discuss various aspects of conducting a preliminary set of experiments on the ISS to determine if such "real-time" crystal growth is feasible, the idea being that astronauts would set up protein crystal growth plates, after a couple of days begin microscopic analysis of crystals, and transmit the microscopic images down to the PI. Based on these results, the PI can then determine new conditions in an effort to optimize the quality of crystals grown.

Over the course of several conference calls, discussions focused on a 2-phase plan. The first phase would consist of just providing the astronauts with several concentrations of frequently-used relatively viscous precipitants to be mixed with water that has been dyed, so that mixing efficiency can be observed using different reagent concentrations. Two types of vapor diffusion hardware will be compared: MiTeGen 96-well plates, a commercially available crystallization plate which has been used in recent CASIS experiments performed on the ISS, and PCAM plates, which have been used numerous times on both the Shuttle and ISS.

If the first phase is successful, the second phase would evaluate the Phase 1 protocol with two readily available purified proteins. The plates would be sealed using commercial sealing tape specifically manufactured for protein crystal growth and analysis, and pictures would be transmitted to the PI during crystal growth. This then could be followed by iterations with varying growth conditions. If this set of experiments are successful, a third phase will be discussed, whereby a number of investigators would be selected via peer-reviewed proposals involving high-value proteins or other macromolecules.

Attachments include a draft of the experimental procedure written by Dr. Lawrence DeLucas, Aerospace Corporation, who flew as a Payload Specialist to perform similar experiments on Shuttle flight STS-50. During Dr. DeLucas' mission it was found that the nucleation and growth phases for proteins was significantly slower in microgravity. As a result, the 14-day space shuttle mission did not provide sufficient time for the majority of proteins for optimization of crystallization. Also included as appendices are information on MiTeGen Plates, PCAM plates, sealing tapes, available ISS microscopes and pipets.

The detailed procedure provided below (pages 6-15) involves two phases; phase-1 (study of mixing protocols for vicous solutions) and phase-2 (crystallization optimization) is tailored for use of the Mitogen plates only. The first flight (RTPCG-1); Dr. DeLucas, PI, will only utilize the commercially available Mitogen protein crystallization plate (image of Mitogen plate available in appendix-1, page 16) to perform two studies labelled as *phase-1* and *phase-2*:

> *Phase-1:* Overall evaluation of the flight procedure to be used for iterative protein crystallization combined with microscopic assessment of two different mixing protocols to accommodate vicous crystallization solutions such polyethylene glycols.
>
> *Phase-2*: Evaluation of the process of iterative protein crystallization using ISS crew in coordination with ground principal investigator (Dr. DeLucas for first flight). This initial flight will use the commercially available protein, hen egg-white lysozyme, as a test protein.

Note: A TBD second flight RTPCG-2 will involve a modified phase-1 and phase-2 study for an alternative flight hardware system (Protein Crystallization Apparatus

for Microgravity, *PCAM*) as noted in Table-1. A third phase (***phase-3***) will occur after successful demonstration of phase-1 and phase-2 goals. A third flight, RTPCG-3 will involve a CASIS request for proposals involving high value proteins as described in Table 1, ***phase-3***. This may involve crystals of inadequate quality for X-ray and/or neutron crystallographic structural analysis or to support drug discovery (this may involve co-crystallization studies with small molecules or peptides). Winning proposals will be selected via peer-review. Continuation of on-orbit "Iterative Protein Crystallization" will depend on crew availability and success rates for a statistically relevant number of high value proteins.

RTPCG-1 Preliminary Concept and Procedures

By: Larry DeLucas, PhD

(January 15th, 2018)

Rationale: The chemical conditions for optimized protein crystallization in microgravity are often different from those experimentally achieved in a 1g environment. This is believed to be due to the differences in protein transport and equilibration kinetics (for both vapor diffusion and liquid diffusion crystallization techniques). Many of the current flight protein crystallization protocols using either of these two experimental techniques require that experiments are prepared prior to launch followed by incubation at -80°C until the payload reaches the ISS. At that point the payload is placed in 20°C or 4°C incubator (causing the protein crystallization experiments to thaw) resulting in activation of the crystallization process. It is currently not possible to intervene in the crystallization experiments or to observe the results of a few preliminary crystallization experiments followed by preparation of new chemical conditions to optimize the microgravity crystallization process for each protein. As a result, investigators typically prepare dozens to hundreds of experiments (each with different chemical conditions), hoping that one or more of the chemical conditions attempted will be close to the optimized crystallization conditions. This means that a significant amount of purified protein must be available to prepare a large number of experiment conditions.

As an alternative, if a protocol were developed whereby an astronaut could downlink microscope images of preliminary protein crystallization results, the science expert on the ground associated with each protein would be able to observe downlinked images of their protein crystals and subsequently, inform the astronaut regarding new chemical conditions to experimentally prepare, to work toward improved or optimized protein crystals. It is possible to accomplish this using existing flight approved hardware. The following protocol provides a viable and relatively inexpensive method to enable iterative microgravity protein crystallization. This protocol assumes that initial vapor diffusion experiments are prepared pre-flight, stored in a -80°C freezer and delivered to the ISS. The frozen experiments are allowed to thaw in a 20°C or 4°C incubator to initiate crystallization. Crystals are allowed to grow during the time SpaceX is docked

followed by a return to Earth for analysis. Initial experiments will be used to refine the overall iterative crystallization protocol while providing an indication of the time required for nucleation and growth of two sample proteins.

A phased approach will be used to develop the final on-orbit experimental procedure. As noted previously, the phase-1 and phase-2 flight procedure shown below will only involve use of Mitogen crystallization plates (mentioned in table-1 & detailed image provided in appendix). Future flights will involve a similar but modified procedure to evaluate phase-1 and phase-2 using PCAM flight hardware. These initial two flights, if successful, will be followed by a phase-3 program that will involve a CASIS solicitation for investigator proposals.

(RTPCG-1) Crew Procedure

Phase-1: Phase-1 is a feasibility study for on-orbit iterative protein crystallization. The study involves experimental evaluation and optimization of a crew procedure using Mitogen 96-well crystallization plates. The crew will assess the ability to accurately extrude and mix (from flight-approved pipettes) different solutions and solution volumes in the two different vapor diffusion hardware systems. Points of concern include: 1) ability to easily handle some of the small hardware components (particularly the PCAM system), pipette tips, and small vials containing the crystallization solutions, 2) ability to adequately mix solutions with different viscosities, 3) ability to prepare the crystallization experiments without introducing an excessive amount of bubbles, 4) ability to use the ISS glovebox or workbench for the experiment preparations and the glovebox microscope (or other ISS microscope) to observe, photo-document and downlink micron-size crystals contained in the experiment chambers of Mitogen plates.

A. Phase I Equipment List

1.) 3 large Velcro bars
2.) Velcro tape strips
3.) Long Compound O-Ring (2 joined 100mm O-rings)
4.) 10 µL Eppendorf pipette with 20 tips
5.) 10 Mitogen plates (96 well) w/ Velcro tape attached to edges

6.) Mitogen plate sealing tape strips

7.) Phase I Vial Bag:

a. Blue-labeled Water baggie
    i. 10 mL of purified water contained in a 15 mL Falcon tube w/ tape loop
    ii. 1,000 µL of purified water that is colored with a dark blue solution dye (food coloring dye is acceptable) in a 1 mL Eppendorf tube w/ patch of Velcro tape

b. Red-labeled Precipitant baggie
    i. 500 µL of the following precipitant solutions are individually contained in 1 mL Eppendorf tubes, all should have Velcro patches.
        1. 20% wt./vol. PEG-6000
        2. 10% wt./vol. PEG-6000
        3. 30% wt./vol. PEG-400
        4. 10% wt./vol. PEG-400

c. White-labeled New Pipette Tip baggie
    i. 20 10µL pipette tips

d. Black-labeled Waste baggie

<u>B. Phase-1 Crew Procedure</u>

Setup

Step 1: Place and secure large Velcro bar VB1 on glovebox floor on the left edge of the working space. Place and secure large Velcro bar VB2 on the floor along the right edge of the working space.

Step 2: Remove one new Mitogen plate from stowage and secure it via attached Velcro onto VB1.

Step 3: Remove two strips of Velcro tape from stowage and press them onto the glovebox floor, near the middle. Ensure the spacing of the strips matches the spacing of the two Velcro strips on the bottom edges of Mitogen plate. Remove the Mitogen plate from VB1 and press onto the tape strips.

Step 4: Position a video camera such that it images a close-up view of each experiment chamber of the mitogen plate (the video camera will need to be moved

as subsequent experiment chambers are utilized). It is important that there is sufficient lighting so that the solutions pipetted and mixed in each experiment chamber are clearly visible.

Step 5: Secure compound O-ring across posts of VB1.

Step 6: Remove the 10 µL pipette from stowage. Attach a tape loop to the pipette handle and secure it on the glovebox floor within easy reach.

Step 7: Remove the Phase 1 bag from stowage. It should contain a red-labeled baggie of precipitant solution tubes, a blue-labeled baggie containing purified water tubes, a white-labeled baggie with 20 new 10µL-sized pipette tips, and a black-labeled used pipette tip baggie (empty).

Step 8: Remove all tubes from their bags and secure them onto Velcro bar VB2 using the Velcro attached to the sides of the tubes. The precipitant bag and water bag will now be used as pipette tip repositories for their respective solutions.

Step 9: Secure the new pipette tip, precipitant tip, and water tip baggies under the O-ring stretched across VB1. Secure the waste baggie somewhere in the workspace. As a reminder, the bags will be color-coded via colored tape: white for new tips, red for precipitant tips, blue for water/protein tips, black for waste.

Stirred Mixing Procedure with First Precipitant

Step 10: Attach a new pipette tip to the syringe. Use the 10 µL pipette with tip attached to withdraw 5.0 µL of the dyed water from the dyed water tube followed by extrusion into the reservoir at the first experiment chamber position of the Mitogen plate.

Step 12: Remove the used pipette tip from the syringe and store inside the blue-labeled water/protein tip baggie. This will tip will be reused later in the procedure.

Step 13: Attach a new, unused pipette tip to the syringe.

Step 14: Withdraw 5 µL of the 20% PEG-6000 solution from the appropriate tube and extrude it into the 5 µL of dyed water already in the reservoir chamber of the Mitogen plate. Avoid introducing bubbles into the drop as much as possible.

Step 15: Gently mix the solutions with the pipette tip. Continue mixing until it appears that the blue dye is equally distributed throughout the solution. Try not to spread out the drop while mixing as it is important that the drop surface area in

contact with the plate is minimized. Record the estimated amount of time required to completely mix the solutions.

Step 16: Rinse tip in the 10 mL of purified rinsing water vial and deposit into the red-labeled precipitant tip baggie. This tip will be used again in later steps.

Step 17: Reattach tip from the water tip baggie.

Step 18: Withdraw 5.0 µL of the dyed water from the dyed water tube followed by extrusion into the reservoir of the next row position of the Mitogen plate. The next position is two wells to the right in the first row of the Mitogen plate, leaving an empty well in between each used well.

Step 19: Return tip to the water/protein tip baggie.

Step 20: Reattach tip from the precipitant tip baggie.

Step 21: Withdraw 5 µL of the 20% PEG-6000 solution from the appropriate tube and extrude it into the 5 µL of dyed water already in the reservoir.

Step 22: Gently mix the solutions with the pipette tip. Continue mixing until it appears that the blue dye is equally distributed throughout the solution. Try not to spread out the drop while mixing as it is important that the drop surface area in contact with the plate is minimized. Record the estimated amount of time required to completely mix the solutions.

Step 23: Rinse tip in the 10 mL of purified rinsing water vial and deposit into the red-labeled precipitant tip baggie.

Step 24: Repeat steps 17-23 using the next position (again skipping one experiment chamber in the row) in the Mitogen plate, for a total of 3 iterations.

Step 25: After completing a row of the Mitogen plate, press a strip of sealing tape onto the row (thereby sealing all of the experiment chambers in the first row).

Extrusion Mixing Procedure with First Precipitant

Step 26: Repeat steps 17-21 in the next row position of the Mitogen plate. Instead of mixing with the tip of the pipette, after adding the PEG solution to the dyed water solution, continuously withdraw and re-extrude 5 µL of the composite drop into the pipette tip until it appears that the blue dye is equally distributed throughout the 10 µL solution. Again, try not to spread out the drop while mixing as it is important that the drop surface area in contact with the plate is minimized.

Record the number of times the liquid must be withdrawn and extruded to completely mix the solutions.
Step 27: Rinse the pipette tip in the rinse tube, being sure to withdraw and extrude 5 µL of purified rinsing water at least twice. Return the tip to the red precipitant tip baggie.
Step 28: Repeat step 26 for the 20% PEG-6000 solution two more times, for a total of 3 iterations of the extrusion mixing procedure. Use the next available position (skipping one experiment chamber along the second row) of the Mitogen plate for each iteration, and seal the row with the sealing tape once all three iterations are completed.

Mixing Procedure with the other Precipitants
Step 29: Repeat steps 10-28 using the next precipitant, 10% PEG-6000, performing 3 iterations of the stirred mixing procedure and 3 iterations of the extrusion mixing procedure. Note: When repeating steps 10 and 13, instead of using new pipette tips, reuse the pipette tips already in the water tip and precipitant tip baggies.
Step 30: Since this is the last time using the PEG-6000 solution, it is now necessary to deposit any pipette tips used into the waste baggie. The next portion of the procedure begins with a new precipitant (PEG-400) and will require new pipette tips.
Step 31: Repeat step 29 for the next precipitant, 30% PEG-400. As noted in step 29, be sure to start with new pipette tips, both for the water solution as well as the precipitant solution.
Step 31: Repeat step 29 for the 10% PEG-400 solution. As in step 30, deposit any tips used in steps 31 or 32 in the waste baggie before moving on.

Phase-2: The initial evaluation will proceed to phase-2 provided a viable procedure (using the Mitogern plates) is developed. Phase-2 involves testing the phase-1 experimental protocol using hen egg-white lysozyme. Hen egg-white lysozyme is non-toxic and available in 50 milligram quantities.

Ground Preparation: In an effort to determine initial conditions as quickly as possible, a small number of experiments are initially prepared prior to launch. This will also provide information regarding the amount of time necessary for

nucleation and sufficient crystal growth to enable microscopic observation to support informed decisions for subsequent experiments (i.e. iterative on-orbit optimization of crystallization conditions). The pre-launch procedure is described below.

Step 1: Purified protein solution (in buffered solution(s) is mixed (solutions are dispensed using pipettes similar to those to be used on the ISS) in separate vapor diffusion experiment chambers with different concentrations of crystallization solutions.

Step 2: A specified volume of different concentrations of reservoir solution is pipetted into the reservoir compartment of the Mitogen plate.

Step 3: The Mitogen plate is sealed by placing the clear plastic tape over the top of the plate.

Step 4: The experiments are immediately frozen at -80°C and once on the ISS these initial experiments are removed from the -80°C freezer and placed in an incubator maintained at +22°C or +4°C depending on the particular protein under investigation (for lysozyme it will be placed in the +22°C incubator).

Step 5: Crew removes each experiment from incubator every 24 hours for a total of 10 days, places experiment chamber in ISS glovebox or workbench for microscopic observation (10X to 30X magnification) to observe and photo-document crystals within the experiment chambers).

Step 6: Downlink crystal images for assessment by principal investigator (PI) followed by uplink of new chemical conditions to be prepared by crew.

Phase-2 Crew Procedure

Required Flight Hardware and Supplies (per protein sample):

1) ~ 10 Mitogen plates.
2) 10µl Eppendorf pipette; 20 pipette tips.
3) Each participating scientist will be required to prepare the following solutions: a) 5mL of concentrated stock solution of the crystallizing agent in the desired buffer, b) 5mL of the desired crystallization buffer, c) 1mL to 5mL of protein solution (at the desired protein concentration) in the crystallization buffer, d) 5 small vials each containing ~2.0mL distilled H_2O

Draft of Initial Flight Procedure for Pre-flight Prepared Experiments:

Step 1: Crewmember removes the bags of pre-prepared frozen (-80°C) Mitogen plates from freezer and places these bags in the +22°C incubator thereby activating crystallization.
Step 2: ~96 hours after activation, remove Mitogen plate bag(s) from +22°C incubator and transport them to the ISS glovebox.
Step 3: Place each experiment chamber under the microscope (i.e. Bioserve microscope, nanorack microscope or other available microscope - 10X to 30X is sufficient magnification) and observe results for subsequent downlink to ground station (any precipitate or non-clear solution or any crystals should be photo-documented). Identification of each Mitogen plate must be included in the photos (this can be accomplished via a bar code or just photograph the name of the PI and the name of the protein contained in each Mitogen plate prior to photo-documentation. At conclusion of observation and photo-documentation of all of the experiments, return bags containing Mitogen plates to the +22°C incubator.
Step 4: Crewmember downlinks all photos to the ground station. Downlinked images are analyzed by the scientists associated with each protein followed by each scientist recommending new volume combinations of the protein and crystallizing solutions to be prepared by the crew.

An example of the preparation instructions for any protein is shown below:
Iterative Crystallization Procedure:
Protein Name: Lysozyme
Identical to Phase I Setup

Setup
Step 1: Place and secure large Velcro bar VB1 on glovebox floor on the left edge of the working space. Place and secure large Velcro bar VB2 on the floor along the right edge of the working space.
Step 2: Remove one new Mitogen plate from stowage and secure it via attached Velcro onto VB1.

Step 3: Remove two strips of Velcro tape from stowage and press them onto the glovebox floor, near the middle. Ensure the spacing of the strips matches the spacing of the two Velcro strips on the bottom edges of Mitogen plate. Remove the Mitogen plate from VB1 and press onto the tape strips.
Step 4: Secure compound O-ring across posts of VB1.
Step 5: Remove the appropriate Eppendorf pipette from stowage (specified by PI). Attach a tape loop to the pipette handles and secure them on the glovebox floor within easy reach.
Step 6: Remove the Phase 2-Lysozyme bag from stowage. It should contain a blue-labeled bag of buffer tubes, a red-labeled baggie of precipitant solution tubes, a white-labeled baggie with a set of new pipette tips, a green-labeled protein tip baggie (empty) and a black-labeled used pipette tip baggie (empty).
Step 7: Remove all tubes from their bags and secure them onto Velcro bar VB2 using the Velcro attached to the sides of the tubes. The precipitant tip bag and protein tip bag will now be used as pipette tip repositories for their respective solutions.
Step 8: Secure the new pipette tip, precipitant tip, and protein tip baggies under the O-ring stretched across VB1. Secure the waste baggie somewhere in the workspace. As a reminder, the bags will be color-coded via colored tape: white for new tips, red for precipitant tips, green for protein tips, black for waste.
Step 9: Remove the zip lock bags containing lysozyme protein solution vials from the freezer or storage and secure them inside the glovebox.

Crystallization Procedure Template

Step 10: Using the appropriate pipette, withdraw the specified crystallizing (precipitant) solution volume (specified by the PI) and extrude the solution into the reservoir portion of the Mitogen plate.
Step 11: Rinse the pipette in the rinse water vial. Be sure to withdraw and extrude rinse water to thoroughly clean the inside of the tip.
Step 12: Withdraw the specified amount of buffer and add it to the crystallizing agent in the Mitogen plate reservoir. Mix the two solutions as specified (this will be determined via the phase-1 results).

Step 13: Rinse the tip in the rinse water vial once more and deposit into the precipitant baggie.
Step 14: Attach a new pipette tip and withdraw the specified amount of protein solution from the protein solution tube.
Step 15: Extrude the protein solution into the protein well in the Mitogen plate. Deposit the tip in the protein tip baggie.
Step 16: Using the pipette tip in the precipitant baggie, withdraw a volume (as specified by the PI) of the mixed reservoir solution and slowly extrude it into the protein solution while gently stirring the drop with the tip of the pipette.

Experiment Procedure

Step 17: Repeat the crystallization procedure as many times as specified by the PI, changing the precipitant, buffer, or solution concentrations as specified in the experiment.
Step 18: After completing one row of the Mitogen plate, press a strip of sealing tape onto the row (thereby sealing all of the experiment chambers in the row). This is repeated for each row once the solutions and mixing process is completed for each experiment chamber in the row.
Step 19: Store the Mitogen plates in the appropriate incubator (+4°C or +22°C depending on the specific protein requirements).

Appendix 1. MiTeGen In Situ-01 Crystallization Plates

Crystal Growth

MiTeGen

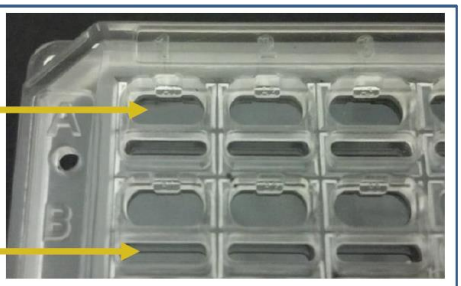
In-Situ-01™ Crystallization Plate

PRODUCT HIGHLIGHTS:

- Low Profile 96-well SBS format
- Screening or Optimization
- Used as Sitting or Hanging Drop
- Highly UV and X-ray Transparent
- Optimized for *in-situ* X-ray screening
- Low birefringence
- Ideal for transporting crystals

Protein Drop Areas:
1 – 6 drops
(2 µl – 50 nl)

Reservoir Wells:
40 µl

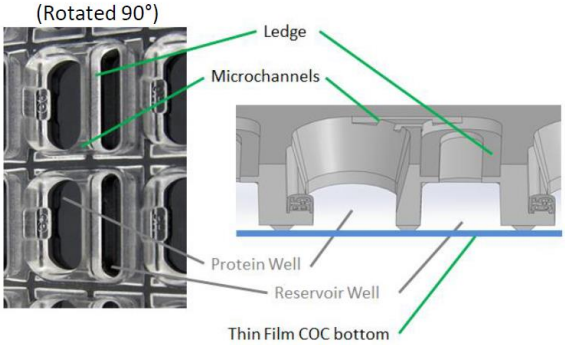


Patent Pending Design Features:

Vapor Communication Microchannels

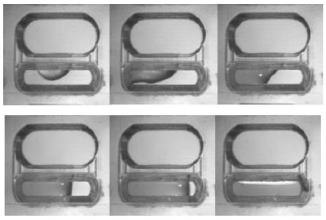
- Allow vapor equilibration between reservoir and protein well, at the same rate as in existing plates.
- **Strongly inhibit liquid transfer** between reservoir and protein wells due to, e.g., rough handling, shipping.
- Plate can be **rotated to any orientation** without liquid transfer out of the reservoir.

(Rotated 90°)



Reservoir Well Liquid retention ledge

- **Prevents liquid motion** out of the well and
- Ensures **uniform well filling**, for larger reservoir volumes in a low profile plate.



Thin Film Bottom

- Ultrasonically welded thin film bottom has the lowest X-Ray diffraction and best UV transparency of any plate.

Copyright© MiTeGen 2013

In-Situ-01™ Crystallization plate

IN-SITU PERFORMANCE:

HEWL crystals were grown in the plate and *in situ* X-ray examination was performed at CHESS. The same crystals were harvested from the plate, placed inside 10 micron-wall quartz capillaries, and again examined by X-rays.

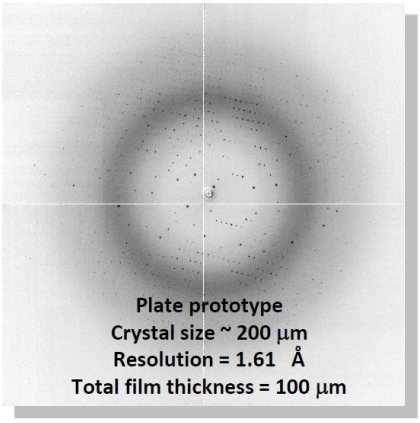
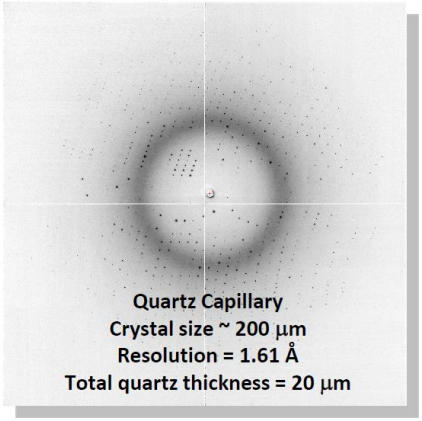


Plate prototype
Crystal size ~ 200 µm
Resolution = 1.61 Å
Total film thickness = 100 µm



Quartz Capillary
Crystal size ~ 200 µm
Resolution = 1.61 Å
Total quartz thickness = 20 µm

Versatility:

Screening or Optimization, Sitting or Hanging

Two 1 µl sitting drops (populated by hand using P2 pipette)

Six 50 nl hanging drops (populated by Labcyte's Echo Liquid Handler)

TECHNICAL SPECIFICATIONS

Frame	
Material	COC (injection molded)
Length	128 mm
Width	85 mm
Height	9.2 mm
Wells	96
Protein drops/well	Single or Multi
Protein drop size	Up to 2 µl
Reservoir volume	Up to 45 µl

Thin film bottom	
Material	COC (rolled film)
Thickness	100 µm

MiTeGen
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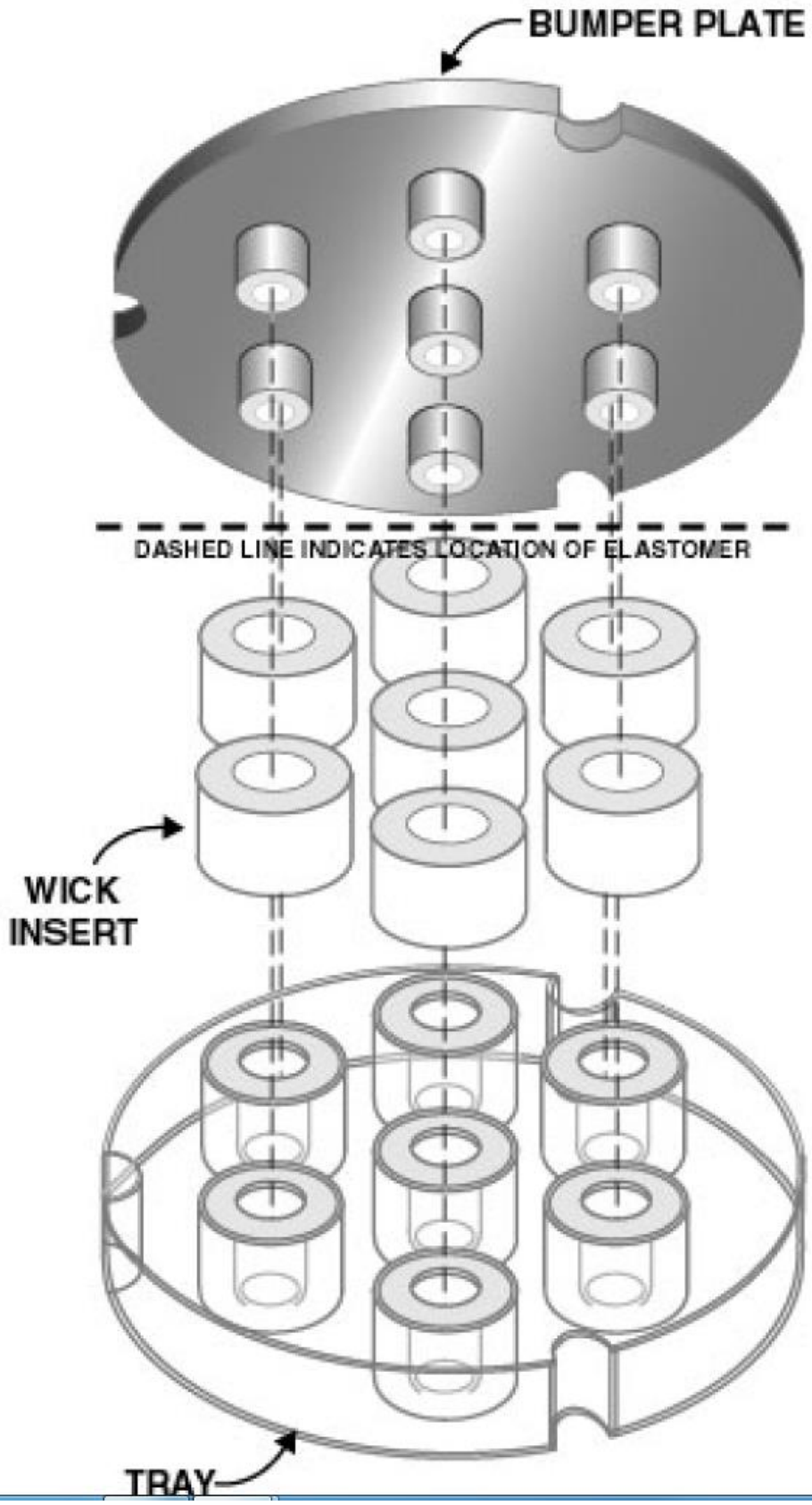
Suite 183
Ithaca, NY 14850
USA

Toll Free: 1-877-648-3436 (US)
Tel: 1-607-266-8877
Fax: 1-607-697-0400
Email: info@mitegen.com

Appendix 2. PCAM



PCAM Tray

Solution droplets are place in wells at the center of each of the seven tiny cups in a PCAM tray. The "motes" around the wells are filled with absorbent material, and an elastomer layer seals the top of the tray. **Note** – for the RTPCG experiment, the tray will be covered with a transparent seal until the protein crystals are ready to be brought back to Earth.

Appendix 3: Granada Crystallization Hardware

Expedition Three Flight Engineer Vladimir N. Dezhurov unpacks a bag with the Granada Crystallization Facility in the Zvezda

The Granada Crystallization Box (GCB) consists of four elements made of polystyrene:

- A reservoir to introduce the gel
- A guide to hold capillaries
- A cover
- A holder to maintain the boxes

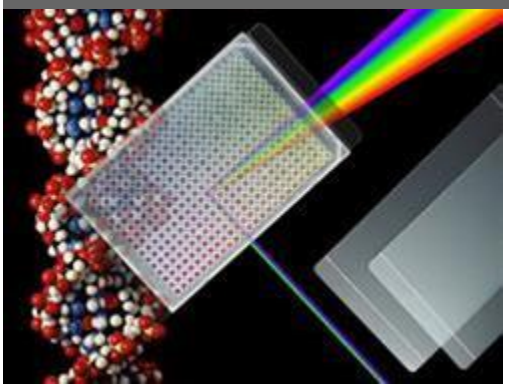
The GCB has been designed to be used in four different ways:

- To grow crystals inside gels under diffusion controlled mass transport.
- To grow protein crystals inside capillaries with un-gelled precipitating agent by the counter-diffusion technique.
- To grow protein crystals inside capillaries with gelled precipitating agent by the counter-diffusion technique.
- To grow protein crystals inside capillaries by the batch method.

Appendix 4: Sealing Film

Crystal Clear Sealing Film

Crystal Clear Sealing Film



Applications

- Sealing film used to seal sitting drop crystallization experiments

Features

- Fits SBS format microplates
- No special applicator required
- Optically clear

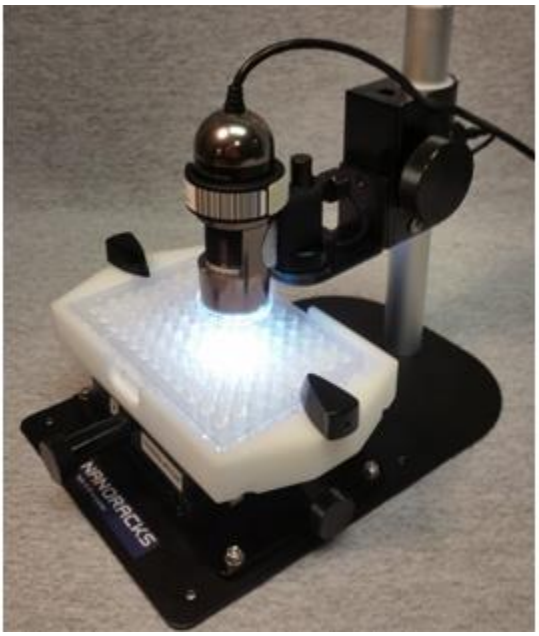
Description

Crystal Clear Sealing Film is an optically transparent sealing film for SBS format 24, 48, 96 and 384 well plates. The 2 mil (0.05 mm) thick, optically clear, non-fluorescing polyester film with 1 mil (0.025 mm) thick acrylic custom adhesive is designed for the convenient and secure sealing of crystallization plates. A split, optically opaque polyester backing with two end tabs assures a clean uniform adhesive layer and allows for easy, accurate positioning on the plate. Before use, peel off the opaque center protective polyester backing to reveal the optically clear sealing film. Film length: 5.625" Film width: 3.125" End tabs: 0.375" Perforations: 0.415" from edge of film Corner radius: 3/32". DNase-, RNase-, and nucleic-acid-free, non-sterile. Recommended temperatures from -40 to +120°C. Not recommended for UV imaging.

ITEMS

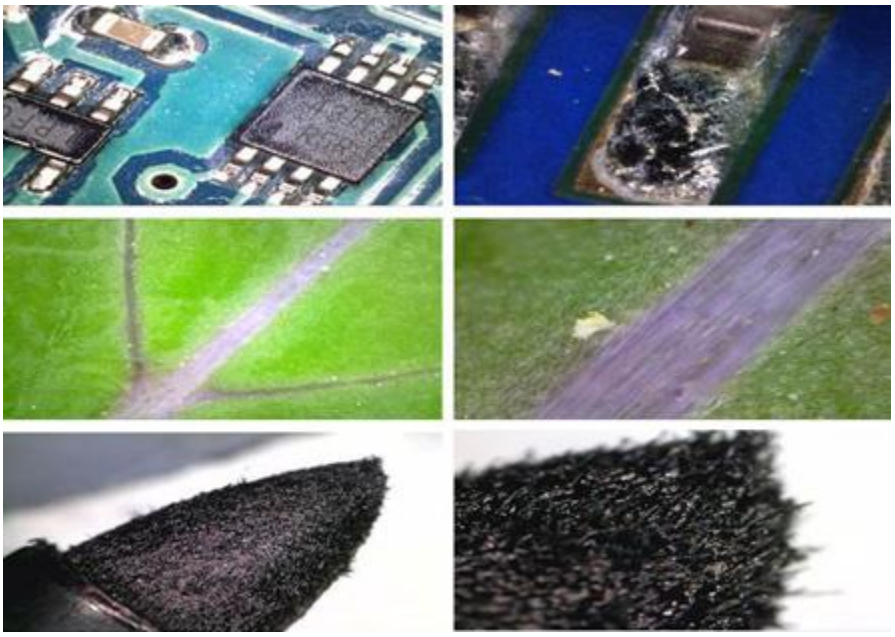
CAT NO	NAME	DESCRIPTION	Price	Quantity
HR3-609	Crystal Clear Sealing Film	100 pack	$140.00	

Appendix 5. NanoRack microscope available on ISS



NanoRacks Microscope

NanoRacks Microscope-3 with sample holder

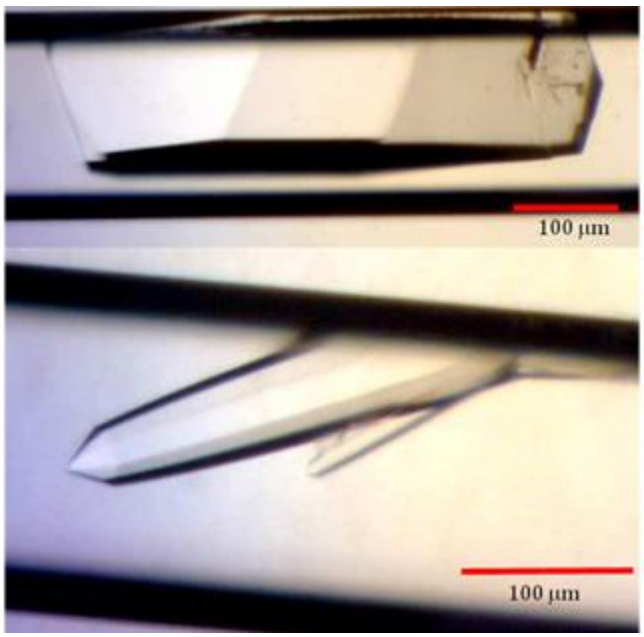


NanoRacks Microscope

Example images from NanoRacks Microscope-3

NanoRacks Microscope

Customer plant incubation chamber image



NanoRacks Microscope

Protein crystal growth microscope image

Appendix 6: NIKON microscope available on ISS

A new standard for inverted microscopes defined by bright high-resolution images and unrivaled usability

Binocular type model TS100

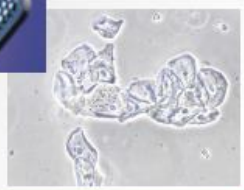
Trinocular type model TS100-F

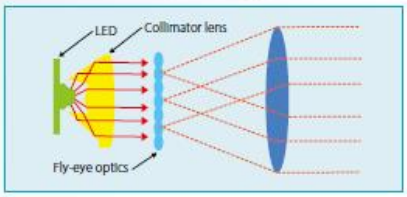
The compact high-performance inverted microscopes ECLIPSE TS100 and TS100-F use Eco-illumination, a newly developed LED illumination. Eco-illumination provides enough brightness for phase contrast and NAMC observations. With a fly-eye lens, uniform brightness is provided in the entire field of view. LEDs are an environmentally friendly low-power-consumption light source. Eco-illumination provides a long lifetime of 60,000 hours and reduces the frequency of lamp replacement. A halogen illumination model is also available.

Nikon's highly acclaimed CFI60 optical system is used, providing flat, sharp and clear images, while achieving longer working distances and higher numerical apertures.

The space-saving body is robust and vibration-resistant, enabling safe specimen observations. The microscope controls are designed for ease of use. The ECLIPSE TS100-F comes with a photo port and is compatible with a variety of cameras. The ECLIPSE TS100 and TS100-F ensure high-quality observations for various areas, such as cell culture, inspections and research.

Fly-eye lens





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BioServe Nikon Microscope

Appendix 7. Available Pipets on ISS

Rainin Positive Displacement Pipettes: Biomolecule Sequencer	
OpNom	**Part Number**
Pipette Kit	**BS-P-KIT**
10 ul Pipette	BS-P-10
10 ul Pipette Tip	BS-PT-10
100 ul Pipette	BS-P-100
100 ul Pipette Tip	BS-PT-100
1000 ul Pipette	BS-P-1000
1000 ul Pipette	BS-P-1000

Traditional Eppendorf Pipettes: **Genes in Space-3**	
OpNom	**Part Number**
Genes Pipette Kit	**BS-PE-KIT**
20 ul Pipette	BS-P-20
200 ul Pipette	BS-P-200
20 – 200 ul Pipette Tip	BS-PT-20-200
1000 ul Pipette Genes	BS-PE-1000
1000 ul Pipette Tip Genes	BS-PET-1000