



National Aeronautics and
Space Administration
Marshall Space Flight Center



Lab-on-a-Chip Based Protein Crystallization

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Why Protein Crystallography?

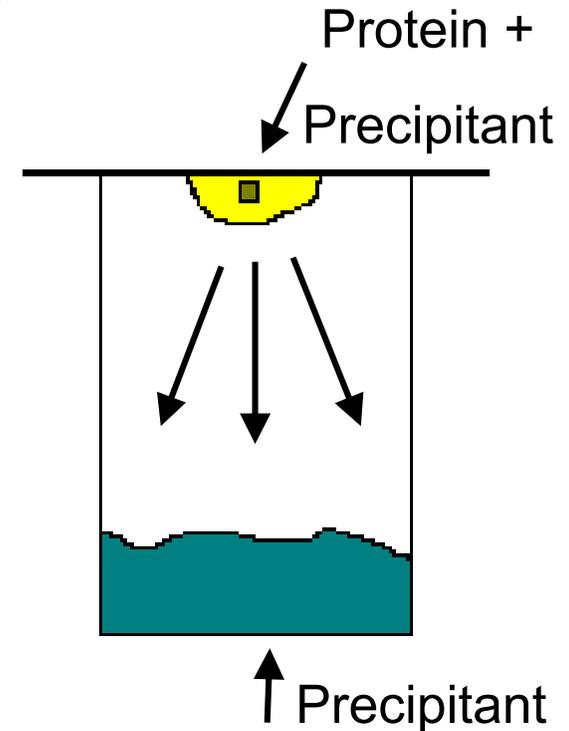


- Protein crystallography provides **access to structural information** for macromolecules (DNA, proteins).
- This **structural information** helps scientists to understand the **function** of the the macromolecular system.
- Understanding structure-function can help scientists **block** or **enhance** the function of a system.
- Examples:
 - Enhancing or copying the function of insulin for treatment of diabetes
 - Blocking the function of critical enzymes in parasites such as trypanosomes that cause Chagas' disease.
- Problems:
 - Growing protein crystals is very difficult—crystallization must be done by trial and error and is the rate limiting step in obtaining structures.
 - Protein crystals are inherently fragile and weak X-ray diffractors. Obtaining detailed information about protein structure is difficult.



How Are Protein Crystals Grown ?

- Proteins must stay hydrated in order to be in their active, native form.
- Proteins are crystallized in a closed system where the protein is made gradually less soluble by adding precipitating agents.
- This is done most frequently using the hanging drop method.
- Hanging drop experiments are traditionally set up in a Linbro tissue culture plate.
- In any structure determination typically several thousand conditions are tried.





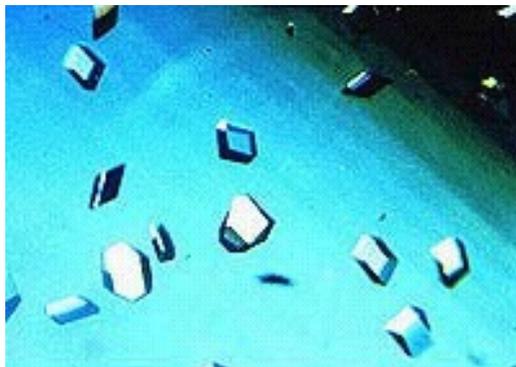
Why Grow Crystals in Space?



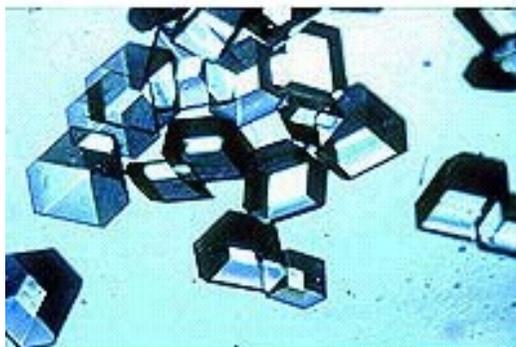
- Some crystals grow to a **higher perfection** in **microgravity** and therefore we can get more data from these crystals.
- **More data** means **more detail** about the protein under investigation.
- Example: Insulin crystals and the data from these crystals after processing.
- Problem: every experiment in space is a one-shot approach with a repeat time of 6-12 months



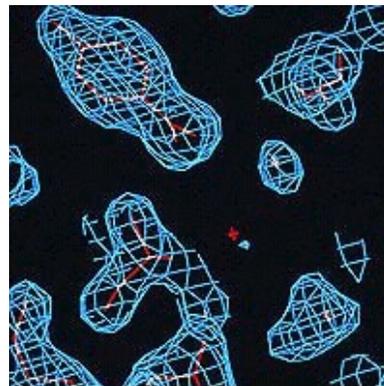
Example of Improved Crystal Quality in Microgravity



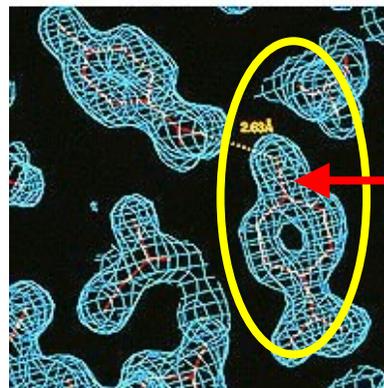
Earth Grown Insulin Crystals



Space Grown Insulin Crystals



Drug is Invisible



Drug is Visible

Reference:

"Crystallographic Studies of Insulin Crystals Grown in Microgravity", G. David Smith, Ewa Ciszak and Walter Pangborn, Proceedings. Conference on ASA Centers for the Commercial Development of Space, M.S. El-Genk and S. Whitten, eds. American Institute of Physics, New York, AIP Conf. Proc. No. 325, 177-182, 1995.



Problems Encountered in Space



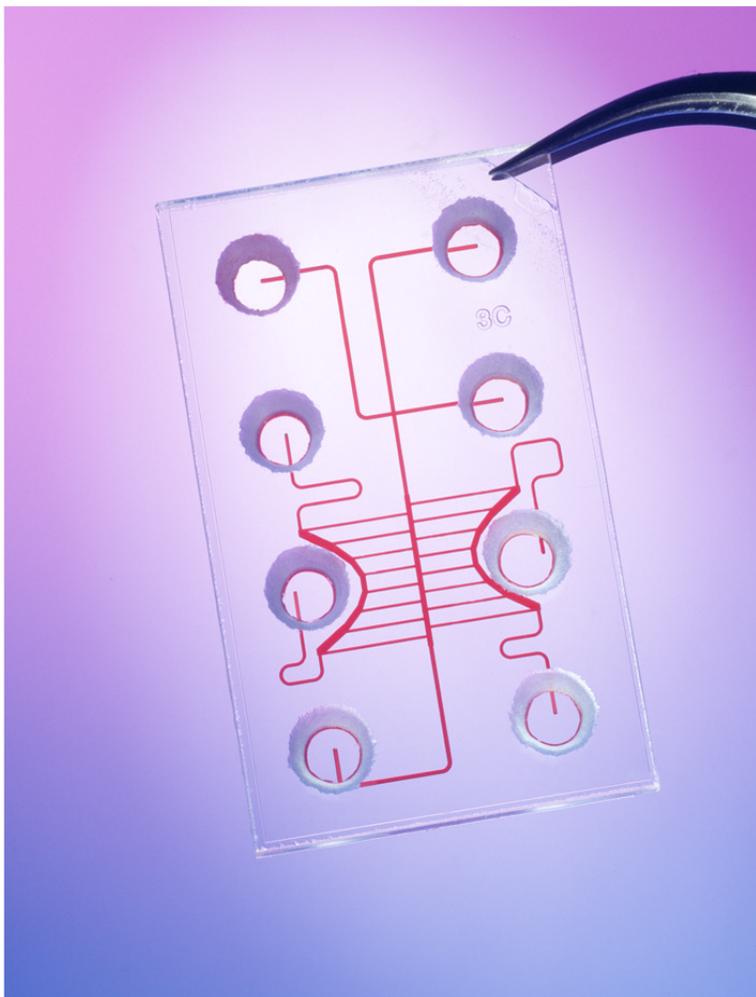
- Mixing of liquids is difficult
- Air bubbles must be avoided
- Space, weight and power are limited
- Little opportunity for human interaction with experiment
- Cannot produce much waste
- Must be safe

Idea: Try LabChip[®] Technology.



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LabChip[®] Microfluidic Device



Customized Networks

Active Fluidic Control

Sealed Environment

Assembly Line Processing

Small volume

Highly accurate

Application Can be automated



Dimensions of Customized Microfluidic Network



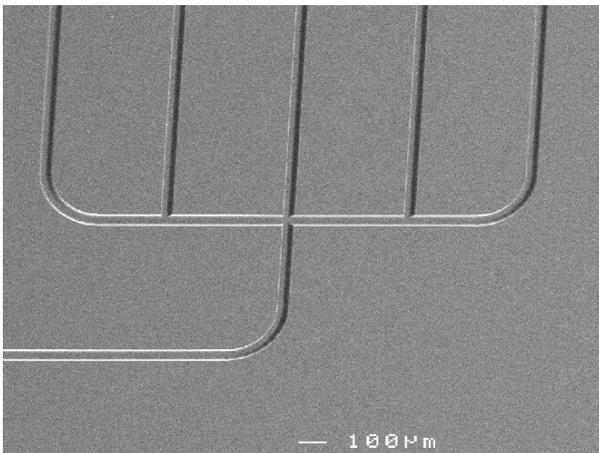
- **Channel: 10 - 25 microns deep**
- **Channel: 25 - 100 microns wide.**
- **1mm of such a channel holds approximately 1 nanoliter of fluid.**
- **Both electrokinetic and pressure gradients are used to actively control fluid and material movement inside the network.**
- **Chip formats:**
 - Planar—research and development**
 - Sipper--high throughput screening**
 - Multisipper--higher throughput**



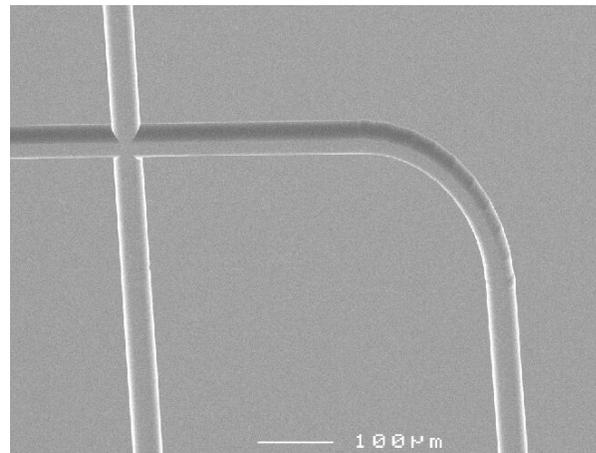
Photolithography & Etching



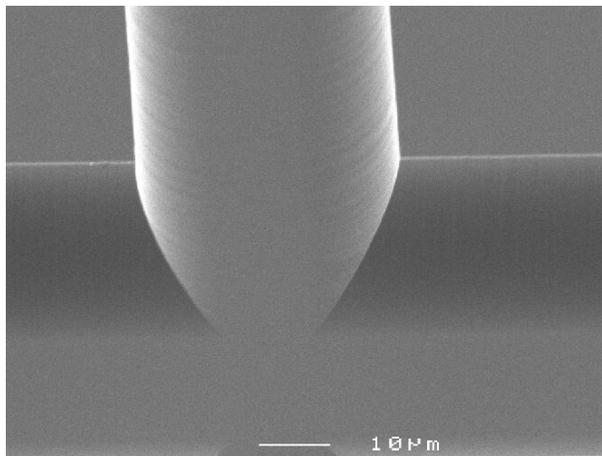
Size Bar 100 μm



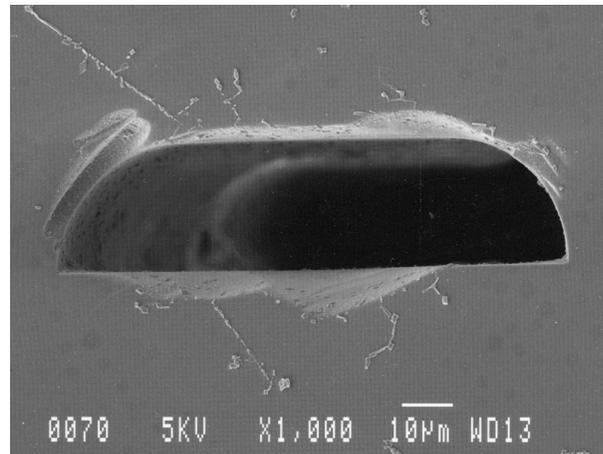
Size Bar 100 μm



Size Bar 10 μm



Size Bar 10 μm



Manufacturing procedures borrowed from the semiconductor world produce highly reproducible devices.



Crystallization in Space



Purpose of Iterative Biological Crystallization

IBC will establish the capability aboard the International Space Station that will enable an iterative aspect into macromolecular crystallization in a microgravity environment.

- This facility will be the first that will allow for iterative experiments in space.
- It will be largely automated.
- It will allow for remote observation and manipulation



(COURTESY NASA)

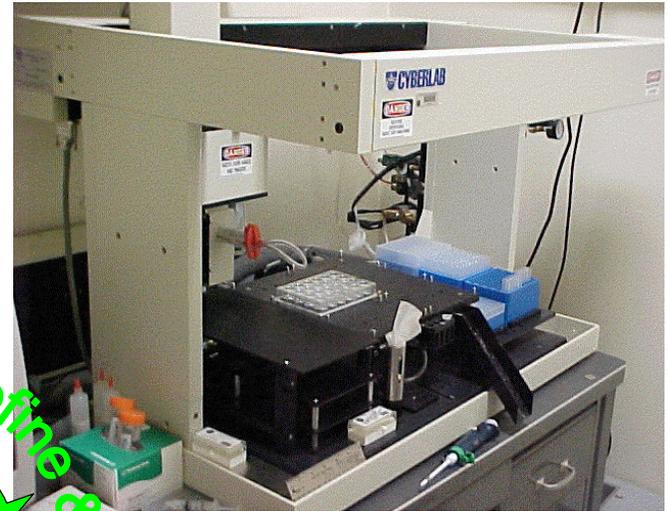


Iterative Biological Crystallization

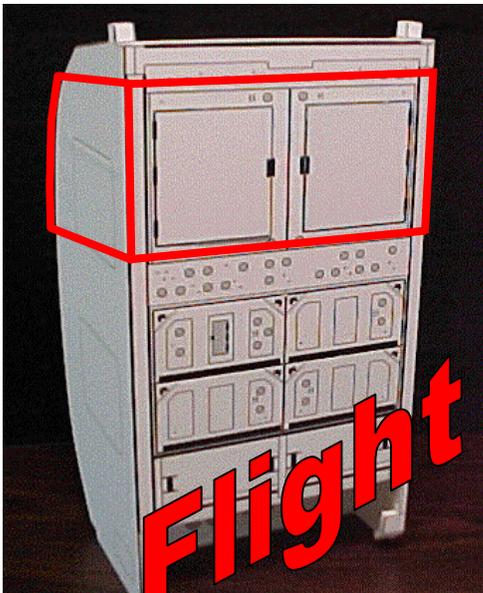


Initial

Traditional,
Manual
Pipetting
Method

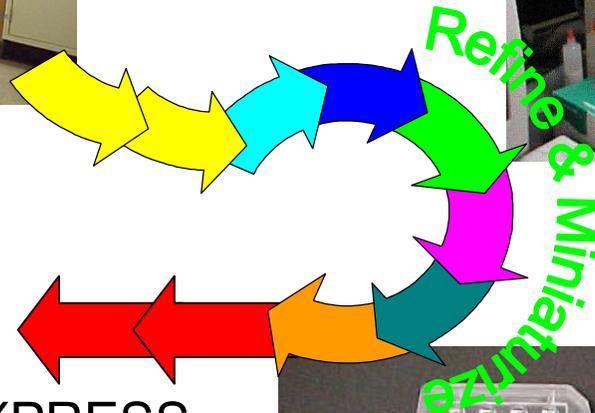


Laboratory Robot
(3 ft x 4 ft x 5 ft)



Flight

EXPRESS
Rack
Volume
(Two @ 17x
20 x 20 in)



LabChip®
Device



Iterative Biological Crystallization



Goal of Iterative Biological Crystallization

The facility will use automated systems, receive instructions from scientists, and prepare sample solutions accordingly. These solutions are to be placed into growth cells, incubated, and remotely observed for results. Selected crystals will be harvested and/or returned to Earth for further study.

- At least 1500 samples per 105 day time period,
- “Wet Bar” with up to 8 solutions per experiment,
- Minimize the amount of solution, sample material and waste,
- Vapor diffusion, batch, and liquid-liquid diffusion crystallization techniques,
- Near real time imaging to assess crystallization,
- Ability to alter and maintain defined growth conditions,
- Remote control of automated mixing/dispensing,
- Minimize inclusion/formation of bubbles in solutions.



Iterative Biological Crystallization



LabChip®

LabChip® investigations with Caliper Technology:

- Determine fluid flow capabilities through micron size channels
- Determine proper fluid mixing of solutions
- Demonstrate crystals can grow in small solution quantities
- Determine if crystals of viable size can be grown (x-ray diffraction)
- Determine potential advancements for chip design to enhance IBC capabilities.



Feasibility Study



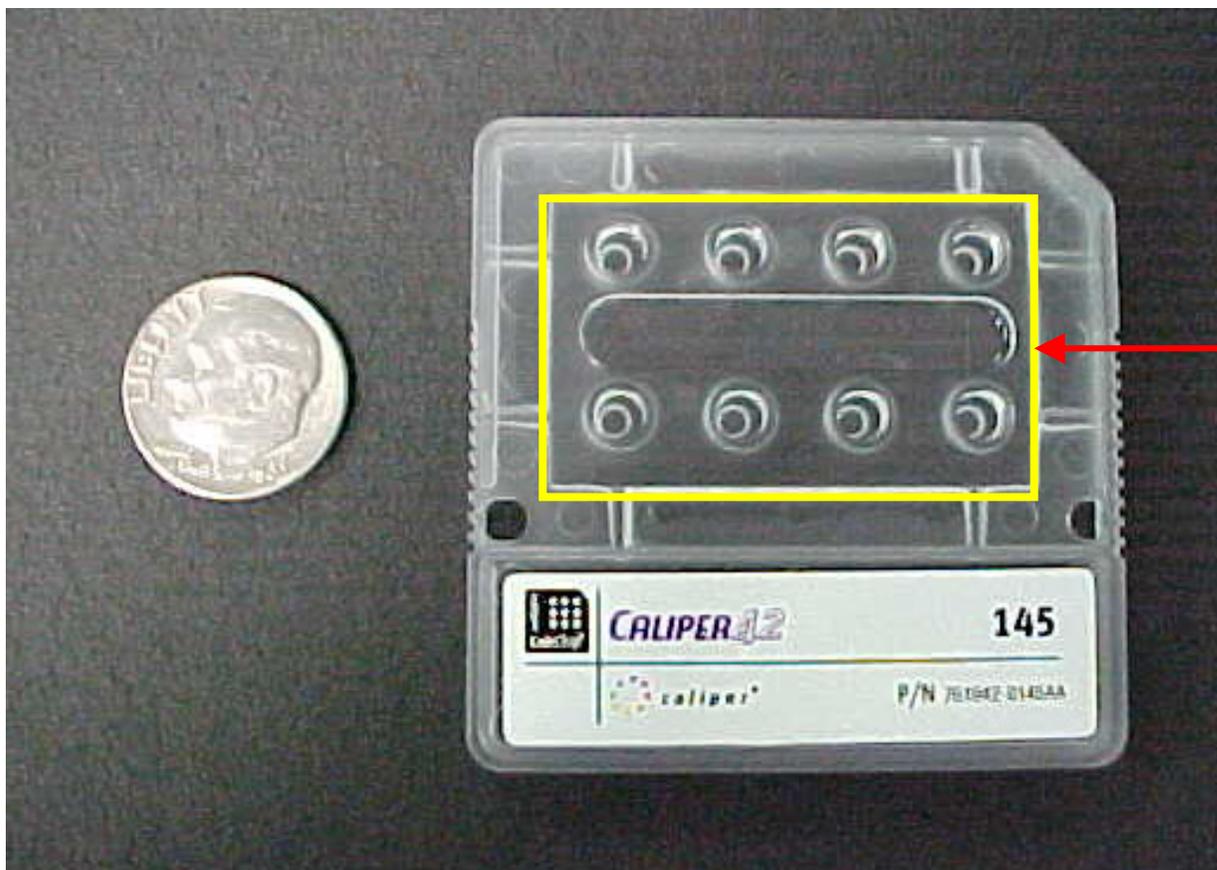
Two Questions:

- Is it possible to grow crystals in a very small volume in a LabChip® device? Specifically, is crystallization volume dependent and will the number of nuclei drop with decreasing volume to numbers so small that no crystallization is observed?
- Is it possible to adequately mix and dispense protein and precipitant solutions, as used in crystallization, in a LabChip® device ?



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Use LabChip[®] Technology to grow protein crystals



Actual
Chip

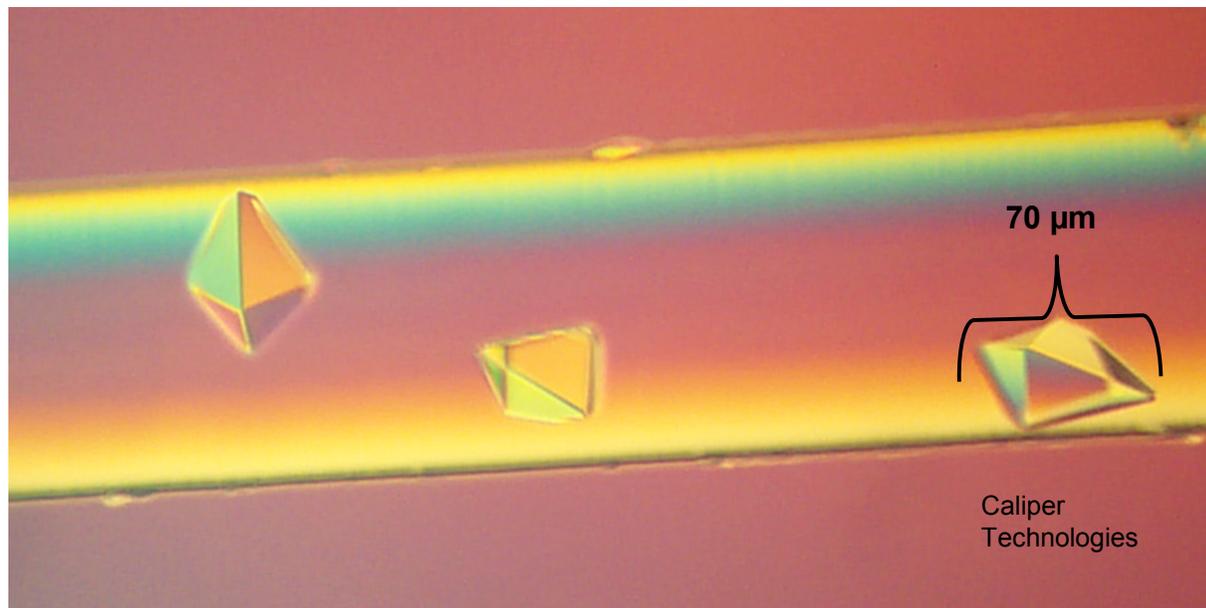


Crystallization in Channels



Experiment: pre-mix a protein and a precipitant, suitable to grow crystals without intervention. Grow crystals in channels on a LabChip[®] device.

Thaumatococcus
crystals in a
channel $50\mu\text{m}$
 \times $141\mu\text{m}$





1st Conclusion



Protein crystals in a LabChip[®] configuration have been demonstrated on a nano-liter scale. Visually the crystals appear to be representative for the protein used. Crystallization of proteins on a very small scale is therefore, in principle, feasible.



Feasibility Study



Two Questions:

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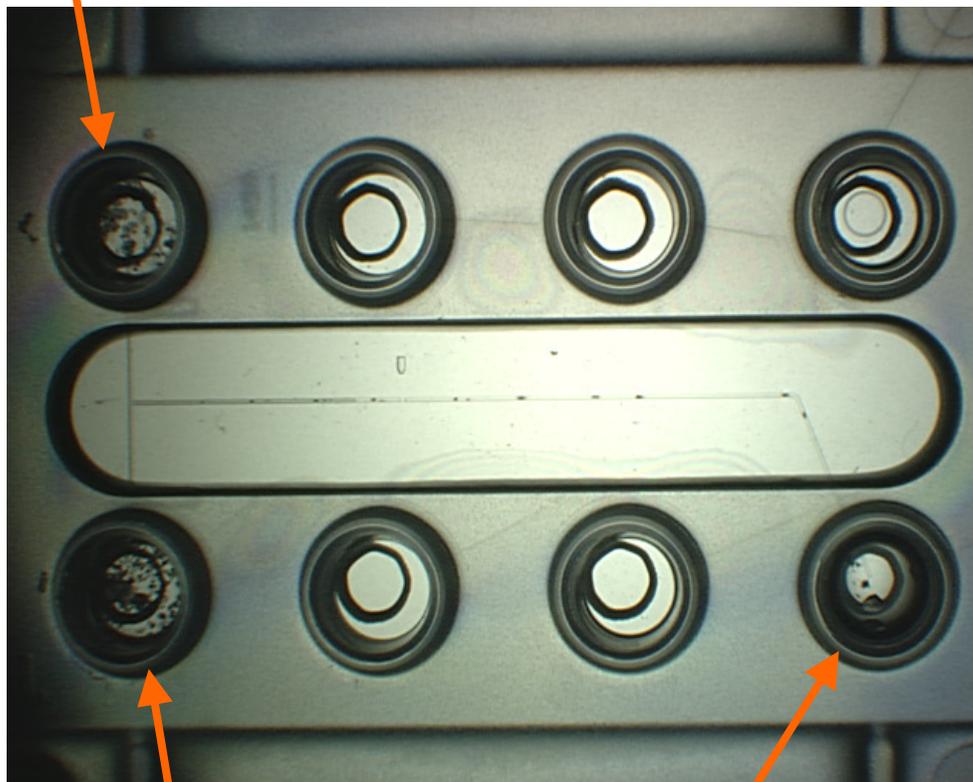


Crystal Growth on Chip



This standard development chip has 3 active wells and channels connecting them. The channels include a T-shaped intersection. The method of growth is batch.

Precipitant

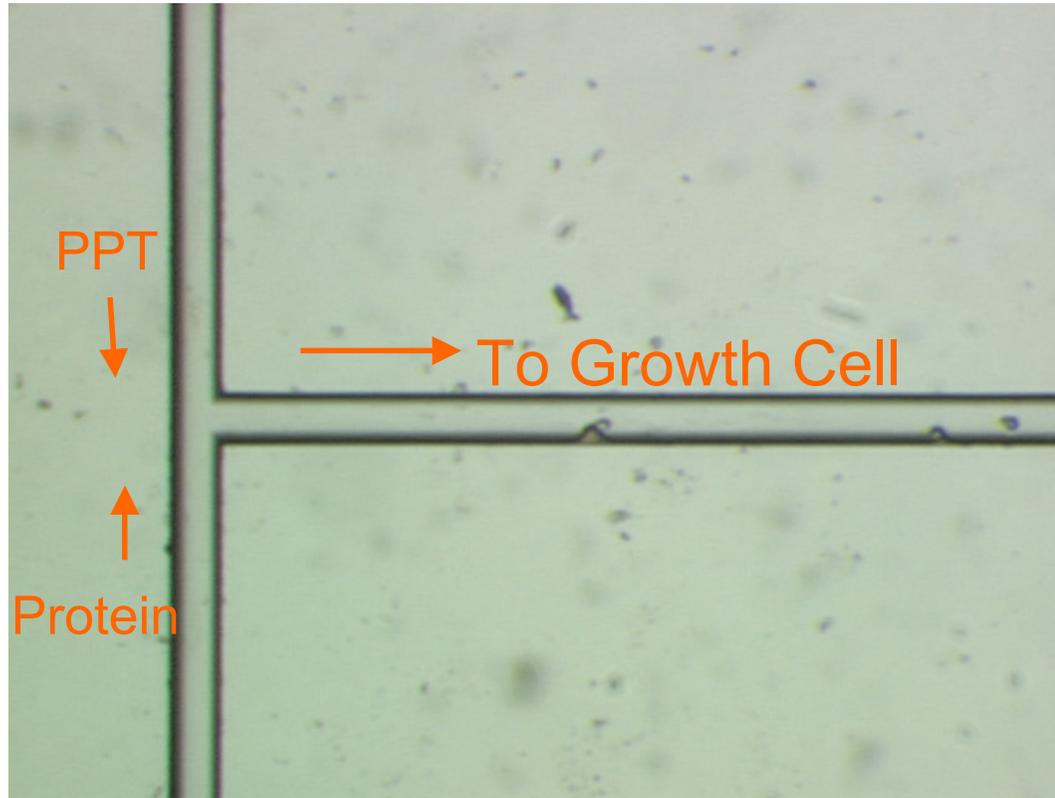


Protein

Growth Well



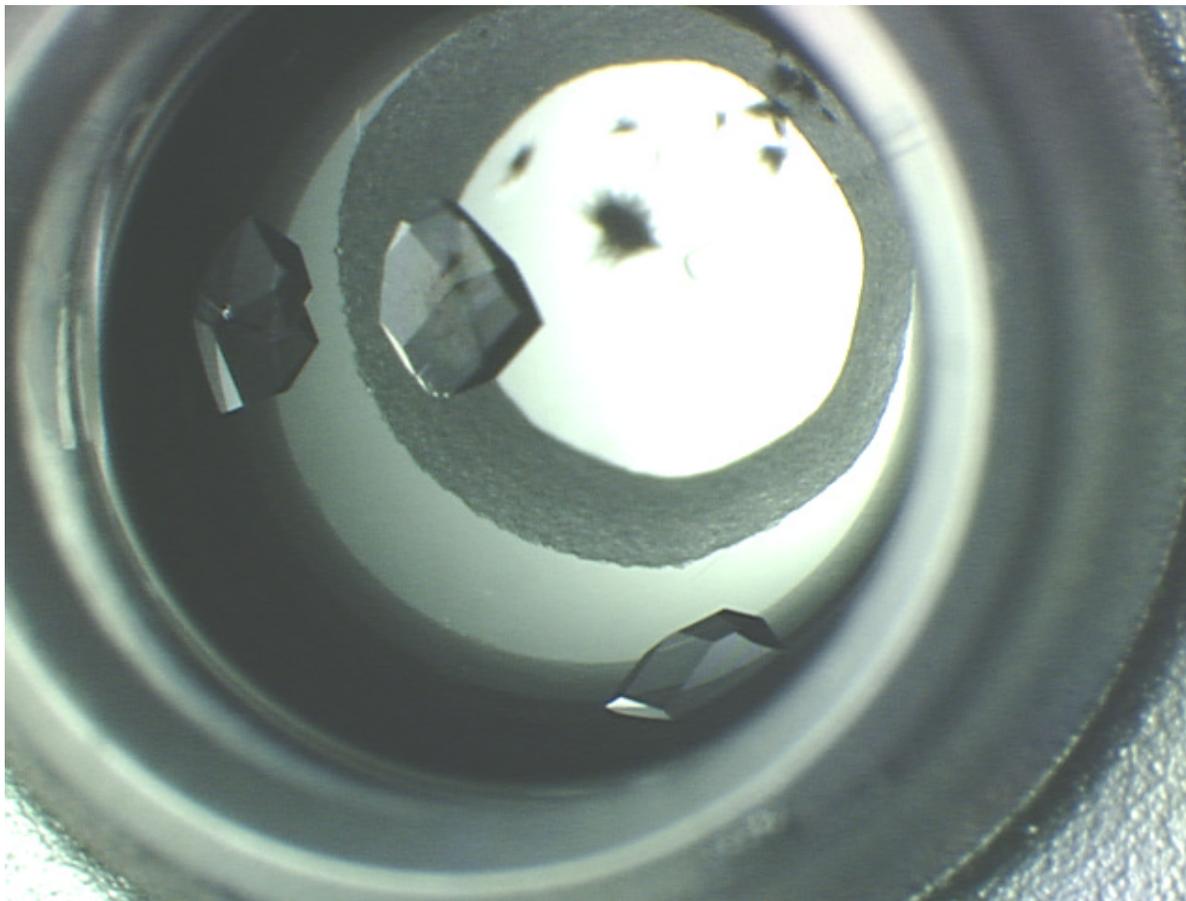
Mixing on Chip



The liquids are moved by a small pressure gradient, and exact amounts of liquid were mixed and deposited into the receiving well, which had the function of a growth cell. Mixing of solutions takes place by means of diffusion across a narrow channel.



Mixing on Chip

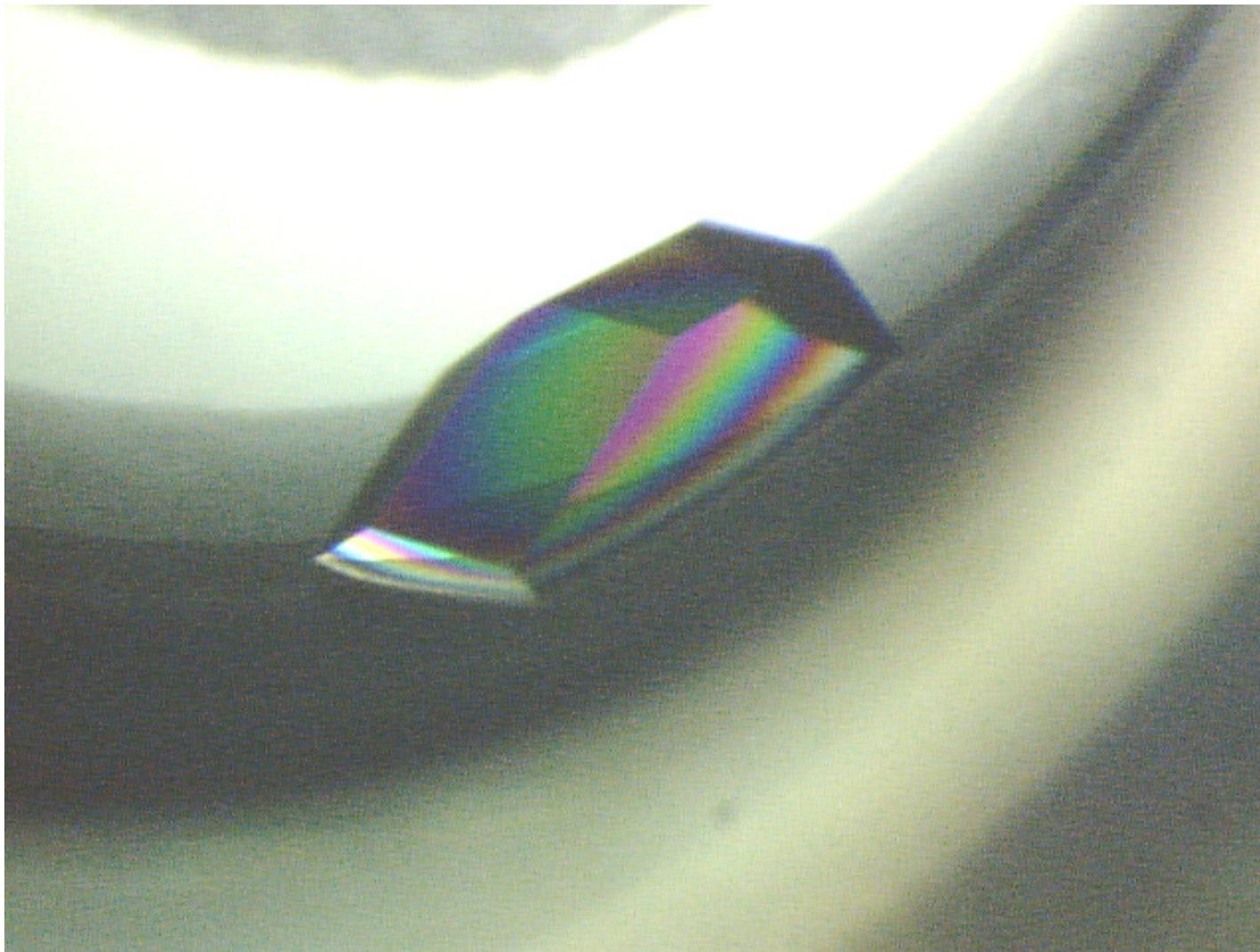


The size of these crystals is $\sim 0.7 \times 0.7 \times 0.4$ mm—perfect dimensions for analysis.



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Mixing on Chip

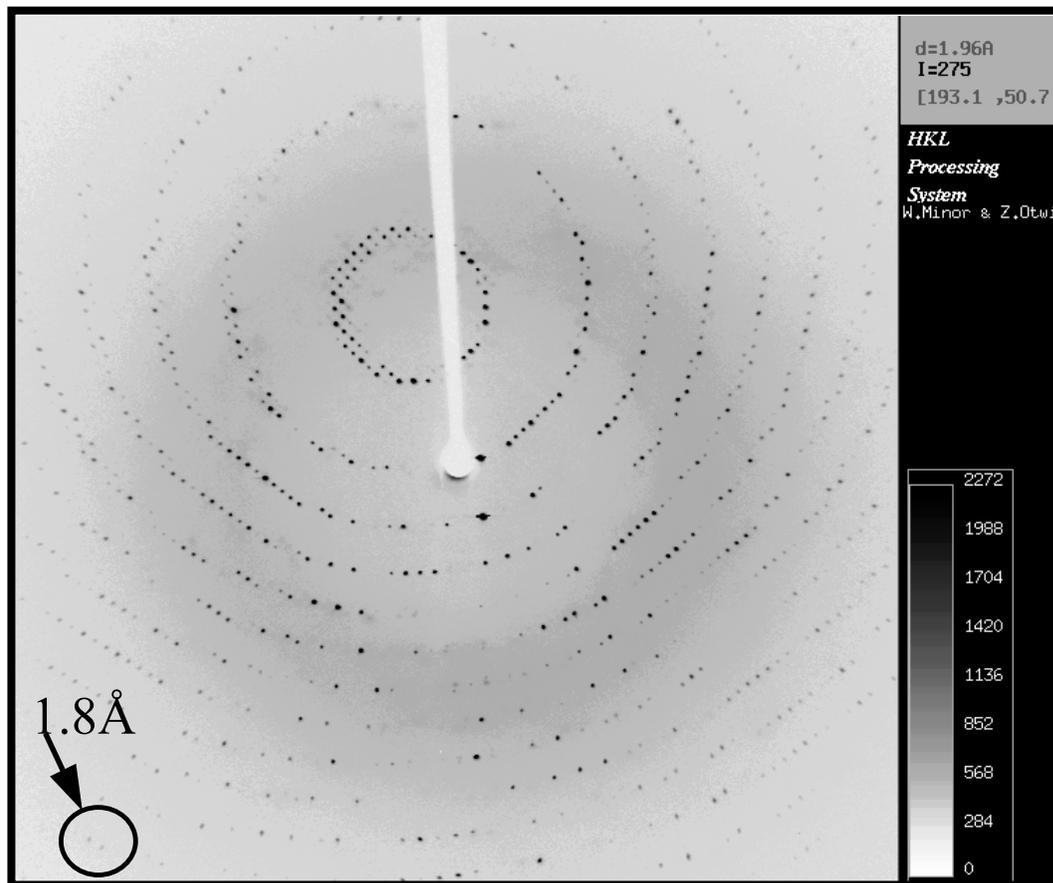




X-ray Data



X-ray diffraction image from the crystal shown in the previous slide. The diffraction spots will ultimately provide the information necessary to resolve the structural information. This image shows that the crystal was of good quality because data points at 1.8Å resolution can be distinguished. The true limit of information for this crystal is actually better than 1.8Å.





2nd Conclusion



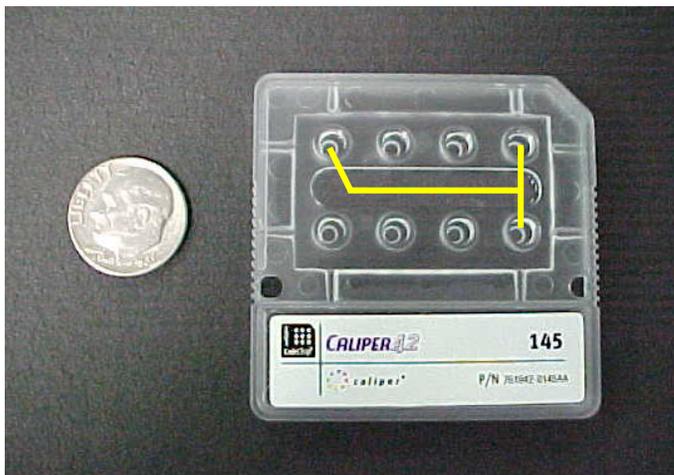
Solutions suitable for protein crystal growth can be dispensed, mixed, and delivered on a LabChip® device. It is possible to grow and harvest single protein crystals, appropriate for X-ray analysis.

The crystals are comparable in quality to crystals grown by 'traditional' methods.



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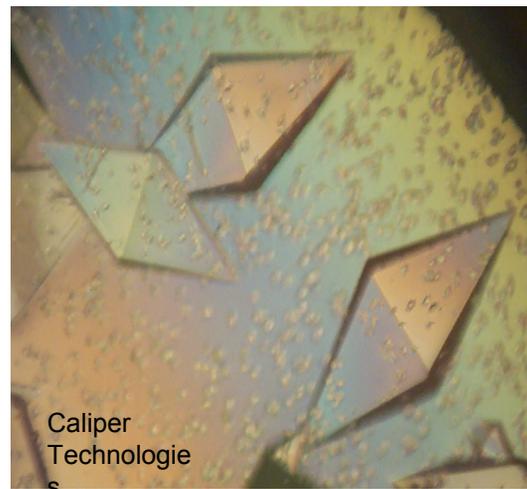
Summary of Experiments



Crystal
Growth
In Well

↓

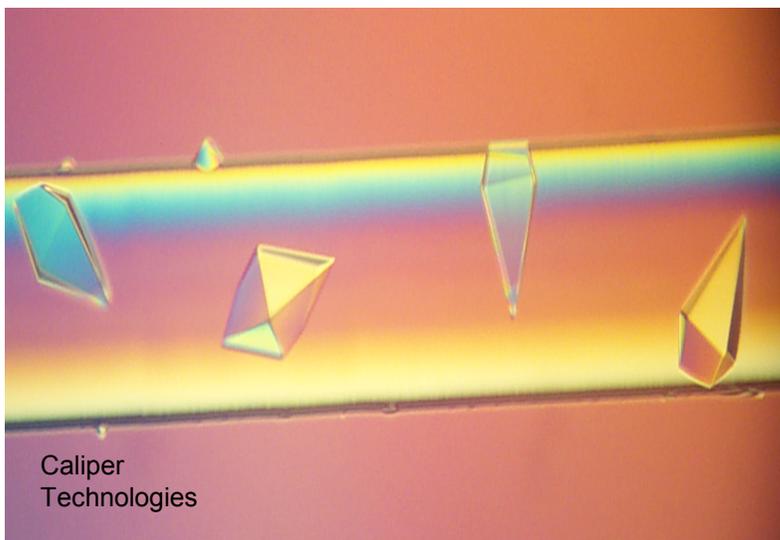
Crystal
Growth In
Channel



Thaumatin Crystals
300 μ m

X-Ray
Diffraction
Analysis

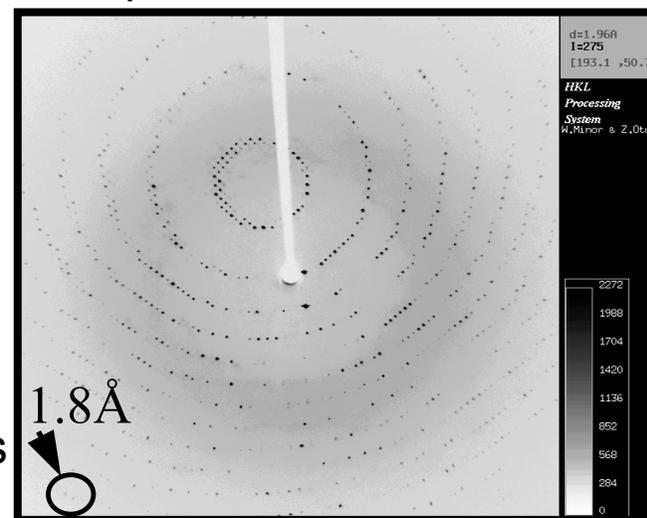
↓



Thaumatin Crystals – 100 μ m

→

Comparable
Quality To
Existing Crystal
Growth Methods





Future Developments



- Remotely operated liquid handling
 - Mixing and dispensing system
 - Crystal growth cells
 - Incubation area with temperature control
 - Optical system for observation and crystal detection
 - System to eliminate vibrations from environment ?
 - Is a method to eliminate vibrations from the environment necessary?
-
- System to preserve crystals ?
 - System to harvest crystals ?

} LabChip® ?



Acknowledgements



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- Two teams were involved in this study. At NASA's Marshall Space Flight Center, the IBC development team, and at Caliper Technologies, a team supporting the Applications Developer Program
- Individuals:
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 - Andrea Chow (Caliper)
 - Cheryl Cathey (Caliper)
 - Erik Gentalen (Caliper)