MicroMounts: Crystal Handling and Mounting in the High-Throughput Era

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Thanks to:

NASA, NIH

Mounting Crystals for Flash Cooling and Low-Temperature X-Ray Data Collection

Present Technology: Nylon Loops

1. Difficult to handle crystals smaller than 50μm

2. Irreproducible hole size and shape

3. Undesirably flexible

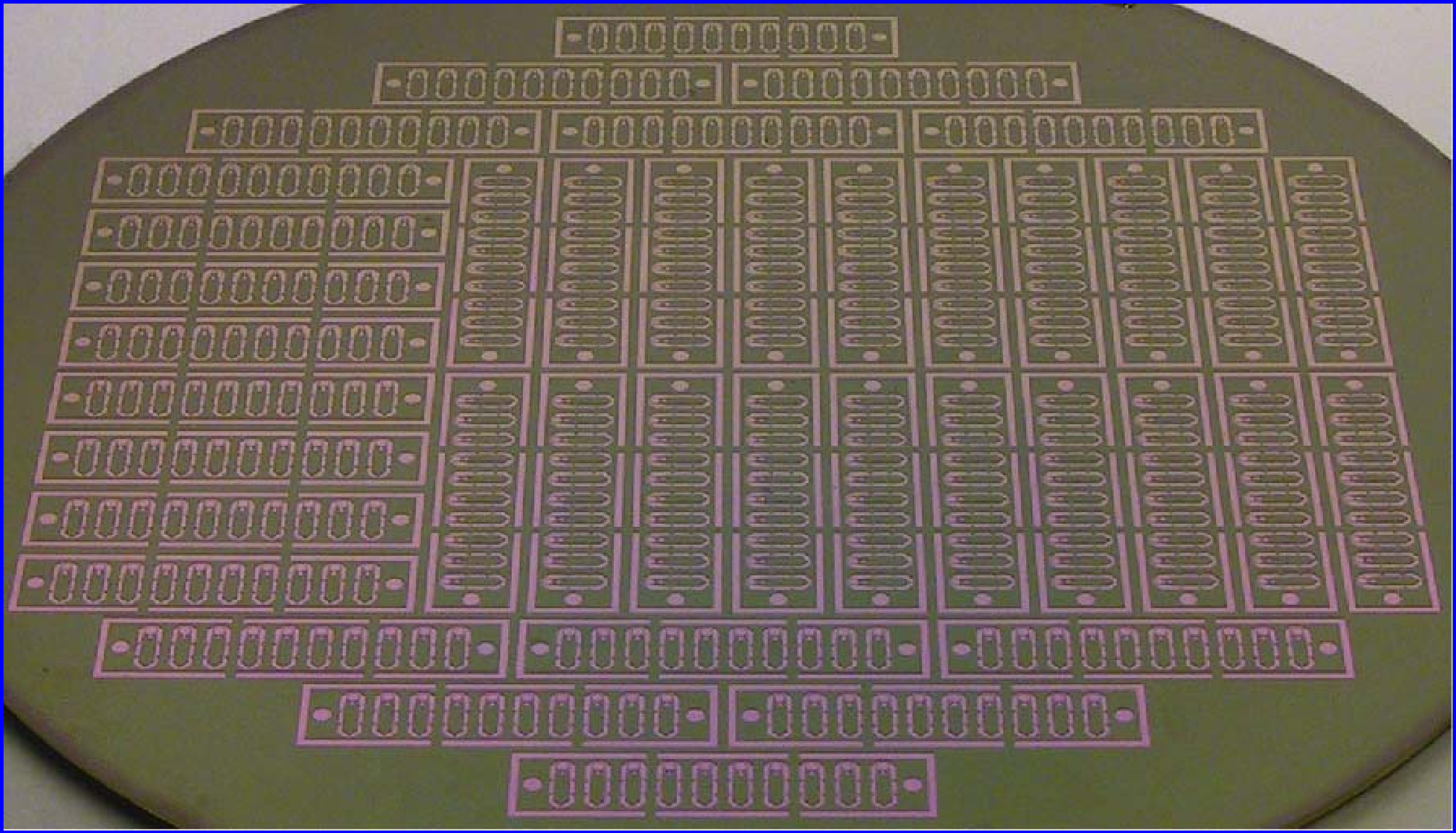
4. Difficult to retrieve and manipulate crystals

4. Irreproducible crystal positioning; difficult automated alignment

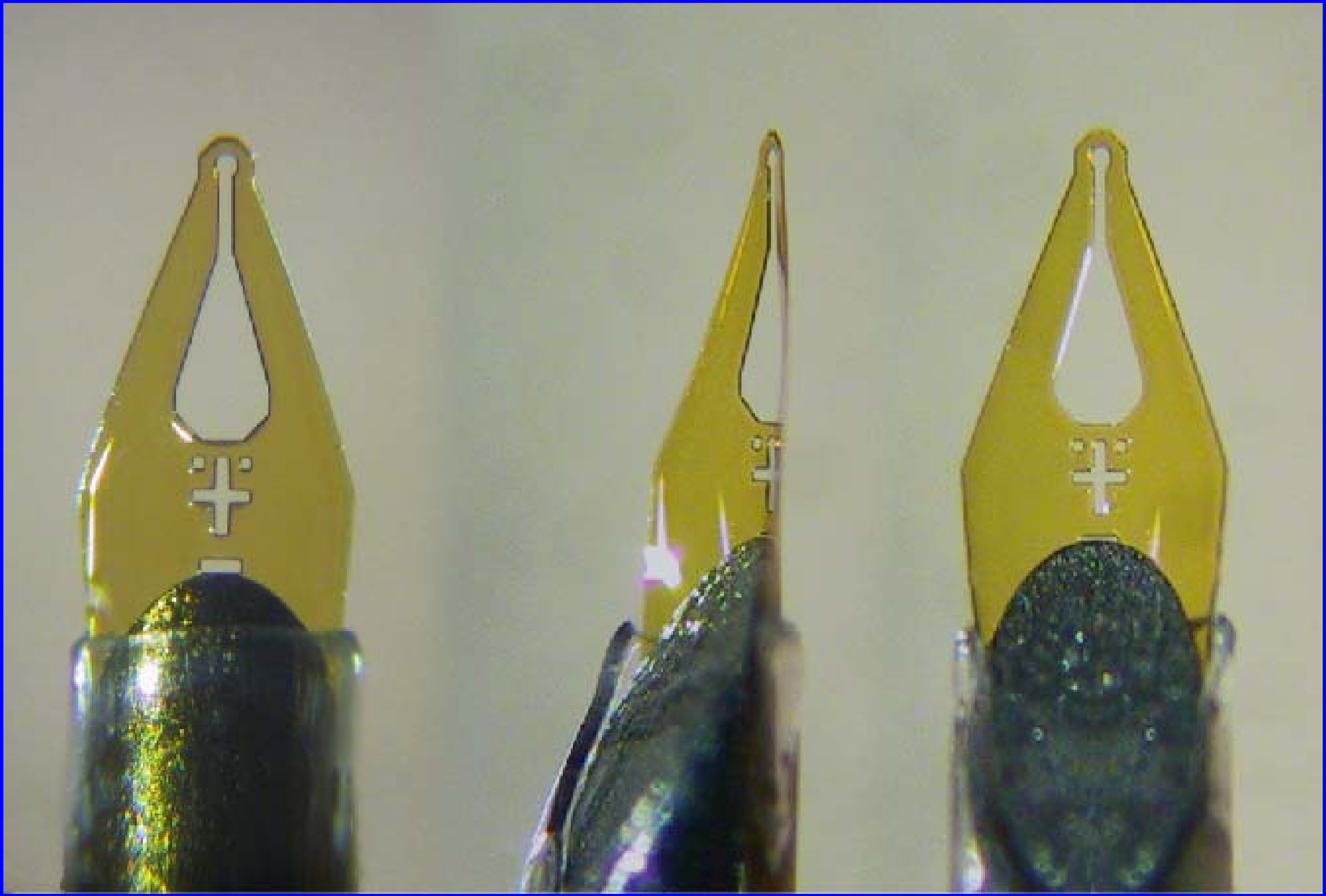
5. Excess liquid complicates cryoprotection, scatters X-rays

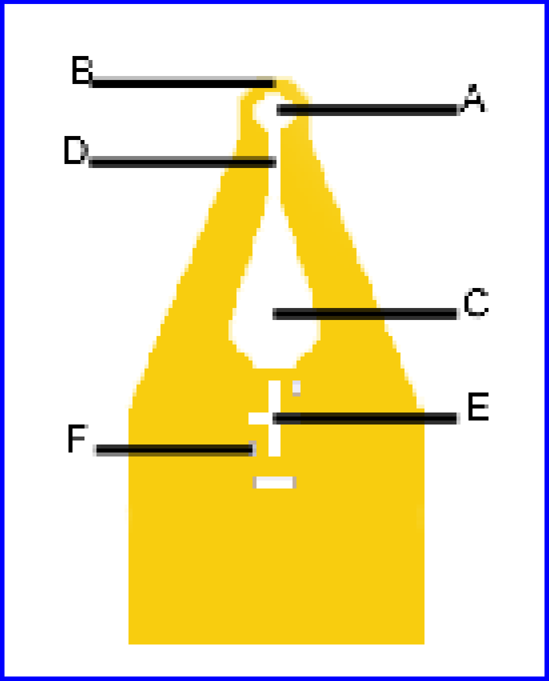
Our Answer: MicroMounts

- Consist of a microfabricated polyimide film attached to a round solid stainless steel post



Oxide-coated Silicon wafer with microfabricated polyimide film





Microfabricated thin film of polyimide:

- low Z consitutuents, low density $\Rightarrow$ less background scatter than nylon

Film curvature:

- provides excellent stiffness even with very thin (5 micron) films, and a convenient scoop-like action in retrieving and handling crystals

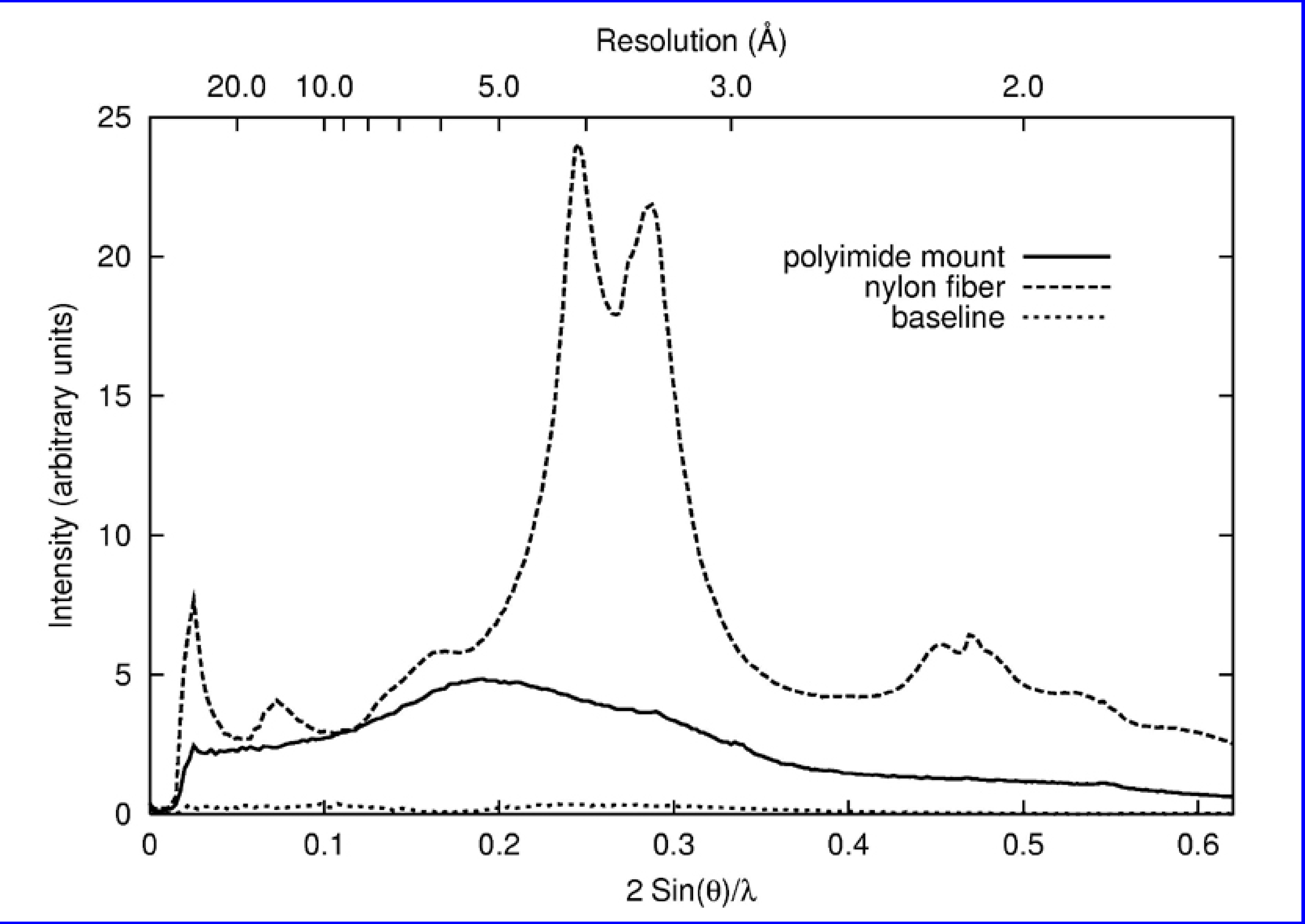
Wicking hole

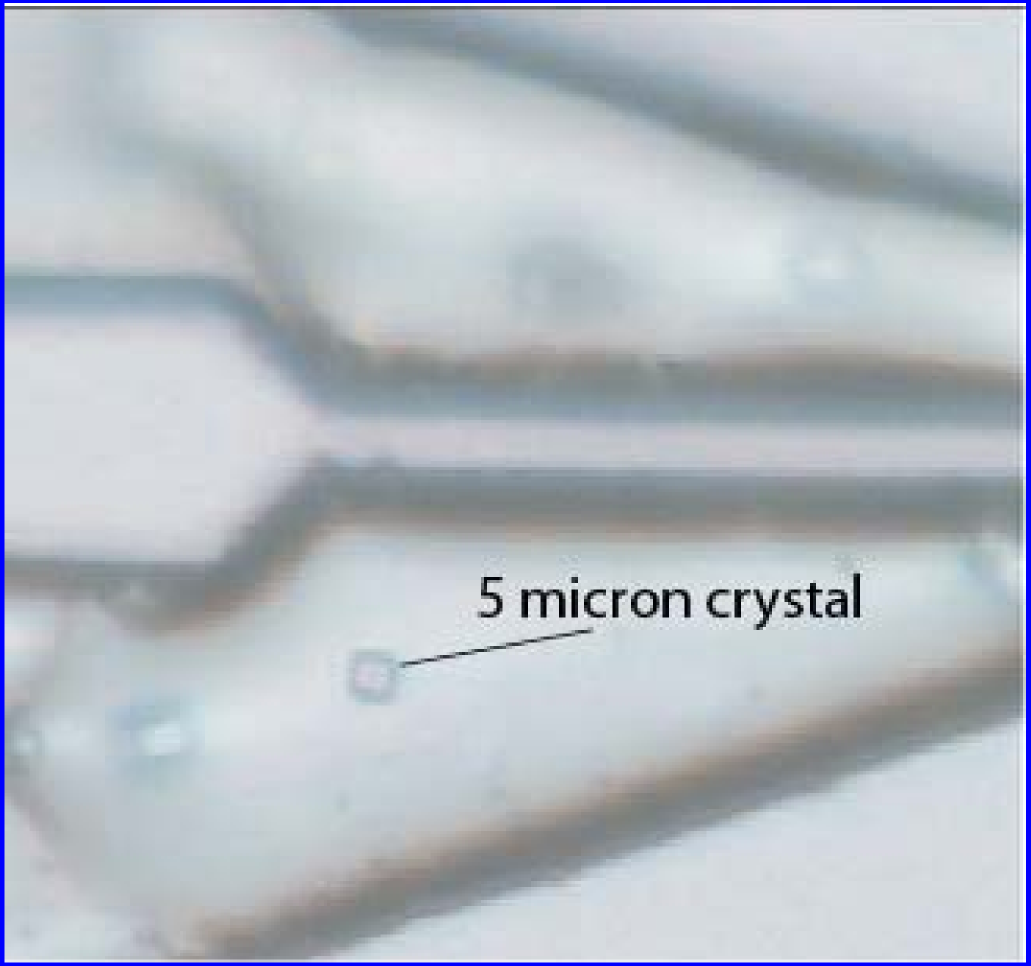
- allows easy removal of excess liquid

Alignment pattern:

- Allows easy manual and automated alignment

Absorption versus resolution:
25 μm nylon versus 10 μm polyimide





Complete data set to 3.1 Å resolution obtained using a single 5 x 7 micron crystal with a 104 Å unit cell

Advantages over CryoLoops:

- Easy handling of crystals as small as 5 μm.
- Rigid yet thin construction
  $\Rightarrow$ reduced bending in cryostreams and viscous solutions
- Convenient, scoop-like action
  $\Rightarrow$ reduced chance of crystal loss or damage.
- Crystal hole size and shape precisely determined
  $\Rightarrow$ easy mounting and alignment
- Easy removal of excess liquid
  $\Rightarrow$ easier cryoprotection, faster freezing

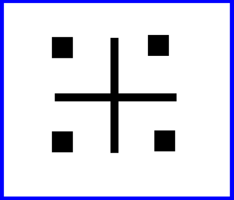
Advantages over CryoLoops:

- Reduced background scatter
  $\Rightarrow$ higher achievable resolution

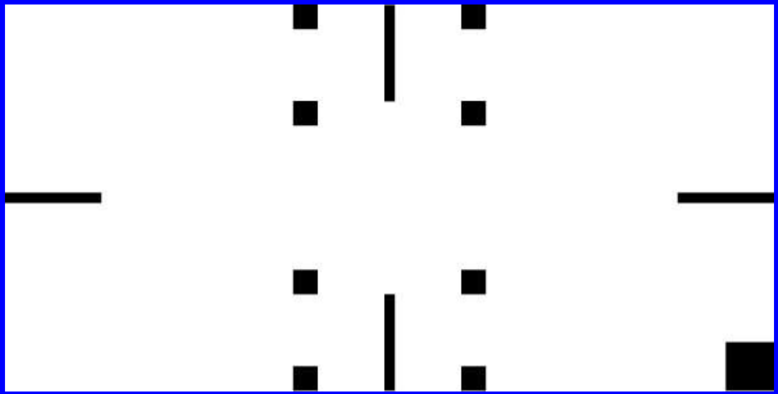
- Solid pins
  $\Rightarrow$ easily bent to place crystal in desired orientation
  $\Rightarrow$ no wicking of liquid inside the pin
  $\Rightarrow$ can be cut to any desired length.

Advantages over CryoLoops:

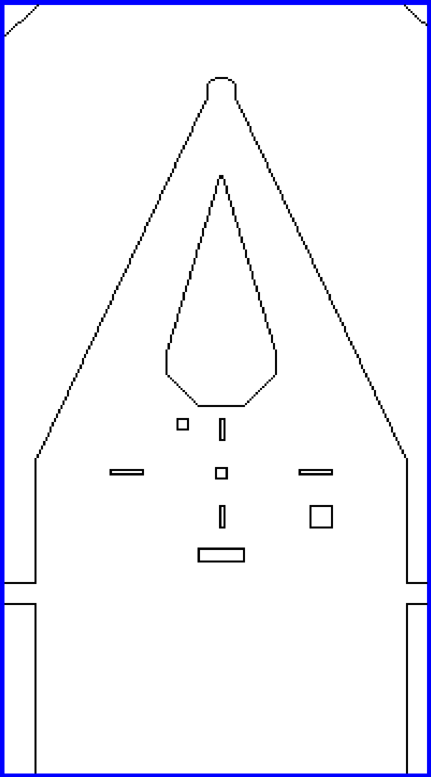
- Easy automated alignment. Beam position and size can be determined without ever optically observing the crystal.



Current alignment pattern

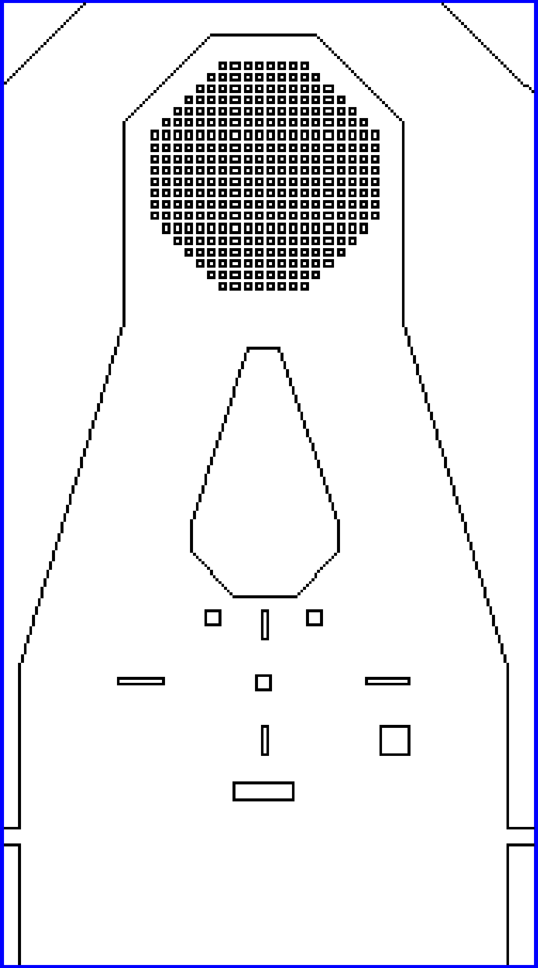


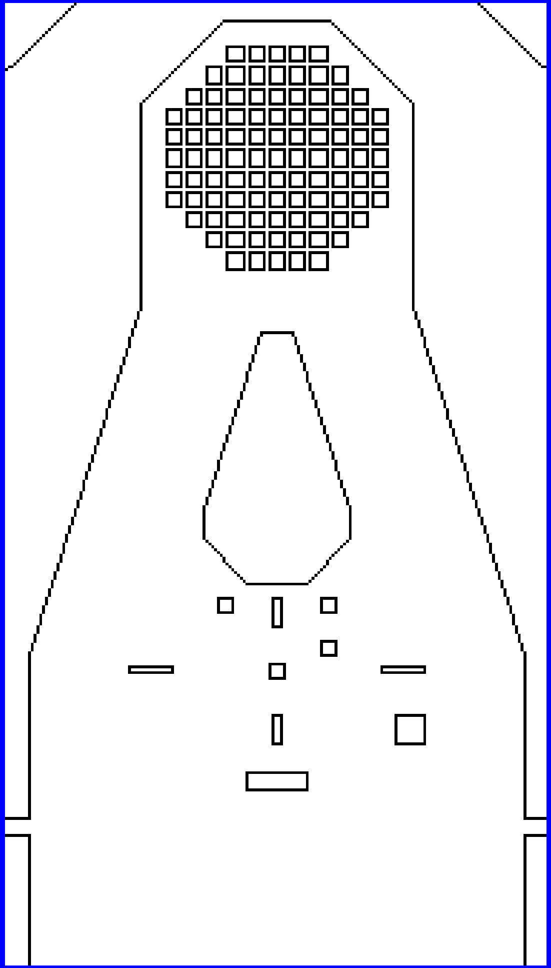
Prototype pattern



Recent Developments:

MicroMesh^{TM} Holders for Rapid Screening:





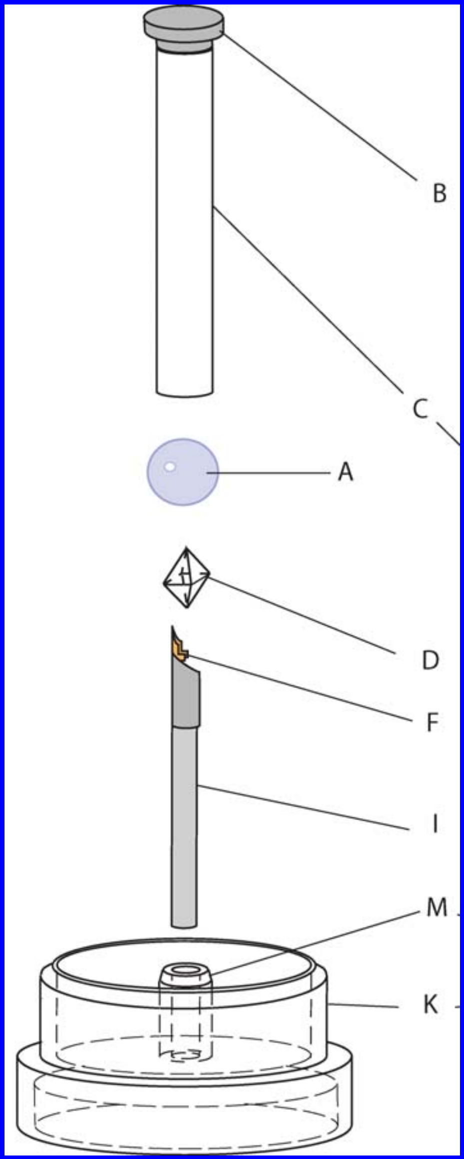
A New Approach to Data Collection at Room Temperature

Why collect data at room temperature?

- Some macromolecular crystals not easily frozen

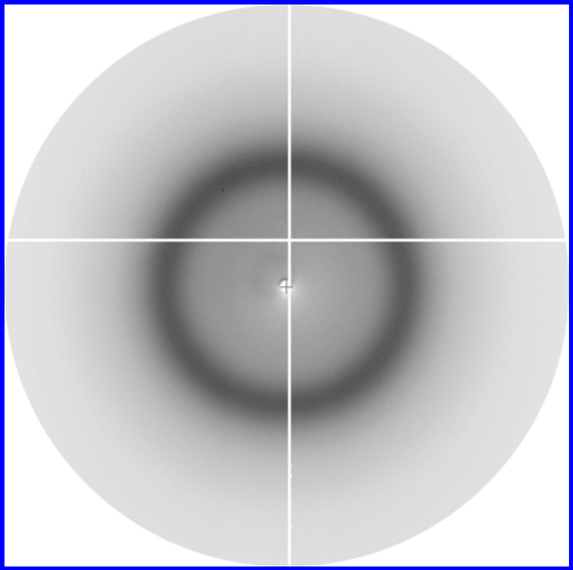
- Diagnose source of poor low-temperature diffraction properties

- Potentially higher resolution and more information (but requires big crystals)

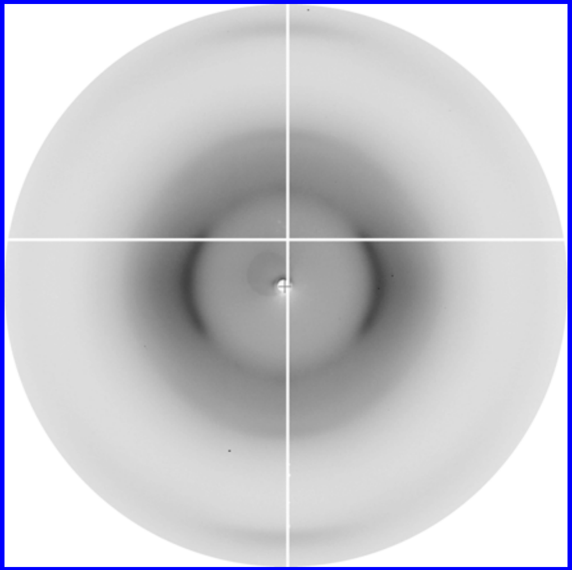


Replace glass with polyethylene (PET) tubing

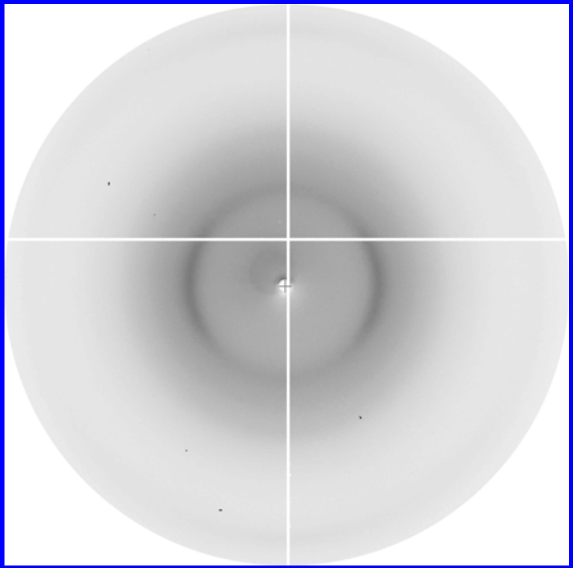

Reduced diffuse scatter:



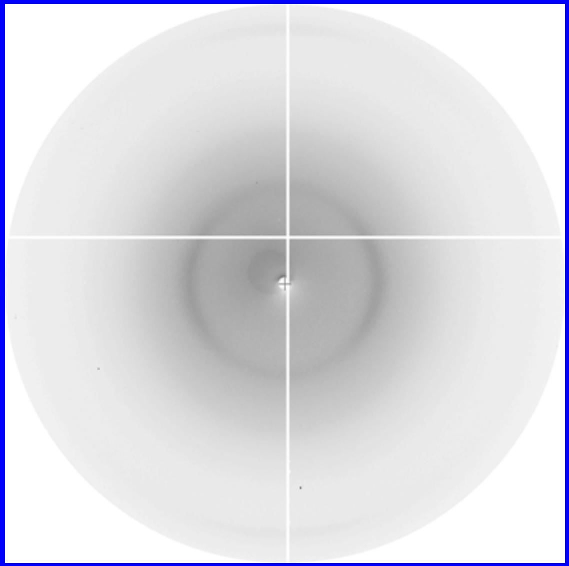
10 μm quartz



50 μm PET

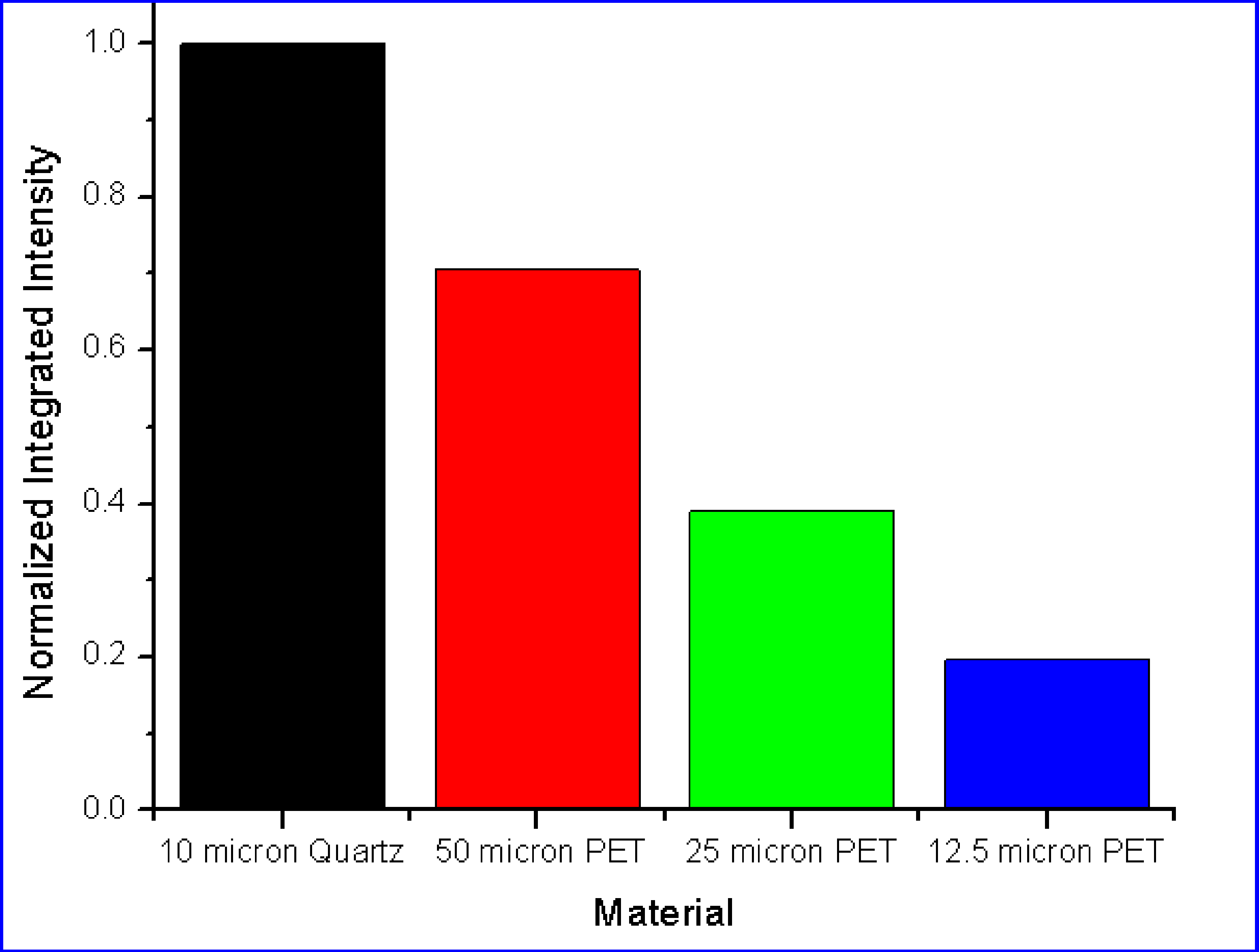


25 μm PET

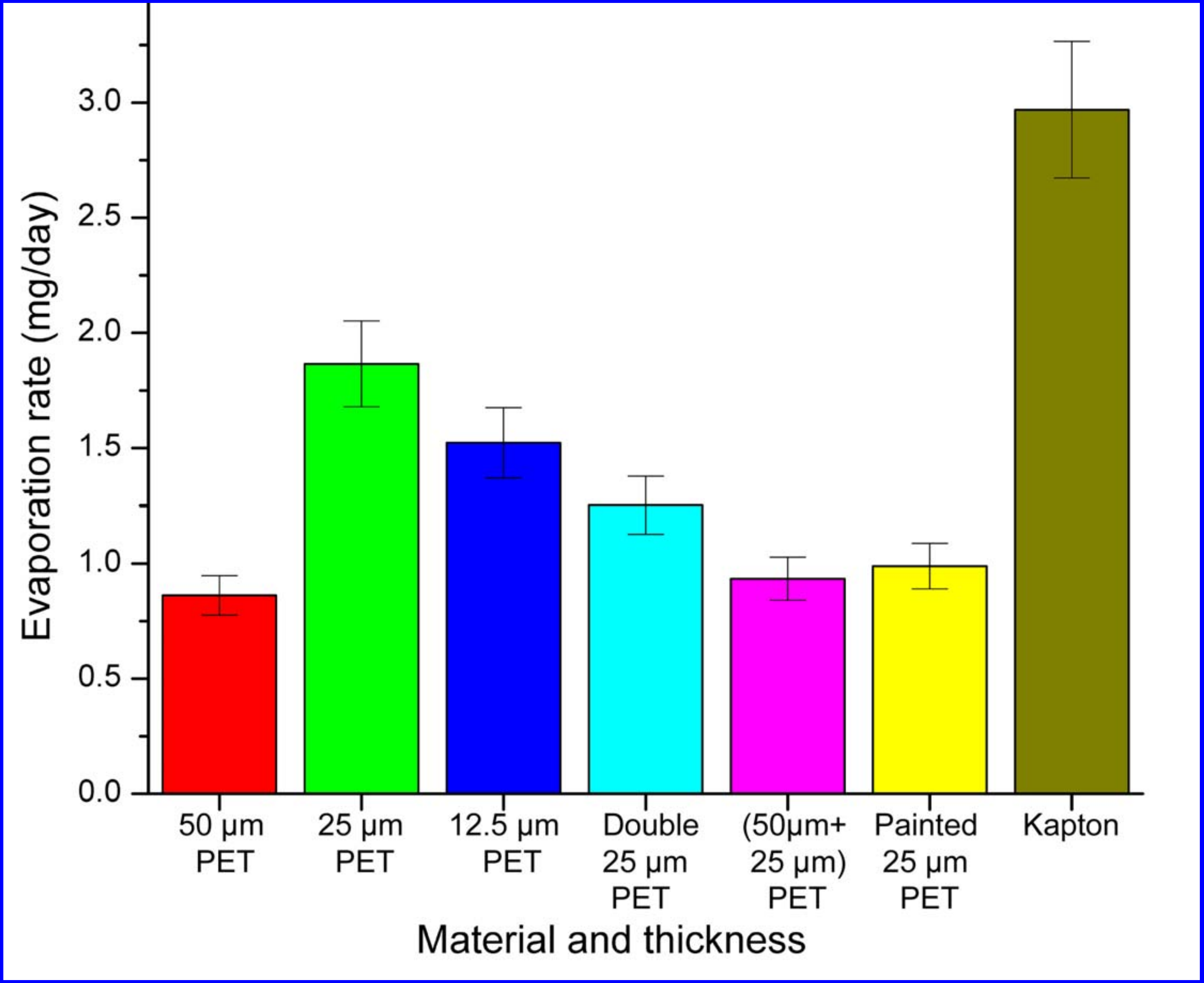


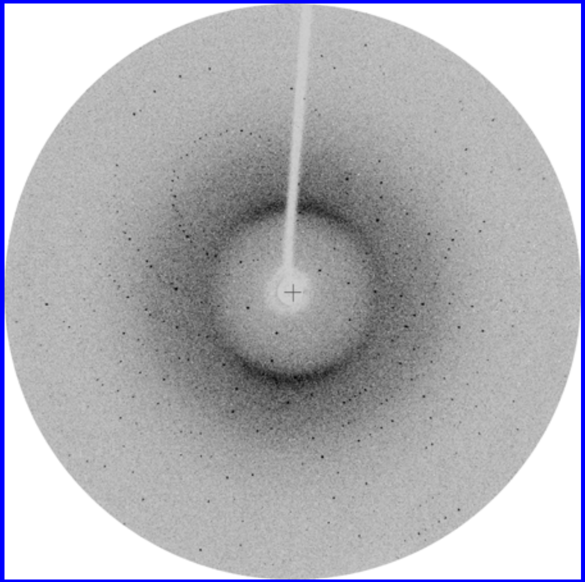
12.5 μm PET

Comparison of integrated diffuse scatter:

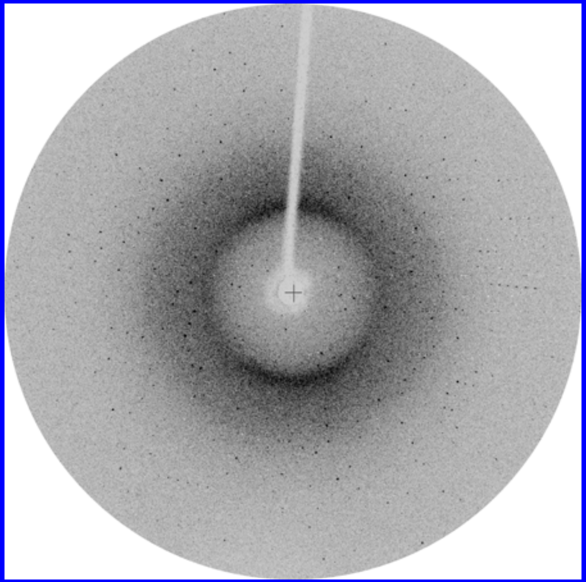


Evaporation through PET tubing:

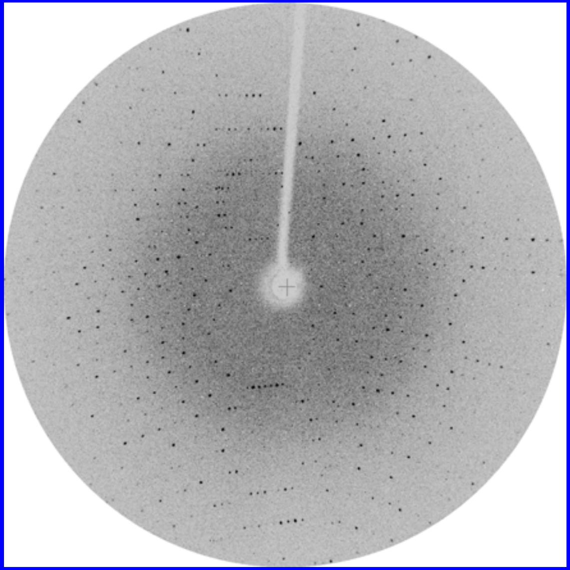




As grown



After cryoprotectant soak



After flash cooling

Thanks to:

MicroMounts:

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Room T Mounts:

Eugene Kalinin, Emil Lobkovsky

Good Advice:

Steve Ealick, Tom Ellenberger