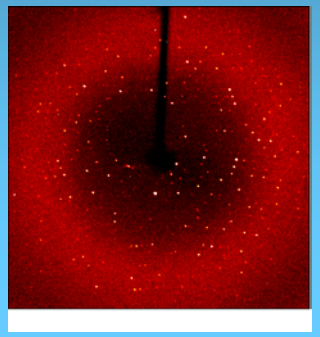
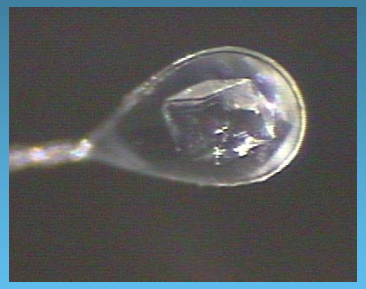
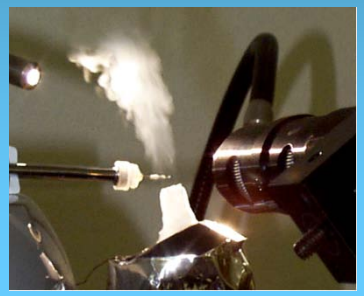
X-Ray Crystallography Facility







XCF Presentation
10/18/00 UAB-CBSE
Engineering Bldg.

University of Alabama at Birmingham
Center for Biophysical Sciences and Engineering

Physical Effects of Microgravity

- Warm air does not rise
- Bubbles do not coalesce
- Surface tension dominates fluid behavior
- Diffusive transport is dominating whereas buoyancy-induced convection is virtually nonexistent

Working in a microgravity environment makes you think differently!

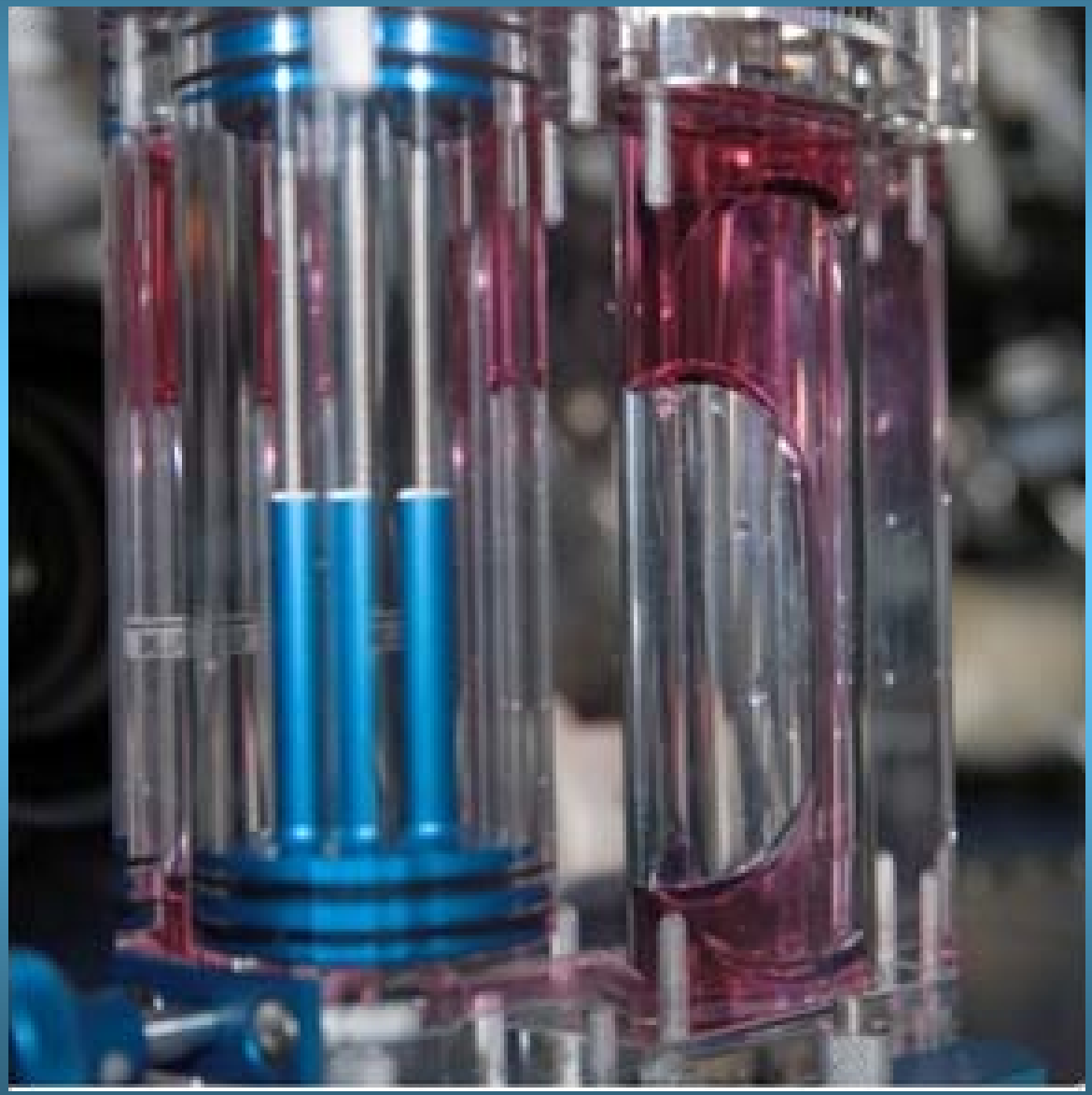
Extend Farraday's 19th Century Lectures

- Would the candle burn in 0g?
- And now the rest of the story…

STS-50: Candle Flames in Microgravity

- Round, as expected – it burned for 45 sec.

Surface Wetting in Microgravity



Summary of Microgravity Vapor Diffusion Experiments

No Crystals	Crystals Too Small for X-ray Data Collection	Diffraction -Sized Crystals	Improved Diffraction Data
16.7%	26.2%	57.1%	27.2%

19 Shuttle Missions - 221 Macromolecules

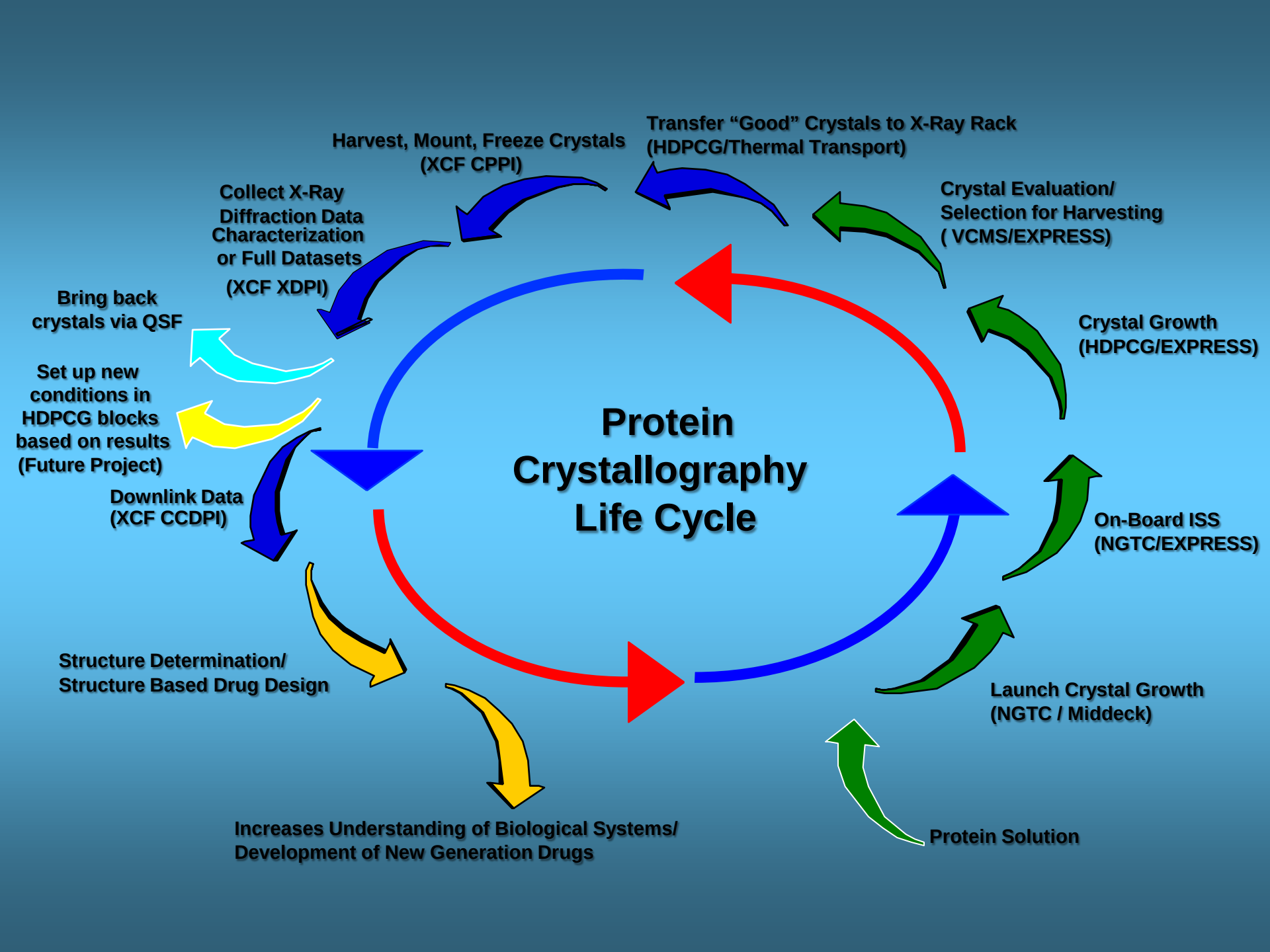
Justification for ISS X-Ray Facility

- Space Shuttle PCG History:
  - Optimum microgravity crystallization conditions are different that 1-g conditions.
  - Protein crystals are labile
  - Crystals are fragile (re-entry g's)
  - Investigators constantly complained about time lapse between growing crystals and collecting data

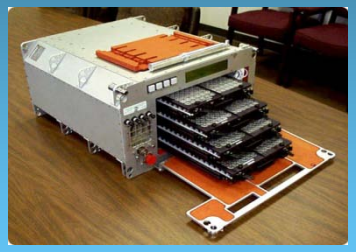
- Conclusion: There is a justifiable need for a crystallographic laboratory in space equivalent to Earth labs.

Purposes and Benefits of the XCF

- Characterization of cryopreservation conditions
- Determine resolution of diffraction (without detrimental re-entry g effect)
- Complete data set collection on unstable crystals or high priority crystals
- Characterization of crystal quality for optimization of crystallization conditions.
- Determine space grown unit cell parameters for new crystal morphologies
- Distinguish protein versus salt crystals
- Detect cracked or twinned crystals

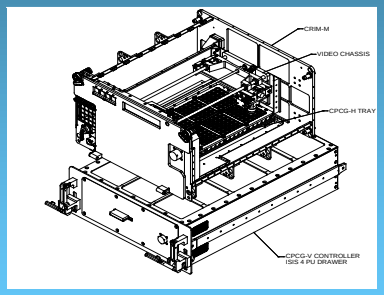

Protein Crystallography Life Cycle
Harvest, Mount, Freeze Crystals (XCF CPPI)
Transfer "Good" Crystals to X-Ray Rack (HDPCG/Thermal Transport)
Collect X-Ray Diffraction Data Characterization or Full Datasets (XCF XDPI)
Crystal Evaluation/ Selection for Harvesting (VCMS/EXPRESS)
Bring back crystals via QSF
Crystal Growth (HDPCG/EXPRESS)
Set up new conditions in HDPCG blocks based on results (Future Project)
Downlink Data (XCF CCDPI)
On-Board ISS (NGTC/EXPRESS)
Structure Determination/ Structure Based Drug Design
Launch Crystal Growth (NGTC / Middeck)
Increases Understanding of Biological Systems/ Development of New Generation Drugs
Protein Solution

Micro-G Protein Crystal Growth: End-to-End Concept



Protein Crystal Growth

- High Density Protein Crystal Growth (HDPCG)
- "Brute-force" method of 1008 sample conditions loaded on the ground



Visual inspection of candidate crystals

- Video Command and Monitor System (VCMS)
- Designed to "look HDPCG growth cells

X-ray Analysis of Crystals

- X-ray Crystallography Facility (XCF)
- Crystal Preparation and Mounting
- Crystal Freezing
- X-ray Analysis (Characterization and full datasets)
- Crystal storage

Future Project

Automated adjustment of chemical growth conditions on-orbit

- High throughput crystal growth and Crystal Optimization

Crystal Growth Hardware

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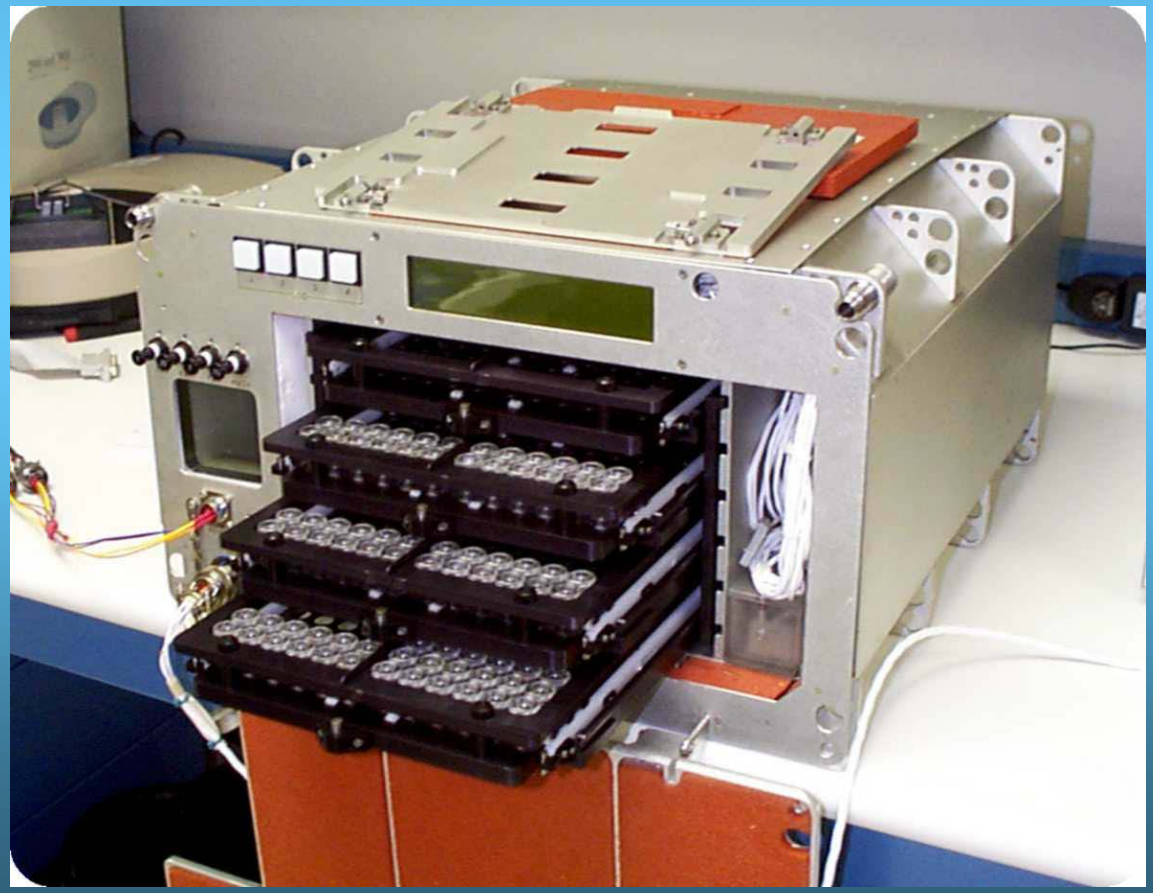
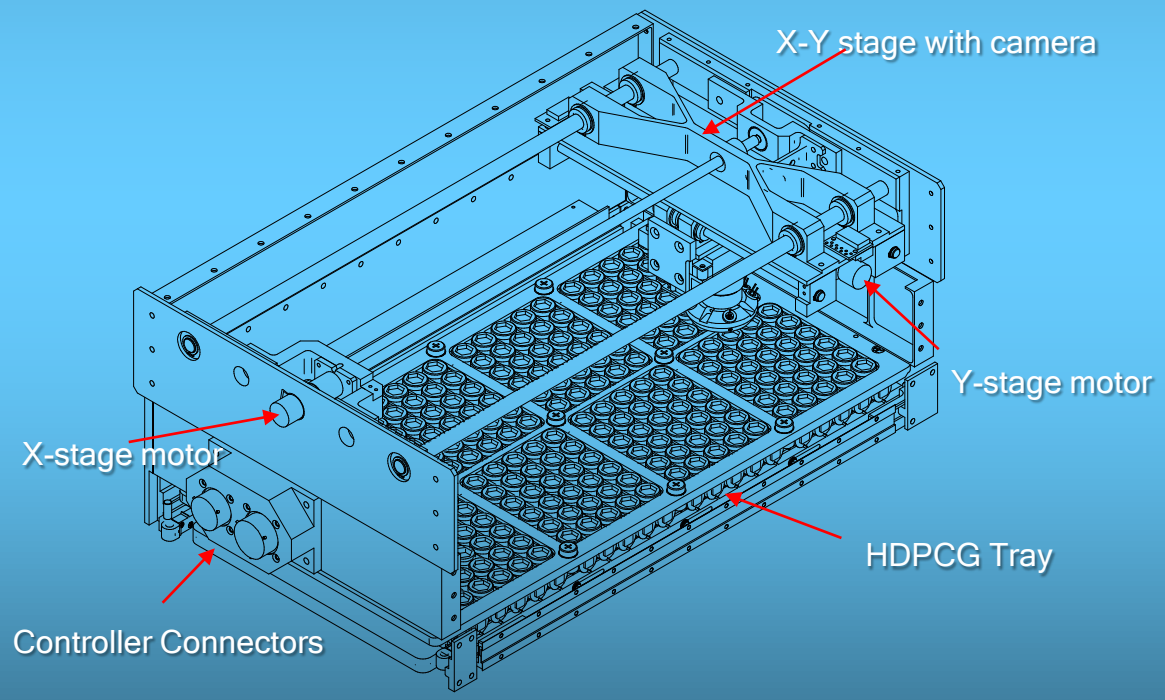
High Density Protein Crystal Growth (HDPCG)/C-RIM Assembly

- **Provides 1008 diffusion crystal growth containers within one middeck locker. Specifically designed to interface with CPPI for automated sample removal and sample loading via future system(s).**



*Video Command and Monitoring System (VCMS)**

- **Provides means of selecting candidate crystal wells through video observation.**



X-Ray Crystallography Facility

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XCF Technology Demonstrator Science Specifications

- Data Resolution - 1.6 Angstroms for up to a 200 Angstrom unit cell size; 2 Angstroms for up to a 300 Angstrom unit cell size.
- Crystal Size Accommodated - .1 mm to .5 mm optimized for .3 mm
- Unit Cell Size Accommodated - Up to 300 Angstroms.
- Mosaic Spread Accommodated - Less than 0.1 degree
- Detector Positioning - +/- 45 degrees at 30 cm; +/- 58 degrees at 10 cm
- Goniometer - ω – sample sweep; χ – sample tilt (fixed at 45 degrees)
  ϕ – sample rotation; x, y, z - fine sample adjustments
- Beam Intensity - 1.0x10^8 x-rays/mm^2-sec or greater
- Divergence angle - Less than 0.05 degrees (1.0 mrad) half angle
- Beam Diameter at crystal location - .3 mm
- Spectral Purity - Kbeta peak <1% of Kalpha peak
- Crystals Cryofrozen in less than 1 sec to -180 degrees C and preserved at -180 degrees C
- Crystalsrobotically harvested, isolated and mounted
- 2K CCD X-ray detector; XCF system will accommodate multiple detectors
- XCF system designed to be telescience capable.

XCF System Description

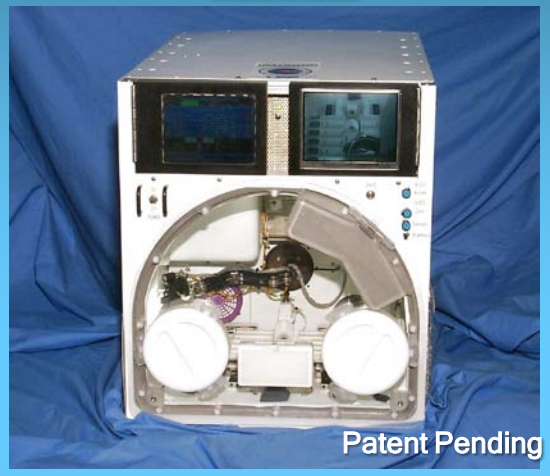
- The X-Ray Crystallography System consists of several key items designed to complete the UAB Lab Based Paradigm. During Phase B, UAB has successfully completed the maturation and validation of the each of these key systems:
  - **X-Ray Diffraction Rack**
    - **Crystal Preparation Prime Item** - Provides all items necessary for harvesting, mounting and preparing samples for analysis.
    - **X-Ray Diffraction Prime Item** - Provides all items necessary for performing x-ray diffraction analysis of samples.
    - **Command, Control, Data Prime Item** - Provides all items necessary for command and control of the XCF, including tele-science activities.

XCF Functional Mapping

Advanced Crystal Handling

- Crystal Harvesting
- Crystal Mounting
  - Thin Film on Loop
  - Capillary
- Crystal Cryofreeze and Storage

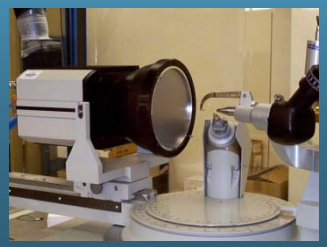
CPPI



X-ray Diffraction Analysis

- X-ray Generation
- Crystal and Detector Positioning
  - 2θ, ω, x, y, z, ϕ, Detector Distance
- X-ray detection
- Crystal cryopreservation

XDPI



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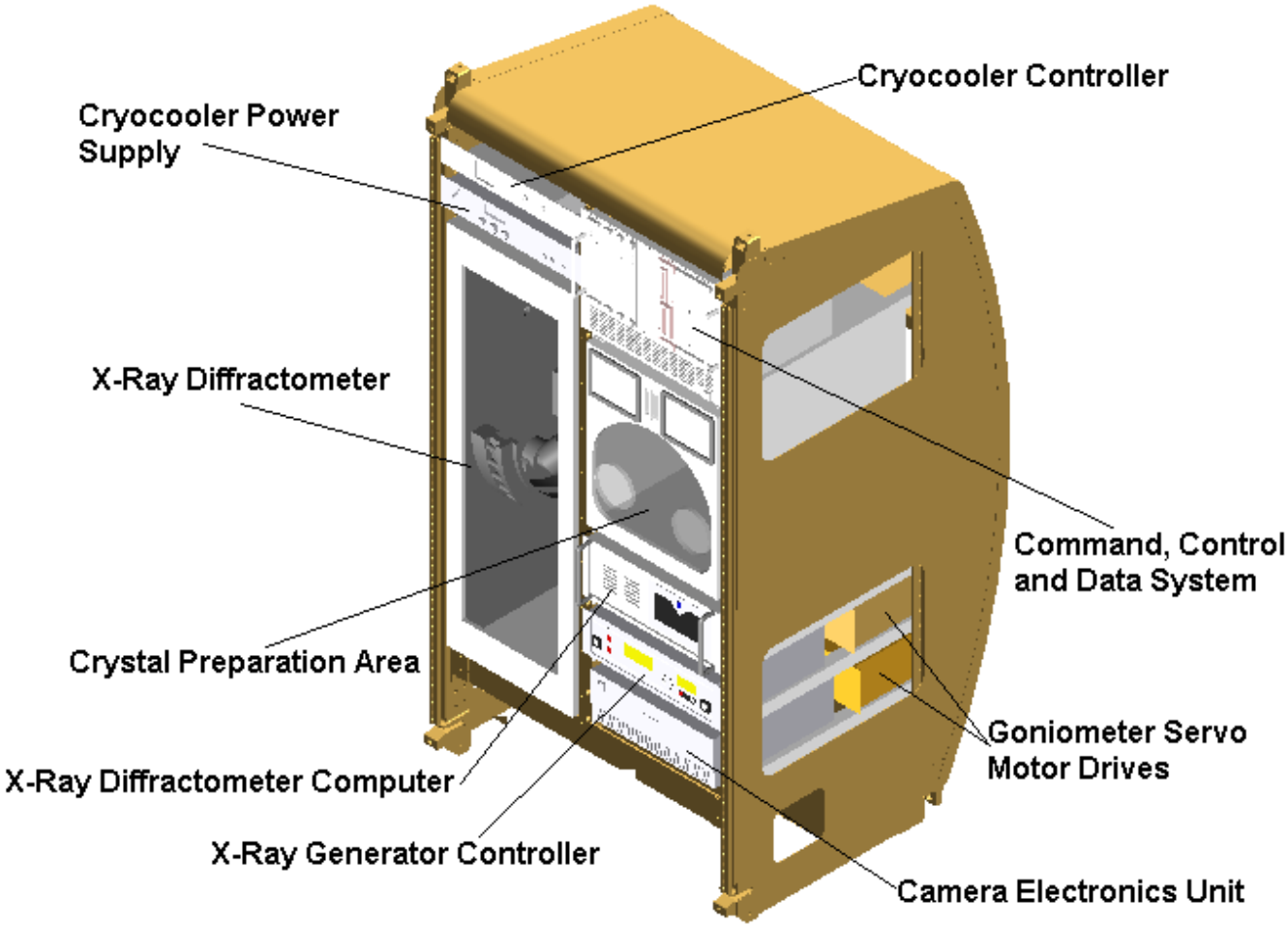

Cryocooler Controller
Cryocooler Power Supply
X-Ray Diffractometer
Command, Control and Data System
Crystal Preparation Area
Goniometer Servo Motor Drives
X-Ray Diffractometer Computer
X-Ray Generator Controller
Camera Electronics Unit

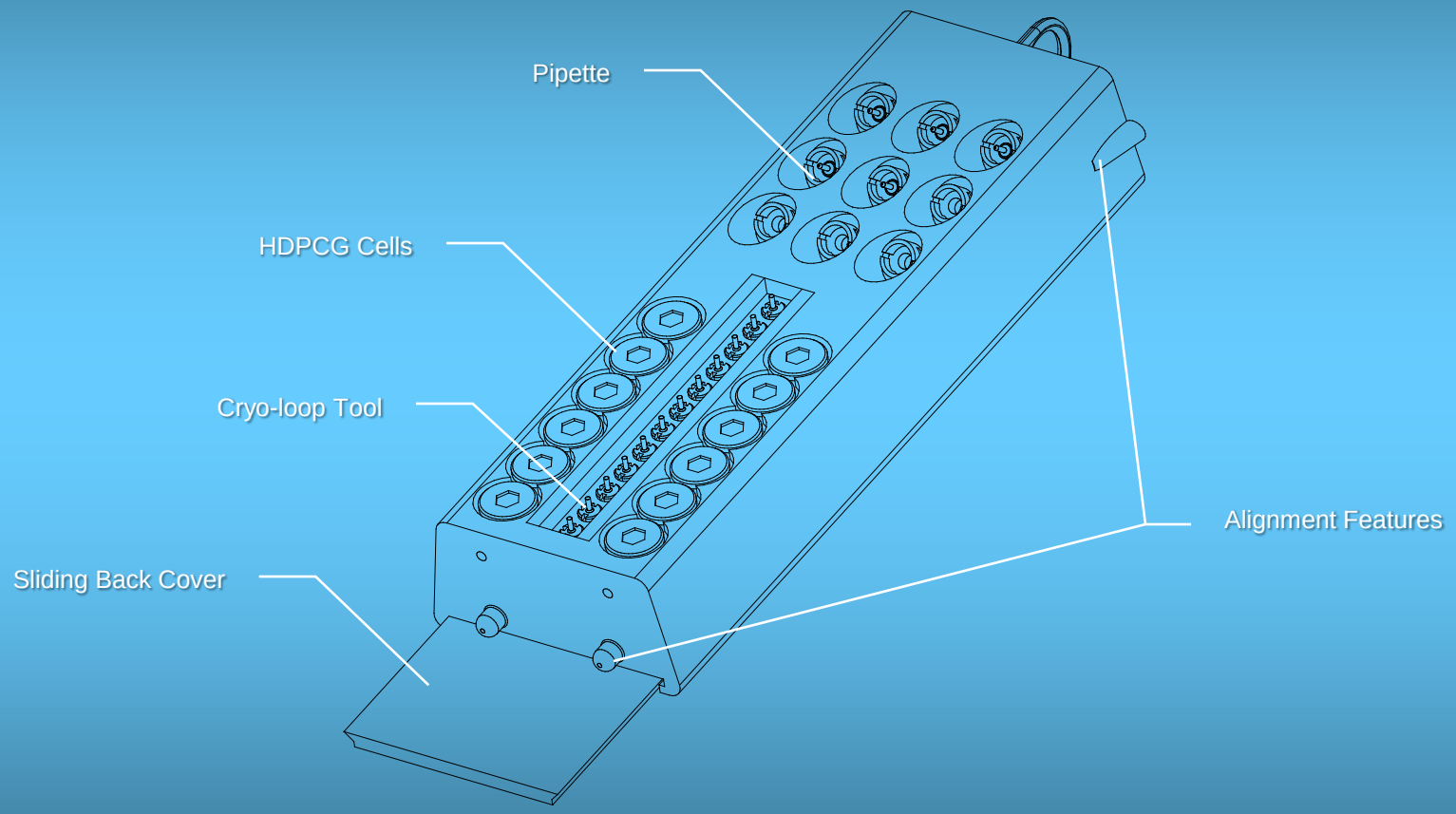
Crystal Preparation Prime Item (CPPI)

- The CPPI prepares individual protein crystals for X-Ray diffraction thru robotic harvesting and cryo-preservation.

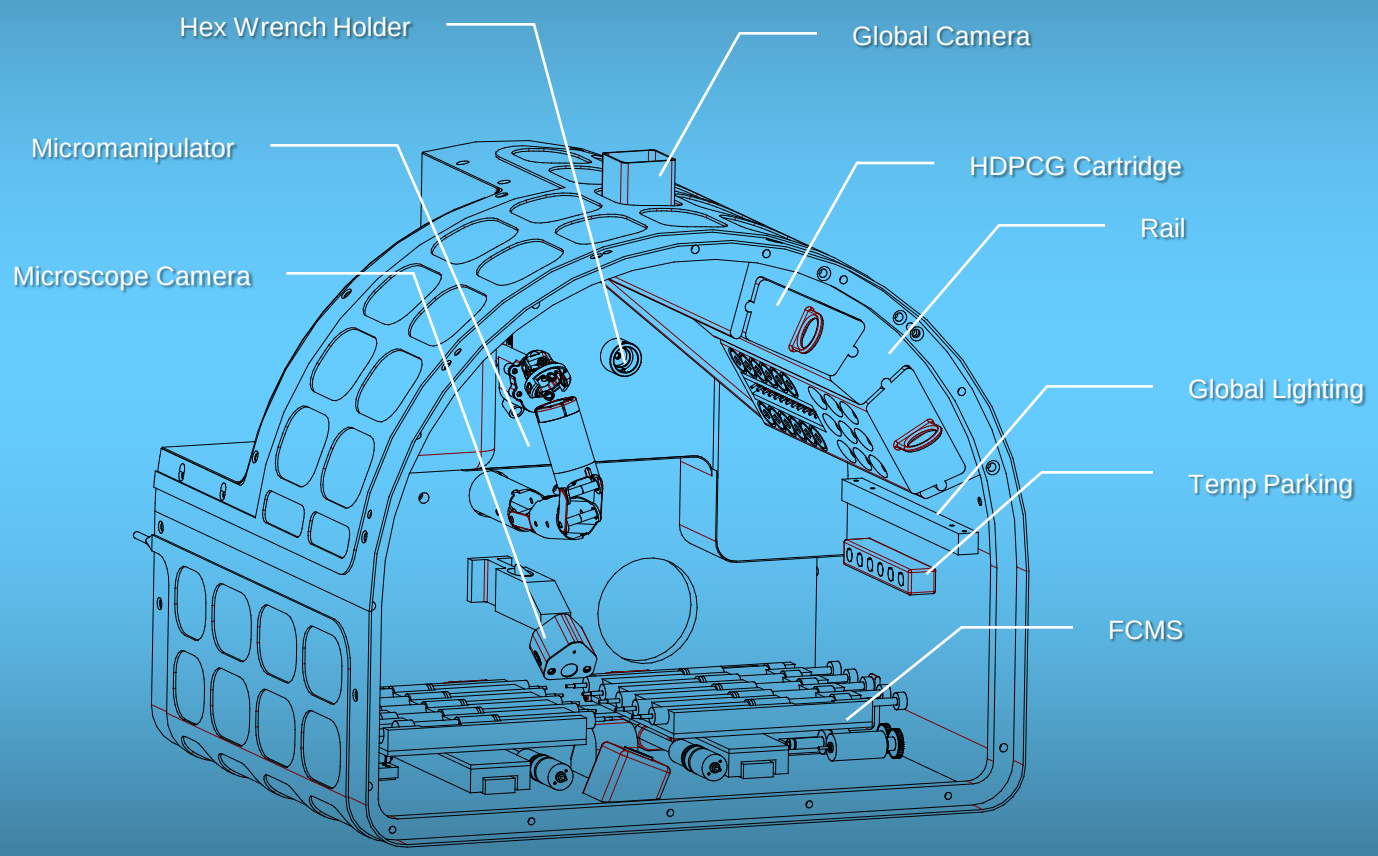
Key Features:

- All activities performed in a thermally controlled, humidity controlled, "clean room" environment.
- Capable of snap freezing crystals at -180 C and maintaining up to 24 frozen cryoloops at this temperature.
    - Freeze rate determined from laboratory practices using standard 5 l/m flow
- Micro-manipulator performs 95% of all activities
- Full tele-science capabilities

HDPCG/Tool Cartridge



Crystal Preparation Chamber



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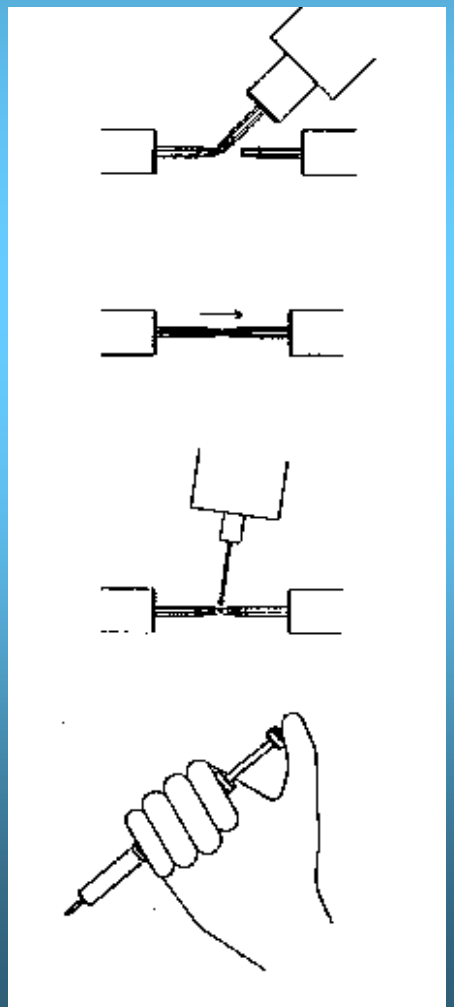
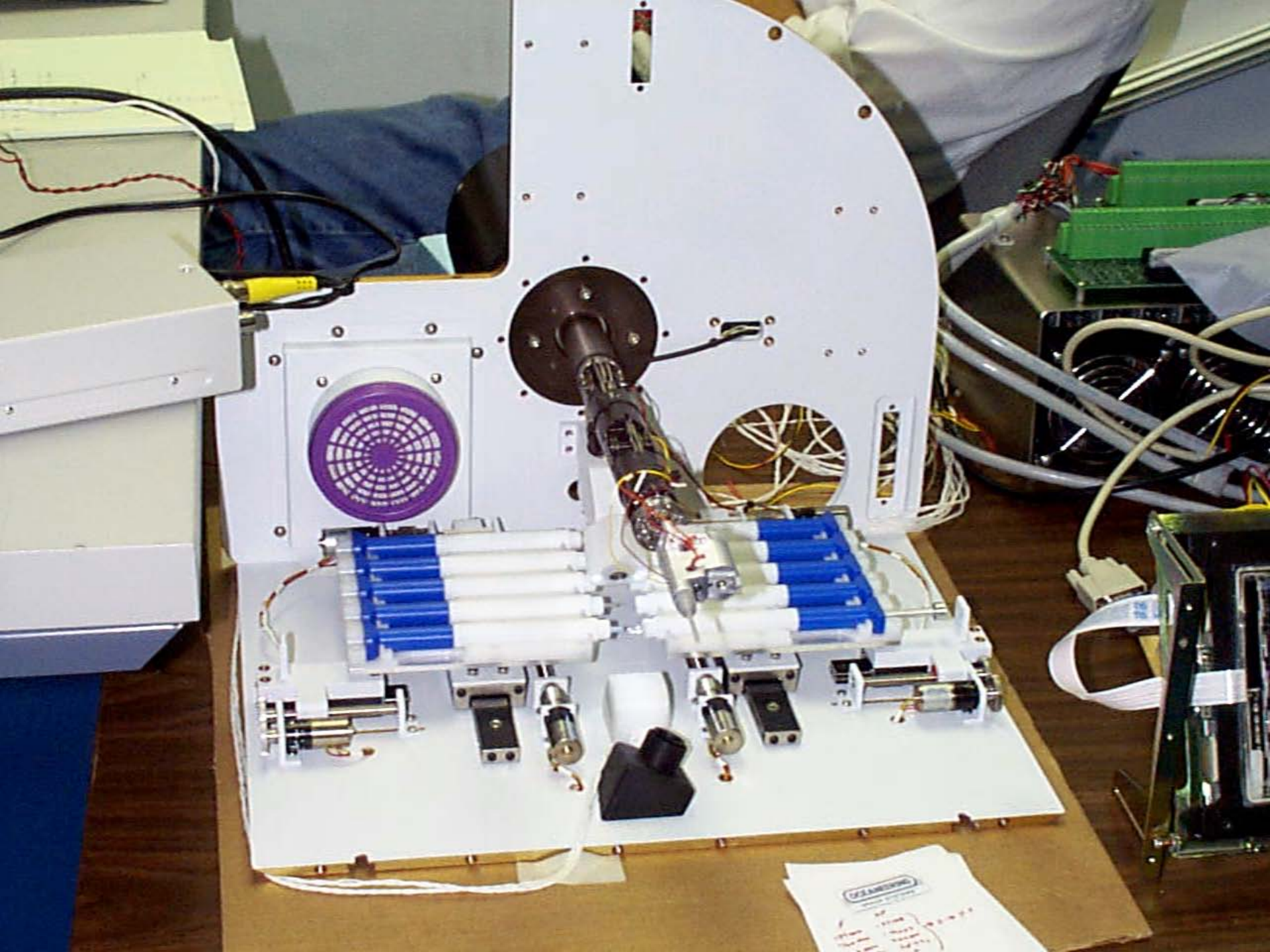
Crystal Harvesting and Mounting

- *Conversion from three-dimensional to two-dimensional problem*

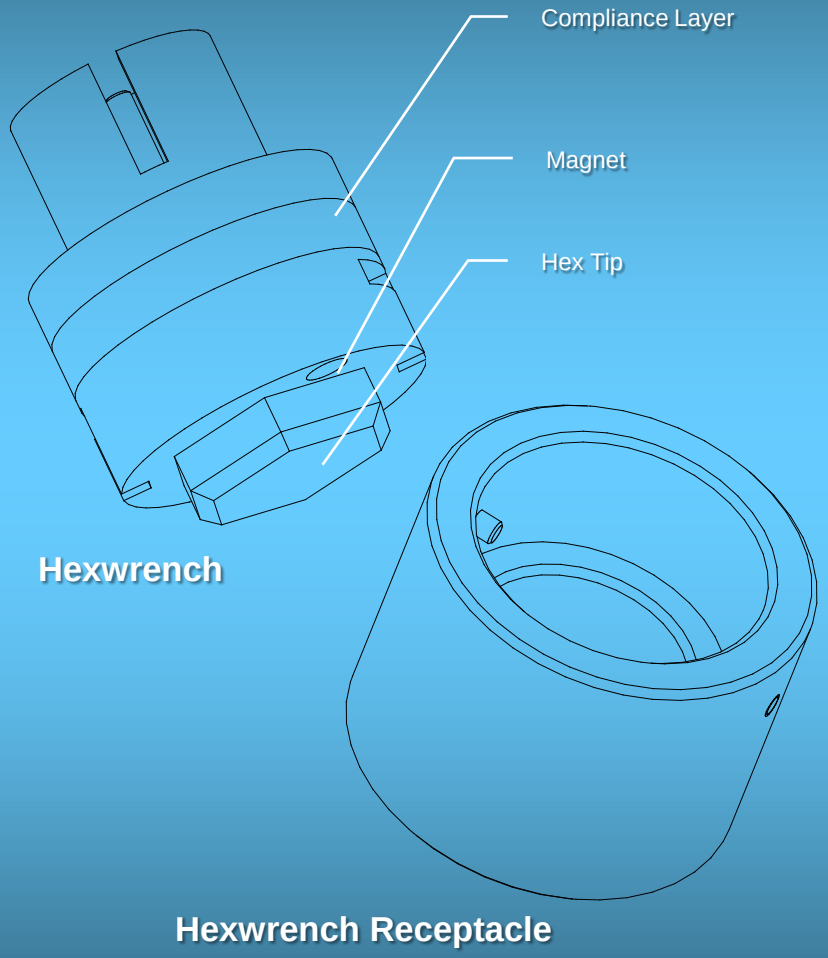
  - Pass crystals and mother liquor from robot pipette.
  - Position target crystal in droplet for mounting in the cryoloop.
  - Add reagents to crystals as required.
  - Mount crystal in loop for cryofreezing or capillary storage.
  - Accommodate manual (crew) loading of crystals from non-robot compatible carriers.





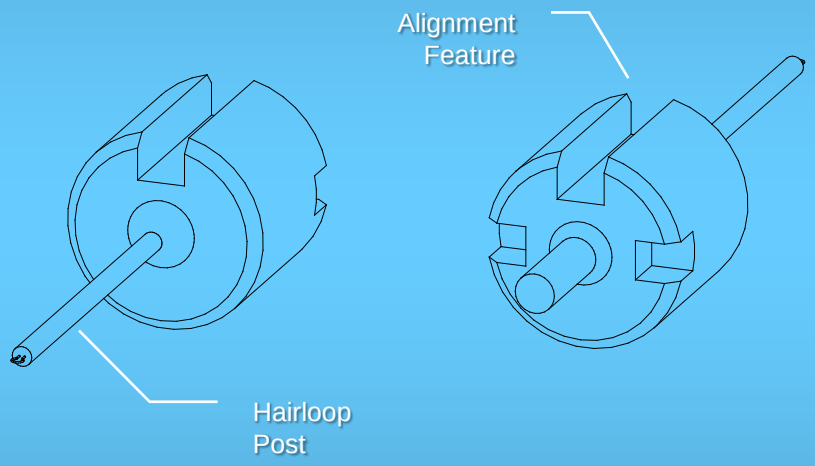
Hex Wrench

- Used to open and close the HDPCG caps
- Contains permanent magnets for holding HDPCG cap on removal
- Permanent holster location inside the CPC
- Compliance layer prevents over exertion of force/torque and accommodates misalignment



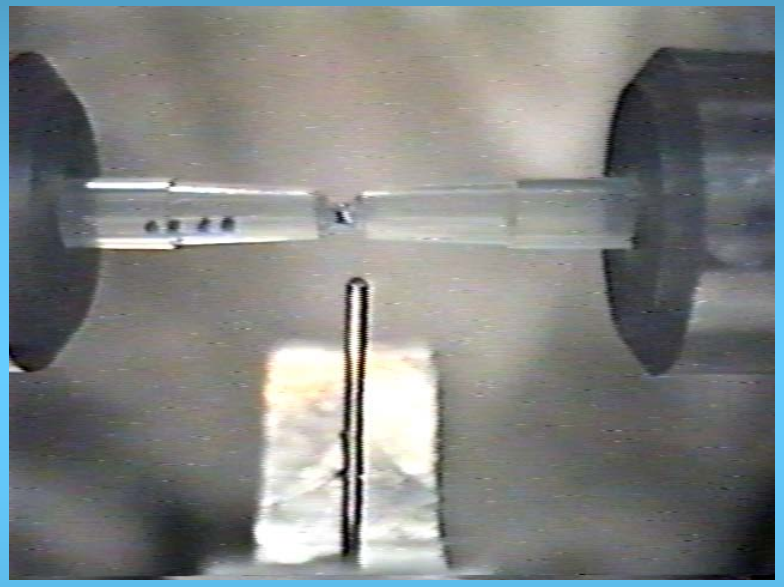
Hairloop Tool

- Standard cryoloops are mounted to a custom base
- Base provides interface between robot, flash storage freezer and cartridge
- Stored in the tool cartridge by a magnet
- Notched for predetermined loop orientation in cartridge



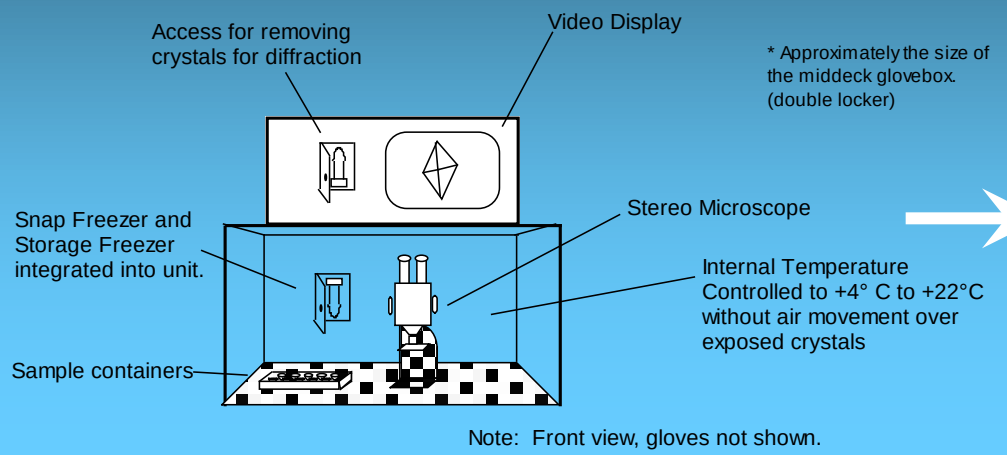
CPPI Technology Development

- A droplet is formed between opposed pipette tips
- Target crystal is moved into droplet
- Cryoloop is passed through droplet at 45°or 90°
- **Results**
  - Crystal movement and positioning was well controlled
  - Easy formation of crystal droplet
  - Pinning edges held droplet in place
  - Mounting demonstrated on KC-135 with 83% success rate on first attempt.

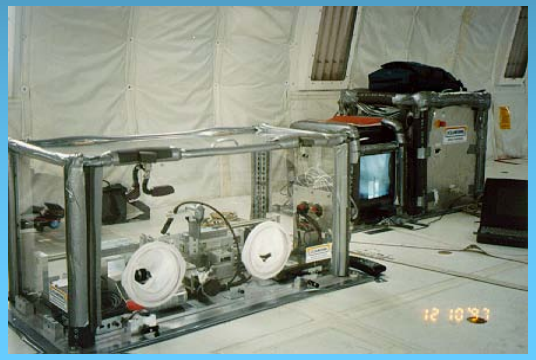


Video Image of Work Area in KC-135

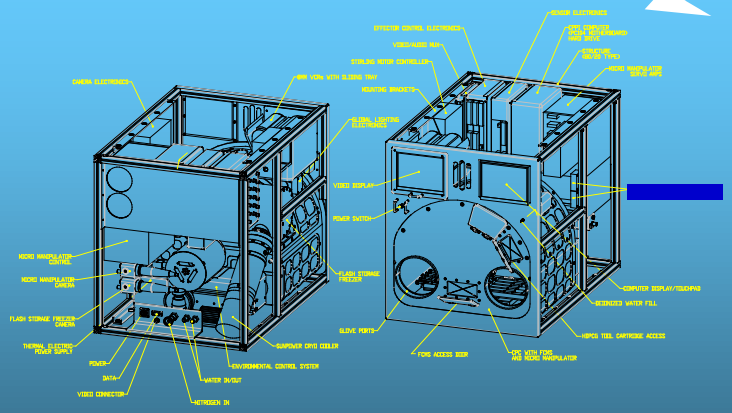
Advanced Protein Crystal Handling Maturation



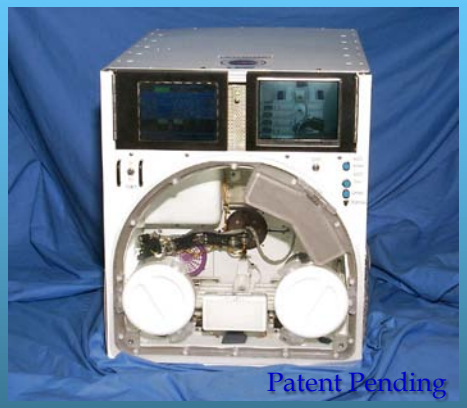
Concept - Fall 1996



Preliminary Design



Detailed Design

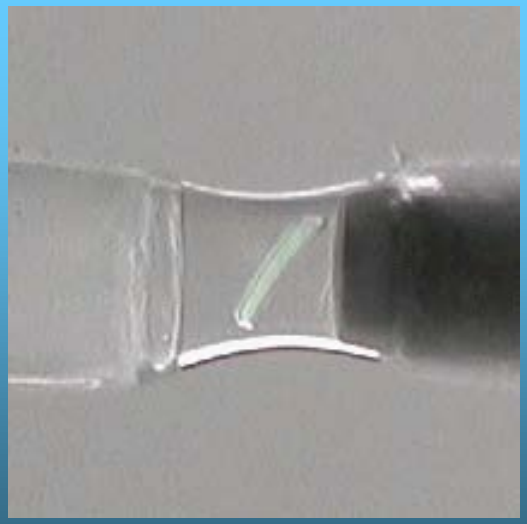


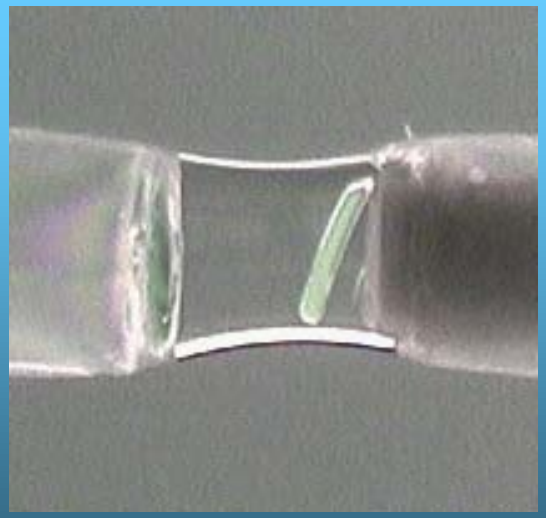
Technology Demonstration Hardware - Fall 1998

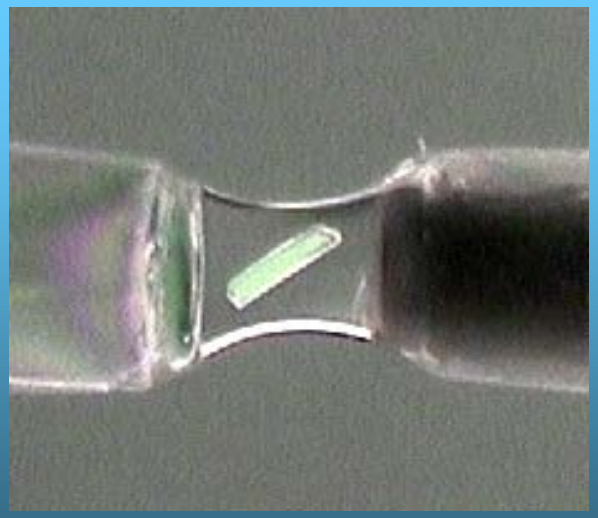
CPPI Testing

- We have successfully manipulated and mounted round, rod-shaped, needle and plate crystals in the CPPI.

- They all have unique characteristics but, in general, the system works with all of these crystals in the same way.







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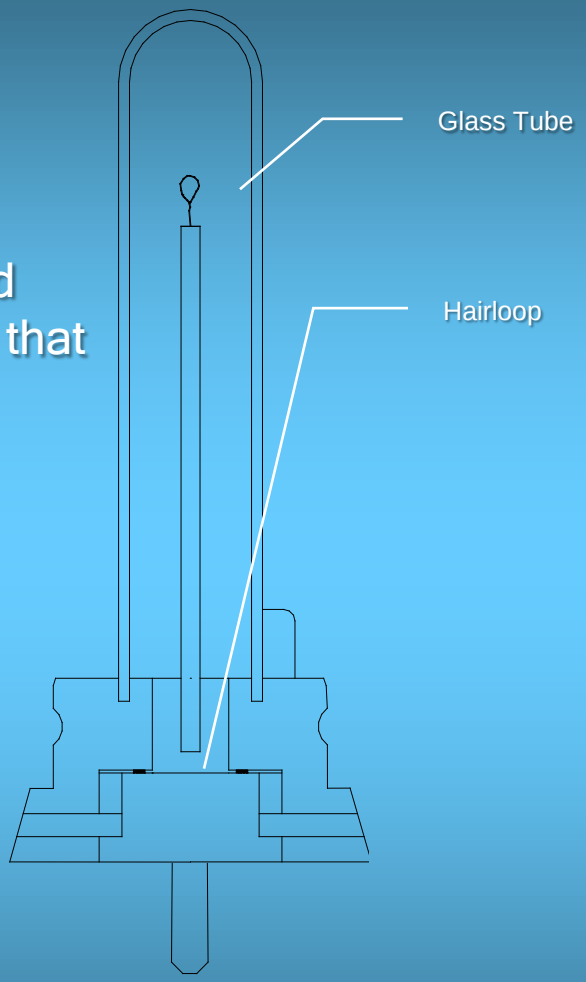
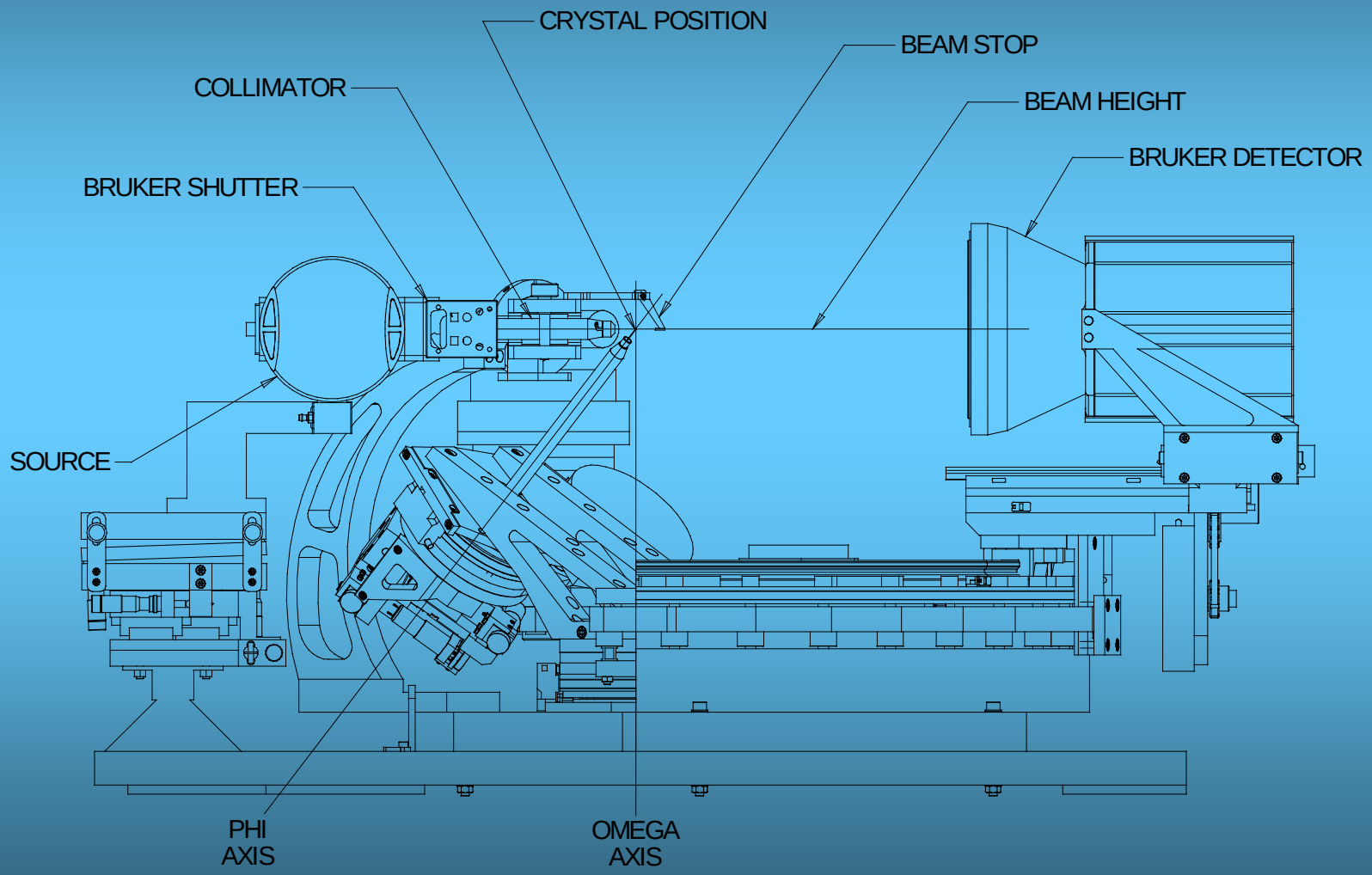
- **Glass Vial (Capillary) Cartridge**
  - A third cartridge with special hair loops enclosed inside glass vials is supplied for storing crystals that will not be flash frozen
  - Glass vial will protect crystals after mounting
  - Glass is contained within the cartridge
  - Individual glass vials can be removed from the cartridge for X-Ray diffraction



Glass Vial (Capillary Tube)

X-ray Diffraction Prime Item



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X-Ray Diffraction Prime Item (XDPI) Description

•The XDPI includes all the necessary equipment to collect x-ray diffraction data on protein crystals. This includes:

- **Advanced X-Ray Generator**
  - **Three Circle Goniometer**
    - **Capillary and Cryoloop Crystal Capability**
- **CCD Detectors**
  - **X-Ray Source Power Supply/Controller**
  - **CCD Detector Frame Buffer**
  - **XDPI Motor Drives**
  - **Cryogenic Crystal Cooler**
  - **XDPI Computer**

Advanced X-Ray Generator

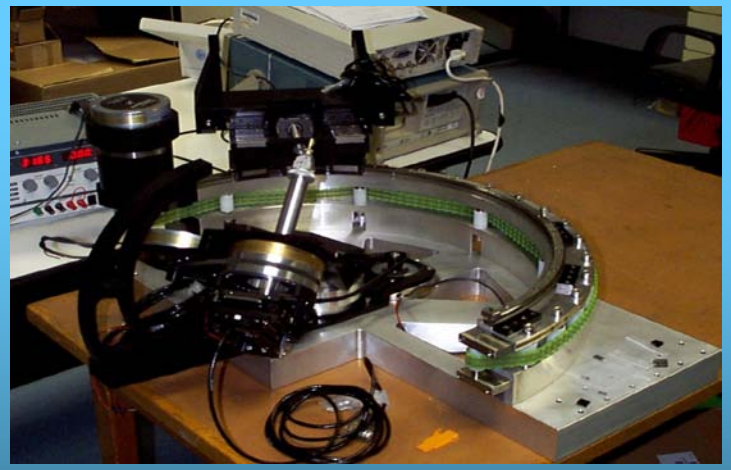
- **Description:** High intensity, monochromatic, nearly parallel Copper x-ray beam.
  - Accomplished by key technology advancements in X-Ray Generation and Beam Forming Optics
- **Current Ground Laboratory X-ray Systems**:
  - Weight ≈ 1-2 Ton
  - Power ≈ 5000 - 10,000 Watts power
  - Volume ≈ 10′ x 10′ x 8′
- Through utilization of unique beam forming optics, Bede Scientific in conjunction with University of Cambridge and UAB has developed an advanced x-ray generator.
  - Weight ≈ 60 lbs. (including controller)
  - Power ≈ 24-80 watts
  - Volume ≈ 18″ x 10″ diameter

Advanced X-Ray Generator and X-Ray Source Power Supply/Controller

Three Circle Goniometer

- **Description:** Provides highly accurate means of positioning crystal during data collection.

- Unique three circle goniometer also designed and developed to support rack packaging and tele-science requirements
- Highly accurate encoded design
- Seven remotely controlled axes
- Low profile to fit within rack packaging
- Works in vertical and horizontal position



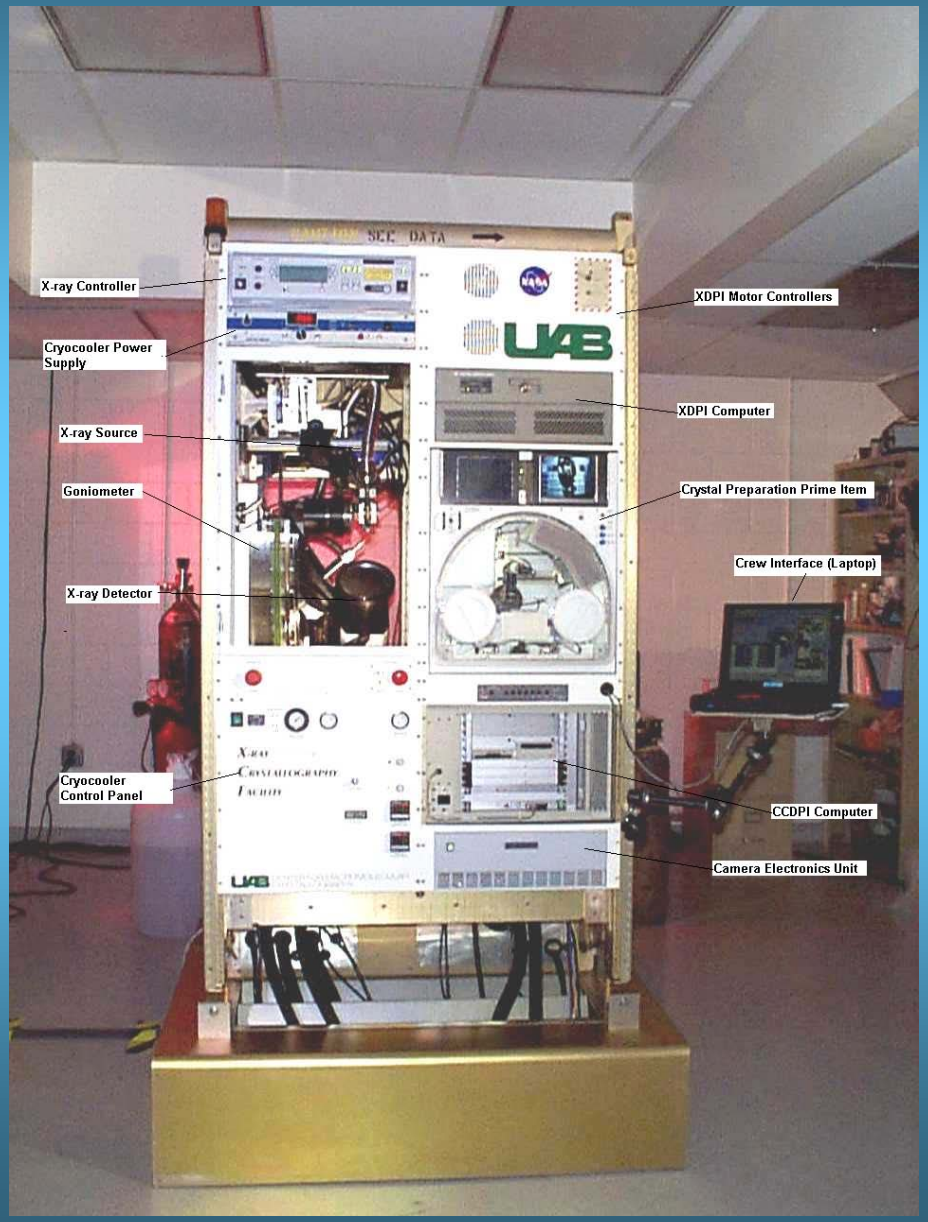
Advanced X-Ray Sensor

- **Description:** Highly sensitive x-ray area detector used to collect Bragg diffraction data.

- A 2048 x 2048 (2 K) CCD X-Ray Detector has been selected to support the XCF Diffraction system.

- Based upon highly advanced commercial unit
- Provides high accuracy data.
- Quick read-out.
- Lighter weight and more reliable than other detectors (in FY2000)
- Prototype unit developed to cool with water (-50 ºC chip) rather than propane/ethanol mixture.

X-Ray Crystallography Facility Technology Demonstrator housed in an Engineering Development Article (Ground Rack)



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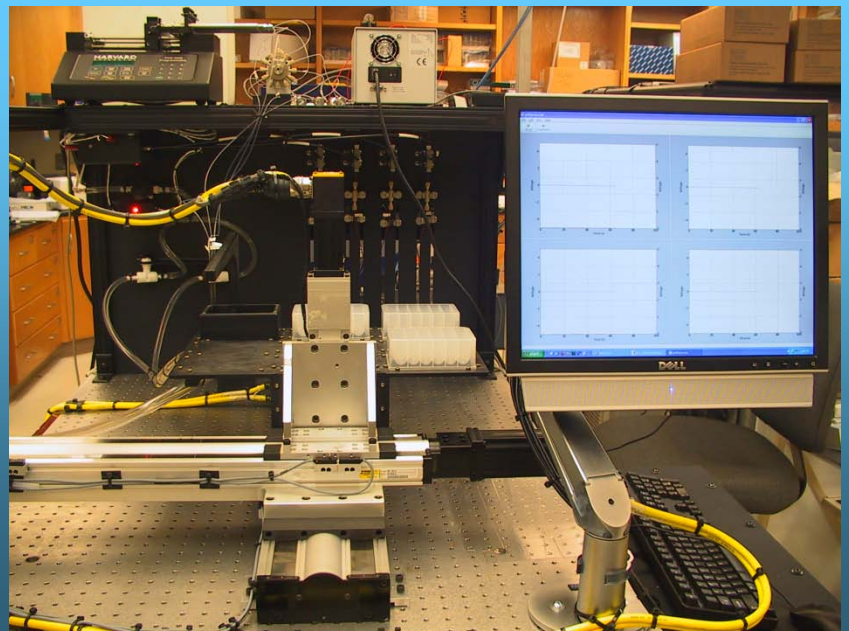
Operational Limitations and Drivers

- ISS Limitations:
  - Crew time
  - Power (2 KW total)
  - Volume (1 rack)
  - Weight (1773 lbs. total)
  - South Atlantic Anomaly
  - Uplink (capacity, availability, and outages)
  - Downlink (capacity, availability, and outages)

- Storage Freezer Limitations
  - Crew time

Future Automation Ideas

1. Automated ***Protein*** Optimization
   - High-throughput system that determines solution conditions that optimize protein solubility and physical/chemical stability:
   High-throughput Self -interaction Chromatography

2. High-throughput Crystal Optimization System
   Based on Snapshot Diffraction Analysis