The Evolution of Automation for Structural Systems Biology

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Structural Systems Biology

Mapping the dynamic interacteome -

understanding the large-scale organizing principles of the cell in space and time at the molecular to cellular level

Crystallography	**atomic resolution structures of biomolecules and their complexes**
Solution x-ray scattering	**molecular envelopes; supramolecular structure changes; HT characterization**
Spectroscopy	**chemical analysis**
Coherent x-ray imaging microscopy	**subcellular localization; cellular architecture**

Synchrotron Radiation as a Source for X-ray Diffraction

G. ROSENBAUM & K. C. HOLMES
Max-Planck-Institut für Medizinische Forschung, Heidelberg

J. WITZ
Laboratoire des Virus des Plantes, Institut de Botanique de la Faculté des Sciences de Strasbourg, Strasbourg

Some preliminary results have been obtained with synchrotron radiation from the 7.5 GeV electron synchrotron Deutsches Elektronen - Synchrotron (DESY) in Hamburg as a source for X-ray diffraction.

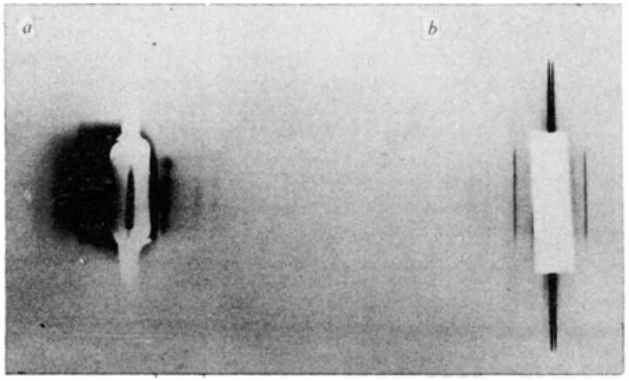


Fig. 3 Equatorial reflexions from dorsolongitudinal flight muscle of *Lethocerus maximus* recorded with: *a*, monochromated synchrotron radiation; electron energy 5 GeV, beam current 8 mA, exposure time 15 min, specimen film distance 40 cm; note the parasitic scattering on the left of the backstop arising from fluorescence from the monochromator holder; *b*, Elliott fine-focus rotating anode tube at 40 kV, 15 mA, exposure time 1 h, specimen film distance 36 cm. The strong line is the 20 reflexion (d=231 Å); the weak lines are the 21, 31 and 32 reflexions.

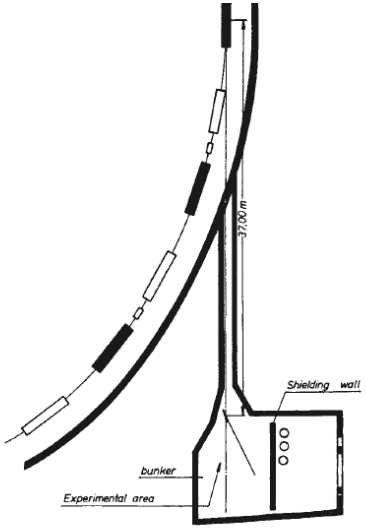


Fig. 1 The F41 bunker at DESY and its position with respect to the synchrotron.

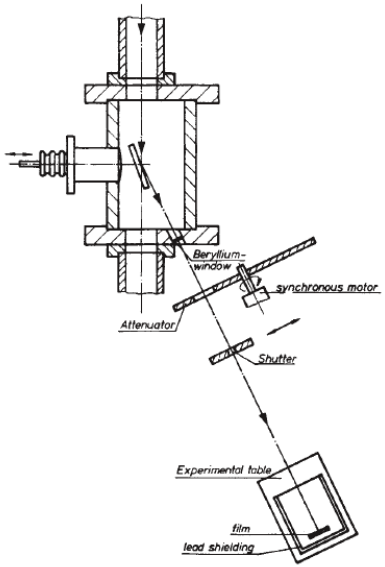
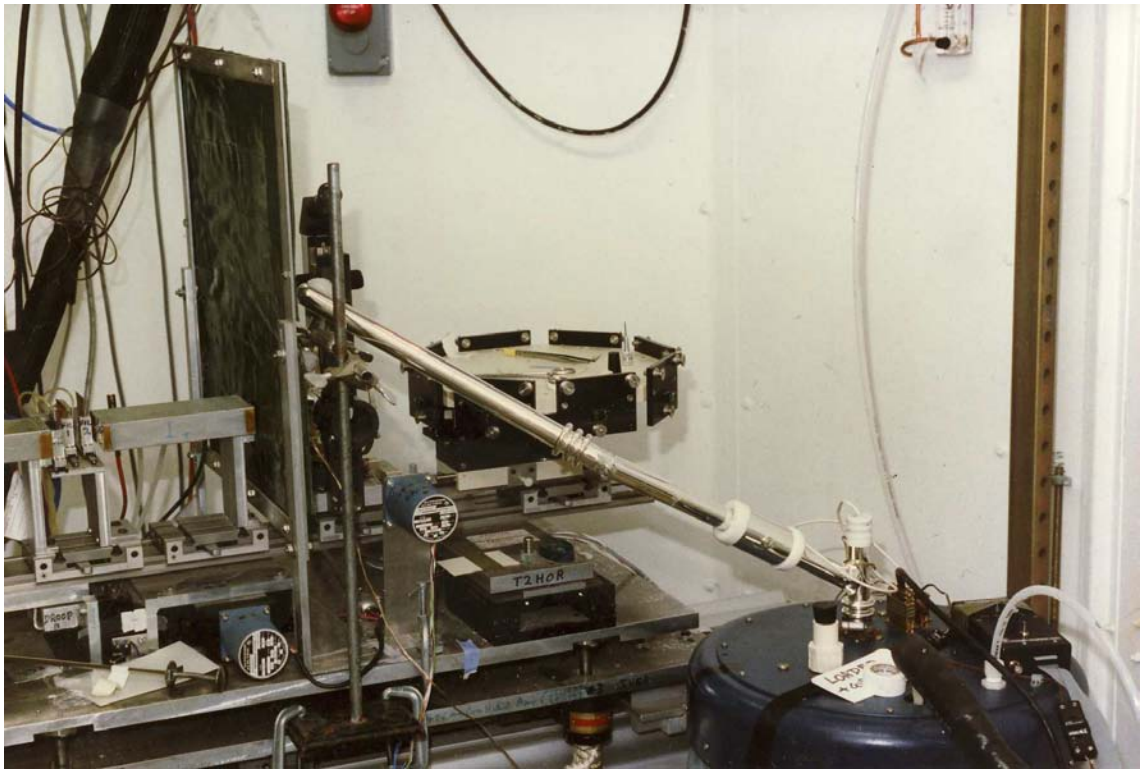
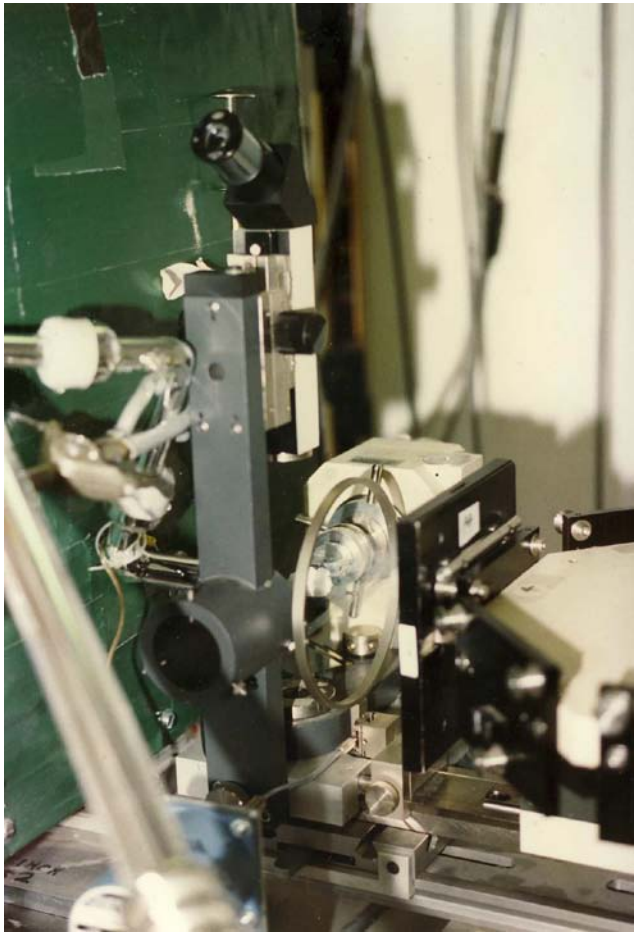


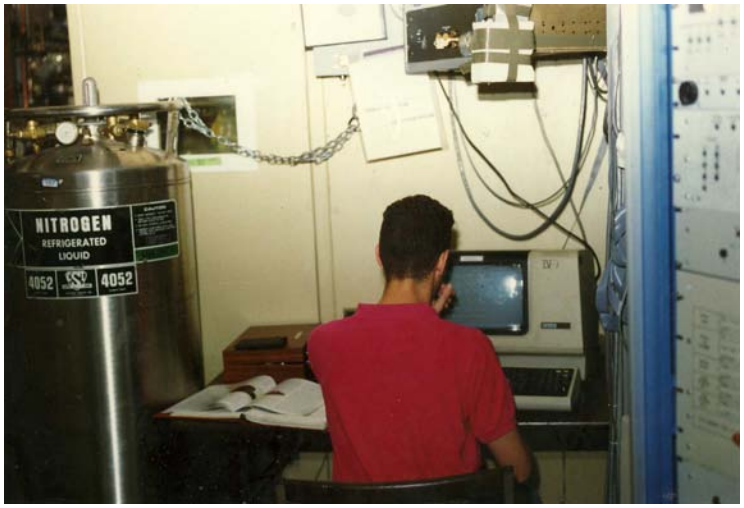
Fig. 2 Monochromator housing and the experimental set-up.

SSRL 1987

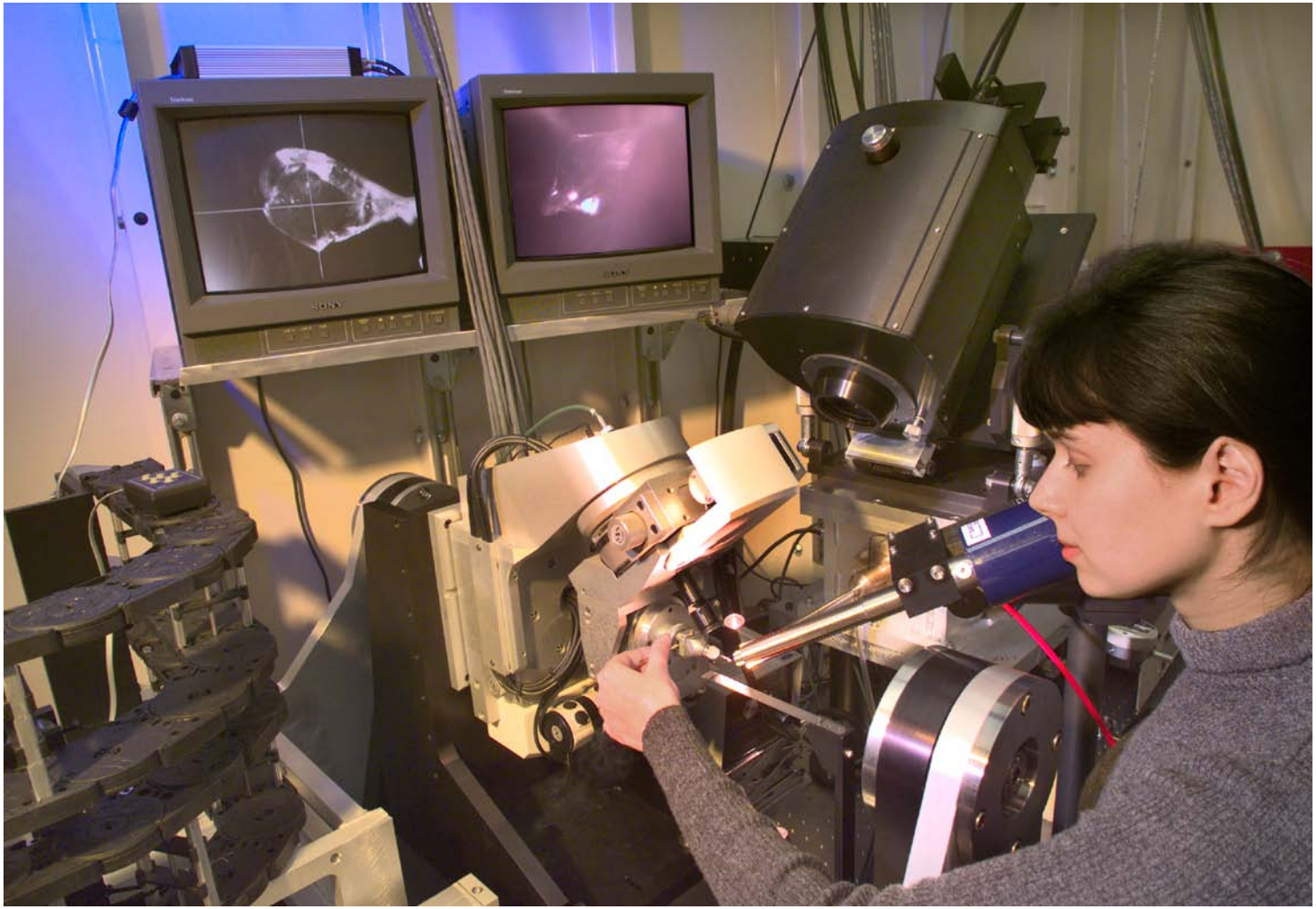




SSRL 1987



Manual mounting and alignment



Stanford Synchrotron Radiation Laboratory

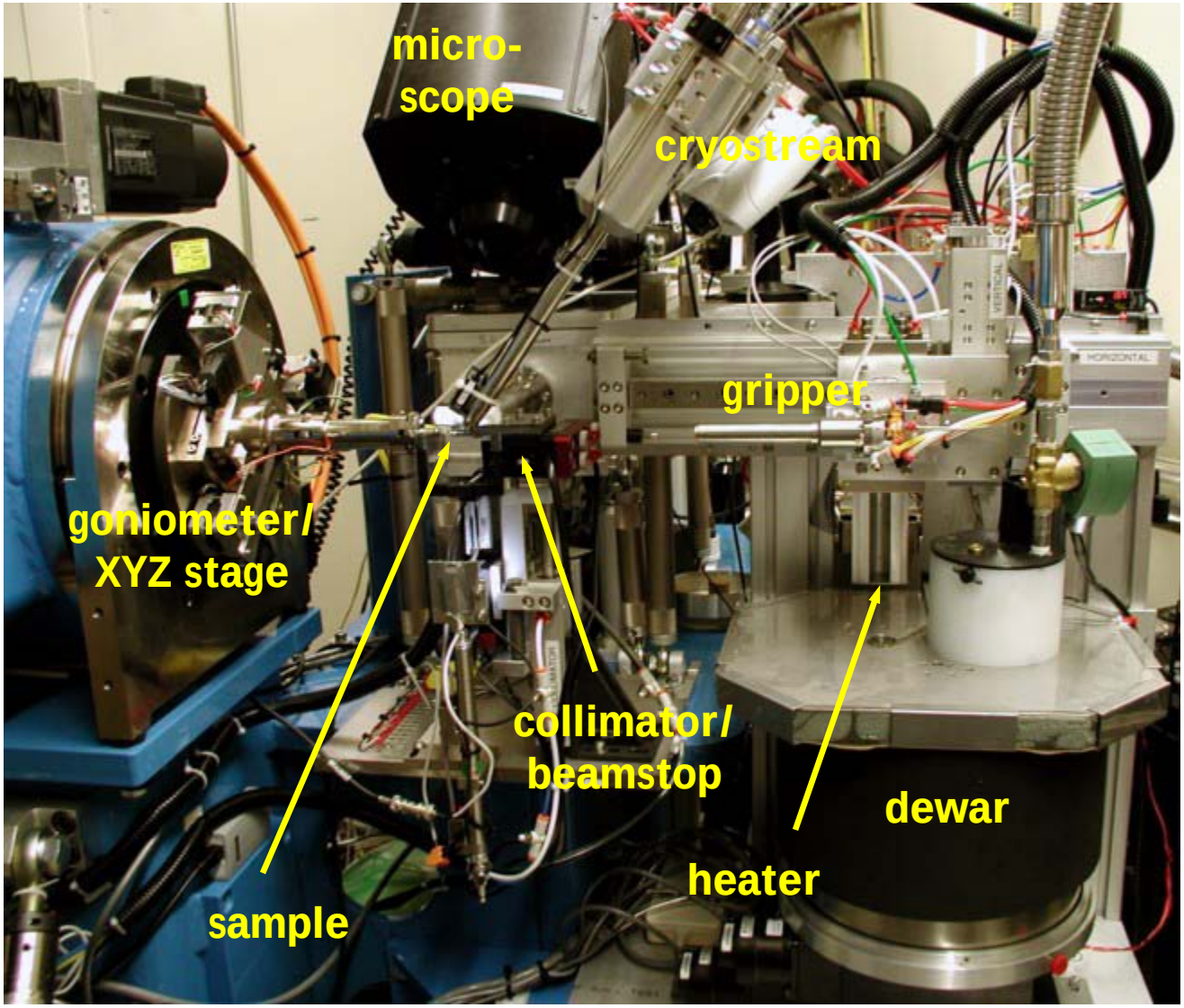
Workshop on techniques for automated mounting, viewing and centering pre-cooled protein crystals

May 11-12, 2000

Workshop Committee:

Paul Phizackerley
Ashley Deacon
Thomas Earnest
Gerd Rosenbaum

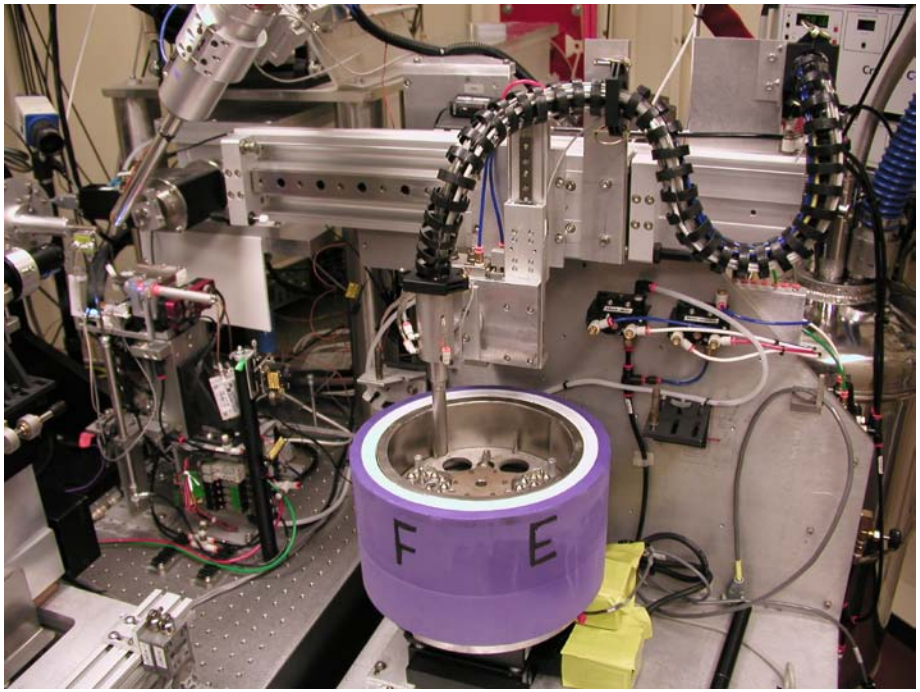
- More efficient use of synchrotron resources
- More reliable mounting / unmounting / remounting
- Rapid evaluation and selection of crystals
- Direct input of information to database and LIMS
- Remote monitoring and control of data collection
- Systematic exploration of experimental protocols
- Ability to intelligently and optimally control data collection / processing / analysis
- Integration with upstream and downstream components to achieve high-output

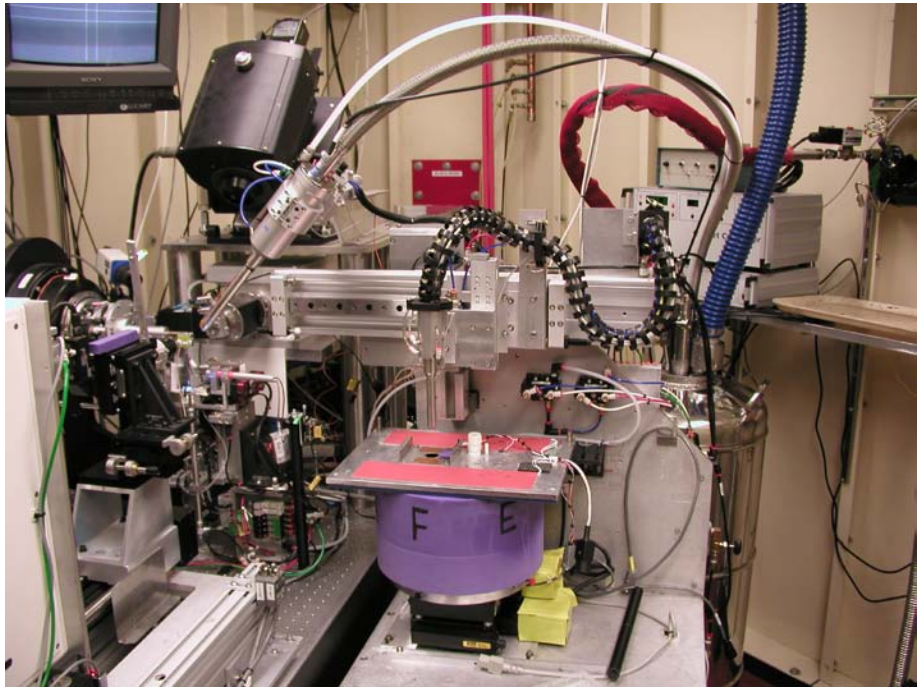


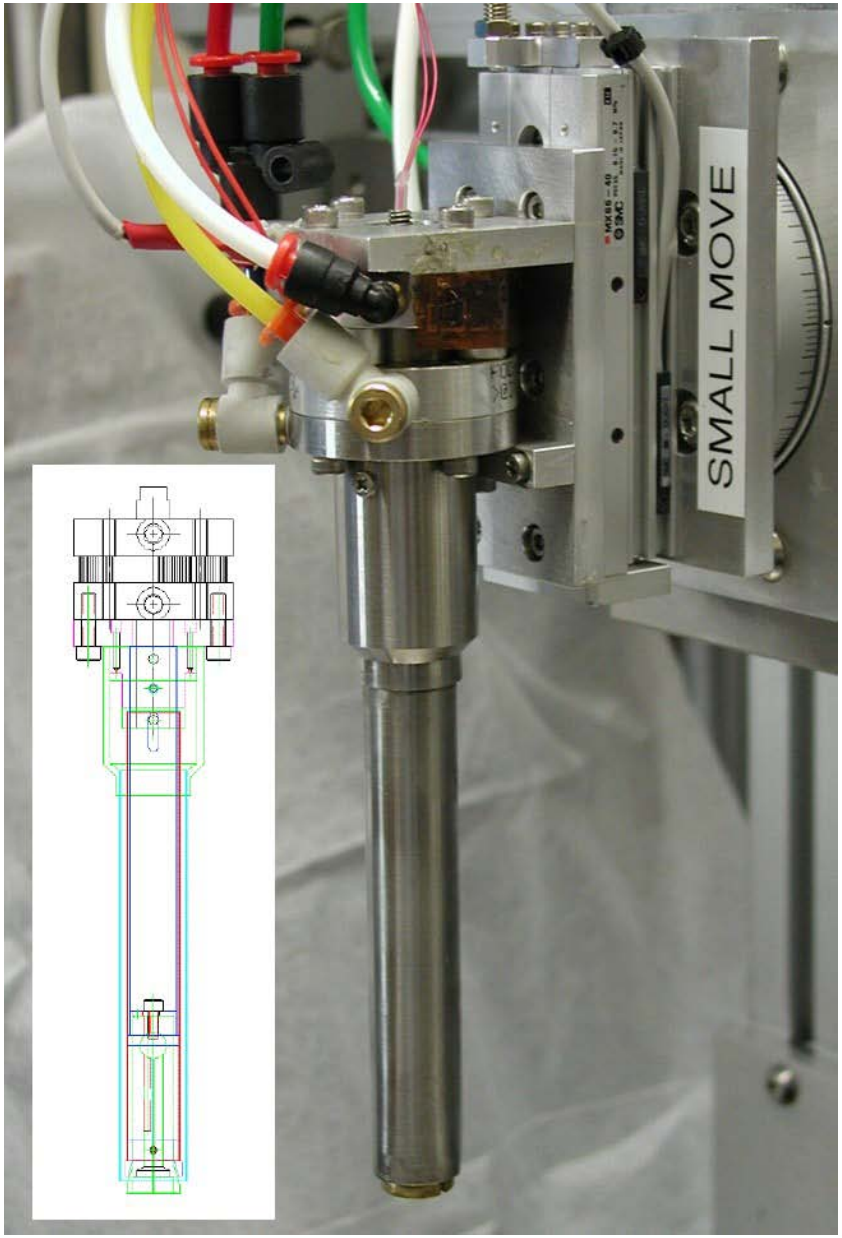
First beamline automounter

ALS 5.0.3
March 2001

ALS Sector 5





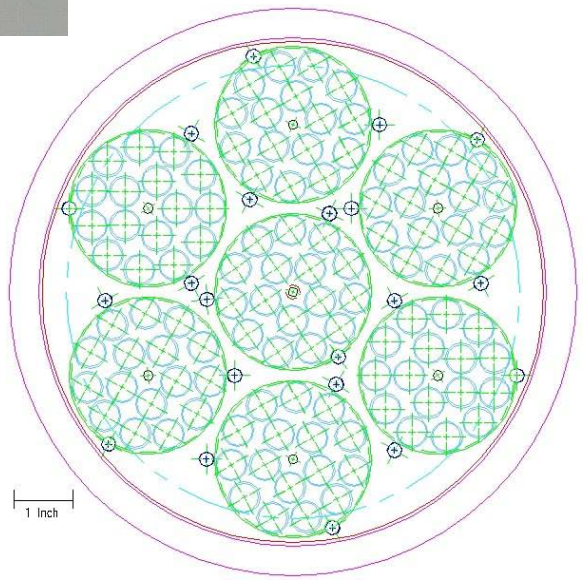

SMALL MOVE

Crystal transport and handling

sample cassette with magnetic base (puck)

puck handling tools

automounter dewar with 112 samples



Next-generation automounter

- Builds on experience of first-generation Berkeley design automounter systems at ALS, APS, NSLS, and CHESS

- Fully-automated, rapid automounter alignment

- Rapid mounting for high-speed crystal screening

- State checking and error handling for ultra-high reliability and fault correction

- Flexibility of layout and capacity

- Redundant safety features to protect personnel and equipment: *i)* proactive absolute motion limits, *ii)* configurable robot envelope, *iii)* kernel-level fast stop-on-digital, *iv)* hardware-level emergency stop circuitry (CAT3 dual channel)

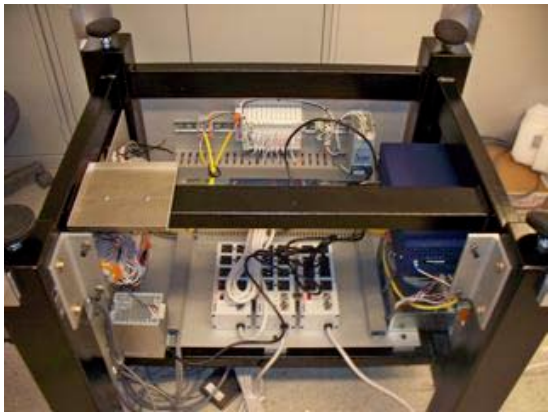
- Ease of software integration through state notation machine

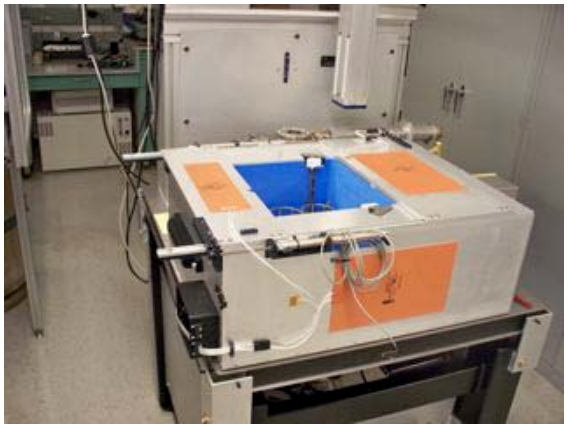
- Technical documents in public domain and freely available

Installation at GMCA-CAT bend magnet beamline
shipped 25 May 2010

also being implemented at MacCHESS

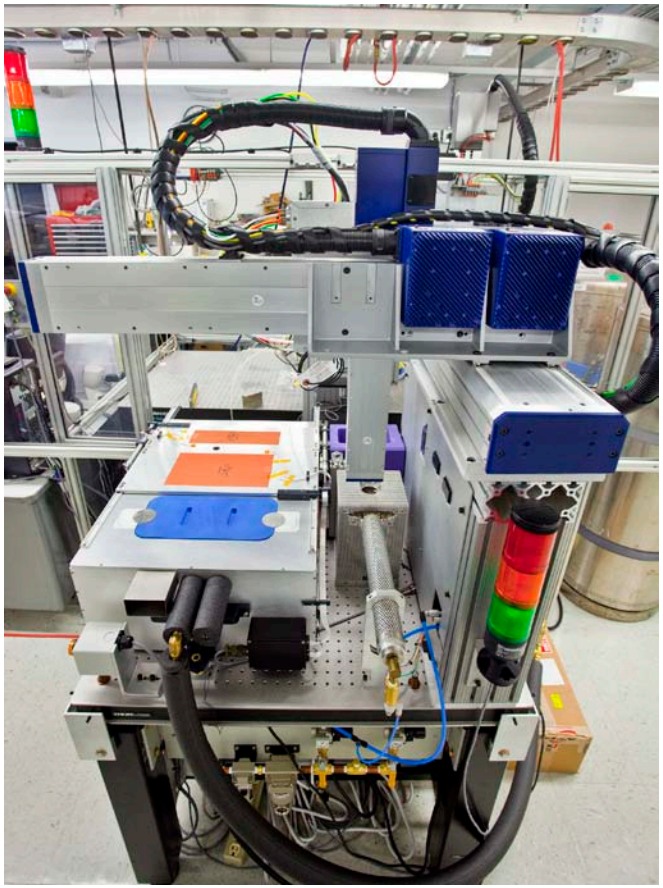
Robot Assembly / March 2010

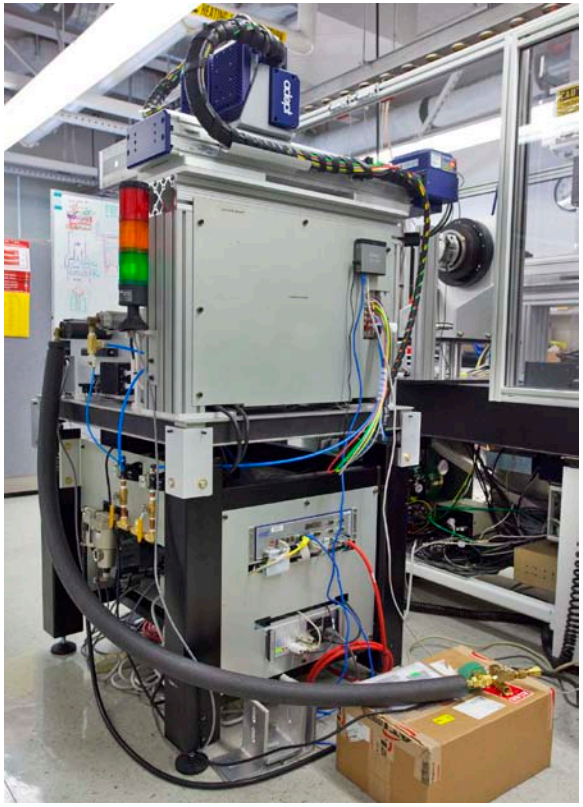


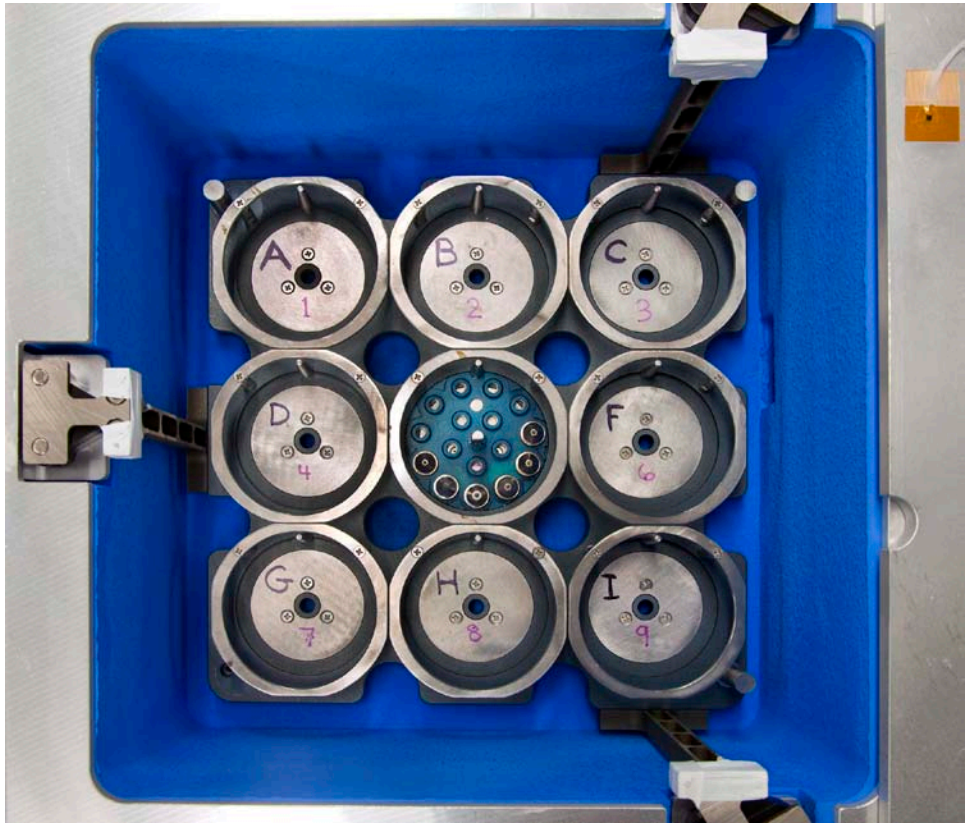


In Development Lab / 21 May 2010

ready to roll



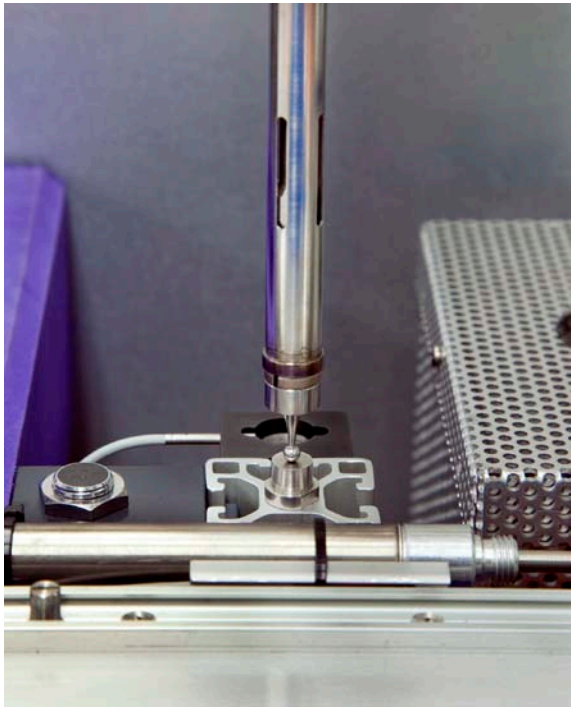




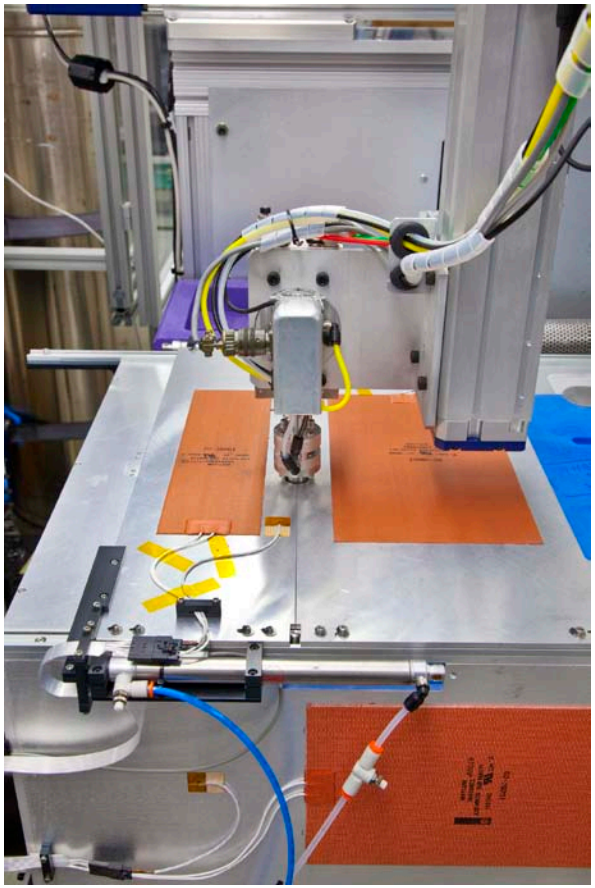
Kinematically-mounted, removable puck plate mounted off lid

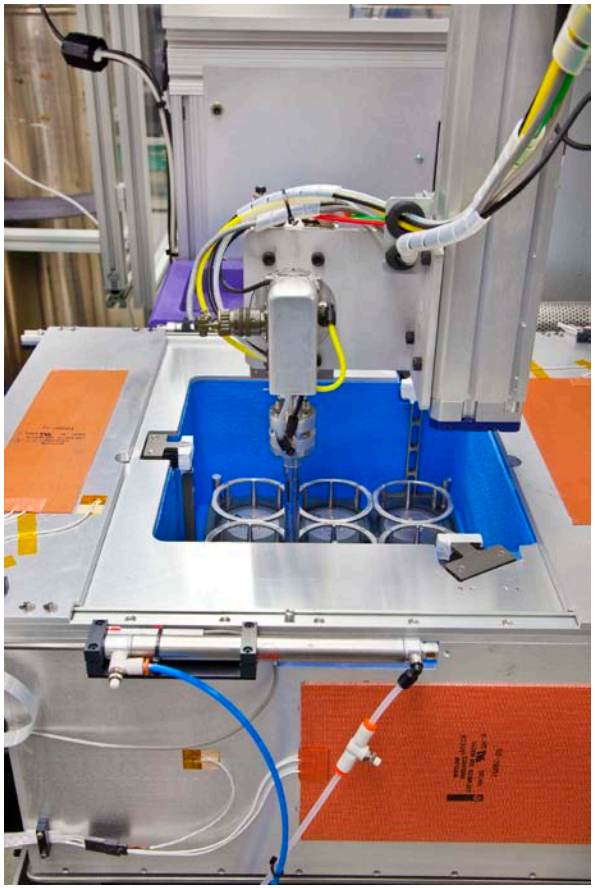
Foam Dewar in metal box

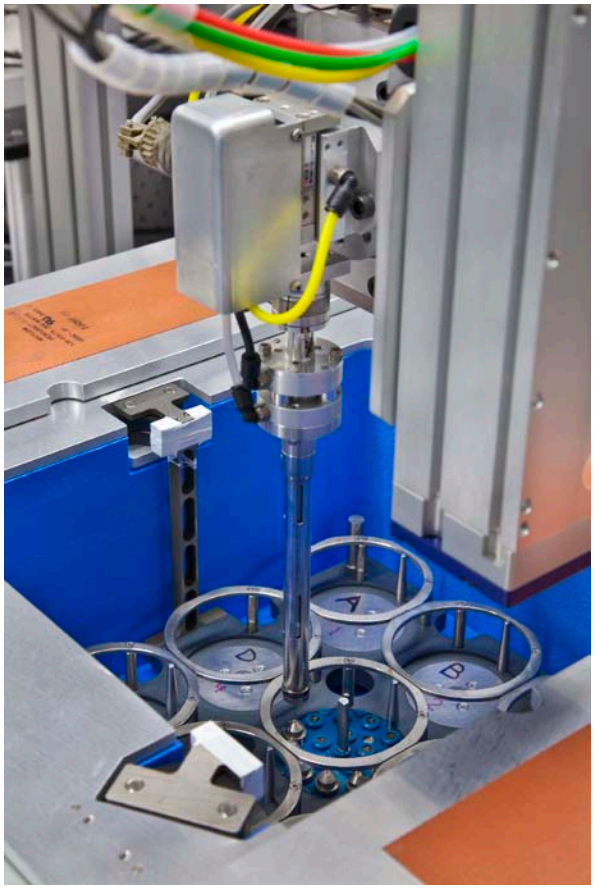
Rapid fully automated robot alignment



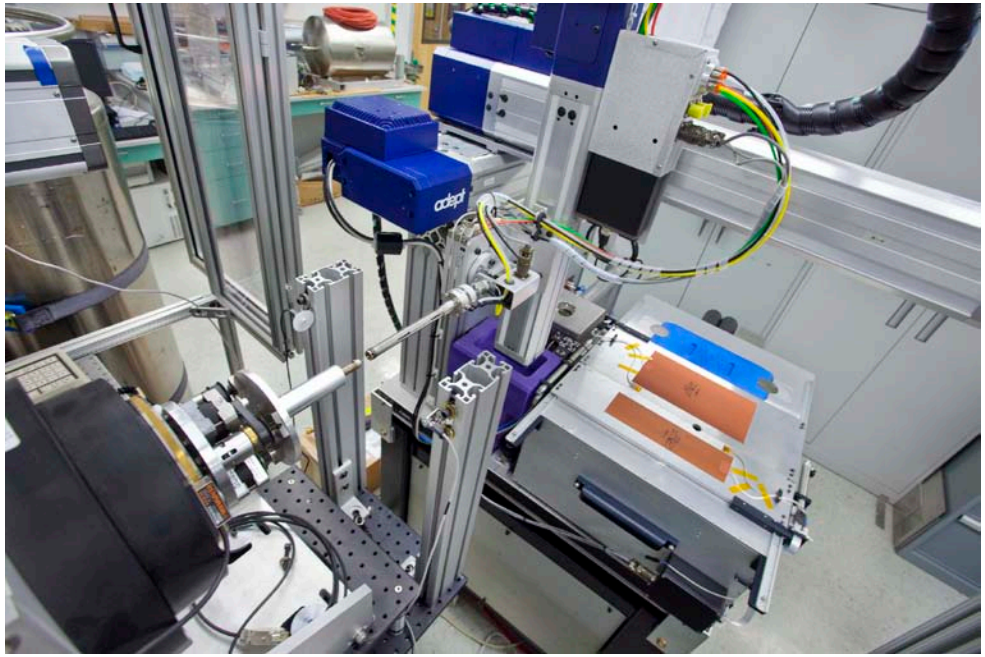
During robot alignment the gripper picks up the alignment tool (ball on a cone) then utilizes fiducial posts and force sensor to determine coordinates of home position, puck plate, puck positions, and goniometer. Can be done at liquid nitrogen or room temperatures.



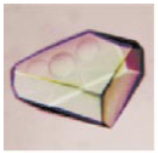
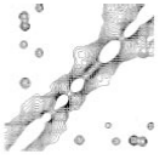
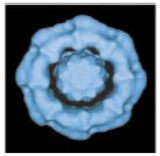
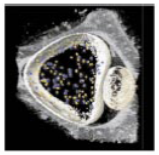
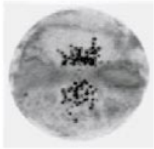




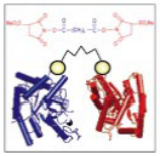
Puck loading and unloading

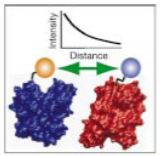


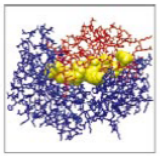
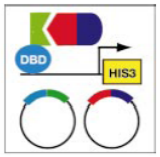
Puck plate can be removed and precisely replaced

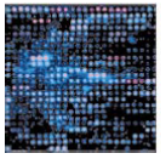
X-ray crystallography	NMR spectroscopy	2D and single-particle electron microscopy	Electron tomography	Immuno-electron microscopy
Subunit structure	Subunit structure			
Subunit shape	Subunit shape	Subunit shape	Subunit shape	
Subunit–subunit contact	Subunit–subunit contact	Subunit–subunit contact	Subunit–subunit contact	
Subunit proximity	Subunit proximity	Subunit proximity	Subunit proximity	Subunit proximity
Subunit stoichiometry	Subunit stoichiometry			
Assembly symmetry	Assembly symmetry	Assembly symmetry	Assembly symmetry	Assembly symmetry
Assembly shape	Assembly shape	Assembly shape	Assembly shape	
Assembly structure	Assembly structure			

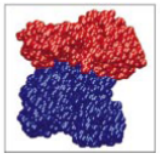
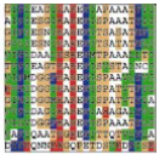




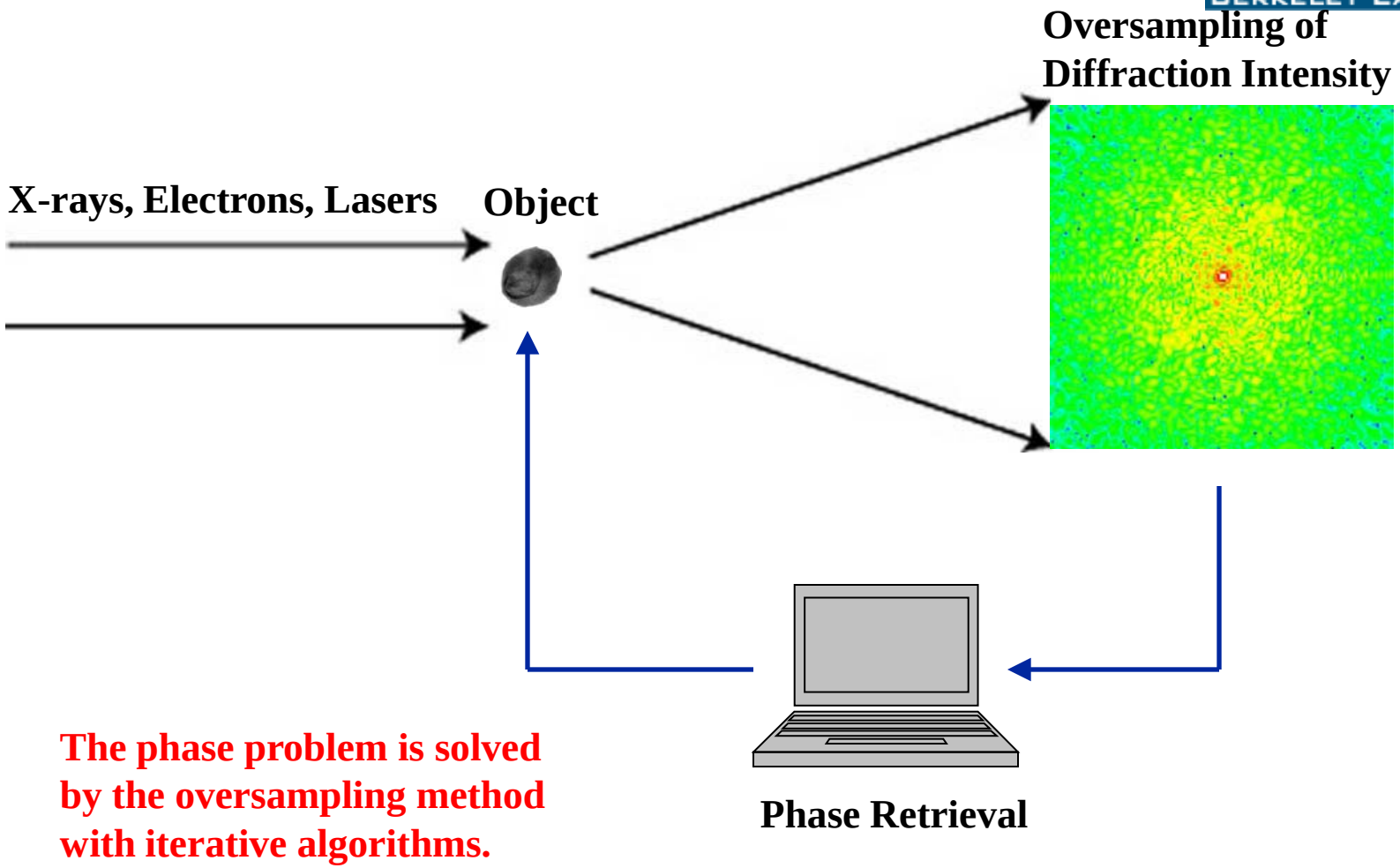



Chemical cross-linking	Affinity purification mass spectroscopy	FRET	Site-directed mutagenesis	Yeast two-hybrid system
Subunit structure				
Subunit–subunit contact	Subunit–subunit contact	Subunit–subunit contact	Subunit–subunit contact	Subunit–subunit contact
Subunit proximity	Subunit proximity	Subunit proximity		Subunit proximity



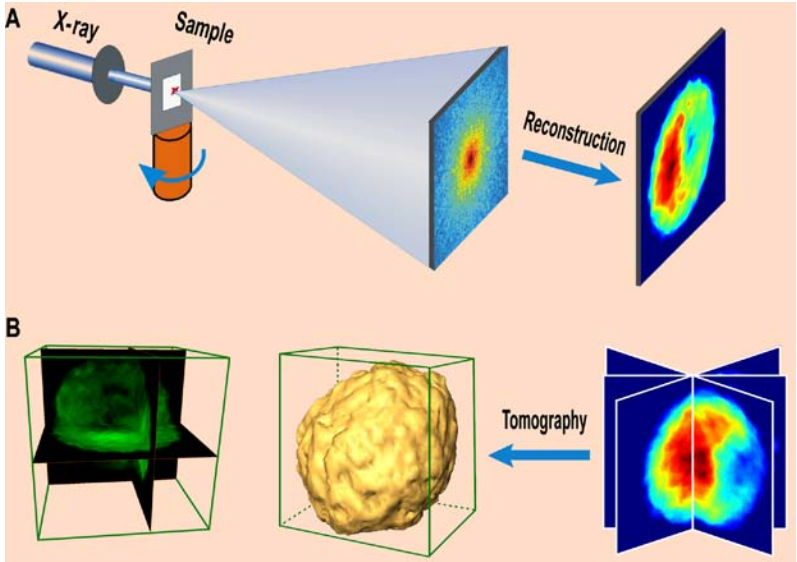



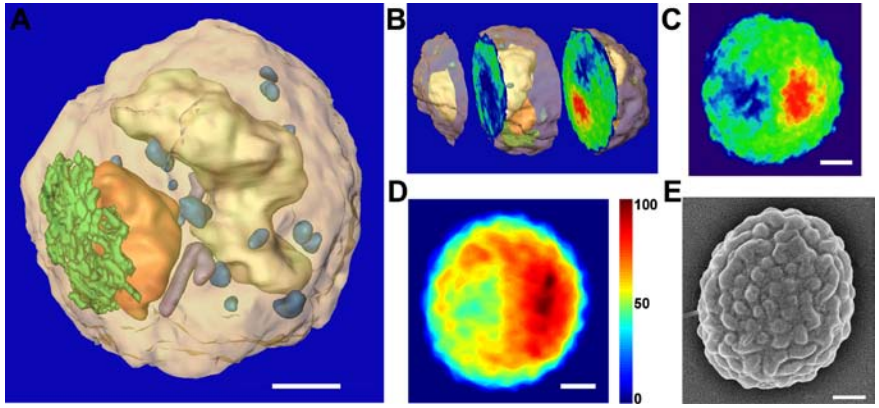
Gene/protein arrays	Protein structure prediction	Computational docking	Bioinformatics
	Subunit structure		
	Subunit shape		
Subunit–subunit contact		Subunit–subunit contact	Subunit–subunit contact
Subunit proximity			



The phase problem is solved by the oversampling method with iterative algorithms.

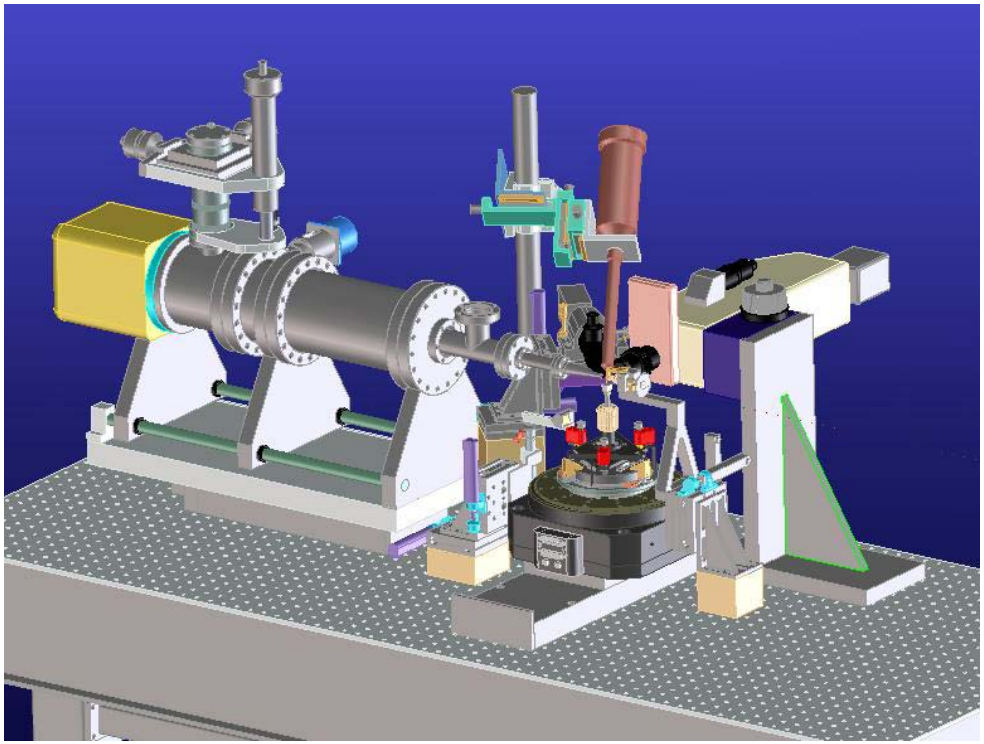
Coherent Diffractive Imaging

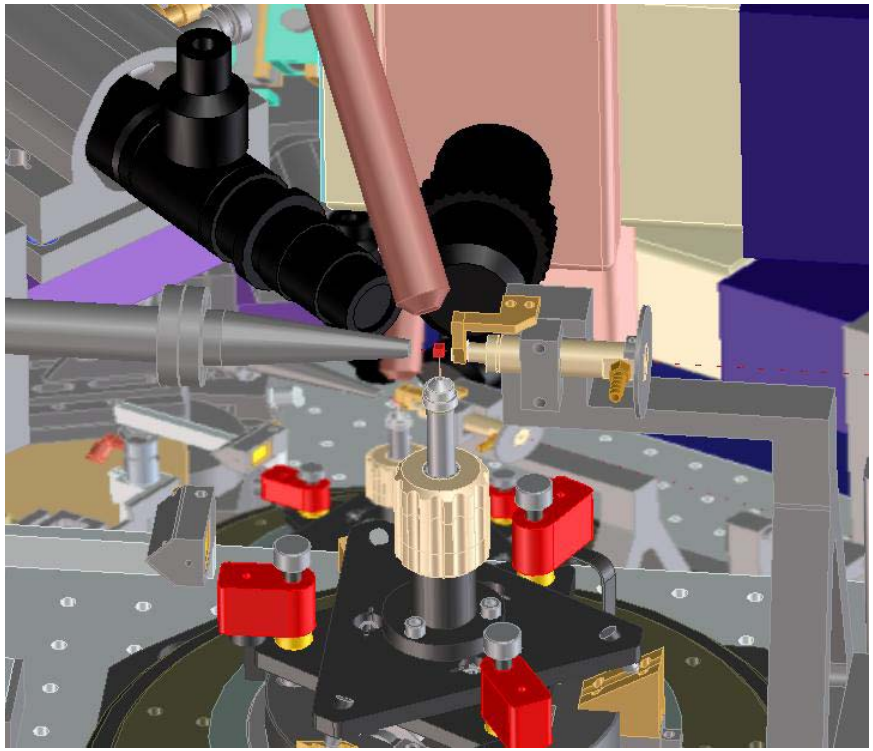




From Miao, et al.
SPring-8

Cryo-tomographic microscope for CXDI of cells and non-periodic samples





- Capability for rapid sample changing and alignment
- High precision stages, vertically mounted
- Inline viewing optical alignment microscope

Acknowledgments

Carl Cork
Jim O'Neill
John Taylor
Gyorgy Snell

Bob Nordmeyer
Earl Cornell
Derek Yegian
Jian Jin
Joe Jaklevic

Ray Stevens
Bernie Santarsiero

Collaborators at APS: GMCA-CAT, NE-CAT, SER-CAT;
ALS: Sector 5; NSLS: PXRR, X6A; MacCHESS

NIH/NIGMS R01, PSI, ARRA

144 samples, no scheduled warm-up

196 minutes, w/ 8 cold retries, 2 warm retries, 0 failures

144 samples, warm-up after every puck (16 samples, ~21 minutes):

216 minutes, w/ 0 cold retries, 0 warm retries, 0 failures

Overhead = 24 minutes for scheduled warmups (8 * 3 minutes).

In both cases, there was a 2 second delay between the end of the load operation and the beginning of the unload. This contributes about 4.8 minutes to the total measured time.

A load and unload each take ~38 seconds with the current robot speed settings.

LOAD:

- Start at SOAK with cold gripper
- Open lid and get sample at specified <slot#,sample#>.
- Transport to proximity sensor and verify pick success. (close lid during transport)
- Transport to goniometer and deposit sample.
- Goto proximity sensor and verify place success.
- Goto SOAK location and wait for unload command.

UNLOAD:

- Start at SOAK with cold gripper
- Goto goniometer and get sample.
- Transport to proximity sensor and verify pick operation.
- Transport to SOAK location and do cooldown of sample base.
- Open lid and place sample at specified <slot#, sample#..
- Goto proximity sensor and verify place success. (close lid during move).
- Goto SOAK location and wait for next load command.