U[sers] Can't Touch This

Remote Access with SSRL at SLAC

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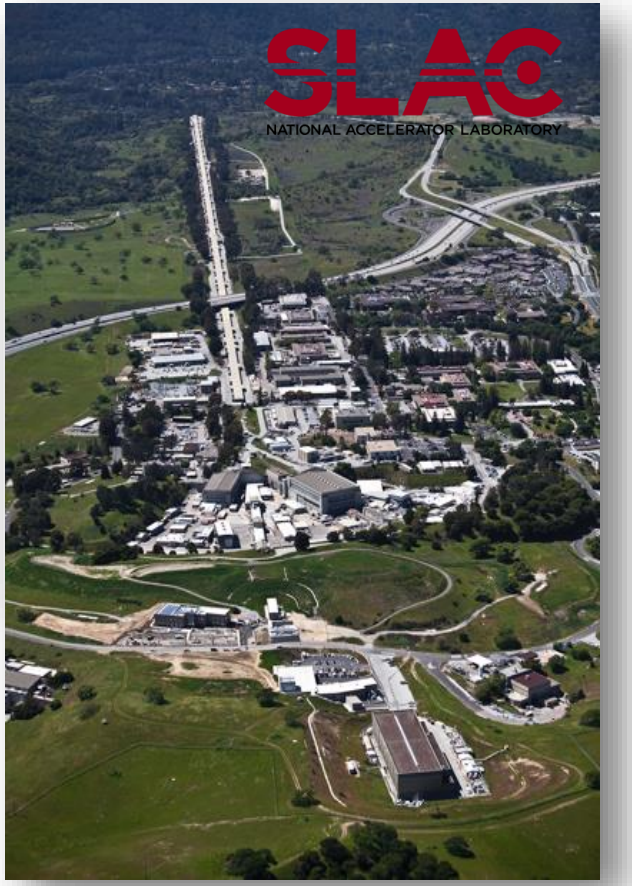
SSRL-SMB-PX talks at MCE 2021

In case you missed it…

Tuesday March 16th

Aina Cohen

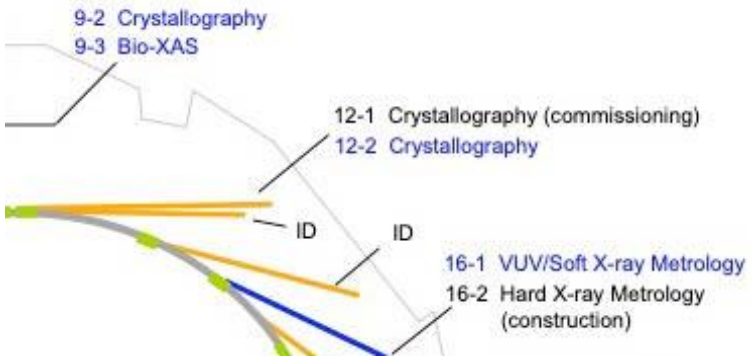
Next Generation Remote Experiments



Wednesday March 17th

Art Lyubimov

Crystallography Live: Processing and Analysis of Xray Diffraction Data in Real Time



Thank you for your attention!

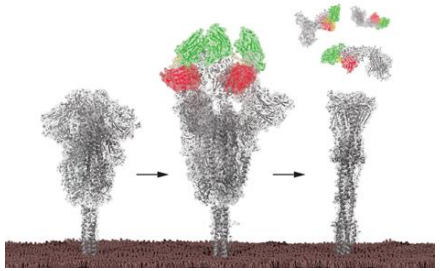
A Rapid Response at SLAC to Combat COVID-19

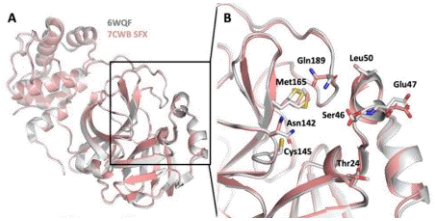
Fast Tracked Research Leads to Drug and Vaccines in Clinical Trials

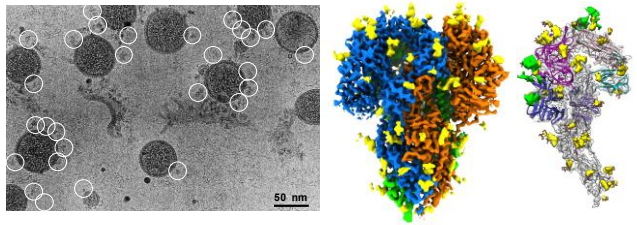
Synchrotron & CryoEM research started one year ago and LCLS in August 2020

so far

Proposals awarded time: **51**
MC Fragments/inhibitors screened: > **1088**
MC PDB deposits: **38**
MC Publications: **11**







SSRL BL12-1: Structure-guided inactivation of the SARS-CoV-2 spike protein using nanobodies (Koenig, Science 2021)

LCLS-MFX: SARS-CoV-2 main protease structures at near-physiological temp to guide drug repurposing (Durdagi, bioRxiv/2020)

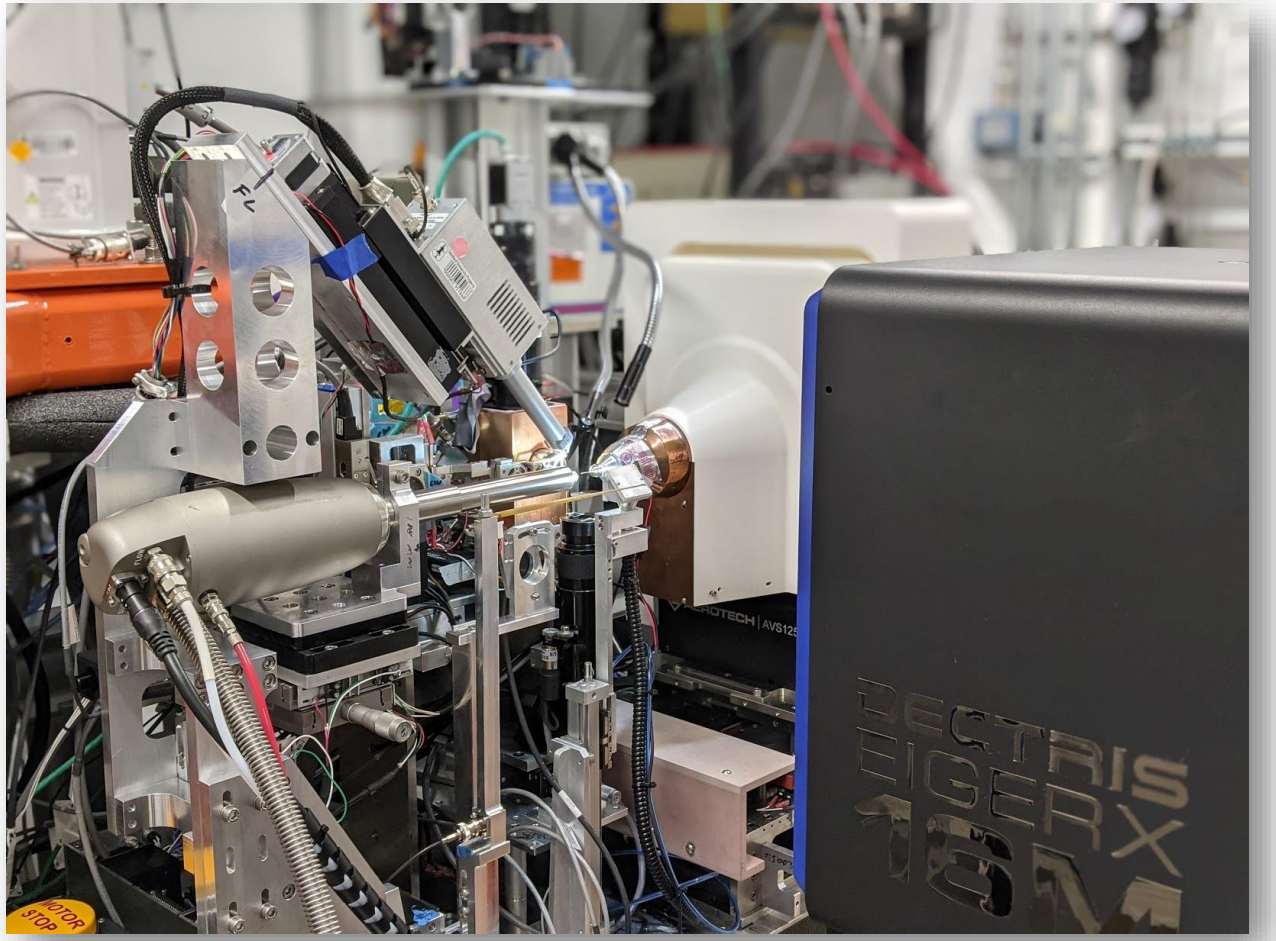
Cryo-EM: Structure of spike proteins and glycans of human coronavirus NL63 directly from virus particles (Zhang, bioRxiv/2020/245696)

Groups from across the US and abroad used SSRL, LCLS and CryoEM facilities at SLAC for COVID-19 related research

Outline

Remote Access with SMB at SLAC

- Standard @ SSRL/SMB
  - Stanford Auto-Mounting (SAM) system (Cohen *et al*., J. Appl. Cryst. 35, 720, 2002)
  - Remote Data Collection from anywhere in the world (Soltis et al., Acta Cryst. D64, 210, 2008)
- Advanced features @ Beamline 12-1
  - Serial and Dynamics
  - Elevated Temperatures
  - Humidity Control
- Look to the future



Standard @ SSRL/SMB – Remote Access

NoMachine NX

- Unix-based
- Access to internal network
  - Beamline control
  - SSRL data storage
  - Data processing

Crystal server

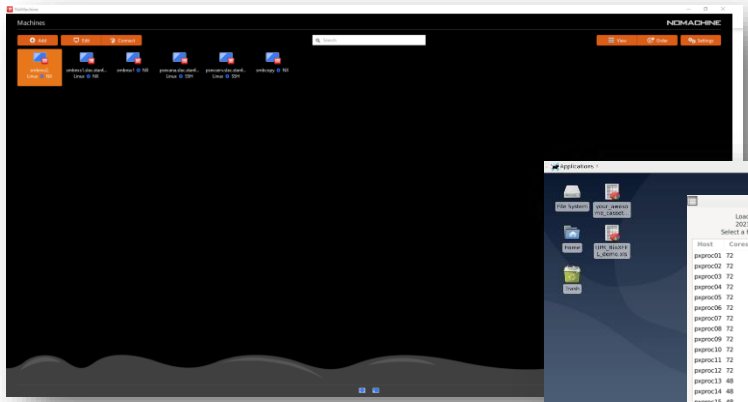
- Sample database
- Spreadsheets

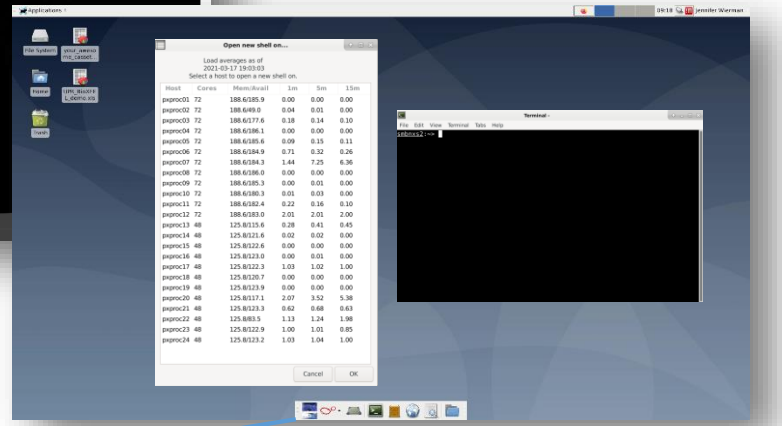
Blu-Ice Control Software

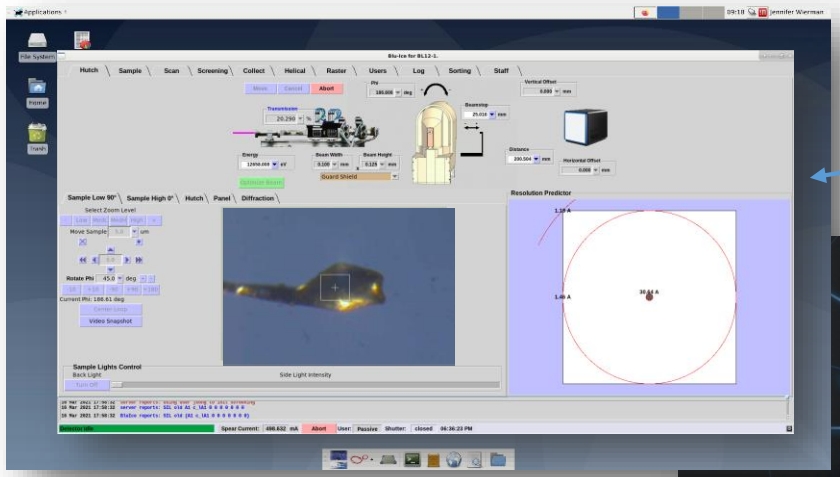
- Experiment control, tcl/tk, C/C++
- Support enables user
- Simple and intuitive graphical interface
- (McPhillips et al., J. Synch. Rad. 9, 401, 2002

User support

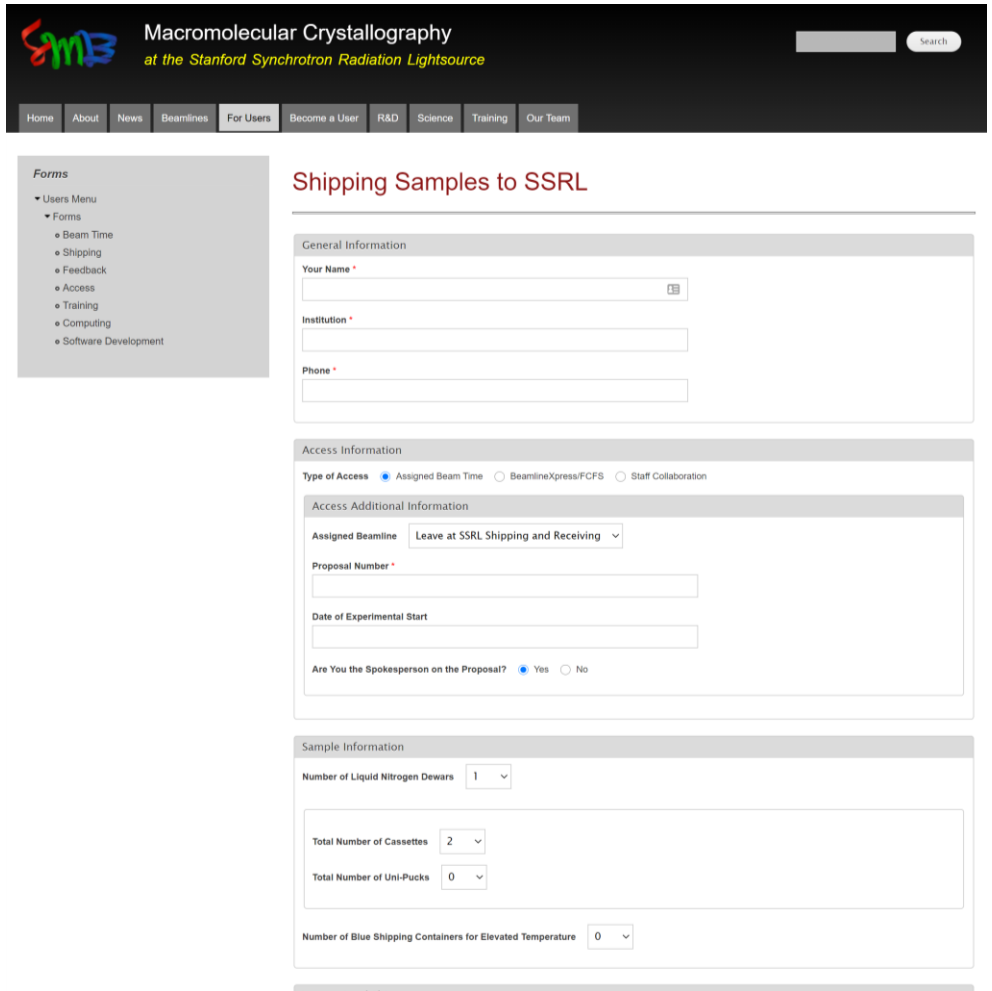
- Zoom, phone, email







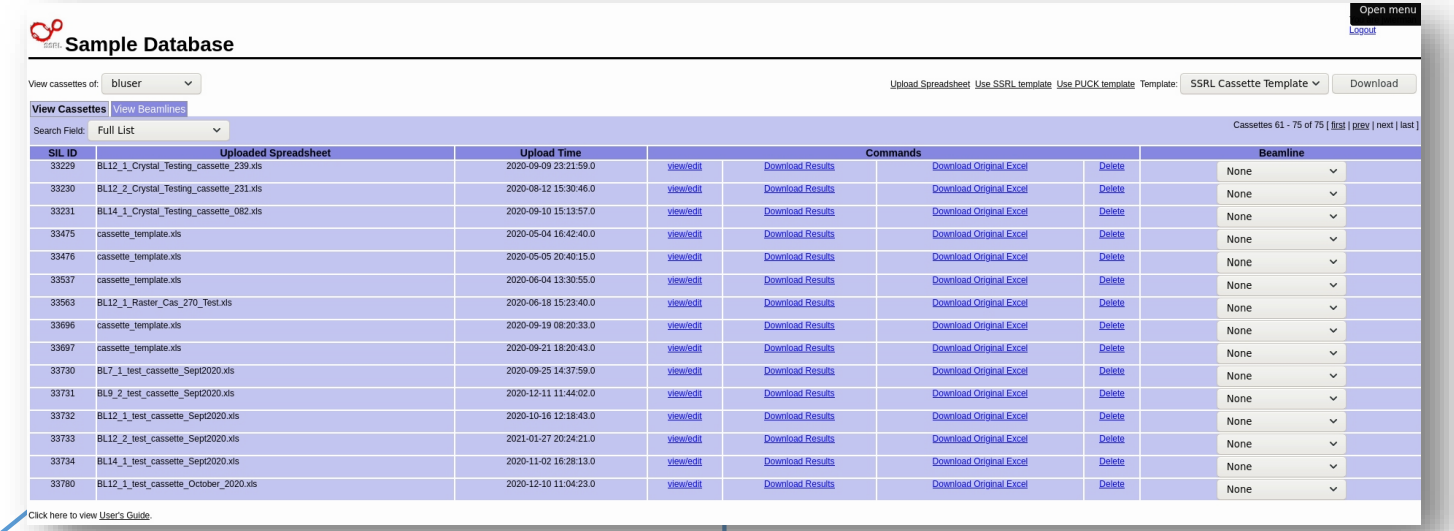
Standard @ SSRL/SMB - Shipping

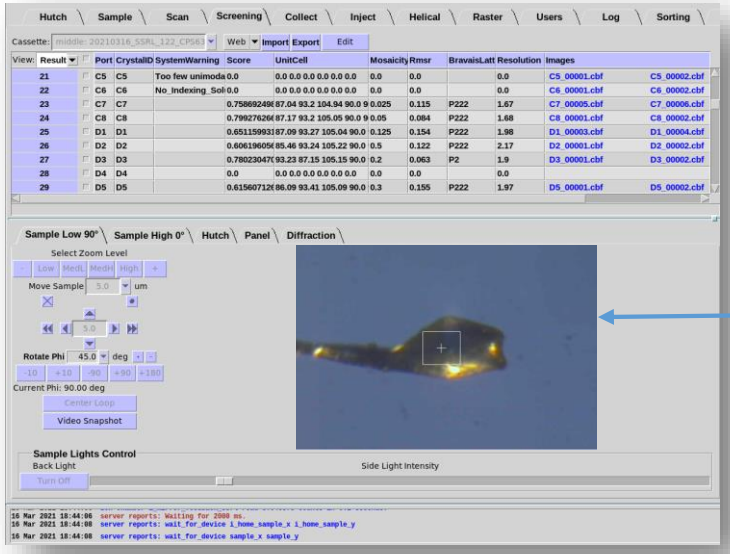


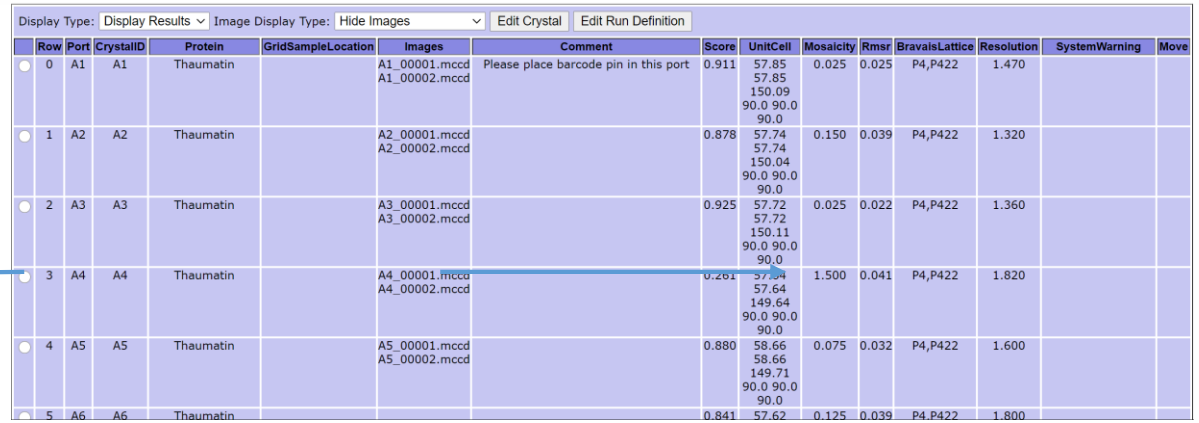
Standard @ SSRL/SMB – Sample Database

Spreadsheet based

- Online accessible
  - Editable
  - Upload/download
- Loads into Blu-Ice
  - Through beamline assignment (database or Blu-Ice)







Standard @ SSRL/SMB - BluIce - Experimental Setup

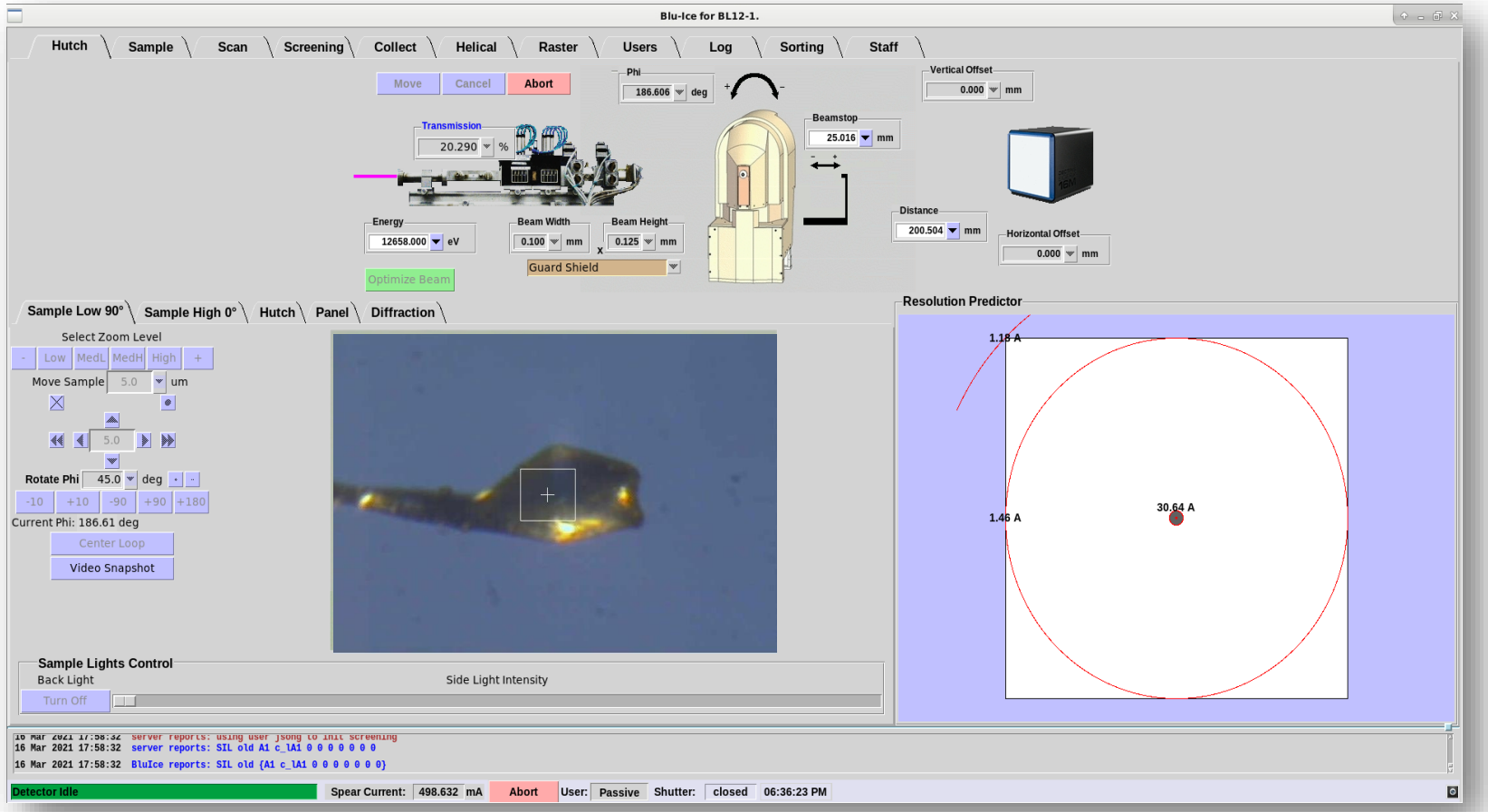
Beamline control

Sample control

- Anneal, wash
- Queue next sample

Scans

- Excitation scans
- MAD scans
  - Runs AutoCHOOCH
  - Displays energies for f', f", plus a calculated remote energy



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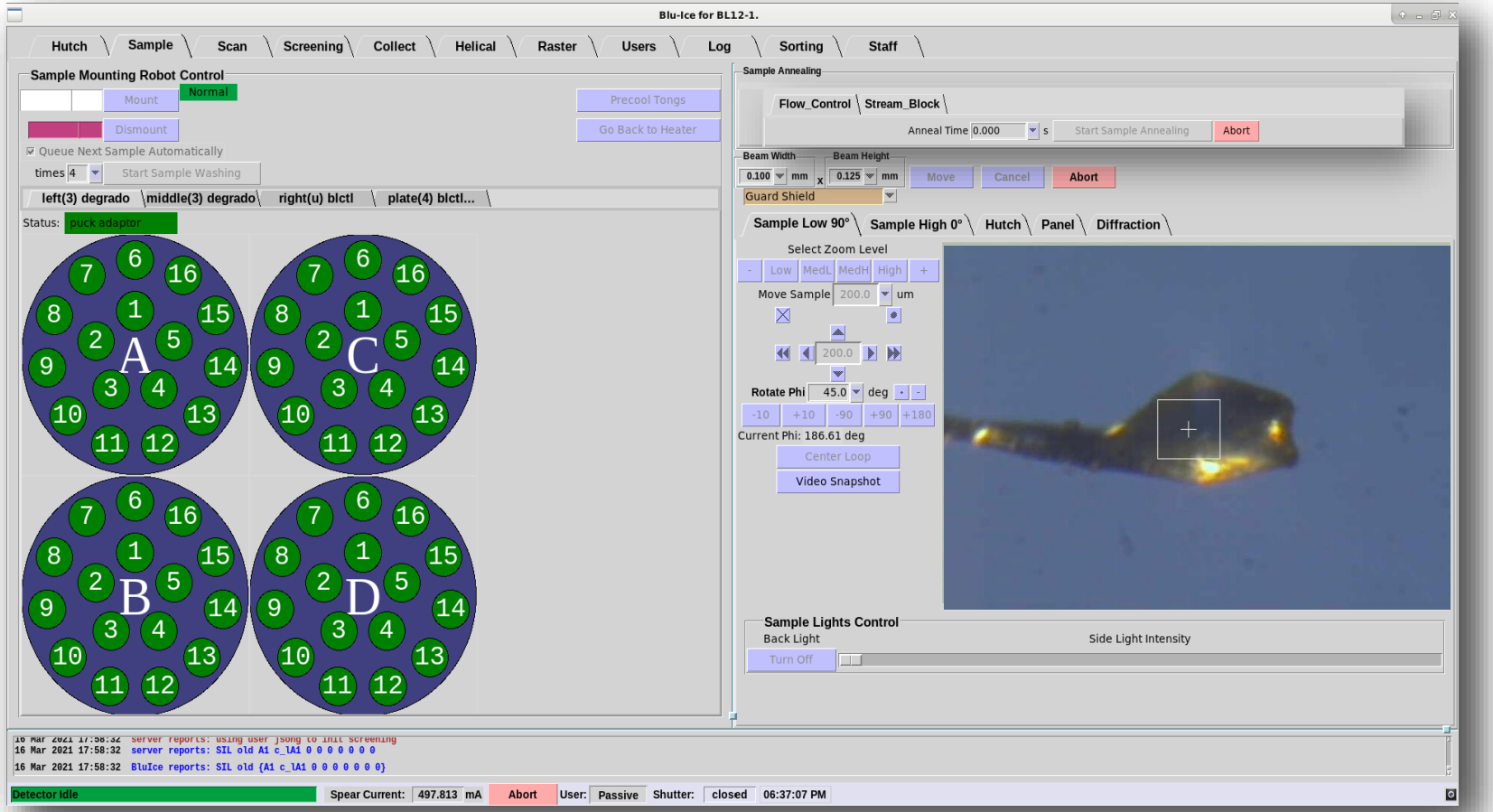

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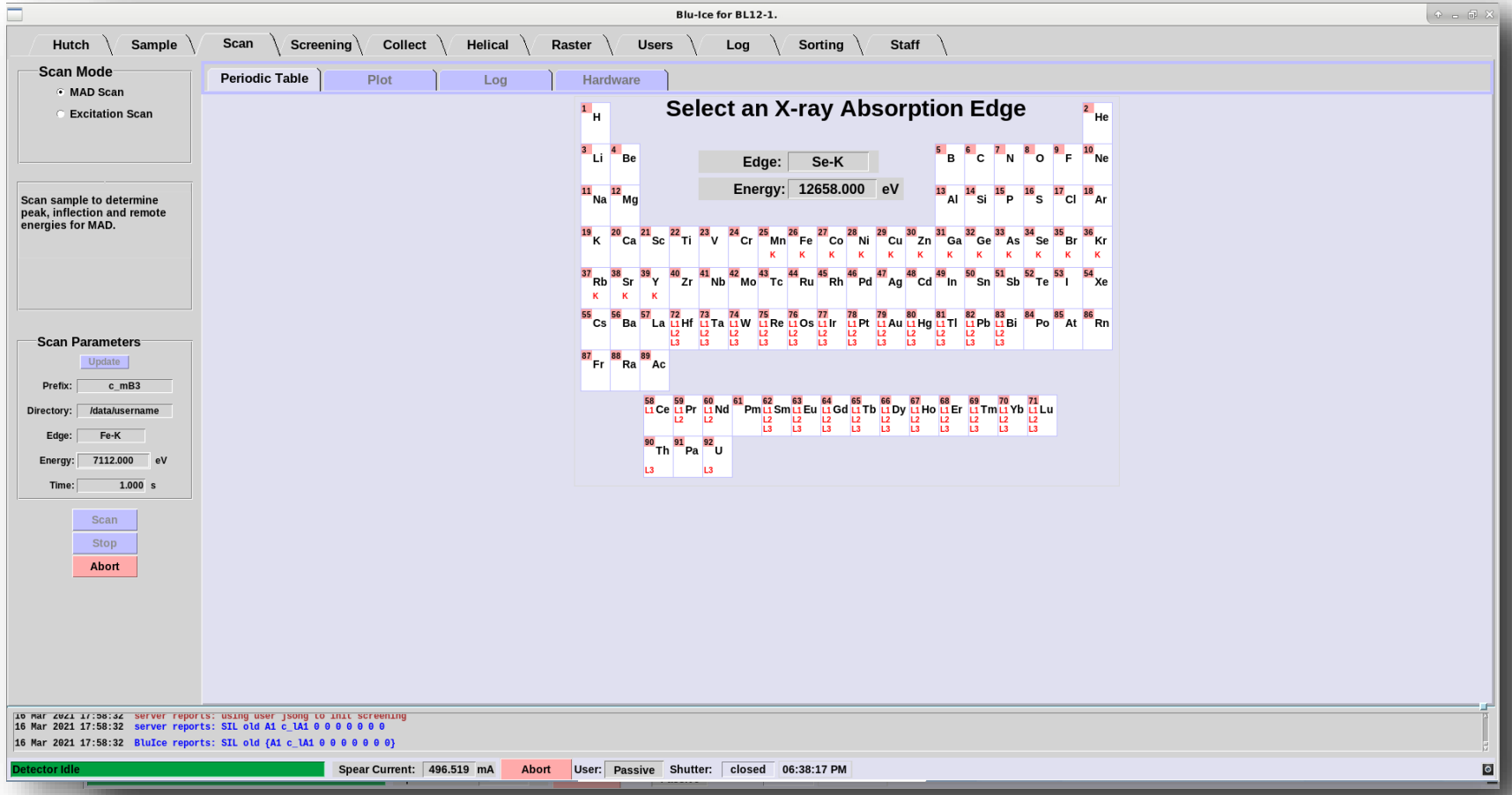

Standard @ SSRL/SMB - BluIce - Experimental Setup

Beamline control

Sample control

- Anneal, wash
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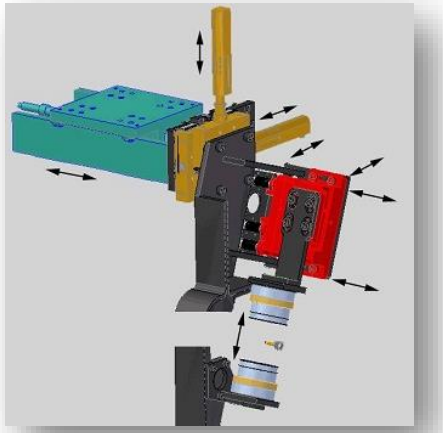
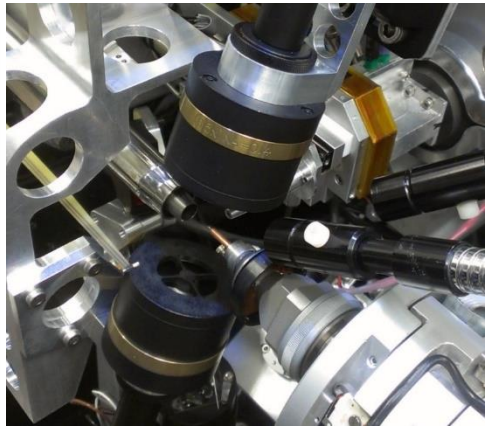
- Excitation scans
- MAD scans
  - Runs AutoCHOOCH
  - Displays energies for f', f", plus a calculated remote energy

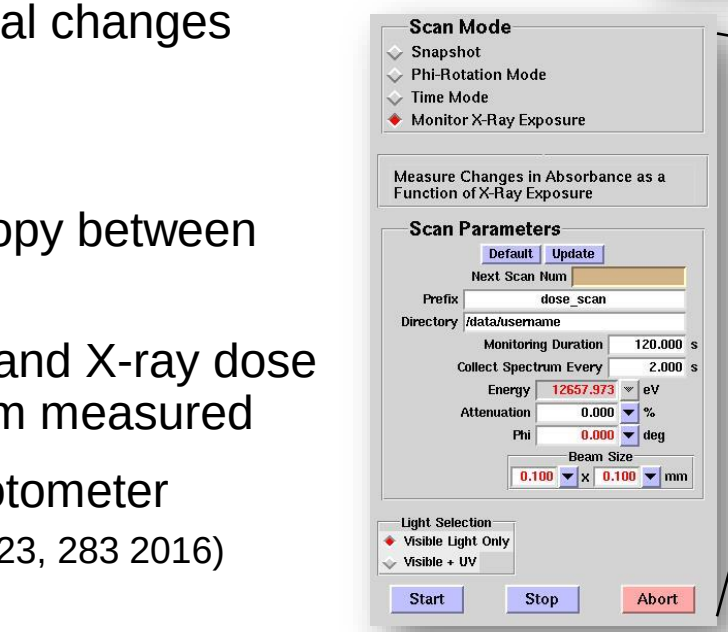
Standard @ SSRL/SMB – Experimental Setup

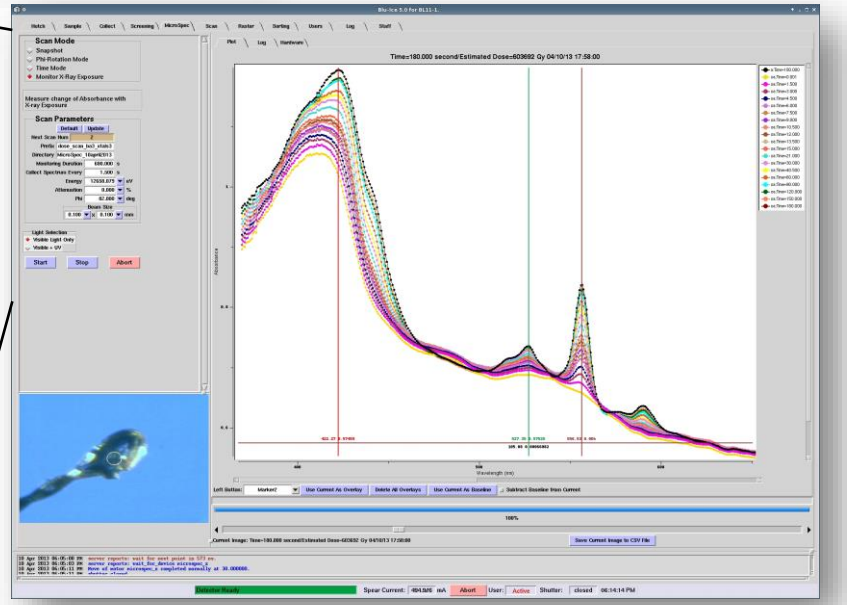
UV-Vis Microspectroscopy @ BL9-2

- Non-invasive
- Confirm identity of reaction intermediates trapped within a crystal
- Monitor reactions occurring in the crystal
  - triggering by lasers or within flow cells
- Monitor X-ray induced structural changes
  - e.g. metalloproteins
  - Radiation damage
- Fully automated for spectroscopy between diffraction data (interweave)
- Both the X-ray exposure time and X-ray dose are recorded for each spectrum measured
- in-situ UV-vis microspectrophotometer
  - (Cohen et al., Protein Pept. Lett. 23, 283 2016)









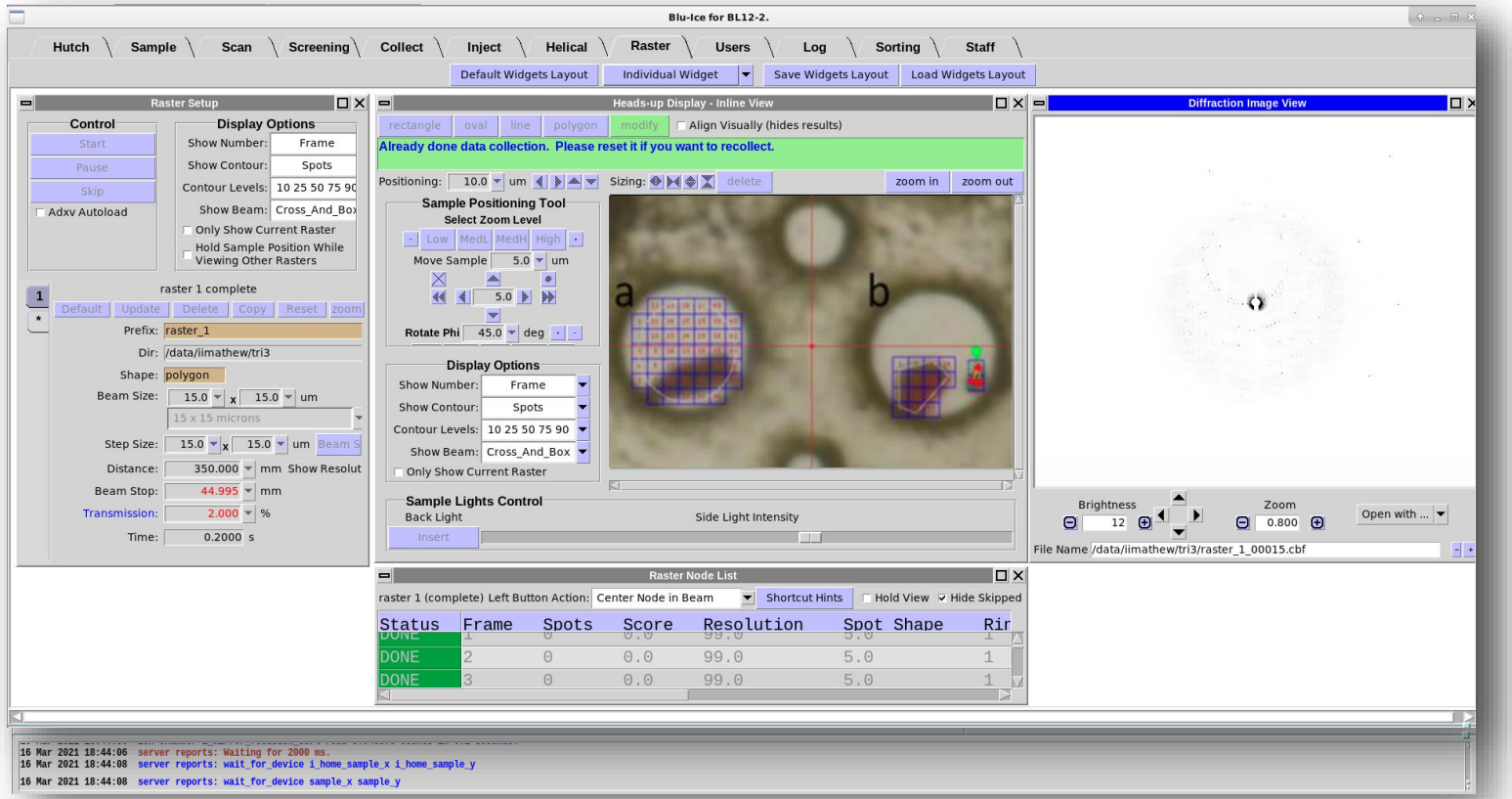
Standard @ SSRL/SMB – Experimental Setup

Screening

- Automatic
  - Exchange samples in 25 seconds
- Loop centering (15 seconds)
- Raster alignment
- WebIce strategy
  - Requires 2 images

Rastering

- Microbeam, matching crystal size
- Polygon, oval, line
- 90° line raster for centering
- Scoring
- Diffraction-based crystal alignment (Song et al., J. Synch. Rad. 14, 191, 2007)



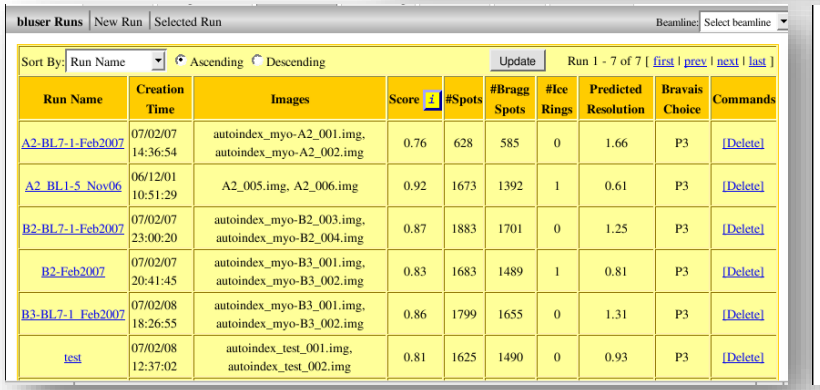
Diffraction images scored automatically
Results are displayed in Blu-Ice and WebIce and saved in spreadsheet

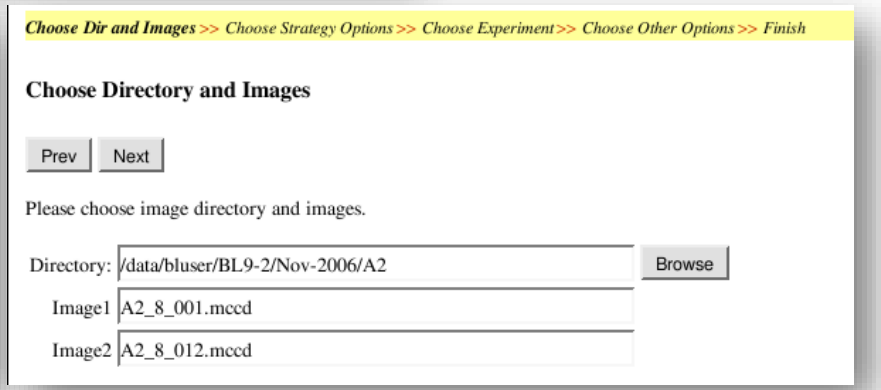
Art Lyubimov
(talked yesterday)

Standard @ SSRL/SMB – WebIce and Strategy

WebIce

- Mosaicity
- Oscillation
- Resolution
- Exposure time
- Number of images
- Detector, beamstop distances
- Energy(s)
- Attenuation
- Inverse beam
- Absorbed dose

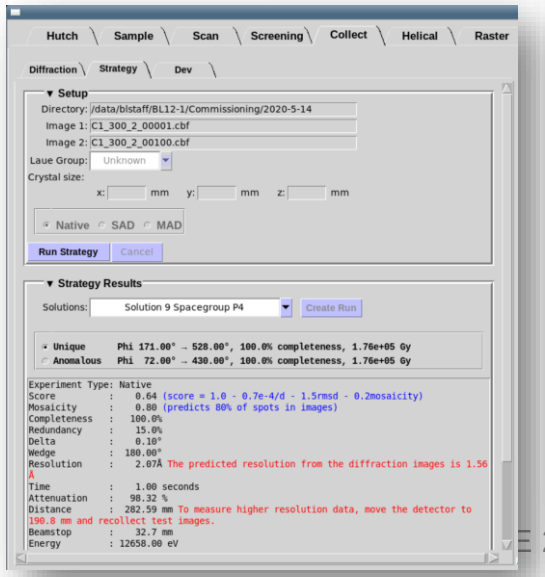


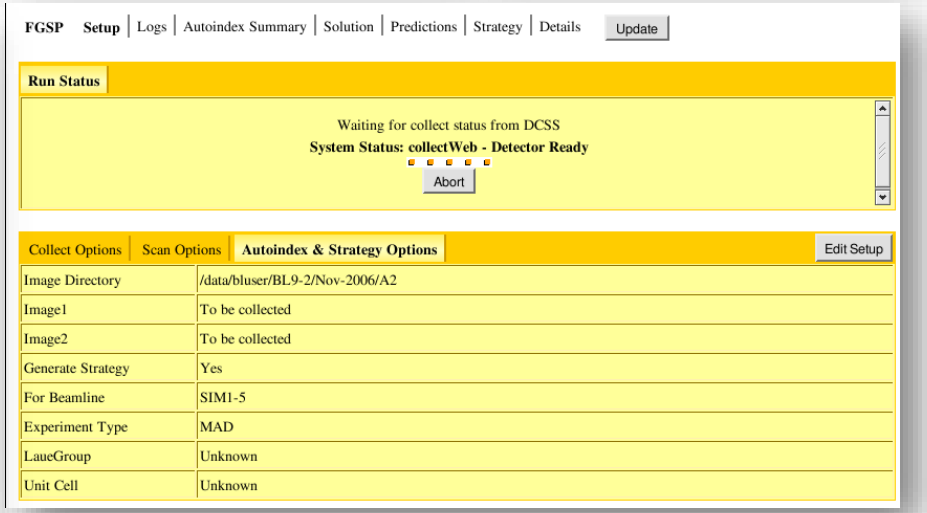


> Import collection strategy into Blu-Ice

Coming Soon

- Integrate into Blu-Ice





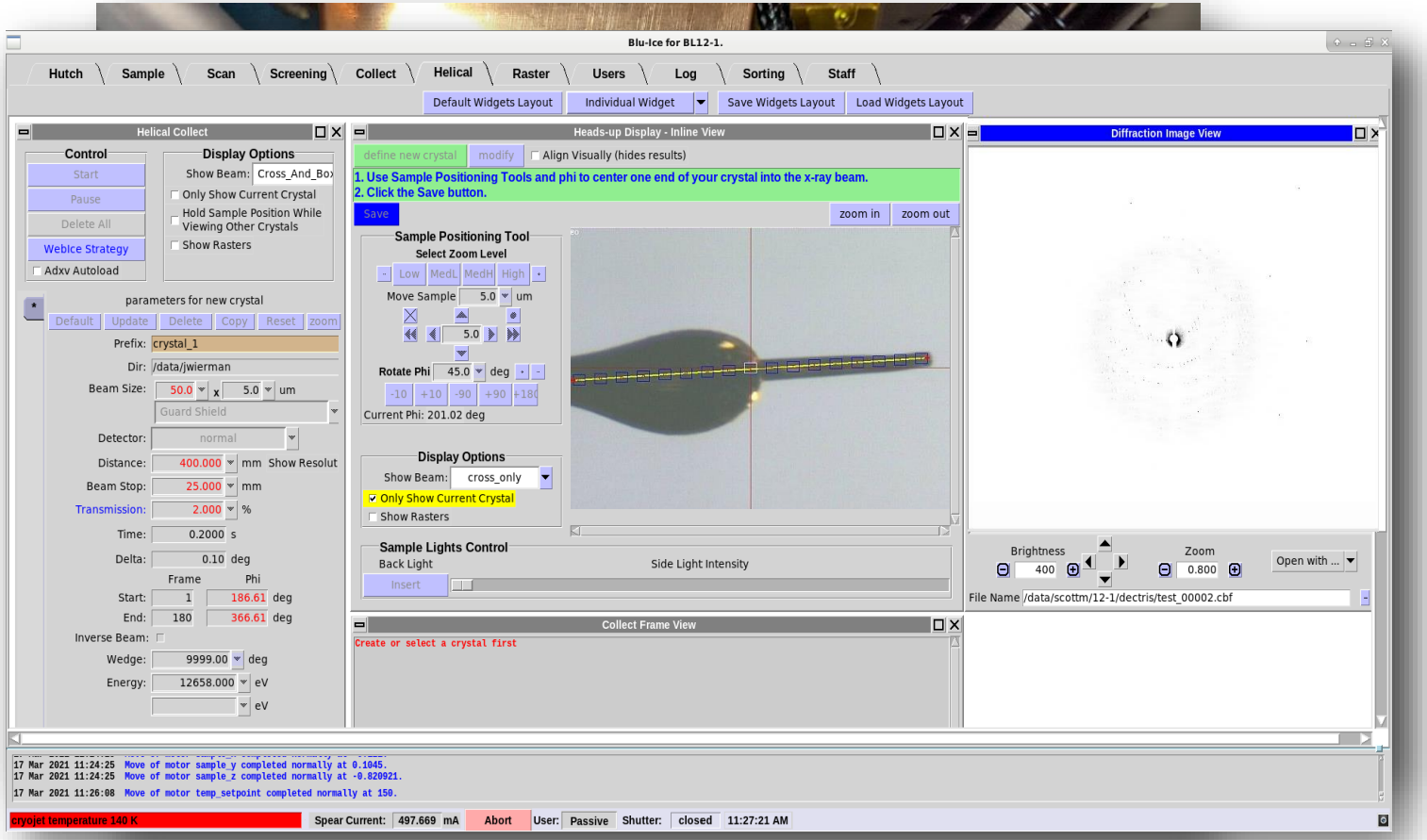
Standard @ SSRL/SMB – Data Collection

Standard data collection

- Rotation, wedge
- Helical

Beamline 12-1

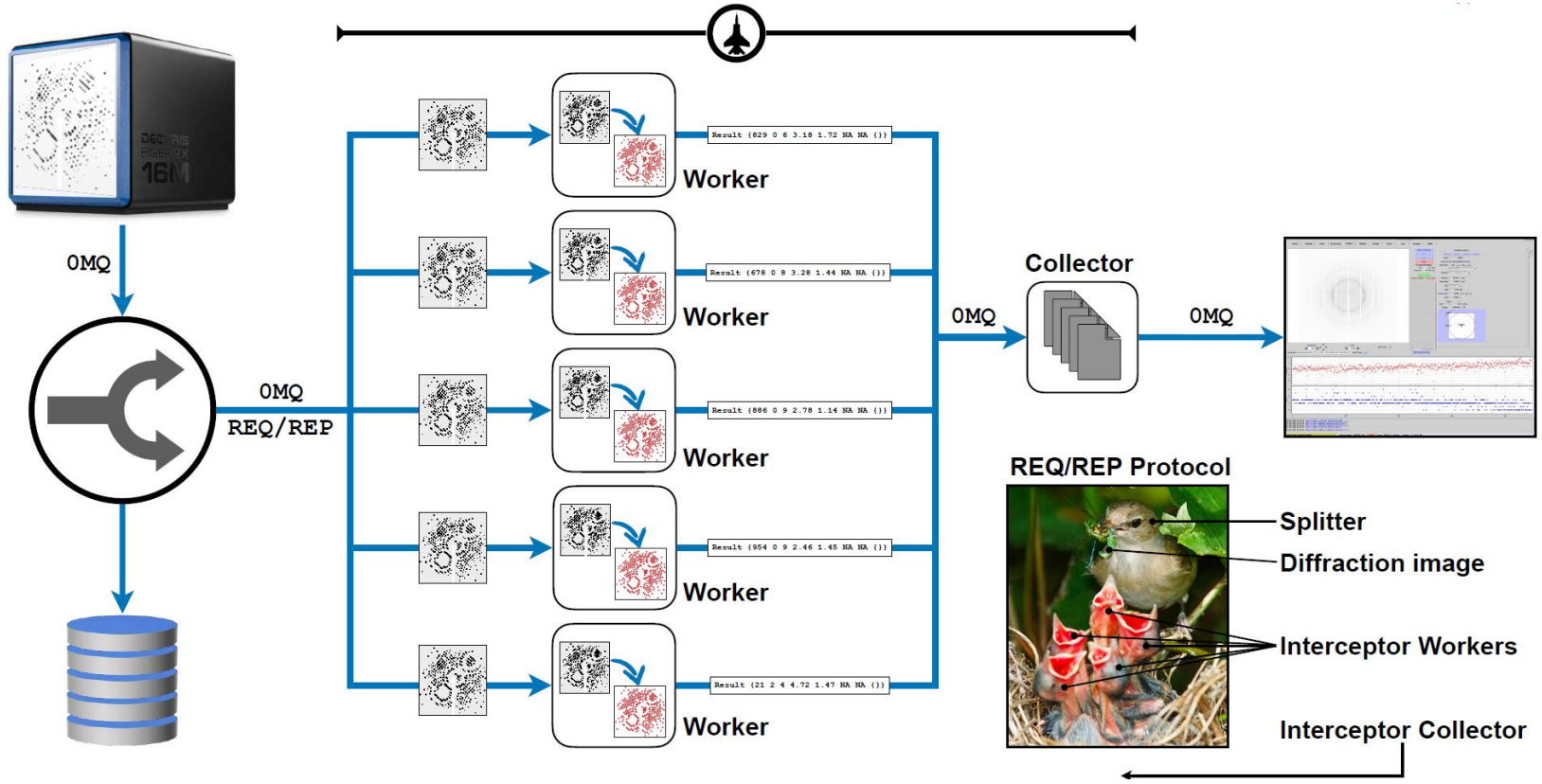
- Microbeam: 5 μm^2 to 100 μm^2
- High Intensity: 5 x10^{12} photons/sec
- 5-15 keV range, S-SAD phasing optimized
- EIGER 16M (133 Hz or 750 ROI)
- Crystal rotation speeds <90°/s
- Instant feedback
- Automated hit finding + data processing
  - Any dataset more than 5 frames is fully autoprocessed in users' directory
  - XDS, POINTLESS, XTRIAGE, AIMLESS, TRUNCATE
  - MOLEREP, REFMAC5



Standard @ SSRL/SMB - Live Data Analysis

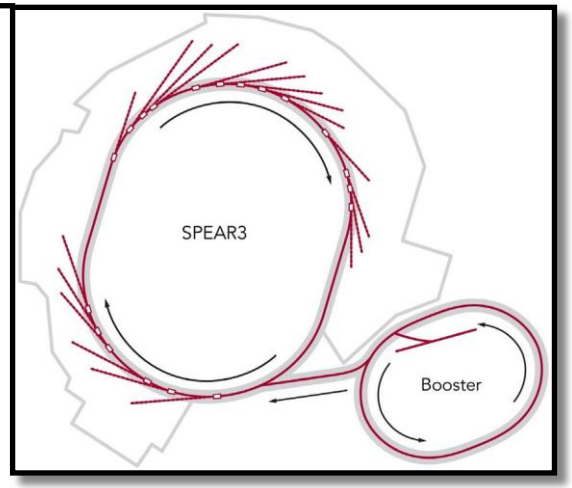
The Interceptor

- Live processing and scoring to track basic diffraction properties of a single crystals in real time
  - Resolution score
  - +max diffraction intensity score
  - Ice ring penalty
  - Elongated spot penalty
- Fully automated, persists in background
- Can handle high-speed bursts (~300 images) up to 100Hz

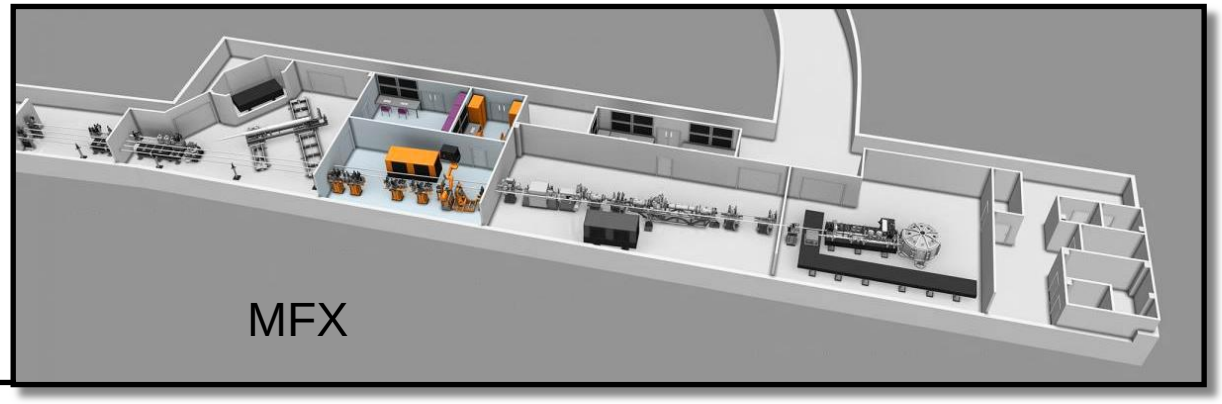


Art Lyubimov
(talked yesterday)

Serial Crystallography at SLAC



Serial Crystallography



SSRL/SMB: 12-1 and MFX

Five Beamlines at SSRL dedicated to Macromolecular Crystallography Research

Aina Cohen
(talked Tuesday)

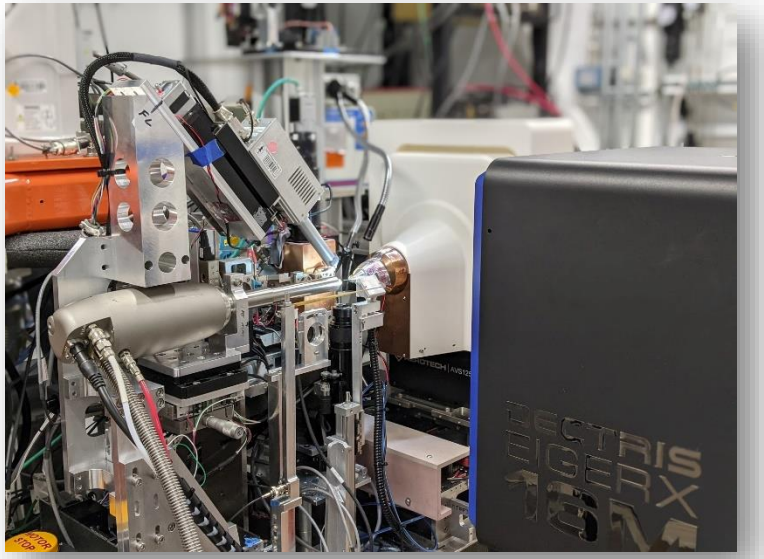
X-ray Free Electron Laser – LCLS-MFX Instrument

SSRL
Beam Line 12-1

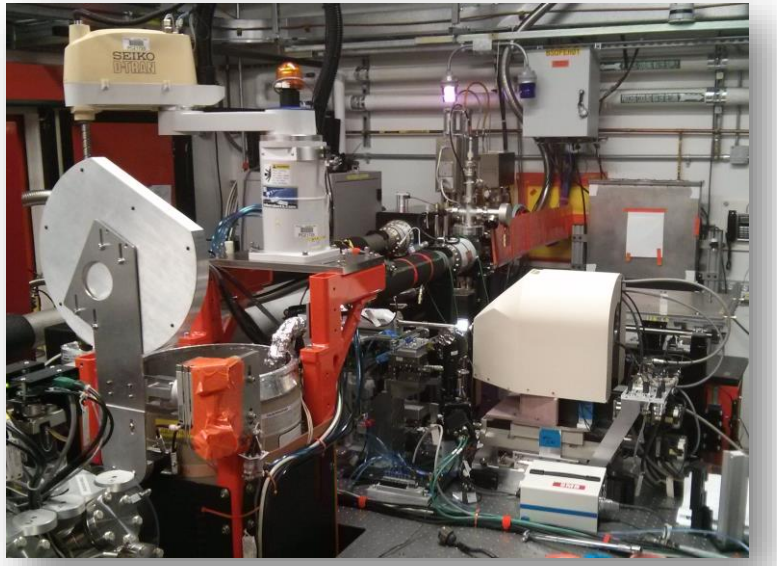
Synergistic User Operations

Similar Equipment & Controls Software

LCLS
MFX



New Rapid Access Proposal Mechanism
BL12-1 / Gateway to LCLS



SSRL/SMB: 12-1 and MFX

Standard setups for goniometer and injector

Collaboration between SSRL-SMB & LCLS-HXR staff

Blu-Ice Control Software (developed at SSRL)

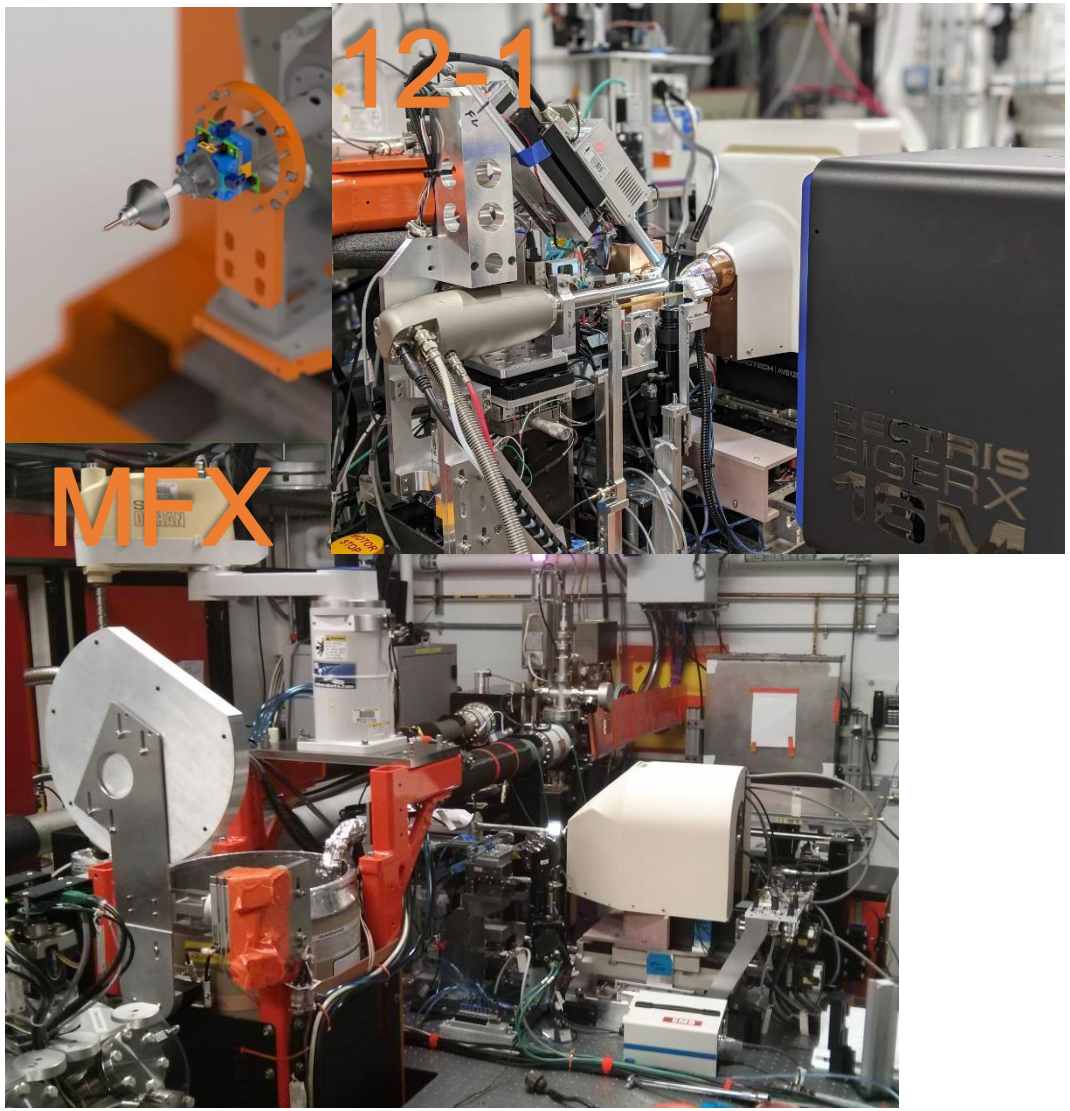
- User friendly - familiar to the user community
- Highly automated

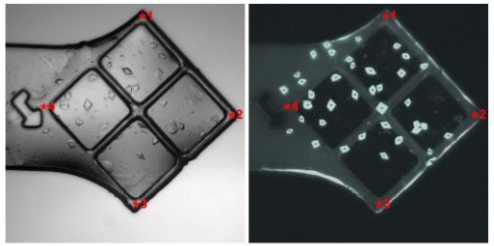
High Speed Microcrystal Goniometer

- Rapid sample motions with +/- 0.5 µm SOC

Automated Sample Exchange with Stanford Robot

- Highly reliable (>1M samples mounted at SSRL)
- Cryogenic studies enable safe transport & storage
- Support room temperature samples at controlled humidity

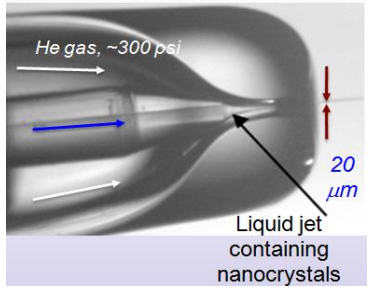




UV-fluorescence microscopy and visual mapping of crystal samples (Barnes et al., PNAS. 116(19), 9333-9339, 2019)

Advanced Features – Serial Crystallography

Gas Dynamic Virtual Nozzle

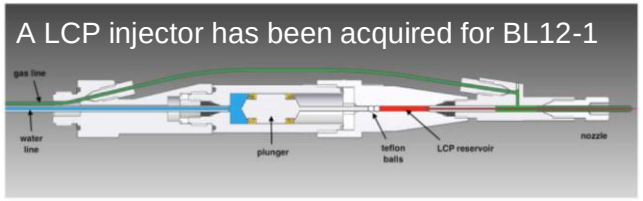


Rev. Sci. Instr. **83**, 35108, 2012

Mobile Electro-spinning Sample Holder (MESH)

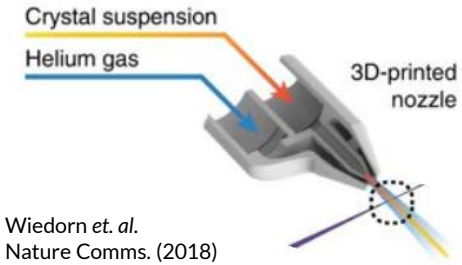
Sierra, *Acta Cryst D* (2012)

LCP/Viscous Media Injector



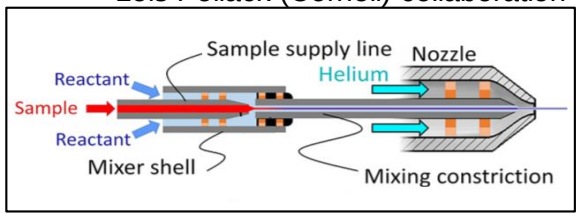
Weierstall, Uwe et al. Nature communications 5 (2014)

Mixing Injectors

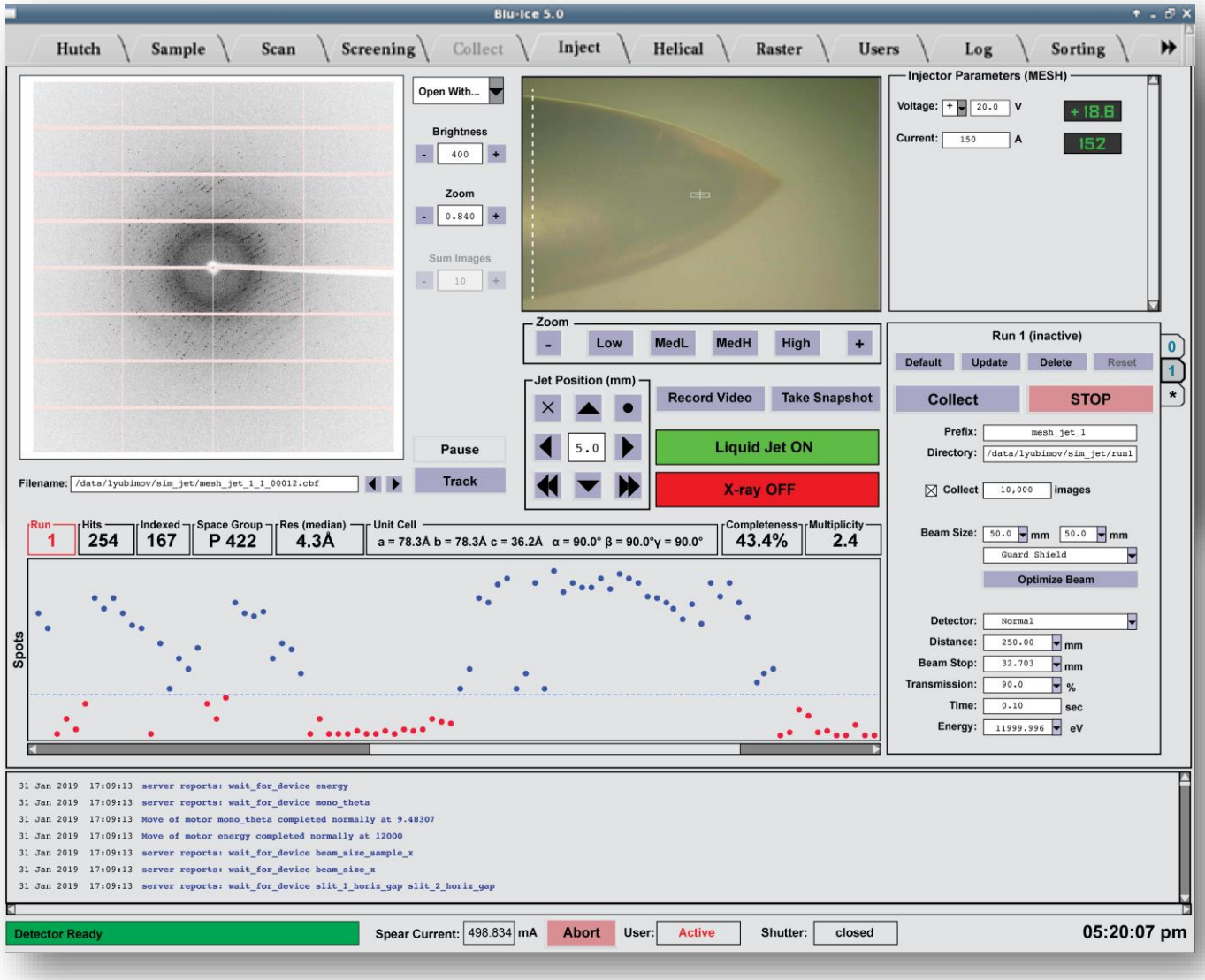


Wiedorn *et. al.* Nature Comms. (2018)

Lois Pollack (Cornell) collaboration

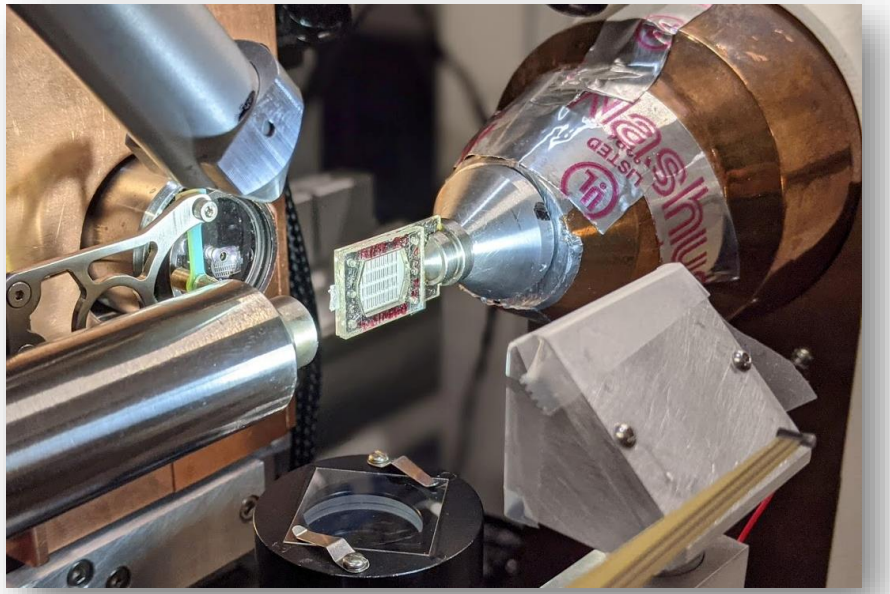


Calvey, Katz, Pollack. Analytical Chemistry 91-11, (2019)



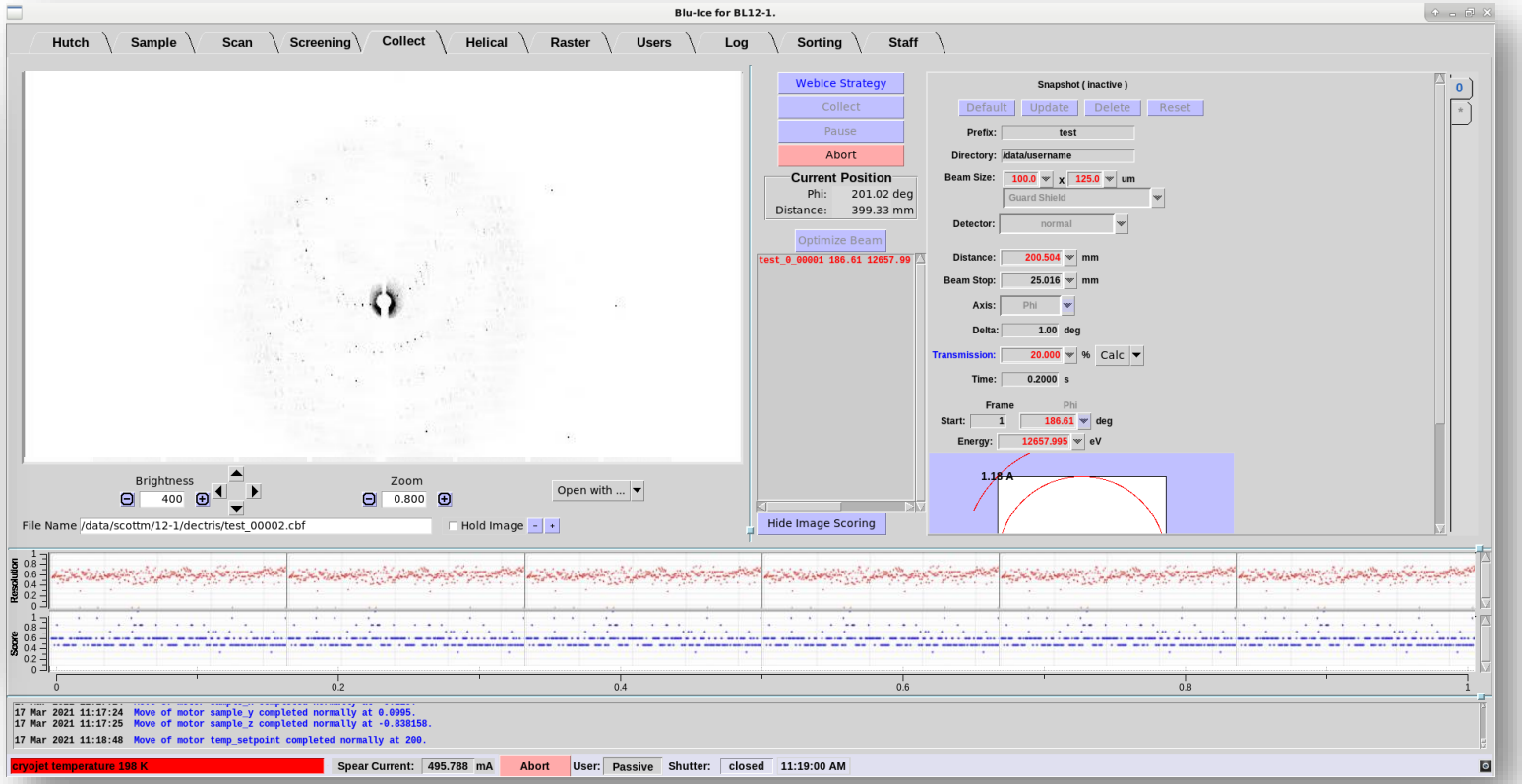
Advanced Features – Serial Crystallography

Thin polymer crystallization chip



Megan Shelby, Deepshika Shamraj Gilbile, Matthew Coleman, Matthias Frank, Tonya Kuhl

LLNL, UC Davis



Advanced Features – Serial Crystallography

Fixed target

Full automation is possible if it fits within the robot gripper

http://www.mitegen.com/

Cohen, *et al.*, *PNAS* (2014)
Gati *et al. IUCrJ* (2014)

Feld, *et al.*, *J Appl Cryst* (2015)

Roedig, *et al.*, *Sci Rep* (2015)

Lyubimov, *et al.*, *Acta Cryst D* (2015)

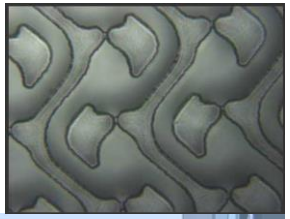
Murray, *et al.*, *Acta Cryst D* (2015)
Gati et al., IUCrJ (2014)
Heymann et al. IUCrJ (2014)
Murray et al. Acta Crys D (2015)
Baxter et al. Acta Crys D (2015)
Sui *et al.* . Lab Chip, 16 (2016)

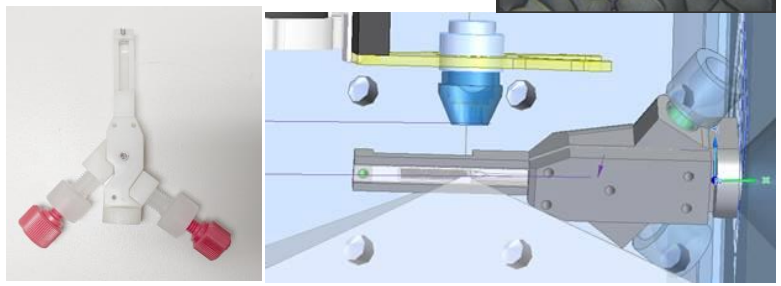
If it has known dimensions and fiducials, we can automate it!

Advanced Features - Studies of Protein Dynamics

Reactions triggered within crystals during (or *prior to*) data collection (**µs to ms** time scale)

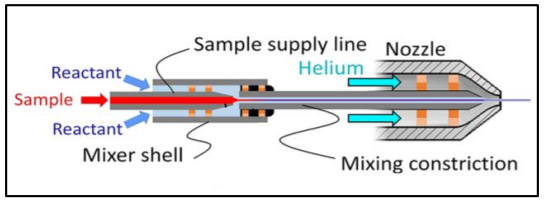
Microfluidics to trap/mix solutions around crystals





Liquid/Crystal Mixing Injectors

Lois Pollack (Cornell) collaboration



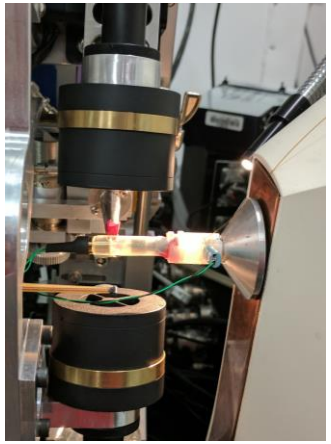
Calvey, Katz, Pollack. Analytical Chemistry 91-11, (2019)

Light activation/Caged Compounds

Jonathan Clinger (Cornell U)
SMB-MX Group (SLAC/SSRL)

High voltage jump

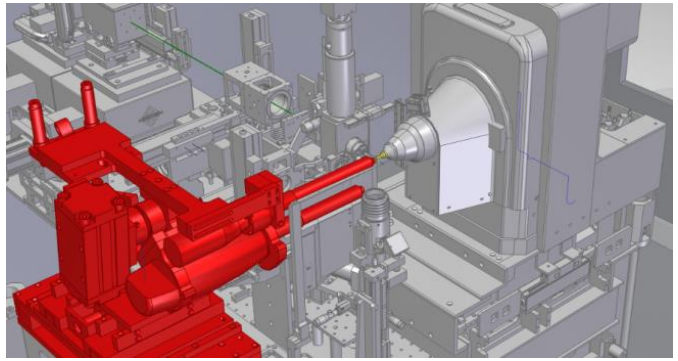
Sarah Perry and Shuo Sui
(UMass Amherst)
SMB-MX Group
(SLAC/SSRL)



"Kinetic crystallography"

Cryo-trap intermediates

In assembly for BL12-1



Advanced Features – UV Fluorescence, Triggering

Validating intermediate states

Freeze-trap reactions in cryostream at fixed time-delays after adding substrate to crystal

Monitor/Follow Radiation Damage

Radiation damage is accrued site-specifically around metal centers

Support for photo-triggered reactions

UV light for uncaging compounds, activating reactions
All automated!

Fully remote pump probe (timing control)

On-axis system to be installed at BL12-1 and in planning for MFX

Option for UV-light (or other light sources) for time-resolved measurements

Advanced Features - UV Source Photo-Triggering

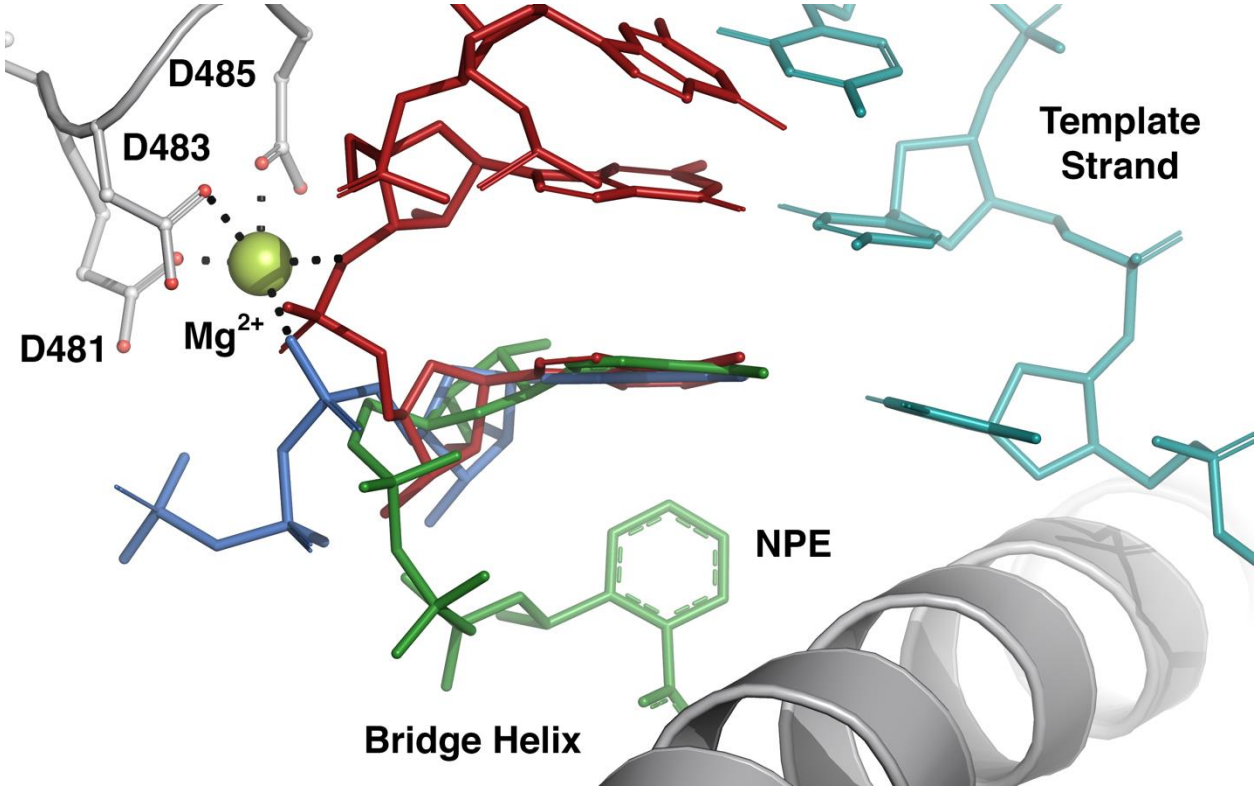
Time-resolved studies of transcription

Remote collection @ 12-1

- crystals exposed to UV light (breaking cage)
- temperature increase from 100K to 170K
- rapid helical data collection (2sec/dataset)

Illumination with the UV source breaks the nitrobenzyl group (NPE) *in crystallo* allowing ATP release from RNA-Pol II, metal coordination (blue) and phosphodiester bond formation (red).

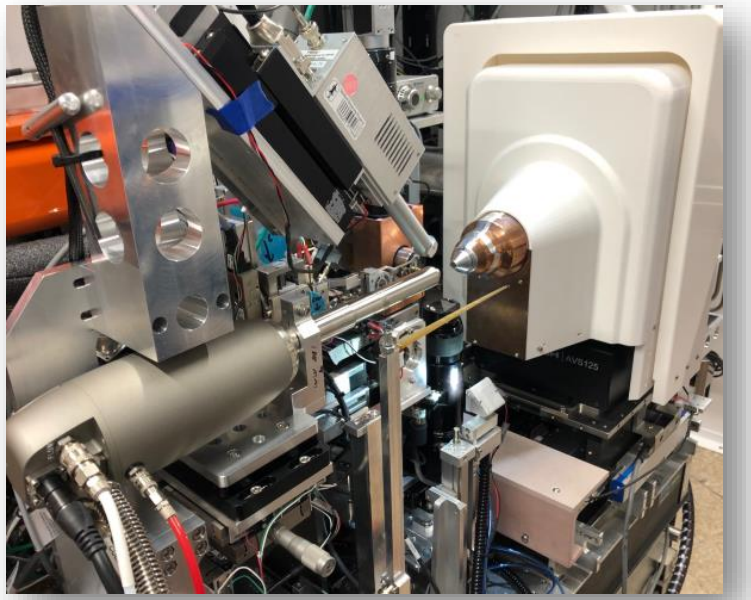
The Calero Lab (University of Pittsburgh)

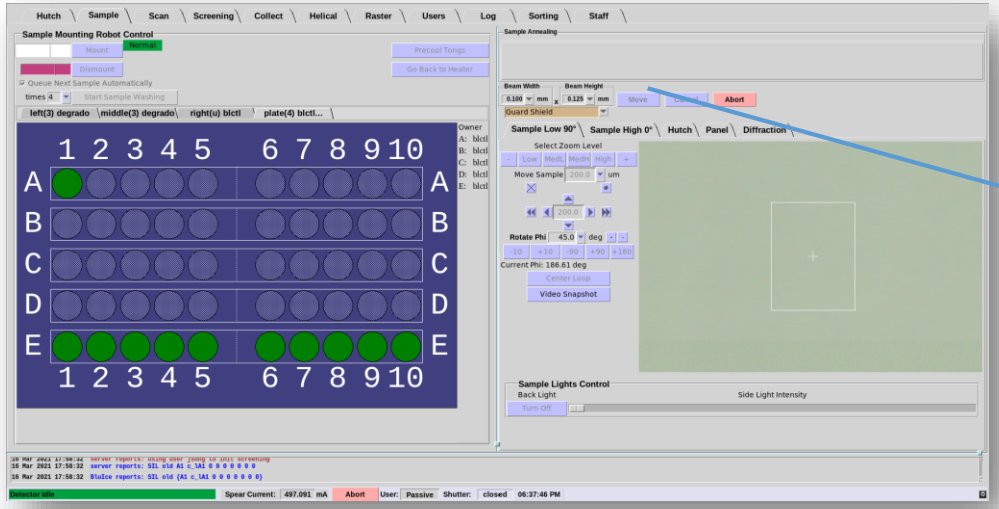


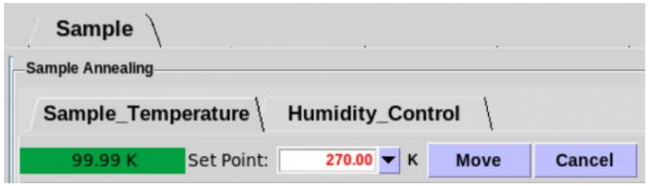
Advanced Features – Elevated Temperatures

- Measurements at physiological temperatures
- Understanding temperature effects in protein structure and interactions

- Potentially no additional disorder from cryoprotectants
- Side chain conformation insights
- Time scale resolution, transient steps







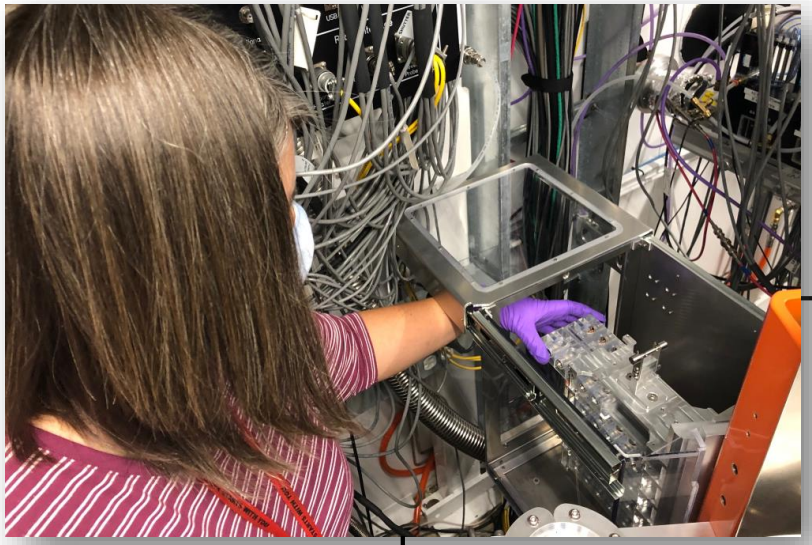
Aina Cohen
(talked Tuesday)

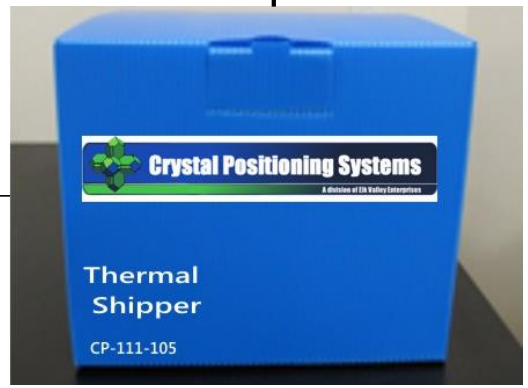
Advanced Features – Elevated Temperatures

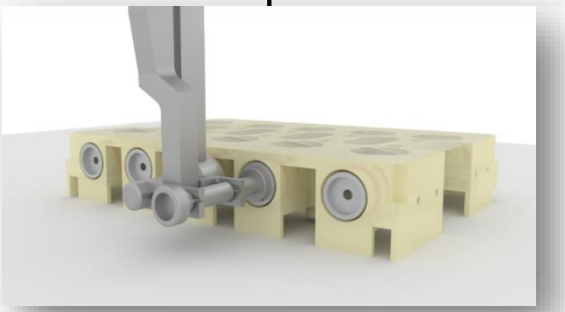
First used at LCLS-MFX, but now standard at 12-1

Aina Cohen
(talked Tuesday)

Samples inside at controlled humidity





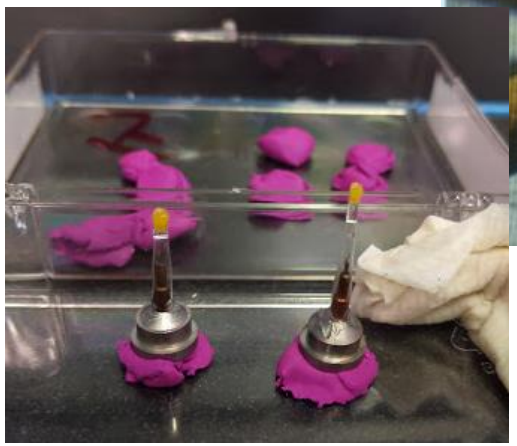


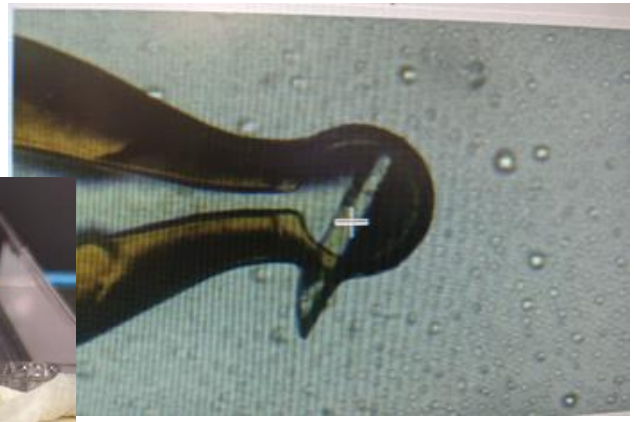
Advanced Features – Elevated Temperatures

Fraser Group (UCSF - QBI Corona Virus Research Group)

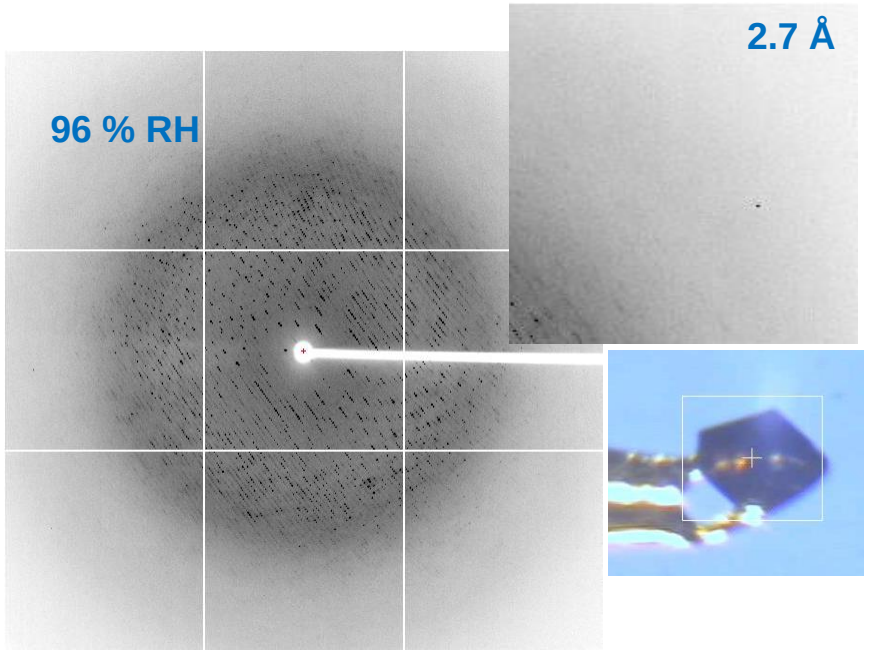
- Studies of conserved “macro domain,” enzyme shown to promote virulence in coronavirus
- Screening of fragments done at BL9-2 and BL12-2
- Measurements at physiological temperatures associated with infection in humans were done at BL12-1
- Understanding how temperature affects the enzyme structure and interactions may provide insight to develop of antiviral therapeutics

James Fraser (PI), Michael Thompson Support: Jeney Wierman and Silvia Russi

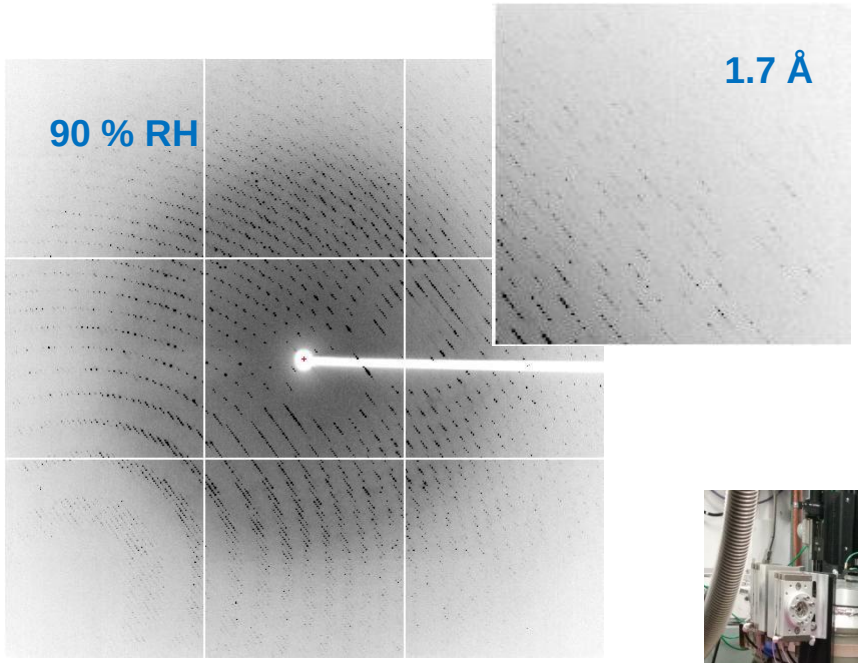




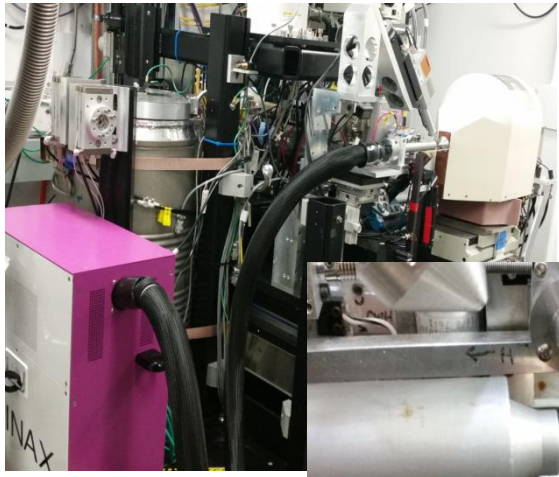
Advanced Features – Hydration/Dehydration

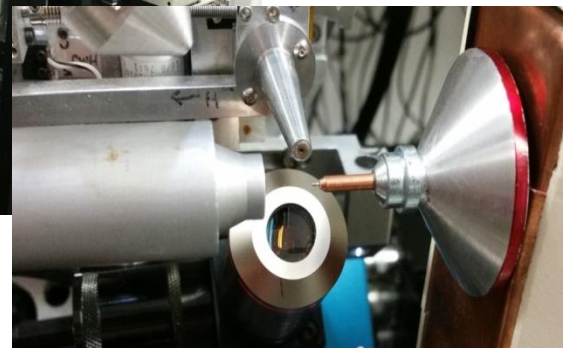


a=87.8 Å, b=140.8 Å, c=233.1 Å, P212121



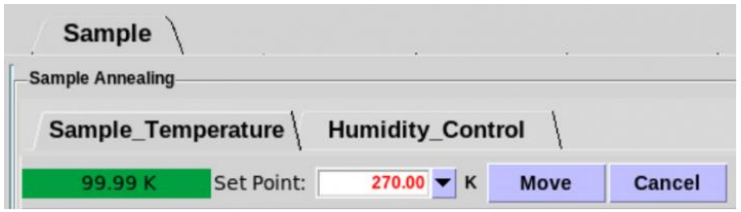
a=84.6 Å, b=142.0 Å, c=233.8 Å, P212121





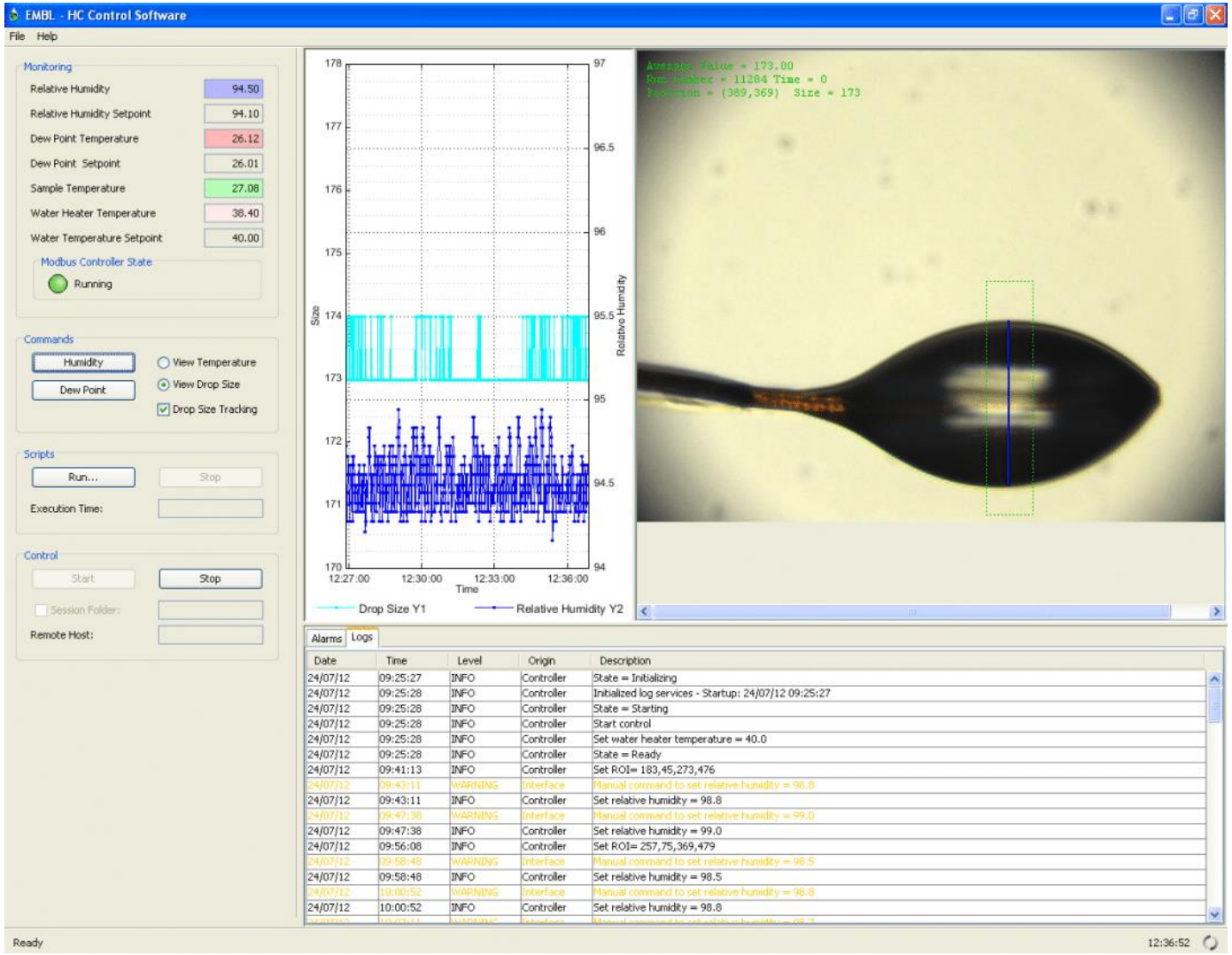
Bovine Liver Catalase

- starting RH=96%, final RH=90%, 1 % RH steps,
- 300 seconds equilibration time between steps

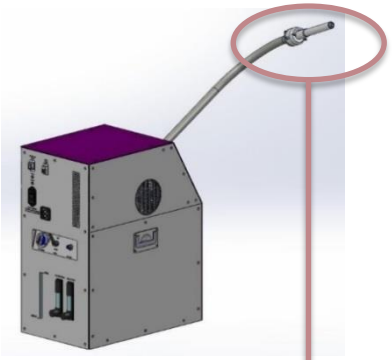


Humidity range of 30.0% to 99.8% ±0.05% RMS

Advanced Features– Hydration/Dehydration



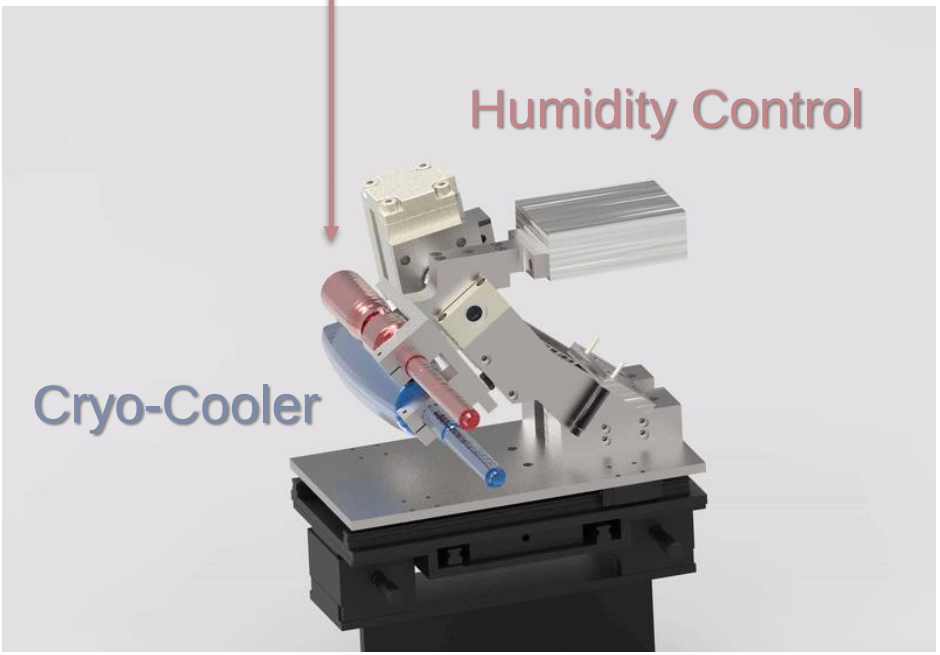
Advanced Features – Nozzle Switcher



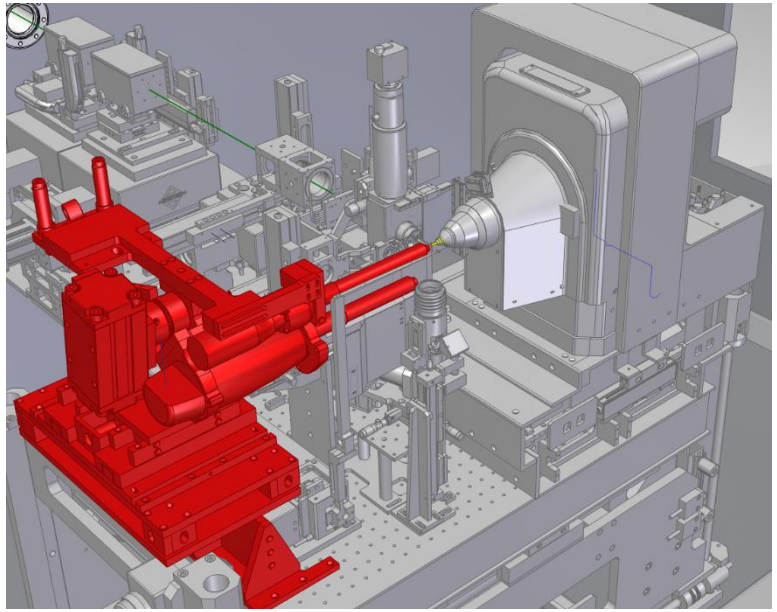
Arinax Humidity Control Device

Automated switch between humid RT or cryo-conditions

- Flash cooling after controlled dehydration experiments
- Samples both cryo and RT supported for same beamtime
- Flash-cooling experiments to trap reaction intermediates



Flash-cooling
60 ms to switch



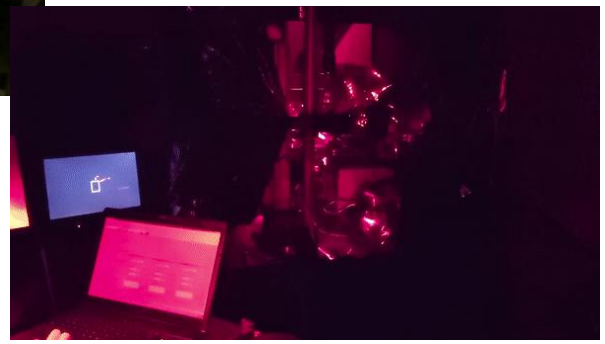
In assembly for BL12-1

Advanced Features – Nozzle Switcher

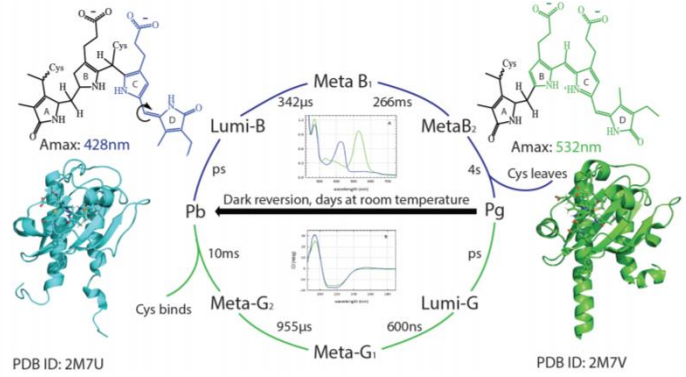
Cryo-trapping Dynamics

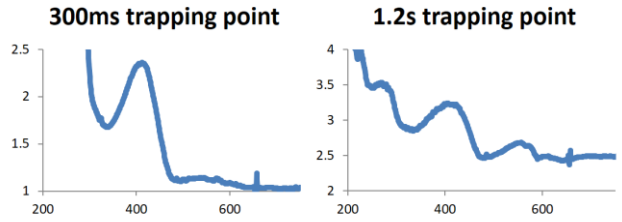
Use in conjunction with light, spray, drop

Jonathan Clinger, Sethe Burgie, Rick Vierstra, and George N. Phillips Jr.



Cryo-trapping Experiment to Observe Phytochrome Photoconversion Intermediates





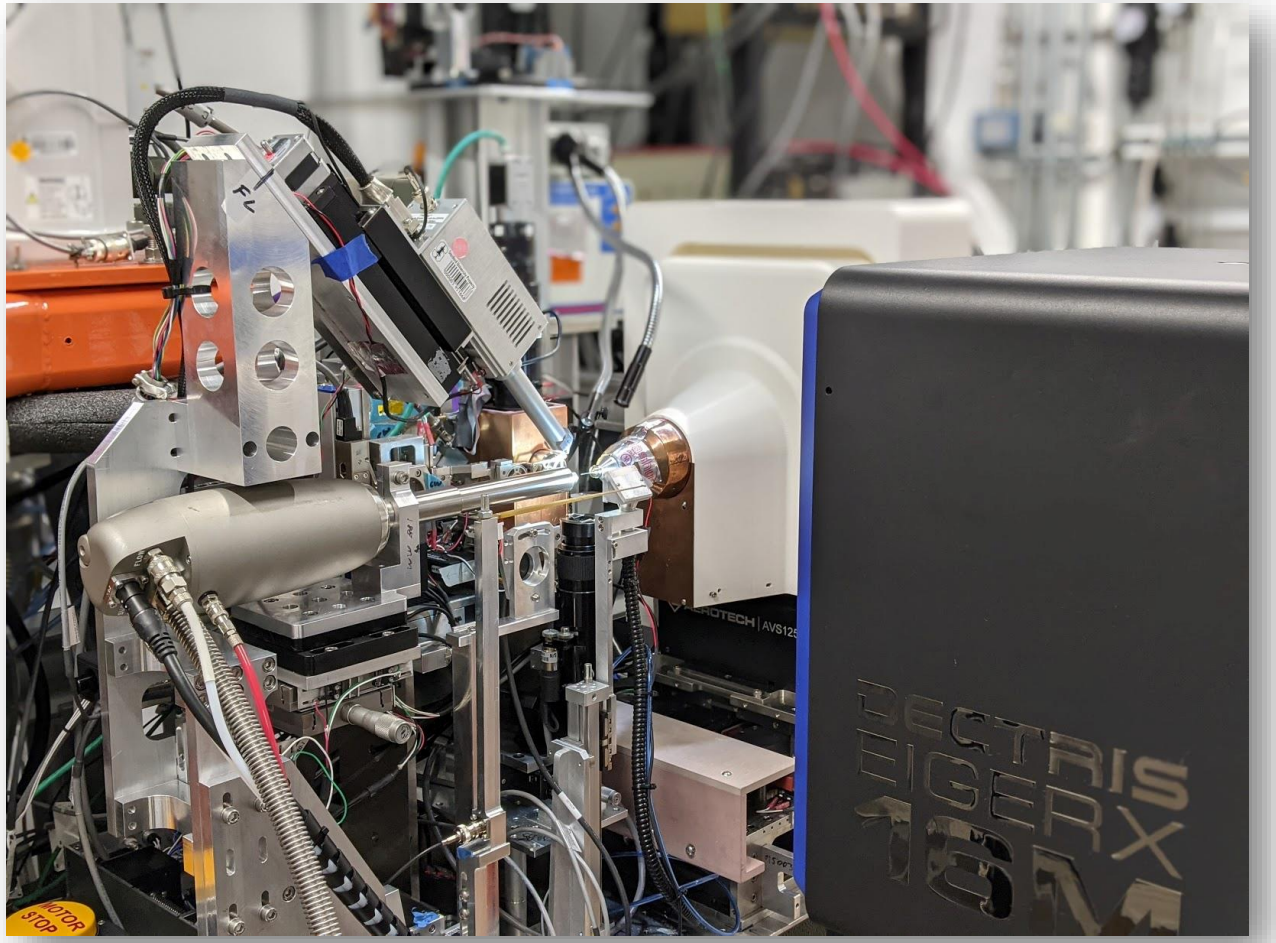
RICE Unconventional Wisdom

WASH U

Summary

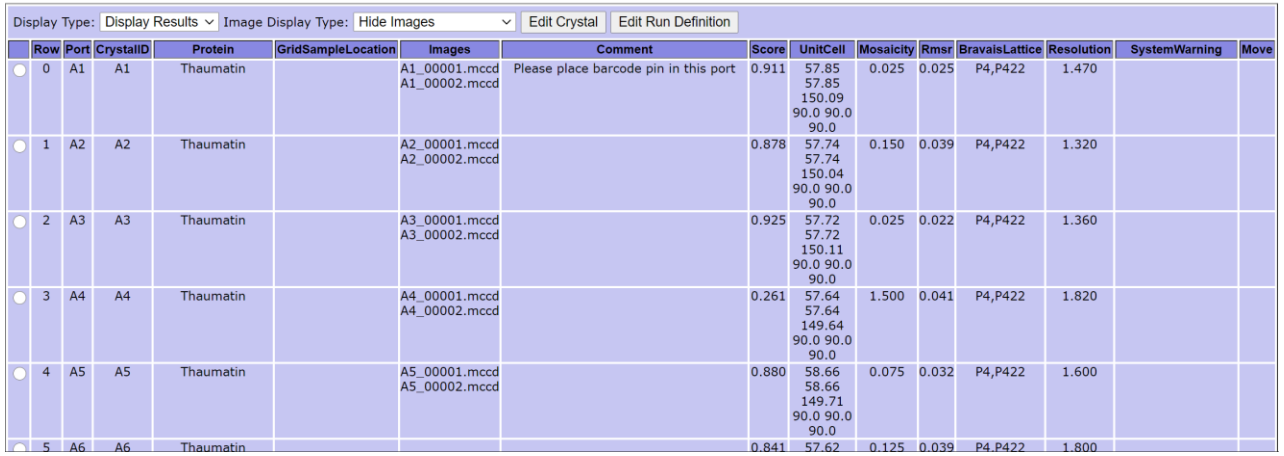
Remote Access with SMB at SLAC

- Standard @ SSRL/SMB
  - Stanford Auto-Mounting (SAM) system (Cohen *et al*., J. Appl. Cryst. 35, 720, 2002)
  - Remote Data Collection from anywhere in the world (Soltis et al., Acta Cryst. D64, 210, 2008)
- Advanced features @ Beamline 12-1
  - Serial and Dynamics
  - Elevated Temperatures
  - Humidity Control
- Look to the future



To the future!

- Multi-crystal database
- Blu-Ice Strategy
- Dynamics automation
  - Timing
  - Automate EIGER with gonio
    - 90°/second, fast framing, all remote
- Optical sources, Raman installment
  - More sources! More fibers!
- Nozzle switcher installment
- New optics: CRLs, multilayer monochromator
- EIGER2 XE 16M
  - move EIGER 16 to BL12-2



Display Type: Display Results | Image Display Type: Hide Images | Edit Crystal | Edit Run Definition

	Row	Port	CrystalID	Protein	GridSampleLocation	Images	Comment	Score	UnitCell	Mosaicity	Rmsr	BravaisLattice	Resolution	SystemWarning	Move
○	0	A1	A1	Thaumatin		A1_00001.mccd A1_00002.mccd	Please place barcode pin in this port	0.911	57.85 57.85 150.09 90.0 90.0 90.0	0.025	0.025	P4,P422	1.470		
○	1	A2	A2	Thaumatin		A2_00001.mccd A2_00002.mccd		0.878	57.74 57.74 150.04 90.0 90.0 90.0	0.150	0.039	P4,P422	1.320		
○	2	A3	A3	Thaumatin		A3_00001.mccd A3_00002.mccd		0.925	57.72 57.72 150.11 90.0 90.0 90.0	0.025	0.022	P4,P422	1.360		
○	3	A4	A4	Thaumatin		A4_00001.mccd A4_00002.mccd		0.261	57.64 57.64 149.64 90.0 90.0 90.0	1.500	0.041	P4,P422	1.820		
○	4	A5	A5	Thaumatin		A5_00001.mccd A5_00002.mccd		0.880	58.66 58.66 149.71 90.0 90.0 90.0	0.075	0.032	P4,P422	1.600		
○	5	A6	A6	Thaumatin				0.841	57.62	0.125	0.039	P4,P422	1.800		

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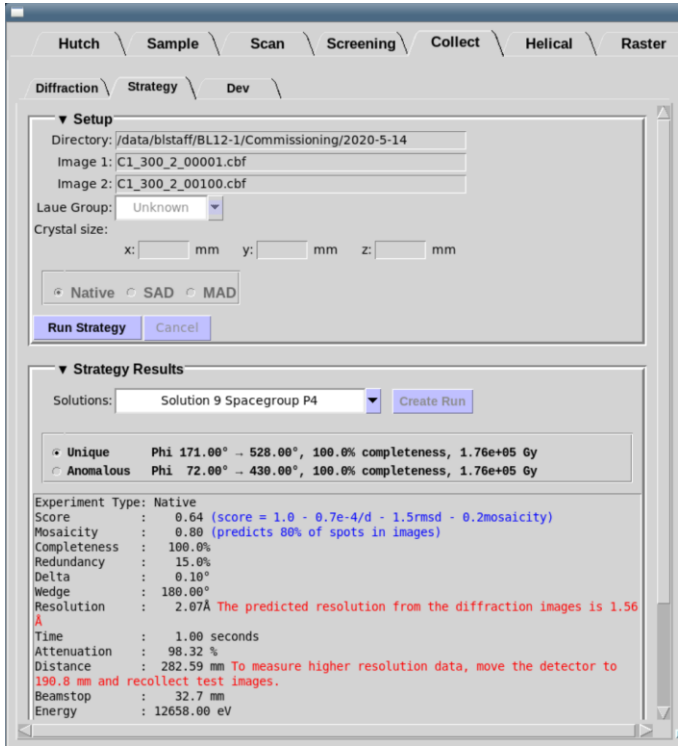

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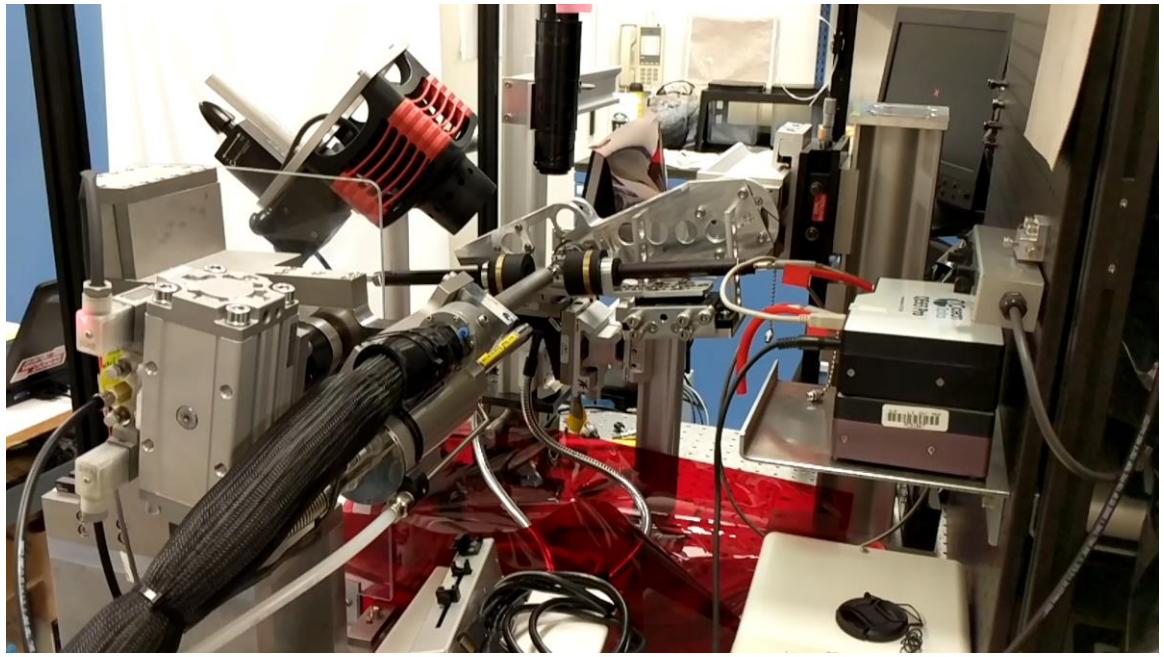

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Acknowledgements

SSRL SMB

Aina Cohen
Mike Soltis
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Scott McPhillips
Mike Hollenbeck
Paul Ehrensberger
Renato Avelar
Robin Frank
Scott Mitchell
Henry Meier

LBNL/ALS

James Holton

If you have additional questions, please email me at jwierman@slac.stanford.edu

Questions we ask ourselves

- How much processing do we automate?
- How much data collection automation/autonomous?
  - The thought: the further the user gets from the nuts and bolts of data collection, the less they appreciate intricacies in the data.
  - “Automation is more work for staff, less for users” - N.P.
- When do we intervene with data collection?
  - *e.g.* “Yikes, they are totally missing the crystal!”
- Where does data come from for strategy?
  - Can users override acquired data?
  - Does every collection generate a strategy?
- How do we handle storing data?
  - Cloud?
  - Massive physical storage?
  - Junk it!
- How to teach new users?
- How much is too much? More widgets? More tabs? More options?

Front End Glamour Shot