The NSLS-II XFP Beamline and Beyond

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X-Ray Footprinting Workshop
2014 ALS Users' Meeting
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NSLS to NSLS-II

- ***NSLS shut down forever on September 30, 2014…***

X28C

XFP

- ***Beginning of operations for NSLS-II declared***

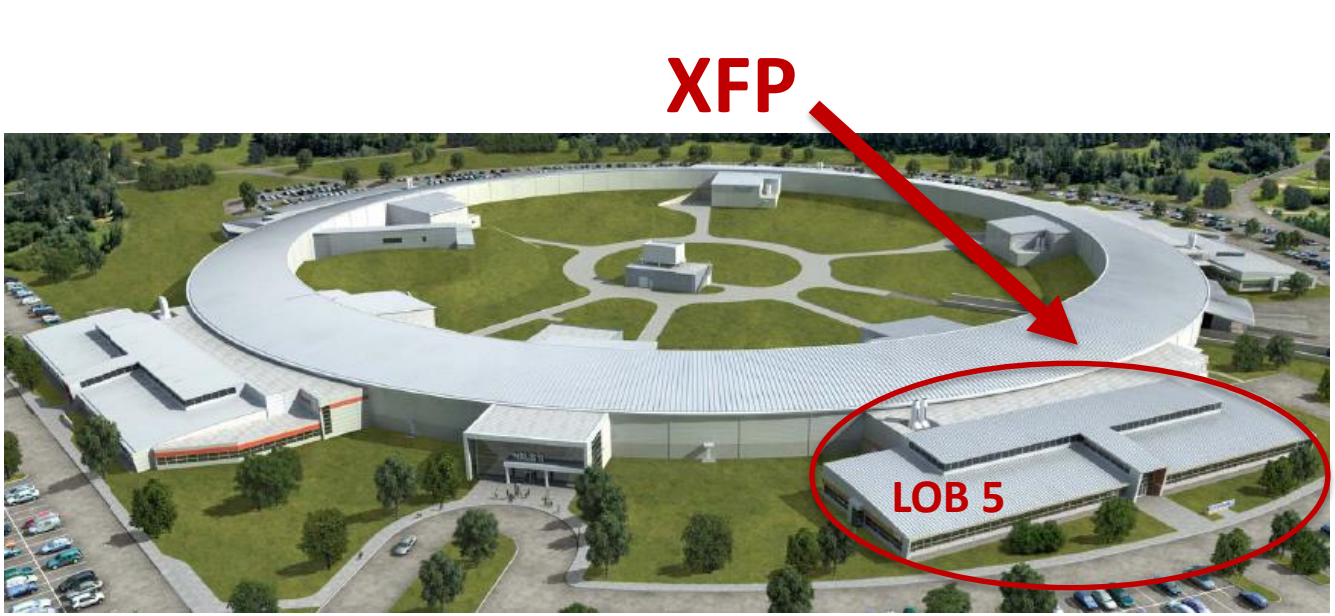
XFP at NSLS-II

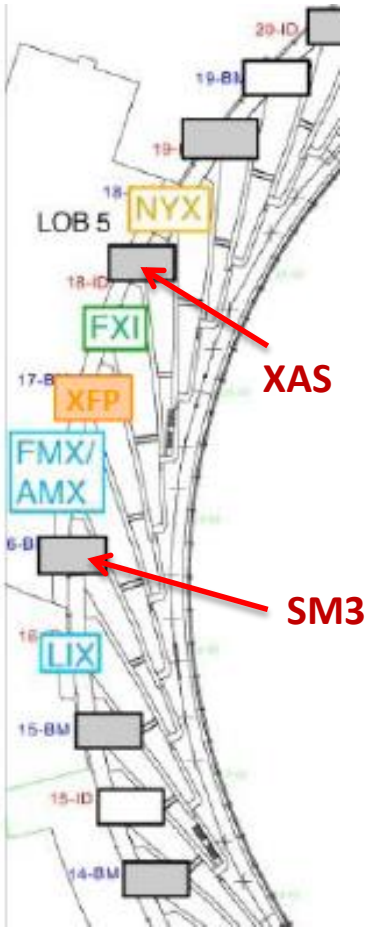
XFP: X-ray Footprinting for *In Vitro* and *In Vivo* Structural Studies of Biological Macromolecules

- ***Partner Beamline – Funding through NSF and CWRU***
- ***Partnership with Photon Sciences Division of BNL***
- ***Operations Funding through NIBIB P30 (5 year)***

- ***Objective: provide access to world class facilities and expertise in x-ray footprinting at NSLS-II***

XFP Location: NSLS-II 17-BM

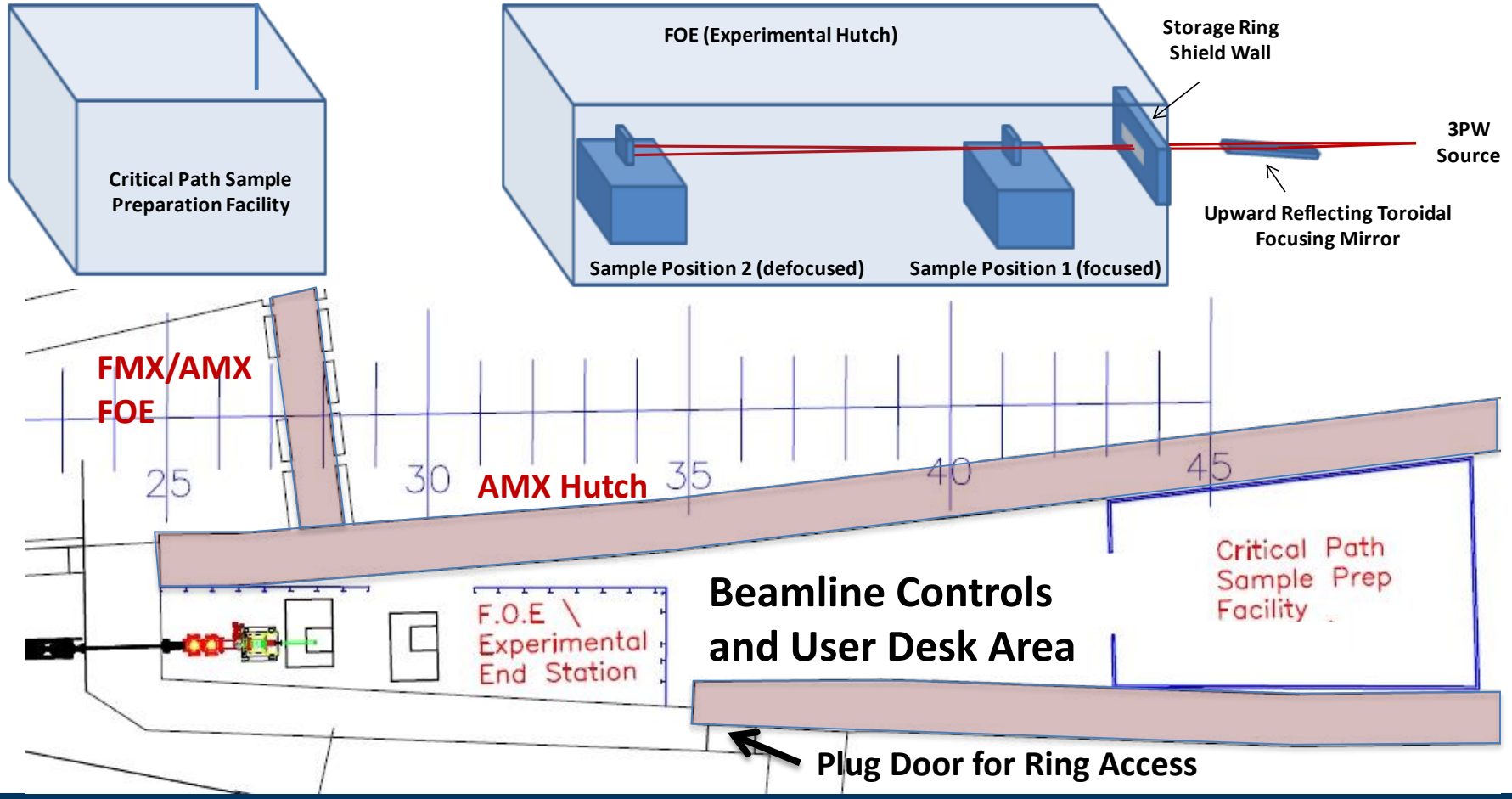




Central to the "biology village"

- Between FMX/AMX on upstream and FXI downstream
- Next to LOB 5: Offices and biology-oriented laboratory space expected in LOB 5 for biology village

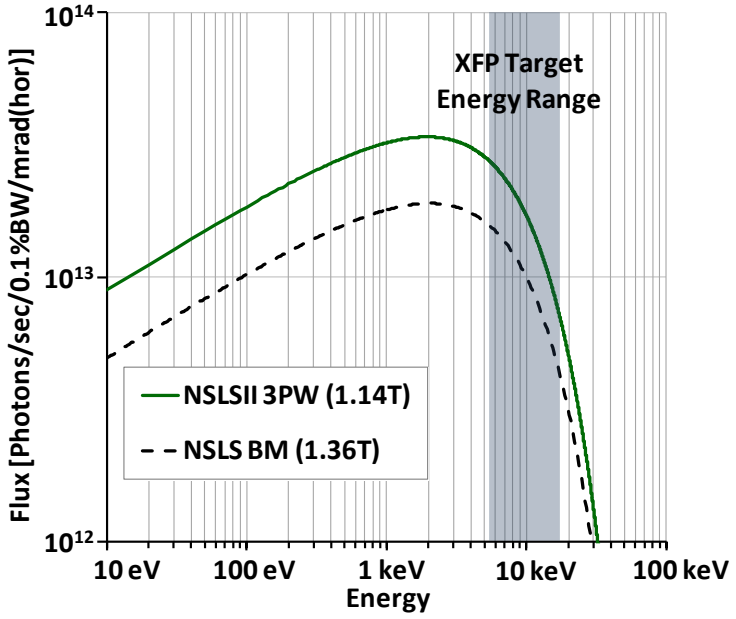
XFP Layout


Critical Path Sample Preparation Facility
FOE (Experimental Hutch)
Storage Ring Shield Wall
3PW Source
Upward Reflecting Toroidal Focusing Mirror
Sample Position 2 (defocused)
Sample Position 1 (focused)
FMX/AMX FOE
25
30
AMX Hutch
35
40
45
F.O.E \ Experimental End Station
Beamline Controls and User Desk Area
Critical Path Sample Prep Facility
Plug Door for Ring Access

Source Properties

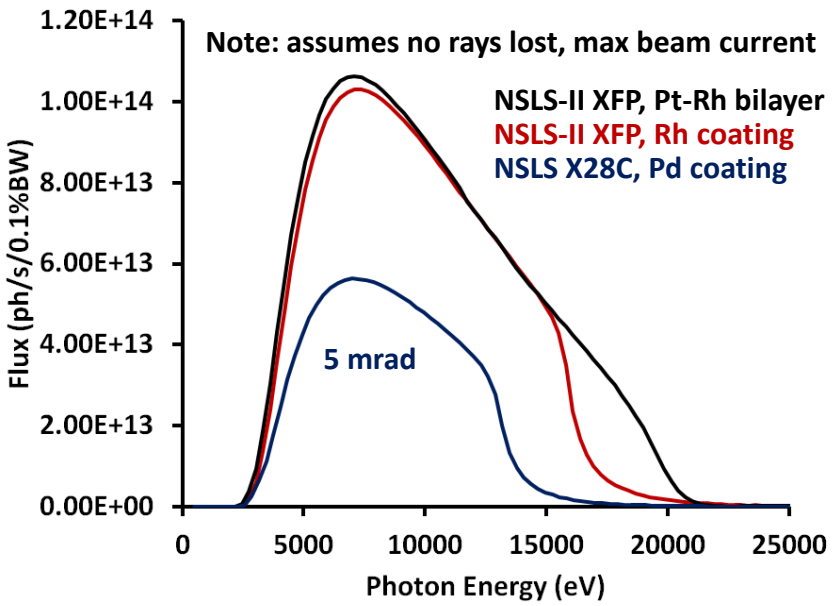
3-Pole Wiggler Source

Source	Hor. Size, Div. [μm, μrad]	Vert. Size, Div. [μm, μrad]
NSLS BM X28C	$\sigma_h = 260$, $\sigma_h' = 300$	$\sigma_v = 57$, $\sigma_v' = 11$
NSLS-II 3PW	$\sigma_h = 167$, $\sigma_h' = 98$	$\sigma_v = 12.3$, $\sigma_v' = 0.82$



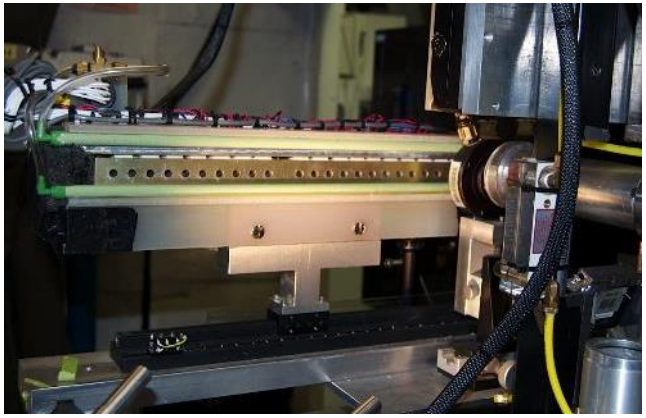
Spectral Flux

- 3 mrad H, 0.33 mrad V from 3PW
- Pt-Rh bilayer coating on toroidal mirror, 4.2 mrad angle

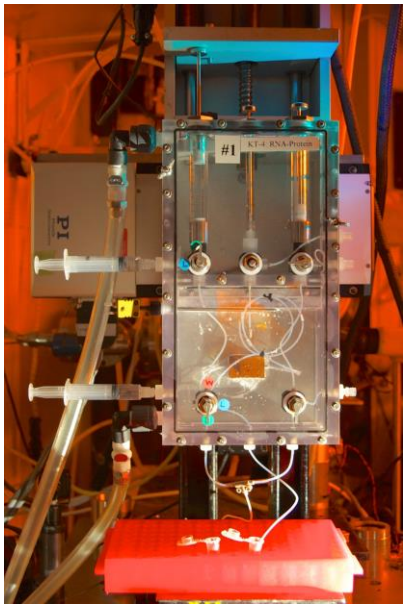


Beamline Modes

- ***Beamline Modes Enabled:***
  - **High Throughput**
  - **High Flux Density**
  - **Large ID Capillary/KinTek**

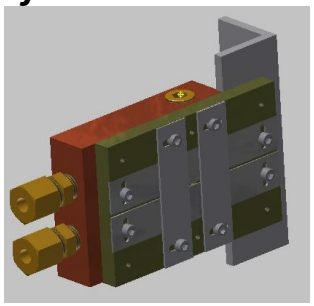


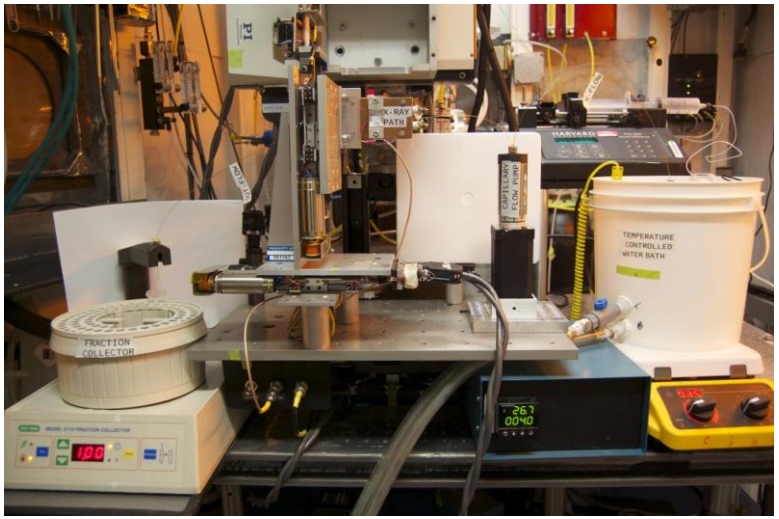
Multi-sample holder – high throughput



KinTek quench – flow (time-resolved mixing)

100 µm capillary flow cell



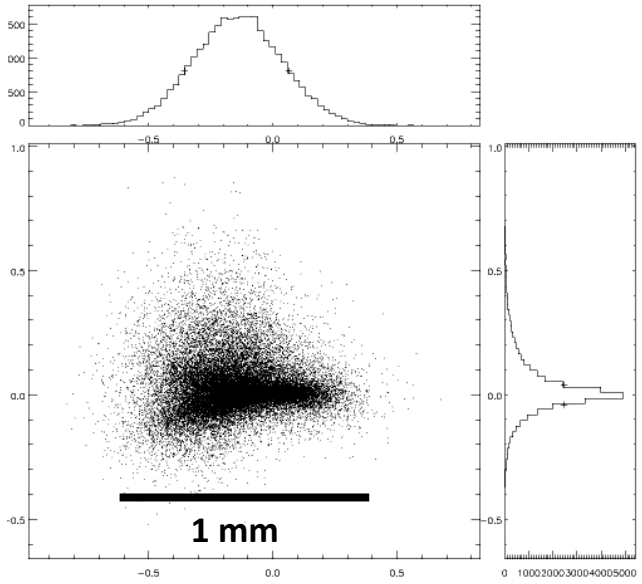


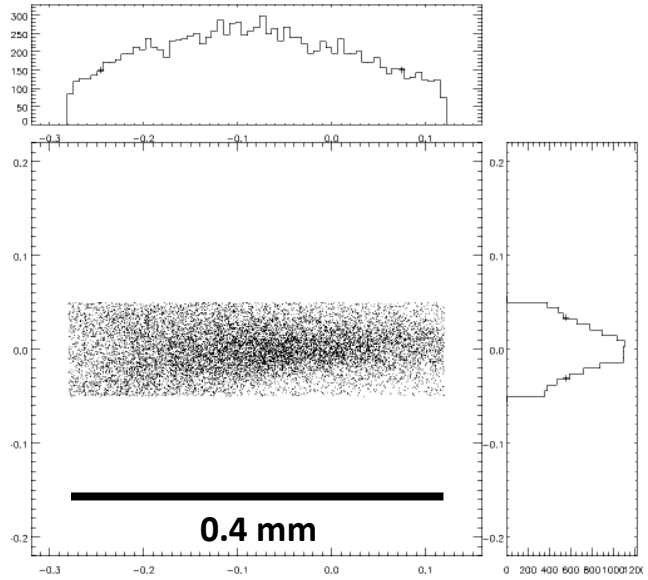
Multi-pump in vivo

XFP Modes: Full Focus

Major Radius: 3.3 km

FWHM V [mm]	FWHM H [mm]	Total Power in Spot [W]	Sample Morphology (V x H, mm)	Power Density on sample [W/mm^2]
0.081	0.425	49	0.1 x 0.4	1228





Full Focused Beam

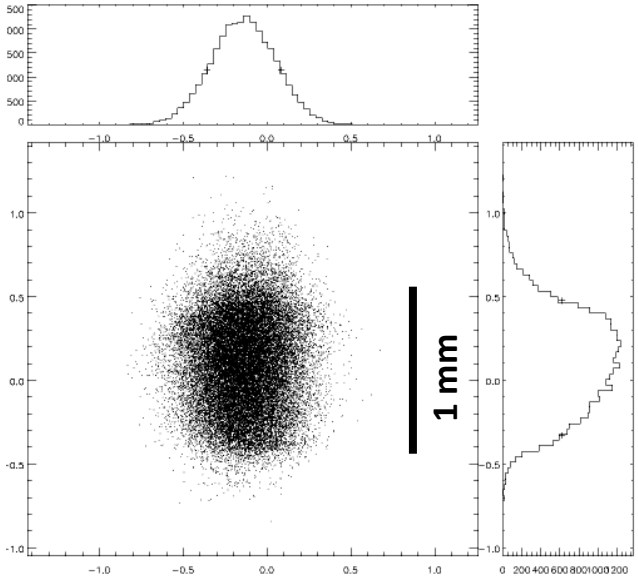
Sample: 100 µm Capillary

Note: mirror figure error not included in calculations

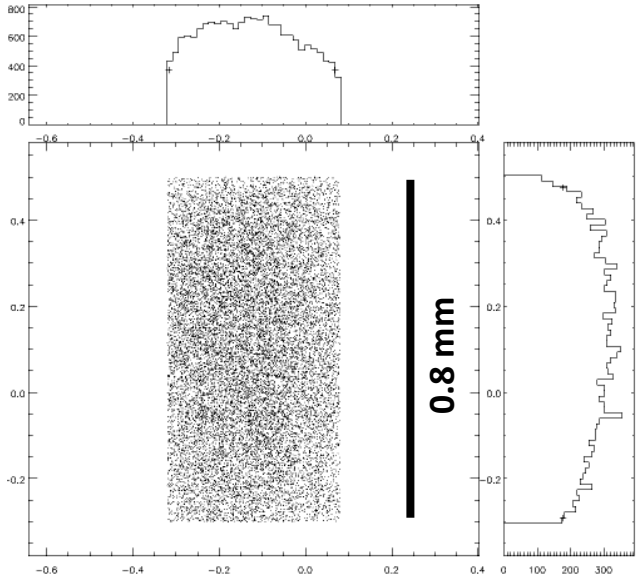
XFP Modes: Large Capillary (&KinTek)

Major Radius: 3.7 km

FWHM V [mm]	FWHM H [mm]	Total Power in Spot [W]	Sample Morphology (V x H, mm)	Power Density on sample [W/mm²]
0.809	0.460	66	0.8 x 0.4	206



Full Beam



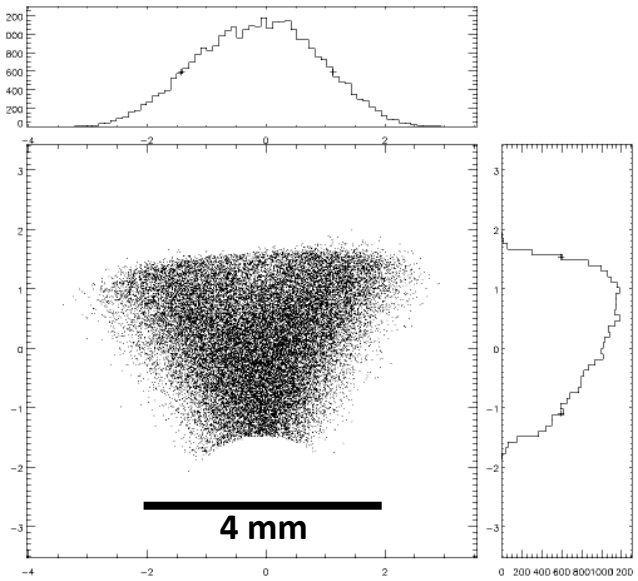
Sample: 800 µm Capillary

Note: mirror figure error not included in calculations

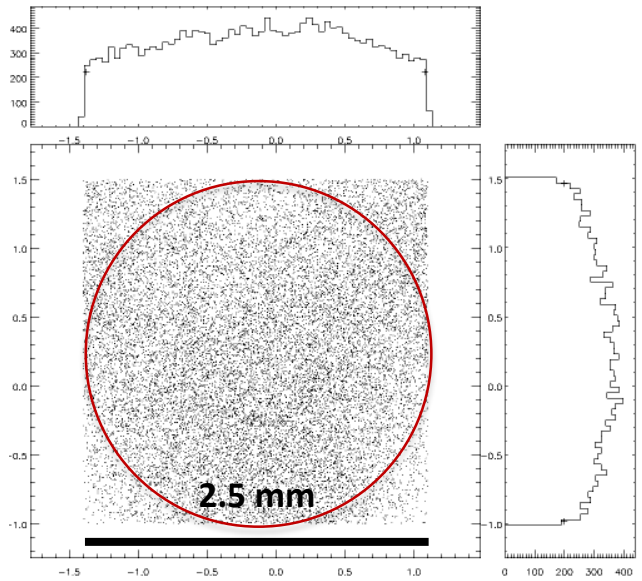
XFP Modes: High Throughput (MSH)

Major Radius: 5.2 km

FWHM V [mm]	FWHM H [mm]	Total Power in Spot [W]	Sample Morphology (Diameter, mm)	Power Density on sample [W/mm²]
2.604	2.646	78	2.5	12.5



Full Beam



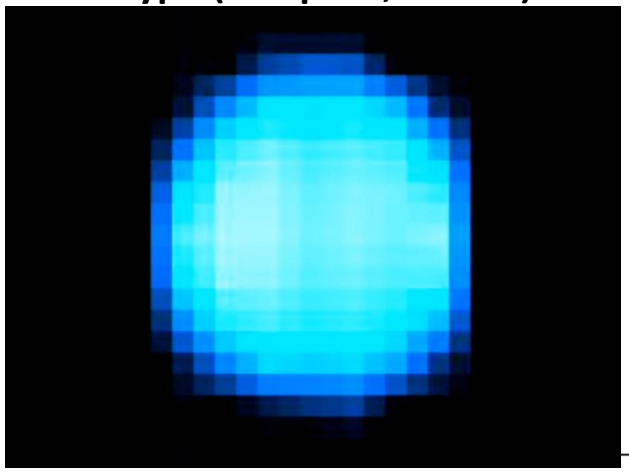
Sample: 5 µL Droplet in PCR Tube

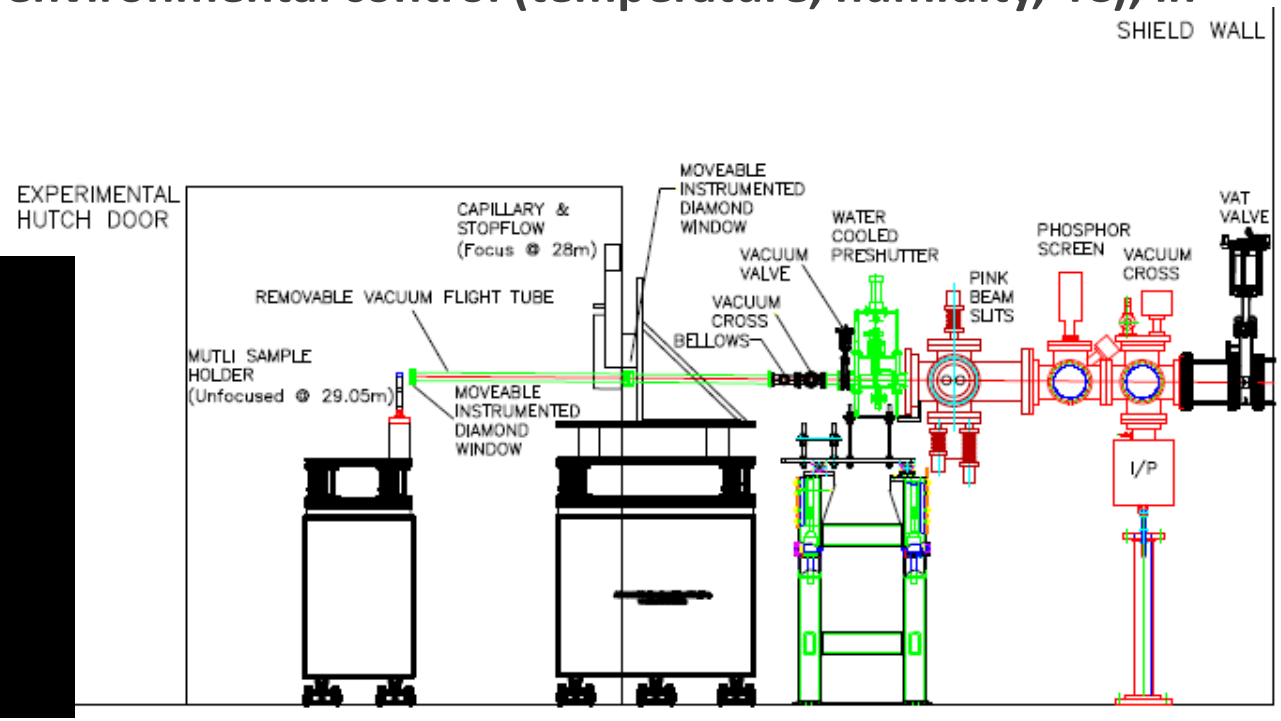
Note: mirror figure error not included in calculations

Endstation

- Current capabilities for transfer: MSH, KinTek, capillary flow cell, *in-vivo* setup, local sample environment control
- Upgrades planned: instrumented diamond window (in progress), 96-well plate system, global environmental control (temperature, humidity, 4C), in-line FPLC capability

Test of Imaging Detector Prototype (256 pixel, 1024…)

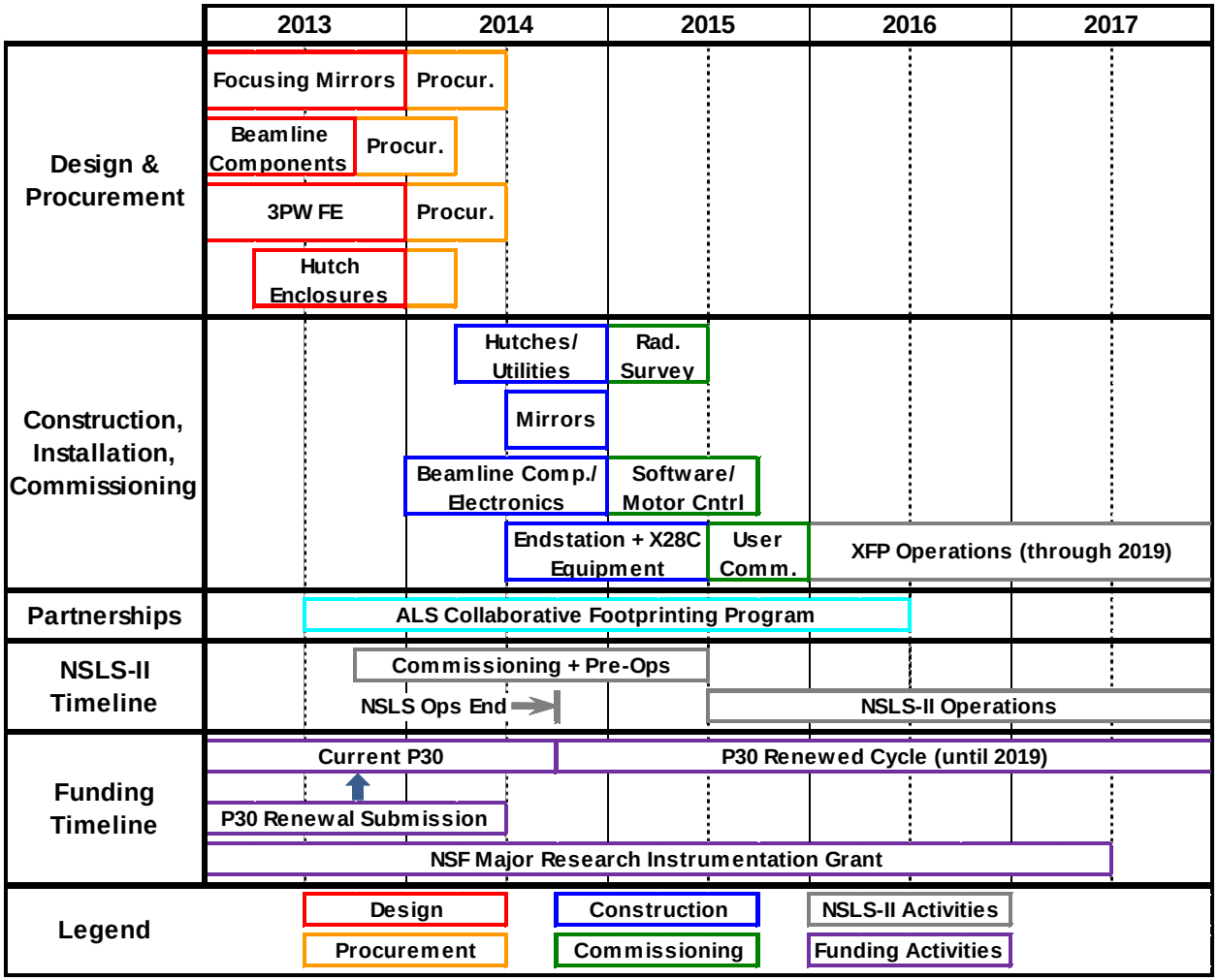




Critical Path Sample Prep Facility

- *Mass spectrometer*
- *High-pressure purification system (FPLC)*
- *Refrigerated Cabinet*
- *UV/Vis Spectrometer*
- *Fluorimeter*
- *-80C Freezer*
- *Incubator*
- *Centrifuge*

XFP Timeline



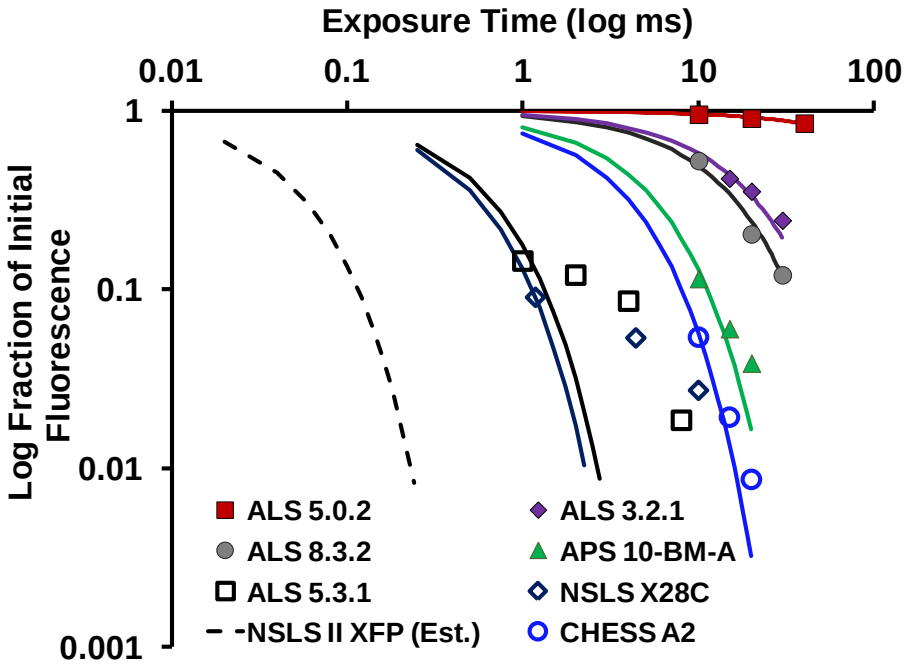
- Beginning to move equipment over to 740 as of October 1, 2014
- Hutch procurement in progress
- Mirror procurement in progress

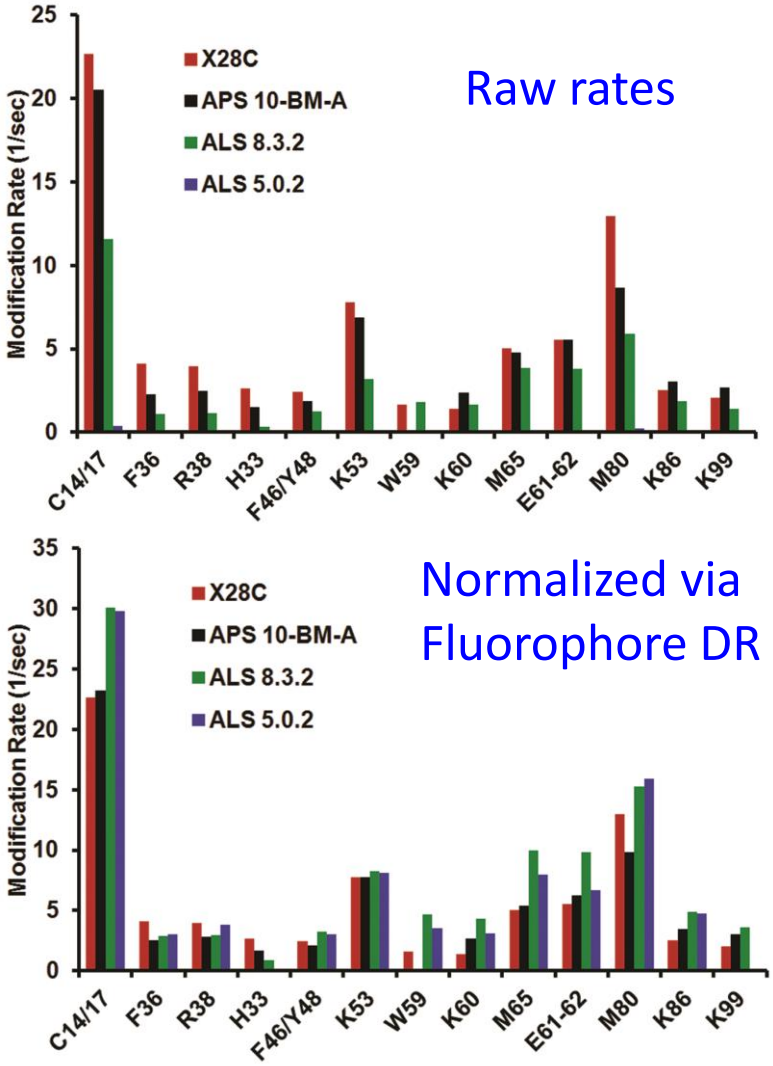
Begin user-assisted commissioning by early 2016

Transition from NSLS

Footprinting on Tour

- Can take portable pump and capillary flow cell system to almost any synchrotron

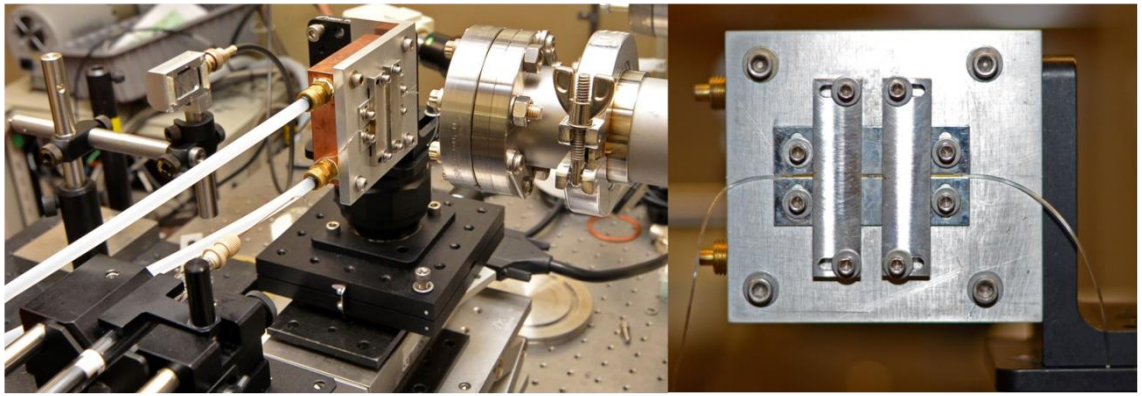




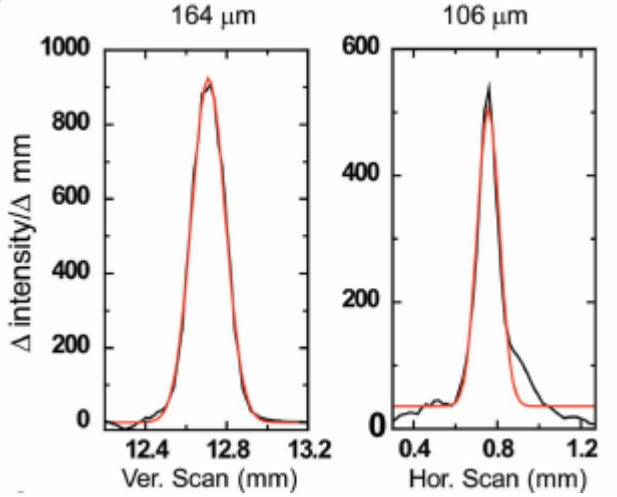
X-Ray Footprinting at the ALS

- CWRU, LBNL partnering to provide resources for user support at ALS
- Beamline 5.3.1 for high-flux experiments, beamline 3.2.1 for less demanding projects (smaller proteins, simpler buffers), beamline 3.3.1 for eventual dedicated Footprinting line
- **Access:**
  - **NSLS X28C users and new collaborations through CWRU coordinated through Dr. Wuxian Shi (local contact: Dr. Sayan Gupta)**
  - **New users (non-CWRU) to contact Dr. Sayan Gupta**
  - **Beamtime at 3.2.1 as needed (mail-in turnaround time of ~2 weeks from request)**
  - **Beamtime at 5.3.1 must be scheduled during allotted time – available ~ 2-4 days every 2 months (calendar will be posted to indicate when time has become available).**
- Maintain footprinting resources on both coasts even after XFP is completed – increase accessibility and disseminate technology to new local community

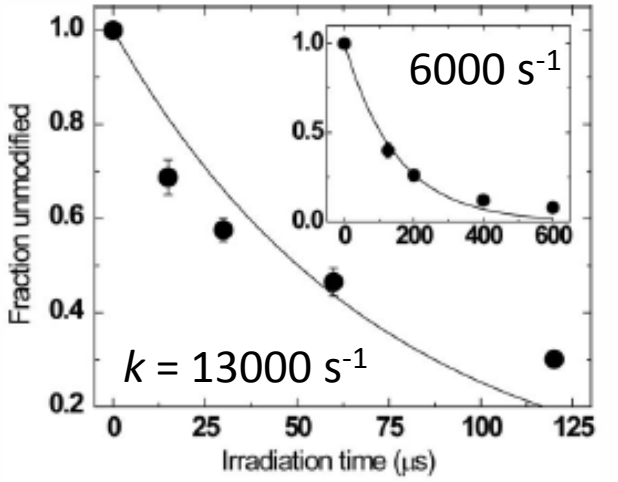
µs X-Ray Footprinting at the ALS



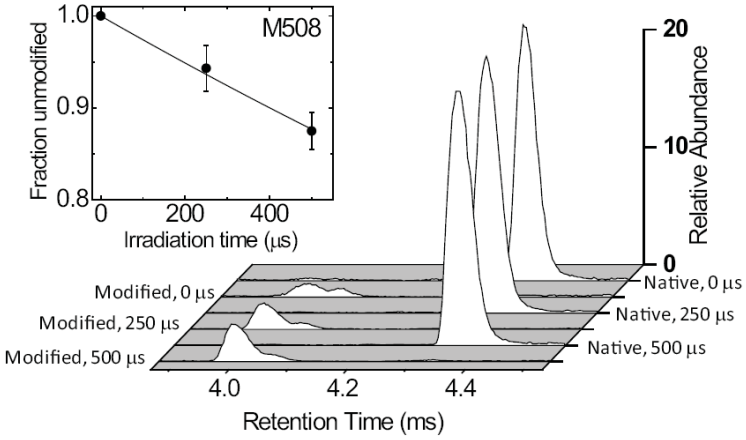
Capillary setup on 5.3.1: 50 µl / irradiation, 100 µm ID



Beam size



High flux density microbeam enables XF to reach µs timescales



Future Directions in X-ray Footprinting Development at the ALS

- **Optics Upgrade for 3.3.1**
  - Beamline 3.3.1 is being commissioned as a dedicated X-ray Footprinting line – with the addition of a focusing mirror, this could become a hub of high-performance XF on the West Coast
- **Automation**
  - Preliminary designs completed for a fully automated flow system (potentially including in-line digestion and MS)

Future of X-ray Footprinting

- **High flux density beams = short exposure times = fast kinetics, high quality data** (how much is too much?)
- Rapid mixing (to actually *do* fast kinetics)
- In-line processing (sample purification/ complex formation, digestion, MS)
- Controlled sample environments, live cells
- Automation, improved data analysis

Goodnight NSLS…