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Update on Redevelopment of 8-BM

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- And many more !!!

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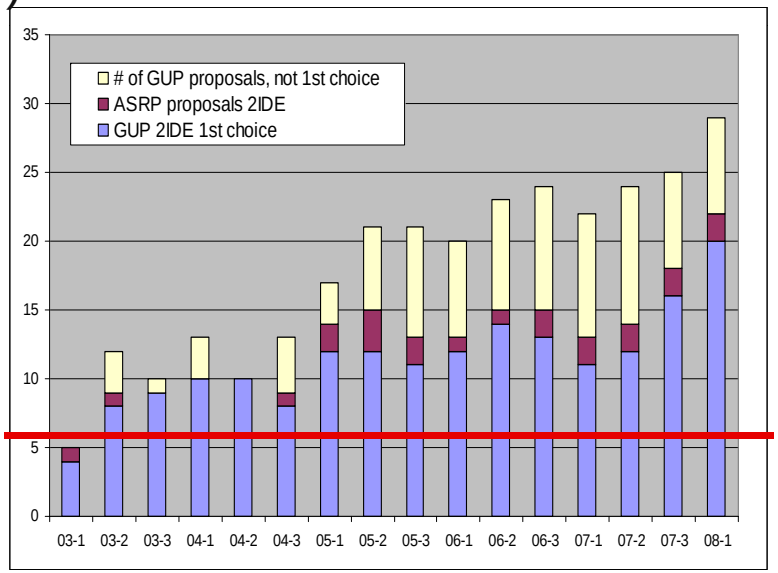
Redeveloping 8-BM: Addressing User Needs for High Throughput Elemental Analysis

- Metals are important to our health and our environment; imaging is in high demand

  Health: At least five million Americans suffer from metal-related diseases (eg, Wilson's diseases, Alzheimer's, Lou Gherig's disease (ALS))
  Environment: Knowing the fates of metals in bacteria would reduce the $150 billion cleanup cost of DOE sites (bioremediation); metal nutrients influence growth of marine micro-organism (climate change)

- Redevelopment of 8-BM addresses user needs
  - Will enable statistical measurements of biological samples
  - Will let us solve problems that cannot be answered elsewhere
  - ⇒ Leverage existing microprobes in high demand with BM based instrument enabling new studies on existing beamlines

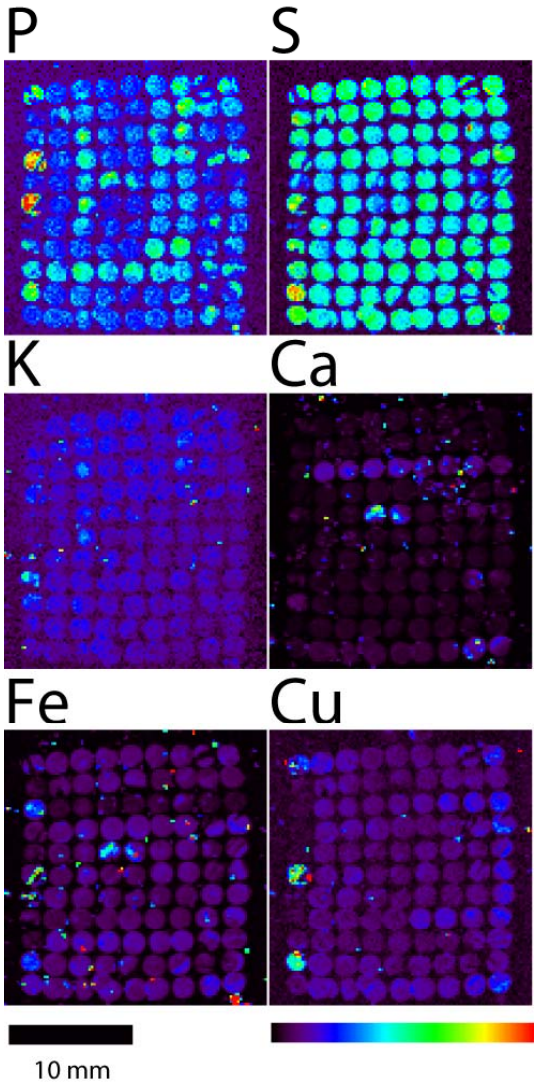


Subscription at BL 2IDE, red line: typical # BAC scheduled experiments

High-throughput analysis of micro-arrays and tissue sections

- Motivation: essential role in health and disease
  - Metals such as copper and zinc play pivotal roles in diseases including: Alzheimer's disease, Lou Gherig's disease, Menkes disease, Wilson disease, as well as cancer.
- New Tool: High-Throughput BM beamline will allow statistically significant data collection on tissues
  - Use 0.3 mm beam to image biopsies directly
  - Probe conventional tissue sections on slides (compatibility with pathology equipment)
  - Accommodate 100s of individual samples
- Example: Tissue Zn concentration and cancer risk

  (C. Abnet *et. al*., J. Nat. Cancer. Inst., 2005)
  - Zinc is an important co-factor in the esophageal-carcinogenic action of nitrosamines in animal studies.
  - Zinc status is difficult to assess in humans. XRF allows direct analysis on thin section of biopsy tissues.



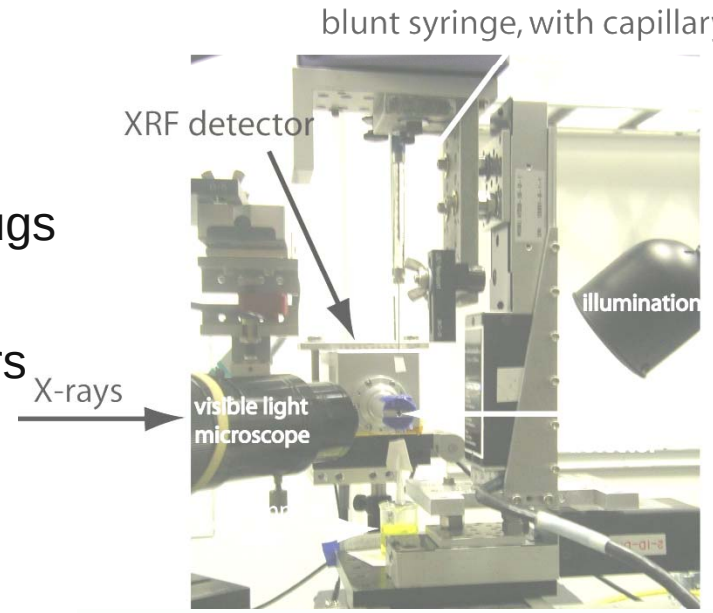
Prototype example

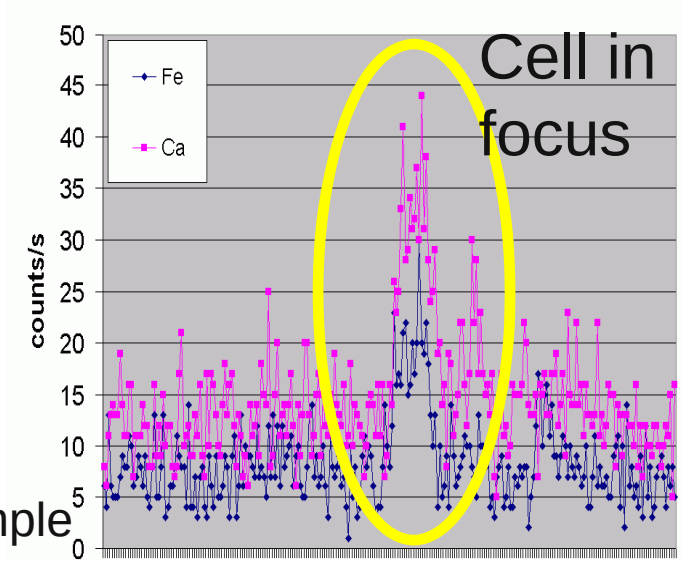
X-ray flow cytometry: High Throughput of Single Cells

- Motivation: metabolic fate of metal contaminants in cells
  - Critical for bioremediation
  - Unparalled information on metabolic fate of drugs

- New Tool: Rapid measurement of massive numbers of suspended cells.
  - give up spatial resolution for high throughput
  - ‘flow’ cells through capillary tubing
  - create line focus, collect XRF of cells as they move through focus
  - enable studies not currently possible

- Example: new nanocomposites as molecular 'tools' to combine functional medical imaging (visualize tumor cells) with intracellular action (kill tumor cells)
  (collab. B. Twining, S. Baines, G. Woloshak)

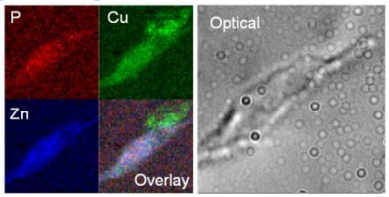




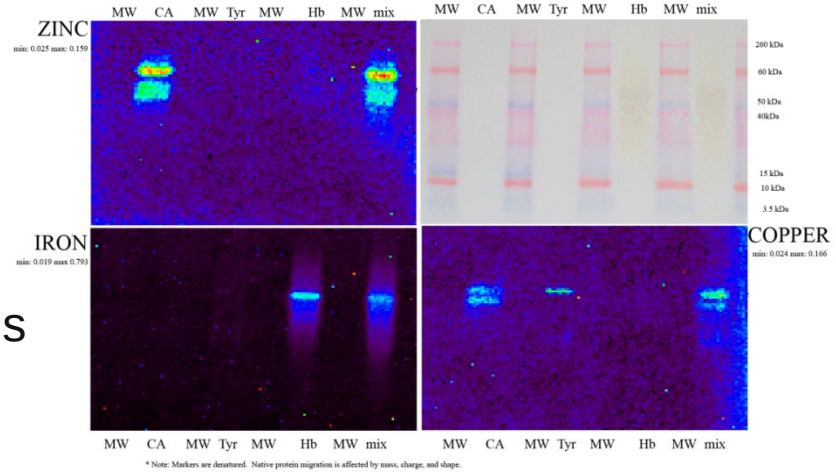
Prototype example

High-Throughput Metalloproteomics: Identifying Metal-Binding Proteins and Pathways

- Motivation: pairing information on metals from images with physiological pathways
  - Proteins with high potential for bioremediation
  - Metalloproteins important in disease
  - Systematic identification of entire complement of proteins binding a metal of interest
- New Tool: XRF scanning capability to identify metals bound to proteins separated by electrophoresis
  - enable rapid advances in multiple fields
  - pairs XRM with conventional electrophoresis used in labs everywhere for studies possible nowhere else
- Example: Copper is exported during angiogenesis (Finney et al PNAS 2007). . . But without this technique, we cannot identify the proteins it is activating that are lucrative, high-specificity anti-cancer targets



"X-ray fluorescence microscopy reveals large-scale relocalization and extracellular translocation of cellular copper during angiogenesis" L. Finney, *et al*, PNAS 104(7): 2247-52. (2007)



Prototype example using purified metalloproteins

■Each metalloprotein shows its bound metal (~50 μg each, carbonic anhydrase w/ Zn), mushroom tyrosinase w/ Cu, and hemoglobin w/ Fe)
■Mixture of proteins, identification of each is possible (~ 50 μg each)

Equipment / financial resources

- Funding: LDRD, MIC group, XSD
- APS project proposal

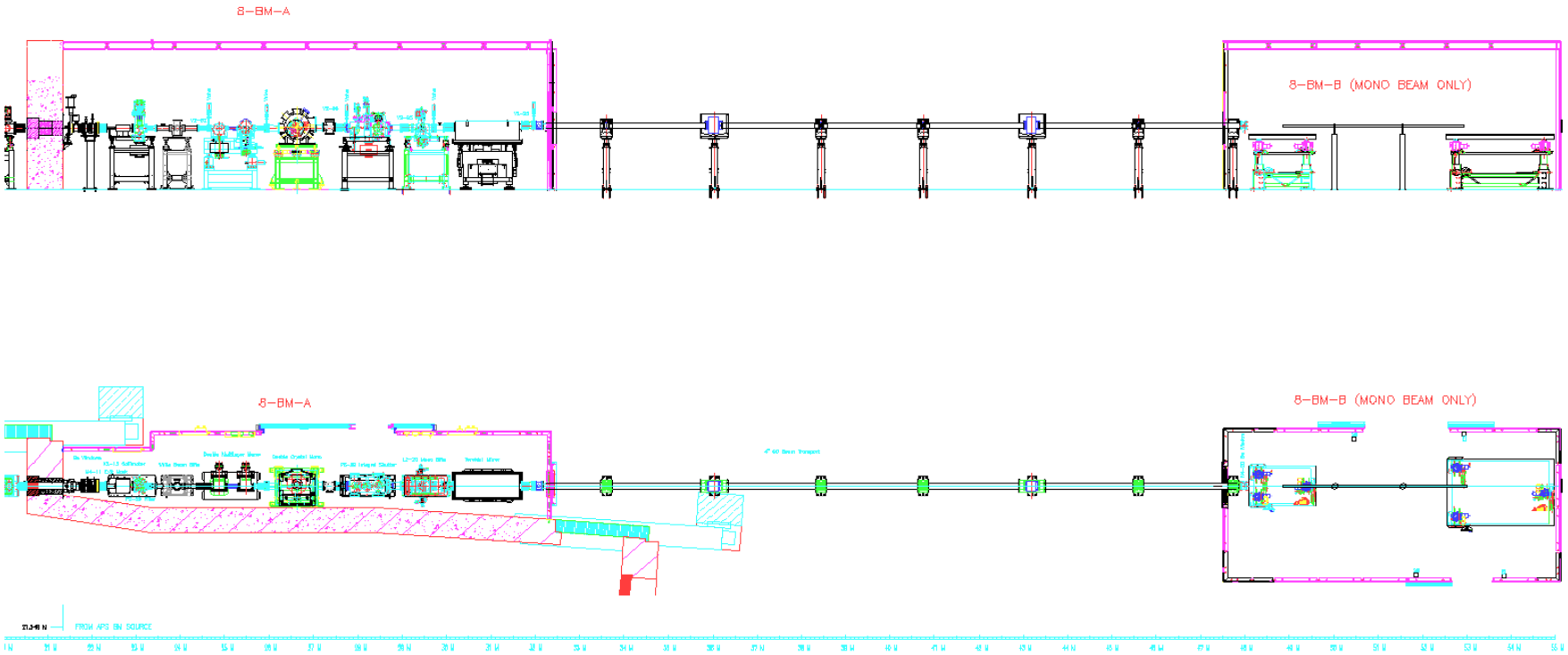
Generous contributions of equipment from other BLs / users:

- White beam mask (12-BM)
- Filters (sector 12)
- white beam slits (NSLS & 11-BM)
- Modified P6-20 shutter (2-BM)
- Mono slits: 1ID
- Be windows (sector 2)
- Double Multilayer Monochromator (2-ID)
- Double Crystal Monochromator (12-BM)
- DCM Table (sector 7)
- Toroidal Mirror Assembly (7-BM, Eric Dufresne, Jim Penner-Hahn, U Michigan)
- Hutches, beamtransport (in place)
- Vacuum Pumps and Controllers (MOM group, various places)
- Controls (existing spares, IPNS)
- Vortex Silicon Drift detector (Christian Abnet, NIH)
- ZP optics (2-ID)
- Prototype flow setup

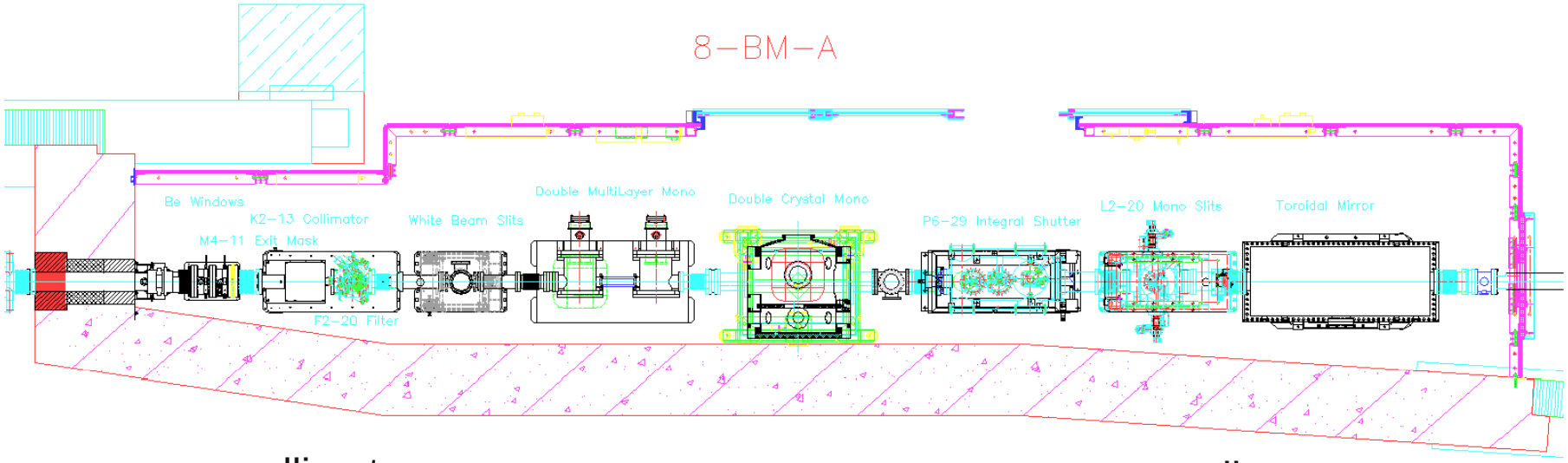
Beamline Layout

8-BM-A

8-BM-B



Beamline Layout - Station A



collimator

DMM

DCM

mono slits

white beam slits

shutter

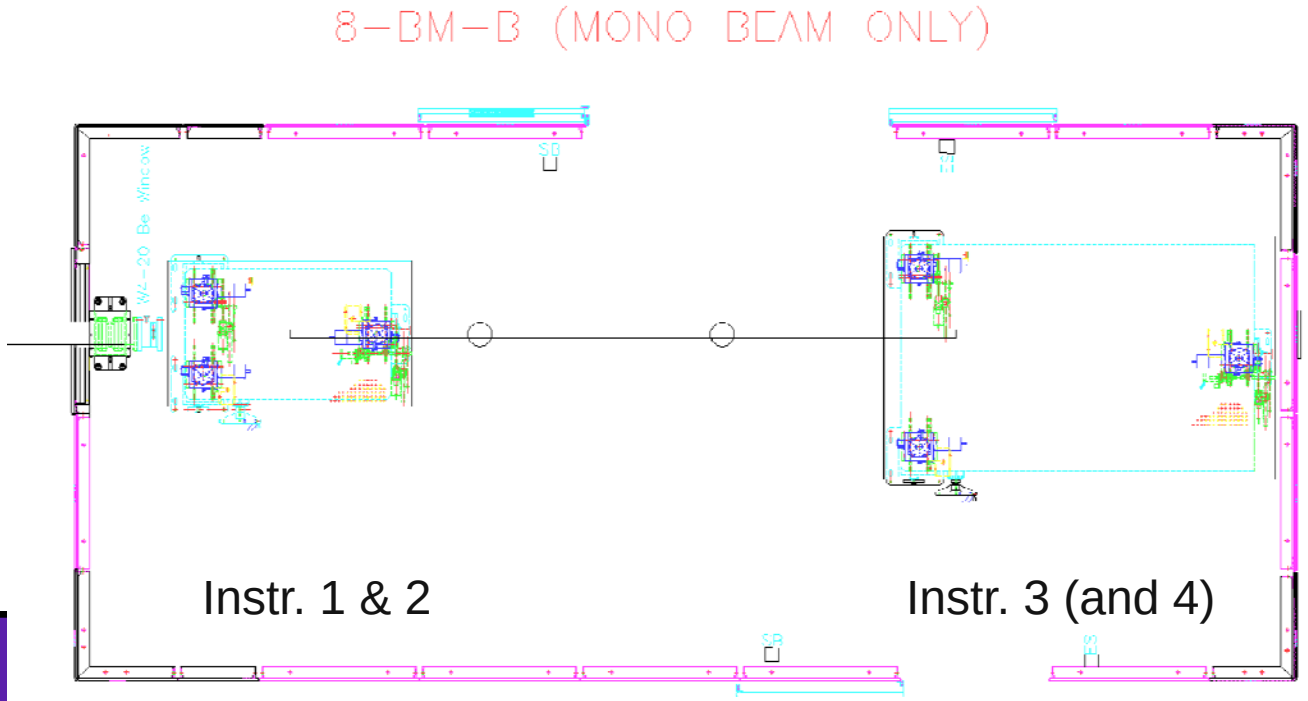
toroidal mirror

Endstation Instrumentation / Layout 8-BM-B

4 similar modes of operation; trivial switch-over

1. High-throughput analysis of micro-arrays and tissues: very low resolution, basic 2D ‘micro’-probe: spatial resolution 0.1 – 3 mm
2. Proteomics: 1D (line focus, for 1D gels) or 2D (point focus for 2D gels) probe with 0.1-0.5 mm beam
3. X-ray flow cytometry: Line focus through which the sample moves, 5x200 micron beam
4. (‘Conventional’ , low resolution, microprobe (10-20 um) for routine preview of tissue samples to be later imaged at higher resolution elsewhere)

From 2BMA



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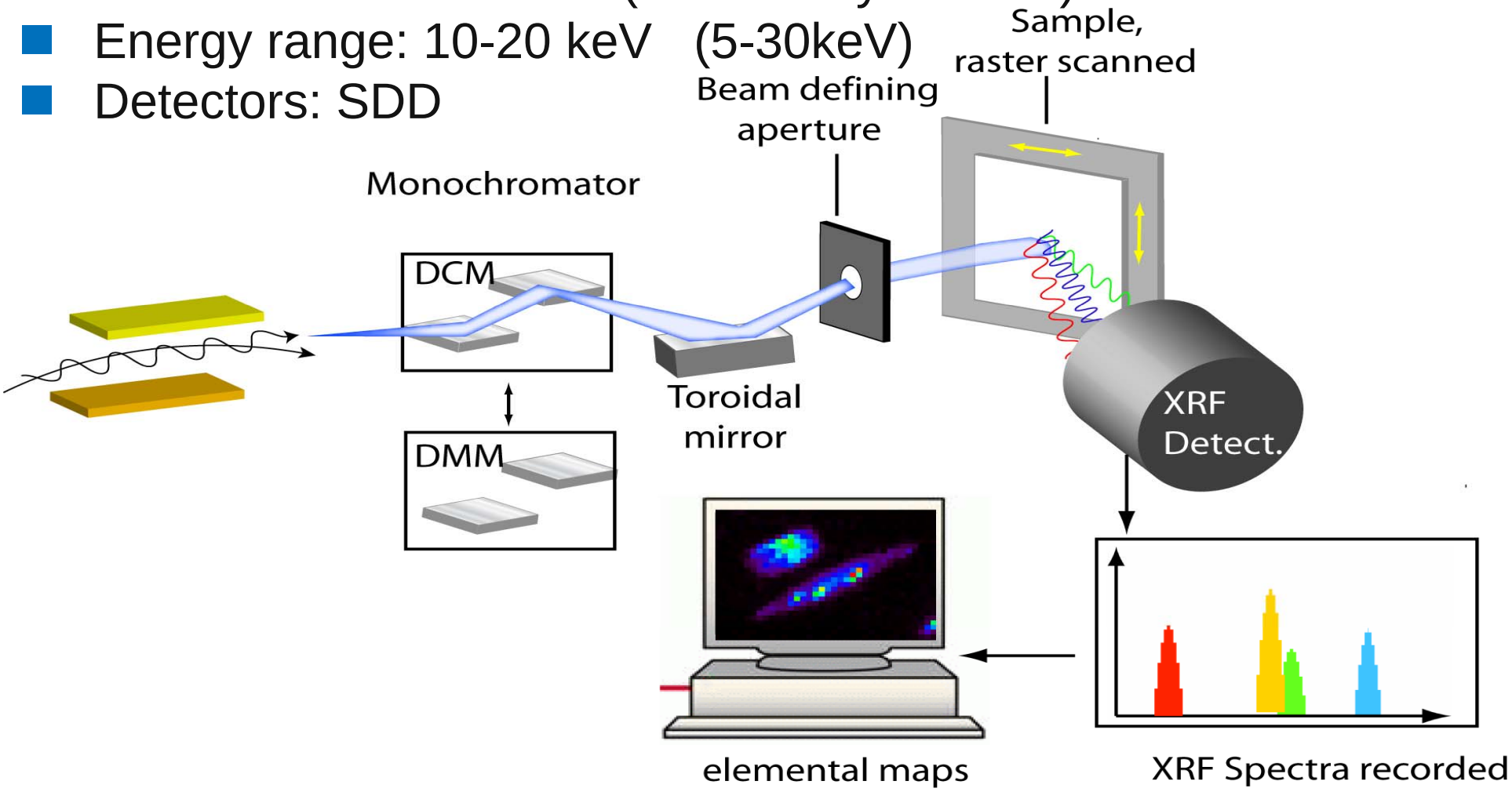
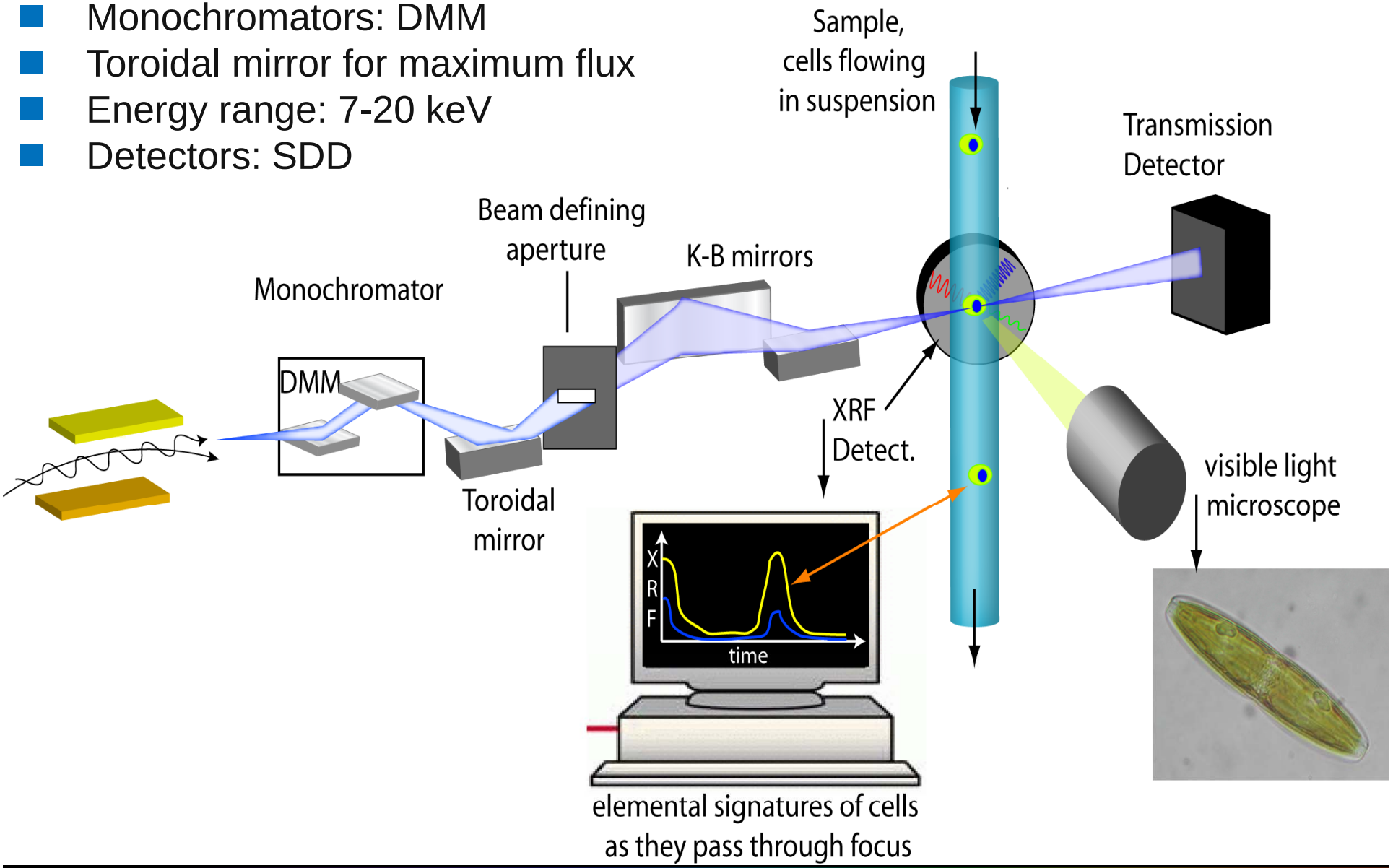
Approach for tissue work and Proteomics:

- 2 monochromators:
  - DMM for metal mapping / quantification
  - DCM for spectroscopy (u-XANES to determine oxidation state)
- Toroidal mirror for maximum flux on sample
- Pinhole to define beam (focused by toroidal)
- Energy range: 10-20 keV (5-30keV)
- Detectors: SDD



X-ray flow cytometry: Schematic

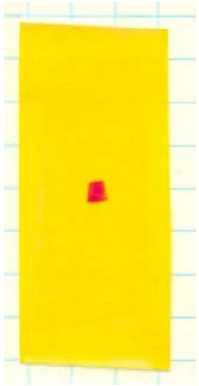
- Monochromators: DMM
- Toroidal mirror for maximum flux
- Energy range: 7-20 keV
- Detectors: SDD



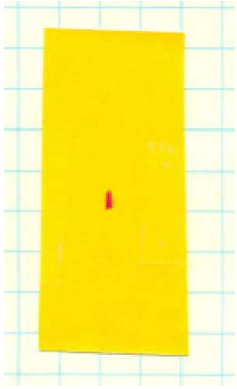
Status:

- Beam delivered into 8-BM-B early August
- DMM and DCM tested (unfocussed flux with DMM: 4 x 10^{11} photons/s)
  - DCM mount vertical stage is being fixed
- Toroidal mirror tested
  - Reopened tank, verified and reworked bender motor setup
- Currently: building up XRF-metalloproteomics setup.

Initial Burn Papers



DMM, unfocussed



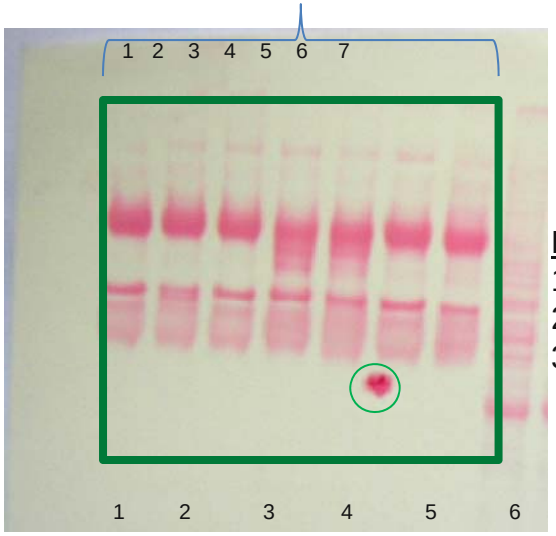
DMM, focussed

Future:

- ‘Pure’ commissioning until mid-October
  - Thoroughly test, calibrate DCM, DMM
  - Focussing with toroidal mirror
- Starting mid-October:
  - Proteomics experiments for overnight
  - Continue commissioning and experiment setup during day
- Replace multilayer (DMM) with one optimized for BM (large bandwidth)
- Use Kirk-Patrick Baez mirrors for improved flux for flow cytometry and low resolution microprobe.

Understanding the Effect of Chromium on Human Health

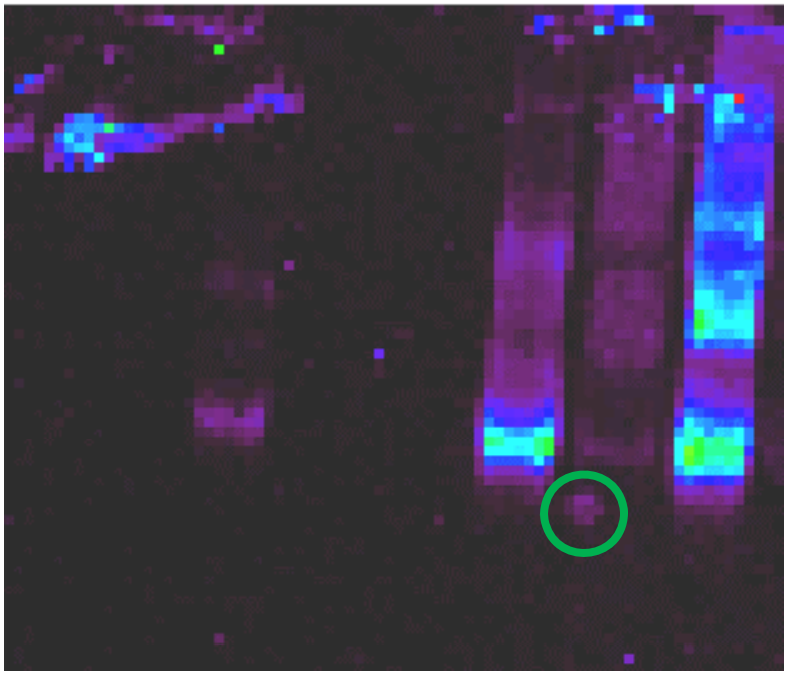
(in collaboration with Peter Lay, Aviva Levina; Univ. of Sydney)



BSA-depleted serum (lines 1-7

1 protein only;
2 Cr(III)-aqua treated;
3 protein and GSH;
4 protein and Cr(IV);
5 *in situ* Cr(III) from reduction of Cr(IV) with GSH;
6 pre-formed Cr(III)-GSH;
7 Cr(III)-propionate.

Finney, L., Chishti, Y., Khare, T., Giometti, C., Levina, A., Lay, P., and Vogt, S. **Imaging the Use and Acquisition of Metals by Proteins: Combining Electrophoresis with Rapid X-ray Fluorescence Imaging.** Proteomics. *In Preparation*

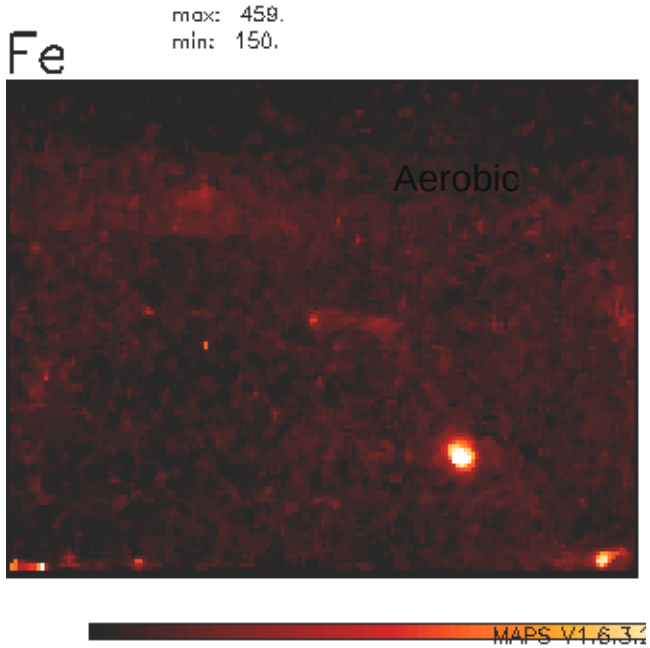


- We identified the interaction of chromium with serum proteins
- These interactions occur only with chromium (III) present
- In coming work, we will investigate the interactions of chromium with intracellular proteins

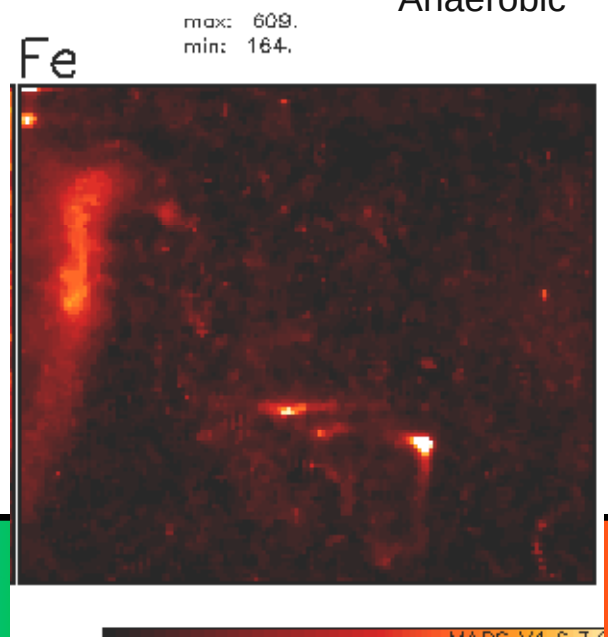
  Thus, variations in the *extent* to which different species of Chromium bind to mixtures of proteins is possible, as well as identifying differences in *which individual proteins* are involved.

Characterizing Critical Proteins in Bioremediative Bacteria *(in collaboration with Carol Giometti, BIO, ANL)*

- *Shewanella Oneidensis* is a reducing bacteria important in bioremediation of sites contaminated with metals.
- We imaged several iron proteins that are expressed under anaerobic conditions and may be important in the bioremediative action of this microbe.
- In coming work, we will be both working to identify these proteins, and also looking at the metal-binding proteins present in other bioremediative bacteria, such as *Anaeromyxobacter dehalogens.*



Anaerobic



Operational perspectives:

- Dedicated setup will allow true optimization of acquired data, as well as data acquisition and analysis strategies.
  - Combined with standardization, it allows for highly efficient operations

Staffing:

- Dedicated Scientific Associate
- Plan for dedicated scientist in FY2010 (winter)
- Contribution from sector 2 staff
- Postdoc just started (50% LDRD, 50% XSD)

General user fraction dictated by our ability to bring instruments into 'production', and provide adequate staffing.

2009-3: only commissioning (and very friendly users)

2010-1: 20% GUP

2010-2: 40% GUP

2010-3: 60% GUP

2011-1: 80% GUP

Note: these numbers rely on hire of a dedicated scientist in FY 2010 (winter)

Summary

- The redevelopment of 8-BM addresses user needs and enables new science important to our health and environment:
  - High-throughput analysis of microarrays and tissue sections
  - X-ray flow cytometry: High-throughput of single cells
  - High-throughput metalloproteomics: Identifying metal-binding proteins and pathways
- Small Investment (<$200k), re-utilize existing equipment (~$5M): big pay-off: 3 unique instruments, all pushing the frontier of possible experiments forward.
- Already have achieved focused beam; planning to begin running overnight experiments mid-October.
- As we enter new phases, with new experimental techniques coming online, we will continue to explore newly enabled science.