X-RAY IMAGING & X-RAY MICROSCOPY

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ACKNOWLEDGEMENTS

▪ Chris Jacobsen, Argonne / Northwestern University
▪ Matt Newville, University of Chicago
▪ Doga Gursoy, Argonne
▪ Francesco De Carlo, Argonne
▪ Microscopy group at Argonne, in particular: Barry Lai, Joerg Maser, Chris Roehrig, Evan Maxey, …. 

▪ Tanja Paunesku, Gayle Woloschak, Northwestern University
▪ Martina Ralle, Oregon Health and Science University
▪ Peter Lay, Hugh Harris, University of Sydney

**Financial support:**
- Department of Energy (Basic Energy Science)
- National Institutes of Health (NIBIB, NCRR)
HIERARCHICAL STRUCTURE OF COMPLEX SYSTEMS

=>
NEED TO VISUALIZE STRUCTURE AND FUNCTION ON ALL RELEVANT LENGTH SCALES

LENGTH SCALE / TIME SCALE

| nm, ns | μm, μs | mm, ms |

- atomistic lattice structure
- discrete dislocation dynamics
- subgrain structures
- polycrystalline grain structure
- macroscopic material behavior
X-RAY IMAGING

Wilhelm Conrad Roentgen: discovered x-rays 1895
Nobel Prize in Physics

Anna Roentgen’s hand with wedding ring
Universität Würzburg
Dec. 1895
ABSORPTION OF X-RAYS

X-rays are absorbed by the Photo-Electric Effect

An x-ray has enough energy to kick out an electron bound to an atom

1. the x-ray is absorbed
2. the core electron leaves the atom and becomes a photo-electron
3. the atom is left without a core electron: in an excited state
X-RAY ABSORPTION COEFFICIENT

The x-ray absorption coefficient $\mu$ of a material depends very strongly on

1. the density
2. the atomic composition
3. the energy of the x-ray

$$\mu \sim \frac{\rho Z^4}{AE^3}$$

Where

$\rho$ = sample density

$Z$ = atomic number (# of electrons)

$A$ = atomic mass

$E$ = energy

$I = I_0 e^{-\mu t}$

This is why x-rays are used in medical imaging:

water (H$_2$O) is almost transparent to x-rays

bone (CaCO$_3$) is much more absorbing

lead is a really good x-ray absorber!
X-RAY ABSORPTION EDGES

X-rays have energies comparable to *binding energies* of electrons in atoms.

Notice the sharp jumps in Absorption:

*Absorption Edges*

These jumps occur at binding energies of core electrons:

x-rays have enough energy to kick out another bound electron.

Some Binding Energies (eV):

<table>
<thead>
<tr>
<th>Element</th>
<th>Binding Energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H 1s</td>
<td>13.6</td>
</tr>
<tr>
<td>O 1s</td>
<td>545</td>
</tr>
<tr>
<td>Fe 1s</td>
<td>7112</td>
</tr>
<tr>
<td>Pb 1s</td>
<td>88005</td>
</tr>
<tr>
<td>Pb 2p3/2</td>
<td>13043</td>
</tr>
</tbody>
</table>

We can select energies to excite particular binding energy levels.

This lets us to adjust the contrast for detecting a particular element.
X RAYS AND ELECTRONS

Consider penetration distance: $1/e$ absorption length for x rays, scattering mean free paths for electrons.

Courtesy C. Jacoben
X-RAY ABSORPTION CONTRAST IN THE WATER WINDOW

- *Drosophila melanogaster* cell, in vitrified ice, imaged @ 0.5 keV with the Goettingen TXM @ BESSY I. S. Vogt, et al

Cy: cytoplasm
V: vesicle
M: nuclear membrane
N: nucleus
DIRECT IMAGING VS SCANNING PROBE IMAGING

**Direct Imaging**
- Direct imaging (radiography)
- Projection microscopy
- Transmission microscope (TXM)

**Scanning Probe**
- Scanning microscope (SXM)
- Ptychography
- Coherent diffractive Imaging (CDI)

Level of coherence required / desired:

++  +  0  -
X-RAY SOURCE
BRIGHTNESS (=BRILLIANCE) VS FLUX / INTENSITY, AND WHAT IS COHERENCE?

100 W incandescent light bulb
(a lot of total flux / intensity, but goes into 4Pi steradian)

5mW laser pointer
Low total intensity, but very bright!!!
All light goes forward.

- Coherent source: cannot distinguish the source from a point source
- You can make any source ‘coherent’ by putting it at infinity, or putting slits in front of it

=> For microprobes: need coherent source to achieve diffraction limited spatial resolution
For microprobes: brightness of sources determines amount of focused flux on sample

\[ B = \frac{\text{Photons in unit spectral range in unit time}}{(\text{source size} \times \text{divergence})^2} \]

Units: photons/s/mm²/mrad²/0.1%BW
SR X-RAY SOURCES

- Typically, SR sources are large horizontally (~1mm), small vertically (~.05mm)
- source is imaged (demagnified) into the specimen – to achieve diffraction limited spatial resolution, need to use (horizontal) slits to define a small ‘virtual’ source (spatially coherent source)
- High brightness sources optimal for microprobes
- NB: typically, X-rays are polarized in horizontal direction
  ⇒ Scattering in plane at 90 degrees is minimized, optimum position for XRF detector is at 90 degrees to the side of the incident beam
A REVOLUTION FOR SR SOURCES: MULTIBEND ACHROMATS

- Reduce Horizontal emittance to match vertical emittance (ie, round source)
- For example, can focus the full flux of APS into a ~250 nm spot!
- Can speed up u-XRF (and u-XRD) by factors of 100 - 1000x
- (Nearly) any technique can become a microscopic technique ....
APS Upgrade multi-bend achromat lattice concept

~50x reduction in horizontal emittance

\[ \varepsilon_x = C_L \frac{E^2}{N_d^3 D} \]

- \( N_d = \) Number of dipoles per sector (\( N_d = 7 \) for APS MBA)
- \( E = \) Beam energy (\( E = 6 \) GeV for APS MBA)

- increase current by 2x, also use optimized insertion devices
- work continues to further increase gains
EXCITING DEVELOPMENTS: MULTIBEND ACHROMATS (MBA)

Curves for APS, ESRF and SP8 upgrades based on present designs, assuming identical undulators.
FULL FIELD IMAGING
2D/3D/4D Imaging with the APS

- Parallel Beam Imaging (PBI)
  - Phase and absorption
  - Spatial resolution: 1 μm
  - Temporal resolution:
    - 3D: kHz projection
    - 2D: 6.5 MHz, 100 ps
Tomographic data acquisition

Detector view as sample rotates

2048 pixels

2048 slices

Slices (sinograms)

1500 projections

uint16

Pixels

Projections
DYNAMIC IMAGING
3D imaging of dynamic systems

Wood adhesive bondline swelling and shrinking
J. Jakes, USDA Forest Service, Forest Products Laboratory, Madison, WI

Xianghui Xiao, APS Imaging Group
**DYNAMIC IMAGING**

*3D imaging of dynamic systems*

- **Parallel Beam Projection**
  - Phase and absorption
  - Spatial resolution: 1 µm
  - Temporal resolution:
    - 3D: 1000 projection/s

Growth of Al-rich dendrite in Al-Cu alloy
Cooling rate 1K/min from 550 K
3D tomographic dataset in 1.6 s


K. Aditya Mohan, Purdue University [http://timbir.readthedocs.io/](http://timbir.readthedocs.io/)
Phase contrast imaging

Refraction of waves:

- **Incident Ray**
- **Normal**
- **Angle of incident**
- **Refraacted Ray**
- **Angle of refraction**

Bottom of the pool on a cloudy day

Bottom of the pool on a sunny day

Refraction (phase-contrast) fringes

Partially coherent illumination (or dedicated optics)

Incoherent illumination

**Refractive index**

\[ n = 1 - \delta - \iota \beta \]
IN VIVO X-RAY PHASE-CONTRAST MICROCTOMOGRAPHY FOR DEVELOPMENTAL BIOLOGY

During gastrulation: series of dramatic, coordinated cell movements drive reorganization of a simple ball or sheet of cells into a complex multi-layered organism.

Use time resolved x-ray tomography to follow structural reorganization during embryonic development.

Simulated images of a water drop (0.4 mm diameter) with an air bubble inside (2 µm diameter) at 15 keV. (a) With current APS lattice, (b) with future MBA lattice. Round source => Significantly improved contrast in the horizontal direction.
HOW TO FOCUS X-RAYS?
INDEX OF REFRACTION FOR X-RAYS

- Because \( n < 1 \) (!) in media, total internal reflection in the visible is total external reflection for X rays.
- Because \((1-n)\) is small, grazing reflection angles only.

refractive index
\[ n = 1 - \delta - \iota \beta \]
FOCUSING FOR SYNCHROTRON X-RAY MICROSCOPY

- **Reflective optics**
  - Efficiency ~90%
  - Achromatic focus
  - Spots down to ~100nm (limited by figure error)
  - Used for 5-20keV

- **Refractive optics**
  - Large working distance for microfocus (0.5-10m)
  - Mechanically robust
  - Spots down to ~100nm (limited by NA, absorption)
  - Used for 5-200keV

- **Diffractive optics**
  - Spots down to ~20nm limited by outer zone width (~1.22dr)
  - Compact optic
  - Efficiency ~2-30%
  - Chromatic focus
  - Used for 0.2-30keV

Courtesy Martin Holt
REFLECTIVE X-RAY OPTICS

Mirror optics are inherently achromatic, i.e., focus position is independent of incident energy.

High efficiency: gain of $\approx 10^5$ with high reflectivity up to $25\,\text{keV}$ ($90\%$ efficiency).

Many efforts have been made in recent years to use achromatic K-B mirrors for hard x-ray sub-100 nm focusing.

What is Montel (or nested K-B) mirror optics?

Two mirrors, mounted side-by-side and perpendicular to each other. Some rays strike one mirror first while others strike the other mirror first.
**COMPOUND REFRACTIVE LENSES**

- Röntgen tried to make lenses, but found no focusing.
- Focal length of one lens is long — so combine many lenses! Tomie; Snigirev et al., *Nature* 384, 49 (1996); Lengeler et al., *J. Synch. Rad.* 9, 119 (2002).
- Resolution approaching 100 nm at 5-10 keV with parabolic beryllium lenses
STANDARD DIFRACTIVE OPTICS: FRESNEL ZONE PLATES

- Circular diffraction grating
  - Radially increasing line density
  - Numerical aperture related to outermost zone width \( dr_n \)
  - Chromatic

Typical Parameters, \( E = 10 \) keV:
\[
dr_n = 100 \text{ nm}, \quad r_n = 160 \mu\text{m},
\]
\[
t = 1.6 \mu\text{m}, \quad \text{(Aspect ratio 16)}
\]
Resolution \( \delta_m = 1.22 \frac{dr_n}{m} \)

Outermost zone width determines spatial resolution, thickness determines efficiency (at a given energy)

\[
NA = \frac{r_n}{f} = \frac{\lambda}{2dr_n}
\]

\[
\delta_R = 1.22 \frac{\lambda}{NA} = 1.22 \cdot dr_n
\]
DOES IT WORK - FRESNEL ZONE PLATE IMAGES

- R. W. Wood (1898): zone plate figure drawn with a pen and a compass!
  Photographically reduced
FULL FIELD NANO IMAGING

3D imaging at 20 nm

- Transmission X-ray Microscope (TXM)
- Resolution: typically 60 nm (30 nm voxels) down to 20 nm
- Energy range: 6 to 12 keV, $\Delta E/E = 10^{-4}$
- Multi-scale approach with an integrated $\mu$-CT module
- In situ: compatible in a wide range of samples environments
  ($T = \text{ambient to } 1500 \, ^\circ\text{C}, P \text{ up to } 100 \, \text{GPa}$), chemical bath, etc.
Energy Science
- Fuel cell
- Battery
- UMo nuclear fuel

Earth and Environmental
- Melt formation
- Rock fracking
- High pressure experiments with DAC
- CO₂ storage
- Pollution / remediation

Material Science
- Metallurgy
- Photonics
- Electronic industry
- Supraconductors

Biology
- Biomaterials
- Wood preservation
- Biology (neurosciences)

Vincent De Andrade, APS Imaging Group
X-RAY MICROSCOPES

Transmission X-ray Microscope

- Full field
- Incoherent illumination; works well with a bending magnet (or lab source), with fast imaging
- More pixels (e.g., $2048^2$)
- Moderate spectral resolution in most cases

Scanning X-ray Microscope

- Coherent illumination; works best with an undulator
- Less dose to sample (~10% efficient ZP)
- Well suited for spectroscopy
- Microprobes: fluorescence etc.
MICROPROBES:
ADDING TRACE ELEMENTAL SENSITIVITY WITH X-RAY FLUORESCENCE
X-RAY INDUCED X-RAY FLUORESCENCE – A BRIEF REMINDER

photo-electric absorption of incident hard X-ray
emission of photo-electron

- Emission of Auger e\(^{-}\) - dominating low Z
- X-ray fluorescence - dominating high Z

Detect XRF using energy dispersive detector

- Energy of X-ray fluorescence photons is characteristic for each element
- XRF is quantitative, i.e., number of XRF photons is directly related to quantity of element
- Photo-electric absorption crosssection straightforward to calculate (monochromatic incident beam)
WHY USE X-RAY-INDUCED FLUORESCENCE TO STUDY TRACE METALS?

- Simultaneously map 10+ elements
- No dyes necessary
- High signal/background ratio
  - sub-ppm (part-per-million) sensitivity, increasing with Z
- Large penetration depth (~> 100 μm)
  - study whole cells, w/o sectioning
  - study ‘thick’ tissue sections
  - possibility to study hydrated “natural” samples using cryo
- Monochromatic incident beam: choose at which Z to stop excitation (e.g., excite As but not Pb)
- Straightforward quantification
- Microspectroscopy / Spectromicroscopy: Map chemical states by u-XANES
- Little radiation damage *

\[
\begin{align*}
\text{Detection Limit for Transition Elements:} \\
\text{for 1 sec. acquisition time, } 0.2 \times 0.2 \, \mu \text{m}^2 \\
\text{spot, } E=10 \, \text{keV}
\end{align*}
\]
A TYPICAL X-RAY FLUORESCENCE SPECTRUM
Periodic table highlighting X-ray fluorescence

Major/minor elements in Biological Systems

Toxic / carcinogenic elements

Used in Imaging, Diagnosis, Therapy, ...

K-line Fluorescence typically used

L-line Fluorescence typically used
SCHEMATIC OF A HARD X-RAY MICROPROBE

- Scan (step or fly) sample through focused X-ray beam
- Record at each scan point
  - Full XRF spectrum
  - Diffraction pattern
  - Ptychography

- $5 - 30$ keV
- $\delta = 150$-500 nm
- $5 \times 10^9$ ph/s

* de Jonge et al, Phys Rev Lett 100(16), 2008
* Holzner et al, Nature Physics 2011

schematic NOT to scale!!
Sample in sample chamber, purge with He
Overview Image of a full HMVEC cell (plunge frozen in liquid ethane, freeze dried), 2 hours after initiating angiogenesis. Cu is localised strongly to areas outside of the cell, comparison to other timepoints suggests the Cu is transported out of the cell, and after a few hours back into the cell.

See also: L. Finney et al, PNAS 104(7): 2247-52. (2007)
GREAT TOOL, BUT IS IT THE RIGHT TOOL FOR THE JOB?

HARRY BELIEVED IN HAVING THE RIGHT TOOL FOR THE WRONG JOB

from http://www.cartoonstock.com/
## COMPARISON OF SOME OTHER TECHNIQUES FOR TRACE ELEMENT MAPPING:

|---------------------------------|---------------|---------------|-------------|--------------------------|
| Light-microsc.                  | 200 nm        | 30 µm         | Wave-length | + changes in living cells can be monitored, but competition w. proteins  
+/- only see ions (in solution), and not total content  
- need dyes  - quantification difficult |
| Hard X-ray-micropr.             | 200 nm-20nm   | 10 µm         | Currently Optics | + no dyes, visualize total elemental content  
+ very high sensitivity, low background, selective excitation  
+ simultaneously detect >10 elements  
+ µ-XANES for chemical state mapping  / - slow |
| Analytical Electron-micropr.    | 20 nm         | 0.1 µm        | object thickn. | + high spatial resolution  
+ simultaneously detect >10 elements  
- thick samples very difficult, sectioning necessary  
- slow  - radiation damage |
| EELS/ EFTEM                    | 2 nm          | 0.005-0.05 µm | Rad. Damage | + very high spatial resolution  
- require ultrathin sections  
- only some elements readily accessible (e.g., P, Fe)  
- co-registration can be difficult (EFTEM), slow (EELS) |
| Proton Micropr. (PIXE)          | ~1um          | ~50um         | Rad. damage Flux limit | + simultaneously detect >20 elements  
+ high sensitivity  
- slow  - radiation damage |

Elemental images of the same air-dried cells from several Sb-treated *Leishmania* amastigotes. Sb is much clearer visible in the x-ray microscope due to its greater sensitivity. Scan width: 10µm.
**APPROACHES TOWARDS SCANNING**

**Step scans:**
- Move to measurement point, settle, start detectors, read out detectors, go to next point.
- Typical overhead ~100 ms/pixel, 1s/line
- Beam utilization ~80%
- Appropriate for long dwell times

**Fly scans:**
- Move sample continuously through focus, reading out detectors ‘continuously’, synchronization via hardware triggers.
- Typical overhead none/pixel, 1s/line
- Beam utilization ~99%
- Permits tweaking spatial resolution vs sensitivity AFTER data acquisition, to optimize results

After David Vine
Fly scanning has been a game changers, uniquely enabled by detector developments:

- enable both high resolution and large field of view
- allow trading spatial resolution against signal to noise ‘after’ data acquisition
- Essential for X-ray fluorescence tomography with full spectral fidelity

YES, IT IS A GAME-CHANGER FOR NANOIMAGING!

Scan of mouse brain section, M Ralle, OHSU

780x400um, 3900x2000 pixel, 200 nm, 10 ms -> 20h
YES, IT IS A GAME-CHANGER FOR NANOIMAGING!

Highest resolution \(\rightarrow\) ‘Needle’
Large field of view \(\rightarrow\) ‘Haystack’

- Finding the ‘needle’ in the ‘haystack’ requires both capabilities
- APS-U enables full, contextual imaging with nanometer resolution

Just one example, applies for stitching nano-CT, … -> leads to big data opportunities
TOMOGRAPHY TO VISUALISE 3D STRUCTURE

- 3D resolution: $\delta=D\alpha$  D specimen size, $\alpha$ tilt angle interval (Crowther et al. 1970)
  - For 10um thick sample, 20 nm desired resolution, need 1600 projections
  - Need automation, must use dose fractionation
  - Use diff. phase contrast for alignment, or fiducials

- Dose fractionation [Hegerl and Hoppe, Z. Natur. 31, 1717 (1976)] provides a way to do fluorescence tomography at higher speed and with lower dose: divide the signal needed for a 2D view among all the 3D projections!

- Differential phase contrast allows you to align low-dose fluorescence projections with ~30x better precision.

New tomography setup at 2-ID-E
Sophie-Charlotte Gleber, et al

Y.P. Hong et al, J Synchrotron Rad (2014). 21, 229-234
LOOKING AT TRACE METALS IN ZEBRAFISH DEVELOPMENT

- XRF tomography becoming routine. Data acquisition fairly automated.
- Field of view ~800x1500μm, 400x750 pixels, 60 projections, dwell:10 ms/pixel. Total data acquisition time: 3-4 days!
- Here resolution limited only by available flux (scan time).

Zebra-fish: metalloprotein cofactor metal distributions correlated with characteristic anatomical features of embryonic development

_D. Bourassa et al, Metallomics, accepted_
DATA ANALYSIS METHODS CAN MAKE A HUGE DIFFERENCE: FILTERED BACKPROJECTION VS ITERATIVE RECONSTRUCTION

**Filtered Backprojection**

**Iterative Reconstruction (MLEM)**

### Intensity Profile

- **Zn** [ng/cm²]
- **Fe** [ng/cm²]
- **Cu** [ng/cm²]
COMBINE LENSLESS IMAGING WITH SCANNING MICROSCOPY: PTYCHOGRAPHY

- Scanning microscopy typically only utilises red area
- Additional information is then 'integrated over' and lost

D. J. Vine, *et al* *Opt. Express* (2012);

See presentation tomorrow, Ross Harder

Deng et al, submitted
RADIATION DAMAGE:

- Exciting optics developments: <10 nm spatial resolution seems achievable, but what about radiation damage?
- In particular with focused x-ray and sensitive samples, radiation damage can be an issue that needs to be taken into account.

Example for radiation damage in a SOI structure, Polvino et al, APPLIED PHYSICS LETTERS 92, 224105 2008

With 200x higher brightness, 10x10 better focusing, can have 10,000 higher flux densities. Fast scanning becomes an absolute must:
ms -> us becomes requirement and opportunity.
APPLICATIONS
Metals can be contaminants that can severely impact device performance
- in multi crystalline Solar cell materials (eg, Bertoni et al., Energy Environ. Sci., 2011)

Metals play a significant role in the semiconductor industry (dopants, structures) (w. BAE systems)

Metals are often the active component in catalysts their behaviour can improve design choices for materials.
- ageing catalysts in the chemical industry

Metals can be used as tracers, e.g., in Cultural Heritage (eg, Picasso paint: Casadio & Rose, Appl. Phys. A (2013))

Facilitate R&D of construction materials
- Fastener corrosion and fungal decay in wood Diffusion of ions through wood as a function of relative humidity (Zelinka et al, Holzforschung, in review)
Multicrystalline solar cells have significant potential for inexpensive energy harvesting

Small quantities of inhomogeneously distributed precipitates, and contaminants affect overall system performance

High spatial resolution (and sensitivity) to detect smallest quantities of metal contaminants

High efficiency to survey large sample areas

Working distance to support in situ environments (heating/cooling, gases, …)


Trace elements (metals) are fundamental, intrinsic components of biological systems. Estimated: 1/3 of all known proteins contain metal cofactors as integral, catalytic components, often with regulatory functions, e.g.,
- Zn in Zinc finger proteins: transcription factors
- Fe in Haemoglobin; and necessary in Chlorophyll synthesis

Metals are linked to diseases
- Endogenous dysregulation, e.g., Alzheimer’s, ALS, Wilson disease (Cu accumulation)
- Exogenous uptake, e.g., Pb, As, Hg (or lack thereof: e.g., Se deficiency)
- Bio-remediation

Metals in therapeutic drugs and diagnostic agents
- Cis-platin in chemotherapy
- Gd in Magnetic resonance imaging (MRI)
- Novel bio-inorganic nanoparticles, in particular Nanomedicine: multifunctional nanovectors ideally combining targeting, therapy (e.g., Pt, TiO2) and diagnosis (e.g., Gd)

**Recent reviews of XFM applications:**
**Imaging:** T. Paunesku *et al.*, J Cell Biochem 99(6), 2006
**Spectroscopy:** C. Fahrni, Curr Opin Chem Biol 11(2), 2007

• Phytoplankton converts dissolved carbon into biomass.
• small fraction (~1%?) is exported from the surface waters into deep ocean (net loss for hundreds of years
• Key limiting factors: micronutrients (Fe), but also Silicon …
SURPRISING ROLE FOR PICOYANOBACTERIA

- Picocyanobacteria make up majority of organisms in ocean. 50+% (!) of O₂ generated by ocean.
- *Synechococcus* can show silicon ratios similar to diatoms
- significant, previously not know Si sink
- mechanism of Si accumulation is not yet known, in part because we cannot resolve the form and precise location of the Si associated with the cell.
WHAT MAKES A GOOD EGG AND HEALTHY EMBRYO?

- Zinc plays an unexpected role in oocyte maturation: Zn content is an order of magnitude higher in eggs than Fe and Cu.
- Zn level increases by 50% during maturation. Zn depletion arrests the maturation process.
- One of the first studies to implicate zinc as a possible signaling molecule in a biological system, not just a protein cofactor.
- Bulk analysis cannot be applied to rare cells such as mammalian oocytes.

In the XFM image a mature (MII) egg retains Zn while polar body is Zn low. This asymmetry is required for correct oocyte maturation. Scale bar 20 um.

APPLICATION: CHROMIUM CARCINOGENESIS

- Cr(III): common dietary supplement, supposed essential role in insulin action. Often claimed to have value as a weight loss or muscle building agent. Dietary supplement: not regulated by FDA
  - US $100 million / year industry

- Cr(VI): has been designated as an established human carcinogen by the IARC.
  - Environmental exposure to Cr(VI), resulting from the poor disposal practices of Cr(VI) into unlined ponds

- Cr(V): Lab studies suggest Cr(VI) exerts its genotoxic effects via reduction into the reactive Cr(V) intermediate – more genotoxic than Cr(VI)?

P. Lay et al, Univ Sydney

Erin Brockovich
IN VITRO MODELLING OF CHROMIUM TREATMENT – 3T3-L1 ADIPOCYTES.

- 3T3-L1 adipocytes (fat cells) easily cultured.

- Do adipocytes take up Cr(III)?
  - If so, does Cr(III) change its oxidation state?
What is the intracellular distribution of Cr in adipocytes treated with Cr?

- Treated with 100 μM trinuclear Cr(III) propionate, 20 hours.
- Cells grown and fixed on silicon nitride windows.
~100 times more Cr than in control
What sort of chromium is present? - Cr K-edge XANES

Typical Cr(III) (propionato complex)

Typical Cr(VI) (glutathione complex)

Typical Cr(V) (2-ethyl-2-hydroxybutanoato complex)

Cr and P Overlay

Hotspot 1
Hotspot 2
Hotspot 3

Cr hotspot in a single adipocyte

Up to 55% Cr(V)
Up to 36% Cr(VI)
(the carcinogenic form)

L. Wu et al, submitted
BIG DATA

▪ Today
  – Manually moving, analyzing data.
  – Ad hoc tools that do not scale to the next generation of instruments
  – algorithms can be “dangerous” if not used carefully

▪ Tomorrow
  – Extensive toolset of scalable algorithms (e.g., machine learning, statistical)
  – Scientific knowledge integrated with analysis, visualization and simulation
  – Automatic Integration of data from multiple sources, cataloguing and transfer
  – Efficient data reduction strategies

Top: X-ray fluorescence maps of different cells. Middle: software automatically identifies and classifies 3 different cell types, enabling further analysis. Comparison of the resulting average elemental content per individual cell.

S. Wang, et al, J Synchrotron Radiation, accepted
HOW CAN YOU MAKE USE OF THESE RESOURCES?

- Beamtime is available on most beamlines at most synchrotrons to outside users through a competitive proposal process.
- Proposal submission deadlines typically 2 or 3 times a year.
- Typically 80% of ‘beamtime’ on any beamline is distributed.

Some types of proposal:
- General User Proposals
  - Open to anyone, just have to write a good proposal. Proposals get reviewed by committee, assigned based on scores. Proposals that don’t quite make the score, ‘age’ so that they have a better chance next time.
  - Users typically come for experiments 3-4 days (9-12 shifts), carry out experiments with help of beamline scientist.
  - No cost for beamtime, the expectation is that results will be published.
- Proprietary Experiments
  - Are also possible. Proposals are rated differently, less detail needed. Results generally not published, but cost recovery of beamtime is required (APS, $373/h at the moment).

Most importantly: try to identify possible beamlines in advance, and contact the beamline scientist well before writing the proposal.

A general resource relating to synchrotron sources world wide:
http://www.lightsources.org/
SUMMARY

- Full field imaging, often in combination with tomography
  - Parallel Beam Imaging (PBI): Phase and absorption, 1 µm spatial resolution, and temporal resolution kHz (3D) to 6.5 MHz, 100 ps (2D). No x-ray optics
  - Nanoimaging: spatial resolution limited by x-ray optics (typically zone plates, CRLs for higher energies), down to 60-20 nm. Time resolution ~Hz

- Scanning probe imaging
  - Resolution limited by x-ray optics (KBs: typically ~microns, can go down to 100 nm, ZPs typically 200 – 20 nm)
  - Typically slow (can only use coherent part of beam for high resolution, need to scan the sample)
  - Access to variety of contrast modes (absorption, phase, fluorescence, diffraction)
    - XRF for trace element detection

- Both can be combined with spectroscopy, but different sensitivities.
APS MBA UPGRADE: A BRIGHT FUTURE

- Brightness increases of 100x and more compared to what we have today
- Micro/nanoprobes directly brightness driven
  ⇒ possible to get nearly 100% of APS flux into a 0.3x0.25 um spot !!!
  ⇒ Upgrade: push for highest direct resolution <=10 nm and augment with CDI/Ptychography

This upgrade will revolutionize scanning microscopies and lensless imaging techniques !!!
Thanks!