

Soft X-ray spectromicroscopy & its analysis with **aXis2000**

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GOALS

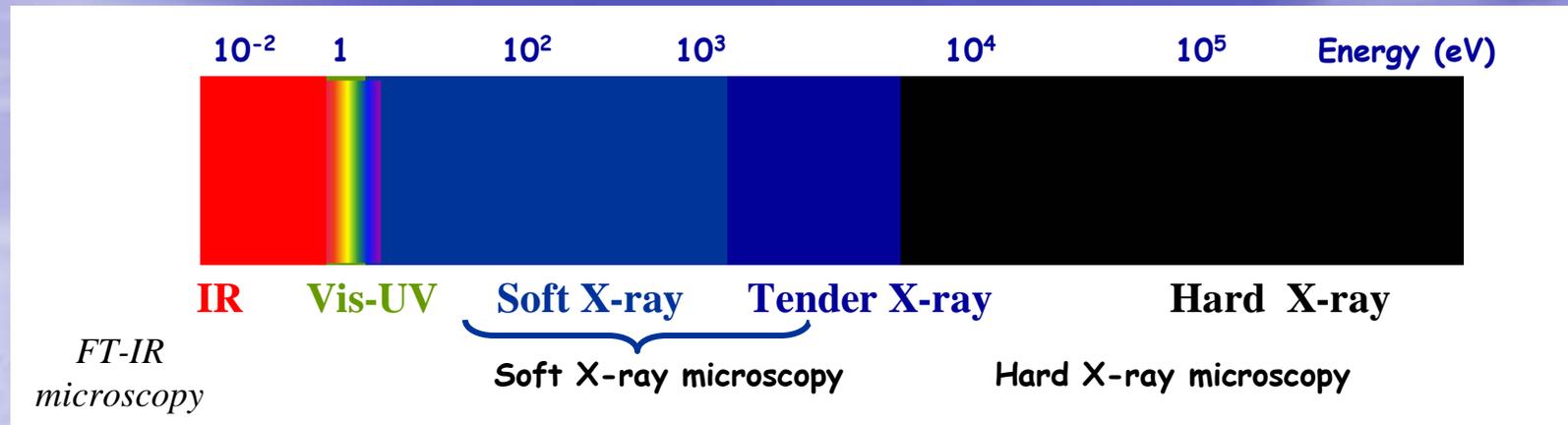
1. familiarization with Synchrotron spectromicroscopy techniques
2. demonstration of data analysis with aXis2000
3. discussion of potential for your application



Outline

- Modes & information from X-ray spectromicroscopy
- Data analysis - example from STXM
chemical mapping of protein on a phase segregated polymer
- structure of aXis2000 widget
- how can you access the power of aXis2000 ?

Basic Principles



- * Use **X-ray absorption contrast** for
 - * chemically sensitive imaging - "X-ray imaging" **NEXAFS microscopy**
 - * spatially resolved chemical analysis - "Micro-probe"
- * Use **penetrating power** of X-rays to study
 - * wet soft matter (biology, polymers, nano-materials) **Soft X-rays ("water window")**
 - * fluorescence microprobe
 - * non-destructive testing; tomography **Hard X-rays**

References:

- J. Kirz, C. Jacobsen and M. Howells, *Quarterly Review of Biophysics*, **33** (1995) 33
- H. Ade, in *Experimental Methods in the Physical Sciences*, Vol. 32, pp. 225, J.A.R. Samson and D.L. Ederer Ed., Academic Press, 1998
- A.P. Hitchcock, *American Laboratory*, **33** (2001) 30; *J. El. spec.* 144 (2005) 259.
- H. Ade and S.G. Urquhart, in "Chemical Applications of Synchrotron Radiation" T. K. Sham, ed. (World Scientific Publishing, 2002)

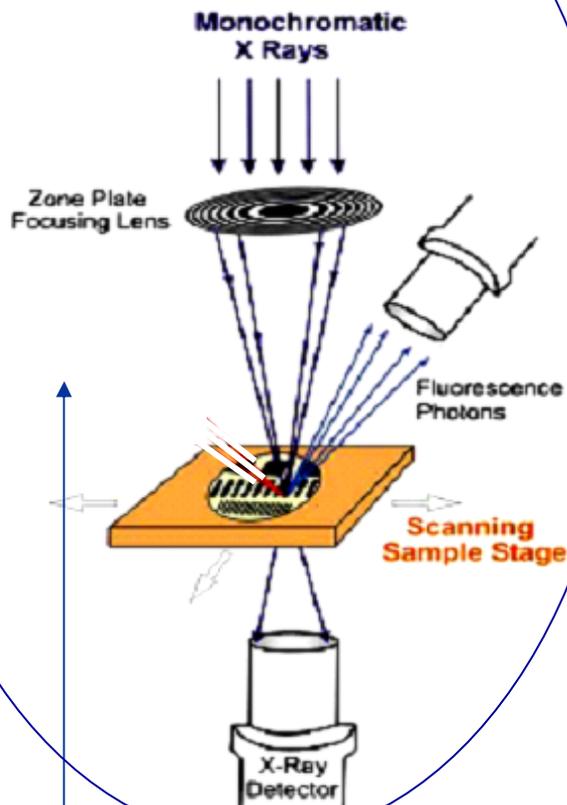
Overview of techniques

STXM

ALS BL 5.3.2; BL11.0, NSLS X1A, BESSY

TXM now at ALS BL 6.1.2 XM-1, BESSY

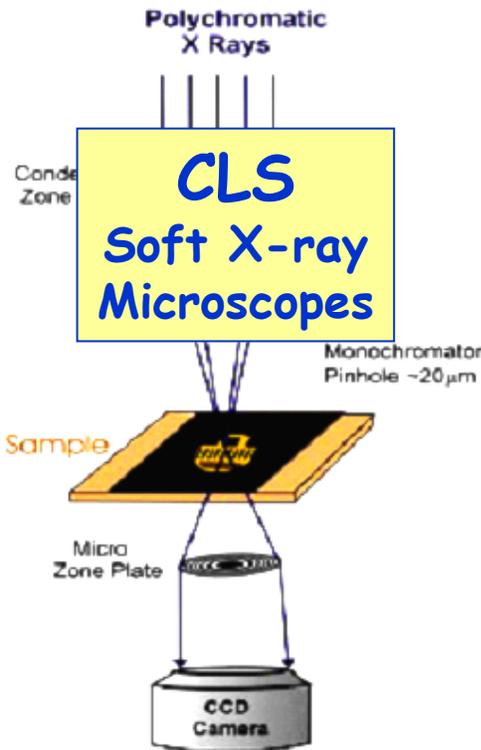
Scanning Transmission X-ray
Microscopy - **STXM**



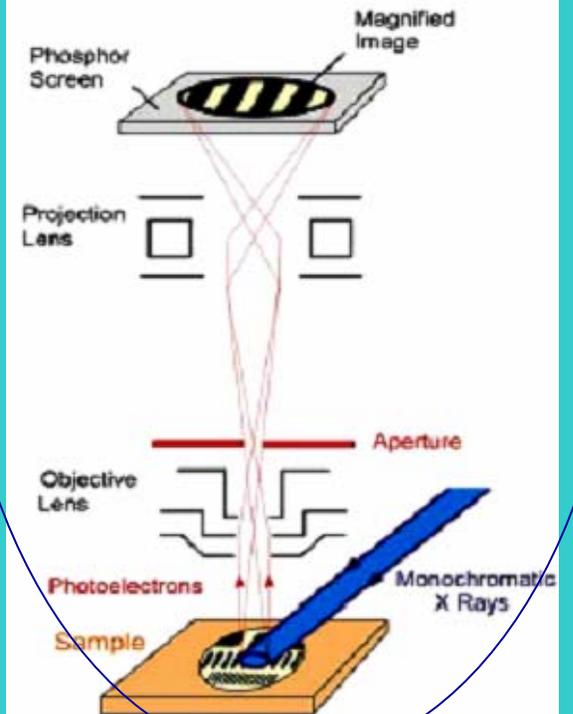
Scanning Photoelectron
Microscopy - **SPEM**

SPEM now at ALS, Trieste, Taiwan, Korea..

Transmission X-ray
Microscopy **TXM**



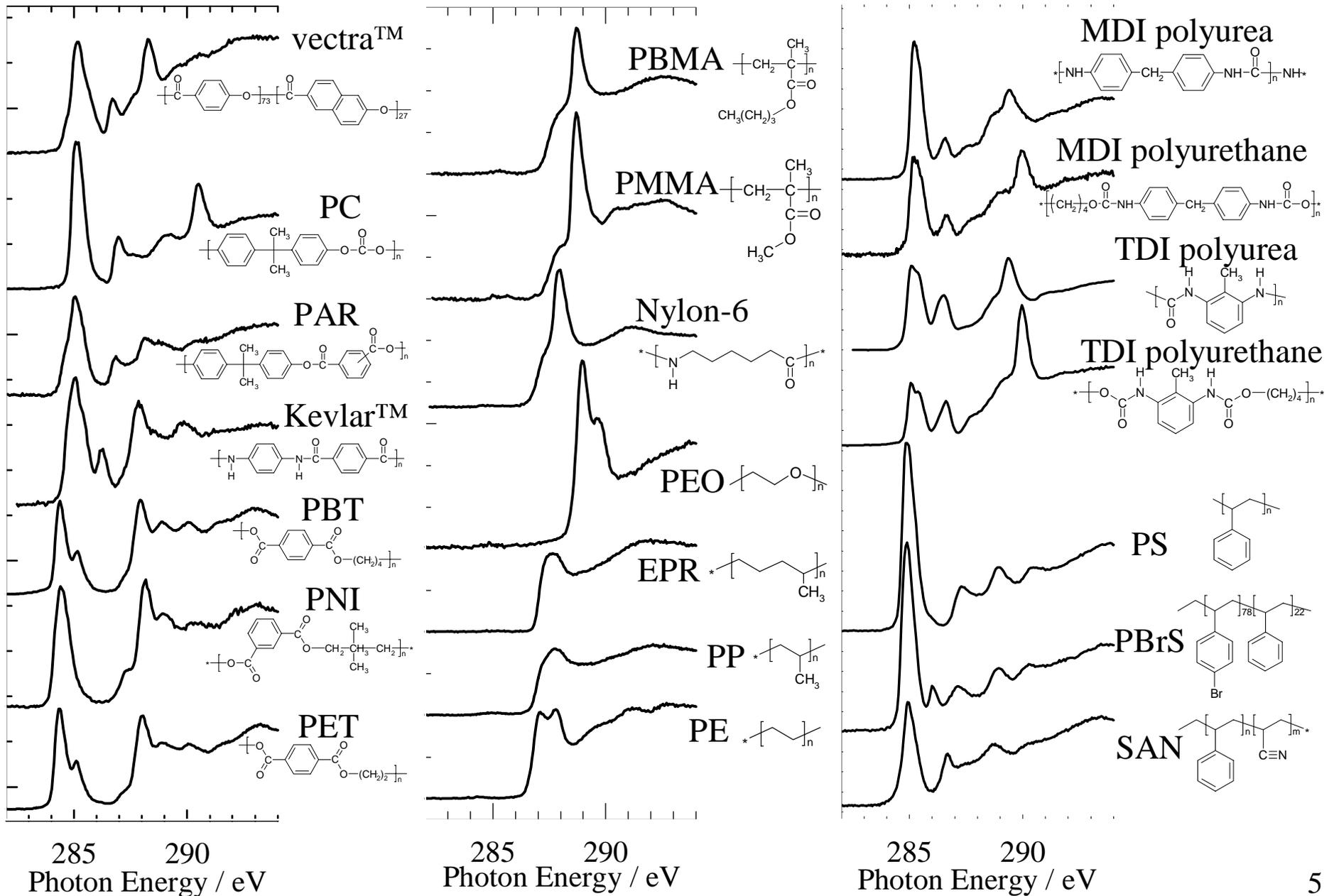
X-ray PhotoEmission Electron
Microscopy - **X-PEEM**



X-PEEM now at ALS BL 7.3.1, SLS, Trieste, SRC

Sensitivity of Polymer NEXAFS Spectroscopy

Ade , Urquhart (1997-99)
(nsls X1A stxm)



Electron yield-based soft X-ray microscopies

Primary XAS process produces - **photoelectrons**

Core hole decay produces

- Auger & **secondary electrons**
- photons
- ions
- luminescence photons

All are being developed as detection channels for analytical X-ray microscopy

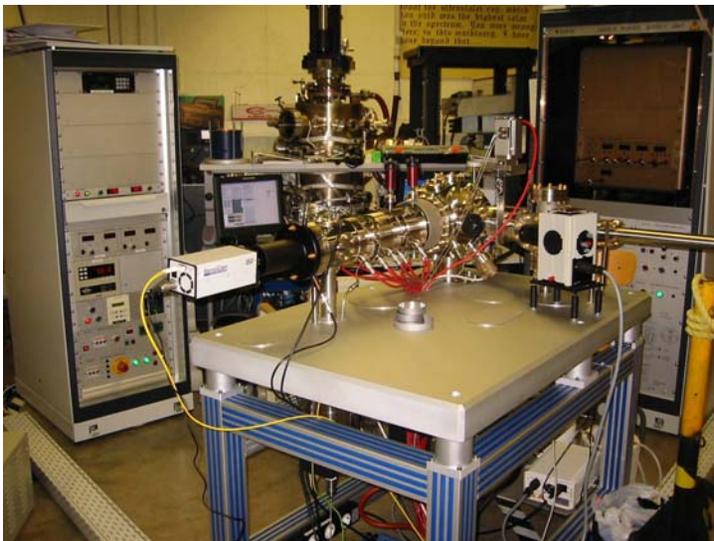
SPEM – Scanning PhotoElectron Microscopy

PEEM - Photo-Emission Electron Microscopy

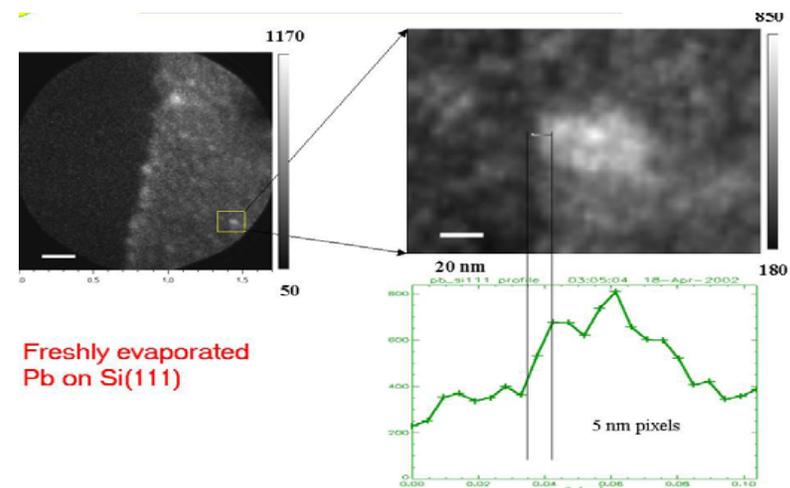
Elettra, Pohang Light Source
ALS BL 7.0.1

Commercially available

* most SR facilities ; BESSY -**SMART**
ALS BL 7.3.1 [PEEM2]



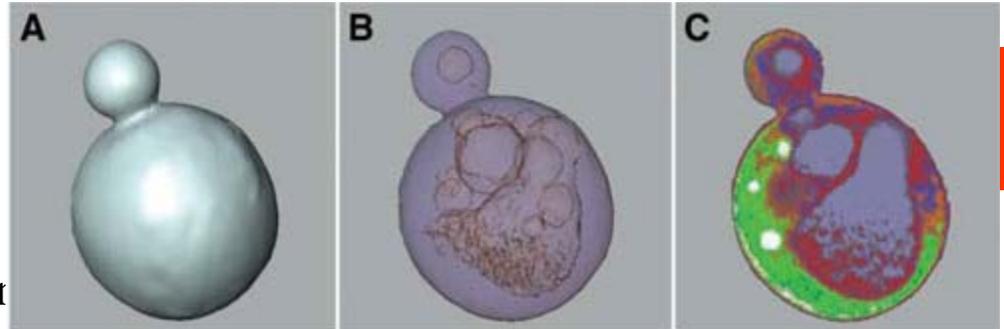
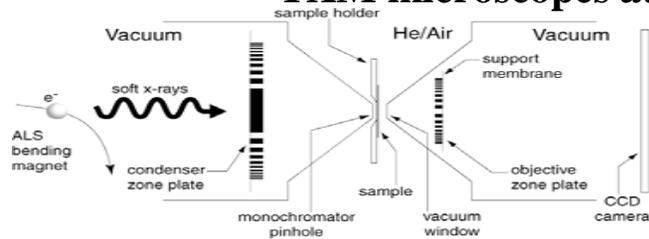
CaPeRS – Canada's Elmitec PEEM
now at CLS



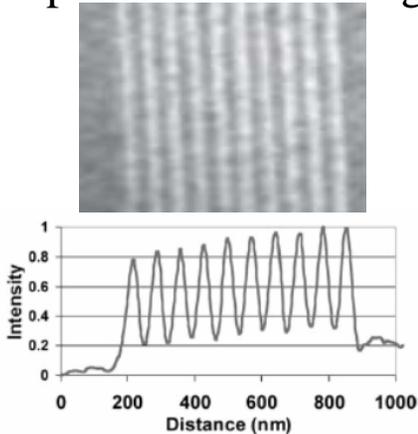
But only ~50 nm with synchrotron light due to e- distribution & chromatic aberration

TXM – biological applications

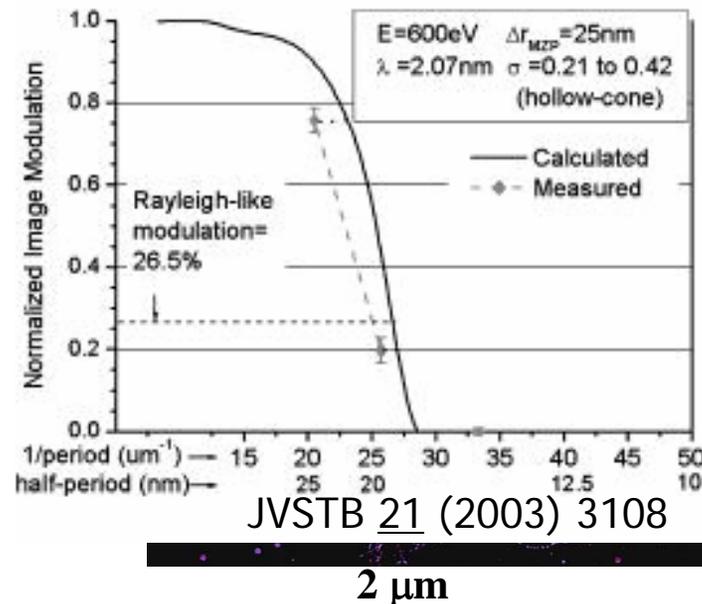
- first demonstrated Gunter Schmäl (Gottenberg) – BESSY (~1986)
- TXM microscopes at ALS (XM-1), Bessy, Aarhus, Elettra



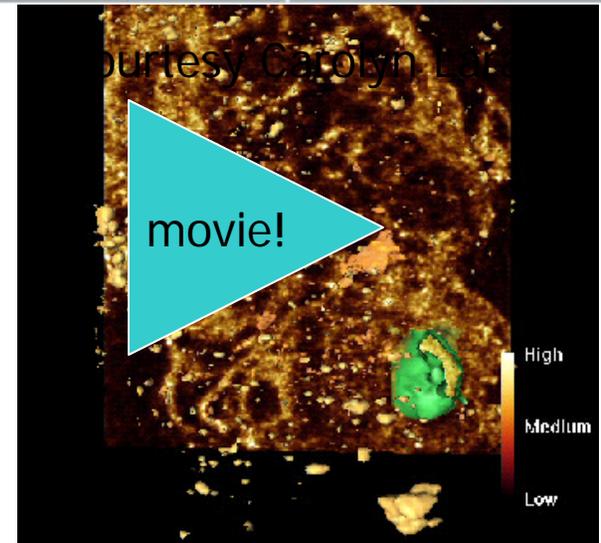
- wet cells: biomaterials & biological imaging
- cryo imaging
- tomography
- XMCD imaging
- phase contrast imaging



Highest spatial resolution
22 nm diffraction limit achieved
 test sample: 15 nm lines 4:1 spacing



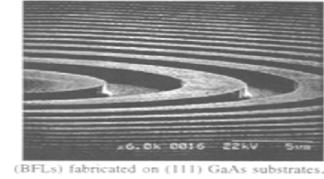
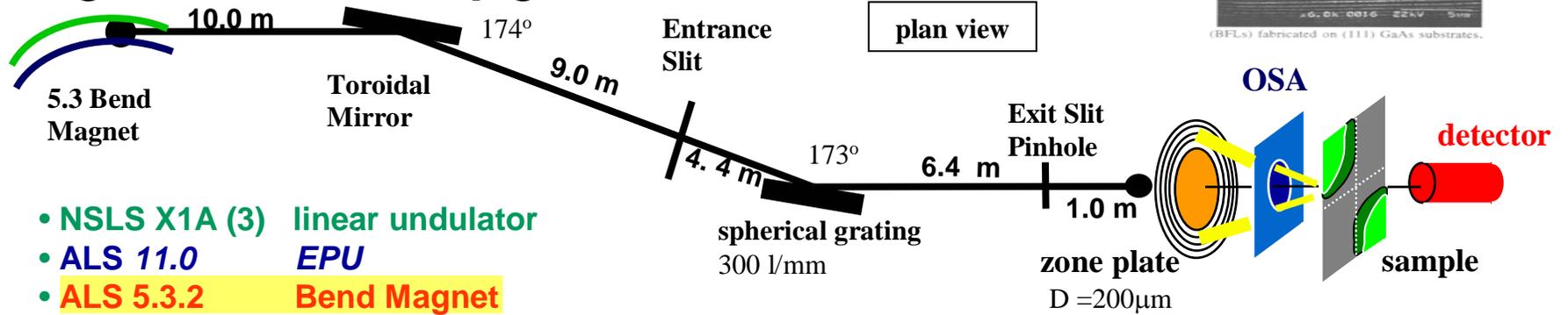
monoclonal antibody / Au-Ag-labeling of
 cytoskeleton proteins
Larabell (UC Davis) ALS 1999



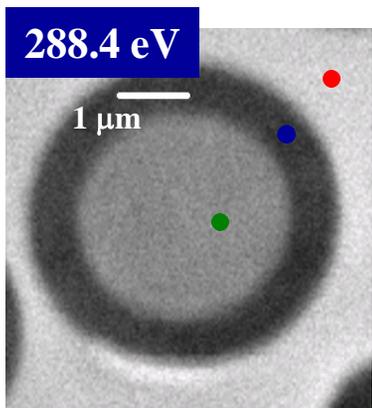
*Gerd Schneider, Carolyn Larabell
 ALS XM-1 2003*

Angle-scan tomography
 (cf. Attwood)

Scanning Transmission X-ray Microscopy (STXM)



Images
transmitted $I(x,y)$



Spectra $\ln(I/I_0)$
{point, line, image}

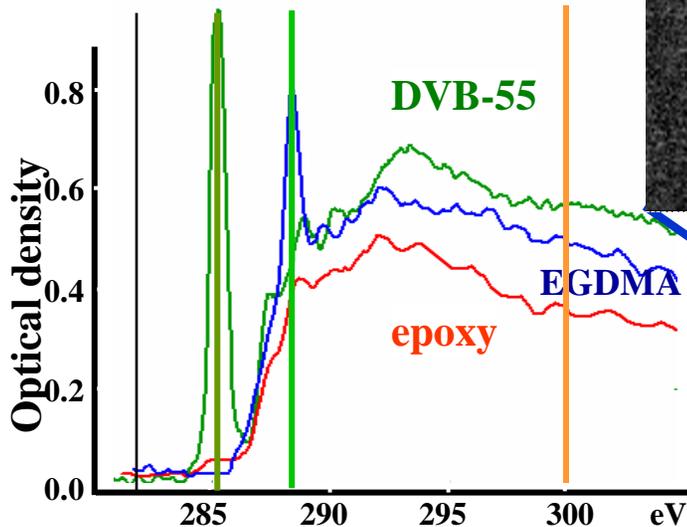
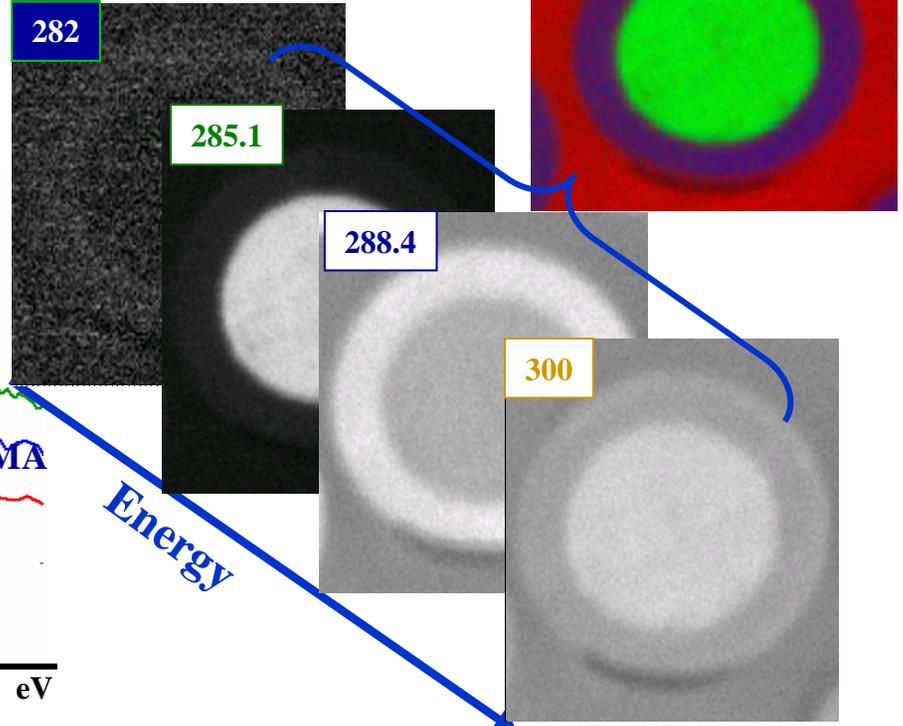


Image sequences (OD format)



STXM_control - ALS (2), CLS, SLS

Polymer STXM User Interface - 18 May 2002

File Setup Window Help

20526002.hdr

Image Scan 300x300 pts 288,198 eV 1 ms Dwell

counter0

Sample X 279.427 Sample Y 260.794 Abs Data 61

20526005.hdr

counter0

Energy counter0

Sample To Cursor

Sample To Cursor Coarse

Sample Z Offset (µm)

-818.24

Starting NEXAFS Point Scan
Saving scan as d:\data\20526\20526004.hdr
NEXAFS Point Scan complete.
Starting NEXAFS Point Scan
Saving scan as d:\data\20526\20526005.hdr
NEXAFS Point Scan complete.

PMT Divider
div by 10

Shutter
Auto

Beamline Control

Current Destination

Energy (eV) 559.986 559.986 Go S

Slits

Entrance (µm) 60 60 Go S

Exit Dispers (µm) 35 35 Go S

Exit NDisp(µm) 20 20 Go S

Shutter Controls
Automatic

Focus
Auto Setup

Current Scan Status

NEXAFS Point Scan Image 1 of -

Estimated Time: 75s Region 1 of 1

Elapsed Time: 120s Line 300 of -

Next Scan #: 6 Point 379 of 379

Microscope Control

Motor

Coarse Z 1218.000 1218.000 Go D Stop S All OFF

Coarse Y 4999.950 4999.950 Go D Stop S In Off

Interferometer
Reset

Sample OSA IN Zone Plate IN Sample IN Stop All

Mover OSA OUT Zone Plate OUT Sample OUT

Scan Controls

START Sample Scan

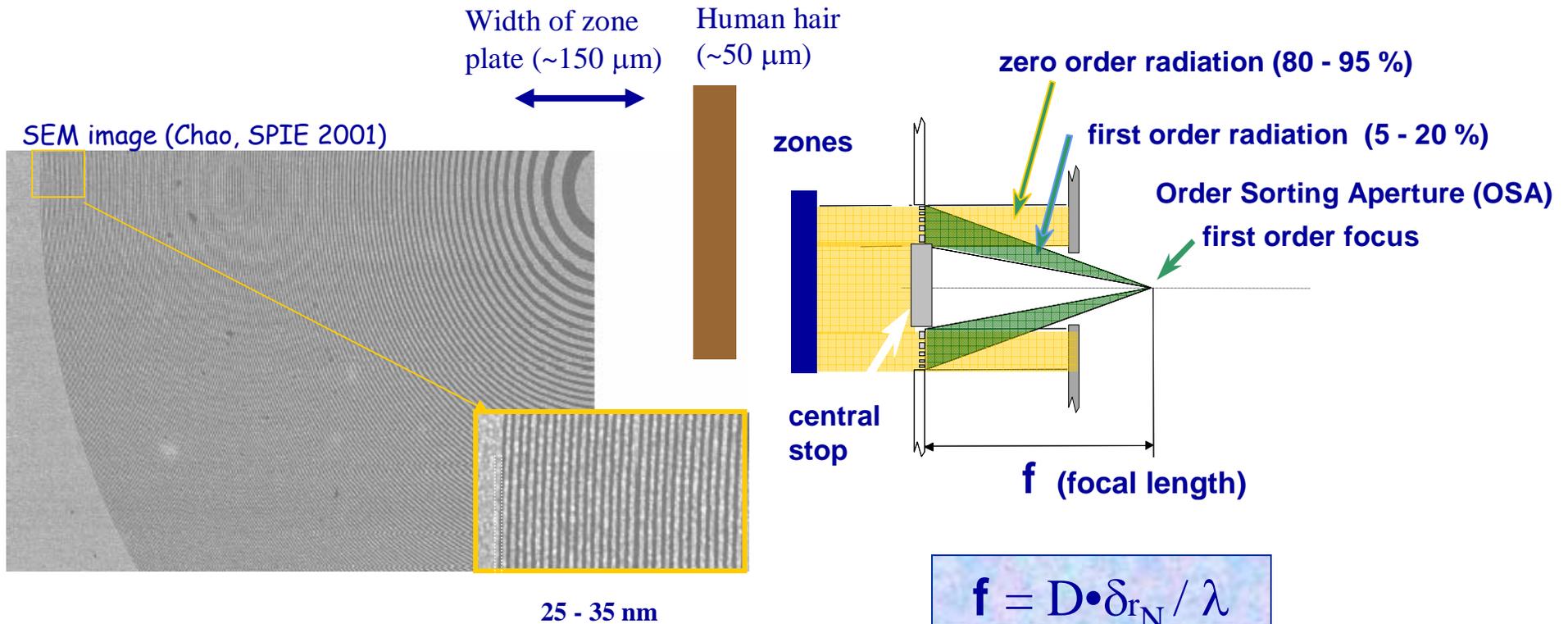
Abort Pause

Microscope Status

| Motor | Updates On |
|--------------|------------|
| Sample X | 279.440 |
| Sample Y | 4999.995 |
| Sample Z | 5.000 |
| Coarse X | 279.400 |
| Coarse Y | 4999.950 |
| Coarse Z | 1218.000 |
| Zone Plate Z | -2053.2 |
| OSA X | 0.000 |
| OSA Y | 0.000 |
| Detector X | 0.0 |
| Detector Y | 0.000 |
| Detector Z | -700.070 |

Fresnel zone plates: diffractive focusing

A.G. Michette, Optical Systems for soft X-rays, Plenum Press, 1986



$$f = D \cdot \delta_{r_N} / \lambda$$

0.9 -1.2 mm in C 1s region

Typical Values (for current ZP in stxm532)

λ (photon wavelength) 1 to 6 nanometers ($\sim 1240/E$)

D (ZP diameter) = 155 microns

δ_{r_N} (outer zone width) = 35 nanometers

Number of zones ~ 1000

Central stop diameter = 80 microns

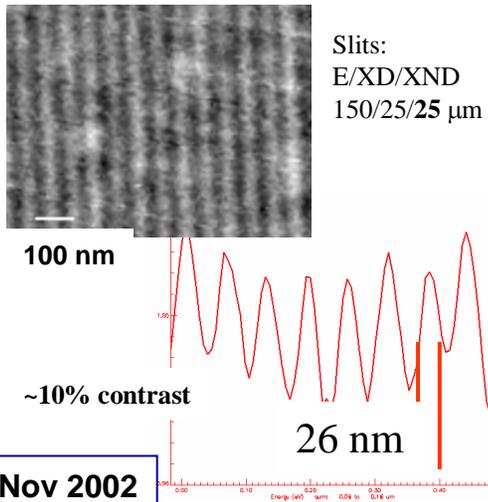
OSA diameter = 55 microns

Spatial resolution
(diffraction limited)

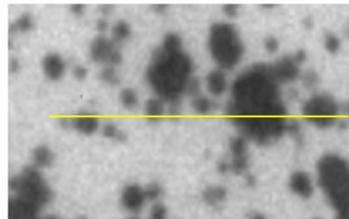
$$\Delta r = 1.22 \cdot \delta_{r_N}$$

5.3.2 STXM Performance

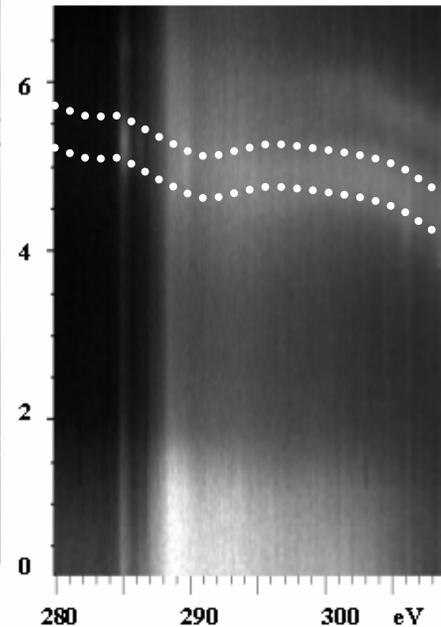
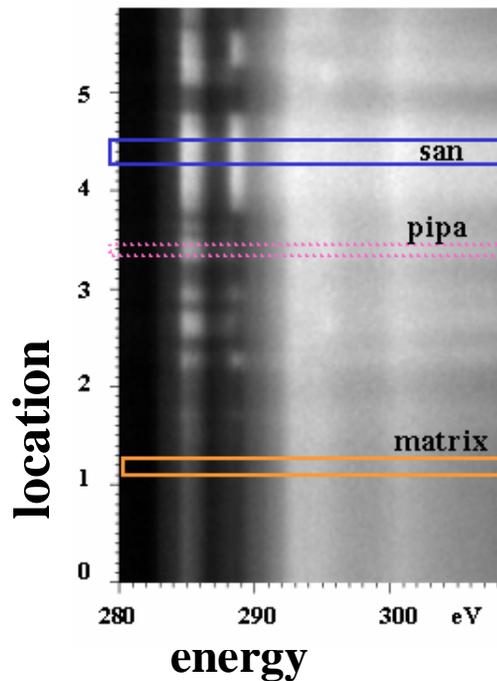
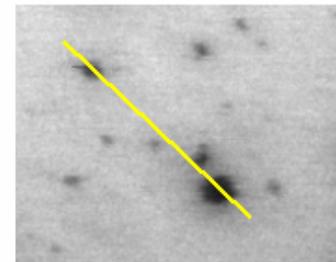
- Diffraction limited spatial resolution (40 nm)
- 50 meV spectral resolution
- 50 nm CHEMICAL resolution via interferometry



ALS BL 5.3.2 (Aug-01)



ALS BL 7.0 (Dec-98)



Advanced Light Source

Tony Warwick
Mike Kirschner
Keith Franck



NC STATE UNIVERSITY

David Kilcoyne
Harald Ade (NCSU)



Tolek Tyliszczak
Adam Hitchcock
Peter Hitchcock



Living.
Improved daily.



STXM-11: state-of-art performance

December 2003 - BREAKTHROUGH in ZP technology !

25 nm diffraction limited zone plates

Recent advances in CXRO zone plate fabrication has resulted in new STXM zone plates with significant improvements in

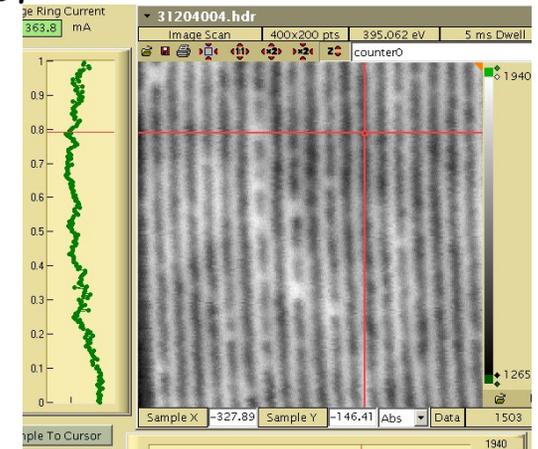
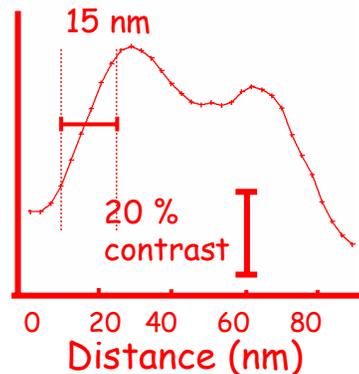
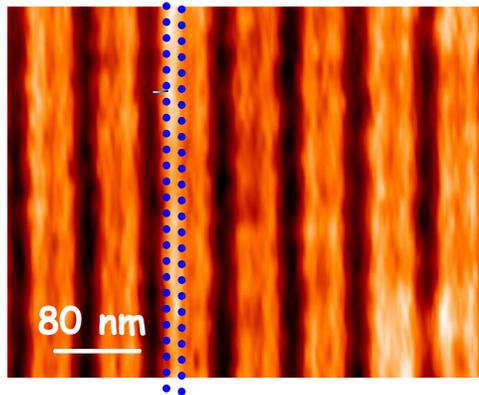
- spatial resolution
 - due to narrower outer most zones (25 nm instead of 35 nm)
- performance at high photon energy
 - due to higher aspect ratio (7:1 instead of 3:1)

The performance of the interferometrically controlled STXM - in particular its thermal and temporal stability, as well as precision of tracking over variable photon energy - has been found sufficient to take advantage of the improved zone plate performance.



25 nm zone plate as test

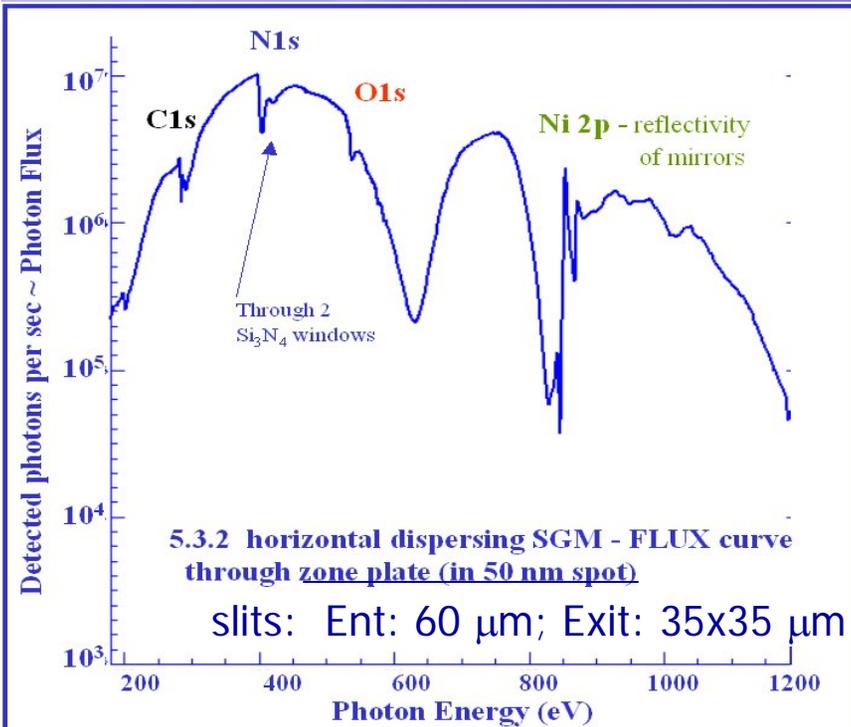
25 nm 1:1 lines as test object



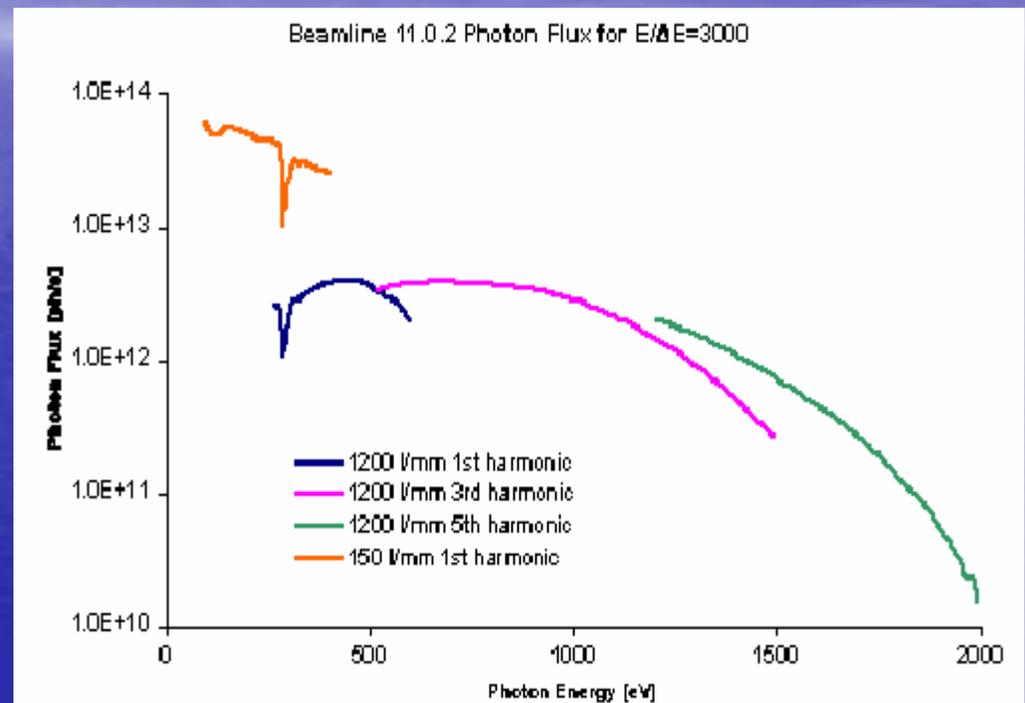
STXM532 - can mount 25 nm ZPs
 - usually 35 nm ZPs (intensity)

ALS STXMs: Energy range, flux

BM STXM 5.3.2



Undulator STXM 11.0.2



with 90/60/60 slits it is easy to get
> 10^8 ph/s in ~60 nm spot on
sample at 390 eV

exit slits are typically 5-30 μm
> 10^9 ph/s ~40 nm spot on
sample at 390 eV

Spectromicroscopy at the CLS

Insertion device: Elliptically Polarized Undulator (EPU)

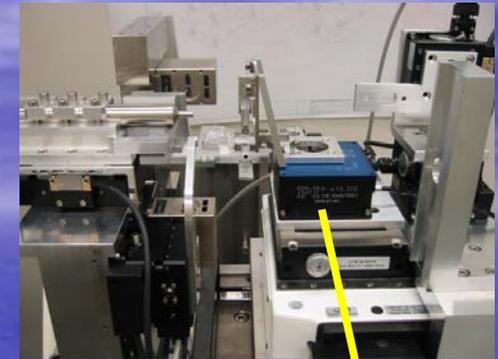
Monochromator: Plane Grating, no entrance slit (modified SX-700)

Energy range: 250 - 1900 eV

Resolving power: 5000

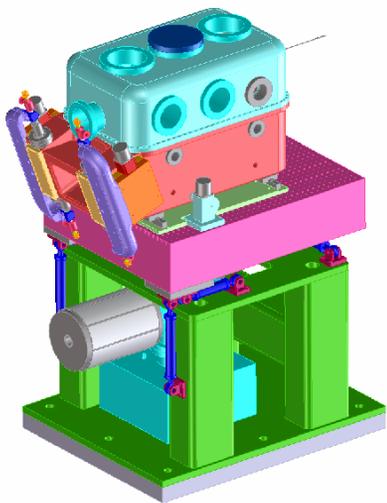
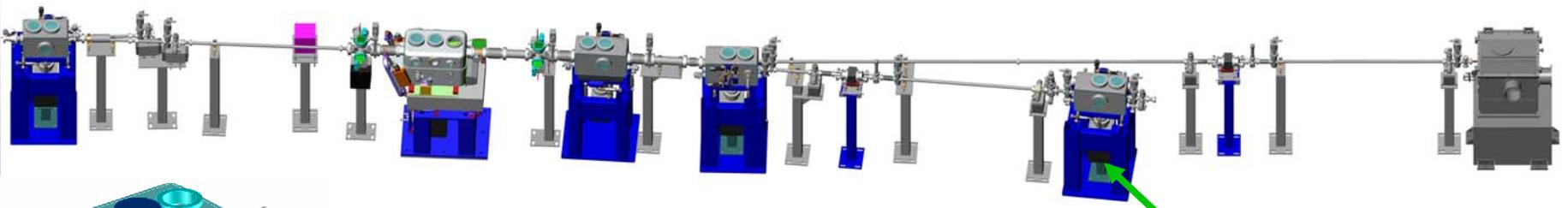
Flux: **PEEM:** 10^{11} - 10^{12} photon/s in 20 micron spot

STXM: 10^8 photon/s in 50 nm spot



STXM: modified 5.3.2 design

ALS assistance: monochromator & mirror holders similar to BL 11.0.1



X-PEEM (Stephen Urquhart)

- operated Apr02-Mar05 at SRC (Madison, WI)
- now at CLS
- to run on SGM summer 2005



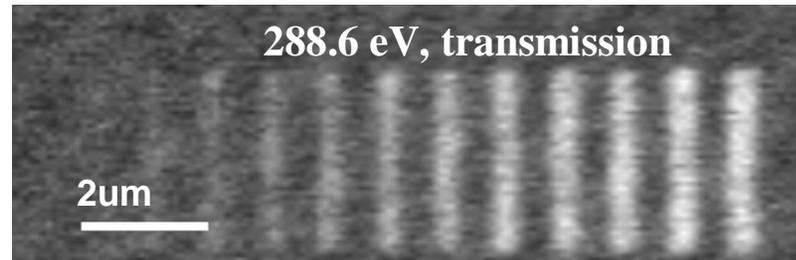
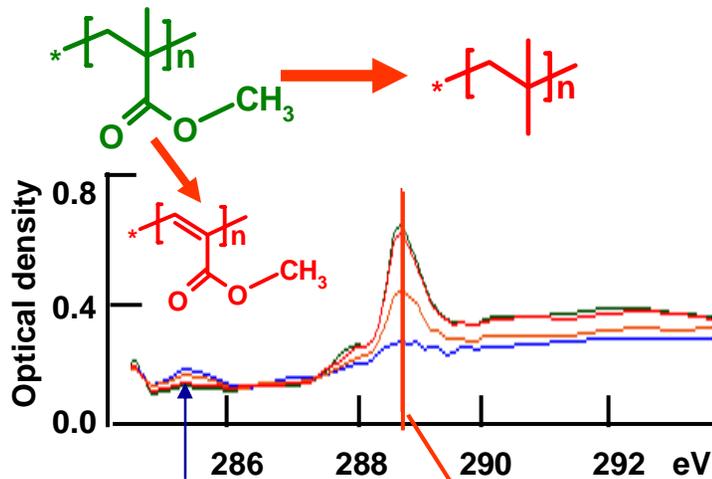
see talk and poster by Kaznatcheev (MSC)

Radiation damage in STXM: PMMA

* 4 μm line pairs; spaced at 1 μm

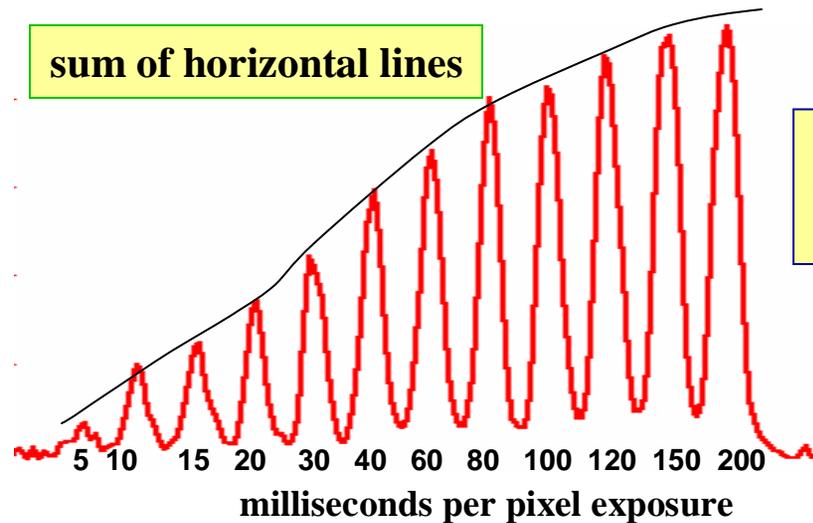
** Exposed in linescan mode at 300 eV;
~2 MHz in detector

PMMA = polymethylmethacrylate



stxm 5.3.2

sum of horizontal lines



Exposure to damage 1/e
~50 ms !!!

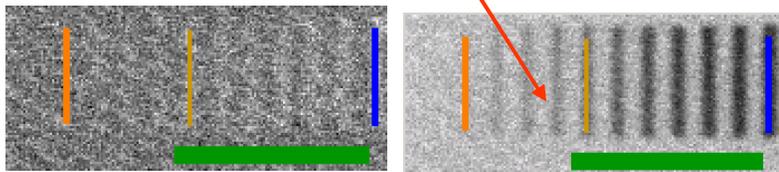
Damage control measures in STXM 5.3.2

a) Hardware

- 1 msec in-vacuum piezo shutter
- Closed between successive scan lines
- fast scanning: 0.2 - 1.0 msec/pixel dwell

b) Acquisition strategy

- Defocused beam (if suitable) – point, line, image modes
- Multi-region acquisition
- Short stacks / SVD



STXM is optimal for quantitative chemical analysis of soft matter

- ✓ **High Spatial Resolution**

Zone plate properties determine resolution. Typically ~30-40 nm

- ✓ **High Spectral Resolution = high chemical resolution**

All instruments achieve ~ natural line width (0.1 eV in C 1s)

- ✓ **Quantitative compositional analysis**

Beer's Law response – Absorbance (OD) proportional to concentration in column / pixel

- ✓ **Adaptable to many environments**

Fully solvated systems – water window

Magnetic fields

Vacuum – surface analysis

- ✓ **Significantly lower radiation damage** than TEM-EELS . . .

Quantitative comparison indicates $10^2 - 10^3$ advantage on basis of information / unit damage

PET – Rightor et al J. Phys. Chem. B 101 (1997) 1950

see poster by Wang (MSC)

. . . and TXM (In STXM, the inefficient Zone Plate optic is BEFORE the sample)

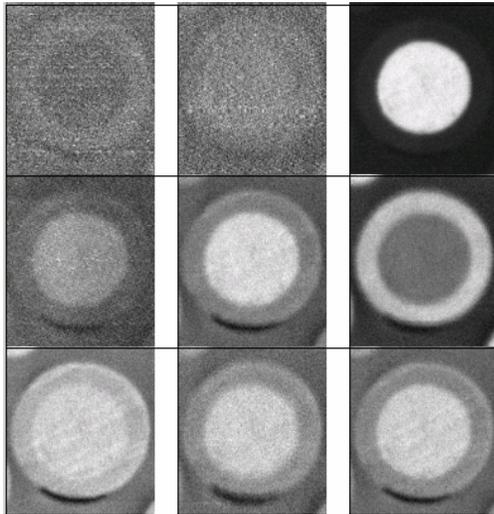
Quantitative Chemical Mapping

From pixel-by-pixel fits to reference spectra

Implemented in *aXis2000*

Core shell particles: (with Stöver)
see *Macromolecules* 34 (2001) 4424

1. Record image sequence (stack)

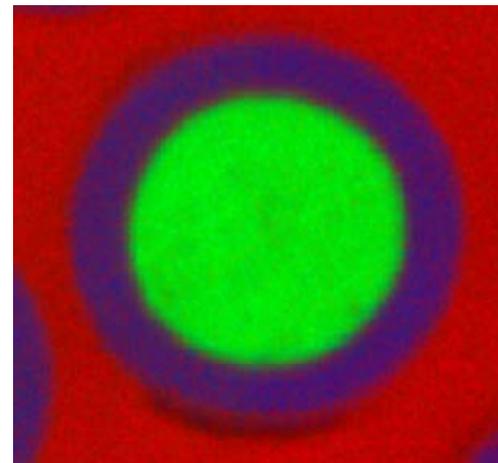


3. Generate component maps

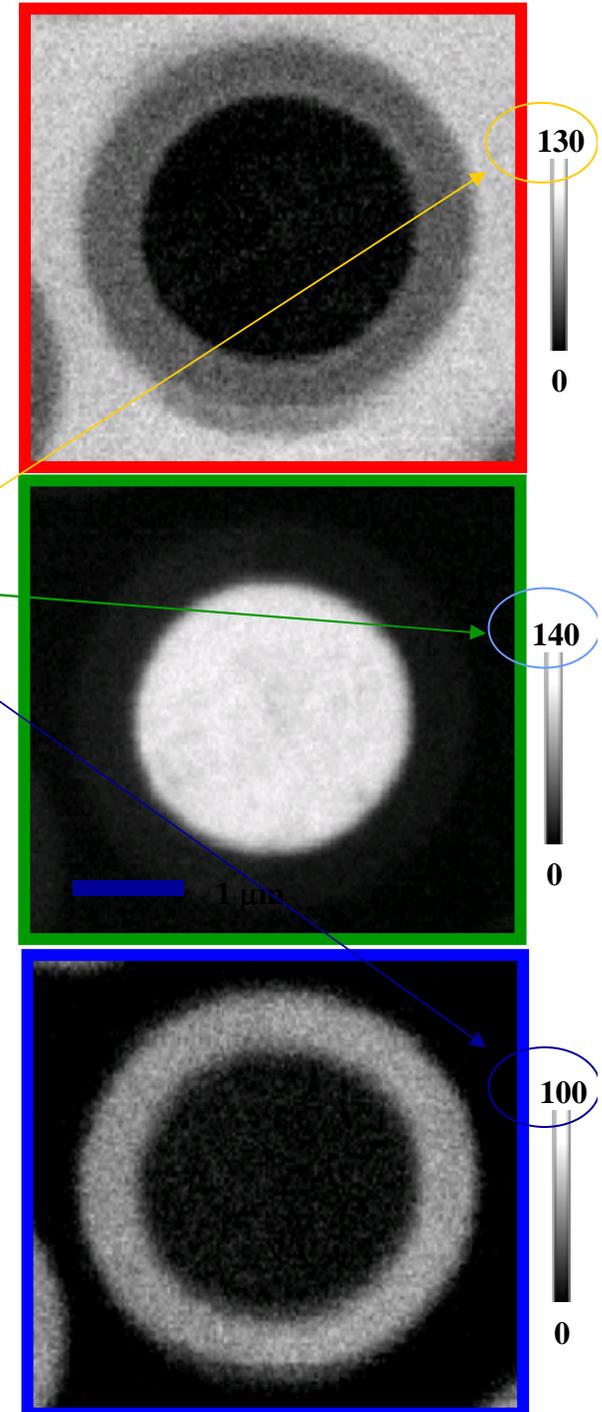
$$OD(j,k) = \sum_i a_i(j,k) * (\text{reference})_i$$

with $a_i(j,k) = \text{THICKNESS (nm)}$ at (jk)

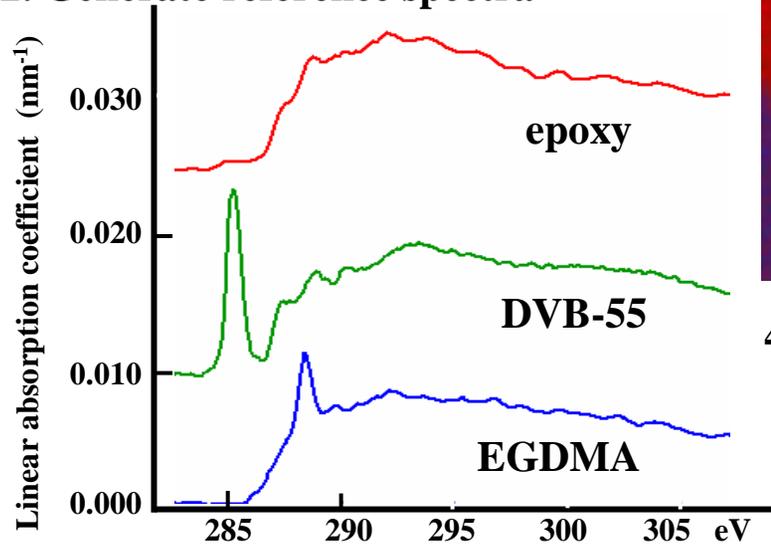
when reference spectra are absolute (nm^{-1})



4. Generate RGB-composite component "image"

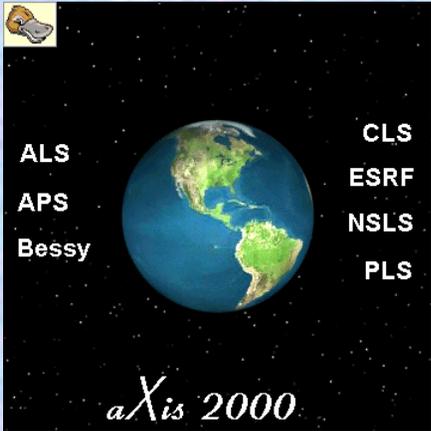


2. Generate reference spectra



Analysis software for soft X-ray spectromicroscopy

aXis2000



(<http://unicon.mcmaster.ca/aXis2000.html>)

IDL VM 6.0
The IDL Virtual Machine™
Distribution Platform for IDL Applications

Upgrade to a development version of IDL and:

- interactively explore your data in the IDL environment
- develop cross-platform applications for distribution
- test custom data analysis algorithms

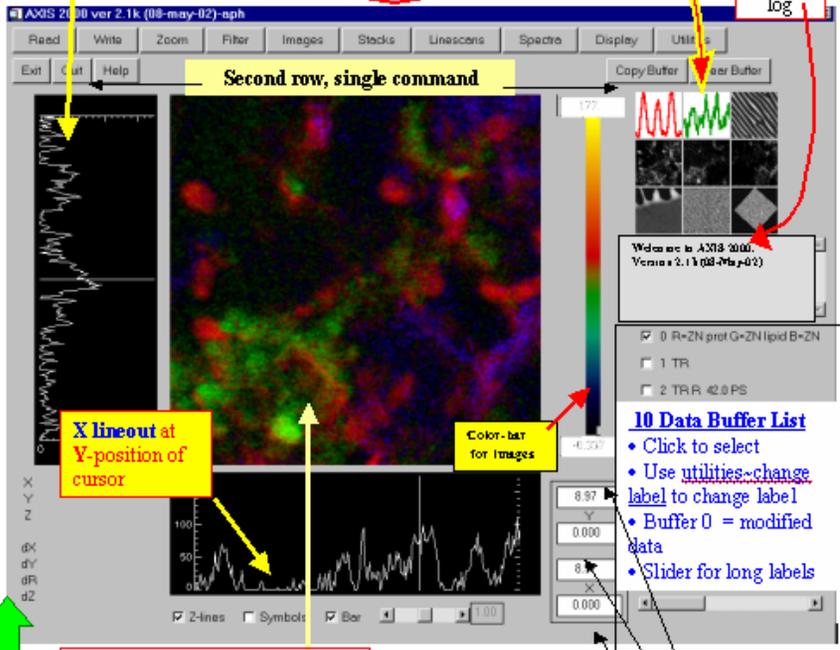
Find out more at www.rsinc.com/IDL

©2003 Research Systems Inc. RSI Research Systems Inc.

aXis2000 is free for non-commercial use

5.3.2 STXM can be viewed and even run remotely (with training & permission) over the net ('Fedex' synchrotron microscopy)

Features of the aXis2000 widget



Y lineout at X-position of cursor

First-row pull-down menus

Thumbnails
• Click to select a buffer

Axis Messages, Hints and log

Second row, single command

X lineout at Y-position of cursor

Color-bar for Images

10 Data Buffer List
• Click to select
• Use utilities-change label to change label
• Buffer 0 = modified data
• Slider for long labels

Lineouts, symbols & scale bar options

Gamma for Images

X, Y, (Z) limits for Images & spectra (display & control)

Cursors
(X, Y, Z) – at cursor
(dX, dY, dZ) – change over line (images) or between cursors (spectra)
dR – distance along line (images only)

Main Image
• Displays currently selected image or selected spectrum (or group of spectra, if Spectra-Overplot used)
• Size of aXis2000 display can be adjusted (0.5 to 2.0) of a nominal size (360x360 pixels in Main Image) by size parameter in axis.ini
Mouse (if Z-lines is selected)
• **First** click – cursor and lineout; arms the line generator
• **Second** click – draws and documents line (image) ;
- reports difference in cursors (spectra)
• **Third** click – clears line and cursor information

BREAK TIME !

