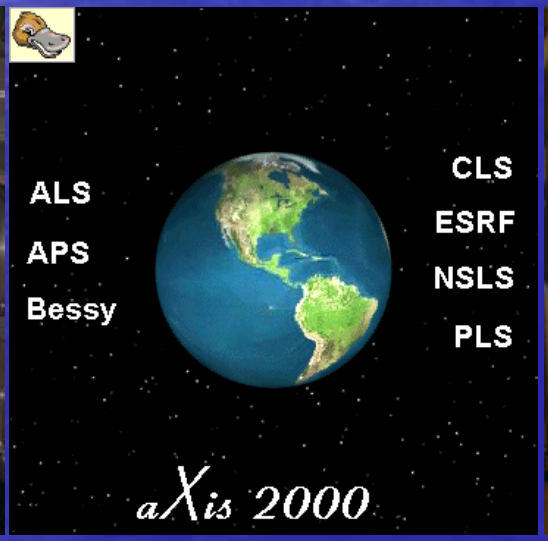
Soft X-ray spectromicroscopy & its analysis with aXis2000 .

Adam Hitchcock - BIMR, McMaster University

GOALS

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

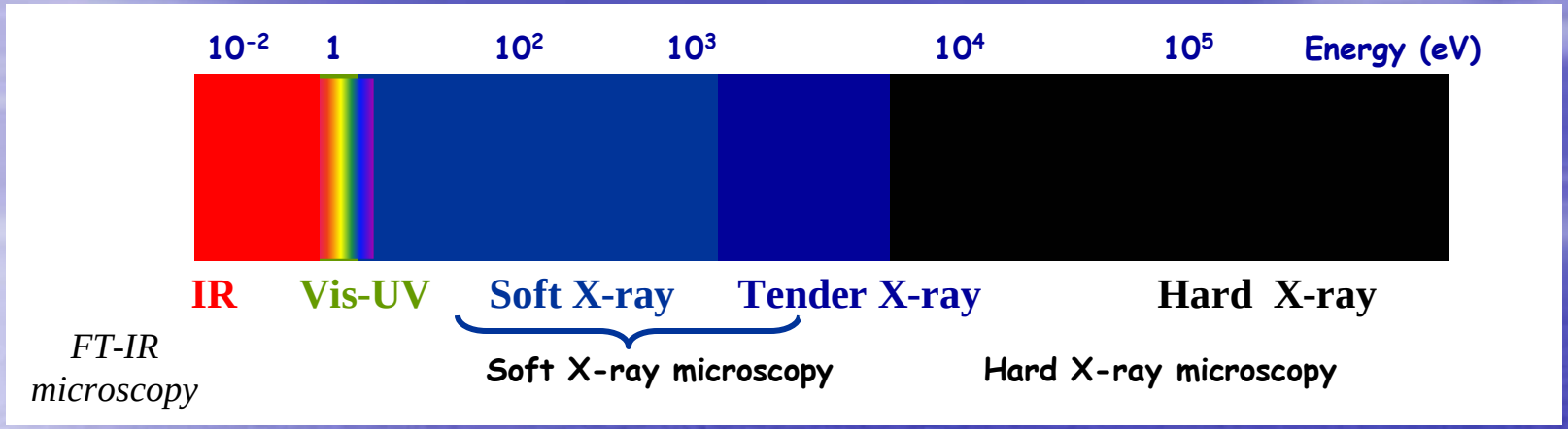


Outline

- 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"
  * spatially resolved chemical analysis - 'Micro-probe"

NEXAFS microscopy

* Use **penetrating power** of X-rays to study
  * wet soft matter (biology, polymers, nano-materials)
  * fluorescence microprobe
  * non-destructive testing; tomography

Soft X-rays ("water window")

Hard X-rays

References:

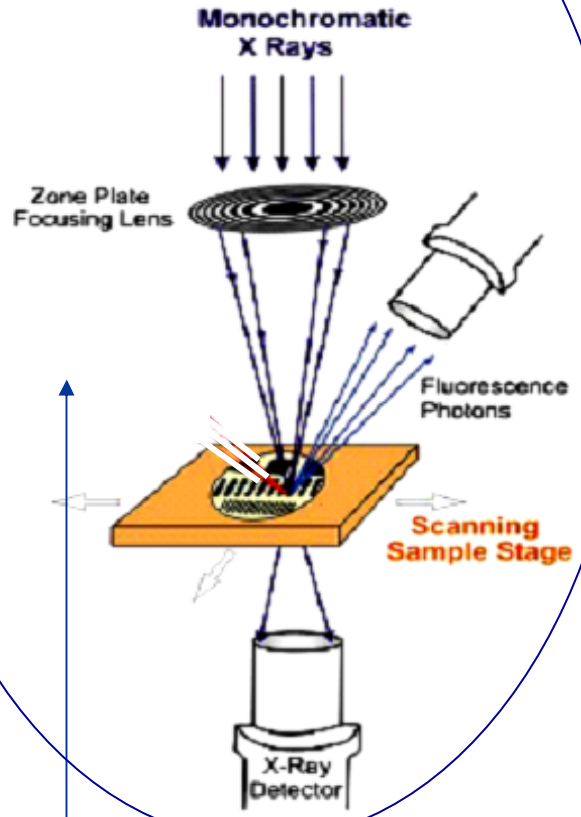
- J. Kirz, C. Jacobsen and M. Howells, Quarterly Review of Biophyisics, 33 (1995) 33
- H. Ade, in *Experimental Methods in the Physical Sciences*, Vol. 32, pp. 225, J.A.R. Samson and D.L. Ederer Ed., Academic Press, 1998
- A.P. Hitchcock, American Laboratory, 33 (2001) 30; J. El. spec. 144 (2005) 259.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

Overview of techniques

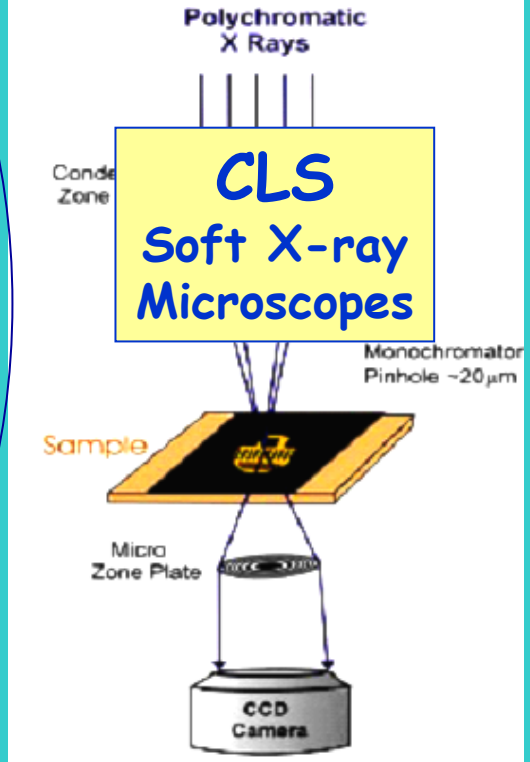
STXM ALS BL 5.3.2; BL11.0, NSLS X1A, BESSY

TXM now at ALS BL 6.1.2 XM-1, BESSY

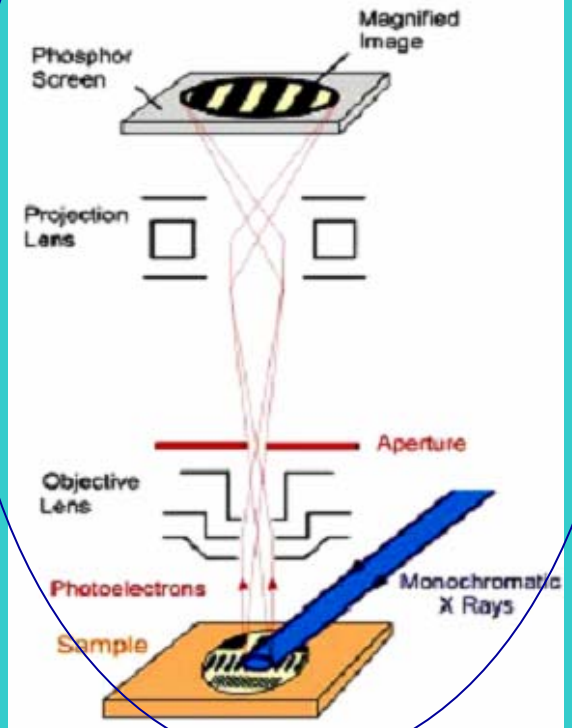
Scanning Transmission X-ray Microscopy - STXM



Transmission X-ray Microscopy TXM



X-ray PhotoEmission Electron Microscopy - X-PEEM



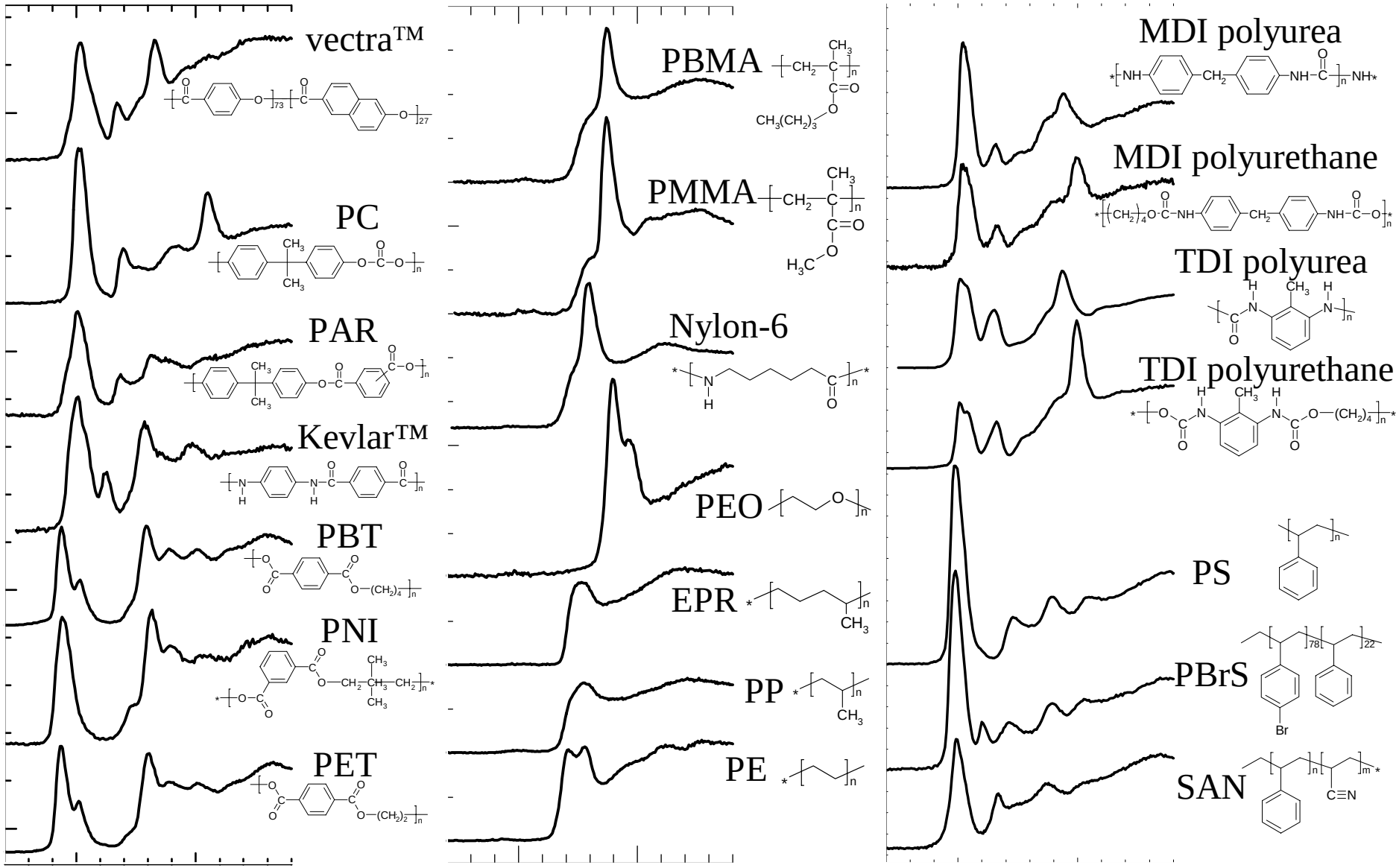
X-PEEM now at ALS BL 7.3.1, SLS, Trieste, SRC

Scanning Photoelectron Microscopy - SPEM

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

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

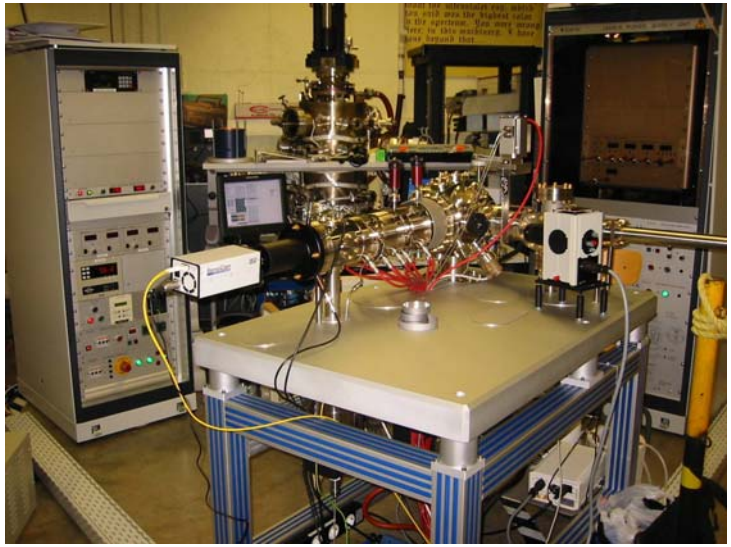
All are being developed as detection channels for analytical X-ray microscopy

SPEM –Scanning PhotoElectron Microscopy

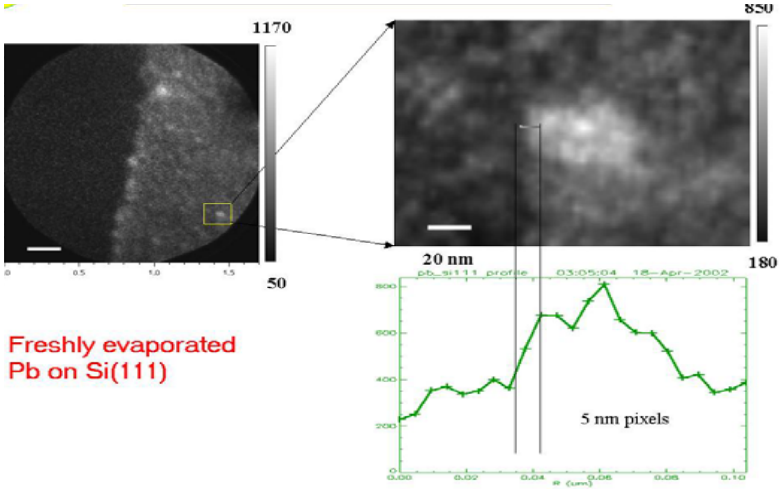
Elettra, Pohang Light Source
ALS BL 7.0.1

PEEM - Photo-Emission Electron Microscopy

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



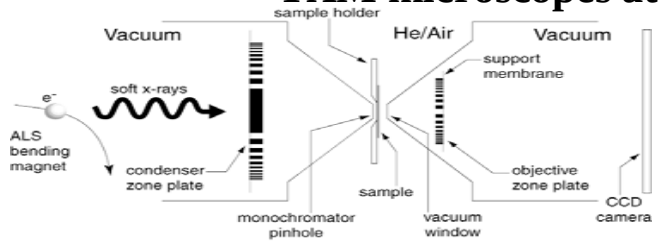
CaPeRS – Canada's Elmitec PEEM
now at CLS

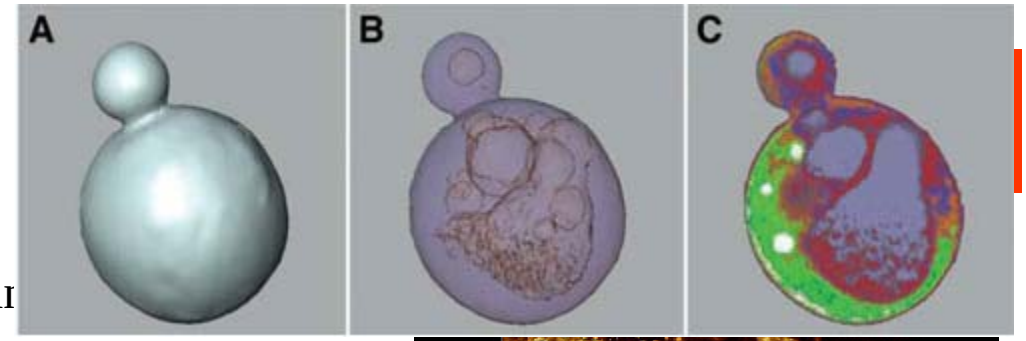


But only ~50 nm with synchrotron light due to e- distribution & chromatic aberration

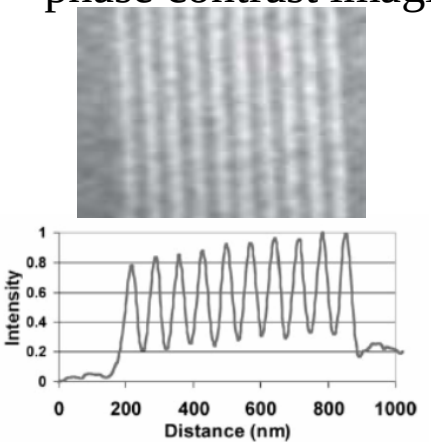
TXM – biological applications

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

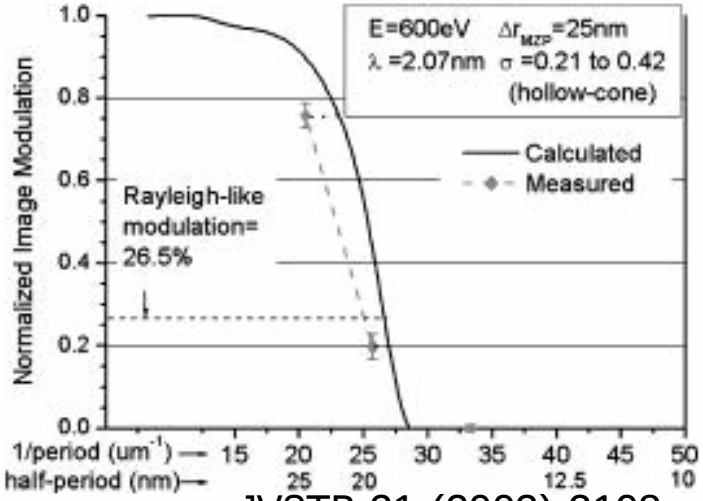




- wet cells: biomaterials & biological imagi
- cryo imaging
- tomography
- XMCD imaging
- phase contrast imaging



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

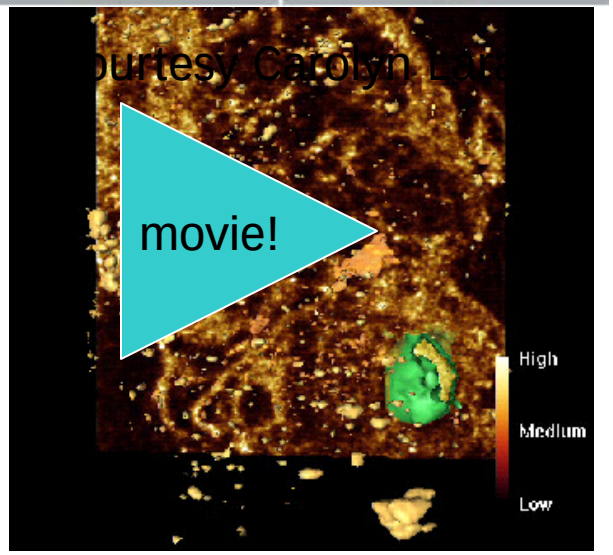


JVSTB 21 (2003) 3108

2 μm

monoclonal antibody / Au-Ag-labeling of cytoskeleton proteins

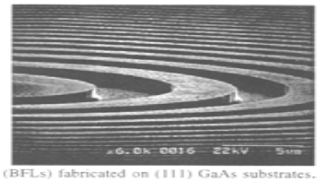
Larabell (UC Davis) ALS 1999

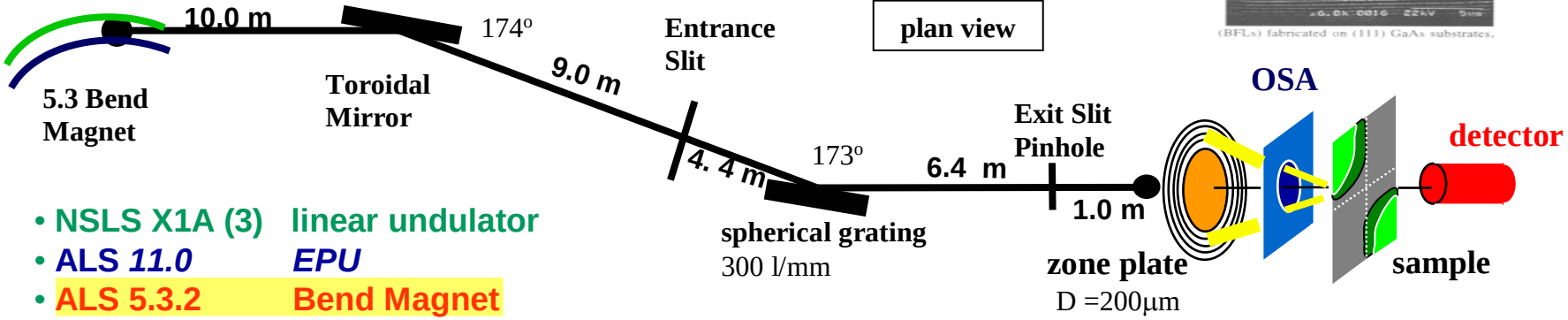


Gerd Schneider, Carolyn Larabel
ALS XM-1 2003

Angle-scan tomography
(cf. Attwood)

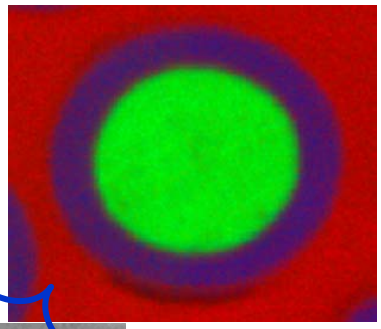
Scanning Transmission X-ray Microscopy (STXM)





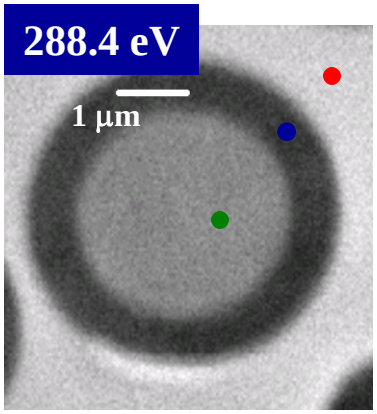
- NSLS X1A (3) linear undulator
- ALS *11.0* *EPU*
- ALS 5.3.2 Bend Magnet

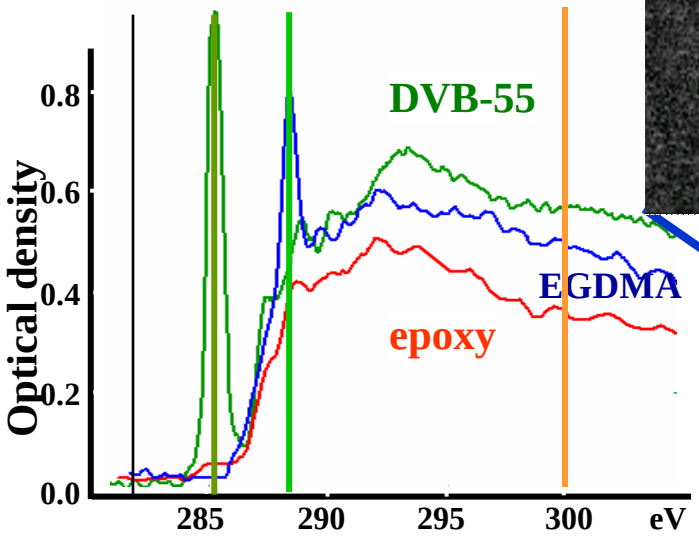
Image sequences *(OD format)*



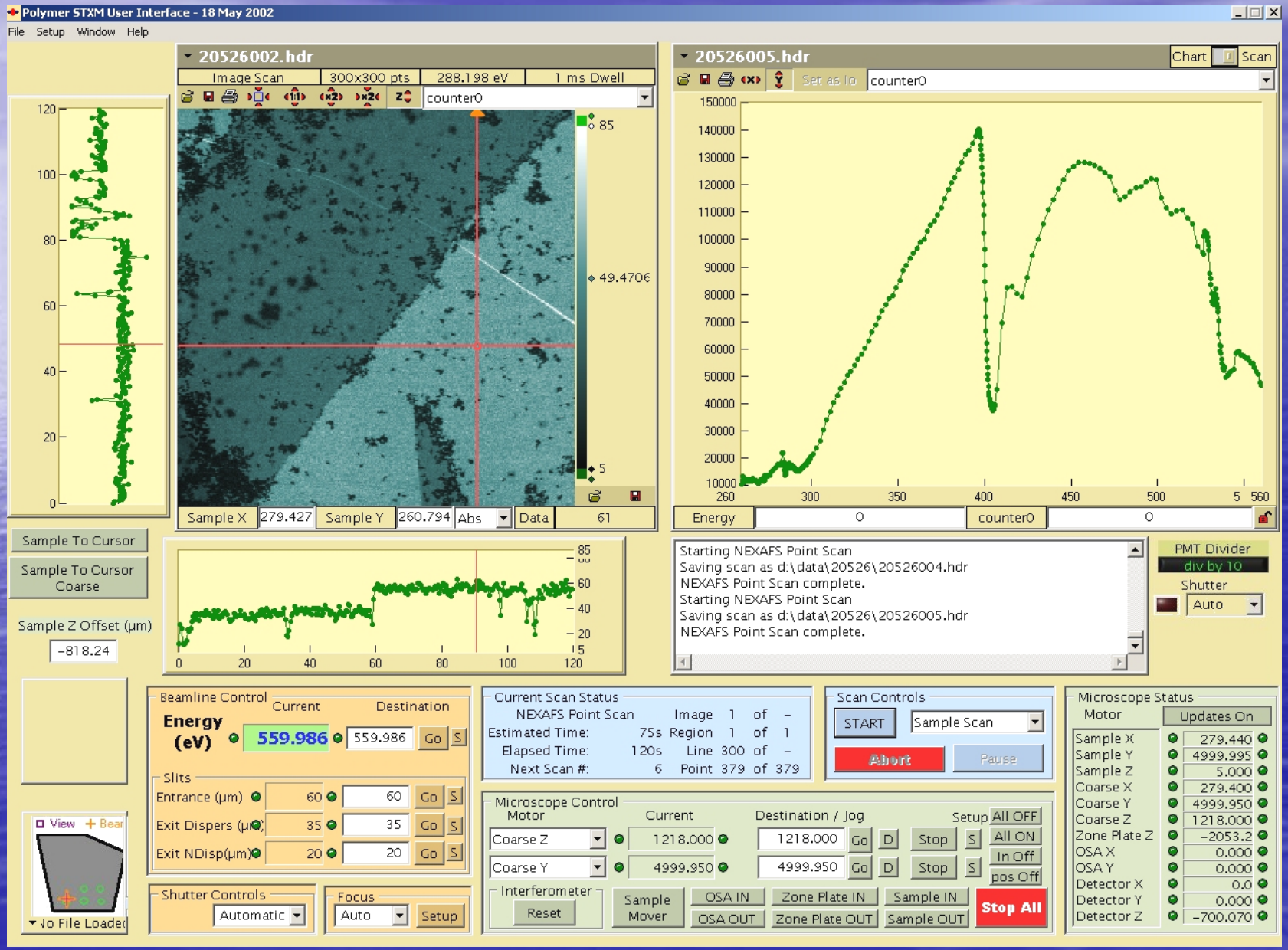
Images
transmitted I(x,y)

Spectra ***ln*(I/Io)**
{point, line, image}



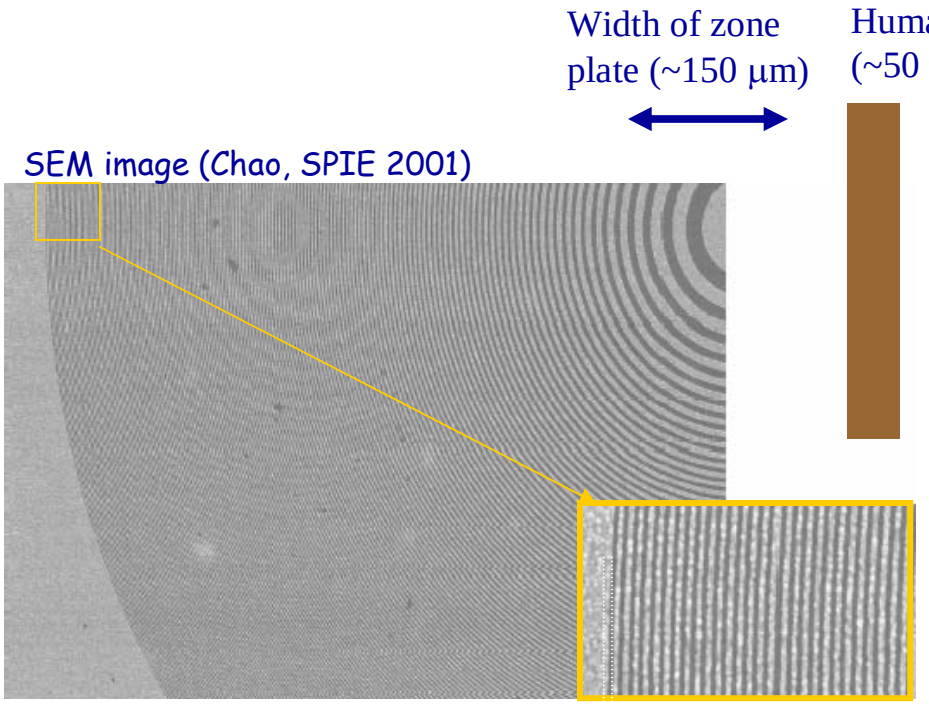


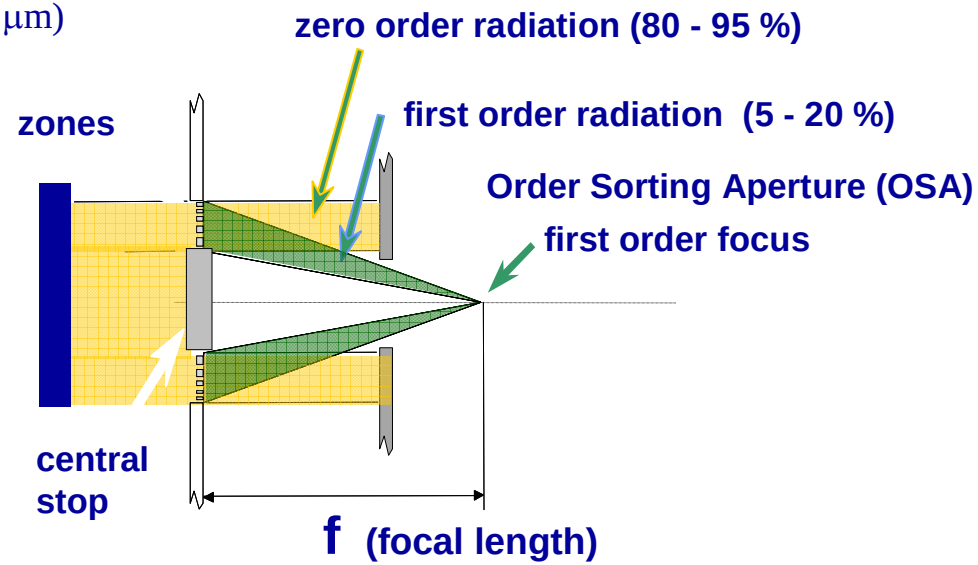
STXM_control - ALS (2), CLS, SLS



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)

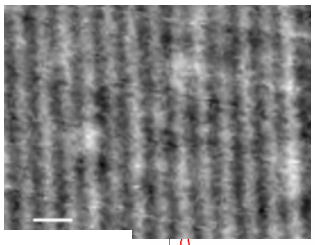
- λ **(photon wavelength)** 1 to 6 nanometers (~1240/**E**)
- **D (ZP diameter)** = 155 microns
- δr_N **(outer zone width)** = 35 nanometers
- **Number of zones** ~ 1000
- **Central stop diameter** = 80 microns
- **OSA diameter** = 55 microns

Spatial resolution (diffraction limited)

$$\Delta r = 1.22 \cdot \delta r_N$$

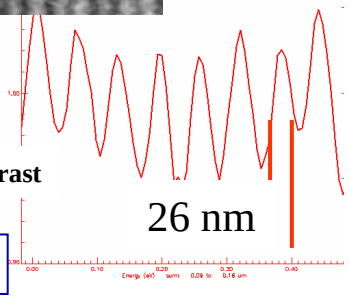
5.3.2 STXM Performance

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



Slits:
E/XD/XND
150/25/**25** μm

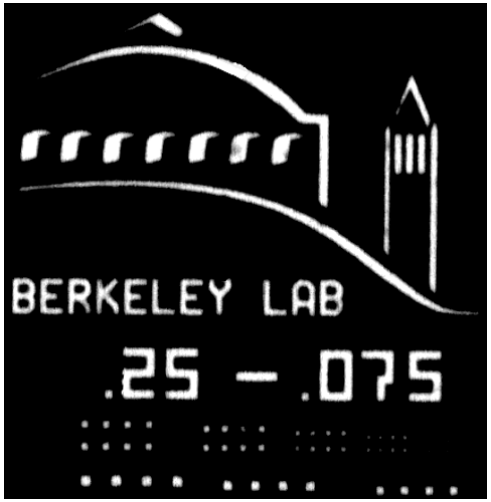
100 nm

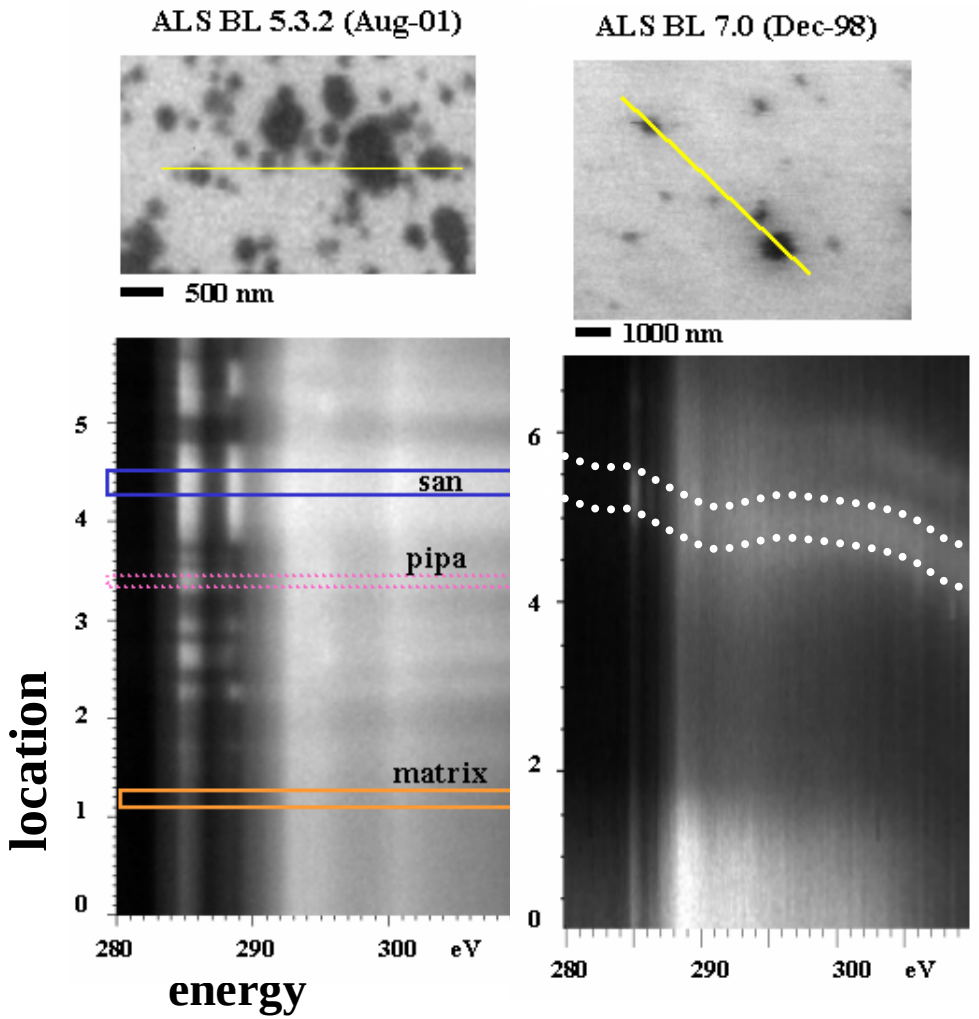


~10% contrast

26 nm

Nov 2002





Advanced Light Source

Tony Warwick
Mike Kirschner
Keith Franck

NC STATE UNIVERSITY

David Kilcoyne
Harald Ade (NCSU)

McMaster University

Tolek Tyliszczak
Adam Hitchcock
Peter Hitchcock

STXM-11: state-of-art performance

December 2003 - BREAKTHROUGH in ZP technology !

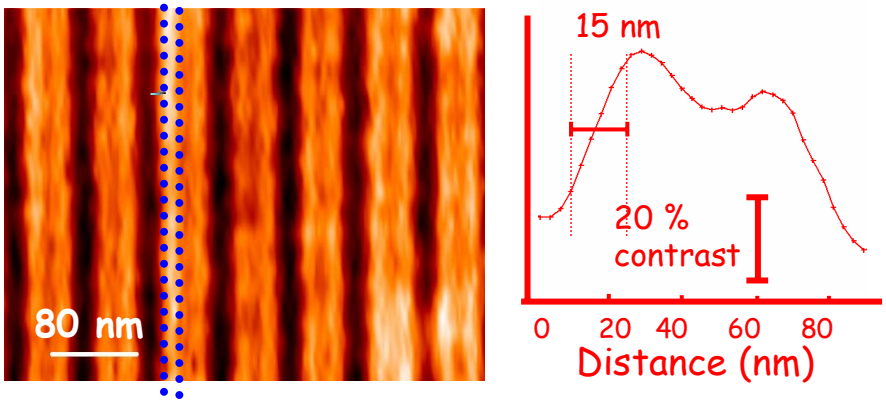
25 nm diffraction limited zone plates

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

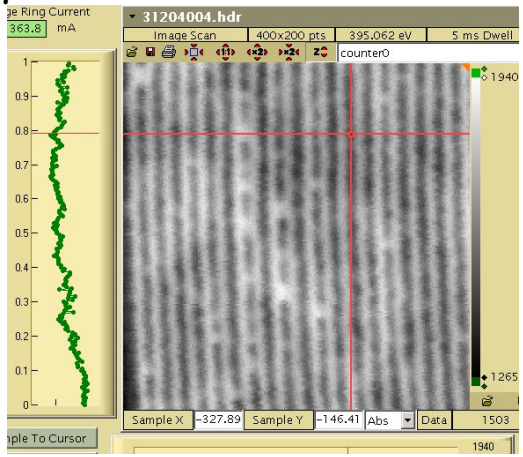
- spatial resolution
  - due to narrower outer most zones (**25 nm** instead of 35 nm)
- performance at high photon energy
  - due to higher aspect ratio (**7:1** instead of 3:1)

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 1:1 lines as test object



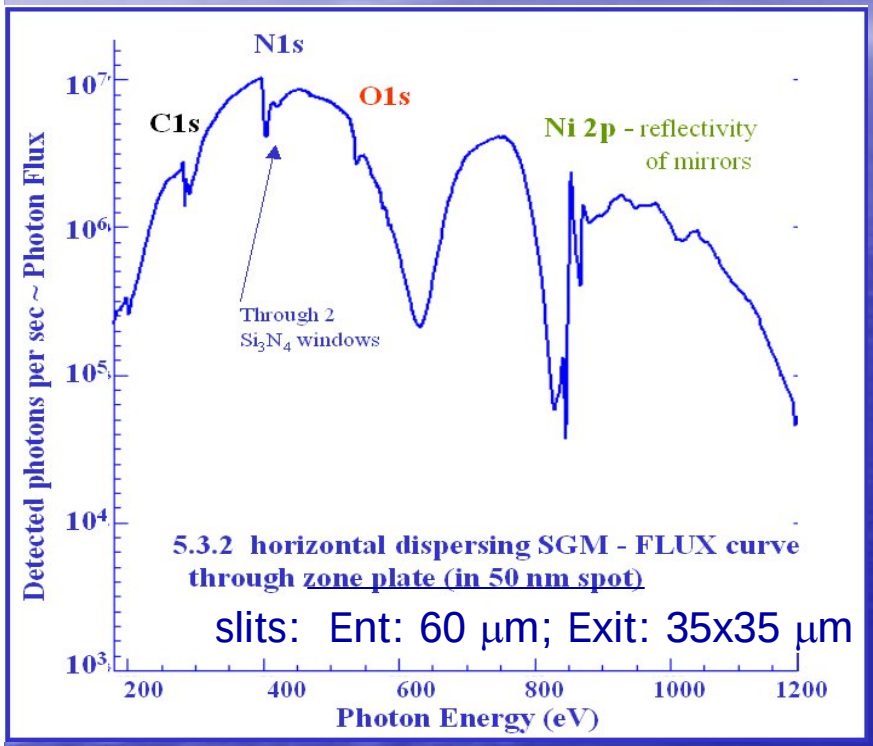
25 nm zone plate as test



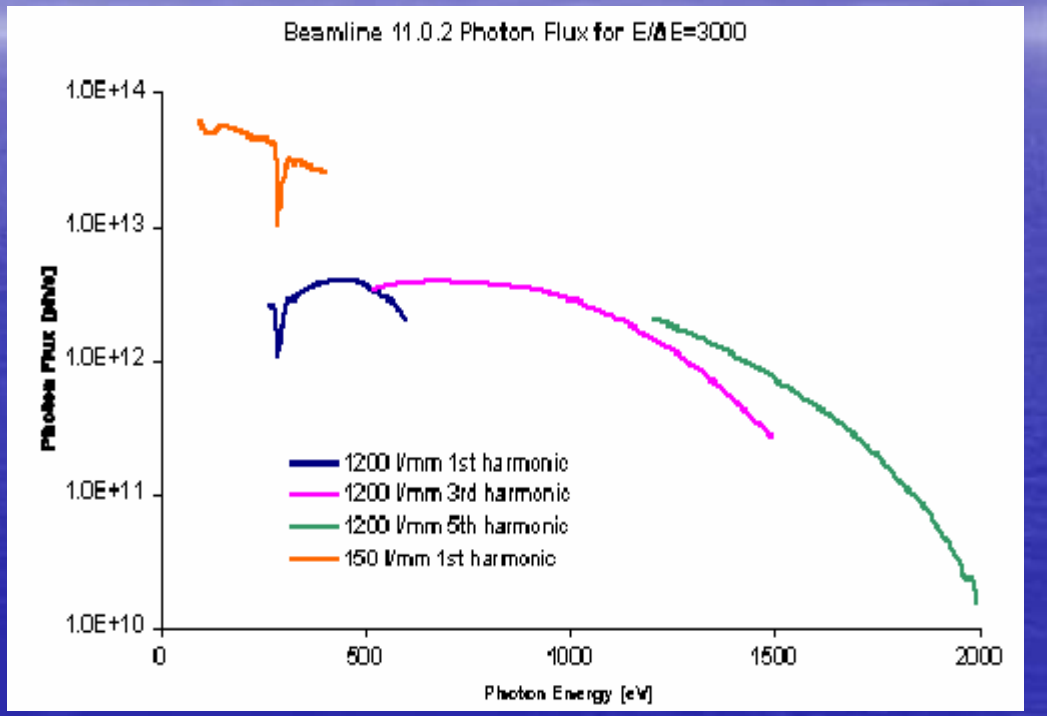
STXM532 - can mount 25 nm ZPs
- usually 35 nm ZPs (intensity)

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)

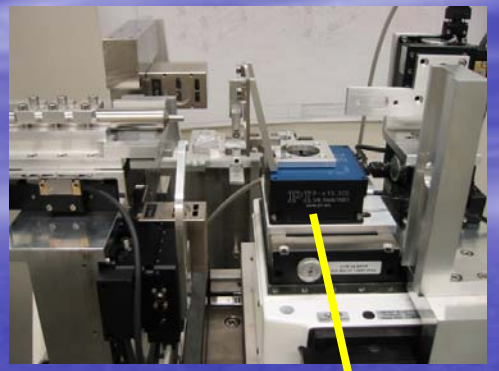
Monochromator: Plane Grating, no entrance slit (modified SX-700)

Energy range: 250 - 1900 eV

Resolving power: 5000

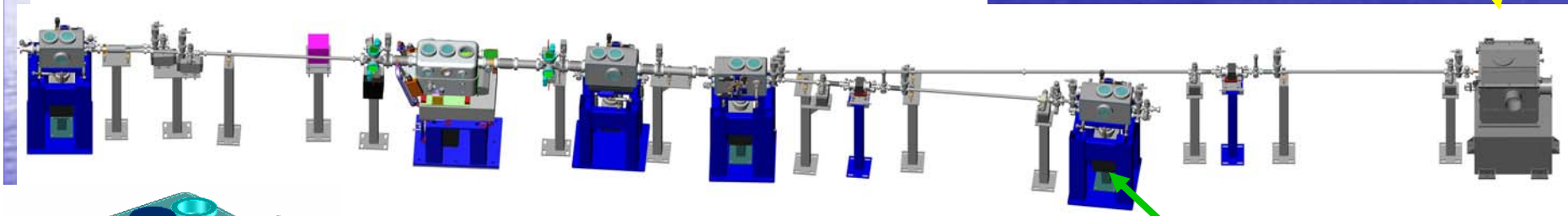
Flux: **PEEM**: 10^{11} - 10^{12} photon/s in 20 micron spot

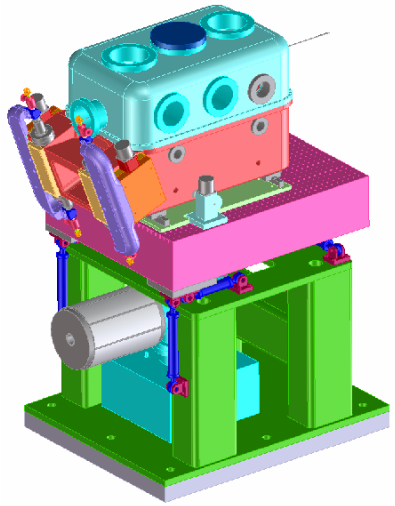
STXM: 10^{8} photon/s in 50 nm spot



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

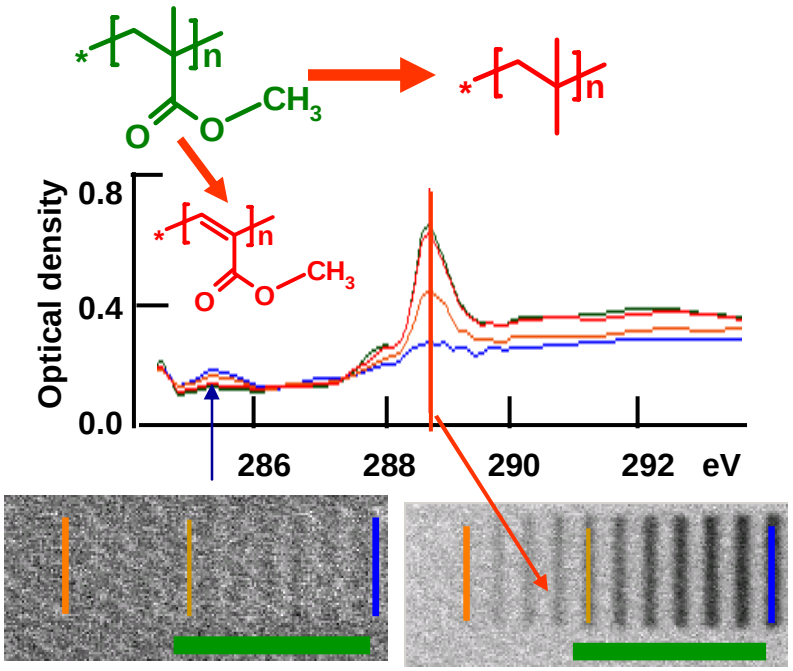
see talk and poster by Kaznatcheev (MSC)

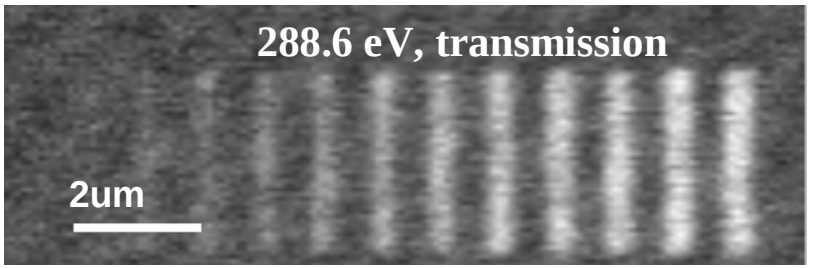
Radiation damage in STXM: PMMA

*** 4 um** line pairs; spaced at 1 um

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

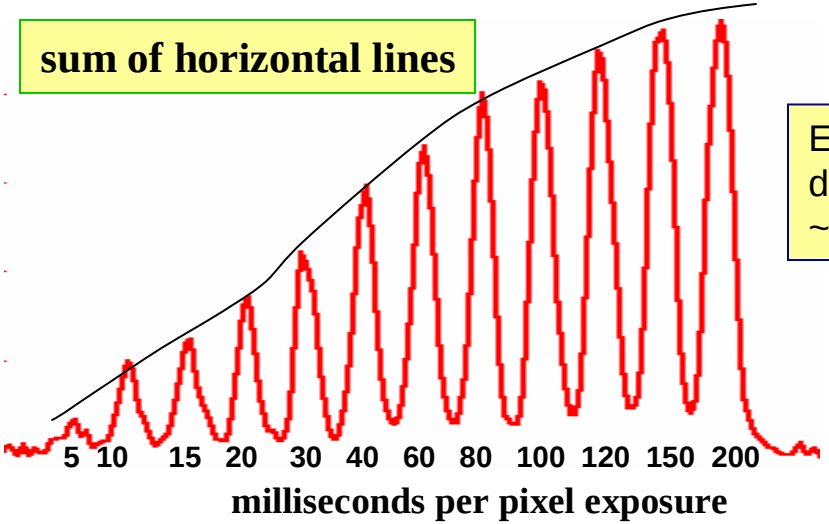
PMMA = polymethylmethacrylate





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)*

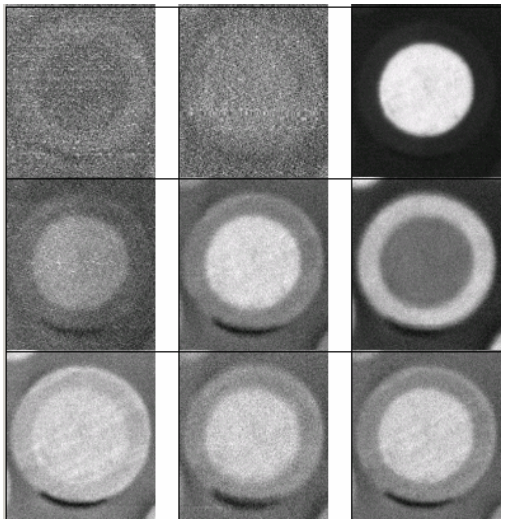
Quantitative Chemical Mapping

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)

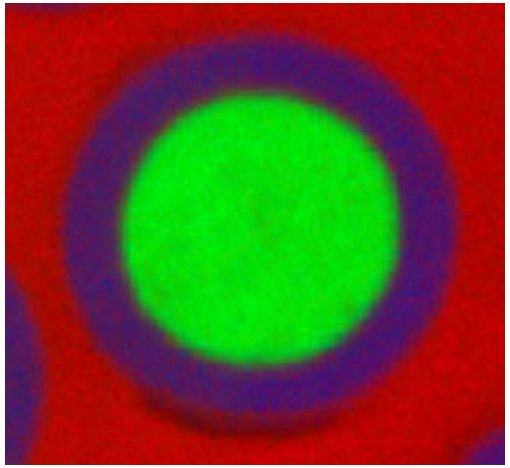


3. Generate component maps

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

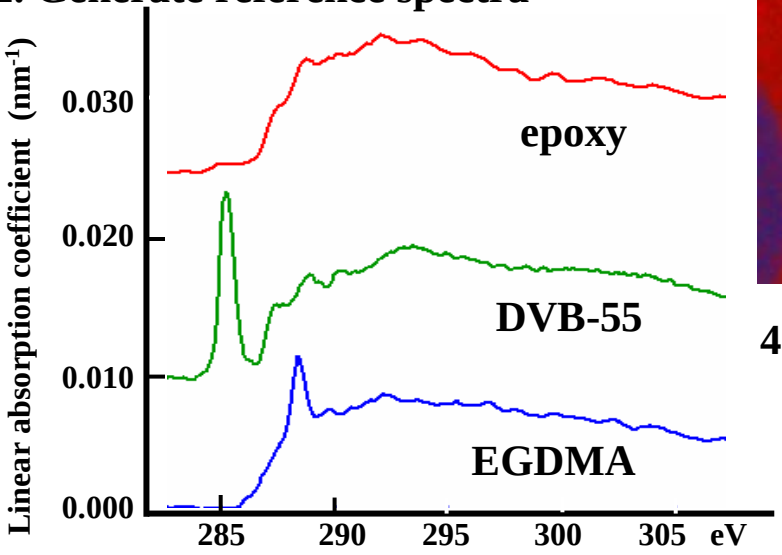
with a_i (j,k) = *THICKNESS* **(nm) at (jk)**

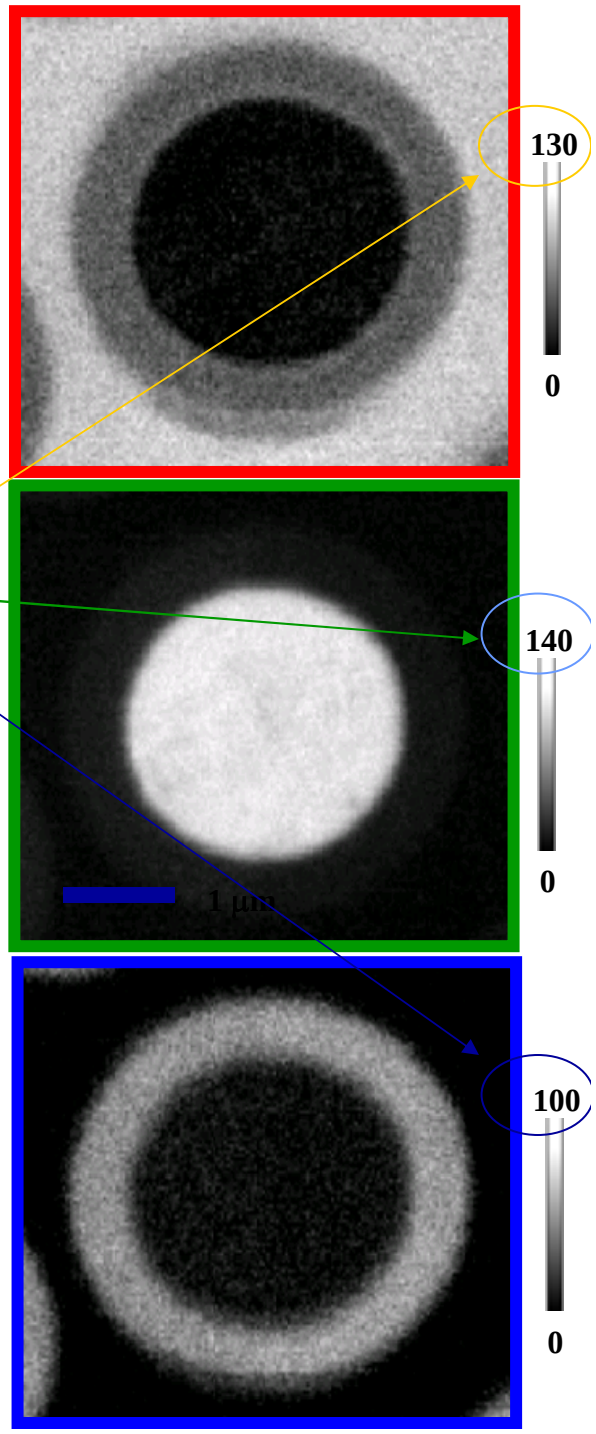
when reference spectra are absolute (nm^{-1})



4. Generate RGB-composite component "image"

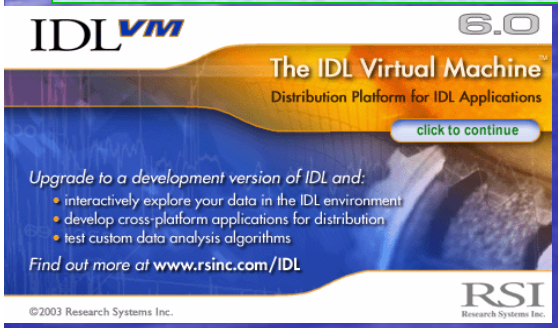
2. Generate reference spectra



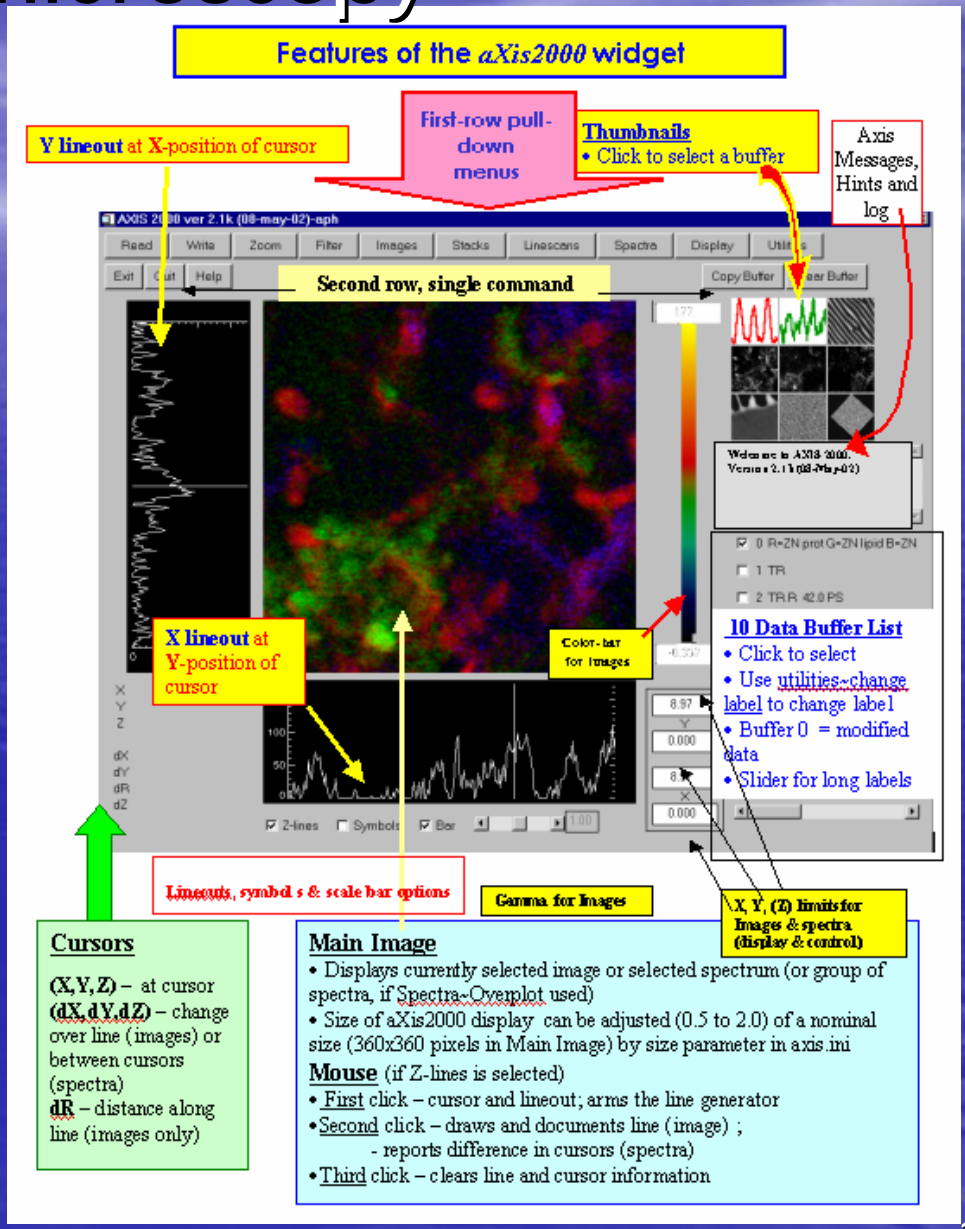


Analysis software for soft X-ray spectromicroscopy

(http:unicron.mcmaster.ca/aXis2000.html)



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)***

BREAK TIME !

Goals of aXis2000

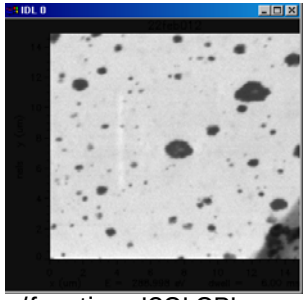
- ‘point and click’ analysis
- relieve analyst of programming
- share tools commonly used in image and spectral analysis
- provide tools specific for

SPECTRO-MICROSCOPY

Prior to aXis2000 .....

1

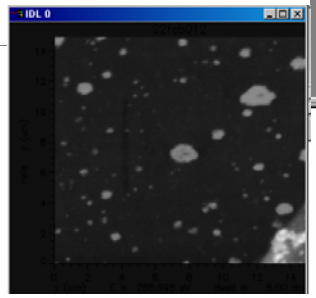
```
IDL> a=read_bnl()
% Compiled module: READ_BNL.
% Loaded DLM: NCDF.
% Compiled module: INIT_SD.
% Compiled module: AX_NAME.
read NSLS image from file: E:\axis-dev\test-data\22FEB012.NC
 250 x  250 pts.  0.0598 x  0.0597 um pixels.
E=  288.998 eV.  Dwell=     6.00 ms.
```



```
IDL> splot2d(a)

splot2d(a)
  ^
% Syntax error.
IDL> splot2d,a
```

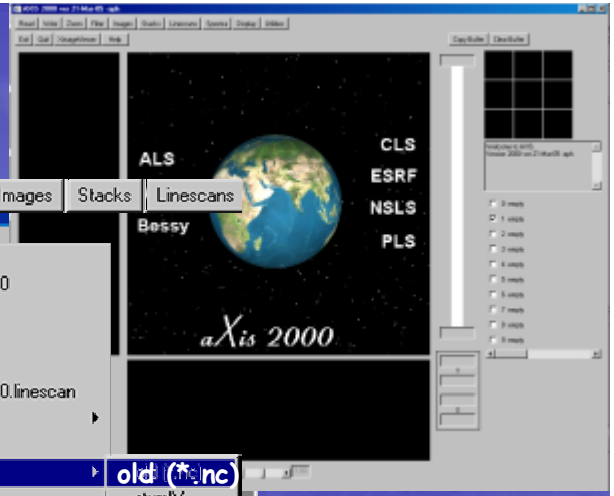
```
IDL> color
% Attempt to call undefined procedure/function: 'COLOR'.
% Execution halted at: $MAIN$
IDL> loadct,0
% LOADCT: Loading table B-W LINEAR
IDL> splot2d,a
```



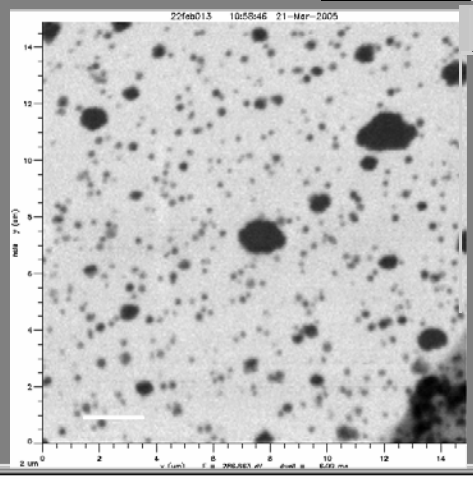
2

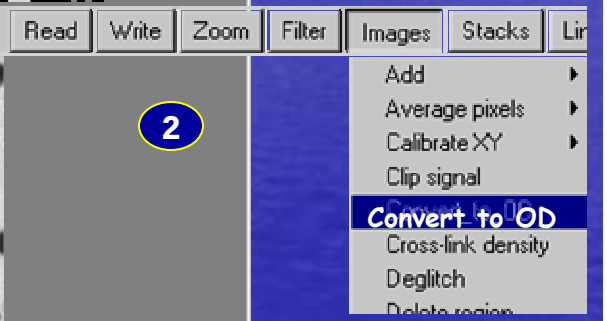
```
IDL> print, median(a.d)
      457.000
IDL> b=a
IDL> b.d = -alog(a.d/600)
```

```
IDL> splot2d, b
IDL>
IDL>
IDL>
IDL>
```

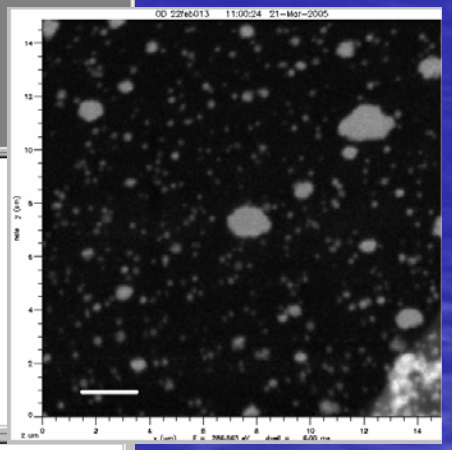


1





2



This is always an alternative, if you have an IDL license ....)

Example: analyzing ALS STXM data

A common process of acquiring chemical analysis data with STXM might be
a) **image** to find area of interest
b) record **point spectra** &/or **linescans** to check chemical identity of regions
c) check for damage
d) record image sequence (**STACK**)
e) check for damage

Th
pa

Description

A polyuretha 0.05 mg/ml solution of fibrinogen. We used C 1s STXM to map the fibrinogen and the components

references:

[1] A.P. Hitchcc ieen, F. Hayes, W. Lidy, R.D. P *s in polyurethanes studied by s*

[2] A.P. Hitchcock, C. Morin, Y.M. Heng, R.M. Cornelius and J.L. Brash, *Towards practical soft X-ray spectromicroscopy of biomaterials*, J. Biomaterials Science, Polymer Ed. 13 (**2002**) 919-938

STXM 5.3.2 User Manual

File: stxm532-manual.doc
Last update: 03-Apr -03 (aph)
History:
Version 1a: 05-sep-02 written by Tohru Araki (no pictures)
Version 1b: 12-Oct-02 update by aph, comments by IK
Version 1c: 03-Apr-03 update by aph

AUTHOR: PETER HITCHCOCK
DATE: JULY 16, 2001

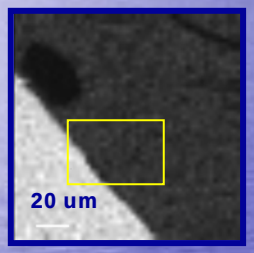
STXM 5.3.2 Interface - User Ergonomics

This report describes in detail the various windows and controls that make up the user interface to the 5.3 STXM.

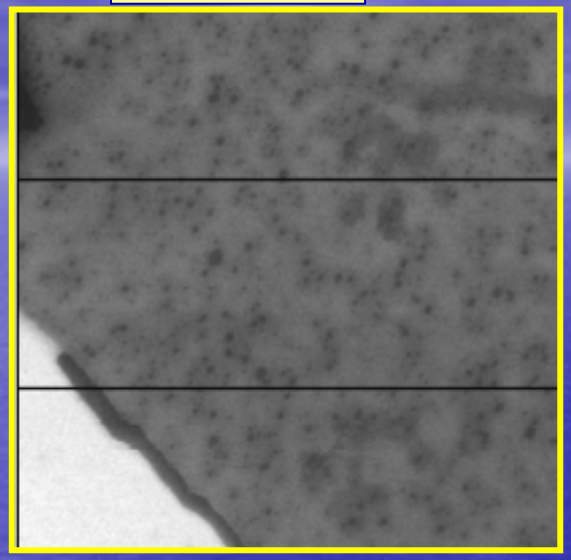
A) Locating region of interest

11103084 out of focus

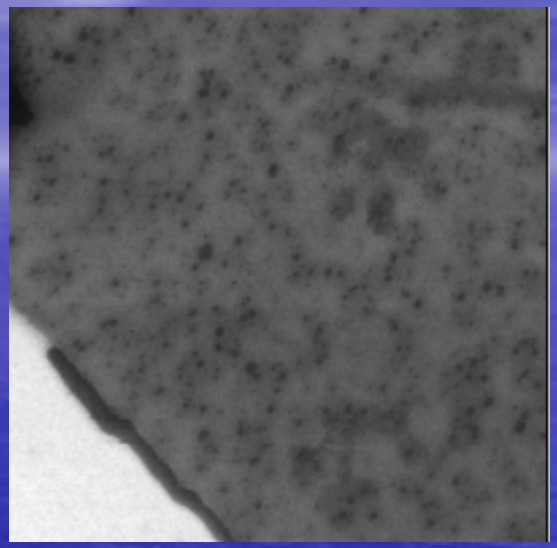
11103085 285.1 eV



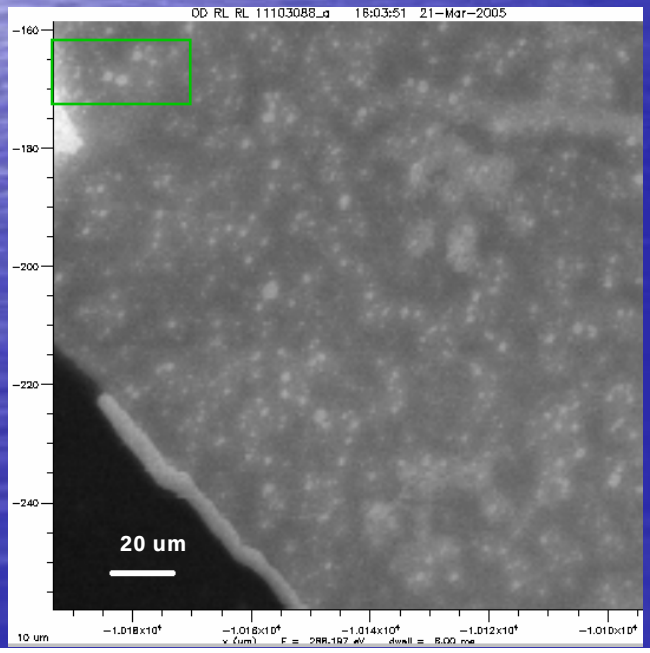
11103088 285.1 eV



replace lines

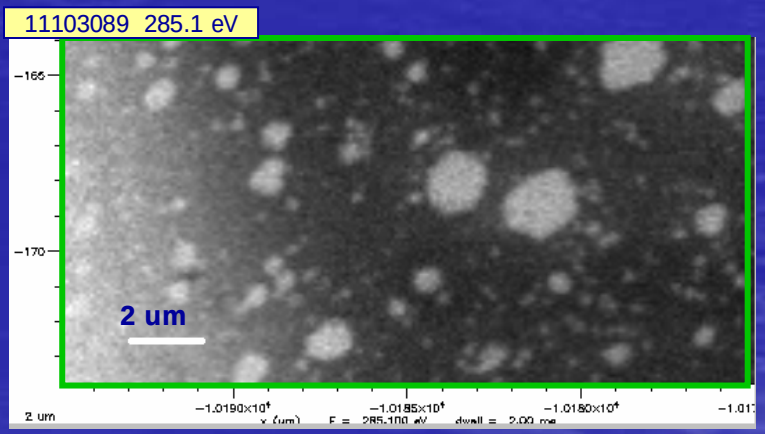


convert to OD

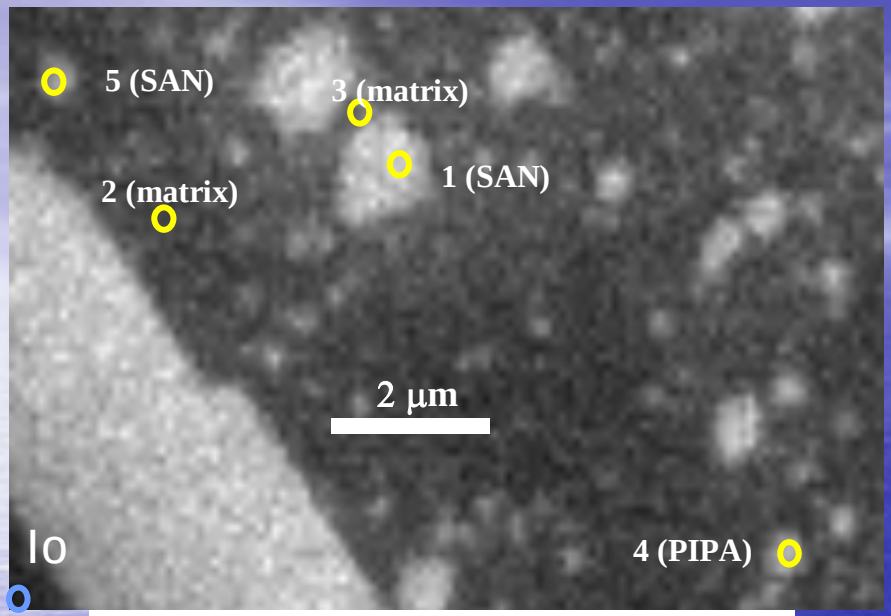


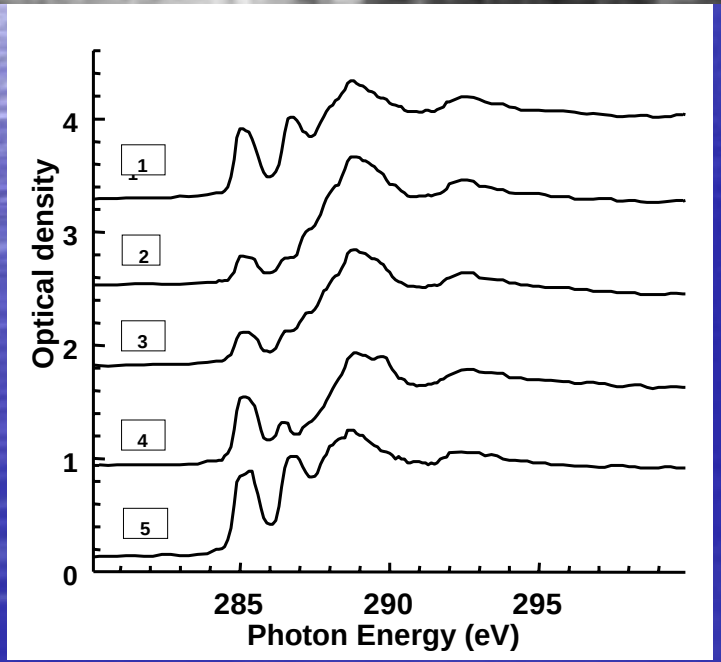
Area selected for stack measurement

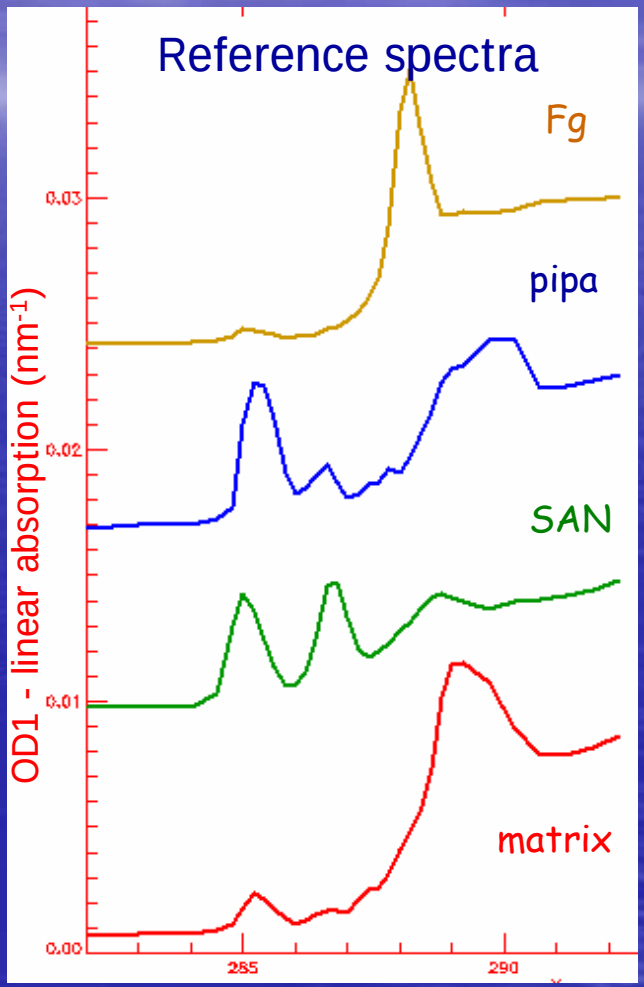
11103089 285.1 eV



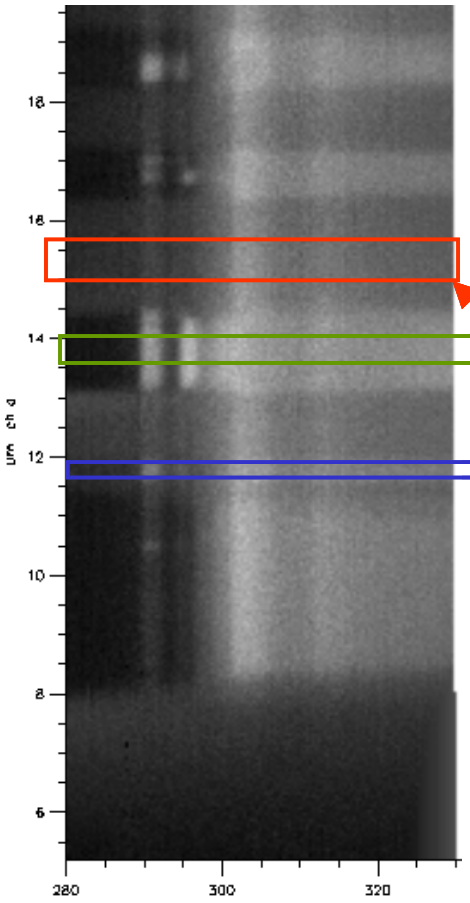
B) Multi-point spectra



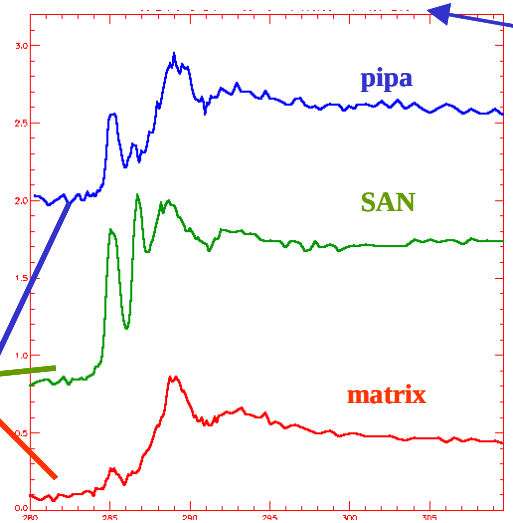




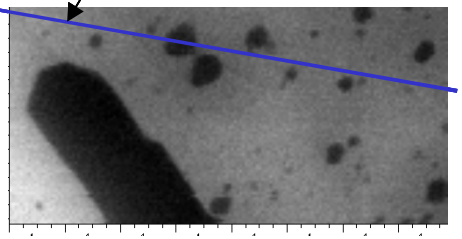
C) Linescan spectra (& damage check)

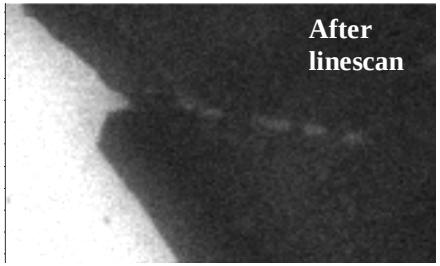


15 msec / point
at end of fill

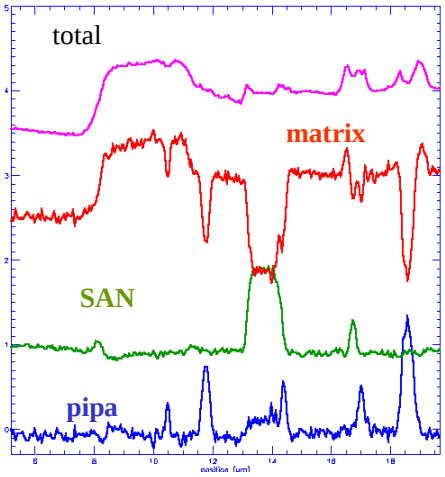


Line
Edge of section thicker



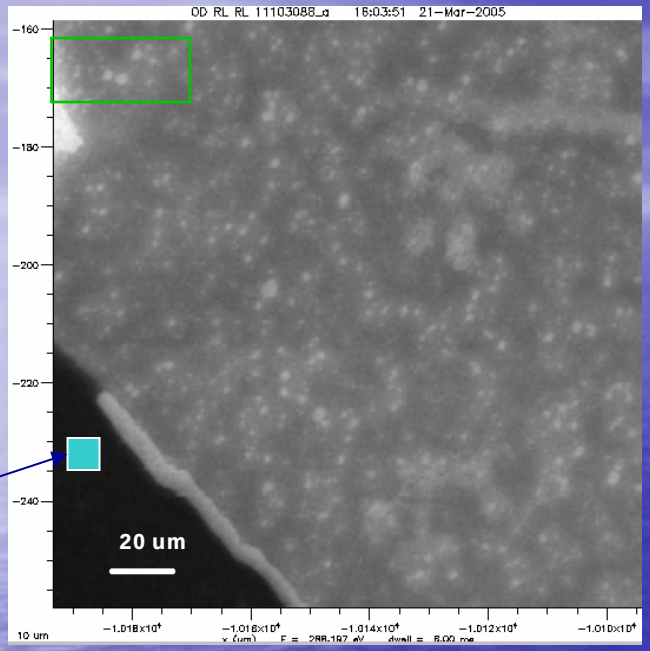


Linescan fit analysis



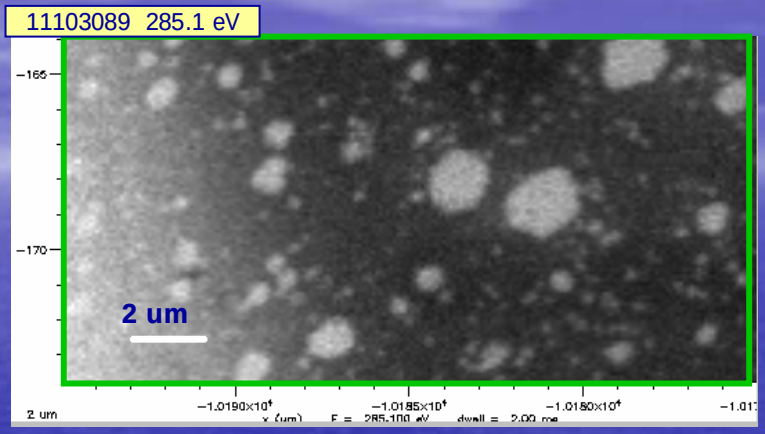
NB models from
pure 355 stack
no meaning to
vertical scale

D) Image sequence (& damage check)

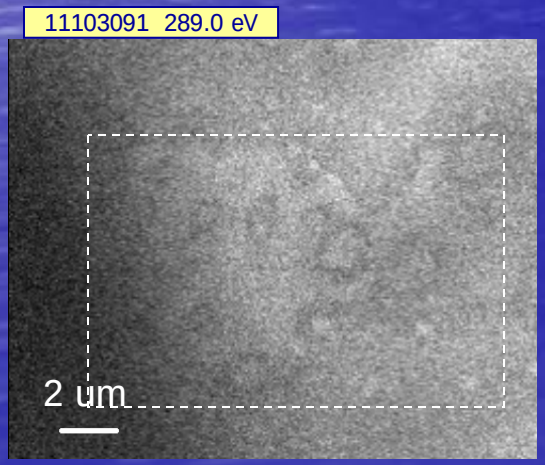


Io area

Area selected for stack measurement



the bright area to the left is known to be a protein deposit so we wanted to be able to get a clear Fg spectral signature to check the analysis in the more dilute regions

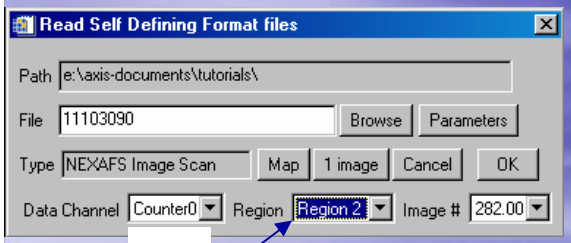


<u>DAMAGE CHECK</u>

image larger than region of stack at damage sensitive energy (289 eV $\sigma^*_{C\text{-}O}$ of ether)

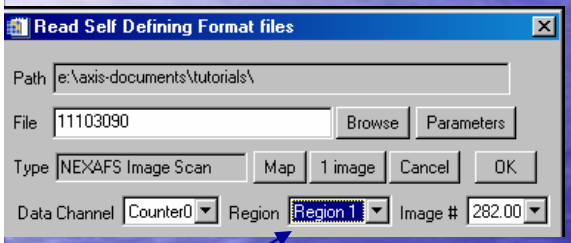
little sign of damage (usually polyether matrix bleaches due to mass loss)

C1s stack - read-in and convert to OD



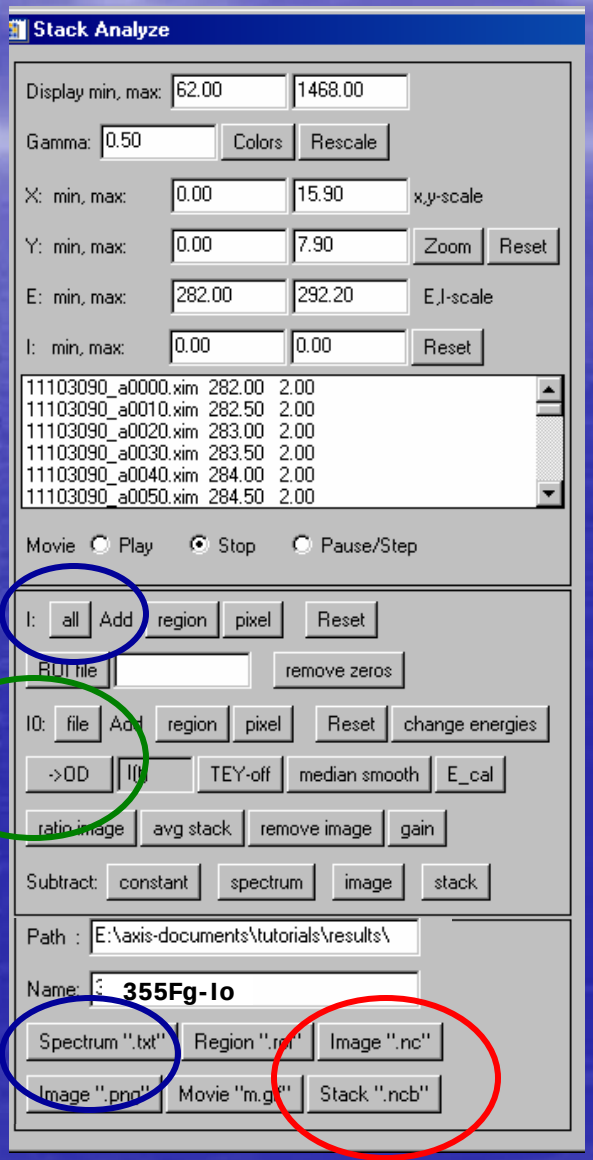
Io

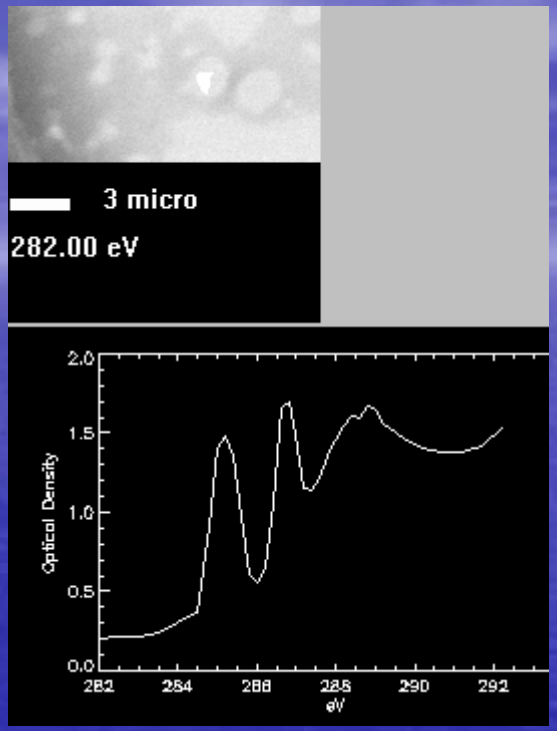
in stack_analyze
1) I: all
2) save Io spectrum

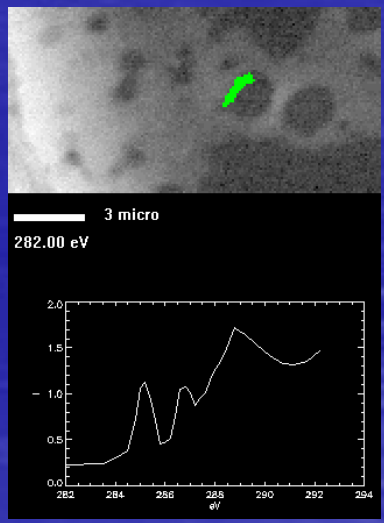


I

in stack_analyze
1) define Io as file from region 2
2) convert to OD
3) type file name
4) save converted stack



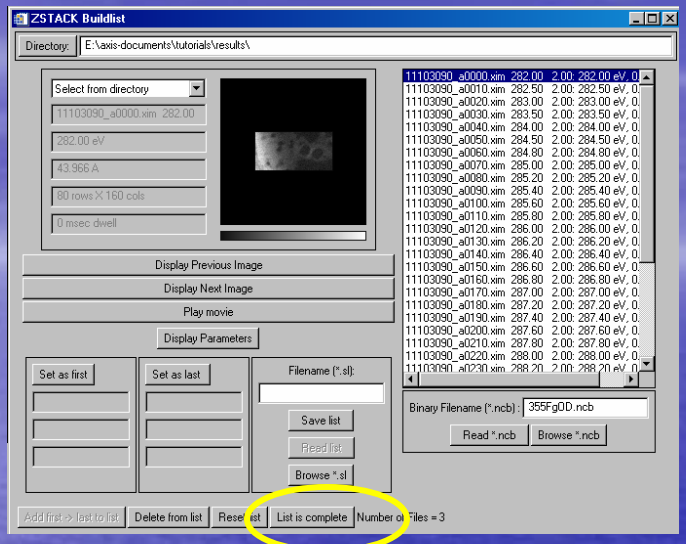


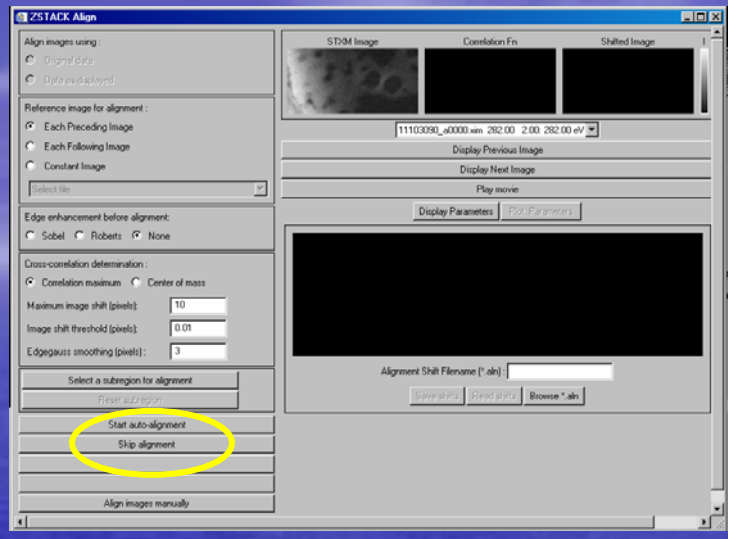


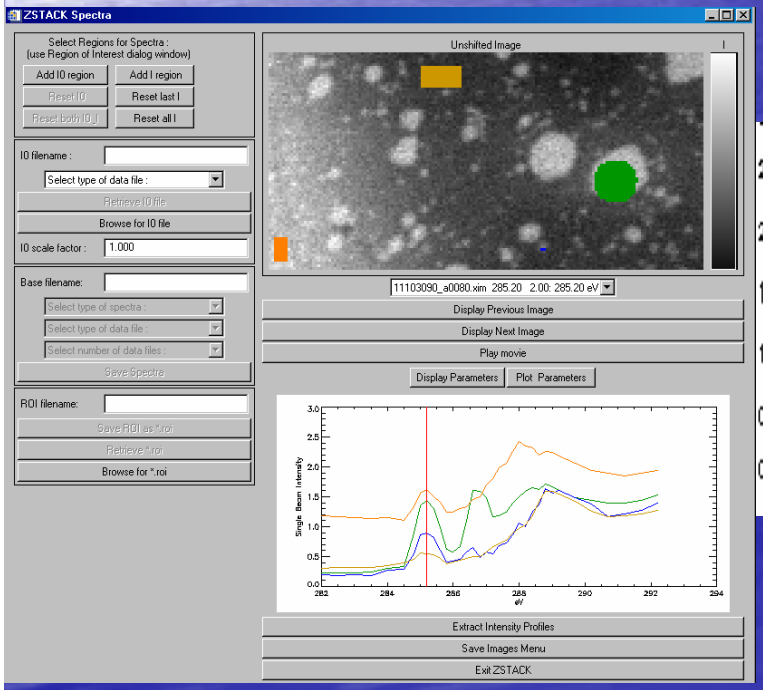
C1s stack - viewing specta of regions

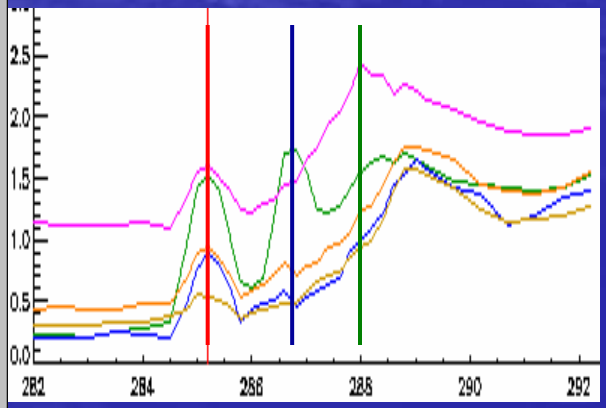
Zstack
sub menu

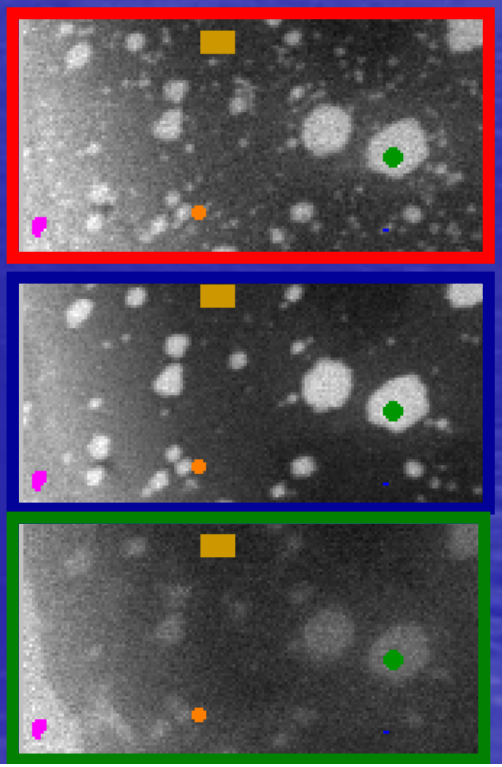
written by Carl Zimba



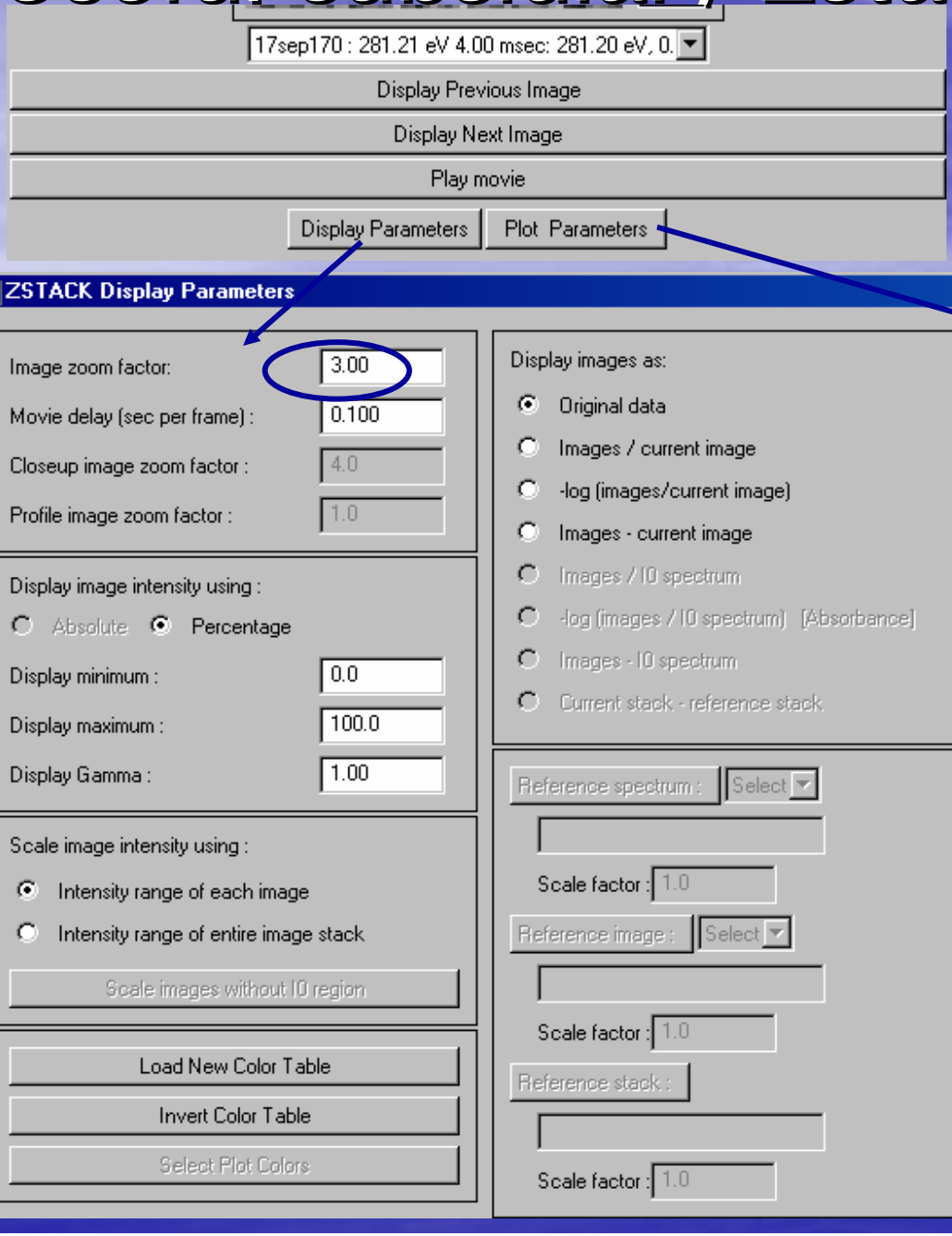








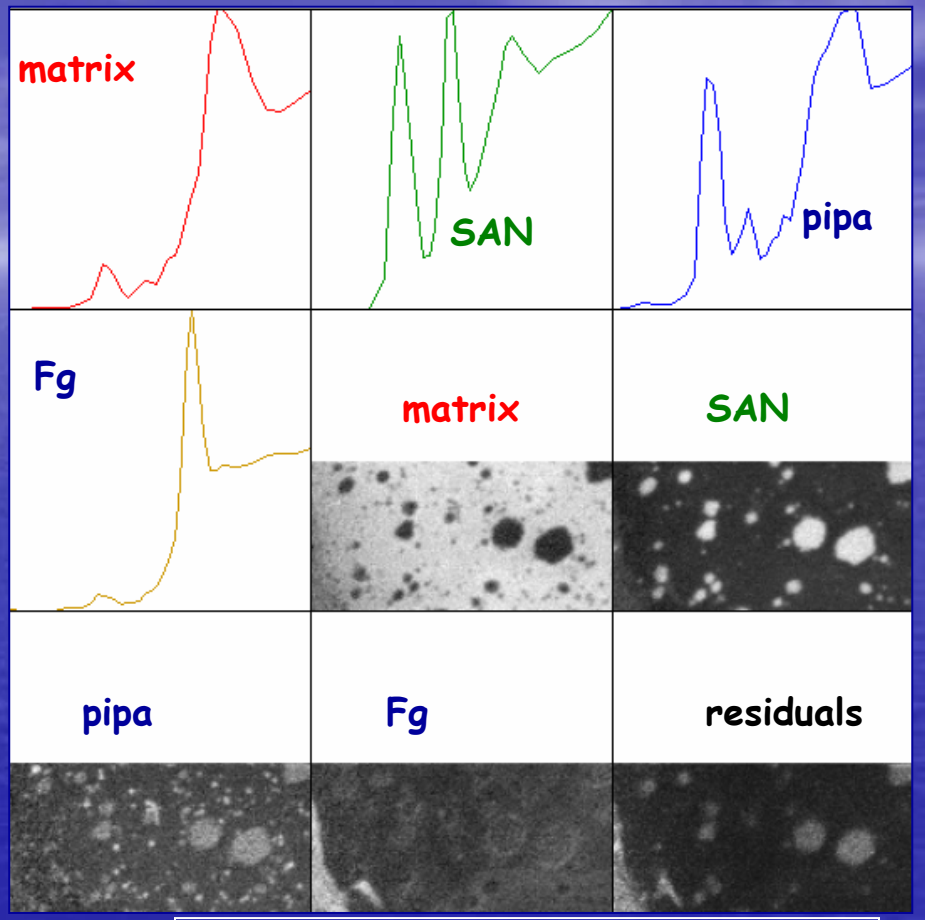
Useful subsidiary Zstack features



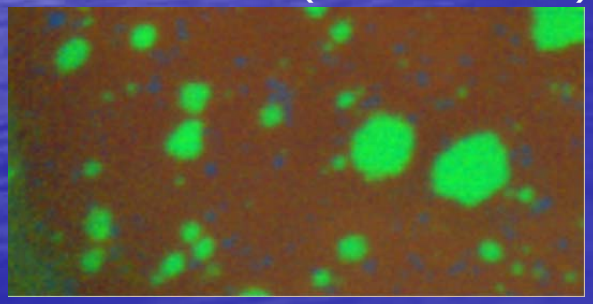
C 1s stack: chemical maps

CONCEPT:

* a stack is a set of 10^4-10^5 spectra (one at each pixel)
* we FIT the spectrum at each pixel to reference spectra of known constituents
* the fit coefficients at each pixel form a **COMPONENT MAP**
* if the reference spectra are on an absolute intensity scale (OD1 = response of 1 nm of pure material) then the grey scale of each map is a quantitative measure of the thickness distribution of that component
* we can display the spatial correlation of the components using an RGB color **COMPOSITE MAP**



not rescaled (absolute nm)



298

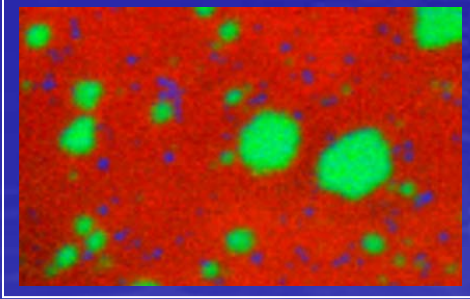
-70

un-clipped data

126 298 114

-70 -17 -59

clipped, rescaled (RELATIVE)

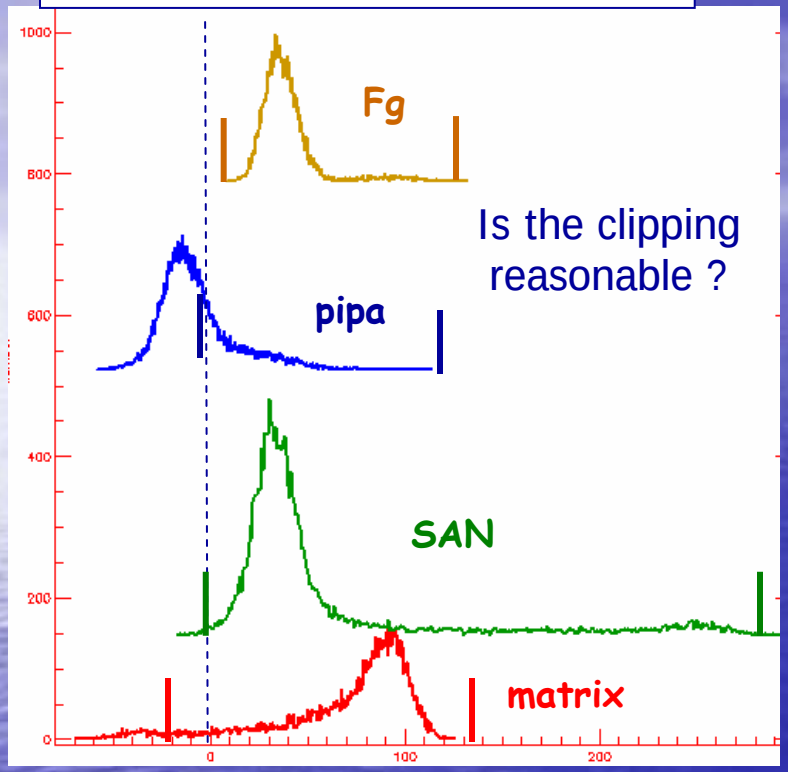


126 283 114

-12 0 0

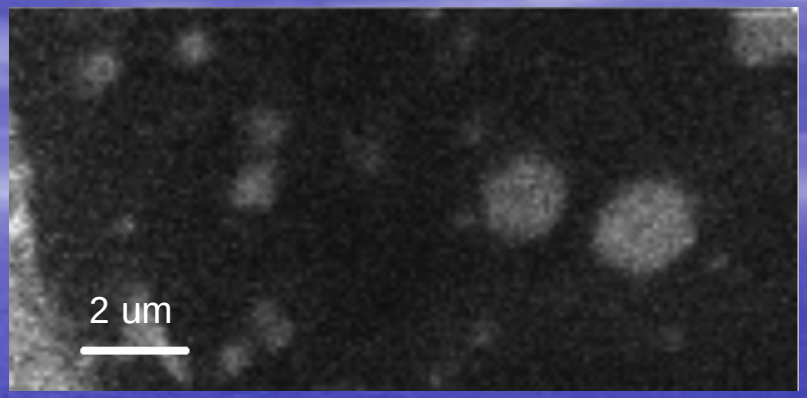
Evaluating the stack analysis

Histograms of the component maps



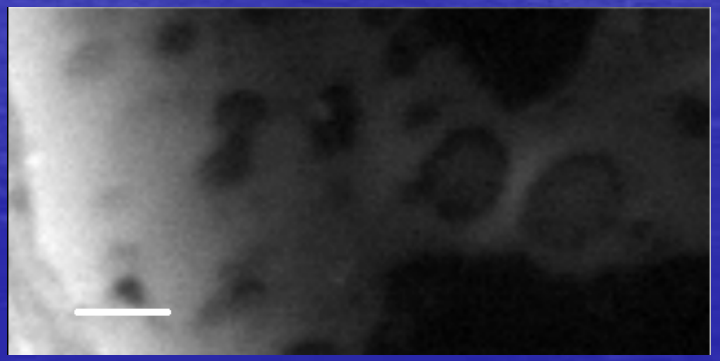
Is the clipping reasonable ?

residuals



SMALL compared to OD of stack (0 to 3)

constant (ideally should be small, +/-)



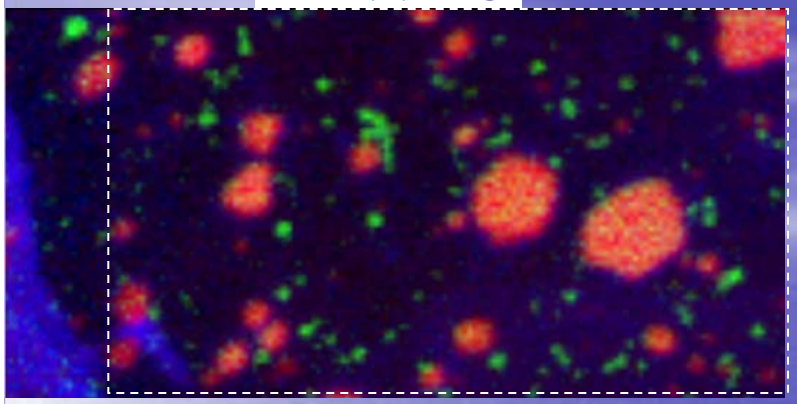
Generally an acceptable result.

PIPA map is probably wrong w.r.t quantity.

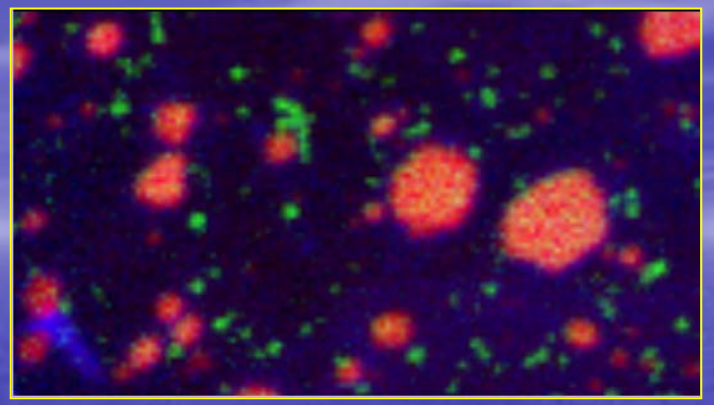
The fit is using “-ve PIPA” signal to accommodate absorption saturation distortions of spectra (region selected in too thick by ~ x2)

Evaluating the result - where is the Fg ?

SAN pipa Fg

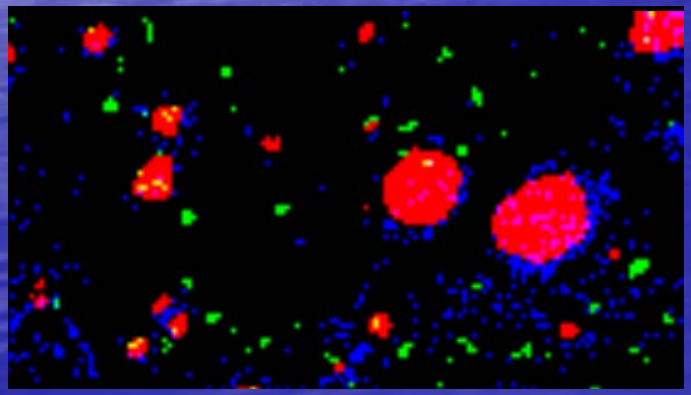


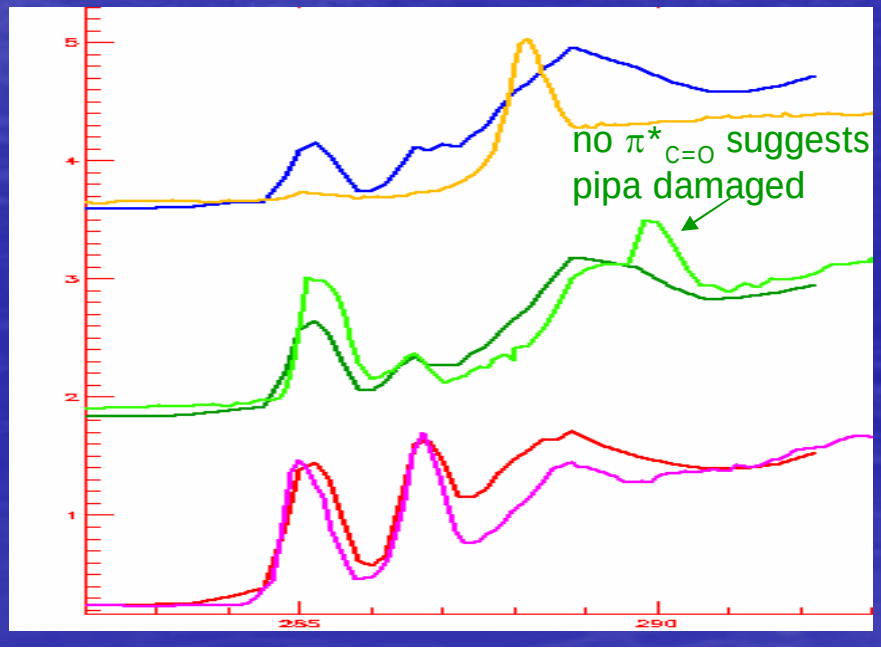
select right 2/3 to get away from 'splat' protein deposit



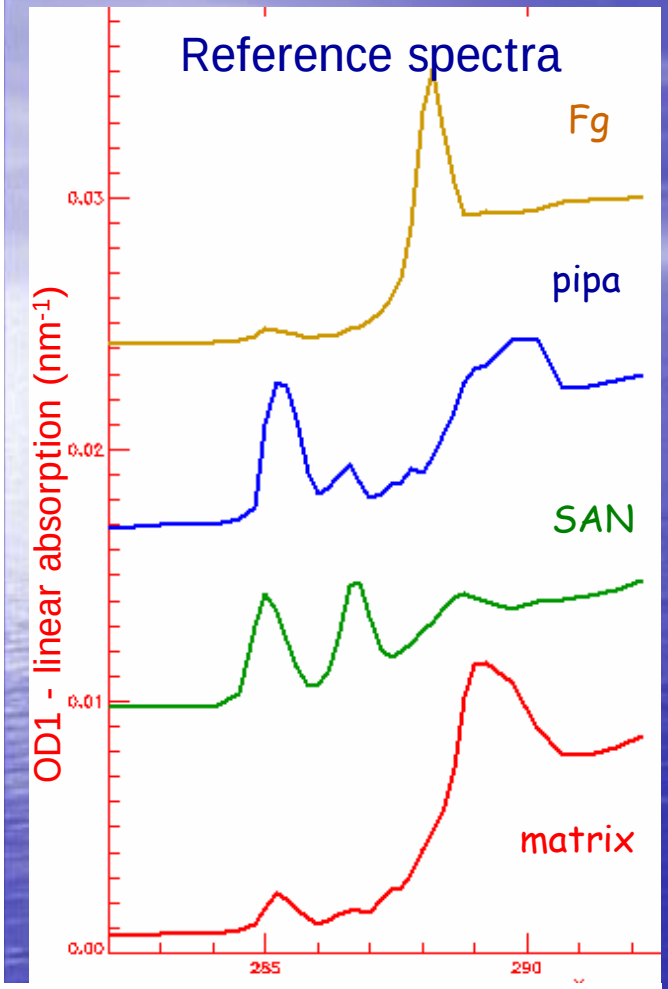
- use image~generate mask to identify pixels of high Fg content
- extract spectrum
- fit that extracted spectrum to reference spectra

SAN >200
pipa > 45
Fg >50, <65

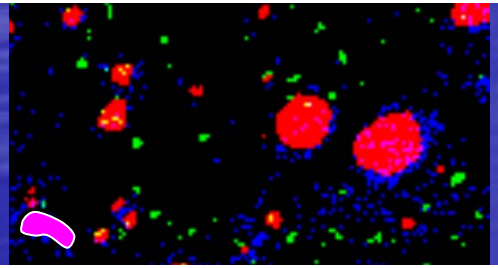


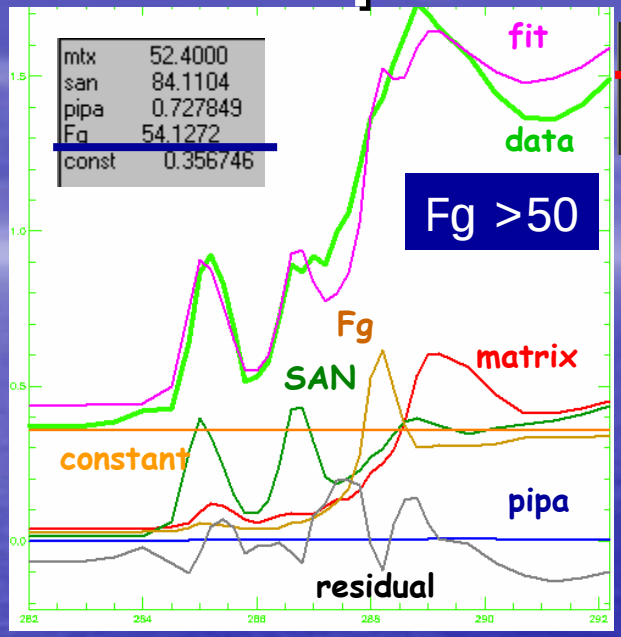


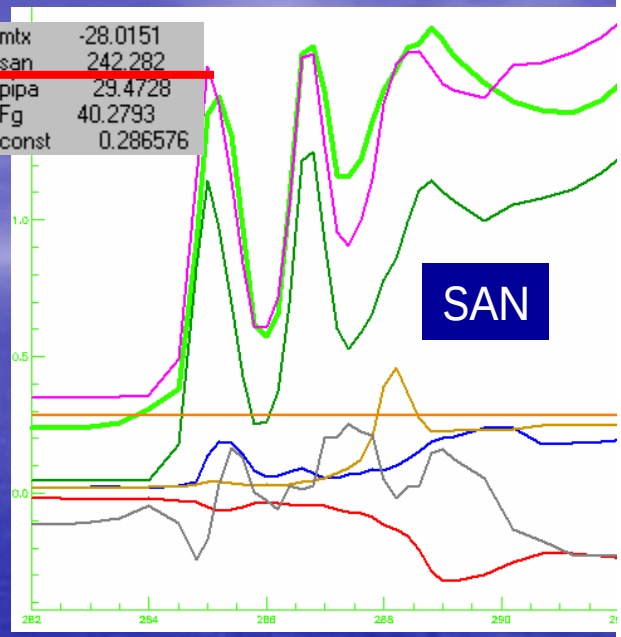
Fits to extracted spectra

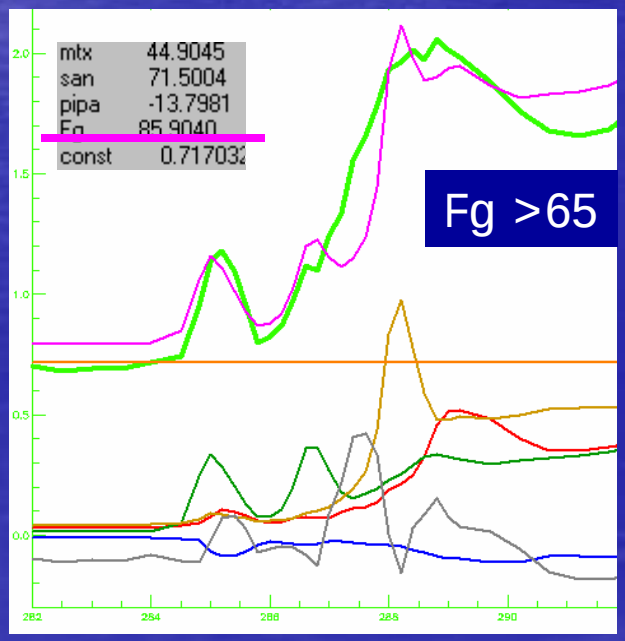


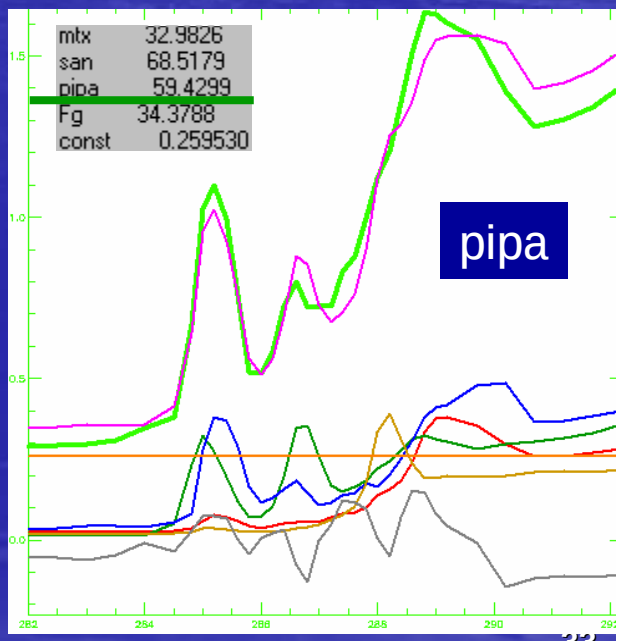
SAN >200; pipa > 45; Fg >50, Fg <65





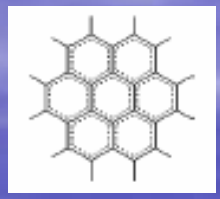


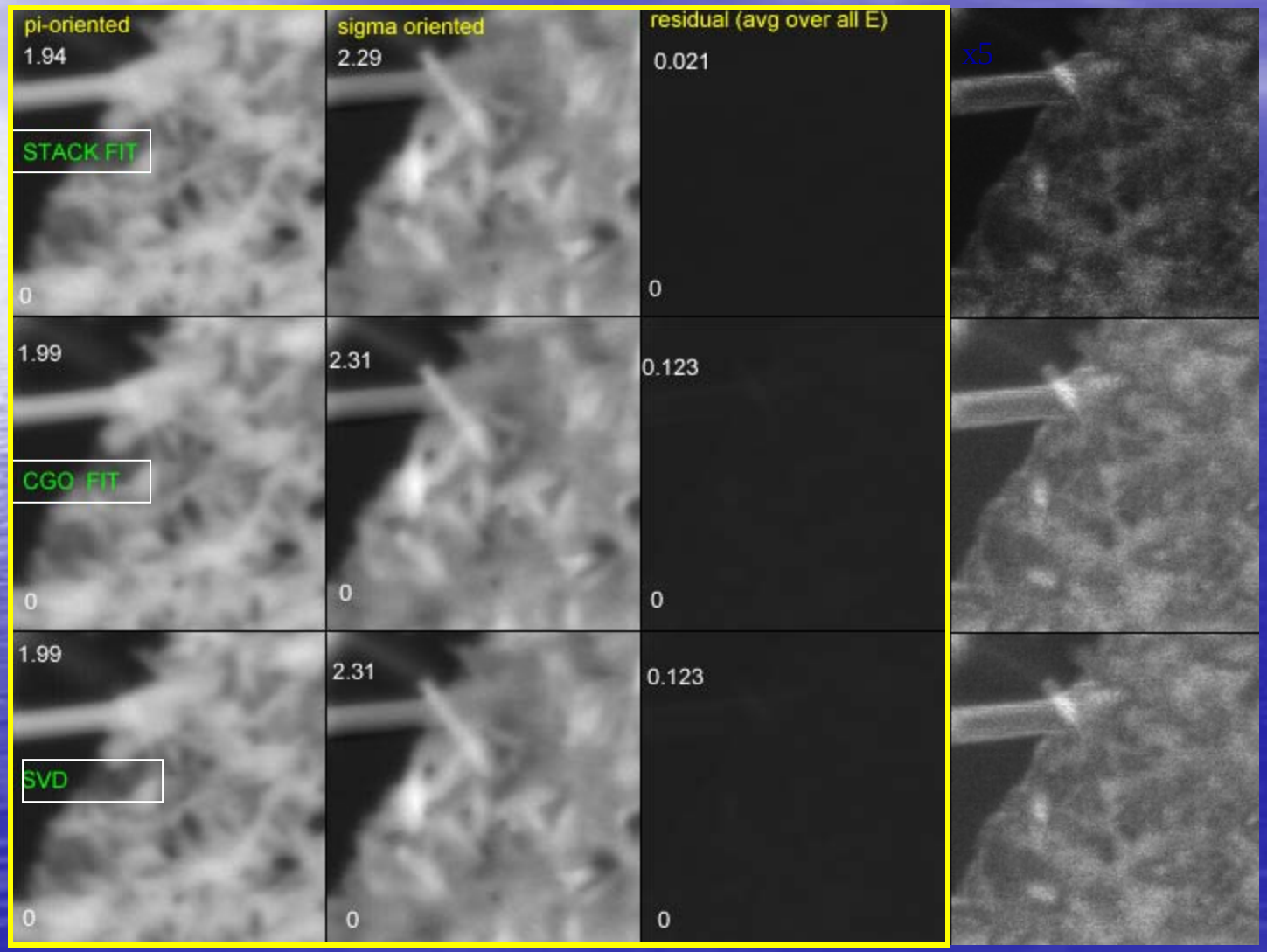


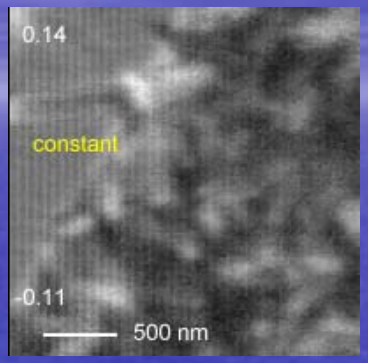


Comparison of 3-methods of deriving chemical maps in aXis2000

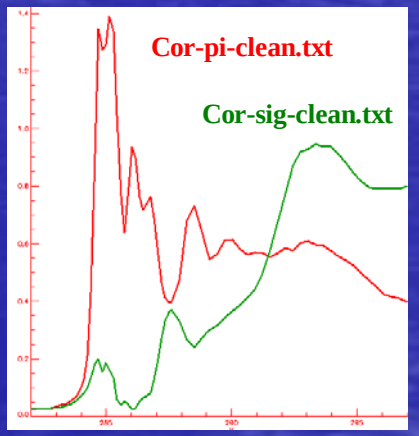
Sample : crystalline thin film of **coronene** (courtesy Ray Egerton, U. Alberta)
Data (67 images in C 1s region) recorded Jul-03 at ALS STXM532
Crystal orientation affects spectrum (linear dichroism)
Extraction of limiting in-plane and out-of-plane spectra used as reference spectra







Model spectra
(derived from stack)



no rescale overplot

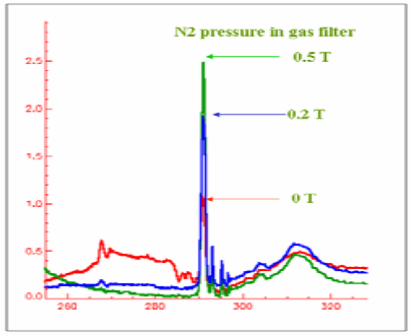
residuals rescaled

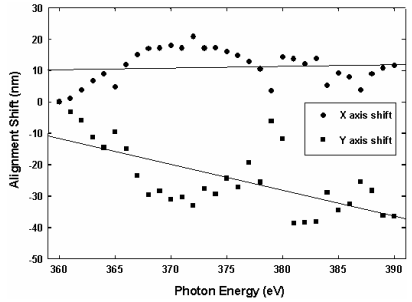
QUESTIONS ?

Some challenges of quantitative mapping

1. Quality of data (spectra, linescan, **image sequence**)

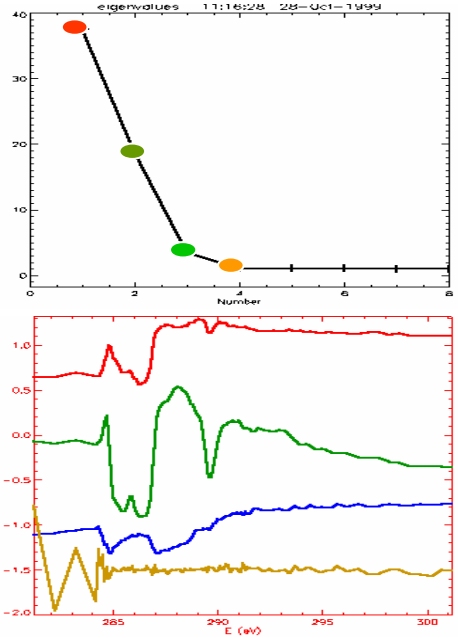
Issue	Recommendation
linearity of absorption	avoid saturation (OD < 2-3)
spectral distortion	reduce or eliminate second order
energy calibration	check regularly; calibrate
alignment	interferometry; careful alignment
radiation damage	use as small a dose as possible
linear E-scale	check with known spectra





2. Suitability & quality of reference spectra

Situation	Recommendation
chemistry well known	record spectra of same or similar pure material (eg monomer unit)
chemistry poorly known but spatially well isolated	internal models guided by external models
chemistry complex	Principle component analysis to place limits on number of components
chemistry unknown	MSA and cluster analysis Lerotic & Jacobsen, J. El. Spec. 2005, 144, 1137
chemistry unknown	internal models by trial and error



Slide courtesy of
Chris Jacobsen
SUNY Stony Brook

Rotating principal components to obtain real spectra using cluster analysis

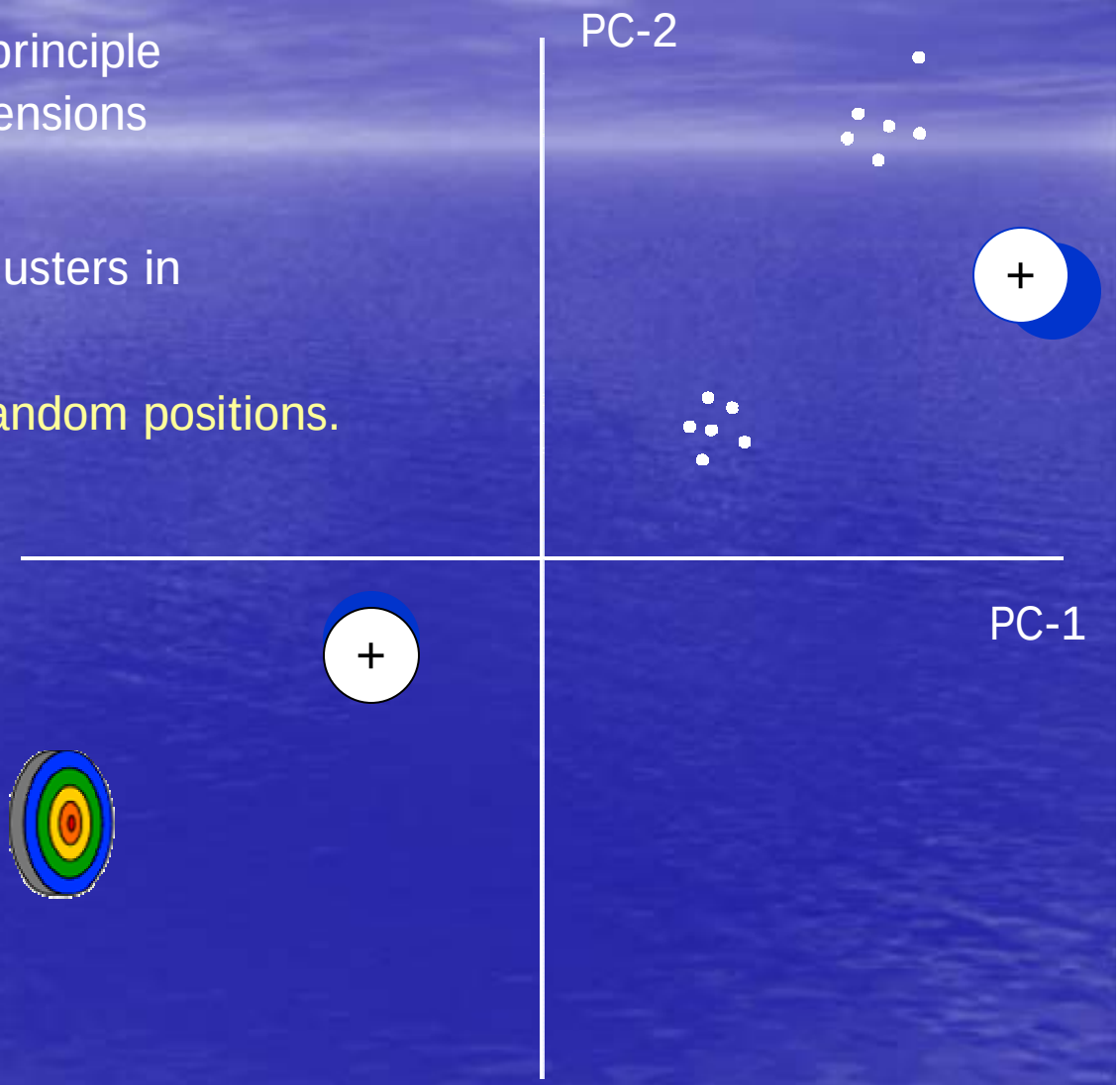
Problem: principal components are abstract. They are mixtures of the actual real spectra of the compounds present.

- PCA provides an orthogonalized representation of the data with less noise, fewer coordinates. Working with only significant components is an effective noise filter.
- We can find groupings of the data in the principal component coordinate system which relate to individual chemical components
- How ? => Cluster analysis or pattern matching

Slide courtesy of
Chris Jacobsen
SUNY Stony Brook

Cluster analysis: Euclidian distance learning algorithm

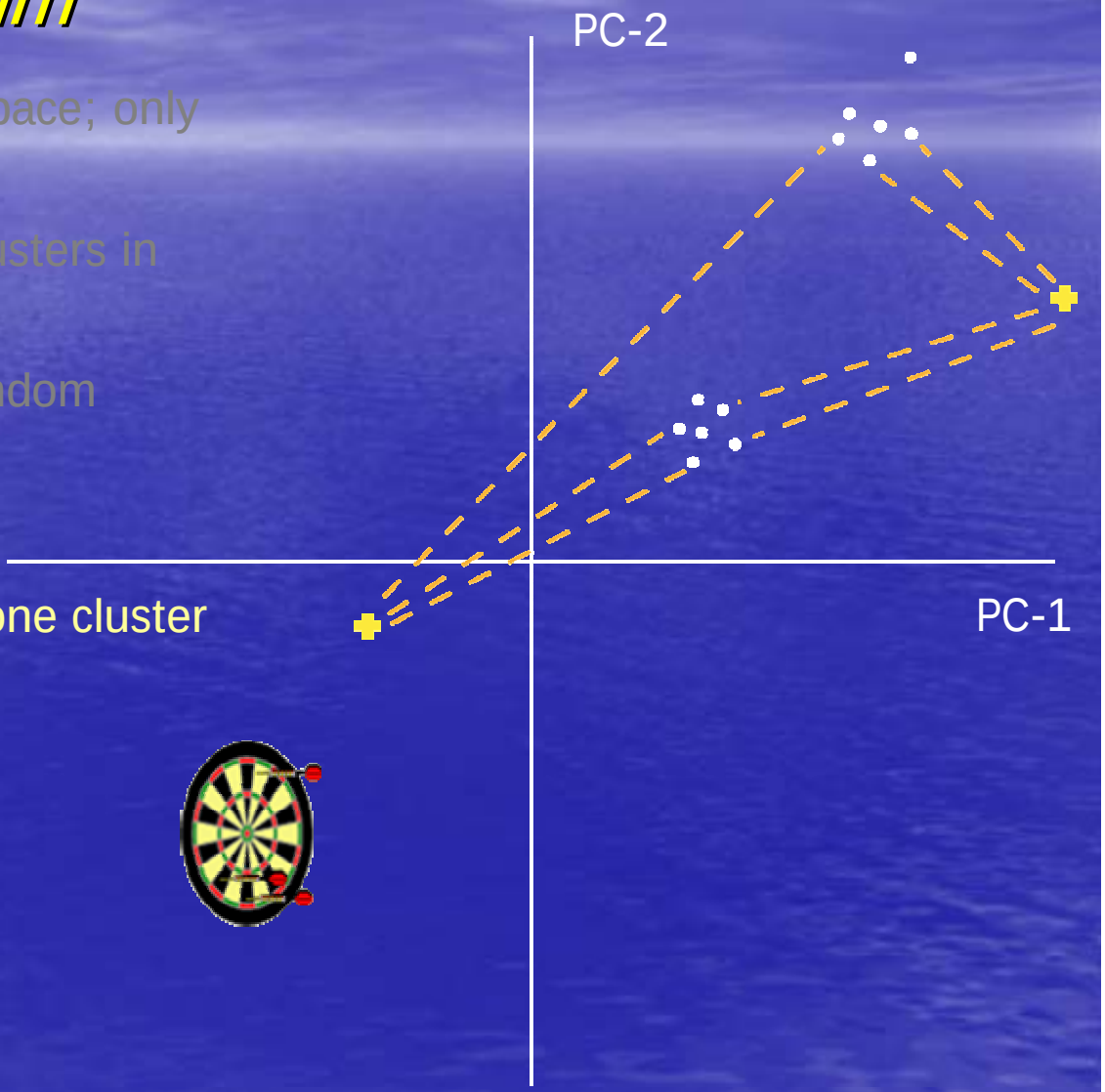
- Data are in multidimensional principle component space; only 2 dimensions shown here.
- Ideally data are arranged in clusters in this space!
- Put down cluster centers at random positions.



Slide courtesy of
Chris Jacobsen
SUNY Stony Brook

Cluster analysis: Euclidian distance learning algorithm

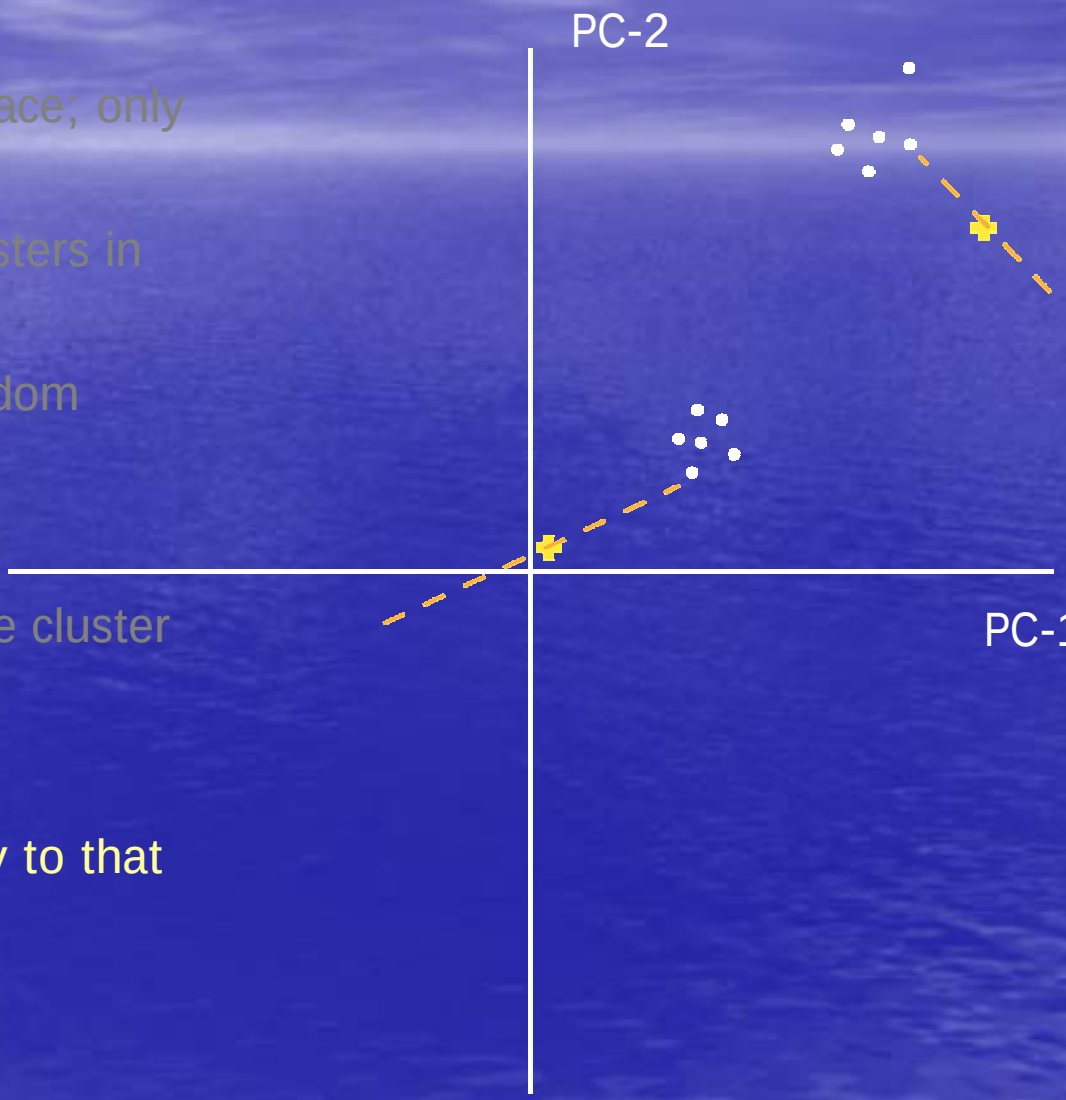
- Data are in multidimensional space; only 2 dimensions shown here.
- Ideally data are arranged in clusters in this space!
- Put down cluster centers at random positions.
- Iterate:
  - Calculate distances from one cluster center to all data points.
  - Pick shortest distance.



Slide courtesy of
Chris Jacobsen
SUNY Stony Brook

Cluster analysis: Euclidian distance learning algorithm

- Data are in multidimensional space; only 2 dimensions shown here.
- Ideally data are arranged in clusters in this space!
- Put down cluster centers at random positions.
- Iterate:
  - Calculate distances from one cluster center to all data points.
  - Pick shortest distance.
  - Move cluster center partway to that nearest point.



Slide courtesy of
Chris Jacobsen
SUNY Stony Brook

Cluster analysis: Euclidian distance learning algorithm

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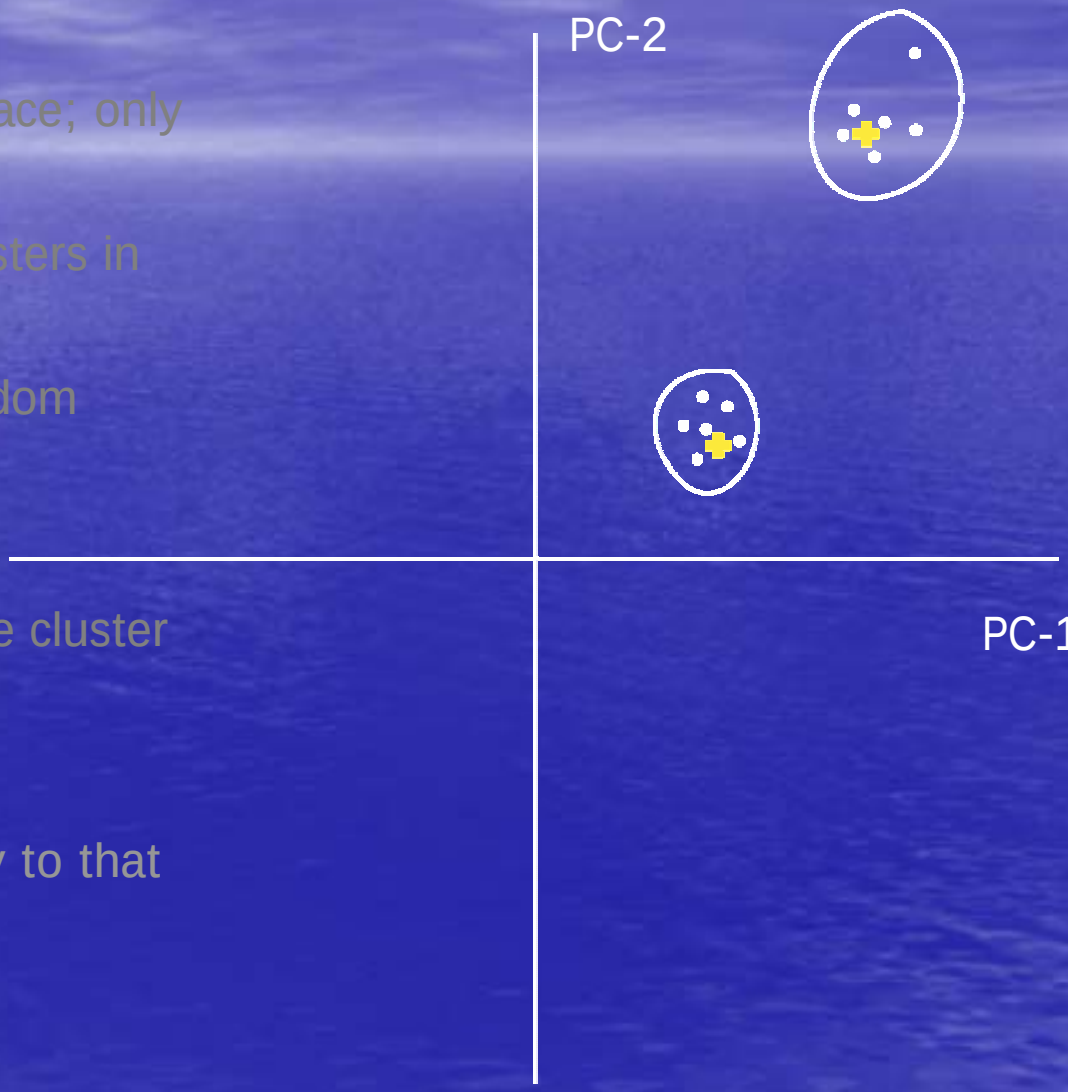
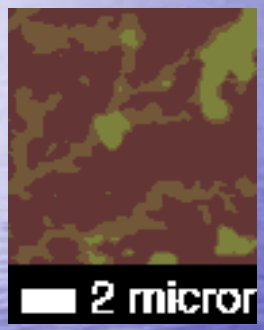
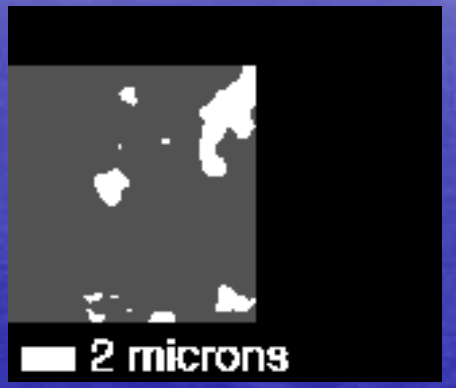


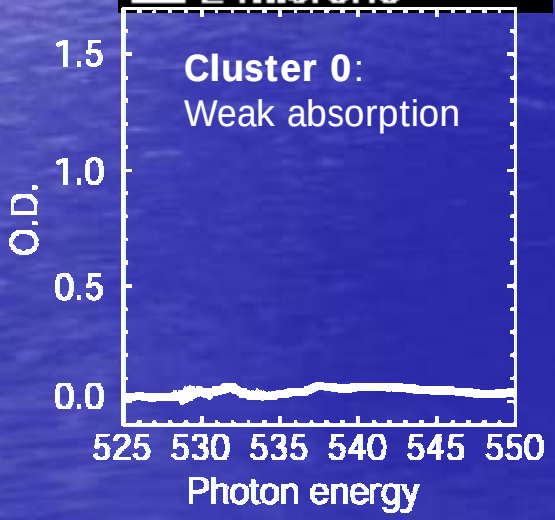
Slide courtesy of
Chris Jacobsen
SUNY Stony Brook

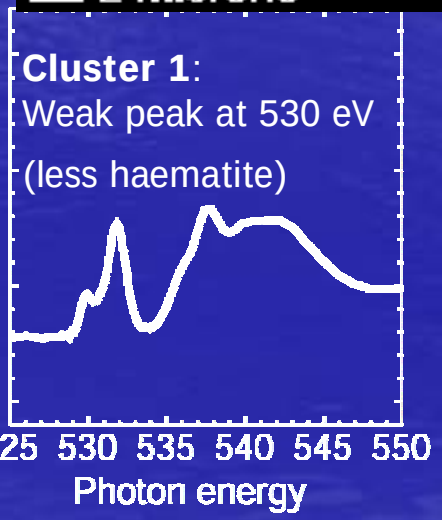
Lu/Haematite structural incorporation

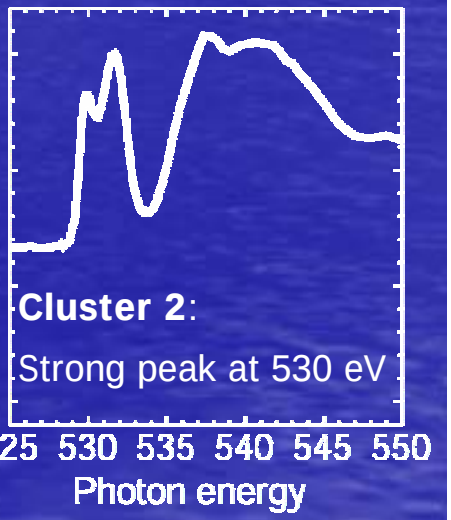
- *T. Schäfer*, INE Karlsruhe
- Lu as homologue for Am, Cm
- 5% Lu in Haematite: transformation in solution, rinsed, dried
- Cluster analysis of O 1s stack ➡ Lu incorporation in Haematite.







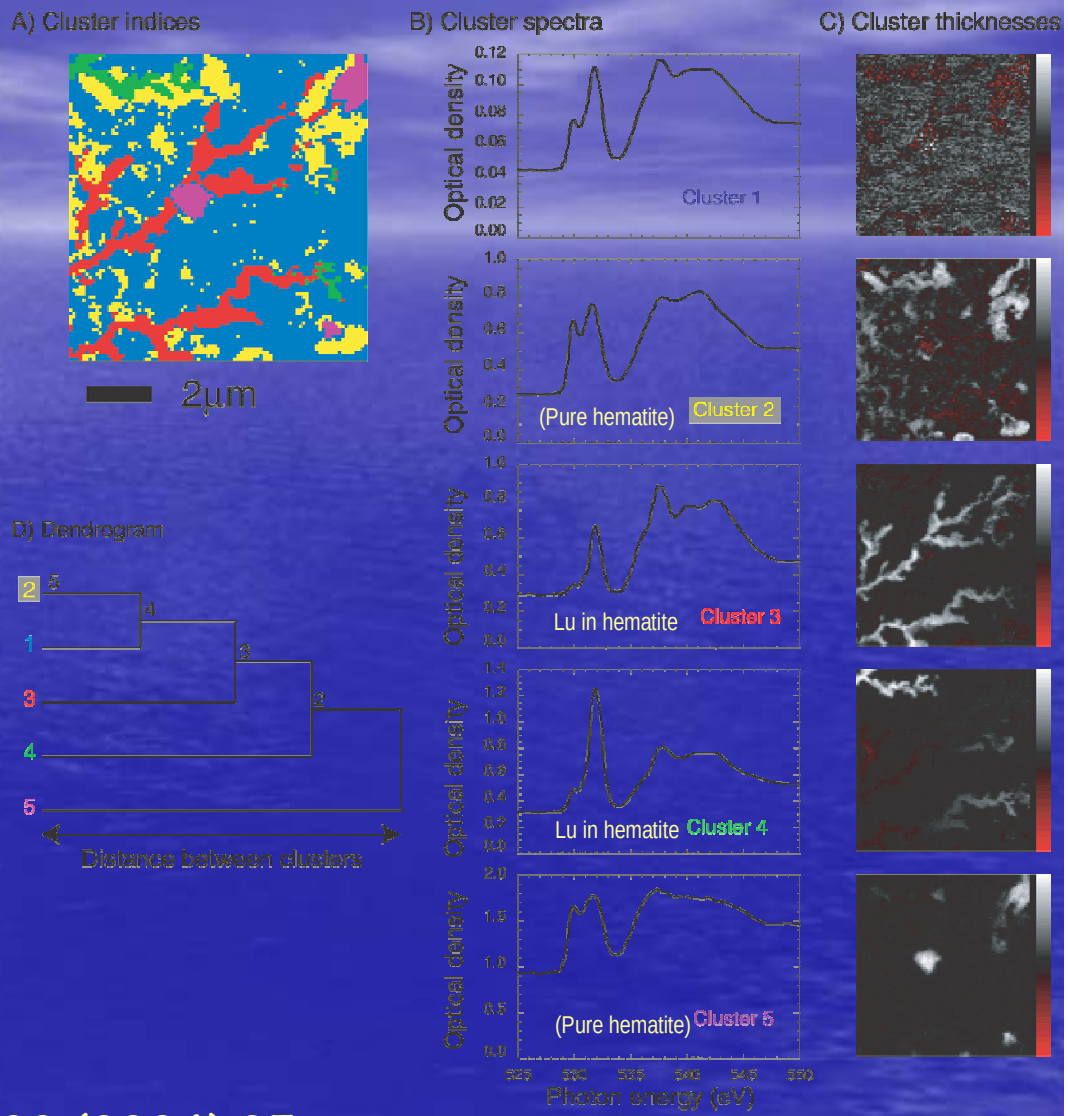




Slide courtesy of
Chris Jacobsen
SUNY Stony Brook

Cluster analysis of spectromicroscopy data

- Study of Lu (stand-in for Am) incorporation in hematite (groundwater transport). T. Schäfer *et al.*, INE Karlsruhe
- Oxygen near-edge spectra: molecular orbital availability, occupancy. Acquire ~150 images across edge.
- Cluster analysis: reveals spectroscopic themes even with a complex, unknown specimen.

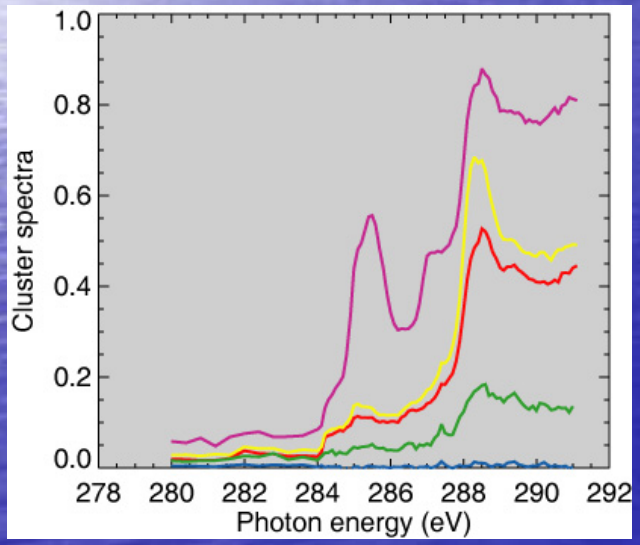


M. Lerotic *et al.*, *Ultramicroscopy* 100 (2004) 35

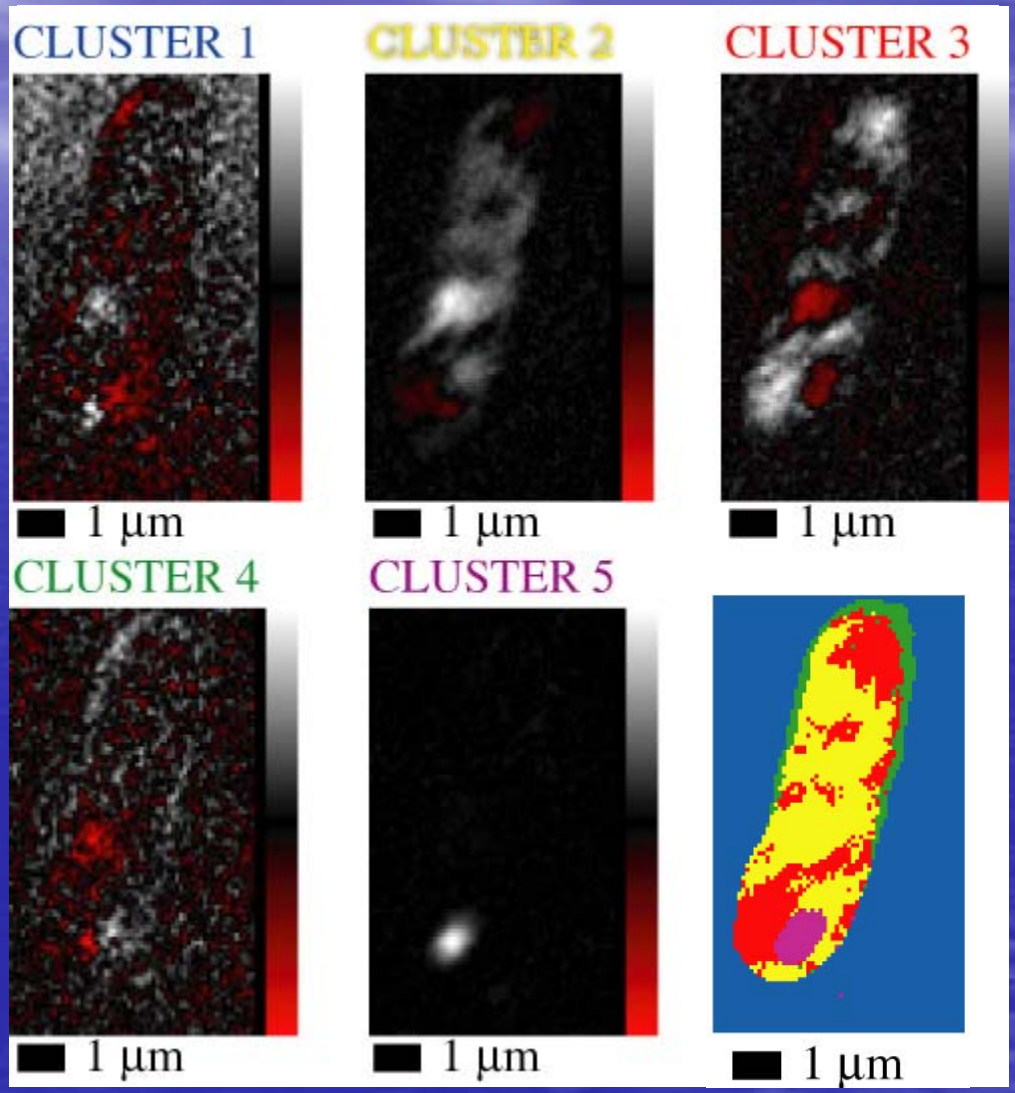
Slide courtesy of
Chris Jacobsen
SUNY Stony Brook

Angle metric improves classification

- Problem: Euclidean clustering confuses composition and thickness information
- Recent advances: use **angle distance measure** to classify based on compositional variations only (insensitive to thickness)
- **Specimen: a spore in a uranium-reducing bacterium (J. Gillow, A.J. Francis, BNL)**
- Use PCA and angle metric clustering to find representative spectra



- SVD with these internal spectra used to derive thickness mapds

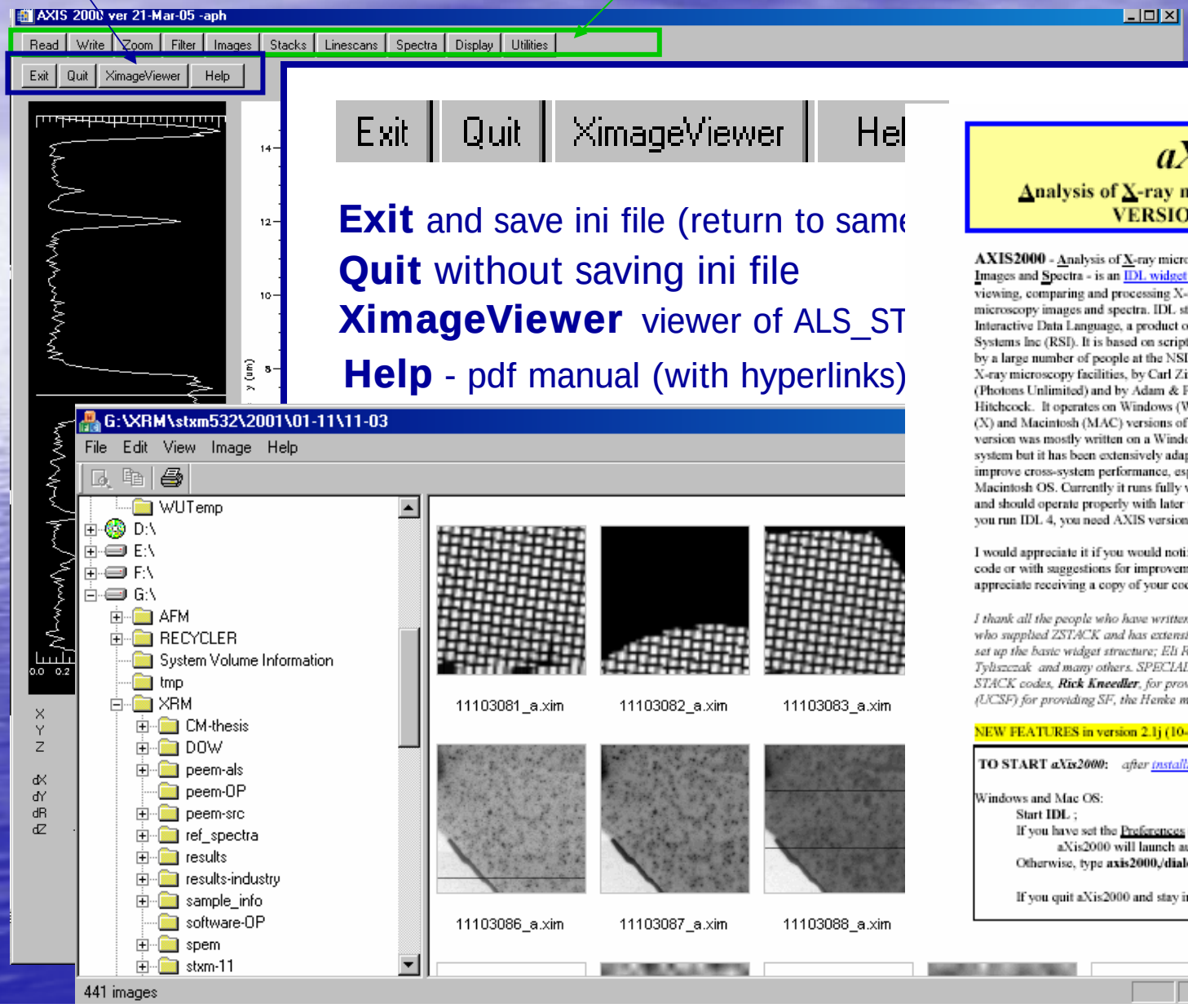


Details: **M. Lerotic** and C. Jacobsen, J .Electron Spectrosc.144 (2005) 1137

Features of aXis2000 widget (1)

single action menus

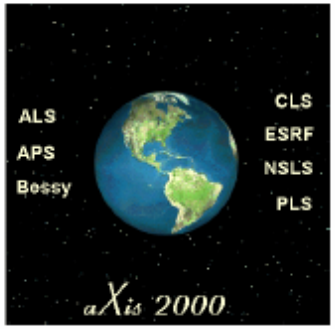
Pull-down menus



aXis 2000

Analysis of X-ray microscopy Images and Spectra
VERSION 2.1k (12-May-02)

AXIS2000 - Analysis of X-ray microscopy Images and Spectra - is an IDL widget for viewing, comparing and processing X-ray microscopy images and spectra. IDL stands for Interactive Data Language, a product of Research Systems Inc (RSI). It is based on scripts developed by a large number of people at the NSLS and ALS X-ray microscopy facilities, by Carl Zimba (Photons Unlimited) and by Adam & Peter Hitchcock. It operates on Windows (WIN), Unix (X) and Macintosh (MAC) versions of IDL. This version was mostly written on a Windows-98 system but it has been extensively adapted to improve cross-system performance, especially for Macintosh OS. Currently it runs fully with IDL 5.2 and should operate properly with later versions If you run IDL 4, you need AXIS version 1.6a or earlier.



I would appreciate it if you would notify me by email (aph@mcmaster.ca) about problems with the code or with suggestions for improvements. If you make extensions or corrections, I would appreciate receiving a copy of your code revisions to incorporate in future versions.

*I thank all the people who have written scripts that went into this. **Carl Zimba** (Photons Unlimited) who supplied ZSTACK and has extensively improved the package overall; my son, **Peter** who helped set up the basic widget structure; Eli Rotenberg, Jonathan Denlinger, Stefano Cerasari, Tolek Tyliszczak and many others. SPECIAL thanks to **Chris Jacobsen** (Stony Brook, nsls) for sharing his STACK codes, **Rick Kneedler**, for providing the basis for the **stack-fit** routine, and **Billy Loo** (UCSF) for providing SF, the Henke mass absorption routine.*

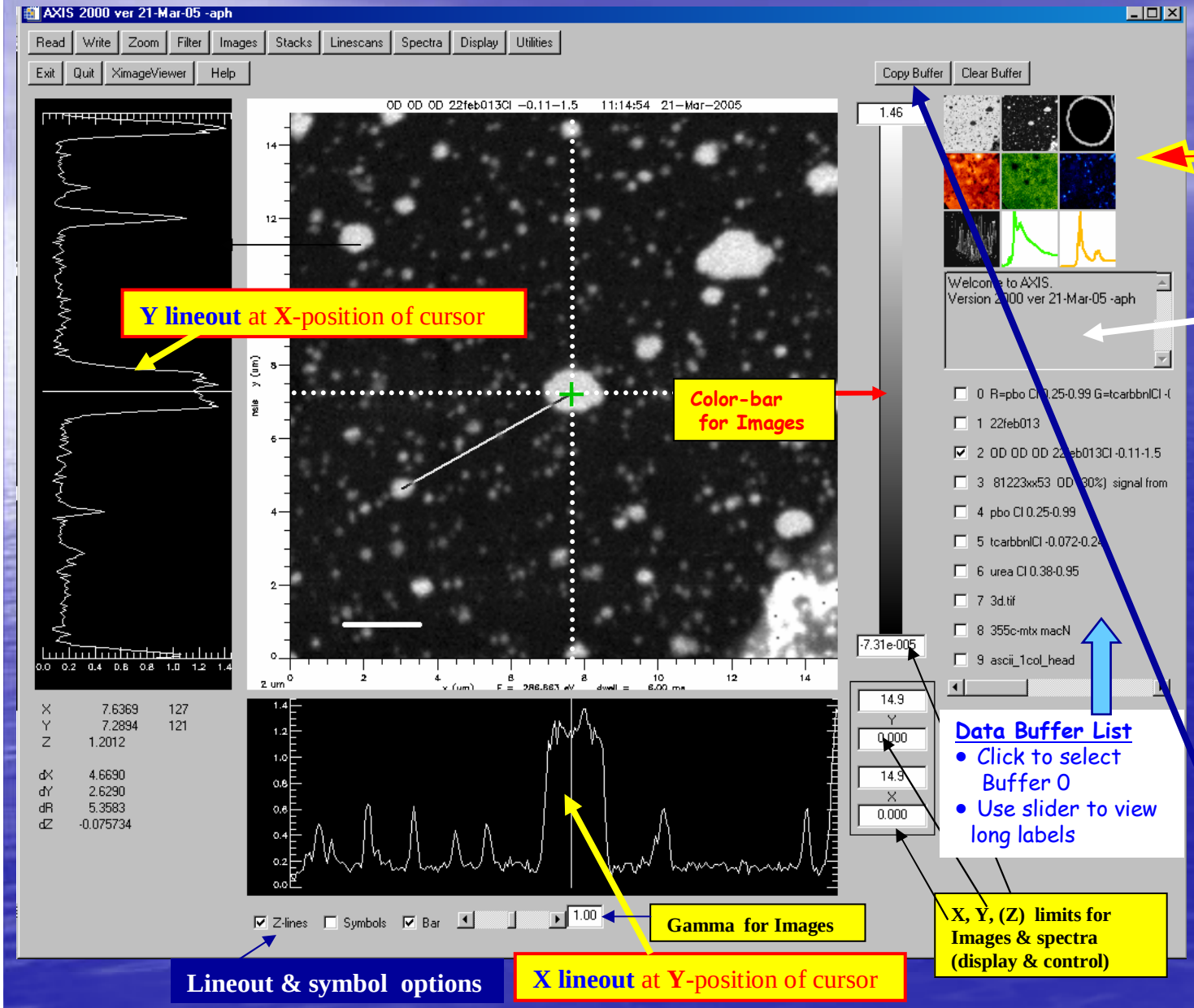
NEW FEATURES in version 2.1j (10-may-02) since 2.1j (28-Feb-02) are HIGHLIGHTED

TO START *aXis2000*: *after installing aXis2000* (see end of this file)

Windows and Mac OS:
Start **IDL** ;
If you have set the Preferences (in IDL) so that **axis2000_batch.pro** is the start file, aXis2000 will launch automatically.
Otherwise, type **axis2000,/dialog** on the IDL command line.

If you quit aXis2000 and stay in IDL, you can restart AXIS by typing **axis2000**

Features of aXis2000 widget (1)



Concept

9 'permanent' data buffers

any process that modifies data places the result in buffer 0 (the 'working' buffer)

if you want to SAVE that result you must Copy it to a permanent buffer

Features of aXis2000 widget (2)

Main Image

- Displays currently selected image or selected spectrum (or groups of either 4 or 9 buffers, if Display~Thumbnails used)
- Size of AXIS can be adjusted from **0.5 to 2.0** of its nominal size (360x360 pixels in Main Image) by size parameter in **axis.ini**

Mouse

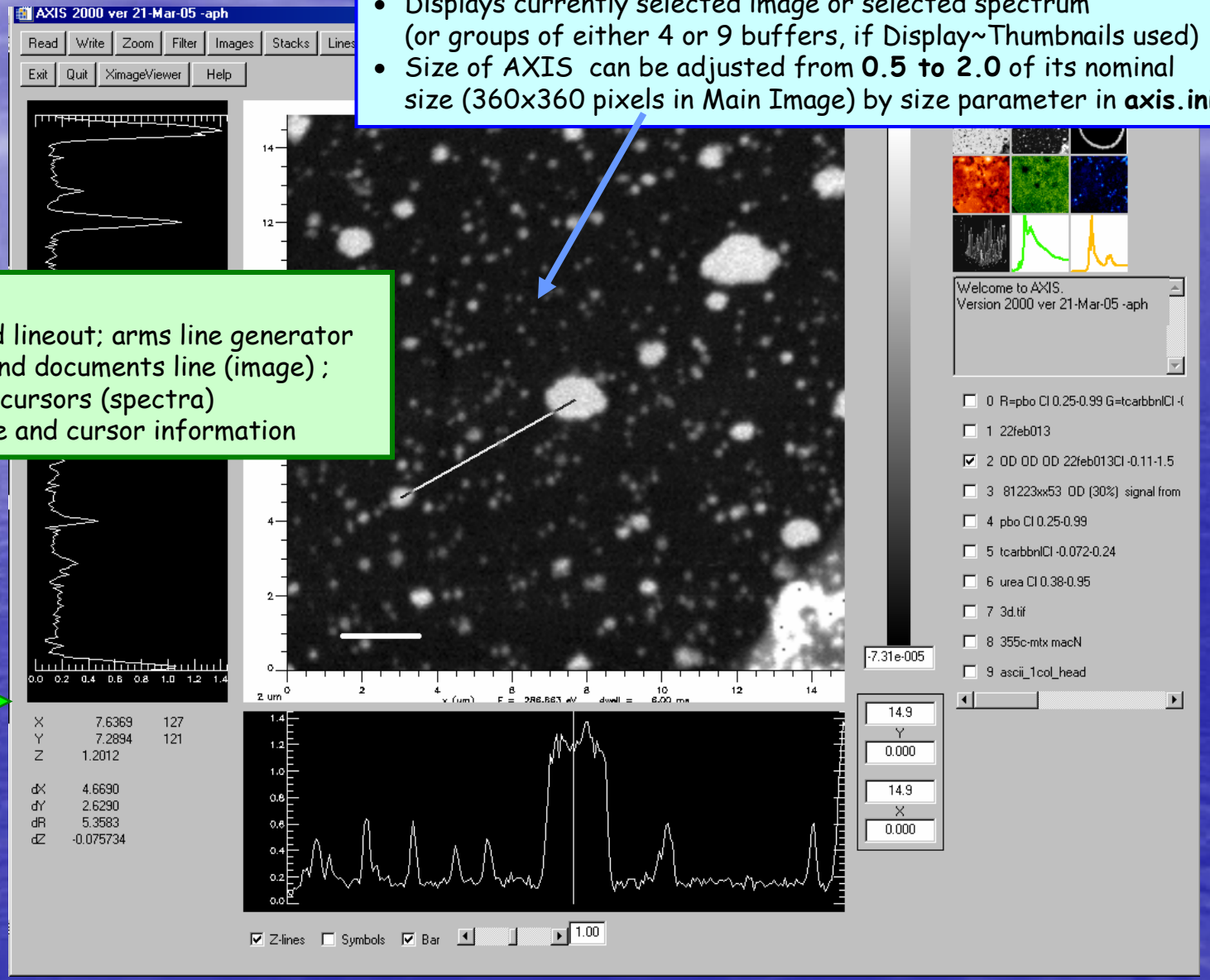
- First click – cursor and lineout; arms line generator
- Second click – draws and documents line (image) ; - reports difference in cursors (spectra)
- Third click – clears line and cursor information

Cursors

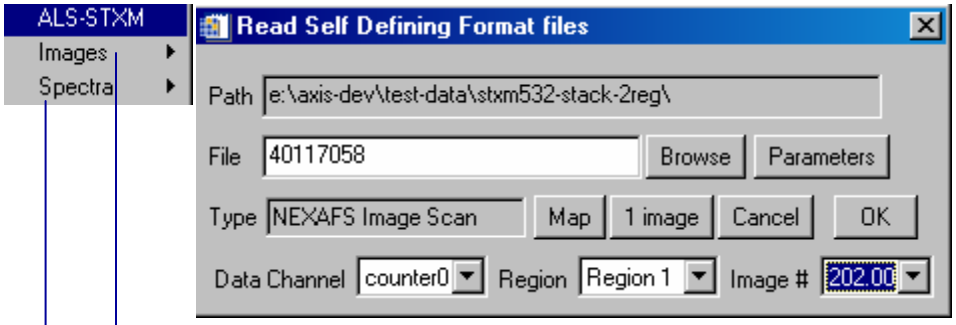
(X,Y,Z) – at cursor pixel indices

(dX,dY,dZ) – change over line (images) or between cursors (spectra)

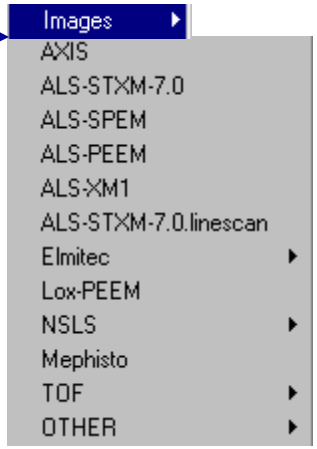
dR – distance along line (images only)



Pull-down menus (1 of 6)

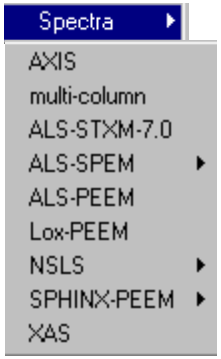


reads all types of ALS STXM files
(images, spectra, stacks, motors, DAQ …)



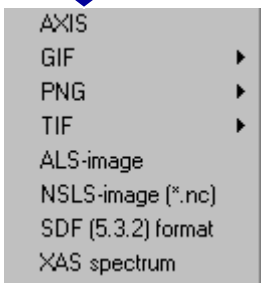
reads image data of many other types of X-ray microscopes
- Axis - internal format
- ALS - PEEM, SPEM, XM1
- SRC - PEEM (Sphinx, Mephisto)
- CLS, SLS, SRC - Elmitec
- CLS - TOF (time-of-flight)

and standard image types
(BMP, PNG, GIF, TIF)



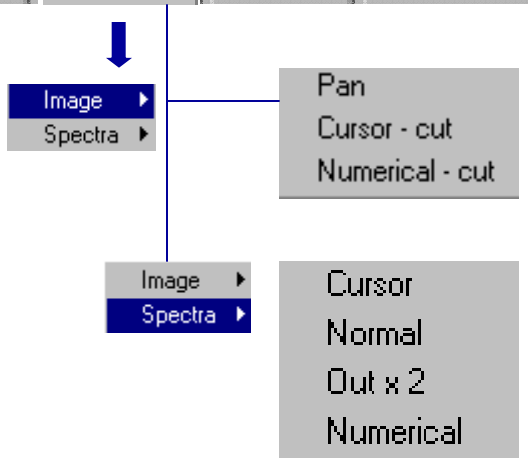
reads many other types of spectral data
- Axis - internal format
- ALS - PEEM, SPEM
- SRC - PEEM (Sphinx, Mephisto)
- CLS - Lox (acq. program for Elmitec)
- CLS - TOF (time-of-flight)
- NSLS - STXM

Pull-down menus (2 of 6)



writes various types of data files

- Axis - internal format (spectra = ascii; images = binary)
- GIF, PNC, TIF - standard image formats
- ALS-image - old (77.0.1) format
- NSLS-image - old and current formats for X1a STXM
- SDF - self defining format used for ALS STXM
- XAS spectrum - fully documentable spectral file (for reference standards)



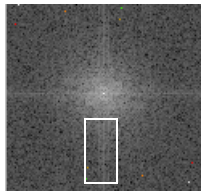
expanded views of currently displayed buffer

Numerical option allows precision extraction of identical regions of images or spectra

Pull-down menus (3 of 6)

- Smooth
- Median
- Lee Filter
- Convol
- Clean (FT image filter)

4 different smoothing routines with adjustable parameters
(apply to both images and spectra)

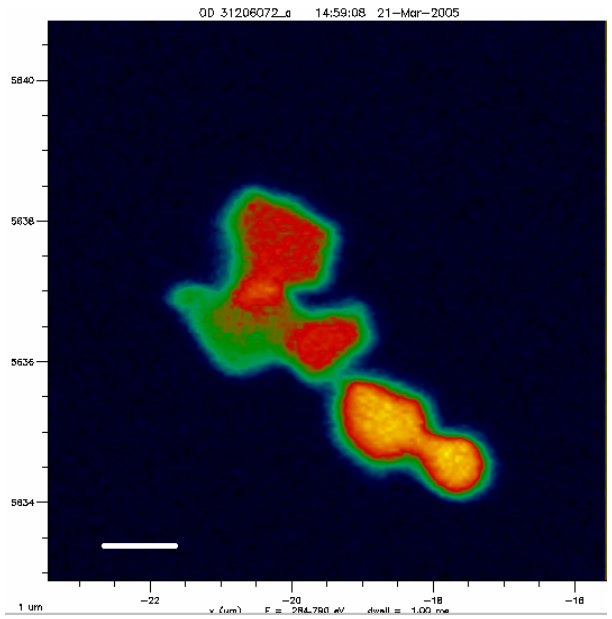


box used to identify signals in 2d FT to be removed. Systematic noise is generally sharp lines)

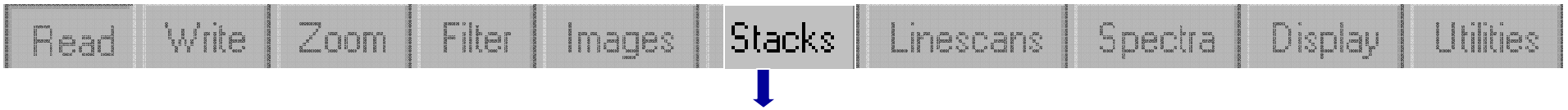
many procedures useful to manipulate images

see manual (accessed by **Help** command in aXis2000) for details

- Add
- Average pixels
- Calibrate XY
- Clip signal
- Convert_to_OD
- Cross-link density
- Deglitch
- Delete region
- Distort XY scale
- exp(Z)
- Fix rollover
- Gain
- histogram
- ln(Z)
- log10(Z)
- generate mask
- Modify one point
- Multiply buffers
- Particle analyze
- Pixels only
- Profiles
- Ratio to
- Remove zeros
- Replace line
- Resize
- RGB composite
- Rotate
- Set XY scale
- Warp

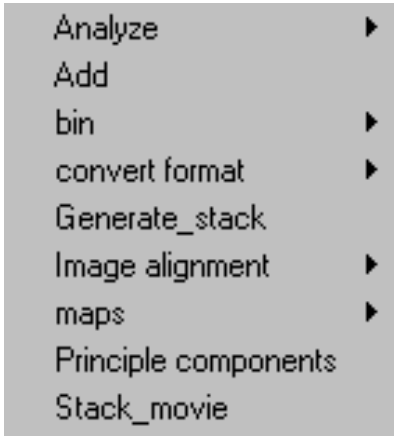


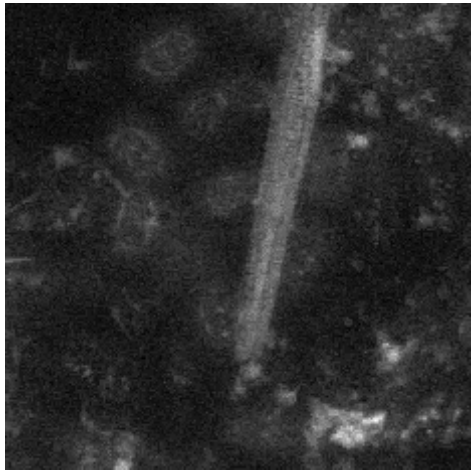
Pull-down menus (4 of 6)

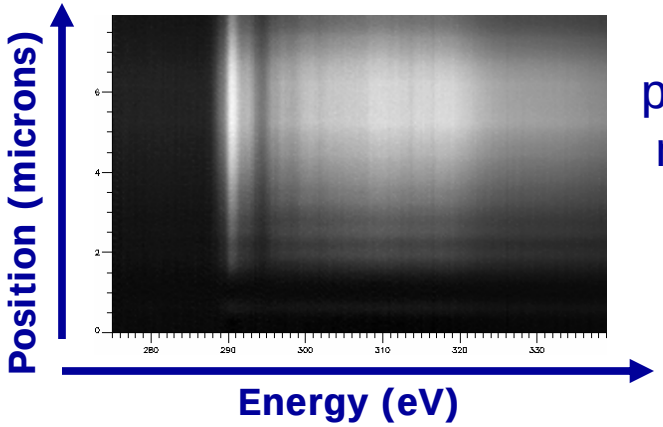


manipulation of image sequences ("STACKS")

see Help file in aXis2000 for details

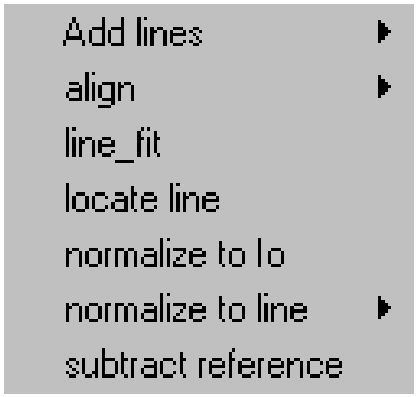




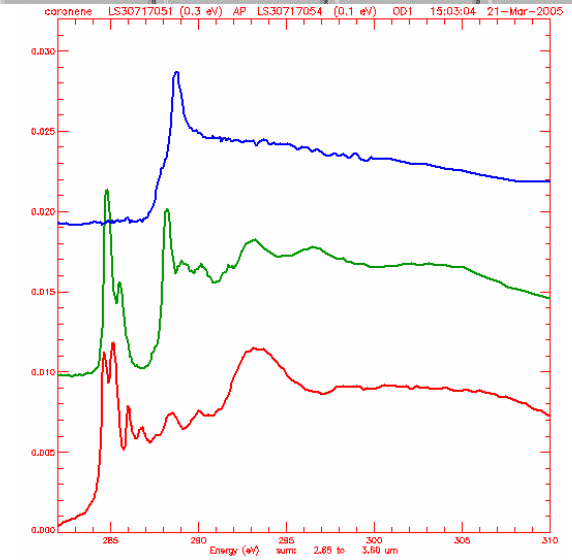


procedures useful to manipulate linescan spectra

see Help file in aXis2000 for details



Pull-down menus (5 of 6)



manipulation of spectra

see Help file in aXis2000 for details

- Absolute value
- Add ▸
- Calibrate ▸
- Curve fit ▸
- Delete
- Differentiate
- Gain ▸
- E_to_Wavelength
- exp(Y)

- Integrate
- ln(Y)
- log10(Y)
- Linear Background
- Modify one point
- Multiply
- Peak area
- Ratio_to
- Reverse values
- Split
- Truncate

Utilities

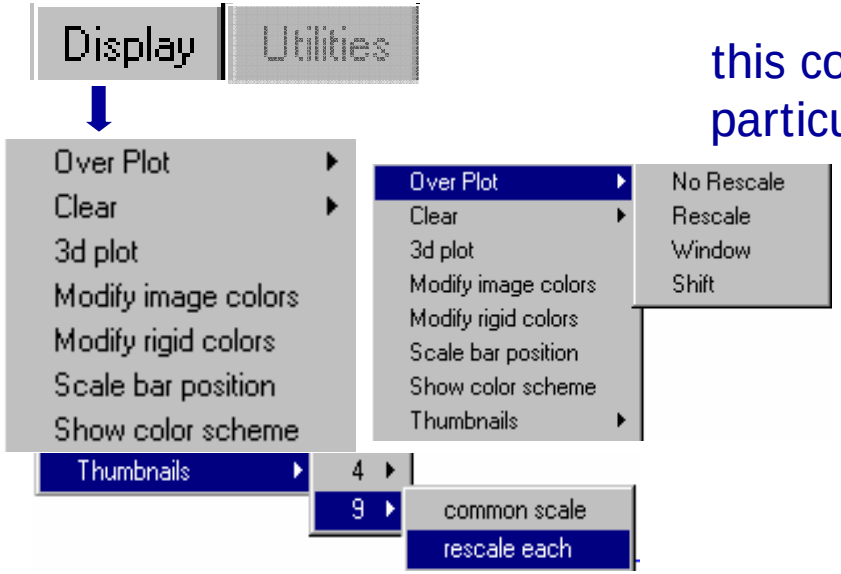
- Print ▸
  - Logbook
  - Annotated
- Change label

miscellaneous procedures

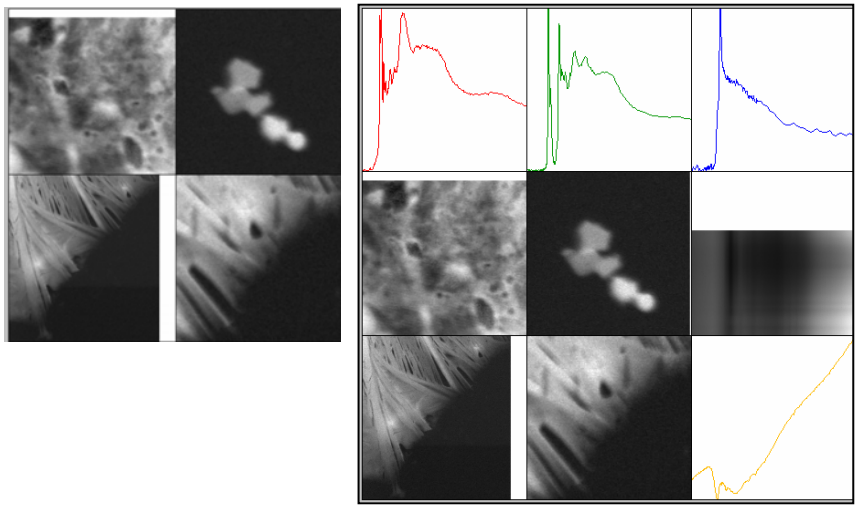
see Help file in aXis2000 for details (modify rigid colors does not yet work)

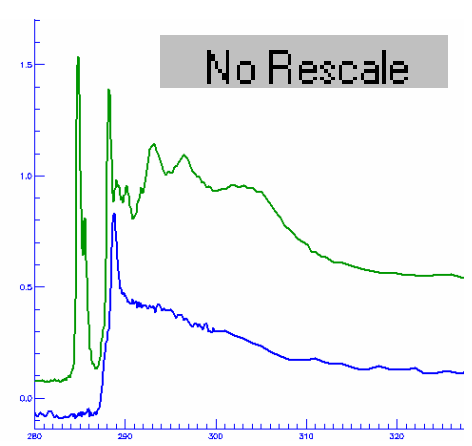
- Print ▸
- Change label
- Change mesh
- Calculate X-ray parameters (SF)
- Execute macro
- Set energy
- Set preferences
- Print SysVar
- Write image ascii ▸

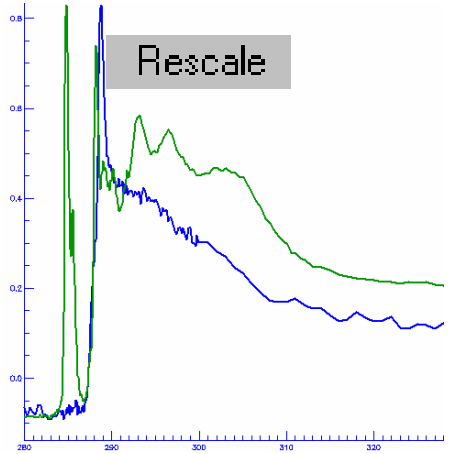
Pull-down menus (6 of 6)

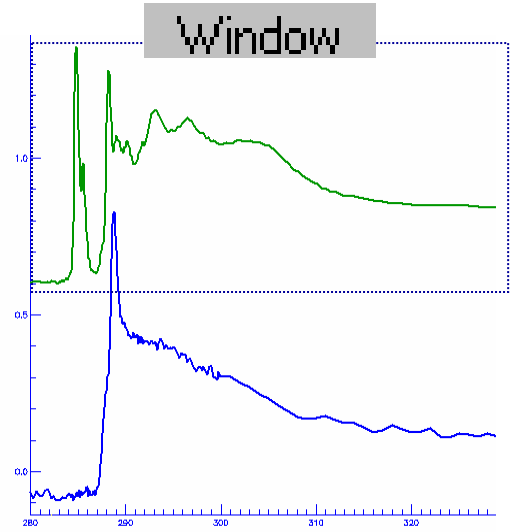


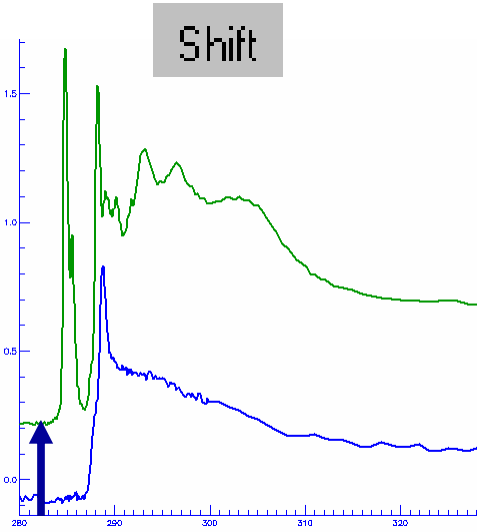
this command menu is used EXTENSIVELY, particularly **OverPlot** and **Thumbnails**











Discussion of possible applications of STXM or X-PEEM