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 .....


```
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
```

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

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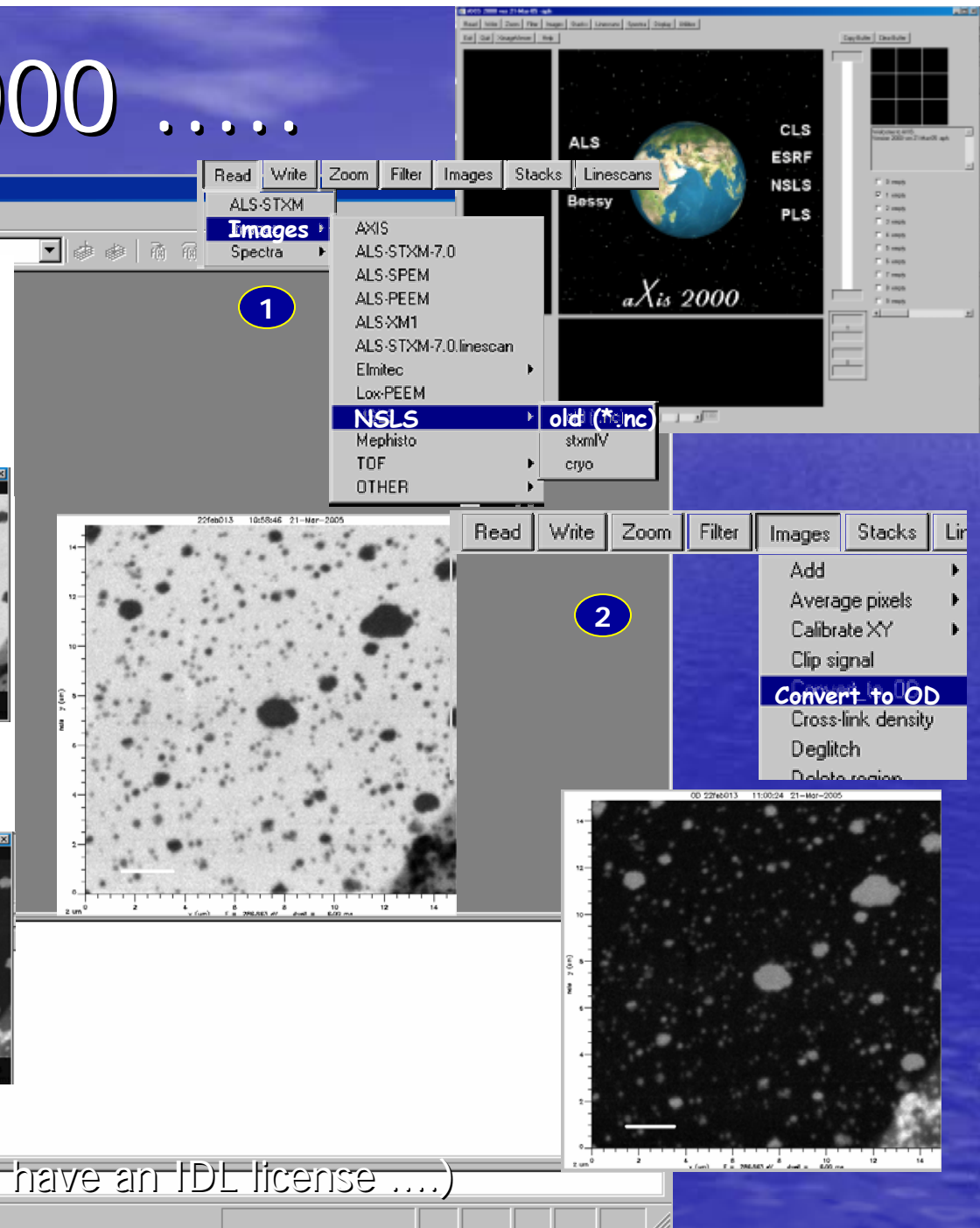
```
IDL>
IDL>
IDL>
```

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

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
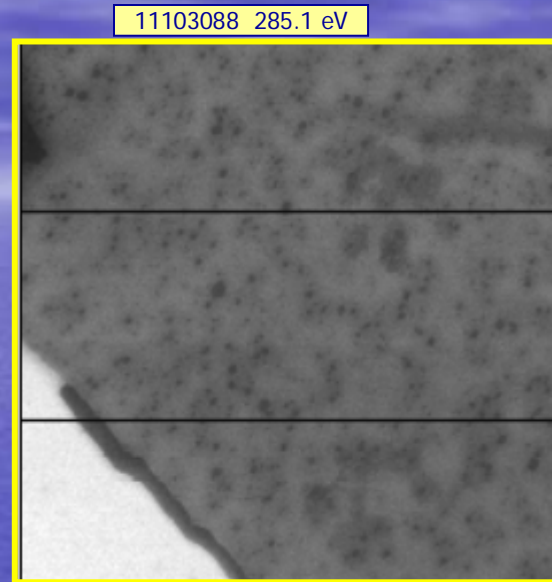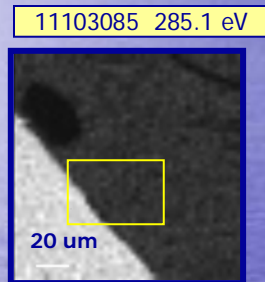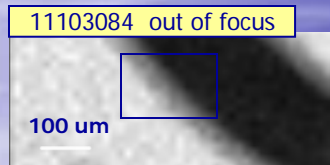up the user interface to the 5.3 STXM.

references:

[1] A.P. Hitchcc   leen, 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

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A) Locating region of interest

B) Multi-point spectra

C) Linescan spectra (& damage check)

Line
Edge of section thicker

pipa

SAN

matrix

After
linescan

15 msec / point
at end of fill

Linescan fit analysis

total

matrix

SAN

pipa

NB models from
pure 355 stack
no meaning to
vertical scale
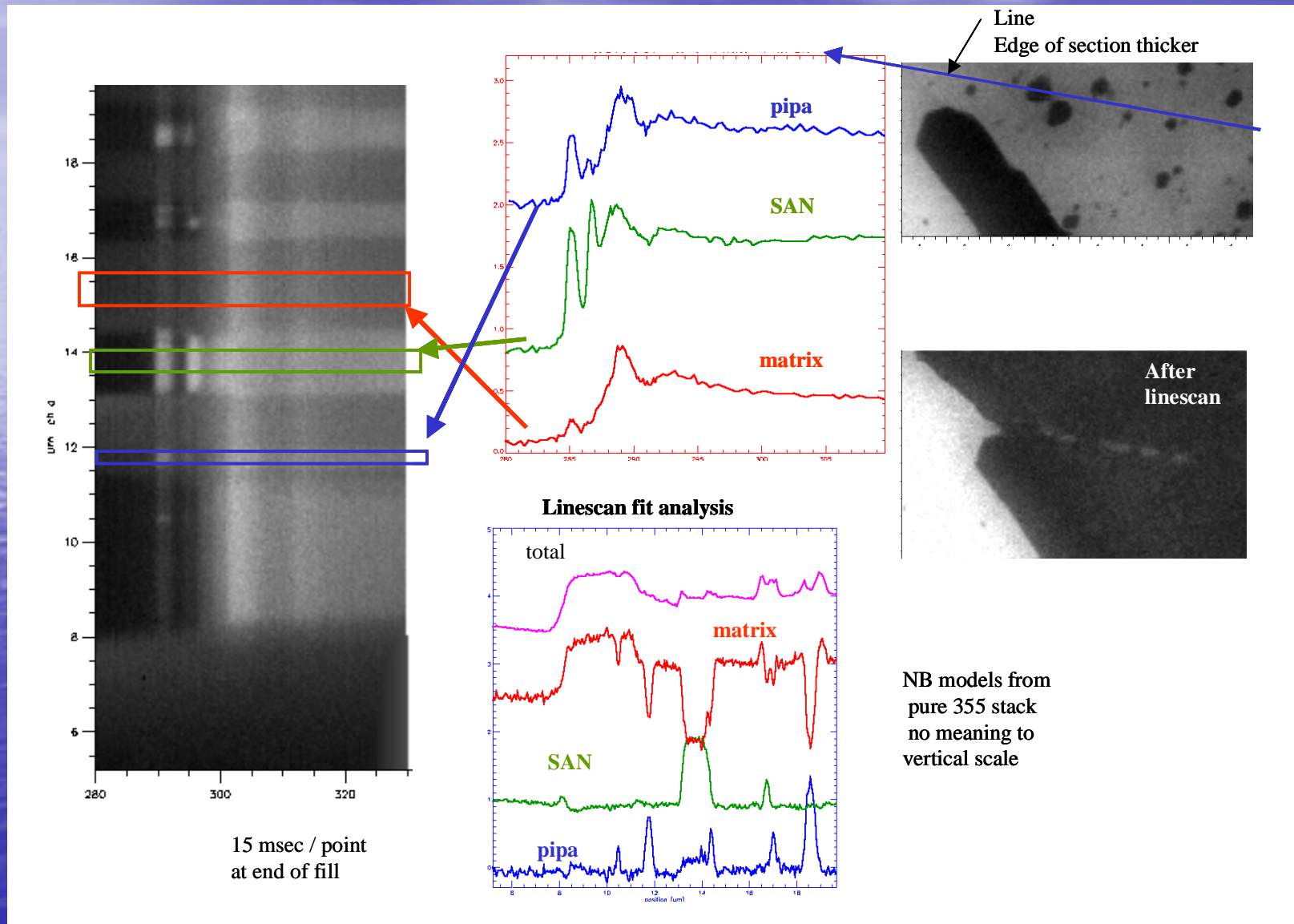
D) Image sequence (& damage check)

Io area

20 um
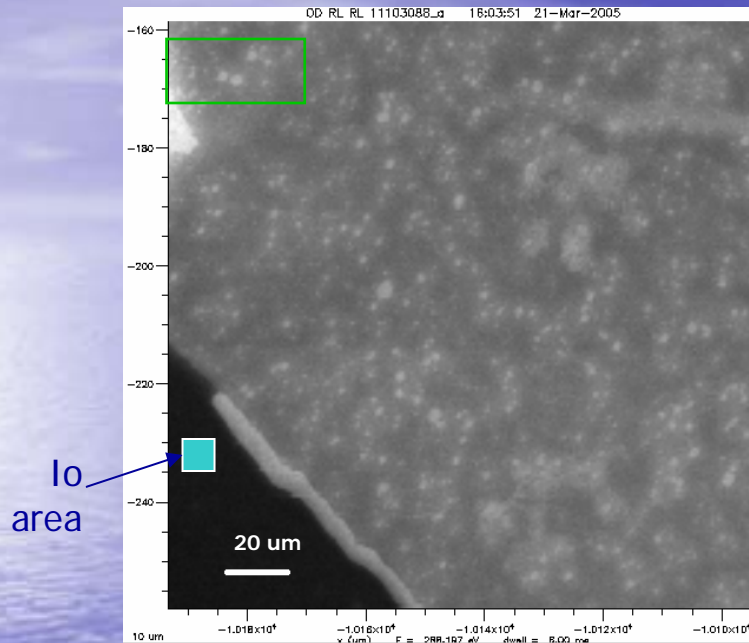
Area selected for stack measurement

11103089 285.1 eV

2 um
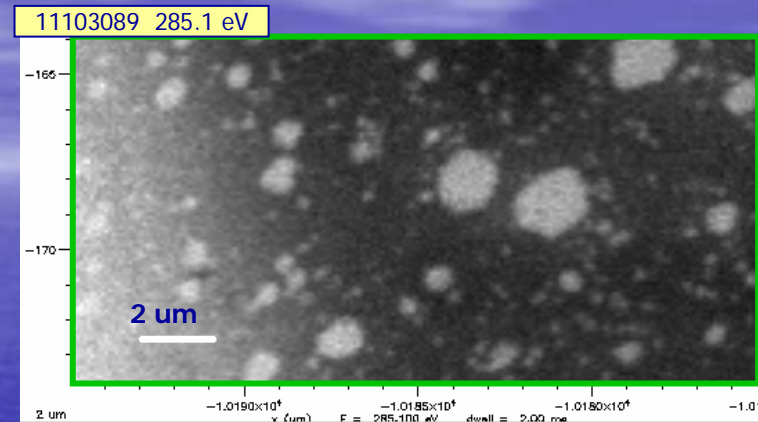
the bright area to the left is known to be a protein deposit so we wanted to be able to get a clear Fg spectral signature to check the analysis in the more dilute regions
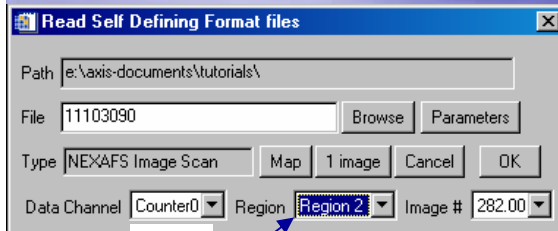
11103091 289.0 eV

2 um
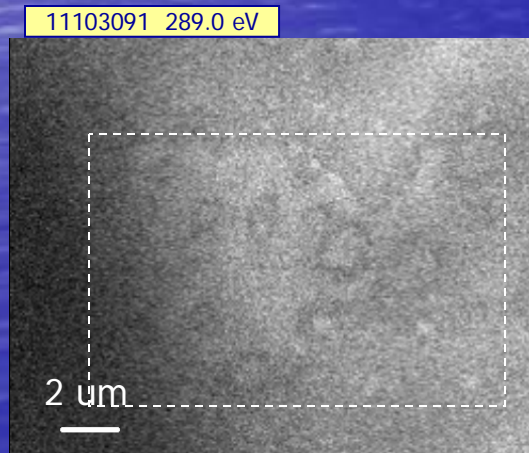
DAMAGE CHECK

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

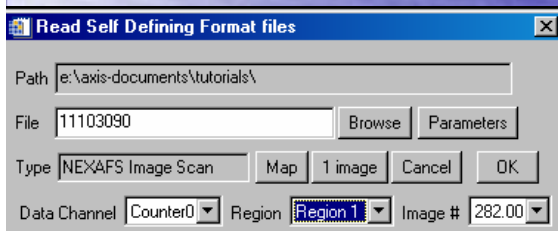little sign of damage (usually polyether matrix bleaches due to mass loss)

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C1s stack - read-in and convert to OD
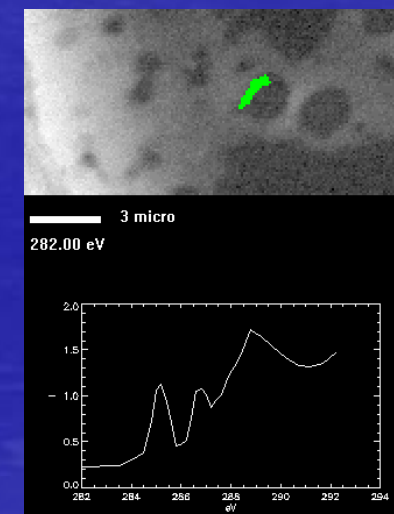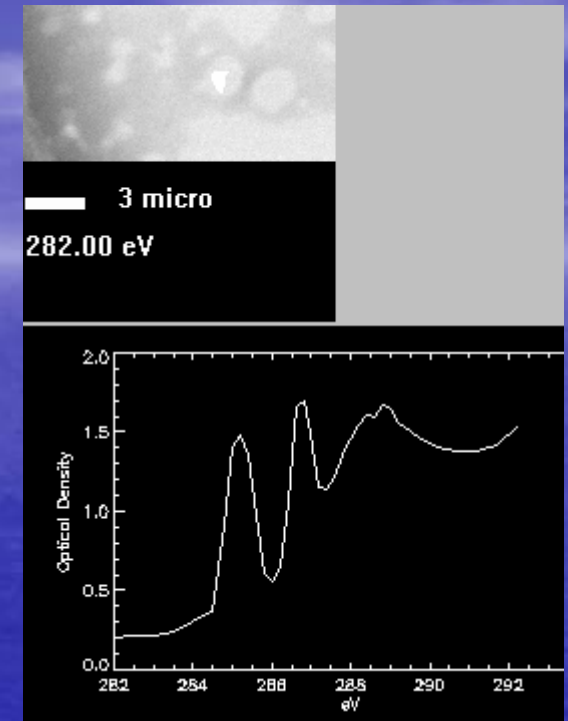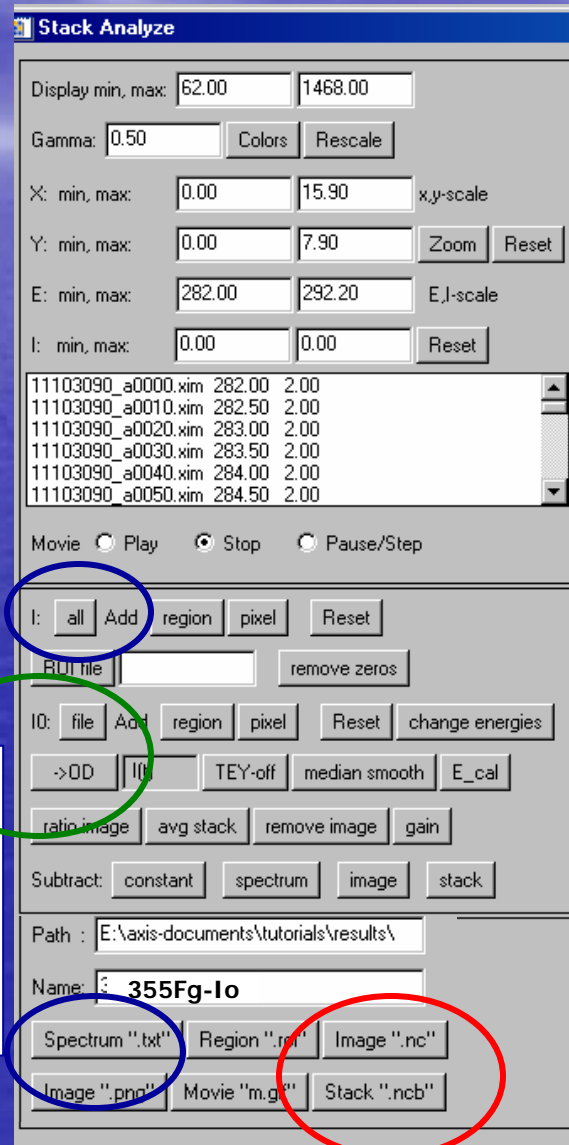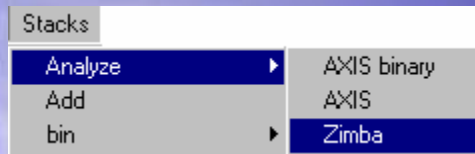

Io

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

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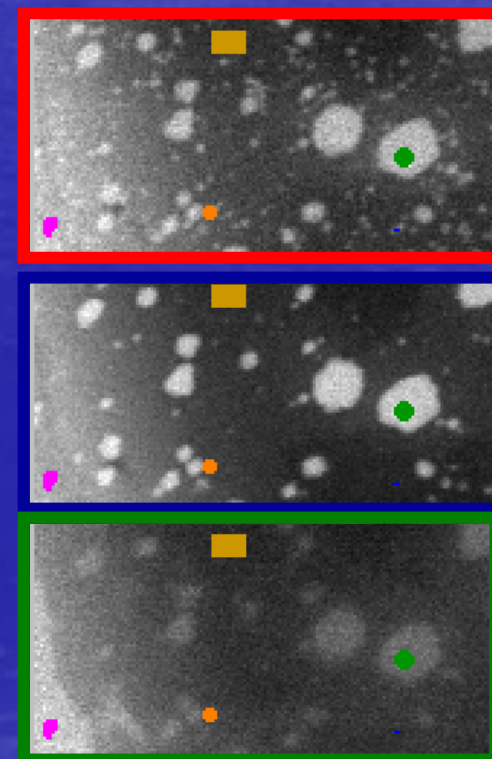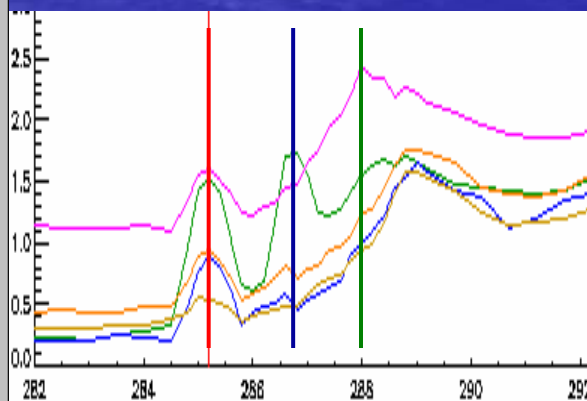2) convert to OD
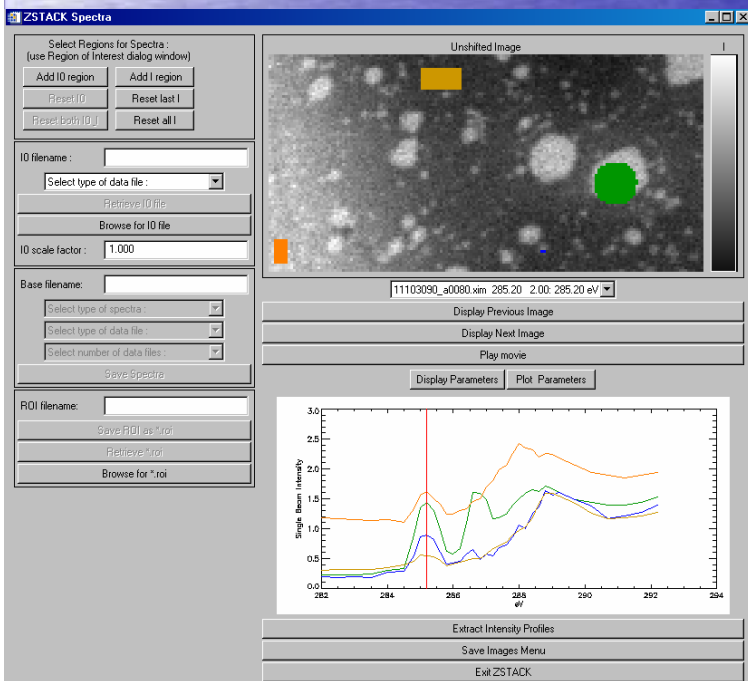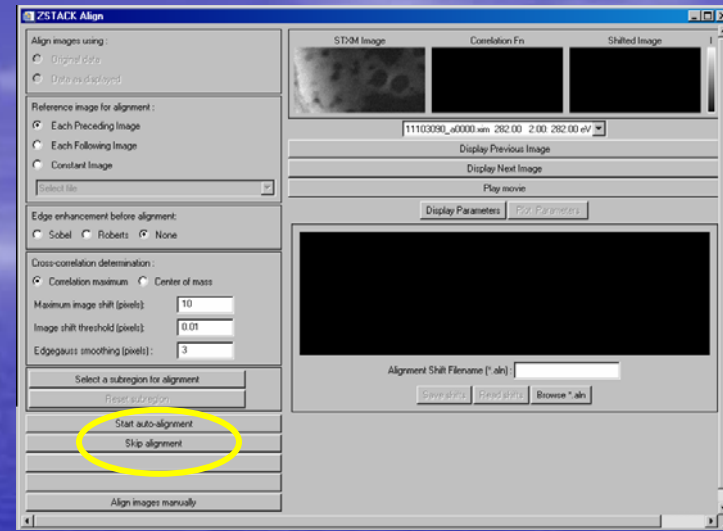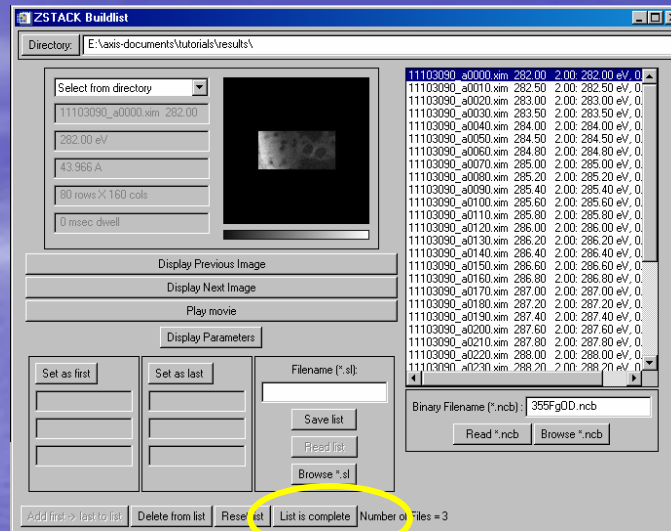3) type file name
4) save converted stack

C1s stack – viewing specta of regions

Zstack
sub menu

written by Carl Zimba

Useful subsidiary Zstack features

17sep170 : 281.21 eV 4.00 msec: 281.20 eV, 0. ▼

Display Previous Image

Display Next Image

Play movie

Display Parameters | Plot Parameters

ZSTACK Display Parameters

Image zoom factor: **3.00**

Movie delay (sec per frame) : 0.100

Closeup image zoom factor : 4.0

Profile image zoom factor : 1.0

Display image intensity using :
- ○ Absolute   ● Percentage

Display minimum : 0.0

Display maximum : 100.0

Display Gamma : 1.00

Scale image intensity using :
- ● Intensity range of each image
- ○ Intensity range of entire image stack

Scale images without I0 region

Load New Color Table

Invert Color Table

Select Plot Colors

Display images as:
- ● Original data
- ○ Images / current image
- ○ -log (images/current image)
- ○ Images - current image
- ○ Images / I0 spectrum
- ○ -log (images / I0 spectrum)   [Absorbance]
- ○ Images - I0 spectrum
- ○ Current stack - reference stack

Reference spectrum : Select ▼

Scale factor : 1.0

Reference image : Select ▼

Scale factor : 1.0

Reference stack :

Scale factor : 1.0

ZSTACK Plot Parameters

Image zoom factor : 3.00

Movie delay (sec per frame) : 0.100

Spectrum Offset: 0.00

Display spectra as:
- ○ Single beam
- ○ % Transmittance
- ● Absorbance

Plot Scaling :

X Range :   ☑ Autoscale

Minimum : **282.000**

Maximum : **294.000**

Y Range :   ☑ Autoscale

Minimum : 0.000

Maximum : 3.000

Load New Color Table

Invert Color Table

Select Plot Colors

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
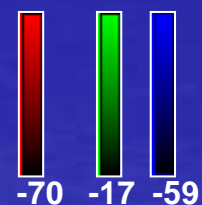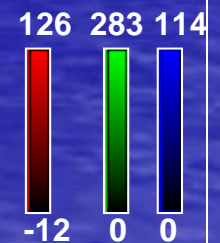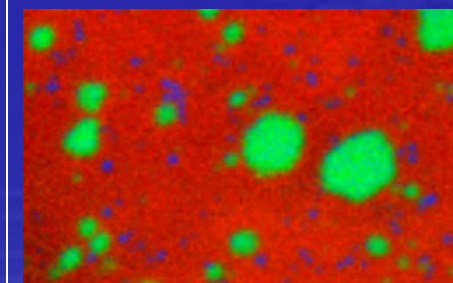* we can display the spatial correlation of the components using an RGB color **COMPOSITE MAP**

matrix   SAN   pipa
Fg   matrix   SAN
pipa   Fg   residuals
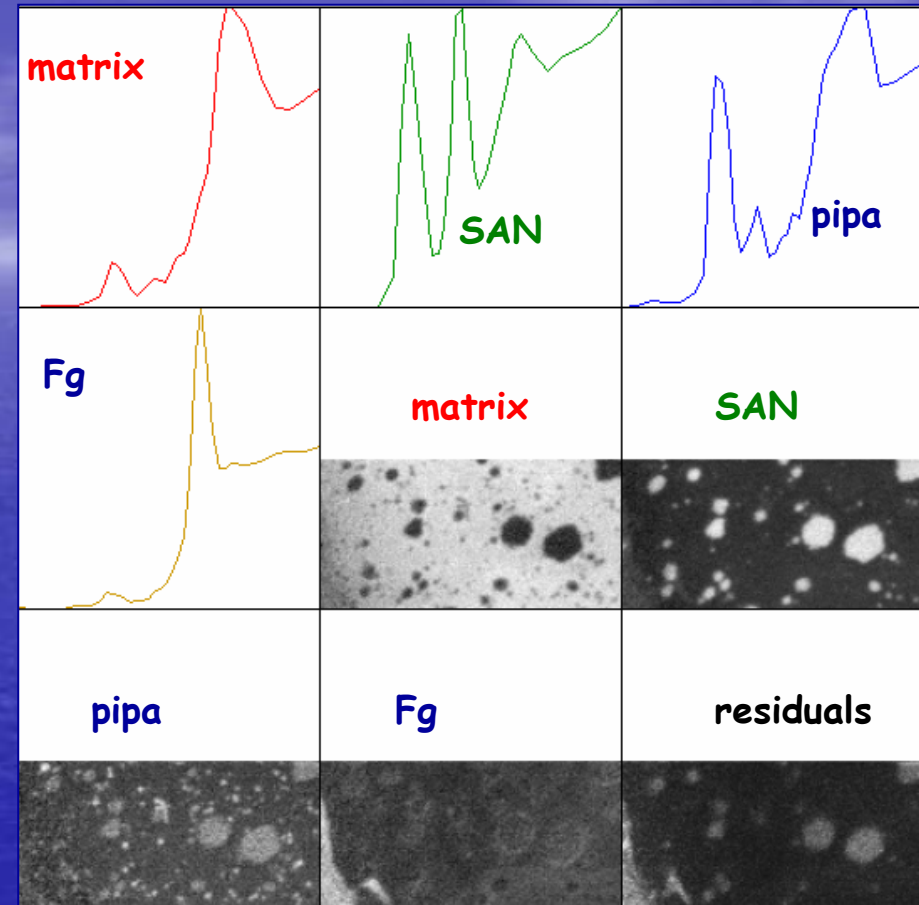
not rescaled (absolute nm)

298
-70
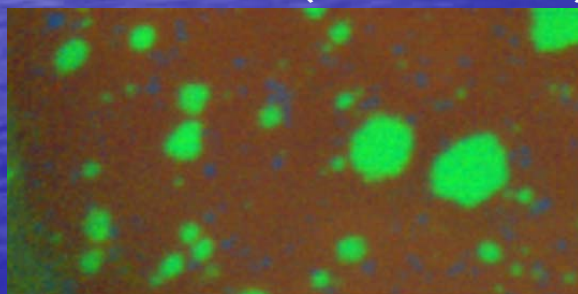
un-clipped data
126 298 114
-70 -17 -59

clipped, rescaled (RELATIVE)

126 283 114
-12   0   0

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Evaluating the stack analysis

Histograms of the component maps

Fg

Is the clipping reasonable ?

pipa

SAN

matrix

residuals

2 um

0.08

0
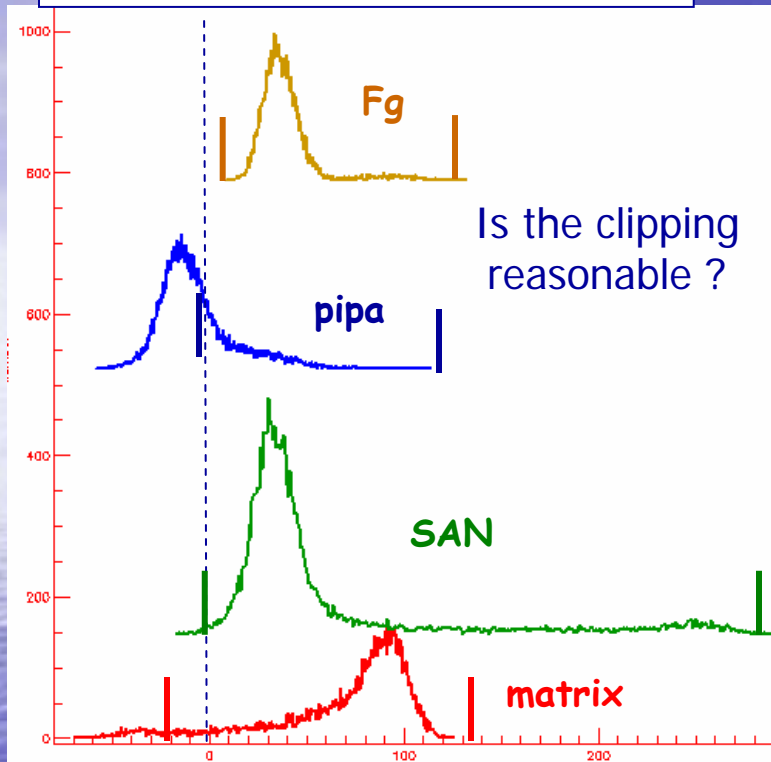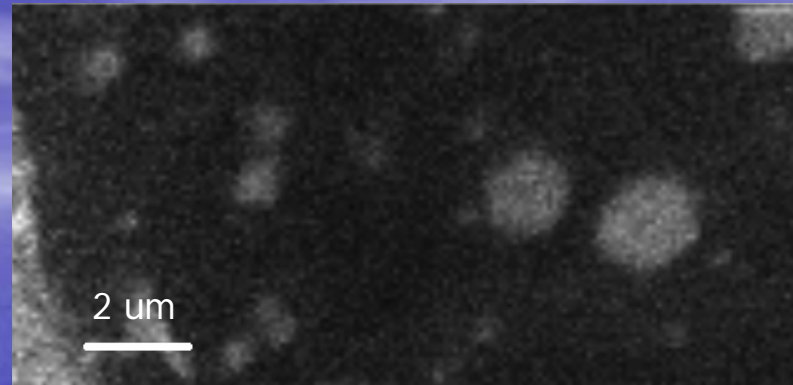
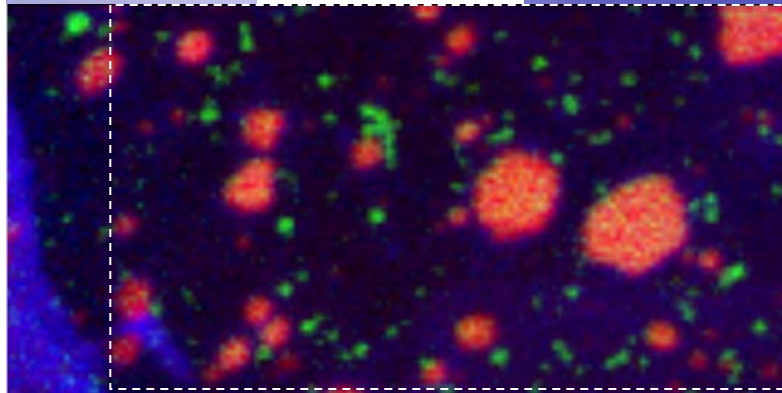constant (ideally should be small, +/-)

1.45

0
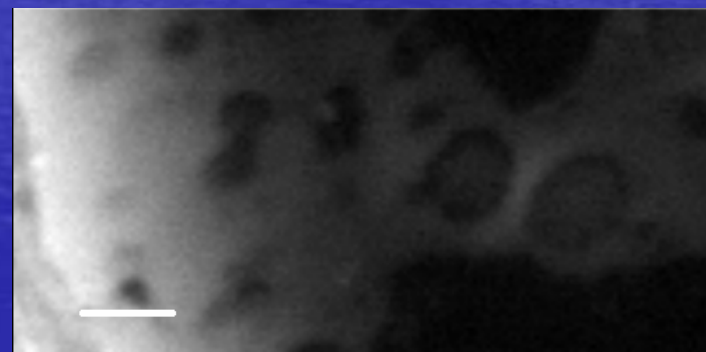
Generally an acceptable result.
PIPA map is probably wrong w.r.t quantity.
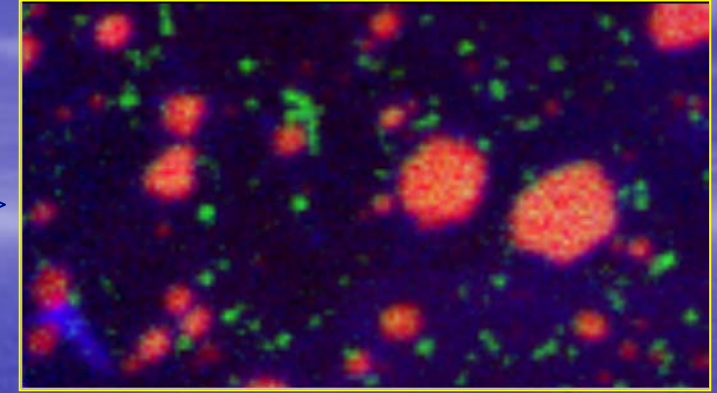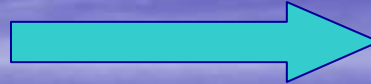The fit is using "-ve PIPA" signal to accommodate absorption saturation distortions of spectra (region selected in too thick by ~ x2)
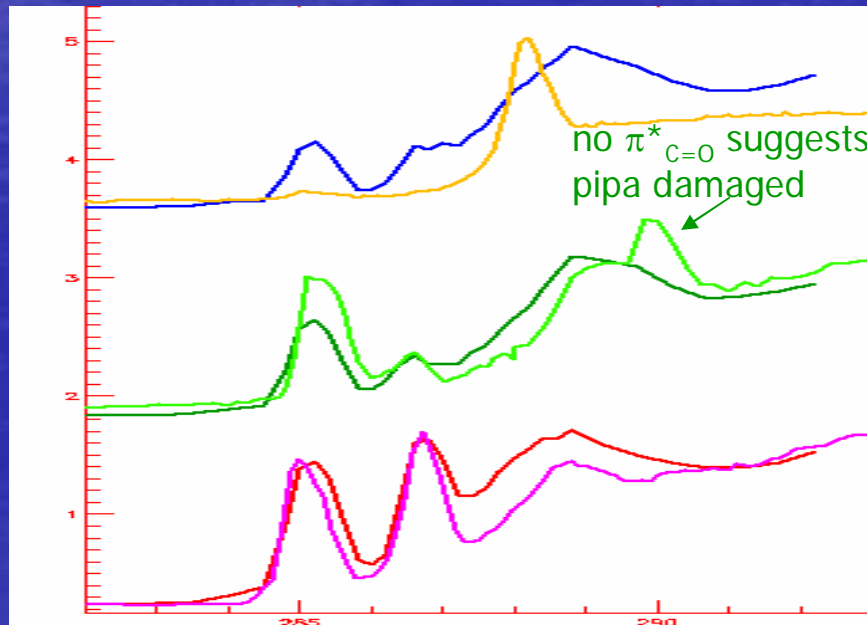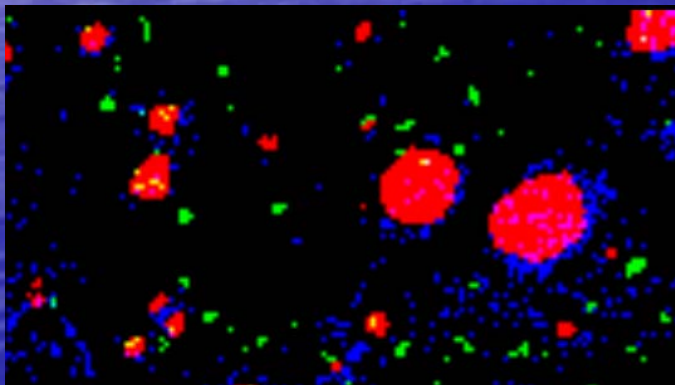
Evaluating the result - where is the Fg ?

SAN pipa Fg

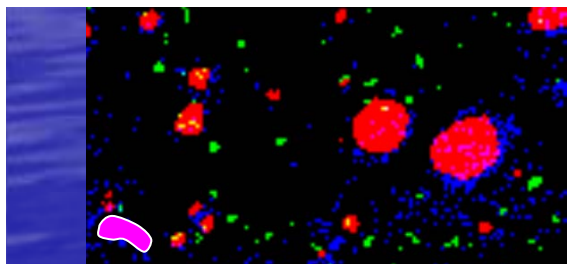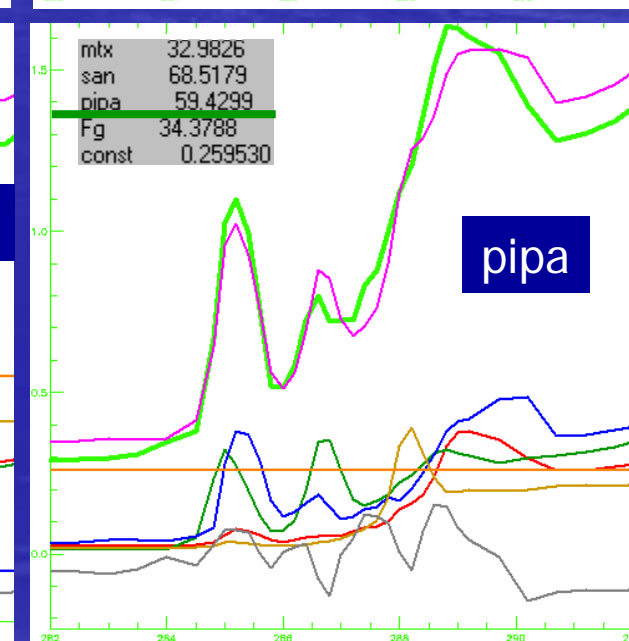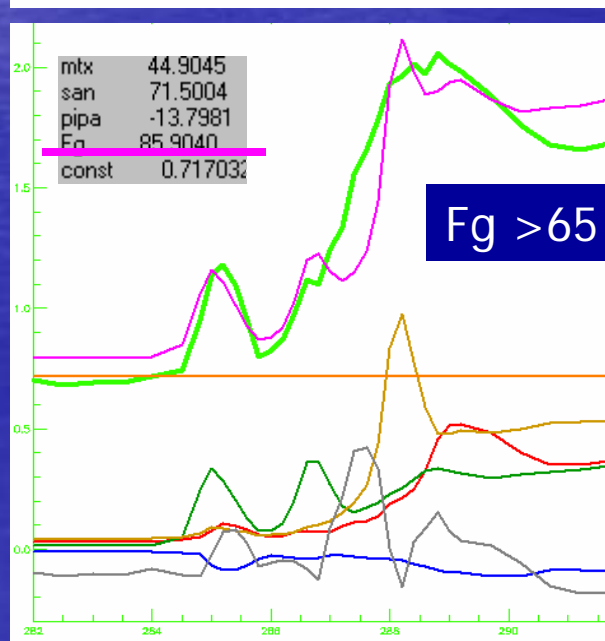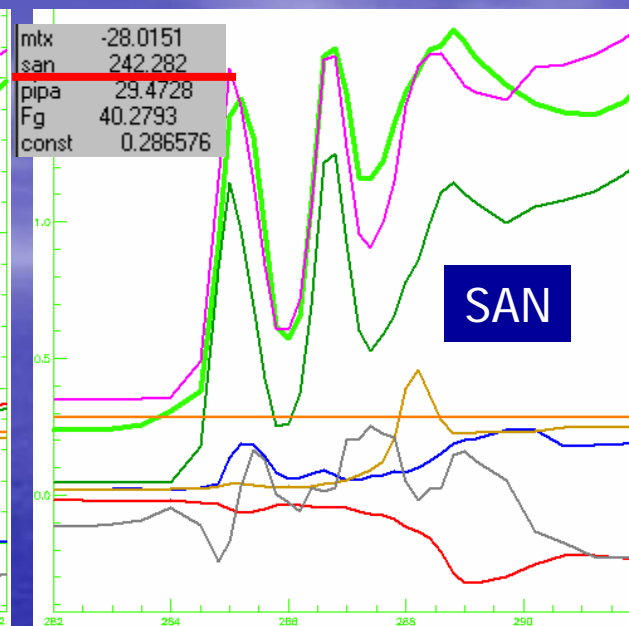select right 2/3 to get away from 'splat' protein deposit

- 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

no $\pi^*_{C=O}$ suggests pipa damaged

Fits to extracted spectra

Reference spectra

Fg
pipa
SAN
matrix

OD1 - linear absorption (nm⁻¹)

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

mtx	52.4000
san	84.1104
pipa	0.727849
Fg	54.1272
const	0.356746

fit
data
Fg
SAN
matrix
constant
pipa
residual

Fg >50

mtx	-28.0151
san	242.282
pipa	29.4728
Fg	40.2793
const	0.286576

SAN

mtx	44.9045
san	71.5004
pipa	-13.7981
Fg	85.9040
const	0.717032

Fg >65

mtx	32.9826
san	68.5179
pipa	59.4299
Fg	34.3788
const	0.259530

pipa

Comparison of 3-methods of deriving chemical maps in aXis2000

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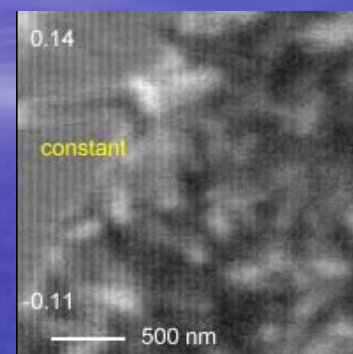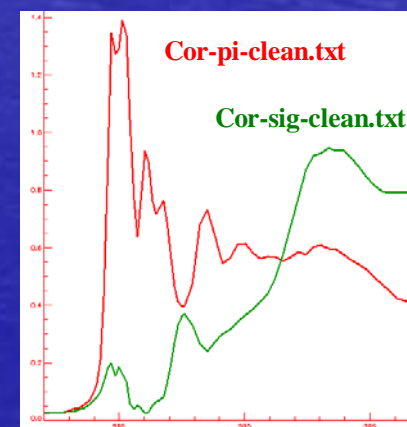Data (67 images in C 1s region) recorded Jul-03 at ALS STXM532
Crystal orientation affects spectrum (linear dichroism)
Extraction of limiting in-plane and out-of-plane spectra used as reference spectra

no rescale overplot

residuals rescaled
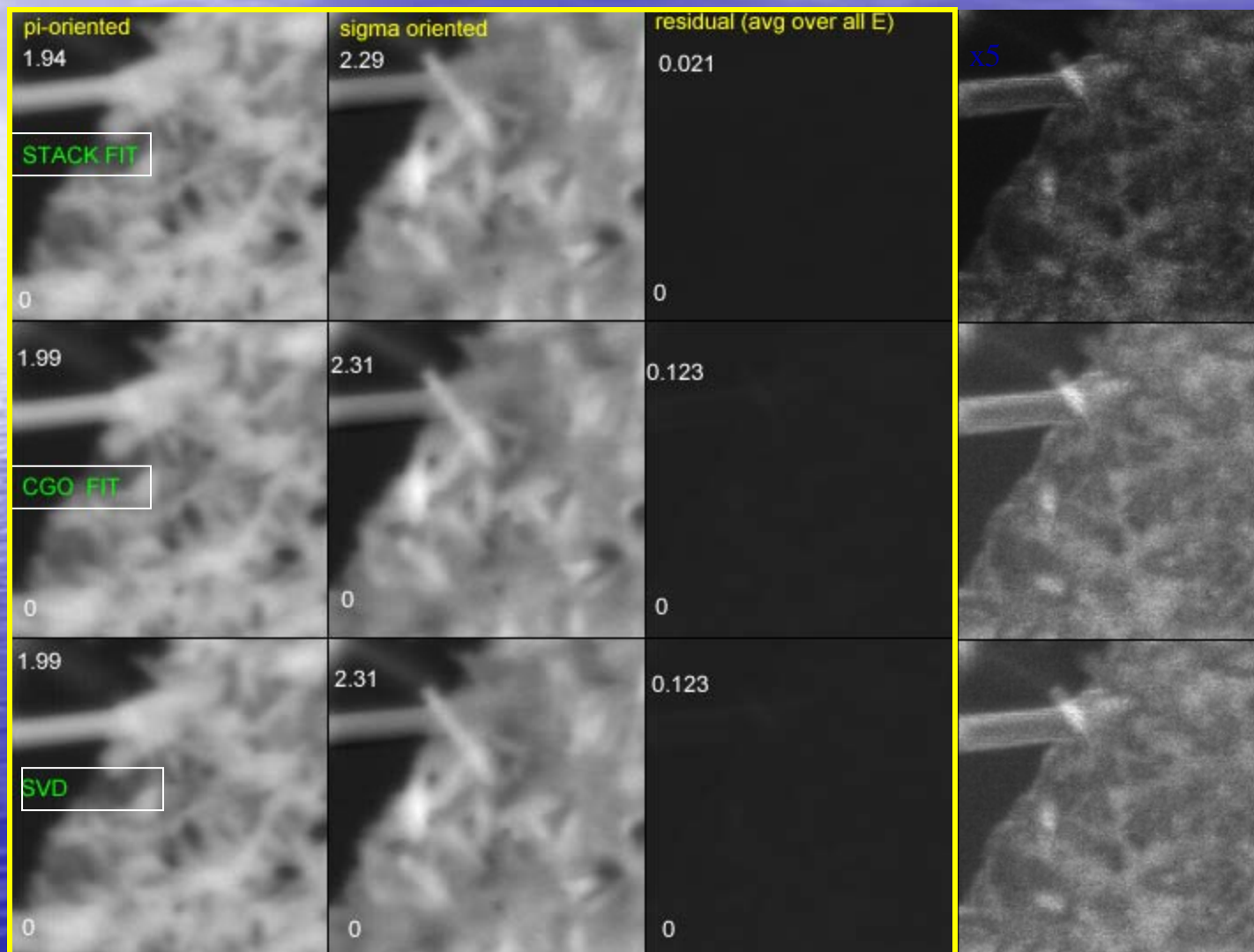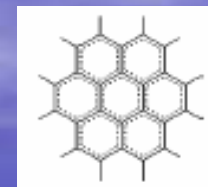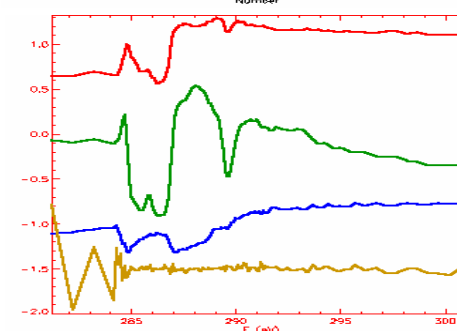
Model spectra
(derived from stack)

QUESTIONS ?

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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
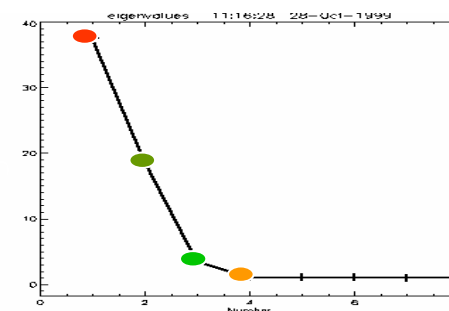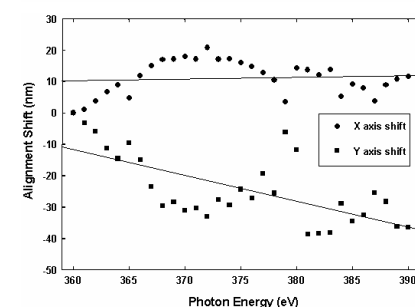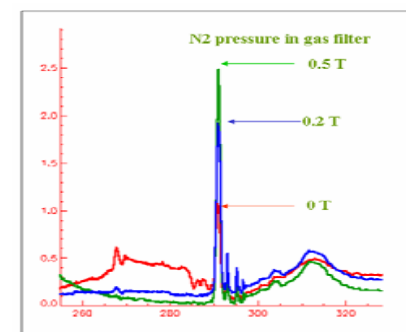
2. Suitability & quality of reference spectra

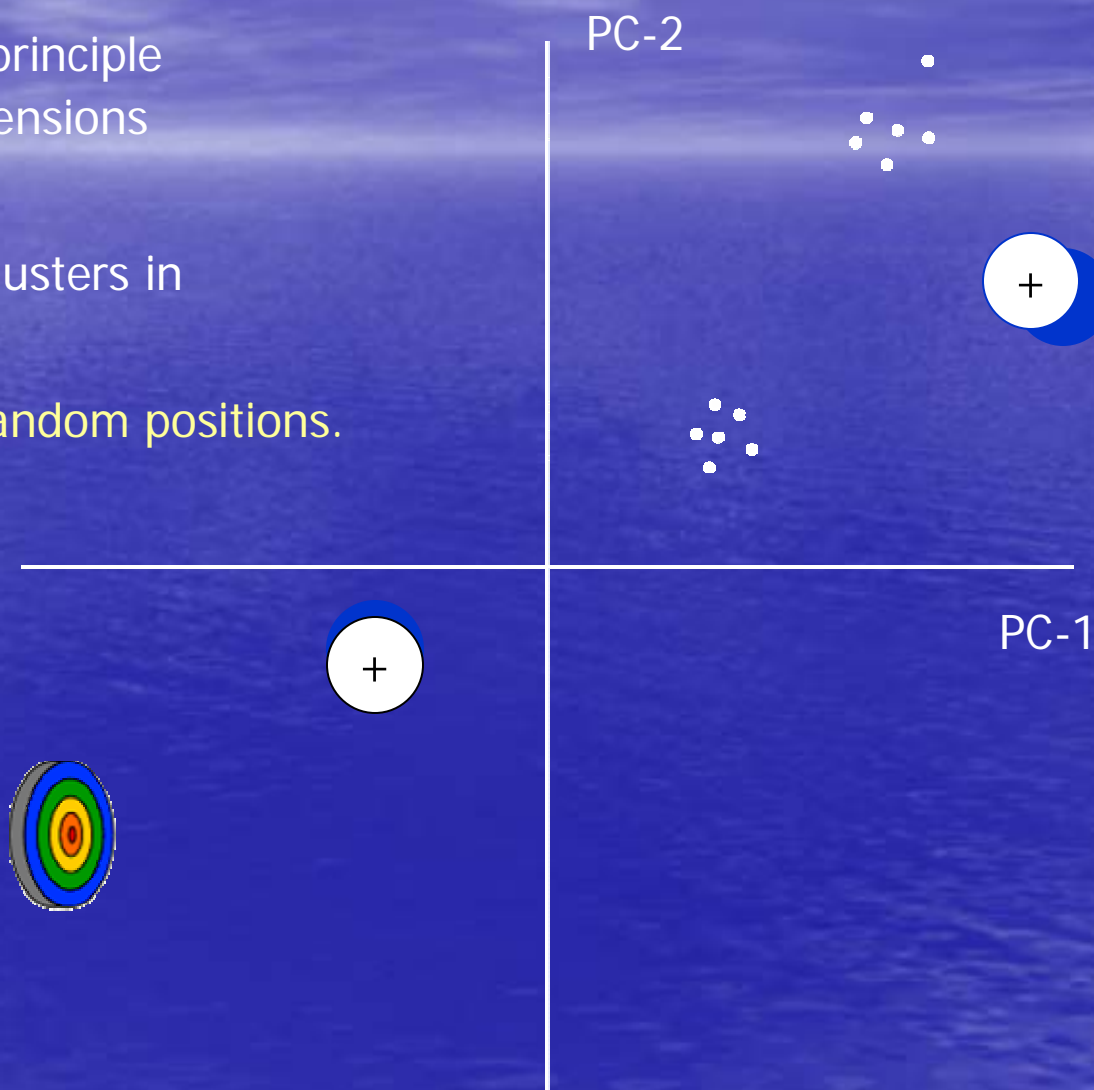| Situation	Recommendation
chemistry well known	record spectra of same or similar pure material (eg monomer unit)
chemistry poorly known but spatially well isolated	internal models guided by external models
chemistry complex	Principle component analysis to place limits on number of components
chemistry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

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

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.

PC-2

PC-1

Cluster analysis: Euclidian distance learning algorithm

PC-2

- 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.

PC-1