TUTORIALS to illustrate use of aXis2000 for the analysis of STXM *.sdf data

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CONTENTS

A. Image manipulation

A.1. Reading an ALS 532STXM image

* start aXis2000
  * Read~STXM (sdf)
    Browse - select ~\sdf-image.hdr
    OK

Notes: The image is a 30x30 micron image of a wet cross-linked gel recorded at 396 eV. The upper right portion of the image is off-the-edge of the gel, passing through 2 silicon nitride windows and about 1 micron of water. (20320063)

A.2. Converting transmission to optical density (OD)

[read in **sdf-image.hdf**] - tutorial #A.1.
* determine average signal in 'white' area (no sample) by either
  * move mouse over 'white area', read value from the cursor
  -or-
  * Images~Average pixels~region- all pixels
  You should find a value of about 4500.
* Images~Convert_to_OD
  * type 4500
  The OD image is computed (OD = ln(4500/image); it is placed in buffer #0 – the default temporary buffer.
  * Copy Buffer, click on any empty thumbnail to copy the image to a permanent buffer.

A.3. Determining profiles across an image

[read in **sdf-image.hdf**] - tutorial #A.1.
[convert to OD] – tutorial #A.2.
*Images~profiles~linear
- click at start of line, move mouse to end of line and click again
- select a buffer to store profile in; provide name if desired
- the OD intensity along the line is displayed

A.4. Removing noise from images

A.4.1 Replacing lines

[read in **sdf-image-noisy.hdf**] - tutorial #1
* Images~Replace line~manual-H
  - move cursor to just above the lower black horizontal line.
The x, and y line number (lower left panel) should be 11
  - click mouse.
  Line 11 is replaced by average of line 10and line 12, and the result is moved to buffer #0.
* Images~Replace line~manual-H
  - click on the upper part of the dark upper line – you will probably identify line 138.
  - select 'no' then take the default in the next interaction box. Which will replace line 138 with lone 139.
  - repeat to replace line 137 with average of lines 136 and 139.
Note in this case, using the default (average adjacent lines) would leave a distortion in the image since two adjacent corrupt lines make up the upper dark line

A.4.2 Smoothing images

[read in **image-to_smooth.axb** using *Read~images~axis*]
* Filter~smooth
  3- or 5-point smoothing reveals details of this image better.

A.4.3 Fourier filtering images

[read in **image-to_clean.axb** using *Read~images~axis*]
* Filter~clean (FT image filter)
  use cursor in rubber band mode (click at start, release, drag to end, click and release) to draw a rectangle top to bottom around the vertical line to the left of the central spot.
NB 2D Fourier transforms ech quadrant is a replica of the other 3, so filtering out a pattern in one quadrant is all that is needed
This is an image of a toner particle. The moiré pattern arises from beating of the frequency of vibrations of beamline optical element(s) against the ~kHz acquisition frequency.

A.4.4 Clipping images

[read in **image-to_clip.axb** using *Read~images~axis*]

* Images~clip signal~histogram

- a histogram of the z-values of this signal will appear. Select lower and upper bounds of data to be retained by moving mouse to lower limit of physically meaningful data (-15), click, then to upper limit (280), click.

- the pixels with values below the lower and above the upper limit are set to the closest limit and the result is placed in buffer #0.

* Copy buffer to move the result into a permanent buffer

This is the map of styrene-co-acrylonitrile (SAN) from a fit a C 1s image sequence of a filled polyurethane. The haze is removed because pixels with large negative values are removed. These negative values from a fit are likely because the reference spectra are slightly wrong. clip signal~histogram *removes these artificial results revealing more clearly the spatial distribution of the SAN particles.*

A.5 DISPLAYING MULTIPLE IMAGES

* Read~images~axis **image-to_clean.axb** into buffer # 1
* Fourier filter this image to remove moiré noise (see Tutorial #A.4.4)
* Copy buffer to move the result into buffer # 2
* Add~buffer #1 with -1 weighting to buffer #2
* Copy buffer to move the difference (removed noise) into buffer # 3
* view buffer # 1 by clicking on thumbnail #1
* Filter~smooth (3-point) the data; move to buffer #4
* Display~thumbnails~4~common scale selecting [1,2,3,4)

This type of comparison can help decide optimum processing. Note that the lineouts are active and give quantitative signal levels of all 4 displayed signals.

B Spectral Manipulation

B.1 READING IN A SPECTRUM

B.1.1 Reading stxm5.3.2 multiple region spectra

- start in buffer #1

* read~STXM (sdf) browse to select **11103106.hdr**

- in Region pull down menu, scroll to bottom, select all regions; OK

Seven spectra will be read. Convert spectra in buffers 1 -6 to OD by:

- select buffer #1 Specrtra~Convert to~OD; buffer 7 (Io)
- transfer result to buffer#1

* - select buffer #2 , 3, ,4, #5 (mtx), buffer #3 (pipa), #2 (san) and convert to OD using buffer #7 as Io. After generating each spectrum, transfer it to a suitable buffer to store for later spectral comparison

This is a set of 7 point spectra acquired at same time on a 355 filler polyurethane sample. region 1: pipa (missed); 2, 6 - SAN, 3 - pipa;, 4 - edge of SAN, 5 - matrix, 7, hole. (Stacks measured on this type of sample are used in the Advanced tutorial).

The points at which these spectra were obtained can be viewed by:

* read~images~graphics~png~image **355-point-spectra.png**

images recorded before and after these point spectra:

11103104 (285 eV) - highlights SAN, PIPA
11103105 (287 eV) - only SAN
11103107 (289 eV) - convert to OD; use Z-scale to check for damage
11103108 (285 eV) - should be same as 104

C. Linescan Spectra processing

C.1 READ, DISPLAY, CONVERT TO OD

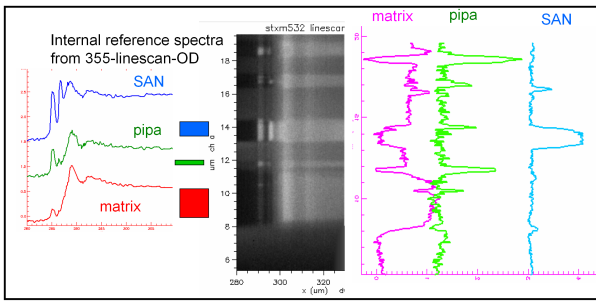
1. Read~STXM (sdf) stxm532-linescan
2. extract Io (in region without sample)
   Linescans~Add lines~horizontal (y-valeus 1.0 – 1.5);
   Store in a permanent buffer (e.g. 6)
3. convert to OD, Linescans~normalize to Io buffer 6

C.2 EXTRACTING AND COMPARING SPECTRA

4. Read~Images~axis 355-linescan-od.axb
(This has already been converted to OD using the lower part of the line, off the polymer sample; it is the same sample as in D)
5.use linescans~Add lines~horizontal to extract the matrix (8.7 – 10.0) , pipa (11.8-12.0) and SAN (13.3-14.00 spectra (numbers are position along the line)
6. write each of these spectra to disk (Write~axis, and put name of the chemical species at start of header)

C.3 Fitting linescan spectra

7. Linescans~linefit 355-linescan-od.axb, the 3 components, select matrix, then pipa, then SAN spectra written our in step 6**.** Store the parameter file - 355linescan_matrix_pipa_SAN.par
8. Spectra are plotted in buffers 1,2 , 3, which the distribution along the line for matrix, pipa and SAN are displated in buffers 4, 5, 6. The residual of the fit is stored to disk (355-linescan-OD_3SVD_res.axb).



D. STACK processing

D.1 Read als5.3.2.2 stack & convert to OD

*532_010817014 is a 38 energy C 1s stack measured on a polyurethane polymer consisting of 2 types of aromatic particles (SAN and pipa) in an aliphatic matrix [*Ultramicroscopy 88 (**2001**) 33*]*

1. Convert STXM-sdf stack to an aXis2000 file
   - Read~STXM (sdf) \532_010817014\10817014.hdr
   region#1 polymer region#2 - Io off microtomed section
   – output is *.ncb,*.dat', where * is user selected name.
   the saved stack is then opened in Stacks~Analyze~stack_process
2. Align if needed (but this stack did not need alignment)
3. After reading region 2, select i: All and save the Io file
4. Stacks~Analyze~stack_process *.ncb. Read Io from file; convert to OD; write out the stack *od.(ncb, *,dat)

D.2 Extract reference spectra from the *od stack

5. Stacks~Analyze~stack_process *od.ncb
6. Play back stack as a movie, Select I-region of largest particle. You should see a spectrum with 2 strong peaks, one at 285.1 eV (π*(C=C)) the other at 286.6 eV (π*(C≡N)) save as ***-SAN**
7. click on the spectrum display a the 286.6 eV peak. Some of the particles should disappear – they are the PIPA particles. Go back to 285.1 eV and select I: region of 1 or more of the small pipa particles. Save as ***-PIPA**.
8. select a region with no particles – save as ***-matrix**.

D.3 Creating chemical maps from SVD fits

9. Stacks~maps~SVD - select *od.ncb
10. define 3 components – select the ***-matrix**, ***-PIPA** and ***-SAN** as the reference spectra; save as matrix-pipa-SAN-14od.par
11. the Singular value decomposition fit will then execute, displaying the 3 reference spectra in buffers 1-3, and the 3 component maps in buffers 4-6, and the residual (data – fit) averaged over all photon energies in buffer 9.
12. Images~clip~histogram each of the component maps to remove –ve tails to improve contrast

12. Display~RBG composite (buffers 4, 5, 6) to make color composite of the 3 components. Select 'Yes' to rescale within each color