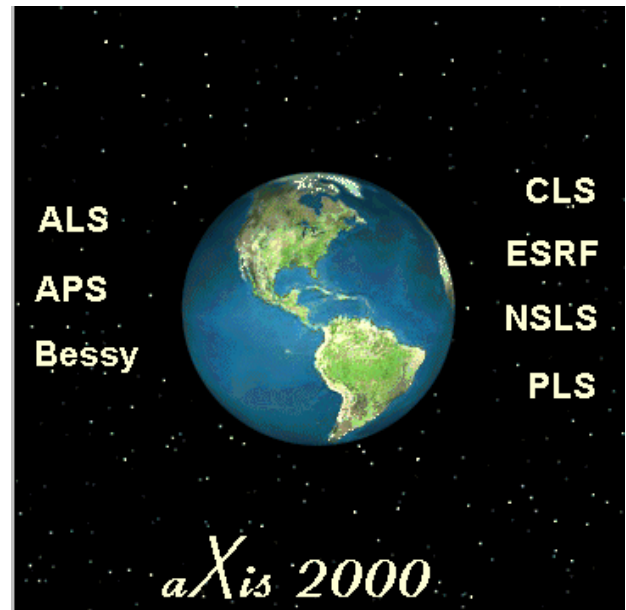


aXis 2000

Analysis of X-ray microscopy Images and Spectra (10 June 2007)

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), currently a part of ITT Visual Information Solutions. 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. In 2002 the code was extensively adapted to improve cross-system performance, especially for Macintosh OS. Currently it runs fully with all IDL 6.x and most features work with IDL5.x. If you run IDL 4, you need AXIS version 1.6a or earlier.



Since May-04 a compiled version (**aXis2000.sav**) for use with [IDL Virtual Machine](#) has been available. This allows access to the power of aXis2000 without needing to purchase an IDL license.

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 extensively improved the overall package in 2000; my son, **Peter** who helped set up the basic widget structure; Eli Rotenberg, Jonathan Denlinger, Stefano Cerasari, Tolek Tyliczszak, Andy Smith, Andreas Scholl, Göran Johansson, Jacob Stewart Ornstein, and many others. SPECIAL thanks to **Chris Jacobsen** (Stony Brook, nsls) for sharing his STACK and PCA_GUI codes, Rick Kneeder, for providing the basis for the stack-fit routine, and Billy Loo (UCSF) for providing SF, the Henke mass absorption routine.*

TO START aXis2000: after [installing aXis2000](#) (see end of this file)

Windows and Mac OS:

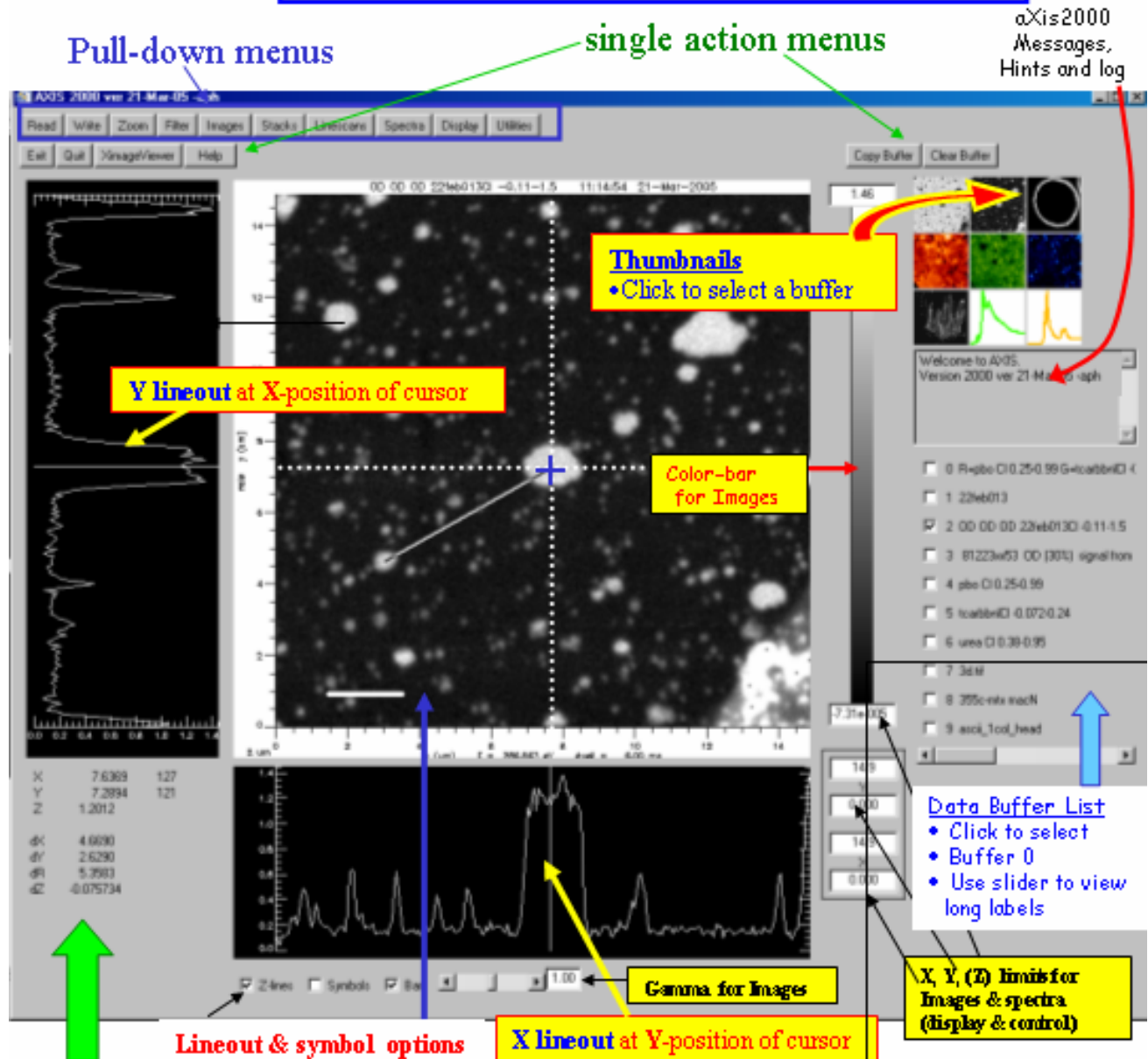
Start **IDL** ;

If you have set the Preferences (in IDL) so that **axis2000_batch.pro** is the start file, aXis2000 will launch automatically.

Otherwise, type **axis2000** on the IDL command line.

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

Features of the *aXis2000* widget



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 SpectraOverplot 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

Hard copy: Printing the displayed signals is achieved by using *Utilities~print*. aXis2000 prints via the IDL Printer virtual device, or by writing Postscript, or PCL-5 format files and copying to appropriate printer, depending on settings in the [axis.ini](#) file. The system-specific print command is defined in the AXIS.INI initialization file. The user can set this and other parameters using the *Utilities~preferences* command in AXIS.

Notation conventions used in this reference manual:

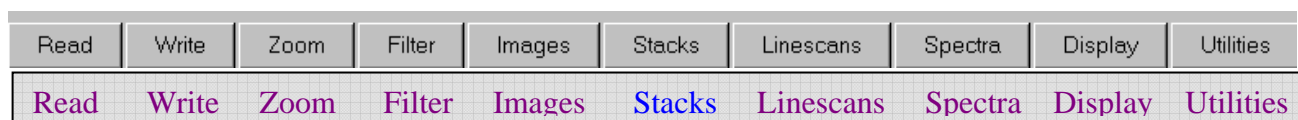
BOLD indicates a pull-down menu command;

TOP LEVEL items are bold, underlined, and light blue highlighted

Utilities~print~annotated is a third level pull-down menu under Utilities first level button

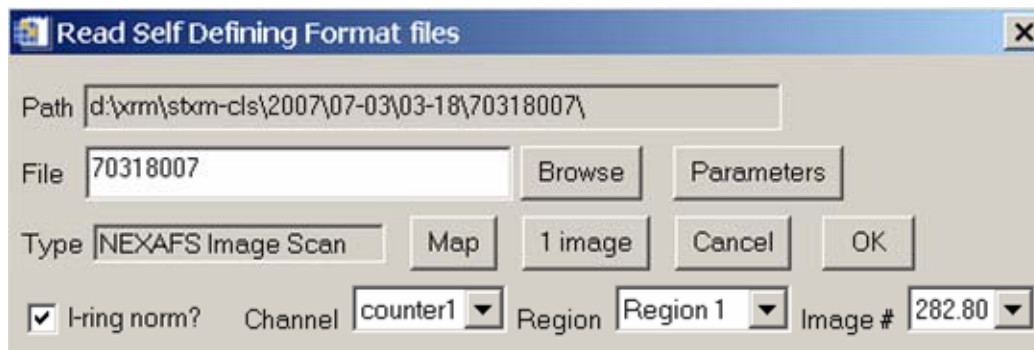
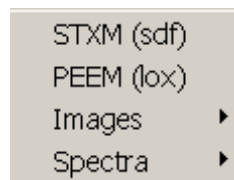
[Linescans](#) indicates an in-manual hyperlink

TOP ROW PULL DOWN MENUS



READ for all types of data (images, linescans, spectra, stacks etc)

STXM(sdf) - all types of [self defining files](#) containing data from the ALS, SLS and CLS STXMs are read by the following [widget](#). (single images; multi-region images, single spectra, multiple point spectra, linescans, image sequences for one region or multiple regions; DAQ, all types of motor scans, etc)



All data written by the ALS, SLS and CLS STXMs is described by a self-defining header file (*.hdr) which contains the information needed to identify the type of data contained in the associated data files. After selecting a file, the widget indicated above can be used to :

Browse – to select the file (or edit the filename box)

Parameters - list the header, which contains all microscope parameters

Channel - select from pull down list, generated from header (up to 26 data channels)

Region - select from pull down list, generated from header

Image# - select specific image of multi-image NEXAFS Image scan

Map – (only for NEXAFS Image Scan) – convert a 2-imagestack into a OD difference map

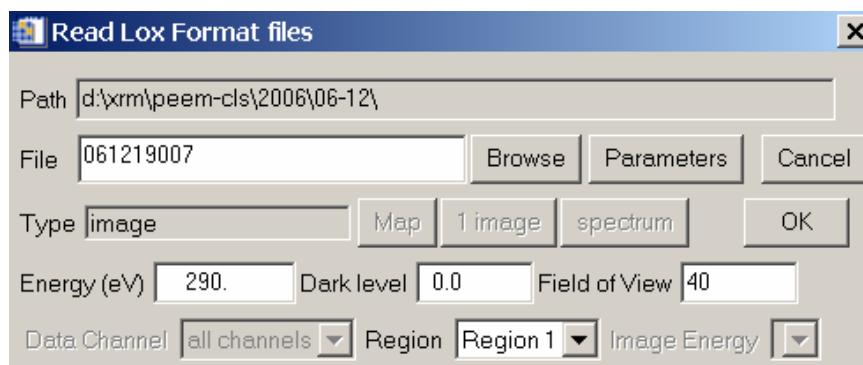
1 image – (only for NEXAFS Image Scan) read only the image identified in the image # pull down list (which displays the photon energy)

READ (continued)

Cancel – dismiss the ALS STXM read in widget

Selecting **OK** then reads the information in the associated image (*.xim) or spectral (*.xsp) ascii files into one or more aXis2000 buffers. Image sequences are converted directly to *.ncb files.

PEEM (Lox) – Reads data written by Lox, the data acquisition for the CLS PEEM (CaPeRS).



Lox data is described by a header file (*.lox) which contains the information needed to identify the type of data contained in the associated data files, along with all data acquisition and PEEM parameters. After selecting a file, the Read Lox widget can be used to :

Browse – to select the file (or edit the filename box)

Parameters - list the header, which contains all microscope parameters

Channel - select from pull down list, generated from header

Region - select from pull down list, generated from header

Image Energy - select specific image of multi-image NEXAFS Image scan

Map – (only for NEXAFS Image Scan) – convert a 2-imagestack into an OD difference map

1 image – (only for NEXAFS Image Scan) read only the image identified in the image energy pull down list (which displays the photon energy)

Energy (eV) - set energy of the image

Dark Level – set camera dark level

Field of View - set field of view of the image (microns)

Cancel – dismiss the ALS STXM read in widget

Selecting **OK** then reads the information in the associated binary image (*.tif) or ascii spectral (*.lox) files into one or more aXis2000 buffers. Image sequences are converted directly to the aXis2000 binary format (*.ncb).

Images

AXIS – images written in aXis2000 **binary** format (*.axb)

This is useful for saving derived results and is the required input for some routines

ALS-xyt – file format for pattern generator (STXM)

AXIS	
ALS-xyt	
Graphics	▶
NSLS	▶
PEEM	▶
ROI	▶
TOF	▶
Other	▶

READ~Images (continued)

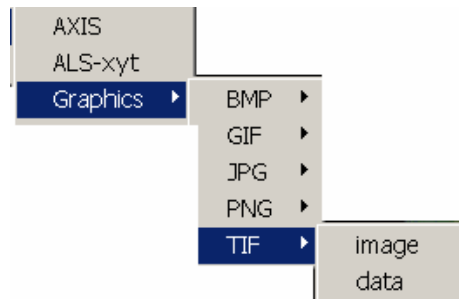
Graphics - - standard graphics formats (BMP, GIF, JPG, PNG, TIF).

TIF format handles 1-channel and 3-channel (RGB) formats

For each image format one can read the image as

image (un-modifiable)

or as **data** (modifiable, pixel indices as (x,y)-values)



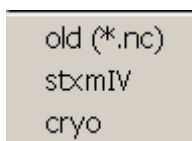
CAUTION: there are known bugs in these routines, with some inversions and strange color distortions

NSLS

Old (*.nc) – prior to ~2002 (NetCDF)

stxmIV – current data format

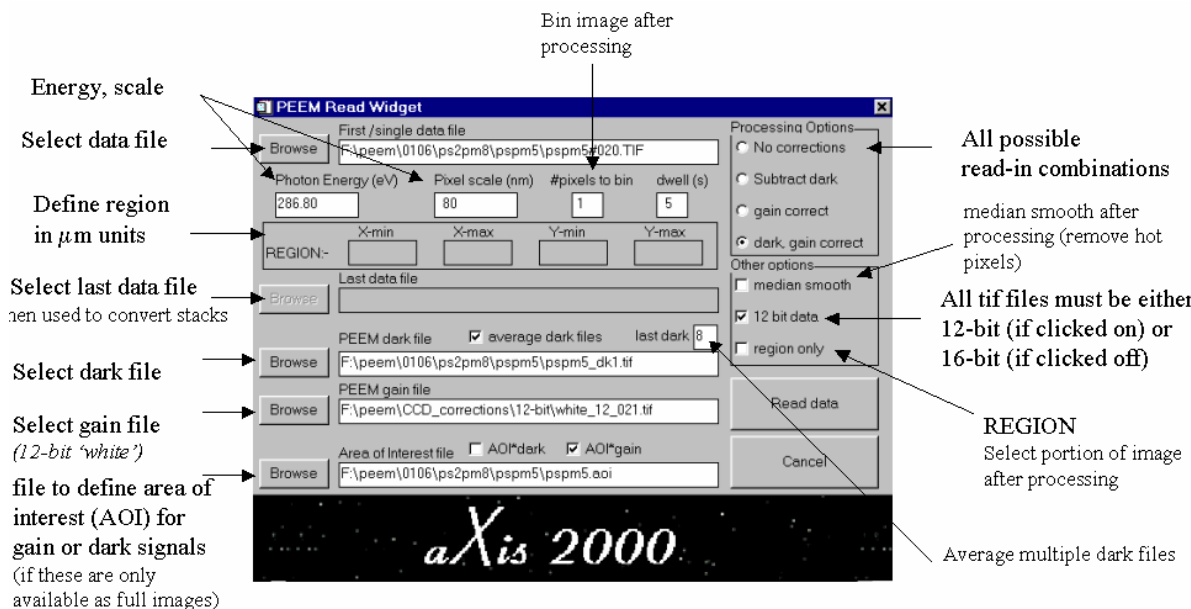
cryo - format from cryo-STXM



PEEM

ALS-PEEM2
ALS-PEEM3
Elmitec
Lox-PEEM
Mephisto

ALS-PEEM2 – reads in 12-bit and 16-bit ALS-specific formats with a **widget**. (NB the **Elmitec** read command uses the same structure to define image and image sequence parameters)



ALS-PEEM3 – reads data format for PEEM3 (implemented Jan-2007, Andreas Scholl)

Elmitec - read PEEM files.

- **dat** – read in 16-bit (12-bit encoded) files written by UView2002

- **tif** - read-in 8-bit (grayscale in 3 channels) exported by UView2002

Lox-PEEM – reads Lox format images (duplicate of Read~PEEM(Lox) menu)

Mephisto – read PEEM data from Mephisto (old binary 512x512 array from a CCD)

READ~Images (continued)

ROI – region of interest definitions for

Lox – ROI for Lox format PEEM

Pem2 – ROI (=AOI) for pem2 data

aXis2000 stack – ROI for stack region selection

lox
pem2
aXis2000 stack

TOF – read McMaster time-of-flight 2-d data in Z-matrix format

- **all (pTa)b** – 2-d as written by the pTa acquisition program
- **img** – Z-matrix data

OTHER -

ALS-SPEM – read in images recorded in XPS or NEXAFS mode using the ALS SPEM

ALS-STXM-7.0 image files (ascii) – data channels:

- im0 = OSA signal (Io);
- im1 = transmitted signal;
- im2 = other (e.g TEY, luminescence, etc)

ALS-STXM-7.0 linescan – linescan files from ALS-STXM7.0.1

ALS-XM1 – data files from full field microscope at the ALS (rudimentary)

ALS-SPEM
ALS-STXM-7.0
ALS-STXM-7.0.linescan
ALS-XM1

Spectra (READ)

AXIS – read spectra from ascii format files (*.txt)

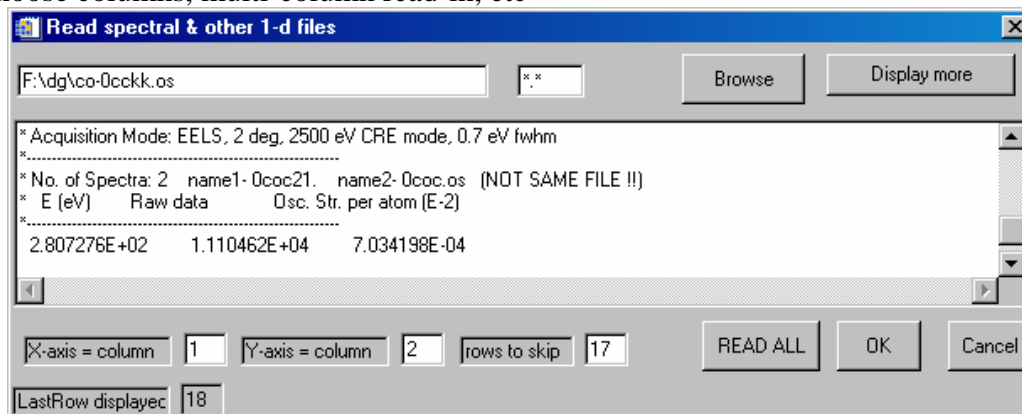
This can be ascii 1-d data written by aXis2000 or many generic formats of multi-column data with or without headers. (default ext. = *.txt)

NB if you wish to write images as a text file – e.g. for import into Origin, Matlab or other data processing packages, please use

Utilities~Write image ascii

AXIS
multi-column
ALS-STXM-7.0
ALS-SPEM ▶
ALS-PEEM
Lox-PEEM
NSLS ▶
SPHINX-PEEM ▶
XAS

multi-column – guided read-in of any ascii data file, with ability to skip lines, choose columns, multi-column read-in, etc



READ~Spectra~multicolumn (continued)

Browse – select file

Display more – add another 6 lines to the display buffer (to get to end of headers)

X-axis column – select data column for x-axis

Y-axis column – select data column for y-axis

Rows to skip – use LastRow displayed to identify which line to start read in

Read all – read all columns in a multicolumn file (x, many y)

OK – read only the identified (x,y) columns

Cancel – abort widget

ALS – STXM-7.0 read in files from ALS BL 7.0 STXM (HISTORICAL)
using the following [widget](#) to select spectral processing options.

ALS spectral read-in

Process sample file

☐ dark + data

☐ no correction

☒ subtract dark counts

☐ normalize to OSA

☐ dark, OSA

Sample: F:\stxm\9812\23xxx\23xxx045.xas col. # 2 Browse

Reference (lo): F:\stxm\9812\23xxx\23xxx047.xas 2 Browse

☒ Update scan parameters on Browse

Process reference file

☐ dark + data

☐ no correction

☒ subtract dark counts

☐ normalize to OSA

☐ dark, OSA

Sample scan parameters

	value
dwell (ms)	200.0
E_min	396.00
E_max	407.80
#sects	1
#cols	2
#dark	10
Avg dark	7.50

Reference scan parameters

	value
dwell (ms)	200.0
E_min	278.00
E_max	304.80
#sects	3
#cols	2
#dark	10
Avg dark	42.50

DATA COLUMNS

1. OSA

2. Transmission

3. Other (e.g. TEV)

GO

Read in:

☐ sample

☐ sample/reference

☒ Absorption: ln(Io/I)

Cancel

Select file name for the sample (and reference if you wish to compute absorption from transmission data, or to normalize yield data); set the data column and process options for the sample and reference; set the read in mode (data, ratio, absorption); then press GO.

For example, to read only column 2, with removal of the [dark count](#) signal from the detector which is pre- and post-appended to the spectral data, but without dark count subtraction, one would chose:

1. *Process sample file*: no correction
2. *Sample*: file name (select via browse or simply type in the name - useful if working through a sequence of files);
3. *col #* 2 ;
4. *Read in*: sample;
5. GO

ALS-SPEM – (ALS BL7.0)

- **XPS** – multi-region XPS spectra recorded with Phi electron spectrometer
- **NEXAFS** – sample current NEXAFS recorded with BL 7.0 SPEM

ALS-PEEM – spectra from ascii file written by ALS PEEM 2

Lox-PEEM – read in multi-column spectra written by Lox (*.lox files)

READ - Spectra *(continued)*

NSLS – read spectral data files in formats used at X1A

- **nc** - as-recorded (BSIF binary)
- **stxmIV** – stxm IV format (nc, but different than old-stxm)
- **ascii** - converted spectral format (generated by an X1A nsls utility)
- **map** - mapper style files (e.g. stack alignment shifts)

SPHINX-PEEM - – read in multi-column spectra written by Sphinx (*.dat files)

- **1** – read 1 column
- **all** – read all columns

XAS – XAS format with user settable header

(used for reference spectra, ZSTACK, NSLS, PCA_GUI and corex bibliography)

WRITE store results (images, spectra, etc) for later use or transfer to other programs.

AXIS - automatically selects file type depending on buffer contents

- spectra written with header in ascii (*.txt)
- images written with header in Z-matrix binary format using IDL system-independent binary coding (*.axb)

If you wish to write images in ascii, use **utilities~Write image ascii**

GIF – writes current Main Image (with any annotation etc) as a GIF file

- **image** (exactly as seen on main screen of aXis2000)
- **data** (the image without axes or labels)

JPG – writes the current Main Image (with any annotation etc) as a JPG file

- **image** (exactly as seen on main screen of aXis 2000)
- **data** (the image without axes or labels)

PNG – writes the current Main Image (with any annotation etc) as a PNG file

- **image** (exactly as seen on main screen of aXis 2000)
- **data** (the image without axes or labels)

TIF - writes the current Main Image (with any annotation etc) to a TIF file.

- **image** (exactly as seen on main screen of aXis 2000)
- **data** (the image without axes or labels)

ALS-image – writes ALS BL7 image format (*.img)

NSLS-image (*.nc) – writes NSLS [netCDF](#) image format (*.nc)

SDF (5.3.2) format – write [self defining file](#) format [*.hdr, *.xim (*.xsp) files for images (spectra)]

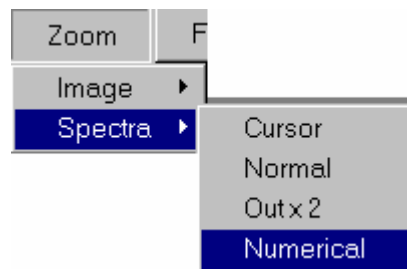
XAS spectrum - write spectrum in XAS format with optional definition of detailed header

AXIS
GIF ▶
JPG ▶
PNG ▶
TIF ▶
ALS-image
ALS-xyt
NSLS-image (*.nc)
SDF (5.3.2) format
XAS spectrum

ZOOM

(for images and spectra)

Image (NB –since expanding the scale is performed by cutting out the region of interest, the cursor-cut and numerical-cut commands can also be used to extract sub images)



Pan – pops-up a zoom window with a ~3x expansion of the region around the cursor. Move the cursor on the main image to look at different areas. Left click to change zoom factor. Right click to end zoom. If the continuous lineouts option button at the bottom of the aXis2000 screen is OFF, then the zoomed image is only updated on each left mouse click.

Cursor – cut - stretchable box cursor used to define region. Data is cut from the displayed image and shifted to the working buffer (0).

Numerical – cut - numerical selection of range. Data is cut from the displayed image and shifted to the working buffer (0).

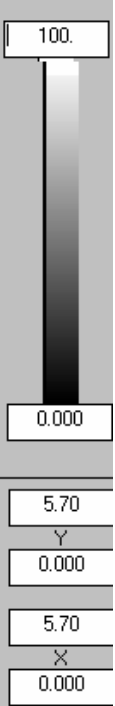
Spectra

Cursor -Stretchable box cursor

Normal - return plot to full X-scale, same Y-scale

Out x2 - useful to "make white space" for comparison overplotting

Numerical - select numerical limits



Z-scale color bar

These boxes display the current minimum and maximum values

X: X-axis of images or spectra (*)

Y: Y-axis of images or spectra (*)

Z: Z-scale of images are indicated at the bottom and top of the color bar

* The scale adjustments work in spectra~ overplot modes, as well as single plot modes, and when Zoom~thumbnails is used.

*** The (x,y,z)-limits for images and (x,y)-limits for spectra can be changed by entering values in the appropriate limit indicator box.**

FILTER

(for images and spectra)

5 types of smoothing with selectable parameters. The first four apply to BOTH images and spectra, but **Clean** works only with images.

Smooth – Boxcar average over n-points (right to edge of images)

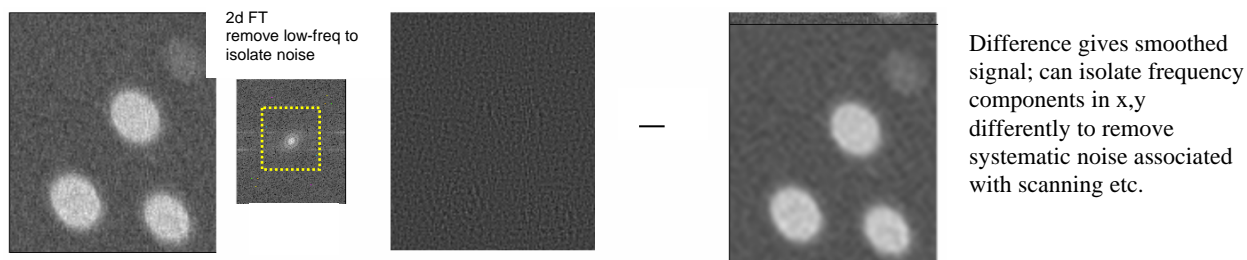
Median – n-point Savitsky-Golay averaging

Lee – Lee filtering smooths additive image noise by generating statistics in a local neighborhood and comparing them to the expected values

Convolution - convolutes with user-definable digital filter (definable frequency response)

Clean (2d Fourier transform image filter) - 2d-FT filter. The FT is displayed on a 1:1 pixel format. Use the rubber-band style cursor (click, drag mouse, click a second time) to define the data in the complementary frequency domain to delete. The reverse transform of all data but the rejected data is displayed in buffer 9. The centre of the FT image corresponds to 0 (dc), while positions farther from the centre correspond to higher frequency. Periodic (moiré) noise associated with aliasing (beating) of a systematic noise signal with stxm sampling can be cleanly removed by deleting strong (typically linear) signals in the FT.

The **Clean (2d-FT Image filter)** can be used with subtraction of the filtered result to perform high-pass or selective band-pass filtering. For example -



IMAGES (processing only valid for images)

Add

- **Append** - append 2 images, matched on basis of (x,y) scales - images can be **tessellated** using append.
- **Buffer** – weighted addition of two buffers (*use a -ve weight to subtract buffers*)
- **Constant** - Add a constant to each pixel of an image (use a negative constant for subtraction)

Average pixels - compute average intensity in a user-defined region

whole image – all pixels - full image

whole image - ignore zeros - same, but does not include zeros

region- all pixels - select pixel region of interest; report average Z-value with statistics

region- ignore zeros - as for all pixels, but does not include zero values. This is useful if an image has been multiplied by a [masked image](#) and the areas of interest are not contiguous. Use to count pixels and thereby determine areas of selected regions of an image.

bin – reduce image size (& improve statistics) by binning (2x2), (3x3), etc pixels to 1 pixel

Calibrate XY - calibrate X,Y scales of images

- **1-point** – shifts current image to make selected point have user-defined (x,y)
- **2 points** - allows linear stretching as well as shifting

NB: The calibration routines are similar to those used in Stacks~Image alignment. They can be used to manually process the first and last images of a sequence in order to ensure that the x,y limits selected will be present in all files.

Clip signal - replace all values outside user-selected limits with a fixed value (default is the average of the in-bounds data)

Convert_to_OD - compute [OD](#) (optical density) representation of a transmission image with user defined Io value. The default is the maximum value pixel in the image (typically this is too large – should use *image~average pixels~region- all pixels* in a hole area)

Cross-link density - use Flory-Huggins equation to compute cross-link density from a polymer volume fraction image.

Deglitch -graphics-driven deglitch routine. Cursors identify lower and upper bounds of data.

Delete region – use box cursor to define a region to replace with user selected value. (this is useful to remove glitches or to select part of a masked region).

Distort XY scale – distort (x,y) scaling. Pixels interpolated to square in new co-ordinates

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

Images *(continued)*

exp(Z) - exponential of z-values (useful to back-convert Absorption to Transmittance)

Fix rollover - for some image files (e.g. ALS PEEM) it is possible to have signed or unsigned formats which leads to 'roll-over' for pixels with above 16 K counts (e.g. if an unsigned integer data set is read using a signed integer). This replaces all pixels with a -ve value with 65,535 plus that value.

1 - fix a single image

many - fix rollover on a series of images (*.lst)

Gain - Multiply - multiply the Z-values by user-supplied constant

Divide - divide the Z-values by user-supplied constant

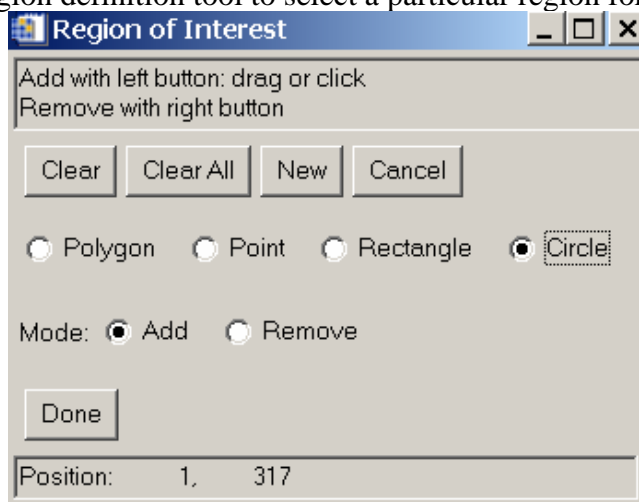
generate mask – convert image pixels to 1 if $Z > \text{threshold}$ and 0 if $Z < \text{threshold}$

- Can be used in conjunction with **Multiply buffers** to select portions of image

- optional write of a region_of_interest file to allow selection in **stack_analyze**

Threshold – allows used to define the threshold value

Manual – use region definition tool to select a particular region for stack area selection



histogram – compute the histogram of the pixels

ln(Z) - natural log of z-values (use to convert Transmittance to Absorbance)

log10(Z) - base-10 log of z-values

Modify one point – allows modification of a pixel (use **Delete region** for are modification)

Left click to add, right click to delete; in each case prompt is a local average

Multiply buffers – take product of current and a second buffer

Particle analyze - when applied to a [masked image](#) (0/1 pixel values only) it analyzes the areas of contiguous regions and reports a distribution of diameters, assuming circular areas.

Pixels only - plot using 1 display pixel per pixel of data

Images (continued)

Profiles - generate and save [intensity profiles](#) from images

Linear – intensity along line defined by two user selected points

Radial – intensity as a function of angle in a circular region. The user defines a diameter which is then rotated about its center. Optionally, the resulting ‘unfolded’ radial distribution can be symmetrized by auto-seeking a threshold level and aligning at that common level. (This routine was written to explore [radial distributions in particles](#). It is also useful for analysis of azimuthal orientation effects probed by linear dichroism.)

Ratio to - computes ratio of 2 buffers with optional scaling. Images are matched by (x,y) scales

Remove zeros – replaces zero value pixels with a local average. (despeckles images with dropouts)

Replace line – use cursor to identify horizontal lines to remove. It can be replaced by the average of 2 adjacent lines (default) or by any selected line. The line suggested is the (n+1) line (one above the selected line). Cursor line value is indicated during replace line selection. This is useful for the ALS STXM data which is acquired line-at-a-time and tends to have errors which affect whole lines. Numerical selection of the line to replace allows access to un-displayed lines in large images

Resize - set size of image in terms of # of pixels explicitly (this is needed to correct rounding errors in re-pixelation steps (e.g. shifts, truncates etc) whenever 2 or more images have to have identical numbers of (x,y) pixels for subsequent processing (same as *utilities~change mesh*)

Rotate - rotate image about a user defined point, by user defined angle. This can be used in conjunction with Images~Distort X,Y scale to remove image distortion by symmetrizing an object of known shape (e.g. circular particles).

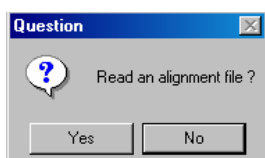
Set XY scale - calibrate distance scale by defining distance between two points

Warp –uses multiple matching point “morphing” capability of IDL. Intended to handle cases where a 2-point manual or simple shift will not track changes in the sample shape in a stack (these typically occur because of radiation damage).

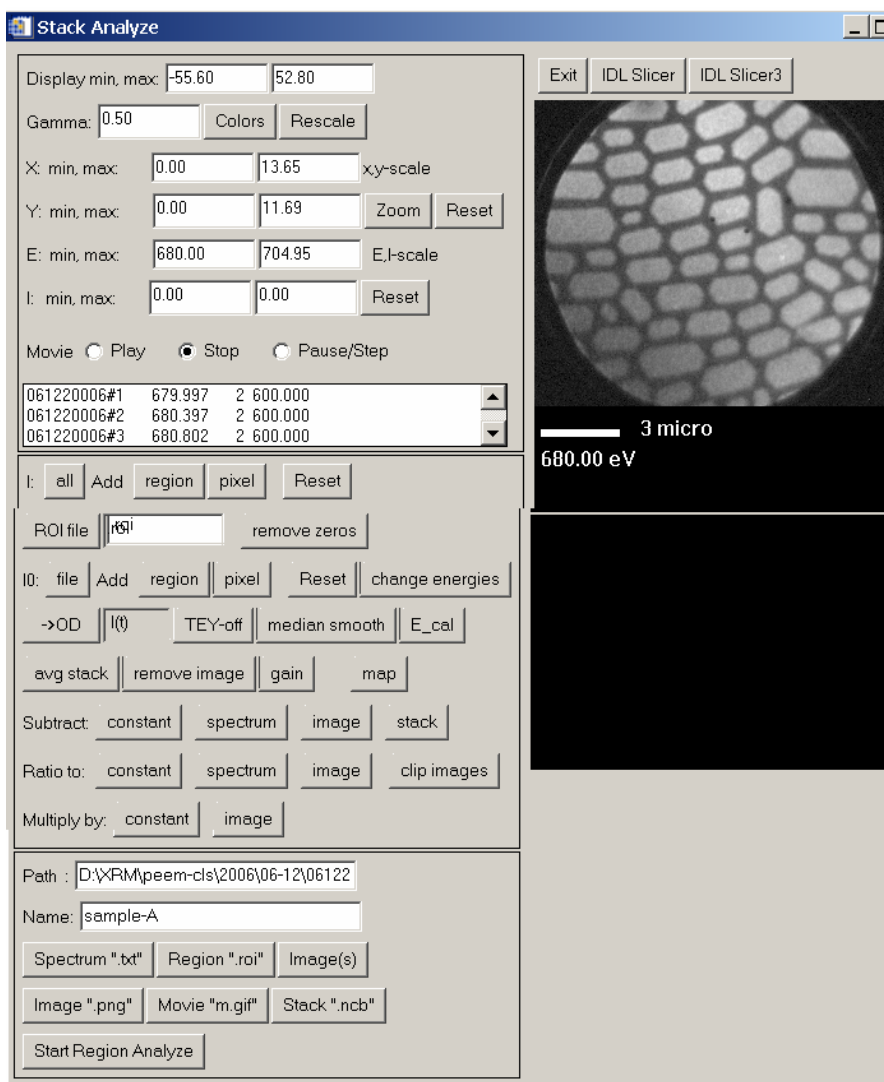
STACKS manipulation of image sequences

Analyze ~ AXIS binary

- loads an AXIS binary stack format file (*.ncb) into the stack_analyze widget. Note that it is convenient to store image sequence data in this binary representation since it is much more compact (by three times) than the original *.nc or sdf formats. Since it is possible to extract images from the binary file and since the IDL cross platform binary formatting is used, no information is lost. The user is promoted for an alignment file (to incorporate alignment shifts into data) and widget size scale factor, before the following widget appears.



- Analyze ▶
- Add
- Append
- bin ▶
- convert format ▶
- Generate_stack
- Image alignment ▶
- maps ▶
- Slicer (3d viewer)
- Stack_movie
- Statistical analysis ▶
- Tomography ▶



STACKS ~Analyze~Stack analyze (continued)

NB click on spectrum to select image at that energy

DISPLAY SECTION

Display min, max – Z-axis limits

Gamma – gamma for Z-axis

Colors – select from palette of standard IDL color tables

Rescale – [ON] min/max each image. [OFF] – use the display min, max settings

X: min, max – use to set the x-scale of image (for precise region selection)

Y: min, max – use to set the y-scale of image

Zoom – graphical expansion of image

E: min, max – use to set the energy scale of the spectral display

I: min, max – use to set the intensity scale of the spectral display

Reset – reset (E, I) scales to full scale values

Movie – play – play the movie

- **stop** – stop playing movie

- **pause/step** – pause, or advance one frame

Image parameter list –select image by clicking or using arrow keys to scroll up/down

DATA MANIPULATION SECTION

I: all - display average spectrum of all pixels (treated as I in Beer's Law or TEY)

Region - display average spectrum of region selected using

Pixel - display average spectrum of a selected pixel

Reset – reset selection of I pixels

ROI file – browse to select *.roi (region of interest) file [click in box to activate]

Remove zeros- replaces zero value pixels with a local average. (despeckle)

Io: file - browse to select spectrum as Io (treated as Io in Beer's Law or TEY)

Region - display average spectrum of Io region selected using

Pixel - display average spectrum of a selected pixel as Io

Reset – reset selection of Io pixels

change energies – exchange current energies of stack for those in a selected file

->OD – convert Z-values from transmission to optical density using currently defined Io

TEY-off [TEY-on] toggle between absorption ($-\ln(I/I_o)$) and TEY (I/I_o) normalization
(the current status is displayed between ->OD and TEY boxes)

median smooth – apply 3-point median smooth to all images

E-cal – calibrate the energy (linear shift by user selected amount)

ratio image – ratio each image in stack to image (*.nc format) selected by user from disk

avg. stack – compute average of all images within the E-min to E-max range
& store as a file and in a selected aXis2000 buffer

remove image – remove currently displayed image from the stack

gain – multiply z-scale by user defined value

map – use 2 (A-B) or 3 (A-(B+C)/2) selected images to take a difference

Subtract – constant – subtract a user defined constant from z-values of each image

- **spectrum** – subtract the selected spectrum (*.txt) from spectrum at each pixel

- **image** – subtract image from file (*.nc) from each image in the stack

- **stack** – subtract a user selected stack (*.ncb) from this stack (NB stacks can be generated from an image and a spectrum using the *stacks~Generate_stack* command)

STACKS ~Analyze~Stack analyze (*continued*)

- Ratio to – constant** – ratio to a user defined constant from z-values of each image
- **spectrum** – ratio to a selected spectrum (*.txt) from spectrum at each pixel
- **image** – ratio to an image from file (*.nc) from each image in the stack

Clip Images – replace z-values in all images which below user-selected minimum or above user-selected maximum with those minimum (maximum) values (can be used to enhance contrast and remove outliers)

- Multiply by** - **constant** – each image multiplied by user selected constant
- **image** – each image multiplied by image from file (*.nc)

OUTPUT SECTION

Path – defines the path for input / output files (default is folder that contained the stack)

Name – filename to be used for output

Spectrum “.txt” – write displayed spectrum

Region “.roi” - write currently selected pixel set to file (multiple regions are supported)

Image “.nc” – write displayed or all images to *.nc files

Image “.png” – write current display (image and spectrum) to a png format file

Movie “.gif” – write images or {images & spectra} as a movie

The stack_analyze routines generate a multi-gif file which is 'clunky' and has licensing problems for use in other applications. The AVIMaker from Platypus software (<http://www.c-point.com/>) makes avi files which are much smoother, at least when used in power point for windows. The AVI Maker can also convert the avif files to other types of movie formats. Note that the individual gif images are required as input to AVImaker so answer 'N' when asked, “delete all *.gif files?”

Stack “.ncb” – write the currently processed stack data as a *.ncb stack

The user is asked to define the lower and upper row and column indices for the saved data, thus allowing removal of the lost regions from the alignment procedure. The *.ncb binary file generated is needed for the **stack-fit** and **stack-SVD** routines. The save stack “.ncb” routine saves whatever is currently displayed in stack_analyze. It is strongly recommended that you normalize to I₀ (i.e. conversion to OD scale for transmission measurements, or divide by an appropriate reference for yield data)

OTHER COMMANDS

Start Region Analyze – Remy Coulombe routine to characterize PEEM data

Exit – exit stack_analyze widget

IDL slicer – enter widget to manipulate 3d display of stack (early version)

IDL slicer3 – advanced 3d manipulation of stack using latest IDL Slicer widget

AXIS - allows user to pick the stack_list (*.sl) and the pixel shift (*.aln) files. If you cancel after first pickfile, then stack_build_list is started. If you select a stack_list and then cancel after the second pickfile, then stack_align is started. Once these choices are made the stack_analyze widget (described above) is started)

This command can be used to re-assemble a stack, which has been written out as separate *.nc files, and has some modified images.

Zimba – Carl Zimba's version of the stack analysis code. Currently these are the highest performing codes for stack analysis, although the code, when run from aXis 2000 often locks up for unknown reasons. Note this widget can be run outside of aXis2000 if you have an IDL license by running *zstack.pro*.

STACKS~Analyze~Zimba (continued)

Some features of the Zimba stack analyze package:

- Reliable alignment (stack_analyze does have an associated alignment widget but the code in Zstack_align is more capable)
- Many different formats for Io data are supported (*.csv, *.txt, *.xas)
- Simultaneous display of original and aligned data
- Excellent manual alignment (ztune)
- Multiple spectral regions defined
- Provision to write full, aligned stack to a single file (*.ncb, *.stk)
- Provision to write image sequences as MPEGs, or a set of image files suitable for avi_maker

Note: The Zimba *.sl and *.aln file formats differ from those for the Jacobsen set. It is not generally possible to 'mix-and-match' the two sets without using a text editor for conversion. In particular, it is essential to delete all letter and non-numerical symbols from the lines listing the shifts in the *.aln file written by Zimba, before using it in the read-in procedure for stack_analyze. The ZSTACK codes can be run independently from AXIS.

Please see separate documentation for ZSTACK supplied by Carl Zimba included at the end of this help file.

Add – add two stacks (useful to take differences to remove dominant contributions to better display minority signals by using negative weighting)

Append – combined 2 stacks (must have the same pixel dimensions !) and order the images according to the photon energy (or ewquivalent variable)

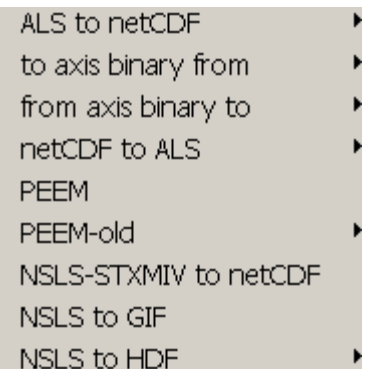
bin - bins (2-2x2 to 1 pixel, 3 = 3,3 to 1 pixel, etc) image data for better statistics. The first character of the original file name is converted to a b to indicate the processing.

- **current** image
- **NSLS - 1** – bin 1 user-selected *.nc file
 - **file** – bin all files listed in the supplied file (typically *.sl files).
- **stack *.ncb** – bin all images in a stack

convert format

For most conversions, the user selects the first file then the last file in a sequence, and the intervening files are converted assuming a standard file numbering convention (e..g last 3 positions before '.' are a file index number).

- **ALS to netCDF** – converts ALS images to NSLS [netCDF](#) files, with E-scale shifts and optional binning (adds squares of 2x2 or 3x3 etc pixels). Generates *.sl file for input to Stack_analyze automatically
 - **1** converts one file selected by pickfile
 - **many** converts many files selected by (first, last) pickfile routine
 - **file** converts files listed in the user-defined file (e.g. *.lst from ALS)



- ALS to netCDF ▶
- to axis binary from ▶
- from axis binary to ▶
- netCDF to ALS ▶
- PEEM ▶
- PEEM-old ▶
- NSLS-STXMIV to netCDF ▶
- NSLS to GIF ▶
- NSLS to HDF ▶

STACKS~convert format (continued)

- to axis binary from >

- bmp
- jpg
- mrc
- NSLS STXMIV
- TOF-all

bmp
jpg
mrc
NSLS STXMIV
TOF-all

- from axis binary to >

- mrc
- mpg

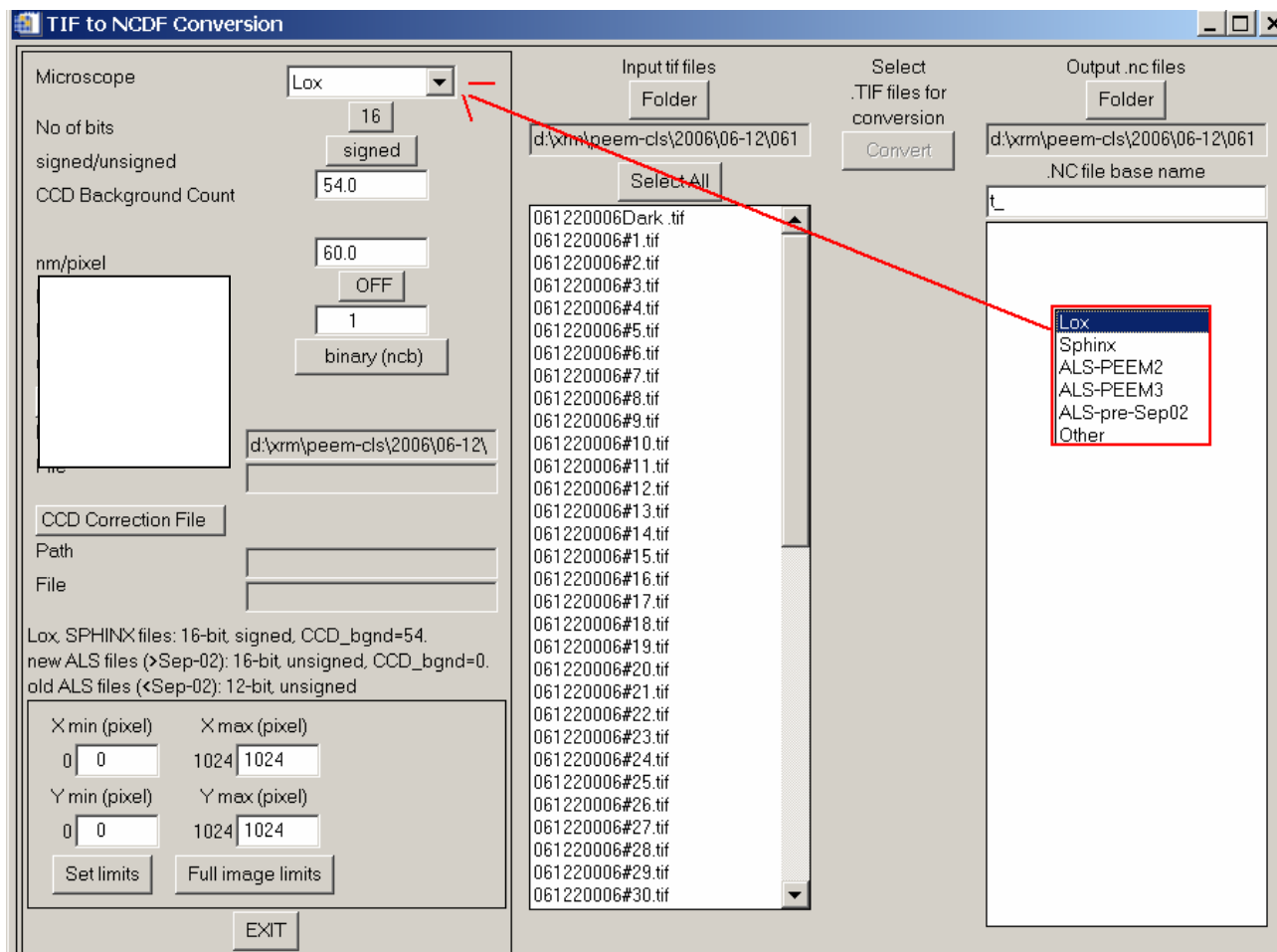
mrc
mpg

- **netCDF to ALS** - converts NSLS [netCDF](#) format files to ALS format files

- **1** converts one file selected by pickfile
- **file** converts files listed in the user-defined file (e.g. *.lst from ALS)

PEEM - converts PEEM stacks written by various instruments (Lox, Sphinx, AALS-PEEM2, ALS-PEEM3, ALS-re-Sep02 (ppem2 old format), Other)

Recommended for ALL types of PEEM data



STACKS /convert format *(continued)*

The output is either as a binary stack (*.ncb) (read with stack_analyze), or as a set of {*.nc} images (read in with Stacks~Analyze~Axis (or Zimba))

The default conditions for SPHINX, ALS-PEEM2 [new (>Sep-02) & old (<Sep-02)], ALS PEEM3 and Lox are indicated in the left centre of the widget.

The upper left part of the widget sets the conversion parameters

nm/pixel – sets scale

No of bits – 12 or 16 bit

Signed/unsigned

Median smoothing – Yes/ No

Bin - reduce size and improve statistics by 2x2 => 1; 3x3 => 1 etc

Lox - Yes / No

Output format {*.nc} set with *.sl stack list, or *.ncb file

CCD Background count – signal in absence of X-rays

Input files **Folder** – select folder with data to convert to stack (lists *.tif files)

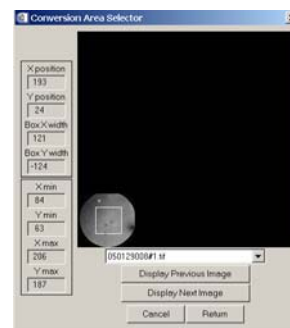
Select all – highlights all files (alternatively select those you wish to convert)

Select CCD Background file – for patterned dark signals

Select spectrum file – get energies of stack from spectrum generated during stack acquisition

Get Limits – identify pixel range to convert

Convert – this button will only become active after an energy spectrum is identified and the files to be converted have been selected. After pushing this button the files are converted to *.nc files, either temporary, or permanent. If Output format is set to *.ncb, only the binary stack is written to disk.



PEEM-old

- ALS-PEEM

to *.ncb – convert to binary stack file, without writing individual netCDF files

to netCDF – convert to set of *.nc (netCDF) files and a stack list (.sl) file

This activates the ALS PEEM read-in widget (see [Read~images~ALS PEEM](#)) and the user selects the first and last files, all conversion options, then executes the conversion.

Note: this widget does not work in the IDL VM version

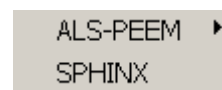
- SPHINX - convert 16-bit TIF files from SPHINX LabView program (Elmitec at SRC)

to *.ncb – convert to binary stack file, without writing individual netCDF files

to netCDF – convert to set of *.nc (netCDF) files and a stack list (.sl) file

This activates the ALS PEEM read-in widget (see [Read~images~ALS PEEM](#)) and the user selects the first and last files, all conversion options, then executes the conversion.

Note: this widget does not work in the IDL VM version



STACKS~Convert format / (continued)

-**NSLS STXMIV to *.ncb** – converts nsls STXMIV image sequence directly to a binary stack file (all images in one file). NB One can use Zimba or Jacobsen align procedures on *.ncb files.

- **NSLS-STXMIV to netCDF** - convert NSLS stxmIV format files to netCDF format files

-**NSLS to GIF** – converts one *.nc file to a GIF image

- **NSLS to HDF** - converts *.nc files to HDF format data files

1 converts one file selected by pickfile

many - converts a sequence of files with first, last identified by pickfile

Generate_stack - a stack is computed and saved from an image and a spectrum, selected by the user from aXis2000 buffers.

Image alignment - tools to manually align a series of sequential files (either ALS or NSLS format; read in after defining first and last of the sequence). *Note this always works whereas the manual align in ZSTACK is somewhat 'tempermental'.*

shift - 1-point calibration of image (x,y-scale).

Stretch/ shift - 2-point calibration of image (x,y-scale)

After shifting or shift/stretching the (x,y) scales for image alignment, this routine then:

1. grids to a user-defined pixel size (should be similar or smaller than the recorded pixel size to avoid loss of information)
2. cuts to a fixed user-defined [xmin, ymin, xmax, ymax].
3. bins to a user defined factor (1=no binning) to trade off S:N versus spatial resolution
4. writes a netCDF file. The names are FORCED to be the same as the input but with the 1st letter of the original filename converted to **s** (shift) or **a** (shift/stretch).
5. generates *.sl file of filenames to be used in stack_analyze. In addition it stores 2 (**s**) or 4 (**a**) files which are the shifts in real-space units (microns) (from buffers 5,6 and 8,9).

The parameters defined in the first pass are saved and applied automatically to all subsequent files. The X,Y shifts and stretch terms for each file are accumulated in buffers 7 (stretch) 5,6 (point 1,x,y) and 8,9 (point 2,x,y) for later use to explore how the microscope or the sample is changing.

Notes for image_alignment:

- *Clear buffers 5-9 (using the **Clear Buffer** above the thumbnails) before starting as they are used for results*
- *Check the extreme limits of a stack sequence to ensure the [xmin, ymin, xmax, ymax] values used to define the common part of all images are valid for all files.*

Warp – Image alignment using a polynomial 2-d transformation to align successive images to the 4 or more fiducial points identified on the first image, and on each subsequent image. This can be useful when the sample distorts during a measurement due to radiation damage

STACKS (continued)

maps

SVD - convert an image sequence (stack) to [component maps](#) using singular value decomposition (SVD) procedures. When accessed from this menu item, the **input must be an AXIS format binary file stack (*.ncb)**.

Typically the input stack consists of a set of images prepared on an optical density (OD) scale with careful alignment. The reference intensities at each energy of the stack are extracted from user identified reference spectra (*.txt, read from disk). The user is prompted to either read the list of reference spectra from a *.par parameter file, or, after identifying the individual spectra, write the list to a *.par file. This is helpful in cases where the same set of reference spectra might be applied to a number of stacks.

The **output** is a set of **composition maps**, automatically written to files with names constructed from a root and the component names (it is wise to keep the component names SHORT to avoid excessively long file names in directory listings). In addition the **residual** signals averaged over all energies is saved as an 'image'. Comparison of the magnitude and spatial distribution of the residual signal is a useful way to evaluate the validity of the analysis.

Optionally a **stack of the residuals** signal can be saved for later examination.

If the input stack is in OD units, and the reference files are on absolute linear absorption scales, the Z-values of the resulting component maps are in absolute thickness units (in nm, assuming the reference spectra are in units of nm^{-1}).

NB the SVD code can also be run from the IDL command line and has different features not implemented directly in aXis2000. See the code file for further details.

SVD
Stack fit
CGO curve fit
polarization fit

Stack-fit – Performs a linear regression analysis (linear least squares fit) of the spectrum at each pixel to a sum of (1 to 8) user-defined model spectra and a constant.

NOTE: Relative to SVD maps, stack-fit adds an additional component to the analysis.

CONSTANT' is a parameter which is flat spectrally (same at all energies) but different at each pixel. This may lead to additional uncertainty in the result. However in some cases, where there can be offsets of reference spectra relative to the data, due to Io errors, stack-fit is the more logical choice.

CGO curve fit . This uses a conjugate gradient optimization method (from Numerical recipes) to perform spectrum-by-spectrum curve fits to reference spectra. The user dialog is similar to those used in the SVD and stack fit routines. The fit of a single spectrum can be carried out using this method with the *spectra~curve fit* command.

In tests on low noise data sets with valid OD scales and with accurate spectral models, essentially identical component maps are generated by SVD maps, stack fit and CGO curve fit

For SVD, Stack fit and CGO-fit analyses with less than 5 reference species, at completion the aXis2000 buffers contain:

Buffers **1-3(5)** – the reference spectra

Buffers **4-6(8)** – the component images - i.e. the spatial distribution of the component

Buffer **7** – the map of the linear term (for **Stack-fit** only)

Buffer **9** – the map of the chi square values of the fit at each pixel (~ fractional uncertainty)

STACKS/map~CGO fit (continued)

For analyses with more than 5 reference species, the component maps are stored in the same buffer number (**1-8**) as the reference spectra and buffer 9 contains the residuals map.

After making the component maps it is often useful to explore the spatial correlations of components by coalesce any 3 of the component maps into a single **color-coded composition map**, by using the *Images~RGB* command. (*If you only want to combine 2 maps, select one of them for 2 colors then use an image processing program such as Paint Shop Pro to set the duplicate color to zero intensity*)

How to evaluate the significance of maps derived using SVD, Stack fit or CGO fit.

The user of *stacks~maps* must realize that the fitting code will always give a result, but the result may not be valid. It is important to evaluate the quality of the fit. AXis2000 provides a number of tools to do this.

One can apply *images~generate_mask* on the **residual map** (in buffer #9) to write out a *region_of_interest* (ROI) file corresponding to the poor fit regions. Using the ROI files in *stack_analyze* of the stack allows the analyst to extract the spectrum of the poor fit region, which may be a 'missing component'.

Examination of the **residual stack** can help to evaluate the validity of the fit. Spectra extracted from various regions of the residual stack should be only noise; if there is a missing chemical component one can sometimes obtain its spectrum in the poor fit regions of the residuals stack.

One can apply *images~generate_mask* on the component maps to identify those pixels with large amounts of components of interest. After extracting the spectrum of those pixels using the ROI files in *stack_analyze* of the stack, the quality of the spectral fit can be examined by applying *spectra~curve fit~{linear regression, or CGO fit}*

Polarization fit

Fits a sequence of images where the control variable is the angle between the E-vector and the image. Such data can be generated by azimuthal angle scanning (e.g. at STXM532), or by rotating the E-vector with an EPU (at STM1102 and CLS-STXM). The polarization signal is fit to

$$I(\theta) = C + A * \cos^2(\theta - B)$$

where C is a non-angle dependent constant, A is the amplitude of the dichroic signal and B is the 'director' (angle of maximum intensity)

Note that the quality of the fit can be evaluated by selecting a region of interest, extracting the angle-dependent signal then using **spectra~curve fit~pol fit**

Stacks (continued)

Slicer – launch IDL's 3d viewer with read in of a binary stack

Stack_movie calls Jacobsen stack_movie routine. Files defined by user-selectable stack list (*.sl) file. If 'cancel' is selected, the stack_build_list routine is initiated.

Statistical analysis

principle components
PCA_GUI (CJJ Dec 2005)

- **Principle components** - applies the IDL principle component analysis package (PCOMP.SAV) to derive the **eigenspectra** (power weighted representation of the data) from a set of OD images (binary stack in an *.ncb file). The routine implemented in aXis2000 provides the **eigenvalues** (fractional contribution of each eigenspectrum) and eigenspectra of the first 8 components. This is useful to obtain a sense of how many independent chemical species might be present in a give data set. The **eigenimages** are readily generated by using the eigenspectra as model spectra in a SVD map
- **PCA_GUI (CJJ Dec 2005)** – combination of principle component, cluster and target analysis allows for unsupervised analysis to extract a set of potential NEXAFS spectra, with extensive capabilities to evaluate and optimize. See the manual for this package for further details.

Tomography

Routines to analyse **4d** (**x,y, E, θ**) data.

Gen_stack from SDF files reads a set of angle varying images to generate an (**x,y, θ**) stack

gen_stack from SDF files
read one image
read map
read stack

Read one image

Read map

Read stack – read full 4-d (**x,y,, E, θ**) data and convert to format suitable for read in to IMOD.

LINESCANS

Processing of [linescan spectra](#). Linescan processing methods are also useful for a variety of image analysis tasks such as:

- obtain averaged **profiles** from an image along orthogonal directions
- remove low frequency noise from images (average all horizontal, or all vertical, then ratio)

If the direction of interest in the image is not oriented along vertical or horizontal, use *Image~rotate* to rotate the direction of interest to horizontal or vertical

Add lines ▶
align ▶
line_fit
locate line
normalize to I₀
normalize to line ▶
subtract reference

Linescans~Add lines / *continued*

Add lines - sums all lines selected with cursor (lower / upper limits)

- **Horizontal** (for Linescans, sum spectra over a range of length along the line)
- **Vertical** (for Linescans, sum line profiles over a range of E)

align - 'tilts' data to user specified line

- **Linear** – straight line defined by 2 points
- **Curve** – curved line defined by multiple section straight lines

line_fit – apply curve fit (as in stack-fit) to a linescan. This is an excellent method to analyze and visualize chemical composition along a line.

locate line – draws (x,y) position of a linescan on the image currently displayed using information from SDF parameter file for the linescan. Be sure to display an image on which the linescan was defined (or at least without any base scan changes).

normalize to Io – divides each **horizontal** line by user-selected buffer then

computes $-\log(\text{Image}/I_o)$. Get I_o from Add lines~Horizontal if there is an open region in the linescan or from a separately recorded point or line spectral scan.

normalize to line

Horizontal - divides each **horizontal** line by content of user-selected buffer

Vertical - divides each **vertical** line by content of user-selected buffer

Hint: To remove line-by-line periodic scan noise, generate a 1-d profile of periodic noise by *Add lines~vertical* (or *Add lines~horizontal*, as appropriate) over all or part of an image then subtract that profile from the image using *Normalize to line*.

subtract reference computes (Image – ref), The reference 1-d profile signal is taken from user selected buffer (this does same thing as *normalize to line ~horizontal*)

SPECTRA

Procedures only for processing 1-d (typically spectral) data

Absolute value – computes absolute value

Add

Append - append 2 data sets - all data points in overlapping region are kept

Buffer - generate SUM of 2 spectra (interpolation used)

Constant - add (or subtract) constant to y-axis

Calibrate (for each of X and Y axes):

Auto - uses last axis recalibration parameters

1 point – shift values by user defined amount from cursor selected point (shift)

2 point – shift values based on 2 points (shift & stretch)

Numerical 1- or 2-point with numerical input

Curve fit

linear regression – use IDL regress function to fit the spectrum in the current buffer to a set of reference spectra. The reference spectra can be identified using a *.par parameter file (identical to that used in *stacks~map* and *linscan~fit*)

CGO fit - use conjugate gradient optimization method (CGO) to fit the spectrum currently displayed in a buffer to a set of reference spectra which the user is prompted to select from the disk. This is a useful method to check and display the fits that are obtained from image stacks. The fit components are listed on the IDL log as well as in the aXis2000 log display.

Pol fit – fit an angular dependent signal to

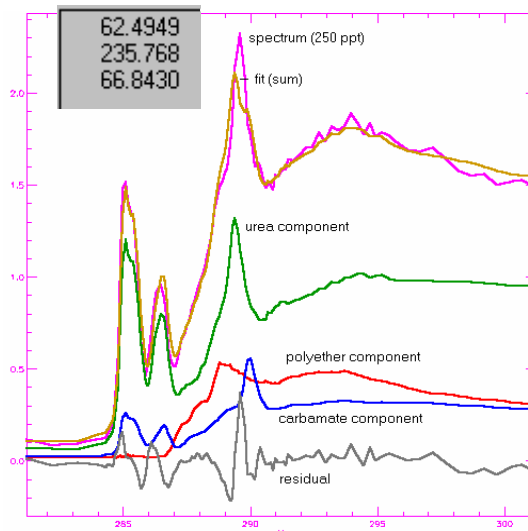
$$I(\theta) = C + A * \cos^2(\theta - B)$$

Convert_to_OD – automatically generate OD spectrum from a transmission spectrum by identifying a second buffer with I₀

Delete - delete all data between 2 cursor-identified positions

Differentiate – take derivative of displayed signal (simple $\Delta Y/\Delta X$ only)

- Absolute value
- Add ▶
- Calibrate ▶
- Curve fit ▶
- Convert_to_OD
- Convert to IP
- 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



Spectra /continued

Gain - **multiply** – multiply y-axis values by a factor
- **divide** – divide y-axis values by a factor

E_to_wavelength - X-axis transformed by $12398/X$ (symmetric conversion)

exp(Y) - computes exponential

Integrate – determine integral of displayed signal

ln(Z) - natural log of z-values (use to convert Transmittance to Absorbance)

log10(Z) - base-10 log of z-values

Linear background - subtract user defined line

Modify one point – left click – add a point, right click – delete the point

Multiply – multiply the spectrum in the current buffer with that from a used selected buffer.

Peak area – determine area under curve between user-selected limits

Ratio_to - takes ratio of 2 buffers (interpolated to same scale)

Reverse values – reverses the (x,y)-values in a spectrum.

Split – separates a multi-valued spectrum into single-valued regions, placed in successive buffers. Data of this type can be generated by acquiring multiple regions which are not in strict increasing-energy order. This can be useful as a means to track radiation damage. Recording the most chemical sensitive part of a NEXAFS spectrum at the beginning and end of a point spectrum is an effective way of having an internal check on radiation damage.

Hint: AXIS auto-detects multi-section spectra recorded with overlapping multiple regions and plots all components in the same buffer. The separate single-valued sections are placed in sequential buffers by this command.

Truncate - truncate spectrum to data between 2 cursor-identified positions

DISPLAY

Over Plot - display **multiple spectra**

No Rescale - select multiple buffers (0-9)

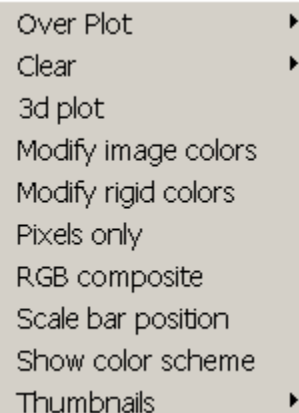
Y-axis of data is preserved

Rescale – select multiple buffers (0-9)

each spectrum is rescaled to full screen

Window - add a single buffer, with data rescaled within y-limits selected by the user using the cursor

Shift - add a single buffer, with bottom y-position selected by cursor; no rescale (ie y-scale is set by previously plotted data)



- Over Plot ▶
- Clear ▶
- 3d plot
- Modify image colors
- Modify rigid colors
- Pixels only
- RGB composite
- Scale bar position
- Show color scheme
- Thumbnails ▶

All modes of *Display~Over Plot* can be combined in any order. All previous *Display~Over Plot* processing is preserved in the main plot window until a single buffer is selected for plotting, by left-clicking on either the thumbnail plot, the buffer label, or the indicator box.

Clear Lines – removes cursor-related lines from Main Image

Current – erases only the currently selected buffer

Selected - any of 0-9 buffers can be erased (also as second row button)

All - resets all buffers to zero (like starting a new version of aXis2000)

NB the **Clear Buffer** single line command accesses the last 2 commands

Hint: If the colors go 'crazy', restore the default color scheme by **Clear Buffer** or *Display~Clear*.

This often occurs on the first use of *stacks~analyze~zimba* – a black display is generated when displaying the first selected spectrum. Use the **Clear Buffer** command above the thumbnails to reset the color scale, and then erase and re-select the regions in *stacks~analyze~zimba*

3d-plot - generate 3a -d shade surface plot from an image with x,y,z axes.

Modify image colors - pop-up widget (XLOADCT) that selects color scheme and adjusts (top, bottom, gamma) variables. *In Win systems, if 256 colors is set, this updates all graphic windows dynamically. If color is set to higher value (16-bit, 24-bit, or true color) one needs to redisplay a graph to change the color scaling. Since each thumbnail sketch (upper left of screen) is displayed independently, the color scale of the thumbnail sketches will only change after they are selected.*

Modify rigid colors - select custom colors for buffer specific spectral colors, background, foreground, etc

RGB composite – takes images in 3 buffers and assigns them to red, green and blue color components of a single image. It is necessary that all images are the same size and are of the same physical region. The composite image is saved to disk in a 3-component TIF format which reads into Paint Shop and Powerpoint. The individual R, B, G components can be read back using the *Read~Images~Other~TIF~data* command. User is given option to use a common scale for all three images (thus preserving information about relative intensities of components), or autoscaling each image independently, which will give equal visibility to each component.

Scale bar position – use cursor to define the left end of the scale bar.

Display / continued

Show color scheme - display current colors assigned. Note that the bottom 16 colors in the 256-color table are assigned to 'hard' colors to allow ready differentiation of different buffers.

Thumbnails – display multiple buffers on main window

4 – user selects any 4 buffers

- **common scale** - plot using identical scale for all images (in B/W for printing)
- **rescale each** – plot with each image byte scaled to its data values (in color)

9 – display all 9 buffers

- **common scale** - plot in B/W (for printing)
- **rescale each** – plot with each image byte scaled to its data values (in color)

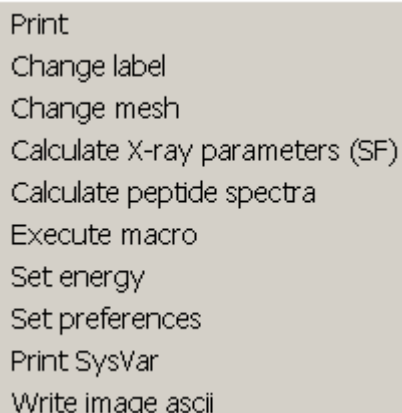
UTILITIES

Print

Logbook (default: 3" x 3") – no annotation

Annotated (default: 4" x 4") – brings up the IDL annotate widget.

***Hint:** (1) A fast alternative to printing is to use **PrtSc** (WIN) to copy the monitor screen to the clipboard, then use an image processing program (e.g. Paint Shop Pro) to cut out the area of interest, and paste that into a presentation program, such as powerpoint.*



***Hint:** (2) Use The PRINTER option in axis.ini to use your default system printer.*

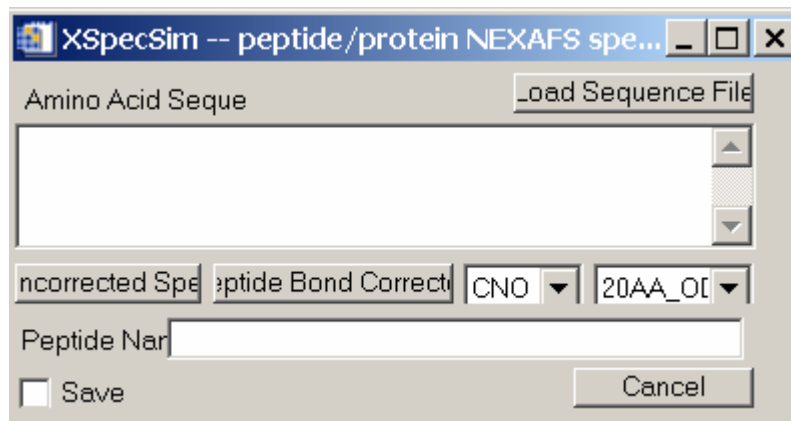
Change label - modify label (this is what will be saved to disk)

Change mesh - redefine numbers of pixels (images) or points (spectra). This is useful to allow matching of sampling of different data sets.

Calculate X-ray parameters (SF) - Determine the **mass absorption** or **transmission** for user defined elemental compositions. The mass absorption atomic signal provides normalization for model spectra used in the SVD map and stack_fit routines. The transmission for a user-defined composition, density and thickness can be useful to evaluate feasibility of a sample. NB The ***sf.pro*** program (in the AXIS directory) is a full implementation of the old CXRO SF program for X-ray constants, which corrected some errors. Run SF at the IDL command line to access all features. (use *sf, /help* for a list of the capabilities)

Utilities / continued

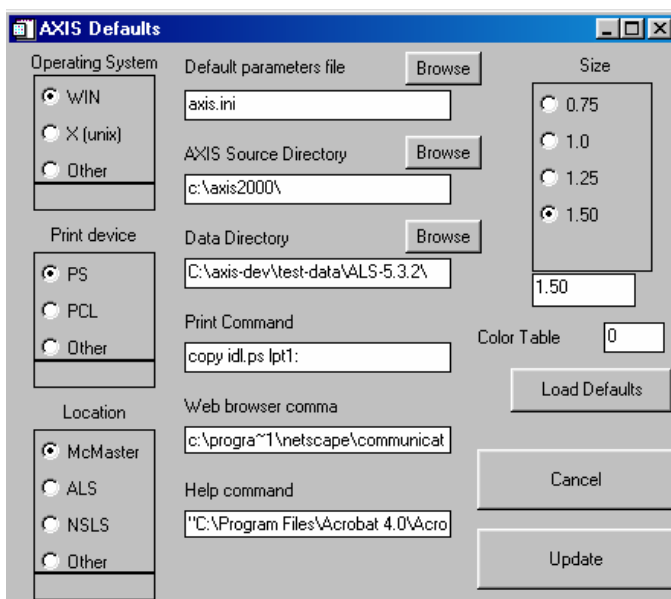
Calculate peptide spectra – launches a widget to generate the NEXAFS spectrum of a user specified amino acid sequence from the C, N, O spectra of the constituent amino acids.



Execute macro – execute a file of standard aXis2000 commands (with parameters) So far only a few of the axis commands are set up to be used in this way.

Set energy - set energy of an image - useful to calibrate the energy of images for, eg. SVD. Also, in some cases *Write~nsls* does not store the correct energy and this fixes those files.

Set preferences – allows user to modify the default parameters which are contained in a file called **axis.ini**. This uses a widget (*see fig*) to let the user define a large number of different parameters. The modified initialization file is written at the end of each normal exit to aXis2000 so that it starts in the same configuration (default directories etc) as in the last use.



On start-up, if AXIS cannot find the default initialization file (**axis.ini** in the subdirectory where the AXIS code files are located), it starts this widget.

Print Sysvar - display on the IDL log window the current values of all IDL graphics parameters (used in code development).

Write_image_ascii

reals – writes the image as ascii floating point numbers (reals), with header and x, y values as 1-d arrays preceding the 2d image data

integers – writes image as integers. (x,y) are reals, but the z-values of the image are scaled by a power of 10 to give adequate dynamic range (0-9,999 or better)

Second row of single command buttons



EXIT write [axis.ini file](#) with parameters then return to main IDL command window.

QUIT return to main IDL command window, without saving current parameters

NOTE:

From the Command Window of IDL type axis2000 to (re-)start AXIS. If you wish to display the rotating AXIS logo, restart AXIS by typing **axis2000, /spin** at the IDL prompt. With or without rotation, if you have a net connection, and you have the command to start your browser identified correctly in the axis.ini file, then when you click on the indicated synchrotron sites or the aXis2000 label, a web connection is made to the X-ray microscopy facility or [aXis2000 web site](#).

XimageViewer

Launch a viewer of image files. In addition to standard image files (jpg, bmp, gif, tif, png, etc) this displays ALS_STXM (SDF) format files in the selected folder. This is useful when you do not have detailed information on data files. This DLL was provided by Ivo Koprinarov.

HELP

Calls Adobe Acrobat reader, using the Unix (Acroread) or Windows command (AcroEx32) to bring up **AXIS.pdf** (a pdf version of **AXIS.doc**). Since the location of the Adobe reader depends on the system, the system command that will start the Acrobat reader and display the AXIS.PDF file is part of the AXIS.INI file and is set by the **help_cmd** line in the **set_preferences** dialog.

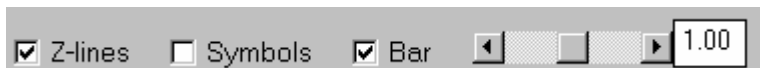
COPY buffer

- moves contents of the current buffer to one of the 10 data buffers in AXIS. The destination buffer is selected by clicking on the thumbnail, or by clicking on the label of the buffer to which you wish to move the data. Note the latter is the only way to move data into buffer 0.

Hint: *To preserve a processed spectrum or image located in Buffer 0 you must use Copy to transfer it to buffer 1-9 to avoid overwriting the result in the next processing step.*

CLEAR buffer pops up multi-buffer widget to let user select those buffers they wish to clear. (The same function is also available as *utilities~clear*).

CONTROLS BELOW THE MAIN IMAGE WINDOW



Z-lines

- ON (default) - X, Y lineouts generated at every mouse move
- OFF - X, Y lineouts generated only on left mouse click

Note: The operation of *Zoom~pan* is also switched with this button. If ON, the zoomed image updates on every mouse motion. If OFF, the zoomed image updates only after a mouse click on the main image

- Symbols**
- ON - plot symbols on lineouts and spectra (main image window only)
 - OFF(default) – no symbols on lineouts or spectra

Note these options allow one to adapt to the speed of the computer. Continuous updating of the lineouts and the zoom/pan, as well as symbol plotting, take large amounts of cpu/graphics resources and can slow aXis2000 to a crawl on slow computers.

Bar – ON – display white scale bar of correct size (1,2,5,10 pattern; within 5-10% of image)
The position of the scale bar can be defined using *Display~scale bar position*

Gamma control – use either the slider or the number box to change the image gamma. Note this control is only active if an image is displayed.

*****XXXXXXXXXXXXXXXXXXXXXXXXXXXX*****

INSTALLATION

*****XXXXXXXXXXXXXXXXXXXXXXXXXXXXX*****

Copy all code into a directory. (use **WinZip** or similar to **expand axis2000.zip** using folder names into your preferred source directory). – I use c:\aXis2000\.

The directory structure should be:

```
C:\aXis2000\    \logo\  
                  \sfdata\
```

To set-up IDL so it starts aXis2000 every time:

- A.1** Start IDL I use version 5.2)
- A.2** Set up preferences
- Path – add the directory with aXis2000 files
 - Start-up- working directory: c:\axis2000\ (or whatever your source directory is)
 - start-up file: axis2000_batch
- A.3** Shut down IDL and restart.

To use IDL without automatically starting axis2000

B. 1 Type `AXIS2000,/dialog` at the IDL command line.

Several different versions of the [INI file](#) are included. Try the one identified for your operating system. If necessary use a text editor to edit the entries for your needs. Typically, this will involve changes to the SIZE parameter (to just fill your graphics screen), the codes used to launch acrobat, command that will run your web browser and command to transfer a postscript to file to your printer. See below for an example and more details.

A. For PC (or other WINDOWS device) machines

Color schemes greater than 256 work fine. In most cases, data is redisplayed, but occasionally you need to refresh a display to get a new color table to be implemented (With color tables above 256 - 16-bit, 32-bit or true color) IDL does not automatically update the display)

B. For Unix (X-windows) based systems

Since Unix is case sensitive, it is important that the lower case of the names of the files be preserved. All the AXIS file names are strictly LOWER CASE. Some FTP programs force the file names to upper case which is a problem. If you have error messages saying a given pro file cannot be found please check the case of the filename and rename it to exactly what the IDL error reports. Use ASCII transfer for all files (except *axis.bmp*, *axis.doc*, *axis.pdf* associated with the stack codes, which must be transferred in binary mode) when transferring code from Windows to UNIX or Macintosh systems to avoid problems with the differences in end-of-line coding in the 3 systems.

- the UNIX version of IDL only likes @bsif_com or @axis_com in the AXIS.PRO file. On the Linux-PC version the batch processing would not work when long names such as axis_com.mon are used for include files. The COMMON include files are now:

axis_com.pro	aligncom.pro
analcom.pro	bsif_com.pro
img_com.pro	

The stack codes which are located in the same subdirectory as the rest of the axis code differ significantly from those currently being supplied by Chris Jacobsen on his web site. These codes are based on the Jun-98 version of his stack widgets, but with considerable enhancements.

Run time hints:

If you quit AXIS but stay in IDL, restart AXIS by executing **axis2000** at the IDL command line.

IDL Virtual machine version

The aXis2000 widget can be run without an IDL license by downloading the IDL VM (virtual machine) code, and running the aXis2000.sav file contained in the aXis2000 package. Instructions for setting up a Windows desktop shortcut to this are given at

<http://unicorn.mcmaster.ca/aXis2000.html>

Please provide feedback (by email by preference) on how you would like to see this program evolve to be more useful to the X-ray SpectroMicroscopy community.

If you report a bug, I will try to fix it as soon as possible. It is most useful if you can send a small data file which exhibits the bug, and, if you are running a full version of IDL, the IDL log window error report.

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FOR THE BEGINNING USERS, a number of **USEFUL DOCUMENTS** are available by download from <http://unicorn.mcmaster.ca/aXis2000.html>

TUTORIALS – a set of unit operations for manipulating X-ray microscopy data.

STACK_ANALYZE_MANUAL.doc - manual to walk-through a typical stack analysis based on ALS STXM or PEEM data. [DATED]

STACK_FIT.DOC - manual to walk through stack fit from a prepared *.ncb aligned image sequence.

FOR THE PROGRAMMERS – SOME USEFUL ADD-INS (in aXis2000.zip)

Content.lst - lists all AXIS routines with a one-line description

Run **ax_make_html** at the IDL prompt. This will extract the header documentation from all the files to generate an html document with internal hyper-links etc. (there is an HTML file in the aXis2000 distribution with this output)

TEST-DATA – a set of files that are used to test the formats AXIS is able to process
(contact Adam Hitchcock to obtain)

The **aXis2000 initialization file** which customizes aXis2000 to your computer.

A sample AXIS.INI file (on a WIN system):

LINE#

```
1  c:\axis2000\  
2  WIN  
3  PRINTER  
4  F:\APH\papers\ICESS9-fg-peem\figures-data\Fg-buffer\  
5    1.50000  
6    0  
7    mac  
8    idl.ps  
9    Arial*14  
10 copy idl.ps lpt1:  
11 "C:\Program Files\Adobe\Acrobat 6.0\Acrobat.exe" c:\axis2000\axis2000.pdf  
12 "C:\Program Files\Internet Explorer\IEXPLORE.EXE"  
-----  
written:  13:19:25 Fri Mar 25  2005  
  
; ***** Initialization file for AXIS Widget *****  
;  
; (aph 31-Jan-00)  Please adapt to your hardware  
; Items set          devices currently supported  
; -----  
0 CodePath           (e.g. c:\axis\) - include final separator  
1 Screen display device (WIN, X)  
2 Print file format   (PRINTERPCL, PS) ; Printer uses system default  
3 Default data directory (any - last path wil be saved on exit of AXIS  
4 graphics scale factor (0.5 - 2) (1 = 360x360 pixels)  
5 color table         (any IDL supports) 0 = B/W; 3 = red temperature  
6 location            (NSLS has special meaning = spooler switch)  
7 spooler or print file (c:\tmp\xla at nsls - special !!)  
8 default font for widget (windows - Arial*14; unix - 6x10 or 8x13 ) NOT USED  
9 Printer command line (command line needed to transfer to printer)  
                        NOT used if PRINTER is set on line 2  
10 Command to view Help pdf (get acrobat reader free from www.adobe.com)  
11 command to run browser  (WIN: must be executable from MS-DOS window)
```

GLOSSARY of terms associated with aXis2000

axis.ini - text file containing default parameters for aXis2000. May need to be edited to optimize performance

component maps - spatial distributions of a chemical species, which can be generated from multiple images (selected energies or a full image sequence) using SVD maps or stack fit

dark count – signal (image or spectral) recorded without X-rays. This is detector specific (e.g. CCD camera leakage, light leakage into PMT etc) and must be subtracted from real signal before any data processing involving ratios (yield or absorption determinations).

IDL Virtual Machine – a version of IDL which allows execution of compiled IDL script. This can be downloaded for free from Research Systems Inc (rsinc.com)

IDL widget - a graphical user interface with pre-programmed data manipulation or other capabilities written in Interactive Data Language, from Research Systems Inc (rsinc.com)

ini file - a text file which contains values of parameters used to customize aXis2000 for your environment

intensity profile - Plot of pixel value across a line defined on an image. Rectilinear profiles are available automatically, updated either on each mouse move, or after a right click, depending on the setting of the continuous lineouts control.

line scan spectra – a 2-d data set in which the intensity along a line is recorded at a series of photon energies. aXis2000 displays the data with energy along the horizontal axis and position along the line as the vertical axis. Thus horizontal lineouts (lower side panel) display spectra at a point; vertical lineouts (left side panel) display the contrast along the physical line at a given photon energy.

masked image - Output of *Images~generate_mask*. It is an image consisting of only 0 or 1 value pixels, based on whether the original pixel was above (1) or below (0) a user-defined threshold. This, along with *Images~multiply buffers* and *Images~average pixels*, is useful to evaluate the intensity in a selected region of a component map.

netCDF – a standard binary scientific data format. The data is read and written by platform dependent routines provided by a standards body. Used for NSLS STXM data.

OD – optical density, or absorbance. In a transmission measurement (STXM, TXM) the recorded data is the intensity of the transmitted X-rays. It is converted to OD by $-\ln(I/I_0)$ where I is the signal transmitted through the sample and I_0 is the signal without the sample in place.

self defining files - A structured, ascii format in which a syntax is used to allow a single program to read (and write) complex data without prior knowledge of the data structure. Read and write routines are available in C++ and IDL.

ZSTACK

Data Analysis for Hyperspectral X-ray Microscopy Imaging v2.1 (5 jan 2001)

Dear Zstack User:

ZSTACK is a suite of IDL procedures for alignment and analysis of a series of x-ray microscopy images that have been acquired at different x-ray energies. It operates on Windows, Macintosh, and UNIX platforms using IDL v5.0 or later.

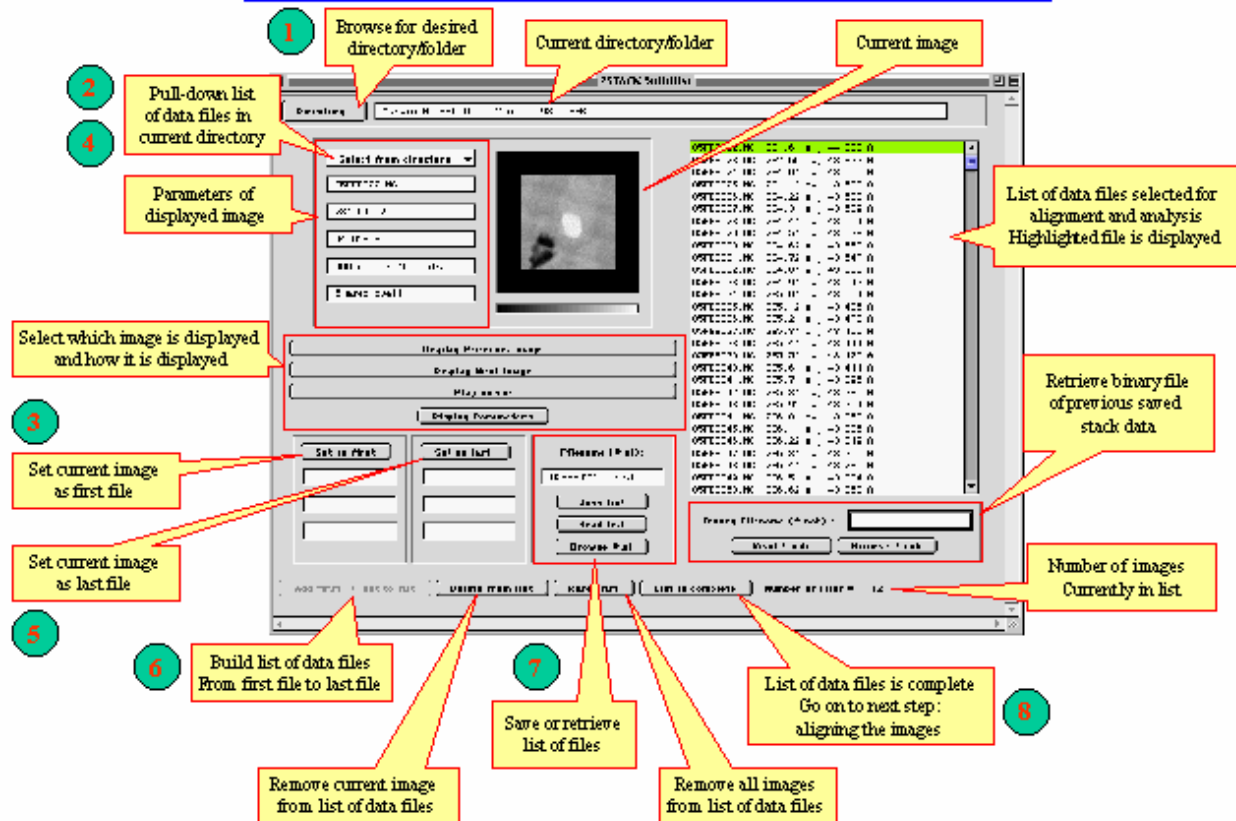
ZSTACK was written originally to satisfy my own needs for analysis of STXM spectral image stacks. This is based upon the original STACK code developed by Chris Jacobsen (SUNY - Stony Brook) but it has been expanded considerably beyond the initial scope, largely due to my analysis needs and feedback from fellow users.

In a collaborative effort with Adam Hitchcock (McMaster University), ZSTACK has been bundled into his aXis2000 data analysis package. ZSTACK can also be used independently of aXis2000. The development of ZSTACK has been an evolutionary process. Please notify me of any bugs, problems, comments, or suggestions for improvements or new features.

Carl G. Zimba

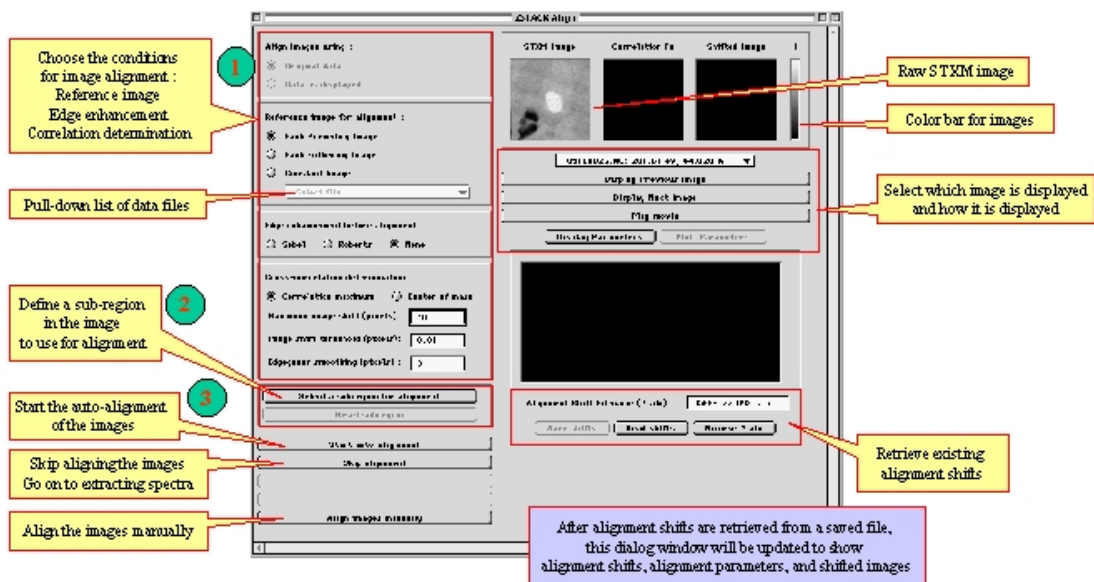
Photons UnLimited
84 Oxford Street
Arlington, MA
photons@mediaone.net

ZSTACK Buildlist



ZSTACK Align

(before alignment)



ZSTACK Align

(after alignment)

This now displays the conditions which were used to obtain current alignment

Subregion used for alignment

Correlation function crosshairs: red for center of corr'n image, blue for maximum position. Red box is edge of corr'n image

Select which image is displayed and how it is displayed

Plot of alignment shifts. Cursor position corresponds to current image and can be moved by clicking mouse in the plot

Save or retrieve alignment shifts

Keep current alignment

Skip the alignment

Redo the alignment erasing the subregion

Redo the alignment keeping the subregion

Manually adjust the alignment

ZSTACK Tune

Adjust the x shift using either the "New" text box or the slide bar

Adjust the y shift using either the "New" text box or the slide bar

Select which image is displayed and how it is displayed

Add fiducial points or shapes to close-up of shifted images

Plot of alignment shifts. Cursor position corresponds to current image and can be moved by clicking mouse in the plot

By placing some fiducial points in the close-up of the shifted images, the quality of the alignment can be inspected while playing the images as a movie

Keep new alignment

Keep old alignment

Reset to old alignment

ZSTACK Spectra

Define regions in image to extract spectra using Region of Interest dialog

If desired, retrieve an I0 spectrum (raw data, *spc, *xas)

Save extracted spectra as single beam, %transmittance, or absorbance in *spc, *xas, or *gif format with all spectra in a single file or one file for each spectrum

Add fiducial points or shapes to close-up of shifted images

Images shifted after alignment

Shifted images clipped of edges where there is no longer any data due to shifting

Select which image is displayed and how it is displayed

Plot of spectra. Cursor position corresponds to current image and can be moved by clicking mouse in the plot

Get intensity vs position

Save images

Finished ? Quit here

Fourteen separate regions of interest can be specified, yielding fourteen I spectra. In addition, a region of interest can be specified in an empty region of the sample to yield an I0 spectrum.

Color of the region of interest in the images corresponds to the same color spectrum.

Region of Interest



ZSTACK Profile

Adjust position of cursor and profile axis

Save intensity profile

Save spectra of current pixel

Save spectra of each pixel along profile axis

Map of intensity as a function of x-ray energy and position along the profile axis

Cursor position and x-ray energy can be moved by clicking mouse in the plot

Cursor position can be moved by clicking mouse in the image

Color of the region of interest in the images corresponds to the same color spectrum

The profile axis is the yellow line in both the shifted image and the profile image. The intensity along this line is plotted as the profile intensity.

Plot of intensity along profile axis

Cursor position corresponds to current position along profile axis and can be moved by clicking mouse in the plot

Plot of spectra

Cursor position corresponds to current image and can be moved by clicking mouse in the plot

Red cursor in profile plot corresponds to position of red line in both the shifted image and the profile image

Finished ? Return to ZSTACK Spectra

Select which image is displayed and how it is displayed

Select which profile is displayed and how it is displayed

ZSTACK Save

Specify base filename and directory to save files

Select a subregion of image to save

Trim images to eliminate edges clipped off during alignment

Save images in a variety of formats, optionally including spectra, roi, and legend info

Save images as a movie, optionally including spectra, roi, and legend info

Save image stack as a binary data file

Images shifted after alignment

Shifted images trimmed of edges clipped during alignment

Select which image is displayed and how it is displayed

Plot of spectra

Cursor position corresponds to current image and can be moved by clicking mouse in the plot

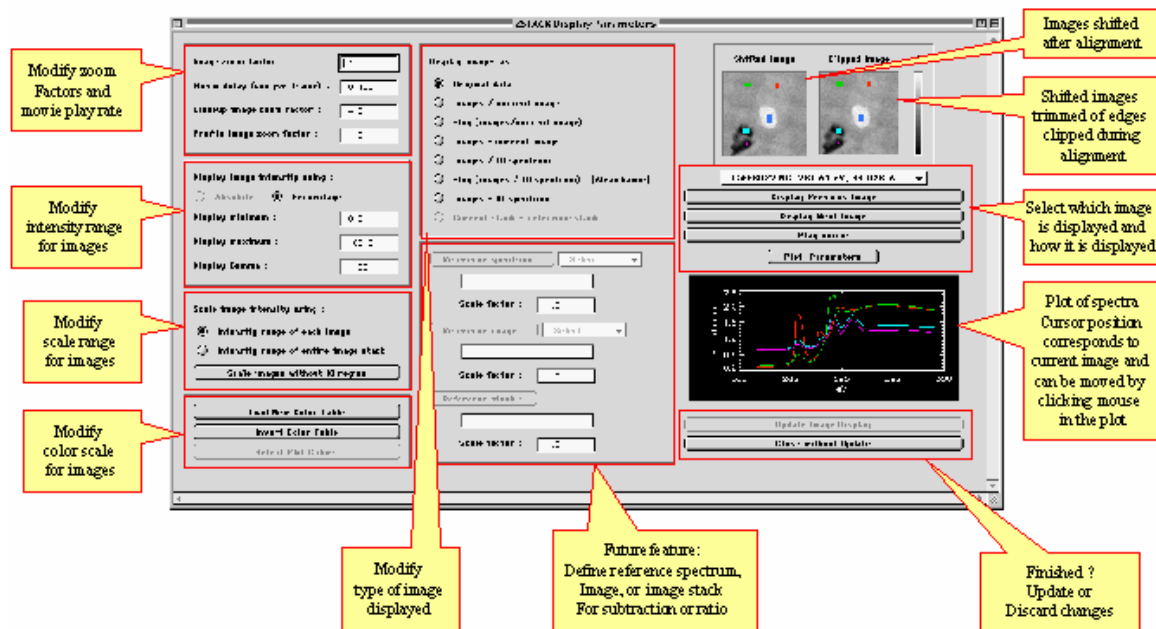
Color of the region of interest in the images corresponds to the same color spectrum

Process data using IDL Slicer3

Finished ? Return to ZSTACK Spectra

After specifying ALL the options above, Click here to save everything (images, movies, binaries) at one time.

ZSTACK Display



ZSTACK Plot

