

# ZSTACK

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

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. The original version was based upon the original STACK code developed by Chris Jacobsen (SUNY - Stony Brook). It has 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 AXIS data analysis package. ZSTACK can also be used independently of AXIS.

The development of ZSTACK has been an evolutionary process. Please notify me of any bugs, problems, comments, or suggestions for improvements or new features.

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# ZSTACK Buildlist

**1** Browse for desired directory/folder

**2** Pull-down list of data files in current directory

**4** Parameters of displayed image

Current directory/folder

Current image

Select from directory

05FEB022.NC

281.61 eV

44.028 Å

100 rows X 100 cols

5 msec dwell

Display Previous Image

Display Next Image

Play movie

Display Parameters

Set as first

Set as last

Filename (\*.sl):

05FEB022\_093.sl

Save list

Read list

Browse \*.sl

Binary Filename (\*.ncb):

Read \*.ncb

Browse \*.ncb

List of data files selected for alignment and analysis  
Highlighted file is displayed

Retrieve binary file of previous saved stack data

Number of images Currently in list

**3** Select which image is displayed and how it is displayed

Set current image as first file

Set current image as last file

**5**

**6** Build list of data files From first file to last file

Remove current image from list of data files

**7** Save or retrieve list of files

Remove all images from list of data files

List of data files is complete  
Go on to next step: aligning the images

**8**

Number of Files = 72

05FEB022.NC: 281.61 eV, 44.028 Å

05FEB023.NC: 282.60 eV, 43.873 Å

05FEB024.NC: 284.01 eV, 43.655 Å

05FEB025.NC: 284.12 eV, 43.639 Å

05FEB026.NC: 284.22 eV, 43.623 Å

05FEB027.NC: 284.31 eV, 43.609 Å

05FEB028.NC: 284.41 eV, 43.594 Å

05FEB029.NC: 284.51 eV, 43.578 Å

05FEB030.NC: 284.62 eV, 43.562 Å

05FEB031.NC: 284.72 eV, 43.547 Å

05FEB032.NC: 284.81 eV, 43.533 Å

05FEB033.NC: 284.91 eV, 43.517 Å

05FEB034.NC: 285.01 eV, 43.501 Å

05FEB035.NC: 285.12 eV, 43.486 Å

05FEB036.NC: 285.21 eV, 43.472 Å

05FEB037.NC: 285.31 eV, 43.456 Å

05FEB038.NC: 285.41 eV, 43.441 Å

05FEB039.NC: 285.52 eV, 43.425 Å

05FEB040.NC: 285.61 eV, 43.411 Å

05FEB041.NC: 285.71 eV, 43.395 Å

05FEB042.NC: 285.81 eV, 43.380 Å

05FEB043.NC: 285.92 eV, 43.364 Å

05FEB044.NC: 286.01 eV, 43.350 Å

05FEB045.NC: 286.11 eV, 43.335 Å

05FEB046.NC: 286.22 eV, 43.319 Å

05FEB047.NC: 286.31 eV, 43.305 Å

05FEB048.NC: 286.41 eV, 43.289 Å

05FEB049.NC: 286.51 eV, 43.274 Å

05FEB050.NC: 286.62 eV, 43.258 Å

Add first -> last to list

Delete from list

Reset list

List is complete

# ZSTACK Align

(before alignment)

The screenshot shows the ZSTACK Align software window. It is divided into several sections:

- Left Panel (Configuration):**
  - Align images using:** Radio buttons for 'Original data' (selected) and 'Data as displayed'.
  - Reference image for alignment:** Radio buttons for 'Each Preceding Image' (selected), 'Each Following Image', and 'Constant Image'. Below is a 'Select file' button.
  - Edge enhancement before alignment:** Radio buttons for 'Sobel', 'Roberts', and 'None' (selected).
  - Cross-correlation determination:** Radio buttons for 'Correlation maximum' (selected) and 'Center of mass'. Below are input fields for 'Maximum image shift (pixels):' (10), 'Image shift threshold (pixels):' (0.01), and 'Edgegauss smoothing (pixels):' (3).
  - Buttons:** 'Select a subregion for alignment', 'Reset subregion', 'Start auto-alignment', 'Skip alignment', and 'Align images manually'.
- Right Panel (Visualization):**
  - Top Row:** Three image windows labeled 'STXM Image', 'Correlation Fn', and 'Shifted Image'. A vertical color bar is on the right.
  - Parameters:** A dropdown menu showing '05FEB022\_NC: 281.61 eV, 44.028 Å'. Below are buttons: 'Display Previous Image', 'Display Next Image', 'Play movie', 'Display Parameters', and 'Plot Parameters'.
  - Bottom:** A large black area for the main image display.
  - Alignment Shifts:** A text field for 'Alignment Shift Filename (\*.aln):' with the value '05FEB022\_093.aln'. Below are buttons: 'Save shifts', 'Read shifts', and 'Browse \*.aln'.

Numbered callouts (1, 2, 3) point to specific sections:

- 1:** Points to the 'Align images using' and 'Reference image for alignment' sections.
- 2:** Points to the 'Cross-correlation determination' section.
- 3:** Points to the 'Start auto-alignment' button.

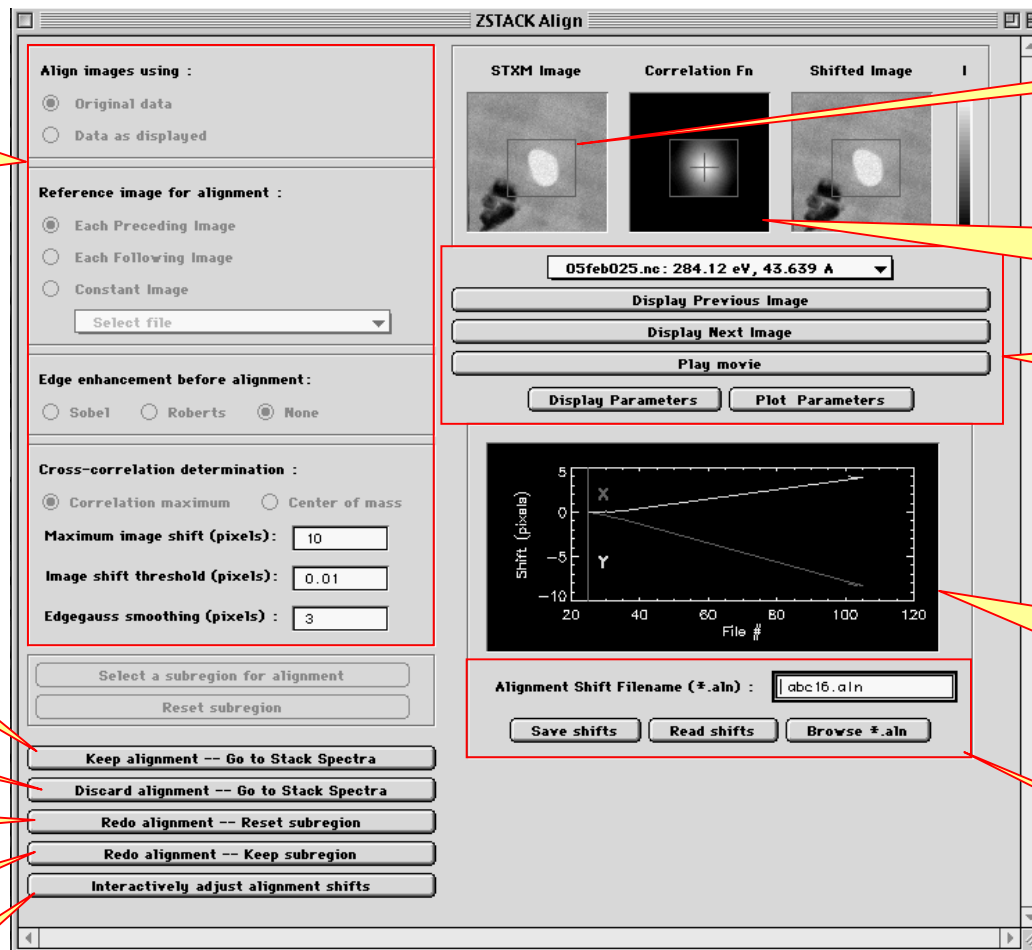
Other callouts describe various elements:

- 'Choose the conditions for image alignment : Reference image Edge enhancement Correlation determination' points to the top-left configuration area.
- 'Pull-down list of data files' points to the 'Select file' button.
- 'Raw STXM image' points to the 'STXM Image' window.
- 'Color bar for images' points to the vertical color bar.
- 'Select which image is displayed and how it is displayed' points to the 'Display Previous Image', 'Display Next Image', and 'Play movie' buttons.
- 'Retrieve existing alignment shifts' points to the 'Read shifts' button.

After alignment shifts are retrieved from a saved file, this dialog window will be updated to show alignment shifts, alignment parameters, and shifted images

# 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

Keep current alignment

Skip the alignment

Redo the alignment  
erasing the subregion

Redo the alignment  
keeping the subregion

Manually adjust  
the alignment

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

# ZSTACK Tune

The screenshot shows the ZSTACK Tune Alignment window. On the left, a control panel includes sliders for X and Y Alignment Shift (both set to 0.000), buttons for image navigation (Previous, Next, Play movie), parameter display, and alignment options (Keep new alignment, Keep old alignment, Reset). On the right, a 2x3 grid displays the STXM Image, Correlation Function, and Shifted Image. Below this is a 'Close-up Images' section with a plot of alignment shifts (Shift in pixels vs. File #) and a cursor for selecting the center of close-up images.

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

Keep new alignment

Keep old alignment

Reset to old alignment

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

# ZSTACK Spectra

The screenshot shows the ZSTACK Spectra software interface. It includes a 'Select Regions for Spectra' panel on the left with buttons for adding and resetting regions. The main area displays 'Shifted Image' and 'Clipped Image' side-by-side. Below these are controls for selecting data files, saving spectra, and a plot of absorbance vs. energy (eV). Callout boxes provide detailed instructions for each major section of the software.

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

The screenshot shows the 'Image Profile' window of the ZSTACK software. The interface includes several panels and plots:

- Generate intensity profile using:** A panel with radio buttons for 'Column (X)' and 'Row (Y)', both currently set to 50. It also includes a text box stating 'YELLOW line is intensity profile', 'RED line is cursor', and 'Bottom Left corner is (0,0)'.
- Scale image intensity using:** A panel with radio buttons for 'Intensity range of each image' (selected) and 'Intensity range of entire image stack'.
- Profile base filename:** A text box containing '05FEB022\_093'.
- Save Profile Intensity as:** A button to save the profile.
- Spectra base filename:** A text box containing '05FEB022\_093'.
- Save Spectra of Current Pixel as:** A button to save the current pixel's spectra.
- Save Spectra along Profile Line as:** A button to save spectra along the profile line.
- Display Previous Image, Display Next Image, Play Movie of Images, Display Parameters, Plot Parameters:** A set of buttons for navigating and analyzing the images.
- Shifted Image and Profile Image:** Two small image windows showing the current image and its profile.
- Intensity Plot:** A plot of 'Image Intensity' (0 to 1200) versus 'Row #' (0 to 100).
- Absorbance Plot:** A plot of 'Absorbance' (0.0 to 3.0) versus 'eV' (280 to 295).

Callout boxes provide additional information:

- Adjust position of cursor and profile axis:** Points to the 'Generate intensity profile using' panel.
- Save intensity profile:** Points to the 'Save Profile Intensity as' button.
- Save spectra of current pixel:** Points to the 'Save Spectra of Current Pixel as' button.
- Save spectra of each pixel along profile axis:** Points to the 'Save Spectra along Profile Line as' button.
- Select which image is displayed and how it is displayed:** Points to the 'Display Previous Image', 'Display Next Image', and 'Play Movie of Images' buttons.
- Select which profile is displayed and how it is displayed:** Points to the 'Display Previous Profile', 'Display Next Profile', and 'Play Movie of Profile' buttons.
- Finished? Return to ZSTACK Spectra:** Points to the 'Return to ZSTACK Spectra' button.
- Plot of spectra:** Points to the 'Absorbance' plot.
- Plot of intensity along profile axis:** Points to the 'Intensity' plot.
- Cursor position corresponds to current position along profile axis and can be moved by clicking mouse in the plot:** Points to the 'Intensity' plot.
- Map of intensity as a function of x-ray energy and position along the profile axis:** Points to the 'Absorbance' plot.
- Cursor position and x-ray energy can be moved by clicking mouse in the plot:** Points to the 'Absorbance' plot.
- Color of the region of interest in the images corresponds to the same color spectrum:** Points to the 'Shifted Image' and 'Profile Image' windows.
- 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.** Points to the 'Shifted Image' and 'Profile Image' windows.
- Red cursor in profile plot corresponds to position of red line in both the shifted image and the profile image.** Points to the 'Intensity' plot.



# 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

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

**ZSTACK Save**

Base filename : 05FEB022\_093  
Directory : Marvin HD:RSI:IDL 5.2:test:98\_05FEB:

Save subregion of data ? ☐ Yes ☒ No  
Select subregion Reset subregion

Save data after clipping edges ? ☒ Yes ☐ No

Save Regions of Interest ? ☐ Yes ☒ No

Image format : ☐ JPEG ☐ GIF ☐ TIFF ☐ PNG  
☒ Current image ☐ All images ☐ Spectra  
 Orientation  
☐ Filename ☐ X-ray Energy ☐ Scale bar  
☒ Spectral Regions of Interest ☐ Color bar

Movie format : ☐ MPEG ☐ MGIF  
☒ Images ☐ Spectra  
 Orientation  
☐ Filenames ☐ X-ray Energies ☐ Scale bar  
☒ Spectral Regions of Interest ☐ Color bar

Binary data format : ☐ \*.STK ☐ \*.NCB ☐ \*.HDF  
☐ Header file

NB : Disabled buttons are features that are not fully implemented

Save

Shifted Image Clipped Image I

05FEB022.NC: 281.61 eV, 44.028 A

Display Previous Image  
Display Next Image  
Play movie  
Display Parameters Plot Parameters

Absorbance  
2.5  
2.0  
1.5  
1.0  
0.5  
0.0  
280 285 290 295 300 eV

IDL Slicer  
Return to ZSTACK Spectra

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

# ZSTACK Display

The screenshot shows the 'ZSTACK Display Parameters' window with several sections and controls:

- Image zoom factor:** A text box with '1.00'.
- Movie delay (sec per frame):** A text box with '0.100'.
- Closeup image zoom factor:** A text box with '4.0'.
- Profile image zoom factor:** A text box with '1.0'.
- Display image intensity using:** Radio buttons for 'Absolute' and 'Percentage' (selected).
- Display minimum:** A text box with '0.0'.
- Display maximum:** A text box with '100.0'.
- Display Gamma:** A text box with '1.00'.
- Scale image intensity using:** Radio buttons for 'Intensity range of each image' (selected) and 'Intensity range of entire image stack'.
- Buttons:** 'Load New Color Table', 'Invert Color Table', and 'Select Plot Colors'.
- Display images as:** Radio buttons for 'Original data' (selected), 'Images / current image', '-log (images/current image)', 'Images - current image', 'Images / I0 spectrum', '-log (images / I0 spectrum) [Absorbance]', 'Images - I0 spectrum', and 'Current stack - reference stack'.
- Reference spectrum:** A 'Select' dropdown menu.
- Scale factor:** A text box with '1.0'.
- Reference image:** A 'Select' dropdown menu.
- Scale factor:** A text box with '1.0'.
- Reference stack:** A 'Select' dropdown menu.
- Scale factor:** A text box with '1.0'.
- Image preview:** Two side-by-side grayscale images labeled 'Shifted Image' and 'Clipped Image'.
- Buttons:** 'Display Previous Image', 'Display Next Image', 'Play movie', and 'Plot Parameters'.
- Spectrum plot:** A line graph showing 'Absorbance' vs 'eV' (280 to 300). A cursor is positioned on the plot.
- Buttons:** 'Update Image Display' and 'Close without Update'.

Callouts provide additional information:

- Modify zoom Factors and movie play rate:** Points to the zoom factor and movie delay controls.
- Modify intensity range for images:** Points to the display minimum, maximum, and gamma controls.
- Modify scale range for images:** Points to the scale image intensity using controls.
- Modify color scale for images:** Points to the color table and plot color selection buttons.
- Images shifted after alignment:** Points to the 'Shifted Image' preview.
- Shifted images trimmed of edges clipped during alignment:** Points to the 'Clipped Image' preview.
- Select which image is displayed and how it is displayed:** Points to the 'Display Previous Image', 'Display Next Image', and 'Plot Parameters' buttons.
- Plot of spectra Cursor position corresponds to current image and can be moved by clicking mouse in the plot:** Points to the spectrum plot.
- Future feature: Define reference spectrum, Image, or image stack For subtraction or ratio:** Points to the reference selection dropdowns.
- Finished ? Update or Discard changes:** Points to the 'Update Image Display' and 'Close without Update' buttons.

# ZSTACK Plot

The screenshot shows the 'ZSTACK Plot Parameters' dialog box. It is divided into several sections with various controls and a central display area.

- Image zoom factor:** A text box with '1.00'.
- Movie delay (sec per frame):** A text box with '0.100'.
- Spectrum Offset:** A text box with '0.00'.
- Display spectra as:** Three radio buttons: 'Single beam', '% Transmittance', and 'Absorbance' (selected).
- Plot Scaling:**
  - X Range:** 'Autoscale' checked. Text boxes for 'Minimum: 280.000' and 'Maximum: 300.000'.
  - Y Range:** 'Autoscale' checked. Text boxes for 'Minimum: 0.000' and 'Maximum: 2.500'.
- Color and Plot Options:** Three buttons: 'Load New Color Table', 'Invert Color Table', and 'Select Plot Colors'.
- Image Display:** Two side-by-side grayscale images labeled 'Shifted Image' and 'Clipped Image'. A vertical color bar is to the right.
- Image Selection:** A dropdown menu showing '05FEB022.NC: 281.61 eV, 44.028 Å'. Below it are buttons: 'Display Previous Image', 'Display Next Image', 'Play movie', and 'Display Parameters'.
- Spectrum Plot:** A line graph showing 'Absorbance' vs 'eV'. The x-axis ranges from 280 to 300 eV, and the y-axis ranges from 0.0 to 2.5. A cursor is positioned on the plot.
- Final Actions:** Two buttons at the bottom: 'Update Image Display' and 'Close without Update'.

Callouts provide additional information:

- Modify zoom factor, movie play rate, spectrum offset:** Points to the top three text boxes.
- Modify options to display spectra:** Points to the 'Display spectra as' radio buttons.
- Modify scale range for spectra:** Points to the 'Plot Scaling' section.
- Modify color scale for images AND plot colors:** Points to the bottom three buttons.
- Images shifted after alignment:** Points to the 'Shifted Image'.
- Shifted images trimmed of edges clipped during alignment:** Points to the 'Clipped Image'.
- Select which image is displayed and how it is displayed:** Points to the dropdown menu and image selection buttons.
- Plot of spectra. Cursor position corresponds to current image and can be moved by clicking mouse in the plot:** Points to the spectrum plot.
- Finished? Update or Discard changes:** Points to the 'Update Image Display' and 'Close without Update' buttons.