STXM 5.3.2 User Manual

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1. Introduction

1.1 Scope of this document
This manual is intended to introduce the novice user to STXM5.3.2 and its safe and efficient operation. A separate document (***stxm_gui_manual.doc***) describes the operating software.

1.2 What is STXM?

In Scanning Transmission X-ray Microscopy (STXM) a micro focused soft x-ray beam is generated by a zone plate, illuminates the sample and the transmitted x-rays are detected. Transmission images are obtained by a raster scan of the sample. Spectra are obtained in point, line or image mode by acquiring signal at multiple photon energies. The 5.3.2STXM is an interferometrically controlled device mounted on a dedicated bending magnet beamline. The user controls both the beamline and the microscope from a single computer.

The primary signal measured in STXM is transmitted intensity (I) as a function of energy (spectra), or position (images). This signal, when converted to optical density (OD), is sensitive to sample thickness, density and composition, according to the following equation. (Io: incident photon flux intensity)
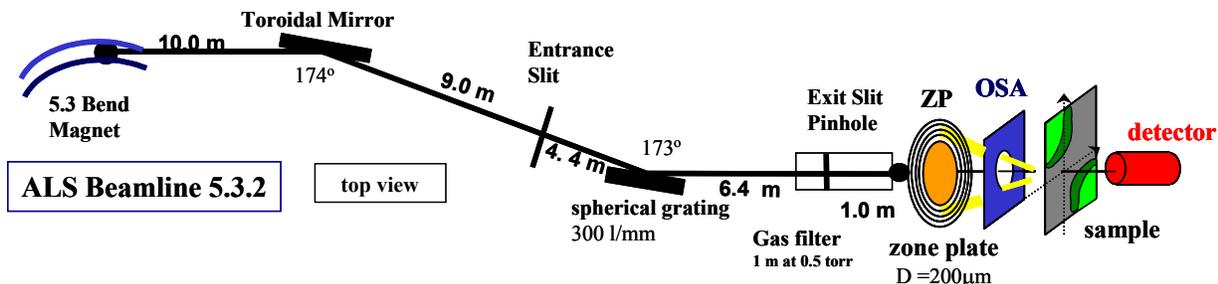
Fig. 1.1 Schematic of the ALS BL 5.3.2 and STXM.

$$OD = -\ln(I/Io) = \sigma.t = \mu.\rho.t \qquad (eqn.\ 1)$$

where σ is the linear absorption coefficient, t is the thickness, μ is the mass absorption coefficient and ρ is the density. The useful range of OD is from 0.1 to 3. If there is too little absorption (sample too thin) the signal is lost in the noise. If the absorption is too great, then various artifacts dominate the observed signal, which is no longer quantitative. For organic material with a density of ~1 samples need to be between 50 and 300 nm. At higher energy edges or lower density samples, somewhat thicker samples are optimum.

Measuring a raster scanned set of pixels and converting with the incident flux (measured through a hole), gives an x-ray OD image at a single photon energy. Changing the photon energy and taking images with other photon energies gives an image sequence (stack), which includes chemical information as well as topographical information. Analyzing the data with suitable reference model spectra can provide chemical maps with sub-100 nm spatial resolution.

1.3 Beamline 5.3.2

Fig 1.1 shows a schematic of beamline 5.3.2. It is a horizontal dispersive bending magnet beamline optimized for C 1s NEXAFS spectromicroscopy. It consists of one toroidal mirror and one spherical grating. Beamline 5.3.2 was optimized for the energy range from 250 eV to 600eV, to cover the C 1s, N 1s, and O 1s absorption edges which are the most important edges for polymer chemistry. As shown in **Fig. 1.2**, the actual usable photon energy range is considerable larger – at least 200 – 1200 eV, and operation as low as 160 eV has been carried out. The typical overall flux intensity in the ~40nm ZP focused spot is ~2 MHz at 300 eV (60/30/30/ slit settings; ~150 meV energy resolution at C1s edge). Beamline 5.3.2 provides high brightness – the photon flux at 390 eV before the beamline is about 10^9 ph/s into a ~0.5 mm spot which overfills the zone plate by several factors. The monochromator has good energy resolution –
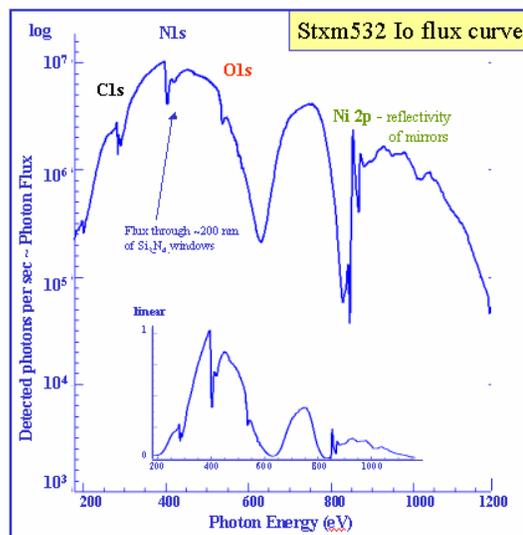
Fig. 1.2 Extended range flux curve of BL 532

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resolving power of better than 6000 with 10x10 micron slits. It has been designed to allow independent trade-off of signal intensity for energy resolution (by changing exit dispersive slit), and signal intensity for spatial resolution (by changing the entrance and non-dispersive exit slits). It features a rapid response active feedback system which stabilizes the position of the beam (H,V) by adjusting the toroid mirror position to equalize current measured on the slits. Overall it is a very stable, fast response soft x-ray beamline highly optimized for STXM.

For more detailed information on the beamline and the design of the STXM please consult:
BEAMLINE: T. Warwick, H. Ade, A.L.D. Kilcoyne, M. Kritscher, T. Tylisczcak, S. Fakra, A. P. Hitchcock, P. Hitchcock, H.A. Padmore, *A New Bend Magnet Beam Line for Scanning Transmission X-ray Microscopy at the Advanced Light Source*, J. Synchrotron Radiation 9 (**2002**) 254-257
MICROSCOPE: A.L.D. Kilcoyne, T. Tylisczak, W.F. Steele, S. Fakra, P. Hitchcock, K. Franck, E. Anderson, B. Harteneck, E.G. Rightor, G.E. Mitchell, A. P. Hitchcock, L. Yang, T. Warwick, H. Ade *Interferometrically controlled Scanning Transmission Microscopes at the Advanced Light Source*, J. Synchrotron Radiation 10 (**2003**) 125-136.

Table 1: Trade-off of slits sizes with energy and spatial resolution

Energy (meV)	Spatial (nm)	Entrance	Exit Dispersive	Exit Nondispersive	Flux (at 320eV)
75	**30**	25	10	35	0.22
100	**30**	40	25	35	1.4
125	**30**	50	30	35	1.8
150	**30**	60	35	35	2.15
175	**30**	75	35	35	2.7
200	**30**	90	35	35	3.2
75	**50**	25	10	60	0.42
100	**50**	40	25	60	2.55
125	**50**	50	30	60	3.95
150	**50**	60	35	60	4.5
175	**50**	70	40	60	5.3
200	**50**	90	45	60	6.85

Table 1 provides guidelines for how the spatial and energy resolution depend on the slit sizes. The typical slit settings of (Ent / Exit (D) / Exit (ND)) = (60μm / 35μm / 35μm) provide approximately 150 meV energy resolution and 50 nm spatial resolution. Note that the slit opening can be off-calibration; it is advisable to recalibrate with a motor scan to find closed position prior to making measurements where spatial and energy resolution is critical.

The main optical and microscope elements are:

Beamline
Toroid mirror - piezo controlled in (x,y); closed cycle water cooled
Grating – 300 l/mm, optimized at C 1s
Entrance slit – oriented horizontally in the dispersive direction
Exit slit – separate control of dispersive (H) and non-dispersive (V) directions
Gas filter – when filled with N_2 (0.2-0.5 torr), decreases second order light in the C1s region

Fast shutter – in-vacuum piezo shutter which minimizes dose to sample

STXM
Window – Si_3N_4 – isolates atmospheric pressure STXM chamber from UHV of beamline.
zone plate (ZP) – supplied by CXRO; critical focusing elements;
                the current ZP parameters can be found by selecting the 'focus parameters'
order sorting aperture (OSA) – when properly positioned this aperture, combined with the central
            stop of the ZP, allows only first order ZP diffracted light to pass. Effective
            second order filter ONLY when both OSA position and ZP focus are set properly
sample motion – fine stage (200x200x20 micron PI piezo stage)
sample motion – coarse stage (stepping motor; range 25 mm in X, 20 mm in Y)
detector – phosphor (fine grained powder on fibre optic) converts to visible light, light pulses
            detected by high performance photomultiplier (PMT) system

2. Key concepts in optimizing STXM

The performance of the x-ray microscope can be characterized by several properties; the spatial resolution, the energy resolution, and the detection limit. This section provides some comments on how to optimize these three properties. As in all scientific instruments, optimizing one property is done at the expense of the others. It is up to the user to select operating conditions that are best suited for their experiment.

2.1 Selecting and optimizing spatial resolution

The principles of operation of Fresnel zone plates are well summarized in the CXRO Handbook of synchrotron radiation. The focal length (f) of a zone plate at a given photon energy (E) is given by.

$$f = D \cdot \delta_{rN} / \lambda \qquad\qquad (eqn.\ 2)$$

where D is the ZP diameter, δ_{rN} is width of the most outer zone (Nth), and λ is the photon wavelength (E (eV) = 12398.52/ λ in Å scale). Typical value of D and δ_{rN} are 155 μm and 35 nm respectively. Thus, at the C 1s edge, 300 eV, the focal length is about 1.6 mm.

The diffracted limit spatial resolution (Δr) is shown as the following equation.

$$\Delta r = 1.22 \cdot \delta_{rN} \qquad\qquad (eqn.\ 3)$$

This equation says the resolution is determined by only the ZP outer zone width. However the ultimate resolution of the zone plate can only be achieved if the microscope is adjusted properly. Thus users have to pay attention to many points about the microscope operational parameters and the beamline settings. In particular, the OSA position, ZP focus, sample-OSA distance, sample focusing, and the accuracy of the energy scale all play a role in getting the highest spatial resolution and keeping it over an extended range of photon energies.

2.2 Energy (spectral) resolution and energy calibration

Energy resolution is determined by the settings of the entrance slit and the dispersive exit slit. It is also important to check and, if necessary, re-calibrate the energy scale, since if this is inaccurate, the equation relating zone plate position to photon energy (eqn. 2) will not be applied correctly.

2.3 Optimizing sensitivity to achieve low detection limits

The detection limit (lower limit of concentration in a mixture that can be detected) depends on the statistical quality of the data, which in turn depends on the incident light intensity, the detector efficiency, and the properties of the sample (total thickness, composition, etc). Longer dwell times and/or increasing the incident flux by using larger slits increases sensitivity, and thus allows a lower detection limit. However for many samples one has to be very careful about the total dose used, in order to minimize sample damage - see section 2.5.

2.4 Principles of focusing to get good quality images

Fig. 2.1 shows the diagram of the x-ray microscope (geometry of main components; Zone Plate, OSA, sample). Zone Plate (ZP) is the diffractive optics to focus. At 532STXM a Fresnel ZP is used and the diffraction efficiency for first order is about 10%. Most of the light is un-diffracted (zeroth order)and this must be excluded as much as possible since, if it is not blocked, it provides an illumination at reduced intensity of the size of the ZP (~200 μm). Even so there is always an irreducible

Fig. 2.1 Geometry of STXM.

Fig. 2.2 Halo signal from higher order and zero order (if central stop partly transparent) reduces contrast and can give spectral errors for small regions. (BL7.0.1 stxm, apr-00; no Ti filter, 8 um pinhole).

zero-order contribution which forms a 'halo' of the size of the ZP, with an integrated strength of 1-5% of the focused beam intensity (see **Fig. 2.2**). In addition, depending on the quality of alignment of the OSA and the central stop, there can be some second-order signal (light in the focused spot at twice the photon nergy). By aligning the Central stop (CS) and Order Sorting Aperture (OSA) properly, the non-first order light can be minimized and higher contrast / more quantitative images are obtained. In order to get the minimum amount of zero order light the center of the OSA must be aligned to the center of the ZP to within 5 μm. Since the OSA position is not very stable, it is necessary to check the OSA every 4-6 hours, or whenever critical measurements are being made.

Fig. 3.1 Toroid mirror feedback control screen

2.5 Measuring strategies to minimize impact of radiation damage

As with all types of ionizing radiation, soft X-rays cause sample damage. At the flux density of the 5.3.2 STXM this can happen quite rapidly, depending on the material. For example, quantitative damage measurements of the damage rate for poly-methylmethacrylate (PMMA) show that 50% of the carbonyl bonds are destroyed in only 50 msec of fully focused beam. If you are studying known or suspected radiation sensitive samples, it is wise to spend some time estimating the dose above which the results would no longer be meaningful to you, and adopt a measurement strategy that keeps the total dose below that pre-determined limit. If the main goal is to obtain a spectrum of a particular region of your sample, consider using a defocused point scan. Linescan or image sequence (stack) modes are the best way to get spectra at the limits of the spatial resolution with minimum damage. It is good practice to take an image over the region studied, at a photon energy sensitive to the structural changes caused by damage (289 eV for aliphatic species, or the energy of a carbonyl peak) to characterize the extent by which your measurements might be compromised by damage.

3. Operation procedure

This section describes the usual procedures for loading, setting up the microscope, and measuring a sample in the 5.3.2 STXM. The first part of this section walks through an actual measurement, with typical times and specific values of the scan parameters. Following that, step-by-step instructions for each operation are provided.

3.0 Anatomy of a typical measurement

The sample is Dow#355 polyurethane with pipa and san filler particles [Hitchcock et al. *Ultramicroscopy* 88 (**2001**) 33]. Times are estimated.

9:00   Open beamline *(How? – see **section 6**)*
*Preview with VLM – index areas for study; write stxm532 format files using **VLM-control** for rapid navigation to areas of interest* (see **section7**)

9:30 *Load sample*

9:35 *Check signal in air*
- 100 kHz (10 kHz with divide-by-10) at 300 eV.

9:40 *Evacuate and refill with He*
- pump to 100 mtorr. Refill to 1/3 atmosphere He. (NB pumping wet cells is not advisable as they may 'explode'. Best to displace air with He flow, with the rapid vent open. 10-20 minutes and 100 bar of He typically reduces N_2 π^* signal to an acceptable level

9:45 *Optimizing photon beam* (**Fig. 3.1**)
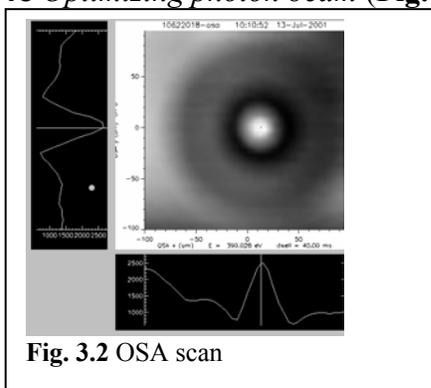
Fig. 3.2 OSA scan
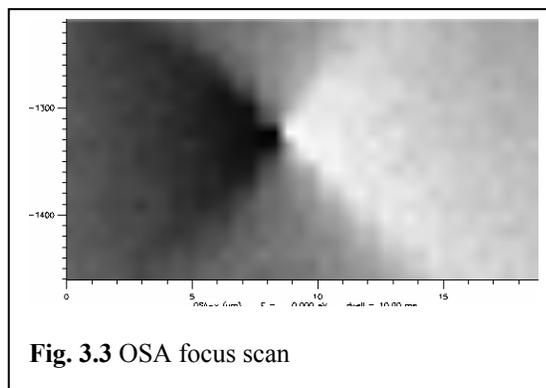
Fig. 3.3 OSA focus scan
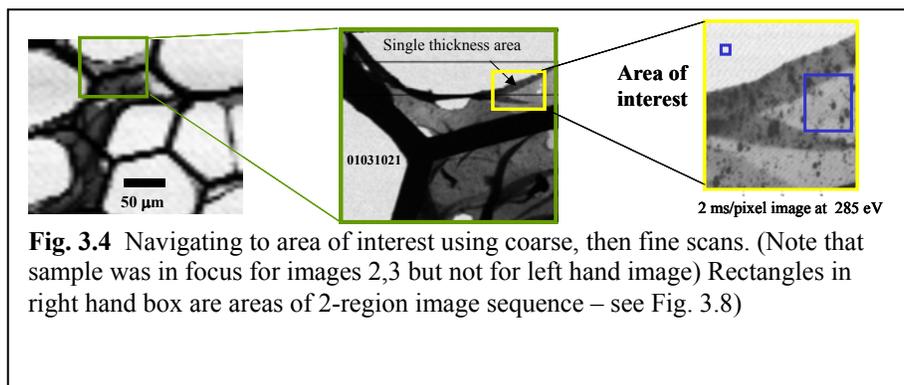
Fig. 3.4 Navigating to area of interest using coarse, then fine scans. (Note that sample was in focus for images 2,3 but not for left hand image) Rectangles in right hand box are areas of 2-region image sequence – see Fig. 3.8)
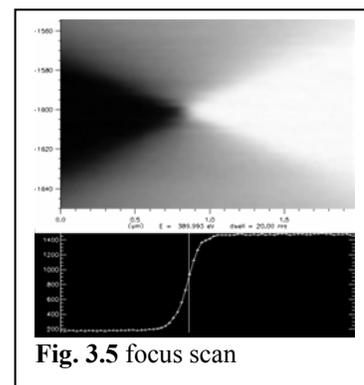
Fig. 3.5 focus scan

9:50 *OSA scan* (**Fig. 3.2**)
- scan 40x40 um, 50x50 pixels, 10 ms/pixel, center (0,0)

9: 55 *OSA focus scan* (**Fig. 3.3**)
-   scan ZP 200um x OSA 10 um, 50x50 pixels, 10 ms/pixel,
ZP center (-0.82 mm), OSA center (0, 25 um)

10:00 *Navigating to find region of interest* (**Fig 3.4**)
- scan 1000x1000 um (600x50) 2 ms dwell
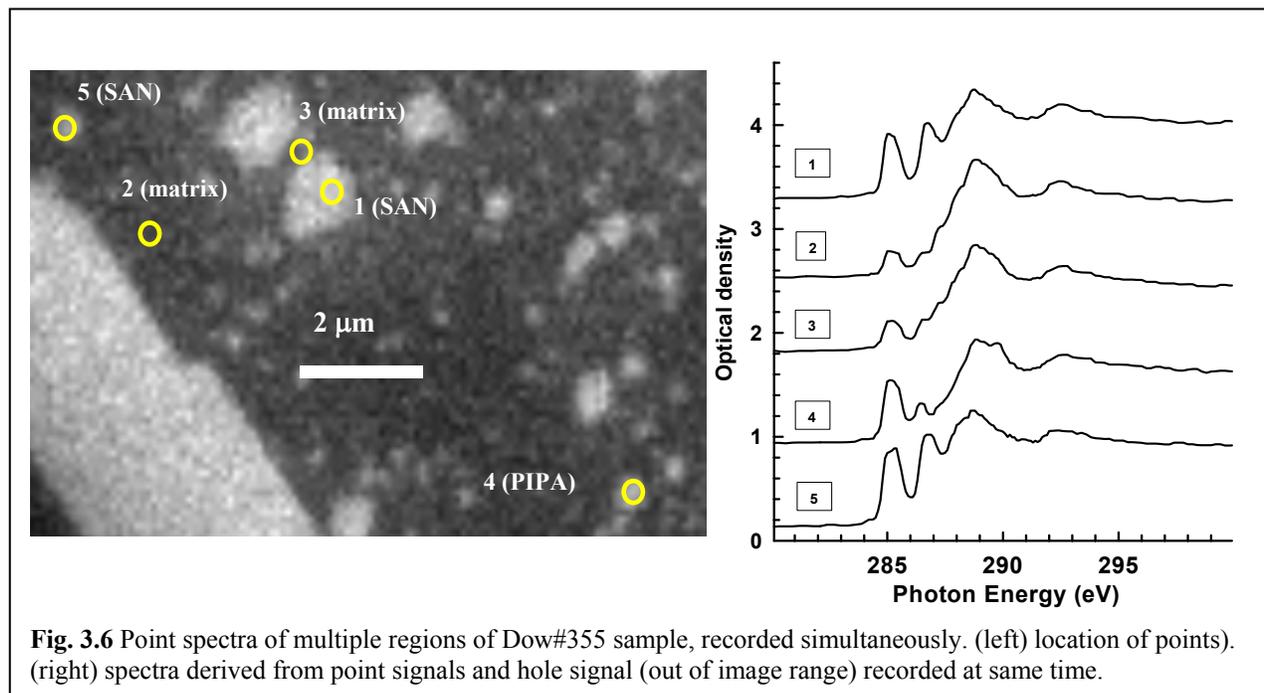
Fig. 3.6 Point spectra of multiple regions of Dow#355 sample, recorded simultaneously. (left) location of points). (right) spectra derived from point signals and hole signal (out of image range) recorded at same time.

10:05 *Focusing on sample*(**Fig 3.5**)

- first time: scan ~200 um about nominal ZP focus (-0.80 to -1.00 mm) and 10-20 um in space as a horizontal line across a high contrast feature. Second time, carried out after region of interest located; is typically ~40 um ZP versus 5 um in space.

10:10 *Recording images* (see **Fig 3.8**)
- scan 20x20um (400x400) pixels 1 ms dwell
-
- chemical identification by comparing images at 285 eV and 287.2 eV.

10.20 *Recording point spectra* (**Fig 3.6**)
- scan 278-330 eV, 4 regions (see **table 3.1**), 20 msec/point, 200 energies,
- 7 spatial points simultaneously (2 Io)

10.25 *Recording linescans* (**Fig 3.7**)
- scan 278 – 330 eV, 5 msec/point, 200 energies, 200 points over 10 μm

Table 3.1 – example of multi-region energy scan				
Start (eV)	End (eV)	Step (eV)	Points	Dwell (msec)
278.0	284.0	0.4	15	1
284.2	292.0	0.2	40	1
292.4	310.0	0.4	45	1

10:30 *Recording image sequences* (**Fig 3.8**)
- scan 282-300 eV, 3 energy regions, 90 energies,
- spatial region 1: 8x8 μm, 120x120 pixel, 1 msec/pixel (sample)
- region 2: 1x10 μm, 10x10 pixel, 1 msec/pixel (Io in hole)

11:15 *End of measurements of C 1s STXM of Dow#355*

9

Total elapsed time ~2 hours. **HINT:** If a one-edge measurement of one region of a sample takes more than 4-5 hours, you should review your procedures, and/or your goals.

Line
Edge of section thicker

pipa

SAN

matrix

After
linescan

Linescan fit analysis

total

matrix

SAN

pipa

NB models from
pure 355 stack
no meaning to
vertical scale

15 msec / point
at end of fill
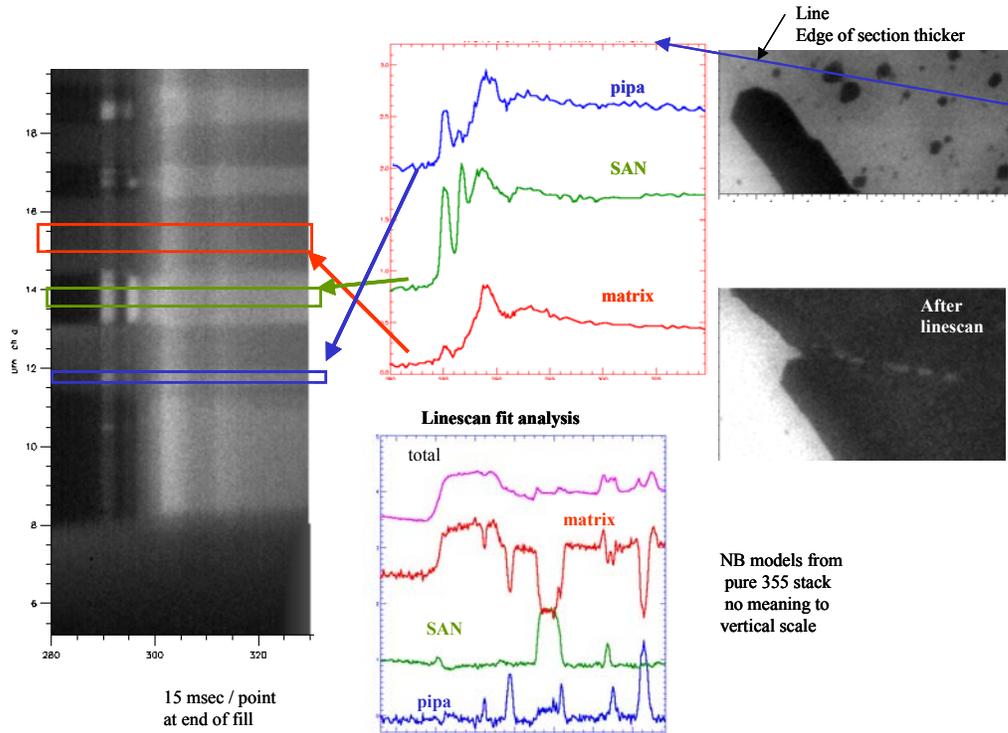
Fig. 3.7 Linescan across indicated morphology of Dow#355 sample.

285.1 eV

287.1 eV

R = SAN
G = pipa
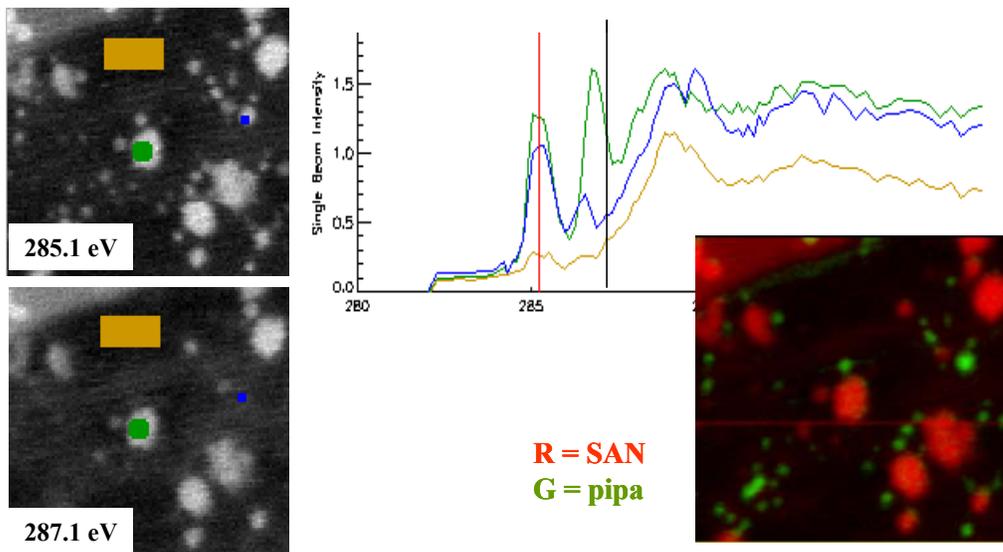
Fig. 3.8 Results of 2-region image sequence (stack) recorded in regions indicated in Fig. 3.5. Selected images at the chemically sensitive energies, selected region spectra, and a composite chemical map are derived from the image sequence data using aXis2000.

3.1 Loading a sample

Samples are mounted on custom machined Al plates (see **Fig. 3.9**) which fit into a kinematic mount in the STXM which provides reproducible positioning at the micron level. There is a system for pre-viewing and identifying / indexing regions of interest in the Leica visible light microscope (VLM). If you use this, it can greatly decrease time to find regions of interest, as well as providing complementary information.

Once your sample(s) are securely fastened to the sample plate, and any indexing / pre-viewing is complete, place the sample plate in the microscope as follows.

1. Turn off the PMT high voltage.
2. Close the shutter.
3. Return the chamber to atmospheric pressure.
4. Open the flange of the chamber.
5. Check the position of ZP, OSA, sample, and the detector, especially you don't have any information about the present situation from the previous user.
6. Push the button "sample out" to move sample back 2 mm and to the inboard side. CAUTION: push the sample out button only ONCE. Manually position the sample if unsure of in/out status.
7. Take out the old sample holder carefully. Do not hit the OSA or ZP!!
8. Install your sample holder. Push the top of the holder firmly (but not too hard!) to make sure the holder is set properly into the kinematic mount.
9. Move the sample to 200μm – 400μm front of OSA. *How to move the sample?* On the "Microscope Control panel" select "Coarse Z" from the Motor pull down menu. Then you have two options. One is set the number directly and push button "Go", the other is push button "D" and use the jog. It is useful to have two people to do this – one to execute small motions (jogs of 100 μm) and the other to look at changes. You can get a good 'feel' for the distances moved if your use the width of the sample holder plate (500 μm) as a guide. Avoid hitting the OSA with your sample !!
10. Close the flange. If you evacuate, you don't need to fasten the bolts too tightly.
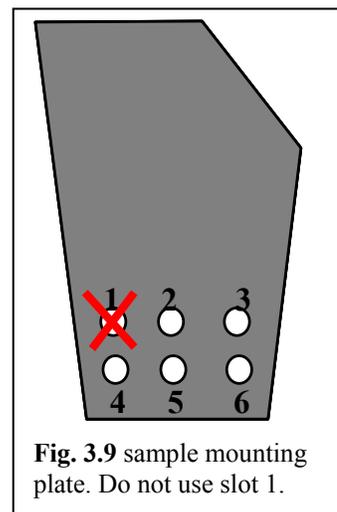
Fig. 3.9 sample mounting plate. Do not use slot 1.

HINT: The samples plate is 200 microns thick – this is a useful gauge. If you are sure of the offset the previous sample had relative to the front of the sample holder plate (grid ~ 30 micron; Si_3N_4 window ~250 micron), then you can use the 'sample in' button to position the sample close to the focal point, as long as you compensate for any difference in the thickness of the previous sample and your sample. E.g. changing from a grid (old) to a Si_3N_4 window (new), one increases the sample coarse-Z by the difference (~200 um), prior to moving 'sample out'.

3.2 Confirm the signal and remove air

With the sample loaded, it is a good idea to check for signal and even start doing rough imaging before evacuating. The microscope will operate in air. The maximum intensity is at 390 eV.

: The chamber must be completely closed to exclude room light before applying high voltage to the photomultiplier tube (PMT).

1. Switch on the high voltage (HV) of PMT and set to 1.0 kV (higher voltages needed for old PMT tubes, maximum is 1200 V).
2. Position the sample to a hole (a slot without any sample) by using coarse X & Y motor control.
3. Switch to chart mode and open the shutter. If all is proper, you will some signal.
4. If you are concerned about the z-position of the sample (e.g. if there is a large difference in sample thickness from the last sample), it might be useful to do some imaging and coarse focusing with air operation. That way, if you need to open up to check things, the time of pumping (3-5 minutes), and the He is not wasted.
5. Confirm both the vent valve and the valve to the He line are closed, then switch on the pump and open the large valve to the chamber slowly. During pumping leave the chart mode on with the shutter open to watch the signal increase.
6. After the vacuum gauge indicates about 200 mTorr (signal should be saturated long before),
    a. close the pump-out valve.
    b. Switch off the pump.
    c. open the valve to flow He. The T gauge will go off scale.
    d. Close the valve when the Bourdon gauge reads about 20 inches of Hg. If you want to do N 1s edge work, it is worthwhile to pump for a longer time. The lowest pressure is about 100 mtorr.
7. If the signal intensity is more than 3×10^6 (MHz) in the energy region you want to use, set the photon counter unit divide-by-ten switch. This mode is needed for all measurements if any region of the energy scanned has > 3 MHz in the Io signal.

NB: If there is no signal, see the trouble shooting section "no signal".

3.3 Optimize the photon beam

1) Set the desired slit sizes - typically (Ent / Exit-D / Exit-ND = 60μm / 30μm / 30μm).
2) Position the beam in a hole. Select a photon energy in the range of interest since the feedback performance depends on the signal intensities, which vary by more than factor of 10 over the photon energy range. Open the piezo shutter.
3) Optimize the toroidal mirror settings
    • Push the "INPUT" button on the monitor to display the Toroidal Mirror Control computer display. This plots the time record of the toroidal mirror feedback system in two different time domains for tracking fast and longer term stability of the photon beam position (see **Fig. 3.1**). Turn off the feedback control of both horizontal (dispersive) and vertical (non dispersive) (buttons will change from green to yellow).
    • Change back to the STXM User Interface computer. Maximize the signal intensity in chart mode by adjusting Toroidal Mirror Piezo voltage controls in the upper part of stxm532 electronics rack. The Toroidal Mirror Piezo Controller has two channels, CH1: Horizontal, CH2: Vertical.
    • Once you get stable signal at the maximum intensity, go back to Toroidal Mirror Control computer. Click the buttons "Auto Horizontal Feedback" and "Auto Vertical Feedback"

to turn on the horizontal, then the vertical feedback. The two feedback buttons will change from yellow ("Off") to green ("On"). Watch the middle traces. The blue and green lines should come to the same value, and fluctuate about that point. Confirm the indicator on the green tracking bar is stable. If its position is far from zero you should adjust the value of the "slit current offset" to make the green bar indicator to be positioned stably around zero (−10 to 10). The feedback switch needs to be turned off and on again to reset the offset, then evaluate the new set point.

- Switch the monitor back to STXM User Interface view.

<mark>NOTE:</mark> The feedback shuts off if the signals are less than 0.05 volts. It is also not stable if the signals are larger than ~7 Volts. If the vertical feedback signals are not within these limits, change the gain on the two amplifier units mounted in the rack above the piezo voltage control. Typically a 10^9 gain is adequate, except when the signal is low, such as with small slits, low (<275 eV) or high (>550 eV) photon energies or weak beam (2-bunch mode) conditions. In the latter case, the gain must be increased to 10^{10}.

3.4 Optimize the OSA and ZP positions

The OSA (x,y) position and the correct ZP-OSA distance (set by the OSA focus scan routine), and OSA-sample distance (controlled by the A0 parameter), must be set properly
- to achieve ultimate spatial resolution,
- to have best higher order filtering, and
- to avoid zero-order related 'ghosts' and 'shadows'.

The present OSA stages do not hold their position well so the OSA position needs to be optimized regularly (every 4-6 hours, or when shadows appear). The OSA position and the OSA-focus needs checking whenever the OSA is bumped.

1. Set the photon energy to 320 eV.
2. Execute "**OSA scan**" to align the center of OSA to the center of the ZP.
   - At "Scan Controls panel" pull down and select "OSA Scan". This will open the OSA scan parameter panel. (IfF OSA scan is the present default scan, click on "START" button)
   - Adjust the OSA scan parameters if needed – usually the parameters are set to suitable values.
   - Check the "OSA in focus". This moves the ZP upstream by the OSA-sample distance (A0) so the focused spot is at the OSA. If not selected, the beam stays focused at the sample position and the OSA image will be blurred and it will be less easy to locate the center.
   - Push the button "Begin Scan"
   - After getting the image of OSA, position the cursor at the center of OSA (click mouse left button on the center position) .
   - Push the button "Set OSA To Cursor + set (0, 0)"
3. Execute "**OSA_focus scan**" to calibrate the ZP home position
   - At "Scan Controls panel" pull down and select "OSA_Focus Scan" and click on "START" button.
   - Move the arrow across the OSA edge by click-lock left mouse button and drag on the middle of the arrow.
   - Push the "Begin Scan" button.

- After the image of the ZP-OSA scan is completed (or at least passed the focus point), position the cursor at the best focused point
- Click "Focus To Cursor + set ZP calibration" button

4. The alignment of the ZP motion and the X-ray optical axis can be checked by making a second OSA scan at a different photon energy – for example, 270 eV, to check across the C 1s edge. If the same OSA center position is not found (within 5 um) , inform the beamline scientist.

(for the experts) <u>Improved performance</u> (lower halo signal) can be achieved by:
1) scan OSA and set the OSA centre position to the center of the hole.
2) Use the motor mover to move the OSA-X position to –5 microns.
3) Use the setup to set that position to 0.
This -5 um offset from the 'apparent' OSA centre, probably compensates for some backlash oin the OSA motor movement. The direction and magnitude of this shift can be found by minimizing the 'non-focused' light in the centre of a grid bar.

3.5 Positioning (navigating on the sample)

At this point you now have the microscope ready to make measurements. However the microscope needs to be focused at the area you are interested in studying, which implies you need to find that area before detailed focusing (since the depth of focus is less than 1 micron, and the OSA-sample distance varies by many microns over the full sample area of 1 mm). Often it is helpful to do a crude focusing on the first high contrast feature you find, and then do a more precise focusing after you identify the region for detailed study.

1. Set the shutter mode "Auto" to minimize sample damage (see **Fig. 3.10**).
2. Take a large image scan at a suitable photon energy. At "Scan Controls panel" pull down and select "Sample Scan" (or click "START" if that is the current scan mode) Set suitable scan parameter. eg. $(800\mu m)^2$ (400x100) 2 ms (*NB*: scan speed is limited to less than 1mm/s. The scan speed changes as the range, pixel number and dwell are changed, and is displayed in **BOLD** if the scan speed is in range the instrument cannot achieve, the results are not reliable and the software may hang up)

Fig. 3.10 Shutter control.

3. If your sample is almost in the center of the slot hole or is very large, go ahead. But if your sample is on a small Si_3N_4 window and you are not sure that the window is in the middle of the slot hole, it can be helpful to do motor scans in X (with Y-centered) and in Y (with X-position at value where you see signal) to find the (x,y) center values for the sample. (choose "Motor Scan" at the scan control panel.)

3.6 Focusing the microscope

1. Find a suitable part of sample to focus. Sharp edged features are required.
2. Select "Focus Scan" at the scan control panel (pull down menu) and click "START".
3. Define the focus scan across a high contrast feature. If possible, choose a horizontal line, since the line-at-a-time mode is faster than the point-by-point image scan mode.

4. You will see a green arrow if the previously defined focus scan was in the same region (unlikely) or a dot at the side (indicating which direction the current focus scan is positioned), or in the middle (indicating the length of the spatial co-ordinate of the focus scan is larger than the current field of view).
5. Click and drag the focus arrow to your region of interest. If the arrow is larger that the displayed field of view, you need to enlarge the field of view using the x2 zoom feature. Once on screen, move the arrow over the high contrast feature
6. You can change the length by clicking on either end of the arrow and dragging. Any angle scan is available, but sometimes positioning errors occur especially on horizontal axis. The horizontal scan (check "horizontal" in the scan parameter panel) is most accurate. If you scan other than horizontally, you must uncheck the "horizontal" box.
7. Before starting the focus scan, confirm the ZP center position in the scan parameter panel is correct. (to check the present ZP position -> Change the photon energy and look the Zone Plate Z value in the Microscope Status panel.)
8. Hit "Begin Scan".
9. After the focus is scanned, locate the best focused line and click on that point by mouse left button
10. Click "Focus To Cursor".
11. If you already obtain an adequately focused image on the above large scan, next zoom in to find the region of your interest and repeat the image/focus procedure with as fine a feature with high contrast. If you did not succeed in focusing (and this sometimes happens if you are far from focus) then you will see an out-of-focus image. At this point it is sometimes helpful to focus on a large object such as a grid bar or edge of Si_3N_4 window.
12. You may have to focus again after zooming in, moving to another region, and/or changing the photon energy by large amounts (>100 eV).
13. After you have focused on the sample successfully, the "Sample Z Offset" value should correspond to your sample thickness.

3.7 Recording images
1. Select desired photon energy
2. Open scan panel
3. Select image – line-at-a-time (NB point-by-point mode should only be used for very slow scanning speeds. It has large overhead to settle the sample position and does not give any more precise images at the present time)
4. Set image center position, image size, pixel density, and dwell. The position of the image can also be defined graphically – see ***stxm_gui_manual.doc*** for details.
5. Push 'start'
6. After the scan is finished, a dialog box will pop-up which asks you if you want to save the result, and allows you to supply a comment line if you wish (the default comment is the filename).

Notes:
1. The filenames are set by the program and cannot be adjusted by the user.
2. In order to find the high contrast energies at which you might wish to record high quality images, it is useful to do spectroscopy of the interesting sample components, by taking the point spectrum or line scan. Previous measurements or the literature spectra of reference materials,

may be useful guides, but it is generally wise to record spectra on the sample on the same day you are studying it, since energy mis-calibration of a fraction of an eV is possible. A good way to check the energy scale is to perform a horizontal line scan over a region of sample and hole.. This minimizes alignment precision needed, gives more information than a point spectrum, and it is easy to check for damage by subsequently recording a image at a damage-sensitive energy.

3.8 Recording point spectra
a) Open scan panel
b) Select point mode
c) Select number of points to record
d) Position the points on the image by click-dragging. Note you can read-in a different image if you want to position points relative to more than one image.
e) Set energy region(s), point spacing, and dwell – see table 3.1 for an example. It is preferable to use a FIXED dwell, and a variable point spacing. The button 'all regions same dwell' is useful to change the dwell in all regions simultaneously.
f) Save the scan parameters if it is likely you will use them again. Since the energy region definition can be changed independently from the spatial parameters, it is useful to save time in setting up complex multi-region energy scans.
g) Push 'start'. The spectra (in transmission mode) will display on the right hand graph.
h) Ultimately there will be provision for real-time computation of absorption spectra using a stored Io. *This mode is not yet implemented.*
i) After the scan is finished, a dialog box will pop-up which asks you if you want to save the result, and allows you to supply a comment line if you wish (the default comment is the filename)

3.9 Recording linescans
1. Open scan panel
2. Select linescan mode – line-at-a-time (if a horizontal line is to be carried out) or linescan-point-by-point (if the line will be at an angle).
3. Use the graphical control to define the line
4. Select number of points along the line
5. Set energy region(s), point spacing, and dwell. It is preferable to use a FIXED dwell, and a variable point spacing. The button 'set same dwell for all regions' is useful to change the dwell in all regions simultaneously.
6. Save the scan parameters if it is likely you will use them again. Since the energy region definition can be changed independently from the spatial parameters, it is useful to save time in setting up complex multi-region energy scans.
7. Push 'start'. The linescan image (spatial is plotted vertically, energy, horizontally) will display on the image panel..
8. After the scan is finished, a dialog box will pop-up which asks you if you want to save the result, and allows you to supply a comment line if you wish (the default comment is the filename)

Note: Multi-region linescans (ie multiple lines on a sample, useful to do Io and I in quite separate regions at the same time) is not enabled at this time.

3.10 Recording image sequences (stacks)
1. Open scan panel
2. Select image mode – line-at-a-time.
3. Select number of image regions to record.
4. Use the graphical control to define each image region
5. Set the number of pixels and dwell for each region
6. Set energy region(s), point spacing, and dwell. It is preferable to use a FIXED dwell, and a variable point spacing. The button 'set same dwell for all regions' is useful to change the dwell in all regions simultaneously.
7. Save the scan parameters if it is likely you will use them again. Since the energy region definition can be changed independently from the spatial parameters, it is useful to save time in setting up complex multi-region energy scans.
8. Push 'start'. Successive images of the image sequence will display on the image panel..
9. After the scan is finished, a dialog box will pop-up which asks you if you want to save the result, and allows you to supply a comment line if you wish (the default comment is the filename)

3.11 Useful tips
        When you should carry out the following operations . . .
 3.11.1 check focus
   o   If the image is out of focus
   o   When the sample position is changed by more than 0.5 mm
   o   When the photon energy is changed more than 100 eV
NB Focus: must be done only in FINE image mode (length of line less than 100 microns), and preferably in horizontal line-at-a-time mode.

3.11.2 Check toroidal mirror feedback
   o   At start of each fill (after a few minutes warm up)
   o   Whenever you change slits
   o   Whenever you change the photon energy range a lot (>100 eV)

The feed back signals must be between 0.1 and 6 if x>6 reduce gain 10^{10} ->10^{9}. if x<0.1 increase gain 10^{9} -> 10^{10} on Keithley 428 current amplifier both)

3.11.3 Check shutter position
   o   If there is lots of signal on the chart when the shutter is 'closed'
   o   every few days
   o   whenever get unexpected noise.

3.11.4 Check OSA
   o   after every re-homing of the ZP or OSA motors – see section 5.1
   o   when there is a shadow or halo signal
   o   when it is hard to focus

3.11.5 PMT electronic settings
   o   Use divide-by-10 on the PMT amplifier whenever the hole intensity is more than 3 MHz

3.11.6 Scan speed

o If you are using the standard, line-at-a-time image scan mode, the scan speed must be such that the velocity indicator is NOT bold. If it converts to a bold font, increase the number of x-axis points, reduce the length of the x-axis, increase the dwell, or some combination of these.

o If you are using point-by-point image scan, you may ignore the bold warning on the scan velocity indicator.

3.11.7 Gas filter

o If you are studying a sample that contains oxygen (especially if only some components do), and you want the best quality C 1s spectra in the 278-284 eV region, check for higher order signal by recording a second order O 1s spectrum (260-270 eV in 0.1 eV steps) of the oxygen containing material. If there is detectible O 1s signal this means the second order light is not being completely filtered by the zone plate. If so, consider using the gas filter, which takes ~15 minutes to set up and stabilize. See section 5.5 for details.

3.11.8 Shutter set up

o The piezo shutter is not large enough to stop light fully and still operate properly. Thus, if you want to check the real dark current (signal from laser light in the tank, and from non-light generated pulses in the PMT), you should close a beamline valve (VVR221). If you feel the shutter is not completely open, or that there is too much residual light when the shutter is closed, follow the procedure to adjust the shutter (section 5.4).

4. Troubleshooting

4.1 Why is there no signal in chart mode? (detector off)

Absolutely no signal (0) – PMT HV is off; cable disconnected
Low signal (<10-50 cps) – shutter closed; OSA blocking light; sample or sample plate in way; detector mis-positioned in (x,y)

4.2 Why is there still signal on the chart even though the shutter is closed? (shutter position)

Low signal (<10-50 cps) – this is normal – it is stray light, laser light, and electronic background
Medium signal (100-1000 cps) – light leak; shielding of PMT form scattered laser not correct; shutter mispositioned.

4.3 Why is the image not sharp? (focusing)
Focusing incorrect; sample does not have sharp features; OSA mis-positioned

4.4 How do I get rid of the diagonal streaky lines? (moiré pattern)
These appear when the toroid feedback is mistuned.

4.5 Why are the peaks in the spectrum flat-topped ?
    Sample is too thick

4.6 Why are there shadows on grid bars ?
    OSA is misaligned

4.7 How can I get good quality spectra when the point spectra are damaged ?
    defocused point spectra; line spectra; stacks; defocused stacks

4.8 How do I carry out a defocused measurement ?
        after focusing on the sample, move the sample coarse Z to more +ve value by 15 microns per micron of defocus desired (exact ration depends on the ZP parameters). E.g. for a 500 nm spot, move sample coarse-Z more +ve by 8 microns. Don't forget to reposition the sample coarse-Z after you have finished with defocused mode.

5. Non-standard operations

5.1 Calibrating the energy scale

In experience so far the 5.3.2 energy scale is stable from day-to-day and fill-to-fill within 0.1-0.2 eV. However occasionally there can be changes - e.g. after shutdowns (new orbits), or after work on the beamline or STXM.
If you find the energy scale of beamline 5.3.2 is wrong (e.g. by recording a sample with spectral features of known energies), use the following procedure to correct the scale. It involves finding the position on the monochromator motor (a linear drive that is converted to rotation) at which the zero order light shines onto the stxm detector.

1. set entrance slit to zero (0). (minimum flux to avoid burning out detector!)
2. execute a motor scan - monochromator axis ; -0.02 to 0.02.
3. use cursor to determine maximum signal
4. open monochromator setup (small S)
5. open calib
6. open parameters
7. set home position to the value of the OLD home position minus the value of the zero order maximum just determined.
    e.g. If the old value of home is 3.524 and the maximum of the zero order signal is at -0.01, then the new home position is 3.534.
8. close monochromator setup
9. home the mono motor
10. repeat mono motor scan (#2) to check that zero now comes at 0 on the mono motor scan scale.
11. check with your spectrum that correct calibration is established.
12. Write an entry in the STXM532 operations log book that you recalibrated the energy scale, and by how much.

5.2 Optimizing detector position
  Use motor scans in detector-X and detector-Y while looking at an open area. The phosphor convert is polycrystalline and the optimum efficiency is found on specific grains of size of order of 30-50 microns. The width of the active area is only ~600 microns.

5.3 Changing zone plate
  Only by STXM532 personnel (currently David Kilcoyne and Tolek Tyliszczak)

5.4 Adjusting the shutter position
a) With the shutter in OPEN position, the sample in a hole, and CHART mode on, move the linear drive towards the beamline until the shutter starts to cut into the beam. Back off so you just get full beam
b) Close the shutter. The signal should drop close to but not exactly zero (it is better to let a few percent of the light go by the shutter and ensure full beam is the OPEN position.
c) Repeat this process until the optimum is found

5.5 Activating (and shutting off) the gas filter
Activating
a) Check the dry pump is on. *How?*
b) Open the N_2 cylinder.
c) Open the leak valve the proscribed number of turns (currently 2-3/4 – should be noted by the leak valve).
d) Watch the pressure rise on the backing pressure gauge (middle of rack outside the hutch). This can be viewed through the hole in the hutch.
e) The pressure should take 10-15 minutes to rise to 0.4 torr and stabilize. Partly shut leak valve if the pressure reaches 0.3 torr and is still rising at a significant rate. A pressure of 0.3-0.5 torr is enough to make second order signal in C 1s region undetectable.
S*hutting down*
a) When finished, shut the leak valve.
b) In many cases, the dropping pressure causes a fault in the gauge controller that turns off the ion gauge upstream of the gas filter. If this happens, turn the ion gauge back on, reset the fault and turn on the beamline valves with the panel mate.

6. Beamline operation

6.1 Opening from normal, 'closed' condition
  Typically four valves separating the main sections of the beamline are closed if the stxm is not going to be used for a period of longer than a few hours.
The panelmate controller is in the rack behind the hutch. Wave the mouse or click to activate the display.
Use the pink navigation buttons to move between pages. Back up to the first panel.
Request beam. Open the beamline front valve
Open successive valves going down the beamline. Make SURE you follow the 5.3.2 beamline !!
Do not accidentally change the states of the valves on the 5.3.1 beamline !! (they are real monsters ☺)

6.2 Resetting beamline control panel in cases of faults
If there is a fault in temperature (toroid cooling), ion gauge trips, or over-pressure conditions the control system will shut all the beamline valves. In such a case (typically detected by 'no signal!' and loss of the display of the photon beam on the TV monitor above the STXM computer), go to the panel mate; find the source of the problem and fix it; reset the faults on the panel mate, then restart the beamline.

7. VLM-control

7.1 Overview

This software is designed for using the Leica Visible Light Microscope (VLM) and associated Nikon CoolPix 995 camera , to provide pre-viewing, image documentation and storage, and region_of_interest location for samples to be studied with the 5.3.2 Scanning Transmission X-ray Microscope (STX)M. The user navigates (manually) with the VLM to find points of interest. The microscope stage is (x,y,z) encoded, and the software reads these signals and converts them into STXM coordinates. When a VLM image is captured from the camera, a high quality jpg format image is saved. In addition, a down-sampled image is written in the stxm532 format (*.xim, *.hdr). The stxm532 file contains the center position and scale of the region being viewed. This file can be read into the stxm532 control software to provide rapid navigation to regions of interest. The software organizes the VLM previewing and indexing information in the form of sessions. The list of images and associated position information is in a configuration file (*ymmdd###.cfg*) wich is read into the stx532 gui. The configuration file cannot be accessed until it is closed by exiting the VLM-control software on the Voylen computer.

7.2 Detailed Instructions

Step 1.
Connect the camera's Video Out to the computer's Video In.
Connect the dual end of the modified USB cable to the camera.
Connect the RS232 cable between the serial port of the USB hub to Com1 on the computer, and the USB cable between port 1 on the USB hub and the computer USB port.
Plug in the custom plug from the motion controller to the power port on top of the USB hub.
Power up the USB port.

Step 2.
Run the video capture software.
Turn on the camera to Manual mode.
Run the VLM Control software.

Step 3
First, the user will be prompt to select a root directory:
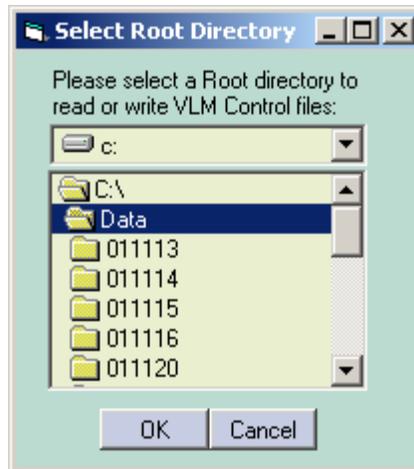
The root directory is where the calibration.txt as well as a few other configuration files for this software is stored. If the user chooses a folder that does not contain these files, the user will be asked to perform calibration before starting any sessions. For details of the calibration procedure please refer to **section 7.3** (how to calibrate VLM-control). Also, the user will have to make sure to manually copy the "DefaultSession.cfg" file to this root directory. Choose OK to proceed, and choose Cancel to abort the software.

Step 4.
Once the root directory is chosen, the user must now select the COM port to which the camera is connected. Then the user can choose to either start a new session or to load an existing session:
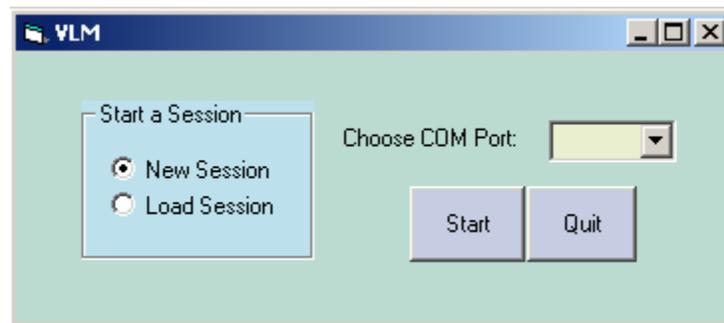
If you choose to start a new session, you can change the session filename and the sample holder used in the new session:
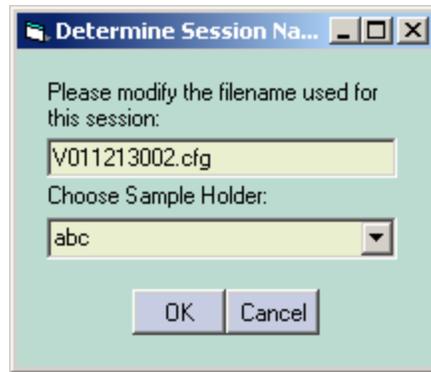
If you choose to load an existing session, you will be able to browse through folders to find the session file of your choice:
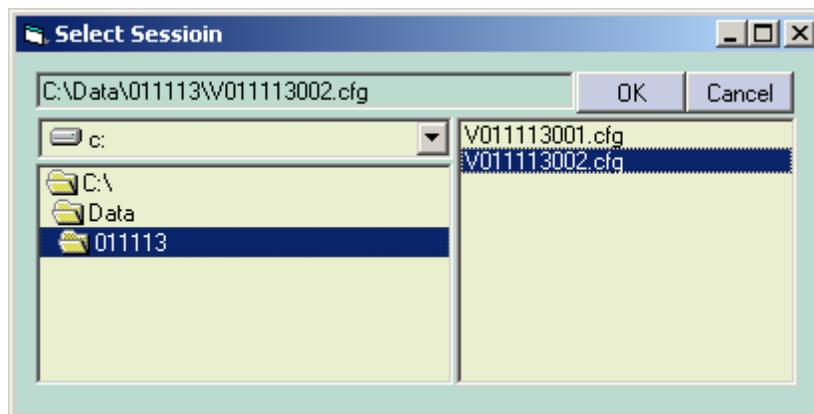
Step 5.
Next the main form of the software will appear, as shown below.

Session V011213001

Session File: `C:\Data\011213\V011213001.cfg`

Image Parameter Files: Image Labels:

[| ▼]   [| ▼]   [Open]

Current VLM Position: **Current STXM Position:**

Sample Holder used in this session:
`abc`

X [202] um   X [202] um

Camera Zoom:
[In ▼]

Y [3] um   Y [3] um

Lens Setting:

Z [3] um   Z [3] um   [lens x20 ▼]   [Calibrate]

Parameters of this image will be saved in:

`V011213001.hdr`   [Get Image]

Please modify the label of this image: [Save & Exit]

`V011213001`

Here you will be able to carry out all routine activities. The title of the form is the label of this session. The top most text box contains the path of the session file. Immediately below there are lists containing (left) the file names of the images in this session and (right) labels associated with the images. Choosing an existing item on either lists then pressing the Open button will open up the associated image, and display its corresponding information. In large fonts below, the current position of the VLM stage and its corresponding STXM co-ordinates are displayed. VLM data are read directly from encoders mounted on the VLM stage, while STXM data are converted from VLM data using the current transformation matrix associated with the selected Sample Holder, Camera Zoom and Lens Setting. In order to get the correct POSITION and SIZE information, it is essential the holder type, zoom setting and lens setting correspond to reality. In addition the VLM-control must be calibrated – see section 7.3. The selections for colder type, zoom setting and lens setting are set by selecting from lists on the right of the current position information. The Sample Holder can not be changed once a session is started. The Camera Zoom and Lens Setting can be changed be selecting different items from the corresponding Combo-Box. Note that by choosing different Camera Zoom levels, the camera will receive commands to automatically change to the new zoom level (only fully zoomed-in and fully zoomed-out are allowed). Near the bottom of the Main form, the two edit boxes contain the Image Header filename and the label of the image. The header file name is fixed to the default value, the user will not be able to modify this. The image label can be modified by the user; the default value is the file name. It is useful to select a label which describes the sample, the magnification (x5, to x100) and the imaging mode (R=reflection, T=transmission)

Step 6.
When a region of interest is observed under the VLM, the user should make sure that the correct camera zoom level and the lens setting are selected. Then, change the image label in the bottom

edit box on the Main form. Then, press Get Image button and <u>wait patiently for about 30 seconds</u>. It is important not to press any other keys while the cursor turns hourglass, implying that the software is busy carrying out commands. When these commands are successfully completed, a modal Preview form will open automatically:
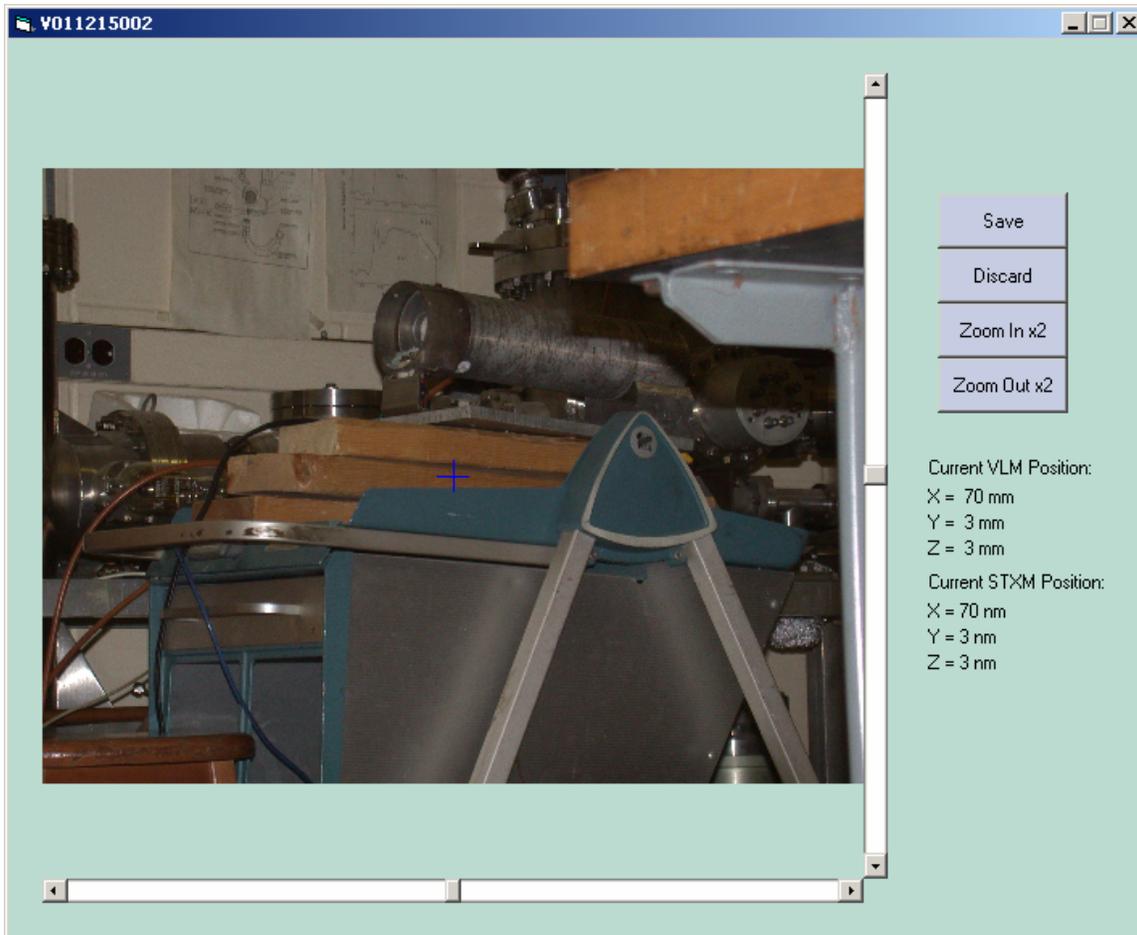
Here the user will be able to view the image that has just been captured by the camera. The user can choose to either save or discard this image. Choosing Save will add this region of interest to the current session file, as well as write the image and header information which is required to transfer the indexing information to the STXM GUI to (*ymmdd###.hdr* and *ymmdd###.xim* files). Choosing Discard will not add the current point to the session file. But these files are stored in the temp directory in the same root directory. The user will be returned to the Main form after pressing Discard button.

<u>Step 7</u>.
When the Save button is pressed, a new form will appear, displaying the same information as on the Preview form:

V011215002

Close

Zoom In
x2

Zoom Out
x2

Current VLM Position:
X = 70 mm
Y = 3 mm
Z = 3 mm

Current STXM Position:
X = 70 nm
Y = 3 nm
Z = 3 nm
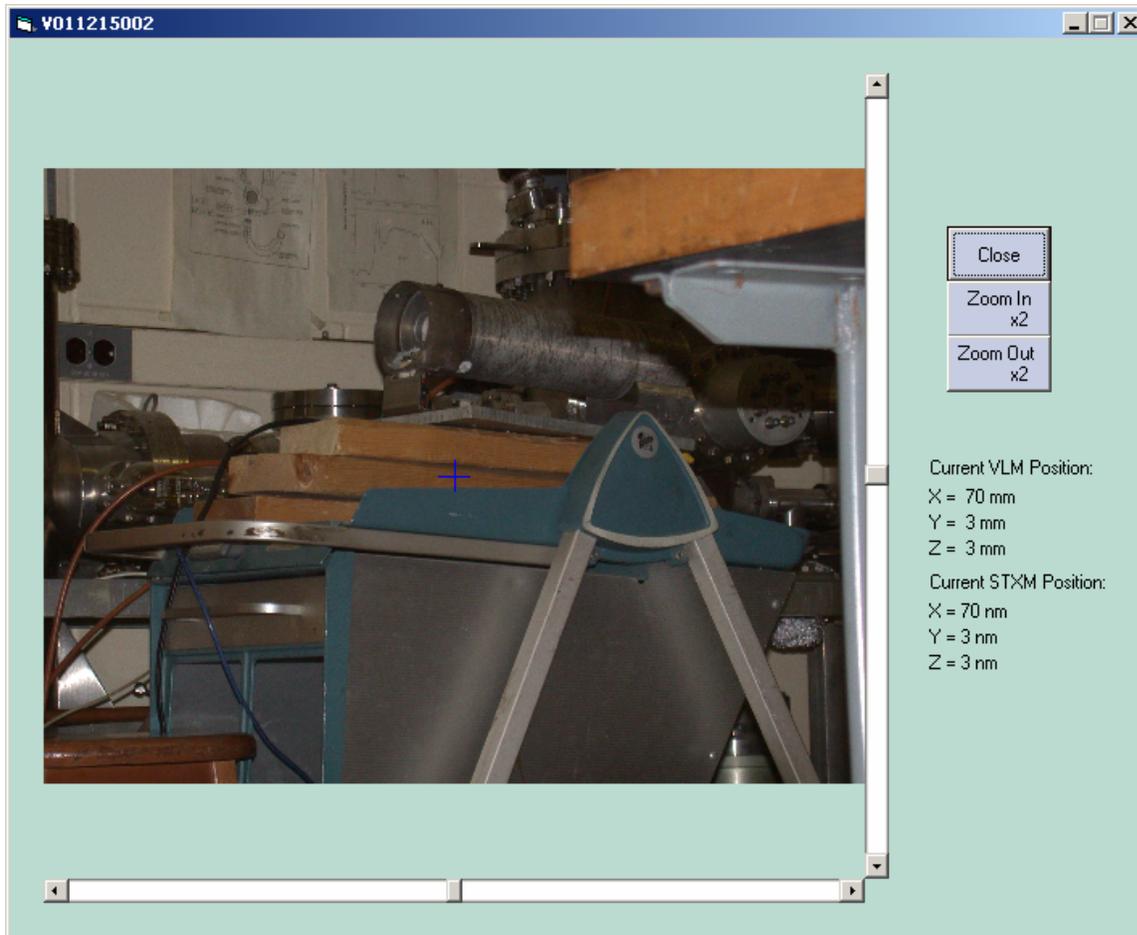
The user can either close this form then go back to the Main form, or just keep this form on the back ground and continue working on the Main form.

Step 8.
When all points of interest on the sample holder have been investigated, the user can choose to Save and Exit this session. Pressing the Save and Exit button will write the session file to disk (*.cfg extension), and bring the user back to the Start form, from which the user can choose to either exit the software, or to work on another session.

7.3 Calibrating the VLM-control program

   7.3.1 Calibrating the VLM encoder stages
Whenever Voylen is restarted or turned off, or the encoder interface is turned off (or other conditions ? – e.g. when VLM-control is shut down ??) the encoders do not read on a sensible scale. In this case, put the 10 micron pinhole sample (typically close by the Leica) into the VLM and position the hole at the center of the image field. In the calibration section of the program, set th (VLM x, y,z) position to (0,0,0).

7.3.2 Calibrating VLM and STXM co-ordinates

The default transformation matrix used by this program is the identity matrix, which will result in the STXM coordinates being exactly the same as the VLM coordinates. Therefore calibration is required to set up the correct correspondence between STXM coordinates and the VLM coordinates. The elements of the calibration matrix is stored for each possible combination of the sample holder, camera zoom, and lens setting. Thus, calibration is needed for each of these combinations. To calibrate, select the desired Camera Zoom and Sample Holder from the main session form, then press the Calibrate button. A modal Calibration form will be opened. Choosing to quit this form before saving will not affect the transformation matrix stored. Choosing to save will overwrite the old transformation matrix with the displayed matrix. If this matrix has been changed in this calibration session, new information will be saved. Else old information will overwrite itself. Pressing the Set Default button will load the identity matrix to the display.

To calculate the calibration matrix, the user needs to input at least 4 sets of measurements in VLM coordinates and the corresponding STXM coordinate. Check the box beside the points to be used in the calculation. If 4 or more points are selected, the Calculate button will be enabled. Press the Calculate button, and the matrix displayed will be updated to the transformation just calculated.

This Calibration form is also the place for adding new Sample Holder, Camera Zoom, or Objective Lens Settings, as well as changing the current information about these items. Beside each of these items, the two buttons will function as above.

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Another collection of edit boxes allow user to perform conversion from VLM coordinates to STXM coordinates. Simply enter the VLM coordinate in the edit boxes on the left, then press the Convert button, the corresponding STXM coordinate will be displayed on the right. This allows user to perform a quick "check".

The calibration information is read from, and stored in the calibration.txt file in the root directory where the data structure is built. Please refer to calibration file.doc for an explanation of each line in the calibration file.

7.4 Typical errors and how to get around them

7.4.1 The TV mode does not work
Check the USB cables – often shorts or disconnects develop. Often the TV signal works only when the camera control USB has been pulled

7.4.2 The images cannot be downloaded from the camera
Camera memory is full. *What to do ?*
Software not working

7.4.3 The center position of the image is wrong when transferred to the STXM
Recalibrate VLM, and/or STXM-VLM.

7.4.4 The size of the image is wrong in the STXM
Recalibrate VLM, and/or STXM-VLM.