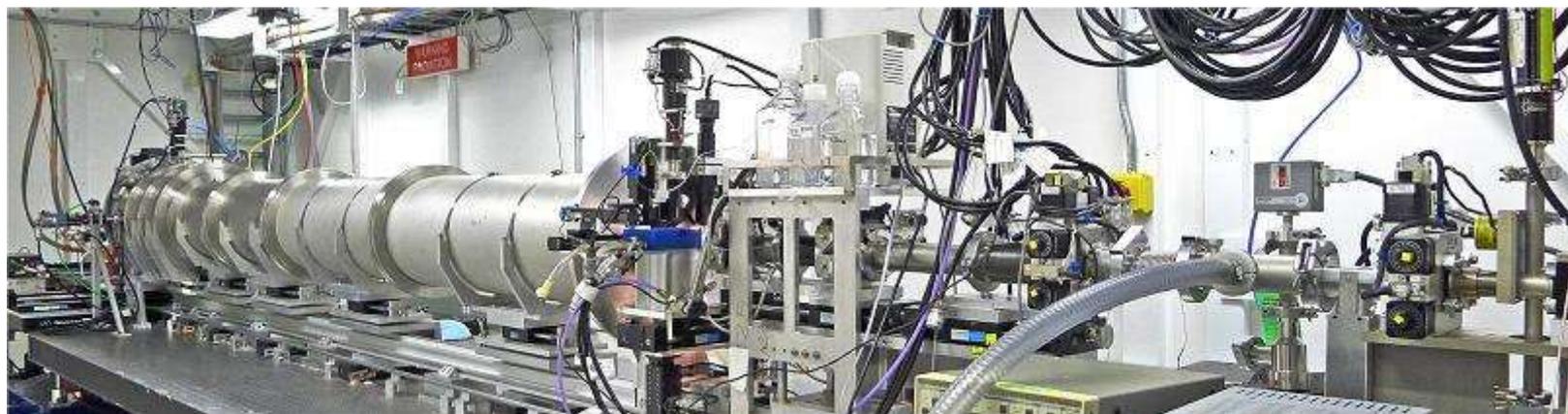


Data reduction and preliminary analysis

Ivan Rajkovic

BioSAXS workshop, March 28, 2016

BL4-2 beam layout



Selecting the right distance

Make sure you put the right info on your proposal

Communicate the Rg and other info to the beamline scientists **BEFORE** the experiment begins

For 11 keV

Distance [m]	Q-range [\AA^{-1}]	Max Rg [\AA]
3.5	0.0035 - 0.25	~130
2.5	0.005 - 0.35	~95
1.7	0.008 - 0.5	~65
1.1	0.012 - 0.85	~42
0.7	0.018 - 1.2	~26

$$q_{\min} * Rg = 0.5$$

$$q * Rg < 1.3 \text{ (Guinier approx.)}$$

Changing the distance

Not like a crystallography beamline!

Steps include:

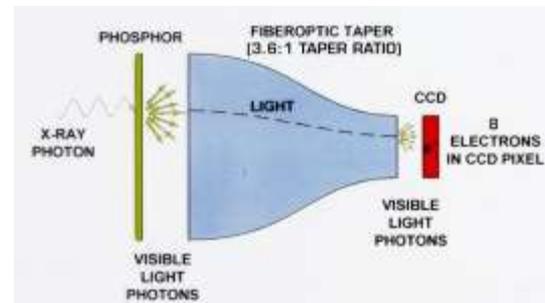
- Vent vacuums
- Move sample stage
- Insert new pipes
- Pump down vacuums
- Change wavelength
- Align beam to center of instrument
- Align slits - s0, s1 and guard slit
- Set-up autosampler again
- Calibrate detector
- Take test data
- 1-2 hours



Collecting data - CCD Detectors

X-Ray CCD detectors work by:

- a) A phosphor screen (to convert X-rays to light)
- b) A fiber-optic taper (to demagnify the light image down to the size of the CCD chip)
- c) A CCD chip to detect the light image as an electric charge on a pixel



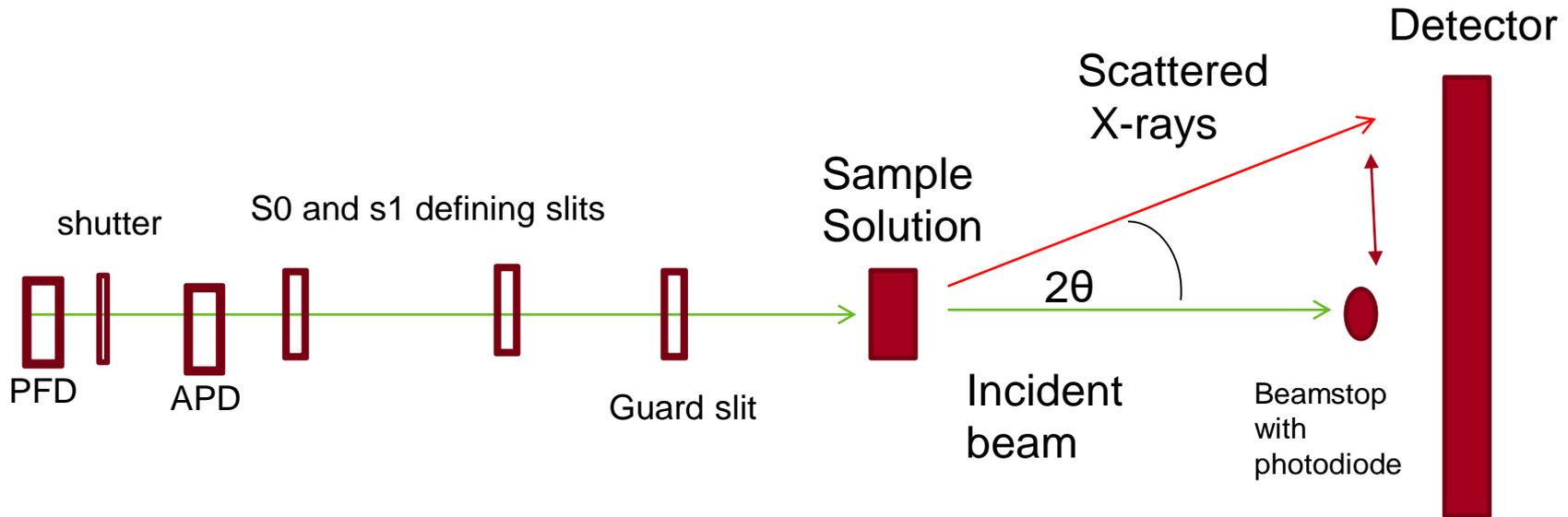
Depending on the 'binning' the count from a number of pixels will be averaged to produce the final reading

Low binning gives higher resolution, but lower accuracy

We generally use the highest possible binning – 8x8 on our Rayonix MX225-He



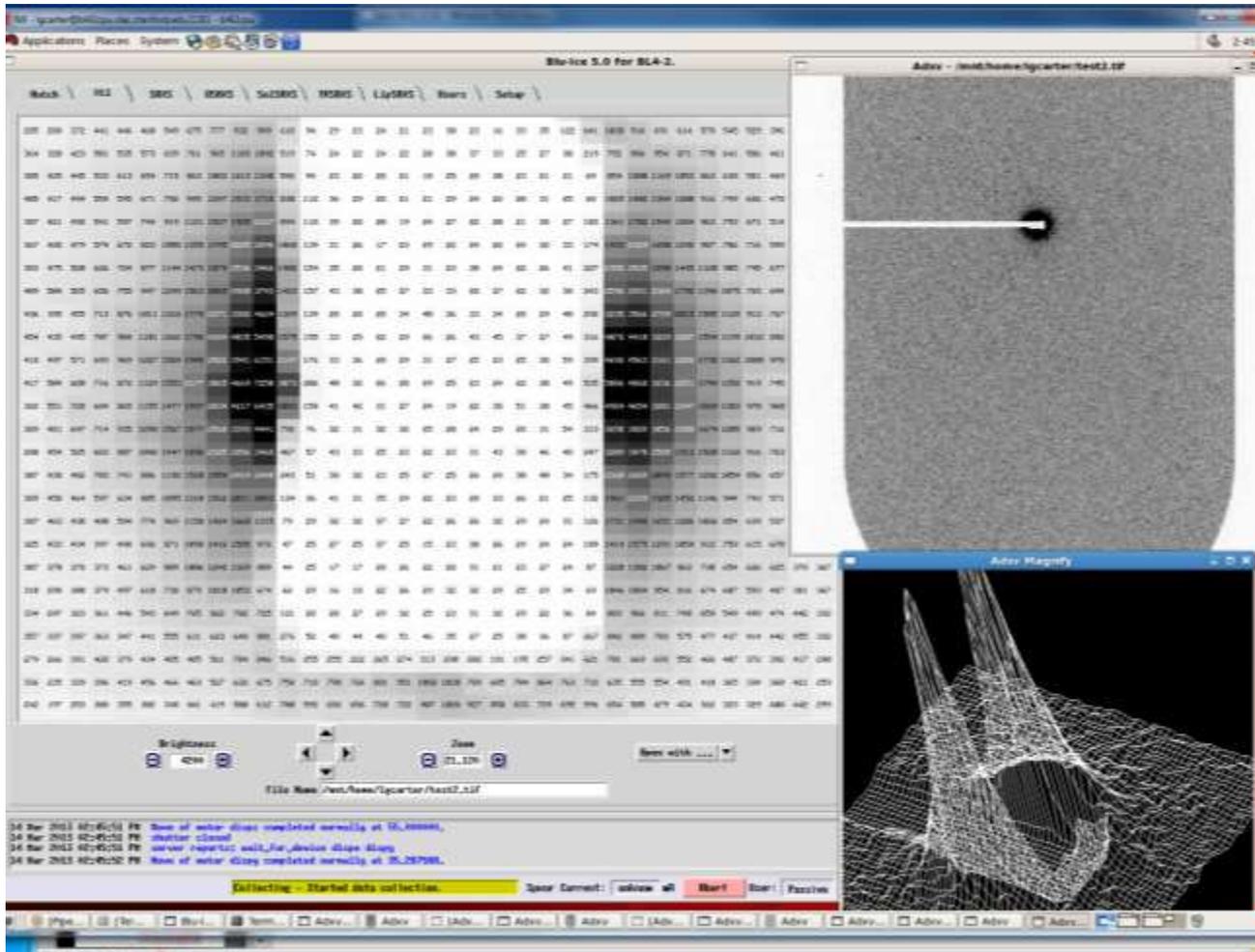
Beam layout



The intensity at q is measured, where q is the angular dependence defined as:

$$q = 4\pi \sin \theta / \lambda$$

Typical images – nothing in the beam



No beam

Blu-Ice 5.0 for BL4-2

Home | XLI | SRMS | USRMS | SuSRMS | TRSRMS | LipSRMS | DeLabel | SEC5RMS | Users | Setup

RebIce Strategy
Collect
Pause
Abort

Current Position
Phi: 0.00 deg
Omega: 0.00 deg
Distance: 655.50 mm

Exposure Control
Enable
Disable
Exposure Factor: 2.0
dmgg_0.0001 0.00 11

Snapshot (inactive)
Default Update History Reset

Profile: dmgg
Directory: /fs/home/bl4c1
Detector: normal

Distance: 100.000 mm
Beam Stop: 38.590 mm
Axis: Phi
Delta: 0.01 deg
Attenuation: 0.000 x
Tilt: 1.00 x

Frame: Phi
Start: 1 0.00 deg
Energy: 11000.001 eV

0.90 m
1.02 m
of the last fork

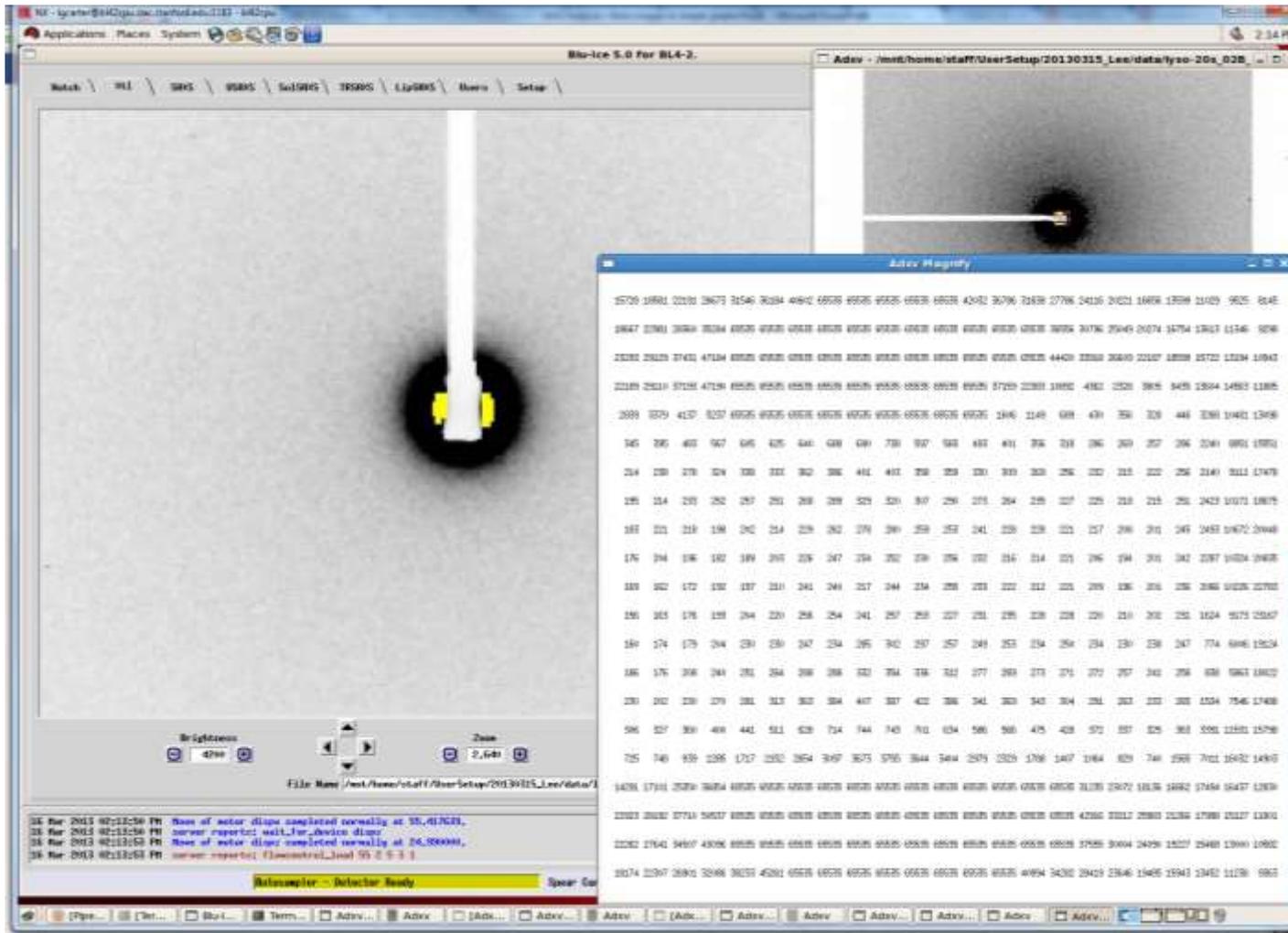
Brightness: 1000
Zoom: 0.000
Open with ...

File Name: /mnt/home/staff/UserSetup/20160325_Natru/data/and

```
25 Mar 2016 03:22:35 PM Ion chaser v2 read -0.0133 counts in 2.5 seconds,
25 Mar 2016 03:22:35 PM Ion chaser v3 read -0.0151 counts in 2.5 seconds,
25 Mar 2016 03:22:35 PM Ion chaser v4 read 5.4294 counts in 2.5 seconds,
25 Mar 2016 03:22:35 PM Ion chaser v5 read 5.0365 counts in 2.5 seconds,
25 Mar 2016 03:22:35 PM Ion chaser v6 read 1.3170 counts in 2.5 seconds,
25 Mar 2016 03:22:35 PM Ion chaser v7 read 1.3176 counts in 2.5 seconds,
25 Mar 2016 03:22:35 PM server reports: wait_for_device 12
```

Collecting - Started data collection. Spear Current: unknown m User: Passive Shutter: closed Vacuum: 4.7674, 3.9788 03:22:34 PM

Overloaded pixels



High background – dirty capillary or windows

The screenshot displays the Blues 5.0 software interface for BL4-2. The main window shows a large grid of numerical data, likely representing detector counts or intensity measurements. The interface includes several control panels on the right side, such as 'Active Strategy' and 'Snapshot (function 3)'. The 'Active Strategy' panel shows 'Current Position' (Phi: 0.00 deg, Omega: 0.00 deg, Distance: 0.00 m) and 'Exposure Control' (Exposure Factor: 2.0). The 'Snapshot' panel shows 'Profile: dummy', 'Detector: WFAuser/SLAC', 'Distance: 0.00 m', 'Beam Size: 0.00 m', 'Beam: Phi', 'Beam: 0.00 deg', 'Wavelength: 0.00 m', 'Time: 1.00 s', 'Start: 0.00', and 'Energy: 11000.000 eV'. At the bottom, a status bar shows 'Detecting - Started data collection.' and 'Open Current: unknown at Abort'. The taskbar at the very bottom shows 'JPetting robot v3...', 'Terminal', 'Blues 5.0 for BL4-2', 'Terminal', 'Adev Control', 'Adev', and 'Adev Magnify'.

Typical buffer image

The screenshot displays the Blu-Ice 5.0 for BL4-2 software interface. The main window is titled "Blu-Ice 5.0 for BL4-2" and features a menu bar with options: Hatch, XLI, SRMS, USRMS, SoLSRMS, TRSRMS, LipSRMS, OnLabel, SECGRMS, Users, and Setup.

The central area shows a large grayscale image of a buffer, which is a circular pattern of data points. Below the image are controls for Brightness (set to 1000) and Zoom (set to 0.800), along with a "Open with ..." button. The file name is displayed as `/mnt/home/staff/UserSetup/20160325_Matsui/data/und`.

On the right side, there are several control panels:

- WebIce Strategy:** Includes buttons for "Callout", "Pause", and "Abort".
- Current Position:** Shows Φ : 0.00 deg, Ω : 0.00 deg, and Distance: 658.50 mm.
- Exposure Control:** Includes "Activate Beam" and "Exposure Control" (with "Enable" and "Disable" options). The Exposure Factor is set to 2.0.
- Snapshot (Inactive):** Includes buttons for "Default", "Monitor", "Delete", and "Reset". It shows settings for Prefix (dummy), Directory (/Fu/home/bl4cl), Detector (normal), Distance (658.000 mm), Beam Stop (38.590 mm), Axis (Phi), Delta (0.01 deg), Attenuation (0.000 x), Time (1.00 s), Start (1, 0.00 deg), and Energy (11000.001 eV).
- Diagram:** A schematic diagram of the detector geometry, showing a circular detector with a diameter of 1.02 m and a radius of 0.51 m. The text "2-View Look Back" is visible below the diagram.

At the bottom, a log window shows the following entries:

```
25 Mar 2016 03:22:41 PH server reports: wait_for_device with
25 Mar 2016 03:22:41 PH server reports: allInSelf 1
25 Mar 2016 03:22:42 PH shutter open
25 Mar 2016 03:22:42 PH Loading /mnt/home/staff/UserSetup/20160325_Matsui/data/under2_01R_0025_0_02.tif...
25 Mar 2016 03:22:42 PH server reports: Loading /mnt/home/staff/UserSetup/20160325_Matsui/data/under2_01R_0025_0_02.tif...
25 Mar 2016 03:22:42 PH server reports: last image event called: waiting list 1
25 Mar 2016 03:22:43 PH shutter closed
```

The status bar at the bottom indicates: Collecting - Exposing under2_01R_0025_0_02.tif... Spear Current: unknow all User: Passive Shutter: closed Vacuum: 4.8330 3.5940 03:22:43 PM

Typical sample image

The screenshot displays the Blu-Ice 5.0 for BL4-2 software interface. The main window is titled "Blu-Ice 5.0 for BL4-2" and features a menu bar with options: Hatch, X11, SRMS, USRMS, GsSRMS, TRSRMS, LipSRMS, OnLabel, SECSRMS, Ibers, and Setup.

The central area shows a grayscale image of a sample, which appears to be a circular feature on a textured background. Below the image are controls for Brightness (set to 1000) and Zoom (set to 0.900), along with an "Open with ..." dropdown menu. The file name is displayed as `/mnt/home/staff/IbersSetup/20160325_Matsumi/data/ind`.

On the right side, there are several control panels:

- WebIce Strategy:** Includes buttons for Collect, Pause, and Abort. Below these are "Current Position" details: Phi: 0.00 deg, Omega: 0.00 deg, and Distance: 855.50 nm. There are also buttons for "Optimize Beam" and "Exposure Control".
- Snapshot (inactive):** Includes buttons for Default, Update, Delete, and Abort. It shows fields for Prefix (dmg), Directory (/fs/home/bl4-2), Detector (normal), Distance (650,000 nm), Beam Stop (30,350 nm), Axis (Phi), Delta (0.01 deg), Intensity (0.000), and Time (1.00 s). It also has fields for Frame (1) and Energy (11000,001 eV).
- Diagram:** A schematic diagram of a circular detector or sample area with a radius of 0.50 m and a diameter of 1.00 m.

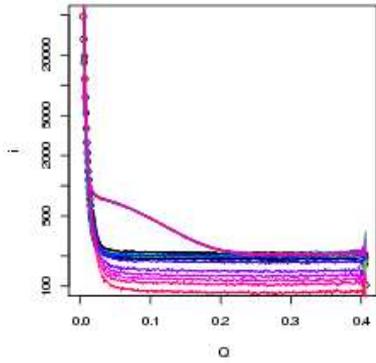
At the bottom of the interface, there is a log window showing system messages:

```
25 Mar 2016 03:14:46 PM server reports: floscontrol_wash 1000 300 1 -4 1
25 Mar 2016 03:14:56 PM server reports: floscontrol_wash completed successfully.
25 Mar 2016 03:14:56 PM server reports: floscontrol_esc 40 20 5 3 1
25 Mar 2016 03:15:24 PM server reports: floscontrol_esc completed successfully.
25 Mar 2016 03:15:24 PM channel2 open
25 Mar 2016 03:15:25 PM server reports: floscontrol_wash 240 100 4 1 1
25 Mar 2016 03:15:25 PM channel2 closed
```

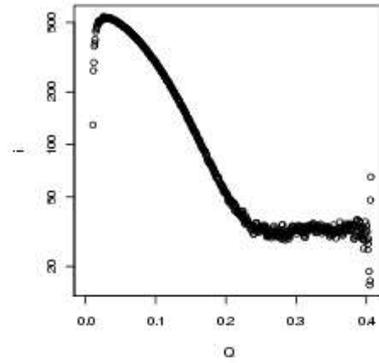
A status bar at the very bottom indicates "Collecting - Started data collection." and provides real-time data: Speed: Current: unknown, User: Passive, Shutter: closed, Vacuum: 4.7854, 4.4680, 03:15:44 PM.

Air bubbles!

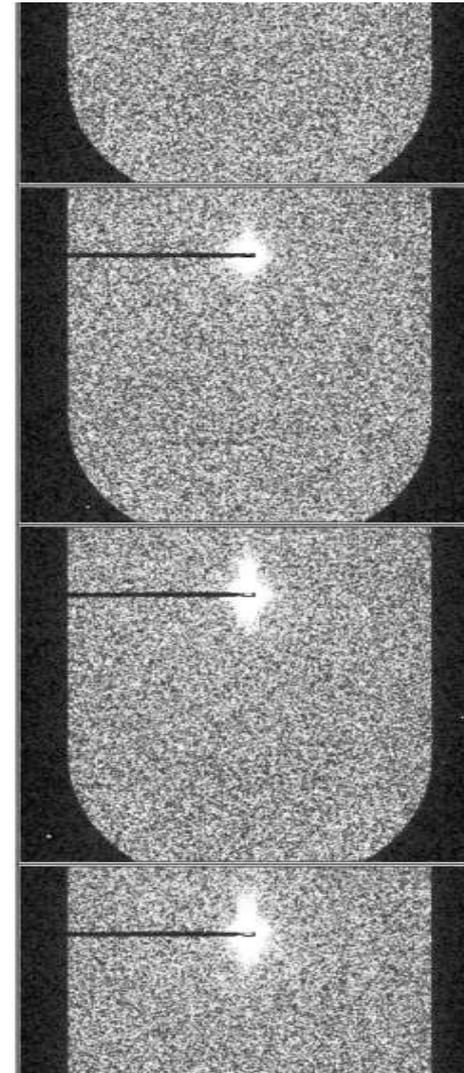
All Images



Log Intensity Curve

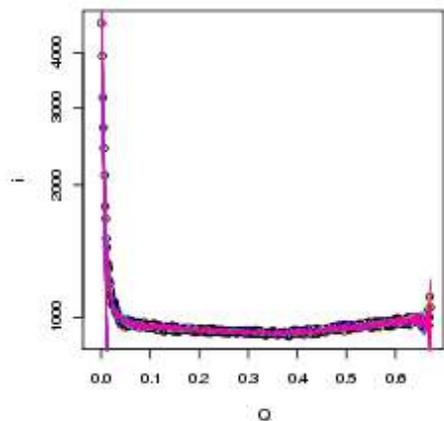


$i \sim Q^{-2}$

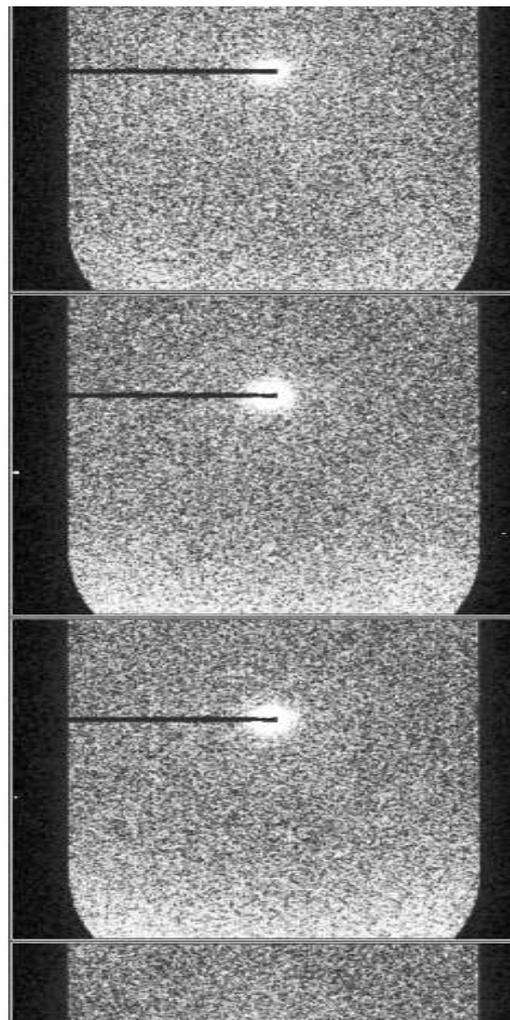
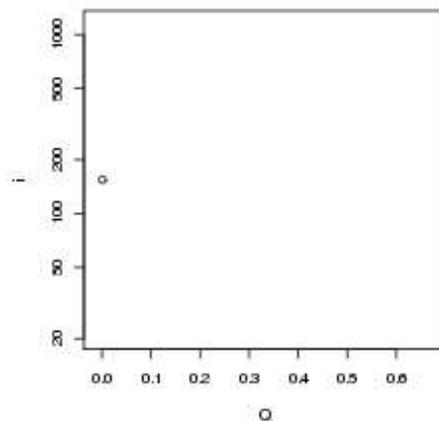


No SAMPLE!

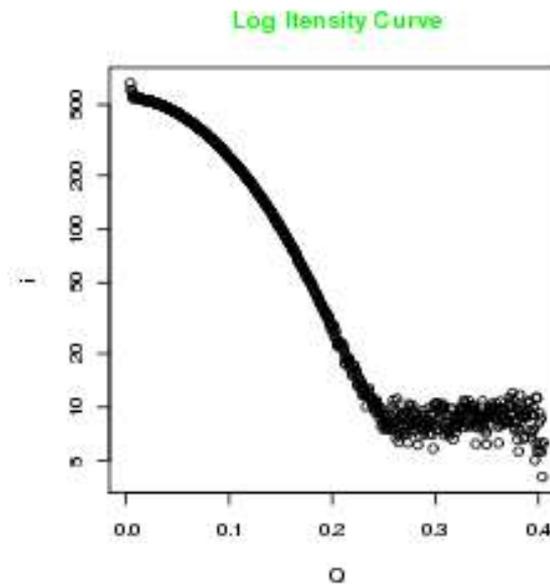
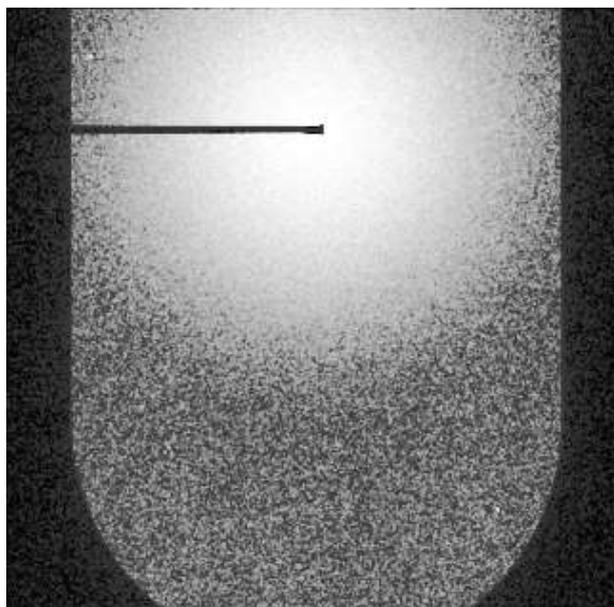
All Images



Log Intensity Curve



The SAXS profile: 2D image -> 1D graph



What we need to calculate the profile?

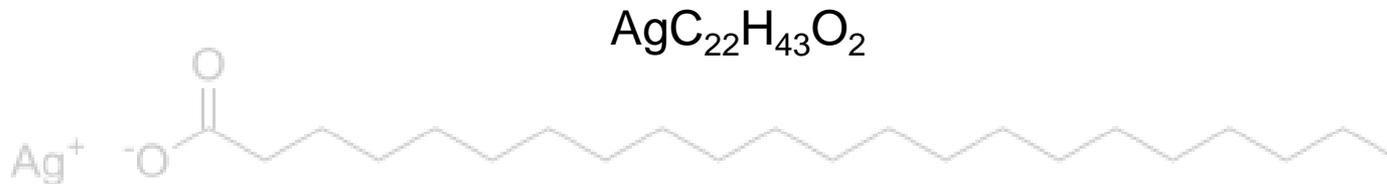
1. The masking out of the beamstop
2. The beam center
3. A mapping of the pixels to a known q value
4. A value to scale each image by

Silver Behenate

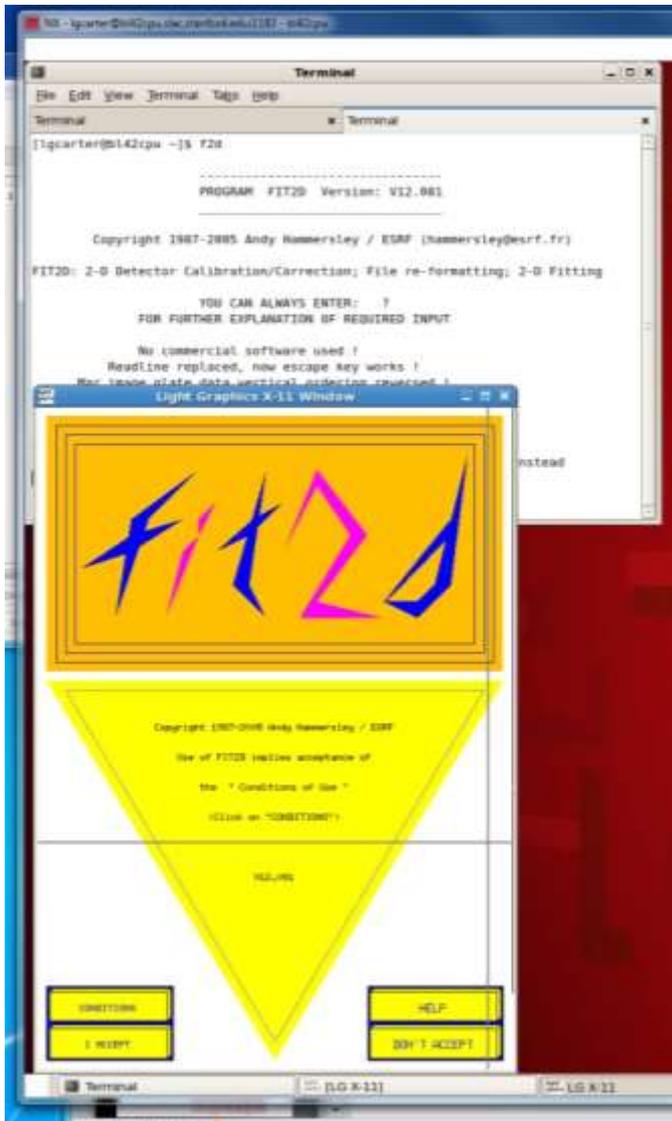
- Silver behenate is a silver salt of the long-chain fatty acid behenic acid
- Powder diffraction from Silver behenate can be used to find the beam center and map pixels to q
- powder diffraction rings of Silver behenate occur at known intervals:

Reciprocal space

58.380 Å	$\xrightarrow{2\pi/\text{Å}}$	0.107	
29.190 Å		0.215	Å ⁻¹
19.40 Å		0.328	



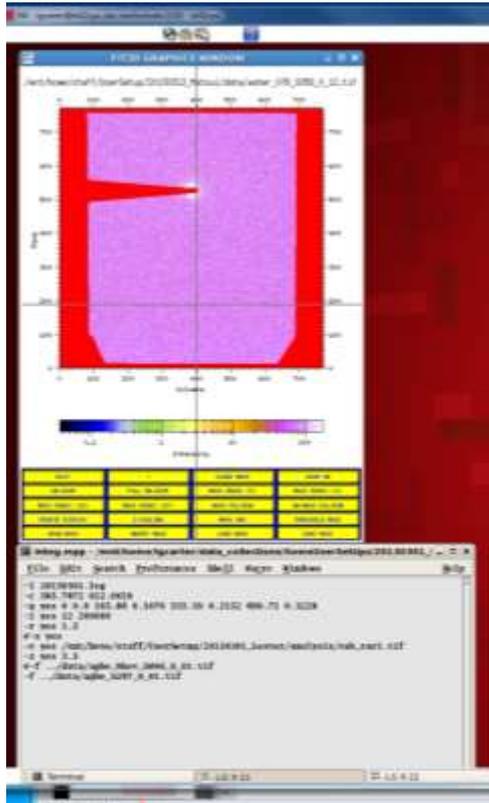
Using FIT2d to examine images



Free software provided by the ESRF

Can be run on bl42cpu by typing f2d

Creating a mask using fit2d



Preferably done on a water image or similar with lower contrast

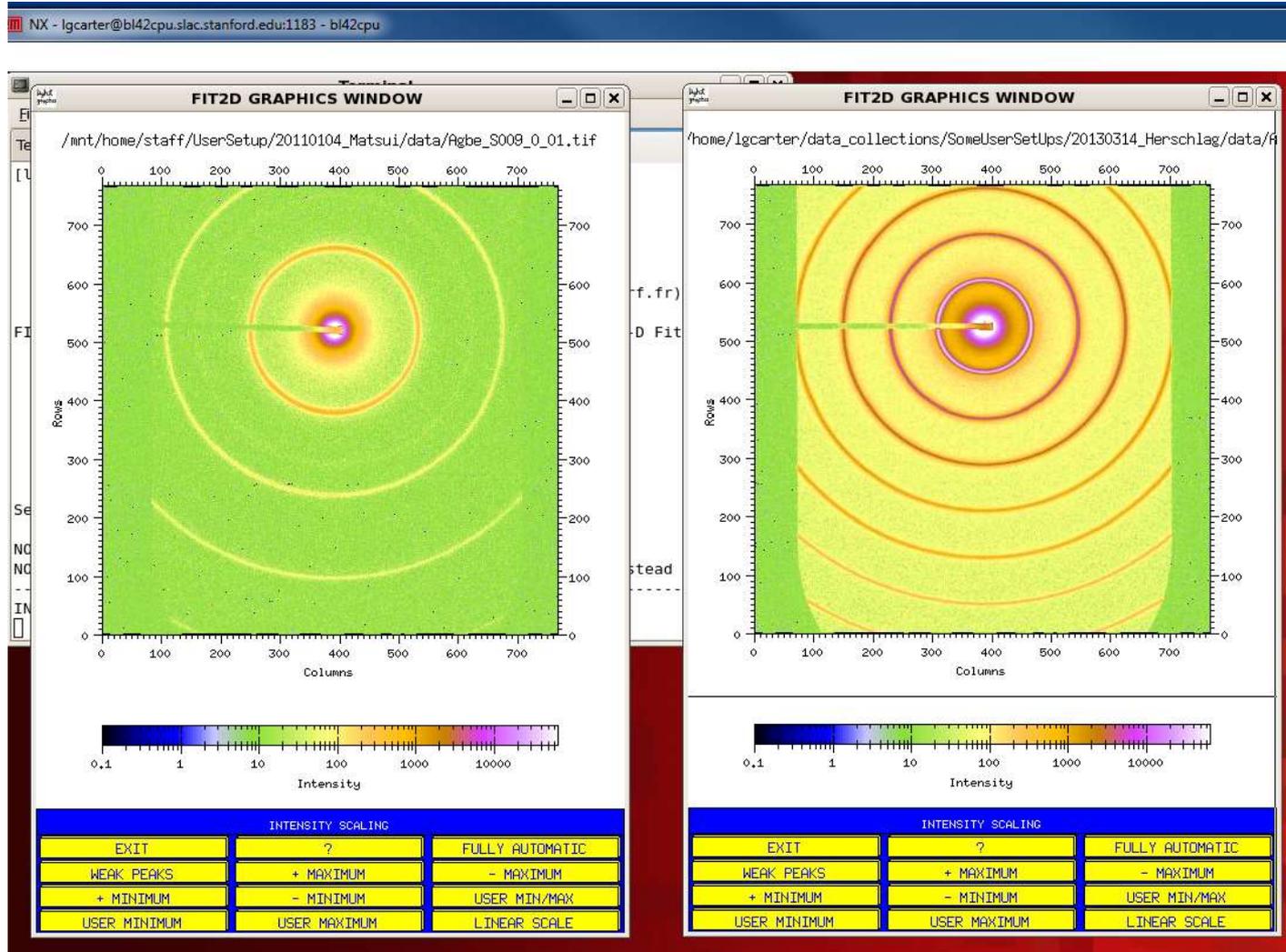
Using the Mask tool in the powder diffraction menu the sides of the detector and beamstop are masked out

The mask is then saved in tif format

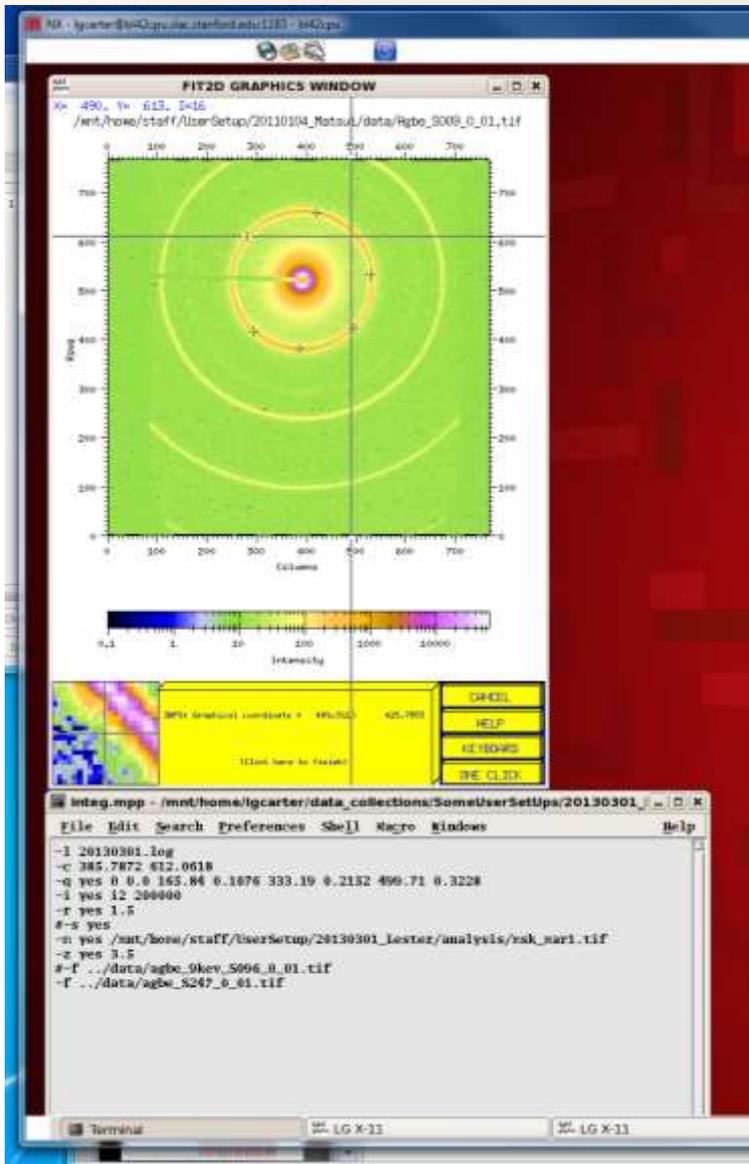
Silver behenate scattering

2.5 m

1.1 m

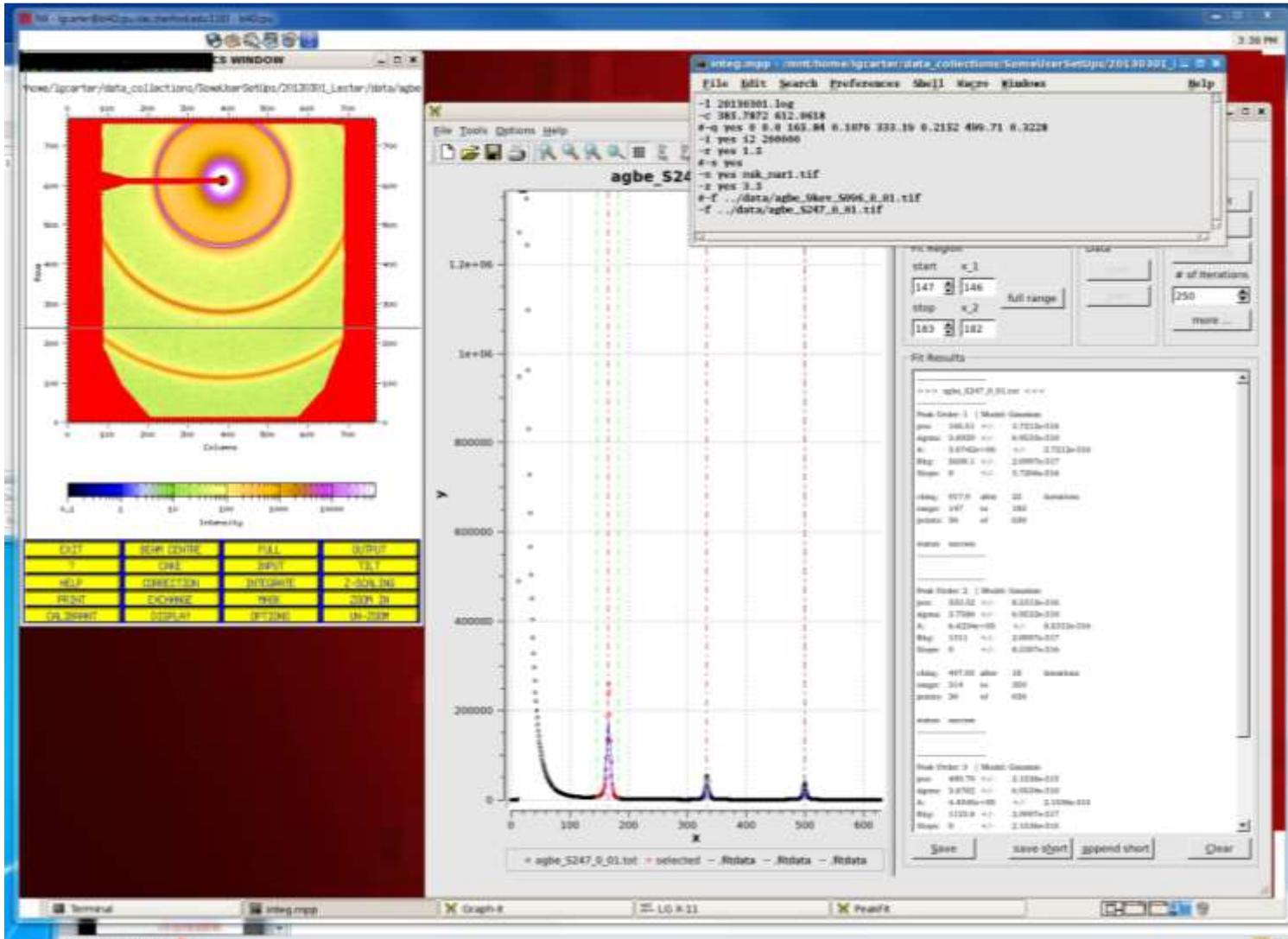


Finding the beam center using AgBE and fit2d



- Once an image is loaded in it can be Z-scaled to make it clearer
- Under the powder diffraction menu is 'beam center' option
- This scan can be used to select several points around the first ring, which can then be used to find the image center

Calibrating the q-space

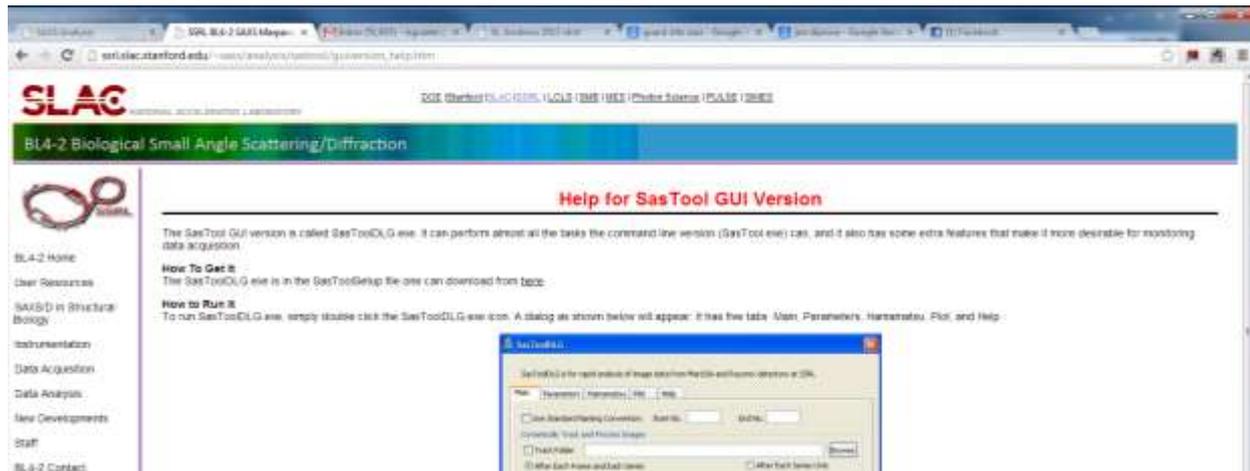


Sastool is our in house tool for convert images to intensity profiles

The is also a Windows GUI version available

Both can be downloaded from:

- <http://ssrl.slac.stanford.edu/~saxs/analysis/sastool.htm>



- reads the tiff raw image file
- obtains and subtracts offsets, normalizes for beam intensity
- converts 2-D grid data to 1-D distance from beam center
- converts distance from pixels to q-space
- calculates statistics for each image frame (average and standard deviation) (this generates a .dat file for each frame)
- compares statistics and use them to include or reject frames in the sum of the whole series (this generates a .tot file for each series)
- and subtracts the buffer intensity from the sample intensity (generating the final .sub file)

Main Sastool parameters

```
-l sample.log          # The log file name.
-c 343.4691 250.5907 # Beam center coordinates in pixels.
-q yes 0 0.0 102 0.1076 205 0.2152 308 0.3228          # Conversion from pixel to q
-i yes i1 1000        # Data normalization factor
-z yes 3.5            # Remove zingers
-r yes 2.0            # Rejection of individual frames from the summation of the whole series
-s yes                # Subtract buffer intensity from individual sample frame.
-m yes ./mask.tif    # Use mask file for data integration
-f ../sample03_0_01.tif ../buffer03_0_01.tif          # Files for SasTool to process.
```

Typical Sastool run

Usually you will have a terminal (right click desktop) open and cd-ed into the analysis directory
You can run gedit in the background to edit integ.mpp:

- gedit integ.mpp &

You will need to type in the buffer and sample pairs at the end of the file

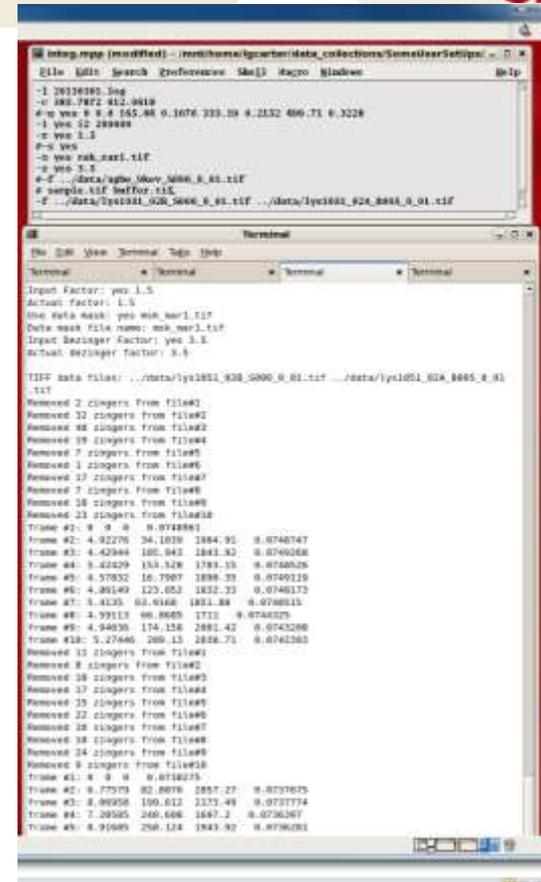
- -f sample.tif buffer.tif

These can be found by searching data directory:

- ls -lrt ../data/*01.tif

Save the file and run sastool

- sastool integ.mpp



Sastool log file

```
20130301.log - /mnt/home/gcafer/data_collection/50meters/201301...
File Edit Search Preferences Shell Macro Windows
~integ.jpg 20130301.log
Parameter file: integ.jpg
Logfile: 20130301.log
Center point: 381.7872 612.8818
Use 11: yes 12 200000
Use 12 for data normalization
Input factor: yes 1.5
Actual factor: 1.5
Use data mask: yes mask_sarl.tif
Data mask file name: mask_sarl.tif
Input detector factor: yes 1.5
Actual detector factor: 1.5
TIFF data files: ../data/lys1051_020_5000_0_01.tif ../data/lys1051_020_5000_0
Variance of frames relative to the first frame for buffer files:
Frame #1: 0 0 0 0.0748061
Frame #2: 4.92276 34.1639 1644.91 0.0748747
Frame #3: 4.42964 105.943 1842.92 0.0748268
Frame #4: 1.42420 153.529 1783.13 0.0748526
Frame #5: 4.57832 14.7907 1899.31 0.0748119
Frame #6: 4.86140 123.892 1832.33 0.0748173
Frame #7: 5.4135 63.9148 1831.48 0.0748315
Frame #8: 4.50113 66.8681 1711 0.0748325
Frame #9: 4.94838 174.158 2001.42 0.0748298
Frame #10: 3.27446 200.13 2038.71 0.0748293
Average variance for buffer:
1873.88
Variance used for rejection: 1873.88
Factor used for rejection: 1.5
Variance of frames relative to the first frame for sample files:
Frame #1: 0 0 0 0.0738275
Frame #2: 6.77370 82.8679 2057.27 0.0737673
Frame #3: 8.00928 106.612 2173.40 0.0737774
Frame #4: 7.20383 240.808 1897.2 0.0738097
Frame #5: 8.01081 258.124 1943.92 0.0738283
Frame #6: 8.91294 280.865 2092.23 0.0738013
Frame #7: 7.34804 177.172 1961.16 0.0738094
Frame #8: 6.94769 187.773 2016.9 0.0738775
Frame #9: 7.48887 246.29 1922.84 0.0738048
Frame #10: 8.48365 193.812 2149.28 0.0737927
Buffer frames:10 Rejected:0
Sample frames:10 Rejected:0
Final sub:./lys1051_020_5000_0_01.sub
Removed 10 Zingers From 711488
```

Input parameters

Buffer files

Samples files

Frame information

```
Data mask file name: msk_mar1.tif
Input dezing factor: yes 3.5
Actual dezing factor: 3.5
TIFF data files: ../data/lys1051_02B_S006_0_01.tif ../data/lys1051_02A_B005_0
Variance of frames relative to the first frame for buffer files:
frame #1: 0 0 0 0.0748961
frame #2: 4.92276 34.1039 1904.91 0.0748747
frame #3: 4.42944 105.943 1843.92 0.0749268
frame #4: 5.42429 153.528 1783.15 0.0748526
frame #5: 4.57832 16.7907 1890.35 0.0749119
frame #6: 4.86149 123.052 1832.33 0.0748173
frame #7: 5.4135 63.9168 1851.88 0.0748515
frame #8: 4.59113 66.8685 1711 0.0744325
frame #9: 4.94036 174.158 2001.42 0.0743208
frame #10: 5.27446 209.13 2038.71 0.0742393
Average variance for buffer:
1873.08
Variance used for rejection: 1873.08
Factor used for rejection: 1.5
Variance of frames relative to the first frame for sample files:
frame #1: 0 0 0 0.0738275
frame #2: 6.77579 82.8076 2057.27 0.0737675
frame #3: 8.06958 199.612 2173.49 0.0737774
frame #4: 7.20585 240.608 1697.2 0.0736397
frame #5: 8.91685 258.124 1943.92 0.0736281
frame #6: 8.91254 240.865 2092.23 0.0734513
frame #7: 7.14494 177.172 1961.16 0.0736094
frame #8: 6.94788 187.773 2056.9 0.0736775
frame #9: 7.48487 246.19 1952.84 0.073648
frame #10: 8.48395 193.812 2149.25 0.0737927
Buffer frames:10 Rejected:0
Sample frames:10 Rejected:0
Final sub:../lys1051_02B_S006_0_01.sub
```

The first number is frame variance

The second number is the summation of difference (normalized by standard error) between the frame and the first frame

The third number is the summation of the square of the difference (normalized by standard error) between the frame and the first frame

The fourth number is the ratio of I2/I1, i.e. transmission

The APD and beamstop photodiode reading



The photodiode is located in the beamstop

The photodiode value is readout and written to the image header. There is usually an offset, so the value will never be 0. If the value falls significantly during data collection (on similar samples) this could be a sign of a problem

You can check the values through the run by using grep:

```
grep "I_1" *.prp  
grep "I_2" *.prp
```

Data .prp files

Contain the recorded intensities from the photodiodes and information about the experimental setup

```
lys1051_02D_S032_0_01.prp
1 Image file name: lys1051_02D_S032_0_01.tif
2 Time this file was written: 01 Mar 2013 06:36:00 PM
3 I_Signal (i.e. I1 in header file) is from: i_apd
4 I_signal=979152.0 (corrected for dark counts)
5 I_0(daqIon3.0)=2.599
6 I_1(daqCounter1.0)=979152.0
7 I_2(daqCounter1.1)=54285.75 (corrected for dark counts)
8 I_apd(daqCounter1.0)=979152.0
9 I_upstream(daqIon3.11)=-0.0135
10 I_downstream(daqIon3.12)=-0.0421
11 v0(daqIon3.0)=2.599
12 v1(daqIon3.1)=2.4625
13 v2(daqIon3.2)=5.5666
14 v3(daqIon3.3)=5.465
15 v4(daqIon3.4)=-0.01
16 v5(daqIon3.5)=0.0131
17 v6(daqIon3.6)=2.5828
18 v7(daqIon3.7)=2.581
19 Detector mode=0; 0 for normal and 1 for dezingered
20 Exposure time=1
21 Counting time=2.5
22 Beam energy=11000.0006542 eV
23 Pipe length=2500 mm
24 Scan motor=NULL
25 Scan range=0 mm
26 Phi position=0.000000
27 Horizontal position=56.563492 mm
28 Vertical position=9.105 mm
29 dispX position=0.487501 mm
30 dispY position=43.912610 mm
31
```



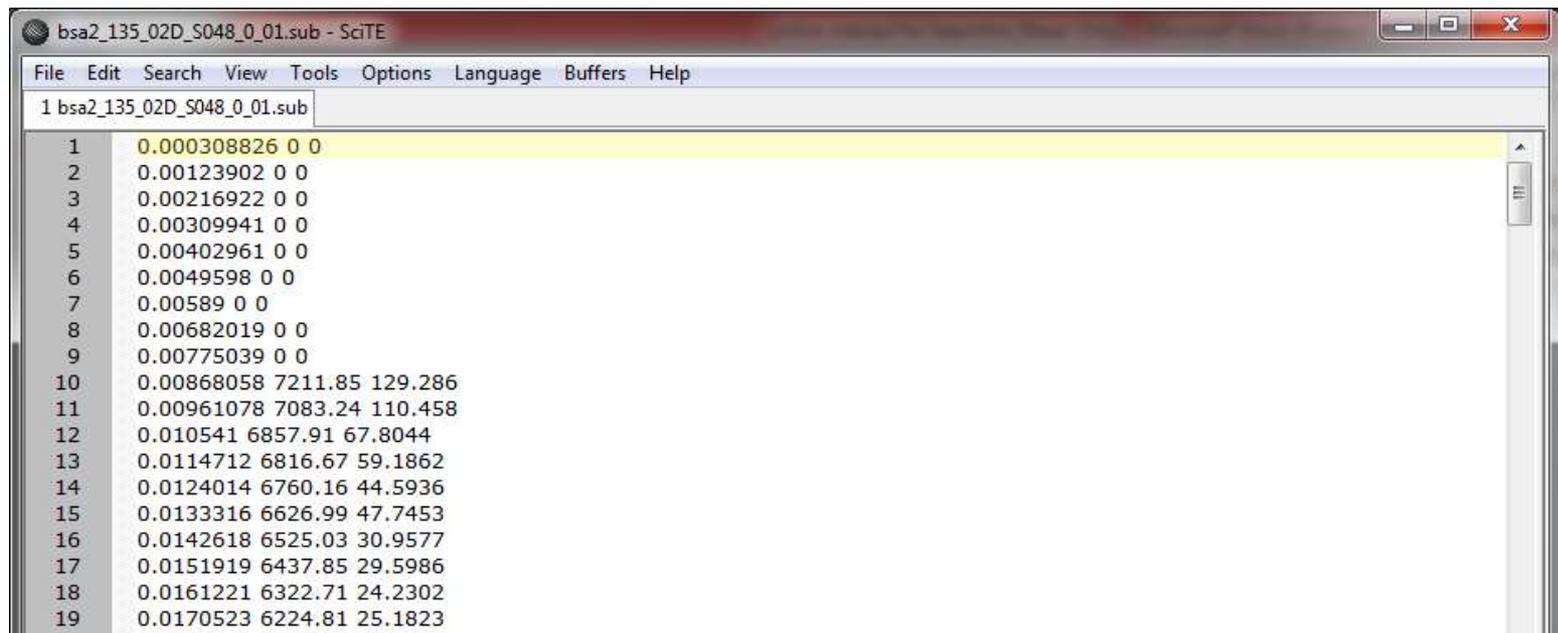
A .dat file for each individual image

A .tot file for the buffer and the sample

A .sub subtracted file

The final .sub file

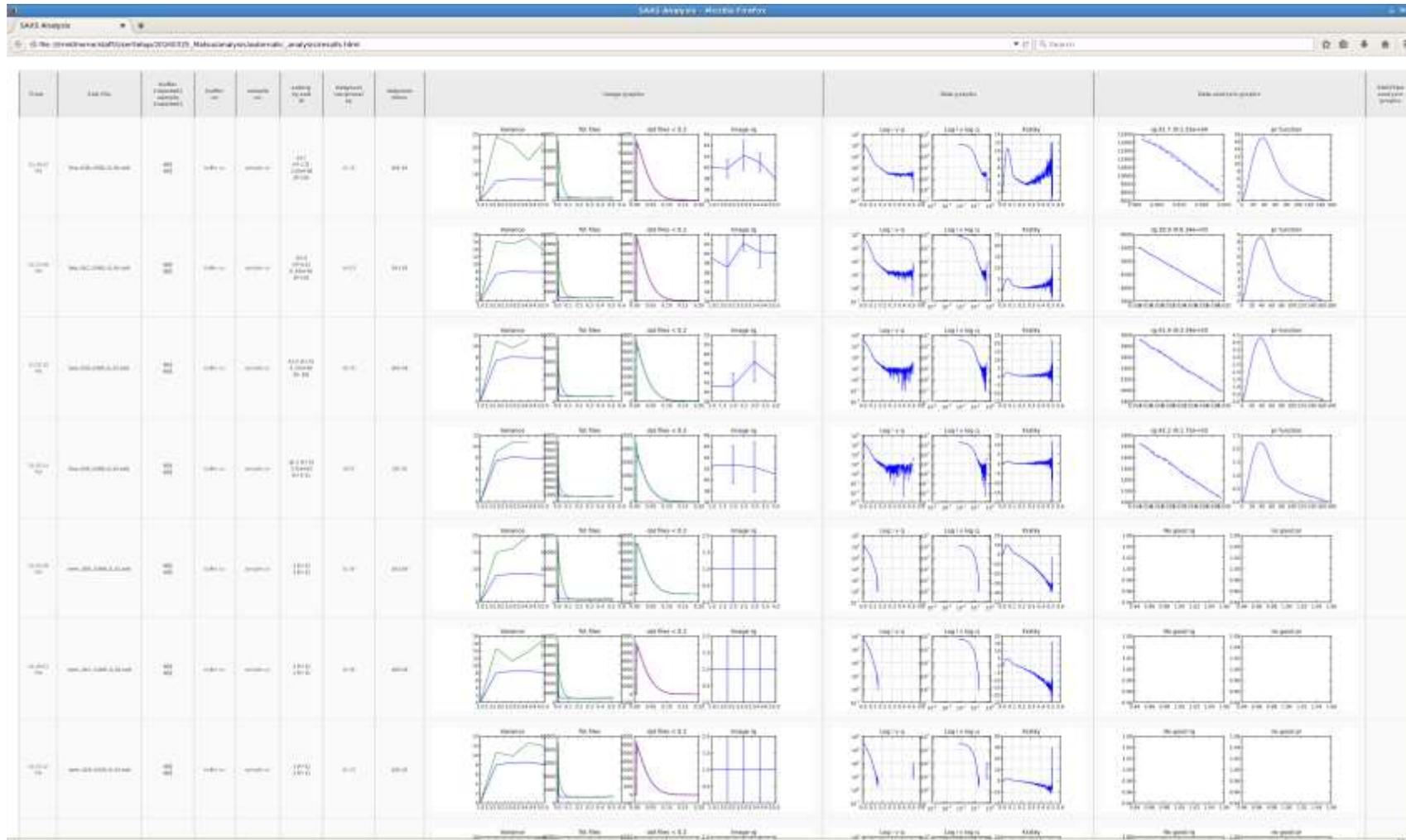
q Int Int_{std}



The screenshot shows a SciTE text editor window titled "bsa2_135_02D_S048_0_01.sub - SciTE". The window contains a single file named "1 bsa2_135_02D_S048_0_01.sub" with 19 lines of data. The first line is highlighted in yellow. The data consists of a line number followed by three numerical values. Lines 10 through 19 have three values, while lines 1 through 9 have only two values.

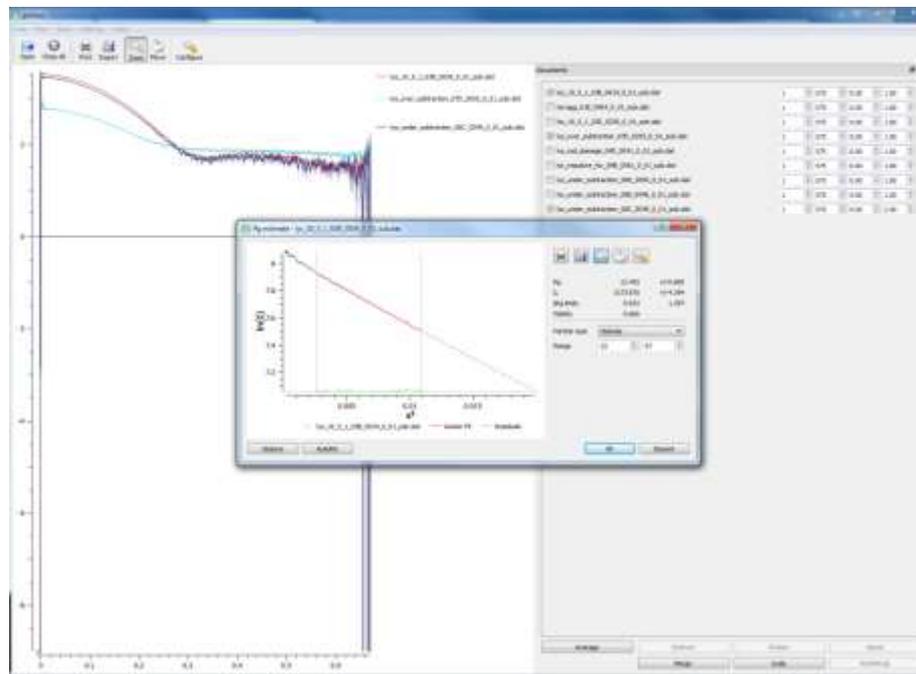
Line	Value 1	Value 2	Value 3
1	0.000308826	0	0
2	0.00123902	0	0
3	0.00216922	0	0
4	0.00309941	0	0
5	0.00402961	0	0
6	0.0049598	0	0
7	0.00589	0	0
8	0.00682019	0	0
9	0.00775039	0	0
10	0.00868058	7211.85	129.286
11	0.00961078	7083.24	110.458
12	0.010541	6857.91	67.8044
13	0.0114712	6816.67	59.1862
14	0.0124014	6760.16	44.5936
15	0.0133316	6626.99	47.7453
16	0.0142618	6525.03	30.9577
17	0.0151919	6437.85	29.5986
18	0.0161221	6322.71	24.2302
19	0.0170523	6224.81	25.1823

SAXSPipe - automatic analysis pipeline

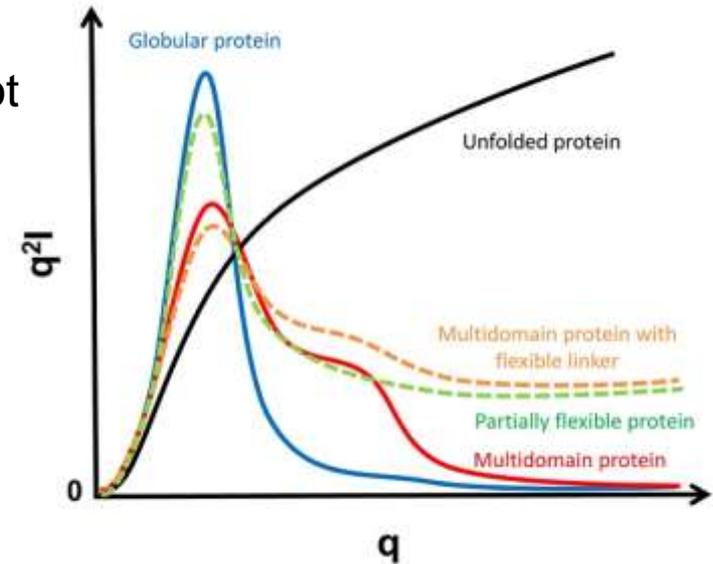


Data quality check

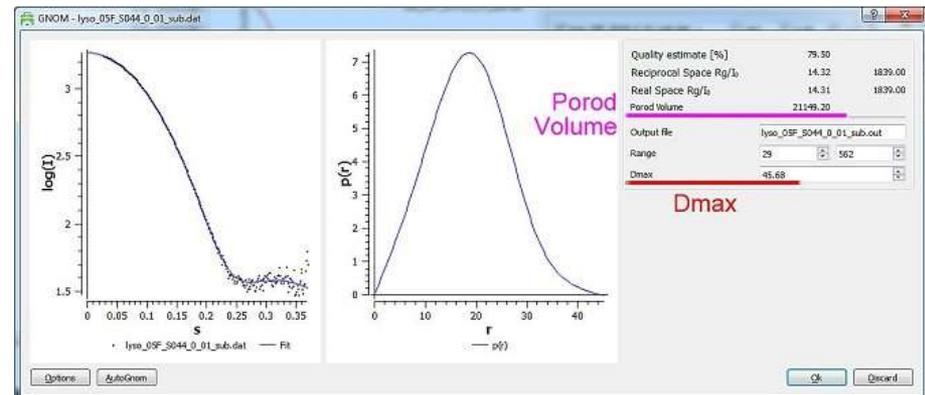
Guinier plot



Kratky plot



Pair-distribution function



Basic analysis process

Check images for issues

Run sastool – check for rejected images

- Air bubbles
- Radiation damage

Plot data

Look for upturn or down turn (aggregation and inter-particle interference)

Check Guinier plot

Check Pr function

Check Kratky plot

Carry out concentration series – are there any concentration effects?

Then move on with analysis

