A Guide to SAXS Data Processing with the Utah SAXS Tools

with

Special Attention to Slit Corrections and Intensity Calibration

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September 2012

Changes

- 6 September 2012
    1. Added feature to saxsFit to allow fitting to a linear combination of two models.
    2. Bug fix to saxsDeSmear involving smoothing function for final itteration.
    3. Miscellaneous tweaks.

Preface

This document provides both background and detailed instructions for a set of computer programs that I have written for processing and analyzing small-angle X-ray scattering (SAXS) data. The programs are primarily intended for users of the the Anton-Paar SAXSess instrument, a commercial line-collimated laboratory-scale camera, but some of the programs may also be useful for SAXS data collected with other instruments, as well as some small-angle neutron scattering (SANS) data. The background information on slit smearing and its correction and on the units and calibration of scattering intensities should be useful to a variety of users.

A variety of programs for different computer platforms are available for processing and analyzing SAXS data. These include both commercial products, such as SAXSquant, which is provided with the Anton Paar SAXSess instrument, and free software, including the extensive set of programs developed by group of Dmitri Svergun at the European Molecular Biology Laboratory (`http://www.embl-hamburg.de/biosaxs/software.html`. Also of special note is the Irena macro package for the commercial data analysis and graphing program Igor Pro (`http://usaxs.xor.aps.anl.gov/staff/ilavsky/irena.html`). One might reasonably ask, then, why develop another set of SAXS data tools? The programs described here are, in fact, much less ambitious than many of those available elsewhere and are in some respects less user friendly. They do have some virtues, however, including the fact that they can be used on almost any platform that supports Python (including the Macintosh with OS X), and they rely only on publicly available software.

There are two major components of the software described here. The first is a set of macros (saxsImage) for the ImageJ program, a widely-used scientific image analysis program developed by Wayne Rasband at the U.S. National Institutes of Health. The saxsImage macros create new menu commands for imageJ that are specifically designed for integrating the two-dimensional image data from the SAXSess camera, as well as analyzing the beam profile. The data from saxsImage are saved in the PDH file format of Glatter *et al.*, with special provisions for storing the beam-profile information.

The second component is a set of programs, written in the Python language, for processing, analyzing and plotting the scattering data. These programs are run using a command-line interface: A shell in Unix-like operating systems (including Mac OS X) or the DOS window in the Windows operating systems. While this approach is, in some respects, less user-friendly than a graphical interface with menus *etc.*, with a bit of experience it can become a very efficient way of working. In particular, the user is freed from the repetitive use of dialog boxes for opening and saving files, as well as other operations. The programs can also be called from scripts that automate some of the data-processing steps.

Acknowledgements

This software and documentation was developed through a collaboration with Prof. Jill Trewhella and her laboratory at the University of Sydney. I am particularly grateful for an International Visiting Research Fellowship from the University of Sydney, which allowed me to spend several weeks in Prof. Trewhella's laboratory in 2009. SAXS studies on unfolded proteins, which led to the development of this software, have been supported by grants from the U.S. National Science Foundation (No. MCB-0749464 to D.P.G.) and the Australian Research Council Discovery Project Scheme (No. DP0770631 to J.T.). SAXS data used for sample files and the examples shown in this document were provided by Cy Jeffries, Daniel Johansen and Brian Argyle.

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Contents

Chapter 1

The Geometry and Mathematics of Smearing and Desmearing

1.1 Geometric Considerations

The simplest geometry for recording SAXS data uses a beam very narrow in both dimensions, as collimated by a series of pinholes, and a linear detector:

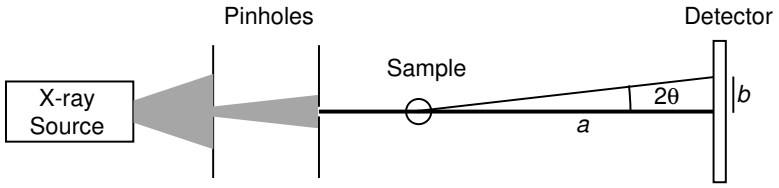

Figure 1.1: A SAXS camera with a point-collimated beam and a one-dimensional detector

By convention, the scattering angle is expressed as the scattering vector magnitude, defined as $q = (4\pi \sin\theta)/\lambda$, where θ is one-half of the scattering angle as shown above, and λ is the X-ray wavelength. In the small-angle regime, the following approximations hold:

$$\begin{aligned} \sin(2\theta) &\approx \tan(2\theta) = b/a \\ \sin\theta &\approx b/(2a) \end{aligned} \tag{1.1}$$

The distance b in the drawing above is related to the corresponding value of q according to:

$$b = q\frac{a\lambda}{2\pi} \tag{1.2}$$

For convenience, it is useful to express other lengths defining the scattering geometry in "q-units". Thus, a length l expressed in ordinary units of length is expressed in q-units as:

$$l\frac{2\pi}{a\lambda} \tag{1.3}$$

The units of q are commonly Å^{-1} or nm^{-1}. Unless otherwise indicated, all of the lengths discussed below are assumed to be expressed in q-units.

With a pinhole-collimated beam, the intensity of scattering at an angle 2θ is simply measured as the intensity detected at the distance, q, from the incident beam.

With a 2-dimensional detector, a point-collimated source gives rise to a radial pattern:

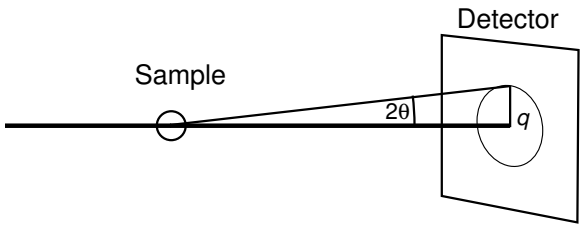

Figure 1.2: A SAXS camera with a point-collimated beam and a two-dimensional detector

The intensity corresponding to the scattering angle 2θ is then determined by integrating over the circle with radius q.

The use of point-collimated beams in SAXS is usually only practical with very bright sources, especially synchrotron beam lines. Laboratory SAXS cameras usually use a beam collimated into a thin line and a matching long sample chamber. This provides much stronger signals for a given beam intensity, making it possible to use weaker sources. However, line collimation significantly complicates data reduction and analysis, and inevitably results in some loss of resolution.

A schematic representation of a line-collimated SAXS camera, with the long dimension of the beam profile (and sample holder) horizontal, is shown below in two views:

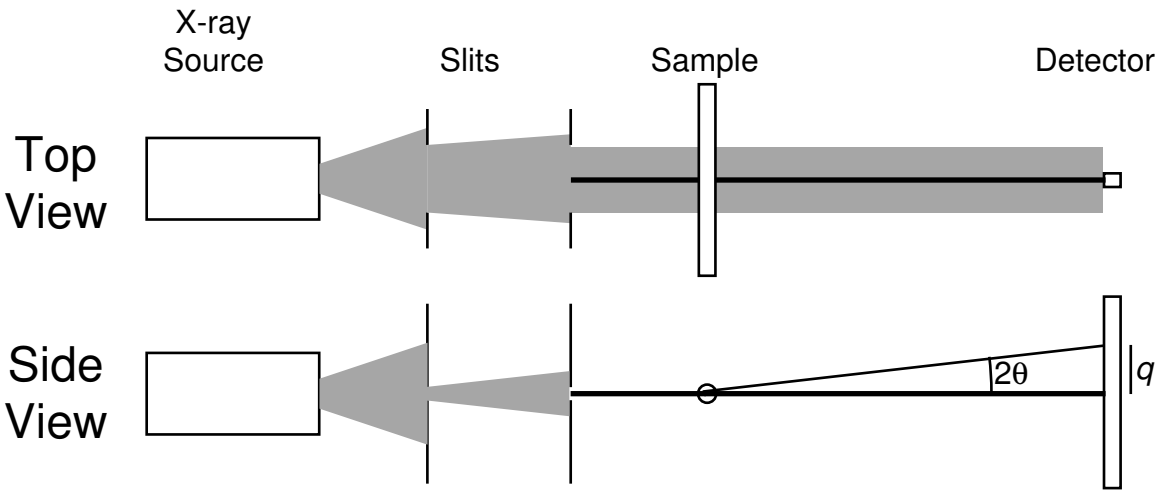

Figure 1.3: A SAXS camera with a line-collimated beam

Although a line-collimated beam can in principle be created using slits as shown above, an arrangement of reflective blocks is usually preferred, so as to minimize parasitic scattering. In the figure above, only a single ray scattered from a single point in the sample is shown, and only a one-dimensional detector is shown. But, each point of the detector will be struck by X-rays scattered from points along the sample:

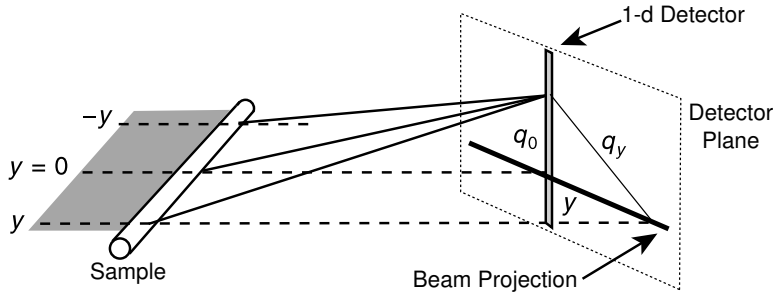

Figure 1.4: Contribution of X-rays scattered from multiple points along the beam length to the intensity detected at point q_0.

The important point is that the rays that reach a single point on the detector from different points in the sample will have been scattered at different angles. In the drawing above, q_0 represents

a position on the detector and corresponds to the angle of the X-rays scattered from the sample point directly aligned with the detector ($y = 0$). [1] X-rays that reach point q_0 from other points along the sample (y) are scattered at a larger angle, corresponding to q_y, given by:

$$q_y = \sqrt{q_0^2 + y^2} \tag{1.4}$$

The same kind of smearing effect can arise from the finite thickness of the beam (as well as from the finite diameter of a pinhole-collimated beam). In general, however, these effects are much smaller than those from the beam length. The diagram below illustrates, with exaggeration, the rays that reach a single point on the detector from different points along the beam thickness, given by the distance x:

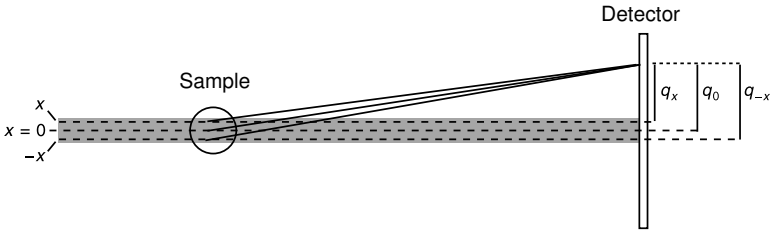

Figure 1.5: Contribution of X-rays scattered from multiple points along the beam width to the intensity detected at point q_0.

Again, q_0 represents the distance from the center of the beam. For rays scattered from a distance x from the beam center and reaching point q_0, the actual value of q, q_x, is given by

$$q_x = q_0 - x \tag{1.5}$$

A further complication is introduced when a two-dimensional detector is employed and the intensities are integrated along the direction perpendicular to the q-axis (and parallel to the beam length), as illustrated below:

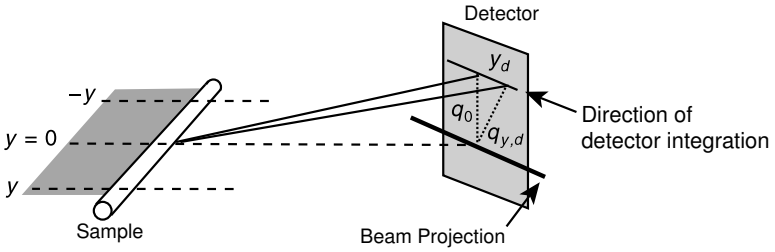

Figure 1.6: Contributions of different scattering angles due to integration along the width of the the detector.

As in the previous diagram, q_0 represents the distance from the center of the beam, directly aligned with the center of the sample, to a point of measurement directly above. Integrating along

[1] The long dimension of the beam will be referred to its length, and displacements along this direction will be expressed using the variable y. The short dimension, or thickness, of the beam will be referred to as the beam width, and distances along this direction will be expressed as x.

the distance y_d increases the signal intensity, but the integrated intensity will include X-rays that were scattered from the sample at angles larger than q_0. Furthermore, each point along the direction of integration will detect X-rays scattered from each point in the sample. For this reason, it is not possible to radially integrate the profile generated by a line-collimated beam. The distance over which the detector is integrated is often identified as a "detector-slit width", referring to earlier arrangements with one-dimensional detectors with significant width in the direction perpendicular to the q-axis.

For the a ray scattered from position $y = 0$ in the sample and detected at a distance q_0 from the beam projection and distance y_d from the center, the actual scattering angle will correspond to:

$$q_{y,d} = \sqrt{q_0^2 + y_d^2} \tag{1.6}$$

This has the same form as the expression for rays scattered from different points along the beam profile length and detected at a single point (Eqn. 1.4), and the two effects can be treated together, as discussed below.

1.2 The Mathematical Description of Smearing

The usual goal in a SAXS measurement is to determine the scattering profile, *i.e.* the scattering intensity, I, as a function of q as it would be measured from a single particle without any smearing effects. For the reasons outlined above, however, the actual intensities measured, especially with a line-collimated beam, will include contributions from multiple scattering angles. We will refer to the idealized scattering profile as $I(q)$ and the actual smeared profile as $I_s(q)$. If the geometry of the various smearing effects is well defined, it is relatively straight forward to predict $I_s(q)$ from $I(q)$, if the latter is already known. In practice, of course, it is usually the reverse transformation that is required, but understanding how to "smear" $I(q)$ provides the basis for "desmearing" $I_s(q)$. In addition, the smearing calculation can be useful for comparing calculated scattering profiles with experimental data.

1.2.1 The beam length

The largest smearing effect is usually due to the contributions of scattering from different points along the beam length, as illustrated in Fig. 1.4, and so we will consider it first, assuming a one-dimensional detector. For a given detector position, q, the measured intensity, $I_s(q)$, will be the sum of intensities from all the illuminated positions in the sample, along the beam length. If the beam has a length l centered at position $y = 0$ (as shown in Fig. 1.4) and has a uniform intensity along its length, then the measured intensity is given by the integral:

$$I_s(q) = \int_{-l/2}^{l/2} I\big(q(y)\big)dy \tag{1.7}$$

where $q(y)$ represents the scattering angle for a ray scattered from position y along the beam length and detected at position q of the detector, and given by Eqn. 1.4. The integral can then be written as:

$$I_s(q) = \int_{-l/2}^{l/2} I\left(\sqrt{q_0^2 + y^2}\right) dy \tag{1.8}$$

In general, however, the beam-length profile will not have a uniform intensity, but rather will fall of gradually at each end. Eqn. 1.8 can be generalized by introducing a weighting function, $w(y)$, and writing the integral as:

$$I_s(q) = \int_{-\infty}^{\infty} w(y) I\left(\sqrt{q_0^2 + y^2}\right) dy \tag{1.9}$$

The weighting function should be normalized so that:

$$\int_{-\infty}^{\infty} w(y) = 1 \tag{1.10}$$

The beam-length profile is often assumed to be symmetrical about $y = 0$, so that Eqn. 1.9 can be written as:

$$I_s(q) = 2\int_{0}^{\infty} w(y) I\left(\sqrt{q_0^2 + y^2}\right) dy \tag{1.11}$$

For the case of a uniform beam of length l, the weighting function is given by:

$$w(y) = \begin{cases} 0 & : \quad y < -l/2 \\ 1/l & : \quad -l/2 \le y \le l/2 \\ 0 & : \quad y > l/2 \end{cases} \tag{1.12}$$

As a better approximation, the beam profile is often represented as a trapezoid:

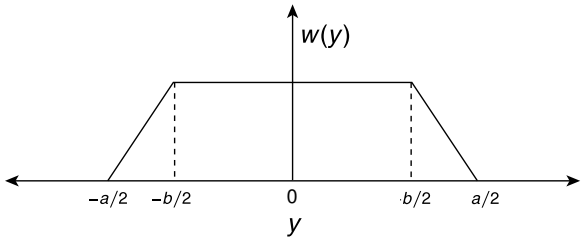

Figure 1.7: trapezoidal weighting function for the beam length profile.

The shape of the profile is defined by two parameters, a and b, which represent the lengths of the long and short parallel sides of the trapezoid. [2] The normalized weighting function is given by:

$$w(y) = \begin{cases} 0 & : \quad y < -a/2 \\ w_0(a + 2y)/(a - b) & : \quad -a/2 \le y < -b/2 \\ w_0 & : \quad -b/2 \le y \le b/2 \\ w_0(a - 2y)/(a - b) & : \quad b/2 < y \le a/2 \\ 0 & : \quad y > a/2 \end{cases} \tag{1.13}$$

where w_0 is the value of $w(y)$ for the central region and is given by:

$$w_0 = 2/(a + b) \tag{1.14}$$

[2] Different authors and computer programs use different parameters to define a trapezoidal beam profile, leading to much confusion. The ones given above have at least the virtue of simplicity for the user and are used in the USToo programs. The relationships to other definitions are given on page 33.

A curvier, if not necessarily sexier, function that can be used to represent the beam length profile is:

$$w(y) = \frac{1+a}{a+e^{|y|/b}} \tag{1.15}$$

where a and b are parameters defining the curve. As given above, the function is not normalized, but it can be normalized by multiplying be the factor w_0:

$$w_0 = \frac{a}{2(1+a)\ln(1+ab)} \tag{1.16}$$

A fit of this function to a measured beam profile is shown below, along with a trapezoidal approximation:

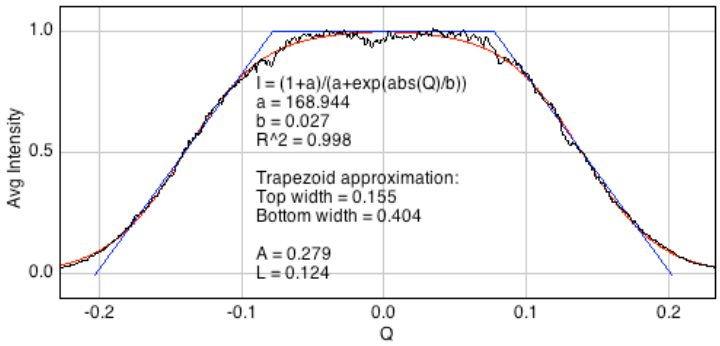

Figure 1.8: A fit of the sigmoidal function to a measured beam-length profile. From ImageJ with the saxsImage plugin.

This profile function is offered as an option, along with the trapezoidal function, in the Utah SAXS Tools, where it is referred to as a sigmoidal beam profile.

1.2.2 The beam width

Here, we initially consider only the effect of a finite beam width, as illustrated in Fig. 1.5, with y set to 0. If q is the distance from the detector point to the center of the beam width, then points above and below the center point will also contribute to the measured intensity, with scattering angles that depend on the position, x, relative to the beam center. The total observed intensity at the nominal q-value will be the integral of the intensity from the points across the beam width:

$$I_s(q) = \int_{-\infty}^{\infty} v(x) I\left(q - X\right) dx \tag{1.17}$$

where $v(x)$ is a weighting function, analogous to $w(y)$ for the y-direction, that describes the intensity profile of the beam across it width. The beam-width profile can be approximated as a trapezoid or as a Gaussian function, as show below for a measured profile:

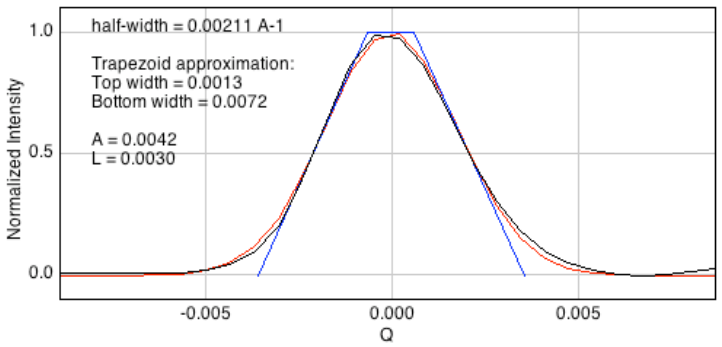

Figure 1.9: A fit of trapezoidal and Gaussian functions to a measured beam-width profile. From ImageJ with the saxsess plugin.

Notice that the width is much smaller than the length, which leads to a much smaller smearing effect. In many cases, the beam-width effect can be ignored.

For a sample point (x, y) that is displaced from the beam center along both axes, the scattering angle will correspond to:

$$q(x, y) = \sqrt{(q - x)^2 + y^2} \tag{1.18}$$

If both smearing effects are significant, the intensity measured at detector position q is calculated as a double integral over both dimensions:

$$I_s(q) = 2 \int_{x=-\infty}^{x=\infty} \int_{y=0}^{x=\infty} v(x)w(y)I\sqrt{(q - x)^2 + y^2}dxdy \tag{1.19}$$

1.2.3 Integration of a two-dimensional detector

As illustrated in Fig. 1.6, an additional smearing effect arises when a two-dimensional detector is used, and a finite width of the detector integrated (referred to as the detector slit-width). This effect can be viewed as an expansion of the effect due to scattering from different points along the beam length (the y-dimension as defined above). The drawing below represents a view onto the beam, with y-dimension of the beam and the second dimension of the detector (labeled y_d) both in the plane of the page.

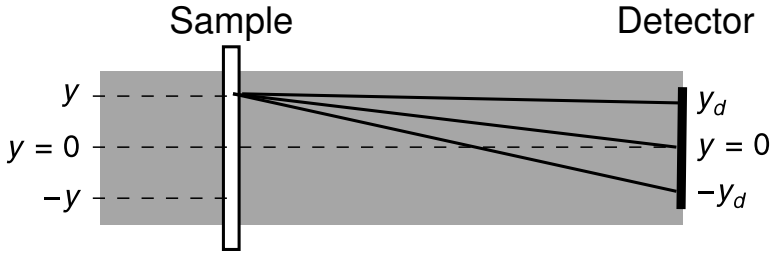

Figure 1.10: Effect of detector position, y_d, on effective scattering angle.

Rays scattered from a given point, y, in the sample will contribute to the detected intensity at positions across the y_d-axis of the detector. For the purposes of calculating the scattering angle,

we can think of an "effective y-position" for a given actual y-position and a detector position, y_d, given by:

$$y_{eff} = y - y_d \tag{1.20}$$

One consequence is that the effective range of smeared y-values is increased by the detector width. To account for this additional effect we must, in essence, integrate over y_d as well as y (and x if the beam width is significant).

A mathematically convenient way of incorporating the additional integration is by modifying the beam-length profile, $w(y)$. For a given value of y, the new weighting function, $W(y)$, is given by:

$$W(y) = \int_{-l_d/2}^{l_d/2} w(y - y_d) dy_d \tag{1.21}$$

where l/d is the length of the segment integrated on the detector. This equation represents a convolution of $w(y)$ by a square pulse function (representing the detector segment length) and has the net effect of broadening $w(x)$.

The figure below shows the effect of the detector correction combined with a rectangular beam-length profile, which results in a trapezoidal weighting function, $W(y)$:

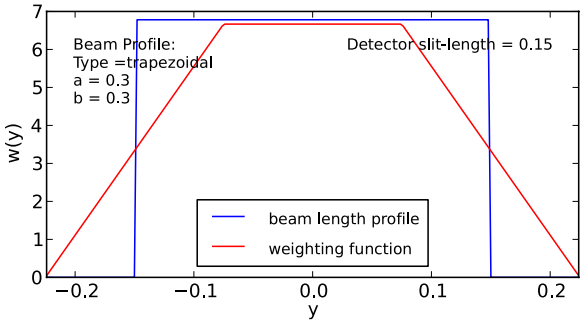

Figure 1.11: Weighting function for a rectangular beam profile, with (red) and without (blue) additional smearing due to integration over a detector slit width of 0.15 Å^{-1}

If the beam profile has a trapezoidal shape, the additional smearing from the detector slit-width will yield a sigmoidal shape:

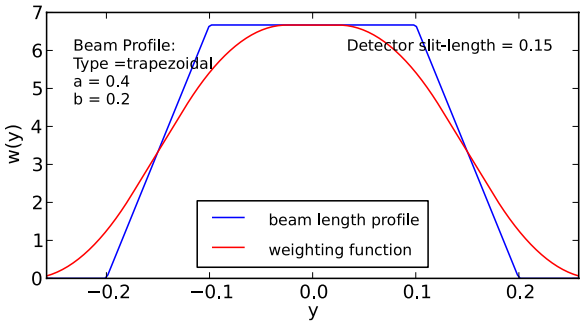

Figure 1.12: Weighting function for a trapezoidal beam profile, with (red) and without (blue) additional smearing due to integration over a detector slit width of 0.15 Å^{-1}

Finally, the effect on a sigmoidal beam profile is shown below:

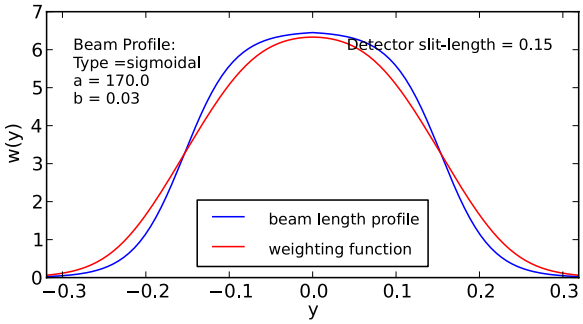

Figure 1.13: Weighting function for a sigmoidal beam profile, with (red) and without (blue) additional smearing due to integration over a detector slit width of 0.15 $\mathrm{\AA}^{-1}$

1.2.4 Some simulated examples

The program saxsSmear, described on page 62, numerically simulates the various smearing effects described above, applying them to either simulated or experimental SAXS curves. Here, a few examples are shown using a simulated profile for hen lysozyme (entry 6LYZ in the Protein Data Bank). The unsmeared simulated profile was generated using the program CRYSOL. The parameters for the beam profile and detector slit width are typical of those pertaining to the Anton-Parr SAXsess instrument with block collimation and a two-dimensional phosphor image plate detector.

The figure below compares the predicted unsmeared profile for lysozyme with those smeared by the beam length, with the beam profile described as a simple rectangle, as a trapezoid and using the sigmoidal function introduced earlier.

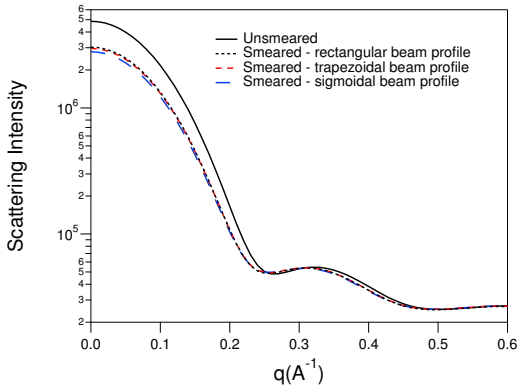

Figure 1.14: Simulated smearing of the SAXS profile predicted for hen lysozyme, with only the contribution of the beam length profile included.

There are a few things to note in these profiles:

1. For a given q, the smearing effect averages the contributions from larger q-values.
2. When the scattering intensity decreases with q, as it generally does, the effect is to reduce the scattering intensity at an apparent q-value from what it would be in the unsmeared profile.

3. The smearing effect is largest in the regions with the largest change in intensity with q. This is generally the lower-q regions of the profile. At larger q-values, the effect becomes very small.

4. The shape of the beam-length profile has only a small effect on the smeared profile.

The next figure compares the predicted smeared profiles with and without a beam-width smearing effect included.

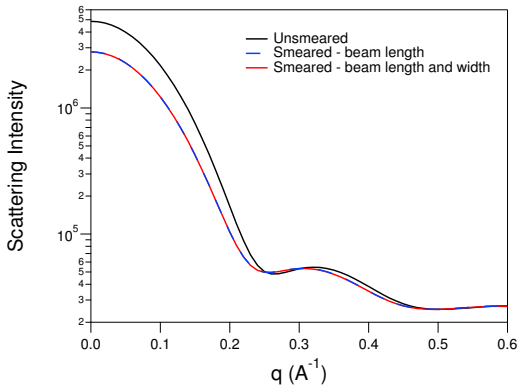

Figure 1.15: Simulated smearing of the SAXS profile predicted for hen lysozyme, with and without the beam-width smearing contribution.

In this simulation, the beam-width profile was represented as a Gaussian function with half-width of $0.003\,\text{Å}^{-1}$, about 1% of the beam length. In this case, the beam-width makes no significant contribution to the total smearing effect.

The detector-slit contribution is also generally much smaller than that from the beam length, as shown below:

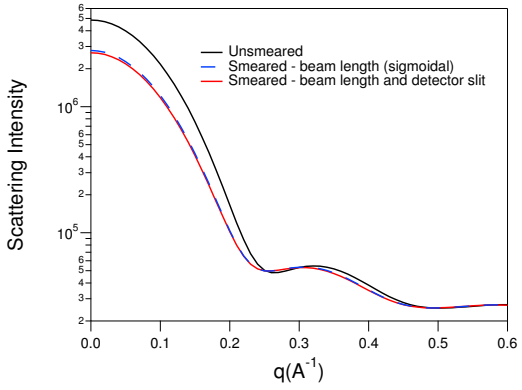

Figure 1.16: Simulated smearing of the SAXS profile predicted for hen lysozyme, with and without the contribution from a detector slit width of $0.15\,\text{Å}^{-1}$.

The major lesson from these examples is that the major smearing factor is the beam length. The shape of the profile, the beam width and the slit detector width all make very small contributions. It should be noted, however, that the actual contributions will depend on the shape of the unsmeared

profile and the specific beam and detector geometries. Though it may be safe in many cases to ignore all but the beam length, the saxsSmear program can be helpful in testing this assumption. For computations involving smearing (or desmearing), addition of the beam width contribution does significantly increase the computational time, because it introduces a second dimension of integration. However, incorporating a more realistic beam-length profile or the detector slit width does not significantly increase the computational cost.

1.3 Desmearing: The Lake method

As noted earlier, the usual goal is to correct the experimental scattering profile, $I_s(q)$, to obtain the profile that would be obtained from an ideal camera with a point-collimated beam $I(q)$. Unfortunately, extracting information that has been smeared out is considerably more difficult than predicting the smearing effect on an ideal profile. A variety of computational techniques have been devised, some of which are coupled to the related problem of calculating a distance distribution function ($p(r)$) for the particles from the scattering profile. The techniques vary in their mathematical sophistication and the assumptions that are made regarding the form of $I(q)$ and the shape of the beam profiles. A general problem with all of these techniques is that they tend to be quite sensitive to the noise present in the experimental profile, and they usually increase the noise.

One of the simplest and most widely used methods is an iterative numerical technique introduced by Lake in 1967. Lake's treatment begins by considering a general iterative technique in which successive trial approximations to the ideal curve are generated, subjected to the smearing function and then compared with the experimental profile. In the following, $I_i(q)$ is the i^{th} approximation to $I(q)$, and $I_{i,s}(q)$ is the smeared form of $I_i(q)$.

As illustrated by the example in Fig. 1.14, the major effect of smearing us usually to decrease the scattering intensity, so that $I_s(q) \leq I(q)$ for most values of q. (Exceptions arise where there are sharp dips and rises with q.) Similarly, an approximation to the unsmeared profile, $I_i(q)$, will generally be larger than $I_{i,s}(q)$. If $I_i(q)$ is, in fact, a reasonable approximation to $I(q)$, then the decrease in intensity due to smearing should be about the same for for $I(q)$ and $I_i(q)$, so that we can write:

$$\begin{aligned} I(q) - I_s(q) &\approx I_i(q) - I_{i,s}(q) \\ I(q) &\approx I_s(q) + \big(I_i(q) - I_{i,s}(q)\big) \end{aligned} \tag{1.22}$$

This then provides a simple algorithm for calculating successive approximations:

$$I_{i+1}(q) = I_i(q) + I_s(q) - I_{i,s}(q) \tag{1.23}$$

As $I_i(q)$ gets closer to $I(q)$, $I_{i,s}(q)$ also gets closer to $I_s(q)$, so that the difference between $I_{i+1}(q)$ and $I_i(q)$ gets smaller. The iteration can be carried out until this difference reaches an chosen value. An important feature of this approach is that the only required mathematical transformations are the smearing functions described in the previous section. Provided that the beam profiles and the detector slit-width are known, the smearing calculation can be applied using numerical integration.

For the initial approximation, $I_1(q)$, the smeared experimental curve, $I_s(q)$ is used, so that the first iteration is given by:

$$I_1(q) = 2I_s(q) - I_{s,s}(q) \tag{1.24}$$

where $I_{s,s}(q)$ is the computationally-smeared form of the smeared experimental profile. [3] The figure below shows two iterations in a simulated desmearing of the calculated SAXS profile for hen lysozyme.

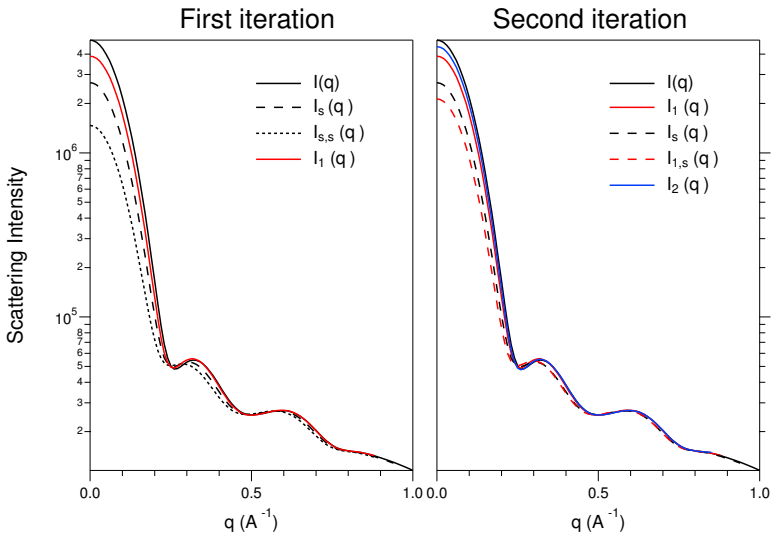

Figure 1.17: Simulated desmearing by the Lake method. In each panel, the solid black curve represents the unsmeared profile, and the black dashed curve is the predicted smeared profile. The simulated smearing is based on a sigmoidal beam-length profile and a detector slit-width of $0.15\,\text{Å}^{-1}$, as in Fig. 1.13 The results of the first and second iterations are shown in red and blue, respectively.

Although Eqn. 1.23 and 1.24 can serve as the basis for a desmearing algorithm, the convergence can be quite slow. An alternative function, also introduced by Lake, is:

$$I_{i+1}(q) = I_i(q)\frac{I_s(q)}{I_{i,s}(q)} \tag{1.25}$$

As for Eqn. 1.23, this equation results in a progressive increase in the iterated approximation to $I(q)$, but the rate of increase is greatest at the lower values of q, where the ratio $I_s(q)/I_{i,s}(q)$ is largest. As $I_i(q)$ approaches $I(q)$, $I_{i,s}(q)/I_s(q)$ approaches 1, so that the process is expected to converge. As before, the first iteration treats the experimental data as the first trial profile, so that:

$$I_1(q) = I_s(q)^2/I_{s,s}(q) \tag{1.26}$$

The improved convergence of this version is illustrated below, again using the predicted lysozyme SAXS profile:

[3] As an aside, this first iteration of the Lake method is very similar to the technique of "unsharp masking" that is widely used for enhancing the apparent sharpness of digital images. In the standard unsharp masking algorithm, the original image is deliberately blurred by averaging adjacent pixels, and the blurred image is subtracted from the original image multiplied by 2. The net effect is to enhance the contrast at edges in the image, much as the desmearing algorithm restores the steepness of the scattering profile.

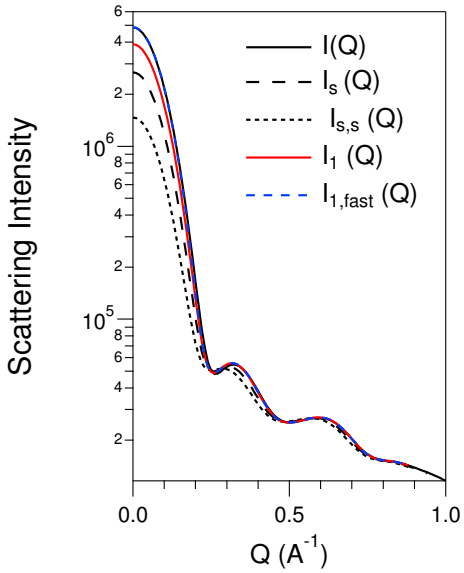

Figure 1.18: Simulated desmearing by the Lake method, comparing the results of the first iteration using Eqns. 1.24 (red) and 1.26 (blue).

In this case (which is, admittedly, artificially favorable because there is no noise in the starting profile), the first iteration yields a desmeared profile that matches $I(q)$ almost perfectly.

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Chapter 2

Absolute Scattering Intensities and Determination of Molecular Weights

For many purposes, it is sufficient to measure and report scattering intensities in arbitrary units, as much of the information from SAXS is derived from the shape of the scattering profile. However, the absolute intensities can be used to determine the molecular weight of a scattering particle, or alternatively the concentration of particles if the molecular weight is known, and can also be of value in comparing experimental and theoretical profiles.

The units of scattering intensity and their interpretation can be quite confusing. The expression "absolute intensity" is used in at least two different ways, but of which are a bit misleading. Another possible source of confusion is that the scattering community generally uses the older cgs system of units, rather than SI units, a convention that is followed here.

2.1 The microscopic differential scattering cross section

First, we consider the scattering from a single atom or a single molecule made up of multiple atoms. In general, the intensity of radiation is expressed as a flux, for instance as particles per second or units of energy (J) per second. If the radiation is viewed as a wave, the intensity is proportional to the square of the wave amplitude. For the irradiating source, which can be visualized as a bundle of parallel rays or a beam of particles all moving in the same direction, it is convenient to express the flux per unit of area:

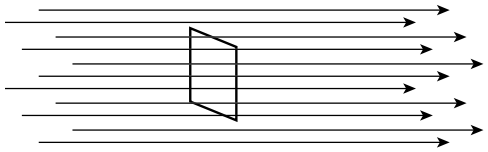

Figure 2.1: Flux of radiation from a source, expressed per unit of cross-sectional area (cm^{-2}).

However, the radiation scattered from a single atom moves in all directions (with the relative intensities determined by polarization effects), and the flux per unit area decreases as the distance from the sample increases:

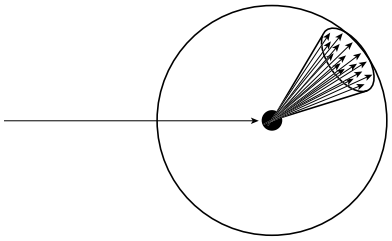

Figure 2.2: Flux of radiation scattered from a point, expressed per unit solid angle (sr−1).

For this reason, it is convenient to express the scattered intensity as a flux per unit of solid

angle,Ω. The SI unit of solid angle is a steradian (abbreviated sr) and is defined such that the total solid angle of a sphere is 4π sr, much like the total angle of a circle is 2π radians. Like the radian, the steradian is independent of the units of length (or any other standard measurement), and measurements of solid angle can be considered dimensionless.

Strictly speaking, the absolute scattering intensity has units of flux per sr. However, because the scattered intensity obviously depends on the intensity of the irradiating beam, by a simple proportionality, it is conventional to normalize the scattered intensity in a given direction, $J(q)$ by the irradiating intensity, J_0:

$$\frac{J(q)}{J_0} \tag{2.1}$$

Since $J(q)$ is expressed per sr, and J_0 is expressed per unit area, this ratio has units of area per sr, or simply area if the solid angle is considered dimensionless. This ratio is also referred to as the differential scattering cross section:

$$\frac{J(q)}{J_0} = \frac{d\sigma}{d\Omega} \tag{2.2}$$

where $d\sigma$ represents a small unit of area and $d\Omega$ is a small unit of solid angle. The ratio $J(q)/J_0$ also represents one of the definitions of absolute intensity. Here, it is assumed that the ratio represents the scattering from a single or molecule and is referred to as a *microscopic* differential scattering cross section.

The total scattering cross section, σ, is obtained by integrating the differential scattering cross section over all directions originating from the scattering atom:

$$\sigma = \int \frac{d\sigma}{d\Omega} d\Omega \tag{2.3}$$

Dividing the scattering cross section by the area of the irradiating beam gives the probability that a given photon will be scattered, in some direction, from the sample. This, then, provides the physical interpretation of σ as an area: The larger the cross section of the target, the larger the probability of scattering. Scattering cross sections are typically very small, on the order of 10^{-30} to $10^{-20}\,\mathrm{cm}^2$, and the quantity $10^{-20}\,\mathrm{cm}^2$ is given the special unit of a "barn" (b), as in ". . . can't hit the side of a barn."

For X-rays scattered by a single atom, the scattering intensity depends on the number of electrons (the atomic number), the angle of scattering and the polarization of the irradiating beam. For the special case of $q = 0$, however, the amplitude of the scattered wave is:

$$A(0) = Zb_e \tag{2.4}$$

where Z is the atomic number and b_e is defined as the scattering length of the electron, and for $q = 0$ is equal to the classical radius of the electron defined as:

$$r_e = \frac{e^2}{mc^2} \tag{2.5}$$

where e and m are the electric charge and mass, respectively, of the electron and c is the speed of light. r_e has the numerical value of $2.81794\times10^{-13}\,\mathrm{cm}$. The differential scattering cross section from a single atom is the square of the amplitude, given by:

$$\frac{d\sigma(0)}{d\Omega}_{atom} = Z^2 b_e^2 \tag{2.6}$$

For a molecule, the X-ray scattering at a given angle depends on the interference of waves scattered from the individual atoms, in addition to the factors mentioned above, but at $q = 0$, the scattering amplitude is the simple sum of the amplitudes from the individual atoms, and the intensity is the square of this sum:

$$\frac{d\sigma(0)}{d\Omega}_m = \left(\sum_{i=1}^{N} Z_i b_e \right)^2 \tag{2.7}$$

where N is the total number of atoms in the molecule and Z_i is the atomic number of atom i.

For neutron scattering, the scattering length of each atom depends on the properties of the individual nuclear types, but the total scattering is determined by an analogous sum of the amplitudes.

2.2 The macroscopic differential scattering cross section

The scattering from a macroscopic sample reflects both the total scattering intensity from all of the molecules and possible interference effects from waves scattered from different molecules. In the limit of high dilution, the effects of interparticle interference can be ignored and the total intensity is the sum of the scattering from the individual molecules. Ignoring, for the moment, the effects of solvent displaced by the molecules, the total scattering can be written as:

$$\frac{J(q)}{J_0} = N_m \frac{d\sigma}{d\Omega}_m \tag{2.8}$$

where N_m is the number of molecules in the irradiated volume. This volume depends on both the cross-sectional area of the beam (or that part of the beam that passes through the sample if the beam cross section is smaller than the sample) and the length of the sample. The cross section of the beam is already accounted for in the definition of the scattering cross section (and leads to the units of area), but the sample length is not. Since the irradiated volume can vary from one instrument to another, even if the sample concentrations are accounted for, it is convenient to express scattering intensities per unit volume:

$$\frac{J(q)}{J_0}\frac{1}{V_s} = \frac{N_m}{V_s}\frac{d\sigma}{d\Omega}_m \tag{2.9}$$

where V_s is the sample volume. This quantity is defined as the macroscopic differential scattering cross section:

$$\frac{d\Sigma}{d\Omega} = \frac{N_m}{V_s}\frac{d\sigma}{d\Omega}_m \tag{2.10}$$

The ratio (N_m/V_s) is the number density of molecules in the sample. Importantly, $\frac{d\Sigma}{d\Omega}$ is a property of the sample (reflecting the concentration of scattering molecules) and should be independent of experimental details such as sample volume, beam intensity, detector efficiency, *etc.*. The units of this quantity are cm^{-1}, and it can be thought of as representing the dimensionless scattering from a sample 1 cm long, after correcting for the flux per unit area of the irradiating beam. The macroscopic differential scattering cross section is the more widely used definition of "absolute scattering intensity", and this definition will be used here.

2.3 Contrast for a solution sample

When SAXS profiles for solution samples are measured, the usual practice is to subtract the signal from a carefully matched reference sample containing everything but the macromolecules of interest. The resulting difference profile represents the differences between the amplitudes of the waves scattered from the macromolecule and those scattered from the solvent molecules displaced by the macromolecule. Importantly, it is the difference in wave amplitudes, rather than intensities, that must be accounted for.

The total amplitude from an isolated molecule, at $q = 0$, is the sum of the atomic scattering lengths:

$$A(0)_m = \sum_{i=1}^{N} Z_i b_e \tag{2.11}$$

where Z_i is the atomic number of the i^{th} atom.

For the solvent, it is convenient to introduce a scattering density, ρ_{solv}, which is the sum of the electron scattering lengths, b_e, per unit volume. The total scattering length is then the product of the density and the volume displaced by the macromolecule. The volume is given by the product of the molecular mass, M_m, and the partial specific volume, $\bar{v}$, which is the amount the solution volume increases with the addition of solute molecules, per gram of solute. For one molecule, the volume is:

$$\begin{aligned} V_m &= M_m \bar{v} \\ &= (M/N_A)\bar{v} \end{aligned} \tag{2.12}$$

where M is the molar mass, and N_A is Avogadro's number. The scattering amplitude (at $q = 0$) from the displaced solvent is then:

$$A(0)_{solv} = (M/N_A)\bar{v}\rho_{solv} \tag{2.13}$$

The scattering amplitude at $q = 0$ for the macromolecule can be similarly expressed in terms of a scattering density:

$$A(0)_{solv} = (M/N_A)\bar{v}\rho_m \tag{2.14}$$

where ρ_m is given by

$$\rho_m = \frac{N_A \sum_{i=1}^{N} Z_i b_e}{M\bar{v}} \tag{2.15}$$

The scattering amplitude difference is then given by:

$$\begin{aligned} \Delta A(0) &= (M/N_A)\bar{v}\rho_m - (M/N_A)\bar{v}\rho_{solv} \\ &= (M/N_A)\bar{v}(\rho_m - \rho_{solv}) \end{aligned} \tag{2.16}$$

The net differential scattering cross section at $q = 0$ is given by the square of the amplitude difference:

$$\begin{aligned} \frac{d\sigma(0)}{d\Omega}_{net} &= \Delta A(0)^2 \\ &= ((M/N_A)\bar{v}(\rho_m - \rho_{solv}))^2 \end{aligned} \tag{2.17}$$

The macroscopic differential scattering cross section (absolute scattering intensity) is:

$$\frac{d\Sigma(0)}{d\Omega} = \frac{N_m}{V_s}\frac{d\sigma(0)}{d\Omega}_m \tag{2.18}$$

The number density of molecules (N_m/V_s) can be expressed in terms of the mass concentration, c in g/cm^3, according to:

$$N_m/V_s = cN_A/M, \tag{2.19}$$

so that the absolute scattering intensity is given by:

$$\begin{aligned}\frac{d\Sigma(0)}{d\Omega} &= (cN_A/M)\frac{d\sigma(0)}{d\Omega} \\ &= (cN_A/M)\left((M/N_A)\bar{v}(\rho_m - \rho_{solv})\right)^2 \\ &= (cM/N_A)\bar{v}^2(\rho_m - \rho_{solv})^2\end{aligned} \tag{2.20}$$

2.4 Calculation of molecular weight or concentration

Eqn. 2.20 can be easily rearranged to an expression for calculating the molar mass from the extrapolated scattering intensity at $q = 0$:

$$M = \frac{d\Sigma(0)}{d\Omega}\frac{N_A}{c\bar{v}^2(\rho_m - \rho_{solv})^2} \tag{2.21}$$

In order to use this equation, the following parameters must be known or estimated:

- The macromolecule concentration, c
- The partial specific volume of the macromolecule, $\bar{v}$
- The scattering density of the macromolecule, ρ_m
- The scattering density of the solvent, ρ_{solv}

The macromolecule concentration is typically determined by UV absorption, and the accuracy of this measurement depends critically on the accuracy of the extinction coefficient used. The partial specific volume can be measured experimentally, but approximations for different classes of molecules, such as proteins or nucleic acids, are often relied upon.

The scattering density of the macromolecule is given by:

$$\rho_m = \frac{N_A \sum_{i=1}^{N} Z_i b_e}{M\bar{v}} \tag{2.22}$$

However, the molar mass (M) is the quantity to be determined, and the total number of electrons ($\sum Z_i$) is similarly unknown. But, a good estimate of the ratio $\sum Z_i/M$ can be made if the general chemical composition is well defined. Among the major elements found in biological macromolecules, hydrogen has approximately one electron per unit molar mass, and this ratio is approximately 0.5 for carbon, nitrogen, oxygen, sulfur and phosphorous. From the average amino acid composition of proteins, the overall ratio of electrons per unit molar mass is 0.534. Thus, ρ_m for proteins can be estimated as:

$$\begin{aligned}\rho_m &\approx \frac{0.534 N_A b_e}{\bar{v}} \\ &\approx 1.22\times10^{11}\ \text{cm}^{-2}\end{aligned} \tag{2.23}$$

This simple approximation ignores the contributions of a hydration layer, which is generally believed to have a scattering density intermediate between that of a protein and bulk water.

The scattering density of the solvent can similarly be estimated from its chemical structure and density. For water, the number of electrons per molecule is 10, and the average molar mass is $M_{solv} \approx 18.015\,\mathrm{g/mol}$. If the mass density is $\rho_{solv,m} \approx 1\,\mathrm{g/cm^3}$, then the scattering density can be calculated as:

$$\begin{aligned}\rho_{solv} &= 10 b_e \rho_{solv,m} N_A / M_{solv} \\ &\approx 9.4{\times}10^{10}\,\mathrm{cm^{-2}}\end{aligned} \tag{2.24}$$

More precise values can be obtained by accounting for the temperature dependence of the density of water (See page 22).

If the molecular mass of the macromolecule is known with confidence, then the concentration can be calculated by rearranging Eqn. 2.20 to a slightly different form:

$$c = \frac{d\Sigma(0)}{d\Omega} \frac{N_A}{M \bar{v}^2 (\rho_m - \rho_{solv})^2} \tag{2.25}$$

The calculated value of the molar mass (or concentration) is very sensitive to the assumed parameter values, especially the partial specific volume of the particle and its scattering density. The plot below shows the apparent molar mass when different values of these parameters are used, assuming that the true molar mass is 10,000 g/mol; the true partial specific volume is $0.73\,\mathrm{cm^3/g}$ and the scattering density difference, $\rho_m - \rho_{solv}$ is $2.5{\times}10^{10}\,\mathrm{cm^{-2}}$

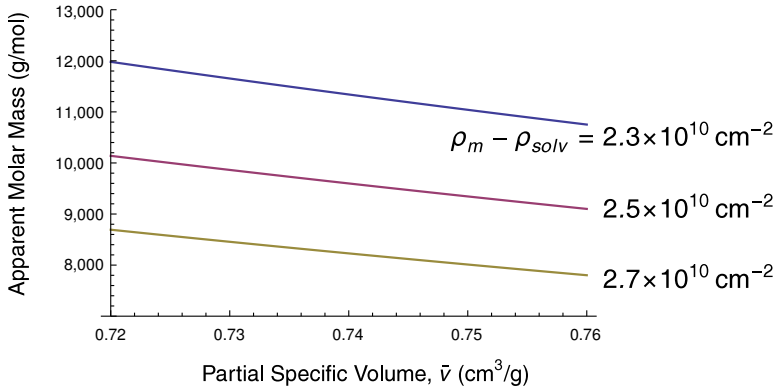

Figure 2.3: Simulation of the effects on the calculated molar mass due to assumed values for the partial specific volume of the protein and and scattering density difference.

The partial specific volume can be measured experimentally, but the measurement typically requires relatively large amounts of material. Another commonly used approach is to calculate $\bar{v}$ from the amino acid composition (as with the NucProt calculator developed by the laboratory of Mark Gerstein, `http://www.molmovdb.org/cgi-bin/psv.cgi` or the program SEDNTERP by John Philo, `http://www.jphilo.mailway.com/download.htm`). These calculations typically yield values of about $0.73\,\mathrm{cm^3/g}$. However, Mylonas and Svergun suggest that the value of $0.7425\,\mathrm{cm^3/g}$ may be more appropriate for SAXS measurements.

2.5 Calibration of "absolute" scattering intensities

As defined on page 16, the macroscopic scattering cross-section is related to the experimental parameters according to:

$$\frac{d\Sigma}{d\Omega} = \frac{J(q)}{J_0}\frac{1}{V_s} \tag{2.26}$$

where $J(q)$ is the scattering flux, J_0 is the incident beam flux, and V_s is the irradiated volume. Although all three of these parameters are, in principle, directly measurable, measurement of both the incident and scattering fluxes on the same absolute scale is often not practical. An alternative is to measure the scattered flux from a reference sample for which $\frac{d\Sigma}{d\Omega}$ is known. Different reference materials have been used for different types of scattering experiments, but liquid reference samples are particularly convenient for calibrating measurements with other liquids, since the cell dimensions can be implicitly accounted for. Pure liquids have the further advantage that their scattering at relatively low q-values arises only from thermal fluctuations. This intensity is nearly independent of q and can be calculated from macroscopic properties of the liquid, according to the relationship:

$$\frac{d\Sigma}{d\Omega} = \rho^2 kT\beta_T \tag{2.27}$$

where ρ and β_T are the scattering density and the isothermal compressibility of the liquid, respectively, k is the Boltzmann constant and T is the temperature. A significant drawback to the use of pure liquids as calibration references is that the scattering intensity is relatively weak, which has limited their use historically. However, with either a very bright source or a line-collimated instrument combined with a very sensitive detector, calibration with a pure liquid becomes quite practical. The use of a semi-transparent beam stop, as in the Anton Par SAXSess instrument, makes this approach particularly convenient, because the effects of sample absorption can be corrected for automatically, as discussed below.

Calibration of absolute scattering intensities also requires corrections for the scattering due to the capillary, as well as absorption of X-rays by the sample and references. Bulk samples give rise to significant absorption of X-rays, analogous to the absorption measured by a UV-visible spectrophotometer. The fraction of energy absorbed by the sample is not available for scattering, so that the actual incident intensity is less that of the beam. The fraction absorbed is usually much greater than the fraction of energy scattered, so that the latter can be ignored, and the absorption can be measured by comparing the beam intensity with the intensity of X-rays that pass directly through the sample. The transmission, T_s, is the fraction of X-ray intensity that is not absorbed by the sample. (For simplicity and consistency with other sources, T is used here to represent both transmission and temperature, but never in the same equation!)

With the transmission accounted for, the actual scattering intensity, relative to the beam intensity, becomes:

$$\frac{J}{J_0} = T_s V_s \frac{d\Sigma}{d\Omega} \tag{2.28}$$

where V_s is the sample volume. In the SAXSess instrument, the X-ray beam stop is semi-transparent and allows a greatly attenuated profile of the beam to reach the detector. If the scattering profiles are normalized with respect to the attenuated beam intensity, the resulting intensities are proportional to $J/(T_s J_0)$, and we can write:

$$I' = CF\frac{d\Sigma}{d\Omega} \tag{2.29}$$

where I' is the normalized intensity, and CF is introduced as a calibration factor that accounts for the sample volume, incident beam intensity and the attenuation factor for the beam stop, all of which are instrumental parameters. The transmission, T_s, is not included in the calibration factor, but is accounted for in the normalized intensity. To determine the calibration factor, scattering data are recorded for both an empty cell and the cell filled with water. The normalized intensity from the cell is given by:

$$I'_c = CF\frac{d\Sigma}{d\Omega}_c \tag{2.30}$$

where the subscript c indicates the empty cell. Similarly, the normalized intensity from the water-filled cell is:

$$I'_{c+w} = CF\left(\frac{d\Sigma}{d\Omega}_c + \frac{d\Sigma}{d\Omega}_w\right) \tag{2.31}$$

The difference between the two normalized intensities is:

$$I'_{c+w} - I'_c = CF\left(\frac{d\Sigma}{d\Omega}_c + \frac{d\Sigma}{d\Omega}_w\right) - CF\frac{d\Sigma}{d\Omega}_c \tag{2.32}$$

$$= CF\frac{d\Sigma}{d\Omega}_w \tag{2.33}$$

and the calibration factor is calculated as:

$$CF = \left(I'_{c+w} - I'_c\right) \div \frac{d\Sigma}{d\Omega}_w \tag{2.34}$$

Since the intensities are normalized, and dimensionless, the calibration factor has units of cm.

Once determined, the calibration factor can be applied to the normalized scattering intensities for other samples, provided that the instrument parameters, including the irradiated sample volume and beam geometry, are unchanged. Typically, scattering is measured for a sample containing the macromolecules of interest and a reference sample containing the same buffer. After normalization, the intensity from the sample is given by:

$$I'_{m+b} = CF\left(\frac{d\Sigma}{d\Omega}_m + \frac{d\Sigma}{d\Omega}_b\right) \tag{2.35}$$

where m and b in the subscripts represent the macromolecule and buffer contributions, respectively. The scattering from the buffer sample is:

$$I'_b = CF\frac{d\Sigma}{d\Omega}_b \tag{2.36}$$

and the difference is:

$$I'_{m+b} - I'_b = CF\frac{d\Sigma}{d\Omega}_m \tag{2.37}$$

The absolute scattering intensity is then calculated as:

$$\frac{d\Sigma}{d\Omega}_m = \left(I'_{m+b} - I'_b\right) \div CF \tag{2.38}$$

2.5.1 Absolute scattering of water

Though other liquids have been used as SAXS calibration standards, water offers the obvious advantage of ready availability, and its physical properties have been well characterized over a range of temperatures. The two parameters that are necessary to calculate the macroscopic differential scattering cross section are the scattering density ρ and the compressibility β_T. The scattering density can be calculated from the mass density, ρ_m, according to Eqn. 2.24. If the compressibility is specified in units of Pa^{-1} and k in units of J/K, the absolute scattering intensity of water, in units of cm-1, is calculated as:

$$\frac{d\Sigma}{d\Omega} = \rho^2 kT\beta_T \tag{2.39}$$

$$= 10^{-6}\left(\frac{\rho_m N_a b_e}{M}\right)^2 kT\beta_T \tag{2.40}$$

where M is the molar mass of water and b_e is the electron scattering length. The factor of 10^{-1} arises because of the conversion from SI units to cm.

Measurements and analysis by Kell (1975) provide the following expression for calculating the mass density and compressibility of pure water. The density (in units of $\mathrm{g/cm}^3$) as a function of temperature is given by:

$$\rho_m(T) = 1{\times}10^{-3}\frac{a_0 + a_1T + a_2T^2 + a_3T^3 + a_4T^4 + a_5T^5}{1 + a_6T} \tag{2.41}$$

where the constants are: $a_0 = 999.83952$, $a_1 = 16.945176$, $a_2 = \text{-}7.9870401{\times}10^{-3}$, $a_3 = \text{-}46.170461{\times}10^{-6}$, $a_4 = 105.56302{\times}10^{-9}$, $a_5 = \text{-}280.54253{\times}10^{-12}$ and $a_6 = 16.878950{\times}10^{-3}$.

Similarly, the compressibility (in units of Pa^{-1}) is given by:

$$\beta_T(T) = 1{\times}10^{-11}\frac{a_0 + a_1T + a_2T^2 + a_3T^3 + a_4T^4 + a_5T^5}{1 + a_6T} \tag{2.42}$$

where the constants are: $a_0 = 50.88496$, $a_1 = 0.6163813$, $a_2 = 1.459187{\times}10^{-3}$, $a_3 = 20.08438{\times}10^{-6}$, $a_4 = \text{-}58.47727{\times}10^{-9}$, $a_5 = 410.4110{\times}10^{-12}$ and $a_6 = 19.67348{\times}10^{-3}$. These empirical functions are incorporated in the USToo programs for calculating absolute scattering intensities from a water reference.

2.6 References on calibration and molecular weight calculations

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Chapter 3

Interparticle Interference and Structure Factor Functions

The scattering profiles from solutions of macromolecules are determined by the the interference of waves scattered from different atoms, either within the same molecule or within different molecules. At sufficiently high dilutions, the interference of waves scattered from different particles can be ignored. But, as the concentration of molecules increases, the interference of waves from different particles becomes more significant, and this effect is generally referred to as interparticle interference. It is important to note that this term refers to the interference of the waves, not interference between the particles themselves, but interactions between the particles will influence the resulting scattering profile.

In the limit of very dilute solution, the scattering profile represents the Fourier transform of the distribution of interatomic distances within the molecule. The distribution of interatomic distances, r, is usually identified as $p(r)$, and its Fourier transform into reciprocal space is called the form factor, $P(q)$. The distribution of intermolecular distances is also described by a probability function, $s(r)$, and the Fourier transform of this function, $S(q)$ is called the structure factor (which is a bit confusing, because one is often more interested in the structure of the molecules, which is represented by the form factor). When interparticle interference is significant, the scattering profile is influenced by both the intramolecular and intermolecular distances, and is the Fourier transform of the convolution of $p(r)$ and $s(r)$. By the convolution theorem, the scattering profile is proportional to the point-wise product of $P(q)$ and $S(q)$:

$$I(q) \propto P(q)S(q) \tag{3.1}$$

This simple relationship in reciprocal space makes it possible, if the system is sufficiently well defined, to separate the intra- and inter-molecular contributions to the scattering profile.

Unfortunately, the exact form of the structure factor, $S(q)$, depends on the details of any intermolecular interactions and typically is quite involved. Here, only the results are given for the simplest treatment, in which the individual molecules are treated as hard spheres. The equations below are based on the statistical-mechanical treatment of hard-sphere fluids by Percus and Yevick, and solution of this equation by Ashcroft and Leckner (1966). The model is defined in terms of two parameters, the radius of the spheres, r, and the volume fraction of solution occupied by the spheres, ϕ. For convenience, two other parameters are defined in terms of ϕ:

$$\begin{aligned}
\alpha &= \frac{(1+2\phi)^2}{(1-\phi)^4} \\
\beta &= -6\phi\frac{-(1+\phi/2)^2}{(1-\phi)^4} \\
\gamma &= (\phi/2)\frac{(1+2\phi)^2}{(1-\phi)^4}
\end{aligned} \tag{3.2}$$

The structure factor is:

$$S(q) = \frac{1}{1-C(q)} \tag{3.3}$$

where $C(q)$ is the Fourier transform of the inter-particle correlation given by:

$$C(q) = -\frac{24\phi}{(2qr)^6}\Big\{\alpha(2qr)^3\left[\sin(2qr) - 2qr\cos(2qr)\right] + \beta(2qr)^2\left[4qr\sin(2qr) - ((2qr)^2 - 2)\cos(2qr) - 2\right] + \gamma\left[(4(2qr)^3 - 48qr)\sin(2qr) - ((2qr)^4 - 12(2qr)^2 + 24)\cos(2qr) + 24\right]\Big\} \tag{3.4}$$

Notice that r and q always appear as the product qr, so that the structure factor functions for different values of r, but the same value of ϕ, have the same shape but are scaled on the q axis. The structure factors calculated for the hard-sphere models with $r = 20\,\text{Å}$ and different values of ϕ are plotted below:

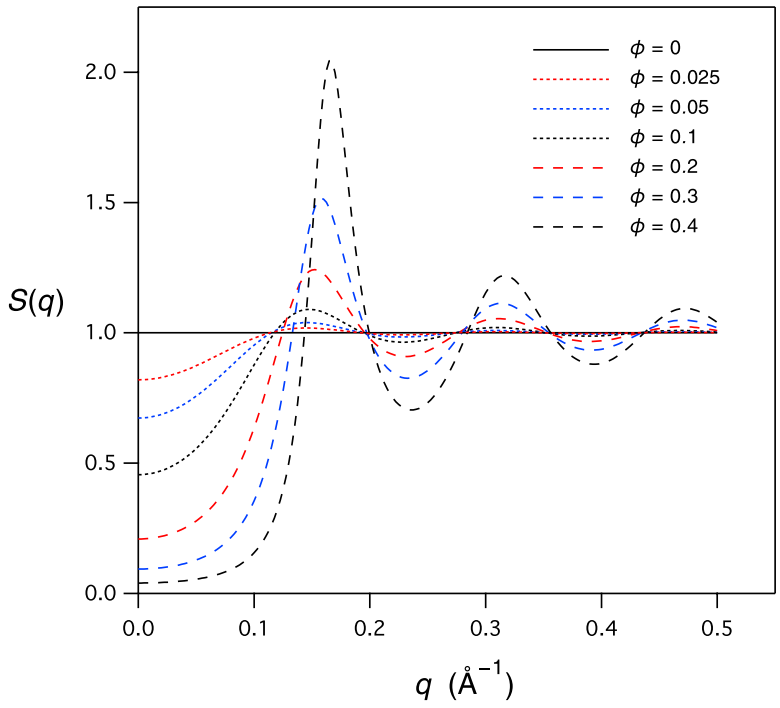

Figure 3.1: Calculated structure factors for the hard-sphere model, using a sphere radius of 20 Å and the indicated values of ϕ, the occupied volume fraction.

Notice that increasing particle concentrations lead to a depression of the scattering intensities at the smallest angles. This reflects the destructive interference between X-rays scattered from different molecules, which becomes more pronounced as the distribution of inter-particle distances becomes more restricted at higher concentrations. Also note the first peak, which becomes more prominent and shifts to larger q as the concentration increases, reflecting smaller intermolecular distances.

More sophisticated treatments of interparticle interference incorporate attractive or repulsive interactions, or both on different length scales. One such treatment that has been used recently in the study of proteins and colloids is the two-Yukawa model, which asumes short-range attraction and long-range repulsion among particles. Unfortunately, the mathematics for this model, and others that incorporate intermolecular interactions, is substantially more difficult than that for

the hard-sphere model, necessitating the use of numerical solutions or Monte Carlo simulations. The structure factor functions calculated for the two-Yukawa model is qualitatively similar to that for the hard-sphere model, with the notable addition of a peak at lower q-values reflecting the short-range attraction between molecules.

Because the form factor ($P(q)$) and the structure factor ($S(q)$) are combined by a simple multiplication to generate the observed intensities, $I(q)$, their contributions can, in principle, be separated from the experimental data. If either $S(q)$ or $P(q)$ is known (or hypothesized), the other can be calculated by dividing the experimental intensities by the known function. Alternatively, model functions with adjustable parameters can be multiplied and used to fit the experimental data directly. It should be noted, however, that the multiplication or division is only valid after any background subtraction or desmearing has been applied. The Utah SAXS Tools includes facilitate for these calculations using the hard-sphere model.

3.1 References on interparticle interference and structure functions

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Chapter 4

The Utah SAXS Tools

I have written a collection of small computer programs that, together, provide many of the necessary tools for processing and analyzing small-angle scattering data, with a particular focus on handling the smearing effects that arise when using a slit-collimated camera. For the present, these are called the Utah SAXS Tools (USToo). These tools were written for use with the data generated using the Anton Paar SAXSess instrument, and they offer much of the functionality of the proprietary SAXSquant software, though with a rather different user interface. Though they were written and have only been tested using the Macintosh OS X operating system, they are based on open-source cross-platform programs, and there should be little difficulty in using them with other computers.

There are two major components of USToo. The first is a set of macros (saxsImage) for the imageJ program, a widely-used scientific image analysis program developed by Wayne Rasband at the U.S. National Institutes of Health. The saxsImage macros create new menu commands for imageJ that are specifically designed for integrating the two-dimensional image data from the SAXSess camera, as well as analyzing the beam profile. The data from saxsImage are saved in the PDH file format of Glatter *et al.*, with special provisions for storing the beam-profile information.

The second component of USToo is a set of programs, written in the Python language, for processing, analyzing and plotting the scattering data. These programs are run using a command-line interface: A shell in Unix-like operating systems (including Mac OS X) or the DOS window in Windows". While this approach is, in some respects, less user-friendly than a graphical interface with menus *etc.*, with a bit of experience it can become a very efficient way of working.

4.1 File Formats

The USToo programs use the PDH (Primary Data Handling) file format for scattering data, with some the "free" data fields used to handle the special information associated with the smearing effects. The PDH format is the native format for the PCG (Physical Chemistry Graz) SAXS software developed at the University of Graz by Prof. Otto Glatter and colleagues. The format is specified in Appendix 8.1 of the PCG manual (page 123 in the 2005 edition).

A PDH file contains SAXS (or SANS) data in a simple three-column text format, with the columns containing the scattering vector magnitude (q), the scattering intensity (I) and the uncertainty in the intensity. The data columns are preceded by five lines of header information:

1. An experiment title or description of the experiment. Fortran format: A80
2. Keywords describing the experiment, read in groups of four characters separated by single spaces. Fortran format: 16(A4,1X).
3. 8 integer constants. Fortran format: 8(I9,1X).
4. 5 floating point constants. Fortran format 5(E14.6,1X).
5. 5 floating point constants. Fortran format 5(E14.6,1X).

The subsequent data lines have fortran format 3(E14.6,1X). The Fortran formats very precisely specify the character positions of the fields, and also specify one blank character (1X) between the fields. When reading PDH files the USToo programs use the blank characters to parse the fields,

which allows some flexibility in the file format. But, this flexibility cannot be assumed for other programs, and the USToo programs use the stricter specification when writing files.

The specification for the PDH format sets aside two of the numerical constant fields for specific purposes:

- The first integer constant in line 3 is the number of data points.
- The fourth floating-point constant in line 4 is a normalization factor, with a default value of 1.0. It must not be 0.0 for the PCG programs.

The other constants in lines 3–5 can be used for special purposes by different programs. The SAXSess software (SAXSquant) sets aside the following floating-point fields in line 4:

- Field 2: The sample-to-detector distance, in mm.
- Field 5: The X-ray wavelength.

Other fields may also be set aside by SAXSquant and other software.

In addition to the fields identified above, the USToo programs use several of the header fields, as follows:

- Field 2 in line 3: An integer specifying the function used to describe the beam-length profile:
  0: None. This usually indicates that there is no smearing, and the other smearing parameters are ignored.
  1: The sigmoidal function. (See page 6.)
  2: The trapezoidal function. (See page 5.)
- Field 3 in line 3: A flag to indicate that the data have been calibrated to absolute intensity in units of cm$^-$1. Set to 1 if the data are calibrated, 0 otherwise.
- Field 1 in line 5: Beam-length profile parameter a.
- Field 2 in line 5: Beam-length profile parameter b.
- Field 3 in line 5: Half-width of the beam-width profile (at half height), in q-units.
- Field 4 in line 5: Detector-slit length (width of integration area), in q-units.
- Field 5 in line 5: Scale for units of q: 1 for Å^{-1}, 10 for nm^{-1}.

An example of the top section of a PDH file, with the SAXSess and USToo specific fields, is shown below:

```
10 mg/mL N protein
SAXS
     1175          1          0          0          0          0          0
0
  0.000000E+00   2.645000E+02   0.000000E+00   1.000000E+00   1.542000E+00
  1.896930E+02   2.664110E-02   2.251900E-03   1.540530E-01   1.000000E+00
  0.000000E+00   1.045670E-02   1.102870E-02
  6.521570E-04   2.993930E-02   1.062280E-02
  1.304310E-03   3.973960E-02   9.684250E-03
```

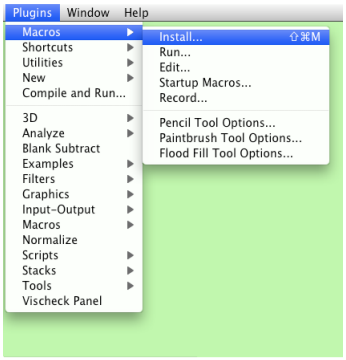
The saxsImage macros (described in the next section) save the integrated SAXS data with the SAXSess- and USToo-specific fields filled with the appropriate information. In addition, utility programs for reading, modifying or creating the header information are provided as part of the USToo package (page 69).

4.2 saxsImage macros for ImageJ

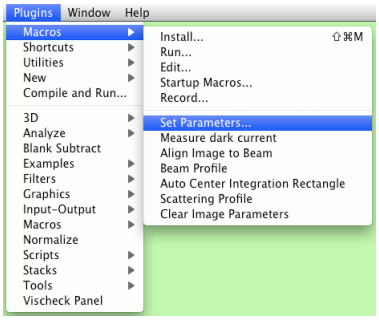
ImageJ is a very powerful and free image processing program written by Wayne Rasband of the U.S. NIH. ImageJ is a successor to NIH Image (which was written for the Macintosh) and is written in Java, making it compatible with any operating system with a Java runtime environment. The program can be enhanced with the addition of custom scripts, macros or plugins; which differ somewhat in their structures and capabilities. The tools provided here are classed as macros, and were written using the ImageJ-specific macro language.
ImageJ can be obtained from: `http://rsbweb.nih.gov/ij/` The program is frequently updated with new features and bug fixes, but is in some regards a victim of its own success, as the updates frequently introduce bugs or conflicts with plugins or macros. As of April 2012, the saxsImage macros appear to work well with ImageJ versions through 1.44o, but I have encountered problems with versions 1.45 and 1.46. Fortunately, older versions remain available at `http://imagej.nih.gov/ij/download/`. A major update of ImageJ (ImageJ 2) is currently being developed as an organized open-source project, as described at: `http://developer.imagej.net/`, and the first beta-version was released in April 2012. The beta does not support the saxsImage macros, but future versions may well, since compatibility with ImageJ 1.x macros is a major goal of the project.

4.2.1 Installing and opening the macros

The macros are provided as a single text file, saxsImage.txt. It can be stored anywhere, but the most convenient place is in the macros folder of the ImageJ application folder. If the file name is changed to "StartpMacros.txt" (and stored in the ImageJ/macros folder) the macros will be automatically loaded when ImageJ is started. If the file is not set up for automatic loading, use the **Install...** command in the ImageJ **Plugins** menu to load the macros.



Once the macros are installed, they become available through the lower part of the **Macros** submenu:

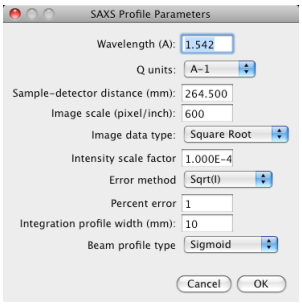


The individual macro commands are listed in the order in which they are normally used in processing a SAXS image, as described on the following pages.

4.2.2 Macro commands

Set Parameters

The **Set Parameters...** command opens a dialog box from which several parameters are set:



These parameters refer primarily to the experimental set up, and are not frequently modified.

- The X-ray wavelength, in Å.
- The choice of units for q in the output file, either Å^{-1} or nm^{-1}, selected from a drop-down menu.
- The sample to detector distance, in mm.
- The pixel scale of the image, in pixels/inch.
- Image type. The Cyclone image plate reader, used in conjunction with the SAXSess instrument, stores its output as the square root of the actual values, to increase the potential dynamic range of the stored data. If this parameter is set to "Square Root", the image values are squared to restore the original intensities. If "Linear" is selected, no correction is applied.
- Intensity scale factor. This value is used to scale the integrated intensity values. The scale factor is arbitrary, but it does influence the relative size of the uncertainties calculated by the square-root method.
- Error method. Three methods are offered for estimating the uncertainties in the intensity measurements for each value of q:
  - "Sqrt(I)": The square root of the integrated intensity.
  - "Std. Dev.": The standard deviation of the pixel values integrated for each value of q.
  - "Percent": A specified percentage of the integrated intensity.

  It is important to note that the intensities recorded by an image plate or a CCD detector are not usually calibrated to provide absolute photon counts. Uncertainties calculated as the square-root of the intensities are expected to be proportional to the counting uncertainty, but do not provide an absolute uncertainty. This can be an issue when calculating relative residuals in fitting the data (*e.g.* the reduced χ^2 statistic).
- Percent error: The percentage of the intensity used as the error, if this option is chosen.
- Integration profile width. This is the width (in mm) of the rectangle used to integrate the image data. The program automatically adjusts the rectangle to the width specified here.

- Beam profile type: The mathematical function used to represent the beam profile in the output file, either “Sigmoid” or “Trapezoid”, selected from a drop-down menu.

Once set, these parameters will be used for all of the macros during a session, but they will not be saved when ImageJ is closed. The macro file can be edited, using a text editor, to change the default values of the parameters. The relevant lines are found near the top of the file:

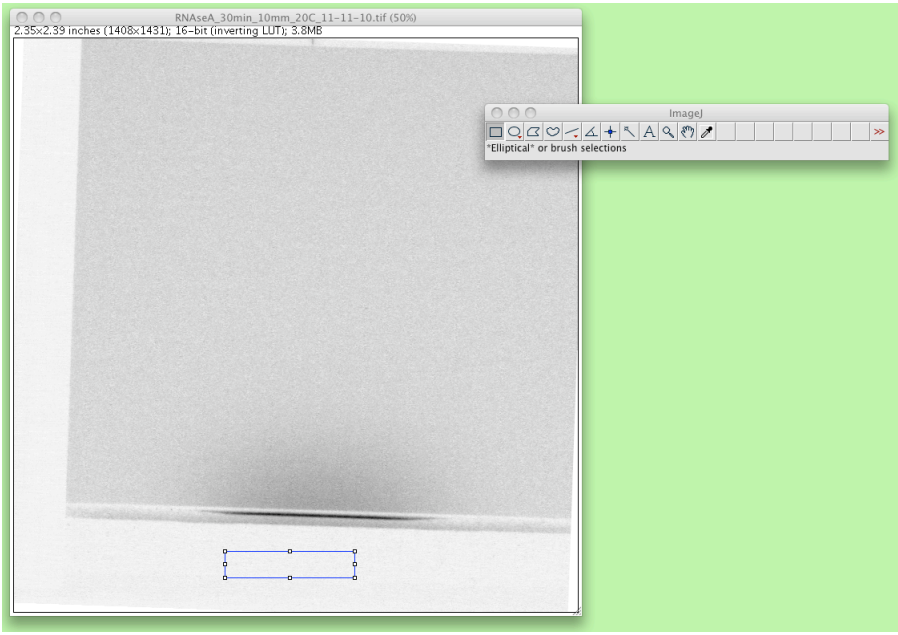
```
var wavelength=1.542; // X-ray wavelength
var qUnits="A-1"; // qUnits - either "A-1" or "nm-1"
var sdDistance=264.5; // sample-detector distance, in mm
var imageScale=600; // pixels per inch
var imageDataType="Square␣Root"; // "Linear" or "Square Root"
var iScale=1E-4; // scaling factor for intensities
var errMethod="Sqrt(I)"; // method for errors, "Sqrt(I)","Std. Dev." or "Percent I"
var errPerc=1.0; // value for calculating error by percent method
var profWidth=10; // width of profile for integration, in mm
var beamProfType = "Sigmoid"; // beam profile type: "Sigmoid" or "Trapezoid"
```

After editing this file, it must be saved and read back into ImageJ, using the **Install...** command in the **Plugins** menu.

Measure Dark Current

Nearly any detector will produce a background signal even in the absence of any exposure, which is commonly referred to as the dark current, even if it is not literally an electric current. For an image derived from an phosphor plate or CCD detector, this “dark current” signal corresponds to a small background intensity in the image that should be subtracted before the image is analyzed further, as described below.

After opening a SAXS image (using the **Open...** command in the **File** menu, use the rectangular selection tool (found in the floating tool menu) to select a region of the image outside of the exposed region:

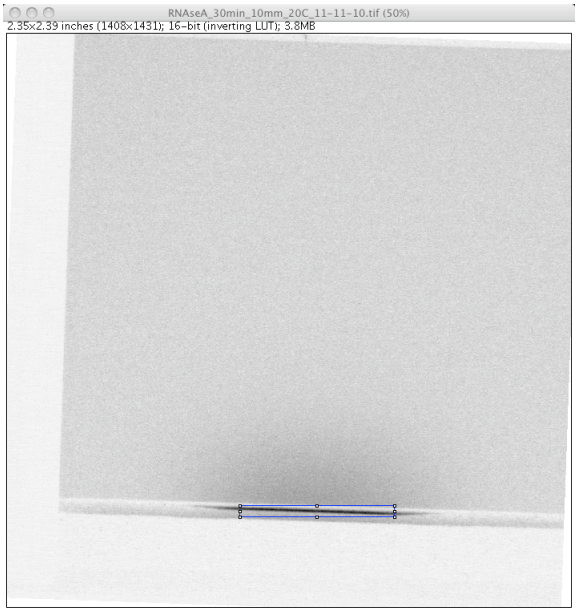


Then, choose the **Measure dark current** command in the **Macro** menu. The intensities of the pixels within the selected region will then be averaged and subtracted from the intensities of all of the

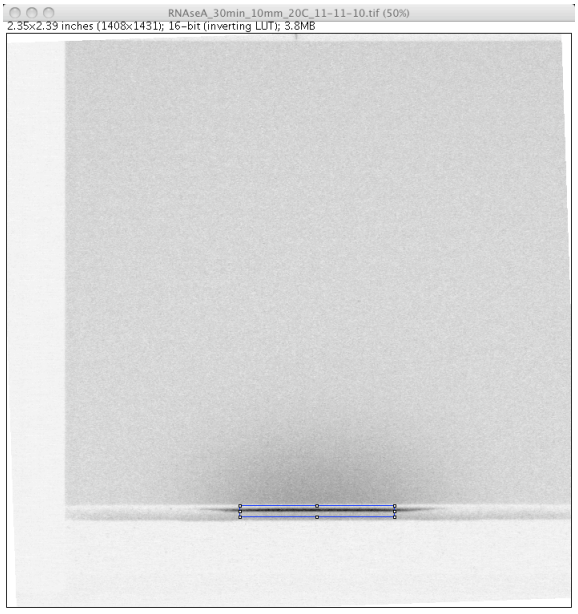
image pixels.

Align Image to Beam

In order to accurately integrate the SAXS data, a rectangular region perpendicular to the beam profile must be selected. Often, the camera image is not quite aligned squarely with the surrounding image, as shown below:



To correct the alignment, first select a rectangular region around the central part of the beam, making the selection as narrow as possible, as in the figure above. Then, choose the **Align Image to Beam** command in the **Macro** menu. The program will identify the peak positions in the beam profile, determine the slope of the beam and rotate the image to make the beam horizontal:

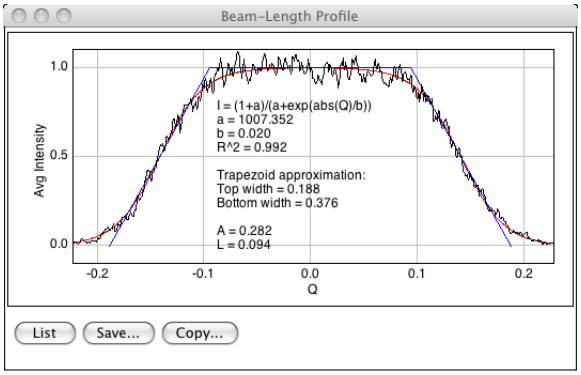


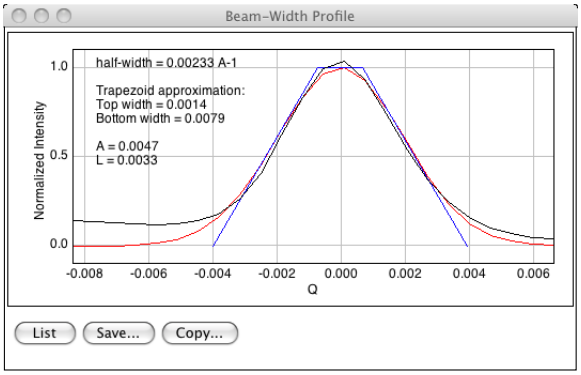
The success of this operation can depend on the selected region and may have to be repeated until the image appears to be well aligned. Usually, the best results are obtained by selecting only the central region of the beam, with approximately uniform intensity, as in the figures above.

Beam Profile

The next step is to measure the beam profile, which is used for desmearing calculations. Again using the rectangular selection tool, select a narrow region that fully encloses the beam:

Then, choose the **Beam Profile** command in the **Macro** menu. After a few moments of thinking, the program will generate two new windows, showing the beam-length and beam-width profiles:

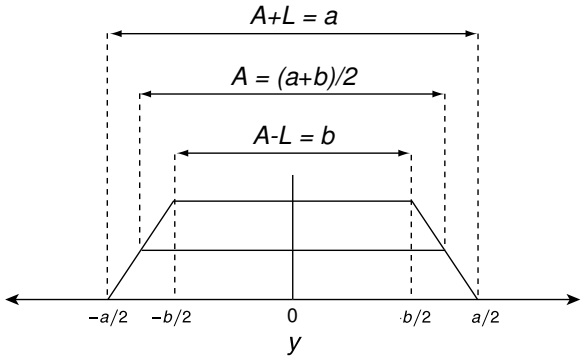




The beam-length profile represents the intensity integrated along the vertical direction in the selected region, and plotted as a function of the horizontal position. For the beam-width profile, intensity is integrated along the horizontal direction and plotted as a function of the vertical position. For the beam-width-profile, however, only the the central region along the horizontal axis is used for the integration.

The beam-length profile is fit to the sigmoidal function described on page 6, and the fitting parameters are shown in the window. This fit is also used to determine the parameters of a trapezoidal profile, which is also plotted. In addition to the lengths of the upper and lower trapezoid sides

(a and b), the window also shows two other parameters sometimes used to describe a trapezoidal beam profile, A and L. A is the width of the profile at half it's height, and L is one half of the difference between the lengths of the long and short sides, as illustrated below:



The parameters A and L are used in the programs from the the laboratory of Dr. Dmitri Svergun, including GNOM. To make things more complicated, the Irena package of data analysis tools (for the Igor Pro program) uses $A/2$ and $L/2$ as the parameters for defining a trapezoidal beam profile. In any case, the beam profile determined using the saxsImage macros can be used, with the appropriate conversion, in the other programs.

The beam-width profile is fit to a Gaussian function, which is also used to derive parameters for a trapezoidal representation.

When the scattering profile is generated (see below), the beam profile information is automatically saved in the PDH-format file, as described on page 27.

Finally, the **Beam Profile** command modifies the selected region to set its width to that specified for the integration rectangle and centers the rectangle over the beam:

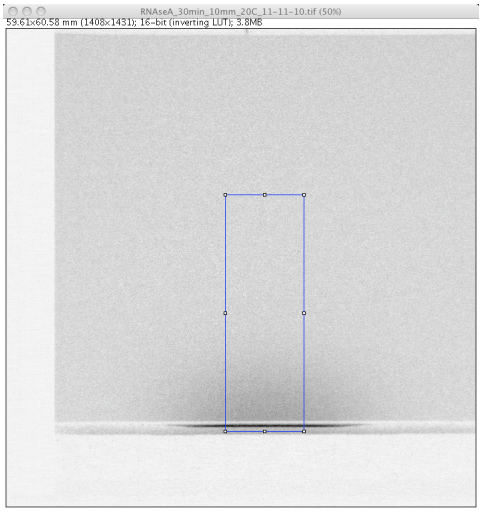
Auto Center Beam Profile

If the integration rectangle has not been centered and set to the proper width by using the **Beam Profile** command described above, the rectangle can be set using the **Auto Center Beam Profile**

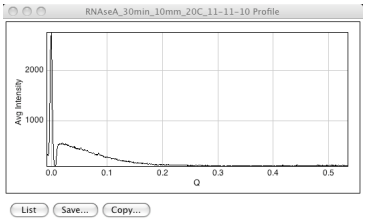
command. First select a rectangular region that includes most of the length of the beam profile and then select the command from the **Plugins** menu. The rectangle will automatically be centered and set to the width specified in the Parameters dialog window.

Scattering Profile

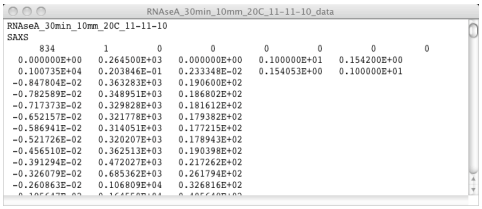
The final step in analyzing the image data is to use the **Scattering Profile** command to integrate across the width of the integration rectangle. First adjust the height of the selected region to cover the range of scattering angles for which there is significant signal intensity above background, as shown below:



Then, select the **Auto Center Beam Profile** command from the **Plugins** menu to generate the profile, which will be automatically plotted in a new window:



The numerical values for the profile will also be written to a new text window:



Writing the full profile to the text window may take a few seconds. As shown above, the text is written in the PDH format, with special information regarding the beam profile and other parameters included in the header lines, as described on page 27.

The contents of the text window can be saved using the **Save** or **Save As...** command in the **File** menu. It is recommended that the file name include the suffix ".pdh" to identify its format.

That's all there is to it!

Clear Image Parameters

The final command in the set simply clears the parameters that have been set for the front image window. These include the dark current value and the beam profile parameters.

4.3 Python programs for processing and plotting SAXS data

4.3.1 Overview, installation instructions and caveats

The tools for processing and plotting SAXS data were written in the Python language and are organized into specialized programs that are executed from a Unix shell (or the Windows DOS environment), which allows quick access to selected functions once the user becomes familiar with the shell and a few commands. The user does not need know anything at all about Python.

To use these programs, the Python interpreter and supporting files must also be installed on the computer. In addition to the basic Python installation, the USToo programs rely on the numpy and scipy packages for numerical and scientific computing, the matplotlib package for plotting and the uncertainties package for handling error propagation. The easiest way, by far, to obtain all of the packages (except the uncertainties package) in a compatible form is to download the Enthought Python Distribution (EPT) from `http://www.enthought.com/products/epd.php`. This distribution is free to academic users. After installing EPD, the separate uncertainties package can be downloaded from `http://pypi.python.org/pypi/uncertainties/` and installed by following the instructions provided. If the EPD is not available for your computer system (as it is not for some older Macintoshes with PowerPC processors), the individual components can be downloaded from the following sources:

- Python: Python is already installed on most Linux systems and on Mac OS X. However, the distribution included with OS X is typically a version or two out of date and will likely not work properly with the other packages required for the USToo programs. Current versions of Python can be downloaded from `http://www.python.org/`. Do *not* use version 3.x, which includes several significant changes from the earlier version that make it incompatible with older programs. Versions 2.6.x or 2.7.x should be fine.

- Numpy and Scipy: These are special packages with functions for numerical and scientific computing. They can be downloaded from `http://www.scipy.org`

- Matplotlib: This is a package of plotting tools, which the USToo programs use for generating graphs. Pre-built binaries for Mac OS X and Windows are available from `http://sourceforge.net/projects/matplotlib/files/`. For other operating systems, it may be necessary to download the source files (available from the same site) and compile them yourself.

- Uncertainties package: This is, so far as I know, not included in any of the packages mentioned above, but can be downloaded from `http://pypi.python.org/pypi/uncertainties/`.

On a unix-based system (including Mac OS X), the USToo Python programs can be placed in any directory that is included in the user's search path. For an individual user, this might by ~/bin, or /usr/local/bin can be used to make the programs available to all users of a computer system. The access permissions must be properly set, including the execute permission. The first line of each program (beginning with the characters "#!") identifies the location of the Python language files and will likely have to be modified for a given system. The location specified in the distributed USToo files is /Library/Frameworks/Python.framework/Versions/Current/bin/python, which is appropriate for Mac OS X with the Enthought Python Distribution installed. If the above

does not make any sense, you will likely need to get help from someone who is familiar with Unix systems!

On Windows systems, there are no file permissions, and the "#!"-line is ignored. Instead the command interpreter relies on a file extension to determine how it should be executed. Thus, the names of all the programs should be modified by adding the extension ".py" to the end.

The USToo Python programs have been developed and tested primarily on a Macintosh OS X system. The testing has not been extensive, however, and there are, no doubt, bugs that have not been caught. In addition, the programs do not include extensive checks for incorrect input data. If the input files do not match the expected format, a program is likely to crash without providing much useful information or, worse, output incorrect results. Use with care, and please report any bugs you discover!

4.3.2 Using a Unix (or Linux) shell: saxsPlot example

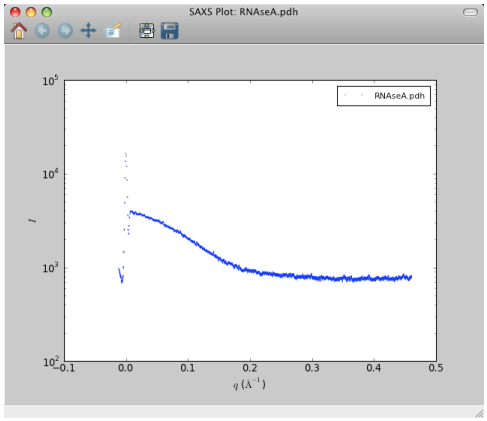
The USToo Python programs are executed from the command line of a Unix, or similar, shell window. This is a rather old fashioned way of interacting with a computer, in which the user types commands (often with arguments or options), and the program returns the output to the same window, or sometimes a new window, as for graphics. To use the programs, you will need to learn some basic commands for things such as moving around within the directory structure, listing and moving files and creating new directories. Numerous books and tutorials can be found on the internet. The tutorial at `http://www.ee.surrey.ac.uk/Teaching/Unix/` provides just about the right level of detail and depth that most users of these programs will need.

When the programs have been properly installed, their names are recognized by the shell as commands. The programs are executed by typing their names at the command line, along with one or more required arguments. Additional options are also specified on the command line. The arguments represent information that is required for execution of the program, such file names or numerical values for processing parameters. The arguments must be typed after the command in a specified order. Options are specified with special keys that begin with one or two dashes, as explained further below. Some options require their own arguments.

To illustrate some of the general features of the programs, we will introduce here the program saxsPlot, which, as its name implies, plots SAXS data. The program can be executed with just a single argument, the name of the data file to be plotted. For instance to plot the data contained in the file RNAaseA.pdh, simply type the following:

```
> saxsPlot RNAseA.pdh
```

Here, the ">" character represents the shell prompt, which may differ on your computer. If everything works correctly, a new window should appear containing the graph:

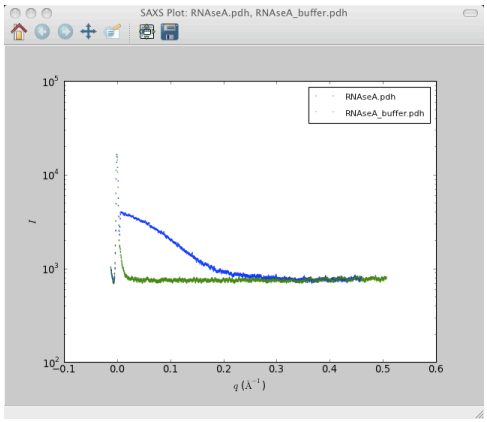


The exact appearance of the plot window may differ on different systems or with different installations of the supporting software. Above the graph (or below it, depending on the matplotlib installation), there is a navigation tool bar, with buttons for changing the view (including magnified views) and saving the figure as a file. Details are available at: `http://matplotlib.sourceforge.net/users/navigation_toolbar.html` To dismiss the plot and exit the program, click on the close button at the top of the window (the red button on Mac OS X, or the box with an "x" on other systems.

saxsPlot can plot multiple data sets at once. Just type additional file names as arguments on the command line. For instance:

```
>  saxsPlot RNAseA.pdh RNAseA_buffer.pdh
```

plots two files, representing an uncorrected profile (RNAseA.pdh) and a buffer reference file:



For any of the USToo programs, information about the available options can be found by typing the command followed by -h (or --help). For saxsPlot, there are several options:

```
>  saxsPlot -h
Usage: saxsPlot [options]

Options:
  -h, --help            show this help message and exit
  -i, --info            Show more help information.
  -r, --raw             Raw input file. Default is pDH format
  -s, --si              Read data from system standard input
  -p IMGFILENAME, --pfile=IMGFILENAME
                        Image file name. Suffix specifies format. Default is
                        None
  -t PLOTTYPE, --type=PLOTTYPE
                        Plot type. Options are linear, log, loglog, guinier,
                        kratky. Default is log.
  --noErr               no I error data.
  --qMin=QMIN           Minimum q-value for plotting
  --qMax=QMAX           Maximum q-value for plotting
  --iMin=IMIN           Minimum i-value for plotting
  --iMax=IMAX           Maximum i-value for plotting
```

Some of the options can be specified using a short form (a single dash followed by a single letter, *e.g.* -h), and all can be specified using a long form (two dashes followed by one or more letters *e.g.* --help). Some of the options (*e.g.* -h, -i, -r, -s) do not require any further input, while others (*e.g.* -p, -t --pMin, --qMax) require additional arguments. When the short form is used, the option is followed by a space and then the argument. If the long form is used, the option is followed by "="

and the argument.

The various options, many of which are shared other programs in USToo are described below.

- -h or --help. Prints the help message with all of the available options and exits the program.
- -i or --info. Prints a more extensive information message and exits the program.
- -r or --raw. By default, the program expects a SAXS data file in the pDH format, as described on page 26. If the -r or --raw option is used, the program accepts a file with a simple three column format, with the columns containing values for q, I and the errors associated with I. If the --noErr option is also used, only two columns are required.
- -s or --si. This option causes the program to read a single data file from standard input, explained further below. With this option, no file names are read as arguments, and the command is executed as:

  ```
  > saxsPlot < inputFileName
  ```

  The "<" indicates that the file is to be read as standard input. For more about file direction, see `http://www.ee.surrey.ac.uk/Teaching/Unix/unix3.html`.
- -p or --pfile. This allows the user to specify the name of a file in which to save an image of the plot. This is an example of an option that requires an argument. It's use looks like this:

  ```
  > saxsPlot -p myPlot.pdf RNAseA.pdh
  ```

  or

  ```
  > saxsPlot --pfile=myPlot.pdf RNAseA.pdh
  ```

  The extension of the file name specifies the image format. If no extension is used, the default format is png. Other options are: emf, eps, pdf, ps, raw, rgba, svg, svgz. An image file can also be saved from the plot window by clicking the disk icon, which brings up a save-file dialog window. Again, the format of the image file is specified by the file name extension.
- -t --type. By default, the SAXS data are plotted as $\log I$ versus q. Other options are: a linear plot of I versus q ('linear'), a plot of $\log I$ versus $\log q$ ('loglog'), a Guinier plot of $\ln I$ versus q^2 ('guinier') and a Kratky plot of Iq^2 versus q (kratky). For instance, to make a Guinier plot, type:

  ```
  > saxsPlot -t Guinier RNAseA.pdh
  ```

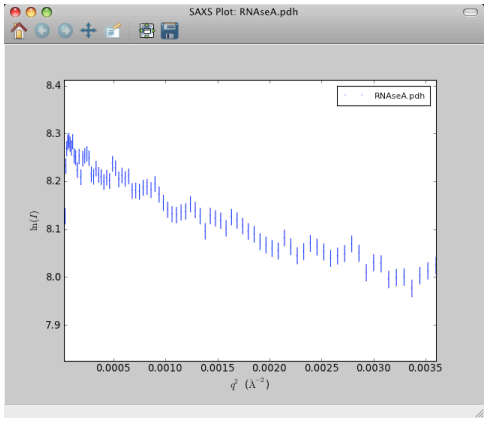
  or

  ```
  saxsPlot --type=Guinier RNAseA.pdh
  ```
- --noErr. By default the program plots the values of I as single points with error error bars. With the --noErr option, no error bars are shown and the individual data points are joined with line segments. To plot data sets that do not contain error bars, use the --noErr and --raw options together.
- --qMin and --qMax. These options can be used separately or together and allow the user to specify the range of q-values included in the plot.
- --iMin and --iMax. These options can be used separately or together and allow the user to specify the range of I-values included in the plot.

The options can be used in combination to customize the plot, as shown in the example below:

```
> saxsPlot --type=guinier --qMax=0.06 --qMin=0.01 --iMin=0.1 --iMax=0.2 RNAseA_diff.pdh
```

Which generates this plot:



The options can be listed in any order and can be typed either before or after the required argument (or arguments). The arguments and options can even be interspersed (except for the arguments associated with specific options, which must follow the option.)

A few additional comments regarding the -s (--si) option are in order. An important concept in Unix-based systems is that of directed input and output. The terms "standard input" and "standard output" are usually meant to refer to input from the keyboard and output to the terminal, respectively. However, the input can also come from a named file, and the output can be directed to a file. The general syntax for directing input from a file is:

```
> myCommand < myInputFile
```

In this example, the contents of myInputFile are read as the expected input of myCommand. If the command normally directs its output to the terminal, it can, instead, be directed to a file with:

```
> myCommand > myOutFile
```

All of the USToo programs (except saxsAvg) include an -s (--si) option. When this option is used, the program expects its input from standard input rather than from a file specified as an argument. With this option, one can, in principle, type the input data after entering the command, but this would rarely be practical. Instead, the input file is directed to standard input, as in the example shown earlier. For those programs that generate an output SAXS data file, the --si option also causes the output to be directed to standard output (the terminal window, unless directed to a file). In addition, the programs that generate output data files also have an option, --so, which directs the output to the terminal, without changing the input behavior.

An extension of this concept is that of the "pipe", in which the output of one program is directed to the input of another, without actually creating an intermediate file. This action is specified using the vertical bar character, "|", as in the example below:

```
> command1 | command2
```

This feature can be used to combine two or more of the USToo programs in a multi-step data processing routine, as discussed further on page 74.

If the --so option is not used with one of the programs that generates an output data file, then the output is written to a file with a name derived from that of the input file, according to the specific program.

4.3.3 Typical workflows

The individual USToo Python programs are described in the following sections. These programs can be used in a variety of ways, but a typical workflow would include the following steps:

1. Use imageJ and the saxsImage macros to to integrate the two-dimensional image data and generate one-dimensional scattering profiles for the sample and buffer, as well as a water sample and empty cell if the data are to be calibrated to absolute intensity.
2. For CCD data, the dark current background can be subtracted with the saxsDarkCurrSub program.
3. If absolute intensities are to be calculated, use the saxsWaterCal program to subtract the empty cell data from the water data and calculate the calibration factor.
4. Use the saxsSubtract program to subtract the buffer data from the water data and calibrate the data to absolute intensity, if the calibration factor has been determined.
5. Check that the difference profile extrapolates to a value close to zero at large q. If not, it may be necessary to use the saxsBkgCorr program to subtract a constant background value from the data. Different options are available for estimating the background.
6. For noisy data, it may be useful at this point to bin the data with the saxsBin program or smooth the data with saxsSmooth.
7. The slit-smeared data can be used directly in the saxsFit program to compare the data with simulated profiles, as generated with CRYSOL.
8. For Guinier, log-log or other direct analysis methods, slit-smeared data should be desmeared using the saxsDeSmear program.
9. Plots can be made with the saxsPlot, saxsGuinier and saxsLoglog programs. The saxsGuinier and saxsLoglog programs automatically fit the data to the Guinier or power-law expressions, respectively. The saxsGuinier program can also calculate either the molecular weight or concentration of the scattering particles, provided that the other parameter is known and the data are calibrated to absolute intensities.

4.3.4 Simple processing programs

This group of data processing programs (nearly) all share the following features and options:

1. The programs read an input SAXS data file and write an output file.
2. In the default mode (without the --si (-s) option), the programs require at least one argument, the name of the input file. The input file name is always the last argument.
3. In the default mode, the output is written to a new file with a name derived from the input file name. The output file name is created by first removing the extension of the input file (the last "." and the following characters, usually .pdh or .txt) and then adding an underscore character, followed by a few characters that indicate the program that generated the file, and then the ".pdh" or other extension is restored.
4. If the --si (-s) option is used, the input file is read from standard input and the output is written to standard output (the terminal or a directed target).
5. If the --so option is used, the input file name is specified as the last argument, as in the default mode, but the output is directed to standard output. One use of the --so option is to specify a different name for the output file.
6. The programs can read and write SAXS files in either the PDH format or a simpler format with three columns containing data for q, I and the uncertainties in I. By default, the program expects the PDH format and writes the output file in that format, modifying the header information as appropriate (usually just changing the number of data points specified). If the --raw (-r) option is used, the programs read the input file looking for lines that contain exactly three white space-separated fields that can be read as floating point numbers (or two fields if the –noErr option is used). All other lines, including those that begin with a "#" character are ignored. The output file is written with just the three columns of data. A PDH file can be input with the --raw option, but the header information will be lost in the output file.
7. The programs all generate plots of the input and output data. The type of plot can be specified using the --type (-t) option, with an argument chosen from: linear, log, loglog, guinier or kratky. The default mode is either log or linear, depending on the program. Plotting can be suppressed by using --type=none.
8. The --help (-h) option generates a short message that lists the command options.
9. The --info (-i) option generates a longer message with information about the program.

The simple data processing programs are described on the following pages.

saxsAvg

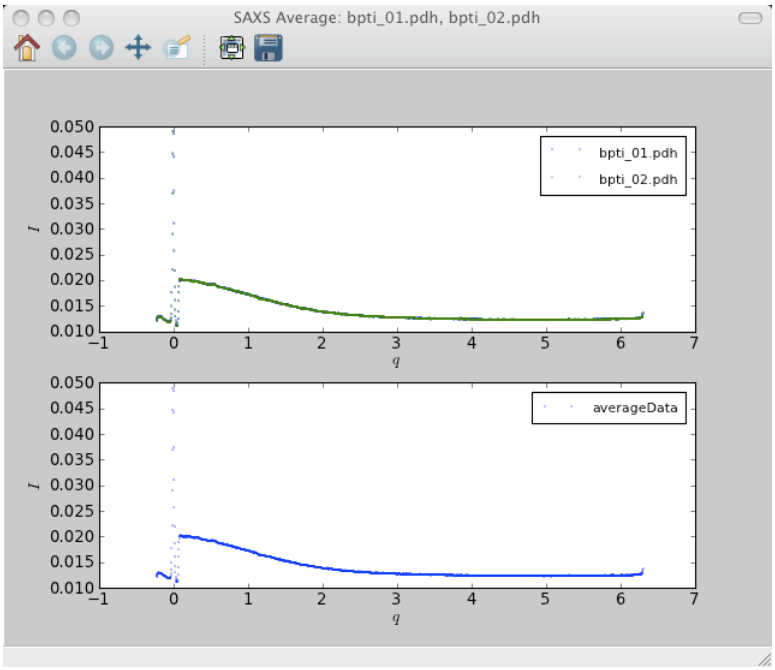
Averages two or more SAXS data files, after trimming them to a common length and range of q-values. The spacings of q-values in the files must be the same.
Required arguments: the names of two or more SAXS files.
Default output file name: inputFile_avg.pdh or inputFile_avg.txt (with --raw option). "inputFile" is the root of the first filename specified.
Options:

```
-h, --help            show this help message and exit
-i, --info            Show more help information.
-r, --raw             Raw input file. Default is pDH format
--so                  Direct output to system standard output
-t PLOTTYPE, --plot=PLOTTYPE
                      Plot type. Options are none, linear, log, loglog,
                      guinier, kratky
```

There is no -s (–si) option, since the program always requires more than one input file.
Example:

```
> saxsAvg  bpti_01.pdh bpti_02.pdh
```

Output file: bpti_01_avg.pdh
Plot:



saxsBinData

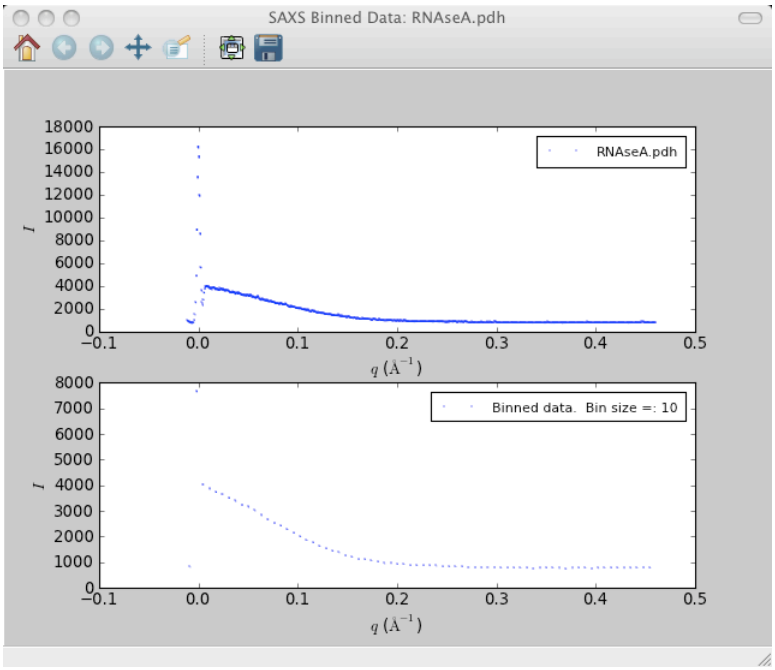
Bins (averages) sequential data points in a SAXS profile. Number of points to average is specified by the --nAvg or --dQ options. Default is to average 4 points. The --dQ option overrides --nAvg.
Required argument: name of input file (or directed input with --si option)
Default output file name: inputFile_bin.pdh or inputFile_bin.txt (with --raw option)
Options:

```
-h, --help            show this help message and exit
-i, --info            Show more help information.
-r, --raw             Raw input file. Default is pDH format
-s, --si              Read data from system standard input and write to
                      standard output
--so                  Direct output to system standard output
-n NAVG, --nAvg=NAVG  Number of points for averaging. Default is 4
-d DQ, --dQ=DQ        Delta Q to determine averaging.  Overrides nAvg.
-t PLOTTYPE, --plot=PLOTTYPE
                      Plot type. Options are none, linear, log, loglog,
                      guinier, kratky. Default is linear
```

Example:

```
> saxsBinData --nAvg=10 RNAseA.pdh
```

Output file: RNAseA_bin.pdh
Plot:



saxsBkgCorr

Subtracts a constant background from a SAXS profile. The constant can be user specified (with the -c option) or based on fitting either a horizontal line or a power-law function over a user-specified range of q-values. The default action is to calculate an average intensity over the defined region and subtract this from the profile. With the --pow option, the intensities are fit to a power-law function, of the form $I = a + bq^p$ with a user-specified value of p, which should be negative. The extrapolated value for large q (a) is subtracted as the background. Use --pow=-4 for the standard "Porod correction" or --pow=-3 for Porod correction of slit-smeared data. If the -c (--const) option is used (with the specified offset value), only the file name is expected as an argument.

This correction is typically applied after subtracting the signal from a buffer blank, to subtract non-specific background that may arise from incoherent scattering or other sources.
Required arguments: lower q-limit for fitting, upper q-limit for fitting, input file name
Default output file name: inputFile_BkgCorr.pdh or inputFile_BkgCorr.txt (with --raw option)
Options:

```
-h, --help            show this help message and exit
-i, --info            Show more help information.
-r, --raw             Raw input file. Default is pdh format
-s, --si              Read data from system standard input and write to
                      standard output
--so                  Direct output to system standard output
-p POWER, --pow=POWER
                      Negative power for fitting function. Default is 0
-c CONSTOFF, --const=CONSTOFF
                      Add a constant offset rather than fitting to data.
                      Default is to use fitting
-t PLOTTYPE, --plot=PLOTTYPE
                      Plot type. Options are none, linear, log, loglog,
                      guinier, kratky
```

Example (default mode with offset determined by average over a defined range of q-values):

```
> saxsBkgCorr 0.5 0.6 bpti_diff.pdh
```

Example (with fitting to a power function):

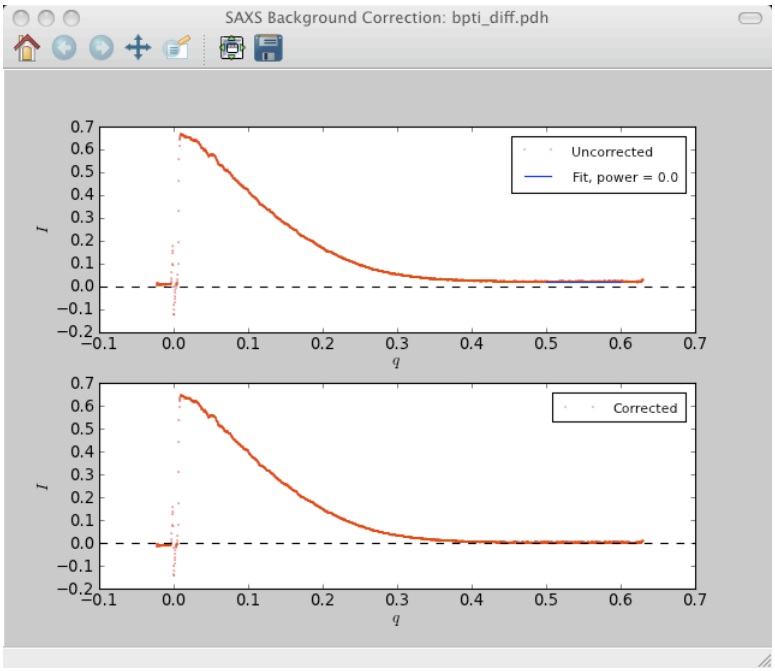
```
> saxsBkgCorr -p -3 0.5 0.6 bpti_diff.pdh
```

Example (using a user specified constant offset):

```
> saxsBkgCorr -c 0.001 0.5 0.6 bpti_diff.pdh
```

Output file: bpti_diff_bcor.pdh
(plot on following page)

Plot:



The region of the original profile used for fitting the background is colored blue.

saxsDarkCurrSub

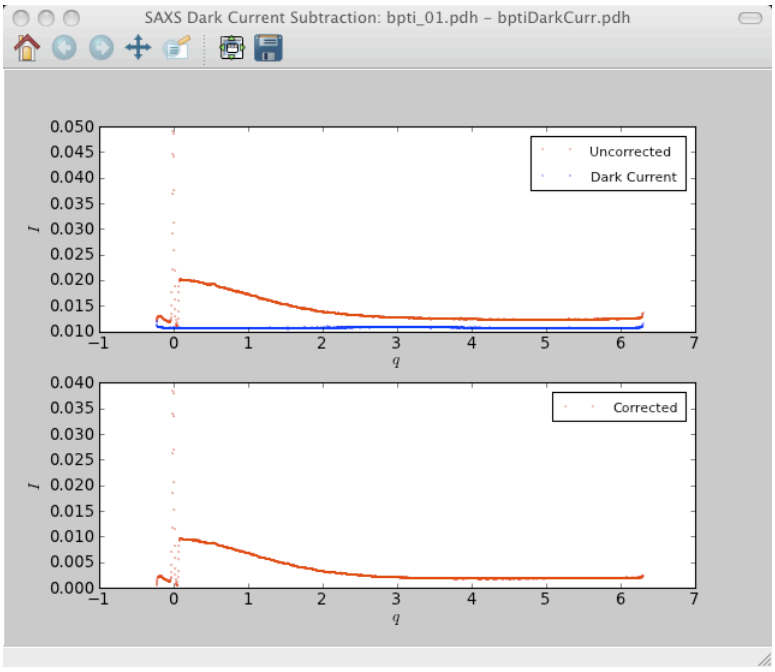
Subtracts a reference file representing the dark current from a SAXS profile. The sample file and the reference file must have the same number of data points and the same q-values. Unlike the saxsSubtract program, there is no alignment or normalization, since the dark-current profile has no beam signal. This is most likely to be used with a CCD detector, where a dark-current image is recorded.
Required arguments: name of reference file, name of data file
Default output file name: inputFile_dcs.pdh or inputFile_dcs.txt (with --raw option)
Options:

```
-h, --help            show this help message and exit
-i, --info            Show more help information.
-r, --raw             Raw input file. Default is pDH format
-s, --si              Read data from system standard input and write to
                      standard output
--so                  Direct output to system standard output
-t PLOTTYPE, --plot=PLOTTYPE
                      Plot type. Options are none, linear, log, loglog,
                      guinier, kratky
```

Example:

```
> saxsDarkCurrSub bptiDarkCurr.pdh bpti_01.pdh
```

Output file: BPTI_01_dcs.pdh
Plot:



saxsDiv

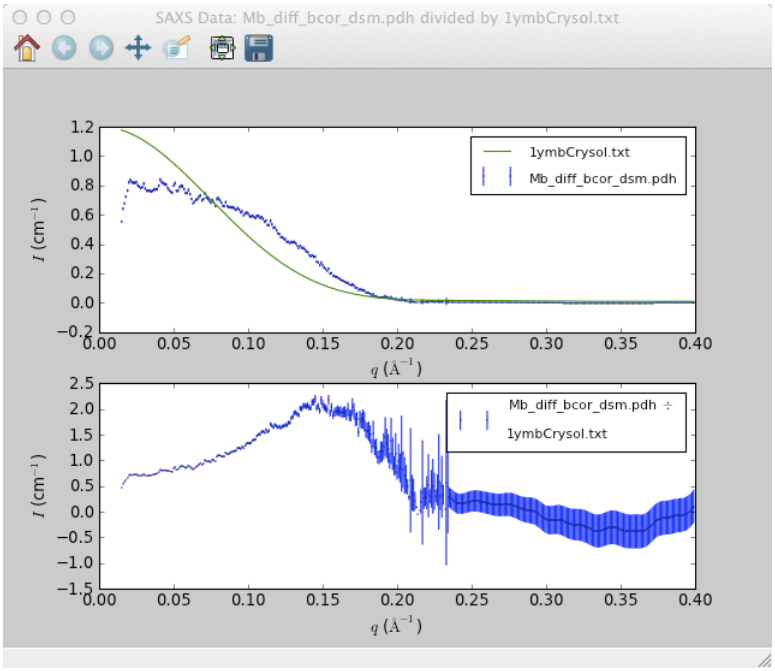
Divides one SAXS profile by another (the divisor file). This can be used to divide experimental data by model data representing a form or structure factor. The divisor file is assumed to be composed of just two columns, I and q. Lines in the divisor file that do not contain exactly two fields with floating point numbers are ignored. A cubic spline is used to represent the divisor data, so that the two files are not required to match one another point for point. If the --scale option is used, the divisor file is first divided by the scale factor. Note that dividing an experimental profile by a form factor or structure factor function is only valid or point-collimated or desmeared line-collimated data.
Required arguments: name of file with divisor data, name of file with data to be divided
Default output file name: inputFile_div.pdh or inputFile_div.txt (with --raw option)
Options:

```
-h, --help            show this help message and exit
-i, --info            Show more help information.
-r, --raw             Raw input file. Default is pdh format
--noErr               no I error data.
--scale=SCALEFACT     Divisor data is divided by scale factor. Default = 1.0
-s, --si              Read data from system standard input and write to
                      standard output
--so                  Direct output to system standard output
-t PLOTTYPE, --plot=PLOTTYPE
                    Plot type. Options are none, linear, log, loglog,
                    guinier, kratky. Default is linear
```

Example:

```
>  saxsDiv --scale=5e6 1ymbCrysol.txt Mb_diff_bcor_dsm.pdh
```

Output file: Mb_diff_bcor_dsm_div.pdh
Plot:



saxsSFcalc

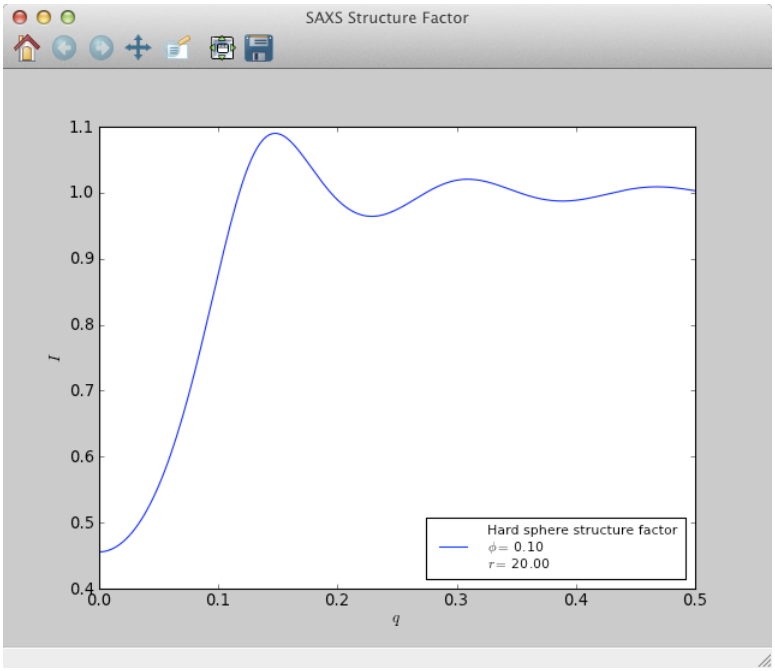
Calculates and plots the structure factor for a hard-sphere fluid, using user specified values for the volume fraction concentration (ϕ) and the sphere radius (r). The units of the sphere radius are assumed to be the reciprocal of the units of q. The hard-sphere structure factor is calculated using the Ashcroft-Lenkner solution to the Percus-Yevick equations, as given on page 24. There is no input file, and the calculated structure factor is direct to standard output.
Required arguments: ϕ, r
Options:

```
-h, --help            show this help message and exit
-i, --info            Show more help information.
--qMin=QMIN           Minimum q-value, default = 0
--qMax=QMAX           Maximum q-value, default = 0.5
--deltaQ=DELTAQ       Spacing of q-values, default = 0.0025
-t PLOTTYPE, --plot=PLOTTYPE
                      Plot type. Options are none, linear, log, loglog,
                      guinier, kratky. Default is linear
```

Example:

```
> saxsSFcalc 0.1 20
```

Output file: standard output
Plot:



saxsScale

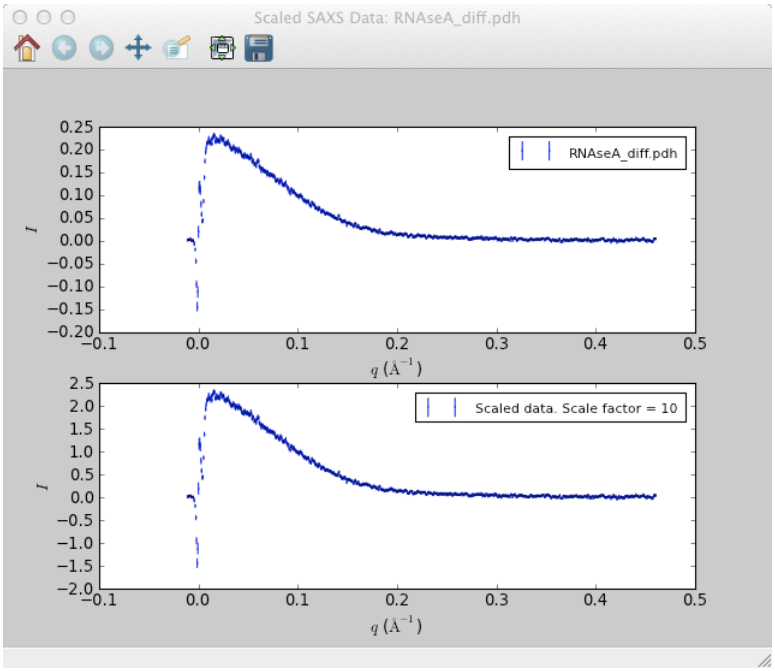
Multiplies a SAXS profile by a user-specified scale factor. Together with the saxsBkgCorr program (using the -c option for to specify the offset), any linear tranformation of a SAXS profile can be made.
Required arguments: scale factor and the name of the input file
Default output file name: inputFile_scale.pdh or inputFile_scale.txt (with --raw option)
Options:

```
-h, --help            show this help message and exit
-i, --info            Show more help information.
-r, --raw             Raw input file. Default is pdh format
-s, --si              Read data from system standard input and write to
                      standard output
--so                  Direct output to system standard output
-t PLOTTYPE, --plot=PLOTTYPE
                      Plot type. Options are none, linear, log, loglog,
                      guinier, kratky. Default is linear
```

Example:

```
> saxsScale 10 RNAseA_diff.pdh
```

Output file: RNAseA_diff_scale.pdh
Plot:



saxsSFdiv

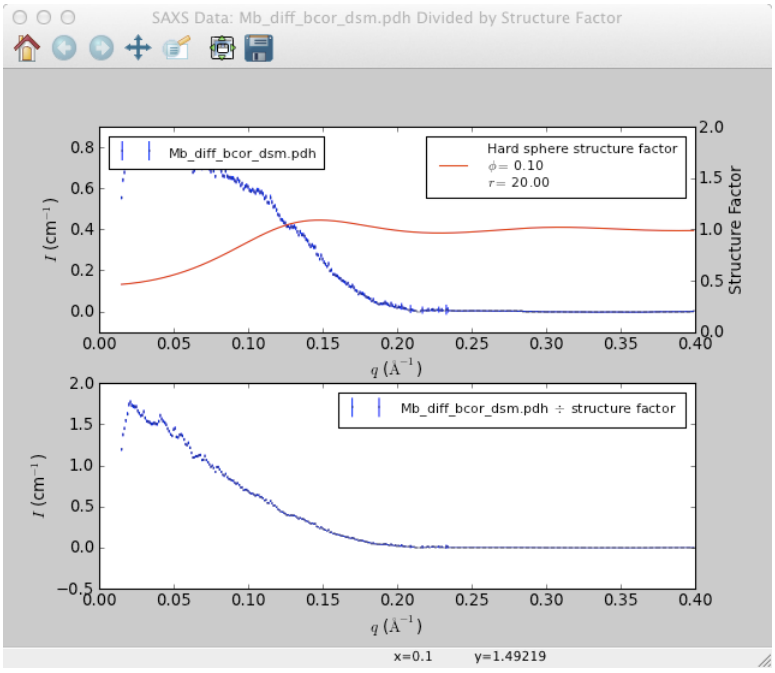
Divides a SAXS profile by the calculated structure factor for a hard-sphere fluid, using user specified values for the volume fraction concentration (ϕ) and the sphere radius (r). The units of sphere radius are assumed to be the reciprocal of the units of q. The hard-sphere structure factor is calculated using the Ashcroft-Lenkner solution to the Percus-Yevick equations, as given on page 24.
Required arguments: ϕ, r, name of input file
Default output file name: inputFile_sfdiv.pdh or inputFile_sfdiv.txt (with --raw option)
Options:

```
-h, --help            show this help message and exit
-i, --info            Show more help information.
-r, --raw             Raw input file. Default is pdh format
-s, --si              Read data from system standard input and write to
                      standard output
--so                  Direct output to system standard output
-t PLOTTYPE, --plot=PLOTTYPE
                      Plot type. Options are none, linear, log, loglog,
                      guinier, kratky. Default is linear
```

Example:

```
> saxsSFdiv 0.1 20 Mb_diff_bcor_dsm.pdh
```

Output file: Mb_diff_bcor_dsm_sfdiv.pdh
Plot:



saxsSmooth

Applies a user-specified smoothing function to the SAXS profile. The various smoothing functions available are described at: `http://www.scipy.org/Cookbook/SignalSmooth`

The default action is a Hanning function with a window length of 5 data points. The choice of smoothing function will not usually change the results much, but the length of the window will.

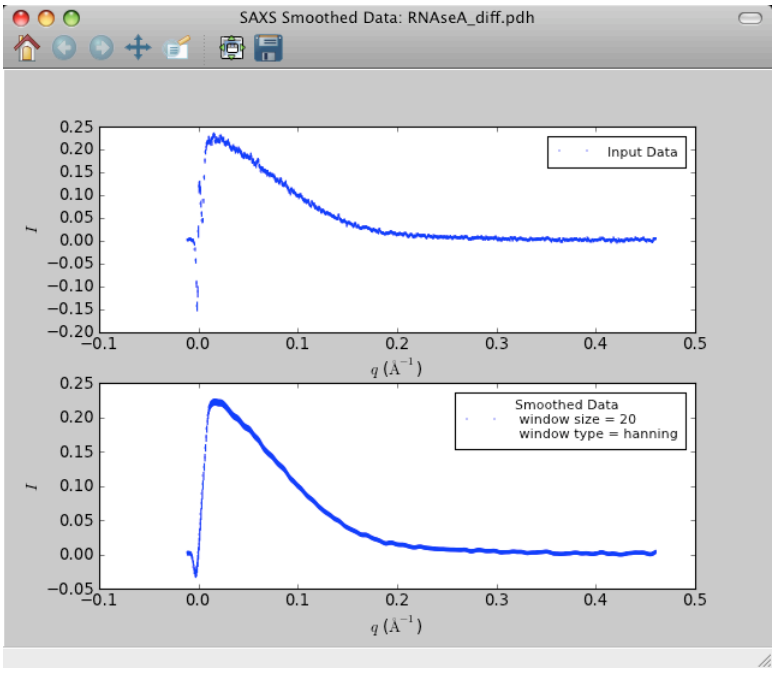
Note that the smoothing introduced by this program should not be confused with the "smearing" applied by saxsSmear, which represents the smearing effect due to finite slit lengths and widths.
Required argument: name of input file
Default output file name: inputFile_smth.pdh or inputFile_smth.txt (with --raw option)
Options:

```
-h, --help            show this help message and exit
-i, --info            Show more help information.
-r, --raw             Raw input file. Default is pDH format
-s, --si              Read data from system standard input and write to
                      standard output
--so                  Direct output to system standard output
-w WIN, --wsize=WIN   Smoothing window length. Default = 5
--wtype=WINTYPE       Smoothing window type. Options are 'flat', 'hanning',
                      'hamming', 'bartlett', and 'blackman'
-t PLOTTYPE, --plot=PLOTTYPE
                      Plot type. Options are none, linear, log, loglog,
                      guinier, kratky. Default is linear
```

Example:

```
> saxsSmooth -w 20 RNAseA_diff.pdh
```

Output file: RNAseA_diff_smth.pdh
Plot:



saxsSubtract

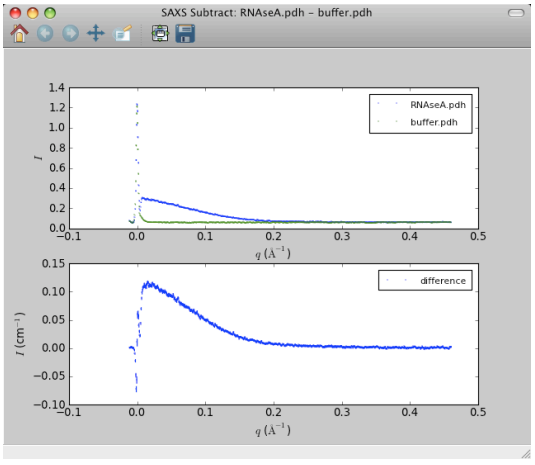
Subtracts a reference profile from a sample profile. This program is specifically intended for data files from the Anton-Paar SAXSess instrument, which include the attenuated beam profile. The peak at $q = 0$ is used to normalize the sample and reference profiles. The two profiles are trimmed to a common length, and the reference file is subtracted from the sample profile.
Required arguments: reference file name and sample file name
Default output file name: inputFile_diff.pdh or inputFile_diff.txt (with --raw option)
Options:

```
Options:
  -h, --help            show this help message and exit
  -i, --info            Show additional information and exit
  -r, --raw             Raw input file. Default is pdh format
  -s, --si              Read data from system standard input and write to
                        standard output
  --so                  Direct output to system standard output
  -t PLOTTYPE, --plot=PLOTTYPE
                        Plot type. Options are none, linear, log,
                        loglog,guinier, kratky. Default is linear
  --cf=CF               Calibration factor used to calculate absolute
                        scattering intensity, with units of cm
  --nonorm              No normalization of sample and reference data.
                        Default is to normalize
```

The --cf option allows the user to specify a calibration factor to scale the profile to absolute intensity (macroscopic differential scattering cross section), with units of cm^{-1}. The calibration factor can be determined by measuring the scattering profiles from a water reference sample and the empty cell, using the saxsWaterCal program. If the data are stored in PDH format (*i.e.*. the --raw option is not used), the header is modified to indicate that the data are calibrated, and the plotting programs show the intensity unit in the y-axis label.
The --nonorm option turns off normalization of the profiles by the attenuated beam intensities. This is useful for processing data from other instrument types.
Example:

```
> saxsSubtract RNAseA_buffer.pdh RNAaseA.pdh --cf=1.98
```

Output file: RNAseA_diff.pdh
Plot:



saxsWaterCal

Determines a calibration factor for calculating absolute scattering intensities (the macroscopic differential scattering cross section) from a scattering measurement of liquid water. This program is specifically intended for data from the Anton-Paar SAXSess instrument, which includes an attenuated beam profile. The peak at $q = 0$ is used to normalize the water and empty-cell profiles. After reading the data files, the program first trims both to a common length and range of q-values. The two files must use the same q-value intervals.

The normalized empty-cell profile is subtracted from the normalized water profile, and an average is calculated over a defined q-range. The default q-range is 0.05 to 0.2 Å^{-1}, but the minimum and maximum values can be set with the --qMin and --qMax options.

The calibration factor is calculated by dividing the average intensity difference by the theoretical macroscopic differential scattering cross section of water, calculated from the density and isothermal compressibility. By default, the water temperature is assumed to be 20°C, but other values between 0 and 100°C can be specified with the –temp option. The density and and isothermal compressibilities are calculated using the empirical equations determined by Kell (see page 22)

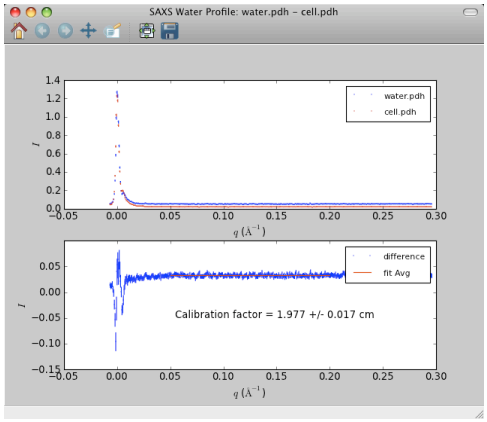
The calibration factor contains implicit information about the beam geometry, the attenuation by the beam stop and the cell dimensions. It is only valid when applied to data recorded under the same experimental conditions and normalized in the same way.
Required arguments: Empty-cell data file name and water data file name.
Output: Writes the calibration factor to standard output, but does not save the calculated difference file.
Options:

```
-h, --help            show this help message and exit
-i, --info            Show additional information and exit
-r, --raw             Raw input file. Default is pdh format
-s, --si              Read data from system standard input
-t PLOTTYPE, --plot=PLOTTYPE
                      Plot type. Options are none, linear, log,
                      loglog,guinier, kratky. Default is linear
--qMin=AVGQMIN        Minimum q-value for calculating average intensity
--qMax=AVGQMAX        Maximum q-value for calculating average intensity
--temp=TEMP           Temperature of water sample, in degrees Celsius, used
                      to calculate calibration factor
```

Example:

```
> saxsWaterCal cell.pdh water.pdh
```

Plot:



4.3.5 Advanced data processing programs

These three programs apply more complex transformations, involving smearing or desmearing, to experimental or simulated scattering profiles and generally require more user specified parameters than do the programs described in the previous section. Rather than using command line arguments for the parameters, each of these programs uses a special parameter file to specify the values. The parameter files are simple text files with the parameter values listed on individual lines, in a specified order. The files can contain blank lines and comment lines that begin with the "#" character. Each parameter line can also contain comments following the parameter value, provided that they are separated by the value by one or more spaces. However, the specified order of the parameter lines must be followed.

For each of these programs, the first required command-line argument is the name of the parameter file The saxsSmear program also includes an option (invoked by the --iMode option) for the user to enter the parameters interactively. The last command-line argument is the name of the input file (unless the standard input option, -s or --si, is used).

saxsDeSmear

Uses the Lake algorithm to correct a SAXS profile recorded using a line-collimated beam. The program can account for the beam length and width, as well as a finite detector slit width. The latter parameter is equivalent to the width of the integration region used for with a two-dimensional detector. The experimental parameters describing the beam profile and detector slit width are read from the header lines of the input SAXS data file, which must be in the pDH format, as described on page 26. The other parameters for desmearing are specified in a parameter file, as described further below. The buffer reference profile should be subtracted from the scattering profile before applying the desmearing, and any background subtraction should be applied before desmearing.
Required arguments: Name of parameter file and name of input data file (must be in pDH format)
Default output file name: inputFile_dsm.pdh and inputFile_diff_dsm.log
Options:

```
-h, --help  show this help message and exit
-i, --info  Show more help information.
-s, --si    Read data from system standard input and write to standard
            output
--so        Direct output to system standard output
```

Example

```
> saxsDeSmear  desmearParam.txt RNAseA_diff.pdh
```

Output files: RNAseA_diff_dsm.pdh and RNAseA_diff_dsm.log

The header of the output pDH file is modified to indicate no smearing (beam type = 0) and to correctly indicate the number of points in the desmeared profile, but the other header fields from the input file defining the beam shape and detector slit width are retained. Details of the desmearing parameters are output to the log file.

The values specified in the parameter file are:

- Desmearing iterations. The Lake procedure is iterative, and the number of optimal number of iterations may require adjustment. The program generates graphs that illustrate the progression of the process, and these can be used to choose the total number of iterations. Only the result of the final specified iteration is saved as the output of the program. A useful strategy

is to run the program with increasing numbers of iterations and then reduce the number once it is clear that the optimum number has been exceeded (as indicated by no further reduction in the residuals or an increase in noise).

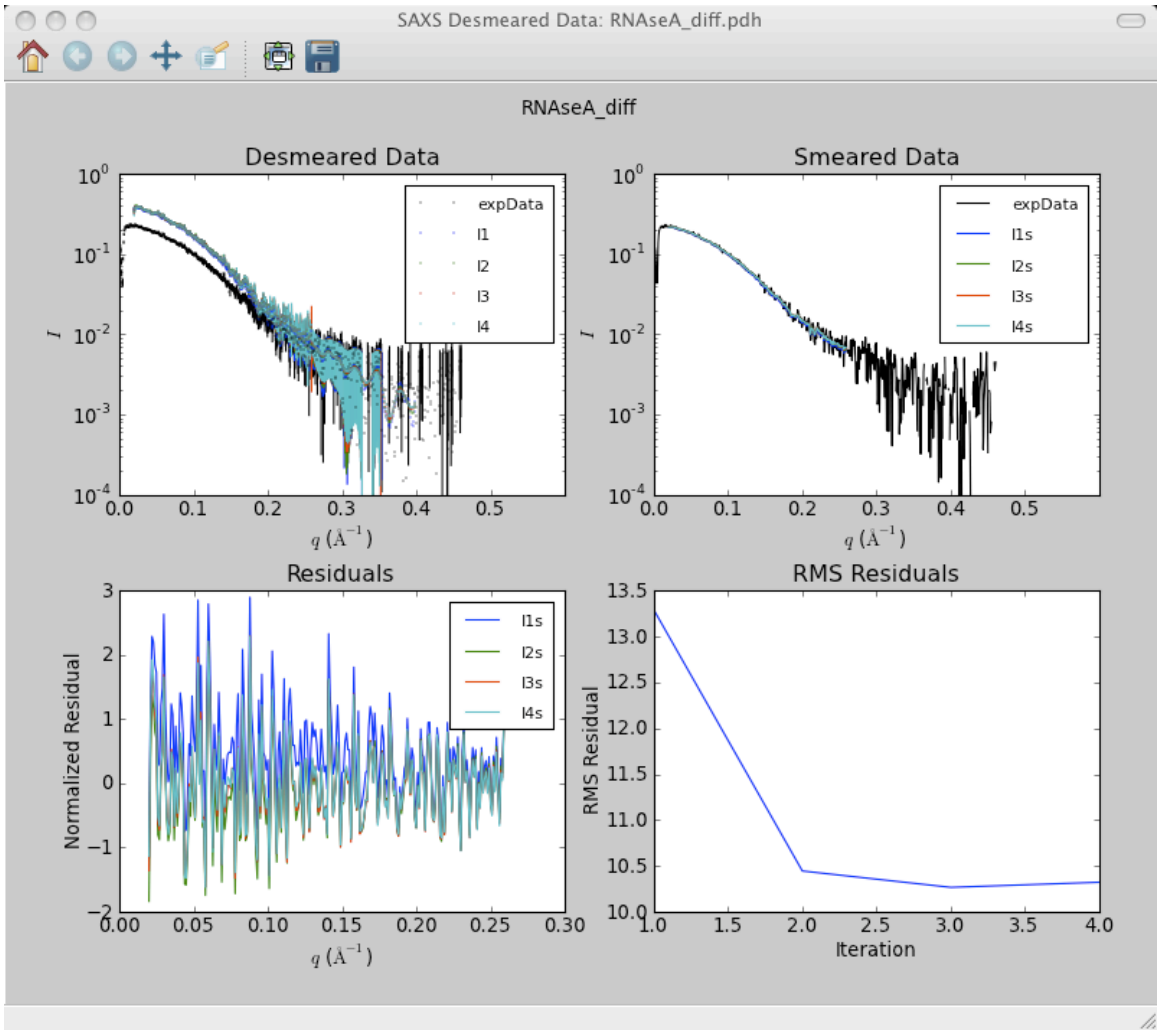
- Smoothing window length for intermediate results. By its nature, the desmearing procedure tends to enhance the noise in the profiles, and this effect increases with more iterations. To limit this effect, the intermediate results can be smoothed, using a window function covering a specified number of data points. A larger window size leads to more pronounced smoothing. For no smoothing, specify a value less than 3. The degree of smoothing is a subjective parameter, but I suggest that the window length be adjusted so that the noise level in the final profile is comparable to that in the input profile.
- Smoothing window length for the final iteration. This is analogous to the parameter described above for the intermediate results.
- Minimum q-value for desmearing. The data for small q-values that are perturbed by the beam stop should be excluded from the desmearing calculation.
- Maximum q-value for desmearing. Data corresponding to intensities close to the baseline will not contribute to the desmeared profile and may increase the noise level. However, also keep in mind that each iteration of the Lake algorithm shortens the data set by reducing the maximum q-value. The saxsDeSmear program fills these points back in with the original smeared values after each iteration except the last.
- Δq, the spacing between q-values in the output profile.
- Include beam width correction ('y' or 'n'). If the beam width is specified in the experimental data file, this parameter determines whether or not the correction is applied. If no beam width is specified in the data file, no correction will be applied regardless of this parameter. The beam width correction is usually very small, relative to the beam length correction, and it adds greatly to the computational time, since it increases the number of dimensions over which the data must be integrated from one to two.
- Minimum q-value for plotting.
- Maximum q-value for plotting.
- Minimum I-value for plotting.
- Maximum I-value for plotting.
- Plot type: 'linear', 'log', 'loglog', 'guinier' or 'kratky'
- Plot the smearing functions ('y' or 'n')
- Show the plots on the screen ('y' or 'n'). One might want to turn off the plots when processing several similar profiles in a batch process.
- Save the plots to a graphics file ('y' or 'n').
- Image format for plots: 'emf','eps','pdf','png','ps','raw','rgba','svg','svgz'

A sample parameter file is listed below:

```
# SAXS desmearing parameters
3               # desmearing iterations
15              # smoothing window for intermediate results (odd integer)
1               # smoothing window for result of last iteration (odd integer)
0.02            # q min for desmearing
```

```
0.4             # q max for desmearing
0.001           # delta q for desmeared curve
n               # include beam width correction: 'y' or 'n'
#       Plot limits
0.0             # q min for plotting
0.6             # q max for plotting
1E-3            # i min for plotting
1               # i max for plotting
#       Plot options
log             # plot type: "linear", "log", "loglog", "guinier" or "kratky"
n               # plot smear functions: 'y' or 'n'
y               # Show plots (on screen): 'y' or 'n'
n               # Save plots: 'y' or 'n'
pdf             # Image format for plots
```

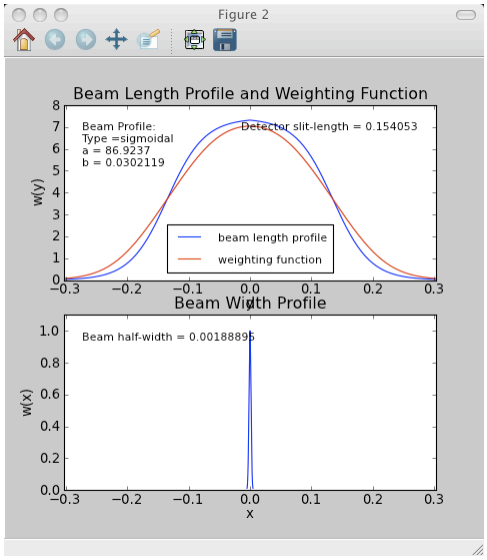
Running the saxsDeSmear program generates a four-paneled figure (unless the "show plots" parameter is set to 'n'), as shown below:



The upper left panel shows the result of each desmearing iteration along with the original experimental data. The upper right panel also shows the original data, along with the result of applying the smearing convolution to each of the desmeared profiles. The progress of the process is indicated by the agreement (or lack thereof) between the experimental data and the smeared-desmeared profile. In this case, it is clear that there is a quite good agreement after the first iteration. The two

lower panels show quantitative measures of the agreement between the smeared-desmeared profiles and the original data. The lower left panel shows a plot of the difference between the original and calculated values for each data point (normalized by the original scattering intensity), and the lower right panel shows the RMS average of the residuals as a function of the iteration number. In this case, the residuals decrease significantly, between the first and second iterations, and a bit between the second and third iteration, but not with the fourth. Thus, it would be sensible to repeat the calculation, stopping after the third cycle. It is not uncommon to see the RMS residual increase with subsequent cycles, because of the inevitable addition of noise. The visual information provided in the plots should be used to guide the user in refining the desmearing parameters until the process appears to yield a stable result.

If the option to plot the smearing functions is set to 'y', then a window like the one below is generated:



The upper panel displays both the beam length profile (blue) and the weighting function (red), which is generated by convolution of the beam length profile with the detector slit (page 8). The lower panel shows the beam width profile. Note that the beam profiles are plotted on the same scale, showing that the length profile dominates the smearing effect. In most cases, the beam width effect can be safely ignored, greatly speeding up the desmearing calculation.

saxsFit

Fits experimental data to model scattering profiles, such as generated by the program CRYSOL (`http://www.embl-hamburg.de/biosaxs/crysol.html`). The program includes a provision to use smeared experimental data. Rather than desmearing the experimental data, the program smears the model data and compares this directly with the experimental data. The only required fitting parameter is a scaling constant to match the intensity of the model data to the experimental data. Optionally, an intensity offset parameter can be introduced and allowed to float. In addition, the theoretical structure factor for a solution of hard spheres, based on the equations on page 24, can be incorporated, with up to two additional fitting factors. The experimental parameters describing the beam profile and detector slit width are read from the header lines of the input SAXS data file, which must be in the pDH format, as described on page 26. The model data are provided in a separate file, described below. The other parameters for desmearing are specified in a parameter file, also described below.

Required arguments: Name of parameter file, name of model SAXS data file and name of input data file
Options:

```
-h, --help  show this help message and exit
-i, --info  Show more help information.
-s, --si    Read data from system standard input and write to standard
            output
--so        Direct output to system standard output
```

The values specified in the parameter file are:

- Minimum q-value for fitting
- Maximum q-value for fitting
- Include beam width correction ('y' or 'n'). If the beam width is specified in the experimental data file, this parameter determines whether or not the correction is applied. If no beam width is specified in the data file, no correction will be applied regardless of this parameter. The beam width correction is usually very small, relative to the beam length correction, and it adds greatly to the computational time, since it increases the number of dimensions over which the data must be integrated from one to two.
- Allow background correction ('y' or 'n'). This introduces a floating offset parameter in the least-squares fitting.
- Fit to a linear combination of the first two models in the model file ('y' or 'n').
- Specified fraction of the first model in the linear combination. Allow to float if 0.
- Include hard-sphere structure factor in fit ('y' or 'n').
- Specified volume-fraction concentration, $0 < \phi < 1$, for hard-sphere structure factor. Allow to float in fitting if 0.
- Specified sphere radius, r, for hard-sphere structure factor. Allow to float in fitting if 0.
- Minimum q-value for plotting.
- Maximum q-value for plotting.
- Minimum I-value for plotting.

- Maximum I-value for plotting.
- Plot type: 'linear', 'log', 'loglog', 'guinier' or 'kratky'
- Plot the smearing functions ('y' or 'n')
- Plot unsmeared model for best fit, form factor and structure factor, ('y' or 'n').
- Show the plots on the screen ('y' or 'n'). One might want to turn off the plots when processing several similar profiles in a batch process.
- Save the plots to a graphics file ('y' or 'n').
- Image format for plots: 'emf','eps','pdf','png','ps','raw','rgba','svg','svgz'

A sample parameter file is listed below:

```
# saxs fit parameters
0.025    # min q-value for fitting
0.4      # max q value for fitting
n        # include beam width correction (y or n)
y        # Allow background correction (fit constant offset: y or n)
# Options for fitting to a linear combination of two models
y        # fit to linear combination of 1st two models in the model file (y or n)
0        # specified fraction of first model in linear comb.  Allow to float if 0
#  Parameters for incorporation of hard-sphere solution structure factor
y        # include structure factor in fit (y or n)
0        # specified volume density (phi) for structure factor (0<phi<0.5).
         #  Allow to float if 0
0        # specified sphere radius for structure factor. Allow to float if 0
#   Plot limits (limits are ignored if max is not greater than min)
0.025    # min q-value for plotting
0.4      # max q-value for plotting
0        # min i-value for plotting
0        # max i-value for plotting
#   Plot options
linear   # plot type: "linear", "log", "loglog", "guinier" or "kratky"
1        # No of model fits to plot (starting with best fit)
n        # plot smear functions (y or n)
n        # Plot unsmeared model for best fit (form factor and structure factor)
#   Output options
y        # Show plots (on screen): 'y' or 'n'
n        # Save plots: 'y' or 'n'
ps       # Image format for plots:  'emf', 'eps', 'pdf', 'png',
         #  'ps', 'raw', 'rgba', 'svg', 'svgz'
```

The model file can contain multiple model scattering profiles. The first line contains names for each of the models. The rest of the file is organized into pairs of columns containing q and intensity data. The first lines of an example file, with two models, are shown below:

```
4pti    1BZ5_dimer
  0.000000E+00  0.894439E+06    0.000000E+00  0.312515E+07
  0.100000E-01  0.890323E+06    0.100000E-01  0.309845E+07
  0.200000E-01  0.878099E+06    0.200000E-01  0.301987E+07
  0.300000E-01  0.858109E+06    0.300000E-01  0.289362E+07
  0.400000E-01  0.830904E+06    0.400000E-01  0.272641E+07
  0.500000E-01  0.797228E+06    0.500000E-01  0.252681E+07
  0.600000E-01  0.757977E+06    0.600000E-01  0.230446E+07
  0.700000E-01  0.714161E+06    0.700000E-01  0.206943E+07
```

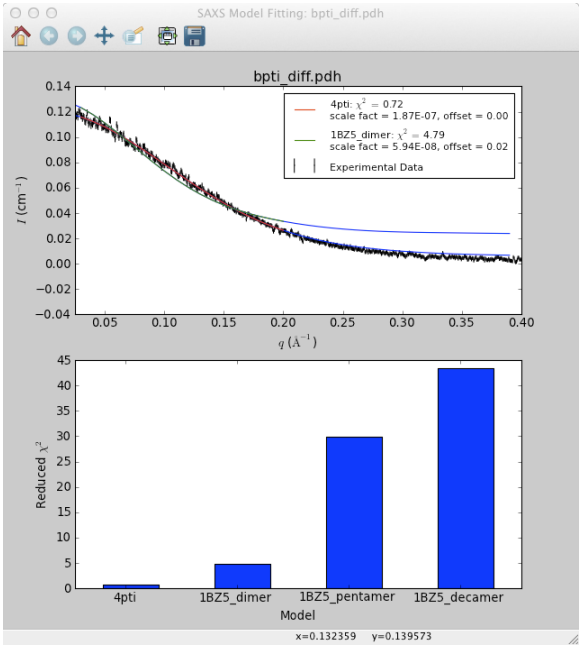
Example

```
> saxsFit fitParam.txt bptiCrysol.txt bpti_diff.pdh
```

Output file: bpti_diff_fit.txt

The output file includes the original experimental data and the scaled fits of the model data, with header information providing details of the fitting procedure.

Running the saxsFit program generates a two-paneled figure (unless the "show plots" parameter is set to 'n'), as shown below:



The top panel shows the experimental SAXS profile along with the number of fit profiles specified in the parameter file, starting with the model that best fits the data. In this case, the experimental data are for a solution of bovine pancreatic trypsin inhibitor (BPTI), a small globular protein, and the model profiles were calculated with CRYSOL for monomeric BPTI and possible oligomeric forms derived from crystal structures. In this case, it is clear that the best fit is obtained for the monomer model.

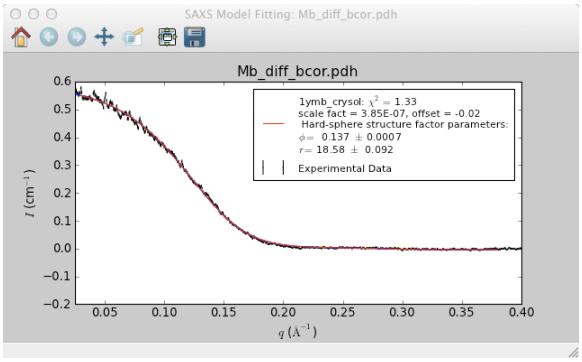
The bottom panel is a bar graph showing the reduced χ^2 statistic for each fit. Reduced χ^2 is calculated as:

$$\chi^2 = \frac{1}{\nu} \sum_{i=1}^{n} \frac{(Y_i - Y_{i,fit})^2}{\sigma_i^2} \tag{4.1}$$

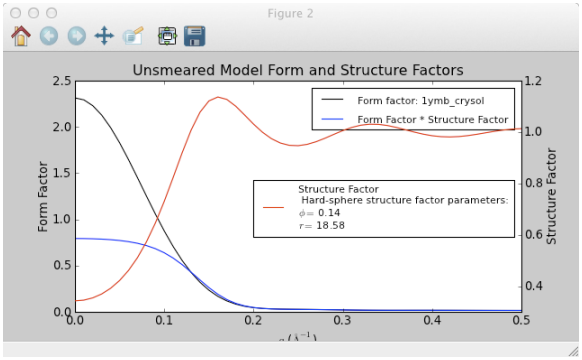
where n is the number of data points; Y_i and $Y_{i,fit}$ are the experimentally observed and fit values for each data point; σ_i is the estimated uncertainty for that point and ν is the number of degrees of freedom. ν is the number of data points, n, minus one plus the number of adjustable parameters in the fitting function (either 1 or 2, depending on whether an adjustable offset is introduced). If the uncertainties are properly scaled, then χ^2 should equal one for a model that describes the data within the measured uncertainties. If the uncertainties arise entirely from counting statistics and are calculated as the square-root of the photon count, then this condition should be satisfied by a good model. There are, however, two (at least) important caveats. First, the detectors used in many instruments (including the image plates and CCD detectors used in the Anton-Paar SAXSess) do not report direct photon counts. The uncertainties, therefore, are arbitrarily scaled.

These uncertainties can be used to weight the individual data points in the fitting, and the relative values of χ^2 obtained for fitting the same experimental data to different models can be compared to identify the best fit. But, the absolute value of χ^2 does not have any real significance. Second, uncertainties derived from either absolute or relative photon counts do not include other sources of experimental error that will contribute to χ^2. The absolute χ^2 values should be treated with caution!

The figure below shows the result of a fit incorporating the hard-sphere structure factor:



In this case, both of the structure-factor parameters, the volume fraction (ϕ) and the sphere radius (r), were treated as adjustable parameters. The program also includes an option to plot the unsmeared form of the model data, including the structure factor, as shown below:



saxsSmear

Applies smearing functions to experimental or model SAXS data, simulating the effects of finite beam length and width, as well as detector slit width. The primary use of this program is to visualize the effects of smearing on model profiles. The program can be used in two modes. By default, a parameter file is used to specify the smearing and plotting options. With the --iMode, these parameters are entered by the user interactively, with an option to save the parameters in a file for additional runs. The interactive mode does not provide options for plot type and axes limits.
Required arguments: parameter file name (unless --iMode is used) and data file name (unless -s is used)
Options:

```
-h, --help  show a help message with options and exit
-i, --info  Show more help information and exit.
-s, --si    Read data from system standard input and write to standard
            output
--so        Direct output to system standard output
--iMode     Interactive mode
```

A sample parameter file is listed below:

```
#  Parameter file for saxsSmear
t                # beam-length profile type: 't' trapezoidal or 's' sigmoidal
0.4              # beam-length parameter a
0.3              # beam-length parameter b
0.01             # half-width of Gaussian beam-width profile
0.1              # detector slit length
#       Plot limits (limits are ignored if max is not greater than min)
0.0              # q min for plotting
0.0              # q max for plotting
0.0              # I min for plotting
0.0              # I max for plotting
#       Plot output options
log              # plot type: 'linear', 'log', 'loglog', 'guinier' or 'kratky'
y                # Show plots (on screen): 'y' or 'n'
y                # Save plots  'y' or 'n'
pdf              # Image format for plots: 'emf', 'eps', 'pdf',
#               'png', 'ps', 'raw', 'rgba', 'svg', 'svgz'
```

The beam profile types and parameters are the same as used in the other programs.

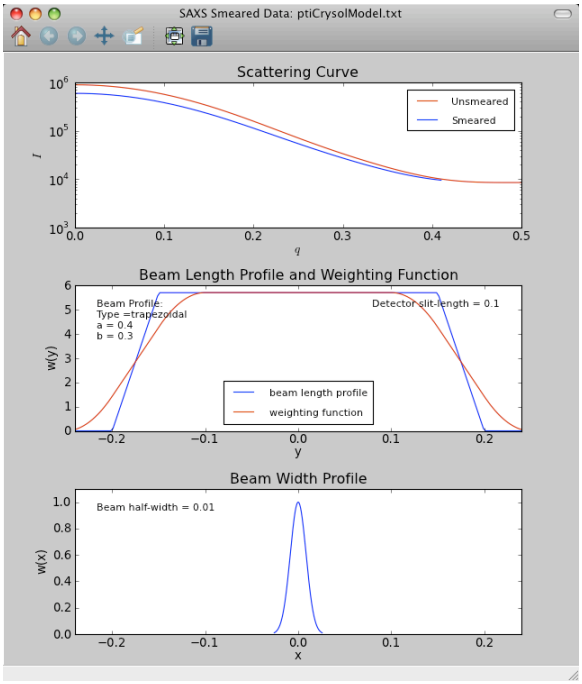
This program is rather indiscriminate in the SAXS profile files that it reads. It simply looks for lines that begin with two fields that can be read as floating point numbers and assumes that these fields represent q and the scattering intensity. It does not look for or read intensity uncertainties. The first line is assumed to be a header and is ignored, and lines that begin with '#' are ignored. If the file has a '.pdh' extension, the first five lines are ignored.

Example

```
> saxsSmear  smearParams.txt bptiCrysolModel.txt
```

Output file: bptiCrysolModel_smear.txt
The output file is a simple two-column text file with q-values in the first column and the smeared intensities in the second.

The program generates plots of the original and smeared profiles, as well as the smearing functions, as shown on the next page.


SAXS Smeared Data: ptiCrysolModel.txt
Scattering Curve
Unsmeared
Smeared
I
q
Beam Length Profile and Weighting Function
Beam Profile:
Type =trapezoidal
a = 0.4
b = 0.3
Detector slit-length = 0.1
beam length profile
weighting function
w(y)
y
Beam Width Profile
Beam half-width = 0.01
w(x)
x

4.3.6 Plotting programs

These programs allow quick plotting of SAXS data from the command line, with a variety of options for plot type and axes limits. Although these programs are unlikely to be suitable for making final figures for publication, they are very useful for initial analyses. One of the programs, saxsPlot, is intended for plotting one or more profiles, without data fitting, while the other two, saxsGuinier and saxsLoglog, make specific plot types and fit the data to the Guinier relationship or a power-law function.

saxsGuinier

Generates a Guinier plot ($\ln(I)$ versus q^2) over a specified range of q-values and fits the data to either the standard Guinier relationship or a double exponential form. Optionally, the program will calculate either the particle molecular weight or the concentration, if the other parameter is specified. This requires that the scattering data be calibrated to absolute intensities.
Required arguments: minimum q-value, maximum q-value and SAXS data file name.
Options:

```
-h, --help            show help message and exit
-i, --info            Show more help information.
-r, --raw             Raw input file. Default is pdh format
-s, --si              Read data from system standard input
-p IMGFILENAME, --pfile=IMGFILENAME
                      Image file name. Suffix specifies format. Default is None
-d, --dexp            Perform double-exponential fit in addition to Guinier fit
--noErr               no I error data.
--conc=CONC           Particle concentration, mg/mL.  If provided, program
                      calculates molecular weight.
--mw=MW               Particle relative molecular mass.  If provided,
                      program calculates concentration.
--vbar=VBAR           Partial specific volume, cm^3/g.  Used for molecular
                      weight or concentration calculation. Default = 0.74.
--rhoSolv=RHOSOLV     Scattering density of solvent, with units cm-2. Used
                      for molecular weight or concentration calculation.
                      Default is 9.4E10 cm-2, X-ray scattering density of water.
--rhoMol=RHOMOL       Scattering density of molecule, with units cm-2. Used
                      for molecular weight or concentration calculation.
                      Default is 1.22E11 cm-2, typical X-ray scattering
                      density of proteins.
--deltaRho=DELTARHO   Scattering density difference with units cm-2. Used
                      for molecular weight or concentration calculation.
                      Default is to calculate difference from
                      molecule and solvent scattering densities.
```

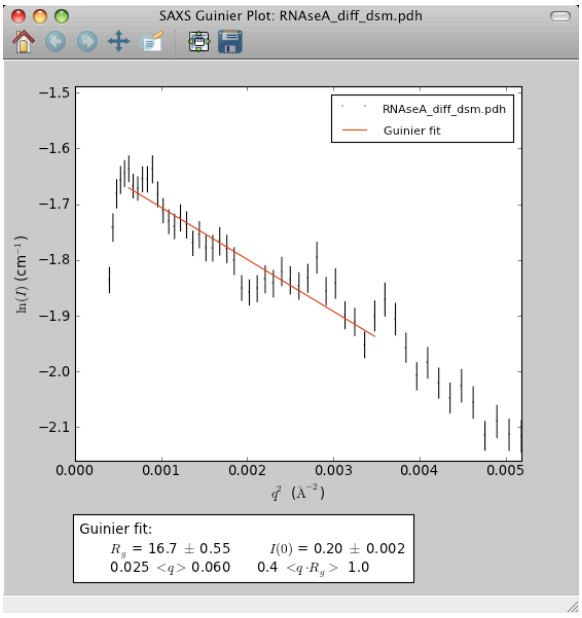
Example

```
> saxsGuinier saxsData.pdh
```

The data are fit to the function:

$$I(q) = I(0)e^{-q^2R_g^2/3}$$

where $I(0)$ is the scattering intensity extrapolated to $q = 0$, and R_g is the radius of gyration. The Guinier approximation is generally valid for values of q such that $q \cdot R_g \leq 1$.
The plot includes the experimental data, the fit curve and resulting parameters, as shown below:

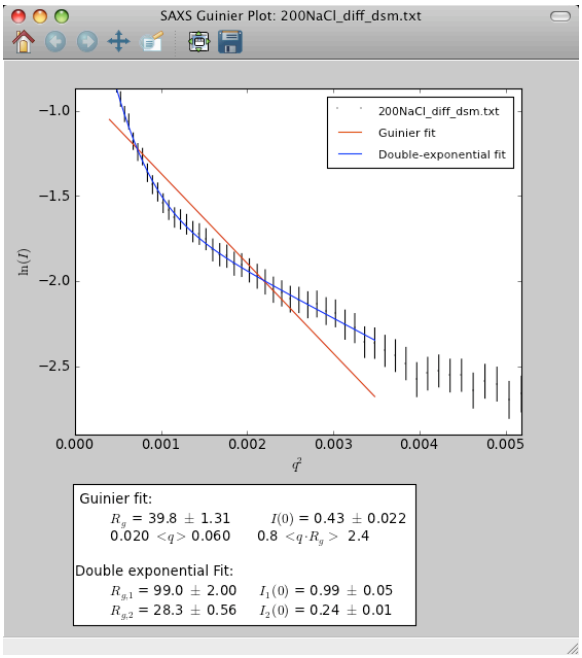


The range of values plotted can be adjusted using the --qpMin, --qpMax, --ipMin and --ipMax parameters.

In some cases, involving sample heterogeneity, it may be useful to fit the data to a double-exponential function of the form:

$$I(q) = I_1(0)e^{-q^2R_{g,1}^2/3} + I_2(0)e^{-q^2R_{g,2}^2/3}$$

This is implemented with the --dexp (-d) option, and generates a plot with both the standard Guinier function and the double-exponential function fit to the data:



This may be especially useful with samples that contain aggregates. In favorable cases, it may be possible to estimate all four of the parameters with some confidence and use these values to

estimate the fraction of molecules present in the two components. In many cases, however, one or more of the parameters may not be well defined.

Executing the saxsGuinier command may generate a warning indicating that the program was not able to fit the data. The usual solution is to extend the range of q-values, most often by increasing the maximum.

If the --conc option is used to specify the particle concentration (in mg/mL), the program calculates the particle molecular weight from the extrapolated intensity at $q = 0$, assuming that the data have been calibrated to absolute in intensities (in units of cm^{-1}). If the the --mw option is used to specify the molecular weight, $I(0)$ is used to calculate the concentration. These calculations are based on the following equation, from page 18:

$$\frac{d\Sigma(0)}{d\Omega} = (cM/N_A)\bar{v}^2(\rho_m - \rho_{solv})^2$$

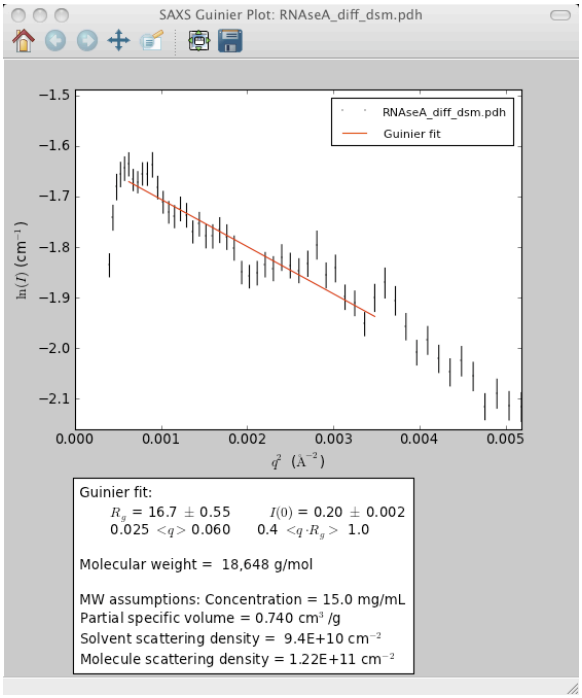
and require values for the following parameters:

- The scattering density of the solvent, ρ_{solv}
- The scattering density of the particle, ρ_m
- The partial specific volume of the particle, $\bar{v}$

The default behavior is to assume that the solvent is water at 25°C, with a scattering density of $9.4\times10^{10}\,\text{cm}^{-2}$, and that the particle is a protein with $\bar{v} = 0.74\,\text{cm}^3/\text{g}$ and a scattering density of $1.22\times10^{11}\,\text{cm}^{-2}$. Other values for $\bar{v}$, ρ_{solv} and ρ_m can be specified using the --vbar, --rhoSolv and --rhoMol options, respectively. The --deltaRho option allows the user to specify only the scattering density difference, $\rho_m - \rho_{solv}$.

An example of using saxsGuinier to estimate the molecular weight:

```
> saxsGuinier RNAseA_diff_dsm.pdh --conc=15
```



saxsLoglog

Generates a plot of $\log(I)$ versus $\log(q)$ over a specified range of q-values and fits the data to a power-law function. This plot is useful for characterizing the Porod-region of the scattering profile and estimating the fractal dimension of a sample.
Required arguments: minimum q-value, maximum q-value and SAXS data file name.
Options:

```
-h, --help            show a help message and exit
-i, --info            Show more help information.
-r, --raw             Raw input file. Default is pDH format
-s, --si              Read data from system standard input
-p IMGFILENAME, --pfile=IMGFILENAME
                      Image file name. Suffix specifies format. Default is
                      None
--noErr               no I error data.
--qpMin=QPMIN         Minimum q-value for plotting
--qpMax=QPMAX         Maximum q-value for plotting
--ipMin=IPMIN         Minimum i-value for plotting
--ipMax=IPMAX         Maximum i-value for plotting
```

Example

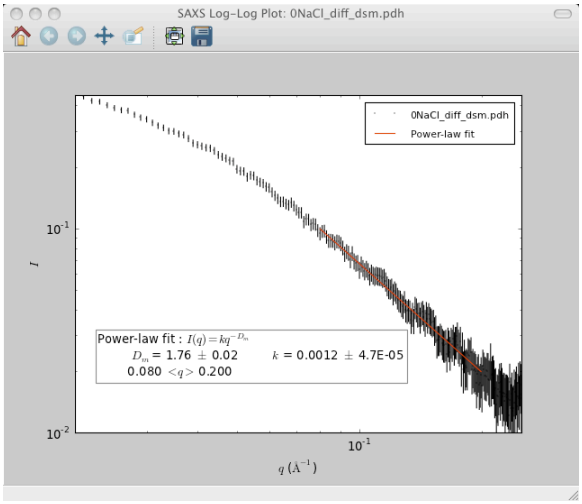
```
> saxsLoglog saxsData.pdh
```

Data are fit to the function:

$$I(q) = kq^{-D_m}$$

The negative of the exponent, D_m, can be interpreted as the mass fractal dimension of the scattering material. For globular particles, $D_m = 4$. For polymers, $D_m = 2$ under θ-conditions, and $D_m = 1.7$ in an athermal solvent.

The plot includes the experimental data, the fit curve and resulting parameters:



The range of values plotted can be adjusted using the --qpMin, --qpMax, --ipMin and --ipMax parameters.

saxsPlot

Plots SAXS data in a variety of formats, with user options to control the axes ranges and other properties. : Required arguments: one or more SAXS data files, in pDH or generic format (with --raw (-r) option.)
Options:

```
-h, --help            show this help message and exit
-i, --info            Show more help information.
-r, --raw             Raw input file. Default is pDH format
-s, --si              Read data from system standard input
-p IMGFILENAME, --pfile=IMGFILENAME
                      Image file name. Suffix specifies format. Default is
                      None
-t PLOTTYPE, --type=PLOTTYPE
                      Plot type. Options are linear, log, loglog, guinier,
                      kratky. Default is log.
--noErr               no I error data.
--qMin=QMIN           Minimum q-value for plotting
--qMax=QMAX           Maximum q-value for plotting
--iMin=IMIN           Minimum i-value for plotting
--iMax=IMAX           Maximum i-value for plotting
```

Example

```
> saxsPlot saxsData.pdh
```

This program is described in more detail on pages 36–39, where it is used an example to illustrate the Unix interface to the USToo Python programs.

4.3.7 PDH file utility programs

These programs provided as simple tools to create, read or manipulate the headers of PDH SAXS data files.

makePdh

Reads a generic scattering profile data file and adds a PDH header, with user provided parameters. Includes standard PDH parameters, Anton-SAXSess specified parameters and parameters used by the USToo programs (including beam profile information for desmearing and fitting).
Required argument: Name of input SAXS file.
Options:

```
  -h, --help            show this help message and exit
  -i, --info            Show additional information and exit
  --noErr               no I error data
  -c NUMBCOLS, --cols=NUMBCOLS
                        Number of columns in input file. Defaut = 3.
```

The default behavior is to look for lines containing exactly 3 columns that can be read as floating point numbers, which are interpretted as containing q, I and I errors. If the -c (--cols) option is set to 2, the program looks for lines with exactly 2 columns and interprets them as containing q and I. I errors are set to zero. If -c option is set to 3 or greater and the --noErr option is not used, the program looks for lines containing the specified number of columns and reads the first three as q, I and I errors. If the -c option is set to three or greater and the --noErr option is used, the program looks for lines containing the specified number of columns, reads the first two as q and I, and sets the I errors to zero.

The user is prompted to provide the various parameters. A typical interactive session looks like:

```
>makePdh RNAseA_avg.txt

Input file name: RNAseA_avg.txt
Output file name (RNAseA_avg.pdh):

PDH-specified parameters
Description: new PDH format file
Key words (SAXS):
No. of data pts (1044):
Normalization factor (1.0):

SAXSess parameters
Sample to detector distance in mm: 254
Wavelength (1.542):

Utah SAXS Tools parameters
Beam length profile type:
         0: None (default)
         1: Sigmoidal
         2: Trapezoidal
2
Trapezoidal beam length parameter a (long side): 0.4
Trapezoidal beam length parameter b (short side): 0.3
Beam half-width in Q-units (0.0): 0.005
Detector slit-width in Q-units (0.0): 0.1
Q-scale:
```

```
         1: A-1 (default)
         10: nm
Absolute intensity scale (cm-1), (y,N)?
```

For most parameters, the user is offered a default value, in parentheses. The user may either accept this default, by pressing the return key, or enter a different value, followed by the return key. The default number of data points is the number of points found in the file. The user may specify a smaller number, which results in truncation of the input data, but the program will not accept a larger number.

modPdhHead

Reads the header information of a PDH file and prompts the user to modify the information. Required argument: Name of input PDH file.

```
  -h, --help  show this help message and exit
  -i, --info  Show additional information and exit
```

The interface to this program is nearly identical to that of the makePdh program, except that the input file must already be a PDH file, and the existing parameters are treated as the default values.

readPdhHead

This program simply reads the header information and parses it for the user according to the SAXSess and USToo definitions for the header fields.
Required argument: Name of input PDH file.
Options:

```
  -h, --help  show this help message and exit
  -i, --info  Show additional information and exit
```

A typical output looks like:

```
> readPdhHead RNAseA_diff.pdh

File name: RNAseA_diff.pdh

PCG-specified parameters
         Description: RNAseA_30min_10mm_20C_11-11-10
         Key words:  SAXS
         No. of data pts: 1048
         Normalization factor: 1.0

SAXSess parameters
         Sample to detector distance: 264.5 mm
         Wavelength: 0.1542

Utah SAXS Tools parameters
         Beam length profile type: Sigmoidal
         Sigmoidal beam length parameter a: 992.61
         Sigmoidal beam length parameter b: 0.0204206
         Beam half-width: 0.00209312
         Detector slit-width: 0.154053
         Q-scale: A-1
```

4.3.8 Utility programs for calculating scattering properties

These programs are used to calculate a variety of useful properties of water (including H_2O/D_2O mixtures) and proteins.

protScattDens

Calculates X-ray and neutron scattering length densities (in units of cm^{-2}) of proteins from the amino acid sequence.
Required argument: The name of a sequence file in FASTA format.
Writes calculated properties to standard output.
Options:

```
-h, --help        show this help message and exit
-i, --info        Show more help information.
-s, --si          Read data from system standard input
--mfd=MFD2O       Mass fraction of water as D2O. Default is 0
--vfd=VFD2O       Volume fraction of water as D2O. Default is 0
--fdeut=FDEUT     Fraction deuteration of non-exchangeable protein hydrogens.
                  Default is 0
--fhexch=FHEXCH   Fraction exchangeable hydrogens exchanged. Default is 1
--vbar=VBAR       Partial specific volume, cm^3/g. Default = 0.74
```

Example (equine myoglobin):

```
> protScattDens 1ymb.fasta
```

Output:

```
Number of residues = 153
Molar mass (all 1H) = 16951.26
Total hydrogens = 1212
Exchangeable peptide hydrogens = 149
Other exchangeable hydrogens = 112
Non-exchangeable hydrogens = 951

Protein X-ray scattering density = 1.227e+11 cm-2
Solvent X-ray scattering density = 9.392e+10 cm-2
X-ray scattering contrast = 2.881e+10 cm-2

Protein neutron scattering density = 1.831e+10 cm-2
Solvent neutron scattering density = -5.589e+09 cm-2
Neutron scattering contrast = 2.39e+10 cm-2
```

By default, the program calculates the molecular weight and scattering densities assuming that all of the hydrogens are ^1H and that the protein is dissolved in 100% H_2O The program also assumes that the ionizable groups are in the predominant state at neutral pH, with no funny pK_as.

The D_2O content of solvent can be specified as either mass fraction, with the --mfd option or volume fraction, with --vfd. Either fraction should be a value between 0 and 1. If a non-zero D_2O concentration is specified, this is used to calculate the appropriate solvent scattering density and the fraction of ^2H in the protein. The number of chemically exchangeable hydrogens is calculated from the amino acid sequence. The fraction of these hydrogens that are actually able to exchange (because of accessibility) can be specified with the --fhexch option. The default behavior is to assume that all of the exchangeable hydrogens do exchange.

If the protein has been biosynthetically deuterated, the fraction of non-exchangeable hydrogens replaced with deuterium can be set with the --fdeut option.

The protein volume is calculated from the molecular weight (of the fully ^{1}H form) and the partial specific volume, $\bar{v}$. The default value for $\bar{v}$ is 0.74 cm^3/g. Other values can be specified with the --vbar option.

Solvent densities are calculated assuming a temperature of 25°C.

ureaProps

Calculates mass density and scattering densities of urea solutions in H_2O and D_2O mixtures, at 25°C.
Required argument: Molar concentration of urea (0–8)
Writes calculated properties to standard output.
Options:

```
-h, --help   show help message and exit
-i, --info   Show more help information.
--vfd=VFD2O  D2O concentration as volume fraction of solution. Default is 0
```

Example:

```
> ureaProps 4
```

Output:

```
    4 M urea at 25 C:
        Mass density = 1.05874 g/cm-3
        Total volume fraction of water = 0.821
        Total fraction of H as deuterium = 0

        X-ray scattering density = 9.8823e+10 cm-2
        Neutron scattering density = -6.8325e+08 cm-2
```

By default, the program assumes that the solution is made with natural-abundance water. A non-zero volume-fraction of D_2O (for the entire solution) can be specified using the --vfd option.

The density of urea solutions in H_2O are calculated using a third-order polynomial fit to the data of Gucker *et al.*:

> Gucker, Jr., F. T., Gage, F. W. & Moser, C. E. (1938). The densities of aqueous solutions of urea at 25 and 30° and the apparent molal volume of urea. *J. Am. Chem. Soc.*, 60, 2582–2588.
> http://dx.doi.org/10.1021/ja01278a008

The densities of H_2O and D_2O are calculated from the data of Kell (see the documentation for the waterProps program). The calculations assume that the volumes of H_2O and D_2O add ideally, with and without urea.

Example for 50% D_2O:

```
> ureaProps 4 --vfd=0.5
    4 M urea in 50 percent D2O (v/v) at 25 C:
        Mass density = 1.11242 g/cm-3
        Total volume fraction of water = 0.821
        Total fraction of H as deuterium = 0.517

        X-ray scattering density = 9.8653e+10 cm-2
        Neutron scattering density = 3.3913e+10 cm-2
```

waterProps

Calculates mass density and scattering densities of H_2O and D_2O mixtures, at temperatures between 0 and 100°C.
Required argument: Temperature, in °C
Writes calculated properties to standard output.
Options:

```
-h, --help   show help message and exit
-i, --info   Show more help information.
--mfd=MFD2O  Mass fraction of water as D2O. Default is 0
--vfd=VFD2O  Volume fraction of water as D2O. Default is 0
```

Example:

```
>  waterProps 20 --vfd=0.5
```

Output:

```
50 percent D2O (v/v) at 20 C:
     Mass fraction D2O = 52.5 percent
     Mass density = 1.05177 g/cm-3
     X-ray scattering density = 9.3843e+10 cm-2
     Neutron scattering density = 2.9032e+10 cm-2
```

By default, the program assumes that the sample is natural-abundance water. A non-zero volume- or mass-fraction of D_2O can be specified using the --vfd or --mfd options, respectively.

The temperature-dependent densities of H_2O and D_2O are calculated from the data and empirical functions of Kell:

> Kell, G. S. (1975). Density, thermal expansivity, and compressibility of liquid water from 0° to 150° C: Correlations and tables for atmospheric pressure and saturation reviewed and expressed on 1968 temperature scale. *J. Chem. Eng. Data*, 20, 97–105.
> http://dx.doi.org/10.1021/je60064a005
>
> Kell, G. S. (1967). Precise representation of volume properties of water at one atmosphere. *J. Chem. Eng. Data*, 12, 66–69.
> http://dx.doi.org/10.1021/je60032a018

The calculations assume that the volumes of H_2O and D_2O add ideally. For justification of this assumption, see:

> Bottomley, G. A. & Scott, R. L. (1976). Excess volumes for H_2O + D_2O mixtures. *Aust. J. Chem.*, 29, 427–428.
> http://dx.doi.org/10.1071/CH9760427

4.3.9 Simple shell scripts for data processing

The USToo Python programs, like other programs that can be executed from a Unix shell, can also be included in shell scripts, providing a means of automating data processing. A shell script is simply a text file containing commands that are executed sequentially when the script, itself, is executed. Shell scripts can be very simple or quite sophisticated, and even a rudimentary treatment is beyond the scope of this document. The examples provided here are only meant to be a starting point, and much more information can be found on the internet.
The following is a simple script that does a background subtraction, bins the data, desmears it and then generates a Guinier plot:

```
#! /bin/bash
saxsSubtract RNAseA_buffer.pdh RNAseA.pdh
saxsBinData -n 5 RNAseA_diff.pdh
saxsDeSmear desmearParam.txt RNAseA_diff_bin.pdh
saxsGuinier 0.03 0.06 RNAseA_diff_bin_dsm.pdh
```

The first line identifies the script as being targeted to the bash shell. There are several different shell environments in the Unix world, each with its own peculiarities and syntax. The bash shell is widely used as the default for both Linux and Mac OS X systems, but others are also available. Each line in the script above executes one of the USToo programs, just as if the line were typed at the shell prompt. The script takes advantage of the default naming scheme used by the programs, so that the name of each output file can be used for the input to the next command. The script assumes that the starting files, RNAseA_buffer.pdh and RNAseA.pdh, as well as the desmearing parameter file (desmearParam.txt), are in the same directory as the script when it is executed. Another important detail is that the final line must include a "line feed" character, created by pressing the return key, so that the final command will be executed. This creates a blank line at the end of the file.

By convention, shell scripts are saved with the extension ".sh", so that this script might be saved as process.sh. After saving the script, it can be executed with the Unix source command:

```
> source process.sh
```

This will cause each of the commands to executed in turn. Each program generates a plot window and pauses until the window is closed. Thus, the programs wait for the user to dismiss the result from the previous one.

Shell scripts can also be made to behave like shell commands by setting the executable bit with the chmod command. The following command will make the script executable by its owner:

```
> chmod u+x process.sh
```

After this is done the script can be executed simply by typing:

```
> process.sh
```

If you prefer not to see the intermediate results of the processing steps, the "none" plot type option can be used:

```
#! /bin/bash
saxsSubtract RNAseA_buffer.pdh RNAseA.pdh -t none
saxsBinData -n 5 RNAseA_diff.pdh -t none
saxsDeSmear desmearParam.txt RNAseA_diff_bin.pdh
saxsGuinier 0.03 0.06 RNAseA_diff_bin_dsm.pdh
```

This suppresses the plots from saxsSubtract and saxsBinData, but the saxsDeSmear plots will still appear, unless the appropriate setting in the parameter file is set to turn the plot display off.

A further refinement involves using the pipe feature of the shell to direct the output of one program to the input of another, without writing the intermediate files to the disk. An example is shown below:

```
#! /bin/bash
saxsSubtract RNAseA_buffer.pdh RNAseA.pdh -t none --so | \
saxsBinData -n 5 --si -t none | \
saxsDeSmear desmearParam.txt --si > RNAseA_diff_bin_dsm.txt
saxsGuinier 0.03 0.06 RNAseA_diff_bin_dsm.txt
```

In this case, the output of the first program, saxsSubtract, is directed to standard output using the --so option and is piped to the input of the second command, saxsBinData, using the "|" symbol. This requires that the --si option be used with the saxsBinData command. Also, note the backslash ("\") character at the ends of the first and second line. When piped commands are typed at the shell prompt, all of the commands are typed on the same line, before the return key is pressed. The backslash tells the interpreter to treat the next line as a continuation of the current line. In the case above the first three lines are all treated as a single line, as they would be typed at the shell prompt. In this script, the output of saxsDeSmear is directed to a new file, with the name specified in the script, which is then used as the input to saxsGuinier.

A final example introduces the use of variables in the shell script:

```
#! /bin/bash
dataFileName=$1
bufferFileName="RNAseA_buffer.pdh"
rootName=${dataFileName%%.pdh}
ext="_diff_bin_dsm.pdh"
outFileName="$rootName$ext"
saxsSubtract $bufferFileName $dataFileName -t none --so | \
saxsBinData -n 5 --si -t none | \
saxsDeSmear desmearParam.txt --si > $outFileName
saxsGuinier 0.03 0.06 $outFileName
```

In the bash shell language, the variables $1, $2, *etc.* correspond to arguments that are typed at the command line when executing the script, just as arguments for other commands are typed. In this case, $1 is used to allow the user to specify the data file name at the command line, so that the script is executed by typing, for instance:

```
> process.sh RNAseA.pdh
```

The string "RNAseA.pdh" is automatically assigned to the variable $1. The role of the "$" in variable names also requires a bit of explanation. When a variables is assigned a value, it is named without the "$", but when the value assigned is later used, the "$" is used. Thus, in the line:

```
dataFileName=$1
```

the value already stored as $1 is assigned to a new variable called dataFileName. In subsequent lines, the value stored in dataFileName is accessed as $dataFileName. Other lines in this script manipulate the string stored in dataFileName (*i.e.* the file name given when the script is executed) to generate the name for the output file, which is stored in the variable outFileName.

Appendix A

List of symbols and numerical constants

β_T - Isothermal compressibility of a liquid, with units of Pa^{-1}.

θ - 1/2 of the scattering angle

λ - X-ray or neutron wavelength

ρ_m - Scattering density (or scattering length density) of the molecule, with units cm^{-2}.

ρ_{solv} - Scattering density of the solvent, with units cm^{-2}.

σ - Scattering cross section, in units of area (cm^2 or barn = $10^{-20}\,\mathrm{cm}^2$).

Ω - Unit of solid angle, with unit of steradian.

$\frac{d\sigma}{d\Omega}$ - Microscopic differential scattering cross section, with units of area (cm^2)

$\frac{d\Sigma}{d\Omega}$ - Macroscopic differential scattering cross section, with units inverse length (cm^{-1}). Common definition of "absolute scattering intensity".

$A(q)$ - Scattering wave amplitude at scattering vector magnitude q.

b_e - Scattering length of an electron = 2.81794×10^{-13} cm.

c - Concentration, in $\mathrm{g/cm}^3$.

$C(q)$ - Fourier transform of the inter-particle correlation function, $c(r)$, used to calculate the structure factor, $S(q)$.

CF - Calibration factor used to convert normalized scattering intensities to absolute intensities, with units of cm.

$I(q)$ - Scattering intensity at scattering vector magnitude q.

$I'(q)$ - Scattering intensity normalized by the attenuated beam intensity.

$I_i(q)$ - In the Lake desmearing algorithm, the i^{th} approximation to the desmeared intensity.

$I_{i,s}(q)$ - In the Lake desmearing algorithm, the smeared form of the i^{th} approximation to the desmeared intensity.

$I_s(q)$ - Scattering intensity, smeared by beam or detector dimensions.

$J(q)$ - Flux of scattered X-rays or neutrons at scattering vector magnitude q, in units per steradian.

J_0 - Flux of the irradiating source, in units of inverse area (cm^{-2}).

k - The Boltzmann constant = 1.380658×10^{-23} J/K

M - Molar mass, with units of g/mol.

N_A - Avogadro's number, 6.0221367×10^{23} molecules/mole.

N_m - Number of molecules in the irradiated volume.

$P(q)$ - Form factor, the contribution of intermolecular distances to the scattering intensity, $I(q)$ at scattering vector magnitude q.

q - Magnitude of the scattering vector, $q = (4\pi \sin\theta)/\lambda$. Also used as a unit of length in describing the instrument geometry.

$S(q)$ - Structure factor, the contribution of interference between waves scattered from different particles (interparticle interference) to the scattering intensity, $I(q)$ at scattering vector magnitude q.

T - Temperature.

T_s - Sample transmission by X-rays or neutrons, expressed as a fraction.

$\bar{v}$ - Partial specific volume, with units cm^3/g.

$v(x)$ - Weighting function of position along the beam width (x), used to describe smearing effect.

V_s - Irradiated sample volume.

$w(y)$ - Weighting function of position along the beam length (y), used to describe smearing effect.

x - Distance along the beam width in a slit-collimated instrument, expressed in q-units.

y - Distance along the beam length in a slit-collimated instrument, expressed in q-units.

y_d - Distance along the detector width, parallel to the direction of the beam length in a slit-collimated instrument, expressed in q-units.

Z - Atomic number