

SAXS Calibrants

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1 Introduction

SAXS (small-angle x-ray scattering) calibrants are used to accurately determine the sample-to-detector distance, which allows one to compute q (reciprocal-space lengths) for the image on an area detector. This document should help you understand how to find and use the various x-ray calibrants available in the laboratory, or make your own.

The essential criteria for any good calibrant are good reproducibility among samples, reasonably strong (intense) scattering, an appropriate length scale, and several orders of diffraction (a well-ordered sample). Broadly speaking, there are two types of calibrants: those that rely on a periodic structure in the calibrant, and those that exploit the geometrical form factor of the calibrant's constituents. These are discussed in turn.

2 Periodic Calibrants

Periodic calibrants are the traditional choice. In the laboratory, we use two types available immediately: silver stearate ($d = 48.68 \text{ \AA}$) and silver behenate [1, 2] ($d = 58.376 \text{ \AA}$). For larger d -spacings, a common biophysical standard is rat collagen. The lattice constant d is given variously in the literature as $668 \text{ \AA} - 670 \text{ \AA}$. (The former number is Eric Eikenberry's value.)

The silver stearate and silver behenate calibrants are easy to prepare. Fill a capillary at least 1 cm tall with the dry powder and seal it. (Flame sealing is easiest in this case, although the powder will burn if you do not leave at least little space). These samples must be stored in light-tight containers

since they will slowly photodegrade. One solution is to wrap a test tube in black electrical tape and store the capillary within. To make the specimen to detector distance more precise, place a 0.1 or 0.2 mm filled capillary within a 1.0 or 1.5 mm capillary. Silver stearate and silver behenate are strong scatterers, so there will still be a sufficient signal.

The rat collagen is more difficult, and considerably more gruesome. (Eric Eikenberry is the expert on this protocol). Take a pair of dull wire cutters and snip a fresh rat tail 3/4 of the way through. Strip out the tendons and drop them into a petri dish containing a standard physiological buffer. The buffer should consist of 20 mM phosphate (pH 7.4) and 150 mM sodium chloride. Keep the tendons wet. Once dry, they will not rehydrate fully, leading to an irreproducible length scale in the range 620 Å to 650 Å.

The tendons are sticky threads which can be threaded through an open (acid-washed) capillary with some difficulty. Be sure to add buffer before sealing the capillary. Because this is animal tissue, the sample will only stay fresh a few days.

2.1 Theory

To calibrate an image, it is sufficient to know the specimen-to-phosphor distance z and the x-ray wavelength λ .

An x-ray diffraction image from periodic calibrants exhibits Bragg scattering peaks centered at θ given by Bragg's law,

$$n\lambda = 2d \sin \theta \tag{1}$$

where d is the lattice spacing mentioned previously. The x-ray scattering vector $\mathbf{q} = \mathbf{k}_f - \mathbf{k}_i$, has a length

$$q = 2k \sin \theta = \frac{4\pi}{\lambda} \sin \theta \tag{2}$$

as shown in figure 1(a). Then (1) is more simply written

$$qd = 2\pi n . \tag{3}$$

An x-ray diffracted through 2θ will strike the detector (phosphor screen or image plate) at a distance p from the center. As shown in figure 1(b),

$$\tan 2\theta = \frac{p}{z} \tag{4}$$

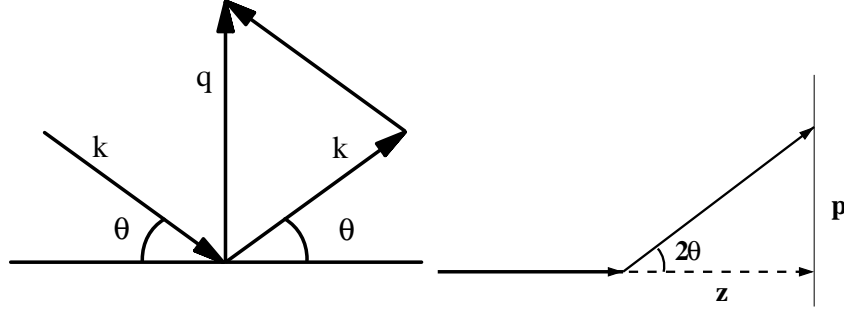


Figure 1: (a) Geometric illustration of $q = 2k \sin \theta$. (b) The beam is diffracted through an angle of 2θ .

where z is the sample-to-detector distance.

Approximating $\theta \approx \sin \theta \approx \tan \theta$ and equating θ in (2) and (4),

$$q = \frac{2\pi p}{\lambda z}. \quad (5)$$

Thus from the wavelength λ and the sample-to-detector distance z , q can be calculated for any transverse length p along the detector. (For Cu K α radiation, $\lambda = 1.54 \text{ \AA}$.)

This procedure for calibrating an image (given z and λ) can be reversed to determine z (given a calibrant of known d and λ). Combining (2) and (5),

$$z = \frac{pd}{n\lambda}. \quad (6)$$

The peaks occur at positions p . Fitting these positions to a straight line, the slope then yields z directly. This same procedure is used by TV6, as described in the next section.

To test the validity of the small-angle approximation, consider silver stearate. This has the smallest d -spacing and hence the largest angle per diffraction order. If you used only the $n = 10$ diffraction peak and the small-angle approximation, you would compute $d = 48.4755 \text{ \AA}$ instead of $d = 48.68 \text{ \AA}$ (a relative error of 0.4%). In general, you would use all visible orders $n = 1, 2, \dots, 10$, so the error would be considerably less. Even in this case the error in z will be small. In contrast, the $n = 10$ diffraction peak for rat collagen produces only a 0.002% relative error by the small-angle approximation.

2.2 Software

Using TV6, the procedure is as follows. Collect a diffraction image from the calibrant. The ideal image will have many orders of diffraction, with both (left and right) first-order peaks visible. Having at least one order on each side of the center helps to determine the $q = 0$ point accurately. Example images are shown in figure 2, and integrated profiles in figure 3.

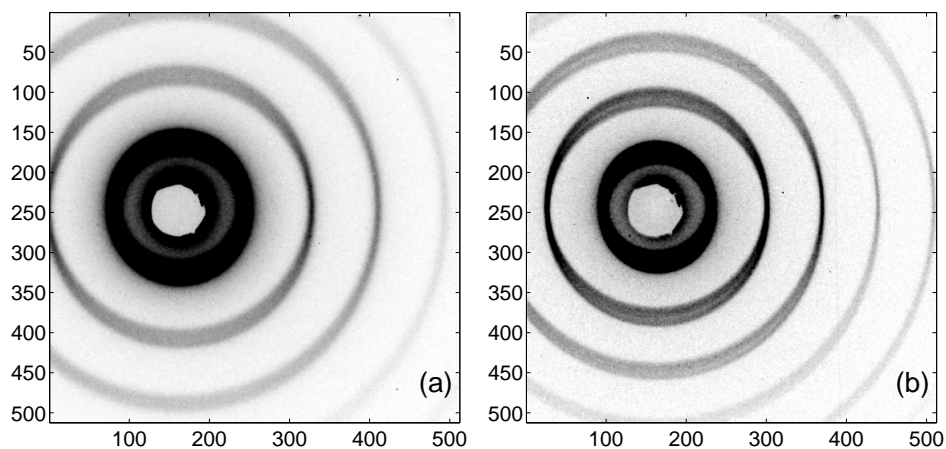


Figure 2: Intensity maps of (a) silver stearate, (b) silver behenate.

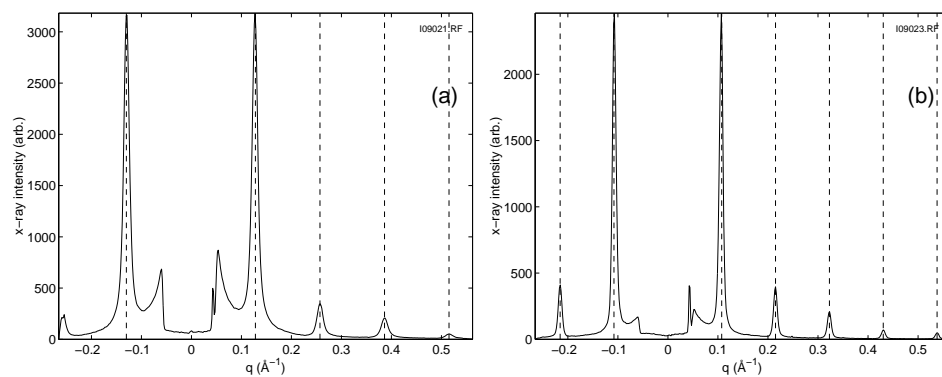


Figure 3: Intensity profiles of (a) silver stearate, (b) silver behenate. These correspond to the images in figure 2.

After creating an intensity profile with `dens`, enter `peak`. Select the peaks

by moving the mouse and entering numbers corresponding to the diffraction order. Press `c` to calibrate, and `d` to find the spec-to-phos distance. Enter the calibration length. You can check this calibration by fitting with `f` and regraphing with `g`, then showing tick marks with `t`. Record z , the spec-to-phos distance in pixels. This enables you to recreate the calibration later without repeating all of these steps.

Alternatively, you can do this in Matlab. In TV6, save your data as an `rfile`; this can be read by Matlab. Assuming the name of your `rfile` is `rfilename.rf`:

```
>> [x,y] = read_rfile('rfilename.rf');  
>> qt_slice(x,y)
```

Then, choose `Calibration -> Define calibration` and answer the prompts. This defines a calibration by determining z given the λ you enter. You can then calibrate this same image by choosing `Calibration -> Calibrate` or select another image first (`File -> Load`).

3 Spherical Calibrants

3.1 Materials

At present, four containers of spherical calibrants are available. First are polystyrene spheres from Duke Scientific (3050A) of radii $25 \text{ nm} \pm 1.0 \text{ nm}$ and 1% concentration. Also from Duke Scientific (5020A) are larger spheres of radii $101.5 \text{ nm} \pm 2.2 \text{ nm}$ and 10% concentration. Rudolf Sprik generously gave us two vials of silica spheres. The one labelled μEC1 is of radii of $27.8 \text{ nm} \pm 0.4 \text{ nm}$, while the $\mu\text{ESiJ3-113}$ has nominal radii of 136 nm.

3.1.1 Procedures

The procedure for handling the colloidal suspensions, as given by Duke Scientific:

“For ease of use, these standards are packages in an aqueous suspension. They must be thoroughly dispersed in the bottle to assure statistically consistent samples. Allow the contents to come to room temperature before use. To disperse the particles, gently invert the bottle several times, then immerse in a low power ultrasonic bath (30 seconds). Do not shake the bottle, as the small bubbles formed may introduce statistical artifacts. Before using, be sure no solid material or clumps are visible inside the bottle. Clear

the dropper tip by dispensing 2–3 drops into a waste container. Dispense immediately after dispersion using the dropper tip.”

With these precautions, filling a capillary with any solution straightforward. I prefer to flame-seal the tops.

3.2 Theory

3.2.1 Derivation of the Spherical Form Factor

Following references [3, 4], consider a sphere with radius R and uniform electronic density ρ_0 (relative to the surrounding medium),

$$\rho(r) = \begin{cases} \rho_0 & r < R \\ 0 & r > R \end{cases} . \quad (7)$$

In general, the Fourier transform is

$$A(\mathbf{q}) = \int d\mathbf{r} e^{-i\mathbf{q}\cdot\mathbf{r}} \rho(\mathbf{r}) , \quad (8)$$

where $\rho(\mathbf{r})$ is the two-point density-density correlation function. However, because $\rho(\mathbf{r})$ is spherically symmetric in this case,

$$A(q) = \int_0^\infty 4\pi r^2 dr \frac{\sin qr}{qr} \rho(r) . \quad (9)$$

Substituting (7) and integrating, the scattering amplitude is

$$A(q) = 4\pi \frac{\sin qR - qR \cos qR}{(qR)^3} , \quad (10)$$

and the measured intensity is

$$I(q) \propto A(q)A^*(q) \propto \Phi^2(qR) . \quad (11)$$

The function $\Phi(x = qR)$ is defined as

$$\Phi(x) = \frac{3}{x} j_1(x) = 3 \frac{\sin x - x \cos x}{x^3} \quad (12)$$

where $j_1(x)$ is the spherical Bessel function. An ideal plot of intensity $I(q)$ is shown in figure 4.

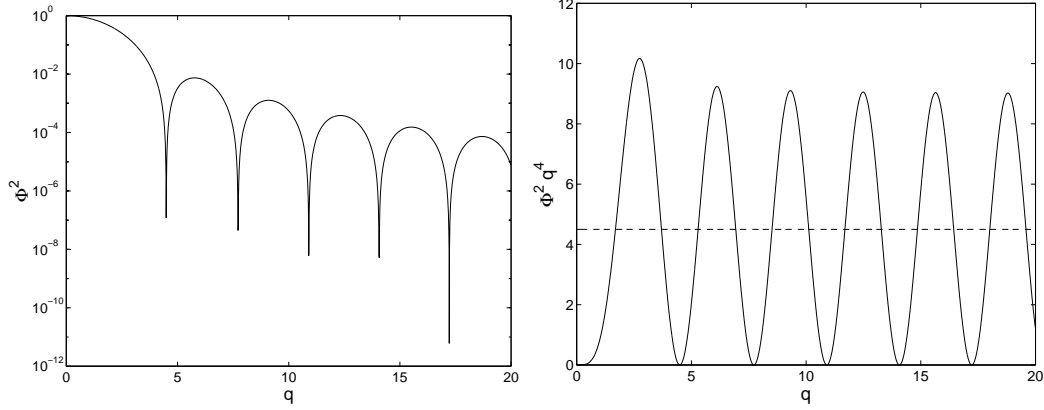


Figure 4: (a) $\Phi^2(q)$ vs. q on a semi-log plot. (b) $\Phi^2(q) \times q^4$ vs. q (a “Porod plot”). The dashed line is $9/(2q^4)$, following (21).

3.2.2 Behavior of $I(q)$

First, examine the limiting forms of the spherical form factor, $\Phi(x)$. At low x , Φ approaches unity.

$$\lim_{x \rightarrow 0} \sin x - x \cos x = (x - x^3/3! + x^5/5! + \dots) - x(1 - x^2/2! + x^4/4! + \dots) \quad (13)$$

$$\approx x - x^3/6 + x^5/120 - x + x^3/2 - x^5/24 \quad (14)$$

$$= x^3/3 - x^5/30 \quad (15)$$

so

$$\lim_{x \rightarrow 0} \Phi(x) = 1 - x^2/10 + \dots \quad (16)$$

The average value of the intensity factor $\Phi^2(x)$ can be found from expanding

$$\Phi^2(x) = 9/x^6 (\sin x - x \cos x)^2 \quad (17)$$

$$= 9/x^6 (\sin^2 x + x^2 \cos^2 x - 2x \sin x \cos x) \quad (18)$$

$$= 9/x^6 (\sin^2 x + x^2 \cos^2 x - x \sin 2x) \quad (19)$$

By taking the average over the oscillating terms,

$$\lim_{x \rightarrow \infty} \langle \Phi^2(x) \rangle = \frac{9}{x^6} \left(\frac{1}{2} + \frac{x^2}{2} \right) \quad (20)$$

$$\approx \frac{9}{2} \frac{1}{x^4} \quad (21)$$

So by multiplying the intensity I by $2/9x^4$, we obtain the forward scattering. This can be seen from figure 4b.

The intensity minima occur where $\Phi(x) = 0$, that is, $x = \tan x$. For large x , this approaches $x = n\pi + \pi/2$. So, if many diffraction minima can be seen, their spacing will yield qR directly. The intensity maxima occur where $d\Phi/dx = 0$, or

$$x^2 \sin x + 3x \cos x - 3 \sin x = 0 . \quad (22)$$

The first zeros are at $x = 0, 5.7635, 9.0950, 12.3229, 15.5146, 18.6890, 21.8539$. For both minima and maxima, $\lim_{qR \rightarrow \infty} \Delta(qR) = \pi$. That is, qR is spaced by approximately π for all but the first few diffraction rings. The maxima for R corresponding to calibrants we have are listed in table

n	qR	$R = 250\text{\AA}$	$R = 278\text{\AA}$	$R = 1015\text{\AA}$	$R = 1360\text{\AA}$
1	5.7635	0.2305	0.2073	0.0568	0.0424
2	9.0950	0.3638	0.3272	0.0896	0.0669
3	12.3229	0.4929	0.4433	0.1214	0.0906
4	15.5146	0.6206	0.5581	0.1529	0.1141
5	18.6890	0.7476	0.6723	0.1841	0.1374
6	21.8539	0.8742	0.7861	0.2153	0.1607
7	25.0128	1.0005	0.8997	0.2464	0.1839
8	28.1678	1.1267	1.0132	0.2775	0.2071
9	31.3201	1.2528	1.1266	0.3086	0.2303
10	34.4705	1.3788	1.2399	0.3396	0.2535

Table 1: Peaks in q for spherical calibrants. q values are all given in nm^{-1} .

With this information, the peak minima or maxima can be fit to determine R . Or if R is known (in the case of a calibrant), then q can be determined. However, one important advantage of spherical calibrants is that the $I(q)$ form has significance, unlike periodic calibrants, which can only provide a single length scale.

If there is any dispersity of radii, this will influence the intensity spectrum. Assuming a Gaussian distribution centered at x_0 with standard deviation σ , the observed intensity is

$$I(q) = \int_0^\infty dx \Phi^2(x) e^{-(x-x_0)^2/2\sigma^2} \quad (23)$$

n	qR	$R = 250\text{\AA}$	$R = 278\text{\AA}$	$R = 1015\text{\AA}$	$R = 1360\text{\AA}$
1	5.7635	272.54	303.07	1106.5	1482.6
2	9.0950	172.71	192.05	701.20	939.54
3	12.3229	127.47	141.75	517.53	693.43
4	15.5146	101.25	112.59	411.06	550.78
5	18.6890	84.049	93.463	341.24	457.23
6	21.8539	71.877	79.927	291.82	391.01
7	25.0128	62.800	69.833	254.97	341.63
8	28.1678	55.766	62.011	226.41	303.36
9	31.3201	50.153	55.770	203.62	272.83
10	34.4705	45.569	50.673	185.01	247.90

Table 2: Peaks in $d = 2\pi/q$ for spherical calibrants. d values are all given in \AA .

As discussed in references [3, 4], the intensity falls off as $\exp(-2\delta qR)$. Because of the reciprocal nature of q and R , a spread in incident wavelength λ will be indistinguishable from a spread in R . For this reason, the spherical calibrant data in this report were all acquired at CHESS where the the x-ray spectral width was narrow. Other effects, such as the size of the beam, will affect all modes equally and can be distinguished from the dispersion.

3.3 Software

The simplest calibration method is to determine the q spacing of peak maxima or minima far from the origin, as this value rapidly approaches π/R as n increases. The next approach is to fit the minima/maxima positions, in analogy to the procedure for periodic calibrants. Here, the peak positions are not linear with q , but can be numerically determined from (22) or table 1.

3.3.1 TV6

With TV6, you can fit the intensity maxima, but not the entire lineshape. Begin with a “finished” image. That is, it should be dezingered, background-subtracted, intensity-corrected, and distortion-corrected. For example, if your data images are `dat1` and `dat2` and backgrounds are `bak1` and `bak2`:

```
>> move im1=dat1:dat2
>> move im2=bak1:bak2
>> move im3=im1-im2
>> move im4=im3!imi
>> move im5=im4!imd
```

This assumes that you have set the distortion correction images (with `setdist`) and intensity correction images (with `setint`).

Display your image with `kut=0` (vary *scal* to taste):

```
>> disp im5 0 scal
```

Perform a `dens` with per-pixel normalization off; this enhances the weaker large- q peaks. Then, enter `peak` and edit the x-ray wavelength with `x`. Choose `y` to enter spherical calibration mode. Enter whole numbers corresponding to each peak order. (If you are running an older version of TV6, the `y` option may not be available. Choose `j` to enter d-spacings, and then pick values from table 2 corresponding to your calibrant.)

The next step is to choose `c` to calibrate and `d` (or `p` in older versions). Now, TV6 will print `z` as the spec-to-phos distance in pixels. Press `f` to show the results (in newer versions), so you can evaluate the quality of the linear fit.

TV6 is now calibrated. Future fits in this TV6 session will use this value for `z`.

3.3.2 Matlab

[Note: as of 2000-01-24, this may no longer be correct. The Matlab code is changing rapidly, and I have not verified this sequence of commands.]

The approach I take here is to fit the entire intensity profile.

To fit these in Matlab, load an `rfile` as described in section 2.2. To best see the intensity oscillations, choose `Options -> Use Circles` (off) and `Options -> Y-axis: Porod Plot`. Typically, the data near $q = 0$ may be overexposed and the data far from $q = 0$ will have a lot of noise and weak oscillations. To select only a portion of the data, choose `Action -> Crop` and select an range in x by clicking and dragging the mouse. (Only the x values are significant; the y -extent of the box is irrelevant.)

Now, choose `Estimate` and a box labeled `qtgui:function` will appear. Choose `Spherical Calibrants` and `constant` background. After the parameter box appears, you can enter parameters directly, or choose `Function -> Estimate` again from the main window. At this point, you can choose

Function -> Fit and check if the parameters are close by comparing the line shapes. Otherwise, enter new values into the parameter edit boxes and either Action -> Redraw or re-fit.

If the fit is successful, then you know the mean mean radius in these arbitrary (pixel) units R_{fit} , as well as the true radius R_{true} . We also have

$$qR_{\text{true}} = pR_{\text{fit}} \quad (24)$$

so

$$z = \frac{2\pi}{\lambda} \frac{R_{\text{true}}}{R_{\text{fit}}} \quad (25)$$

where z is in pixels.

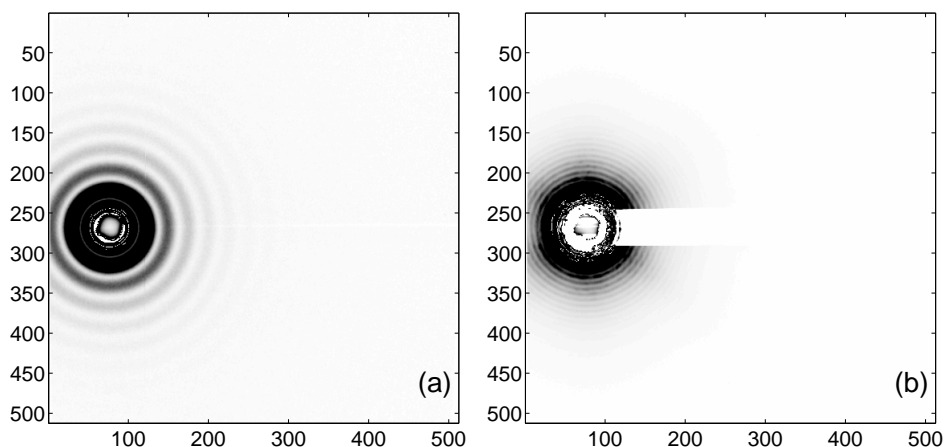


Figure 5: Intensity maps of (a) Silica spheres (“synthetic rat”), (b) Polystyrene spheres (“ACF p.58-1 2A”). Because of the strong scattering near the center, there is significant streaking towards the right in both images. Azimuthal integrations were performed vertically ($\theta = \pm\pi/2 \pm 25^\circ$).

4 Calibrant Inventory

All pre-made capillaries are in the top drawer of the first bench as you enter the wet room. These capillaries are detailed in table 3 below. Also in that drawer are materials for making spherical calibrant capillaries. In the dry box to the left are silver stearate and silver behenate.

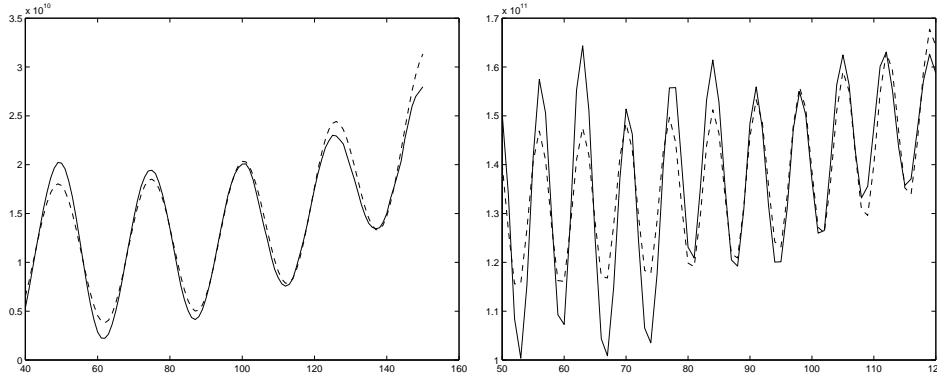


Figure 6: Porod plots of (a) Silica spheres (“synthetic rat”), (b) Polystyrene spheres (“ACF p.58-1 2A”). These correspond to the images in figure 5.

calibrant	length scale (Å)	quantity	keyword
Ag-stearate	48.68	6	ACF Ag-ST 3 ... 7; MWT 3-5-98
Ag-behenate	58.376 ± 0.006	5	ACF Ag-BE 1 ... 5
silica spheres	278 ± 4	6	“synthetic rat”; ACF p.41-2 3A ... 3E
silica spheres	1360	5	ACF p.41-2 4A ... 4E
polystyrene spheres	250 ± 10	2	ACF p.58-1 1B; ACF p.131-1 1C
polystyrene spheres	1015 ± 22	6	ACF p.58-1 2A; ACF p.41-2 2A ... 2E

Table 3: Inventory of calibrants available immediately. Length scales are nominal. Keywords match the labels on the test tubes holding the capillaries.

5 Cross-Calibrations

Cross calibrations are given in tables 4 and 5. These table were constructed by calibrating with the standard in the leftmost column and calculating the length scale (d -spacing or mean radius) of the other standards.

	Ag-ST	syn-rat	2A
Ag-ST	48.68	281.8	1012
syn-rat	47.82	278	994
2A	48.81	283	1015

Table 4: Data acquired on CHESS D-1 by Karen Edler (see labbook Finnefrock #2, pp. 29–32).

	Ag-stearate ACF #3	Ag-behenate ACF #5
Ag-stearate ACF #3	48.6799	58.3398
Ag-behenate ACF #5	48.7099	58.3757

Table 5: Data acquired on PhotoMetrics (see labbook Finnefrock #2, pp. 1–2).

6 Acknowledgements

Thanks to Karen Edler for taking time out of her CHESS run and acquiring so many exposures on the spherical calibrants. This was a tedious job. Thanks also to Krysta Levac for supplying me with rat tails, and sparing me the most gruesome task. Rudolf Sprik kindly gave us the silica spheres, and Lois Pollack lent them to me.

7 Conclusion

If you notice any errors in this document or have trouble using/making calibrants, please let me know.

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