DHR Series and AR Series

Small Angle Light-Scattering (SALS) Accessory

Getting Started Guide

Revision G Issued November 2013

Notice

TA Instruments — Waters LLC
159 Lukens Drive
New Castle, DE 19720

Introduction

Important: TA Instruments Manual Supplement

Please click the TA Manual Supplement link to access the following important information supplemental to this Getting Started Guide:

- TA Instruments Trademarks
- TA Instruments Patents
- Other Trademarks
- TA Instruments End-User License Agreement
- TA Instruments Offices

Notes, Cautions, and Warnings

This manual uses NOTES, CAUTIONS, and WARNINGS to emphasize important and critical instructions. In the body of the manual these may be found in the shaded box on the outside of the page.

NOTE: A NOTE highlights important information about equipment or procedures.

CAUTION: A CAUTION emphasizes a procedure that may damage equipment or cause loss of data if not followed correctly.

MISE EN GARDE: UNE MISE EN GARDE met l'accent sur une procédure susceptible d'endommager l'équipement ou de causer la perte des données si elle n'est pas correctement suivie.

A WARNING indicates a procedure that may be hazardous to the operator or to the environment if not followed correctly.

Un AVERTISSEMENT indique une procédure qui peut être dangereuse pour l'opérateur ou l'environnement si elle n'est pas correctement suivie.

Regulatory Compliance

Safety Standards

For Canada

CAN/CSA-C22.2 No. 61010-1 Safety requirements for electrical equipment for measurement, control, and laboratory use, Part 1: General Requirements.

CAN/CSA-C22.2 No. 61010-2-010 Particular requirements for laboratory equipment for the heating of materials.

For European Economic Area

(In accordance with Council Directive 2006/95/EC of 12 December 2006 on the harmonization of the laws of Member States relating to electrical equipment designed for use within certain voltage limits.)

EN 61010-1:2001 Safety requirements for electrical equipment for measurement, control, and laboratory use, Part 1: General Requirements + Amendments.

EN 61010-2-010:2003 Particular requirements for laboratory equipment for the heating of materials + Amendments.

For United States

UL61010-1:2004 Electrical Equipment for Laboratory Use; Part 1: General Requirements.

UL61010A-2-010:2002 Particular requirements for laboratory equipment for the heating of materials + Amendments.

Electromagnetic Compatibility Standards

For Australia and New Zealand

AS/NZS CISPR11:2004 Limits and methods of measurement of electronic disturbance characteristics of industrial, scientific and medical (ISM) radio frequency equipment.

For Canada

ICES-001 Issue 4 June 2006 Interference-Causing Equipment Standard: Industrial, Scientific, and Medical Radio Frequency Generators.

For the European Economic Area

(In accordance with Council Directive 2004/108/EC of 15 December 2004 on the approximation of the laws of the Member States relating to electromagnetic compatibility.)

EN61326-1:2006 Electrical equipment for measurement, control, and laboratory use-EMC requirements-Part 1: General Requirements. Emissions: Meets Class A requirements per CISPR 11. Immunity: Per Table 1 - Basic immunity test requirements.

For the United States

CFR Title 47 Telecommunication Chapter I Federal Communications Commission, Part 15 Radio frequency devices (FCC regulation pertaining to radio frequency emissions).

Safety

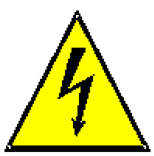
Do not attempt to service this accessory, as it contains no user-serviceable components.

Required Equipment

While operating this accessory, you must wear eye protection that either meets or exceeds ANSI Z87.1 standards. Additionally, wear protective clothing that has been approved for protection against the materials under test and the test temperatures.

Electrical Safety

You must unplug the instrument before doing any maintenance or repair work; voltages as high as 120/240 volts AC are present in this system.



WARNING: High voltages are present in this instrument. Maintenance and repair of internal parts must be performed only by TA Instruments or other qualified service personnel.

AVERTISSEMENT: Présence de tensions élevées dans cet instrument. La maintenance et la réparation des pièces internes doivent être effectuées uniquement par TA Instruments ou tout autre personnel d'entretien qualifié.

Radiation Danger

WARNING: The SALS Accessory uses a Class 2 laser product. Do not stare into beam.

AVERTISSEMENT: L'accessoire SALS utilise un produit laser de classe 2. Ne regardez pas fixement le faisceau.

Instrument Symbols

The following label is displayed on the accessory for your protection:

Symbol	**Explanation**
	This symbol on the front of the SALS Accessory warns that laser light is present during operation of this instrument. The SALS Accessory uses a Class 2 laser product. Do not stare into the beam. Ce symbole affiché à l'avant de l'accessoire SALS avertit de la présence d'un rayonnement laser pendant l'utilisation de cet instrument. L'accessoire SALS utilise un produit laser de classe 2. Ne regardez pas fixement le faisceau.

Please heed the warning labels and take the necessary precautions when dealing with those parts of the instrument. This manual is intended to be used in conjunction with the *DHR Getting Started Guide* and the *AR-G2/AR 2000ex/AR 1500ex Rheometer Getting Started Guide*. For full details on the operation of the rheometer and safety information, please refer to that manual. Both manuals contain cautions and warnings that must be followed for your own safety.

Table of Contents

Chapter 1:

Introducing the SALS Accessory

Overview

Small Angle Light Scattering (SALS) Accessory is a powerful tool for studying the properties of static and sheared dispersions. A beam of laser light is trained on the sample and is scattered by interactions with the electrons of objects within the sample. If the light is scattered through a small angle, the pattern formed can be observed using a two-dimensional sensor such as a USB camera. From this pattern, called the scattering pattern, information about the size, shape, orientation, and spatial distribution of the particles of the dispersion can be inferred.

Figure 1 SALS Accessory.

The TA Instruments SALS Accessory is a Smart Swap™ system designed for use with the DHR-3, DHR-3, and AR-G2/AR2000ex Rheometers. A patented, Peltier temperature-controlled lower plate, with sapphire window, allows the laser to be trained on the sample. The resultant scattered light is transmitted through the upper transparent quartz geometry and is observed by a lens and camera assembly. The camera images are stored with the data points and can be exported for additional treatment and analysis.

Scattering Theory

When electromagnetic radiation such as visible light encounters an object, interference between the radiation and the electrons of the object occurs. The result is a change in the direction of the radiation. The effect is most noticeable when the size of the object is similar in scale to the wavelength of the radiation, in which case the effect is called diffraction, or scattering. The pattern produced by the scattered radiation can be used to gain information on the size, shape, orientation and spatial distribution of the objects responsible for the scattering. Many of the structures produced by surfactant molecules, for example vesicles and wormlike micelles, have at least one dimension in the range that will scatter visible light, and the technique is well suited to their study.

In scattering theory, light is described by its angular wavenumber, k, defined as $2\pi/\lambda$ where λ is the wave-length of the light in the scattering medium. Since $\lambda = \lambda_0 / n$ where λ_0 is the wavelength of the light in a vacuum, and n is the medium refractive index, $k = 2\pi n/\lambda_0$. The dimensions of k are therefore (radians x) inverse length.

The light wavevector, k, is defined as k times a unit vector in the direction of the light. Since the scattering involved in SALS is almost completely elastic, the wavevector of the incident light, k_i, will have the same magnitude as the scattered light, k_s, such that $|k_i| = |k_s| = k$.

The scattering vector, **q**, is defined by $\mathbf{q} = k_s - k_i i$ (see the figure below).

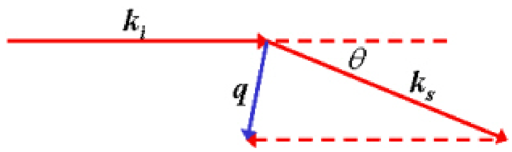


Figure 2 Scattering vector, q, and scattering angle, θ.

It can be shown by simple trigonometry that $q = 4\pi \sin(\theta/2)/\lambda$, where $q = |\mathbf{q}|$ and the scattering angle θ is the angle by which the direction of the light has changed.

The approximate idea of the length scales probed by the light can be obtained from Bragg's law:

$\lambda = 2 \text{ L } sin\phi$

where L is the length scale and the angle ϕ defined for Bragg's law is half the angle ϕ defined for SALS (by convention the symbol θ is used rather than ϕ for Bragg's law, but in this context that would be confusing). So:

$L = \lambda / 2 \ sin\phi = \lambda / 2 \ sin\ (\theta/2)$

So $L = 2\pi / q$

Thus the greater the length scale, the lower the value of q, and hence the smaller the scattering angle. In TA Instruments SALS system, the range of angles over which measurements can be made is about 6° to 26.8°. The wavelength of the laser used is 635 nm, and if the sample refractive index is taken to be that of water, i.e., 1.332, the q range is 1.38 μm^{-1} to 6.11 μm^{-1} and the length scale range is about 1.03 µm to about 4.6 µm.

The scattering angle can therefore be used to gain an idea of the length scale of the structures present in the sample. The intensity of the light and the scattering pattern give some idea of the spatial distributions of the particles, and their number density.

The Direction of Incident Light

In principle, the incident light can be in any direction relative to the shear direction, but it is usual to arrange it along one of the principle axes. In the TA Instruments system the shear gradient direction is used, so scattering occurs in the plane of the vorticity (neutral) direction - shear direction (see below).

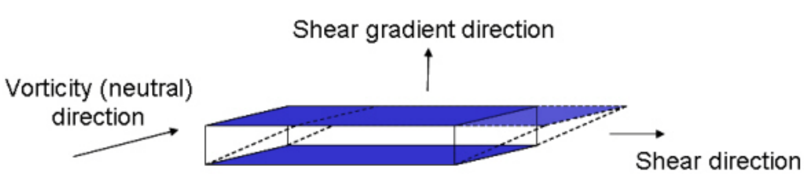


Figure 3 The three directions of a sheared sample.

Product Description

The laser used in the SALS Accessory is a Class II 0.95 mW diode laser, wavelength 635 nm, circular beam with diameter of 1.1 mm, beam divergence (typical) 0.7 mrad.

The upper geometry is a 50 mm diameter x 2 mm thick optical quartz disk, refractive index 1.457 at 635 nm. To comply with the criterion for the single-point correction for the parallel plate geometry [M.S. Carvalho, M. Padmanabhan and C.W. Macosko, *J. Rheol.* 38 (1994) 1925-1936], the laser is set at 0.76 x the quartz disk diameter, i.e., 19 mm, from the axis of rotation of the disk.

The image sensor's effective area is 6.6 mm x 5.3 mm, effective pixels 1280 x 1024, pixel size 5.2 µm square.

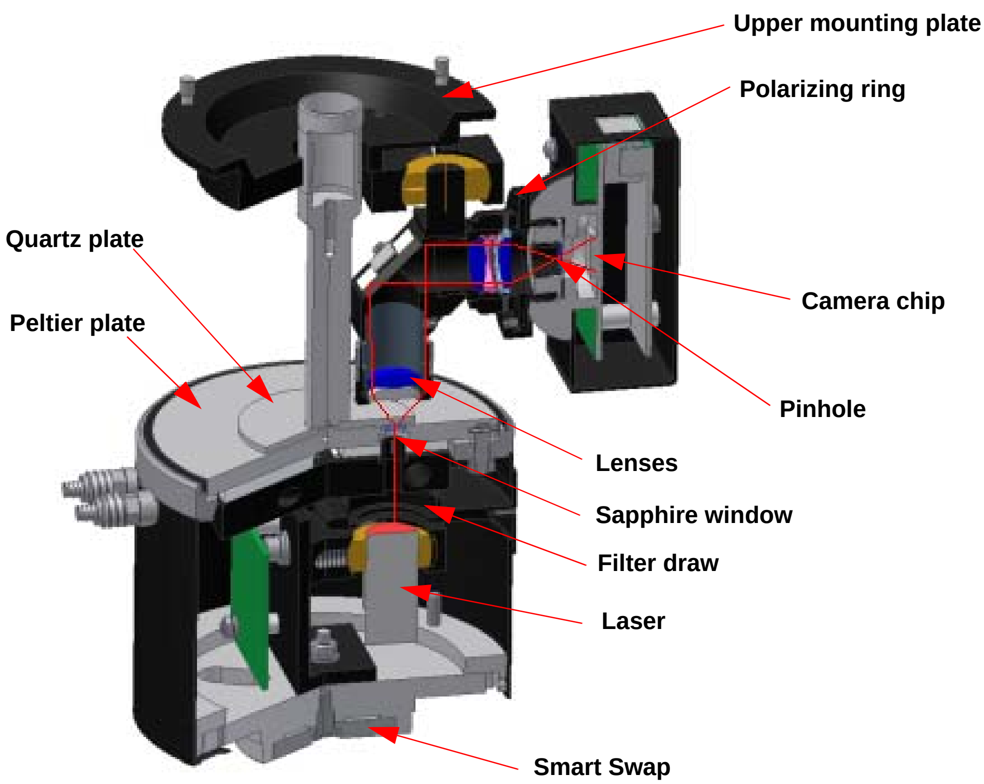


Figure 4 SALS Accessory components.

Figure 4 (above) provides a schematic of the SALS accessory components. A schematic of the optical system is shown in Figure 5. The pinhole is set at the focal point for 635 nm parallel light of convex lens 2; divergent or convergent light will not pass through to the sensor. The image point will, therefore, be at the focal point for parallel light of the convex (DCX) lens 1 – planoconvex (PCX) lens – quartz disc – sample system. The depth within the sample of this point will depend on the refractive index of the sample, and the position of the adjustable planoconvex lens. It should be noted that as a pin hole has a finite size, the sensor 'sees' light from a cylinder rather than a single point.

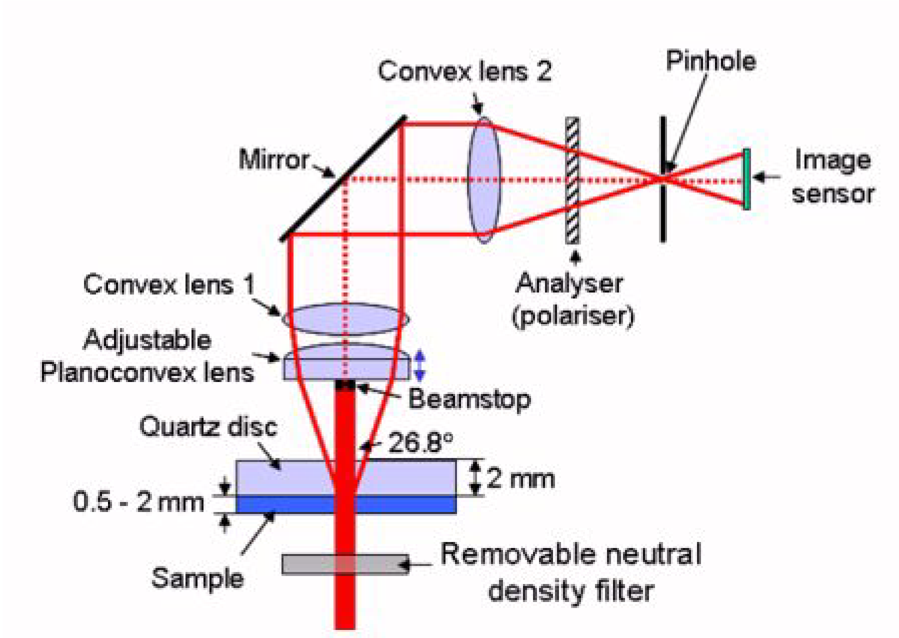


Figure 5 Schematic of TA Instruments SALS optics.

The position of the PCX lens is indicated by the inscribed scale. One rotation of the lens holder is equivalent to a travel distance of 1 mm. The zero position is assumed to be where the DCX and PCX lens are in contact.

A simplified view of the optics is given in Figure 6, in which the deflection through 90° is ignored (this does not affect principle of the optical system).

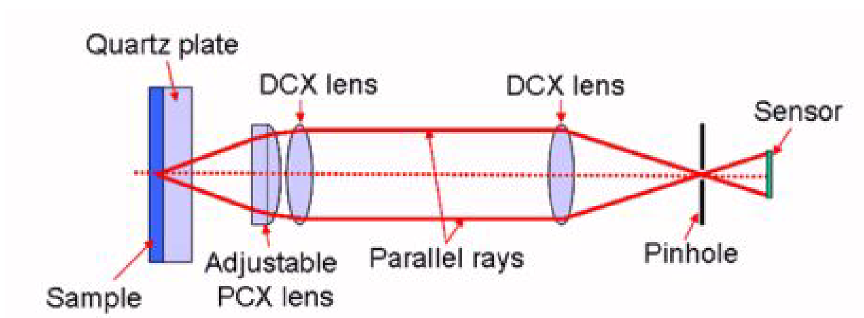


Figure 6 Simplified view of the SALS optics.

The maximum scattering angle will also depend on the position of the planoconvex lens and the refractive index of the sample.

Adjusting the Plane of Focus

The position of the planoconvex lens indicated in Figure 6 can be adjusted to alter the image point within the sample. It is expected that the plane of focus will be at or close to the sample midheight. The position of the lens that gives this will depend on the refractive index of the sample.

Polarization

The diode laser used produces plane polarized light. In the TA Instruments system, the direction of polarization is aligned with the shear direction. An analyzer (i.e., a second polarizer) is placed in the path of the diffracted light. There are two positions for the analyzer, parallel or perpendicular to the direction of polarization of the incident beam. The degree of polarization produced in the sample by shearing can be investigated by comparing the scattering patterns produced with analyzer in the parallel and perpendicular positions.

Intensity Adjustment

The intensity of the laser light itself cannot be adjusted, but the intensity of the incident light can be reduced by placing a neutral density filter between the laser and the sample.

Intensity Corrections

Corrections to the intensity are only required for level 3 experiments (see Chapter 3). The scattered light falls on a flat sensor consisting of an array of pixels. The absolute intensity of the light, I_{abs} is obtained from the intensity reported by the sensor, I_{sensor} through the relationship:

$$I_{abs} = \left(2^{bit} - 1\right)\left(\frac{I_{sensor}}{(2^{bit} - 1)}\right)^{1/\gamma}$$

where bit is the bit depth of the chip. The sensor used has a gamma (λ) value of 1.0, so this correction is not required.

However, a correction does need to be made for the fact that the flux of protons on each individual pixel will depend on the angle at which it hits, and the distance of the pixel from the scattering center. It can be shown that the correction for the angle is given by:

$I_{angcorr} = I_{abs} / \cos\theta$

where $I_{angcorr}$ is the intensity of the light corrected for the angle at which it hits.

The flux of protons on a fixed area is also inversely proportional to the square of its distance, D, from the light source, and since $D^2 = d^2 / \cos^2\theta$, where d is the distance between the source and the plate in the normal direction (Figure 7), the total corrected intensity, I_{corr} is given by:

$I_{corr} = I_{sensor} / \cos^3\theta$

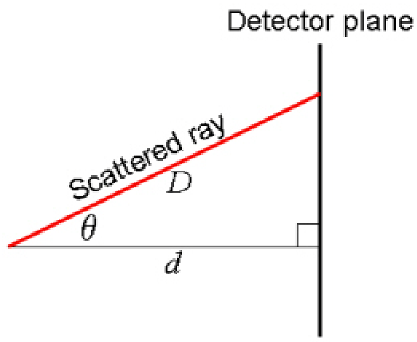


Figure 7 Paths for scattered (D) and unscattered (d) light.

Specifications

Table 1: SALS Accessory Specifications

q Vector Range	~1.38μm^{-1} to 6.11μm^{-1}
Scattering Angle	~6° to 26.8°
Laser	635nm 0.95mW class 2
Peltier Temperature Controlled	5 to 95°C

WARNING: The upper temperature limit is set by the optical adhesive that retains the sapphire window. Do no exceed this limit.

Avertissement: Le limite de température supérieure est réglée par l'adhésif optique qui retient la fenêtre de saphir. Ne dépassez pas cette limite.

Chapter 2:

Installing the SALS Accessory

Installing the SALS Accessory

The steps needed to attach the SALS Accessory to the rheometer involve the following:

1 Mounting the upper and lower fixtures

2 Attaching the upper geometry

3 Connecting camera and installing drivers

4 Aligning the system

Mounting the Upper and Lower Fixtures

Follow these steps to mount the fixtures:

1 Ensure that the rheometer is turned on. Raise the head to the maximum travel.

2 Attach the upper fixture to the mounting ring on the underside of the instrument head using the three captive screws provided.

NOTE: The camera should project to the right of the instrument when viewed from the front.

3 Mount the lower fixture on the rheometer using the Smart Swap connection.

4 Remove lens cover from upper assembly.

Attaching the Upper Geometry

The procedure for attaching a geometry to the instrument spindle is as follows:

1 Push the geometry up the spindle and hold it while locating the draw rod in the screw thread of the geometry.

2 Screw the draw rod down, turning it clockwise. It should be screwed finger tight but not forced.

To remove the geometry, perform this operation in reverse.

Connecting the Camera and Installing the Drivers

To get your camera up and running, follow these steps before plugging in the camera USB cable:

1 Download the LuCam Software V6.0.3 or higher from the link below:

http://www.lumenera.com/support/downloads/industrial-downloads.php

2 Run the downloadable executable LuCamSoftware-v6.0.3.exe and follow the instructions given.

3 Once installed, plug in the camera to a spare USB port and follow the instructions to install from the “recommended” location.

Aligning the System

The laser in the base and the surface of the Peltier Plate are factory aligned and should not require any adjustment.

A small amount of adjustment will be necessary to align the upper fixture whenever it is fitted. This is most easily achieved by using a sample with a known scatter pattern.

The scattering from a random arrangement of spherical particles, with diameters greater than the wavelength of the incident light, results in series of concentric rings that diminish in intensity as the scattering angle increases (Mie scattering). Polystyrene micro particles of nominal diameter (3 µm) are suitable for this purpose, and these can be obtained from Sigma-Aldrich (Fluka), product no. 80304. They are supplied at a solids content of 2% by weight and should be diluted by a factor of about 200 using purified water to eliminate multiple scattering.

Follow these steps to adjust the alignment:

1 Turn on the rheometer and start TRIOS Software.

2 Set the temperature of the Peltier Plate to 25°C.

3 Ensure upper geometry and lower plate are clean and free from lint.

4 Zero the gap.

5 Raise the head.

6 Set the focal point to 500 µm by setting the adjustable front lens to 1.35. See the figure below.

Figure 8 SALS front lens.

7 Ensure the polarizer ring (analyzer) on the upper fixture is set to “in parallel.” This is when the ring is rotated fully up, as shown in the image below.

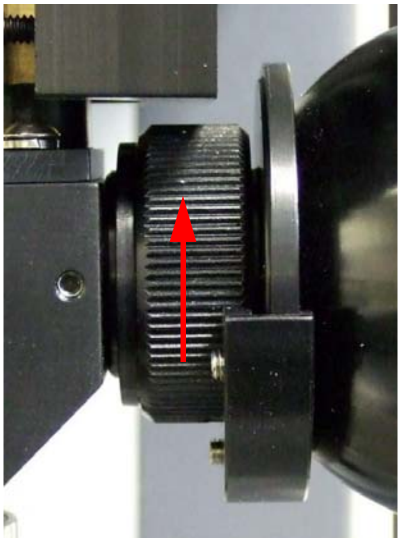


Figure 9 Polarizer ring.

8 Remove any neutral density filter from the drawer in the lower fixture. See Figure 10 below.

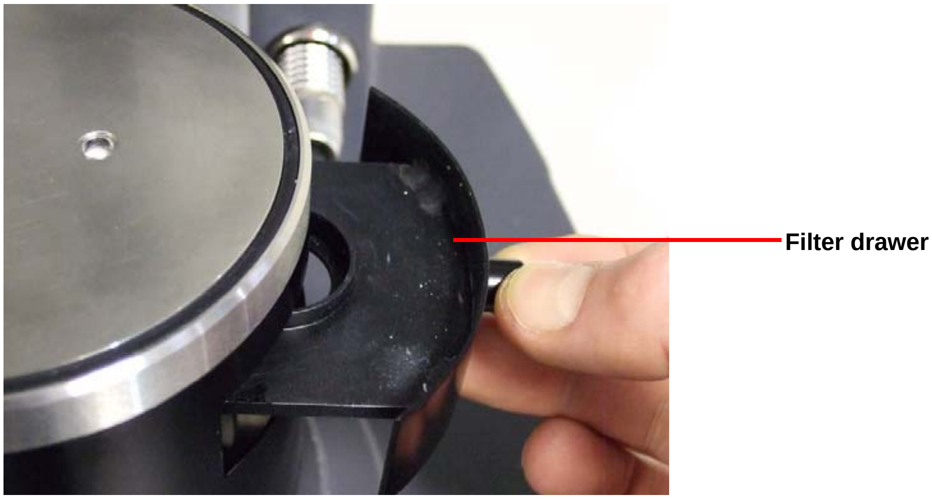


Figure 10 Removing the filter.

9 Place two or three drops of the alignment solution on the window in the Peltier Plate.

10 Turn on the bearing lock and close the geometry gap to 1000 µm.

11 Turn on the laser from the TRIOS Control panel.

12 Turn on the camera image by using the camera icon on the **View** ribbon. For ease of use, it is convenient to have a PC in a dual monitor configuration so that the scatter image can be viewed on a separate screen.

13 Adjust the body of the upper fixture in the swivel mount (see below, left) until you obtain a pattern of concentric rings similar to those shown in the below-right image.

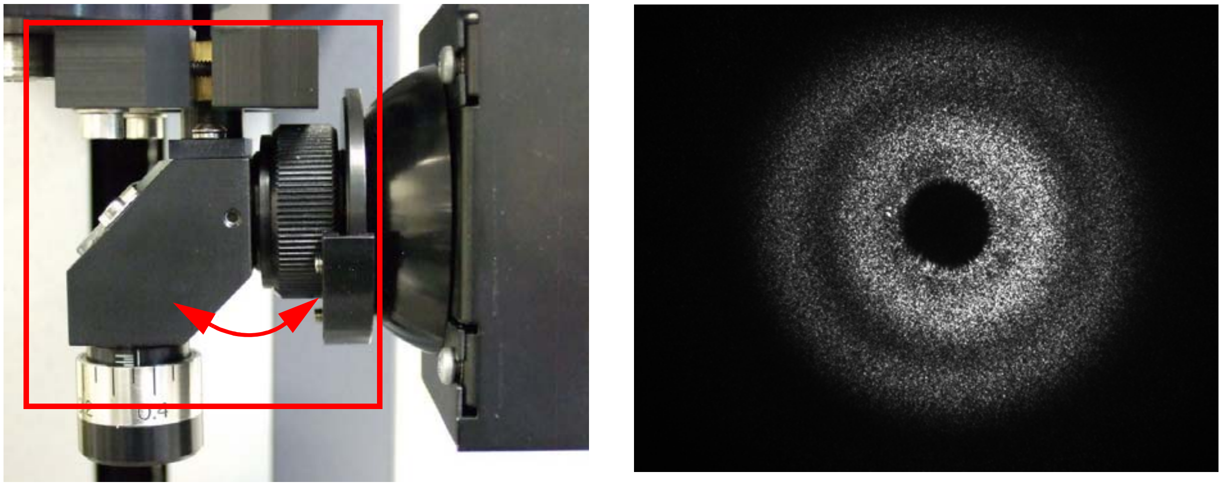


Figure 11 Swivel mount.

NOTE: It may be necessary to adjust the camera settings such as exposure, brightness, and contrast by accessing the Camera properties on the camera setup screen (right-click on image). The lighting controls are not used by this system.

14 Once you are happy with the image and the system is aligned, raise the rheometer head. The sample can then be cleaned from the plates.

Chapter 3:

Calibrating the SALS Accessory

Preparing the SALS

Before starting an experiment you need to decide what you want to learn from the scattering data as this will impact greatly on the amount of calibration and data treatment required. The simplest use (level 1) might be to collect the scattering image during an experiment, and then look at anisotropy in the image to infer structure changes within the sample that match features in the rheological data. The next level in complexity (level 2) might be to process the images to provide better clarity. Finally, you may wish to gather absolute data for numerical analysis (level 3).

Each level requires a different amount of preparation and data handling. Images can be saved as JPEG or Bitmap format. The level of experiment will determine the choice of format.

Level 1 Experiments

Images can be collected together with the rheological data and viewed in the Data Analysis package. Images can be collected in JPEG format to reduce file size.

Level 2 Experiments

The rheological test needs to be designed to allow multiple images to be collected at fixed shear conditions (for example, a series of peak hold steps). These images can be exported, averaged, have backgrounds subtracted, and a false color applied. A program such as ImageJ, a public domain Java image processing program available at http://rsb.info.nih.gov/ij/, is a suitable tool. Images should be collected and exported in bitmap format to avoid any loss due to compression. It is also important that the camera exposure is locked and other system settings remain unchanged, if backgrounds are to be subtracted.

Level 3 Experiments

In addition to the requirements detailed in Level 2, it will also be necessary to calibrate the system. See the next section, "Calibrating the Scattering Angle" .

Calibrating the Scattering Angle

Calculation of the Scattering Angle

The relationship between the scattering angle and the pixel position on the sensor can, in principle, be calculated directly from the characteristics of the optical system, but variables such as the lens and sensor positions are not known sufficiently precisely to make this a reliable way of determining that relationship.

Mie Scattering

A more accurate method of obtaining the relationship between the scattering angle and the pixel position is to calibrate the system using the scattering pattern obtained from a well-characterized sample.

The Mie scattering pattern from the sample previously used for system alignment can be used for this purpose.

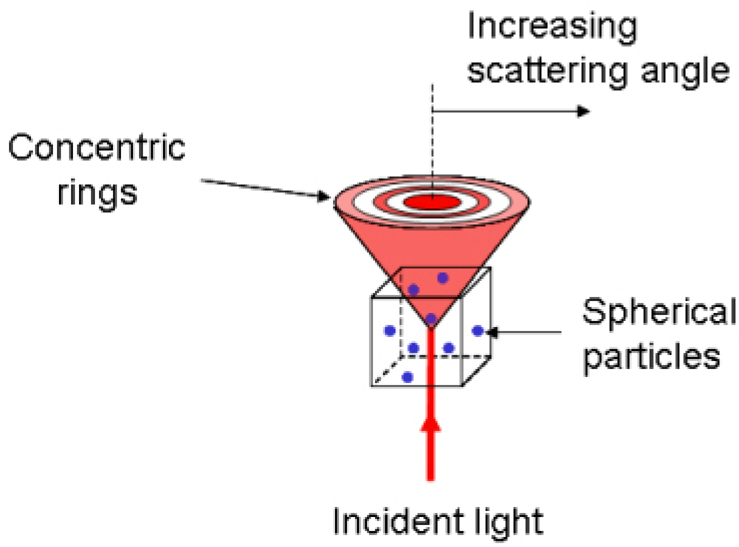


Figure 12 Mie scattering.

Using the Laven Program

The scattering pattern for microparticles of this size can be calculated using a public domain program available at http://www.philiplaven.com/mieplot.htm. The author is Philip Laven, of the European Broadcasting Union, Geneva, Switzerland. Unfortunately, the program was not designed to be used with SALS systems, so the option of plotting scattering intensity, *I*, against *q* value is not available. This means that *q* values have to be calculated using, for example, Microsoft® Excel.

The Laven program main window is shown below. Some of the information that needs to be entered is given on the Certificate of Analysis supplied with the microparticles. The other entries are either standard values or are specific to the TA Instruments SALS system.

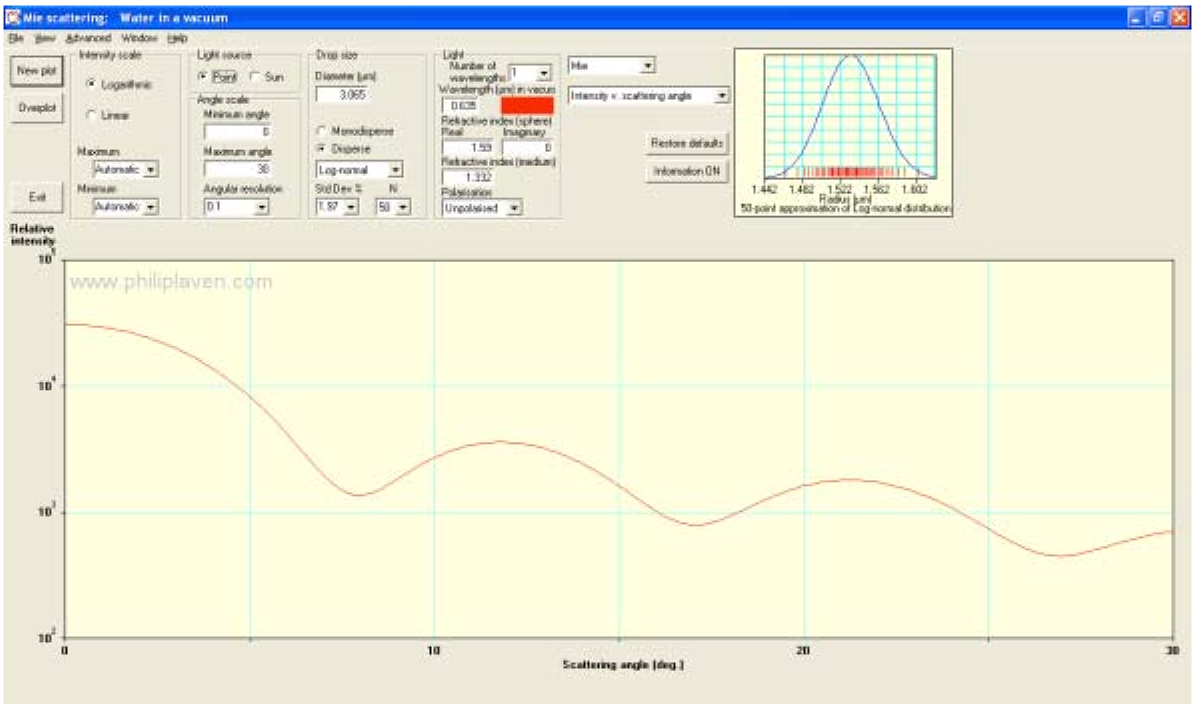


Figure 13 Laven program main window.

To use the Laven program:

1 Select from the following parameters:

Parameter	Instructions for SALS
Intensity Scale	Select **Logarithmic**. Leave **Maximum** and **Minimum** as **Automatic**.
Light Source	Select **Point**.
Angle Scale	**Minimum angle** and **Maximum angle**. These refer to the scattering angle in degrees; typically 0 and 30 respectively seem appropriate. **Angular resolution**. This is the resolution to which the Mie curve will be calculated. If it is set too high, the curve will not be smooth. If it is set too low, the calculation will be excessively time-consuming. A value of 0.1 gives a reasonable compromise.
Drop Size	**Diameter (µm)**. Enter the value for **Calibrated Particle Diameter** from the Certificate of Analysis, but note that the default for the Mie program is radius. Diameter can be selected from the **Advanced** menu. Select **Disperse** and scroll to either **Normal** or **Log-normal** (it doesn't seem to matter which is selected). **Std Dev %** (i.e., standard deviation %). Enter the value given on the Certificate as **CV** (i.e., coefficient of variance), which is the same thing. **N** is the number of slices in the particle size distribution. The higher this is set, the longer the calculation takes, but it is fast enough when left on 50.
Light	**Number of wavelengths** leave as 1, as laser light is monochromatic. **Wavelength (µm) in vacuo**. Enter the wavelength of the laser light, which is 0.635 µm. **Refractive index (sphere)**. For **Real** use the value given on the Certificate for Refractive index (strictly speaking, this was not measured at the correct wavelength or possibly temperature, but the difference will be negligible). For **Imaginary** use zero. **Refractive index (medium)**. Use the refractive index of water, which is about 1.332 (as with the spheres, this varies with wavelength and temperature, but the effect is negligible). **Polarization**. Select as appropriate. Either **perpendicular** or **parallel** options can be used with TA Instruments SALS system.

2 For the remaining two text boxes, select **Mie** and **Intensity v. scattering angle**.

3 Click **New plot** to perform the calculation. The results can be exported as a text file from the **File** menu, which can then be opened directly by Microsoft Excel.

The first column in the results text file is headed Angle theta (these values are in degrees). The data in this column can be converted to q values using the formula $q = 4 \pi n / \lambda_0 \sin(\theta / 2)$, where n is the medium refractive index (~ 1.332 for water), and λ_0 is the wavelength of the laser light in a vacuum. Since the units of q are usually μm^{-1}, the laser wavelength should also be in µm, i.e. 0.6328 (NB Excel uses radians for angles). This line works:
=(SIN(A20*2*PI()/360))*4*PI()*1.332/0.6328

Preparing and Loading the Calibration Sample

Follow steps 1 to 12 of the alignment procedure, lock the camera exposure, and then capture the image. To improve the data quality, it is best to record several images at this point, for later averaging. An oscillatory time sweep at the minimum stress in continuous controlled stress mode is suitable for this task.

Once the Mie scattering images have been recorded, a background image of purified water should be recorded, which will later be subtracted from the Mie scattering image. Raise the instrument head and remove the sample of spheres, clean the upper and lower plates, and replace the sample with two or three drops of purified water. Close the geometry gap to 1000 µm, and record the image (as with the Mie scattering images, it is best to record several images for later averaging). It is critical that the focussing and camera exposure are not adjusted between recording the Mie and water images. To prepare images for manipulation, load the results files into the Data Analysis package and export the images.

Manipulation of the measured images can be done using a program such as ImageJ, a public domain Java image processing program available at http://rsb.info.nih.gov/ij/. The plug-in Radial Profile Angle should also be downloaded.

Using ImageJ

Follow these steps:

1 Start ImageJ. The program main window appears.

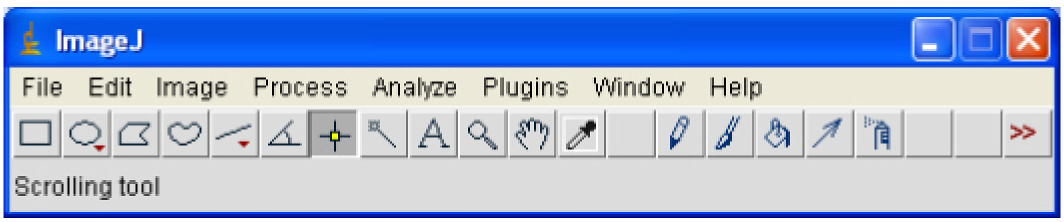


Figure 14 ImageJ main window.

2 Choose **File** > **Open**. Navigate to where the Mie scattering files are and open them.

3 Select **Image** > **Stacks** > **Convert Images to Stack**. This collects all the open images ("slices") so that they can be manipulated together. For example, to calculate the average intensity of each pixel for all the images in the stack, from the menu bar choose **Image** > **Stacks** > **Z Project**. The window shown below displays.

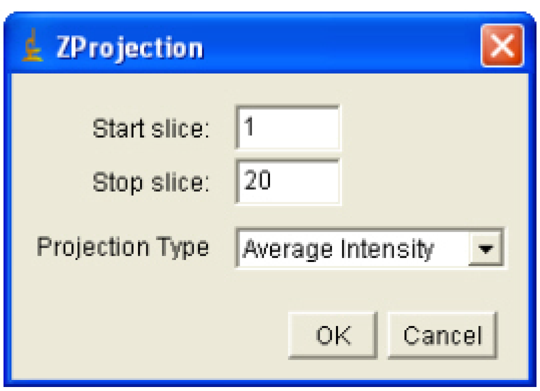


Figure 15 ImageJ ZProjection window.

4 Enter the desired values and click **OK**.

The averaged image should resemble that shown below. A similar process can be used to obtain a background image for water.

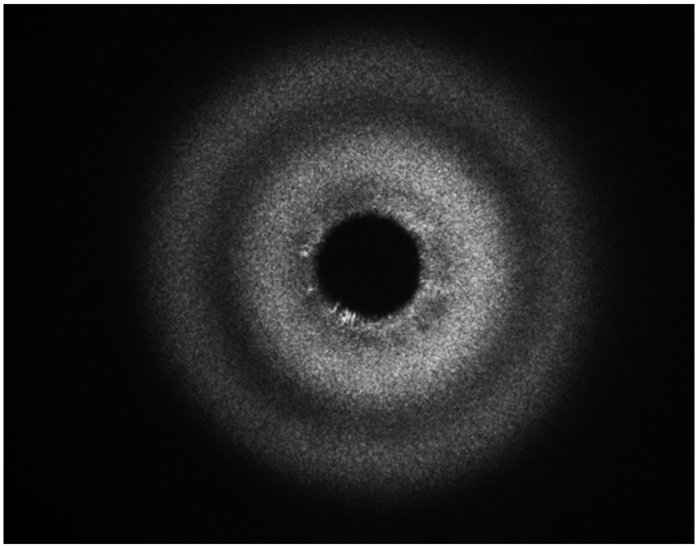


Figure 16 Averaged Mie scattering image in ImageJ.

Once you have both averaged images, the background is subtracted using the **Image Calculator** window (shown below) selected from the **Process** menu.

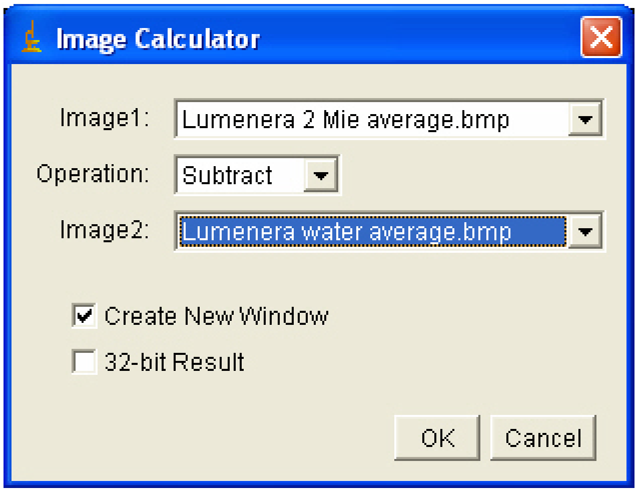
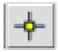


Figure 17 Image Calculator window in ImageJ.

5 When the background correction has been made, click the Point selections icon  on the toolbar.

6 Place the crosswires as close to the center of the beamstop as possible, and click to fix (the position can be adjusted later, if it is not correct).

7 Choose **Plugins**>**Radial profile angle**. The window shown below displays.

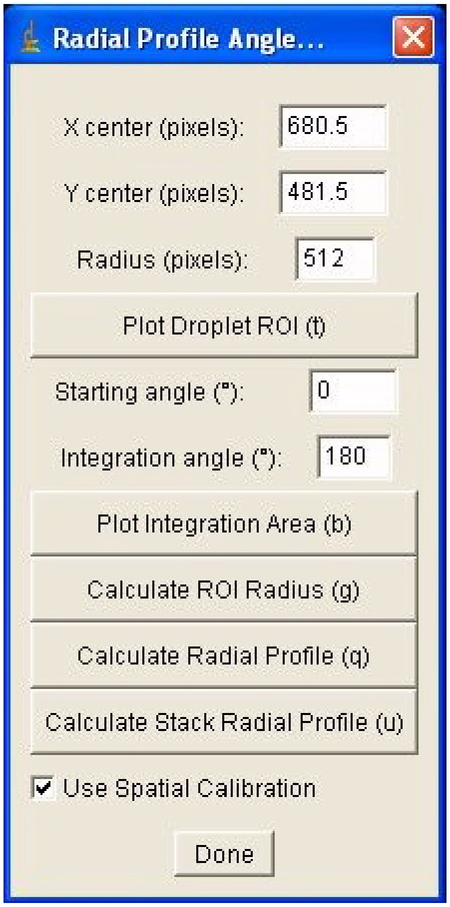


Figure 18 Radial Profile Angle window from ImageJ.

8 Input the radius over which the integration is to be performed, in pixels. To take full advantage of the camera chip, this should be set to 512. The integration angle is on both sides of the starting angle, so the full angle is twice that entered. To integrate over the whole plot, use a starting angle of 0° and an integration angle of 180°.

9 Click **Plot Integration Area**. The radii between which the integration is to be performed will appear as yellow lines, as shown in Figure 20. The x and y centers and the angles can be adjusted at this point.

10 Click **Calculate Radial Profile**, and an image similar to the one below displays. The plot is intensity against distance from the center point, in pixels. The intensity is adjusted to account for the increase in the number of pixels with increasing radius, but further corrections need to be made for the sensor properties and solid angle. This can be done using Microsoft Excel, for example.

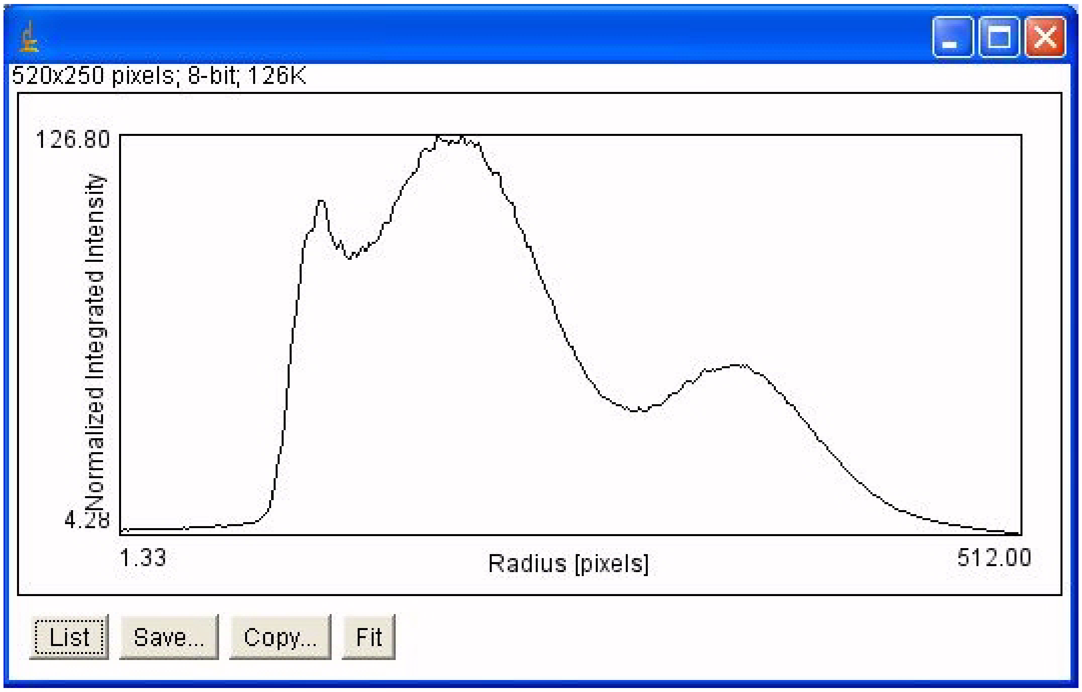


Figure 19 Intensity against pixel radius plot from ImageJ

11 Save the data as a text file by selecting **List** > **File** > **Save as**. The data can then be compared with that obtained from the Mie calculation to obtain intensity correction factors that can be applied to sample test data. An example using Microsoft Excel is shown in the next section.

Calculating the Optics Correction Factor

The two system calibration factors are determined using a worked example in Excel.

This section shows a worked example using Microsoft Excel, Laven data and Intensity vs. Radius (pixels) date from ImageJ.

Open the .txt file created by the Laven program in Excel. See the figure below.

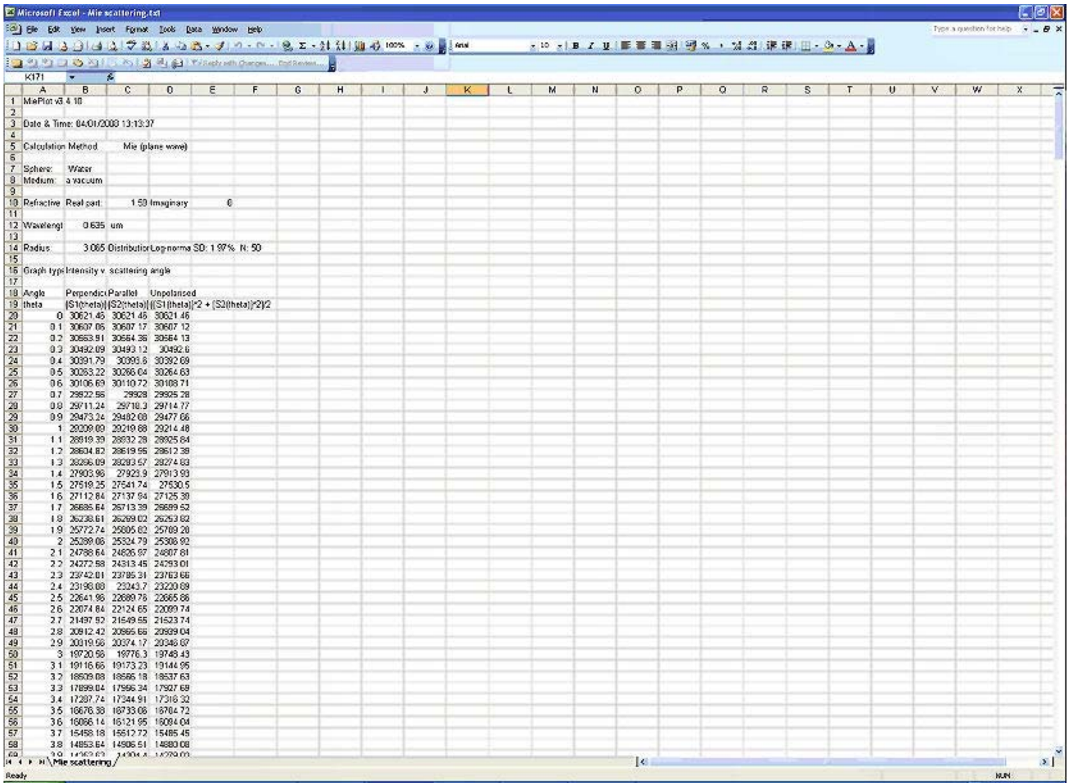


Figure 20 Excel spreadsheet displaying Laven data.

Add the data below into the cells indicated, copying the formulae down to line 385.

	J	K	L	M	N	O	P
1	y shift	Radius [pixels]	Intensity	Angle	I_{corr}		Correction Factor
2	25			=(180/ PI())*ATAN(K2*G$5)	=(L2*J$2)/P2		=(COS(M2*PI()/ 180))^3
385				=(180/ PI())*ATAN(K2*G$5)	=(L2*J$2)/P2		=(COS(m2*PI()/ 180))^3

Also add the optics factor at G5. This value, together with the y shift, is approximate and will be modified later.

	G
4	Optics Factor
5	1.1e-3

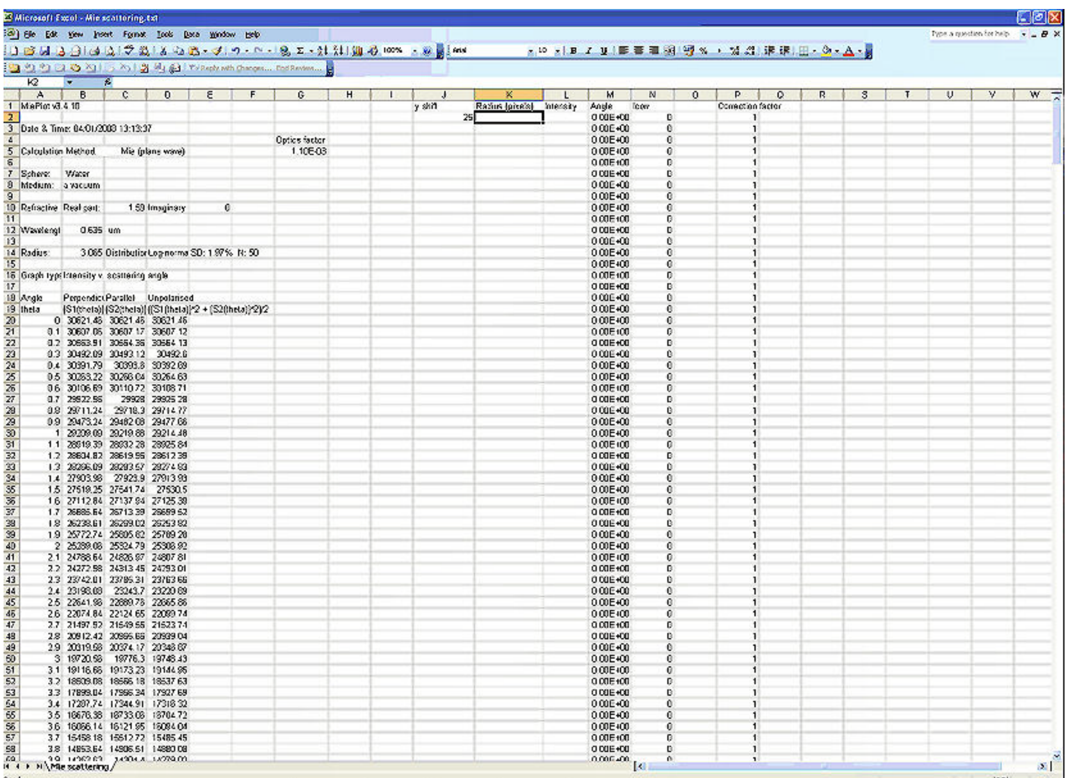


Figure 21 Adjusted data.

Paste the data from ImageJ into columns K and L.

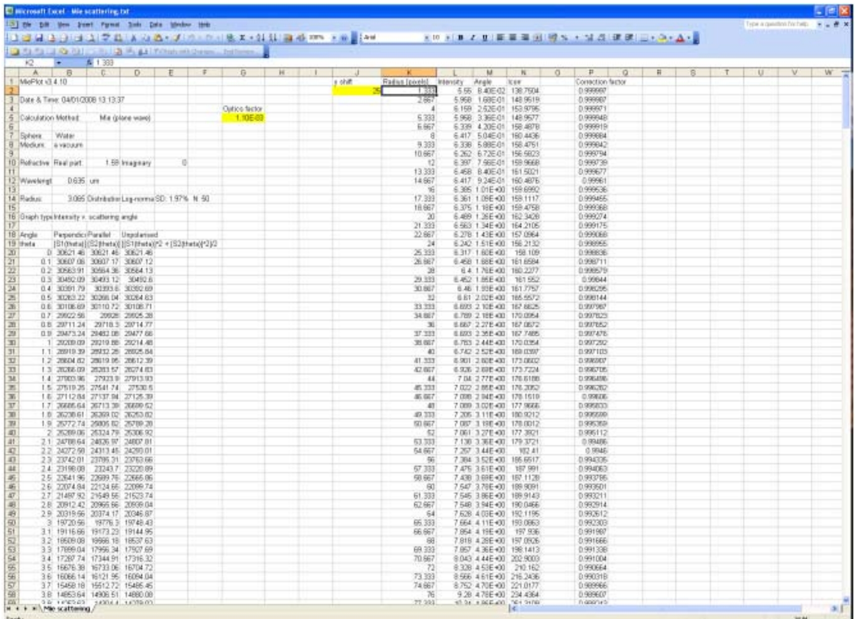


Figure 22 Data pasted.

Create a xy overlay graph of D vs. A and N vs. M. Scale the y axis to 5000 maximum.

Adjust the y shift so that the first peaks of each curve have the same maximum.

Adjust the **Optics Factor** so that the first peaks of each curve overlap. The quality of the overlap of the second peak will depend on the dilution factor of the spheres solution. See the figure below.

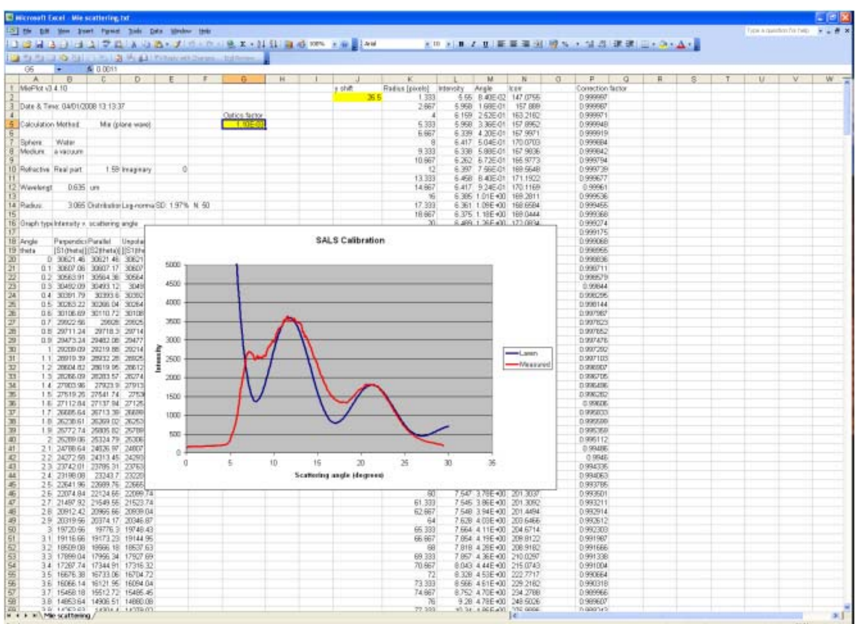


Figure 23 Adjusting the optics factor.

In this example the y-shift is 26.5 and the Optics Factor 1.1e-3. These values can now be used to correct sample data, as long as all the mechanical and camera settings remain unchanged, by simply pasting ImageJ data into columns K and L.

Chapter 4:

Using the SALS Accessory

Overview

Once the SALS accessory has been properly installed, aligned, and calibrated (if needed), follow the directions in this chapter to load your samples, set the focal positions, lock the exposure, and select the desired image format for your results.

Loading the Sample

The sample can be loaded in the usual way for a parallel plate, i.e., by raising the instrument head, placing the sample on the lower plate, bringing the head back to the required geometry gap, and trimming the sample to the edge of the upper plate. But it is important when performing a SALS experiment that there are no entrained bubbles in the sample, as these will tend to disrupt the scattering pattern. Unfortunately, many of the samples that are likely to be used with the SALS system are particularly susceptible to this problem. Some way to ensure that no bubbles are formed or a way to remove them must be found.

With some samples you can close the geometry gap to about twice that normally used for the experiment, and inject the sample very gently into the gap using a wide-tipped plastic pipette to prevent trapped bubbles. The gap can then be slowly brought to its working position. If any bubbles do become entrapped, they can sometimes be removed using a needle pipette.

Setting the Focal Position

It is not essential to use a geometry gap of 1000 microns, but it is important that the focal position be within the sample. The table below shows the approximate depth of the focal point within the sample, predicted for a sample of refractive index 1.333 (i.e., equal to that of water), for the adjustable front lens positions taken from the scale.

Table 2: Depth of Focus and Maximum Scattering Angle for Front Lens Positions

Adjustable Lens Position	Depth with Sample (mm)
1.0	0.29
1.35	0.49
1.5	0.68
2.0	1.07
2.5	1.44
3.0	1.81
3.5	2.17
4.0	2.52

Locking Exposure

Exposure, as well as other camera settings, are made by accessing the camera **Settings** on the **Image** panel in TRIOS Help.

Selecting the Image Format

See *Using the Camera* in TRIOS Software Online Help for more information.

Exporting Images

See *Using the Camera* in TRIOS software Online Help for more information.

Index

C

I

L

N

P

R

S

T

W