Anton Paar

Measure
what is measurable
and make measurable
that which is not.

Galileo Galilei (1564-1642)

Instruction Manual

Litesizer™ 500

Light-Scattering Instrument
for Particle Analysis

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Disclaimer

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Contents

1 About the Instruction Manual

This instruction manual informs you about the installation and the safe handling and use of the product. Pay special attention to the safety instructions and warnings in the manual and on the product.

The instruction manual is a part of the product. Keep this instruction manual for the complete working life of the product and make sure it is easily accessible to all people involved with the product. If you receive any additions or revisions to this instruction manual from Anton Paar GmbH, these must be treated as part of the instruction manual.

Conventions for Safety Messages

The following conventions for safety messages are used in this instruction manual:

WARNING

Description of risk
Warning indicates a hazardous situation which, if not avoided, **could** result in death or serious injury.

CAUTION

Description of risk
Caution indicates a hazardous situation which, if not avoided, could result in minor or moderate injury.

NOTICE
Description of risk
Notice indicates a situation which, if not avoided, could result in damage to property.

CAUTION

Hot surface
This symbol calls attention to the fact that the respective **surface can get very hot**. Do not touch this surface without adequate protective measures.

CAUTION

Laser radiation
The instrument is equipped with a laser of the laser class 3B, which is integrated in the Litesizer™ 500, and therefore conforms to the laser class 1. Follow your national safety regulations.

TIP. *Tip gives extra information about the situation at hand.*

Typographical Conventions

The following typographical conventions are used in this instruction manual:

Table 1-1: Typographical Conventions

Convention	Description
<key>	The names of keys and buttons are written inside angle brackets.
"Menu Level 1 > Menu Level 2"	Menu paths are written in bold, inside straight quotation marks. The menu levels are connected using a closing angle bracket.

Software Screenshots

The screenshots depicted in this manual are representative only. They were taken from Kalliope Version 1.2.0, and may not reflect the latest version of the software.

2 Safety Instructions

- Read this instruction manual before using the Litesizer™ 500.
- Follow all hints and instructions contained in this instruction manual to ensure the correct use and safe functioning of the Litesizer™ 500.

2.1 General Safety Instructions

2.1.1 Liability

- This instruction manual does not claim to address all safety issues associated with the use of the instrument and samples. It is your responsibility to establish health and safety practices and determine the applicability of regulatory limitations.
- Anton Paar GmbH only warrants the proper functioning of the Litesizer™ 500 if no modifications have been made to the mechanics, electronics, or firmware.
- Only use the Litesizer™ 500 for the purpose described in this instruction manual. Anton Paar GmbH is not liable for damages caused by incorrect use of the Litesizer™ 500.

2.1.2 Installation and Use

- The installation procedure should only be carried out by authorized personnel who are familiar with the installation instructions.
- Do not use any accessories or spare parts other than those supplied or approved by Anton Paar GmbH (see section 4, "Supplied Parts").
- Make sure all operators are trained to use the instrument safely and correctly before starting any applicable operations.
- In case of damage or malfunction, do not continue operating the Litesizer™ 500. Do not operate the instrument under conditions which could result in damage to goods and/or injuries and loss of life.

2.1.3 Moving the Litesizer™ 500

To move the Litesizer™ 500:

1. Switch off the appliance and unplug all cables.
2. Open the module.
3. Remove the module, as shown in Fig. 2-1.

Fig. 2-1: Removing the module

4. Lift the instrument by placing hands in the middle of each side under the base plate (see Fig. 2-2).
5. Place the module back into its holder by first reinserting it (with the lid open) as shown in Fig. 2-1, and then with a tilting action, firmly push it into place so that it properly engages.

Do not lift the Litesizer™ 500 by the module or leads or other external plastic parts. The instrument could be damaged and injury may arise.

Fig. 2-2: The correct way to lift the Litesizer™ 500

2.1.4 Maintenance and Service

- The results delivered by the Litesizer™ 500 not only depend on the correct functioning of the instrument, but also on various other factors. We therefore recommend you have the results checked (e.g. plausibility tested) by skilled personnel before consequential actions are taken based on the results.
- Service and repair procedures may only be carried out by authorized personnel or by Anton Paar GmbH, and must be carried out at least

every 10 years to ensure product safety.

2.1.5 Disposal

- Concerning the disposal of the Litesizer™ 500, observe the legal requirements in your country.

2.1.6 Returns

- For repairs send the Litesizer™ 500 to your Anton Paar representative. Return the instrument and module together with the filled out "Maintenance/Error Report" and the "Standard Maintenance Contract". Find the applicable forms on the Anton Paar homepage (www.anton-paar.com).

NOTICE: *Before packing the Litesizer™ 500 for transport, the transport safety lock must be engaged, as described below.*

2.1.6.1 Engaging the Transport Safety Lock

1. Switch off the instrument, remove the cuvette module, and unplug all cables.
2. Carefully lift the front of the instrument so that it stands on its rear surface, as shown in Fig. 2-3.

Fig. 2-3: The Litesizer™ 500 standing on its rear surface

3. Insert the transport safety lock Torx key into one of the two small holes next to the module bay, as shown in Fig. 2-4.

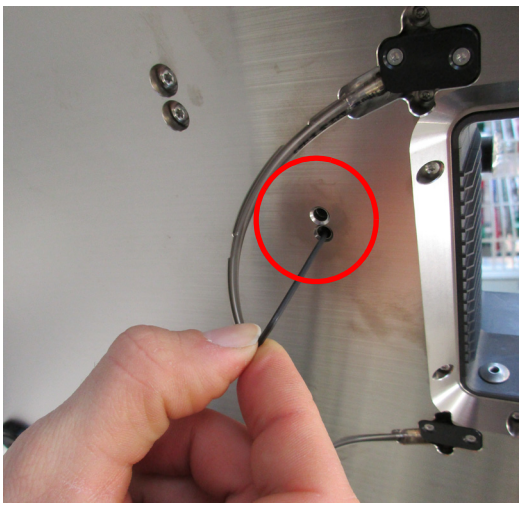


Fig. 2-4: Insertion of Torx key

4. Gently turn the screw clockwise until it stops turning.
5. Repeat for the second hole.

The transport safety lock is now engaged.

2.2 Safety Signs on the Litesizer™ 500

CAUTION

Laser radiation
Symbol position: Module cavity surface (Fig. 2-5).
The Litesizer™ 500 is equipped with a laser of class 3B, which is integrated into the Litesizer™ 500, and therefore conforms to laser class 1 regulations. There is no exposure to laser radiation in the normal operation of this instrument. Follow your national safety regulations.

WARNING

Danger of laser radiation
Symbol position: Module cavity surface (Fig. 2-5).
Switch instrument off before handling any tools containing metal or other conducting materials in this area.

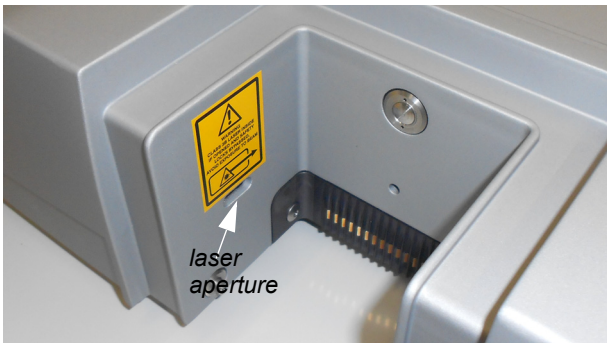


Fig. 2-5: Safety signs and laser aperture on the front surface of the module cavity

CAUTION

Risk of damage to instrument
Symbol position: Litesizer™ 500 rear surface (Fig. 2-6).
Use dry air (ISO 8573.1, class 1.3.1) or nitrogen at 0.4 to 0.8 bar overpressure (1.4 to 1.8 bar total input).
Use a 6 x 4 mm hose to connect the air/nitrogen.
Failure to adhere to these specifications may result in damage to the instrument.

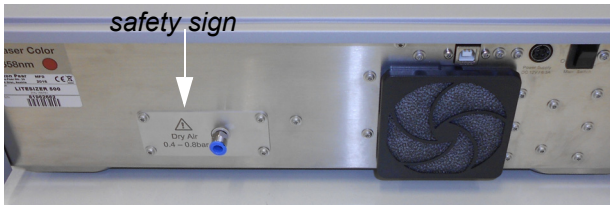


Fig. 2-6: Safety sign on the rear surface of the instrument

CAUTION

Hot surface
Symbol position: On the inside surface of the cuvette module lid and on top of the thermal insulation cover (see Fig. 2-7).
When making high-temperature measurements (above 45 ˚C), the cell area should be allowed to cool before removing the thermal insulation cover and cuvette.

Fig. 2-7: "Hot surface" safety sign on the inside surface of the cuvette module lid (left) and on top of the thermal insulation cover (right)

2.3 Precautions for Using Flammable Samples or Cleaning Agents

- Place the unit in a well-ventilated area.
- Always use the purge port (see Fig. 12-2) with nitrogen as purge gas when measuring flammable samples (min. flow rate: 2 L/min).
- Do not measure any sample with a spontaneous ignition temperature of <279.87 ˚C.
- Flammable samples should be measured in quartz cuvettes at the lowest feasible measuring temperature.
- Fill the cuvette at a safe distance from the instrument in a well ventilated area, using only the minimum sample volume, and close the cuvette.
- Check the drain hole at the bottom of the cuvette module periodically using a pipe cleaner.
- Safely dispose of the sample as soon as possible after the measurement.
- If a flammable sample is spilled in or near the instrument:
  - Switch off and unplug the instrument
  - Remove cuvette module
  - If necessary let it cool down in a well-ventilated place
  - Remove the vial from the cuvette
  - Remove all visible residue sample with a dry cloth
  - Remove any remaining residue by blowing with nitrogen or dry air
  - Do not reassemble the Litesizer™ 500 or turn it on until all traces of sample residue have been removed.
  - If in doubt, contact your Anton Paar service representative.
- Observe and adhere to your national safety regulations for handling samples and solvents (e.g. use of safety goggles, gloves, respiratory protection etc.).
- Only store the minimum required amount of sample, cleaning agents and other inflammable materials near the Litesizer™ 500.
- Connect the Litesizer™ 500 to the mains via a safety switch located at a safe distance from the instrument. In an emergency, turn off the power using this switch instead of the power switch on the Litesizer™ 500.
- Supply a fire extinguisher.

3 Litesizer™ 500 - An Overview

The Litesizer™ 500 is an instrument for characterizing particles in liquid dispersions. It determines particle size, zeta potential, and molecular mass by measuring dynamic (DLS), electrophoretic (ELS), and static light scattering (SLS). The Litesizer™ 500 should not be used in any other way than described in this manual.

cmPALS, a new ELS technology: Unique to the Litesizer™ 500 is cmPALS, a novel patented PALS technology (European Patent 2 735 870) that provides unprecedented accuracy in ELS measurements. Also, the incorporation of autoadjustment optics lends further stability to the Litesizer™ 500's optics, particularly in the long term. Despite these features, the Litesizer™ 500 is especially compact and lightweight.

Simple software: Particularly convenient is the Litesizer™ 500's accompanying software program, Kalliope, which sets a new standard in user-friendliness. The user sees all important information on a single clear display, including input parameters, results, and final calculated values, as well as expert advice. Experiments can be performed in series with DLS and ELS, allowing the user to observe changes in particle properties while varying pH, time or temperature, for example.

Reporting: The Litesizer™ 500 enables fast and customizable analysis and reporting, and complies with the US FDA's Regulation 21 CFR Part 11 concerning electronic records and signatures.

Transmittance: An extra capability of the Litesizer™ 500 includes continuous transmittance measurement. Transmittance measurements provide a fast indication of a sample's suitability for light-scattering measurements. In addition, this measurement allows the Litesizer™ 500 to select the best parameters for your sample (focus position, measuring angle, measurement duration).

3.1 Litesizer™ 500 Measurement Specifications

Technology	• Dynamic light scattering (DLS) • Electrophoretic light scattering (ELS) • Static light scattering (SLS) • Transmittance
Light source	Laser light of wavelength 658 nm from a single-frequency laser diode, providing 40 mW.
Laser warm-up time	• 6 min
Detection angles	• 15˚, 90˚, 175˚ (particle size) • 15˚ (zeta potential) • 90˚ (molecular mass)
Particle size range	• 0.3 nm – 10 µm* (particle size) • 3.8 nm – 100 µm (zeta potential) • 980 Da – 20 MDa (molecular mass)
Minimum concentration	• 0.1 mg/mL (lysozyme) (particle size) • 1 mg/mL (lysozyme) (zeta potential) • 0.1 mg/mL (lysozyme) (molecular mass)
Temperature range	0–90 ˚C (32–194 ˚F)
Minimum volume	• 20 µL (particle size) • 350 µL (zeta potential) • 20 µL (molecular mass)

* *under laboratory conditions*

3.2 Measurement Principles

3.2.1 Particle Size

Particle size is measured by dynamic light scattering (DLS). Particles suspended in a liquid are constantly undergoing random motion, and the speed of this motion depends on the size of the particles: smaller particles move faster than larger ones. In DLS, light is scattered by the sample, and the scattering is then detected and recorded many times. Comparison of those records with each other reveals how much the particles have moved in the time between each record (and therefore how fast they are moving). From this information, the average size of the particles can be calculated, as can the size distribution.

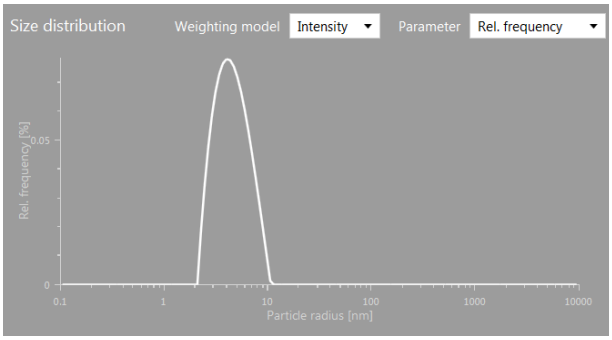


Fig. 3-1: Relative frequency vs. particle radius

3.2.2 Zeta Potential

Zeta potential is measured by electrophoretic light scattering (ELS), which measures the speed of the particles in the presence of an electric field. How fast the particles move depends on the surface charge, or zeta potential, of the particles. In general, the greater the magnitude of the zeta potential, the more stable the colloid.

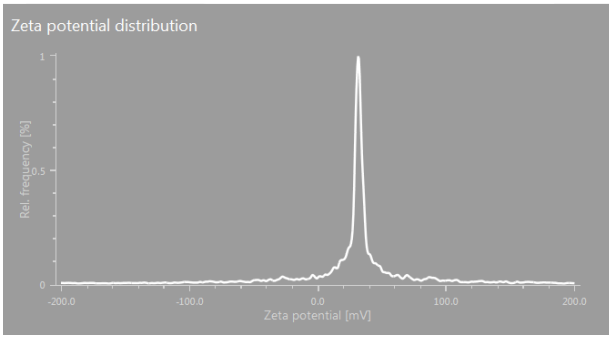


Fig. 3-2: Relative frequency vs. zeta potential

3.2.3 Molecular Mass

Molecular mass is measured by static light scattering (SLS). In this case, the intensity of the scattered light is directly related to molecular mass. If the scattering intensity is measured at several different concentrations, then a Debye plot can be generated, the intercept of which provides the molecular mass.

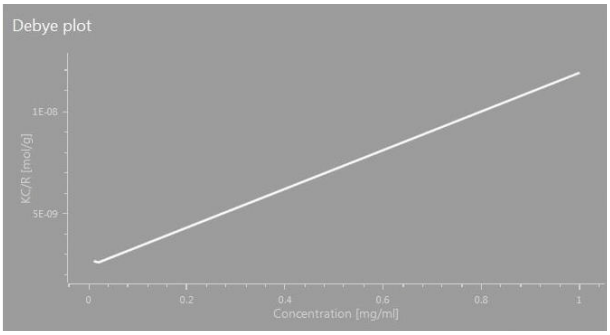


Fig. 3-3: Debye plot (KC/R [mol/g] vs concentration)

3.2.4 Transmittance

Transmittance is measured by detecting the fraction of light that passes through the sample. The Litesizer™ 500 measures the transmittance continuously for every sample. The value is reported in real time and appears in the top right corner of the display any time the Litesizer™ 500 is in operation (see Fig. 3-4).

Fig. 3-4: Screen shot showing continuous transmittance reading

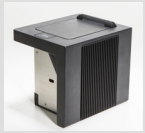
The transmittance of a sample can also be reported relative to the solvent, by first setting the solvent as the reference.

4 Supplied Parts

The Litesizer™ 500 was tested and packed carefully before shipment. However, damage may occur during transport. Therefore:

1. Keep the packaging material (box, packing foam) for possible returns and further questions from the transport and insurance company.
2. Check the delivery for completeness by comparing the supplied parts to those noted in the table, below.
3. If a part is missing, contact your Anton Paar representative.
4. If a part is damaged, contact the transport company and your Anton Paar representative.

Table 4-1: Supplied Parts

Symbol	Pcs	Article description	Mat. no.
	1	Litesizer™ 500	155761
	1	Batch module: Two options: - BM10 (high-end batch module) - BM20 (batch module)	 155764 166281
	1	Software package, Kalliope™ on USB stick. Three options: - Kalliope™ Professional Enables access for three users - Kalliope™ Professional Single Addition. Enables access for one additional user - Kalliope™ Professional Unlimited. Enables access for an unlimited number of users	 166116 170677 170678
	1	Instruction manual	166212
	1	Power cable (3x1.0 mm^2, 10 A), according to country - CEE - UK - USA - China - CH - Thailand - Brasil	 52112 61865 52656 27011 93408 79730 130117
	1	Power supply 12 V / 7.5 A	156217
	1	USB cable	94228
	1	Torx key T6 for transport safety lock	170679*
	1	Starter kit (see table below)	155766

* *Not available for separate sale*

Table 4-2: Starter Kit

Symbol	Pcs.	Article description	Mat. No.
	1	Cuvette rack	163383
	100	Disposable cuvettes (pack of 100) PS 10 x 10 x 45 mm	164435
	100	Disposable cuvette lids (pack of 100)	163395
	5	Omega cuvettes for zeta potential (box of 5; 20 male Luer plugs included)	155765
	1	Thermal insulation cover	157666
	1	Syringe filter – Anotop 10 Anotop type 10 0.02µm syringe filter	163385
	1	Syringe filter – Anotop 25 Anotop type 25 0.02µm syringe filter	163388

5 Installation of the Litesizer™ 500

5.1 Installation Requirements

The instrument should be placed on a stable, flat lab bench in a clean environment that is free from mechanical vibrations and excessive noise. Ensure that nothing is placed on top of the instrument.

To ensure temperature stability, place the Litesizer™ 500:

- away from heating
- away from direct sunlight
- away from open windows

Make sure that the power plug and the power switch are always easily accessible so that the instrument can be disconnected from the mains at any time.

A strong built-in cooling fan dissipates heat through the bottom and the back of the instrument (see Fig. 5-1). Ensure that the airflow is not blocked.

Read the Safety Instructions in Chapter 2.

Find all technical data in Appendix A - Technical Data.

PC requirements:

- Dual core system (or better)
- 2 GB RAM (Windows 7 - 32 Bit) / 4 GB RAM (Windows 7 - 64 Bit) (or better)
- Windows 7 - Servicepack 1 (or better)
- 5 GB HDD

5.2 Opening the Transport Safety Lock

1. Before switching the Litesizer™ 500 on or inserting the module or connecting any cables, carefully lift the front of the instrument so that it stands on its rear surface, as shown in Fig. 5-5.

Fig. 5-5: The Litesizer™ 500 on its rear surface

2. Insert the transport safety lock T6 Torx key into one of the two small holes next to the module bay, as shown in Fig. 5-6.

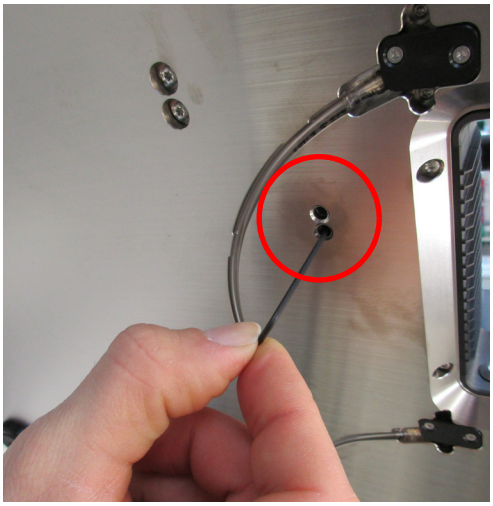


Fig. 5-6: Insertion of Torx key

3. Gently turn the screw anticlockwise until it stops turning.
4. Repeat for the second hole.

The transport safety lock is now open.

5.3 Connecting the Instrument

Make sure the power switch is turned off. Make sure the transport safety lock is removed (see section 5.2). Connect the instrument to the computer via USB cable (see Fig. 5-1). Connect the AC/DC adapter to the instrument at the power socket. Make sure that mains voltage and frequency comply with the specified data (110/230 VAC, 50/60 Hz) of the AC/DC adapter. Connect the AC/DC Adapter to the mains voltage. Switch the instrument on at the main switch.

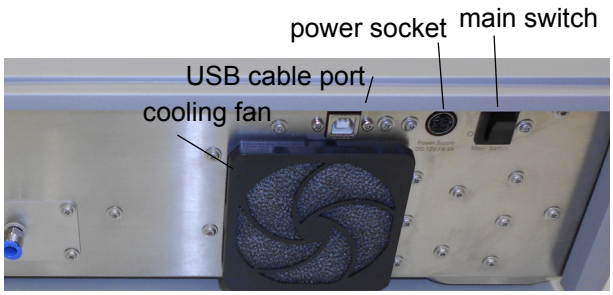


Fig. 5-1: Litesizer™ 500 rear surface

NOTE: *The Litesizer™ 500 has a stand-by mode and a deep stand-by mode, which are activated after 15 min and 180 min of inactivity, respectively. For details, see section 6.2 below.*

6 How to Use the Litesizer™ 500

6.1 Switching On

Switch the Litesizer™ 500 on at least ten minutes before you begin measurements. The power switch is at the back of the instrument.

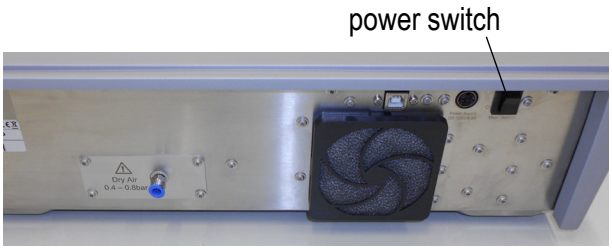


Fig. 6-1: Litesizer™ 500 rear surface

6.2 Status and Stand-by Modes

When the Litesizer™ 500 is switched on, the POWER and STATUS indicator lights (Fig. 6-2) can each show two different colors, as listed in Table 6-1. After 15 min of inactivity, the instrument will go into stand-by mode, which means the laser will be switched off. After a further 180 min of inactivity, the instrument will go into deep stand-by mode, in which the temperature control is also switched off.

The device can be woken up from stand-by or deep stand-by mode by pressing the ⏻ button, opening the module cover or removing/inserting a module.

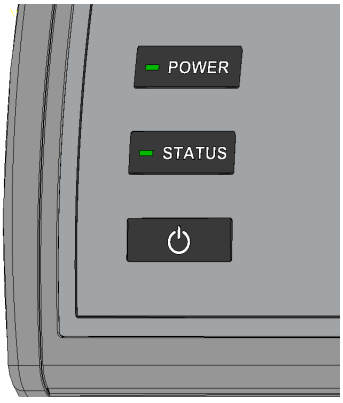


Fig. 6-2: The POWER and STATUS indicator lights

Table 6-1: POWER and STATUS indicator lights

POWER indicator	STATUS indicator	Meaning
blue	green blinking	currently booting
green	green	successful boot, instrument in operation
blue	red blinking	failure during boot
blue	off	stand-by (laser off)
blue "breathing"	off	deep stand-by (temp. control off)

6.3 Starting the Software System, Kalliope

Install the software system, Kalliope. Start Kalliope by selecting "Programs > Kalliope" in the windows start menu or use the shortcut on the desktop.

6.4 Activating the Software License

When Kalliope is first started, you will be asked to enter a license code, as shown in Fig. 6-3. There are two ways to activate the software; either online or manually. For both, a valid license code is required.

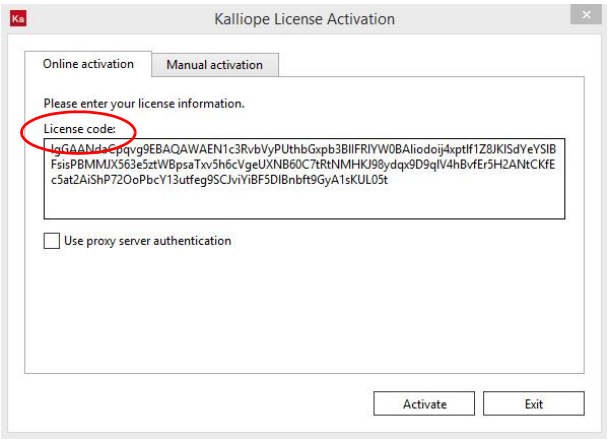


Fig. 6-3: Entering the software license code

6.4.1 Online Activation

For online activation, an internet connection is required.

1. In the "Online activation" tab, enter the valid license code provided with the delivery, as shown in Fig. 6-3.
2. Click Activate, then click Exit and restart the software.

6.4.2 Manual Activation

1. On the License Activation page, click the Manual activation tab at the top of the page (Fig. 6-4). Note the Machine code (Fig. 6-4, b) and the internet address (Fig. 6-4, a).

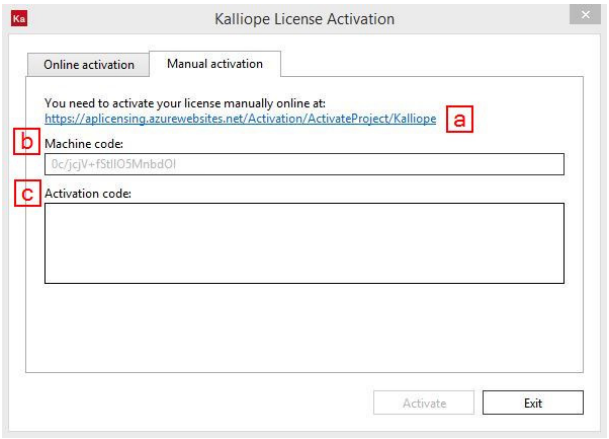
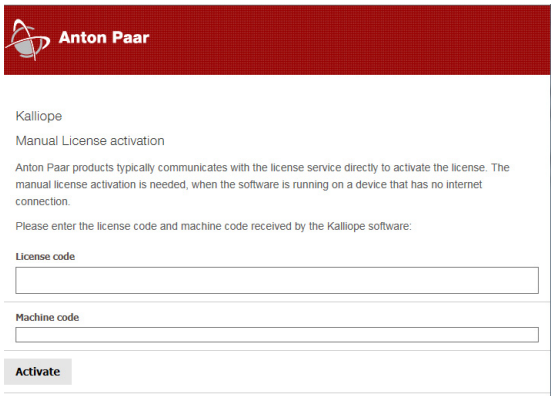


Fig. 6-4: Manual software license activation

2. On a PC with internet connection, go to the Anton Paar licensing webpage (https://aplicensing.azurewebsites.net/Activation/ActivateProject/Kalliope; Fig. 4).
3. 

4. Retrieving the activation code for manual license activation
5. Enter the valid license code provided with the delivery, and the machine code of the PC on which Kalliope is installed (Fig. 6-4, b), then click Activate. This will generate a valid activation code.
6. Go back to the offline PC on which Kalliope is installed, and enter this activation code (Fig. 6-4, c).
7. Click Activate, then click Exit and restart the software. The display should now appear as in Fig. 6-5. If the instrument is connected to the computer and the software, a small green tick will appear in the top right corner.

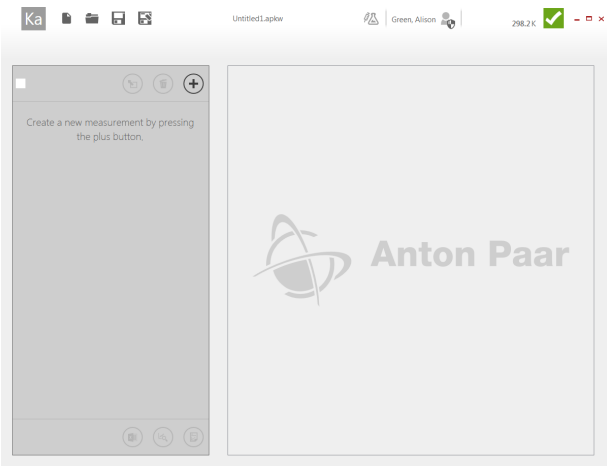


Fig. 6-5: Kalliope start-up screen

NOTE RE SOFTWARE UPDATES: *When uploading a new version of the software, the Litesizer™ 500 must be switched off.*

6.5 Checking the System

Clicking on the Ka icon in the top left-hand corner opens the following menu (Fig. 6-6):

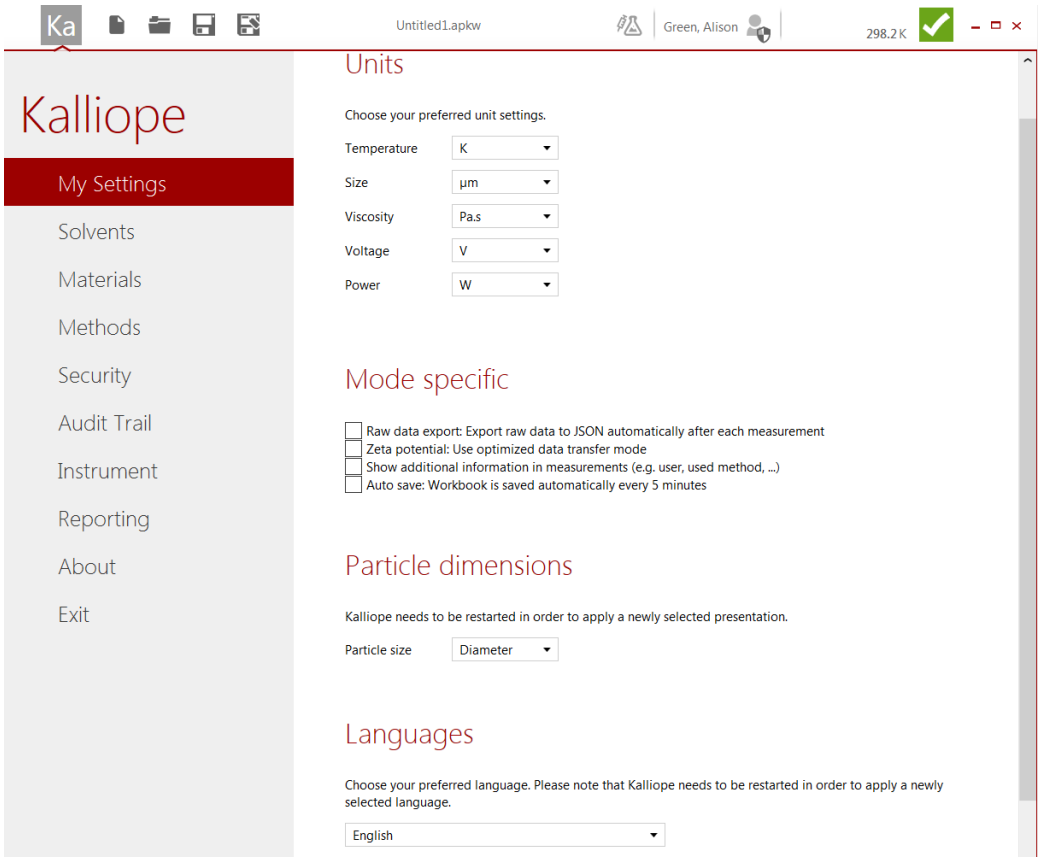


Fig. 6-6: Kalliope menu

6.6 Adjusting the Settings

Under the Kalliope menu, select "My settings" to open the menu shown in Fig. 6-6, which allows the user to select the preferred units, the data-handling mode, the particle-size dimensions that are reported (radius or diameter) and the preferred language.

Note that Kalliope needs to be restarted in order to apply new selections. Click again on the Ka icon to close the window and return to the measurement window, above (Fig. 6-5).

Note regarding decimal points or commas: Kalliope will display either decimal commas or decimal points, according to your computer's settings. This can only be changed in the user's own computer settings, not within Kalliope itself.

6.7 User Management

Under the Kalliope menu, select "Security" to open the menu shown in Fig. 6-7. To change the rights of existing users, or to add new users, check the "Enable user management" box. The Users list will then appear. New users can be added by clicking on the + icon at the bottom of the screen, which brings up the list of names registered on the connected operating system. Users must be registered on the operating system (e.g. Windows 7) before they can be added to the instrument user list. Users' rights can be changed by selecting "Basic", "Advanced" or "Administrator" from the drop-down menu, while users can be removed from the list by clicking on the icon.

Note: *Checking or unchecking the User Management box results in a change in the settings, and Kalliope must be restarted in order to apply the changes.*

Note: *A user must be logged in on the computer that is connected to the instrument in order to access the system and use the instrument.*

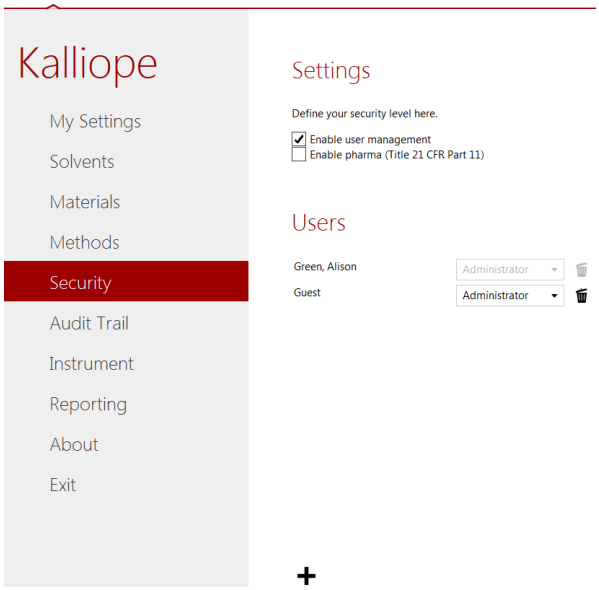


Fig. 6-7: User management

6.8 Pharma Option (21 CFR Part 11)

In the "Security" menu shown in Fig. 6-7, check the second box under "Settings" to enable the pharma option. Note that "User Management" (section 6.7) must first be enabled before the pharma option can be enabled. Kalliope must be restarted to apply the new settings.

When using the pharma option, a workbook must be saved manually before a measurement can be started. Otherwise Autosave will not be enabled.

All documentation must be saved in a defined folder.

Once a measurement has started, the filename can no longer be changed. Furthermore, the measurement and its associated files cannot be deleted.

6.9 Performing a Measurement

On the start-up screen (Fig. 6-5), click on the ⊕ icon to select a new measurement. The measurement options are displayed as follows (Fig. 6-8), with measurement modes on the left, and measurement series options on the right.

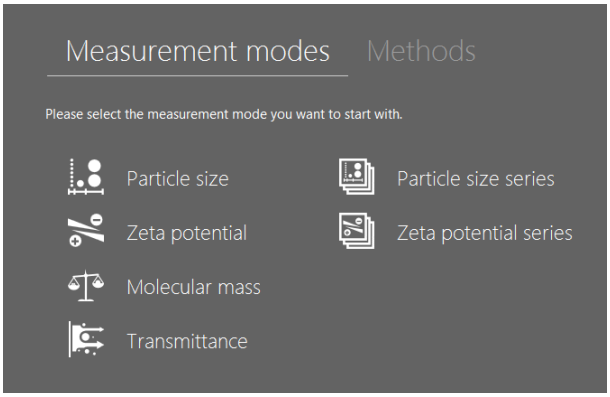


Fig. 6-8: Selection of measurement mode

Details for running each measurement mode can be found in the following chapters.

7 Particle-Size Measurements

7.1 Sample Preparation

Making a measurement with the Litesizer™ 500 is simple and straightforward. Nonetheless, the accuracy and precision of the results depend significantly on correct sample preparation for each type of measurement. Thus, it is important to follow the guidelines for preparing each type of sample, including choice of cuvette, choice of solvent, sample concentration, and filling of the cuvette.

7.1.1 Concentration

Particle-size measurements are significantly influenced by particle concentration. If the concentration is too low, then not enough light will be scattered to make a measurement. If the concentration is too high, then the light scattered by each particle may be further scattered by other particles (this effect is referred to as multiple scattering). A too-high concentration can also lead to distorted measurements because the particles can no longer undergo Brownian motion (free diffusion). Nonetheless, the ideal concentration varies for different particle sizes - see table below. For very small particles (<10 nm), there is no real maximum concentration.

For larger particles (>1 m), there is an additional effect to consider; i.e., that of number fluctuation. At low concentrations, the scattering intensity may still be high because larger particles scatter more effectively. But the number of particles in the scattering volume (the volume of overlap between the incident and detector beams) may be so small that number fluctuations significantly affect the results. Thus, the lower concentration limit for larger particles is such that there are at least 500 particles in the scattering volume (approximate scattering volumes are 10^6 µm^3 for back scattering, 10^4 µm^3 for side scattering, and 5 x 10^4 µm^3 for forward scattering).

The following table shows the suitable range of particle concentration according to the expected particle size:

Expected particle size	Minimum concentration
<10 nm	0.5 mg/mL
10 – 100 nm	0.1 mg/mL
100 nm – 1 µm	0.01 mg/mL
>1 µm	0.1 mg/mL

If the concentration is completely unknown, then in some cases, the visual appearance of a sample can be used as an indicator of concentration. Ideally, the sample should be prepared so that it is slightly cloudy, or turbid. Note, however, that samples of very small particles (<20 nm) will never become cloudy, even at high concentrations; whereas samples of very large particles will look turbid even at very low concentrations. Thus, in such cases, it is necessary to prepare and measure samples at several different concentrations in order to find the concentration range within which the results do not differ from each other more than the normal minor deviations. As a guide, the particle-size measurement should generate a mean detected light intensity of at least 20 kcounts/s. A further indicator is the filter optical density (attenuation level) used. For automatic measurements, a filter optical density of 0 also suggests that the sample concentration is low, although the results may still be meaningful if the count rate is still sufficiently high.

7.1.2 Solvent (or Dispersant) Selection

The solvent or dispersant should be distilled, deionized and/or filtered prior to use to ensure that it contains no unwanted particles, such as ions or dust, which may interfere with measurements.

All solvents or dispersants should be purified by filtration using a pore size of 10 or 20 nm. As an additional check, a particle-size measurement should be carried out on the solvent before mixing it with the material to ensure that it contains no unwanted particles.

The sample itself should not be filtered, because this may remove the particles to be measured. Samples should only be filtered if it is intended to remove large particles or agglomerates because they are not of interest or disturb the measurement.

7.1.3 Ultrasonication

Ultrasonication can be used to dissolve agglomerates or remove gas bubbles from the sample; however, ultrasonication should be used carefully, because it may initiate chemical reactions, thus distorting measurement results. Ideally, the effect of ultrasonication on the light-scattering properties of a sample should be checked by making measurements on a sample before and after ultrasonication.

7.2 Cuvettes

The standard cuvettes, both quartz and polystyrene, have inner dimensions of 10 mm x 10 mm x 45 mm. Ideally, the sample volume should be approximately 1 mL, but it must not be less than 0.85 mL or greater than 3 mL, as explained below.

Fig. 7-1: A standard cuvette showing the ideal sample volume.

The measurement is made 6.5 mm from the bottom of the cell, and the meniscus must be at least 2 mm above the measurement height (8.5 mm). For reliable measurements, the depth must be between 8.5 and 30 mm, and thus, the volume must be between 0.85 and 3 mL. If the sample volume is <0.85 mL, then the laser may be too close to the meniscus; if the sample volume is >3 mL, then the thermal equilibrium may not be stable.

7.2.1 Small-Volume Cuvette

The small-volume quartz cell is designed to be used when little sample is available. The maximum volume is 45 µL, while the minimum that can be used is 20 µL.

Fig. 7-2: Small-volume cell showing the minimum sample volume (20 µl)

7.2.2 Filling the Cuvettes

Disposable, powder-free latex gloves should be worn throughout all procedures; both to prevent skin contact with any samples or solvent, but also to protect the measurement cells and glassware from contaminants on/in the skin.

To fill a cuvette, place the tip of the pipette at the bottom of the cell so that it fills from the bottom up, thereby avoiding bubble formation. Check the sample through the windows for tiny bubbles, and tap the cell to dislodge any that have formed.

Place the lid firmly on the cuvette, and ensure that the outer surface is clean and dry before inserting it into the Litesizer™ 500.

7.2.3 Inserting the Cuvette

Open the chamber by pushing the OPEN button. Insert the cell firmly as far as it will go (see Fig. 7-3). Close the chamber.

Fig. 7-3: Inserting the cuvette

7.3 Making a Measurement

On the start-up screen (Fig. 6-5), click on the ⊕ icon to select a new measurement. Select Particle size. Input parameters can be entered on the left-hand side of the display, as follows (see Fig. 7-4). An explanation of the input parameters can be found in Table 7-2.

7.3.1 Input Parameters for Particle Size

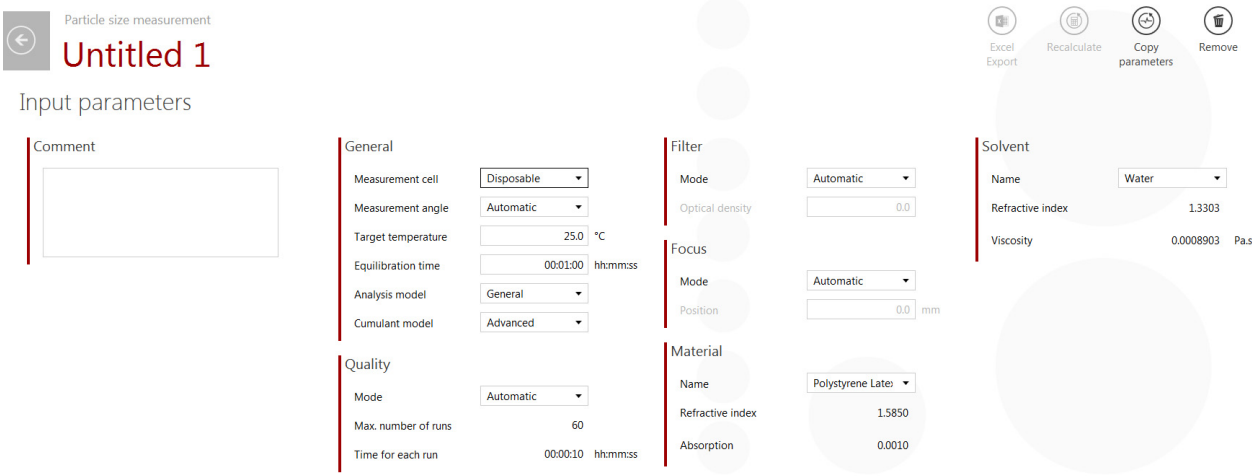


Fig. 7-4: Input parameters for particle size

Table 7-2: Explanation of input parameters for particle size

Title

Experiment name	At the top is the name of the current measurement ("Untitled", in red). The name can be changed by clicking in any part of "Untitled". The back button will switch back to the overview display.
Comment	Describe here the sample and/or conditions.

General

Measurement cell	**Disposable cell**: suitable for all water-based samples (except proteins and particle sizes greater than 3 micrometers) for measurements up to 70 ˚C. **Quartz cell**: suitable for all non-water-based solvents/dispersants, for proteins and large-particle samples, and for high-temperature (up to 90 ˚C) measurements **Small-volume cell**: when a limited amount of sample is available.
Measurement angle	**Automatic**: the optimum angle is automatically found for the sample, based on the transmittance, which is continuously measured. **Back scatter (175˚)**: suitable for strongly scattering samples, including large particles, highly concentrated or turbid samples. Also suitable for weaker scatters and at low concentrations **Side scatter (90˚)**: suitable for weakly scattering samples, including small particles and transparent samples. It can be used for particles ranging from 0.3 nm up to 1 µm. **Forward scatter (15˚)**: suitable for detection of large particles at low concentration; e.g., protein aggregates, infusion solutions
Target temperature	Can be set from 0 to 90 ˚C. Note that for measurements above 70 ˚C, the quartz cell must be used.
TIP: *Before making high- or low-temperature measurements, see Chapter 12, "Using the Litesizer™ 500 at High and Low Temperature".*	
Equilibration time	For measurements close to ambient temperature, the equilibration time should be set at two minutes. The further the measuring temperature from ambient temperature, the longer the equilibration required (a common rule of thumb is to add one minute for every ˚C different from ambient temperature, based on a 1 mL sample).
Analysis model	**General**: suitable if the sample is not well known, or if a single (broad) peak is expected. **Narrow**: Suitable if one or more narrow peaks is expected.
Cumulant model	**ISO 22412**: default model. **Advanced**: suitable if any of the following features is visible in the correlation function: - elevated baseline (the correlation function decays to >1) - the correlation function is noisy - a second shoulder is visible (if you are sure that the second shoulder is an artifact or caused by dirt).

Table 7-2: Explanation of input parameters for particle size

Quality

Mode	**Automatic**: time for each run (measurement time) will be 10 s, and the measurements will continue until the threshold number of counts has been accumulated (10 x 10^6) or until 60 runs have been made. **Quick**: the threshold number of counts is 3 x 10^6, with maximum 30 runs. **Manual**: Number of runs and Time for each run must be manually selected.
Number of runs	from 1 to 100
Time for each run [hh:mm:ss]	from 1 s to 30 min

Filter

Mode	**Automatic**: the optical filter density will be automatically optimized based on the detected scattered intensity. **Manual**: the optical filter density must be manually selected.
Optical density	from 0 to 6.5.

Focus

Mode	**Automatic**: the focus position will be optimized automatically. **Manual**: the focus position must be manually selected.
Position	From -4.5 to 3.5 mm, where 0 is the center of the cuvette. The optimal focus position varies not only for different samples, but also for the different scattering angles.

Material

Name	Polystyrene latex, proteins, metal nanoparticles, etc.

The material must be selected from the user's database. New materials can be entered on the database by the user by clicking on the Ka icon. The material's refractive index and absorption must also be entered into the database before they can be selected for a particle-size measurement. Once the material is selected, then the refractive index and absorption will be automatically filled.

Note: *the temperature used in the measurement must be the same as that entered in the database.*

Solvent

Name	The solvent must be selected from the database.

Once the solvent is selected, then the refractive index and viscosity will be automatically filled. New solvents can be entered in the database by the user by clicking on the Ka icon.

Note: *the temperature used in the measurement must be the same as that entered in the database.*

7.3.2 Starting a Measurement

Once the input parameters are complete, the Start icon in the bottom right-hand corner of the screen will be activated, and can be clicked to start the measurement.

Following temperature adjustment, equilibration and optical adjustment, the measurement will be displayed on the screen while it is running, as shown below, while the run number is displayed at the bottom of the screen. The Litesizer™ 500 will keep performing runs until a threshold number of counts has been accumulated (10 x 10^6 for automatic, or 3 x 10^6 for Quick), or until the specified number of runs has been reached. Once the measurements are finished, all the measured and calculated values (see Table below for explanation) appear in the gray boxes to the right of the graphs, with the Mean hydrodynamic radius box appearing in green.

7.3.3 Measurement Output Screen

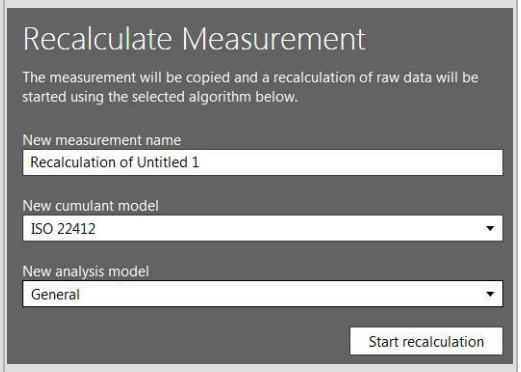
The measurement output screen retains a display of the input parameters on the left, a series of action icons at the top right, and the results (plots, automatic values and calculated values - see Fig. 7-5 to Fig. 7-8 and Table 7-4 to Table 7-6 below) on the main part of the screen at the right.

7.3.4 Action Icons

Fig. 7-5: Action icons (see top right-hand corner of screen)

Table 7-3: Explanation of the Action Icons

Excel Export	Exports current results to Excel file for further processing.
Recalculate	Recalculates the hydrodynamic radius. A new screen appears (see below), allowing the user to choose between the two possible cumulant models and the two analysis models. See appendix for further details.
Copy parameters	Creates a copy of the input parameters of the current measurement. The name of the original measurement is retained, but with the prefix “New”.
Remove	Deletes current measurement, and switches back to starting display.



7.3.5 Particle-Size Results - Intensity Trace and Related Values

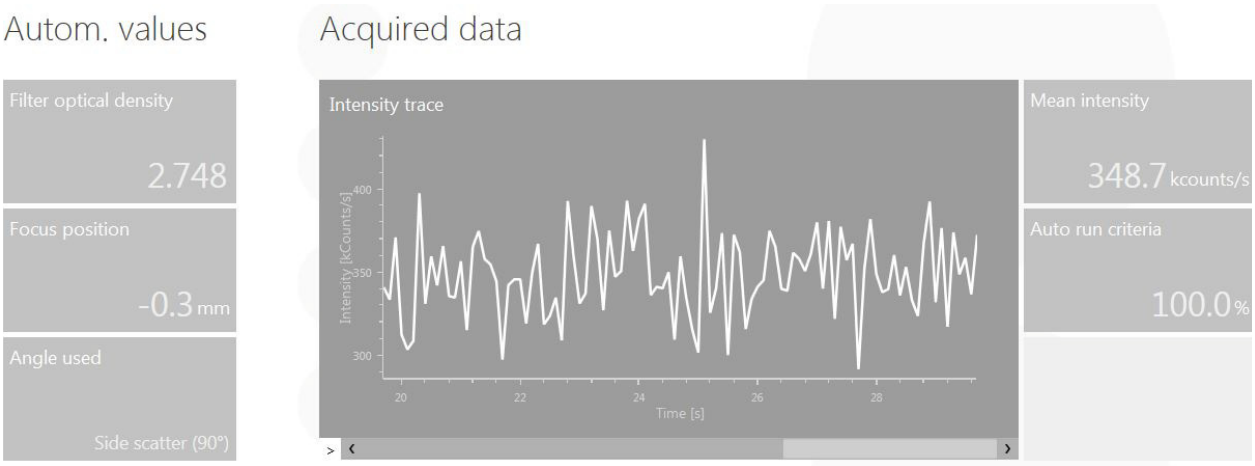


Fig. 7-6: Particle size output: Intensity trace, filter optical density, focus position, angle used, mean intensity, and auto run criteria

Table 7-4: Explanation of Intensity Trace and Related Values

Filter optical density	Shows attenuation level used. For automatic measurements, a value of 0 suggests that the sample concentration is low, although the results may still be meaningful if the count rate is still sufficiently high.
Focus position	Indicates the position of the optical focus used in the measurement.
Angle used	Back scatter (175˚), forward scatter (15˚) or side scatter (90˚)
Intensity trace	Plots intensity vs time. Appears as soon as the measurement has begun.
Mean intensity	Displays mean detected light intensity in kcounts/s. If less than 20 kcounts/s, then increase the concentration.
Autorun criteria	Displays the % of the threshold counts accumulated (10 x 10^6 counts for Automatic, 3 x 10^6 for Quick). Nothing displayed for manual measurements.

7.3.6 Particle-Size Results - Correlation Function and Hydrodynamic Radius

Further to the right on the screen is presented the correlation function, along with the mean hydrodynamic radius, the polydispersity index, the $g1^2$ intercept and the baseline (see Fig. 7-7).

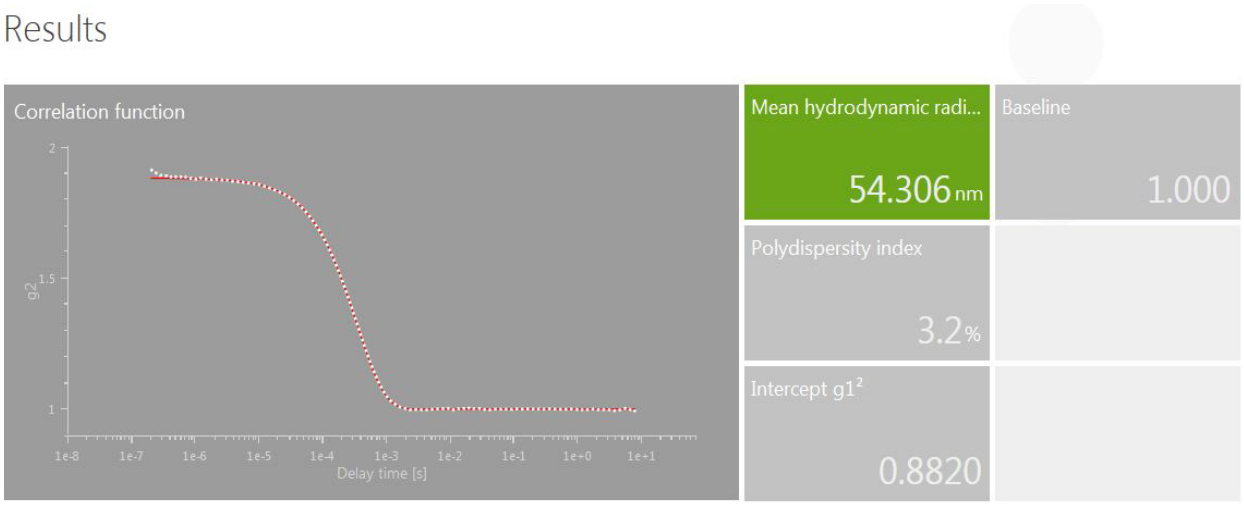


Fig. 7-7: Particle size output: mean hydrodynamic radius, polydispersity index, $g1^2$ intercept and baseline

Table 7-5: Explanation of Correlation Function and Related Values

Correlation function	The measured autocorrelation function is plotted in white and the cumulant fit function in red.
Mean hydrodynamic radius	Shown in green for a successful measurement, or in red in case of an erroneous measurement.
Polydispersity index	Indicates the breadth of the size distribution. A value of 10 % or less indicates that the sample is monodisperse, according to ISO 22412:2008(E) for 100 nm latex.
Intercept $g1^2$	The value of is $g1^2$ is g2 - 1 (where g2 is the correlation function intercept). For a good measurement, $g1^2$ will lie between 0.85 and 0.95. If g(1) > 1, there may be dust in the sample. Lower values indicate weak scattering or turbidity.
Baseline	The baseline should ideally be 1.000. If the measured baseline deviates by more than 0.01, then according to ISO 22412:2008(E), the measurement is unreliable.

7.3.7 Particle-Size Results - Size Distribution Function and Related Values

On the right-hand end of the screen is presented the particle radius distribution function, along with a list of the peak values, and the number of processed runs.

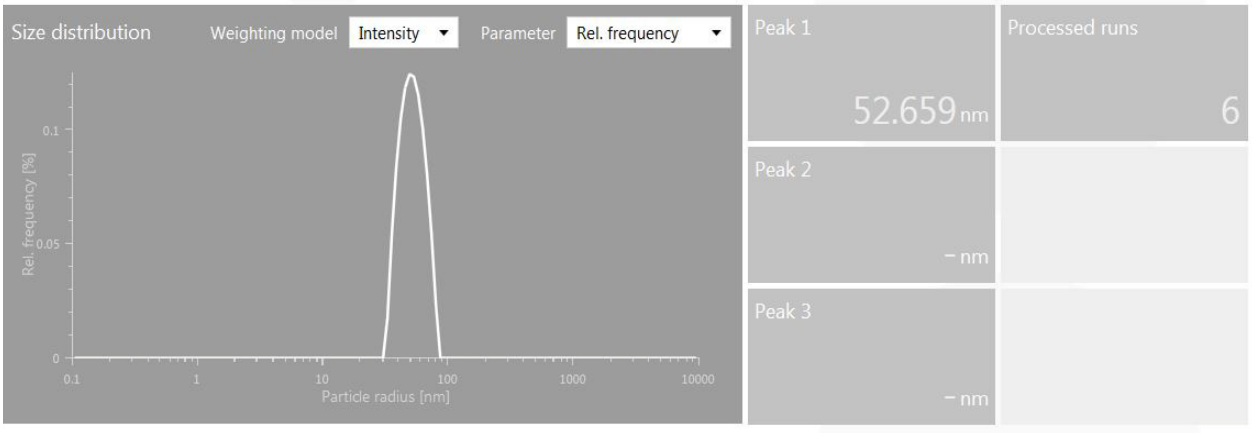


Fig. 7-8: Particle size output: particle radius distribution function, peak values, and number of processed runs

Table 7-6: Explanation of Size Distribution Function and Related Values

Size distribution plot	For the size distribution, the weighting model and parameter can be selected at the top of the graph field.
Peaks 1, 2 and 3	Up to three peaks from the size distribution plot will be listed here, indicating the most prevalent hydrodynamic radius of the particles.
Processed runs	The number of runs measured. Note that only half of the measured runs are analyzed.

7.4 Particle Size - Measurement Series

A series of particle-size measurements can be made by selecting New Measurement in Kalliope and then Particle size series, (see Fig. 7-9).

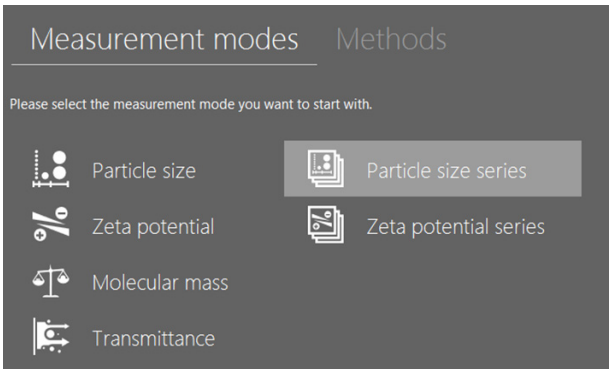


Fig. 7-9: Selecting a measurement series for particle size

7.4.1 Measurement Series Input Parameters

In addition to all of the input parameters, as described in section 7.3.1, the measurement series input parameters will appear at the right-hand end of the Input parameters screen (see Fig. 7-10).

Five parameters can be varied: temperature, concentration, pH, angle, or measurement focus (see Fig. 7-11). Alternatively, measurements can simply be repeated, as "repetition".

To set the number of measurements in the series, click on the + icon until the required number of fields appears (see Fig. 7-12).

Once the input parameters are complete, click Start in the bottom right-hand corner of the screen.

For Temperature, Angle, Measurement focus and Repetition series, the measurements will continue automatically one after the other. For Concentration and pH measurement series, individual samples must be prepared for each measurement. The user will be asked to change the sample as each measurement is finished.

NOTE: *For a temperature series, ensure that sufficient equilibration time is allowed for each measurement. A general recommendation is to add 1 min for every ˚C difference between measurement temperatures, based on a 1 mL sample; e.g., for a series of three measurements at 20, 25, and 30 ˚C, at least 5 min equilibration time should be selected.*

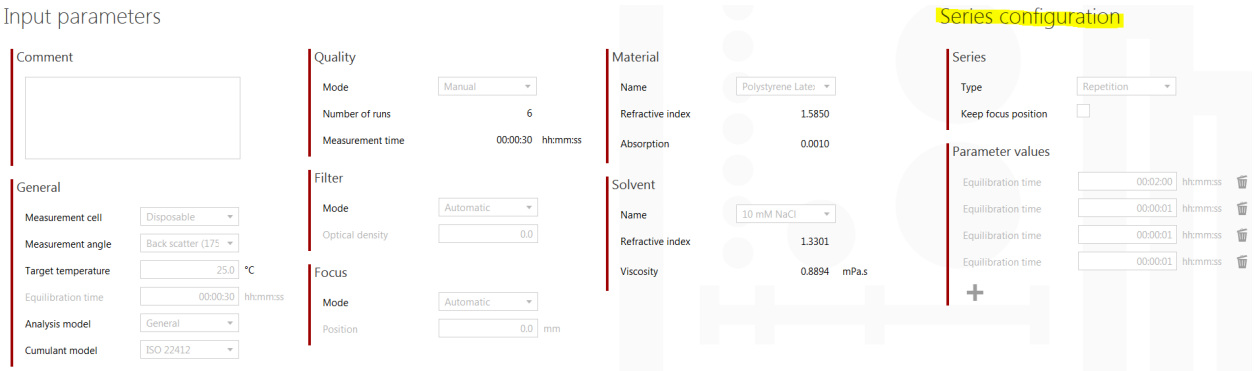
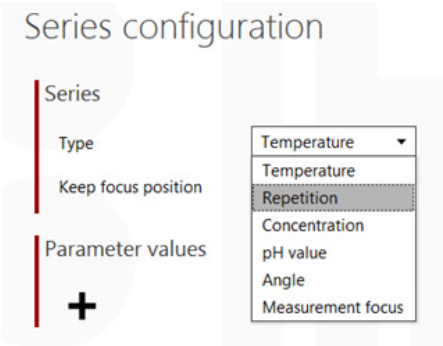


Fig. 7-10: Input parameters for a measurement series



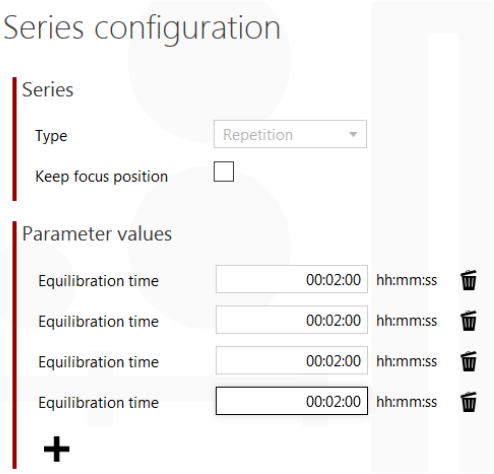


Fig. 7-11: Series parameter selection

Fig. 7-12: Setting the number of measurements

7.4.2 Measurement Series Output

Data for the measurement series appears in a table (see Fig. 7-13), with input parameters and measured and calculated values appearing in columns side by side.

To the right of the results table are two plots graphically summarizing the results (see Fig. 7-14): The first plot depicts relative frequency vs particle radius, with each separate measurement plotted in a different color. The second plot depicts hydrodynamic radius vs repetition number. The same color is used for the two plots, the color code being listed in the table.

Individual measurements can be viewed in more detail by clicking on the icon in the table. The overview can then be returned by clicking on the Show series icon. Results can be further analyzed by exporting the data to an Excel file (click on the Excel Export icon). The current measurement can be repeated by clicking on the Copy parameters icon, which creates a copy of the input parameters of the current measurement. The name of the original measurement is retained, but with the prefix “New”.

Particle size series measurement

Latex100nm_0,1mgml_S1#

Create ISO analysis | Excel Export | Copy parameters | Remove

Results

Index	Name	Status	Equilibration time	Temperature	Measurement position	Processed runs	Polydispersity index	Hydrodynamic diameter	Peak 1 (intensity)	Peak 2 (intensity)	Peak 3 (intensity)	Color	Go to
1	Latex100nm_0,1mgml_S1# 1	Succeeded	00:02:00	25.0 °C	0.0 mm	0	3.3456	0.1027 µm	0.1051 µm	- µm	- µm		
2	Latex100nm_0,1mgml_S1# 2	Succeeded	00:00:01	25.0 °C	0.0 mm	0	3.0388	0.1013 µm	0.1042 µm	- µm	- µm		
3	Latex100nm_0,1mgml_S1# 3	Succeeded	00:00:01	25.0 °C	0.0 mm	0	2.5830	0.1013 µm	0.1040 µm	- µm	- µm		
4	Latex100nm_0,1mgml_S1# 4	Succeeded	00:00:01	25.0 °C	0.0 mm	0	2.4841	0.1008 µm	0.1033 µm	- µm	- µm		

Fig. 7-13: Measurement series output data

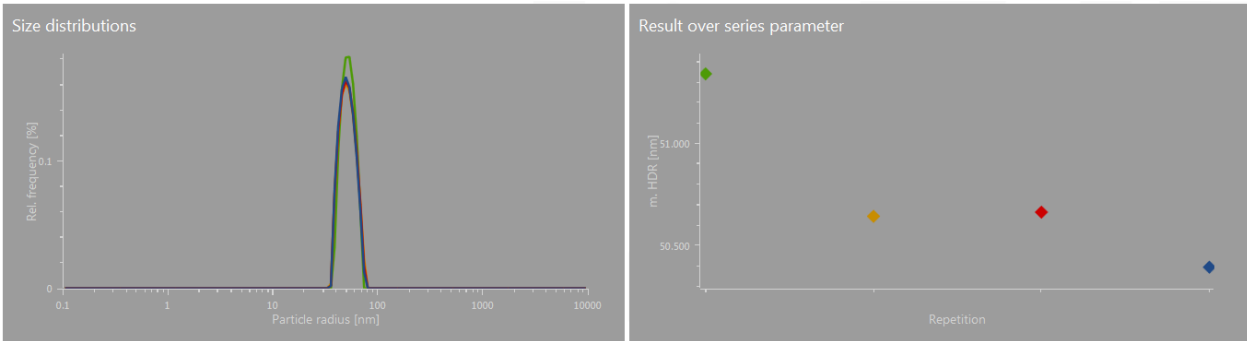


Fig. 7-14: Measurement series plots

7.4.3 Comparison and Analysis of Data

Comparison of measurements from separate experiments is also possible through the Analyze function (although the measurements must be from the same workbook). The results of interest must first be open in Kalliope; results can be measured directly, or previously measured results can be accessed by clicking on the open workbook icon . Once the results of interest are open in Kalliope, individual measurements can be selected for comparison and analysis by checking the white boxes in the left-hand column (see Fig. 7-15), which then contain a red tick. When at least two measurements have been selected, then the Analyze icon will be activated. Clicking on the icon produces a graph of the relative frequency vs particle radius, with each separate measurement plotted in a different color, as well as a table of the data output, including measured and calculated values appearing in columns side by side (see Fig. 7-16). The weighting model used in the calculation can be selected by using the drop-down menu in the top right-hand corner of the graph.

As for measurement series (section 7.4.2, above), individual measurements can be viewed in more detail by clicking on the icon in the table. The Analysis overview can then be returned by clicking on the green back arrow icon at the left of the screen (or on the Show series icon).

7.4.4 Reporting

To present measurements or analyses as reports, see section 11, "Reporting".

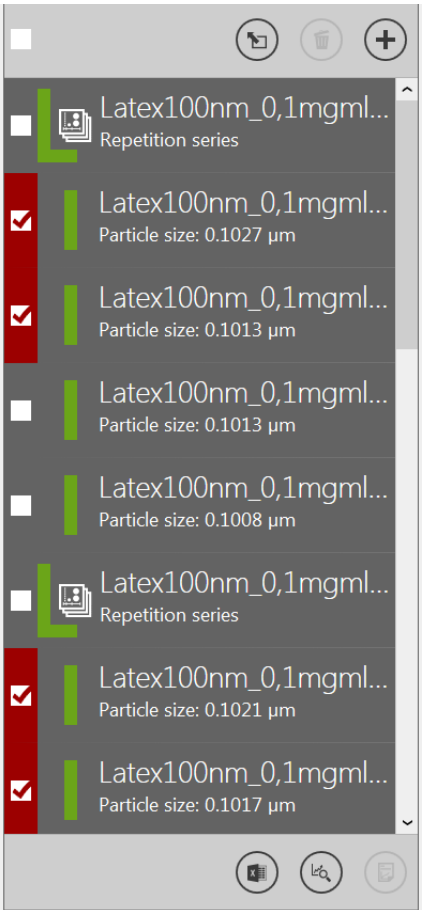
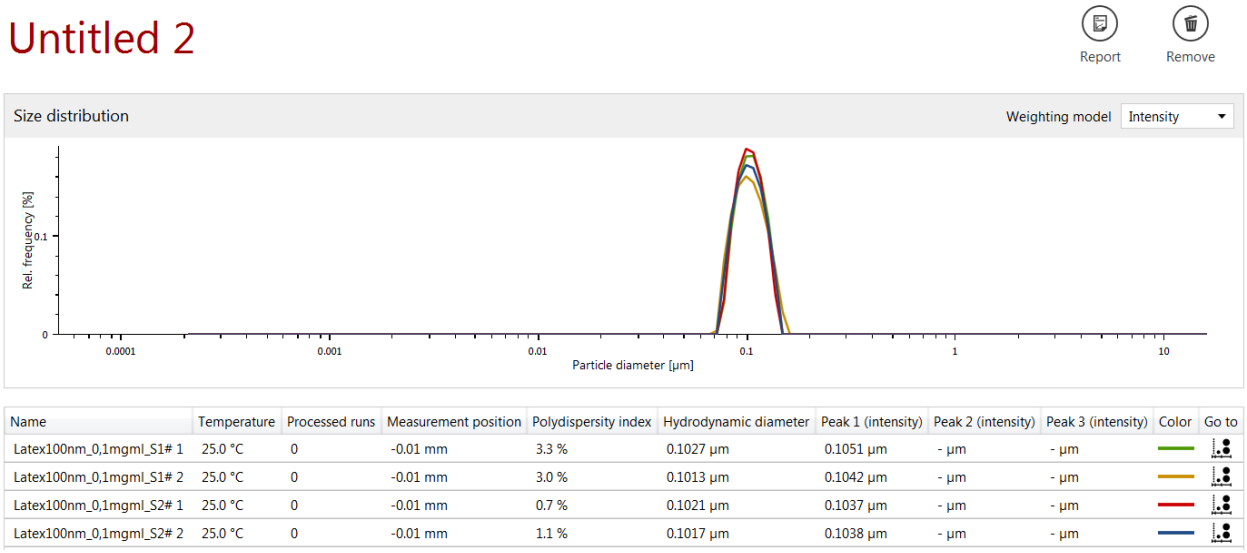


Fig. 7-15: Selecting measurements to compare by using the Analyze function



Name	Temperature	Processed runs	Measurement position	Polydispersity index	Hydrodynamic diameter	Peak 1 (intensity)	Peak 2 (intensity)	Peak 3 (intensity)	Color	Go to
Latex100nm_0,1mgml_S1# 1	25.0 °C	0	-0.01 mm	3.3 %	0.1027 µm	0.1051 µm	- µm	- µm		
Latex100nm_0,1mgml_S1# 2	25.0 °C	0	-0.01 mm	3.0 %	0.1013 µm	0.1042 µm	- µm	- µm		
Latex100nm_0,1mgml_S2# 1	25.0 °C	0	-0.01 mm	0.7 %	0.1021 µm	0.1037 µm	- µm	- µm		
Latex100nm_0,1mgml_S2# 2	25.0 °C	0	-0.01 mm	1.1 %	0.1017 µm	0.1038 µm	- µm	- µm		

Fig. 7-16: Output of the measurements selected for comparison by using the Analyze function

8 Zeta-Potential Measurements

8.1 Sample Preparation

Zeta-potential measurements provide information about the stability of a colloidal dispersion. The zeta potential depends not only on the charge of the particles themselves, but also on the solvent in which the particles are dispersed. Therefore sample manipulation is only recommended for samples that cannot be measured in their original condition; for example, because the sample concentration is not within the required limits. Sample preparation may also be necessary for specific applications or special standardized measurement procedures, but every sample manipulation must be done carefully in order to not falsify the results.

8.1.1 Concentration

Zeta-potential measurements are not as sensitive to particle concentration as are particle-size measurements. Most importantly, the concentration must be high enough that sufficient light is scattered to provide meaningful measurements. As for particle size measurements, if a zeta potential measurement generates a mean detected light intensity of <20 kcounts/s, then the concentration should be increased if possible. Likewise, for automatic measurements, a filter optical density of 0 suggests that the sample concentration is low, although the results may still be meaningful if the count rate is still sufficiently high.

8.1.2 Diluting the Sample

Equilibrium dilution procedure: If the particle concentration is too high, the sample must be diluted, preferably by adding more of the solvent that is already in the sample, because the zeta potential will not be affected. This method is referred to as "equilibrium dilution procedure."

Solvent extraction/filtration: If no original solvent is available, it must be extracted from the original solution, either by sedimentation or centrifugation, but this only works well for large particles with sufficient density contrast. For small particles, dialysis is necessary with membranes that are not penetrable by the sample particles.

Solvent imitation: If the same solvent cannot be used, then a solvent should be sought whose properties match the original solvent as closely as possible, in terms of viscosity, polarity, pH, electrolyte concentration.

8.2 Cuvettes

The Omega cuvettes for zeta potential are made from polycarbonate, and thus only aqueous samples may be used. The sample volume is 350 µl, and the entire cell should be filled.

Disposable, powder-free latex gloves should be worn throughout all procedures; both to prevent skin contact with any samples or solvent, but also to protect the measurement cells and glassware from contaminants on/in the skin.

To fill the Omega cuvette, place the tip of the syringe snugly inside one of the sample ports (see Fig. 8-1, top). To stop bubbles forming, the filling direction should always be upwards. Thus, for the first half, the cell should be upside down. Gently inject the sample into the cuvette. Once the liquid reaches halfway (see Fig. 8-1, middle - see arrow), carefully turn the cell upright and continue to inject the sample until the cuvette is full (see Fig. 8-1, bottom).

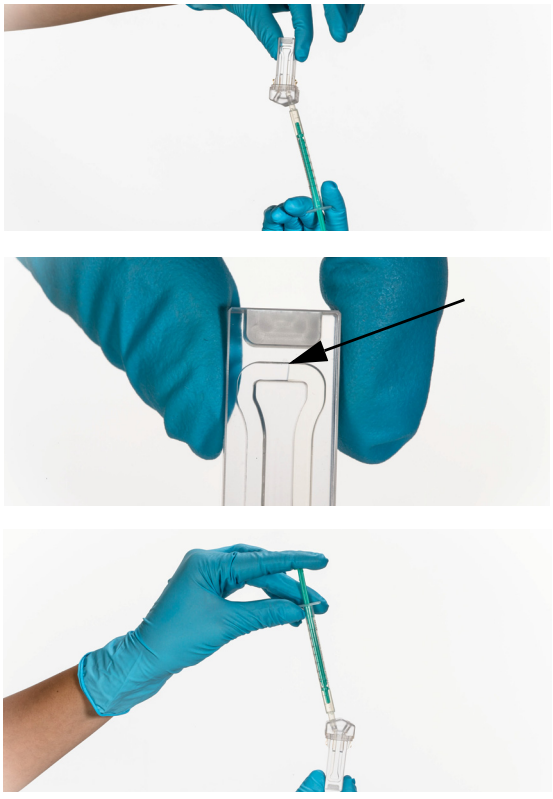


Fig. 8-1: Filling the Omega cuvette: The cuvette should be held upside down until it is half full, so that the filling direction is always upwards.

Ensure that both electrodes are covered by the sample. Check for tiny air bubbles and tap the cell to dislodge any that have formed. Insert the cuvette stoppers (see Fig. 8-2).

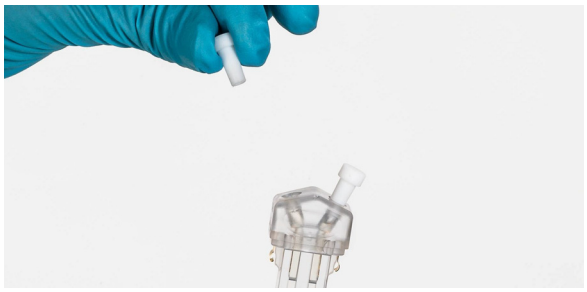


Fig. 8-2: Inserting the cuvette caps

8.2.1 Inserting the Cuvette

Ensure that the outer surface of the cuvette is clean and dry before inserting it into the Litesizer™ 500.

Open the chamber by pushing the OPEN button on top of the black module. Insert the cell firmly until it stops, with the electrodes and sample ports pointing to the sides (see Fig. 8-3). Close the chamber.

Fig. 8-3: Inserting the Omega cuvette

TIP: For advice on how to clean the Omega cuvettes, see Chapter 12, "Using the Litesizer™ 500 at High and Low Temperature".

8.3 Making a Measurement

On the start-up screen (Fig. 6-5), click on the ⊕ icon to select a new measurement. Select % Zeta potential. Input parameters can be entered on the left-hand side of the display, as follows (Fig. 8-4):

8.3.1 Input Parameters for Zeta Potential

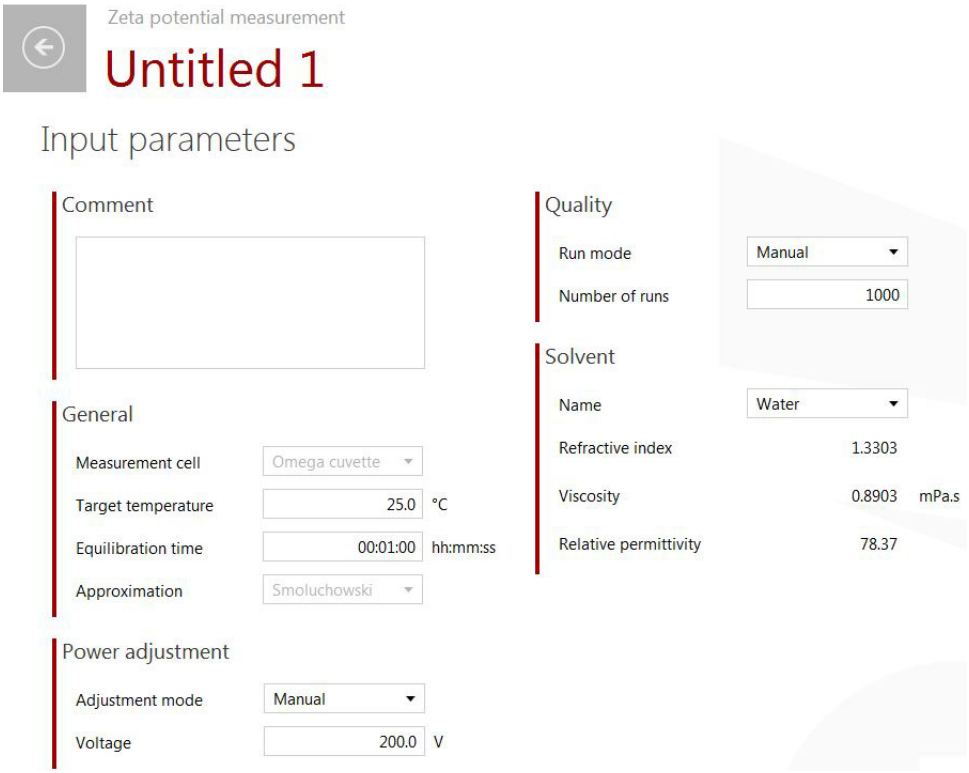


Fig. 8-4: Zeta potential input parameters

Table 8-1: Explanation of Input Parameters for Zeta-potential Measurement

Title

Experiment name	At the top is the name of the current measurement ("Untitled", in red). The name can be changed by clicking in any part of "Untitled". The back button will switch back to the overview display.
Comment	Describe here the sample and/or conditions.

General

Measurement cell	**Omega cuvette**: The Omega cuvette is made from polycarbonate; thus, only aqueous solvents may be used for these measurements.
Target temperature	Measuring temperature must be manually entered, and must be set between 0 and 70 ˚C.
Equilibration time	For measurements close to ambient temperature, the equilibration time should be set at two minutes. The further the measuring temperature from ambient temperature, the longer the equilibration required (a common rule of thumb is to add one minute for every ˚C different from ambient temperature, based on a 1 mL sample).
TIP: *Before making high- or low-temperature measurements, see Chapter 12, "Using the Litesizer™ 500 at High and Low Temperature".*	
Approximation	The Smoluchowski approximation is suitable for water-based samples.

Power adjustment

Adjustment mode	**Automatic**: The voltage is increased in increments until the maximum voltage is reached, for which the maximum power is not exceeded. **Manual**: The voltage can be set from 0.1 to 200 V.
Voltage	from 0.1 to 200 V

Quality

Run mode	**Automatic**: the experiment will stop when the standard deviation reaches the threshold value. **Manual**: The number of runs can be set from 20 to 1000.
Number of runs	from 20 to 1000

Solvent

Name	The solvent must be selected from the database.

Note: Once the solvent is selected, then the Refractive index, viscosity and relative permittivity will be automatically filled. New solvents can be entered in the database by the user by clicking on the Ka icon.

8.3.2 Starting a Measurement

Once the input parameters are complete, the Start icon in the bottom right-hand corner of the screen will be activated, and can be clicked to start the measurement.

Following temperature adjustment, equilibration and optical adjustment, the measurement will be displayed on the screen while it is running, as shown below, while the run number is displayed at the bottom of the screen. The Litesizer™ 500 will keep performing runs until the standard deviation reaches the threshold value, or until the specified maximum number of runs has been reached. Once the measurements are finished, all the measured and calculated values (see Table 8-3 and Table 8-4 below for explanation) appear in the gray boxes to the right of the graphs, with the zeta potential box appearing in green.

8.4 Measurement Output Screen

The measurement output screen retains a display of the input parameters on the left, a series of action icons at the top right, and the results (plots, automatic values and calculated values - see Fig. 8-5 to Fig. 8-7 and Table 8-2 to Table 8-4 below) on the main part of the screen at the right.

8.4.1 Action Icons

Fig. 8-5: Action icons (see top right-hand corner of screen)

Table 8-2: Explanation of Action Icons

Excel Export	Exports current results to Excel file for further processing.
Copy parameters	Creates a copy of the input parameters of the current measurement. The name of the original measurement is retained, but with the prefix “New”.
Remove	Deletes current measurement, and switches back to starting display.

8.4.2 Zeta-Potential Results - Intensity Trace and Related Values

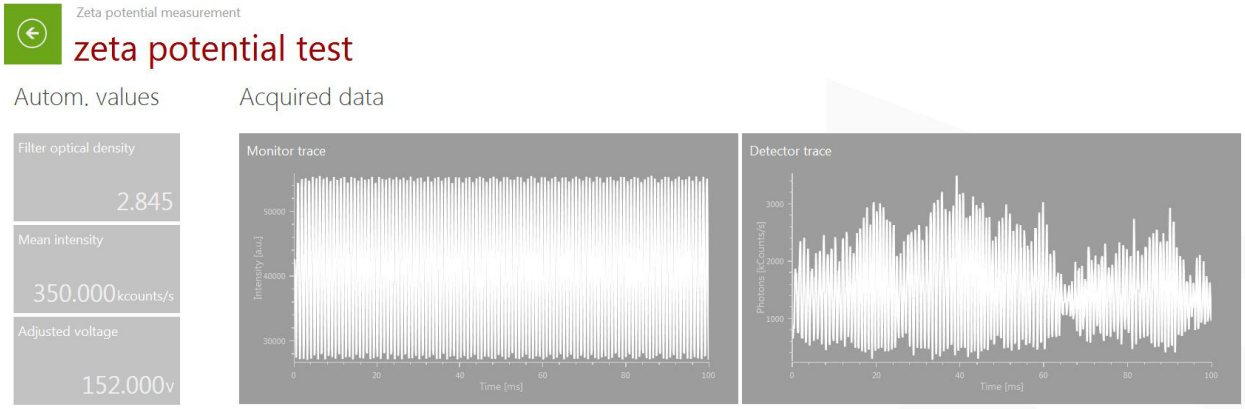


Fig. 8-6: Zeta potential output I

Table 8-3: Explanation of Intensity Trace and Related Values

Filter optical density	Indicates the attenuation level used in the measurement. For automatic measurements, a filter optical density of 0 also suggests that the sample concentration is low, although the results may still be meaningful if the count rate is sufficiently high.
Mean intensity	Displays the mean detected light intensity in kcounts/s. If the mean intensity is less than 20 kcounts/s, then increasing the concentration should give better results.
Adjusted voltage	The voltage applied in the measurement.
Monitor trace	Shows the interference between the modulated and unmodulated reference beams. A good measurement will show a stable signal with constant amplitude.
Detector trace	Shows interference between scattered light from the sample and the modulated reference beam. An amplitude of at least 1000 is required to obtain meaningful results.

8.4.3 Zeta-Potential Results - Phase Plot and Zeta-Potential Distribution

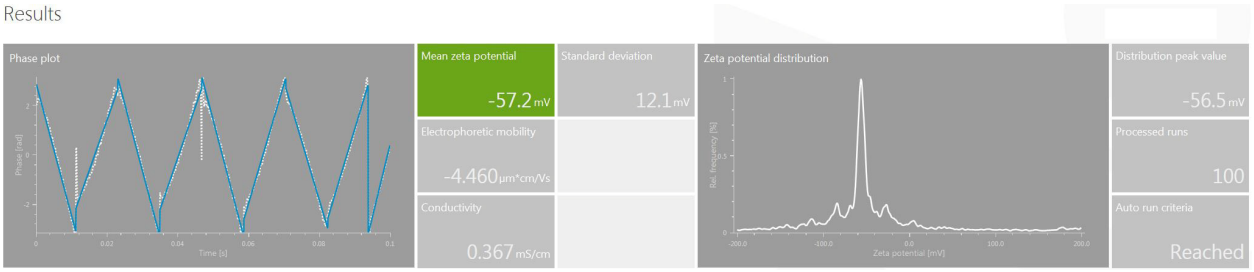


Fig. 8-7: Zeta potential output II

Table 8-4: Explanation of Phase Plot, Zeta Potential Distribution and Related Values

Phase plot	Plots the phase analysis light scattering (PALS), which shows the phase difference between the detector trace and the monitor trace in white. The blue line shows the fit of the PALS to the data.
Mean zeta potential	The mean zeta potential is calculated from the phase analysis light scattering (PALS).
Electrophoretic mobility	Calculated from the phase analysis light scattering (PALS).
Conductivity	The conductivity should ideally be less than 1 mS/cm. The Litesizer™ 500 can measure zeta potential of samples with conductivities up to 200 mS/cm; however, such samples may be degraded or damaged by the voltage applied.
Standard deviation	The standard deviation of the mean zeta potential.
Zeta potential distribution	The Fourier transform of the detector trace is used to generate the zeta potential distribution as a function of frequency.
Distribution peak value	The distribution peak is the maximum of the zeta potential distribution. The value should be similar to that generated from the PALS.
Processed runs	Number of runs carried out in the measurement.
Auto run criteria	"Reached" indicates that the threshold standard deviation was reached.

8.5 Zeta Potential - Measurement Series

A series of zeta potential measurements can be made by selecting New Measurement in Kalliope and then selecting Zeta potential series (Fig. 8-8).

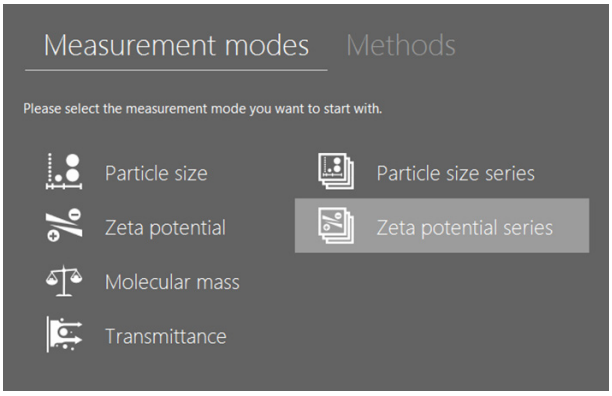


Fig. 8-8: Selecting a measurement series for zeta potential

8.5.1 Measurement Series Input Parameters

In addition to all of the input parameters, as described in section 8.3.1, the measurement series input parameters will appear at the right-hand end of the Input parameters screen (see Fig. 8-9).

The series can be based on variation of any one of four parameters: temperature, concentration, pH, (see Fig. 8-10); alternatively, the measurement can simply be repeated by selecting Repetition.

To set the number of measurements in the series, click on the + icon until the required number of fields appears (see Fig. 8-11).

Once the input parameters are complete, click the Start icon in the bottom right-hand corner of the screen to start the measurements.

For Temperature and Repetition series, the measurements will continue automatically one after the other. For Concentration and pH measurement series, individual samples must be prepared for each measurement. The user will be asked to change the sample as each measurement is finished.

NOTE: *For a temperature series, ensure that sufficient equilibration time is allowed for each measurement. A general recommendation is to add one minute for every ˚C difference between measurement temperatures, based on a 1 mL sample; e.g., for a series of three measurements at 20, 25, and 30 ˚C, at least 5 min equilibration time should be selected.*

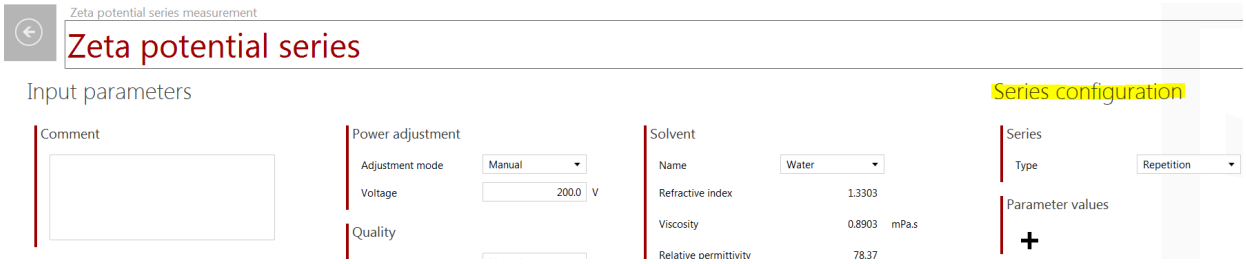
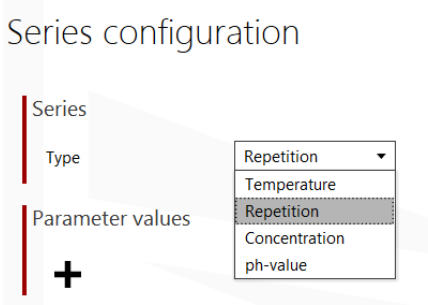


Fig. 8-9: Input parameters for a measurement series



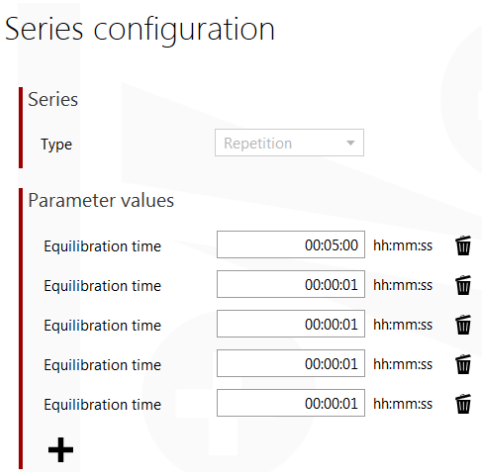


Fig. 8-10: Series parameter selection

Fig. 8-11: Setting the number of measurements

8.5.2 Measurement Series Output

Data for the measurement series appears in a table (see Fig. 8-12), with input parameters and measured and calculated values appearing side by side. The order of the values can be changed by clicking on the column heading and dragging it to the desired position.

To the right of the results table are two plots graphically summarizing the results (see Fig. 8-13): The first plot depicts relative frequency vs zeta potential, with each separate measurement plotted in a different color. The second plot depicts mean zeta potential vs repetition number. The same color is used for the two plots, the color code being listed in the table.

Individual measurements can be viewed in more detail by clicking on the icon in the table. The overview can then be returned by clicking on the Show series icon. Results can be further analyzed by exporting the data to an Excel file (click on the Excel Export icon). The current measurement can be repeated by clicking on the Copy parameters icon, which creates a copy of the input parameters of the current measurement. The name of the original measurement is retained, but with the prefix “New”.

Zeta potential series measurement

Zeta potential: Repetition

Results

Index	Name	Status	Temperature	Equilibration time	Mean zeta potential	Detector peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
1	Latex220nm-5mgml 1	Succeeded	25.0 °C	00:05:00	-77.4 mV	-77.5 mV	1.789 mS/cm	-6.030 µm*cm/Vs	200	150.000 V		
2	Latex220nm-5mgml 2	Succeeded	25.0 °C	00:00:01	-79.3 mV	-82.5 mV	1.803 mS/cm	-6.178 µm*cm/Vs	200	150.000 V		
3	Latex220nm-5mgml 3	Succeeded	25.0 °C	00:00:01	-78.8 mV	-81.0 mV	1.829 mS/cm	-6.143 µm*cm/Vs	200	150.000 V		
4	Latex220nm-5mgml 4	Succeeded	25.0 °C	00:00:01	-79.9 mV	-80.2 mV	1.836 mS/cm	-6.226 µm*cm/Vs	200	150.000 V		
5	Latex220nm-5mgml 5	Succeeded	25.0 °C	00:00:01	-79.9 mV	-76.8 mV	1.843 mS/cm	-6.227 µm*cm/Vs	200	150.000 V		

Fig. 8-12: Measurement series output data

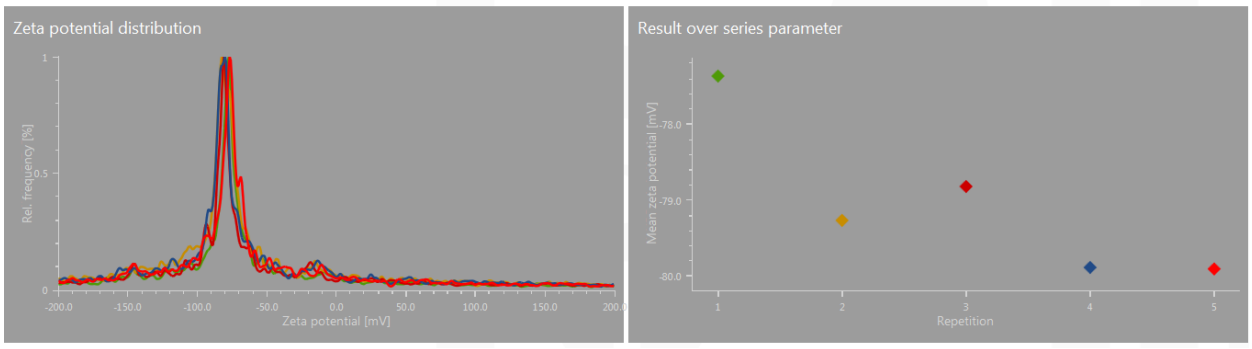


Fig. 8-13: Measurement series plots

8.5.3 Comparison and Analysis of Data

Comparison of measurements from separate experiments is also possible through the Analyze function. The results of interest must first be open in Kalliope. Results can be measured directly; or previously measured results can be accessed by clicking on the open workbook icon . Once the results of interest are open in Kalliope, individual measurements can be selected for comparison and analysis by checking the white boxes in the left-hand column (see Fig. 8-14), which then contain a red tick. When at least two measurements have been selected, then the Analyze icon will be activated. Clicking on the icon plots a graph of the relative frequency vs zeta potential, with each separate measurement plotted in a different color, as well as a table of the data output, including measured and calculated values (see Fig. 8-15).

As for measurement series (Chapter 8.5.3, "Comparison and Analysis of Data", above), individual measurements can be viewed in more detail by clicking on the icon in the table. The Analysis overview can then be returned by clicking on the green back arrow icon at the left of the screen (or by clicking on the Show series icon).

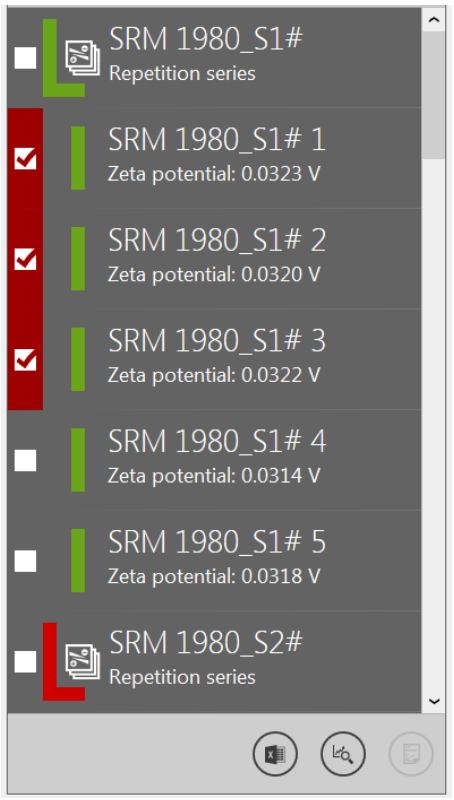
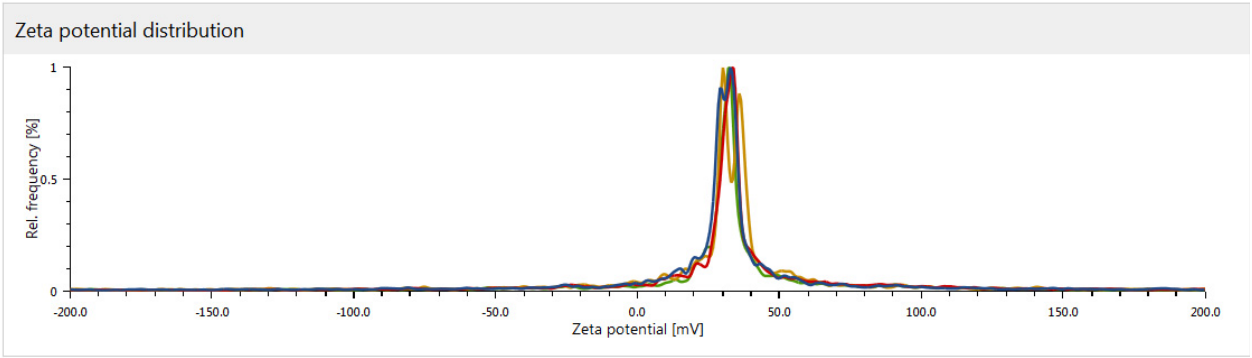


Fig. 8-14: Selecting measurements to compare by using the Analyze function

8.5.4 Reporting

To present measurements or analyses as reports, see section 11, "Reporting".



Name	Temperature	Mean zeta potential	Detector peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
SRM 1980_S3# (2) 4	25.0 °C	30.9 mV	32.5 mV	0.867 mS/cm	2.406 µm*cm/Vs	100	200.000 V		%
SRM 1980_S3# (2) 5	25.0 °C	31.0 mV	30.1 mV	0.867 mS/cm	2.416 µm*cm/Vs	100	200.000 V		%
SRM 1980_S3# (3) 1	25.0 °C	30.7 mV	33.2 mV	0.858 mS/cm	2.394 µm*cm/Vs	100	200.000 V		%
SRM 1980_S3# (3) 2	25.0 °C	30.7 mV	32.7 mV	0.860 mS/cm	2.396 µm*cm/Vs	100	200.000 V		%

Fig. 8-15: Output of the measurements selected for comparison by using the Analyze function

9 Molecular-Mass Measurements

9.1 Sample Preparation

For molecular mass measurements, the sample preparation is similar to that for particle size measurements, in that mass measurements are particularly sensitive to dust or other contaminants in the solvent. Thus, solvents should be filtered several times before making up samples. All glassware and equipment used for sample preparation should be scrupulously clean and free of dust and scratches.

It is advisable to use at least three samples of different concentrations in order to generate the Debye plot. Furthermore, a sample of the solvent, and of the reference must also be made up fresh. The solvent sample must include all solutes (such as buffer or salt) that are contained in the sample other than the particles to be measured.

As for particle-size and zeta-potential measurements, if a molecular-mass measurement generates a mean detected light intensity of <20 kcounts/s, then the concentration should be increased.

9.2 Cuvettes

The standard cells for molecular mass measurements are quartz, with inner dimensions 10 mm x 10 mm x 45 mm. Ideally, the sample volume should be approximately 1 mL.

Fig. 9-1: A standard cuvette showing the ideal sample volume.

The measurement is made 6.5 mm from the bottom of the cell, and the meniscus must be at least 2 mm above the measurement height (8.5 mm). For reliable measurements, the depth must be between 8.5 and 30 mm, and thus, the volume must be between 0.85 and 3 mL.

9.2.1 Small-Volume Cuvette

The small-volume quartz cell is designed to be used when little sample is available. The maximum volume is 45 µl, while the minimum that can be used is 20 µl.

Fig. 9-2: Small-volume cell showing the minimum sample volume (20 µl)

9.2.2 Filling the Cuvettes

Disposable, powder-free latex gloves should be worn throughout all procedures; both to prevent skin contact with any samples or solvent, but also to protect the measurement cells and glassware from contaminants on/in the skin.

To fill a cuvette, place the tip of the pipette at the bottom of the cell so that it fills from the bottom up, thereby avoiding bubble formation. Check the sample through the windows for tiny bubbles, and tap the cell to dislodge any that have formed.

Place the lid firmly on the cuvette, and ensure that the outer surface of the cuvette is clean and dry before inserting it into the Litesizer™ 500.

9.2.3 Inserting the Cuvette

Open the chamber by pushing the OPEN button. Insert the cell firmly until it stops (see Fig. 9-3). Close the chamber.

Fig. 9-3: Inserting the cuvette

9.3 Making a Measurement

On the start-up screen (Fig. 6-5), click on the ⊕ icon to select a new measurement. Select Molecular mass. Input parameters can be entered on the left-hand side of the display, as described in Fig. 9-4 and Table 9-1, below.

9.3.1 Input Parameters for Molecular Mass

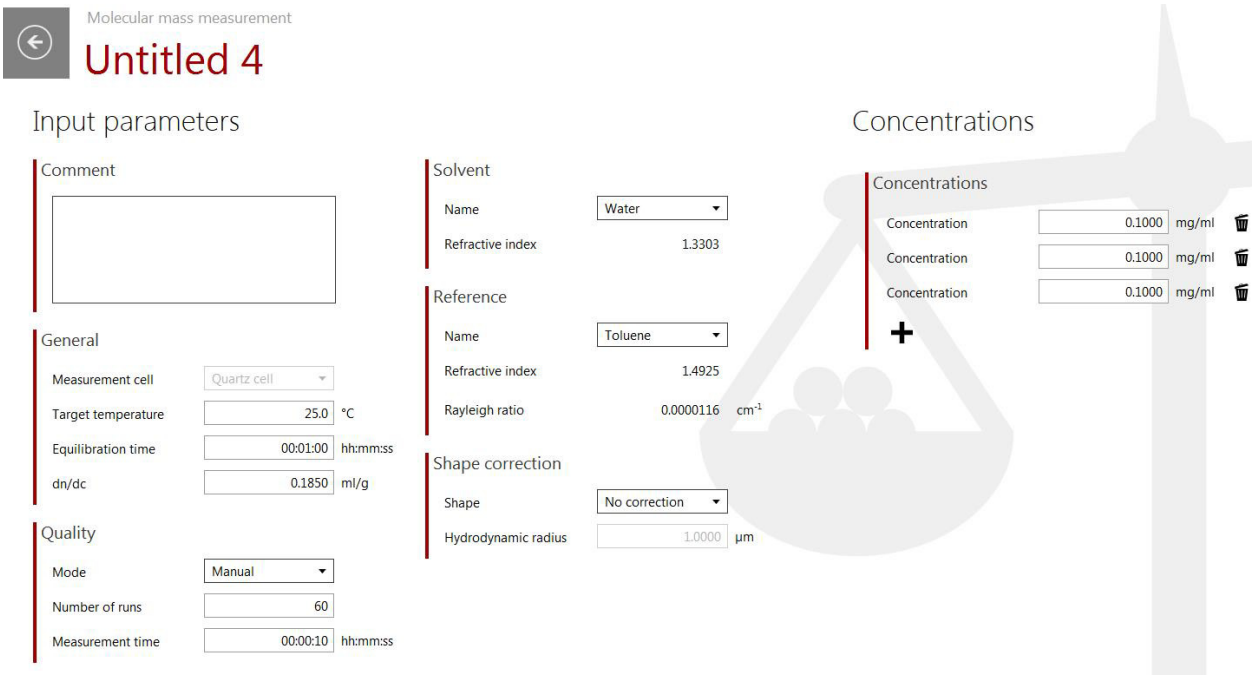


Fig. 9-4: Molecular-mass input parameters

Table 9-1: Explanation of Input Parameters for Molecular Mass

Title

Experiment name	At the top is the name of the measurement. Click in any part of "Untitled" to change the name. The back button switches back to the overview display.
Comment	Describe here the sample and/or conditions.

General

Measurement cell	**Quartz**: the only cell that can be used for molecular mass measurements
Target temperature	Measuring temperature must be set between 0 and 90 ˚C (entered manually)
TIP: *Before making high- or low-temperature measurements, see Chapter 12, "Using the Litesizer™ 500 at High and Low Temperature".*	
Equilibration time	For measurements close to ambient temperature, the equilibration time should be set at two minutes. The further the measuring temperature from ambient temperature, the longer the equilibration required (add one minute for every ˚C different from ambient temperature, based on a 1 mL sample).
dn/dc	This is the refractive index increment. It represents the change in refractive index as a function of the change in concentration.

Quality

Mode	automatic or manual
Number of runs	from 1 to 100
Time for each run	must be set between 1 s and 30 min

Solvent

Name	Select a solvent from the database.
Note: Once the solvent is selected, then the Refractive index will be automatically filled. New solvents can be entered in the database by the user by clicking on the Ka icon.	

Reference

Name	Toluene is generally used as the reference. Once the reference is selected, the refractive index and Rayleigh ratio will be automatically imported from the solvent database. New references can be entered in the solvent database by clicking on the Ka icon.

Shape correction

Shape	A shape correction should be selected if the particles do not scatter isotropically. Spherical, spiral, cylindrical, or "no correction" can be selected.
Hydrodynamic radius	The hydrodynamic radius must be inserted if known, or it must first be measured by performing a particle-size measurement.

Concentrations

At least three concentrations are required to make a measurement. Additional concentrations can be entered by clicking on the + icon, while a concentration can be deleted by clicking on the icon. Concentrations can be selected between 0.001 and 500 mg/mL.

Table 9-1: Explanation of Input Parameters for Molecular Mass

Note: regardless of input order, the Litesizer™ 500 will measure in order of concentration, starting from the most concentrated sample.

9.3.2 Starting a Measurement

Once the input parameters are complete, the Start icon in the bottom right-hand corner of the screen will be activated, and can be clicked to start the measurement. You will then be asked to insert each sample in succession, starting from the most concentrated sample down to the least concentrated sample (regardless of which order they are entered in the Input), followed by the solvent sample, and finally the reference. Sample measurement starts after the optics adjustment and sample equilibration. Once sample measurement begins, the results will appear on the right-hand side of the screen. Once the measurements are finished, all the measured and calculated values can be seen in the gray boxes to the right of the graphs.

9.4 Measurement Output Screen

The measurement output screen retains a display of the input parameters on the left, a series of action icons at the top right, and the results (plots, automatic values and calculated values—see Fig. 9-5 to Fig. 9-7 and Table 9-2 to Table 9-4 below) on the main part of the screen at the right.

9.4.1 Action Icons

Fig. 9-5: Action icons (see top right-hand corner of screen)

Table 9-2: Explanation of Action Icons

Excel Export	Exports current results to Excel file for further processing.
Copy parameters	Creates a copy of the input parameters of the current measurement. The name of the original measurement is retained, but with the prefix “New”.
Remove	Deletes current measurement, and switches back to starting display.

9.4.2 Molecular-Mass Results - Intensity Trace and Related Values

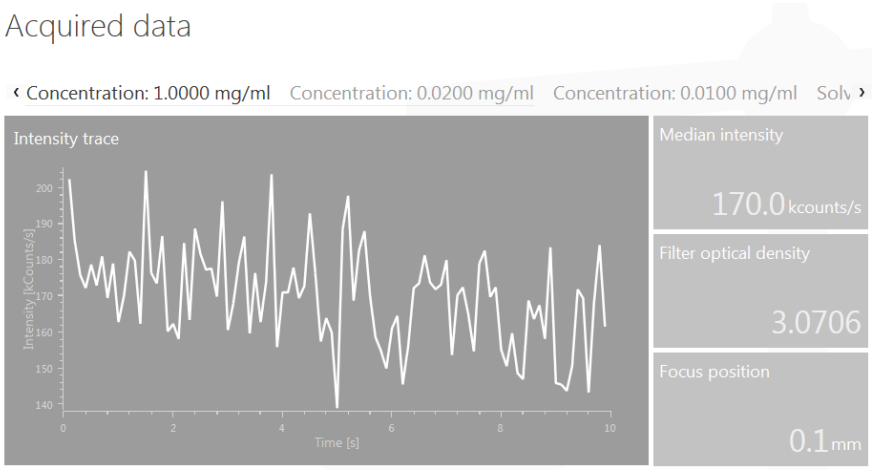


Fig. 9-6: Molecular mass output I

Table 9-3: Explanation of Intensity Trace and Related Values

Intensity trace	The intensity trace displays the current measurement as soon as it begins.
Mean intensity	Displays the mean detected light intensity in kcounts/s. If the mean intensity is less than 20 kcounts/s, then increasing the concentration should give better results.
Filter optical density	Indicates the attenuation level used in the measurement. For automatic measurements, a filter optical density of 0 also suggests that the sample concentration is low, although the results may still be meaningful if the count rate is still sufficiently high.
Focus position	Indicates the position of the optical focus used in the measurement.

9.4.3 Molecular-Mass Results - Debye Plot, Molecular Mass and 2nd Virial Coefficient

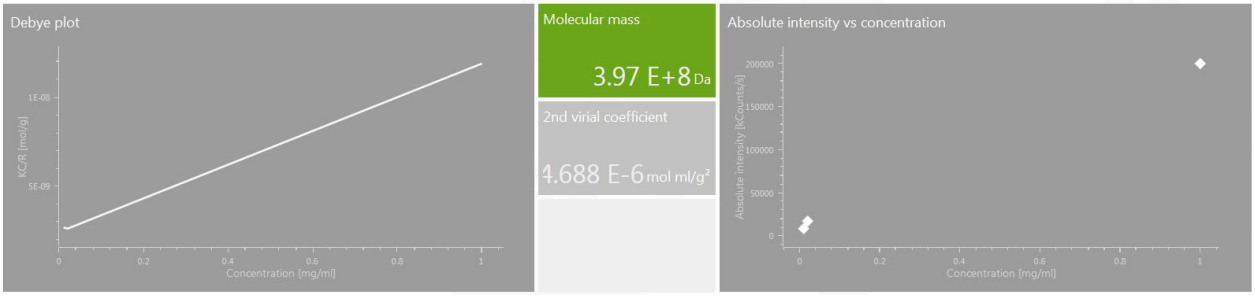


Fig. 9-7: Molecular mass output II

Table 9-4: Explanation of Debye Plot, Molecular Mass and 2nd Virial Coefficient

Debye plot	The Debye plot relates the intensity of the scattered light (KC/R) to the particle concentration.
Molecular mass	Calculated as the inverse of the Y-intercept of the Debye plot.
2nd virial coefficient	Calculated from the slope of the Debye plot. Reflects the interaction between particles and solvent, and between the particles. A positive gradient indicates that the particle–solvent interactions are stronger than the particle–particle interactions, so the dispersion or solution is stable. Vice versa, a negative gradient indicates that the particle–particle interactions are stronger than the particle–solvent interactions, so the particles will tend to aggregate.
Intensity vs concentration	The mean intensity from each sample is plotted vs concentration, which should give a linear relationship. Deviations from linearity may indicate that one or more of the measurements is erroneous; for example, the sample may contain dust or other contaminants.

10 Transmittance Measurements

10.1 Sample Preparation

There are no real restrictions on the type of samples that can undergo transmittance measurements. For aqueous samples, the disposable polystyrene cuvettes can be used. For all other solvents, the quartz cells should be used.

10.1.1 Reference Sample

Along with the sample to be measured, a sample of the neat solvent should be prepared with the same sample volume, and in the same type of cuvette. The solvent sample can then be used as the reference.

10.2 Cuvettes

The standard cells, both quartz and polystyrene, have inner dimensions of 10 mm x 10 mm x 45 mm. Ideally, the sample volume should be approximately 1 mL.

Fig. 10-1: A standard cuvette showing the ideal sample volume.

The measurement is made 6.5 mm from the bottom of the cell, and the meniscus must be at least two mm above the measurement height (8.5 mm). For reliable measurements, the depth must be between 8.5 and 30 mm, and thus, the volume must be between 0.85 and 3 mL.

10.2.1 Filling the Cuvettes

Disposable, powder-free latex gloves should be worn throughout all procedures to protect the cuvettes and glassware from natural oils and contaminants on/in the skin, but also to prevent skin contact with any samples or solvent.

Hold the cuvette at an angle and allow the sample to run slowly down the internal corner. Such a technique should stop bubbles forming. Place the lid firmly on the cuvette, and ensure that the outer surface of the cuvette is clean and dry before inserting it into the Litesizer™ 500.

10.2.2 Inserting the Cuvette

Open the chamber by pushing the OPEN button. Insert the cell firmly until it stops (see Fig. 9-3). Close the chamber.

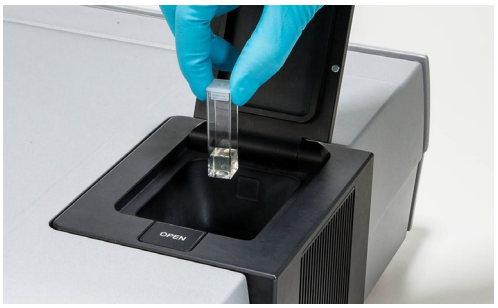


Fig. 10-2: Inserting the cuvette

10.3 Making a Measurement

On the start-up screen (Fig. 6-5), click on the ⊕ icon to select a new measurement. Select Transmittance. Input parameters can be entered on the left-hand side of the display, as described in Fig. 10-3 and Table 10-1, below:

10.3.1 Input Parameters for Transmittance

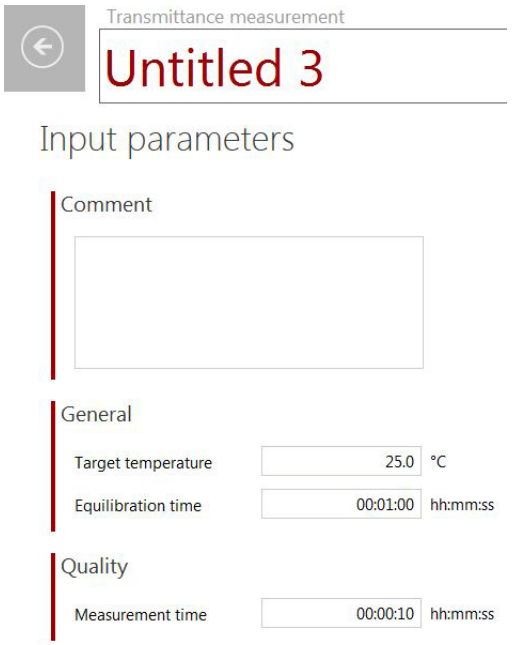


Fig. 10-3: Input parameters for transmittance

Table 10-1: Input Parameters

Title	
Experiment name	At the top is the name of the current measurement ("Untitled", in red). The name can be changed by clicking in any part of "Untitled". The back button ⊖ will switch back to the overview display.
Comment	Describe here the sample and/or conditions.
General	
Target temperature	Measuring temperature must be manually entered, and must be set between 0 and 90 ˚C (or 273 and 363 K). **Note**: for measurements above 70 ˚C, the quartz cell must be used.
TIP: *Before making high- or low-temperature measurements, see Chapter 12, "Using the Litesizer™ 500 at High and Low Temperature".*	
Equilibration time	For measurements close to ambient temperature, the equilibration time should be set at 2 min. The further the measuring temperature from ambient temperature, the longer the equilibration required (a common rule of thumb is to add 1 min for every ˚C different from ambient temperature, for a 1 mL sample).
Quality	
Time for each run	must be set between 1 s and 30 min. The recommended initial Time for each run is 10 s.

Table 10-1: Input Parameters

Note: To set the reference, a sample of the neat solvent or dispersant should be used. Once the transmittance is measured, click on the Set as reference icon. Then use the back button to start a new measurement with the new reference. If no reference measurement is made, then the transmittance of the empty chamber (with no cuvette) will be taken as the reference value.

10.3.2 Starting a Measurement

Once the input parameters are complete, the Start icon in the bottom right-hand corner of the screen will be activated, and can be clicked to start the measurement. Sample measurement starts after the optics adjustment and sample equilibration. Once sample measurement begins, the results will appear on the right-hand side of the screen. Once the measurement is finished, the results can be seen in the gray boxes to the right of the graphs.

10.4 Measurement Output Screen

The measurement output screen retains a display of the input parameters on the left, a series of action icons at the top right, and the results (plots, automatic values and calculated values—see Fig. 10-4 to Fig. 10-5 and Table 10-2 to Table 10-3 below) on the main part of the screen at the right.

10.4.1 Action Icons

Fig. 10-4: Action icons (see top right hand corner of screen)

Table 10-2: Explanation of Action Icons

Set as reference	Sets the transmittance of the current sample as the reference.
Excel Export	Exports current results to Excel file for further processing.
Copy parameters	Creates a copy of the input parameters of the current measurement. The name of the original measurement is retained, but with the prefix “New”.
Remove	Deletes current measurement, and switches back to starting display.

10.4.2 Transmittance Results

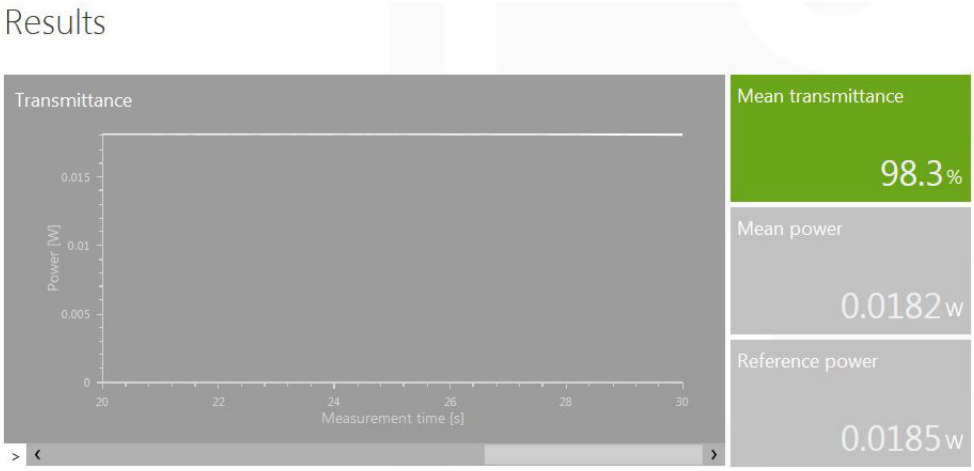


Fig. 10-5: Transmittance output

Table 10-3: Explanation of Transmittance Results

Mean transmittance	Colored green. Transmittance relative to reference power, which is set to 100 %.
Mean power	Mean transmitted power of the current measurement, in watts.
Reference power	Mean transmitted power of the reference measurement, in watts. If no extra reference measurement is made, then the reference will be taken as the power transmitted through an empty chamber.

Note: To find out whether a sample changes over time (e.g. through sedimentation or aggregation), then it is advisable to repeat the transmittance measurement at certain time intervals

11 Reporting

11.1 Reports of Individual Measurements

To present an individual measurement as a reports, the measurement must first be selected from an open workbook by checking the white box in the left-hand column (see Fig. 11-1). The box then contains a red tick and the report icon in the bottom right-hand corner is activated. Clicking on the icon allows you to select a report template (which can be customized as described in), which then produces a comprehensive summary of the data output, including measured and calculated values, in a convenient report format. Once created, the report can be forwarded for Review and/or Approval, either electronically by clicking on the Sign icon Sign , or in hard copy by collecting the appropriate signatures at the end of the report.

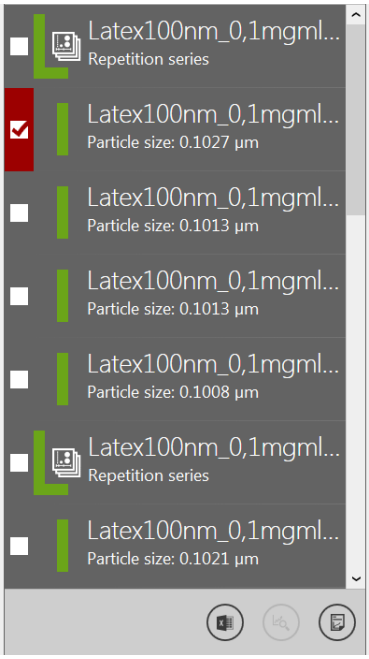


Fig. 11-1: Selecting a measurement for creating a report

11.2 Reports of Measurement Series

Measurement series can also be presented as reports. This can be done by either selecting a measurement series, in the same way as described above for reports of individual measurements, or from within an analysis. Once an analysis is open in Kalliope, the report icon can be found in the top-right corner of the page.

11.3 Customizing Reports

Customized reports can be produced by opening the Kalliope menu and selecting Reporting. The simplest way to proceed is to select the Standard report under the appropriate mode (see Fig. 11-2), then click on the copy icon in the top right-hand corner.

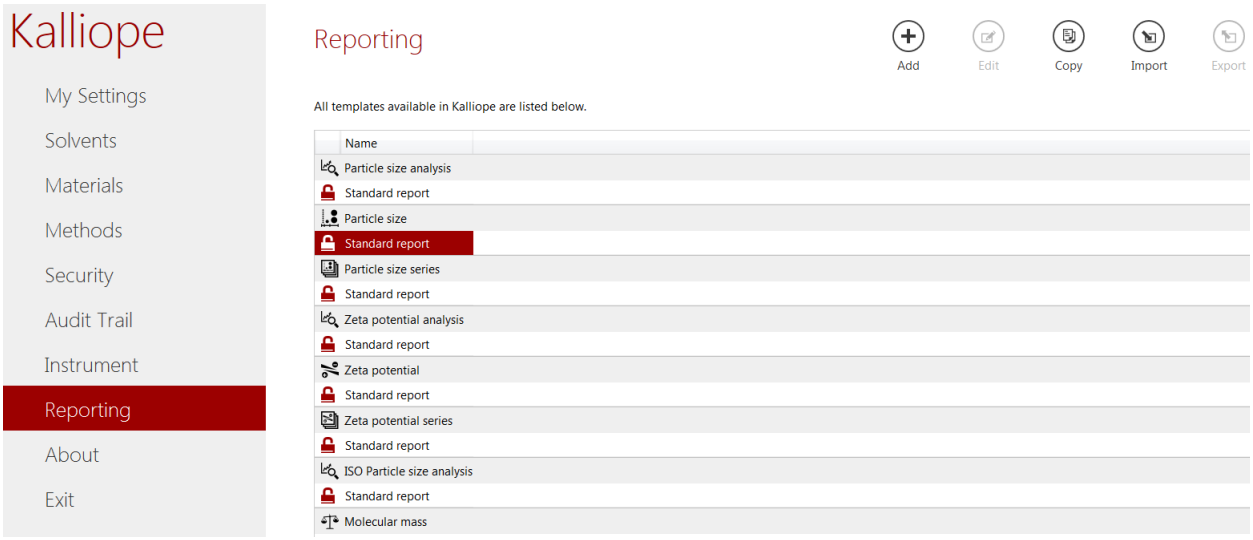


Fig. 11-2: Customizing a report

This creates a "Copy of Standard report". Clicking on the edit icon (next to the Copy icon) opens the report template designer (see Fig. 11-3). The report can now be renamed, while items can be added or removed by clicking on the the + icon or the delete icon, respectively. The template can be saved and closed by clicking on the close icon in the top right-hand corner. To access the template, click on the report icon, as described in section 11.1 or section 11.2, above.

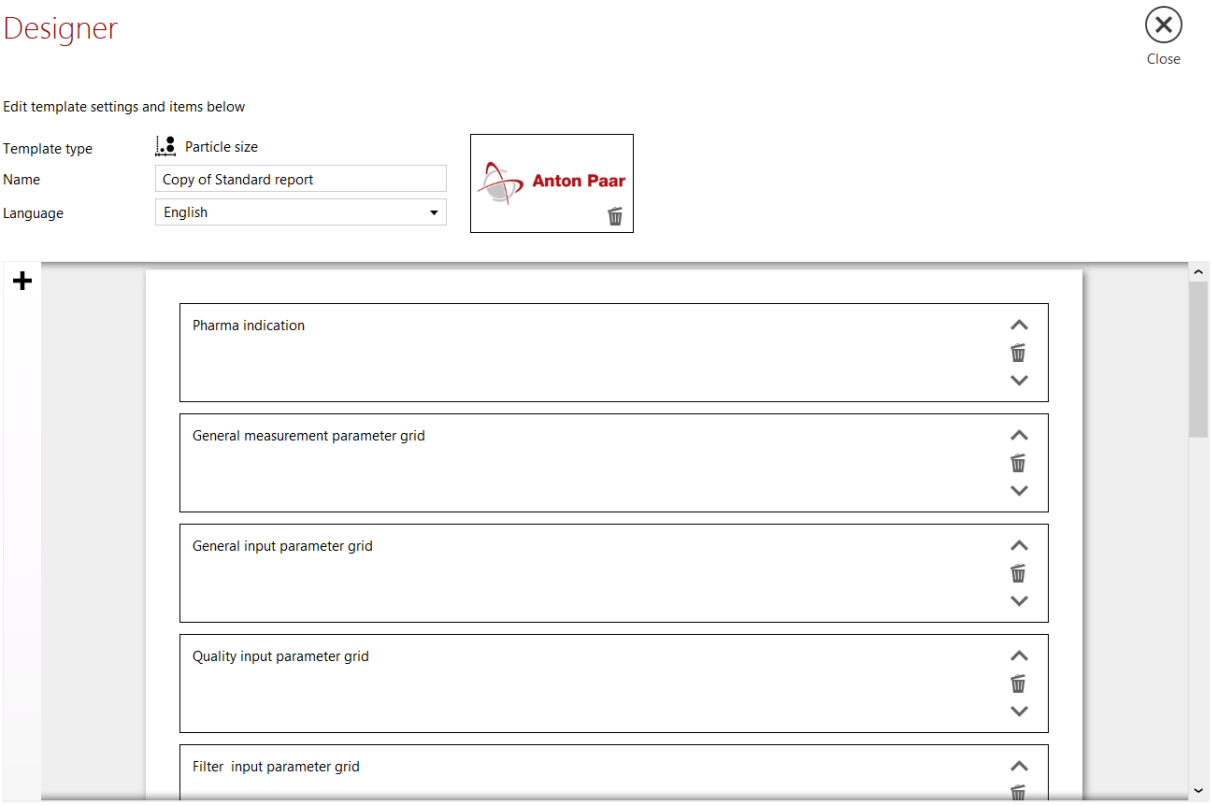


Fig. 11-3: Report template designer

12 Using the Litesizer™ 500 at High and Low Temperature

The Litesizer™ 500 is capable of making measurements at any temperature from 0 to 90 ˚C. Nonetheless, to ensure reliable measurements at temperatures other than room temperature, it is important to incorporate extra equilibration time, and to use the thermal insulation cover (Fig. 12-1), which will help to maintain a constant temperature throughout the sample and cuvette, thereby minimizing thermal currents in the sample.

Fig. 12-1: Thermal insulation cover

Once the cuvette is in place in the module, insert the thermal insulation cover into the module above the cuvette until it clicks into place.

Note: *The thermal insulation cover cannot be used with the zeta-potential cell.*

12.1 Equilibration Time

For a 1 mL sample, add one minute extra equilibration time for every ˚C different from ambient temperature. E.g., if a measurement is to be carried out at 35 ˚C when the room temperature is 25 ˚C, then an extra 10 min equilibration time should be incorporated into the experiment.

12.2 Special Considerations for Low-Temperature Measurements

When making measurements below room temperature, there is a risk of atmospheric water vapor condensing on the cuvette windows, which could significantly affect measurement results. Thus, it is advisable to connect a dry air source (ISO 8573.1, class 1.3.1, 0.4 to 0.8 bar overpressure, or 1.4 to 1.8 bar total input) to the port (Fig. 12-2), which will keep the air in the cuvette chamber dry, and thereby prevent condensation.

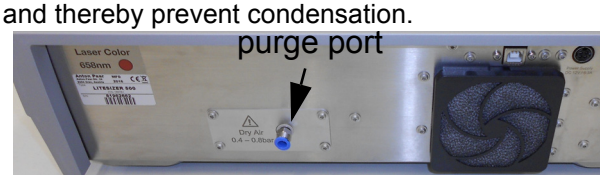


Fig. 12-2: Litesizer™ 500 rear surface with purge port

CAUTION

Use dry air (ISO 8573.1, class 1.3.1) or nitrogen at 0.4 to 0.8 bar overpressure (1.4 to 1.8 bar total input). Failure to adhere to these specifications may damage the instrument.

CAUTION

The purge air must not be too cold, or condensation can occur inside the instrument, which may damage the instrument.
The purge air temperature can easily be checked by visually inspecting the compressed-air supply hose; if there is no condensation visible on the outside of the hose for at least the last two meters of hosing before the purge port, then the air temperature is OK.

12.3 Special Considerations for High-Temperature Measurements

For measurements above 70 ˚C, the quartz cell must be used. Zeta-potential measurements cannot be made above 70 ˚C.

If flammable solvents are to be used at high temperature, it is recommended that the purge port (see Chapter 12.2, "Special Considerations for Low-Temperature Measurements", above) be used with nitrogen as purge gas to prevent any build up of flammable vapors in the module. Before using flammable solvents, see Chapter 2.2, "Safety Signs on the Litesizer™ 500" (Precautions for Using Flammable Samples or Cleaning Agents).

CAUTION

Hot surface
When performing high-temperature measurements (above 45 ˚C), the cell area should be allowed to cool before removing the thermal insulation cover and cuvette. A warning symbol can be found on the inside cover of the module and on top of the thermal insulation cover.

13 Cleaning the Litesizer™ 500 and Cuvettes

13.1 Cleaning the Instrument

To clean the instrument housing, use warm water with a mild cleaning agent (pH<10), or ethanol.

It is not advisable to apply cleaning agents or any other liquids inside the module of the Litesizer™ 500.

NOTICE
In case of a spillage inside the cuvette module, use a lens cleaning cloth or equivalent material that does not leave fibers behind. Wet the lens cloth or lens tissue with water and gently wipe out the module. Wet a further cloth with ethanol or acetone to remove any residual water, as well as any water-insoluble residue.

NOTICE
Never use:

- organic solvents (e.g. toluene, hexane, acetone, chloroform)
- strong acids or bases (e.g. nitric acid, sulfuric acid, hydrochloric acid, caustic soda)
- strong mechanical action (steel brush)

13.2 Cleaning the Cuvettes

13.2.1 Disposable Cuvettes

Do not clean and reuse the disposable cuvettes.

They are likely to give erroneous results on subsequent uses.

13.2.2 Quartz Cuvettes

1. Rinse with the solvent that was used for the measurement.
2. Rinse with purified water.
3. Rinse with acetone or ethanol and dry in a warm oven (50–60 ˚C).
4. If any residue remains, a pipe cleaner may be used to dislodge the residue before repeating steps 2 and 3.

13.2.3 Omega Cuvettes

1. Flush three times with purified water by using a syringe with the tip inserted into one of the sample ports (see Fig. 8-1, top)
2. Flush with dry air.

NOTICE
Never use organic solvents (e.g. toluene, hexane, acetone, chloroform) to clean the Omega cuvettes.
Never use ultrasound to clean the Omega cuvettes.

14 Validation Protocol

This procedure is for validating the performance of the Litesizer™ 500. The validation should be carried out every six months or when the device is moved to a different location.

Table 14-1: Equipment Required for Validation Protocol

Latex 220 nm standard (Mat. No. 165156)*
Ludox TM-40 (Mat. No. 165157)
NaCl solution (0.9 %)
Toluene, anhydrous 99.7 %
Deionized water
Disposable cuvettes (Mat. No. 164435)
1 Glass cuvette
1 Omega cuvette for zeta potential (Mat. No. 155765)
Anotop type 25 0.02 µm syringe filter (Mat. No. 163388)

* *the stated size may change according to the supplier batch*

14.1 Preparation of Latex Samples for Particle-size Measurements (see Methods in Kalliope)

14.1.1 Sample for Validation A: Concentrated 220 nm Latex for Back-Angle Measurement

1. A laminar-flow cabinet should be used for these steps where possible.
2. Discard the first drop of latex from the bottle and add 1 drop of standard to a disposable cuvette.
3. Add 1 mL of 0.9 % NaCl solution.
4. Place a cap on the cuvette and mix for a few seconds prior to measurement.

14.1.2 Sample for Validation B: Dilute 220 nm Latex for Side-Angle Measurement

1. A laminar-flow cabinet should be used for these steps where possible.
2. Using a syringe, transfer 100 µL of validation sample A into a disposable cuvette.
3. Add 2 mL of 0.9 % NaCl solution.
4. Place a cap on the cuvette and mix for a few seconds.
5. Transfer 1 mL to a new disposable cuvette, place the cap on, and measure it.

14.1.3 Sample for Validation C: Preparation of Ludox TM40 for Zeta Potential

1. Rinse the Omega cuvette with water, as described in section 13.2.3.
2. Dilute the stock solution (40 % w/v) to a final concentration of 4 % (w/v).
3. Fill the cell with the stock solution (350 µL).
4. Ensure that there are no bubbles in the cell and close it with the stoppers.

14.2 Protocol for Validation Testing

14.2.1 Size Measurements (DLS) Validation A-B

Two latex samples (200 nm) are required for calibrating the Litesizer™ 500.

1. Switch on the instrument 6 minutes before measuring to ensure laser stability.
2. Fill a clean cuvette with the sample prepared as described in section 14.1.1 or section 14.1.2 above and insert the cuvette into the instrument.
3. Start the software program, Kalliope, and choose "Particle size series".
4. Select the parameters indicated in Table 14-2 and then click Start:

Table 14-2: Input Parameters for Validation A and Validation B

Measurement cell:	disposable
Measurement angle:	back (Validation A) or side (Validation B)
Target temperature:	25 °C
Equilibration time:	grayed out when selecting a repetition series
Analysis model:	general
Cumulant model:	ISO 22412
Quality Mode:	automatic
Filter:	automatic
Focus:	automatic
Material name:	polystyrene latex
Solvent:	1.54 mM NaCl
Series type:	repetition
Keep focus position:	tick this option
Number of repetitions:	3
Equilibration time for first repetition:	2 min
Equilibration time for subsequent measurements:	10 seconds

When the series is finished, select the three repetitions and click the Analysis button. The table under the size-distribution graph will display the mean size of each measurement as well as the average across all measurements, and the corresponding standard deviation.

Table 14-3: Pass Criteria for Particle-Size Validation

Mean size diameter	certified mean diameter range ±2 %
Repeatability	better than 5 %
Polydispersity index	<10 %

14.2.2 Zeta-Potential Measurements (ELS) Validation C

1. Switch on the instrument 6 minutes before measuring to ensure laser stability.
2. Fill a clean cuvette with the sample prepared as described in section 14.1.3 and insert the cuvette into the instrument.
3. Start the software program, Kalliope, and choose "zeta potential series".
4. Choose the test parameters as indicated in Table 14-4 below
5. Click Start

Table 14-4: Input Parameters for Validation C

Measurement cell	Omega cuvette
Target temperature	25 °C
Equilibration time	grayed out when selecting a repetition series
Adjustment mode	automatic
Run mode	Automatic
Solvent	Water
Number of repetitions	3
Equilibration time for first repetition	2 min
Equilibration time for subsequent measurements	10 seconds

When the series has finished, select the three repetitions and click on the Analysis button. The table under the zeta-potential distribution graph will display the mean zeta potential of every measurement as well as the average across all measurements and the corresponding standard deviation.

Table 14-5: Pass Criterion for Zeta-Potential Validation

Mean zeta potential	-38 mV ± 10 %

14.2.3 Molecular-Mass Measurement (SLS) Validation D

1. Switch on the instrument 6 minutes before measuring to ensure laser stability.
2. Prepare a sample of toluene by filtering through a 0.02 µm membrane filter into a quartz cuvette.
3. Start the software program, Kalliope, and choose "molecular mass".
4. Choose the test parameters as indicated in Table 14-6 below.
5. Click Start.

Table 14-6: Input Parameters for Validation D

Measurement cell	Quartz
Target temperature	25 °C
Equilibration time	3 min
dn/dc	1 mL/g
Quality mode	manual
Number of runs	3
Measurement time	10 seconds
Solvent	toluene
Reference	toluene
Shape correction	no correction
Concentrations	1 mg/mL; 2 mg/mL; 3 mg/mL

Leave the cell in the instrument until the measurement is finished. When indicated by the Litesizer, open the module, remove the cell and insert the next sample. Close the module and click ok.

Table 14-7: Pass Criterion for Molecular-Mass Validation

Molecular mass	<10 Da*

* *0 is a valid number*

Appendix A: Technical Data

Physical Specifications

Dimensions (w x d x h)	460 mm x 485 mm x 135 mm
Weight	18 kg

Housing Material

Front, top & side cover	Noryl™ FN215X: polyphenylene ether/polystyrene
Back, bottom	stainless steel
Module	PPS GF 40: polyphenylene sulfide + glass fibers
Omega cuvettes	Cuvette body: polycarbonate/ Electrodes: gold Caps: poly(ethene-co-tetrafluoroethene)

Power

Power supply at instrument	12 V via external adapter
Power supply for AC adapter	230 VAC, 50/60 Hz
Power consumption	50 W

Environmental Conditions

Ambient temperature	+10 to +35 °C
Ambient humidity	up to 90 % non-condensing
Airborne noise emission	< 70 dB
Degree of pollution	2

Air/Gas Supply

Quality	clean, dry and oil-free (ISO 8573.1, class 1.3.1)
Pressure	0.4 to 0.8 bar overpressure, or 1.4 to 1.8 bar total input

Wetted Parts

The following materials are in contact, or may come into contact, with samples

Cuvettes

Disposable cuvettes	polystyrene
Quartz cuvettes	quartz
Cuvette caps	polypropylene
Zeta potential cuvettes	Cuvette body: polycarbonate/ Electrodes: gold Caps: poly(ethene-co-tetrafluoroethene)

Module

Module body	polyphenylene sulfide + glass fibers
Electrical contacts	gold

Appendix B: Declaration of Conformity

CE Declaration of Conformity

Anton Paar

Anton Paar GmbH hereby declares that the product listed below in the version offered for sale meets all the basic requirements of the applicable sections of the relevant EU directives in design and type.

This declaration will be deemed invalid should any unauthorized modifications be made to the product. Follow the information given in the instruction manual when setting up and operating the instrument.

Product designation:	**Particle Analyzer**
Model:	**Litesizer 500**
Manufacturer:	**Anton Paar GmbH**

The product meets the requirements of the following directives:

- **Electromagnetic Compatibility 2004/108/EC**

  Applied standards:

EN 61326-1:2013	Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 1: General requirements

The product is classified as a Class B equipment and is not intended for the use in industrial area.

- **Low Voltage Directive 2006/95/EC**

  Applied standards:

EN 61010-1:2010	Safety requirements for electrical equipment for measurement, control and laboratory use Part 1: General requirements
EN 61010-2-010:2014	Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials
EN 62233:2008	Measurement methods for electromagnetic fields of household appliances and similar apparatus with regard to human exposure

DI(FH) Dr. Jakob Santner
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