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PARTICLE SIZE

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NANOSIGHT **NS300**
USER MANUAL

NANOSIGHT NS300
USER MANUAL

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CONTENTS

1 INTRODUCTION

Welcome to this NanoSight NS300 User manual.

The following topics are covered in this introductory chapter:

Introduction

The NanoSight NS300 instrument is a patented, laser-based, light scattering system which provides an easy-to-use, reproducible platform for specific and general nanoparticle characterization.

Particles suspended in a liquid are loaded into the laser module sample chamber and viewed in close proximity to the optical element. The NS300 device illuminates the particles using a specially aligned and focused laser beam. This allows extremely small particles (down to 10 nm, dependent on refractive index) to be seen directly and individually by conventional microscopy.

Particles in the liquid sample which pass through the beam path are seen as small points of light moving rapidly under Brownian motion, allowing information on particle properties to be obtained. With the NS300 you can analyze the presence, size distribution, concentration and fluorescence of all types of nanoparticles from 10 nm to 2000 nm, depending on the instrument configuration and sample type.

The laser module contains thermoelectric Peltier elements, allowing the sample temperature to be controlled. This is fully programmable using the NTA software suite.

Two sample chamber plates are supplied. The O-Ring Top Plate confers greater solvent compatibility. The Low Volume Flow Cell (LVFC) and installed tubing allow the use of all non-flammable, water-based solvents with neutral pH. Other solvents may not be compatible with wetted surfaces and should not be used without confirming suitability. If in doubt about the choice of solvent and its compatibility with any part of the device, please contact Malvern Instruments (helpdesk@malvern.com) for further information.

This manual is designed to help familiarise the user with the NanoSight NS300 operation. Further advice and help to get the best out of your instrument can be found in the NanoSight Application Notes, Technical Notes and User Training Videos. These can be found on our website (www.malvern.com) or by contacting Malvern Instruments.

For technical assistance, please contact Malvern Instruments.

Malvern Instruments Technical Support:

- Tel: + [44] (0)1684-892456
- Email: helpdesk@malvern.com

About this manual

This manual gives information needed for safe and efficient operation of NanoSight NS300.

The aims of this manual are to:

- Provide essential health and safety information, which all users must read.
- Give information on the site requirements of the system.
- Describe the NanoSight NS300 hardware components.
- Describe the major NTA 3.2 software features and how to use them.
- Describe the measurement process.
- Set out various maintenance and troubleshooting procedures.
- Provide the specifications of the instrument.

Product documentation structure

This manual fits into the following information structure for this product:

- **NanoSight NS300 User Manual** — provides detailed information on how to use and administer the system. The Manual also gives Health and Safety, Maintenance, Troubleshooting and other vital information which all users must read.
- **Help system** — integrated with the NanoSight NS300 software, provides information on all software features.

Warning! The instrument and the samples measured may be hazardous if misused. Users must read Health and safety on page 13 before operating this system.

About this manual

The operation and maintenance of the following units is covered:

Item	Model number
NanoSight NS300	NTA3300

Naming convention

The NanoSight NS300 is referred to either in full as the NanoSight NS300, or as 'the instrument'.

Menu commands

Menu commands in the NanoSight NS300software are always shown in bold text in the form:

main menu-menu item-submenu item

As an example, the command **File-Open-Data** file refers to selecting the Data file sub-menu item under Open in the File menu.

Where to get help

This section gives information on the various channels in place to get help with your system.

Help desk

All queries regarding the system should initially be directed to your local Malvern Instruments representative, quoting the following information:

- **Model and serial number** of the instrument (located on the rear panel or casing of the instrument).
- **Software version** (see **Help-About** in the software).

Contact the International Helpdesk if the local Malvern Instruments representative is not available:

Telephone: +44 (0) 1684 891800 or **email: helpdesk@malvern.com**.

Note: This help line is primarily English speaking.

Remote support

Malvern Instruments offers a remote support service, delivered by an Internet connection. Benefits include fast and efficient fault diagnosis, reducing downtime and costs.

Malvern website — www.malvern.com

The Malvern Instruments website offers a comprehensive range of particle characterization resources for use by customers 24 hours a day, seven days a week.

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HEALTH AND SAFETY

This section provides vital health and safety information which all users of the system must read and understand.

The following topics are covered in this section:

General safety warnings

Warning!
The instrument must not be used in hazardous areas.

Warning!
The instrument is for use in moderate climates only. Never use the equipment in damp or wet conditions.

Warning!
Avoid excessive heat, humidity, dust and vibration.

Warning!
Do not place liquid filled containers on the equipment.

Warning!
Do not use where the equipment may be subjected to dripping or splashing liquids.

Warning!
If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Warning!
The instrument can be used with all non-flammable water-based solvents of neutral pH. Other solvents may not be compatible and must not be used without first contacting Malvern Instruments to confirm suitability.

Warning!
When using biologically or chemically hazardous sample material, it is the responsibility of the operator to determine the requisite protection for each application.

Warning!
The NS300 system contains no user serviceable parts. It should not be modified in any way. Any modification will void the warranty and could make the device unsafe.

Warning!
Use of controls, adjustments or performance of procedures other than those specified herein may result in hazardous laser radiation exposure.

Warning!
The laser module may become hot when operating the temperature control. Caution should be taken when handling the device.

Warning!
The temperature control should always be turned off when the instrument is unattended.

Warning!
The NS300 must only be serviced by qualified Malvern Instruments personnel, or Malvern Instruments approved agents.

Site requirements

The NanoSight NS300 has specific site requirements that must be enforced to ensure safe operation of the instrument. Please see Site requirements on page 19 for more information.

Warning! Safety may be compromised if the system is used in an environment not conforming to the site requirements.

Laser safety warnings

Warning!
Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Warning!
The NanoSight NS300 device is classified (to BS EN 60825-1 (2001)) as a Class 1 laser device. Removal of instrument casing or opening the housing of the laser module voids all warranties and could expose users to hazardous voltages or Class 3B laser radiation.

Warning!
The laser module should not be plugged into the main body of the NS300 without the flow cell or top-plate being secured in position with the screws supplied.

Warning!
The laser module must always be removed from the main body of the NS300 (cutting power to the laser) prior to removing the flow cell or top-plate screws.

Electrical safety warnings

The following electrical specifications must be adhered to for safe operation of the NanoSight NS300.

- Fuses must only be replaced with a fuse of the type and rating — F5AH250V Ø5 mm x 20 mm long.
- Only use the power adapter and other accessories supplied with the instrument.
- If the mains cordset needs to be replaced, please select another cordset which conforms to the following specifications:

Item	Specification
Voltage rating	• 125 V AC if being used with a 100-120 V supply. • 250 V AC if being used with a 220-240 V supply.
Current Rating	6 A minimum.
Temperature rating	60 °C minimum.
Length	3 m Maximum.
Fittings	• Grounded plug for attachment to power outlet. • IEC appliance coupling.

In addition to the above specifications, the mains cordset should be certified by one of the following institutions.

UK – BSI

USA – UL

Europe – VDE

Japan – JET, JQA, TUV

- If the appliance is being used outside of the above areas the local regulations for mains power cords must be checked and a cordset which complies with the relevant standards should be sourced for use with the equipment.
- The safety of the equipment can no longer be warranted if a non-approved cordset is used.

Sample handling warnings

- Always handle all substances in accordance with the COSHH (Control Of Substances Hazardous to Health) regulations (UK) or any local regulations concerning sample handling safety.
- Before using any substance, check the *Safety Data Sheets (SDS)* for safe handling information.
- Use the instrument in a well ventilated room, or preferably within a fume cupboard, if the fumes from the sample or dispersant are toxic or noxious.
- Wear personal protective equipment as recommended by the Safety Data Sheets if toxic or hazardous samples are being handled, particularly during sample preparation and measurement.
- Wear protective gloves when handling hazardous materials, or those that cause skin infections or irritations.
- Do not smoke during measurement procedures, particularly where inflammable samples are used or stored.
- Do not eat or drink during measurement procedures, particularly where hazardous samples are used or stored.
- Take care when handling glass (e.g. microscope slides and beakers). Hazardous materials may enter a wound caused by broken glass.
- Always test a new sample or dispersant for chemical compatibility before use.
- After measuring hazardous samples, scrupulously clean the system to remove any contaminants before making another measurement.
- Always label samples for analysis using industry standard labelling, particularly if they are handled by a number of staff or stored for long periods. Clearly mark any operator hazard and associated safety precautions that are required for the handling of dangerous materials.
- Keep a record of all hazardous substances used in the system for protection of service and maintenance personnel.
- Always adopt responsible procedures for the disposal of waste samples. Most local laws forbid the disposal of many chemicals in such a manner as to allow their entry into the water system. The user is advised to seek local advice as to the means available for disposal of chemical wastes in the area of use. Refer to the *Safety Data Sheets*.
- The surfaces of the system may be permanently damaged if samples are spilt on them. If a spillage does occur, disconnect the system from the power supply before scrupulously cleaning up the spillage.

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SITE REQUIREMENTS

This section provides information on the typical site requirements for the NanoSight NS300.

The following topics are covered:

Siting the instrument

When siting the NanoSight NS300 make sure that the following environmental conditions are satisfied:

- The NanoSight NS300 system and any accompanying accessories must be sited indoors.
- It must be away from strong light sources (e.g. windows).
- It must be away from strong heat sources (e.g. radiators).
- It must be in well ventilated areas void of excessive humidity and dust.
- It must be on a horizontal vibration-free bench built to support the weight of the system (shown below):

Component	Weight
NanoSight NS300 instrument housing	12 kg
NanoSight NS300 Laser module	0.65 kg
Computer and printer	See manufacturer's documentation

Warning! Do not position the system so that power sockets are obstructed. They may need to be disconnected during an emergency.

Operating environment

Store/operate the system in the following conditions:

Parameter	Specification
Operating temperature:	
Instrument housing	15 °C to 35 °C
Laser module	5 °C below ambient to 50 °C
Storage conditions (instrument housing)	5 °C to 35 °C
Humidity:	
Instrument housing	Maximum humidity 80% for temperatures up to 31 °C, decreasing linearly to 50% relative humidity at 40 °C
Laser module	5 to 95% non-condensing

Parameter	Specification
Usage	Indoor use only
Altitude	Up to 2000 m
Mains supply voltage fluctuations	Up to ± 10% of nominal voltage

In addition:

- Do not obstruct power sockets as they may need to be disconnected during an emergency.
- Avoid passing electrical cables through areas where liquids can be spilled.
- Rooms must be well ventilated if noxious samples or dispersants are used.

Space required

Provide enough space to allow easy access to all components and connections. Allow at least 25 mm for ventilation at the rear. Also allow 150 mm at the front of the instrument and 100 mm to the left for the door to open.

Component dimensions are:

Component	Width	Depth	Height
NanoSight NS300 Instrument Housing	250 mm	400 mm	400 mm
Computer and Printer	See manufacturer's documentation		

Services

This section describes the services required.

General

The following services are required for each component:

Component	Power sockets required
NanoSight NS300 instrument	1
Computer	1
Monitor	1

Power specification

The mains power supply must be clean and filtered. If necessary, fit an un-interruptible power supply (UPS) to remove any spikes or noise.

The power requirements are:

Unit	Power requirement
NanoSight NS300 instrument	Power requirement -100-240 V, 50-60 Hz

Note: Only use the PSU/cables provided. Using another PSU voids any warranty and may be unsafe.

4

INSTALLATION

This chapter covers the following topics:

Handling

The NanoSight NS300 incorporates a rugged housing designed to protect the integrity of the instrument. However, it is a sensitive scientific instrument and should be treated as such. The laser module contains a static-sensitive laser diode and should never be used in circumstances when a static discharge may damage the diode.

Between uses of the NanoSight NS300, or for longer term storage, the unit must be cleaned and dried as described in General maintenance on page 140.

Unpacking and initial inspection

The standard instrument is supplied with the following accessories:

- Mains power lead
- USB cable
- CMOS Firewire cable
- Spare NS300 tubing kit
- Spare Low Volume Flow Cell (LVFC) gasket component
- Instruction manuals
- Hex-key set
- Particle size standards.

Always do the following when unpacking your NS300:

- Inspect the shipping container when the NS300 is received.
- Carefully check the contents for completeness and condition.

The NS300 is supplied in a specially designed packing case which should be returned to Malvern Instruments after delivery. The packing case can be kept if required, although an additional charge will be made.

Notify Malvern Instruments (helpdesk@malvern.com) if the contents are incomplete, or if the instrument or accessories appear to be damaged in any way. Keep all damaged packaging, materials and goods for inspection by the carrier.

Installation and relocation

Note: Initial installation will be carried out by qualified Malvern Instruments personnel/ representatives.

If the instrument needs relocating, always perform the following steps:

- Disconnect the equipment from the mains and ancillary units before moving.
- Locate the instrument in an area of good ventilation and with sufficient space for safe and efficient operation and maintenance.
- Site the supplied PC so that danger from spillage and splashing is avoided.
- Connect equipment to an earthed power supply with a voltage corresponding to that on the power adapter.
- Make sure that you can get access to the mains plug to allow the unit to be disconnected from the mains supply.
- Always use the mains lead supplied. Your sales representative can provide a lead suitable for your country.

5 HARDWARE SETUP

This section provides information on the typical hardware setup of a NanoSight NS300 and gives more detail on the connections available.

The following topics are covered:

The instrument

The NanoSight NS300 is illustrated:

1. Instrument connections
2. External fluidics inlet and outlet
3. Door

1. Objective
2. Laser module
3. Lever to release/secure laser module

Instrument communications

The NS300 instrument communicates with the PC via a USB connection. The following steps outline how to correctly setup instrument communications.

1. Plug the USB cable into the USB port on the right side of the NS300 instrument casing (labeled USB-1) and connect it to one of the USB ports on the supplied PC.

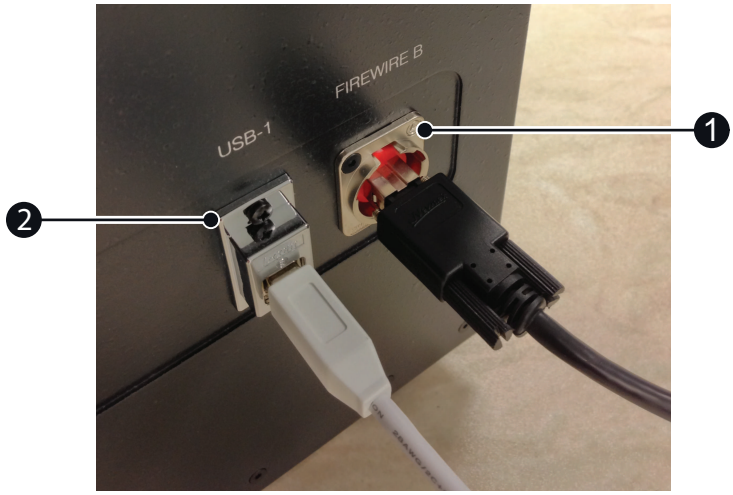


Figure 5-1: USB and Camera connection

1. Camera connection
2. USB connection

2. Plug the firewire camera cable into the firewire port on the side of the NS300 (labelled Firewire B) and connect it to the PC firewire port.
3. Set up the monitor, keyboard and mouse and turn on the PC.
4. Connect the mains lead to the NS300 instrument and turn the instrument on at the power switch, which is located on the left side of the case.

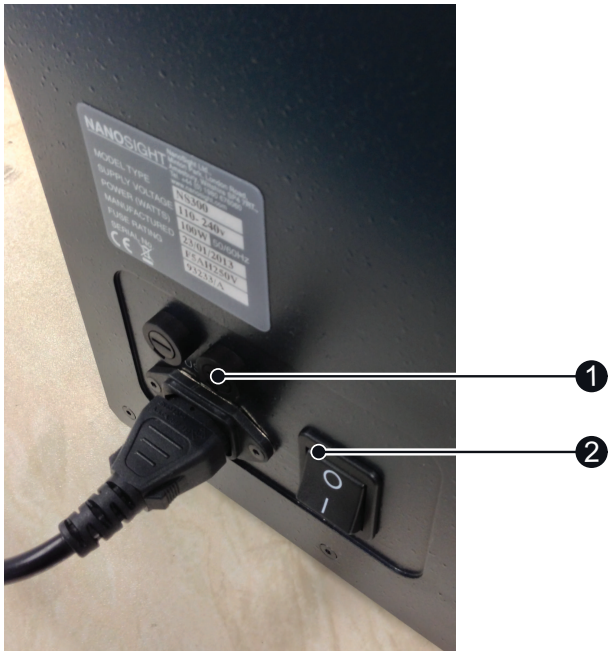


Figure 5-2: Power connection

1. Power connection
2. Power switch

5. Start the NTA software and confirm that no hardware detection error messages are displayed in the status panel. See Getting started on page 66 for more information.

The laser module

The laser module contains a specially configured and focused laser source, which is mounted within a sealed housing. An optical flat element **(1)** is fixed in place on the top cover of the housing. The electrical contacts **(2)** on the end of the housing connect to the contacts within the NS300 to provide the laser module with power.

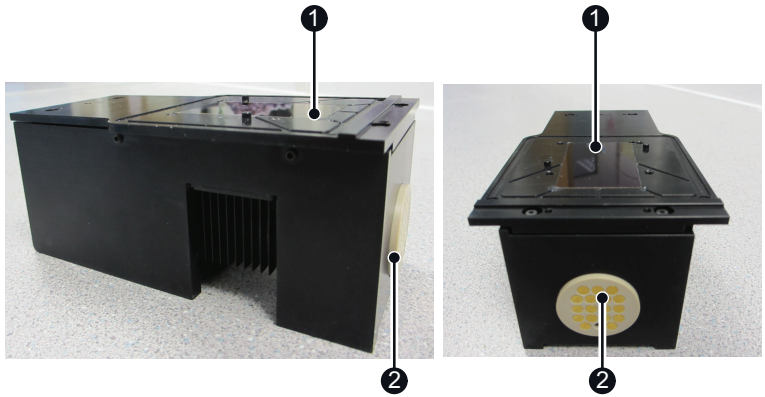


Figure 5-3: NS300 laser module.

Top-plate selection

The NS300 is supplied with two different top-plates that can be mounted on the laser module. The assembly, fluidic connections and the instructions for cleaning will vary according to which design of top-plate is being used. See The Low Volume Flow Cell on the facing page and The O-Ring on page 39 for more information.

The Low Volume Flow Cell

The Low Volume Flow Cell (LVFC) top-plate is suitable for use with chemically compatible aqueous solutions. It can be cleaned by flushing wash fluid through the chamber, which avoids the need to manually disassemble and clean the chamber after every sample.

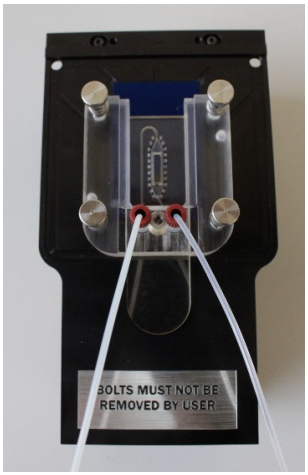


Figure 5-4: Low volume Flow Cell

Warning!
The LVFC is suitable for use with non-flammable, water-based solvents with neutral pH. Ethanol and other solvents may not be compatible with wetted surfaces and should not be used without confirming suitability with the listed materials.

Setup

The LVFC top-plate consists of two separate parts:

1. The manifold, which has ports to attach the tubing fittings.
2. A gasket component, which contains an embedded microchannel and chamber seal.

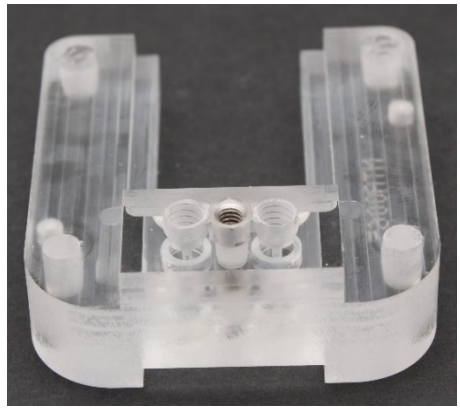


Figure 5-5: Low Volume Flow Cell manifold (NTA0065).

Figure 5-6: Low Volume Flow Cell gasket component (NTA0066).

The LVFC is supplied with NS300 systems with the two components already assembled:

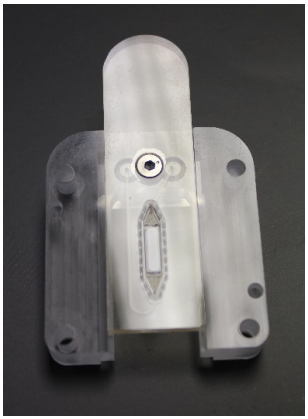


Figure 5-7: Low Volume Flow Cell assembly with PDMS chamber seal on the underside of the gasket component.

The following steps tell you how to load the LVFC top-plate onto the laser module:

1. Use the supplied sprung bolts to connect the top-plate to the laser module.
2. Gently finger tighten the bolts until an increased resistance is felt when the bolts reach the end of the screw thread. This attaches the gasket component with PDMS chamber seal to the optical glass flat, forming a chamber in which the sample can be measured with the NS300.

Caution!
Check that there are no fibers or dust particles on either sealing surface before attaching the flow cell to the laser module. Contamination of the sealing area can cause fluid leaks. If such contamination is present do not attempt to physically remove the contamination. Instead, follow instructions for cleaning, described in Low Volume Flow Cell maintenance on page 142.

Caution!
Do not overtighten the fastening screws as this can cause damage to the screw threads.

Figure 5-8: Low Volume Flow Cell assembly mounted on laser module with sprung fastening bolts.

When using the LVFC, liquid is loaded into the system through fluidic tubing connected to ports on the flow cell manifold, set up as described in Low Volume Flow Cell tubing connection below.

Low Volume Flow Cell tubing connection

When using the Low Volume Flow Cell, liquid is always loaded into the system using a disposable 1 ml syringe.

Inside the casing, inlet and outlet tubing is connected from the corresponding ports on the inside of the tubing holder to ports on the flow cell using the fittings supplied.

A schematic of the LVFC tubing configuration is shown alongside the actual configuration:

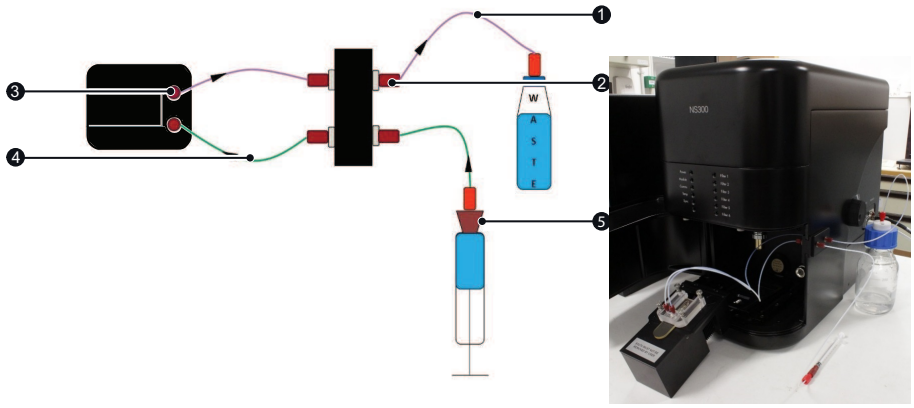


Figure 5-9: Low Volume Flow Cell tubing configuration.

1. Outlet tubing (TUB0288) — connects to the tubing holder port nearest the back of the NS300 and the right port of the low volume flow cell manifold (looking from the tubing connection end of the manifold).
2. FTG0972
3. FTG0971
4. Inlet tubing (TUB0281) — connects to the tubing holder port nearest the front of the NS300 and the left port of the low volume flow cell manifold.
5. The syringe (FTG0988) — connects via a Luer fitting to the inlet fluidic tubing on the left port of the tubing holder on the outside of the NS300 casing.

Note: Before using the system to load samples, rinse out the inlet tubing with water or buffer to remove any trapped air before connecting the inlet fitting to the flow cell (see Priming the tubing on the next page).

Caution!
Make sure there are no kinks or blockages in the outlet tubing. This will cause an increase in pressure inside the flow cell, and could cause the seal to leak.

Priming the tubing

Important! When using the LVFC, the inlet fluidic tubing should be rinsed out with water or buffer before the tubing is connected to the top-plate and the top-plate primed for use. This improves bubble clearance from the tubing on initial priming, reducing the likelihood of air bubbles entering the sample chamber and causing problems in subsequent measurements.

To rinse through the inlet tubing with buffer or sample:

1. Make sure that the inlet tubing fitted to the inside of the NS300 casing is **not connected** to the top-plate.
2. Place the end of the inlet tubing into a suitable waste container.
3. Insert a 1 ml syringe of liquid into the Luer port and push ~900 µl of the liquid through the inlet tubing as fast as the back pressure will allow — this should take approximately 5-10 seconds.
4. Leave the syringe containing the remaining liquid attached to the Luer port to prevent any air being introduced.

Warning!
Initially rinsing the inlet tubing at higher speeds allows you to better remove any air initially trapped in the tubing or connectors. Make sure that the pressure generated does not force the syringe out of the Luer port.

Changing the inlet tubing syringe

It is important to make sure that liquid to liquid contact is always maintained at the syringe port when changing over the syringe fitted to the inlet tubing e.g. changing between buffer and sample, or when replacing an empty syringe.

Before changing syringes:

- Make sure there are no air pockets at the tip of the new syringe, and that there is a small positive meniscus protruding from the syringe.
- Keep the Luer port at bench level when changing syringes to prevent liquid draining from the Luer port.
- Remove the old syringe from the Luer port and insert the new syringe into the Luer port (keeping syringes and Luer port horizontal) such that the two meniscuses combine without trapping an air bubble.

The O-Ring

The O-Ring top-plate (ORTP) provides greater chemical compatibility for non-aqueous solvents, or samples which are more viscous or contain larger particles, which may block the flow-cell.

To prevent particle carryover, always detach the ORTP from the laser module and manually clean it after each sample.

Figure 5-10: O-Ring top-plate.

Setup

The O-Ring top-plate contains an embedded O-Ring seal.

1. Use the supplied sprung bolts to connect the top-plate to the laser module. This forms the sample chamber.
2. Gently finger-tighten the bolts until an increased resistance is felt when the bolts reach the end of the screw thread.

Warning!
Do not over-tighten the fastening screws as this can cause damage to the screw threads.

The O-Ring top-plate can be setup in two different ways, according to how samples are to be loaded into the system. See Manual injection below and Injection using the syringe pump on page 42 for more information.

Manual injection

A push-fit elbow Luer connector is provided so that the laser module fits into the NS300 with the syringe remaining in place.

- Load samples into the chamber using a disposable 1 ml syringe, which is directly connected to a Luer port on the top-plate.

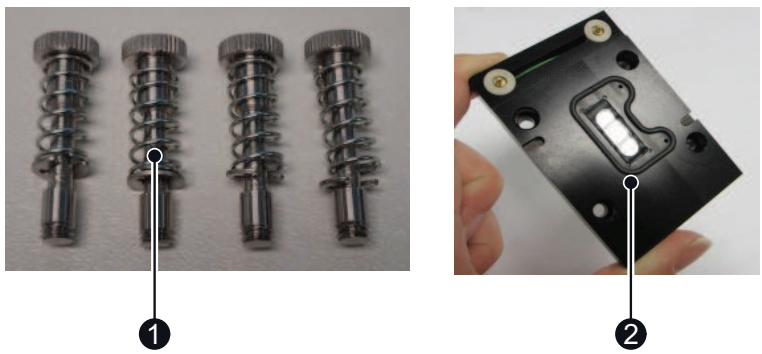


Figure 5-11: Sprung fastening bolts **(1)** and O-Ring top-plate **(2)**.

1. Sprung fastening screws (spring held by circlip attached to the bolt).
2. O-Ring seal.

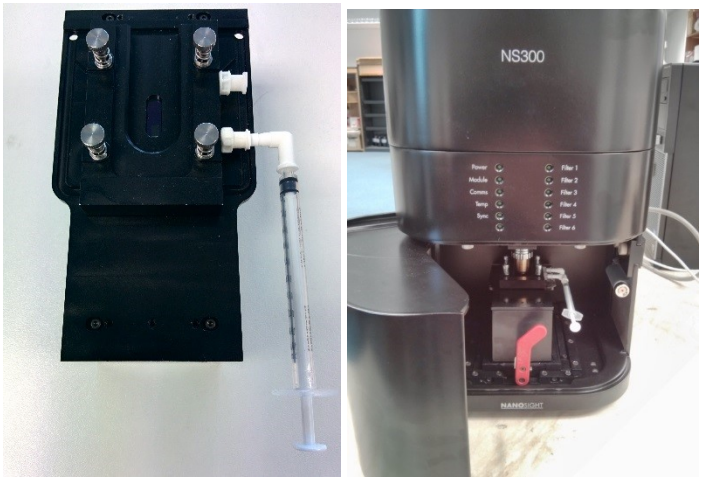


Figure 5-12: O-Ring top-plate with Luer ports and push fit elbow Luer connector (left). O-Ring top-plate mounted on the laser module inside the NS300 (right).

Injection using the syringe pump

A sample can also be loaded into the O-Ring top-plate through the NS300 fluidic tubing for use with a syringe pump accessory.

In this case:

- Remove the Luer ports and the tubing connected using the fittings supplied, as described in O-Ring syringe pump tubing connection below.

Figure 5-13: O-Ring top-plate with tubing fittings for syringe pump use.

O-Ring syringe pump tubing connection

When using the O-Ring top-plate with the Syringe Pump accessory, liquid is loaded into the system using a disposable 1 ml syringe.

Inside the casing, inlet and outlet tubing is connected from the corresponding ports on the inside of the tubing holder to ports on the O-Ring top-plate using the fittings supplied.

A schematic of the O-Ring syringe pump tubing configuration is illustrated, alongside the actual configuration:

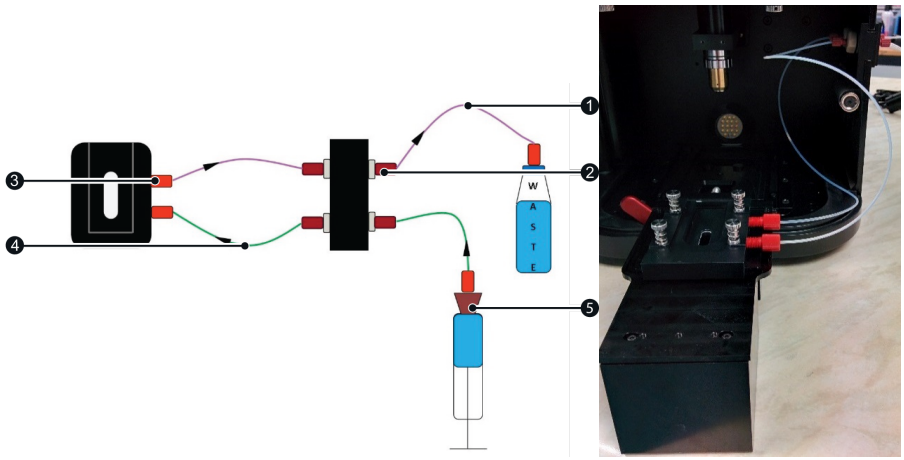


Figure 5-14: O-Ring top-plate syringe pump tubing configuration.

1. Outlet tubing (TUB0288) — connects to the tubing holder port nearest the back of the NS300 and the right port of the O-Ring top-plate.
2. FTG0972
3. FTG0971
4. Inlet tubing (TUB0281) — connects to the tubing holder port nearest the front of the NS300 and the left port of the O-Ring top-plate.
5. Syringe (FTG0988) — connects via a Luer fitting to the inlet fluidic tubing on the left port of the tubing holder on the outside of the NS300 casing.

Note: Before using the system to load samples, rinse out the inlet tubing with sample or buffer to remove any trapped air before connecting the fitting to the O-Ring top-plate (see Changing samples on page 63).

Caution!
Make sure there are no kinks or blockages in the outlet tubing. This will cause an increase in pressure inside the flow cell, and could cause the seal to leak.

Caution!
The fluidic tubing connection is intended for use with the NanoSight NS300 syringe pump accessory only. Manually loading sample through a syringe with the waste tubing connected exerts higher pressures on the chamber seal. This can result in sample leakage or damage to the top-plate.

External fluidic setup

The fluidic tubing can be used with both top plates, and attaches to a tubing port holder on the right-hand side of the NanoSight NS300 casing.

Slide the holder in and out of the case for easy access to the fluidic connectors. The tubing port holder is held in place with a black nylon screw on the inside of the casing, accessed by opening the door.

Tubing is supplied for use with the low volume flow cell or when the O-Ring top-plate is being used with the syringe pump accessory. In both cases, the left-hand port on the tubing holder is the inlet port, used to introduce sample or wash fluid into the system:

1. Connect the inlet tubing TUB0281 to the left port with a Luer port fitting on the end to load sample into the system via a disposable syringe.
2. Connect the waste outlet tubing TUB0288 to the right port. Connect the other end to one of the ports on the waste bottle cap using the supplied fittings. This is important as raising the waste prevents syphoning of liquid when changing sample syringes.
3. Attach the internal tubing to the top-plate inside the casing as described in The Low Volume Flow Cell on page 33 and The O-Ring on page 39.

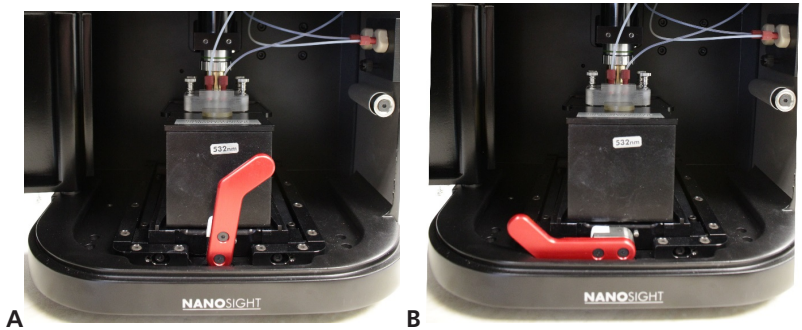
Figure 5-15: External NS300 fluidic connections.

Mounting the laser module

Mount the laser module within the main instrument housing:

Note: Make sure the sample is loaded without any air pockets or bubbles before mounting the laser module.

1. Rotate the red lever inside the NS300 to the left to allow the laser module to be mounted.
2. Place the laser module into the slide and gently push forward until it mates with the power connector inside.
3. Rotate the red lever inside until vertical to lock the laser module in place, ensuring reproducible positioning.
4. Close the access door.



A B

Figure 5-16: A: Laser module locked in place **B**: Laser module removal.

The sample is then ready for NTA measurement — see Making a measurement on page 65 for more information.

6

SOFTWARE

This section provides information on the application software used with the NanoSight NS300 instrument.

The following topics are covered in this section:

About the NanoSight NTA software

NTA is Malvern's software for the NanoSight range of instruments. It allows videos of particles moving under Brownian motion to be captured and analyzed to generate high resolution size and concentration data.

Measurements are run via SOP-type procedures with default options for standard measurements and user defined options for additional flexibility.

Hardware control is integrated into the software for control of temperature, flow rate, focus etc… when appropriate hardware is available.

NTA key features

- High resolution particle size distribution algorithm.
- Advanced image analysis, particle detection and tracking.
- Integrated scripting option for SOP development.
- Basic statistical parameter output.
- Vibration detection and correction.
- Integrated hardware control and communication.
- PDF and CSV document export options.

Software map

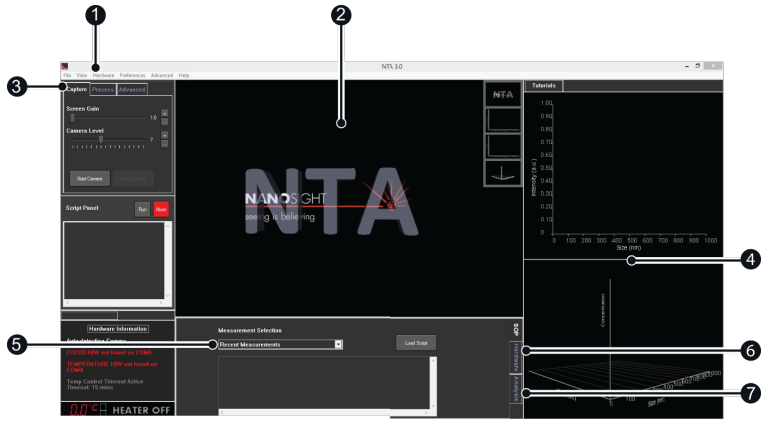


Figure 6-1: Software map.

1. Top level menu bar — includes File, View, Hardware, Preferences, Advanced, and Help menus.

2. Live/video view.

3. Capture, Process, and Advanced settings tabs.

4. Results graphs.

5. Measurement selection dropdown in the SOP tab.

6. Hardware tab.

7. Analysis tab.

NanoSight NTA 3.2 scripting

Script control

All NanoSight measurements are controlled by standard operating procedures (SOPs) or scripts. A number of frequently used SOPs are provided with the instrument software.

The script control feature in NTA 3.2 allows you to program a list of hardware or software commands for the software to follow. This can be useful in creating Standard Operating Procedures. For time based studies the commands can be programmed in and the instrument left to run automatically. Scripts be run at the time of writing or created and saved for later use.

NTA script panel

The *Script panel* can be found on the left hand side of the screen, on opening the NTA 3.2 software.

Place the mouse icon over the *Script panel* text and drag the panel out into the main NTA window area. The panel will expand as shown:

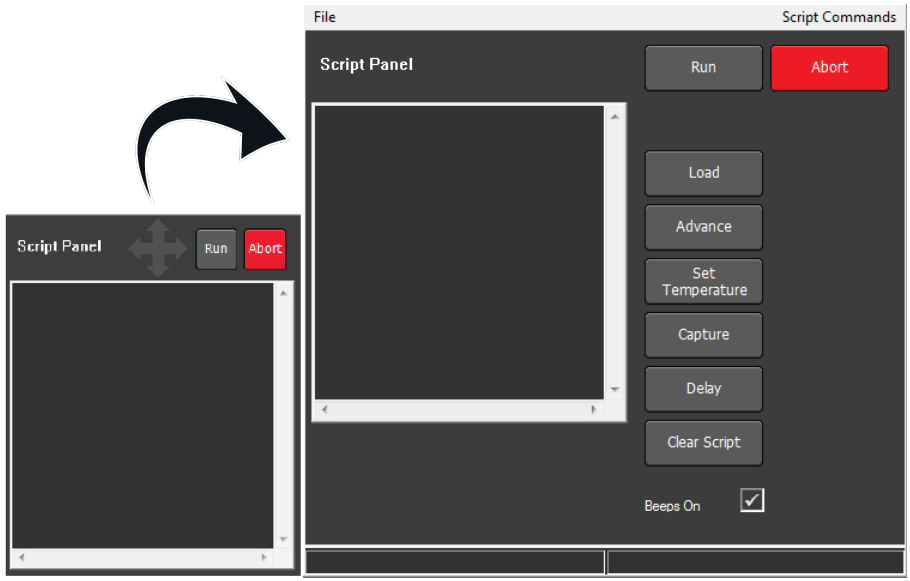


Figure 6-2: The *Script panel* in NTA opening screen (left), script panel expanded (right).

Shortcuts to commonly used commands are shown to the right of the commands window within the *Script panel*.

1. Click on the **Script Commands** menu at the top right of the panel to display a directory structure, listing all the commands available for your specific NanoSight system configuration.
2. Click on a command to add it to the commands window within the *Script panel.* Some commands will then also require an additional argument to be added.
3. Commands can also be entered by typing directly in the window, or by copying and pasting from elsewhere in the commands window or from other text editors.

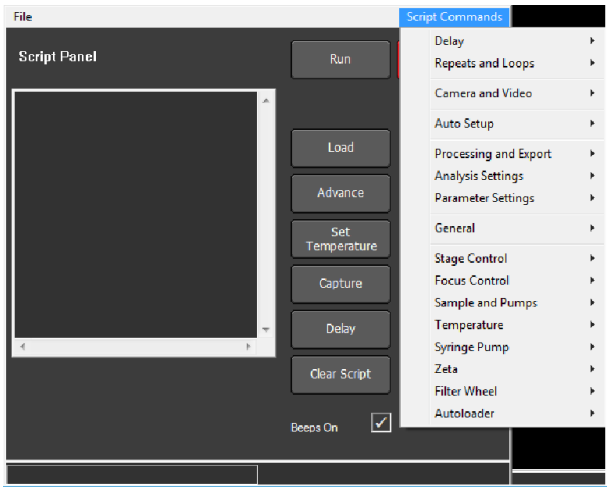


Figure 6-3: Script commands directory structure.

Notes:

- A script run can be started or aborted with the **Run** and **Abort** buttons, respectively.
- To save a script or load a previously saved script back into the command window click on the **File** menu at the top left of the *Script panel*.
- The *Script panel* can be re-docked back into the main NTA software screen by

double clicking on any blank area of the panel.

- All data captured using the script control will be saved according to the folder and filename currently selected in the SOP tab.

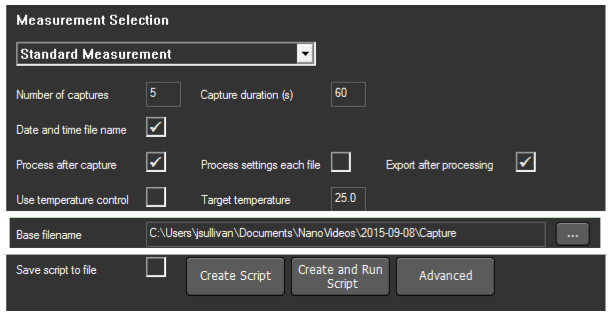


Figure 6-4: SOP tab showing the *Base Filename location* in NTA 3.1 software.

Script window auto-docking

Double click the centre of un-docked script to re-dock the window within the NTA screen layout.

Script commands (NS300)

The usage and syntax for all NTA 3.2 commands for the NanoSight NS300 are listed below; the commands that can be used will depend on the specific system configuration. Any additional parameters required should be entered with a space following the command. Required parameters are indicated by the highlighted fields with an example value shown.

Delays

DELAY **5**	Wait **5** seconds before moving onto the next command

Repeats and Loops

REPEATALL	Add to the end of a script to repeat indefinitely. Abort the script to halt
REPEAT **4**	Repeat script **4** times from the beginning, or from the pos-

	ition of a REPEATSTART command (see below). The repeated portion of the script will be run 5 times in total
REPEATSTART	Start position for a repeated section of the script Any following REPEAT command will repeat commands between REPEATSTART and REPEAT
SETLOOPVARSTART **1**	Set initial value of incremental loop variable (accessed via VAR) to **1**
SETLOOPVAREND **9**	Set final stop value of incremental loop variable to **9**
SETLOOPVARINCREMENT **1**	Set the increment step size for the loop counter to **1** Example: with an initial value 1, a stop value 9, and an increment of 2, the loop will run 5 times with the counter variable VAR having values of 1, 3, 5, 7 and 9
LOOPGO	Start position of incremental loop Loop between LOOPGO and LOOPSTOP repeats until VAR has reached stop value
LOOPSTOP	End-point of incremental loop

Camera and video

CAMERAON	Turn the camera on
CAMERAOFF	Turn the camera off
CAMERASHUTTER **165**	Set camera shutter to **165**
CAMERAGAIN **15**	Set camera gain to **15**
CAMERALEVEL **5**	Set camera level to **5**
CAPTURE **60**	Capture video of **60** seconds length
CAMERAHILIM **16000**	Set the maximum threshold limit of the camera histogram to **16000**
CAMERALOLIM **1000**	Set the minimum threshold limit of the camera histogram to **1000**
CAMERASETTINGSMSG	Display a dialog box asking the user to confirm the camera settings and then click ok

Auto setup

AUTOSETUP	Run the full automatic image setup routine – sets the camera level for autofocus, runs the autofocus and then sets the optimum camera level for NTA measurement

FOCUSLEVELRUN	Set the camera level appropriately for autofocus
AUTOFOCUSRUN	Run the autofocus routine at the currently set camera level
AUTOLEVELRUN	Sets the optimum camera level for NTA measurement (image must already be in focus)

Processing and export

PROCESSSINGLESETTING	Display a dialog box for the user to choose the detection threshold and then process all the videos
PROCESSMULTISETTING	Display dialog boxes for the user to choose the detection threshold for all the captured files for the current experiment. Process videos using individual settings chosen for each analysis
PROCESSBASIC	Process all the captured files for the current experiment using the last used or scripted settings
PROCESSSELECTEDSINGLESETTING	As above but for selected captured files in the current experiment
PROCESSSELECTEDMULTISETTING	As above but for selected captured files in the current experiment
PROCESSSELECTEDBASIC	As above but for selected captured files in the current experiment
EXPORTRESULTS	Display the Export dialog box, for the user to choose export formats and save files
NEWSAMPLE	Create a new .nano experiment file for subsequent captures. If using this option to save data into several experiment files within one script, a NEWSAMPLE command must be added before the first capture in the script. The option to time and date filenames must be turned on to prevent overwriting the experiment filenames
EXPERIMENTNAME **testname**	Save all subsequent captures under a new experiment file with the name **testname**
MAKEWMV	Convert entire current video to WMV format
MAKEWMV10	Convert first 10 seconds of current video into WMV format

Analysis settings

DETECTTHRESHOLD **5**	Set detection threshold to **5** (available range 1 to 255)
AUTOBLUR **ON/OFF**	Option to turn the automatically optimised blur setting on or off

BLUR **3**	Set a fixed blur value - 0 (off), 1(3x3), 2(5x5), 3(7x7) or 4(9x9)
AUTOMINTRACKLENGTH **ON/OFF**	Option to turn the automatically optimised track length setting on or off
MINTRACKLENGTH **10**	Sets a fixed minimum track length of **10** (available range 1-180)

Parameter settings

SETVISCOSITY **1.3**	Set viscosity to **1.3** (available range 0.0 - 1.8)
RECORDDILUTION	Set the dilution factor information before capture. This will be recorded in the experiment file and displayed in the results output

General

MESSAGE **text**	Convey information to a user via the script. Leave a space after MESSAGE then type the required entry. Message is displayed to user in a text box when command is run
SUBSCRIPT **filename.txt**	Call the commands from another saved script (**filename.txt**) into the current script. Main script and called scripts should both be in the default Scripts folder set in NTA
$ **text**	Insert a single line comment into a script. Text on any line starting with the $ symbol will not be run as a script command

Focus control

FOCUS **25**	Move the focus position to **25** (arbitrary units)

Temperature (Instruments with temperature controlled laser units only)

SETTEMP **37**	Turn temperature control on, set at **37** degrees
TEMPERATURECONTROLOFF	Turn temperature control off

Syringe pump

SYRINGELOAD **20**	Infuse sample with the syringe pump at speed **20** (arbitrary units)

SYRINGEWITHDRAW **20**	Withdraw sample with the syringe pump at speed **20** (arbitrary units)
SYRINGESTOP	Stop syringe pump movement

Filter wheel

FILTER **2**	Move the filter wheel to filter position **2**
FILTERADV	Move the filter wheel to the filter position one higher than the current position
FILTERRET	Move the filter wheel to the filter position one lower than the current position

Autoloader — not available with NS300

Script example

Requirement:
Scripted process to hold the loaded sample temperature at 37 °C, and run an NTA measurement of 60 seconds capture duration every 5 minutes for half an hour, starting immediately after the sample temperature is initially set. All videos are captured at camera level 10 and analyzed at detection threshold 5.

MESSAGE Ensure sample is loaded into the chamber and instrument is set at the measurement position ready for analysis

SETTEMP 37

CAMERALEVEL 10

CAPTURE 60 REPEATSTART DELAY 240

CAMERALEVEL 10

CAPTURE 60

REPEAT 5

TEMPERATURECONTROLOFF

DETECTTHRESHOLD 5

PROCESSBASIC

EXPORTRESULTS

7

SAMPLE LOADING

This chapter covers sample loading instructions for the Low Volume Flow Cell and the O-Ring top-plate.

Sample loading

Before proceeding with analysis, check a sample of any buffer or diluent to confirm that it doesn't contain any contaminating nanoparticles. When loading sample using the Low Volume Flow Cell or the O-Ring top-plate:

- Load the sample with the laser module outside of the instrument.
- As the sample is loaded, detect the presence of air pockets or bubbles and remove them.
- Load the sample into the top-plate using a 1 ml disposable syringe connected directly to the Luer fittings on the top-plate.

The O-Ring top-plate should be manually cleaned and dried before loading a sample. See Manual cleaning procedures on page 142 and Drying the O-Ring top-plate on page 149.

Caution! Both the Low Volume Flow Cell and the O-Ring top plates must only be used with disposable **1 ml** syringes with Luer port fitting. Using syringes with a larger volume than 1 ml, or exceeding the maximum rated flow speed of 0.05 ml per second (1 ml total in 20 seconds) may result in leaking or damage to components.

Important: The laser module should always be removed from the NS300 and held vertically when first loading the sample chamber from dry. Failure to do this can result in incomplete filling of the chamber and imaging problems.

Important! Bubbles in the path of the laser within the chamber can cause specular reflection of the laser beam off the bubble surface. This will degrade image quality and should be removed before analysis. If bubbles are routinely observed when a sample is loaded, clean the top-plate manually to remove any contamination which might be preventing good wetting of the surface. Certain samples may generate bubbles through out-gassing of dissolved gases over extended periods. In both cases, the image collected by the camera will show a high intensity region (or may be completely saturated) indicative of the presence of such bubbles. If samples repeatedly show evidence of bubble formation, it is advisable to de-gas the sample before analysis.

Low Volume Flow Cell

Loading an initial sample

Once the LVFC is mounted onto the laser module and the inlet tubing has been pre-rinsed with buffer or sample, connect the inlet tubing to the flow cell manifold to load liquid into the sample chamber.

1. Connect the end of the inlet tubing inside the NS300 casing to the left port of the low volume flow cell manifold.
2. Fill a 1 ml disposable syringe with the appropriate buffer or sample.
3. Remove any air bubbles from the syringe.
4. Insert the new syringe into the Luer port, ensuring liquid-to-liquid contact is maintained. See Changing the inlet tubing syringe on page 38 for additional guidance on changing the syringe.
5. Introduce the buffer or sample slowly into the chamber. The flow cell should not be loaded at speeds exceeding 0.05 ml per second (1 ml total in 20 seconds).
6. The LVFC is now loaded ready for use. If using a syringe pump, place the syringe into the syringe pump holder and operate as described in the *NanoSight Syringe Pump Operating Manual*.

Figure 7-1: Loading a sample with the LVFC.

Changing samples

The Low Volume Flow Cell top-plate has been designed so that the system can be flushed clean between samples (dependent on sample type) with particle carryover of less than 1%. It is not necessary to remove the flow cell or disconnect the tubing for flush cleaning.

1. Flush the system by loading a 1 ml syringe of clean water or buffer solution. The flow cell should not be flushed at speeds exceeding 0.05 ml per second (1 ml total in 20 seconds).

Note: Make sure that liquid to liquid contact is maintained at the Luer port when changing over the syringe.

Note: When using samples in buffer, flush the fluidic path with particle-free diluent. Using water to flush between samples-in-buffer may cause particles to stick due to changes in osmolarity or pH.

2. Confirm the cleanliness of the chamber by checking for any particles present in the NTA software image.
3. Repeat the flush if necessary to remove remaining sample particles.
4. Load the next sample syringe into the system. Make sure you load at least 800 µl of the new sample through the system to prevent significant dilution of the sample with wash fluid remaining in the outlet waste tubing.

If sample particles persist in the image after rinse through cleaning, or if many particles stuck to the optical flat are visible, increasing the background noise on the images, the flow-cell should be cleaned manually as described in Drying the Low Volume Flow Cell on page 145.

When using samples which commonly adhere to the optical glass surfaces, it is more appropriate to use the O-Ring top-plate, which is disassembled and thoroughly cleaned between each measurement.

O-Ring top plate

Manual injection of a sample

1. Fill a 1 ml disposable syringe with the appropriate buffer or sample.
2. Remove any air bubbles from the syringe.
3. Hold the laser module vertically, so that the front inlet port is at a lower level than the back outlet port.
4. Fill the chamber slowly against gravity, allowing any bubbles to escape.
5. Insert the 1 ml syringe into the front inlet port and introduce the sample slowly into the chamber. The top-plate should not be loaded at speeds exceeding 0.05 ml per second (1 ml total in 20 seconds).

Note: Loading the sample slowly limits the introduction of bubbles. The O-Ring top-plate must always be dried between each sample or buffer load, and is not suitable for liquid changeover without disassembly.

Loading a sample for use with a syringe pump

Note: The syringe pump is an optional extra that can be purchased from Malvern Instruments.

Priming the tubing

Important! When connected for syringe pump use, rinse the inlet fluidic tubing with buffer, or sample, before the tubing is connected to the top-plate and the top-plate primed for use. This improves bubble clearance from the tubing on initial priming, reducing the likelihood of air bubbles entering the sample chamber and causing problems in subsequent measurements.

To rinse through the inlet tubing with buffer or sample:

1. Make sure the inlet tubing fitted to the inside of the NS300 casing is not connected to the top-plate.
2. Place the end of the inlet tubing into a suitable waste container.

3. Insert a 1 ml syringe of liquid into the Luer port and push ~900 µl of the liquid through the inlet tubing as fast as the back pressure will allow (this should take 5–10 seconds).
4. Leave the syringe with the remaining liquid attached to the Luer port to prevent any air being introduced.

Caution!
Initially rinsing the inlet tubing at higher speeds allows better removal of any air initially trapped in the tubing or connectors. However, make sure that the pressure generated does not force the syringe out of the Luer port.

Changing the inlet tubing syringe

When using the fluidic tubing connections, it's important to ensure that liquid to liquid contact is always maintained at the syringe port when replacing an empty syringe.

Before changing syringes:

- Have the next syringe prepared, ensuring there are no air pockets present at the tip and that there is a small positive meniscus protruding from the syringe.
- Keep the Luer port at bench level when changing syringes to prevent the liquid level dropping inside the Luer port due to siphoning.
- Remove the old syringe from the Luer port and insert the new syringe into the Luer port (keeping syringes and Luer port horizontal) such that the two menisicuses combine without trapping an air bubble.

Note: The O-Ring top-plate must always be disassembled, cleaned and dried between different samples or when changing between buffer and sample loads. The O-Ring top-plate is not suitable for liquid changeover without disassembly, which can lead to significant particle carryover or sample dilution in situ.

Loading the sample through the syringe pump tubing

After pre-rinsing the inlet tubing with buffer or sample (see Priming the tubing on the previous page) attach the tubing fittings to the top-plate before mounting the top-plate on the NS300 laser module (the tubing fittings are harder to access with the top-plate attached).

1. Connect the end of the inlet tubing inside the NS300 casing to the left port of the O-Ring top-plate, and the outlet tubing to the right port.
2. Using the sprung fastening bolts, mount the top-plate with attached inlet and outlet tubing onto the laser module.
3. Fill a 1 ml disposable syringe with the appropriate buffer or sample.
4. Remove any air bubbles from the syringe.
5. Replace the pre-rinse syringe connected to the Luer port with the new syringe, ensuring liquid-to-liquid contact. (See Changing the inlet tubing syringe on page 38 for additional guidance on changing the syringe).
6. Hold the laser module vertically, so that the front inlet port is at a lower level than the back outlet port. The chamber can then be filled slowly against gravity, allowing any bubbles to escape.
7. Introduce the buffer or sample slowly into the chamber. The flow cell should not be loaded at speeds exceeding 0.05 ml per second (1 ml total in 20 seconds).

To use the loaded O-Ring top-plate with the syringe pump, place the syringe into the syringe pump holder and operate as described in the *NanoSight Syringe Pump Operating Manual*.

Changing samples

When using the O-Ring top-plate, remember the following;

- The chamber configuration is not suitable for flush through cleaning.
- The top-plate should always be disassembled and manually cleaned between samples, as described in Manual cleaning procedures on page 147, to avoid any sample carry-over.

8

MAKING A MEASUREMENT

This chapter outlines the basic steps involved in making an NS300 measurement. For more information about the software and its features, please see Software on page 47.

The following topics are covered:

Getting started

There are two main steps involved in making a NanoSight NS300 measurement. These are:

- Optimizing the image
- Taking a measurement.

The NanoSight NTA software is pre-installed on all computers supplied by Malvern Instruments.

1. Double click the NanoSight NTA software icon on the desktop:

This will open a typical home screen:

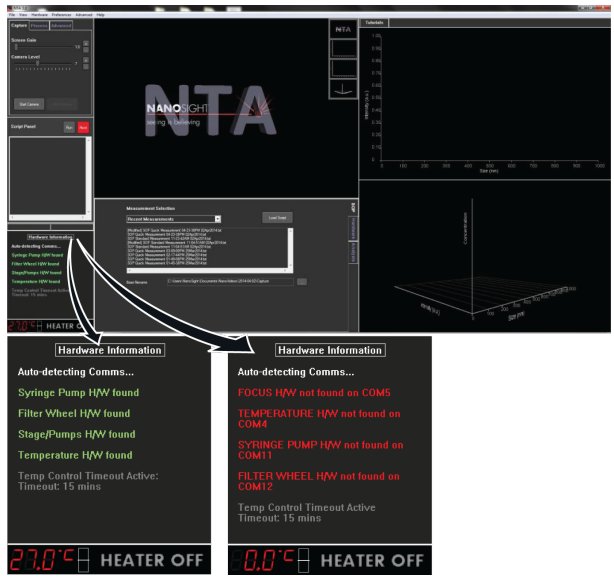


Figure 8-1: A typical NTA homescreen with *Hardware information* examples.

Connection status is detailed in the *Hardware Information* window. Any hardware attached to the equipment, if available, such as the syringe pump will be automatically detected by the software.

2. Check that the required hardware is detected. If the required hardware is shown as 'not found', check connections and power.

Note: For users new to the NanoSight Instrument and NTA software, Malvern Instruments recommends making first measurements using size-calibrated standard particles.

Obtaining an initial live image for optimization

1. For multi-laser systems, select the correct laser from the *Hardware* tab at the top of the screen.

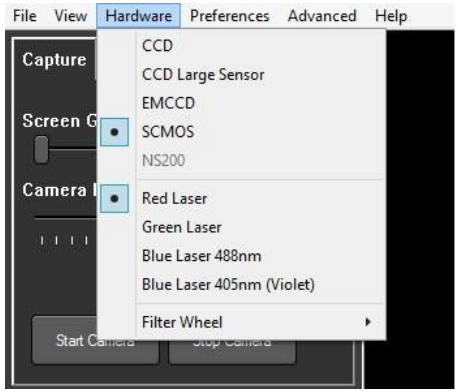


Figure 8-2: Select correct laser from the *Hardware* tab.

2. Select **Capture**.

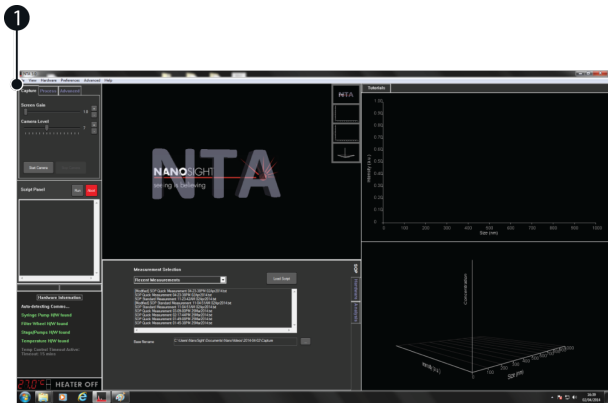


Figure 8-3: Select the capture tab **(1)**.

This will show the *Capture settings* tab.

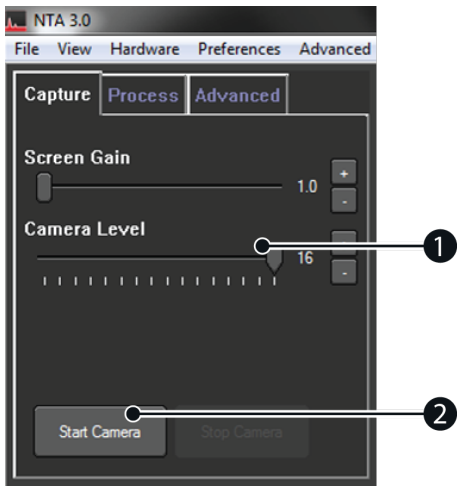


Figure 8-4: Capture settings tab.

3. Set **Camera Level (1)** to **16 (Max)**.
4. Click **Start Camera (2)**.

Optimizing the image

This is an iterative process between beam position **(1)**, camera level **(2)**, focus **(3)**, and concentration **(4)**.

Different sample types will require different final settings.

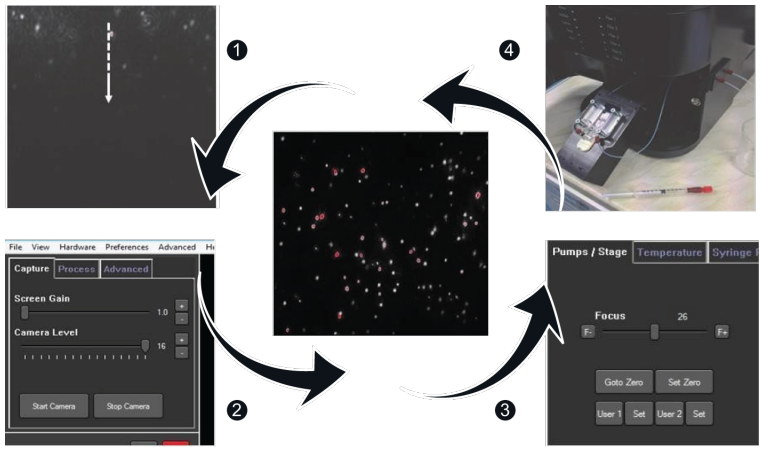


Figure 8-5: The iterative process of optimizing an image.

Laser beam position

The NS300 imaging position is set-up and calibrated by Malvern. The system is designed to have good beam relocation once set-up, although small adjustments may be necessary to optimize the image. If the beam is not central in the field of view on screen, i.e. not filling the top or the bottom of the screen, the image can be adjusted up and down by a small amount.

The illuminated particles need to fill the field of view.

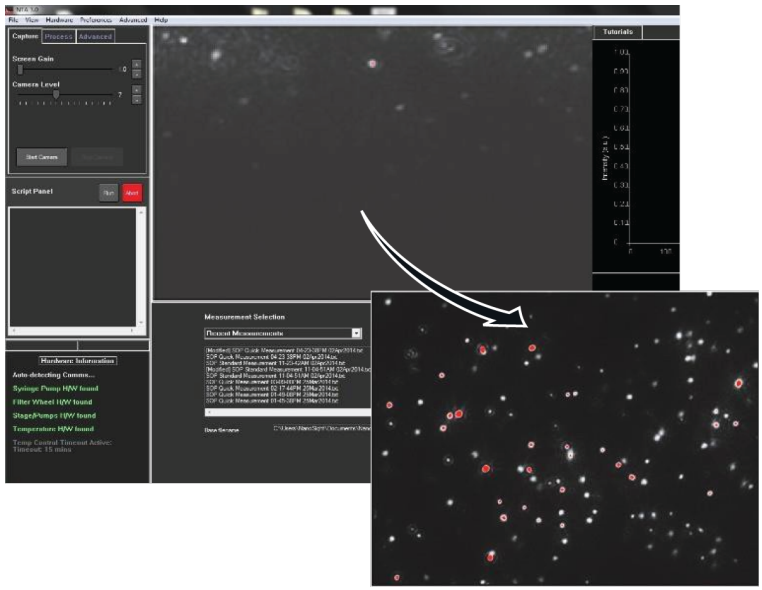


Figure 8-6: Centering image to show illuminated particles in the field of view.

Use left mouse button to drag and center the image:

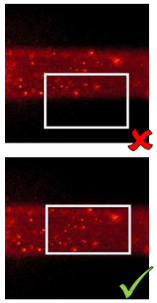


Figure 8-7: Centering the image.

Adjusting the camera level

Correctly setting up the camera and sample image prior to capturing the video are essential to achieving valid results.

Note: The software will provide warnings at the extremes of operation. Lack of warning does not mean the camera settings are OK. You must make sure that manual camera level settings are as close to perfect as possible to achieve optimal results.

Adjust the **Camera Level** until all of the particles in the sample can be seen clearly but no more than 20% are saturated (colored pixels).

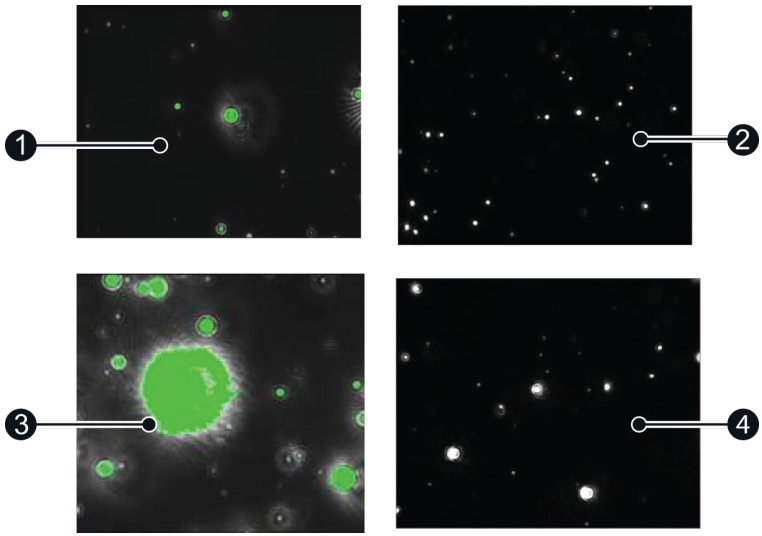


Figure 8-8: Good versus poor mono and polydisperse samples. Images obtained with a 532 nm (green) laser.

1. Poor monodisperse sample
2. Good monodisperse sample
3. Poor polydisperse sample
4. Good polydiserse sample

Using old camera levels

The camera brightness levels give a linear increase in brightness across the imaging range as the camera levels are incremented. In NTA software version 3.0 and earlier the camera levels were less linear, meaning that the same sample at the same camera level will not appear at the same brightness in NTA 3.0 and 3.1.

To view a sample using the old camera levels, i.e. for use in comparing image brightness to previously captured data, there is an option in the software to temporarily revert back to NTA 3.0 levels:

- Select **Preferences > Hardware Preferences**.
- Information is saved as a user preference and can be defined on a per Windows User Account basis **(1)**.
- If videos are recorded using NTA 3.0 levels, this is displayed in any exported files containing camera level values.
- When using NTA 3.0 camera levels this will be displayed in the capture tab **(2)**.

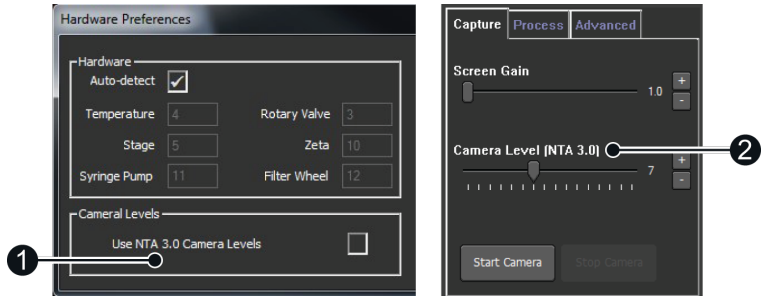


Figure 8-9: Old camera levels shown in capture tab.

Image focus

1. Initially, make coarse focus adjustments using the focus dial on the right hand side of the instrument casing.
2. Make fine focus adjustments using the slider found within the *Pump/Stage* tab in the software:

Figure 8-10: Fine focus adjustment in the NTA software.

Because the particles are constantly moving it can be difficult to achieve a uniform perfect spherical focus.

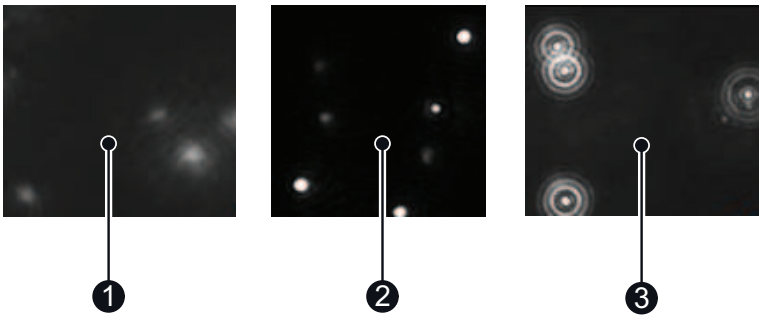


Figure 8-11: Examples of indistinct **(1)**, ideal **(2)** and satisfactory **(3)** particle images.

Indistinct particles, as in **(1)** will give inaccurate results.

Ideally, particles should appear as in **(2)** but those in **(3)** will be satisfactory if that is the best image that can be achieved.

If you are unable to locate the illuminated particles, or obtain a clearly focused image with your instrument, please contact Malvern Instruments on + [44] (0)1684-892456, or email us at helpdesk@malvern.com.

Concentration

The NanoSight instruments can work with particle concentrations in the range of ~10^7–10^9 particles/ml, which is approximately 20–100 particles in the field of view.

- Too high a sample concentration may prevent accurate particle tracking.
- Lower concentrations require longer capture and analysis time to produce statistically significant results.

Use the NanoSight syringe pump for low concentration samples, as this will improve results by sampling more particles.

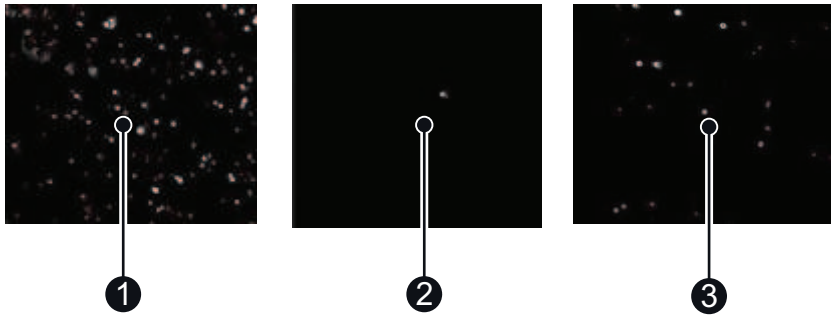


Figure 8-12: High, low and ideal concentrations.

1. Concentration too high — 208 particles identified
2. Concentration too low — 1 particle identified
3. Ideal concentration — 44 particles identified

Auto-setup

Auto setup can be used before the NTA Standard Measurement SOP for fully automated capture settings.

For **non-polydisperse** samples, with brightness equivalent to 50 nm – 400 nm latex for HS systems, or 100 nm – 400 nm latex for non-HS systems, NTA software version 3.1 and above can automatically set the image focus and select an appropriate camera level for standard measurement.

- Click the **Auto Setup** button in the Capture tab to focus the image and set an optimal camera level for measurement.

Figure 8-13: Auto-setup for fully automated capture settings.

1. Set an initial camera level.
2. Focus image.
3. Set capture camera level.

Note: Auto-focus is only specified for monodisperse samples which are well within the detection range of the instrument and within the concentration range $2x10^8$ to $1x10^9$ particles / ml. Some polydisperse particle suspensions may also be suitable for auto-focus, but samples with aggregates present can cause significant focus errors.

Note: For the auto focus to work, the *Scatter* position of your instrument must be initially set at the correct stage position and approximately the correct focus position to get a clear image of particles. If the current *Scatter* focus is not set correctly, the focus should be adjusted using the focus slider bar. Also correct the *Scatter* focus position by clicking the **Set** option next to the *Scatter* button.

For samples unsuitable for auto-focus, adjust the image focus manually using the slider control.

- Select the **Auto setup camera level only** checkbox to skip autofocus on subsequent **Auto Setup** runs and manually set the camera level.

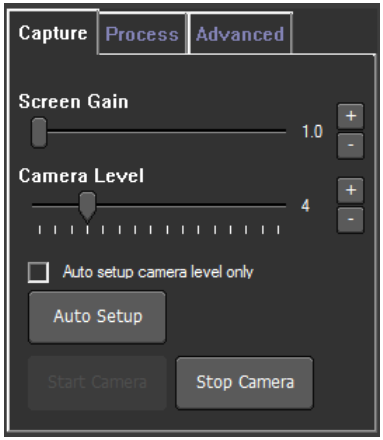


Figure 8-14: Auto set-up camera level only check box.

Thresholding pixels

If the pre-defined camera levels are not suitable for a sample, e.g. if it is very dim relative to the image background, use the histogram displayed underneath the capture screen to optimize the image settings when using the Scientific CMOS camera.

The histogram determines how the range of intensities captured by the camera, during the recording, is displayed as pixel grayscale values on the screen.

The grey histogram shows the range of intensities detected by the camera. To control the range displayed:

- Move the gray cursors using the left and right mouse buttons respectively. The range of pixel intensities to be displayed is then redefined as the range between the cursors. The grayscale values recorded as black (no signal) or white (saturated) are shown in blue on the histogram.

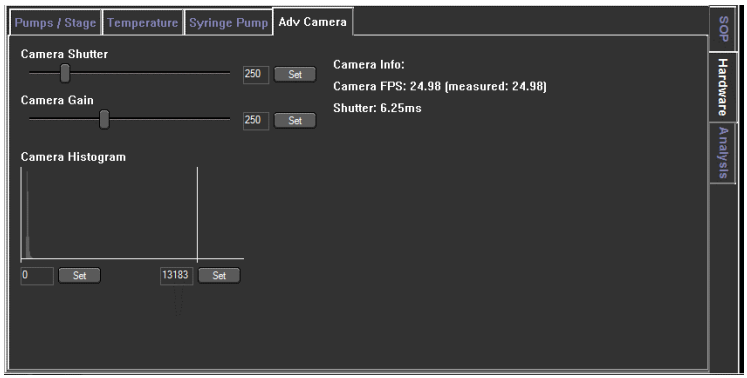


Figure 8-15: Advanced camera settings.

Restrict pixel thresholds as little as possible, whilst still allowing all particles to be seen.

To achieve the optimal threshold range:

1. Set the minimum to a level which allows the dimmest particles to still be seen (move the gray cursor into position with the left-hand mouse button).
2. Set the maximum to a level at which the largest particles do not contain many saturated pixels (move the other gray cursor into its maximum position using the right-hand mouse button).

Taking a measurement

The NTA software contains options for:

- **Standard Measurement** — Suitable for most size and concentration measurement within instrument specifications.
- **Quick Measurement** — Creates a single video and analysis.
- **Recent Measurements** — A list of the last 10 measurements taken allowing the rapid repeat of readings.
- **Zeta Measurement** — Not applicable for NS300 instruments.

Once an image can be seen on the capture screen, fine-tune the focus as appropriate. To record a movie:

1. Select **Standard** measurement. This scripted workflow captures videos, before moving on to data processing and export.

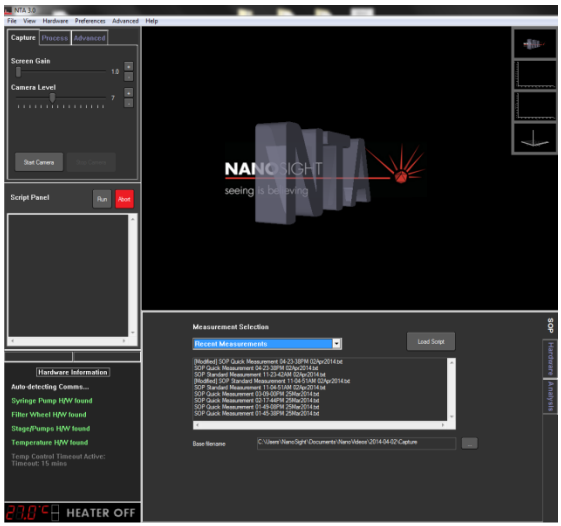


Figure 8-16: Selecting standard measurement.

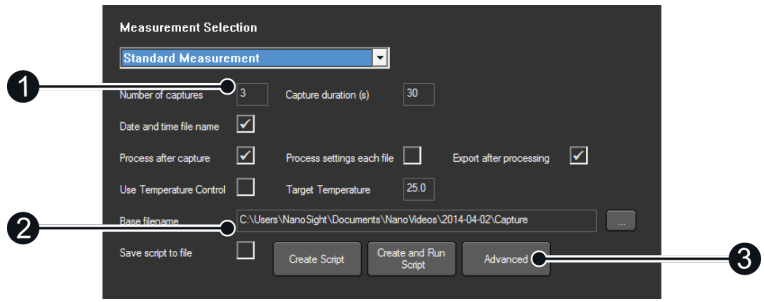


Figure 8-17: Measurement selection window.

2. Input the following:
    - **Number and duration of Captures (1)**: The default number and length of video captures are suitable for most samples.
    - **Base Filename (2)**: Select the name and location for the captured video files.
3. For dilution and viscosity settings click **Advanced (3)**.

The *Advanced capture settings* window now opens:

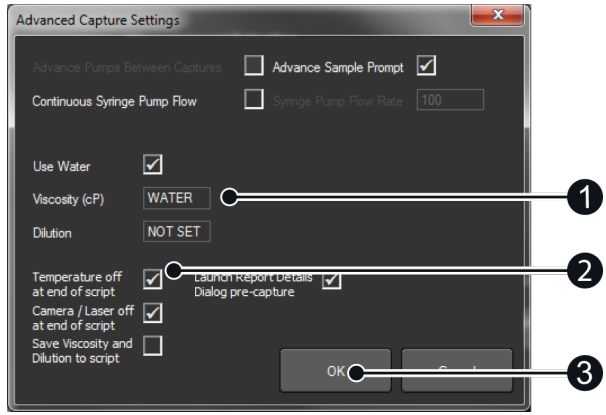


Figure 8-18: Advanced dilution and viscosity settings.

- Sample **dilution** factor can be entered. Where the diluent is not water, **solvent viscosity** must be entered here **(1)**.
- It is recommended that the lower check boxes **(2)** are left checked to avoid heat build up between readings (**Temperature off**..., **Camera/laser off**…, **Save Viscosity**…).

Note: The NTA software contains viscosity information for water. For all other liquids the correct viscosity information must be entered for accurate size data to be calculated.

---

4. Click **OK (3)**. The sample is ready to be measured.
5. Click **Create and Run script**.

Cancel the recording at any time by clicking the red **Abort** button.

Before capturing a video, you are prompted for sample details to be included with the video files and output files (optional).

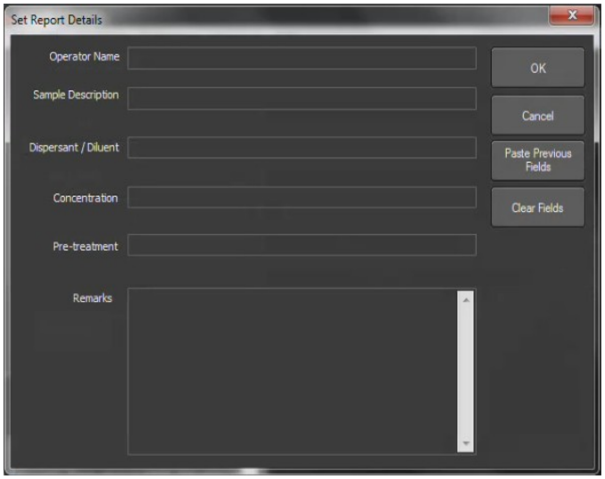


Figure 8-19: Additional sample details option.

1. Enter any details you wish to include with the files.
2. Click **OK**.

The software will prompt for the sample to be injected (this will be requested at the start of each repeat capture according to the script). The NanoSight Syringe Pump accessory provides the alternative of continuous sample flow.

As long as the temperature communications program is running, the sample temperature will be displayed in the NTA capture screen and automatically saved with the video.

After video capture the software defaults to immediate processing.

The following screen shows the captured video rather than the live sample image.

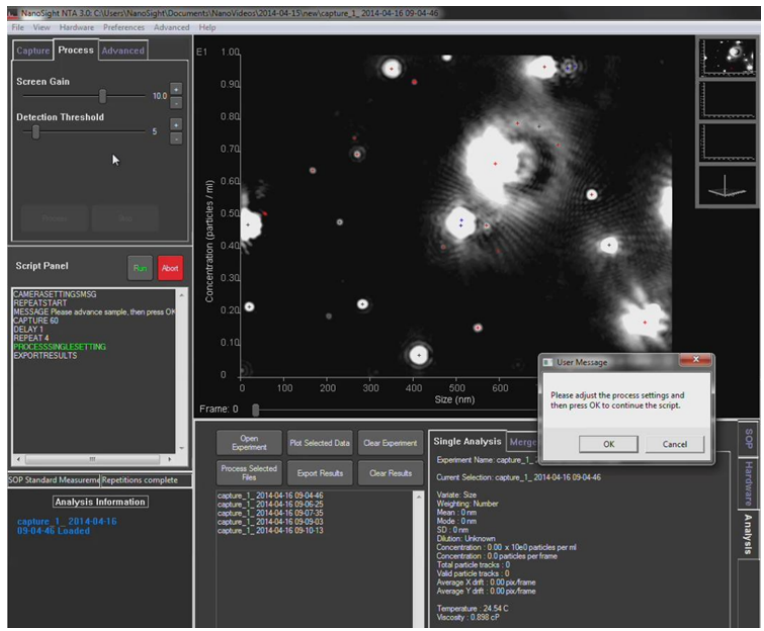


Figure 8-20: Captured playback.

Detection threshold must now be set before starting video processing.

Setting detection threshold

The detection threshold sets the minimum brightness of pixels to be considered for tracking.

The lower the setting the more centers will be found. However if it is too low, 'noise' can be tracked. If the setting is too high, particles will be excluded.

For the best analysis, identify the center of each particle by reducing the detection threshold to a level that includes as many particles as possible. The following are restrictions that should help you to achieve this:

- Count number of red crosses should be between 10 and 100. This count can be found in the bottom right of the image.
- When considering the image by eye, some of the red crosses may not appear to be distinct particles. Ideally there should be <10 such crosses.
- There may also be blue crosses on the screen. Ideally there should be <5.

IMPORTANT: The detection threshold setting must not be altered while the video is processing, nor between videos of the same sample measurement. For example, five videos of a sample recorded with the same capture settings should be analyzed with the same detection threshold.

Set detection threshold

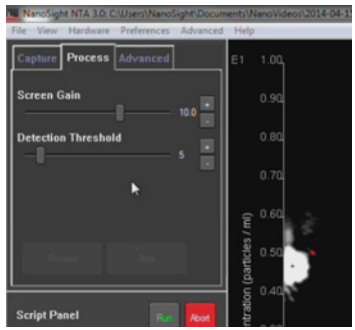


Figure 8-21: Setting detection threshold.

1. Adjust the detection threshold using the **slider** or the **+** or **–** buttons.
2. Check the selected setting with multiple frames of the video.
3. Move the slider under the main screen to check the image quality in additional frames.

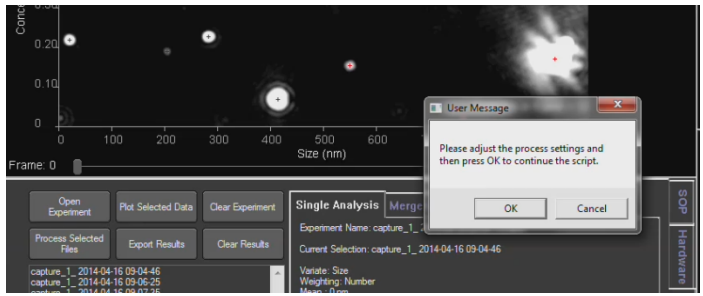


Figure 8-22: Checking video footage one frame at a time.

Low detection threshold

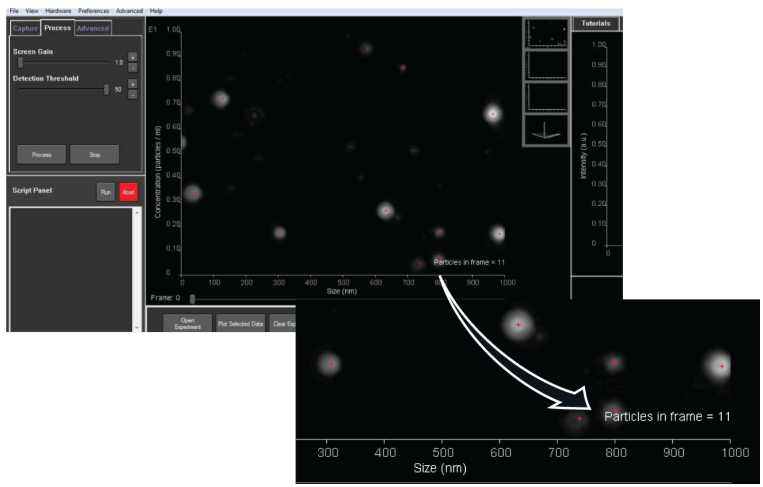


Figure 8-23: Low detection threshold.

The frame particle count value is shown in the bottom right of the main graph window.

High detection threshold

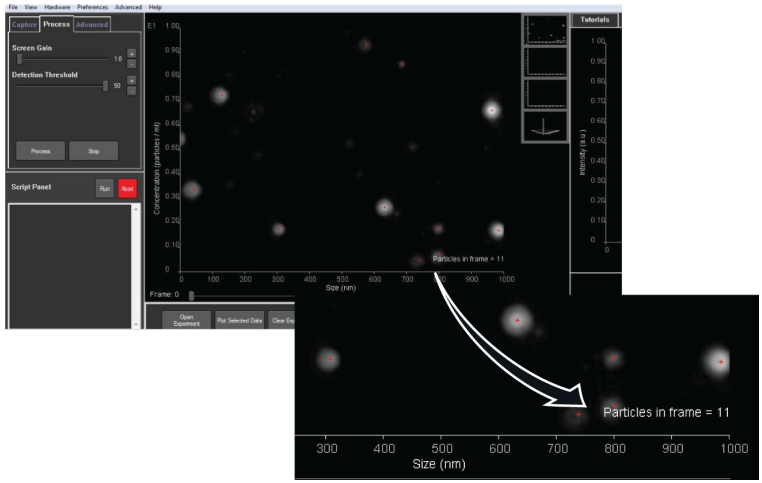


Figure 8-24: High detection threshold.

When the particle detection threshold has been set, click **OK** to start the measurement process.

Processing

During processing the image will appear to be brighter than during the set-up phase.

As the software processes the video images, red 'tracks' appear on the screen depicting the Brownian Motion of the particles.

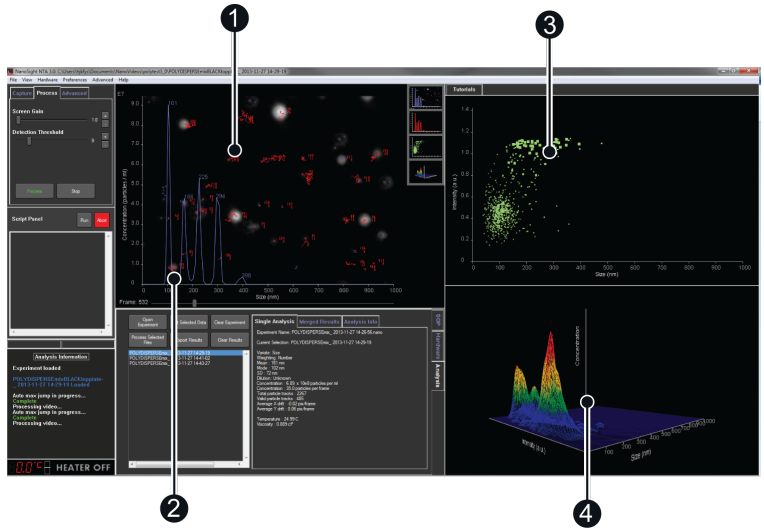


Figure 8-25: The NTA software during image processing.

- Size (nm) vs Concentration (particles/ml) measurements are shown on the blue (default) graph **(1 and 2)**, overlaying the particle screen as the video(s) are processed.
- The same measurements are additionally displayed as a **scatter plot (3)** (Size (nm) vs Intensity (a.u.)) and as a **3D plot (4)** Size (nm) vs Concentration (particles / ml) vs Intensity (a.u.)).
- Any vibration will affect the motion of the particles which can influence sizing accuracy.
- Although the NTA 3.0 software versions or above compensate for some interference, best results are achieved with zero vibration.

Example: Screen view at the end of processing for n=5 captures from one sample. Individual size distribution profiles of the 5 captures for the sample are over-plotted.

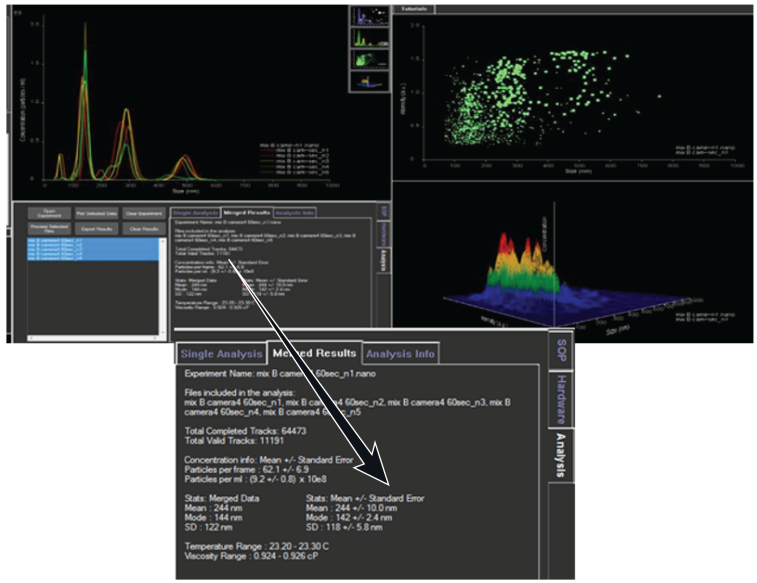


Figure 8-26: Software view after processing.

Mean ± SEM for the concentration, mean size, modal size and SD of the sample are shown.

The x-axis can be re-scaled during data processing, by left-clicking and dragging on the axis.

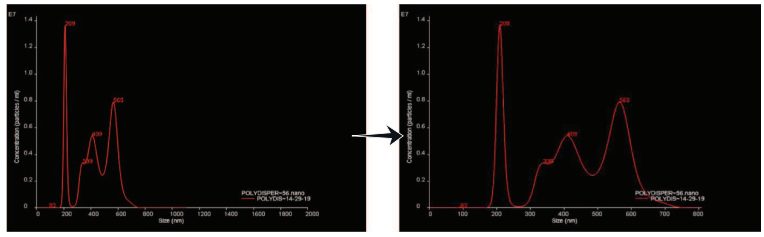
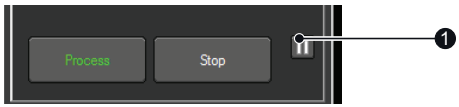


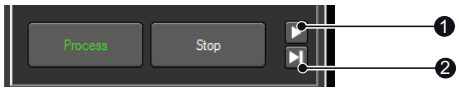
Figure 8-27: Re-scaling the x-axis.

Processing can be paused, and stepped through frame by frame.

- When processing is running, a **pause button (1)** becomes visible in the 'Process' tab.



- When processing is paused, a **play button (1)** and a **step frame button (2)** become visible.



Information symbols

During processing various information symbols may be displayed, hovering the mouse over these will give more information about the warning.

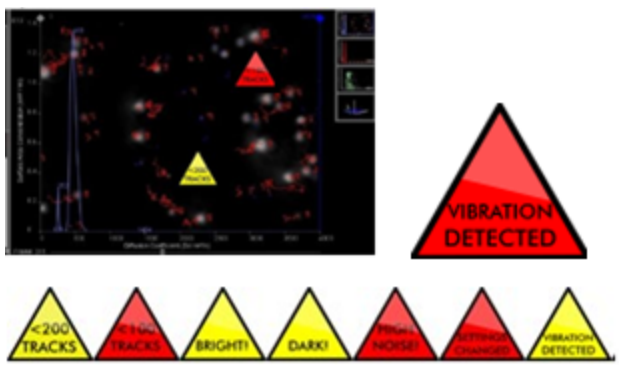


Figure 8-28: Information symbols.

Exporting

At the completion of processing the software automatically opens the *Export Settings* window.

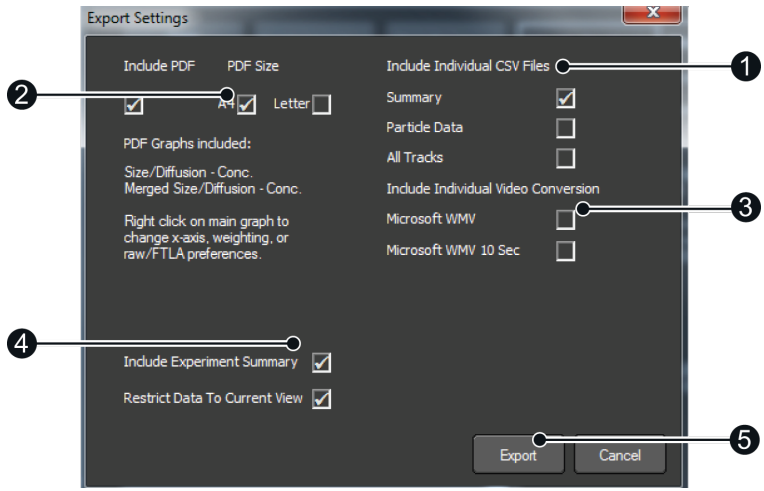


Figure 8-29: Export settings window.

- The defaults are for PDF graphs and batch summaries **(2)** and **(4)**.
- Raw data for further processing can be exported as CSV files **(1)**.
- The AVI files used to capture the video data are very large (e.g. a 60 sec video uses ~0.5 GB of data). NTA Software gives the option to export the videos as compressed WMV files **(3)** for customer support, demonstration and presentation purposes, including a 10 sec option.
- The **Export** button **(5)**.

Note: Only **.AVI** files can be compressed by NTA software.

PDF data export

Example of exported PDF report for n=5 data from one sample:

- The size distribution profile data are shown over-plotted **(1)**.
- Mean ± SEM data are shown, with the size of the key peaks annotated.

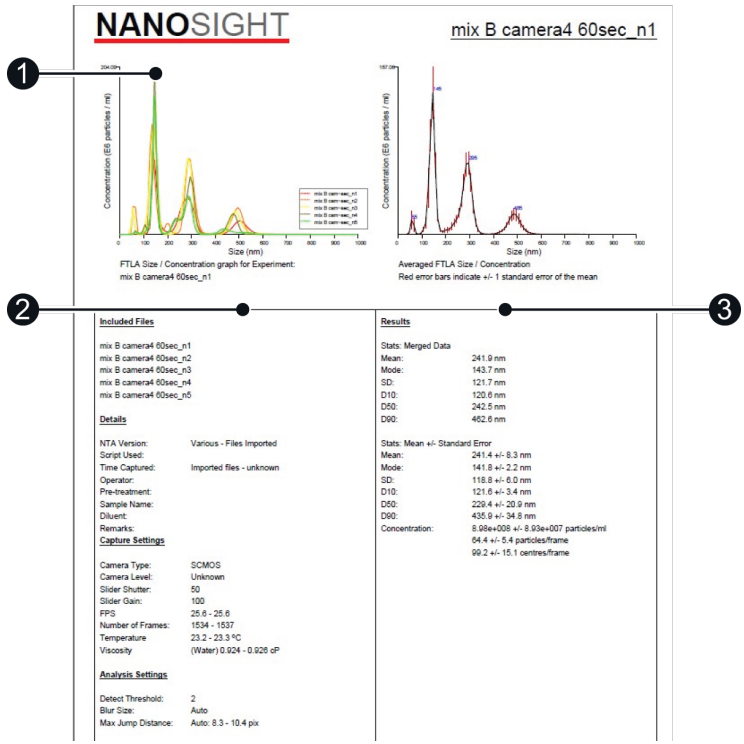
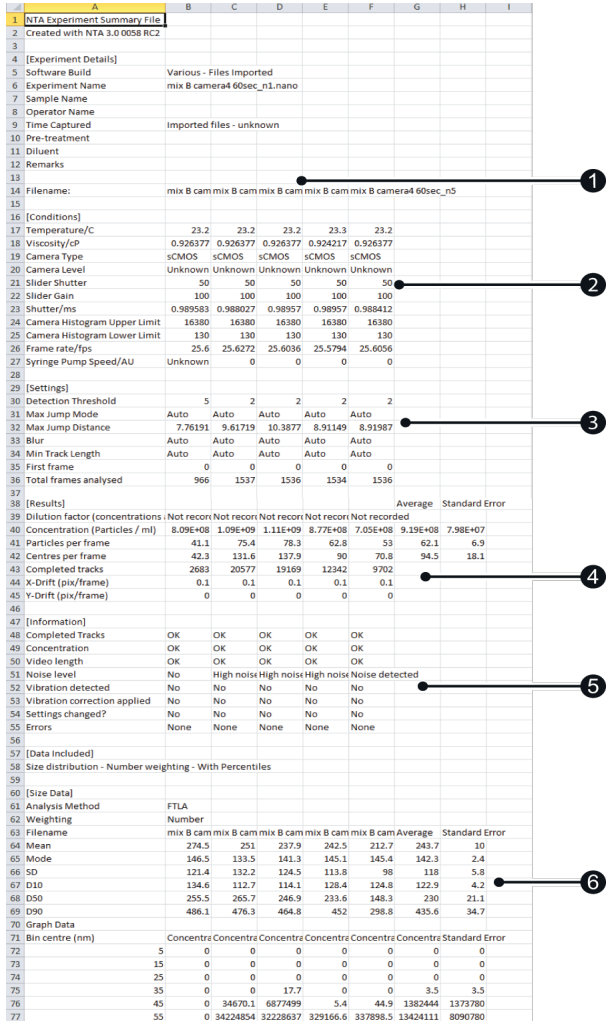


Figure 8-30: PDF data export.

- The lower panels include data on the settings used **(2)** along with a results summary **(3)**.
- Typically the **modal particle size** is used to describe the sample.
- The **SD** is a measure of the width (spread) of the size distribution profile.

- **D10**, **D50** and **D90** values indicate percent undersize, for example 50% particles are 229 nm or smaller, giving another indication of the spread of particle sizes within the sample.

CSV file data export



	A	B	C	D	E	F	G	H	I
1	NTA Experiment Summary File								
2	Created with NTA 3.0 0058 RC2								
3									
4	[Experiment Details]								
5	Software Build	Various - Files Imported							
6	Experiment Name	mix B camera4 60sec_n1.nano							
7	Sample Name								
8	Operator Name								
9	Time Captured	Imported files - unknown							
10	Pre-treatment								
11	Diluent								
12	Remarks								
13									
14	Filename:	mix B cam	mix B cam	mix B cam	mix B cam	mix B camera4 60sec_n5			
15									
16	[Conditions]								
17	Temperature/C	23.2	23.2	23.2	23.3	23.2			
18	Viscosity/cP	0.926377	0.926377	0.926377	0.924217	0.926377			
19	Camera Type	sCMOS	sCMOS	sCMOS	sCMOS	sCMOS			
20	Camera Level	Unknown	Unknown	Unknown	Unknown	Unknown			
21	Slider Shutter	50	50	50	50	50			
22	Slider Gain	100	100	100	100	100			
23	Shutter/ms	0.989583	0.988027	0.98957	0.98957	0.988412			
24	Camera Histogram Upper Limit	16380	16380	16380	16380	16380			
25	Camera Histogram Lower Limit	130	130	130	130	130			
26	Frame rate/fps	25.6	25.6272	25.6036	25.5794	25.6056			
27	Syringe Pump Speed/AU	Unknown	0	0	0	0			
28									
29	[Settings]								
30	Detection Threshold	5	2	2	2	2			
31	Max Jump Mode	Auto	Auto	Auto	Auto	Auto			
32	Max Jump Distance	7.76191	9.61719	10.3877	8.91149	8.91987			
33	Blur	Auto	Auto	Auto	Auto	Auto			
34	Min Track Length	Auto	Auto	Auto	Auto	Auto			
35	First frame	0	0	0	0	0			
36	Total frames analysed	966	1537	1536	1534	1536			
37									
38	[Results]						Average	Standard Error	
39	Dilution factor (concentrations	Not recor	Not recor	Not recor	Not recor	Not recorded			
40	Concentration (Particles / ml)	8.09E+08	1.09E+09	1.11E+09	8.77E+08	7.05E+08	9.19E+08	7.98E+07	
41	Particles per frame	41.1	75.4	78.3	62.8	53	62.1	6.9	
42	Centres per frame	42.3	131.6	137.9	90	70.8	94.5	18.1	
43	Completed tracks	2683	20577	19169	12342	9702			
44	X-Drift (pix/frame)	0.1	0.1	0.1	0.1	0.1			
45	Y-Drift (pix/frame)	0	0	0	0	0			
46									
47	[Information]								
48	Completed Tracks	OK	OK	OK	OK	OK			
49	Concentration	OK	OK	OK	OK	OK			
50	Video length	OK	OK	OK	OK	OK			
51	Noise level	No	High noise	High noise	High noise	Noise detected			
52	Vibration detected	No	No	No	No	No			
53	Vibration correction applied	No	No	No	No	No			
54	Settings changed?	No	No	No	No	No			
55	Errors	None	None	None	None	None			
56									
57	[Data Included]								
58	Size distribution - Number weighting - With Percentiles								
59									
60	[Size Data]								
61	Analysis Method	FTLA							
62	Weighting	Number							
63	Filename	mix B cam	mix B cam	mix B cam	mix B cam	mix B cam	Average	Standard Error	
64	Mean	274.5	251	237.9	242.5	212.7	243.7	10	
65	Mode	146.5	133.5	141.3	145.1	145.4	142.3	2.4	
66	SD	121.4	132.2	124.5	113.8	98	118	5.8	
67	D10	134.6	112.7	114.1	128.4	124.8	122.9	4.2	
68	D50	255.5	265.7	246.9	233.6	148.3	230	21.1	
69	D90	486.1	476.3	464.8	452	298.8	435.6	34.7	
70	Graph Data								
71	Bin centre (nm)	Concentra	Concentra	Concentra	Concentra	Concentra	Concentra	Standard Error	
72	5	0	0	0	0	0	0	0	
73	15	0	0	0	0	0	0	0	
74	25	0	0	0	0	0	0	0	
75	35	0	0	17.7	0	0	3.5	3.5	
76	45	0	34670.1	6877499	5.4	44.9	1382444	1373780	
77	55	0	34224854	32228637	329166.6	337898.5	13424111	8090780	

Figure 8-31: CSV file data export.

Note: When vibration has been detected during the measurement, the size reported will be smaller than the true size of the particles in the sample.

1. **Each column represents a captured file**
2. **Initial camera settings and capture information**
3. **Processing settings**
4. **Concentration and included particles**
5. **Processing and warnings history**
6. **Analysis results**

For additional data views see Viewing data on page 108.

Shutting down the system

Instrument switch off reminder — When closing the software, the system reminds you to switch off the instrument. Click **No** to stop this message appearing for the next 10 uses.

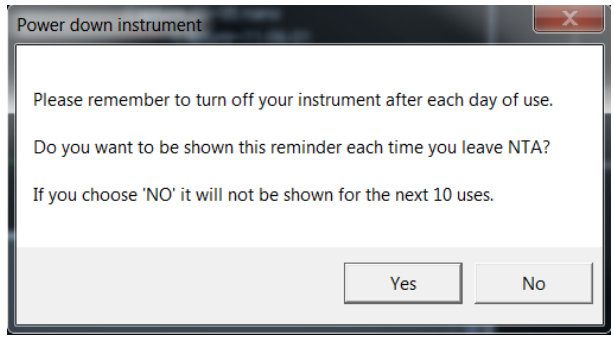


Figure 8-32: Instrument switch off reminder.

Top-plate screws reminder — When closing the software, you will be reminded to loosen or remove the top-plate fastening screws after each day of use. Click **No** to stop this message appearing for the next 10 uses.

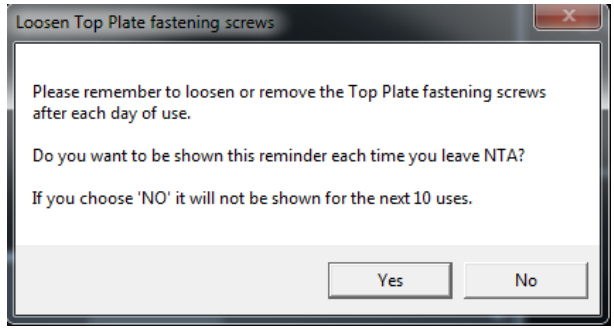


Figure 8-33: Top-plate screws reminder.

9 FLUORESCENCE MEASUREMENTS

This chapter briefly gives information about measuring fluorescent particles.

It covers the following topics:

About fluorescence mode

Nanoparticle Tracking Analysis (NTA) technology from Malvern can be used to analyze the size and concentration of particles from the light they scatter. With appropriate experimental design, NTA can also be used to characterize fluorescent particles. This is done by detecting the fluorescence signal, which is emitted naturally by particles or as a result of fluorescence labeling or tagging.

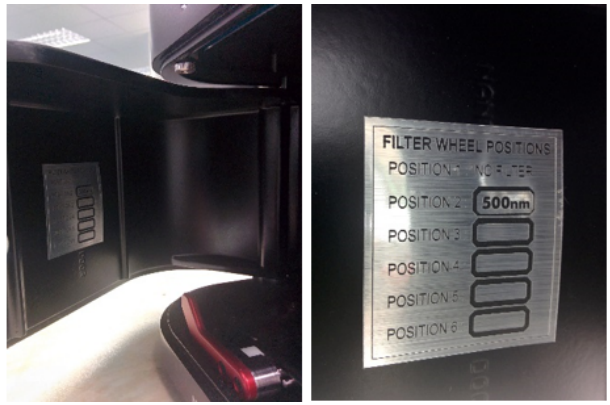
Additionally, the NS300 system can also be used to measure a range of fluorescent particles. The appropriate combination of laser excitation and suitable fluorescent filters is used to block scattered laser light whilst imaging the fluorescent signal from particles.

Measuring fluorescent nanoparticles is a more advanced measurement approach compared to measuring particles by the light they scatter. When measuring nanoparticles, the concentration of fluorophore on the surface or within the particle may be low. This in turn means that the fluorescence signal may be quite dim. Alongside this, the number of fluorescence cycles through which a fluorophore can pass before it's no longer able to emit a fluorescence signal is limited. This phenomenon is known as photobleaching. This means that for some fluorophores the fluorescence signal is lost before the nanoparticle has been tracked for sufficient frames of the captured video for it to be included in the dataset.

To make measurements in fluorescence mode, the instrument must be fitted with the high sensitivity Scientific CMOS camera, a trigger cable and a syringe pump.

For high sensitivity systems with fluorescence capability, the laser automatically pulses on and off in sync with the camera shutter. This reduces photo-bleaching of fluorescent particles. Additionally, the syringe pump delivers a constant supply of new unbleached particles to the sample chamber.

Videos captured through the fluorescence filter require a high camera level and may need optimization of the thresholding pixels to give an image suitable for analysis. The optical path for recording videos under fluorescence is also different, so you may need to adjust the focus.



Fluorescence filters are mounted on an integrated filter wheel within the instrument housing. Introduction of filters into the optical path is controlled by the NTA software. The position of filters installed in the filter wheel is displayed on a sticker inside the access hatch for each NS300 system.

The table below shows the available laser wavelengths and standard filters supplied with fluorescent NanoSight systems.

Available Laser Wavelengths (nm)	Standard Filter Supplied (nm)
Violet 405	430 long pass
Blue 488	500 long pass
Green 532	565 long pass

Note: For users wanting to use the Red 642 nm laser (for fluorescence measurements), please contact your local Malvern representative.

Once an optimum dilution for measurement in scatter mode has been determined, the sample can be checked in fluorescence mode.

- If no signal is seen, continue to inject sample at a higher concentration until fluorescent particles become visible.
- It is not uncommon to run fluorescence measurements at a much higher concentration than scatter measurements.

Preparing to make a fluorescence measurement

Before sample analysis:

- Generate a dilution series of the fluorescent sample, between 1x and 1000x that used for light scatter measurements.
- Protect samples from the direct light exposure by storing the sample in non-transparent sample container (or wrapped in aluminium foil) and also protect the sample in the syringe from light.
- Connect the NanoSight syringe pump to the instrument and set the flow rate so particles cross field of view within 5 – 10 seconds, see NanoSight Syringe Pump manual for further information. For comparing scatter and fluorescence measurements the same flow speed should be used.

How to make a fluorescence measurement

To make a fluorescence measurement, perform the following steps:

1. Identify the correct position under the light scatter mode.
2. Make sure the sample is flowing under the recommended flow rate (particles crossing the field of view within 5 – 10 seconds).
3. Increase the camera level to the maximum setting.
4. Insert the fluorescence filter into the optical path: under the *Hardware Filter Wheel* menu item, select the relevant filter position for your system.

Figure 9-1: The *Filter Wheel* tab, as shown for NTA software version 3.0 and above.

5. View particles by adjusting the focus in the *Hardware Pump/Stage* tab: click the **F-** and **F+** buttons to incrementally adjust the focus, or use the slider bar for larger focus steps.

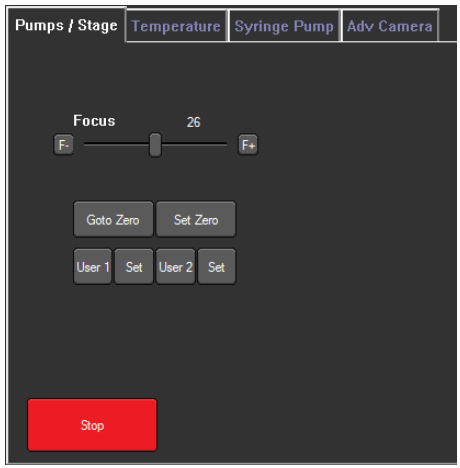


Figure 9-2: Camera focus control found within the *Pumps/Stage* tab.

6. Adjust the camera level. When working with fluorescent samples that are very dim (relative compared to the image background), you may need to adjust the *Advanced Camera greyscale histogram* settings to increase the brightness of the particles relative to the background (optimize contrast):

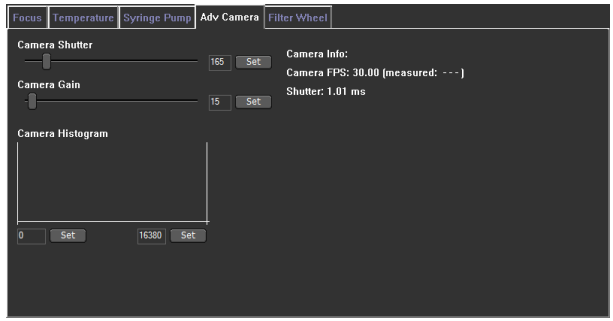


Figure 9-3: Camera greyscale histogram within the *Advanced Camera* tab.

To achieve optimal threshold range:

1. Set the minimum value to a level that allows the dimmest particles to be seen — move the grey cursor (blue arrow — left side) into position with the left-hand mouse button.
2. Set the maximum value to a level where large particles don't contain saturated pixels — move the other grey cursor (red arrow — right side) into its maximum position using the right-hand mouse button.
3. Collect the data under the fluorescence mode by running a script of 5 repeats of 60 or 90 seconds.
4. Measure the sample in light scatter mode by manually removing the filter from within the software. Run the script.
5. Compare results from both modes (by overlaying size distributions) and calculate the labeling efficiency (by comparing the concentration values).

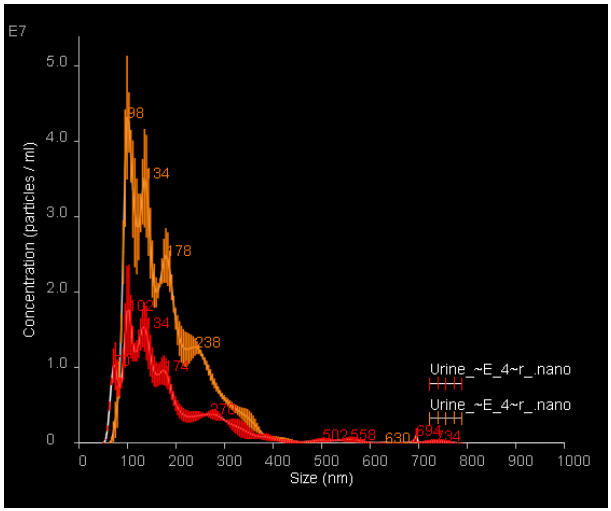


Figure 9-4: Overlay of NanoSight size distribution data for fluorescently labelled urinary exosomes, light scatter data are shown in orange with fluorescence data shown in red. Data are mean ± SEM.

6. Compare the size profile distribution with unlabelled sample.

10

TEMPERATURE CONTROLLED MEASUREMENTS

This chapter details information regarding performing measurements at non-ambient temperatures.

It covers the following topics:

Operation

Peltier elements within the laser module operate as a heat pump; transferring heat from one side of the Peltier to the other (i.e. they push heat from one face to the other making one side hot, the other cold). They are electrically powered and the power can be controlled by precisely varying the voltage (and therefore current) through the Peltier elements. There is a thermistor to measure the temperature positioned within the laser module body. The temperature measurement is fed back to the controller card and the power adjusted to attain the desired temperature.

Available temperature control	from 5 °C below ambient up to 50 °C
Temperature Accuracy	+/-1 °C
Time to temperature:	3 minutes (to indication of within 1 °C)

Warning!
Before using the temperature controller it is imperative that all warnings associated with its operation, as described below, are read and understood.

Warning!
The temperature controller may become hot. Caution should be taken when handling the device. The temperature controller should always be turned off when unattended.

All users must read and understand the following:

1. The temperature controller must be turned off if leaving it unattended.
2. The main body of the viewing unit must be monitored to make sure it doesn't overheat. This is possible if the temperature control is set to a high temperature (>40 °C) or a low temperature (<ambient) for a long period of time. In either case the laser module will heat up significantly, which can cause fluctuations in the laser power and possible long-term damage. Should this happen, turn the temperature controller off in NTA. Alternatively, in an extreme case, remove the power jack from the control box.
3. When cooling the sample to below ambient temperature, the temperature control must only be employed for a maximum of 15 minutes at a time, before being switched off for at least 15 minutes.

Before using the temperature controller it is imperative that all the warnings mentioned above are read and understood.

Initializing

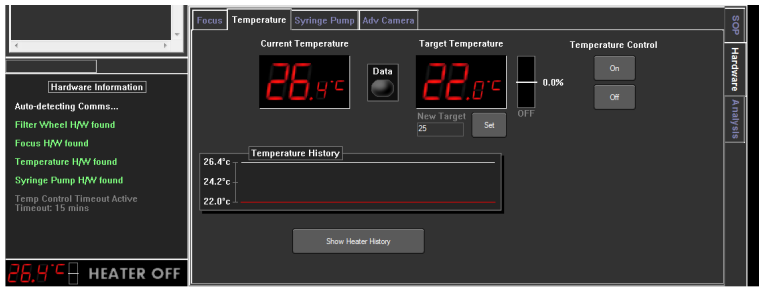


Figure 10-1: The *Temperature* tab.

1. Switch on power to the NS300.
2. Switch on the PC.
3. Load the NanoSight NTA software.
4. The temperature sensor box is in the lower *Hardware* tab, under the temperature tab.

Temperature setting

The temperature can be set manually and through a measurement.

Manually

Temperature control can be turned on and off.

1. When the temperature sensor is initialized, click the **On/Off** button.
2. The default 'set temperature' is 25 °C. To change this temperature, click the **New target** box and type in the required temperature.
3. Click **Set**.

The temperature controller will now work to set the temperature of the top-plate to the target temperature. Some overshoot should be expected. The angled line shows a trace of the temperature over the last 200 seconds. This line is autoscaling but has no labels.

A new temperature can be programmed to change the temperature (by repeating steps 2 and 3).

4. Press the **Off** button in NTA to turn off the temperature controller. The temperature readout will continue.

Through measurement

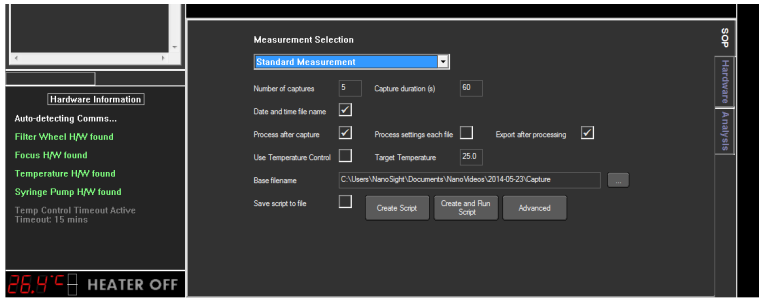


Figure 10-2: Setting temperature through measurement.

1. When planning a measurement, the temperature at which the measurement should be taken can be set by clicking in the **Target temperature** box under the SOP tab.
2. Type in the temperature required.
3. Tick the **Use Temperature Control** button.

Manual temperature input override

If no temperature readout is available select the **Manual Temperature (1)** option in the Standard Measurement SOP page. This lets you choose to manually input a temperature to be used for all captures recorded using the script (the temperature input window will not appear after each captured video).

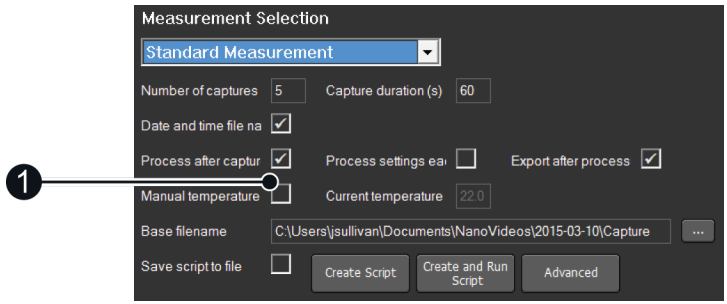


Figure 10-3: Selecting manual temperature.

11

ANALYZING DATA

This chapter gives information on viewing and analyzing your measurement data, as well as some examples of typical data profiles:

Viewing data

To view the combined data profile ± SEM

1. Place the cursor in the main graph area and right click to open a graph display options menu.
2. Select **Switch Multigraphs/Average**.

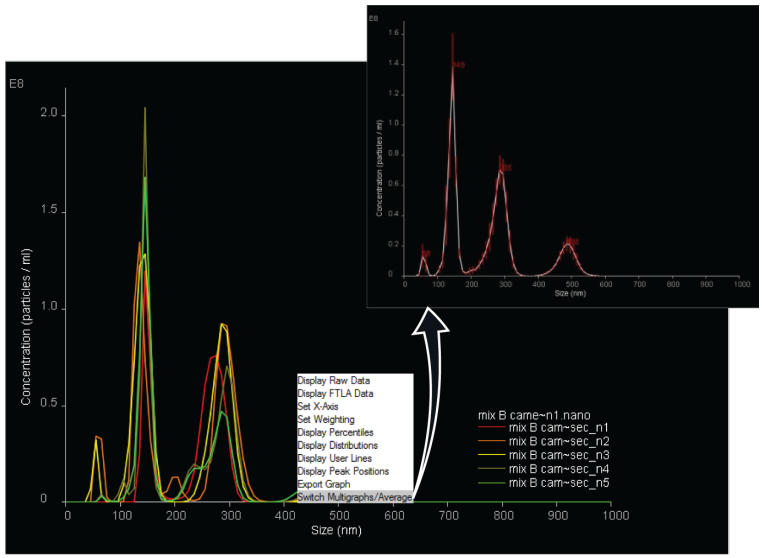


Figure 11-1: Viewing the combined data profile.

To view the data for a single capture.

1. Double click the chosen file **(1)**.
2. The size distribution data for the chosen capture is shown in the *Single analysis* tab **(2)**.

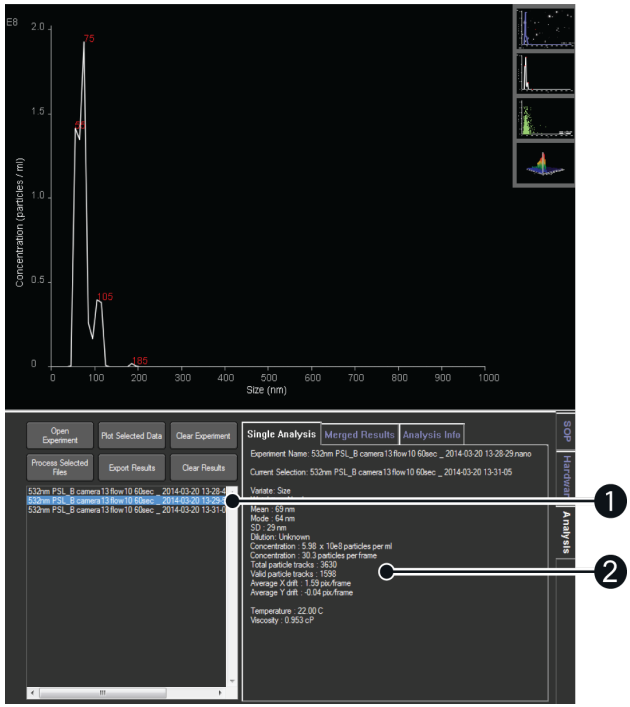


Figure 11-2: Data from a single capture.

Plotting and analysis

Open/close an experiment

1. Click on the *Recent Experiments* tab to open a previous experiment. The most recent experiments are listed in the window underneath.
2. Double click to load the experiment and automatically display the results for the files selected.
3. Open older experiments by using the **Open Experiment** button or the **File menu > Open Experiment** command.

The files available for the loaded experiment are listed in the *Current Experiment* tab. File names currently selected for data display and export are displayed in bold type, and have a tick-mark displayed to the right.

- Click the *Close Experiment* tab to close the current experiment and clear graphs and results from the screen.

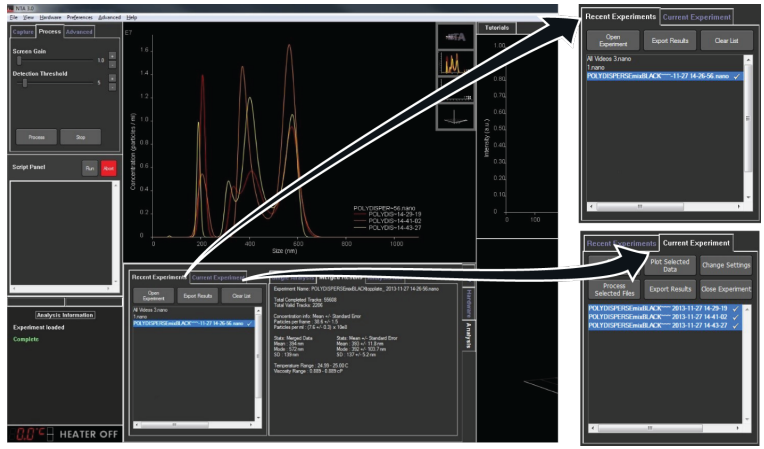


Figure 11-3: Current and Recent experiment tabs.

Combine experiments

1. Click on **File > Combine Experiments** to combine multiple experiments. The experiments to combine (and associated video files) must reside in the same folder.
2. In the file browser window that opens, select the experiments to combine.

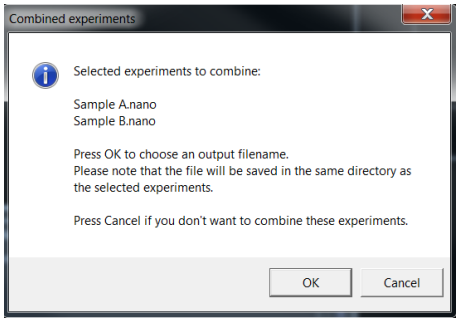


Figure 11-4: Combine experiments prompt.

3. A prompt will show. Click **OK** to combine the selected experiments into one file and choose an output filename.

Graph plots

As for NTA 3.1 and above, click and drag available plots from the graph viewport into graph windows **(2)** and **(3)**. This displays them alongside the plot in the main window **(1)**.

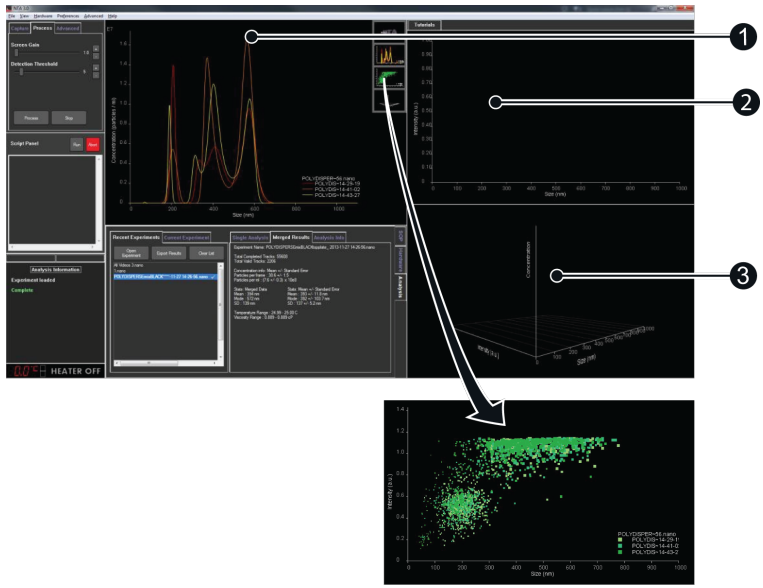


Figure 11-5: Graph windows.

When an experiment is loaded, graph windows **(2)** and **(3)** are empty. These can be used for any overlay plots to compare graphs between different experiments/datasets.

Experiment intensity scatter plot

When loading an experiment, the scatter plot contains data from all selected analyzes.

Exclusion regions

Scatter plot exclusion regions have been implemented to replicate functionality in previous versions of NTA.

Note: Exclusion regions can only be applied to a single measurement.

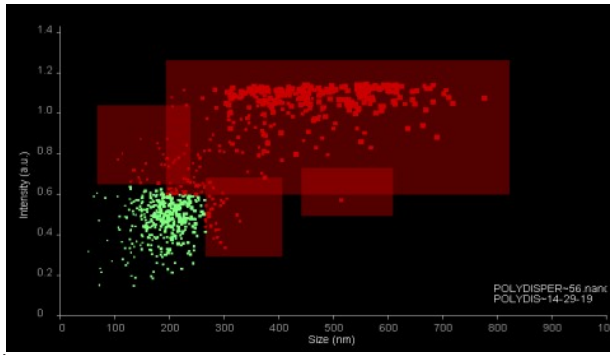


Figure 11-6: Scatter plot exclusion regions.

- Left mouse click and drag to add a region to the scatter plot.
- Multiple square regions can be added to remove areas of a distribution.
- Chosen regions are saved and are reloaded back in when looking at the analysis again.
- Regions are recorded in the summary .csv file.
- Other graphs (e.g. size distribution, 3D size-intensity distribution) are updated as regions are added.
- Regions must be added per analysis (i.e. not for the entire experiment).

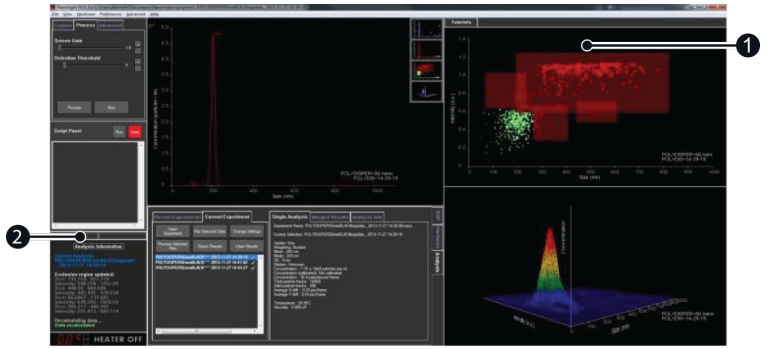


Figure 11-7: Main window displaying exclusion regions.

- During processing excluded particles are highlighted using a square **(1)**.

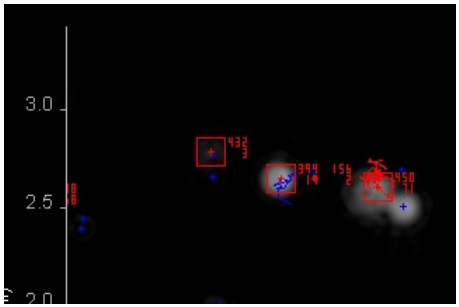


Figure 11-8: Exclusion regions highlighted by a square.

- Exclusion regions are shown in the status window **(2).**

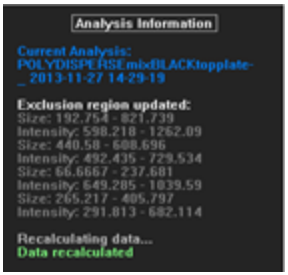


Figure 11-9: Exclusion regions shown in the status window.

An example is shown here of exclusion regions added in a size vs intensity plot (left), and viewed in a size vs intensity plot (right). Excluded regions are shown in red.

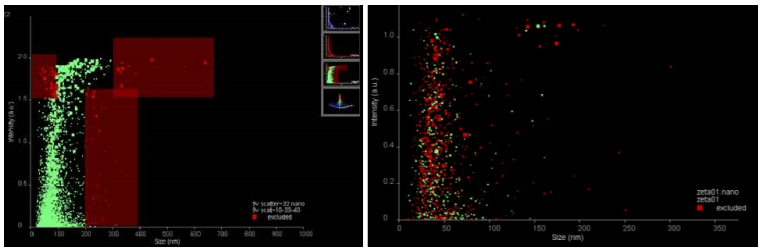


Figure 11-10: Exclusion regions added in a size vs intensity plot (left), and viewed in a size vs intensity plot (right).

When viewing an experiment/multi-plot scatterplot, any excluded points are shown in red.

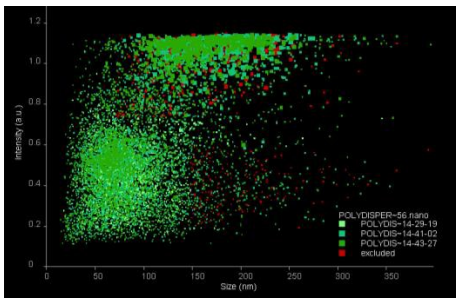


Figure 11-11: Excluded regions shown in red.

Selected concentration

User lines

Set user lines as markers on a plot to obtain concentration information about a selected part of the distribution.

Note: User lines can only be applied to an average or a single line from a multigraph. Do not attempt to apply user lines to an over-plotted multigraph.

To display user lines:

1. Select the **Display User Lines** option from the right-click context menu on the graph.
2. Move the lines to the required value by left clicking and dragging on the diamond symbols **(1)** at the top of the lines.

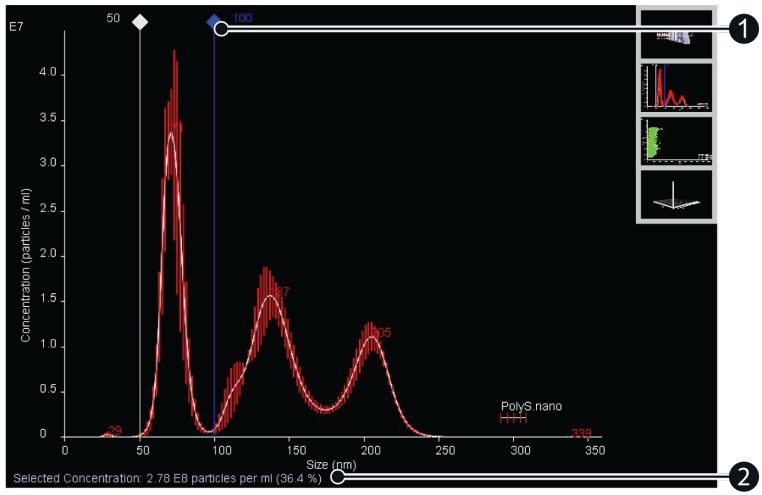


Figure 11-12: User lines.

When user lines are shown on the single graph plot (merged plot or single experiment), a selected concentration **(2)** is shown underneath the graph and exported.

- This selected concentration is the estimated concentration of the sample between the values given by the two user lines.
- It's shown as an absolute value in particles per ml, and as a percentage of the total concentration.

Selected concentration is available in size and diffusion, vs concentration graph views.

Change settings window

Alter the settings for viscosity and sample dilution in the software to automatically update results and graphs, without the need for re-processing.

To change viscosity or dilution settings for a loaded experiment:

1. Navigate to the *Current Experiment* tab.
2. Click the **Change Settings** button.

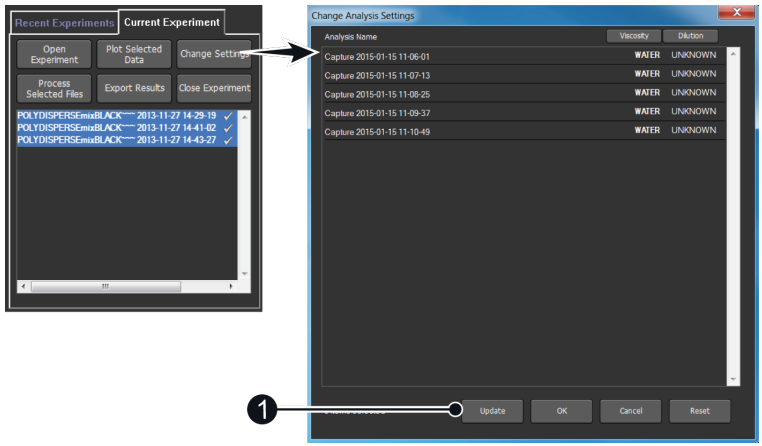


Figure 11-13: Change experiment settings windows.

The buttons at the bottom of the *Change Settings* window can be used to save or clear any changes.

- **Update**: Sets the new settings and updates the graphs and results in the NTA main window.
- **OK**: Same as **Update**, but then closes the window.
- **Cancel**: Clears any changes since last update, and closes the window.
- **Reset**: Resets any values back to as they were at the time of the last update.

To change settings:

1. Select the settings in a single column which you wish to change.
2. Click individual cells to select, or click the column header button to select all.

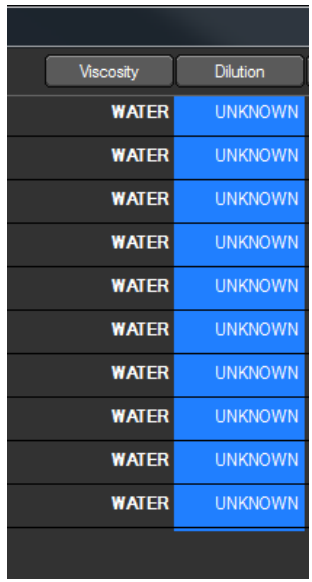


Figure 11-14: Selecting cells to change.

3. Double click any selected cell to edit the value. If Dilution is set to 0 it will display 'Unknown' and the concentration will not be adjusted.

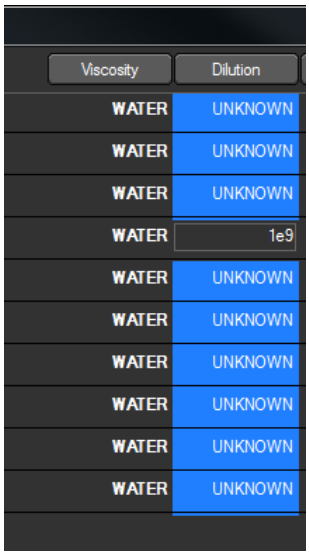


Figure 11-15: Enter new value for all selected cells.

4. Press the **Enter** key to automatically update all values in the column.

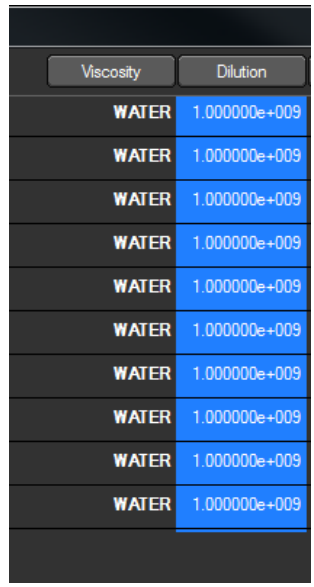


Figure 11-16: Updated highlighted cells.

Viscosity setting

When selected, viscosity cells display a tick box to switch between water or a manual viscosity.

1. Select the tick box to set the viscosity of the sample for water at the measured temperature.
2. When using other solvents, un-tick the box to manually enter the appropriate viscosity.

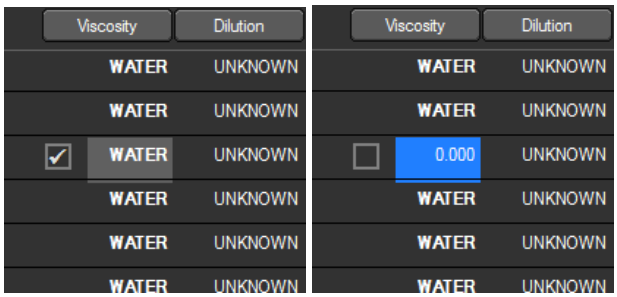


Figure 11-17: Tick box to change from water to manual viscosity.

Draw all tracks

On a processed individual analysis, a diagram of all tracked movements of particles can be generated. This can be useful when checking the alignment of the laser module and ensuring that illuminated particles are evenly filling the field of view for concentration measurements.

1. Display the video window plot (top graph in the viewport panel) for a single analysis in the graph window.
2. Right click on the graph window and select **Draw All Tracks** from the context menu.

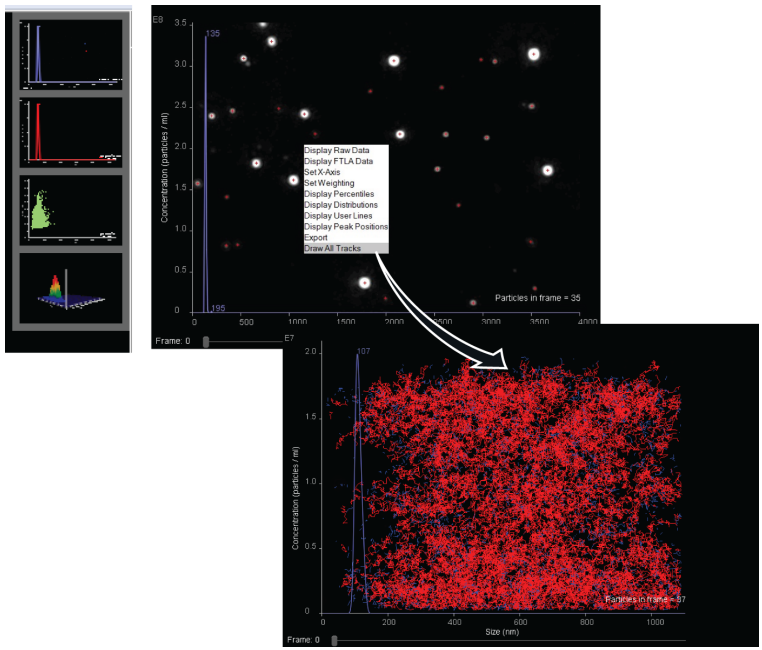


Figure 11-18: Draw all tracks.

After a few seconds an image should appear, as shown on the right. Red tracks are 'valid tracks' (i.e. included in the measurement) while blue tracks are rejected tracks.

3. **Right click > Export** can be used to export the image to file.

Graph overlay

Drag experiment graphs from the viewport windows to graph windows 2 and 3.

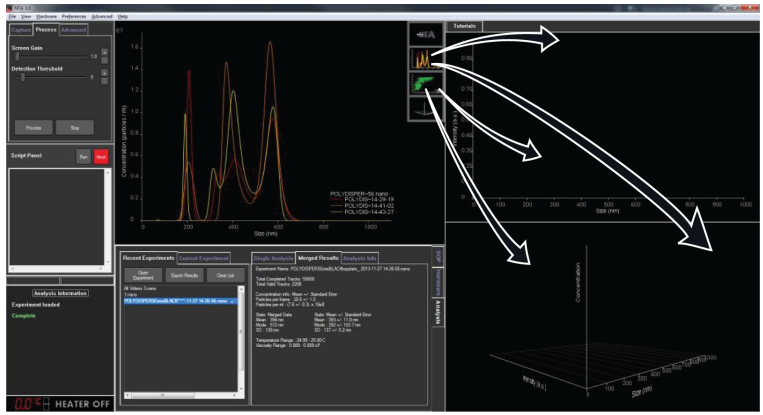


Figure 11-19: Drag graphs from viewport windows to graph windows.

- The new plot will automatically be transferred to a blank graph window.
- If the window is not blank, options to replace or overlay are shown (depending on the graph types).

Replace/Cancel buttons — these become visible when dropping a graph on top of another, but they are not compatible for overlay (e.g. different axes).

- **Select Replace** to overwrite the previous graph with the new one.

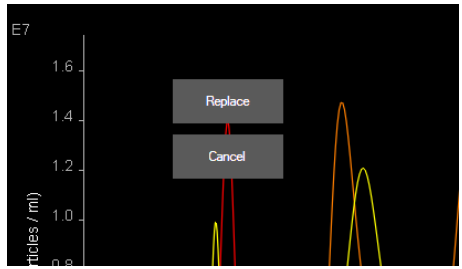


Figure 11-20: Replace/ Cancel buttons.

Overlay button — this becomes visible when datasets are compatible for overlay.

- Select **Overlay** to overlay the two graphs on a common axis.

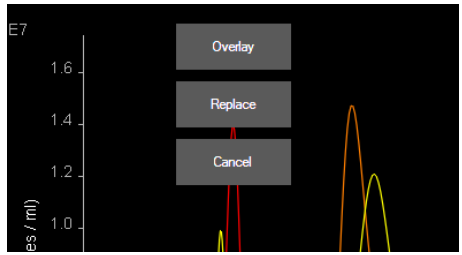


Figure 11-21: Replace/ Cancel/ Overlay buttons.

This feature can be used to overlay data from a subset of files captured in a single experiment or to overlay data from multiple different experiments on the same plot.

1. Open an experiment and drag a single file or multi-plot graph from the viewport panel into Window 2 or 3.
2. Open another data file or the next experiment, plot the data and overlay the corresponding graph. Up to 10 data sets can be overlaid in this way.
3. Right-click and select **Export Graph** from the *Context* menu to export overlaid graphs as PDF reports or BMP images. Graphs will be exported into the same location as the currently loaded experiments.

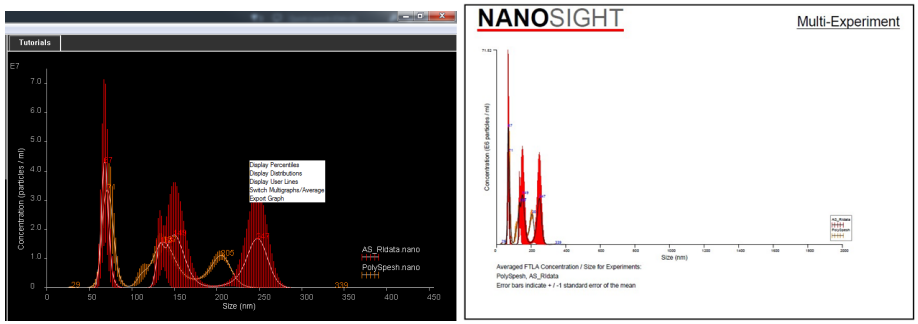
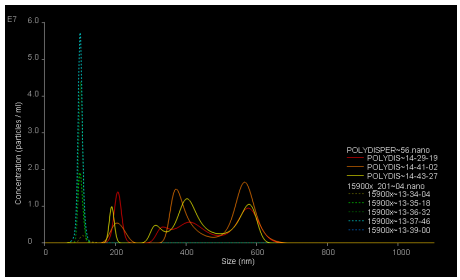
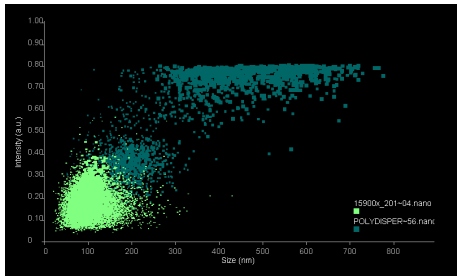


Figure 11-22: Export graph as PDF.

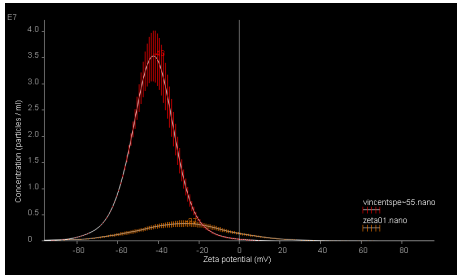
Examples of possible overlaid graph options are shown below:



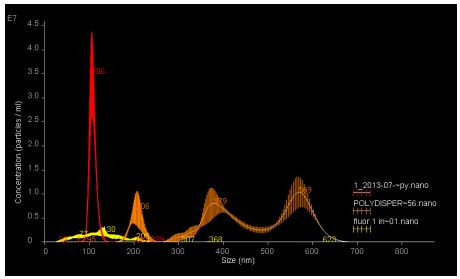
Two sets of size vs concentration multi-plots.



Two sets of size vs intensity scatterplots.



Two sets of zeta vs concentration plots with error bars.



Three sets of size vs concentration plots with error bars.

Graph settings

Navigate to the **Preferences > Graph Settings** option to adjust the current graph settings.

- Bin widths can be chosen separately for size and diffusion graphs, and depending on whether raw or FTLA data is used.
- These settings can be defined on a per windows user account basis.
- These settings are used for all default graph displays and standard export files (from the *Export* window).

Figure 11-23: Graph settings.

For size and diffusion, log spaced bins can be used:

- **Default X Axis (4)**: This the x-axis used when opening an experiment and when exporting data (for zeta experiments, zeta potential is automatically set as the x-axis).
- **Reset (3)**: Reset resets all graph settings back to their default values.
- **Update (2)**: This saves the graph settings and updates any graph currently displayed by the software.
- The **Range (1)** of the graph is displayed and updated as changes are made to the **No. of Bins** and the **Bin Width**. Any incompatibilities with settings are shown in red.

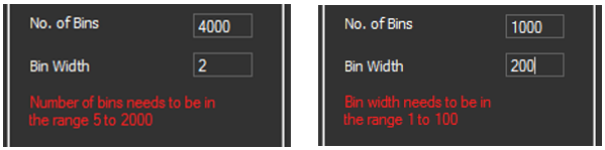


Figure 11-24: Bin number and width.

Graphics customisation

Navigate to the **Preferences > Adjust Graph Colors** option to adjust the current colours used for NTA graph plotting. All color preferences will be saved and re-loaded when NTA is restarted. These settings can be defined on a per windows user account basis.

The color maps shown in the window for Sets 1, 2 and 3 are used for different plot types within NTA.

- Set 1 – 2-D distribution profiles (e.g particle size histogram).
- Set 2 – Scatter plots (e.g. intensity vs size).
- Set 3 – Overlaying graphs.

To change graph colors:

1. Click on a color to display a selection box.
2. Select a new color to redefine the color used for NTA graph plotting. The colors can be reset to their default setting by clicking **Reset**.

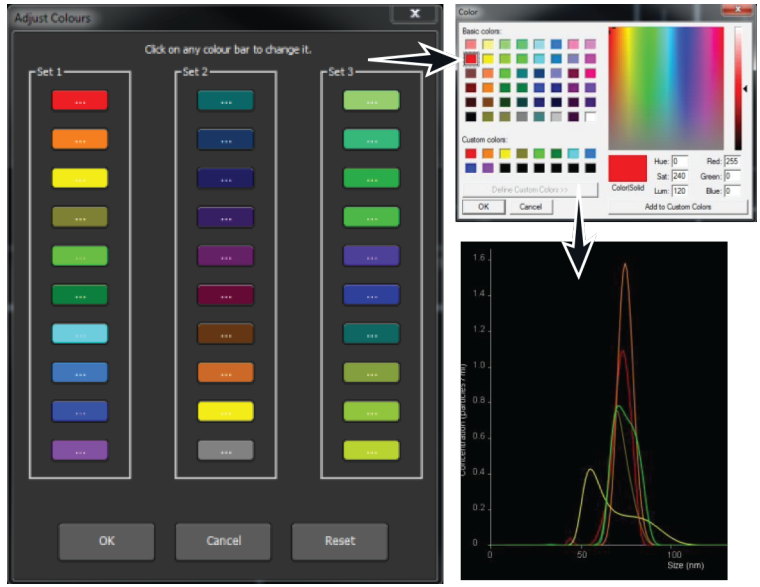


Figure 11-25: Changing graph color.

Example results and guidance

Typical data profiles

The profile obtained depends upon the type of sample measured.

When the particle size of the sample is more controlled, e.g. size standards, extruded liposomes etc., a narrow size distribution profile with a single peak should be obtained, indicating a mono dispersed sample:

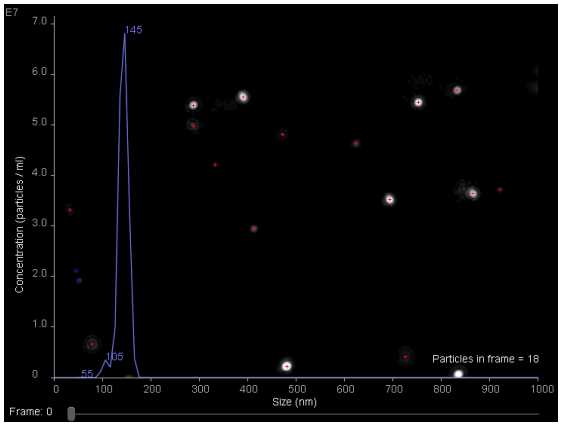


Figure 11-26: Monodispersed sample.

For samples such as extracellular vesicles purified with a sucrose gradient, it is likely that the main peak of the profile is broader with one or more peaks identified:

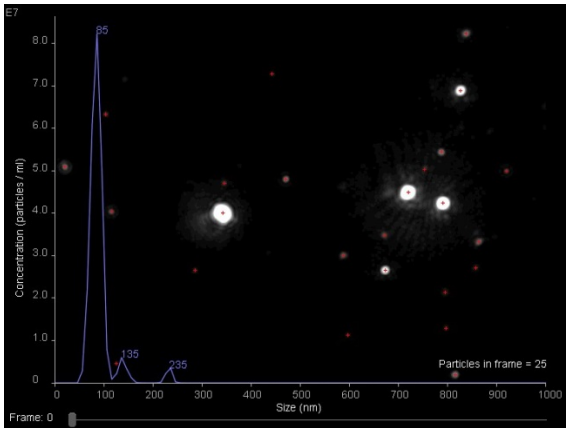


Figure 11-27: Multiple, broader peaks.

For polydispersed samples e.g. aggregated protein, a very wide size distribution profile might be expected with many peaks identified, which typically decrease in peak-height as size increases:

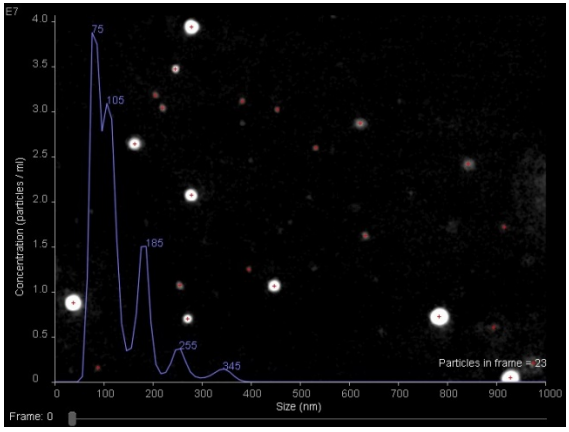


Figure 11-28: Polydisperse sample.

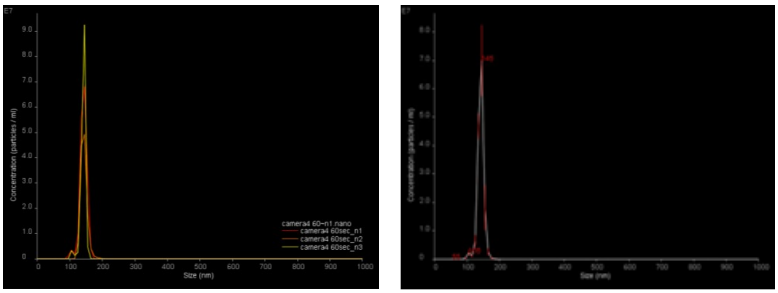
Guidance for accepting data

Accepting the data depends on the type of sample you are measuring.

When multiple profiles for the same sample measurement are plotted together, the similarity of the profiles should be in keeping with the level of polydispersity, providing no sampling errors are present, the capture on processing setting were constant, and the sample was measured for long enough:

i.e. a monodispersed sample should over-lay closely for the data to be acceptable, whilst a polydisperse sample can have less reproducible profiles and the data may still be acceptable.

Monodisperse sample:



Polydisperse sample:

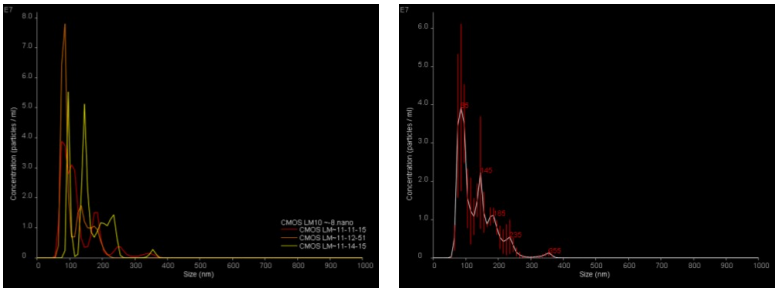


Figure 11-29: Overlays of monodisperse and polydisperse measurements.

Notes:

- Samples of the same type should be measured using the same settings for comparison.
- Polydispersed samples will typically need to be captured for longer to generate good data.
- Samples with a naturally low level of particles/ml will need to be captured for longer to generate good data.
- Users are advised to refer to the PDF and CSV data output files to check for warnings that could result in invalid results.

Note: NTA works to meet ISO19430. For more information on this standard, see the International Organisation for Standardization website.

Working towards ISO19430

The International Organization for Standardization (ISO) provides ISO standards, which are guidelines that are manufacturer agnostic, but are an international agreement between manufacturers. Every manufacturer can produce a set of specifications on how to operate their equipment.

ISO19430 — Particle Tracking Analysis (PTA) method — describes limitations, quantification parameters as well as instructions on how to operate the instrument in a certified manner. In terms of the guidance Particle Tracking Analysis (PTA) is also known as Nanoparticle Tracking Analysis (NTA), since the term "nanomaterial" has not been defined or adopted globally.

To meet the criteria, when measuring 100 nm latex size-traceable standard spheres, acceptable modal size is 100 nm ±6 nm m (± 6%) and for 150 nm latex acceptable modal size is 150 nm ±9 nm.

A number of criteria of ISO19430 are given below along with additional comments.

	ISO19430 criteria	Comment

Sample and sampling volume	• ~1 ml sample.
	• Measurement volume based on 0.1 –1.0 nl.
	• Field of View (FoV) 100 x100 µm.
	• Focal depth 10 µm = ~0.1 nl.
	• Measurement representative sample. This increases overall volume measured by sampling multiple parts of the sample, especially for low concentration samples.
	• Maximum sample concentration $1x10^9$ particles/ml.
	• Minimum sample concentration $1x10^6$ particles/ml for systems with wide optical FoV.
		• For sample volume, criteria are based on the approximate sample viewing area provided by the technology.
		• For sample concentration:
		  • Lower limit of 10^6 recognized that the count will be low but the particles will be well dispersed, so number of tracks will be low and statistics will be poor.
		  • For sample concentration, upper limit of 10^9, the denser sample will mean particles are close together, tracks may cross, particles may appear to merge and be unresolvable by software leading to poor tracking.

Measurement precision and uncertainties	• Precision – number of tracks (for monomodal 100 nm): • 400 tracks- CV <10%	• Counting efficiency — smaller particles move more and so are more likely to leave the FoV compared to larger which can lead to bias. This bias can be overcome if

	ISO19430 criteria	Comment
	• 2000 tracks- CV <3% • Counting efficiency — automatic or manual thresholding of minimum number of tracks.	the number of particles of a givens size per frame are reported.
Sizing accuracy	• Sample viscosity and measurement temperature are key parameters — the standard gives guidance on acceptable accuracy. • As a diffusion coefficient is being measured, a solid, vibration-free environment is required.	• The required particle numbers for a certain cumulative variance were evaluated on common monomodal particle types. • The number of tracks achieved, not the number of particles, are important. • Counting limits can be influenced by the size of particles. • Small particles (low scattering levels) may be too dim and fall below the LOD. Large particles may be too bright to allow the faint ones to be seen. • Sizing accuracy needs information on sample viscosity and temperature. • For precision for temperature, the accepted range is ± 3 °C and confers a ±1% sizing accuracy. • Measurement should take place in a vibration free environment. • Although compensation algorithms are available, it is better to have no external vibrations for the measurement.
Equipment	• Sample cell • Laser/illumination • Optical microscope • Camera • Computer	• Well focused illumination of the sample is required. • Several measurements of the same sample must be made. • Re-sample adding a new dispersion into the instrument sampling volume. • Minimum of three repeats required.
OQ/PV	• Measurement should take place in a vibration free environment. • Instrument should be left clean and dry when not in use. • Flush with diluent between samples.	• For 100 nm latex spheres acceptable modal size is ± 6 nm m = ± 6%. • If you achieve this then you are allowed to claim that your equipment is within the range of precision of ISO19430 . • The standard also recommends that you run 150 nm latex for ± 9 nm.

	ISO19430 criteria	Comment
	• When using saline, flush with water to prevent crystal formation.	• In terms of minimum numbers of tracks used to evaluate the sample, see ISO13322-1:2014 – industry.
DATA	• More tracked particle equates to better data. See ISO 13322 - 1:2014. • Key parameters are D10, D50 and D90. • Size limits for different RI particles: Au 15 nm, silica 75 nm	• Must report D10, D50, D90 data. • Size limits – depend on sample and its scattering properties. • Gold is a strong light scatter so has a smaller size limit of 15 nm, whereas for Silica it's 75 nm

How Nanosight NTA meets the requirements of ISO19430

	ISO19430	NanoSight NS300
Sample and Sample volume	• Volume 0.1-1 nl FoV 100 x100 x10 µm. • Representative sampling, repeat sampling. • Concentration $1x10^6$ – $1x10^9$/ml.	• FoV ~ 100 x100 x10 µm = 0.1 nl. • Standard SOP equals 5 repeats (with sample advance, or flow mode (with syringe pump). • Working range = $1x10^6$ – $1x10^9$ particles/ml.
Measurement precision	• Temperature. • Viscosity. • No drift. • 10-60 frames/sec.	• Temperature measured automatically or manually, and recorded in datasets. • Water data default. Alternative data added before or after data capture/processing Recoded in datasets. • Automatically monitored within software and calculated live during video processing. Reported in datasets. • Video captures at 25 frames/second.
OQ	• OQ procedure. • Vibration free. • Cleaning methods.	• Utilizes size verified standards. • Monitored within software auto-correction and recorded within datasets. • Training at install visual verification.
Data	• Standards 100 nm ± 6 nm. • D10, D50, D90. • Au 15 nm.	• Comply, ± 5nm. • Calculated live during video processing and reported in datasets.

ISO19430	NanoSight NS300
• Silica 75 nm.	• Comply. • Comply.

Example data

Example data for 10 nm Gold nanoparticles:

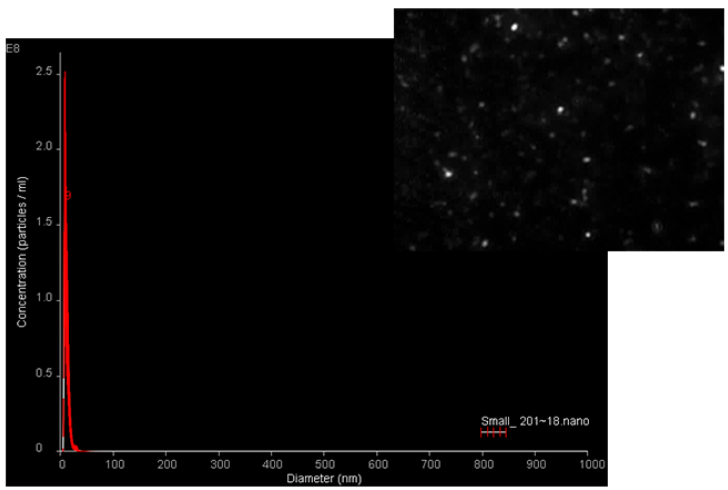


Figure 11-30: Size distribution graph and image of Gold nanoparticles as seen in the NanoSight instrument.

Summary of the key data parameters obtained:

	Mean	Standard error		Mean	Standard error
Mode	10.1	0.1	**D10**	6.0	0.1
Mean	11.3	0.2	**D50**	10.1	0.1
Valid tracks	23862	298	**D90**	14.6	0.3

Example data for 70 nm Silica nanoparticles:

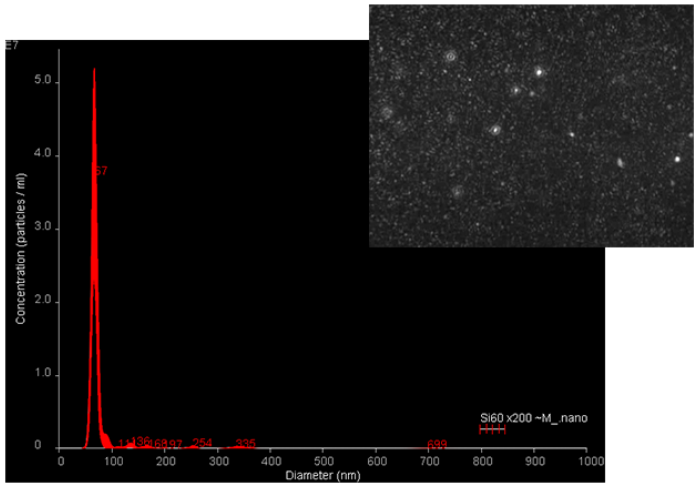


Figure 11-31: Size distribution graph and image ofSilica nanoparticles as seen in the NanoSight instrument.

Summary of the key data parameters obtained:

	Mean	Standard error		Mean	Standard error
Mode	69.4	2.9	**D10**	62.3	3.0
Mean	74.6	2.7	**D50**	68.7	2.9
Valid tracks	2329	291	**D90**	78.3	2.9

12 MAINTENANCE

This section describes the various maintenance and cleaning procedures which can be carried out on the NanoSight NS300.

Warning!
The NS300 system contains no user serviceable parts and the instrument casing should not be opened by any user. The instrument should not be modified in any way. Any modification will void the warranty and could make the device unsafe.

Warning! The NS300 must only be serviced by qualified Malvern Instruments personnel, or Malvern Instruments approved agents.

It covers the following topics:

General maintenance

Instrument casing

Clean the casing of NS300 body and laser module using a damp cloth.

Caution! Although the unit is splash-proof, do not wet or allow excess moisture or liquid to penetrate any part of the system. Do not use solvents. This can cause irreparable damage to the unit, which is not covered under warranty.

Optical elements

- Do not abrade or scratch the optical flat surfaces.
- Do not introduce particulates or contaminants onto the surface.
- Treat all optical surfaces with the same care as would be employed with equivalent surfaces on your microscope.

Note: Should the optical flat in the laser module become damaged, it can be replaced by Malvern Instruments (pricing on request).

Fluidics

Clean the sample cell and tubing as described in Low Volume Flow Cell maintenance on page 142 and O-Ring maintenance on page 147. This will help maintain best functionality and protect against cross contamination of the parts exposed to the sample during and following use. Failure to follow these procedures can result in poor performance and a decrease in lifetime of replaceable parts.

- Regularly inspect fluidic tubing and replace any that show signs of wear. Please contact Malvern Instruments for additional sets of tubing (P/N NTA4161 NS300 Complete Tubing Kit).
- When not in use, remove the top plate from the laser module. The top plate, along with any tubing, must be left clean and dry.

- When emptying the sample chamber at the end of use;
    1. Flush the final sample from the chamber by 2ml of diluent followed by 2 ml water.
    2. Slowly push 2 ml of air through the sample chamber before the top plate is removed from the laser.
- For best practice, once disassembled, thoroughly dry the surfaces exposed to sample under a gentle dry-air stream, e.g. aerosol air duster.

Low Volume Flow Cell maintenance

This section gives information on how to maintain the system and deal with problems that may arise.

Manual cleaning procedures

A manual clean is only needed if there is visible cloudiness or sample residue stuck to the optical flat or the glass window in the gasket component. The LVFC must be removed from the laser module but does not need any further disassembly for manual cleaning.

The recommended practice for manually cleaning the optical flat on the laser module and the Low Volume Flow Cell top-plate is given below:

1. Before performing a manual clean, the system should be flushed to clean sample from the tubing and fittings.
2. Once all tubing assemblies are flushed clean, empty the fluidics by loading a 1 ml syringe full of air through the system (do not exceed maximum rated flow speed of 0.05 ml per second).
3. Open the door on the NS300 instrument and rotate the red lever to the left to release the laser module. Slide out the laser module from inside the NS300 so that the LVFC can be easily accessed.

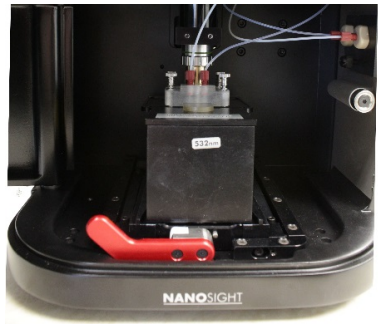


Figure 12-1: Removing the laser module from the NS300.

4. Disconnect the tubing fittings from the Low Volume Flow Cell top-plate, remove the sprung fastening bolts, and lift the flow cell gently off the laser module.

Warning!
Do not wipe or touch the chamber seal on the underside of the gasket component.

5. Wet a tissue with water, (or a solution of up to 10% ethanol if needed), and use this to wipe the optical flat on the laser module.

Warning!
Do not pour any liquid over the laser module, as this could penetrate the casing and damage the laser inside.

6. Wipe the flat gently with a soft dry tissue (e.g. Mediwipes) to remove any streaks from the optical surface and then dry with compressed air.

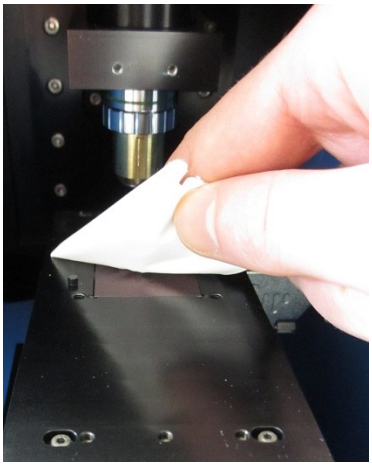


Figure 12-2: Cleaning the optical flat on the laser module.

7. Using a dripping or low-pressure source, e.g. a water bottle, rinse the manifold ports and the area inside the chamber seal on the underside of the gasket with water, (or a solution of up to 10% ethanol if needed), to remove any remaining sample particles.

8. If any sample residue is visible on the glass window surface, gently clean the underside of the glass. Use a small cotton bud dampened with **water** or a solution of up to **10% ethanol**. When cleaning the glass window inside the chamber seal area, limit any rubbing of the surrounding soft PDMS seal.

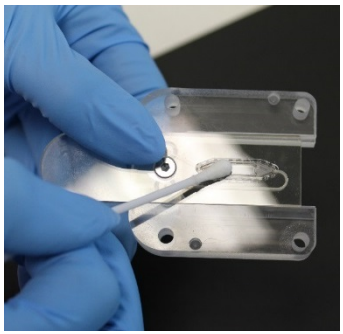


Figure 12-3: Cleaning the underside of the glass window in the gasket component.

Warning!
The glass window in the top-plate is fragile, and should be treated with care during manual cleaning. Do not apply any pressure to the top surface of the glass as this may cause the viewing window to become separated from the gasket component.

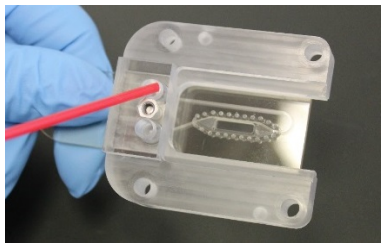
Warning!
Do not use an ethanol solution more concentrated than 10% as this may damage LVFC components.

Important! If the gasket component repeatedly leaks after a manual clean, it should be replaced (See Gasket component replacement on the facing page).

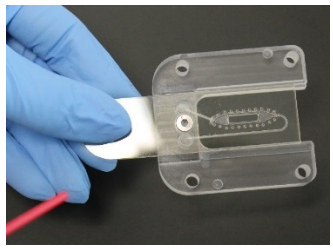
9. After manual cleaning, the low volume flow cell must be dried as described in Drying the Low Volume Flow Cell on the facing page before it can be remounted on the laser module.

Drying the Low Volume Flow Cell

1. Inject compressed air through the tubing ports on the manifold to thoroughly dry the embedded microchannel inside the gasket component.
2. Once the channel is dry, turn the flow cell assembly over and lightly dry the underside of the component and the area inside the chamber seal. Keep the nozzle of the compressed air at least 15 cm from the seal.
3. Repeat as necessary until the gasket component is completely dry.



A: Drying gasket component through manifold tubing ports



B: Drying the chamber seal on the underside of the gasket component

Figure 12-4: Drying gasket and chamber.

Warning!
Avoid touching or rubbing the seal when drying the underside of the gasket component. Keep the compressed air nozzle at least 15 cm away, to avoid the seal being damaged by high pressures.

Once the low volume flow cell is dry, check that there are no fibers or dust particles on the gasket component seal. The flow cell is then ready for reattaching to the NS300 laser module.

Gasket component replacement

When supplied with new NS300 systems, the LVFC is fully assembled and does not need to be disassembled for normal usage and cleaning. The lifetime of the gasket component will depend on the types of samples measured. It's recommended that the gasket component is used for no longer than 12 months. Replacements are available from the Malvern Instruments eStore.

1. If the flow cell needs to be disassembled, i.e. if the gasket component seal becomes worn or damaged and needs replacing, use a 2 mm Hex key (part contained in NTA4111) to undo the fixing bolt on the underside of the flow cell and disconnect the gasket component from the manifold.

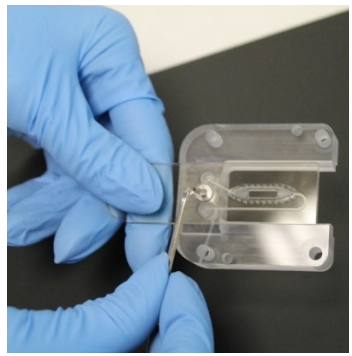


Figure 12-5: Disassembly of the flow cell.

2. Before reassembling with a new gasket component, make sure that the circular seals are bedded fully down in the ports on the manifold (Gasket component replacement above).

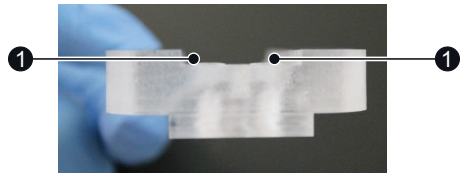


Figure 12-6: Circular seals **(1)** pressed fully down into the manifold (only just protruding from the surface).

3. If the circular seals have lifted out during disassembly, wet the seals with deionised water and push them fully into the sockets on the manifold. Make sure that they are fully seated and level. If the circular seals need replacing, two spare seals are provided with each gasket component supplied.
4. Lay the gasket component into the recess on the manifold, making sure the holes in the gasket line up with those in the manifold. Then hold in place by inserting the fixing bolt.

5. Tighten the bolt until the gasket component is held securely — the circular seal contact area can be viewed from the underside of the manifold and should be checked to confirm a visibly unbroken seal contact.

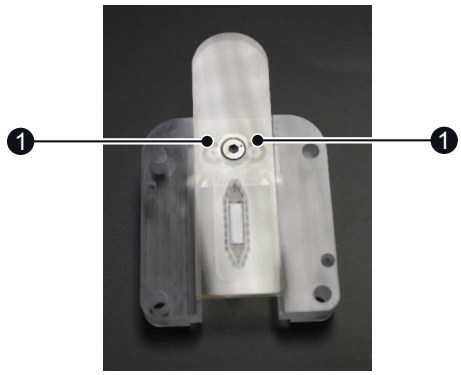


Figure 12-7: Visual of the circular seal **(1)** contact areas from underneath, confirming a good connection of the gasket component to the manifold.

6. Check that there are no fibers or dust particles on the PDMS chamber seal on the underside of the gasket component. The flow cell is then ready for reattaching to the NS300 laser module.

O-Ring maintenance

This section gives information on how to maintain the system and deal with problems that may arise.

Manual cleaning procedures

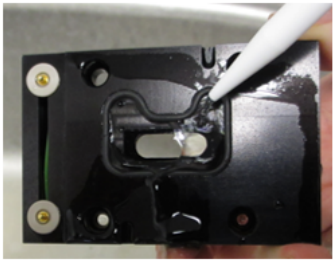
The O-Ring top-plate should always be manually cleaned between samples.

The recommended practice for manually cleaning the optical flat on the laser module and the O-Ring top-plate is given below. If necessary, other cleaning agents may be used such as dilute mild detergents, or dilute acid washes prior to a final solvent cleaning step. If in doubt about the choice of solvent and its compatibility with the top-plate, optical flat or any other part of the NanoSight device, please contact helpdesk@malvern.com for further information.

1. If any tubing is connected for the syringe pump, flush a 1 ml syringe of clean water through the system to clean sample from the tubing and fittings.
2. Once all tubing assemblies are flushed clean, empty any fluidic tubing by loading a 1 ml syringe full of air through the system (do not exceed maximum rated flow speed of 0.05 ml per second).
3. Open the door on the NS300 instrument and rotate the red lever to the left to release the laser module. Slide out the laser module from inside the NS300 so that the O-Ring top-plate can be easily accessed.
4. Unscrew the sprung fastening screws securing the top-plate and lift the top-plate gently off the laser module.

Figure 12-8: Laser module with O-Ring TopPlate unmounted from the NS300.

5. Rinse the top-plate with **water**, or **water** then **ethanol** if needed, including the O-Ring seal and inside the fluidic ports.



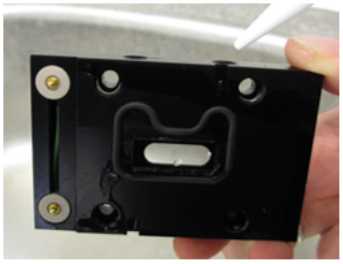


Figure 12-9: Cleaning the O-Ring top-plate.

6. Clean the inside of the window by gently wiping it with a tissue wet with **water**, or **water** then **ethanol** if needed, to remove all traces of the sample being analysed.
7. Wet a tissue with **water**, or **water** then **ethanol** if needed, and use this to wipe the optical flat on the laser module. Do not pour any liquid over the laser module, as this could penetrate the casing and damage the laser inside. Wipe the flat gently with a soft dry tissue to remove any streaks from the optical surface, then dry with compressed air.

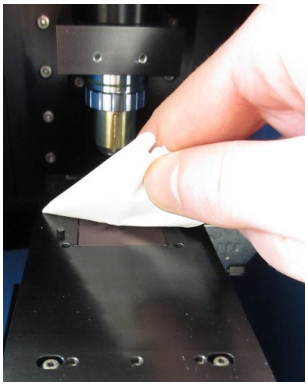


Figure 12-10: Cleaning the optical flat on the laser module.

Drying the O-Ring top-plate

The O-Ring top-plate should always be thoroughly dry before attaching it to the laser module for use. Failure to do this can result in incomplete filling of the chamber and imaging problems.

1. Use compressed air to blow any residual liquid from the inlet and outlet channels (Luer ports) through which the sample is introduced into the chamber.
2. Wipe the glass window of the top-plate gently with a soft dry tissue (e.g. Mediwipes) to remove any surface streaks, then dry with compressed air.

O-Ring replacement

The O-Rings should be replaced relatively regularly depending on the frequency of use, i.e. monthly for high usage instruments, every 3 to 6 months for lower usage instruments. O-Ring packs are available on the Malvern Instruments eStore.

13 IMPORTANT INFORMATION

The following topics are covered:

NanoSight NS300 specification

NS300 instrument housing

Item	Specification
Size	400 mm x 400 mm x 250 mm
Weight	~12 kg
Operating temperature	10 °C to 40 °C
Humidity	Up to 80% rH at 31 °C then decreasing linearly to 50% at 40 °C
Casing material	Plastic, aluminium
Voltage	100-240 V rms +/- 10%
Frequency range	50 – 60 Hz
Max power	60 VA

NS300 laser module

Item	Specification
Size	140 mm x 74 mm x 68 mm
Weight	650 g
Temperature control range	from 5 °C below ambient to 50 °C
Temperature control accuracy	+/-1 °C
Time to temperature	3 minutes (to indication of within 1 °C)
Humidity	5-95% non-condensing
Casing material	Anodised aluminium alloy
Top-plate materials	**Low Volume Flow Cell**: PMMA, silicone, glass, PDMS, SU8 epoxy, Norland Optical Adhesive 61 **O-Ring top-plate**: Anodised aluminium alloy, glass and viton rubber
Wetted Parts	PEEK connectors, Delrin connectors, PTFE tubing **Low Volume Flow Cell**: PMMA, silicone, glass, PDMS, SU8 epoxy, Norland Optical Adhesive 61 **O-Ring top-plate**: Anodised aluminium alloy, glass, viton rubber

Laser classification

Item	Specification
Embedded laser	Red 642 nm CW, max power < 50 mW Green 532 nm CW, max power < 60 mW Blue 488 nm CW, max power < 55 mW Violet 405 nm CW, max power < 70 mW

Chemical compatibility

Although the NanoSight NS300 has been manufactured from materials considered to give the widest protection from chemical attack, it is important to check the chemical compatibility of any sample with any material it may come into contact with in the system.

Warning!
It is advisable that the chemical compatibility is checked against the materials identified in this manual before inserting a sample. It is also recommended that a test is performed on the material with the sample before more permanent usage is undertaken.

Any cleaning and maintenance procedures necessary are described in Maintenance on page 139.

Returning equipment

If, for any reason, you experience problems with your instrument, contact Malvern Instruments (helpdesk@malvern.com).

In the unlikely event you experience a problem with the NS300 system that requires returning the unit for repair, please contact Malvern Instruments for instructions and documentation:

The following information will need to be supplied:

- Sender's name and address;
- Sender's contact telephone number and email address;
- Complete list of equipment being returned including serial numbers;
- A detailed description of the problem or reason why the equipment is being returned;

- Declaration that, if the instrument has been used with biologically or chemically hazardous sample material, all equipment has been fully decontaminated before return.

On receipt of this information Malvern Instruments will provide a Return Merchandise Authorization (RMA) number. An RMA number must be obtained from Malvern Instruments before returning any equipment, and should be clearly displayed on the return shipment and included on all subsequent correspondence.

If the original shipping case is not available, shipping cases will be supplied by Malvern Instruments as required to ensure safe return of the system.

Warranty

Malvern Instruments warrants that the NS300 system as supplied with its accessories is free from defects in materials and workmanship for a period of one year from shipping to the customer. During this warranty period, Malvern Instruments will, at its discretion, repair or replace defective products.

Any liability under this warranty extends only to the replacement value of the equipment.

This warranty is void if:

- The NS300 or its accessories have been partly or completely disassembled, modified or repaired by persons not authorized by Malvern Instruments
- The instrument or instrument system is installed or operated other than in accordance with these operating instructions.

No other warranty is expressed or implied. Malvern Instruments is not liable for consequential damages except as limited by English law.

Regulatory information

This section provides details of all applicable regulatory information.

EU Declaration of Conformity

The CE badge on this product signifies conformance to the relevant European Directives - consult the Declaration of Conformity certificate for the product for more information.

VCCI acceptance (Japan only)

The Voluntary Control Council for Interference (VCCI) mark on this product signifies compliance to Japanese EMC regulations as specified by VCCI.

この装置は、クラスA情報技術装置です。この装置を家庭環境で使用すると電波妨害を引き起こすことがあります。この場合には使用者が適切な対策を講ずるよう要求されることがあります。 VCCI-A

Translation:

This is a Class A product based on the standard of the Voluntary Control Council for Interference by Information Technology Equipment (VCCI). If this equipment is used in a domestic environment, radio interference may occur, in which case the user may be required to take corrective actions.

Canadian Regulatory Information (Canada only)

This digital apparatus does not exceed the Class A limits for radio noise emissions from digital apparatus set out in the Radio Interference Regulations of the Canadian Department of Communications.

Note that Canadian Department of Communications (DOC) regulations provide, that changes or modifications not expressly approved by Malvern Instruments Limited could void your authority to operate this equipment.

This Class A digital apparatus complies with Canadian ICES-003.

Cet appareil numérique de la classe A est conforme à la norme NMB-003 du Canada.

FCC Notice (US only)

The Federal Communications Commission (FCC) mark on this product signifies conformance to FCC regulations relating to Radio Frequency Devices. These have been satisfied by testing the product against, and being found to be compliant with:

FCC CFR 47 Part 15:October 2011.Class A digital device.

The device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions:

1. This device may not cause harmful interference, and
2. This device must accept any interference received, including interference that may cause undesired operation.

Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

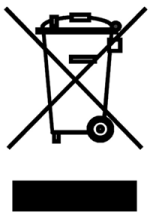
Note: Changes or modifications not expressly approved by Malvern Instruments Limited could void the user's authority to operate the equipment.

Disposal of Electrical & Electronic Equipment

When the need arises to dispose of the system, this should be done in a responsible manner. Follow these guidelines:

- Refer to local regulations on disposal of equipment; in Europe refer to the information below.
- Seek advice from the local Malvern Instruments representative for details.
- Decontaminate the instrument if hazardous materials have been used in it.

The following is applicable in the European Union and other European countries with separate collection systems.



This symbol on the product or on its packaging indicates that when the last user wishes to discard this product it must not be treated as general waste. Instead it shall be handed over to the appropriate facility for the recovery and recycling of electrical and electronic equipment.

By not discarding this product along with other household-type waste, the volume of waste sent to incinerators or landfills will be reduced and natural resources will be conserved.

For more detailed information about recycling of this product, please contact your local city office, your waste disposal service, or your Malvern Instruments representative.

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