

NANOSIGHT NS300 OPERATING MANUAL

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NANOSIGHT NS300 OPERATING MANUAL

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1.1 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 characterisation.

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 focussed laser beam. This allows extremely small particles (down to 10 nm) 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 analyse 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.

The installed tubing and the viewing chamber surfaces 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 (helpdesk@Malvern.com) for further information.

This manual is designed to provide an introduction 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, NTA Software Operation Manual, Quick-start Guides and User Training Videos. These can be found on our website (www.Malvern.com) or by contacting Malvern.

For technical assistance, please contact Malvern

Malvern Technical Support: Tel: + [44] (0)1684-892456 or Email: helpdesk@Malvern.com

1.2 Explanation of Warning Symbols



Warning: read instructions to understand possible hazard prior to use.



Electric shock hazard

1.3 Important Notes



1.3.1 Safety

1.3.1.1 Laser Safety

- The NanoSight NS300 device is classified (to BS EN 60825-1 (2001)) as a Class 1 laser device. The instrument contains a Class 3B laser which must never be removed from the laser module housing.
- The laser module should not be plugged into the main body of the NS300 without the top plate being secured in position with the screws supplied.
- The laser module should always be removed from the main body of the NS300 (cutting power to the laser) prior to removing the top-plate screws.

1.3.1.2 Electrical Safety

- Fuses must be only replaced with a fuse of the type and rating - F5AH250V Ø5mm x 20mm long.
- Use only 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:

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.

1.3.1.3 General Safety

- The instrument must not be used in hazardous areas.
- The instrument is for use in moderate climates only. Never use the equipment in damp or wet conditions.
- Avoid excessive heat, humidity, dust and vibration.
- **Do not** place liquid filled containers on the equipment.
- **Do not** use where the equipment may be subjected to dripping or splashing liquids.
- If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.
- 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 to confirm suitability.
- When using bio or chemically hazardous sample material, it is the responsibility of the operator to determine the requisite protection for each application.
- The NS300 system contains no user serviceable part. It should not be modified in any way. Any modification will void the warranty and could make the device unsafe.
- Use of controls, adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

- The laser module may become hot when operating the temperature control. Caution should be taken when handling the device.
- The temperature control should always be turned off when the instrument is unattended.



1.3.2 Servicing

- The NS300 must only be serviced by qualified Malvern personnel, or Malvern approved agents.
- The NS300 contains no user-serviceable parts. The instrument casing should not be opened by any user.



Removing the 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.



1.3.3 Maintenance

- 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.
- The casing of the NS300 body and the laser module should be kept clean with the use of a damp cloth. **Do not** wet or allow excess moisture to penetrate any part of the system. **Do not** use solvents.
- To maintain best functionality and to protect against cross contamination during and following use, the sample flow cell and tubing should be cleaned as described in this manual.
- Regularly inspect fluidic tubing and replace any that show signs of wear. Please contact Malvern for additional sets of tubing (P/N NTA4038 NS300 Tubing Kit).

1.3.4 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.

During use and when cleaning it is important to ensure that liquid does not enter the inside of the laser module. Use only a damp tissue for cleaning, never expose the laser module to excess fluids. During sample loading and instrument operation, ensure that the sample chamber is properly sealed and that there is no evidence of any leaking fluids.

Between uses of the NanoSight NS300, or for longer term storage, the unit must be cleaned and dried as described in this manual.

1.3.5 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 gasket component
- Instruction manuals
- 2mm Allen/Hex-key with flow-cell nut

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 after delivery. The packing case can be kept if required, although an additional charge will be made.

Notify Malvern (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.

1.3.6 Installation and Relocation

Initial installation will be carried out by qualified Malvern personnel.

In the event that the instrument needs to be relocated the following instructions must be adhered to:

Always disconnect the equipment from the mains and ancillary units before moving.

The instrument should be located in an area of good ventilation and with sufficient space for safe and efficient operation and maintenance.

The supplied PC should be sited to avoid dangers from spillage and splashing.

The equipment must be connected to an earthed power supply with a voltage corresponding to that on the power adapter.

Ensure that the mains plug is easily accessible 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.

1.3.7 Returning Equipment

If, for any reason, you experience problems with your instrument, contact Malvern (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 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 bio or chemically hazardous sample material, all equipment has been fully decontaminated before return.

On receipt of this information Malvern will provide a Return Merchandise Authorization (RMA) number. An RMA number must be obtained from Malvern 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 as required to ensure safe return of the system.

1.3.8 Warranty

Malvern 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 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 authorised by Malvern.
- 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 is not liable for consequential damages except as limited by English law.

2. NanoSight NS300 Hardware

2.1 NS300 Technical Specifications

2.1.1 NS300 Instrument Housing

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

2.1.2 NS300 Laser Module

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:	Flow-cell top-plate: PMMA, glass, PDMS SU8 epoxy, Norland Optical Adhesive 61 O-ring top-plate: Anodised aluminium alloy, glass and viton rubber
Wetted Parts:	PTFE tubing Flow-cell top-plate: PMMA, silicone, glass, PDMS, PEEK connectors SU8 epoxy, Norland Optical Adhesive 61 O-ring top-plate: Anodised aluminium alloy, glass, Viton rubber and Delrin connectors

2.1.3 Laser Classification

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

2.2 NS300 Operation

2.2.1 Instrument Set-up

1) The NS300 instrument communicates with the controlling PC via a USB connection. Plug the USB cable into the USB port on the right side of the NS300 instrument casing (labelled USB-1) and connect it to one of the USB ports on the supplied PC.

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.

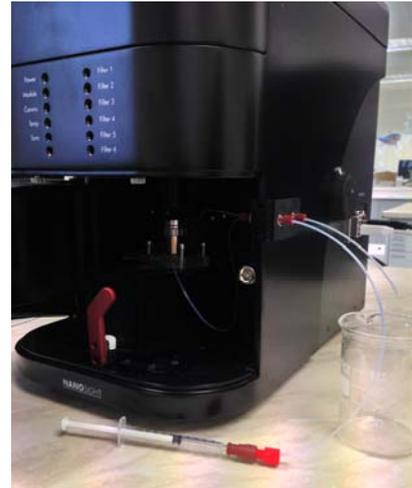
3) On the right-hand side of the NS300 casing is a tubing port holder. The tubing can be used to introduce sample when using the flow-cell top-plate or a syringe pump accessory. The holder can be slid in and out of the case for easy access to the fluidic connectors. The tubing port holder is held in place with a black nylon screw on the inside of the casing, accessed by opening the front hatch.

4) The left-hand port on the tubing holder is the inlet port, used to introduce sample into the cell. The right-hand tubing port is the waste port:

- i) For injection from a disposable syringe, connect the tubing line with luer attachment to the left-hand port.
- ii) For use with the syringe pump accessory, connect the sample inlet tubing from the syringe pump to the left-hand port.
- iii) Connect a length of waste tubing to the right-hand port and put the other end in a suitable waste receptacle.

5) Set up and turn on the PC.

6) Connect the mains lead to the NS300 instrument and turn the instrument on at the power switch, located on the left side of the case.



2.2.2 Daily Use

The NS300 should always be left clean and dry whenever the system is not in use. The NTA software should be shut down and the NS300 power switch turned off.

After use, the system should be flushed through with clean diluent to remove all traces of sample from the tubing and optical surfaces. If using diluent with a high concentration of dissolved solids (i.e saline solution), flush clean water through the system after use.

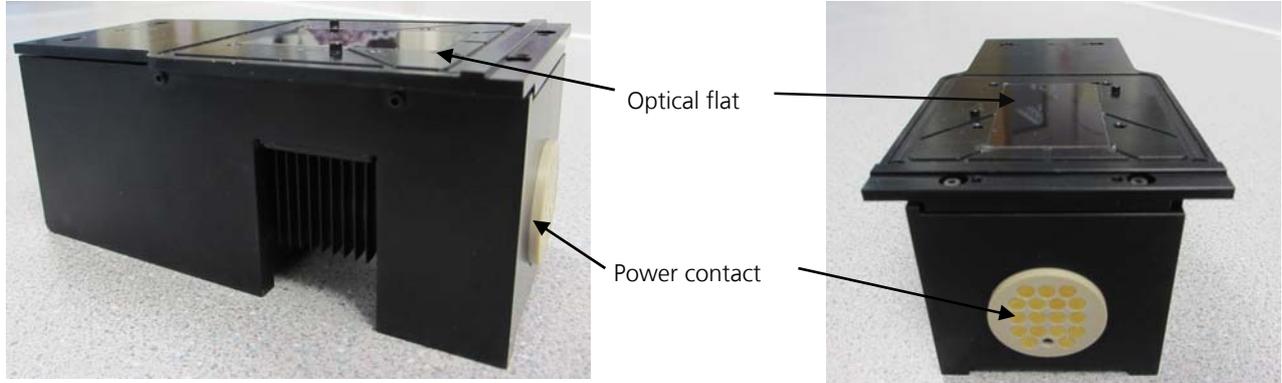
After the fluidic system is clean, empty the top-plate by extracting all liquid with a syringe, and then manually disassemble, clean and dry the sample chamber as described in Section 2.2.4.



Between uses of the instrument, the sprung fastening screws holding the top-plate down should be fully loosened, so that the optical flat is not held under any pressure. Failure to loosen the top-plate screws between uses can result in damage to the optical flat.

2.2.3 Laser Module Configuration

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

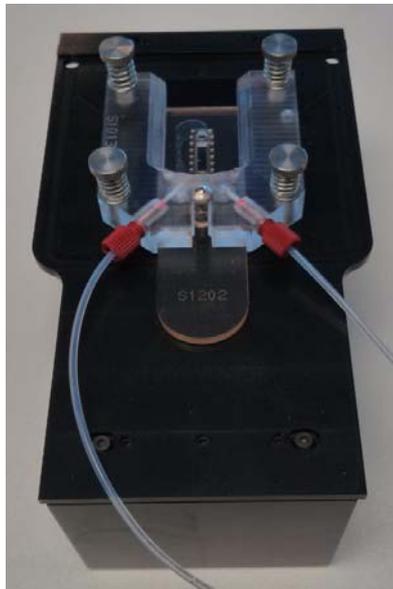


The laser module can be supplied with a choice of two different top-plates, as shown below:

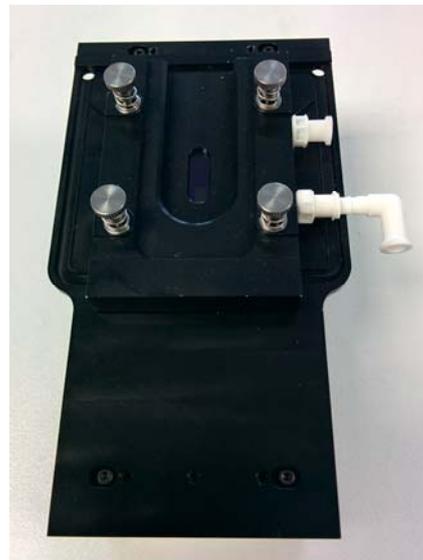
The **flow-cell top-plate**, can be cleaned by flushing wash fluid through the chamber, avoiding the need to manually disassemble and clean the chamber after every sample.

The **O-ring top-plate** requires disassembly and manual cleaning after each sample, but provides greater chemical compatibility for non-aqueous solvents, or samples which are more viscous or contain larger particles, which may block the flow-cell.

The sample chamber configuration and the instructions for cleaning will vary according to which design of top-plate is being used. Please refer to Section 2.2.3.1 and 2.2.4.1 for the flow-cell top-plate and Section 2.2.3.2 and 2.2.4.2 for the O-ring top-plate, as appropriate.



Flow-cell top plate

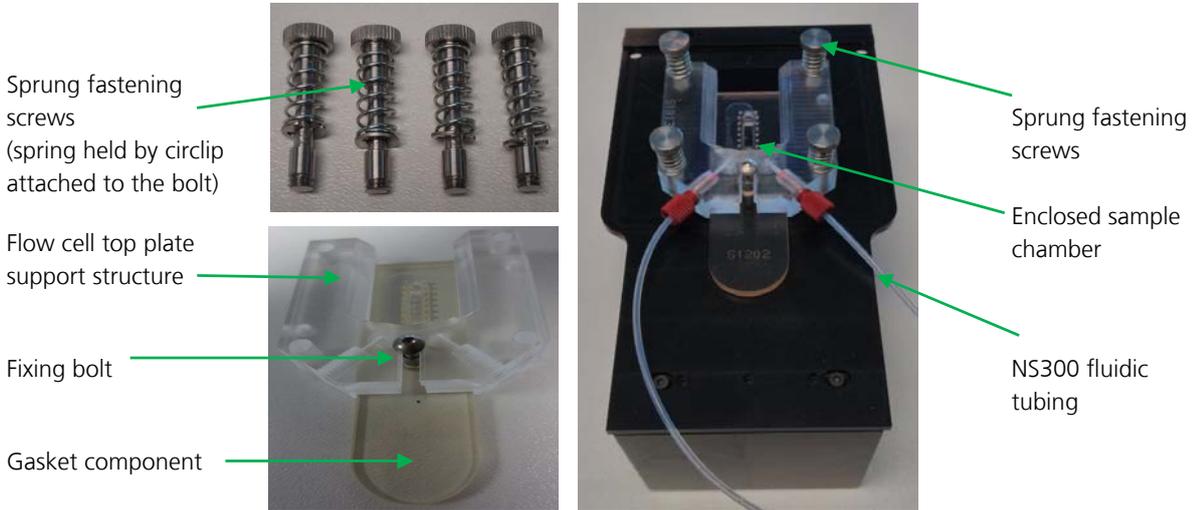


O-ring top-plate

2.2.3.1 Flow-cell top-plate

For the flow-cell top-plate, the NS300 fluidic tubing is connected to ports on the top-plate, which is mounted onto the optical flat on the laser module to form a sample chamber.

Flow cell Top-Plate



The sample chamber is formed by two parts which are assembled and then attached on top of the optical flat on the laser module.

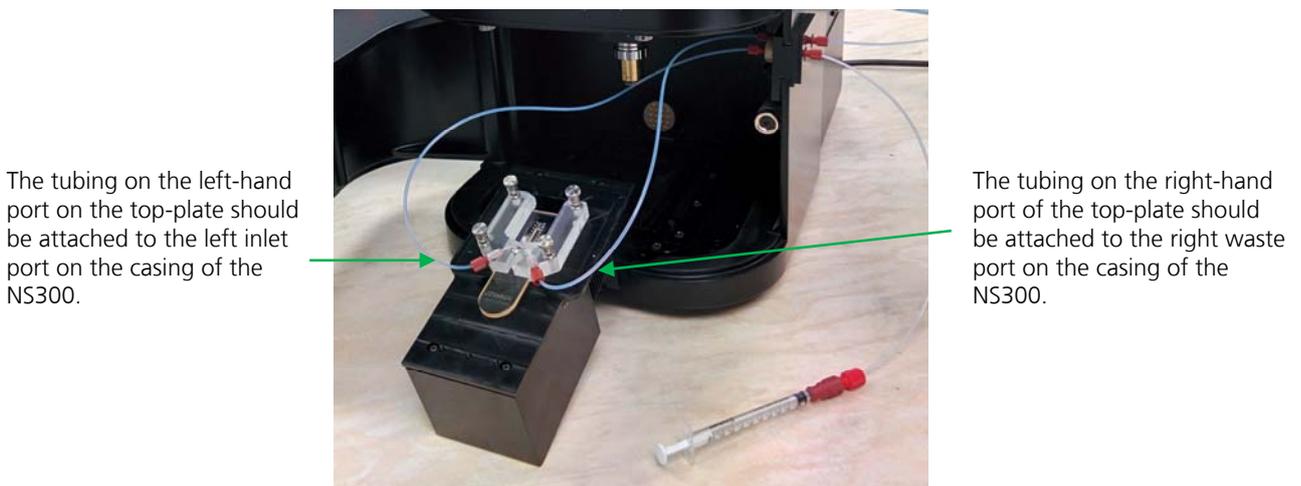
A gasket component, with an embedded microchannel, is connected onto the flow cell top-plate support structure using a fixing bolt, tightened using an Allen / Hex key.

Two circular seals on the underside of the top-plate support structure seal the gasket component onto the support structure

The combined top-plate is then sealed onto the laser module using four sprung fastening screws

The gasket component seal is compressed onto the optical flat to make an enclosed sample chamber.

The top-plate support structure circular seals (Part No NTA4012 and the gasket component (Part No. NTA4057) can be replaced if the seals become worn or damaged.



For instructions on how to clean and assemble the flow-cell top plate see Section 2.2.4.1

2.2.3.2 O-ring top-plate

The O-ring top-plate contains an embedded O-ring seal which is mounted onto the laser optical flat to form the sample chamber. The top-plate is sealed onto the laser module using four sprung fastening screws. The O-ring top-plate can be setup in two different ways, according to how samples are to be loaded into the system.

2.2.3.2.1 Direct syringe connection

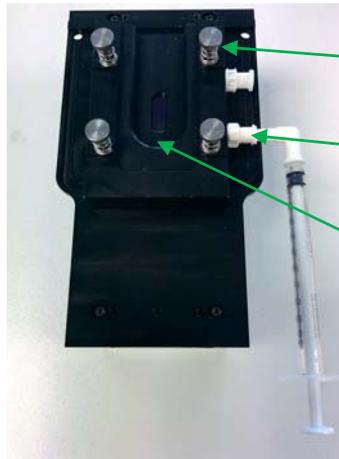
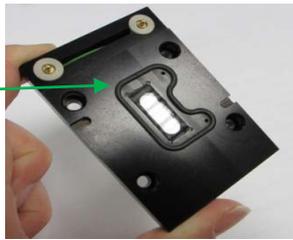
Samples can be loaded into the chamber using a disposable syringe, directly connected to a luer port on the top-plate. A push-fit elbow luer connector is provided so that the laser module fits into the NS300 with the syringe remaining in place.

O-ring Top-Plate

Sprung fastening screws
(spring held by circlip attached to the bolt)



O-ring seal



Sprung fastening screws

Push-fit luer connectors

Enclosed sample chamber



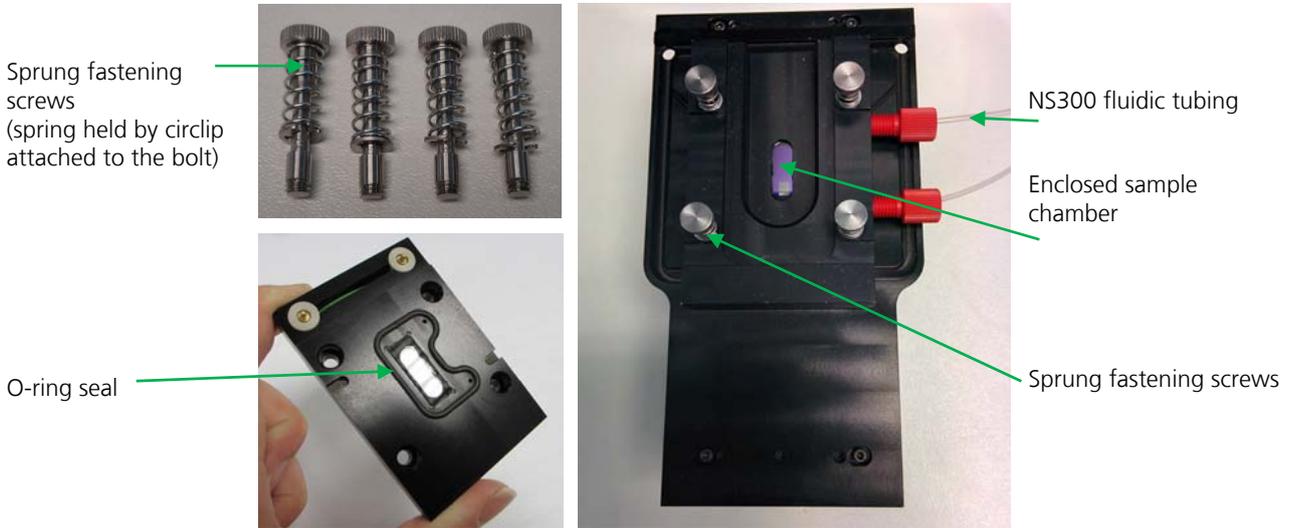
Insert the disposable syringe into the front luer port for sample loading (see Section 2.2.5).

Once the sample has been loaded, the laser module should be placed into the NS300, keeping the syringe connected

For instructions on how to assemble and clean the O-ring top plate see Section 2.2.4.2

2.2.3.2.2 Fluidic tubing connection

A sample can also be loaded into the O-ring top-plate through the NS300 fluidic tubing when using a syringe pump accessory. In this case, the luer ports should be removed and the tubing from the tubing port holder attached to the ports on the top-plate, using the connectors provided. The syringe pump can then be connected externally to the tubing port holder on the outside of the NS300 casing.



The outlet tubing (wider bore) should be attached to the back port of the top-plate and to the right waste port on the casing of the NS300.

The inlet tubing (narrower bore) should be attached to the front port of the top-plate and to the left inlet port on the casing of the NS300.

For instructions on how to assemble and clean the O-ring top plate see Section 2.2.4.2



Important!

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

2.2.4 Assembling and Cleaning the Laser Module



Whilst the unit is splash-proof, under no circumstances must excess liquid be allowed to enter the housing of the laser module at any time. This will cause irreparable damage to the laser mounted within the unit.



Between uses of the instrument, the sprung fastening screws holding the top-plate down should be fully loosened, so that the optical flat is not held under any pressure. Failure to loosen the top-plate screws between uses can result in damage to the optical flat.

It is important to take care not to abrade or scratch the optical flat surface, or 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. Should the optical flat in the laser module become damaged, it can be replaced by Malvern (pricing on request).

The following paragraphs provide recommended guidelines for cleaning the laser module. If necessary, but dependent on the top-plate in use, other cleaning agents may be employed such as dilute mild detergents, or even 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.

2.2.4.1 Flow-cell top-plate

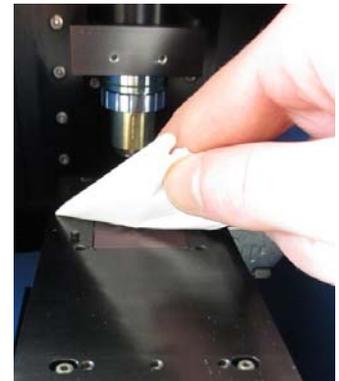
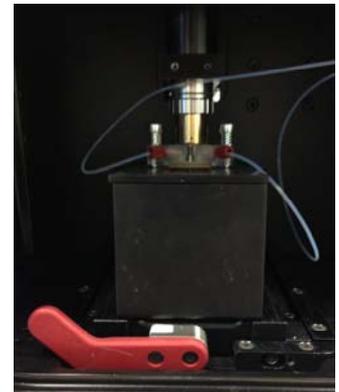
The flow-cell top plate has been designed to not need manual cleaning between every sample. Between samples, push water or diluent through the sample chamber, to remove any particles present.

A manual clean is only necessary if there is visible cloudiness or sample residue stuck to the optical flat or to the glass window in the flow-cell top-plate.



The glass window in the top-plate is fragile, and should be treated with care during manual cleaning.

- 1) Before manual cleaning, flush water or cleaning solution through the sample chamber.
- 2) Extract the liquid in the sample chamber using the inlet syringe, making sure that no liquid is sucked back up from the waste bottle.
- 3) The cell can then be disassembled, cleaned and reassembled as follows.
 - i) 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 sample chamber can be easily accessed. There is no need to remove the tubing for the purposes of cleaning.
 - ii) Unscrew the metal screws and springs securing the top plate and lift the top plate gently off the laser module. **Important:** Avoid touching the channel seal on the bottom of the gasket component.
 - iii) 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 (e.g. Mediwipes) to remove any streaks from the optical surface and then dry with compressed air.



- iv) Avoiding touching the chamber seal on the bottom of the gasket component, undo the fixing bolt with the 2 mm Allen/Hex-key until it is loose enough to slide out the gasket component. Avoid completely unscrewing the bolt



- v) Using a dripping or low-pressure source, e.g. a water bottle, to avoid causing any damage, rinse the circular seals on the underside of the top-plate support with **water**, or **water** then an **up to 10% ethanol-water** solution if needed, to remove any sample particles.

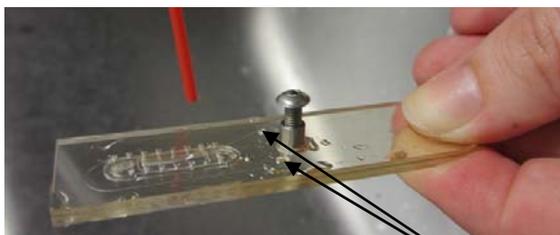
- vi) Rinse the seal and the glass on the underside of the gasket component with a low-pressure source of **water**, or **water** then an **up to 10% ethanol-water** solution. Take care to limit any rubbing of the channel seal surrounding the glass window,

If any sample residue is visible on the gasket viewing window, the **underside** of the glass can be wiped from the bottom of the gasket component with a small cotton bud



Do not apply any pressure to the top surface of the glass, as this may cause the viewing window to be pushed out and separated from the gasket component

- vii) Direct compressed air through the embedded micro-channel via the holes on the top of the gasket component (i.e. from the side without the attached seal) to dry the channel.



a) Dry channel thoroughly through the holes on the top of the gasket component

b) Dry underside of the gasket component, keeping the air duster nozzle away from the seal

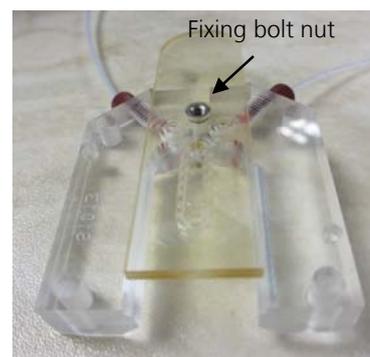
- viii) Keeping the nozzle of the compressed air can at least 5 cm from the seal, lightly dry the underside of the component and the glass surface section using compressed air.



Important: Avoid touching or rubbing the seal when drying the underside. Keep the compressed air nozzle at least 5cm away from the seal, to avoid high pressure damaging it

Important: If the gasket component repeatedly leaks after a manual clean, it should be replaced (Part No. NTA4027).

ix) When all parts of the top plate are clean and dry, reattach the gasket component to the top-plate support using the fixing bolt. Use an Allen/Hex-key to tighten the bolt, just until the bolt nut on the underside is fully recessed into the gasket component with no protrusion. You should not be able to feel the nut if you run your finger over it.



x) Place the top-plate back onto the optical flat on the laser module. Position the top plate using the locating pins on the laser module and the corresponding holes in the top plate.

xi) Place the four metal screws and springs into the screw holes. For each screw in turn, gently push down each screw head, compressing the spring, and twist clockwise until the just screw locks in place in the screw thread underneath. Tighten the four screws evenly in **diagonal pairs** until all are finger-tight. **Do not** use Allen/Hex-keys or over-tighten the screws as this may cause damage to the screw threads or damage the optical flat.



xii) Ensure there are no kinks in the waste outlet capillary tubing - this can lead to an increase in pressure in the chamber.

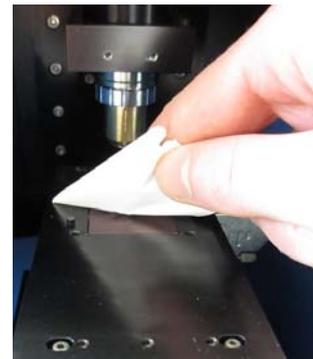
2.2.4.2 O-ring top-plate

For the O-ring top-plate, a manual clean is required between samples to ensure there is no carry-over.

- i. If the top-plate is connected to the NS300 fluidic tubing and a syringe pump, load a syringe of water or cleaning solution through the sample chamber and to waste, to remove any sample from the tubing lines and the top-plate connectors.
- ii. Extract the liquid in the sample chamber using the inlet syringe, ensuring no liquid is sucked back up from the waste bottle if the syringe pump tubing is connected
- iii. 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 the sample chamber can be easily accessed. There is no need to remove any tubing for the purposes of cleaning.
- iv. Unscrew the sprung fastening screws securing the top plate and lift the top plate gently off the laser module.
- v. Rinse the top-plate with **water**, or **water** then **ethanol** if needed, including the O-ring seal and inside the fluidic ports.



- vi. 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.
- vii. 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.
- viii. 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.
- ix. When all optical surfaces are clean and dry, place the top-plate back onto the laser module. Position the top plate using the locating pins on the laser module and the corresponding holes in the top plate.
- x. Place the four sprung fastening screws into the screw holes. For each screw in turn, gently push down each screw head, compressing the spring, and twist clockwise until the screw locks in place in the screw thread underneath. Tighten the four screws evenly in **diagonal pairs** until all are finger-tight. **Do not** over-tighten the screws as this may cause damage to the screw threads or damage the optical flat.
- xi. If using the waste outlet tubing ensure there are no kinks - this can lead to an increase in pressure in the chamber.



2.2.5 Loading a Sample

Once the top-plate is in place and any tubing connected, the system is ready to load a sample. We recommend that before proceeding with analysis, a sample of any buffer or diluent is checked to confirm that it doesn't contain any contaminating nanoparticles.

For both top-plates, the first sample should be loaded with the laser module outside of the instrument. As the sample is loaded, the presence of any air pockets or bubbles can be detected and removed.

Sample can be loaded into the chamber with a syringe connected to the inlet tubing for the flow-cell top-plate, or directly connected to the luer fitting on the O-ring top-plate. Samples can also be loaded using a syringe pump accessory connected to the inlet tubing.



Important!  Occasionally a bubble may be present in the chamber and in the path of the laser beam. This might cause some degree of specular reflection of the laser beam off the bubble surface. This will degrade image quality and should be removed before analysis. Certain samples may generate bubbles through out-gassing of dissolved gases over extended periods. In both cases, the image collected by the camera supplied will show a high intensity region (or may be completely saturated) indicative of the presence of such bubbles. These can sometimes be removed by carefully moving fresh sample through the chamber, allowing air bubbles to disperse. If samples repeatedly show evidence of bubble formation, it is advisable to de-gas the sample before analysis.

2.2.5.1 Using the flow-cell top-plate

- i. Fill a syringe with the appropriate sample. The top-plates are designed for use with a 1 ml syringe. Large bore size syringes (>2.5ml) can inadvertently produce high pressures which may damage the top-plate, and must not be used.
- ii. Remove any air bubbles from the syringe
- iii. Place the syringe into the luer port on the end of the inlet tubing, ensuring liquid-to-liquid contact if the system has already been loaded with buffer solution.
- iv. Introduce the sample slowly into the chamber. The top-plate should not be loaded at speeds exceeding 1ml in 10 seconds.

Note: When loading the sample cell from dry, liquid should be introduced as slowly as possible. This avoids generating pressures which might result in the sample bypassing the seals in the sample chamber or damaging the top-plate. Loading the sample slowly also limits the introduction of bubbles.

2.2.5.2 Using the O-ring top-plate



Important!

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

- i. Fill a syringe with the appropriate sample. The top-plates are designed for use with a 1 ml syringe. Large bore size syringes (>2.5ml) can inadvertently produce high pressures which may damage the top-plate, and must not be used.
- ii. Remove any air bubbles from the syringe
- iii. 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.
- v. Insert the syringe into the front inlet port and introduce the sample slowly into the chamber. The top-plate should not be loaded at speeds exceeding 1ml in 10 seconds.

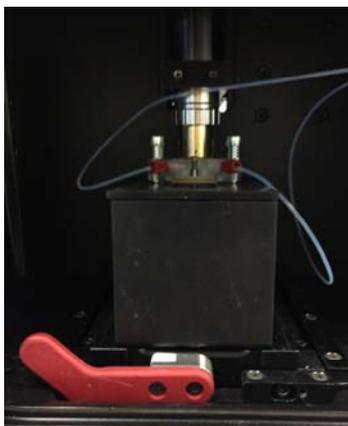
Note: The O-ring top-plate must always be dried between each sample or buffer load, and is not suitable for liquid changeover without disassembly. When loading the chamber from dry, liquid should be introduced as slowly as possible. This avoids generating pressures which might result in the sample bypassing the seals in the sample chamber or damaging the top-plate. Loading the sample slowly also limits the introduction of bubbles.

2.2.5.3 Using the syringe-pump

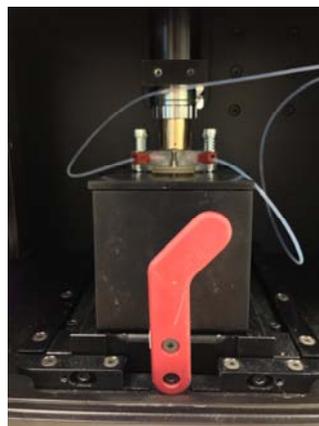
- i. Load a syringe with sample and connect it to the luer fitting on the end of the inlet tubing.
- ii. Manually load sample into the chamber, following the loading procedure in Section 2.2.5.1 for the flow-cell. For the O-ring top-plate, the procedures described in Section 2.2.5.2 apply, with the exception that the sample will be loaded through the inlet tubing rather than via the direct luer fitting on the top-plate.
- iii. Once liquid is seen emerging in the waste tubing, place the syringe into the syringe pump holder as described in the NanoSight Syringe Pump Operating Manual
- iv. Operate the syringe pump as described in the manual.

2.2.6 Mounting the Laser Module

Once the sample is loaded without any air pockets or bubbles, the laser module should be mounted within the main instrument housing. Rotate the red lever inside the NS300 to the left to allow the laser module to be mounted. Place the laser module into the slide and gently push forward until it mates with the power connector inside. The red lever inside should be rotated until vertical to lock the laser module in place, ensuring reproducible positioning. The access door should then be closed.



a) Laser module mounting / removal



b) Laser module locked in position

2.2.7 Loading a New Sample

2.2.7.1 Flow-cell top plate

The flow-cell top-plate has been optimised so that the sample chamber can be cleaned in situ between samples.

- i. Rinse the chamber by loading clean water or a suitable cleaning solution. The top-plate should not be loaded at speeds exceeding 1ml in 10 seconds. Please contact Malvern for advice on additional customised cleaning procedures.
- ii. Confirm the cleanliness of the chamber by checking for any particles present in the NTA software image, before loading the next sample as described in Section 2.2.5.1.

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 Section 2.2.4.1.

After samples which may adhere to the optical glass surfaces or between samples which differ significantly in terms of solvent, particle type or particle loading, it is advisable to disassemble the top plate and rinse and dry thoroughly the optical element and top plate, to ensure there is no carry-over.

2.2.7.2 O-Ring top plate

When using the O-ring top-plate, the chamber configuration is not optimised for effective flush through cleaning. The top-plate should always be disassembled and manually cleaned between samples, as described in Section 2.2.4.2, to avoid any sample carry-over.

Once the sample chamber and any connected tubing have been cleaned, load the next sample as described in Section 2.2.5.2.

3. NanoSight NS300 Control Software

3.1 NTA Software Startup

Run the NTA Software.

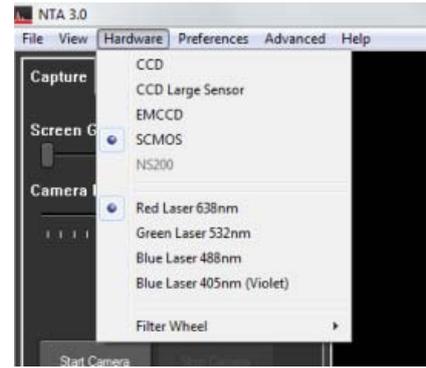


The NanoSight NTA software comes pre-installed on Malvern-supplied computers and can be run either from the desktop icon or via the start menu (under **All Programs > NanoSight**). On opening the NTA Program, the following screen appears:

3.2 Camera Setup

Run the NTA software and select CCD or 'Scientific CMOS Trigger' from the **Hardware** menu, as appropriate for your system. Note that on NS300 CMOS systems, a trigger cable will be connected internally, and the trigger mode should always be used. When using the trigger mode it is important to ensure that the trigger cable is correctly connected. Laser pulsing should be visible by eye on camera level 6. Running in trigger mode without the triggering cable attached will cause errors in the measured results.

Click on Capture in the top left corner of the screen to enter capture mode, the camera will take a few seconds to initialize.



Adjust the camera level so all particles are visible. When the image starts to show colored pixels, the light from image is saturating the camera and the camera level should be reduced. The color of the saturated pixels will reflect the color of the laser in use. With high sensitivity camera systems, it is also possible to adjust the brightness of the particles by pixel thresholding see 3.4.

The image will still be acceptable with approximately 10% particles displaying coloured pixels.



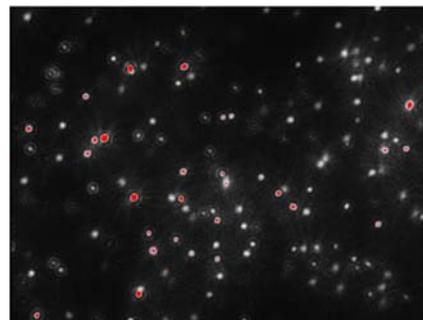
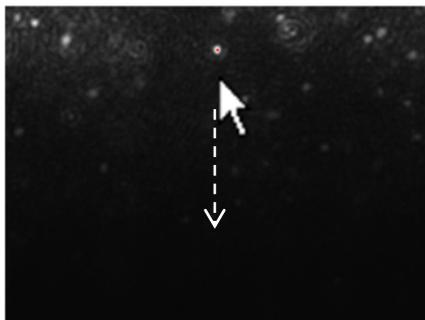
Operational Care

High-sensitivity cameras should not be left with the camera level at a high value for a prolonged period (i.e. when the camera is not being used) to restrict current flow and thus extend camera lifetime.

3.3 Adjusting the NS300 to obtain a video

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 optimise the image. If the beam appears not to be 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.

- i. With a view of the particles on the NTA screen in capture mode, left-click the mouse button on the video image.
- ii. Hold the left mouse button down and drag the image up or down until the laser beam is illuminating particles over the whole field of view



The focus should always be optimised to give a clear sharp image of the particles. The focus can be adjusted either by using the slider control in the NTA software, or manually using the focus dial on the right hand side of the instrument casing. To adjust the focus in the software, use the focus slider bar in the Hardware Control Panel. The focus can also be adjusted using the mouse wheel.

Dragging the slider on the left gives fine adjustments, while using the slider on the right gives coarser adjustments.

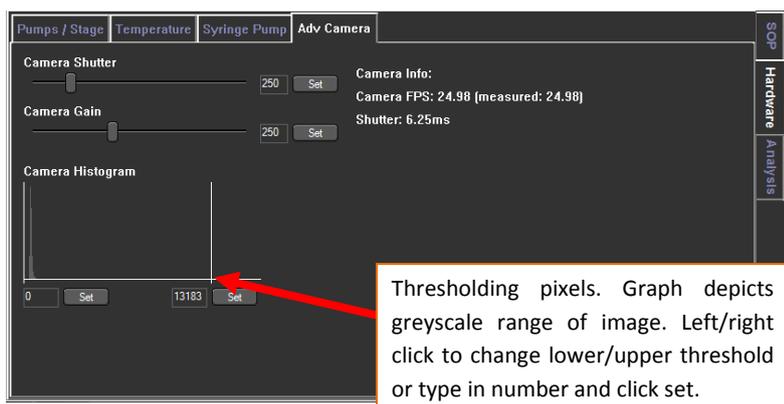
If you are unable to locate the illuminated particles, or obtain a clearly focussed image with your instrument, please contact Malvern on + [44] (0)1684-892456, or email us at helpdesk@Malvern.com.



3.4 Thresholding Pixels (High-sensitivity systems only)

If the pre-defined camera levels are not suitable for a particular sample, e.g. if a sample is very dim relative to the image background, the histogram displayed underneath the capture screen can be used to optimise 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 being detected by the camera. The range of pixel intensities displayed is user-controlled and is set by altering the positions of the grey 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.



When setting the pixel thresholds, it is desirable for the range selected to be restricted as small as possible whilst still allowing all particles to be visualised.

The optimal threshold range is achieved by setting the minimum to a level which allows the dimmest particles to still be visualised (move the grey cursor into position with the left-hand mouse button) and the maximum to a level at which the largest particles do not contain many saturated pixels (move the other grey cursor into its maximum position using the right-hand mouse button).

3.5 Fluorescence Mode

The NS300 system can also be used to specifically measure fluorescent particles, using suitable fluorescent filters to block out the scattered laser light and only image the fluorescent signal coming from the particles. For high sensitivity systems with fluorescence capability, the laser power is automatically pulsed on and off, triggered in sync with the Scientific CMOS camera shutter in order to reduce photo-bleaching of fluorescent particles.

Videos captured through the fluorescence filter will require a high camera level and may need optimization of the thresholding pixels, as described above, in order to give an image suitable for analysis. When recording videos under fluorescence, the focus may need adjusting accordingly, due to the optical path difference through the filter.

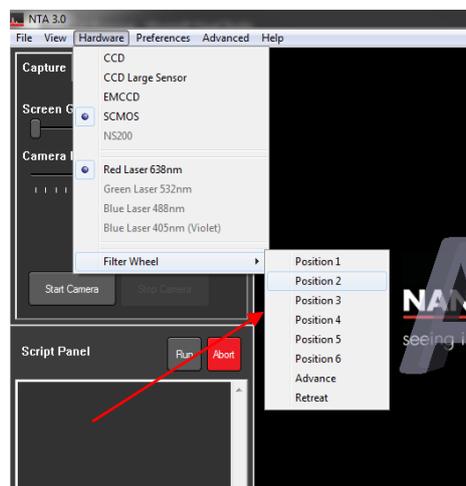
Fluorescent filters are mounted on an integrated filter wheel within the instrument housing and can be introduced into the optical path, controlled by the NTA software.

The position of filters installed in the filter wheel is displayed on a sticker inside the access hatch for each NS300 system.



3.5.1 Viewing fluorescently labelled particles

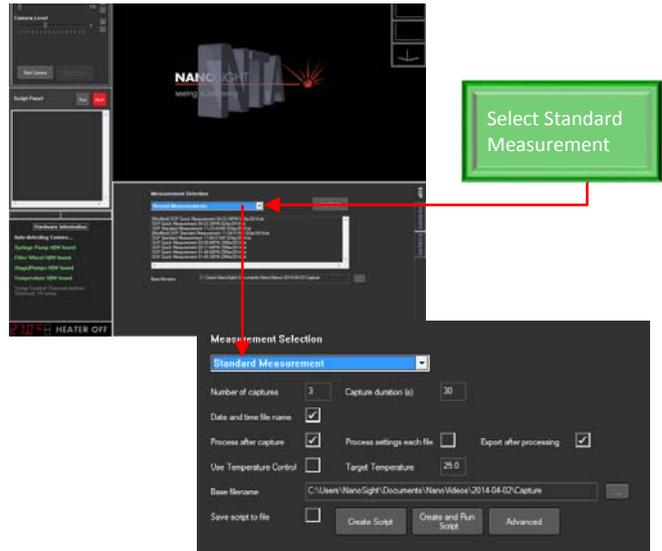
- 1) Increase camera level to level 16.
- 2) If applicable, start the syringe pump (see the syringe pump manual)
- 3) To engage a filter, open the Camera menu and click on Filter 1, Filter 2 etc. to move the selected filter into the field of view.
- 4) Adjust the grey histogram (see Section 3.4) so that the image intensity scale is restricted as much as possible.
- 5) Adjust the focus as necessary and begin video capture.



3.6 Capture Videos

Once an image can be seen on the capture screen, fine-tune the focus as appropriate. A movie can be recorded using suitable camera settings. Select Standard Measurement, adjust number and duration of capture as needed. Start the capture using 'Create and Run Script'. The recording can be cancelled at any time by using the red 'Abort' button.

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. If the temperature sensor readout is not correctly displayed in NTA, please see the Troubleshooting section of this manual.



3.7 Temperature Control

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)



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



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

3.7.1 Operation

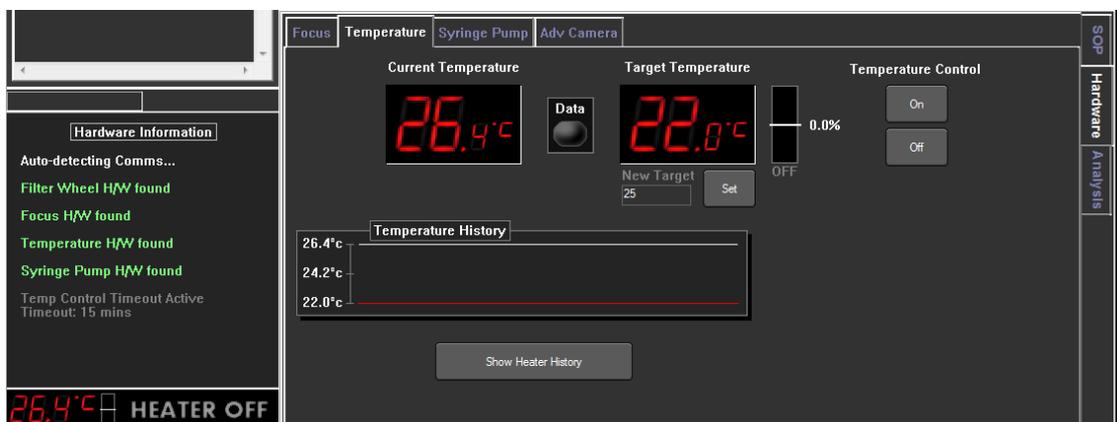
It is imperative that all users have read and understood these concerns:

1. The temperature controller should be turned off if left unattended.
2. The main body of the viewing unit should be monitored to ensure this does not overheat. This is possible if the temperature control is set to a high temperature (>40degC) or a low temperature (<ambient) for a long period of time.
3. It is important that the heating is not able to get out of control. This can happen if a high temperature or low temperature is set for a long time. In either case the laser module will heat up significantly. Should this happen you should turn the temperature controller off in NTA. Alternatively in an extreme case the power jack to the control box can be removed.
Note: if this occurs it may not be sufficient to set the temperature lower, the temperature controller should be switched OFF.
4. When cooling the sample to below ambient temperature, the temperature control should only be employed for a maximum of 15 minutes at a time, before being switched off for at least 15 minutes. Prolonged cooling can lead to an excessive build up of heat around the laser module which can cause fluctuations in the laser power and possible long-term damage.

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

3.7.2 Initialising

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



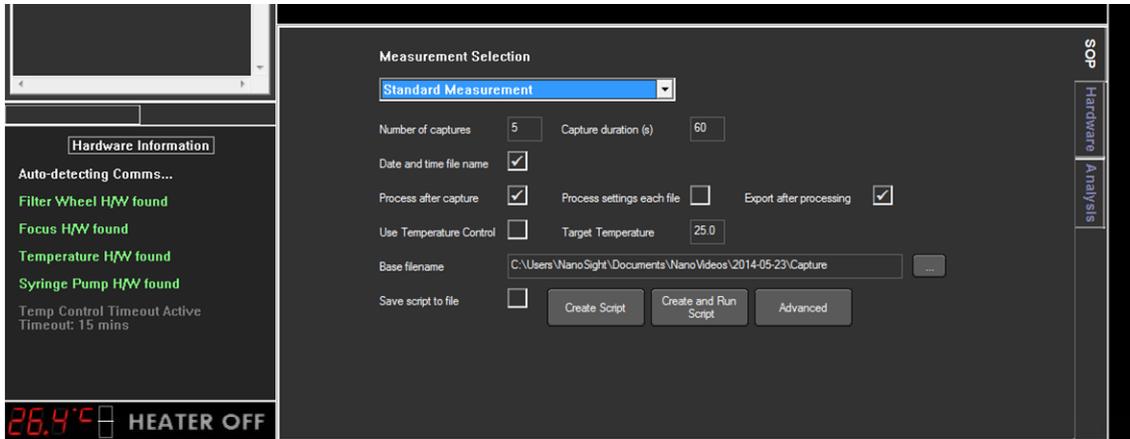
3.7.3 Temperature Setting

Manually

1. Temperature control can be turned on and off.
2. Default 'set temperature' is 25degC. To change this temperature, click the 'New target' box and type in the required temperature e.g. click on the small 25.0 in the image above. Type in the temperature you require and click 'Set'.
3. The temperature controller will now work to set the temperature of the top plate to the target temperature. Some overshoot should be expected. Dependent on the accuracy of temperature required up to 30 minutes should be allowed for the sample temperature to fully stabilize. The angled line shows a trace of the temperature over the last 200 seconds. This line is autoscaling but has no labels.
4. A new temperature can be programmed to change the temperature (by repeating steps 2 and 3).
5. When finished with the system press the 'off' button in NTA to turn off the temperature controller. The temperature readout will continue.
6. When the temperature sensor is initialized, click the On/Off button.
7. Default 'set temperature' is 25degC. To change this temperature, click the box to the right of the temperature sensor and type in the required temperature e.g. click on the small 23.0 in the image above. Type in the temperature you require and click 'OK'.
8. Click the 'Set' button below this.
9. The temperature controller will now work to set the temperature of the top plate to the target temperature. Some overshoot should be expected. Dependent on the accuracy of temperature required up to 30 minutes should be allowed for the sample temperature to fully stabilize. The angled line shows a trace of the temperature over the last 200 seconds. This line is autoscaling but has no labels.
10. A new temperature can be programmed to change the temperature (by repeating steps 2 and 3).
11. When finished with the system press the 'off' button in NTA to turn off the temperature controller. The temperature readout will continue.

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.



5. Spare Parts List

Part Number	Description
NTA4038	NS300 Complete Tubing Kit
NTA4112	Complete set of NS300 connectors
NTA4050	NS300 Inlet Tubing for Flow Cell Top Plate x10 (inc 1 set of connectors)
NTA4051	NS300 Top-Plate Waste Tubing x10 (inc 1 set of connectors)
NTA4110	NS300 Inlet Tubing for O-ring Top Plate x10 (inc 1 set of connectors)
NTA4113	NS300 exterior tubing x10 (inc 1 set of connectors)
CAB0380	USB Cable
NTA0380	CMOS Firewire cable
NTA4010	Flow-cell top plate
NTA4009	O-ring top plate
NTA4075	Viton O-Rings Pack of 10
NTA4076	Nitrile O-Rings Pack of 10
NTA4077	EPDM O-Rings Pack of 10
NTA4078	Silicon O-Rings Pack of 10
NTA4012	Flow-cell top-plate circular seals
NTA4084	Replacement O-ring top-plate luer ports straight (Pack of 10)
NTA4086	Replacement O-ring top-plate luer ports angled (Pack of 10)
NTA4027	Flow-cell gasket component
NTA4049	Flow-cell fixing bolt and nut (2) & 2mm Allen key
NTA4109	Top-plate sprung fastening screws (Pack of 4)
BOT0048	NS300 250ml Waste Bottle