NANOSIGHT NS300
OPERATING MANUAL

MAN0516-08-EN-00 January 2016

Malvern

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Contents

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, 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 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 Instruments (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 Instruments.

For technical assistance, please contact Malvern Instruments.

Malvern Instruments 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 flow cell or 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 flow cell or 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 Instruments to confirm suitability.
- When using biologically 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 laser 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 Instruments personnel, or Malvern Instruments 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 Instruments for additional sets of tubing (P/N NTA4161 NS300 Complete 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 low volume flow cell gasket component
- Instruction manuals
- Allen/Hex-key set
- Particle size standards

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.

1.3.6 Installation and Relocation

Initial installation will be carried out by qualified Malvern Instruments 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 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.

1.3.8 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 authorised 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.

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:	**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

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 Setup

2.2.1 Instrument Communications

Figure 1: USB and camera connection

1) The NS300 instrument communicates with the 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) 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, located on the left side of the case.

5) Start the NTA software, and confirm that no hardware detection error messages are displayed in the status panel (see also Section 7 of this manual and NanoSight NS300 NTA Software guide)

Figure 2: Power connection

2.2.2 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.

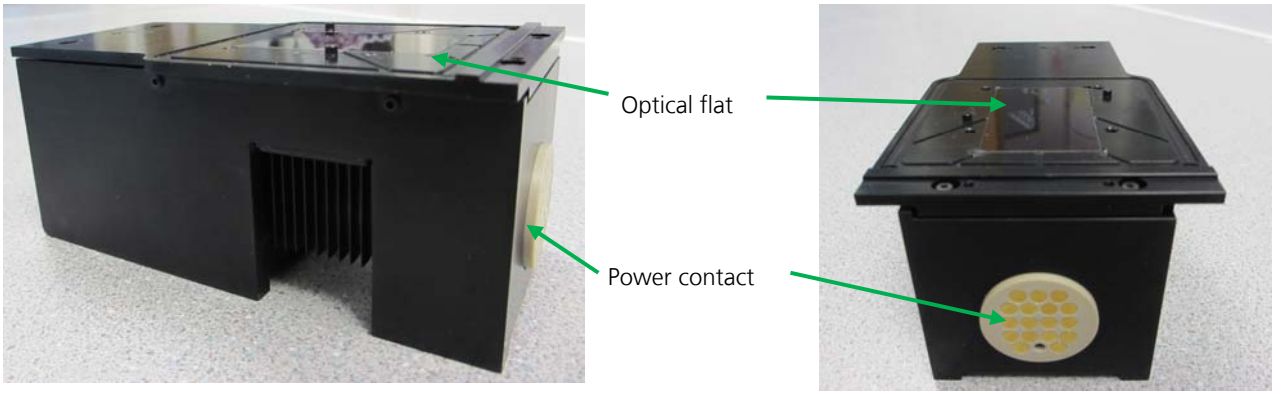


Figure 3: NS300 laser module

2.2.3 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. Please refer to the appropriate section of this manual for the low volume flow-cell top-plate or the O-ring top-plate.

- The **low volume flow-cell** (LVFC) top-plate (Fig. 4) is suitable for use with chemically compatible aqueous solutions and can be cleaned by flushing wash fluid through the chamber, avoiding the need to manually disassemble and clean the chamber after every sample.

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.

Figure 4: Low Volume Flow Cell

- 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.

  The ORTP (Fig. 5) must be disassembled from the laser module and manually cleaned after each sample to prevent particle carryover.

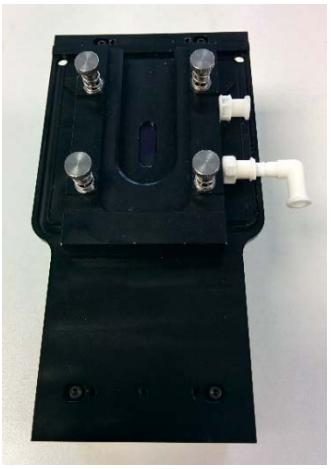


Figure 5: O-Ring Top Plate

2.2.4 External Fluidic Setup

Both top-plates can be used with fluidic tubing which attaches to a tubing port holder on the right-hand side of the NS300 casing (Fig 6). 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.

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. 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.

The right-hand tubing port is the waste outlet. Connect the outlet tubing TUB0288 to the right port and to one of the ports on the waste bottle cap using the fittings supplied. This is important as raising the waste prevents syphoning of liquid when changing sample syringes.

The internal tubing should be attached to the top-plate inside the casing as described in the following sections for the low volume flow cell or the O-ring top-plate.

Figure 6: External NS300 Fluidic Connections

3. Low Volume Flow Cell

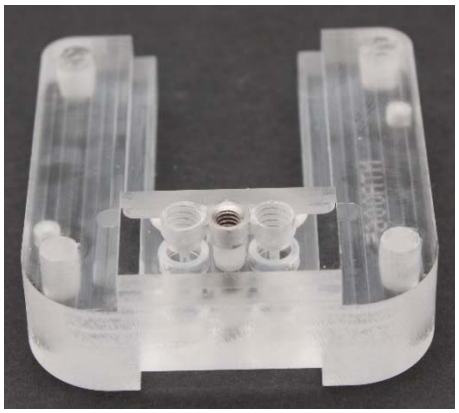
3.1 System Setup

3.1.1 Flow Cell Assembly

The low volume flow cell top-plate consists of two separate parts – the manifold (NTA0065), with ports to attach the tubing fittings (Fig 7A), and a gasket component (NTA0066) which contains an embedded microchannel and chamber seal (Fig. 7B). The LVFC is supplied with NS300 systems with the two components already assembled. The complete flow cell assembly should be mounted on the optical glass flat in the NS300 laser module using the four sprung fastening bolts supplied.

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 remove physically but follow instructions for cleaning, described later.

A

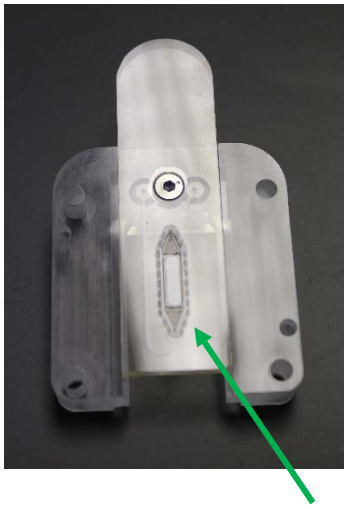


B

Figure 7; A: Low volume flow cell manifold (NTA0065)

B: Low volume flow cell gasket component (NTA0066)

C



D

C: LVFC assembly with PDMS chamber seal on the underside of the gasket component

D: LVFC assembly mounted on laser module with sprung fastening bolts

The supplied sprung bolts should be used to connect the flow cell to the laser module. The bolts should be gently finger tightened 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 (Fig 7C).

Do not overrtighten the fastening screws as this can cause damage to the screw threads

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 Section 3.1.2.

Important!
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. If in doubt about the choice of solvent and its compatibility with any part of the Malvern device, please contact helpdesk@malvern.com for further information.

3.1.2 Flow Cell Tubing Connection

When using the low volume flow-cell, liquid is always loaded into the system using a disposable 1ml syringe. The syringe 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.

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. The inlet and outlet tubing connections are as shown in Fig. 8.

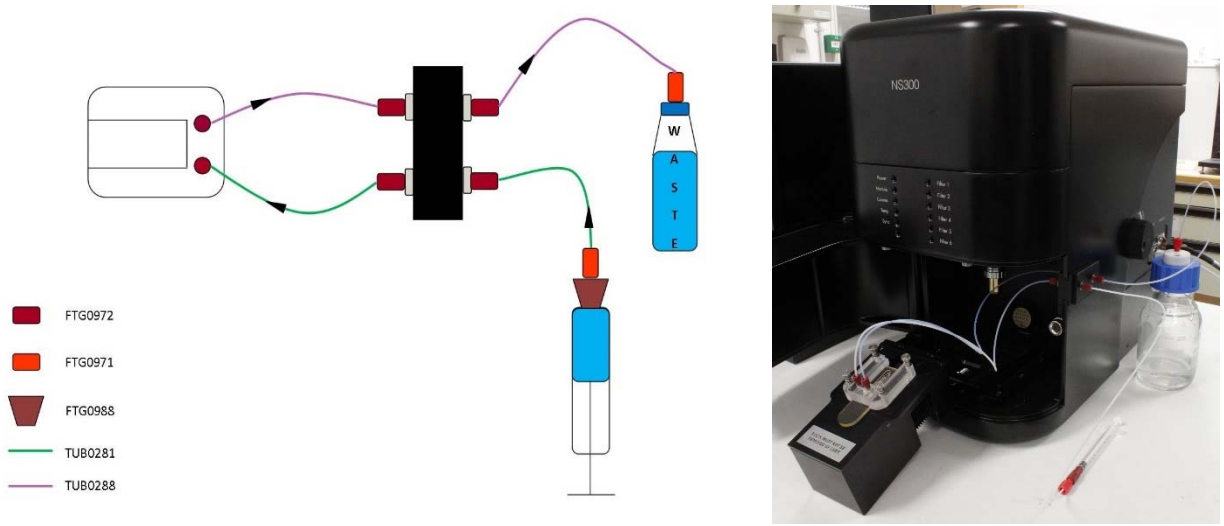


Figure 8: Low Volume Flow Cell Tubing Configuration

The 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 (looking from the tubing connection end of the manifold). Note that before using the system to load samples, it is recommended to rinse out the inlet tubing with sample or buffer to remove any trapped air before connecting the inlet fitting to the flow cell (see Section 3.2.1 – Priming Tubing)

The 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.

Ensure 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.

3.2 Flow Cell Usage

3.2.1 Priming Tubing

Important: For the low volume flow cell, the inlet fluidic tubing should be rinsed out 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:

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

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

3.2.2 Changing Inlet Tubing Syringe

It is important to ensure 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, have the next syringe prepared, ensuring there are no air pockets present at the tip and that there is a small positive meniscus (bead of liquid) protruding from the syringe. Keep the Luer port as low as possible (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.

3.2.3 Loading an Initial 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.

The 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 is loaded into the flow cell chamber with a 1ml disposable syringe connected to the Luer fitting on the inlet tubing.

Important: The low volume flow cell must only be used with disposable **1ml** syringes with Luer port fitting. Using syringes with larger volume than 1ml, or exceeding the maximum rated flow speed of 0.05ml per second (1ml total in 20 seconds) may result in leaking or damage to components.

Once the flow cell is mounted onto the laser module and the inlet tubing has been pre-rinsed with buffer or sample (See Section 3.2.1), the inlet tubing can then be connected to the flow cell manifold to load liquid into the sample chamber.

i. Connect the end of the inlet tubing inside the NS300 casing to the **LEFT** port of the low volume flow cell manifold

ii. Fill a 1ml disposable syringe with the appropriate buffer or sample.

iii. Remove any air bubbles from the syringe

iv. Insert the new syringe into the Luer port, ensuring liquid-to-liquid contact is maintained (See Section 3.2.2 for additional guidance on changing the syringe)

v. Introduce the buffer or sample slowly into the chamber. The flow cell should not be loaded at speeds exceeding 0.05ml per second (1ml total in 20 seconds).

vi. The low volume flow cell 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 9: Loading a sample with the low volume flow cell

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. If bubbles are routinely observed when a sample is firsty loaded, the flow cell should be cleaned 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 supplied 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,

3.2.4 Changing Samples

The 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.

i. Flush the system by loading a 1ml syringe of clean water or buffer solution. The flow cell should not be flushed at speeds exceeding 0.05ml per second (1ml total in 20 seconds).
Note: Ensure that liquid to liquid contact is maintained at the Luer port when changing over the syringe

ii. Confirm the cleanliness of the chamber by checking for any particles present in the NTA software image

iii. Repeat the flush if necessary to remove remaining sample particles

iv. Load the next sample syringe into the system. Make sure to load at least 800ul of the new sample through the system to prevent significant dilution of the sample with wash fluid remaining in the outlet waste tubing.

Important: Using syringes with larger volume than 1ml, or exceeding the maximum rated flow speed of 0.05ml per second (1ml total in 20 seconds) may result in leaking or damage to components.

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 3.2.5.

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

3.2.5 Manual Cleaning Procedures

The LVFC has been designed not to require manual cleaning between samples (dependent on sample type). Between samples, push at least 1 ml of water or diluent through the sample chamber (following the advice in Section 3.2.4), to remove any particles present. This ensures carry over of less than 1 %.

A manual clean is only necessary 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.

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.

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. If the optical flat in the laser module becomes damaged, it can be replaced by Malvern Instruments (pricing on request).

The recommended practice for manually cleaning the optical flat on the laser module and the low volume flow cell top-plate is given below:

- Before performing a manual clean, the system should be flushed to clean sample from the tubing and fittings.
- Once all tubing assemblies are flushed clean, empty the fluidics by loading a 1ml syringe full of air through the system (do not exceed maximum rated flow speed of 0.05ml per second).
- 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
- 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

Figure 10: Unmounting the laser module from the NS300

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

- 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.

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.

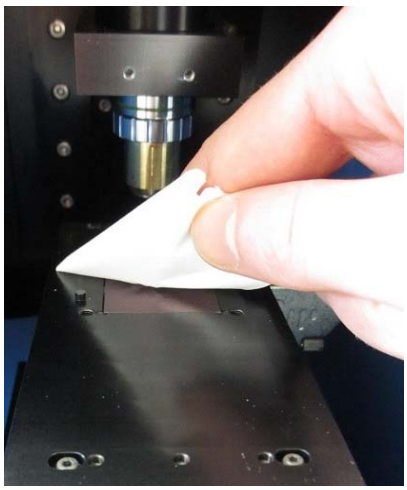


Figure 11: Cleaning the optical flat on the laser module

- 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.

- If, and only if, any sample residue is visible on the glass window surface, the underside of the glass can be gently cleaned using a small cotton bud dampened with **water** or a solution of up to **10% ethanol** (Fig. 12). When cleaning the glass window inside the chamber seal area, take care to limit any rubbing of the surrounding soft PDMS seal.

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

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.

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

Important: If the gasket component repeatedly leaks after a manual clean, it should be replaced (See Section 3.3.1)

- After manual cleaning, the low volume flow cell must be dried as described in Section 3.2.5 before it can be remounted on the laser module.

3.2.5 Drying the Low-Volume Flow Cell

- Direct compressed air through the tubing ports on the manifold to thoroughly dry the embedded microchannel inside the gasket component (Fig. 13A).
- Once the channel is dry, turn the flow cell assembly over and, keeping the nozzle of the compressed air at least **15 cm** from the seal, lightly dry the underside of the component and the area inside the chamber seal (Fig. 13B).
- Repeat as necessary until the gasket component is completely dry.

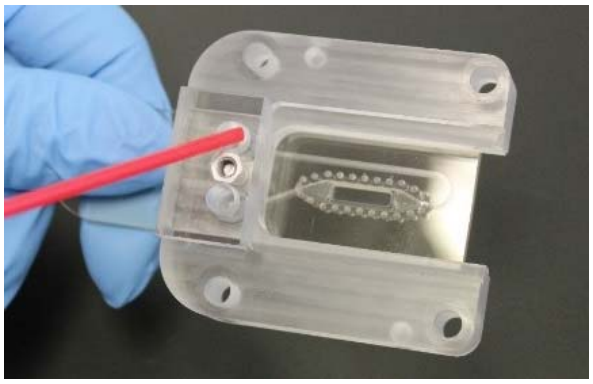


Figure 13, A: Drying gasket component through manifold tubing ports

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

Avoid touching or rubbing the seal when drying the underside of the gasket component. Keep the compressed air nozzle at least 15cm 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

3.3 Care and Maintenance

3.3.1 Gasket Component Replacement

When supplied with new NS300 systems, the LVFC is supplied fully assembled and does not need to be disassembled for normal usage and cleaning.

- 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 Allen / 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 (Fig. 14).

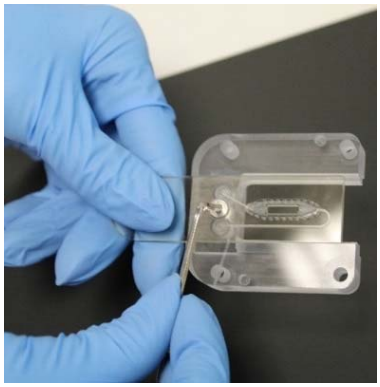


Figure 14: Disassembly of the flow cell

- Before reassembling with a new gasket component, ensure that the circular seals are bedded fully down in the ports on the manifold (Fig. 15).
- 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, ensuring that they are fully seated and level. If the circular seals need replacing, 2 spare seals are provided with each gasket component supplied.

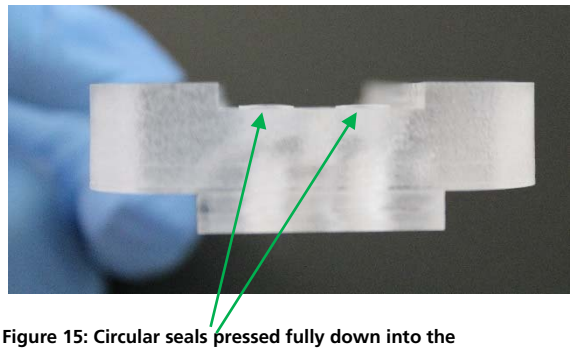


Figure 15: Circular seals pressed fully down into the manifold (only just protruding from the surface)

- Lay the gasket component into the recess on the manifold ensuring the holes in the gasket line up with those in the manifold and then hold in place by inserting the fixing bolt.
- 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 an visibly unbroken seal contact (Fig. 16)
- Check that there are no fibers or dust particles on the PDMS chamber seal on the underside of the gasket component (Fig.7C). The flow cell is then ready for reattaching to the NS300 laser module.

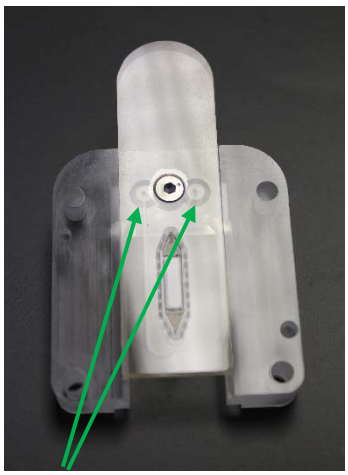


Figure 16: Visual of the circular seal contact areas from underneath, confirming a good connection of the gasket component to the manifold

4. O-Ring Top Plate

4.1 System Set-Up

The O-ring top-plate contains an embedded O-ring seal. The supplied sprung bolts should be used to connect the top-plate to the laser module, forming the sample chamber. The bolts should be gently finger tightened until an increased resistance is felt when the bolts reach the end of the screw thread.

Do not overrtighten 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.

4.1.1 O-Ring Top-Plate Assembly for Manual Injection

Samples can be loaded into the chamber using a disposable 1ml 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.

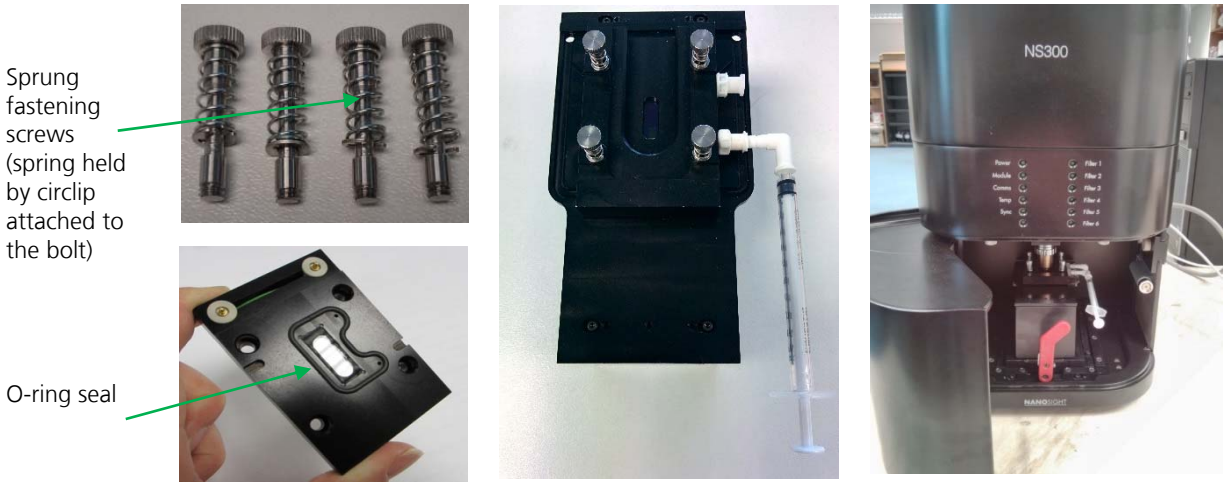


Figure 17; A Sprung fastening bolts and O-Ring Top-plate (ORTP)

B: ORTP with Luer ports and push fit elbow Luer connector

C: ORTP mounted on the laser module inside the NS300

4.1.2. O-Ring Top-Plate Assembly for Syringe Pump Use

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, the Luer ports should be removed and the tubing connected using the fittings supplied, as described in Section 4.1.3.

Figure 18: O-ring Top-Plate with tubing fittings for syringe pump use

4.1.3 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 1ml syringe. The syringe 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.

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. The inlet and outlet tubing connections are as shown below.

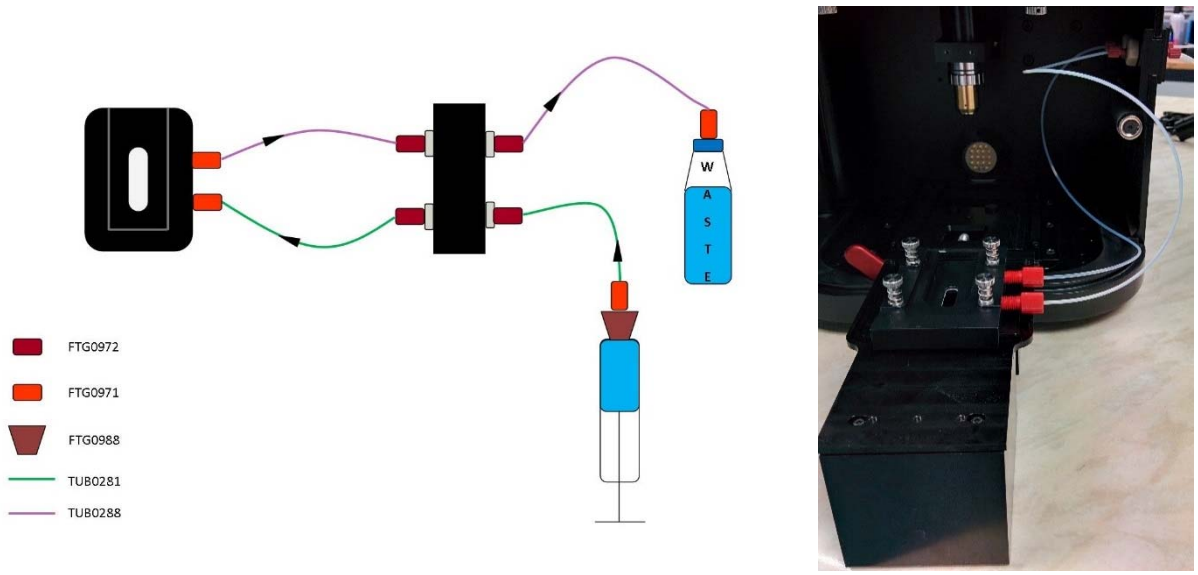


Figure 19: O-Ring Top-Plate Syringe Pump Tubing Configuration

The inlet tubing TUB0281 connects to the tubing holder port nearest the front of the NS300 and the left port of the the O-Ring top-plate. Note that before using the system to load samples, it is recommended to 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 Section 4.2.3 – Loading an Initial Sample)

The 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.

Ensure 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.

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 exerts higher pressures on the chamber seal. This can result in sample leakage or damage to the top-plate.

4.2 O-Ring Top-Plate Usage

4.2.1 Manual Injection of 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. The 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 is loaded into the O-Ring Top-plate with a 1ml disposable syringe connected directly to the Luer fittings on the top-plate.

Important: The O-Ring Top-plate must only be used with disposable **1ml** syringes with Luer port fitting. Using syringes with larger volume than 1ml, or exceeding the maximum rated flow speed of 0.05ml per second (1ml total in 20 seconds) may result in leaking or damage to components.

The O-ring top-plate should be manually cleaned and dried (See Sections 4.2.4 and 4.2.5), and assembled on the NS300 laser module before loading a sample directly into the chamber via the Luer ports on the top-plate.

i. Fill a 1ml disposable syringe with the appropriate buffer or sample.

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.

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

iv. Insert the 1ml syringe into the front inlet port and introduce the sample slowly into the chamber. The top-plate should not be loaded at speeds exceeding 0.05ml per second (1ml 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.

4.2.2 Loading a Sample for Use with a Syringe Pump

4.2.2.1 Priming Tubing

Important: For the O-ring top-plate when connected for syringe pump use, the inlet fluidic tubing should be rinsed out 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:

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

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

4.2.2.2 Changing Inlet Tubing Syringe

When using the the fluidic tubing connections, it is 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 (bead of liquid) protruding from the syringe. Keep the Luer port as low as possible (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 meniscuses 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.

4.2.2.3 Loading the Sample through the Syringe Pump Tubing

Once any connected inlet tubing has been pre-rinsed, 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.

The 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 is loaded into the O-Ring Top-plate with a 1ml disposable syringe connected to the Luer fitting on the inlet tubing.

Important: The O-Ring Top-plate must only be used with disposable **1ml** syringes with Luer port fitting. Using syringes with larger volume than 1ml, or exceeding the maximum rated flow speed of 0.05ml per second (1ml total in 20 seconds) may result in leaking or damage to components.

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.

The O-ring top-plate should be manually cleaned and dried (See Sections 4.2.4 and 4.2.5), before loading a sample.

After pre-rinsing the inlet tubing with buffer or sample (See Section 4.2.2.1), 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)

i. 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.

ii. Using the sprung fastening bolts, mount the top-plate with attached inlet and outlet tubing onto the laser module

iii. Fill a 1ml disposable syringe with the appropriate buffer or sample.

iv. Remove any air bubbles from the syringe

v. Replace the pre-rinse syringe connected to the Luer port with the new syringe, ensuring liquid-to-liquid contact (See Section 4.2.2.2 for additional guidance on changing the syringe)

vi. 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.

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

vii. Introduce the buffer or sample slowly into the chamber. The flow cell should not be loaded at speeds exceeding 0.05ml per second (1ml total in 20 seconds).

Manually loading sample through a syringe with the outlet tubing connected exerts higher pressures on the O-Ring Top-plate seal. This can result in sample leakage or damage to the top-plate. Take care to load sample slowly when the outlet tubing is connected.

The O-ring top-plate is now loaded ready for use with the syringe pump. Place the syringe into the syringe pump holder and operate as described in the NanoSight Syringe Pump Operating Manual.

4.2.3 Changing Samples

When using the O-ring top-plate, 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 Section 4.2.4, to avoid any sample carry-over.

4.2.4 Manual Cleaning Procedures

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

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.

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 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 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.

- If any tubing is connected for the syringe pump, flush a 1ml syringe of clean water through the system to clean sample from the tubing and fittings.
- Once all tubing assemblies are flushed clean, empty any fluidic tubing by loading a 1ml syringe full of air through the system (do not exceed maximum rated flow speed of 0.05ml per second).
- 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.
- Unscrew the sprung fastening screws securing the top plate and lift the top plate gently off the laser module.

Figure 20: Laser module with O-Ring top-plate unmounted from the NS300

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

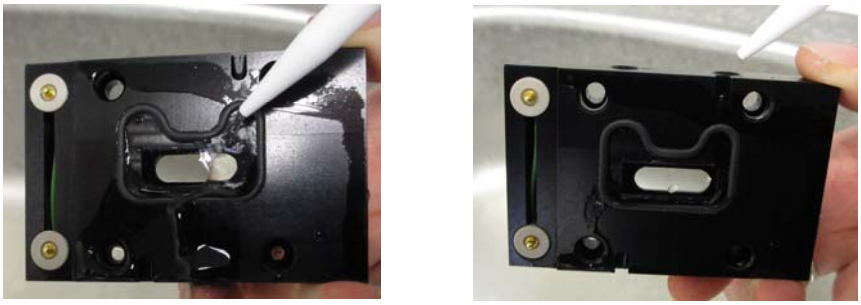


Figure 21: Cleaning the O-Ring Top-plate

- 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.

- 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.

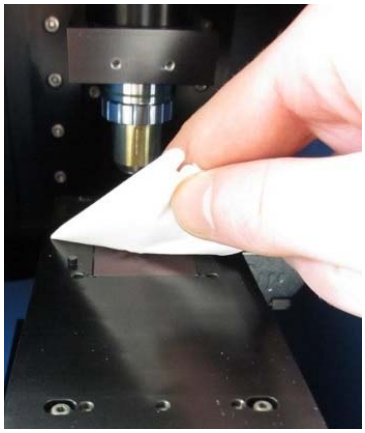


Figure 22: Cleaning the optical flat on the laser module

4.2.5 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.

- Use an air stream (e.g. from a can of compressed air) to blow any residual liquid from the inlet and outlet channels (Luer ports) through which the sample is introduced into the chamber.

- 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.

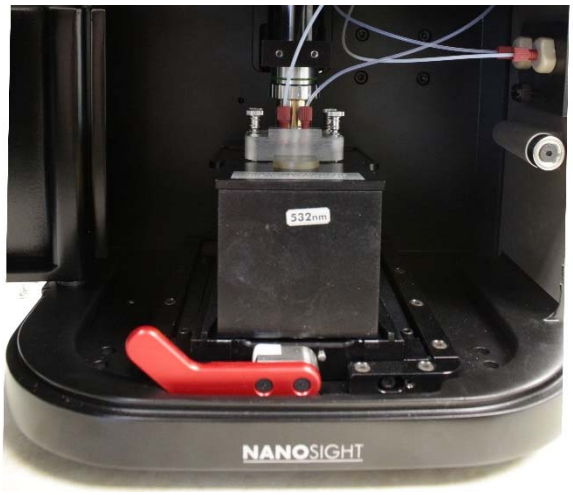
4.3 Care and Maintenance

4.3.1 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.

5. 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.



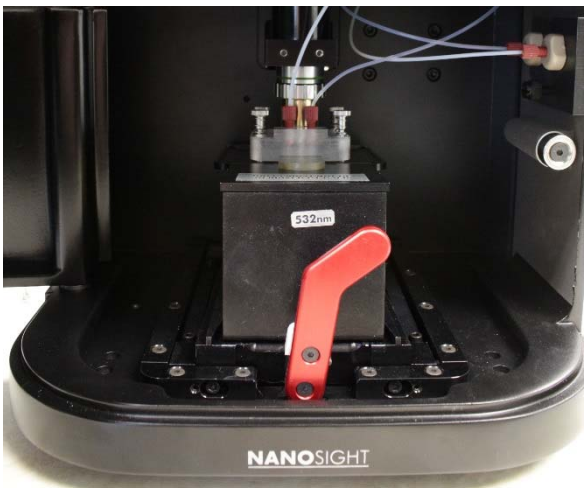


Fig 23; A: Laser module mounting / removal **B: Laser module locked in position**

The sample is then ready for NTA measurement (See Section 7 of this manual and the NanoSight NS300 NTA Software guide).

6. Daily Use

After use, the system should be flushed to clean sample from the tubing and optical surfaces. If using diluent with a high concentration of dissolved solids (i.e PBS or other saline solutions), always flush clean water through any tubing and fittings connected after use.

Once all tubing assemblies are flushed clean, empty the fluidics by loading a 1ml syringe full of air through the system (do not exceed maximum rated flow speed of 0.05ml per second).

Disconnect the tubing fittings, remove the sprung fastening bolts and gently lift the top-plate off the laser module. Follow the procedures for a manual clean if necessary, and then dry the flow cell or O-ring top-plate ready for the next 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.

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

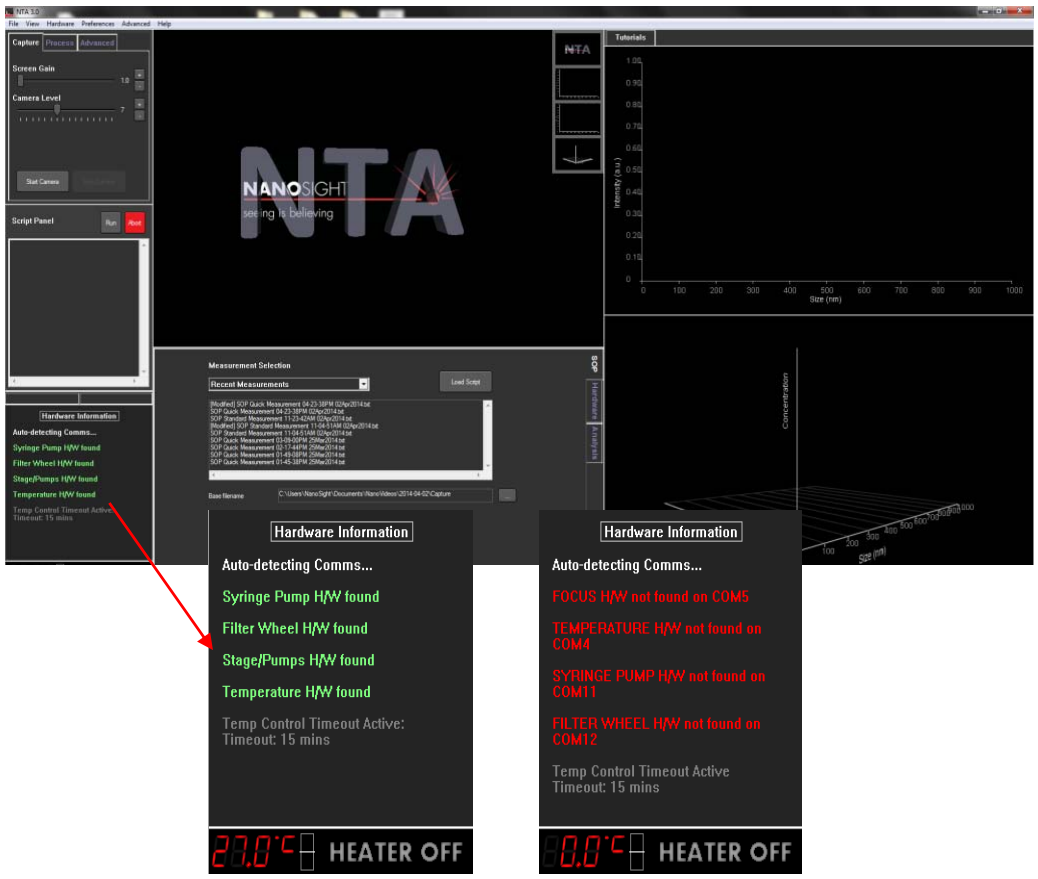
7. NanoSight NS300 Control Software

The key software features are summarised here. For further information on the NTA software functionality with the NS300, please see the NanoSight NS300 Software Guide.

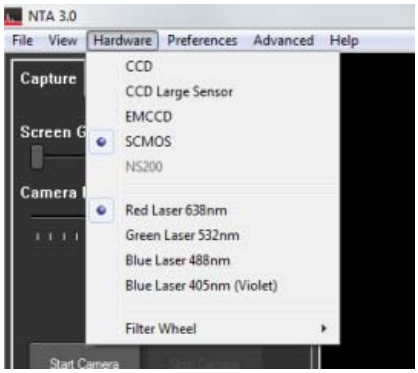
7.1 NTA Software Startup

Run the NTA Software.

The NanoSight NTA software comes pre-installed on all computer supplied by Malvern Instruments 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:



7.2 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 7.4.

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

Operational Care

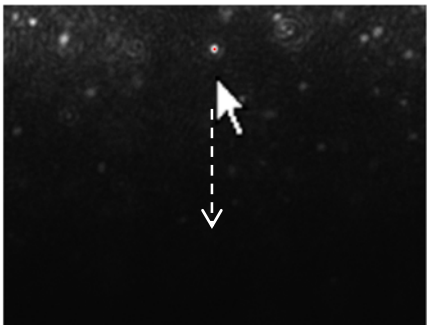
High-sensitivity cameras should not be left with the camera level at a high value for a prolonged period (i.e. when the camera is not being used) to restrict current flow and thus extend camera lifetime.

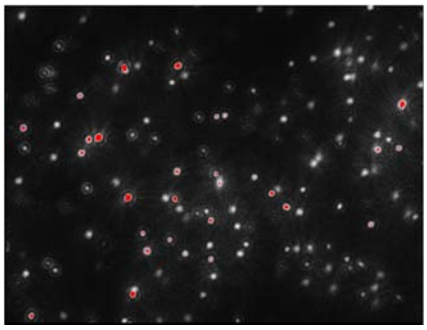
7.3 Adjusting the NS300 to obtain a video

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.

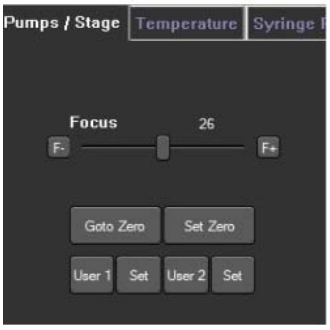
i. With a view of the particles on the NTA screen in capture mode, left-click the mouse button on the video image.

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.



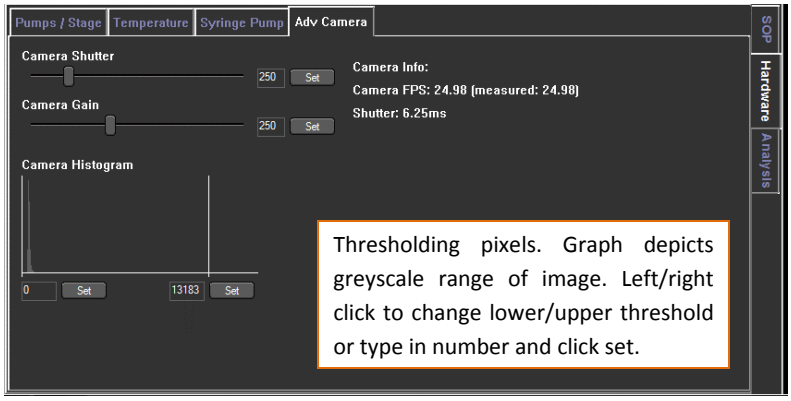
Dragging the slider on the left gives fine adjustments, while using the slider on the right gives courser adjustments.

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

7.4 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).

7.5 Fluorescence Mode

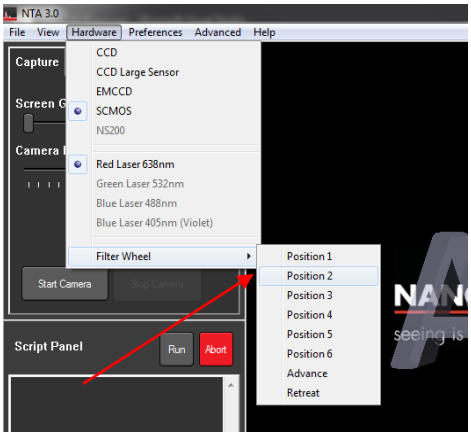
The NS300 system can also be used to specifically measure fluorescent particles, using suitable fluorescent filters to block out the scattered laser light and only image the fluorescent signal coming from the particles. For high sensitivity systems with fluorescence capability, the laser power is automatically 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.

7.5.1 Viewing fluorescently labelled particles

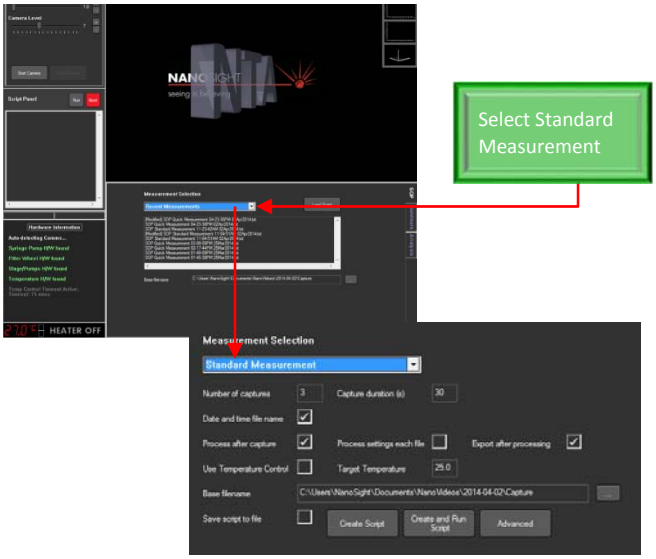
1) Increase camera level to level 16.
2) If applicable, start the syringe pump (see the syringe pump manual)
3) To engage a filter, open the Camera menu and click on Filter 1, Filter 2 etc. to move the selected filter into the field of view.
4) Adjust the grey histogram (see Section 7.4) so that the image intensity scale is restricted as much as possible.
5) Adjust the focus as necessary and begin video capture.



7.6 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.



7.7 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.

7.7.1 Operation

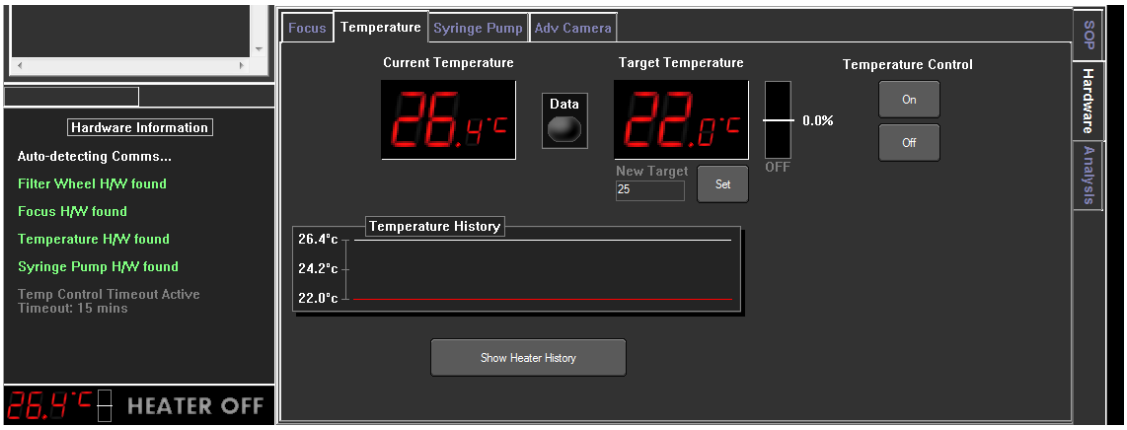
It is imperative that all users have read and understood these concerns:

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.

7.7.2 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.



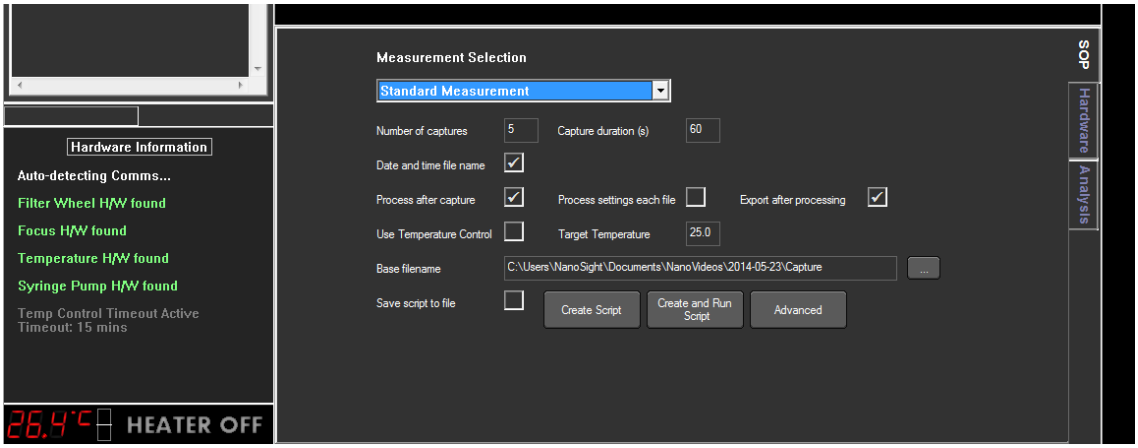
7.7.3 Temperature Setting

Manually

1. Temperature control can be turned on and off. When the temperature sensor is initialized, click the On/Off button.
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. 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.

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.



8. Spare Parts List

Part Number	Description
NTA4171	NS300 LVFC ORTP Tubing and connector Kit
NTA4161	NS300 LVFC ORTP Complete Tubing Kit only
NTA4112	NS300 Connector kit only
NTA4162	NS300 Waste bottle with cap
BOT0049	NS300 Waste bottle only
BOT0059	NS300 Waste bottle cap only
NTA0065	Low volume flow cell manifold
NTA4169	2x low volume flow cell gasket components and box (gasket components not available to buy separately)
NTA4111	Hex key set
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
NTA4084	Replacement O-ring top-plate luer ports straight (Pack of 10)
NTA4086	Replacement O-ring top-plate luer ports angled (Pack of 10)
NTA4109	Top-plate sprung fastening screws (Pack of 4)
CAB0380	USB Cable
NTA0380	CMOS Firewire cable