

## Nanosight NS300 NBTC User Instructions

### Preliminary notes:

-The laser module is delicate and expensive. Handle it with care and do not leave it on the bench unattended. Only leave it in place in the instrument with the lever locking the laser module in place.

-Sample requirements: At least 1mL of sample (more for very polydisperse samples or those requiring flow). Recommended concentration range is  $10^6$ - $10^9$  particles/mL, and particles in the ~20-800nm size range. Smaller particles will not be visible in the cell, larger particles will drown out signal from smaller particles and can not be reliably measured over reasonable time frames. Sample can be recovered if needed though may be diluted in the process.

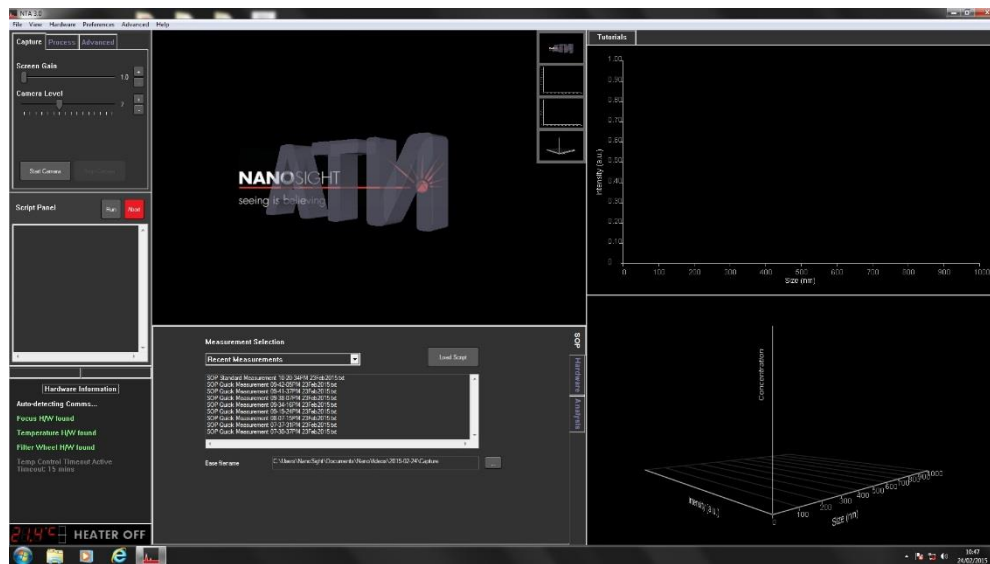
-Use only 1mL syringes with the liquid cells. This minimizes the chances of a leak by controlling the maximum force that can be used to push liquid through the system.

- 1) Log into the instrument at the login station.
- 2) Remove the laser module and insert the o ring cell. (MORE PICTURES AND DETAILS)
- Check that the tubing connects from the inlet to outlet are finger tight.
  - Replace the laser module and lock into place by lifting the lever while pushing in on the laser module.
- 3) Rest the back laser module on the front of the Nanosight so that it sits at a slight angle. Fill a 1mL syringe with filtered DI water from the MilliQ. Remove any bubbles by flicking the syringe. Insert the syringe into the inlet port and gently push the fluid through. After about 0.5 mL, liquid will start to fill up the cell. Continue until there is 0.1-0.2mL remaining in the syringe.

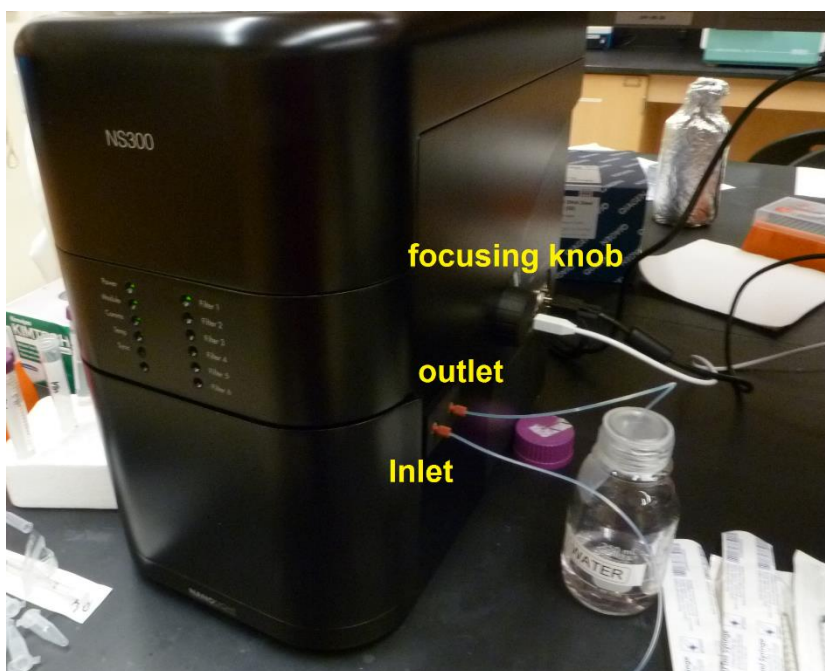
(Note: This technique minimizes bubbles within the measurement chamber.)

- 4) If the instrument is off, the power button is on the left side of the instrument. If the power is on, the “Power” and “Module” lights at the front of the instrument should be lit.

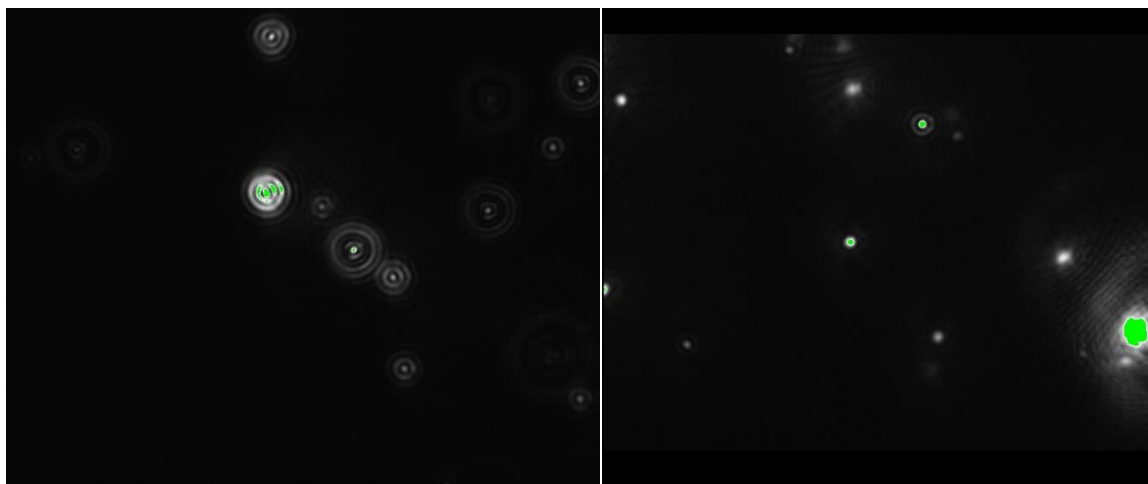
5) Start up the Nanosight NTA software.



- 6) Start the camera with the “start camera” button, and increase the “Camera Level” until you can see spots moving around the screen. This is scattering from particles in the water. Push in more liquid from the syringe, and confirm that you see scattering from an occasional particle moving across the screen from right to left. (n.b.: if particles are moving at a significantly different angle, or if there is a large section of unmoving diffraction, check the troubleshooting section at the end of these instructions.
- 7) Fill a 1mL syringe with the sample. Remove any bubbles by flicking the syringe and leave a small bead of water on the tip of the syringe to prevent an air pocket between the old and new sample. Insert the syringe into the inlet port and gently push the fluid through. After about 0.5mL, you should see an increase in particle number due to the sample going into the sample cell. Flow a bit more sample in, until the number of particles seems stable, but do not push all of your sample in.



- 8) Bring the particles into focus by turning the focusing knob on the instrument (IMAGE) or on the software. Note that, because of the depth of the liquid cell, that not all particles will be perfectly in focus at one time. The first image below is out of focus, the second one will yield reliable results.



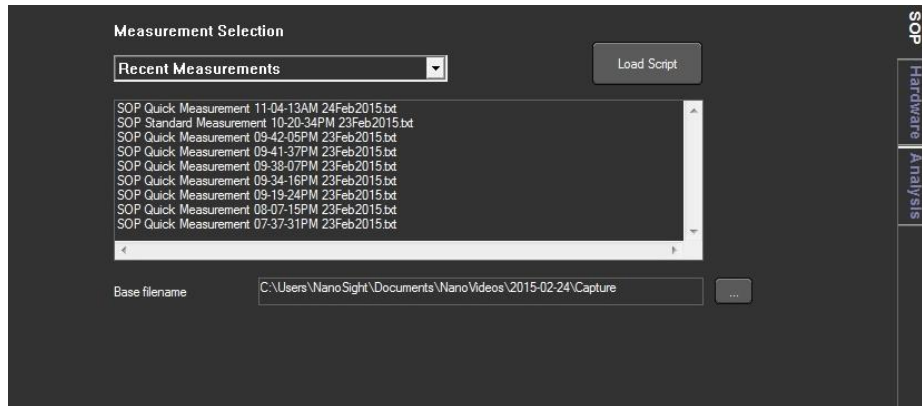
*Out of focus particles (left), in focus particles (right).*

- 9) Decrease the camera level to 1-2 clicks above the level that gives a “dark” error message. The sample is now ready to be measured.



- 10) Set up a measurement:

The Measurement window is at the bottom center of the screen. Click the “SOP” tab.



The software gives you three choices: “Recent Measurement”, where you can repeat a measurement setup from a recent experiment, “Quick Measurement”, which gives you fewer options and takes a single measurement, and “Standard Measurement”, which gives you a wider variety of options and the choice to take multiple measurements on a single sample. (Note: “Zeta Measurement,” while listed as an option, is not available on the NBTC NS300. If you would like zeta potential information about your particles, please contact NBTC staff for training on the Zetasizer.)

Notes on Standard Measurement Options:

-We recommend taking 3-5 measurements per sample using the Standard Measurement dialog. Further measurements are taken by pushing more sample through with the syringe and measuring the new population of particles visible in the window.

-A measurement time of 30-60 seconds is appropriate for most samples. Longer measurements will lead to increased accuracy of the size estimations for the population visible within the window. Increased number of measurements will lead to more robust statistics on the overall population of particles within the sample.

-Temperature: The sample can be held between about 20 and 50C. (nb zetasizer users: a long temperature equilibration time is not necessary on this instrument due to the small volume in the flow cell. The cell is typically equilibrated as soon as the temperature is stable.)

-Save data to your user folder. If you do not choose a folder, the data will automatically be saved to a folder with today’s date.

-Viscosity can be changed in the Advanced settings. Viscosity can be determined using the viscometer. Please note that there is a temperature dependence on viscosity, so the measurement obtained on the viscometer should be at the same temperature as the measurement.

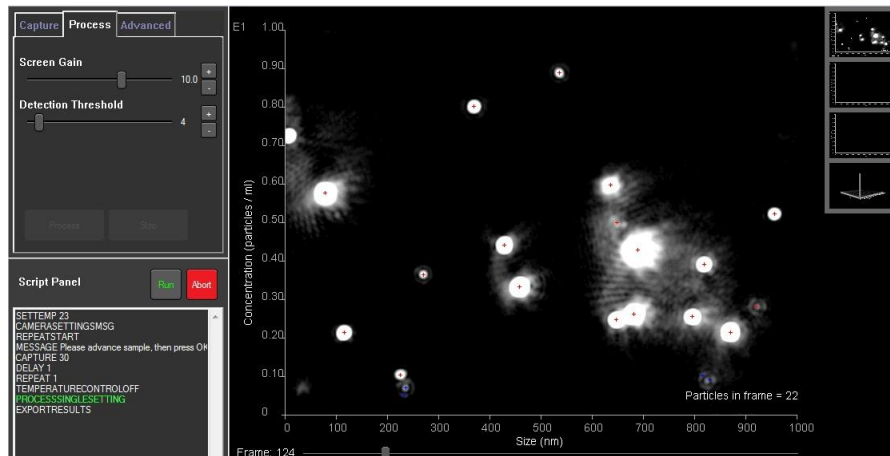
When you have set the options as desired, click “Run.”

The software will give you the choice to readjust the camera levels. If they are still set appropriately as in Step 9, you should not need to make further adjustments. Click OK.

11) The Nanosight will now take video for the sample for the length of time specified in the

measurement settings. If multiple measurements are going to be taken, the software will pause and ask you to advance the sample. Gently push more sample through the cell until a new set of particles can be seen, then press “continue”.

- 12) When the data collection is complete, the camera screen will display the first frame of the first measurement with a message to adjust the process settings. The Process settings tab can be adjusted in the upper left window:

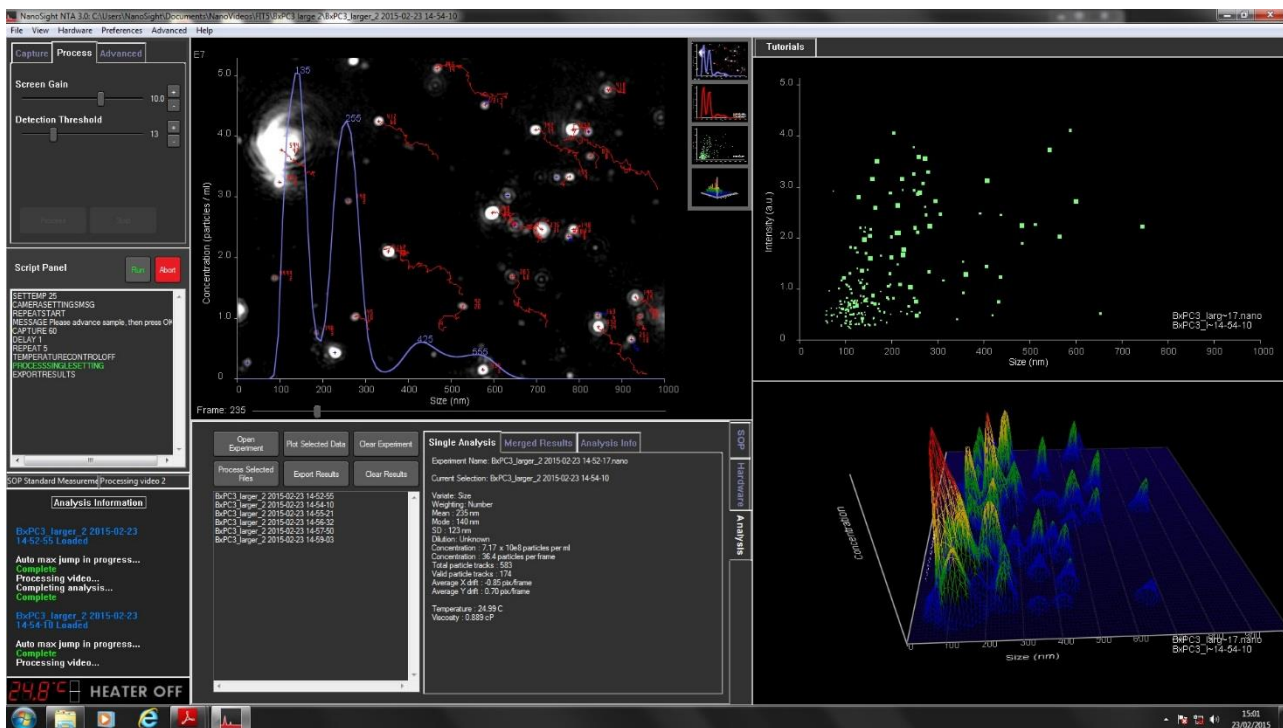


The Screen Gain adjusts the brightness of the data that has been collected and does not affect the data measurement itself.

The Detection Threshold sets the sensitivity of the software's particle-detection algorithm. Red crosses indicate particles that the software considers more reliable, blue crosses indicate particles that are detected but with less confidence. Set the detection threshold to a level where you are confident in most of the software's determinations. A value between 4 and 7 seems to be most common.

To scroll through the complete video, use the slider at the bottom of the image window. (Set to frame 124 in the screenshot above.)

- 13) Click OK to run the measurement. You will see the software track individual particles and the data will appear in the windows.



*Analysis in a polydisperse data set.*

-If multiple measurements were taken, the software will ask you to adjust the process settings of each data set in turn.

#### 14) Export data:

The overall report can be exported as a pdf. A complete or partial video of the data taken can also be obtained (n.b.: we strongly recommend saving any .wmv files to a jump drive and deleting them from the NanoSight computer; the files created are large and can add up, and if we run out of disk space we may need to institute regular data cleanouts.)

#### 15) Performing another run:

Fill a syringe with 1mL DI water, remove any bubbles, and gently push the liquid through the cell. Repeat this two more times (3mL of DI water in total). Confirm that the fluid running through the cell is relatively free of particles. Repeat the measurement with a new sample.

#### 16) Shutting down:

Fill a syringe with 1mL DI water, remove any bubbles, and gently push the liquid through the cell. Repeat this two more times (3mL of DI water in total). Fill the syringe with 70% ethanol and push this through the cell. You should see increased scattering. Pull the syringe back to fill with air, and push it through the cell two or three times, until no more liquid comes out of the exit port.

Dispose of all liquid in the exit port. Sterilize the waste container, if necessary. Open the cell. Put the screws and cell into their designated containers. Dry the surface of the laser module with a clean Kimwipe.

Check that there is no liquid that leaked out of cell onto the laser module or its underside. Remove any liquid with a Kimwipe. Replace the laser module into the NS300 unit and lock it into place by lifting the lever while pressing the laser module into place.

Log off.

Fluorescence notes:

- The fluorescence filter for the 532 nm laser (the only one currently available) is filter #2.
- The manufacturer recommends using a syringe filter to measure fluorescent samples so as to minimize sample photobleaching. For assistance with the syringe filter please consult with NBTC staff.