

University of Minnesota Nano Center Standard Operating Procedure

Equipment Name: Nanoparticle Tracking Analyzer

Model: NanoSight LM-10

Location: PAN 185

Badger Name: Not on Badger

Revision Number: 1

Revisionist: J. Marti

Last Revision Date: 3/7/16

A. Introduction

- 1. Tool Description.** The NanoSight is a nanoparticle characterization tool that tracks the motion of nanoparticles suspended in a liquid and calculates size distributions for particle dispersions in the size range 10 nanometers to about 1 micrometer. Nanoparticle samples may be suspended in aqueous or organic liquids, as long as the viscosity of the material is known.
- 2. Tool Components.** Figure 1 shows a front view of the NanoSight. Key components visible in this view are

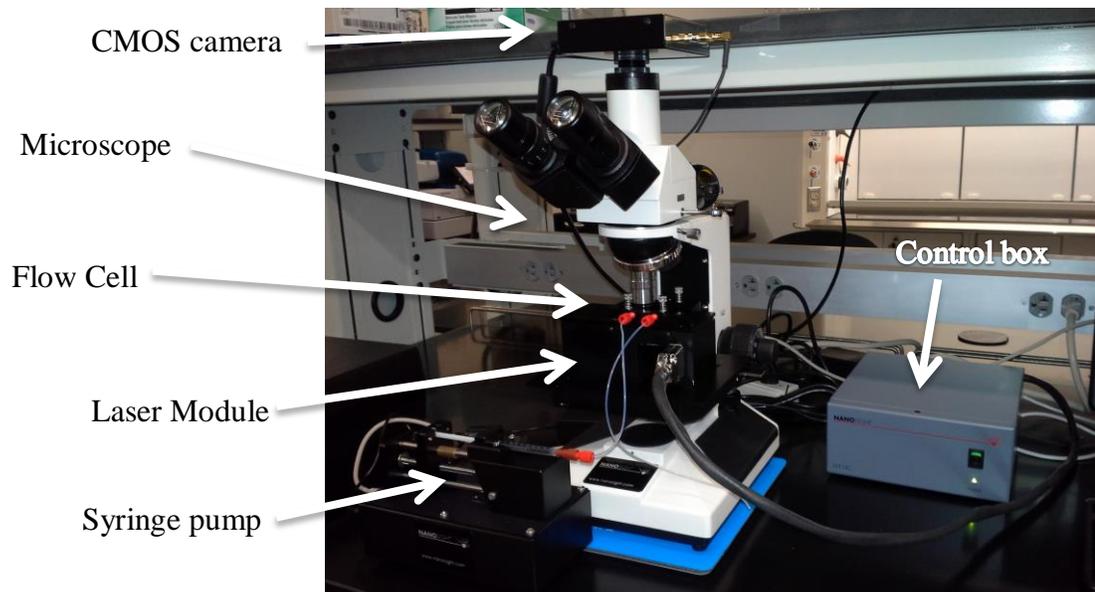


Figure 1. The NanoSight tool.

- 3. Safety.** The NanoSight uses a 400nm (near UV) laser inside a flow cell. Under normal operating conditions with the top-mounted flow cell in place, it is not possible to directly view this laser radiation; any light escaping the flow cell is scattered light and is not harmful. However, DO NOT attempt to operate the laser without the flow cell in place and with liquid in the flow cell. This would potentially expose the viewer to short wavelength laser radiation.
- 4. Restrictions/requirements**
 - a. Samples must be suspended in a liquid medium.

- b. The standard flow cell may be used with water, methanol, ethanol, and isopropanol. Contact staff if your sample is in acetone or any other solvent (special O-rings must be used with these solvents).
- c. Particles should be smaller than 1000nm with little or no settling observed in the sample. If your sample contains particles that are mostly larger than one micron, it should be run on the Bluewave laser diffraction analyzer.
- d. Particles must be at relatively low concentrations, i.e., the dispersion should appear clear or very slightly cloudy. If not, dilute the sample.

5. Required Facilities. Several connections to 120V AC power, and connection to a computer running Windows 7 or higher. The system must be placed on a secure support free of vibration. Anti-vibration pads or a vibration-cancelling platform should be used.

6. Definitions.

- a. Dispersion: particles that are stably suspended in a liquid medium. Note: this is distinct from a *solution*, in which one or more species are dissolved in a solvent.
- b. Particle size distribution (PSD): a plot of particle concentration versus size. PSDs may be relative or absolute, and can be expressed in terms of particle count per size channel (number distribution), particle surface area per size channel (area distribution), or total particle volume per size channel (volume distribution). The NanoSight gives an absolute number distribution.

Theory of Operation. Nanoparticle tracking analysis (NTA) is a technique to measure the sizes of particles under a micron in size. The NTA process directs intense, short wavelength laser light at particles suspended in a liquid. The particles are undergoing Brownian motion, with the smaller lighter particles moving more quickly than the larger, more massive particles. In the NanoSight, these particles scatter a small amount of light out through an optical window where it is collected by a microscope objective and directed to a very sensitive camera. The camera records video of the moving particles at 30 frames per second, and image analysis is used to track the motion of each particle over several seconds worth of video. The software then calculates a diffusion coefficient based on the recorded particle motion, which is used to calculate a hydrodynamic size of each particle. This size is plotted as a histogram. Note that unlike other light scattering-based instruments, nanotracking analysis gives an absolute, rather than relative, size distribution.

B. Using the NanoSight

1. Equipment Setup

- a. Begin a new entry on the tool log sheet. List your name, the date, the materials you are working with (particle material(s) and suspending fluid), and any issues you encountered during your run.
- b. Log on to the computer using your X500 user name and password.
- c. Turn on the power strip located behind the microscope.
- d. Remove the laser module from the black plastic storage case. Place it flat on the lab bench.
- e. Remove the top-mounted flow cell from the storage case and place it on the laser module, lining up the mounting holes. Locate the four mounting screws in the storage case and use them to attach the cell to the module. The syringe fittings should be facing

- you. Tighten the screws until they bottom in the mounting holes.
- f. Screw the threaded Luer fittings into the flow cell as shown in Figure 2a. If using the syringe pump, screw the threaded Luer fittings into the flow cell as shown in Figure 2b.
 - g. Open the operating software. The software home page will load (Figure 3).

2. Rinsing the sample cell and loading a sample.

- a. Obtain a 100 ml beaker for sample waste and a 50 ml beaker with clean, DI water.
- b. Obtain two 1 ml syringes, located on the shelf above the NanoSight or the drawers beneath it.

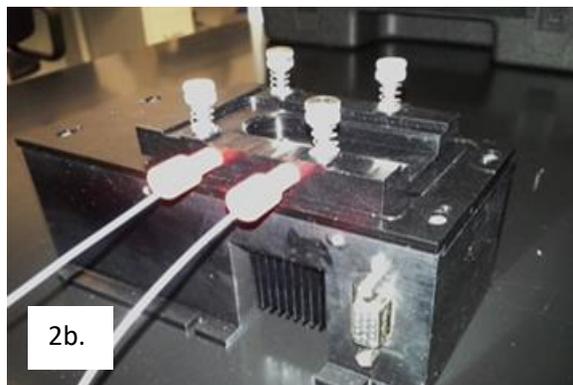
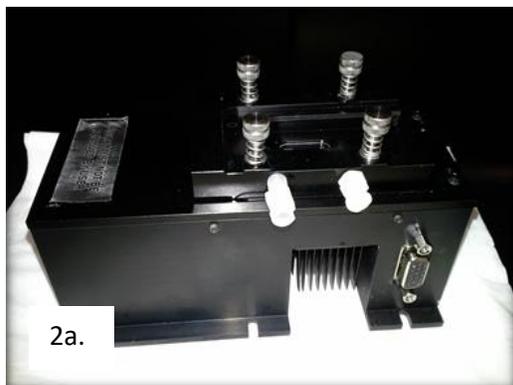


Figure 2. Laser module with flow cell and Luer plumbing attached.
 Fig 2a. Luer syringe fittings Fig 2b. Luer fitting plus 1/16" tubing for syringe pump.

- c. Stand the laser module on end for sample filling, so that the exhaust port on the flow cell is on top.
- d. Fill a syringe with clean DI water and slip the end of the syringe into the lower Luer fitting
- e. Slowly depress the syringe plunger to fill the sample cell with clean water. Watch the liquid fill the cell as it wets the inside of the cell window. Continue to fill the cell until liquid passes out of the drain (upper) port.
- f. Flush the cell with four full syringes of DI water.
- g. Remove the remaining DI water fill by slowly withdrawing the syringe. Try to remove all the water.
- h. Fill a second 1 ml syringe with your sample dispersion. Fit it into the input Luer fitting and slowly depress the plunger, while watching the flow cell window. Continue to fill the cell until liquid just begins to pass out of the drain (upper) port. Leave the syringe in the port.
- i. Mount the laser module/flow cell on the grooved portion of the microscope stage by sliding it laterally from left to right until it can go no further. The objective lens should be positioned above the flow cell window. If not, use the stage motion controls to position it correctly.

3. Aligning the Optics. To make a measurement, the flow cell window must be aligned precisely in the microscope field of view. To align, follow these steps. In the following, the

microscope stage movement is discussed in terms of the “x direction” (left-right), the “y direction” (toward the front or rear of the microscope), and the “z direction” (vertical position).

- a. Plug the multipin connector into the laser module.
- b. Turn on the laser power using the switch on the front of the control box.
- c. Power up the camera by selecting “Start Camera” in the camera pane (upper right hand panel). Adjust the camera gain to 12.
- d. Slide the light path lever on the microscope so that the light path is directed to the eyepieces, not the camera.
- e. Look into the eyepieces. Using the microscope stage motion controls, move the laser module in the x and y directions so that the left edge of the flow cell window just enters the microscope field. This will look like a bright purple band of light.
- f. Slowly move the module in the x direction leftward while observing through the eyepieces. As the cell window passes beneath the objective, you should see a prominent laser diffraction spot that looks somewhat like a thumbprint.
- g. After moving the cell sufficiently leftward, you will see a violet line running through the visual field from top to bottom. This is the edge of the view window. Use the microscope focus knob to bring this line into focus. If the line is dim, turn up the camera setting.
- h. If you do not see the window edge, repeat steps e-h. Check to ensure that the stage is positioned so that the center of the objective is aligned with the y-direction midline of the flow cell window. Also verify that the stage is in the correct z position by looking at the reference marks on the microscope arm.
- i. Once the line has been located, position it in the center of the eyepiece view. Slide the light path lever on the microscope so that the light path is directed to the camera. You should now see the line in the monitor view. If not, use the stage x direction controls to get it into the camera field of view.
- j. Turn down the camera setting to 7 or 8. Adjust the stage in the y direction to find the

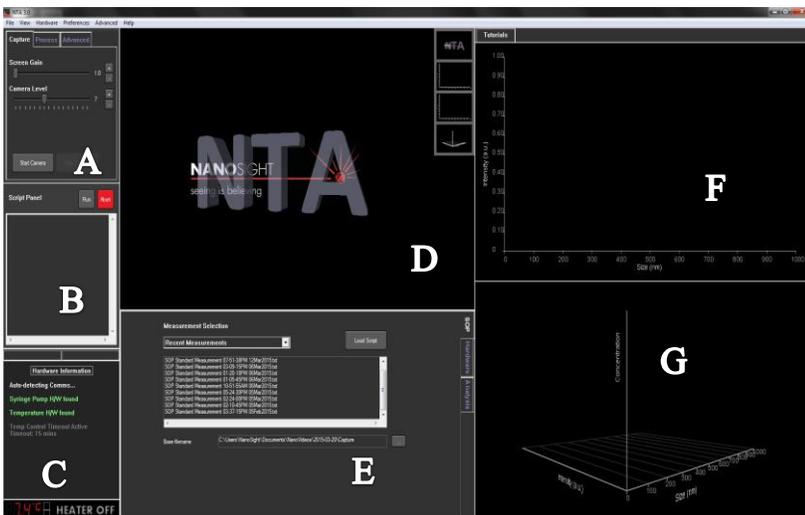


Figure 3. The NTA software main screen. Components:

- A. Camera and image processing controls
- B. Script window
- C. Equipment status window
- D. Results plot and camera output
- E. Measurement window (SOP and hardware controls)
- F. Results plot #2
- G. Results plot #3

area of brightest laser intensity. This is the path of the laser through the cell. Center this beam path in the camera field of view.

- k. Move the stage further left until the line moves just out of the field of view. You are

now focused on the correct observation volume of the flow cell.

- l. You should now see the particles of your sample come into view. If your particles do not scatter light strongly, a higher value of camera gain can be used.
- m. At this point you can deliver your sample into the NanoSight manually, or place the syringe into the syringe pump and set it up for automatic delivery. See staff for information on using the syringe pump.

4. Using a Standard Operating Procedure (SOP). To make a measurement, you must design an SOP (run recipe) or load a previously saved recipe script. The SOP defines the processing steps used by the system, sets observing and analysis parameters, and controls accessories like the syringe pump and the temperature block on the laser module.

- a. SOPs are managed in the Measurement panel, in the lower center portion of the control screen. In the panel, select the SOP tab. The dropdown menu offers the choices of
 - i. Recent measurement: a list of previously used SOPs is shown. Any of these SOPs can be loaded by double clicking on them. This is useful if you repeatedly use the same SOP.
 - ii. Quick measurement: this is for a fast single look at your sample. You'll need to specify the measurement duration, the file name, whether you wish to use temperature control, and how to process the data.
 - iii. Standard measurement: gives you the most flexibility in designing measurements. In addition to measurement duration, file name, temperature control, and data processing, you can specify the number of repeated measurements; by clicking on "Advanced", you can set the NanoSight to use the syringe pump and to run in liquids other than water.
 - iv. Zeta measurement: not enabled in our system.
- b. If using a pre-existing SOP from the Recent Measurement list, double click on the SOP to load it. The steps of the SOP will appear in the Script window to the left.
- c. If you need to design a new SOP, make the selections in the Standard Measurement window. If you plan to use the syringe pump, use the Advanced tab to set up the pump feed speed. If using temperature control, select that box in the SOP window and enter the desired temperature. More controls for the syringe pump and temperature control are found in the Hardware tab of the Measurement panel. When done, click on "Create Script" to load the script into the script window.
- d. **IMPORTANT:** Specify the file name and path to store files where you want them. The default location is on the D drive under "MNC Users". If there isn't already a folder in MNC Users with your last name, create one now.

5. Running the sample. Once your sample has been loaded into the flow cell, and an SOP has been loaded into the script window, you are ready to run your sample.

- a. In the script panel, click on "Run".
- b. You will be prompted to set the camera gain so that you can see particles in the live image screen. Set the gain and fine-focus the microscope so that the majority of the particles are in focus. Also ensure that the flow cell's measurement volume is centered in the field of view. If not, use the microscope's stage controls to re-center it.
- c. When the image is centered and in focus, click "Okay" to continue the SOP script.
- d. If you are using a manual syringe feed, you will be prompted to "Advance the sample" by depressing syringe slightly to supply new sample.

- e. The NanoSight will begin a video recording of particle motion. This will be recorded for as long as you specified in the SOP.
- f. If you specified multiple captures in your SOP, you will be prompted to “Advance the sample” after each measurement.

6. Data Analysis. When the recording is complete, the NanoSight goes into data processing mode. You will be prompted to input the process parameters, namely, the detection threshold (set in the Camera and Image Processing Controls window at upper left). This tells the NanoSight how low a scattered light signal should be counted as a real particle. The threshold value should generally be between 5 and 10; above 10 the system will miss the small particles, and lower than 5 leads to counting noise and spurious signals.

After setting the threshold value, click OK on the dialog box. The NanoSight software then analyzes the video recording frame by frame and shows particle diffusion tracks. From this data the software derives the particle size distribution and plots the distribution over the particle image as a number distribution plot, that is, the concentration of particles (per ml) versus particle size in the range 1 to 1000 nanometers. Several other representations of the distribution are also plotted on the screen.

Measurement results are listed in the “Merged Results” tab of the Measurement window below the results graph. The particle concentration and mean and peak particle size (mode) will be given in this panel. More information about the run will be found under the “Analysis” tab.

7. Exporting your data. When the data is displayed on the screen, you can right click on the plot to save the graph on the hard drive as a PDF or Bitmap file. The measurement data may be exported in comma separated variable (CSV) format by clicking the “Export Results” button. The data is written to the folder you specify.

C. Tool Shutdown

When your work is complete:

1. Turn off the laser control switch and the power strip.
2. Remove the connector from the laser module. Remove the laser module from the stage and place it on the bench top.
3. Withdraw your sample from the flow cell by drawing it out with the syringe. Discard it into the waste container
4. Fill a syringe with clean water and connect it to the flow cell. Slowly depress the syringe plunger to flush the cell with clean water. Rinse with 3-4 syringes full of clean water.
5. Remove the Luer fittings and return them to the black plastic storage case.
6. Unscrew the top bolts and disassemble the flow cell. Use a Kim wipe to wick up the bulk liquid.
7. Using lens paper ONLY, GENTLY wipe the optical windows of the laser module and flow cell. These are optically coated surfaces, so do not rub hard--wipe in a straight line across the window using minimal pressure. If you are unable to remove a spot from window after 1-2 gentle passes with lens cleaning paper, STOP and inform MNC staff.
8. Replace the laser module and flow cell components in the black plastic storage case.

9. Clean the work area and place used beakers near the sink for cleaning. Replace the dust cover on the microscope.
10. Log out of the computer.