

University of Minnesota Nano Center Standard Operating Procedure

Equipment Name: DLS Particle Analyzer

Model: Microtrac NanoFlex

Location: PAN 185

Revision Number: 4

Revisionist: J. Marti

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A. Introduction

1. **Tool Description.** The Microtrac NanoFlex is a nanoparticle characterization tool that uses dynamic light scattering to derive size distributions for particle dispersions in the size range 1 nanometer to 6 micrometers. Nanoparticle samples may be suspended in aqueous or organic liquids, as long as the viscosity and refractive index of the material are known.

2. **Tool Components.** Figure 1 shows a front view of the NanoFlex.

Key components visible in this view are

- a. On/off indicator
- b. Sample probe
- c. Sample stage
- d. On/off switch
(on back panel)



Fig. 1. The NanoFlex DLS.

3. **Safety.** No special safety precautions apply.

4. **Restrictions/requirements.** Samples must be suspended in a liquid medium, and must be in the correct concentration range. The exact range will depend on the material being analyzed; the NanoFlex will indicate when the correct range is reached.

5. **Required Facilities.** 120V AC power; connection to a Windows-based computer; a source of deionized lab-grade water.

6. Definitions.

- a. 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).
- b. Dynamic light scattering (DLS) is a technique to estimate the size distribution of particles under a micron in size. The DLS process directs 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. A small amount of light is scattered from each moving particle back into the detector; this scattered light will be very slightly changed in frequency due to Doppler shifting by the moving particles. This frequency shift is used to calculate the Brownian motion of the particle, and by extension, its mass. Knowing the particle material allows the system to calculate particle volume, surface area, and diameter.

- c. Dispersion: particles stably suspended in a liquid medium.

B. Using the NanoFlex

1. 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. If not already on, turn on the NanoFlex, then the computer. Log into the computer using your own X500 user name and password. This enables you to store files in your own My Documents folder, secure from other users.
- c. Open the operating software, Microtrac Flex. The software home page will load (Figure 2).

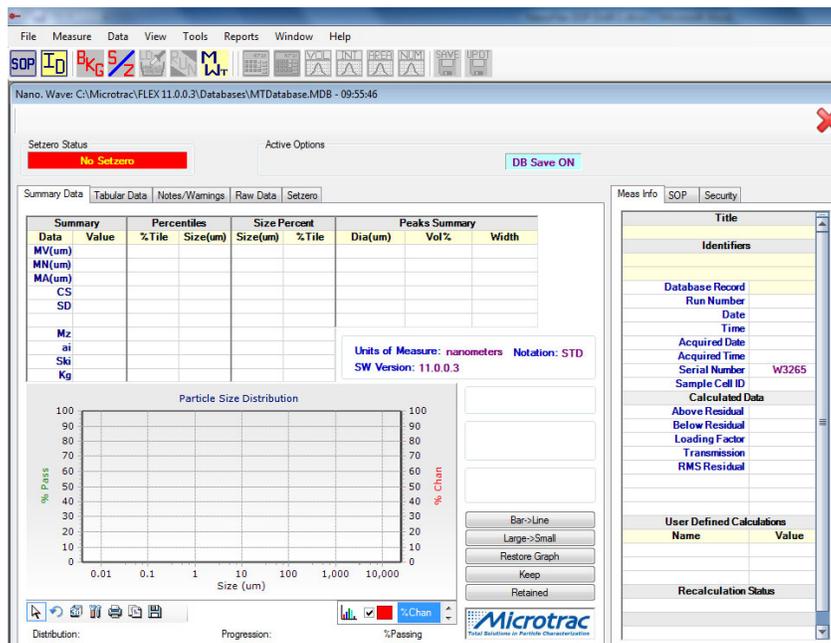


Figure 2. Flex software home page.

2. Sample preparation

- a. Particles to be measured can be any material and can be suspended in any liquid, although the use of high viscosity liquids will limit the available measurement range.
- b. Particles should be small enough to be readily dispersed in the fluid, that is, there should be little or no settling 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.
- c. The dispersion must be at a sufficient concentration to generate an acceptable signal. The NanoFlex system will indicate whether there are enough particles present for a good measurement. See “Running the Sample” below.

- 3. **Using a Standard Operating Procedure (SOP).** Before making a measurement, you must select an SOP (run recipe) from a list of pre-defined procedures. The SOP defines the material properties of your sample (particle and liquid), how many times the measurement is to be carried and for how long, and several other required parameters. If none of the

pre-defined SOPs meet your needs, you can edit an existing SOP and save it under a new name.

- a. In the Flex operating screen, click on the “SOP” button at the upper left. The “Measurement SOP” dialog box will appear. The top part of the box shows which SOP is currently loaded. The middle portion provides two drop-down menus; the upper menu allows you to select and load a pre-defined SOP. If you are using an SOP you’ve previously defined, select that SOP and then close the “Measurement SOP” box.
- b. If you need to define a new SOP, type a name for your new SOP in the second drop-down menu.
- c. Click the “Options” button in the lower right. This brings up a series of screens with tabs that allow you to customize the SOP. The main tabs to modify are Timing and Analysis.
- d. Under the Timing tab, input the number of seconds to measure your sample; 30 seconds is the default value, but 60 seconds is a better choice for small particles (under 100nm). As a rule of thumb, larger particles (> 500 nm) will require longer run times (90 sec or more). You can also specify repeated measurements to determine repeatability and uncertainty.
- e. The Timing tab also provides options for setting the zero (background) signal. See section B 4 for more information on this feature.
- f. Under the Analysis tab, there are several sub-tabs. The first is Particle Information, where you enter particle parameters.
 - i. Check the Save/Recall drop down menu to see if your particle has a pre-defined parameter set; if so, select it with the drop-down menu, and go to item f below.
 - ii. If your particle does not have a pre-defined parameter set, enter the name of the particle material in the second drop down menu.
 - iii. Indicate if the material is expected to be absorbing or transparent. If the latter, provide an estimate of the refractive index of the material. For your convenience, refractive index values can be obtained for hundreds of materials by clicking on the “Particle Reference” folder icon.
 - iv. Indicate whether this material is expected to be spherical (usually the case for particles synthesized chemically) or irregular (the case for ground or milled particles).
 - v. After setting these parameters, click the Save button to make this particle parameter set a permanent part of the program database. You may now call it up for future measurements.
- g. The next sub-tab under Analysis is for entering information on the fluid suspension medium. Check the Save/Recall drop down menu to see if your suspension liquid has a pre-defined parameter set; if so, select it with the drop-down menu. If not, enter the name of the suspension liquid in the second drop down menu. The main numbers needed to define this material are index of refractive and liquid viscosity.
 - i. Refractive index values for most common suspension liquids can be obtained by clicking on the “Fluid Reference” folder icon.
 - ii. The same reference table gives information on viscosity vs. temperature for these liquids.
- h. Enter the information as required, then click the Save button to make this fluid parameter set a permanent part of the program database.
- i. The last box to update is the Sample ID information. The ID has four data fields to

fill in. The first is for the sample name. Field 2 may be used for any sample label or descriptor you choose. Field 3 should be your last name. This enables easier searching of data records after the measurements have been taken. Field 4 allows for up to 255 characters of sample description. The sample ID can be updated prior to each measurement.

- j. After Timing, Analysis, and ID parameters have been set, select OK to close the SOP Options box and return to the Measurement SOP dialogue box. Click on the floppy disk icon to save the new SOP. You will be returned to the software home page. The SOP is now saved or updated and is loaded for running.
- 4. Setting zero.** The detector of the NanoFlex has some intrinsic electronic noise that must be subtracted from the signal. This is done via the Setzero function. If you have checked “Enable Auto-Setzero” in your SOP, this will be done automatically at startup.
- a. If you’d like to manually determine the zero level, open the Timing tab on the SOP Options box, uncheck the Enable Auto-Setzero box, enter 60 seconds as the setzero time, and close the SOP Options box.
 - b. Place the NanoFlex probe in a small beaker of clean, DI water.
 - c. At the top of the main user interface, click on “SZ”. You will go to the Setzero screen.
 - d. The system will automatically collect a signal blank, and calculate a correction factor. After 60 sec, the system will announce whether it has acquired a sufficient set zero value.

If the zero value was not set successfully, check the following.

- e. Blank sample—was it clean and particle free? If not, discard and replace with a clean beaker full of fresh filtered DI water from the lab DI water supply, and repeat the set zero measurement..
 - f. Lab bench—were there vibrations during the zero acquisition? Simply drumming fingers on the table or moving the keyboard could degrade the signal. Repeat the set zero test, taking care to minimize all vibrations.
 - g. Is the probe dirty from a previous measurement? To check, place the sample probe in a fresh clean water sample, then click on the “LD” (Load) button in the main interface. In the Load screen, select the “Rinse Sample Cell” button. The system will return a vertical bar graph which shows the amount of reflected laser power from the blank sample. Any level above 0.01 indicates a dirty probe. A clean probe window should give a loading index value below 0.001 and yield a “Rinse Complete” status indicator.
 - h. To clean the probe: first try a small amount of isopropanol on a lab wipe, then collect another background measurement. If this fails to fully clean the probe, add 1-2 drops of Dawn detergent to 30ml of DI water, and use this detergent solution and a soft brush to clean the probe. Rinse the probe by immersing in 400 ml of DI water.
 - i. Recheck the background to ensure it is below the 0.01 level; if not, repeat step iv. When the probe is sufficiently clean, repeat the set zero process.
- 5. Loading the sample.** Once the SOP has been selected and loaded, and the zero level has been set, you are ready to test your sample. The NanoFlex requires a certain range of signal in order to get a reliable measurement, which depends on liquid viscosity and particle

concentration. This loading range is determined using the LD (Load) screen.

- a. Click on the LD button on the main interface. You will see a vertical scale indicating reflected laser power, with the optimum range indicated in green.
 - b. If your sample is optically opaque (high concentration), place the probe in a beaker of clean DI water, and add your sample dropwise to the beaker. Watch the loading scale and add sample until the scale is in the green range; ensure that the loading does not exceed the maximum level.
 - c. If your sample is optically clear or nearly so (low concentration), position the probe directly in your sample. Verify that the reflected power is above the minimum required level.
 - d. If the reflected power is under the minimum required level and you cannot add more sample, you may need to concentrate your sample by centrifugation. Consult the lab manager for assistance.
- 6. Running the sample.** Once the correct loading has been achieved, click on the “Run” button (either in the LD screen or on the main interface). You will be prompted to review or update the sample ID info. Once you hit okay, the system will run a measurement for the time you selected in the SOP.

The results will be plotted on the screen as a distribution plot of the percentage of the particles in the sample versus particle size in the range 0.1 to 6000 nanometers. You have several options on how to plot this data.

- a. Number distribution. This gives the percentage of all particles in the sample that lie in each size channel (a channel corresponds to a narrow diameter range, which can be defined in the operating software).
- b. Volume distribution gives the percentage of the total particle volume in the sample that lies in each size channel. Since large particles in a sample represent most of the total particulate volume, the volume distribution is dominated by the larger particles.
- c. Size distributions can also be given in terms of particle surface area.

Which measure of size distribution to use will depend on what you are looking to find out about your sample—consult MNC staff to learn more. After the data has been collected, the Microtrac software can switch between number, area, and volume distributions without having to re-test the sample.

- 7. Exporting your data.** When the data is displayed on the screen, you can right click on the plot to either print the graph in room 185 or export the data digitally. The data may be exported as comma separated variable (CSV) format, which makes it easy to export into spreadsheet or scientific graphing programs. The graph may be exported as a PDF file, if you would like to insert the graph into a word processing or presentation file.
- 8. Using the database.** All measurements taken with the NanoFlex are recorded in a database named MTDatabase. To access a data record, go to the File tab on the home page, select the “Open Database for Recall” command, and select the current database file (see MNC staff for the file in current use). A new Database screen will appear. Click on the highlighted “DBR” (data base retrieval) button. You will be given a query form which enables you to select the data sets from previous measurements (collectively known as “records”). You

may search by record date range, or by any value in any of the record label fields. Click the “enable” box to allow searching by data field. Note that since the third data field in the sample ID should consist of the last name of the operator, you should be able to search for all your records at once by using this field.

After you have entered the data on the query form, clicking on the next tab (“Query Results”) will perform the search and bring up the records matching your queries. If no search fields are used, or if you forget to click the “enable” box, all records in the database will be returned, sorted by date.

Select the records that you wish to view or export by highlighting them with the mouse, then click “Retrieve”. Multiple records may be selected using the mouse while depressing the shift and control keys. Retrieved records will be plotted using the same format as that of live measurements, except that a few new buttons are enabled. If you selected more than one record, you can scan through them using the “Next” and “Prev” buttons. The recalled data may be used to recalculate a distribution (for example, from volume to number distribution), exported to another data format, or printed using the local printer.

C. Tool Shutdown

When your work is complete:

1. Remove the probe from your sample and remove your sample from the stage.
2. Clean the probe with isopropanol and a lab wipe, as described in section B 4h above.
3. Immerse the probe in a small beaker with clean DI water.
4. Clean the work area and place used beakers near the sink for cleaning.
5. Log out of the computer.
6. Update the NanoFlex log book if you need to note any issues you encountered during your run.