# University of Minnesota Nano Center Standard Operating Procedure

**Equipment Name:** Particle Analyzer
**Model:** Malvern Zetasizer Nano ZS
**Location:** PAN 185
**Revision Number:** 2
**Revisionist**: J. Marti
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## A. Introduction

1. **Tool Description.** The Malvern Zetasizer is a characterization tool that measures particle size distributions and zeta potentials. The size detection uses the dynamic light scattering (DLS) method for particles in the 3 nanometers to 6 micrometers size range. Zeta measurements use electrophoretic mobility and cover particles between 3 nm and 100 um.

2. **Tool Components**. The figure below shows a front view of the Zetasizer. Key components visible are
   a. Sample well (open)
   b. Ready indicator
   c. Side drawer for cuvette storage

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.

5. **Required Facilities.** 120V AC power; connection to a Windows-based computer.

6. **Definitions**
   a. *Particle size distribution* (PSD) is 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. *Zeta potential.* When small particles are placed in a polar medium like water, their surfaces will acquire an electrostatic charge via several mechanisms. This surface charge will attract oppositely charged counterions from the liquid, leading to a structure called the double layer. The ions close to the surface will move with the particle, while those farther away will move with the liquid; the boundary between these ion populations is called the slipping plane. The electric field difference between the particle surface and the slipping plane, when expressed as a voltage, is called the zeta potential of the particles, often represented as the Greek letter ⊗. Zeta potential depends on the material, the medium, and the ionic environment of the particle )(pH, salinity, etc). Values of ⊗ typically range from near zero to ±200 millivolts (mV).

d. *Electrophoresis* is the application of an oscillating electric field to a volume containing charged particles. As the particles move in response to the changing field, their motion is measured and their zeta potential is derived.

## B. Preparing the Zetasizer

### 1. Setup

a. Begin a new entry on the tool log sheet. List your name, the date, the machine mode you are using (size, zeta potential, or both), the materials you are working with (particle material(s) and dispersing fluid), and any issues you encountered during your run.

b. The Zetasizer will normally be left on. If it has been turned off, allow it to warm up for 30 minutes prior to use.

c. 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.

d. Open the Zetasizer operating software. You will prompted to enter a name that will identify your measurement records.

e. The first page that loads will be a measurement file (Figure 2), which is where all your measurement results will be stored. Upon first use, the program will show a default file. You will want to set up a new measurement file for your own data, as follows:

   i. Go to File\New\Measurement File.

   ii. Set up and name your measurement and select its location.

iii. This measurement file will open by default on your next use. You can always access other measurement files by selecting File\Open\Measurement File.

2. **The Measurement File Screen: Menus, Toolbars, and Tabs.** The top of the measurement screen has the standard Windows menu bar (File, Edit, View, etc). Below the menu bar are several toolbars that control what records can be viewed and what operating procedures are to be used.
   a. The first toolbar selects the *Workspace*, which has a dropdown menu to allow you to select the type of measurement results to be displayed. You will typically choose between Size, Zeta, and Surface Zeta (not all of the measurement modes have been enabled on our system).
   b. Also in the Workspace toolbar is a dropdown menu of defined SOPs.
   c. Below the Workspace are a set of tabs which determine how the results are displayed. The Records View provides an overview listing of all records in the measurement file. Each record line displays the measurements for that sample, including sample name, measurement date and time, temperature, and items relevant to the measurement (e.g., Z-average particle size, zeta potential, etc.). Other columns can be added by the user.
   d. The remaining tabs will vary according to the function selection in the Workspace. For example, with Size selected, the tabs allow the user to plot the size results of a given record as am intensity distribution, a volume distribution, or a number distribution; additional tabs provide informaiton on the details of the signal collection and processing. For Zeta measurements, tabs allow you to plot the ZP distribution and judge the quality of the results.
   e. You may add or remove tabs from any workspace by going to Tools\Settings\Configure Workspace, then selecting which workspace (Size, Zeta) you wish to modify. A screen will come up with three tabs. Click on Report Pages to see a list of tabs that can be added to the records page.
   f. If not already present, add Volume PSD and Number PSD tabs to the measurement file screen by checking the appropriate boxes.

3. **Working with a Standard Operating Procedure (SOP)**. Before making a measurement, you must select or write an SOP (i.e., a run recipe). 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. The software has several pre-defined SOPs; if none of these meet your needs, you can edit an existing SOP and save it under a new name.
   a. To draft a new SOP, go to File\New\SOP (or select the icon in the Menu bar). To modify an existing SOP, go to File\Open\SOP. Both these steps open the SOP Editor
   b. The SOP editor brings you to a Windows file explorer screen where you will choose the type of measurement (size, zeta potential, etc.) desired.
   c. The SOP editor screen has a list structure for the parameters that need to be added. Go down the list and add the information. Some fields are optional, but you must first specify the measurement type (size, zeta, etc.)
   d. Under “Sample”, provide a sample name. Add any details you like in the text box.
      i. Provide the properties of the dispersed material (refractive index, absorption) and the dispersant liquid (refractive index, viscosity, dielectric constant). Many

of these data are available in drop-down menus on the SOP editor screen. If you do not have this info for your sample, consult lab staff.

ii. Under "Temperature" you can set the sample cell at any temperature between 10 and 70°C. A minimum equilibration time of 60 sec is recommended; use a longer time for higher temps.

iii. Under "Cell type" you will be given options on the type of sample cell to use, depending on the measurement type.

e. Under "Measurement" you will specify the duration and number of measurements.

f. Under "Data" you can set up reports and control how the data is to be exported in text or comma separated variable (CSV) formats.

g. When done setting up the SOP, click on the "Save" (for a new SOP) or "Save as" (for a modified SOP). Name your SOP and select a file location.

## C. Measuring Particle Size Distribution

### 1. 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 Zetasizer software will indicate whether there was sufficient concentration *after* measurement has been completed. See "Running the Sample" below.

**2. Loading the sample.** There are two cuvette options.

a. If you have > 1 ml of sample, use the standard 10mm polystyrene cuvettes. Add about 1ml dispersion to the cuvette with a pipet. The instrument lid has a diagram guide to how much to add. Place a cap on the cuvette after filling.

b. For very small volume samples, use a small volume polystyrene cuvette, which allows as little as 25 uL of dispersion to be analyzed. Place a cap on the cuvette after filling.

c. The Zetasizer can measure at high temperatures (up to 90°C). Do not use polystyrene cuvettes above 70°C; use glass or quartz for these cases.

### 3. Running the sample

a. Place the cuvette securely into the well and close the lid. Note that the small volume cuvette has an orientation mark which must face the front of the analyzer.

b. Select "Measure" and select the desired SOP.

c. Select the green "Play" button to start the measurement.

d. The system will show measurement progress and display the results of each run.

e. A tone will sound when measurement is complete.

## D. Measuring Zeta Potential

### 1. Sample preparation

a. Particles should be suspended in aqueous solutions with a minimum level of conductivity. Malvern recommends a dispersant with 10 mM NaCl, equivalent to 0.1x PBS. This may require that your samples are diluted with PBS or other electrolyte prior to measurement. Because of its very low conductivity, deionized water should be avoided as a dispersant.
b. If you wish to measure the zeta potential of particles suspended in nonpolar media, a different fitting model will be needed. Consult MNC lab staff.
c. Zeta potential is function of dispersion pH. For a complete measurement, use a meter to establish the pH of your dispersion. If you need to map out the zeta potential vs pH curve, see lab staff for assistance.

**2. Loading the sample.** There are two cuvette options.

a. Reusable dip cell. Ensure that the dip cell is cleaned before use and in between dissimilar samples.
   i. To clean, place the dip cell in a clean square cuvette with 1-2 ml of DI water. Stand the cuvette up in a 20 ml beaker with water, and bath sonicate the beaker for 5 minutes.
   ii. Pipet your dispersion into a square cuvette and insert the dip cell into the cuvette. A minimum of 1 mL is needed for this cell. iii. Insert the cuvette/dip cell securely into the cuvette well. Ensure that the green Malvern logo on the top of the dip cell is upright.
b. Folded capillary cell (minimum capacity 0.75 ml): must be rinsed prior to use, as follows.
   i. Flush the cell with methanol.
   ii. Fill a 30 ml syringe with DI water and attach to one of the cell's Luer ports. iii. Attach a second empty 30 ml syringe to the other Luer port.
   iv. Depress the syringe plunger to force water through the cell to fill the other syringe.
   v. When this is full, force the water back through the cell into the first syringe. Repeat this 4 times to thoroughly flush the cell.
   vi. After the folded capillary cell has been flushed, withdraw the water using a syringe. Add your sample through one of the ports using a pipet or syringe. vii. Place the plug caps in both Luer ports.
   viii. Attach the small metal plates to the front and back of the cell. These help maintain a uniform electric field during measurement. ix. Insert the folded cell with the attached plates securely into the cuvette well. Ensure that the Luer ports face the front of the Zetasizer.

### 3. Running the sample

a. After inserting the cuvette or cell fully into the well, close the lid.
b. Select "Measure" and select the desired SOP.
f. Select the green "Play" button to start the measurement.

g. The system will show measurement progress and display the results of each run.
c. A tone will sound when measurement is complete.

## E. Other Measurement Types

1. Other types of measurements that the Zetasizer is capable of include
   a. Molecular weight
   b. Zeta potential of flat surfaces
2. Consult with lab staff if you'd like to do these measurements.

## F. Displaying and Exporting Results

**1.** All measurement results will be listed on the Measurement File, one measurement record per line. Selecting a record and a tab will display the results; by selecting and opening multiple records of the same type, you can get a plot of all the results on the same graph.

**2.** The system software has error checking routines that can flag questionable or low quality results. Details on any problems with the measurement will be found by Quality Report tab. If you receive an error warning, consult lab staff.

**3.** Particle size measurements
   a. The default distribution given is the *intensity distribution*, which is the most basic output of the DLS method. This distribution gives two primary measurements, the Z average size and a polydispersity index (PDI) value.
   b. The intensity distribution can be greatly affected by the presence of a small number of larger particles, such as contaminants. For a more useful view of the particle distribution, obtain the *volume distribution*, which can be viewed by selecting the *Volume PSD* tab.
   c. The *Volume distribution* gives the percentage of the total particle volume in the sample that lies in each size channel (a channel corresponds to a narrow diameter range, which can be defined in the operating software). Since large particles in a sample represent most of the total particulate volume, the volume distribution is dominated by the larger particles.
   d. A second alternative way to look at your data is the *number distribution*. This gives the percentage of all particles in the sample that lie in each size channel. Because this is a derived distribution that is two steps away from the original data, it is subject to large errors, especially if the original data set is noisy, and so is not recommended.
   e. 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.
   f. The Expert Advice tab reports on the quality of the measurement. It will show whether the data display any unwanted attributes, such as particle aggregation.

**4.** Zeta potential measurements
   a. The default result displayed is the electrophoretic mobility distribution. To see this plot, add the zeta potential distribution tab if it is not already present.
   b. Other useful tabs for zeta potential measurements include the count rate and phase plot, which allow the user to judge measurement quality.
   c. The Expert Advice tab reports on the quality of the measurement.
   d. A zeta measurement within 1-2 mV of zero is probably a sign of a defective measurement or problems with sample prep. Consult lab staff for guidance.

5. **Exporting data.** Data from one or more records, as well as graph and table data, can be exported to other applications such as Excel, Word, or Wordpad, as well as printed to a PDF document. There are several ways to export the required information.
    a. A one page summary record in PDF format can be produced by opening one or more records in one of the PSD tabs (Intensity, Volume, or Number), then printing the page via the Control-P key. When prompted for the destination printer, select "Microsoft PDF". You'll be prompted for a name and folder in which to store the document.
    b. To export the X-Y values for one or more size distribution graphs, select the graphs and view in your preferred distribution (intensity, volume, or number). Then select Edit\Copy Size Values. The X-Y data is copied to a clipboard, and may be directly added to an open spreadsheet by selecting any cell and using the Paste (Control V) function. NOTE: This does not work for zeta potential graphs.
    c. A table or graph can be dragged and dropped into another application by holding the Ctrl key, selecting the item and dragging into the application.
    d. A table or graph can be copied by selecting the item then selecting Edit\Copy. The item can then be pasted into another application.
    e. Any of the parameters from one or more records can be exported as a text file, which can then be loaded into a Word document or a spreadsheet.
    f. Export templates can be used to specify which record parameters are exported. The Tools\Settings\Data export templates window allows users to create and modify templates.
    g. To export the summary data shown in the Records View of the Measurement file, select one or more records, then select File\Export. This opens a dialog box which gives you the option of copying to a new text file, or copying onto the clipboard for later pasting.

## F. Tool Shutdown

When your work is complete:

1. Remove the cuvette from the Zetasizer. Drain the sample and dispose of appropriately. Plastic cuvettes are single-use and can be discarded after rinsing. Place reusable glass or quartz cuvettes in the used cuvette beaker
2. If the dip cell was used, place it in a clean square cuvette with 1-2 ml of DI water. Stand the cuvette up in a 20 ml beaker about ½ filled with water, and bath-sonicate the beaker for 5 minutes. When done, remove the cell and allow to air dry.
3. Clean the work area.
4. Log out of the computer; leave the Zetasizer power on.
5. Update the Zetasizer log book if you need to note any issues you encountered during your run.