NanoSight Zeta Measurement

Quick Start Guide

P573B

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This quick start guide is designed to accompany the 'NTA Capture and Analysis Quick Start Guide' and the 'NS500 Quick Start Guide'. A sound understanding of standard size measurements using the NS500 is an absolute prerequisite to run zeta analysis.

Instrument initialisation and sample loading

Zeta mode must be enabled in the NTA Software to perform zeta capture and analysis; this option can be found in the **Advanced** menu. Please refer to the NS500 quick start guide for details on instrument initialisation and sample loading. In addition to the NTA stage and temperature communications, the zeta communications must also be started prior to opening the software.

System check

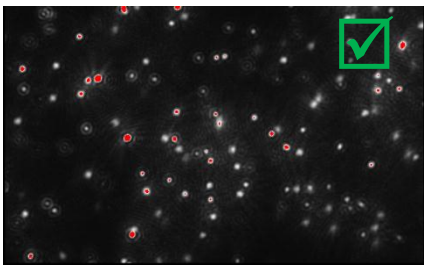
! The following steps are extremely important to ensure that the diluent flow profile is calculated accurately. Errors here can have a significant impact on the measurement of the zeta potential. Please refer to the document **P575 Zeta Potential Analysis Troubleshooting Guide** if you have any problems during these steps.

Zero position check

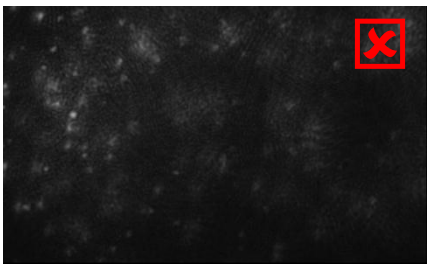
Go to the zero stage and focus position (click **GOTO ZERO** in the NS500 Control window). The 'thumbprint' region where the laser emerges should be in the centre of the field of view and the static 'particles' seen should be in focus; if not, correct the stage and focus manually as required and **Set Focus/Stage Zero** in the **Advanced** menu.

Capture positions check

Check zeta positions 1 to 6; in each position the majority of particles should be in focus. If the zero position appears correct but the programmed points do not display particles in focus, it may be that the refractive index of your diluent used is significantly different to the diluent used on setup (water) or that the system has become misaligned.



Majority of particles in focus



Particles out of focus

Electrode and voltage check

Go to different user positions, apply positive and negative voltages and check if there is any visible particle motion. At user position 3 (near the middle of the sample cell) electro-osmosis dominates for most samples, and significant particle motion should generally be seen.

High conductivity samples: When using high conductivity samples thermal flow may be seen when the voltage is switched on. This causes fast flow speeds and sometimes a change in direction of flow after a few seconds of switching the voltage on. In this case it is recommended that voltage is reduced (minimum of 10V is recommended) and delay times are increased to allow the flow to stabilise (to around 20 seconds).

If no particle motion is visible or the current is unstable or lower than normal, an air pocket may be trapped under the electrodes preventing electrical contact. The electrodes should be removed to remove any air trapped underneath.

Disconnect the electrodes from the wired connector and unscrew the electrodes from the top plate using the tool provided. Advance sample until diluent is seen filling the electrode ports and there are no bubbles present in the channel. Screw the electrodes back in, absorbing any excess sample with a tissue, and reconnect the electrode wires.

Video capture

Before capturing a video you should set the di-electric constant of the diluent in the **Electrophoretic Mobility window** if necessary. It is set to $\varepsilon_r = 80.1$ (water at 24°C) by default.

The camera level should be set to give sufficient contrast to identify particles at all profile depths (zeta positions 1-6)

Video capture for zeta potential analysis is done via the **Script Control** capability**,** found in the **Advanced** menu. It is suggested that you start with a pre-built zeta script that is located in the *Scripts* folder in *My Documents* on a NanoSight supplied PC; this can be modified as necessary and saved for future use.

A zeta script should run a positive and negative capture at each of the 6 positions for 12 videos in total. All videos are used to calculate the diluent profile, but only the first video (captured at the first profile depth, labelled position 1 in the zeta positions bar) is used for the final zeta potential result.

To avoid overwriting existing files a new base file name should be set for each analysis. This can be done by clicking **Set Base File Name** in the script window.

Below is an explanation of the lines in the Zeta script:

```
SETTEMP 24                  // Set the temperature to 24°C
SETUSERVOLT 24              // Predefine the voltage as 24V
PUMPADV                     // Pump through some fresh sample
DELAY 10                    // Wait 10 seconds for the flow to stabilise
PINCHCLOSE                  // Close the pinch valve to close the system
DELAY 10

DEPTH 0                     // Proceeding videos are at depth 0 (position 1)
STAGE GA, -15500            // Move the stage to position 1
STAGE GZ, 43                // Move the focus to position 1
USERVOLTPOS                 // Set the voltage to positive
DELAY 10                    // Wait 10 seconds for the flow to stabilise (increase in case of thermal flow)
CAPTURE 60                  // Capture for 30 seconds
VOLTAGE 0                   // Set voltage to 0 (turn voltage off)
DELAY 10                    // Wait 10 seconds for the flow to stabilise
USERVOLTNEG                 // Set voltage to negative
…                           // Continue as for positive
DEPTH 1                     // Proceeding videos are at depth 1 (position 2)
…                           // Continue as for depth 0
CAPTURE 30                  // Captured videos can be shorter to save time
…                           // Continue through subsequent depths

PINCHOPEN                   // Open the pinch valve
```

For repeat runs on the same sample, it is recommended to pump fresh sample through between analysis, using a full **LOAD** command in the control panel. This ensures that all previously analysed sample in the system is replaced with fresh, avoiding the possibility of errors due to sample degradation under the applied electric field.

Analysis

Step 1 – Diluent profile analysis

In the first step of the analysis, the average drift velocity of the particles in each video is calculated to build up the profile of the electro-osmosis of the diluent. The complete video sequence should be loaded for analysis using the **Batch Process** window. The analysis settings for each file should be set to track the brightest and most in-focus particles in each video.

As the files are processed the profile will build up in the **Electrophoretic Mobility window**. When the profile is complete, a parabolic curve is fitted to the data, displayed in green on the right-hand graph.

An estimate of the average zeta potential, obtained from the combined videos is given at this point and displayed in the bottom right of the **Electrophoretic Mobility window**

If the profile obtained does not have the form of a smooth curve or if the difference in velocities at opposite polarities is less than 5000nm/s at all positions, please refer to the document **P575 Zeta Potential Analysis Troubleshooting Guide**, which describes possible causes and gives advice for improving data quality.

Step 2 – Zeta potential analysis

After the profile has been calculated and is displayed in the **Electrophoretic Mobility** window of the NTA software, the electro-osmosis can be removed to calculate the zeta potential of every particle tracked. Re-load the first video (at the optimal imaging location) to display the results on a particle-by-particle basis.
Analysis settings can be optimised at this point and the video re-processed to ensure that all particles seen in the video are being accurately tracked.

Results

After the zeta potential has been calculated for each particle the main graph can display either particle size vs. concentration or zeta potential vs. concentration; these options are available in the **Graphs** menu. Sample statistics are given in the bottom left hand corner and relate to the current graph being displayed. Additionally the 2D scatter plot displays zeta potential vs. particle size and the 3D scatter plot displays zeta potential vs. particle size vs. relative intensity.

To export a sample report while in Zeta mode, ensure that Report Style 1 (4 figures) is selected from the **Export** menu, and then **Set Sample Details** and **Print,** as for sizing analysis. If **Include Summary in Export** is selected, a summary file will be created when the first video is re-processed for the final zeta potential analysis. This file will contain 0.1mV resolution bin data for Zeta Potential against concentration, as well as the 1nm resolution bin data for size against concentration, surface area and volume.

For more detailed information and troubleshooting please refer to the documents **P559 Zeta Analysis Manual** and **P575 Zeta Potential Analysis Troubleshooting Guide**.

Quick start guides are also available for capture and analysis in NTA and for general use of the NS500; these can be found in the help menu of the NanoSight NTA Software.

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