SOFTWARE UPDATE NOTIFICATION (SUN) – NANOSIGHT NTA 3.1 (Iss1) SOFTWARE

PARTICLE SIZE

ZETA POTENTIAL

Introduction

This document details the release of the NanoSight NTA software version 3.1 (Iss1). It covers the upgrade and installation instructions, as well as additions and improvements in this release of the software.

Overview of new features

Below is a summary of the new improvements and features of NTA 3.1 compared to NTA 3.0. More details and instructions on the new features are available in the appendix at the end of this document.

Improved Automation
- Automatic focus available for NS300 and NS500
- Automatic camera level for all instruments

Data Display and Manipulation
- Flexible data overlays for line graphs and scatter plots
- CSV and PDF export options for overlaid data
- Ability to report concentrations between specific size limits
- Option to 'exclude' regions of particles from the scatter plot

Interface changes
- Quick access to recent experiments through dedicated tabbed window
- Experiments display overlaid graphs and scatterplots on load
- Improved flexibility and usability for graph settings
- Filter wheel has its own hardware tab, with customisable buttons
- Procedure guide for electrode air removal
- Warnings to remind user of essential hardware checks/maintenance

Additional improvements
- Refined camera levels to give more suitable settings for brighter particles
- Speed optimised size distribution algorithm runs up to 20 times faster
- Refined focus and stage controls for ease-of-use
- Option to correct viscosity and dilution after making a measurement

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Comparison of features between NTA 2.3, 3.0 and 3.1

Feature	NTA 2.3	NTA 3.0	NTA 3.1
Interface			
SOPs/Script Generator	No	Yes	Yes
Quick Load of Recent Scripts	No	Yes	Yes
Quick Load of Recent Experiments	No	No	Yes
Pause/Step Frame Processing	Yes	No	Yes
Capture			
Automatic Focus	No	No	Yes (monodispersed only)
Automatic Camera Level	No	No	Yes
Live Analysis	Yes	No	No
EDR Capture	Yes	No	No
Algorithms			
High Resolution Size Algorithm (FTLA)	No	Yes	Yes (speed optimised)
Vibration Correction	Yes	Yes	Yes
Graphs			
Graph Overlays	Maximum 10	No	Unlimited, with grouping
Scatterplot Overlays	Maximum 2	No	Unlimited, with grouping
Graph Overlay Exports	Bitmaps	No	Bitmaps and PDFs
Custom Graph Colours	No	No	Yes
Data Manipulation			
Exclusion Regions	Yes	No	Yes
Selected Concentration	Yes	No	Yes
Additional			
Draw 'All Tracks'	Yes	No	Yes

Recommended System Requirements

Any computer currently running NTA 2.3 or NTA 3.0 will be able to run NTA 3.1. 1GB of free space is required and a 1680 x 1050 or higher screen resolution is strongly recommended. Full computer specifications are available in a separate document.

Supported Languages

- English

Software Installation Procedure

This section will explain how to update your NTA software to the latest version.

Instructions are provided on the next page for upgrades from NTA 2.3 and above.

If you a running a version of NTA earlier than 2.3, follow the instructions for upgrading from NTA 2.3. If you do encounter issues with camera drivers or software activation, please contact helpdesk for advice.

You should contact the helpdesk for advice if you match any of the following criteria:

- You are running NTA 1.5 or earlier

- You are running NTA 2.3 and have multiple laser modules

- You are installing the software on a blank computer to run with the NanoSight instrument (you will need to make sure system settings are copied across)

It is assumed that you have authority to install or update software within your company's SOPs. If you do not have this authority please consult with your I.T. support department before proceeding.

It is assumed that you have Administrator rights for the computer. This is required by the installation process.

It is not necessary to remove the previous version of software to install and run this software release.

Required or Supplementary Files

The software, manuals and related documentation are available from Malvern or your local distributor. Please contact helpdesk@malvern.com for information on how to obtain the software.

Technical Support

For questions regarding the operation of the software consult the latest software quick start guide (included with software).

If you have any questions or problems with this installation, or if the software does not work as expected, please contact in the first instance your local distributor.

As a backup you may contact helpdesk@malvern.com or phone on +44 (0) 1684 892456 during UK office hours (9am to 5pm).
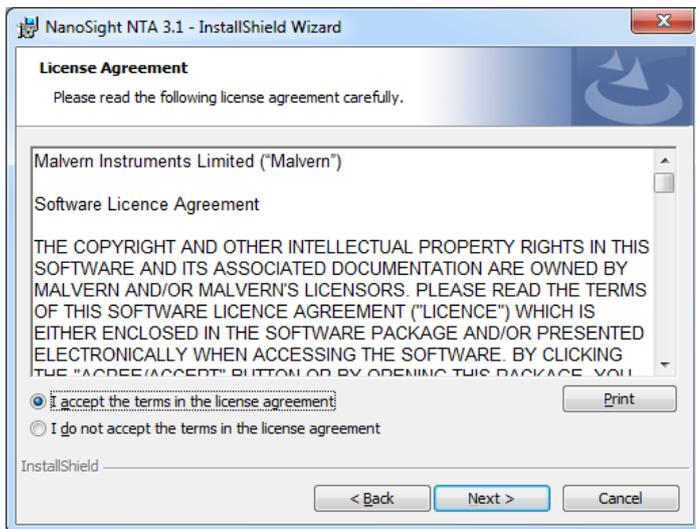
Installation Instructions

In order to install the software you will need the installer file called 'NanoSight NTA 3.1 Installer.exe'. Previous versions of software will not need to be removed during this process with multiple software versions able to run on the same computer. Ensure that sufficient space is available on the computer (approximately 1GB of space is recommended for installation).

If the installer is contained in a zipped file, extract to a convenient location and double click '**NanoSight NTA 3.1 Installer.exe**' to start. The installation Wizard will then run through initial installation of NTA 3.1 presenting the dialog window below.
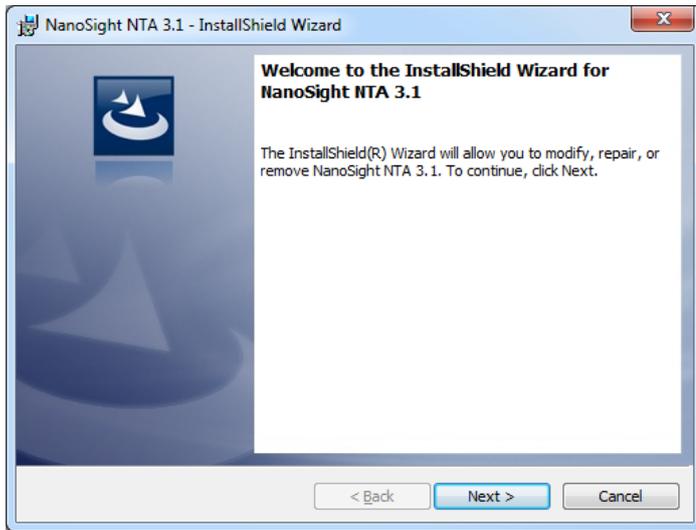
1. Select '***Next***', and review the license agreement. To continue you must confirm acceptance by selecting the appropriate option.

2. Selecting '***Next***' in this window will install NTA 3.1. Administration rights may be required.

3. After installation is complete select '***Finish***'

The NTA 3.1 software has now been installed on the computer. This will open the NTA Installation Manager to check further details of your individual system.
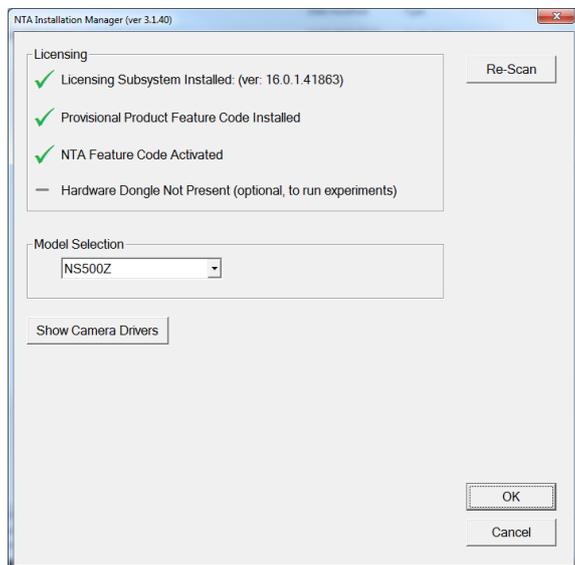
The NTA Installation Manager controls security, model selection and camera drivers. When upgrading from NTA 2.3, NTA 3.0 or another version of NTA 3.1 all license features should show a green tick with the correct instrument model selected.

Some users may see a message instructing you to uninstall and reinstall the Scientific CMOS drivers. If so, click 'Show Camera Drivers' and click 'Install/Remove' next to Scientific CMOS, follow instructions on screen, restart the computer, and then open the installation manager from the start menu and install the Scientific CMOS drivers again.

Selecting '**OK**' will close the dialog box and complete the installation of NTA 3.1.

Uninstall Procedure

The software can be uninstalled using the standard Add/Remove feature in the Windows Control Panel.

Known Issues

- After running 'Make Background' on a single file with no background associated with it, the on-screen image of the video will not update until the file is loaded back in.

- If the computer sleeps during camera recording, measurements will not be completed and upon waking the camera driver and further recordings will have issues. A PC restart is required. Switching off automatic sleep/hibernate is strongly recommended when running NTA.

Appendix I: NTA 3.1 Supplementary Software Guide

Document Summary

The NTA 3.1 Software Release incorporates several new features designed to extend functionality and improve ease of use. This guide is intended to give an overview of the key changes in comparison to the NTA 3.0 software package and hence assumes prior knowledge and experience of NTA 3.0. We would recommend using this guide alongside the NTA 3.0 Software Guides for a full overview of the software.

NTA 3.1 Key Features

- Improved experiment and hardware control interface

- Automated image setup

- Extended functionality for on-board NTA result processing and data output

- Experiment overlays and merged results

- Customization options for graphics display and output

- Integrated user guidance on instrument use and system care

Preparing the System

NS300 Systems

NS300 Focus Tab has user defined Scatter and Fluorescence position buttons

These are used to move to the required measurement position and replace the original zero and user 1 and 2 buttons
Scatter is assigned as the 'zero' position internally, and is set to give a focused image of standard particles under scattered laser light.
Fluorescence can be set at a different focus position to account for any focus shift seen when imaging at different wavelengths.
These positions can be defined on a per Windows User Account basis.

The current focus position can be changed manually by typing into the box or by adjusting the slider.

Filter Wheel control is now also in a Hardware tab, rather than in the menu.
The availability of filter buttons and their names are defined in the **NTA Admin Tool**, and can be customized as required (examples shown).
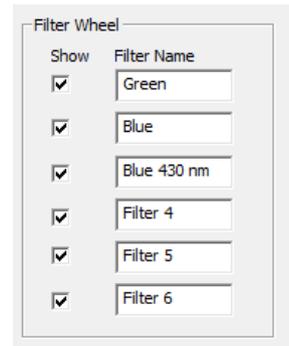
NS500 Systems

Priming the Fluidics

Press *Prime Fluidics* on the Pumps / Stage tab to prime the NS500 for use. Prompts will display to run a two-stage process, to slowly fill the system fluidics with diluent and then automatically run a short wash cycle.

The first time the camera is started, the software will remind the user to ensure the *Prime Fluidics* sequence has been completed, before beginning data capture. This message will only be seen once each time you run the software.
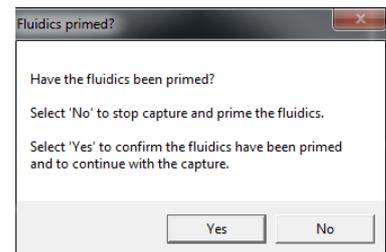
Setting Measurement Position

The *Goto Zero* position is assigned as the 'zero' position internally, and is set to give a focused image of the centre of the static laser thumbprint pattern. *Scatter* and *Fluorescence* are set to the standard sample measurement positions, under scattered laser light and fluorescence wavelengths respectively. These positions can be defined on a per Windows User Account basis.
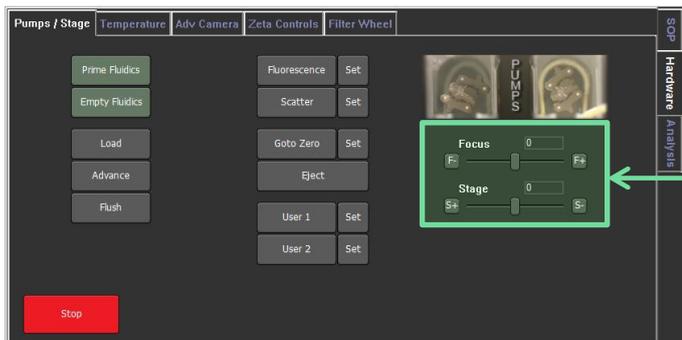
Refined Focus and Stage control

The current stage and focus position can be changed manually by typing into the boxes or adjusting the sliders.

The movement of the slider bars has been adjusted to allow finer control for slow speeds

Making a Measurement

Optimising the Image

Camera Levels

The camera brightness levels have been adjusted for CCD, sCMOS and EMCCD camera types to give a more linear increase in brightness across the imaging range. This means that the same sample at the same camera level will not appear at the same brightness in NTA 3.0 and 3.1. The new brightness settings give greater flexibility when setting low camera levels, improving the performance for large or high refractive index particles, and give the same performance on particles near the lower limit of detection.

 To view a sample using the old camera levels, i.e. for use in comparing image brightness to previously captured data, there is an option in the software to temporarily revert back to NTA 3.0 levels:

- Option found in *Preferences* → *Hardware Preferences*

- This is saved as a user preference and can be defined on a per Windows User Account basis.

- If videos are recorded using NTA 3.0 levels, this is displayed in any exported files containing camera level values

- When using NTA 3.0 camera levels this will be displayed in the capture tab
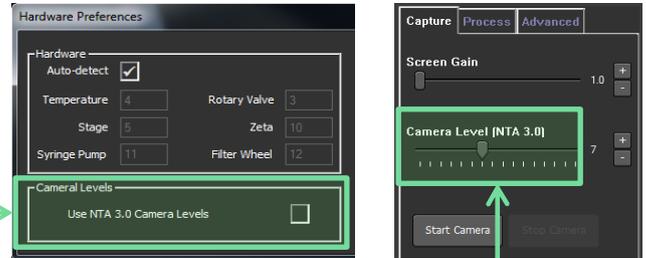
Auto-Setup

The *Auto Setup* function can be used prior to the NTA Standard Measurement SOP for fully automated capture settings.

NS300 and NS500 systems

For **non-polydisperse** samples, with brightness equivalent to 50nm – 400nm latex for HS systems, or 100nm - 400nm latex for non-HS systems, NTA 3.1 can automatically set the image focus and select an appropriate camera level for standard measurement.
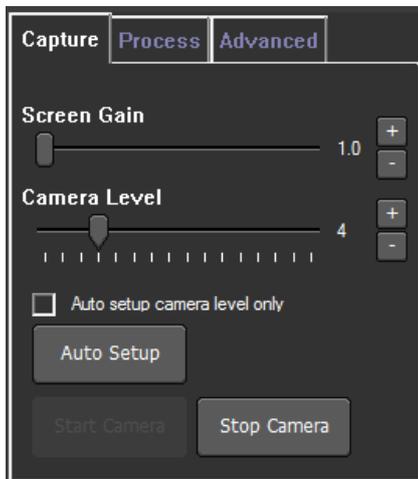
Click the *Auto Setup* button in the *Capture* tab to focus the image and set an optimal camera level for measurement

1. Sets an initial camera level

2. Focuses image

3. Sets capture camera level
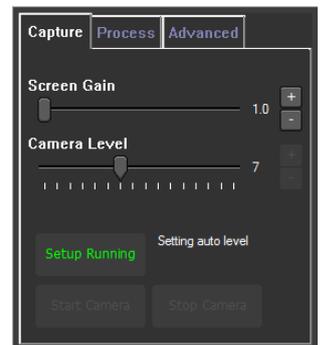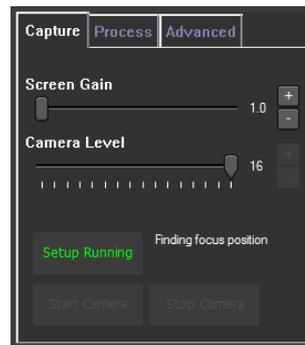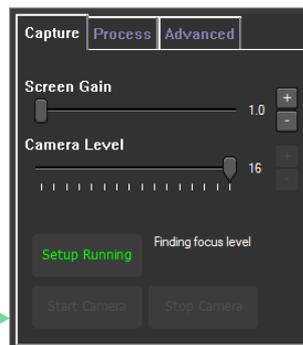
Note:

Auto-focus is only specified for monodisperse samples which are well within the detection range of the instrument and within the concentration range 2×10^8 to 1×10^9 particles / ml. Some polydisperse particle suspensions may also be suitable for auto-focus, but samples with aggregates present can cause significant focus errors.

Note:

The *'Scatter'* position of your instrument must be initially set at the correct stage position and at the approximately correct focus position to get a clear image of particles, for the Auto-focus to operate. If the current *'Scatter'* focus is not set correctly, the focus should be adjusted using the focus slider bar and the *'Scatter'* focus position corrected by clicking the *'Set'* option next to the Scatter button.

For samples unsuitable for auto-focus, the image focus should be adjusted manually as required using the slider control. Select the '*Auto setup camera level only*' tickbox to skip autofocus on subsequent *Auto Setup* runs and just set the camera level.

All other systems (including LM10, LM20)

For systems without motorised focus control, the focus should be adjusted manually as required. Click the *Auto Setup* button in the *Capture* tab to set an optimal camera level for measurement.
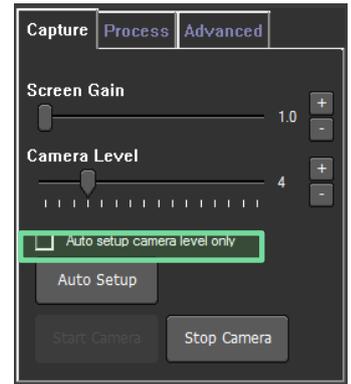
SOP and Script Control

Script Window Auto-Docking

Double click the centre of un-docked script to re-dock the window within the NTA screen layout.

Manual Temperature Input Override

In the Standard Measurement SOP page, if no temperature readout is available, select the *Manual Temperature* option to choose to manually input a temperature to be used for all captures recorded with the script (the temperature input dialogue box will not appear after each captured video).
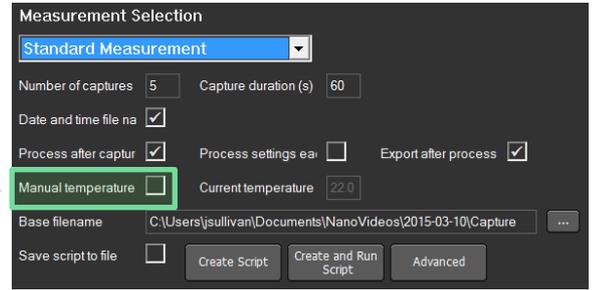
Processing

The x-axis can now be re-scaled during data processing, by left-clicking and dragging on the axis
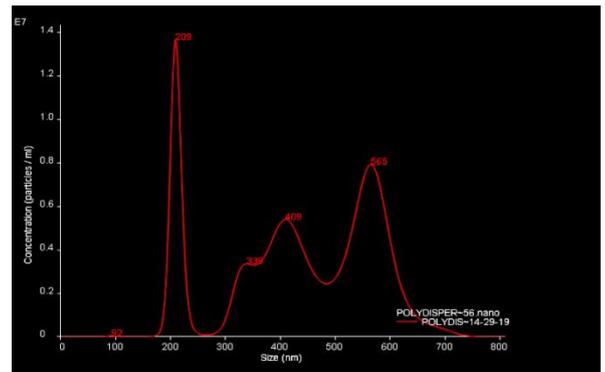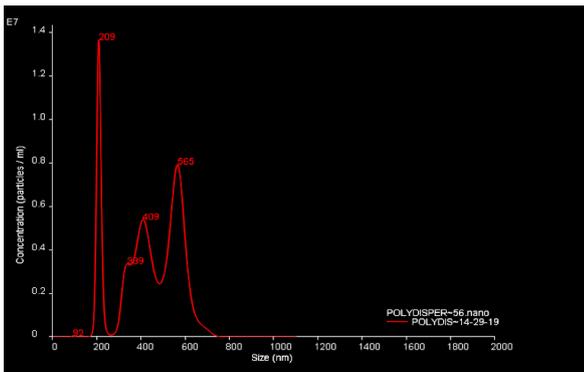
Processing can be paused, and stepped through frame by frame.
When processing is running, **a pause button** becomes visible in the 'Process' tab
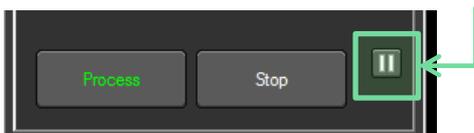
When processing is paused, a **play button** and a **step frame button** become visible.

Plotting and Analysis

Experiment Interface

Open an Experiment

Click on the *Recent Experiments* tab to open a previous experiment. The most recent experiments are listed in the window underneath – double click to load the experiment and automatically display the results for the files selected. Older experiments can be opened using the *Open Experiment* button or the *File* menu → *Open Experiment* command.

The files available for the loaded experiment are listed in the *Current Experiment* tab. Filenames currently selected for data display and export are displayed in bold type, and have a tick mark displayed to the right.
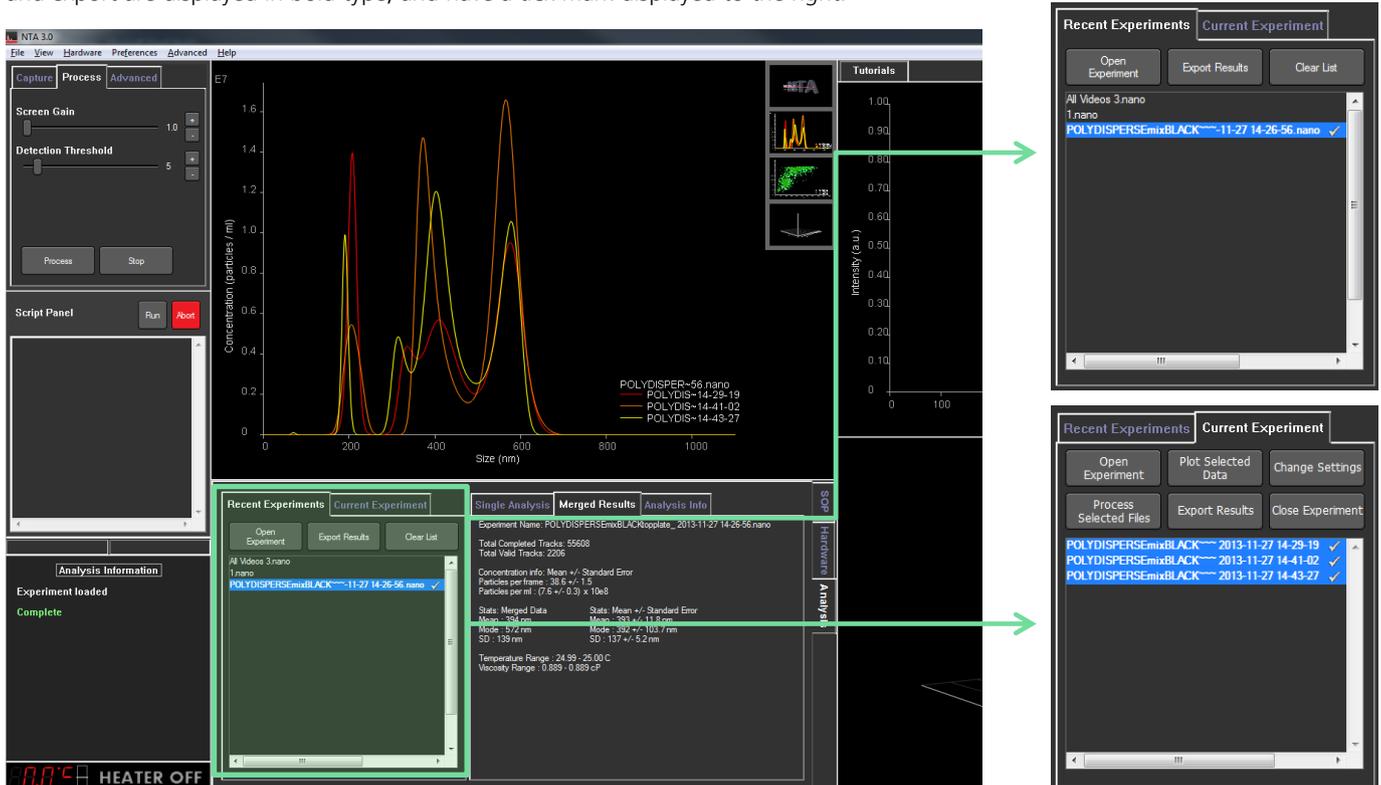
Close Experiment

Click the Close Experiment tab to close the current experiment and clear graphs and results from the screen

Combine Experiments

Multiple experiments can be combined into a single experiment file, by clicking on *File* → *Combine Experiments*. The experiments to combine (and associated video files) must reside in the same folder.

In the file browser window that opens, select the experiments to combine (drag a box around all files with the left mouse button, or *ctrl + left click* to highlight multiple files).
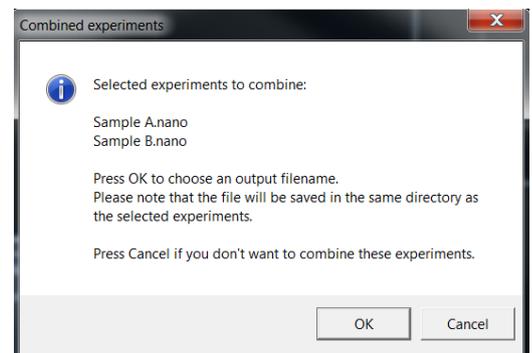
A prompt will appear as shown. Click OK to combine the selected experiments into one file and choose an output filename.

Graph Plots

As for NTA 3.0, available plots from the graph viewport panel can be dragged (left mouse and click) into Graph Windows 2 and 3 for display alongside the plot in the main window.
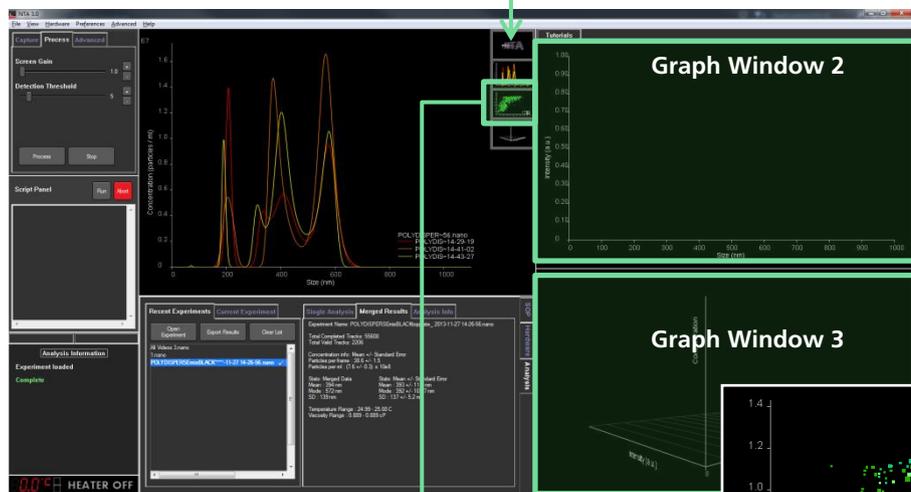
When an experiment is loaded, graph windows 2 and 3 are left empty and so can be used for any overlay plots to compare graphs between different experiments/datasets (described later in this guide)

Experiment Intensity Scatter Plot

When loading an experiment, the scatter plot now contains data from all selected analyses.
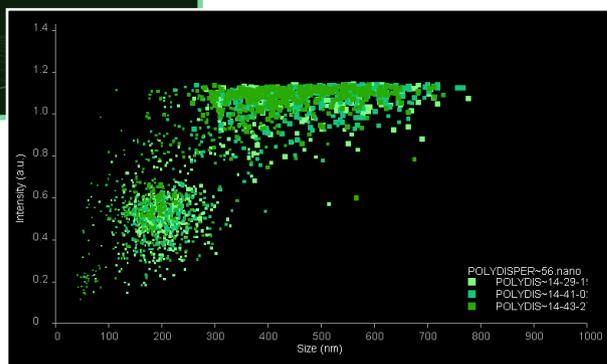
Exclusion Regions

Scatter plot exclusion regions have been implemented to replicate functionality in previous versions of NTA.

- Left mouse click and drag to add a region to the scatter plot
- Multiple square regions can be added to remove areas of a distribution
- Chosen regions are saved and are reloaded back in when looking at the analysis again
- Regions are recorded in the summary.csv file
- Other graphs (e.g. size distribution, 3D size-intensity distribution) are updated as regions are added
- Must be added per analysis (i.e. not for the entire experiment)
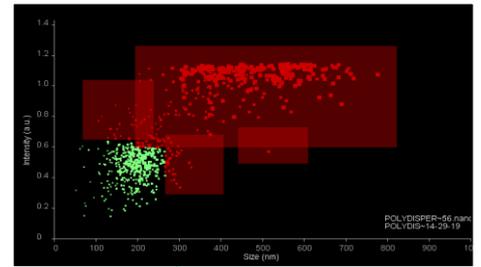
During processing, excluded particles are highlighted using a square
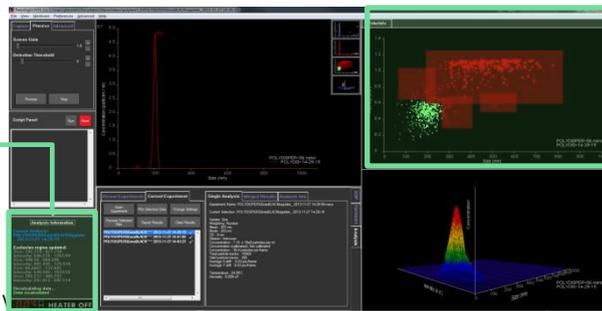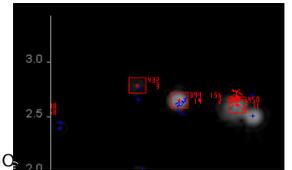
Exclusion regions are shown in the status window

When viewing a different set of axes to which the exclusion regions are applied but excluded points are present this is also shown on the legend of the displayed plot.

An example is shown here of exclusion regions added in a size vs zeta potential plot (left), and viewed in a size vs intensity plot (right).
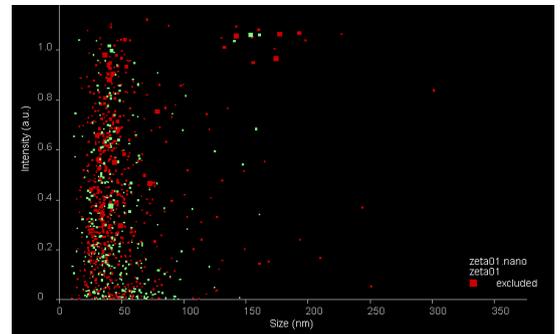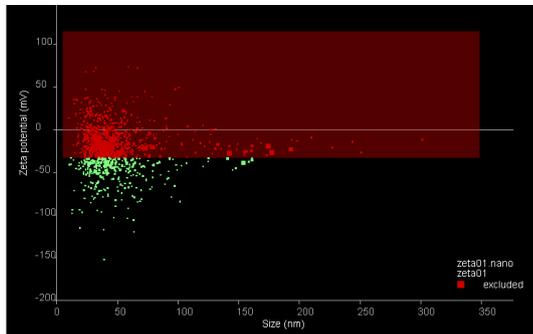
When viewing an experiment/multi-plot scatterplot, any excluded points are shown in red.

Material relationships
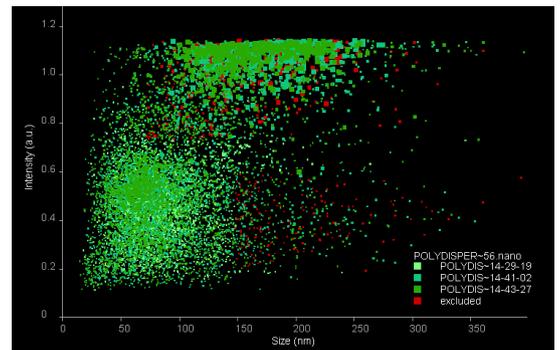
Selected Concentration

User Lines

User lines can be set as markers on a plot to obtain concentration information about a selected part of the distribution. To display user lines, select the '*Display User Lines'* option from the right-click context menu on the graph.

Move the lines to the required value by left clicking and dragging on the diamond symbols at the top of the lines.

When user lines are displayed, on the single graph plot (merged plot or single experiment), a 'Selected Concentration' will be displayed underneath the graph and exported. This selected concentration is the estimated concentration of the sample between the values given by the two user lines, and is displayed as an absolute value in particles per ml, and as a percentage of the total concentration.

Selected concentration is available in size and diffusion, vs concentration graph views.
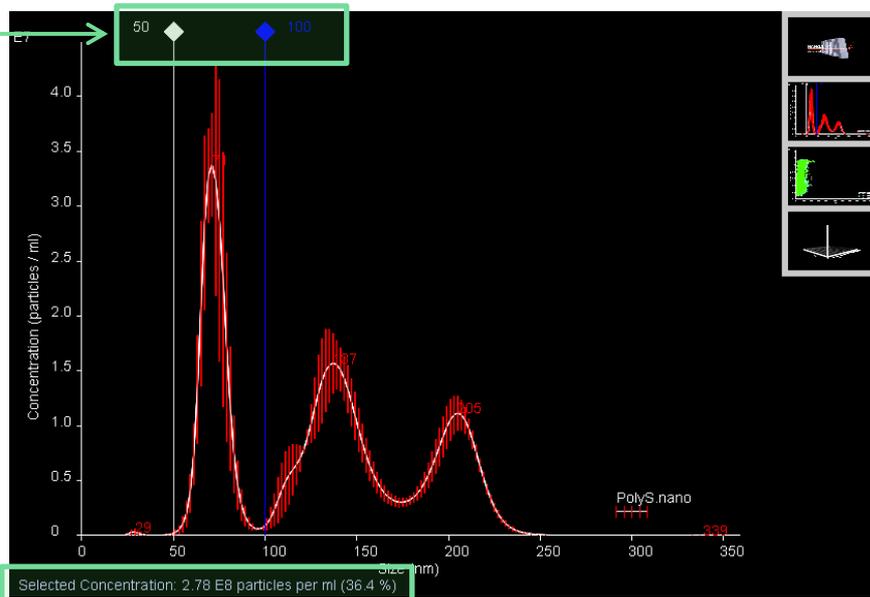
Change Settings Dialogue

The settings for viscosity and sample dilution can now be altered in the software, for example if viscosity was set incorrectly at the time of capture, and the results and graphs automatically updated accordingly without the need for re-processing.

To change viscosity or dilution settings for a loaded experiment, navigate to the *Current Experiment* tab, and click the *Change Settings* button.
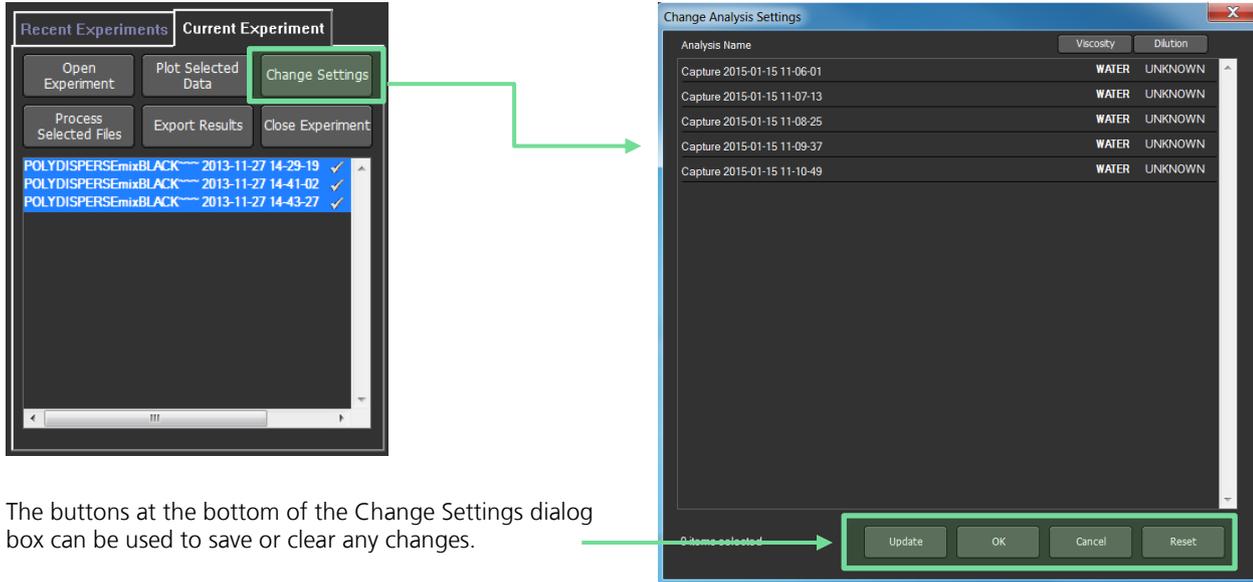
The buttons at the bottom of the Change Settings dialog box can be used to save or clear any changes.

Update
Sets the new settings and updates the graphs and results in the NTA main window

OK
Same as *Update*, but then closes the window

Cancel
Clears any changes since last update, and closes the window

Reset
Resets any values back to as they were at the time of the last update

To change settings:

1. Select the settings in a single column which you wish to change. Click individual cells to select, or click the column header button to select all

2. Double click any selected cell to edit the value. If Dilution is set to 0 it will display 'Unknown' and concentration will not be adjusted

3. Press the Enter key to automatically update all values in the column

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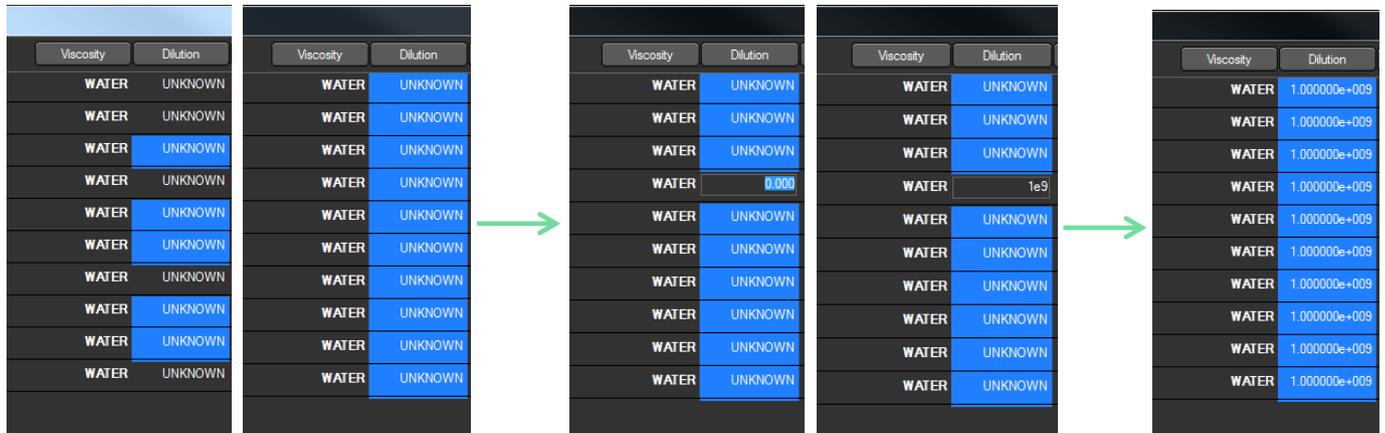
Viscosity Setting

When selected, viscosity cells display a tick box to switch between water or manual viscosity.

Select the tick box to set the viscosity of the sample for water at the measured temperature. When using other solvents, un-tick the box to manually enter the appropriate viscosity.
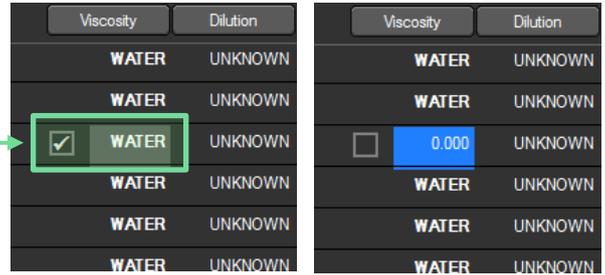
Draw All Tracks

On a processed individual analysis, a diagram of all tracked movements of particles can be generated. This can be useful when checking the alignment of the laser module and ensuring that illuminated particles are evenly filling the field of view for concentration measurements.

Ensure that the video window plot (top graph in the viewport panel) for a single analysis is being displayed in the graph window. Right click on the graph window and select '*Draw All Tracks*' from the context menu.
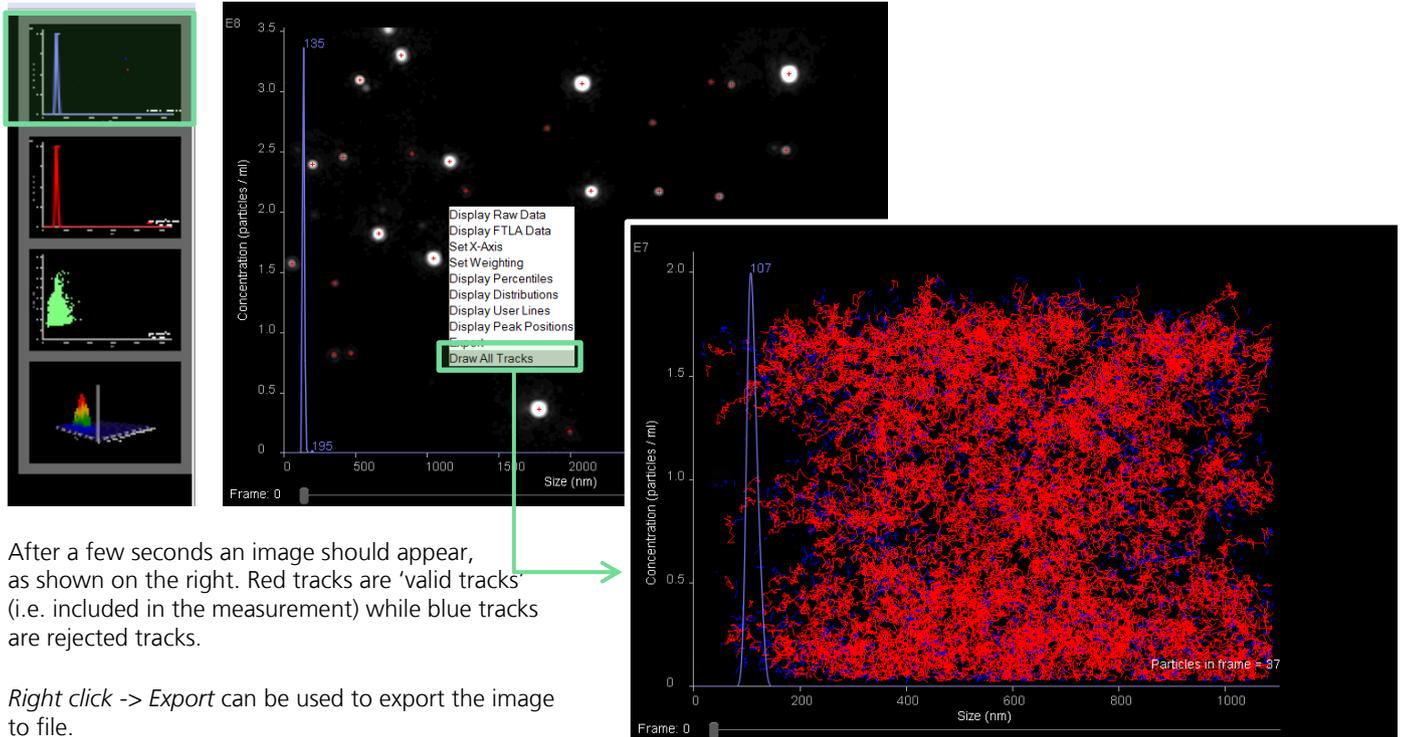
After a few seconds an image should appear, as shown on the right. Red tracks are 'valid tracks' (i.e. included in the measurement) while blue tracks are rejected tracks.

Right click -> Export can be used to export the image to file.

Graph Overlay

Displayed experiment graphs (line graphs or scatter plots) can be dragged from the viewport windows to graph windows 2 and 3.
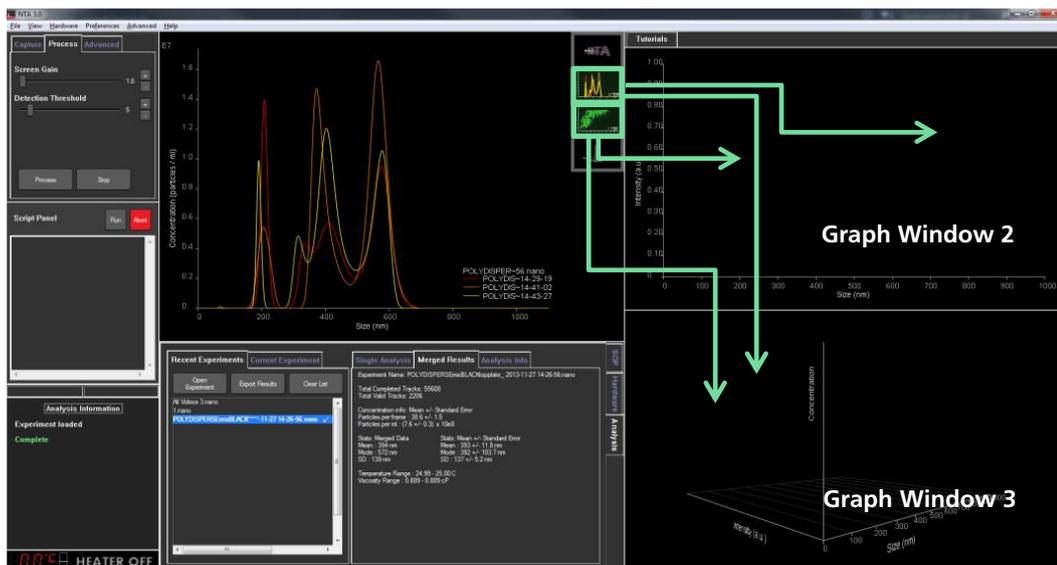
If the window that the plot is being dragged to is currently blank (i.e. on loading a new experiment), or displaying a different graph type (i.e. dragging a size distribution into a window displaying a scatter plot), the new plot will automatically be transferred to the graph window. If a plot is dragged to a window where a graph of the same type is already displayed, options to replace or overlay plots will be shown depending on the graph types used.
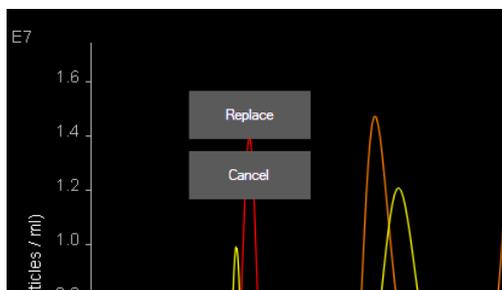
Replace/Cancel buttons become visible

This happens when dropping a graph on top of another, but they are not compatible for overlay (e.g. different axes). Select *Replace* to overwrite the previous graph with the new one.

Overlay/Replace/Cancel buttons become visible

As above, but with **Overlay** button, for when datasets are compatible for overlay. Select *Overlay* to overlay the two graphs on a common axis.

This feature can be used to overlay data from a subset of files captured in a single experiment or to overlay data from multiple different experiments on the same plot. Open an experiment and drag a single file or multi-plot graph from the viewport panel into

Window 2 or 3. Open another data file or the next experiment, plot the data and overlay the corresponding graph. Up to 10 data sets can be overlaid in this way.

Overlaid graphs can be exported as PDF reports or BMP images, by right-clicking on the graph and selecting *Export Graph* from the context menu. Graphs will be exported into the same location as the currently loaded experiments.
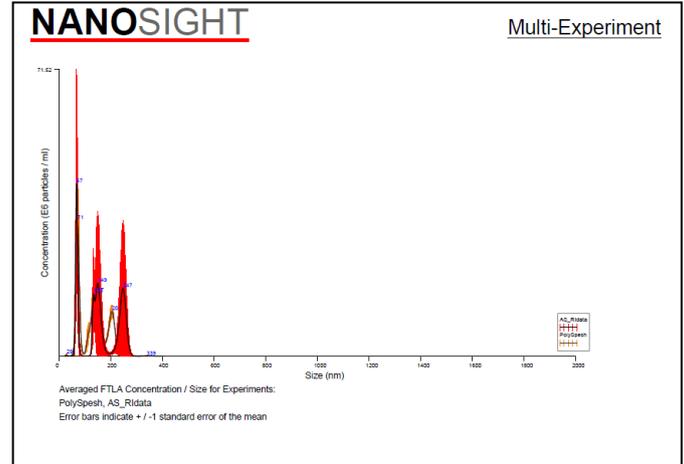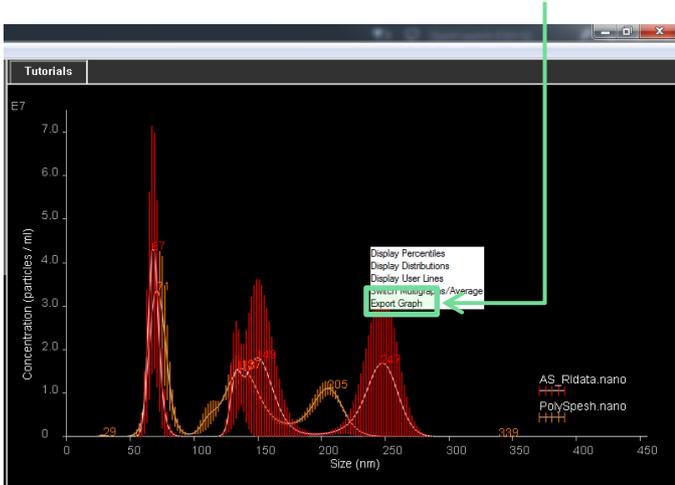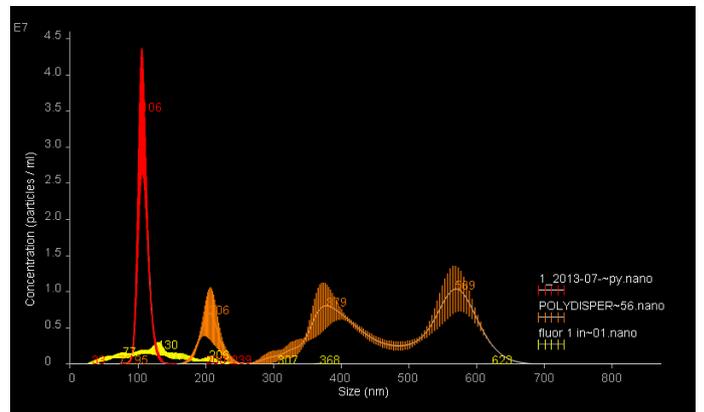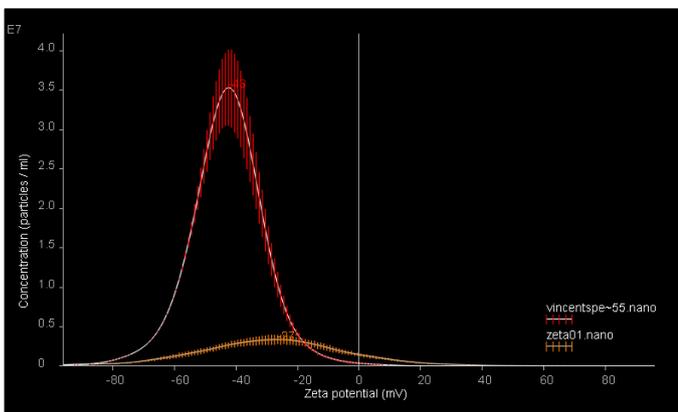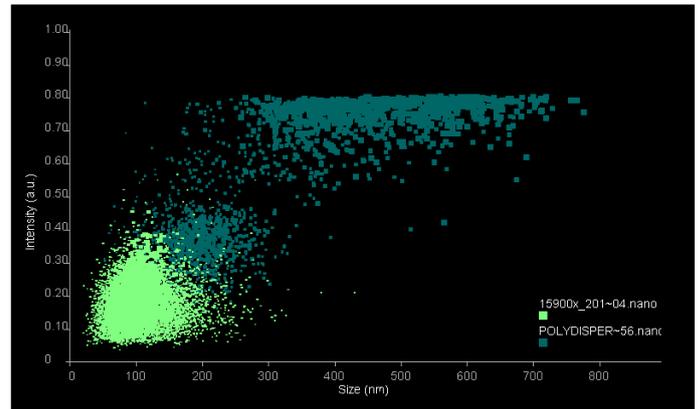

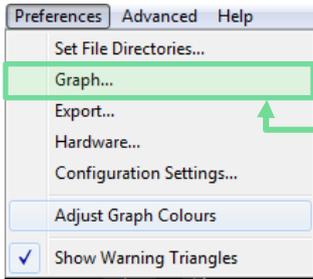Examples of possible overlaid graph options are shown below:

Two sets of size vs concentration multi-plots
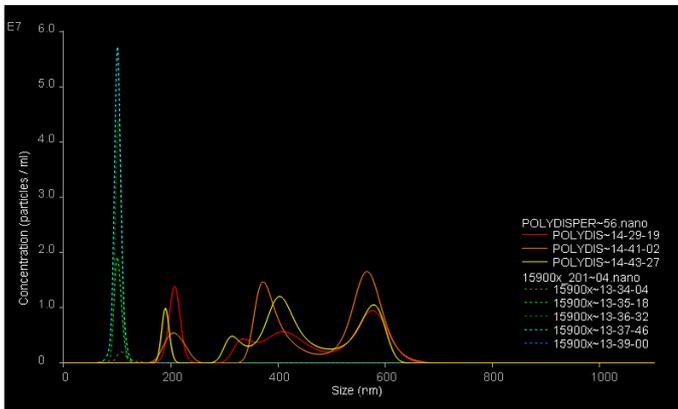
Two sets of size vs intensity scatterplots

Graph Settings Dialogue

Navigate to the *Preferences* → *Graph Settings* option to adjust the current graph settings. Bin widths can be chosen separately for size, diffusion and zeta graphs, and depending on whether raw or FTLA data is used. These settings can be defined on a per Windows User Account basis.

These settings are used for all default graph displays and standard export files (from the Export Dialogue)
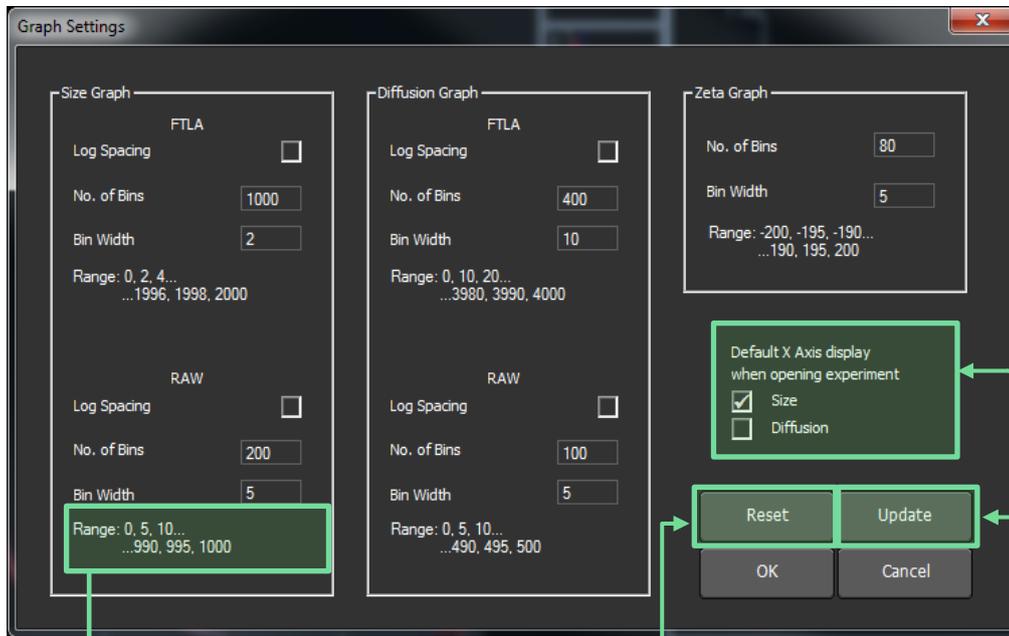
For size and diffusion, log spaced bins can be used

Default X Axis

This the x-axis used when opening an experiment and when exporting data (for zeta experiments, zeta potential is automatically set as the x-axis).

Reset
Reset resets all graph settings back to their default values

Update
This saves the graph settings and updates any graph currently displayed by the software

The range of the graph is displayed and updated as changes are made to the **No. of Bins** and the **Bin Width**

Any incompatibilities with settings are shown in red
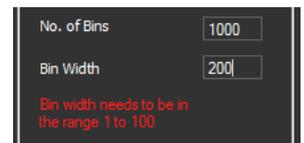
Graphics Customisation
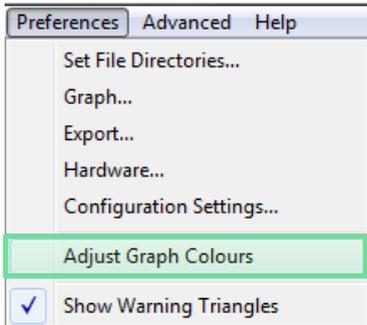
Navigate to the *Preferences → Adjust Graph Colours* option to adjust the current colours used for NTA graph plotting. All colour preferences will be saved and re-loaded when NTA is restarted. These settings can be defined on a per Windows User Account basis.

The colour maps shown in the dialogue box for Sets 1, 2 and 3 are used for different plot types within NTA.

Set 1 – 2-D distribution profiles (e.g particle size histogram)

Set 2 – Scatter plots (e.g. intensity vs size)

Set 3 – Overlaying graphs

Click on a colour to bring up a selection box. Select a new colour to redefine the colour used for NTA graph plotting. The colours can be reset to their default setting by clicking *Reset*.
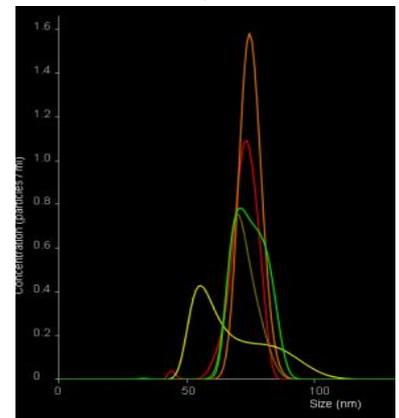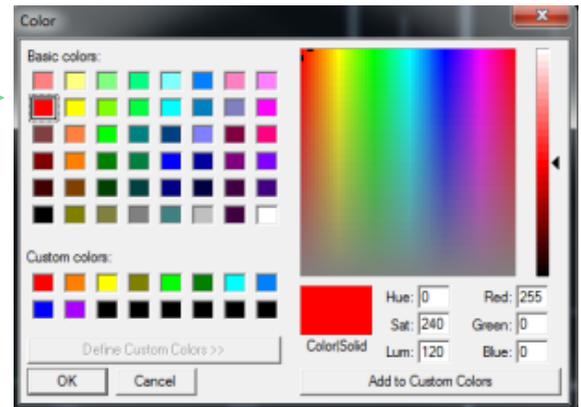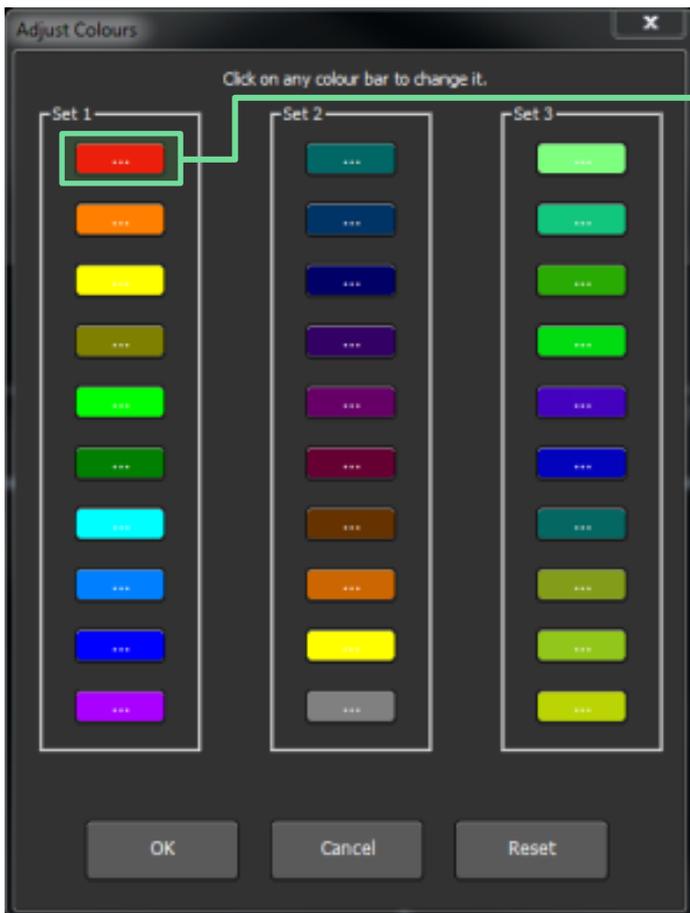
Shutting Down System

NS500 Systems

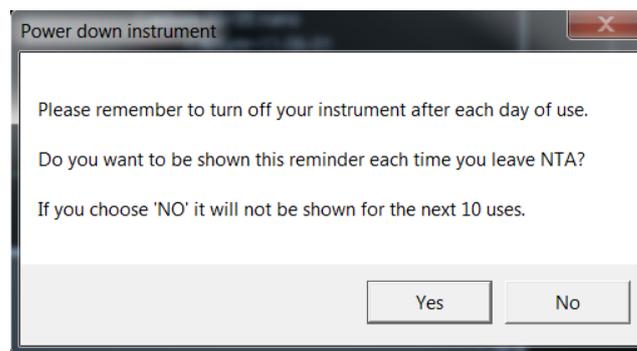### Emptying the Fluidics

Press *Empty Fluidics* on the Pumps / Stage tab to empty the NS500 after use. The camera will automatically turn off if running and prompts will display to run a two-stage process to empty the system. A short wash cycle will run before the user is prompted to disconnect the diluent tubing and the fluidic system is slowly emptied.
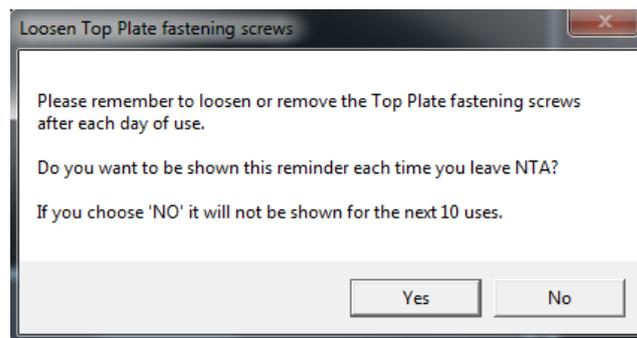
NS500 and NS300 Systems

Instrument Switch Off Reminder

A message will appear when shutting down the software to remind the user to switch off the instrument. The user can click "No" on the prompt window to choose to stop this message appearing for the next 10 uses.

Power down instrument

Please remember to turn off your instrument after each day of use.

Do you want to be shown this reminder each time you leave NTA?

If you choose 'NO' it will not be shown for the next 10 uses.

Yes   No

All Systems

Top-plate Screws Reminder

When closing down the software, the user will be reminded to loosen or remove the top plate fastening screws after each day of use. The user can click "No" on the prompt window to choose to stop this message appearing for the next 10 uses.

Loosen Top Plate fastening screws

Please remember to loosen or remove the Top Plate fastening screws after each day of use.

Do you want to be shown this reminder each time you leave NTA?

If you choose 'NO' it will not be shown for the next 10 uses.

Yes   No

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# Addendum to Appendix 5 in NTA 3.0 Software Guide: Making a Zeta Measurement

Electrode Air Removal

NTA 3.1 includes integrated guidance on the recommended procedure to ensure any air is removed from the flow cell before running a zeta potential measurement.

Once the sample has been loaded, click the *Electrode Air Removal* button on the Zeta Hardware tab for easy to follow instructions on this procedure.
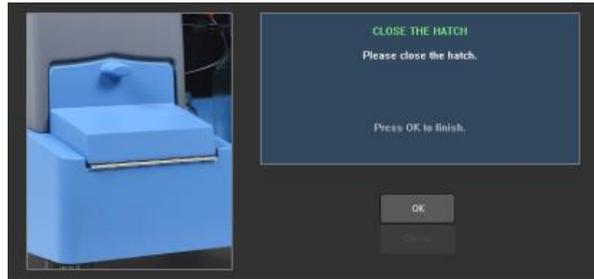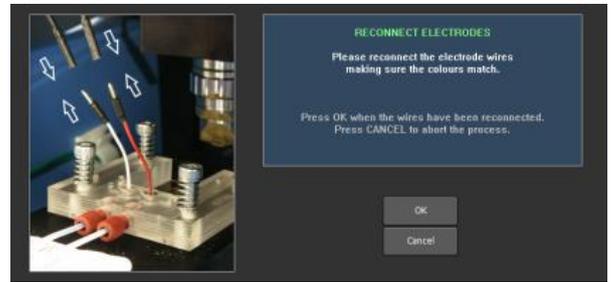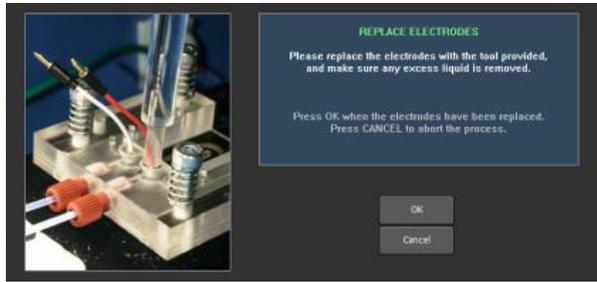
Disconnect and remove the electrodes from the top-plate:
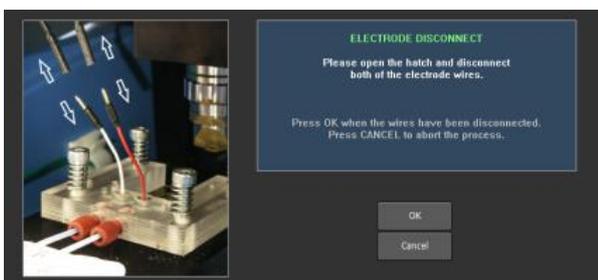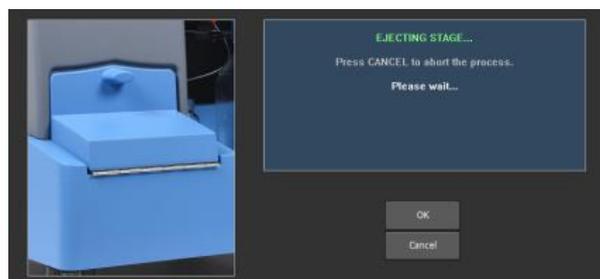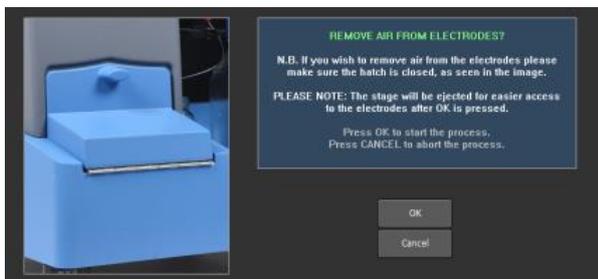
Push some sample through the system to remove any air from the electrode ports:
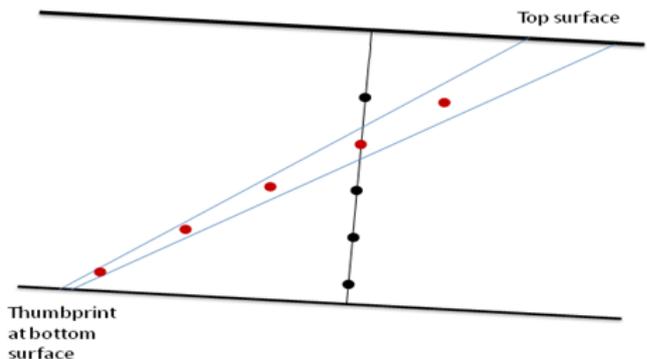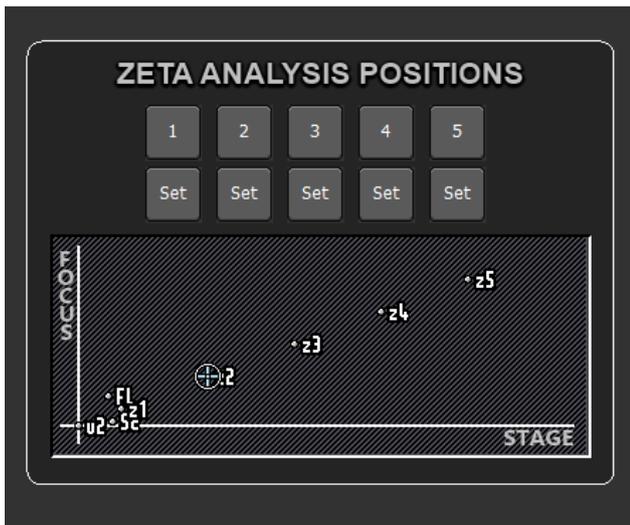
Replace and reconnect the electrodes:
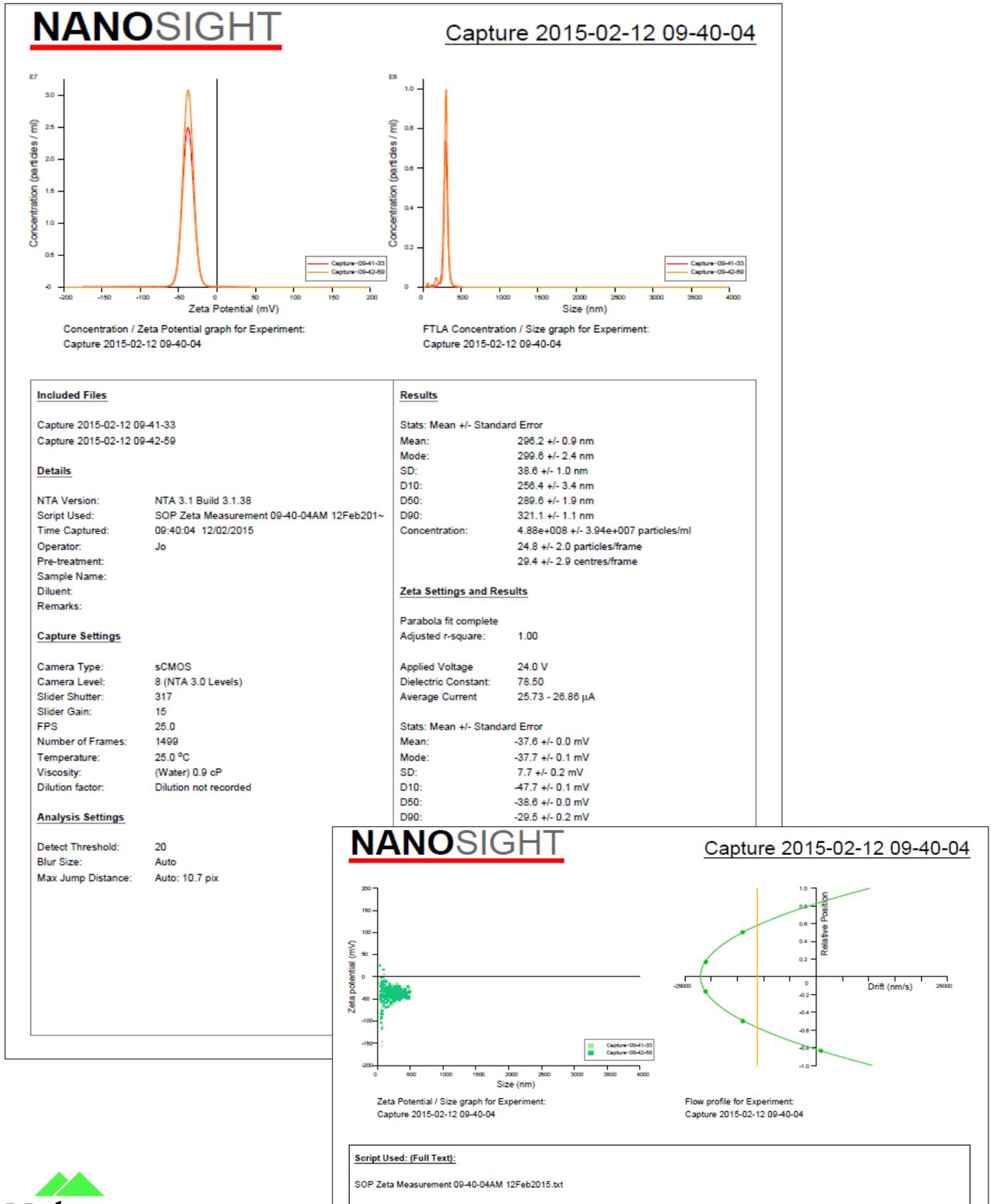

Zeta Measurement Positions

The standard zeta potential experiment uses 5 measurement positions, corresponding to the different depths with the sample chamber. The Zeta Controls tab displays buttons to move to each of these 5 measurement positions, with the current position being highlighted in the graph below.

The illustration above shows the pre-set tilt to the stage and how the measurement positions correspond to different sample depths.

PDF Data Export

Example of exported PDF report for zeta measurement of one sample;

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