

SOFTWARE UPDATE NOTIFICATION (SUN) – NANOSIGHT NTA 3.2 SOFTWARE

**PARTICLE SIZE****ZETA POTENTIAL**

Introduction

This document details the release of the NanoSight NTA software version 3.2. 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 3.2 compared to NTA 3.1. More details and instructions on the new features are available in the appendix at the end of this document.

Improved Automation

- Auto-setup, auto-level, focus-level, and auto-focus script commands available
- Script command to change experiment filename
- Make background script commands added
- Changes to continuous flow SOP scripts to ensure smooth flow

Exports

- Laser color added to nanolog.csv and other outputs

Interface changes

- Run button on Recent Measurements page
- User direction messages for capture and process settings improved
- User Positions 1 and 2 removed

Additional Features

- Optional CFR feature available – audited operation, secure login, data integrity checking, user level program configuration
- CFR feature can be enabled by adding feature code to CFR security dongle
- The CFR feature is available as a chargeable upgrade – Part No. PSS0054 for new builds, Part No. NTA4165 as an upgrade for customers running NTA 3.2 software

Other

- Extended camera log for diagnostic purposes, can revert to daily usage via nano.ini

Comparison of features between NTA 2.3, 3.0, 3.1, and 3.2

Feature	NTA 2.3	NTA 3.0	NTA 3.1	NTA 3.2
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	
Optional CFR Feature	No	No	No	Yes

Recommended System Requirements

Any computer currently running NTA 2.3, NTA 3.0, or NTA 3.1 will be able to run NTA 3.2. 1GB of free space is required and a 1680 x 1050 or higher screen resolution is strongly recommended. Full computer specifications are as follows:

Windows 7 Ultimate 32 bit), Intel Core i5-4570s Processor (Quad Core, 2.90GHz, 6MB, HD4600 Graphics), 4GB (1x4GB) RAM, 1TB Hard Drive, IEEE 1394 adapter card, Serial port Adapter card, Mouse
Physical dimensions 360x420x75mm

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 (with a single laser module) and above. For new installs or for upgrades from software versions prior to NTA2.3, follow instructions in Appendix II.

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 should be packaged with this document. If you do not have these, you can download them from the Malvern website (www.malvern.com). Please contact your local distributor or helpdesk@malvern.com for information on how to obtain ancillary manuals, specifying the serial number of the instrument and camera information, if available.

Technical Support

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

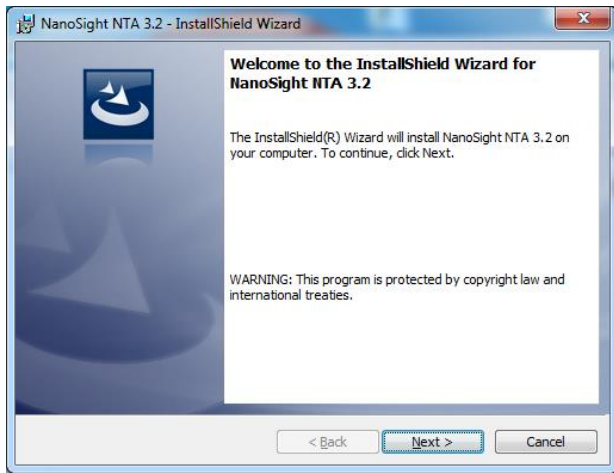
If you have any questions or problems with this installation, or if the software does not work as expected, please contact in the first instance your local distributor.

As a backup you may contact helpdesk@malvern.com or phone on +44 (0) 1684 892456 during UK office hours (9am to 5pm). Please supply the instrument serial number, camera type if known, and details of any peripheral equipment.

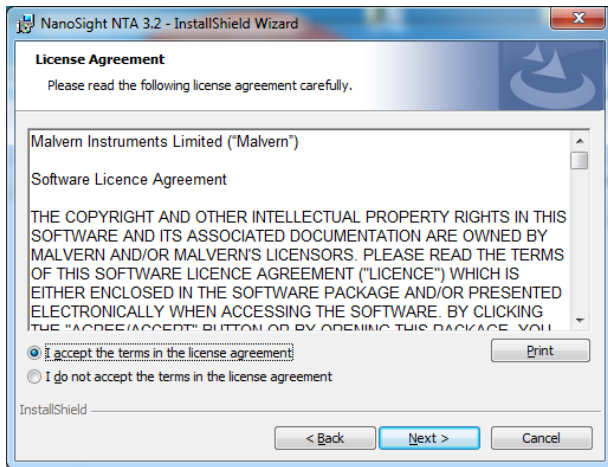
Installation Instructions

In order to install the software you will need the installer file called 'NanoSight NTA 3.2 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 3.2 Installer.exe**' to start. The installation Wizard will then run through initial installation of NTA 3.2 presenting the dialog window below.

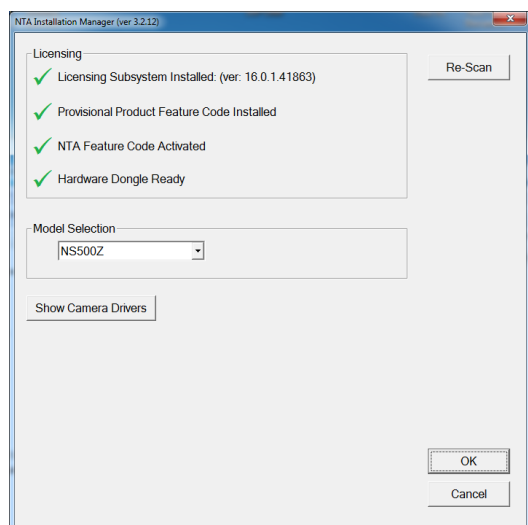


1. Select '**Next**', and review the license agreement. To continue you must confirm acceptance by selecting the appropriate option.



2. Selecting '**Next**' in this window will install NTA 3.2. Administration rights may be required.
3. After installation is complete select '**Finish**'

The NTA 3.2 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 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 3.2.

Uninstall Procedure

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

Known Issues

- 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.
- The FTLA size graph x-axis maximum may be set to 4000 nm if upgrading from NTA 3.0. The desired bin number and bin widths can be set in Preferences. By default, NTA 3.2 uses the following values:
Size Graph - FTLA bins 1000, bin width 1 nm; Raw bins 200, bin width 5.
Diffusion Graph – FTLA bins 1000, bin width 4; Raw bins 400, bin width 10.
Zeta Graph – bins 80, bin width 5.
- When viewing raw data on the main graph, FTLA data may still be output in the CSV files. This is due to the way NTA saves data in individual .nta files. Workaround:
Double click on the first video file in the Current Experiment files list to open, and select Display Raw Data on the right click menu option on the graph. Double click on each of the remaining files in turn. Select all files required for export, and plot selected data. Select Display Raw Data again to change the view for the whole experiment and export.

Appendix I: NTA 3.2 Supplementary Software Guide

Document Summary

The NTA 3.2 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 3.2 Key Features

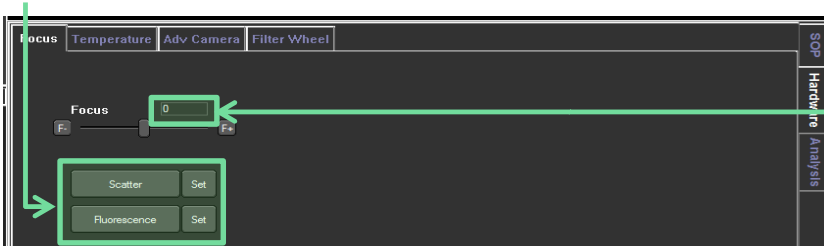
- Extended scripting functionality
- Optional 21 CFR Part 11 compliance feature. This is a chargeable upgrade to the existing NTA 3.2 software, Part No. NTA4165 for upgrade, or Part No. PSS0054 when purchasing a new instrument.

Preparing the System

NS300 Systems

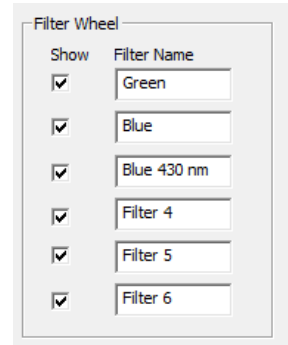
NS300 Focus Tab has user defined Scatter and Fluorescence position buttons

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).

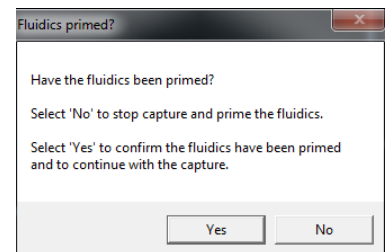


NS500 Systems

Priming the Fluidics

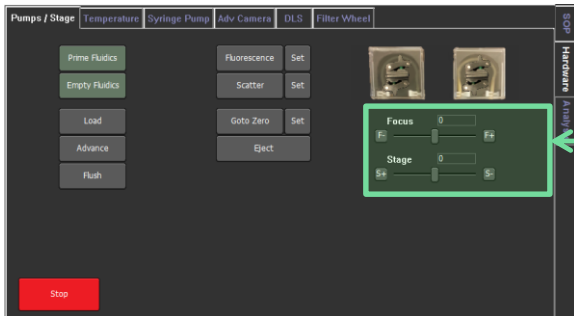
Press *Prime Fluidics* on the Pumps / Stage tab to prime the NS500 for use. Prompts will display to run a two-stage process, to slowly fill the system fluidics with diluent and then automatically run a short wash cycle.

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

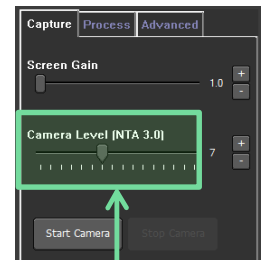
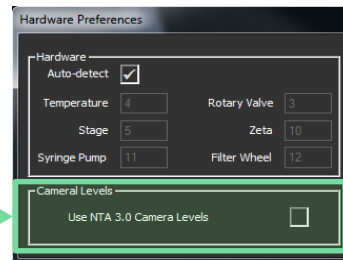
Optimising the Image

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

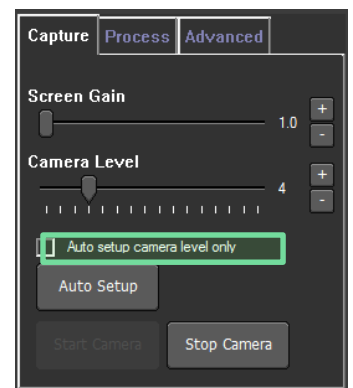
Note:

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.

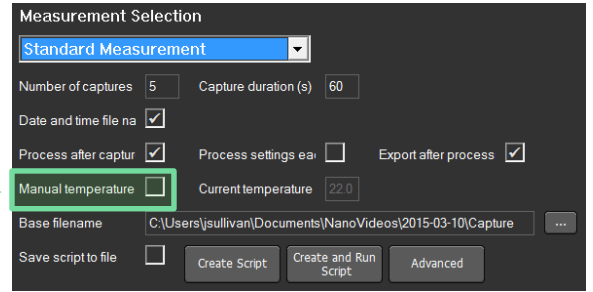
SOP and Script Control

Script Window Auto-Docking

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

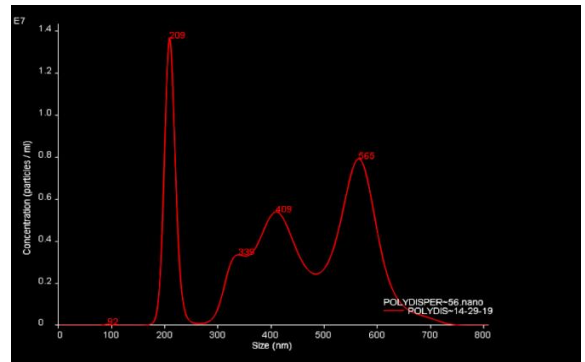
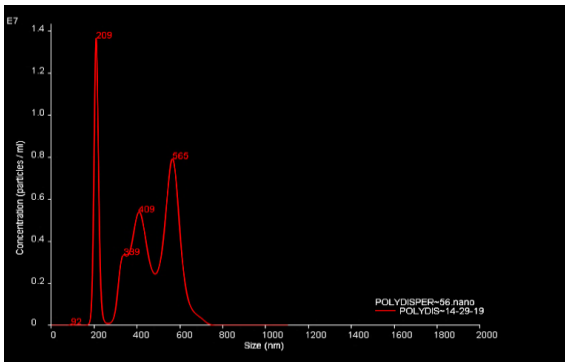
Manual Temperature Input Override

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).

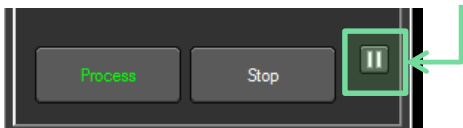


Processing

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.



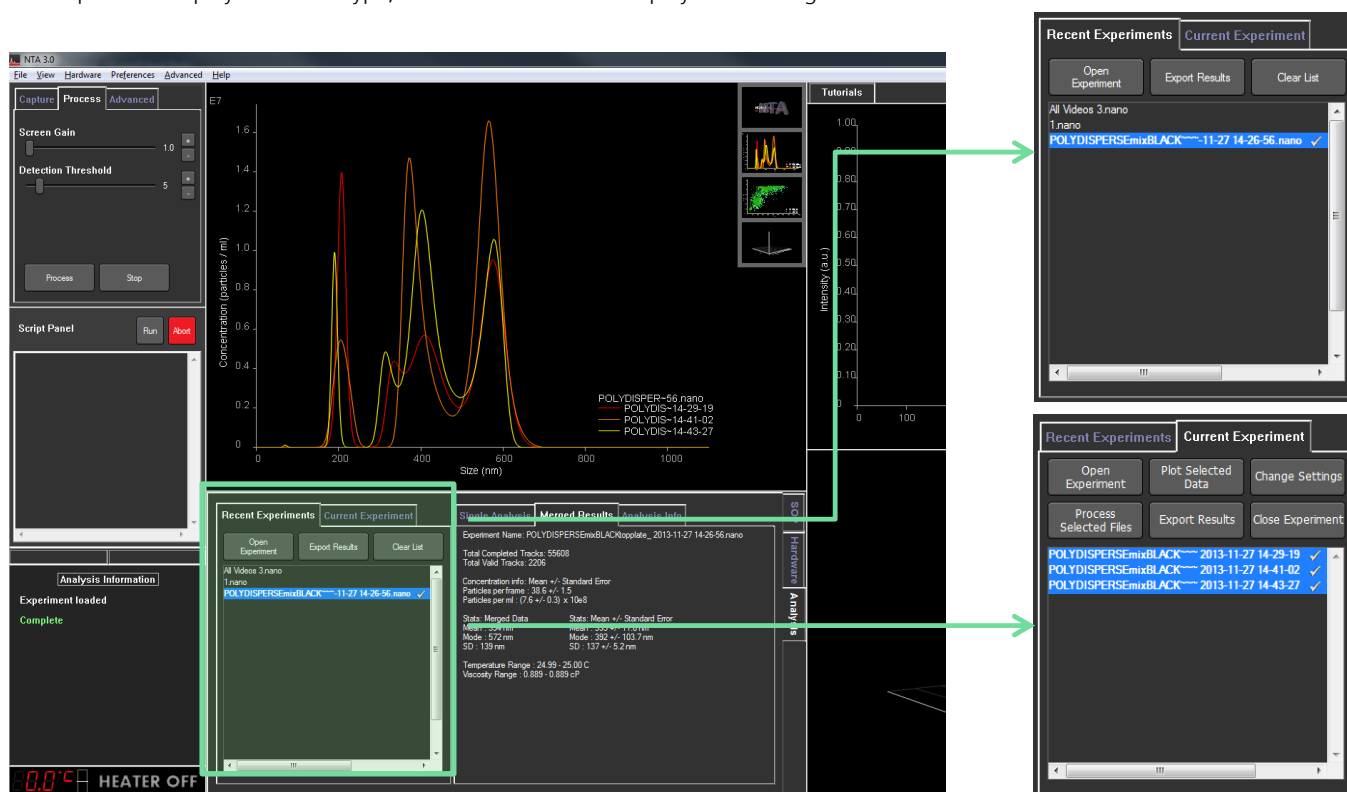
Plotting and Analysis

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.



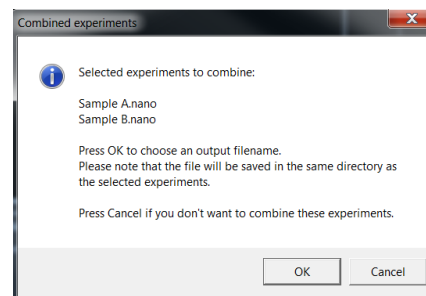
Close Experiment

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.

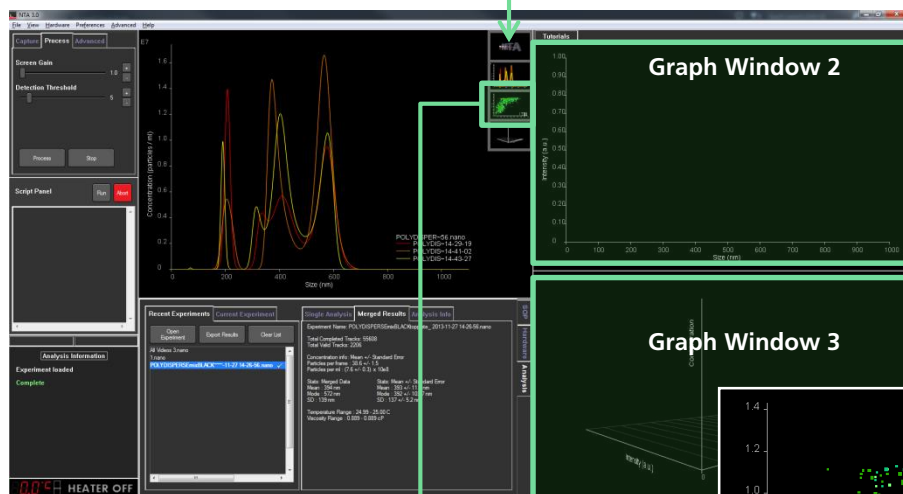
In the file browser window that opens, select the experiments to combine (drag a box around all files with the left mouse button, or *ctrl* + left click to highlight multiple files).



A prompt will appear as shown. Click OK to combine the selected experiments into one file and choose an output filename.

Graph Plots

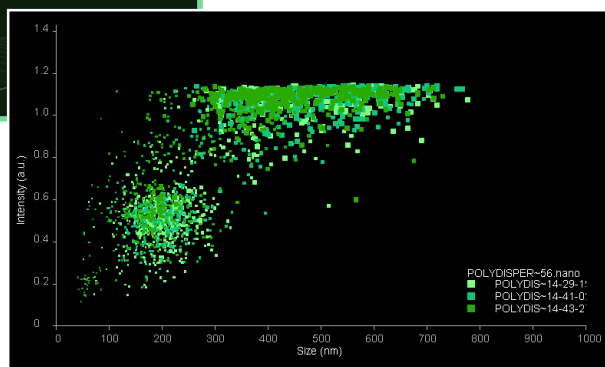
As for NTA 3.0, available plots from the graph viewport panel can be dragged (left mouse and click) into Graph Windows 2 and 3 for display alongside the plot in the main window.



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

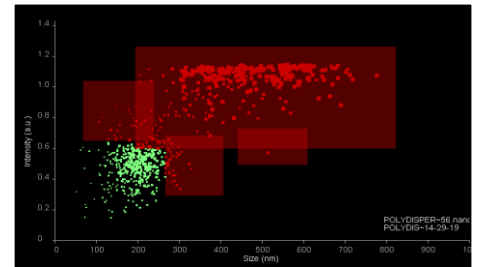
When loading an experiment, the scatter plot now contains data from all selected analyses.



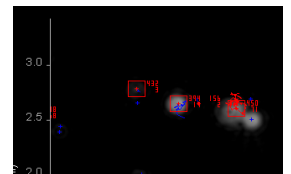
Exclusion Regions

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



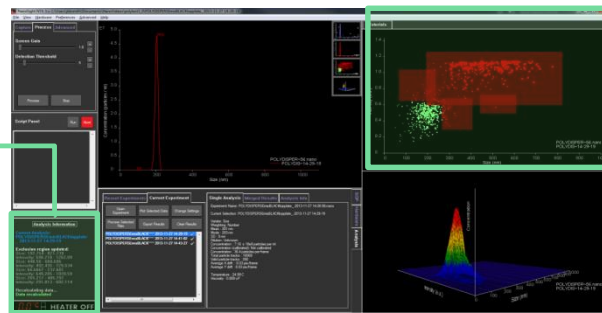
Analysis Information

Current Analysis:
POLYDISPERSE mixBLACKtopplate-
_2013-11-27 14-29-19

Exclusion region updated:
Size: 192.754 - 821.739
Intensity: 598.218 - 1262.09
Size: 440.58 - 608.696
Intensity: 492.435 - 729.534
Size: 66.8687 - 237.681
Intensity: 649.295 - 1039.59
Size: 265.217 - 405.797
Intensity: 291.813 - 682.114

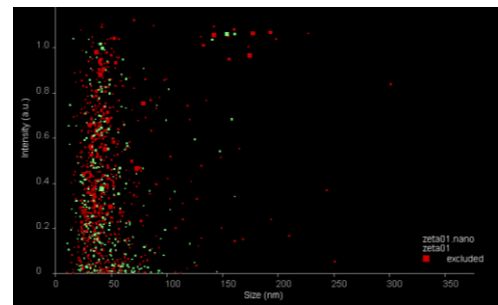
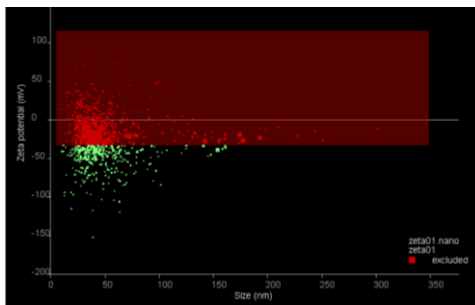
Recalculating data...
Data recalculated

Exclusion regions are shown in the status window

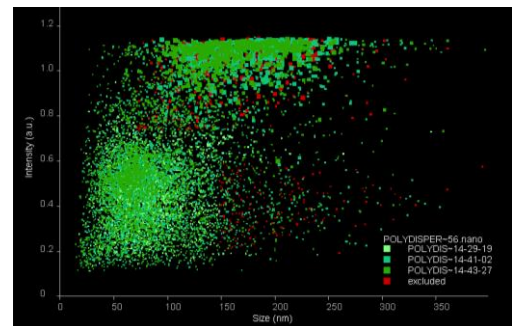


When viewing a different set of axes to where exclusion regions have been added, excluded points are shown in red. When excluded points are present this is also shown on the legend of the displayed plot.

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.



Selected Concentration

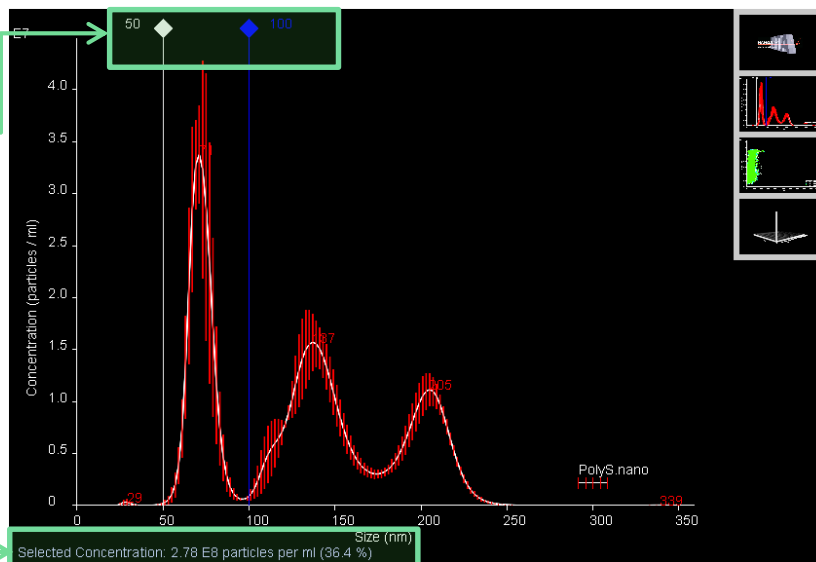
User Lines

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.

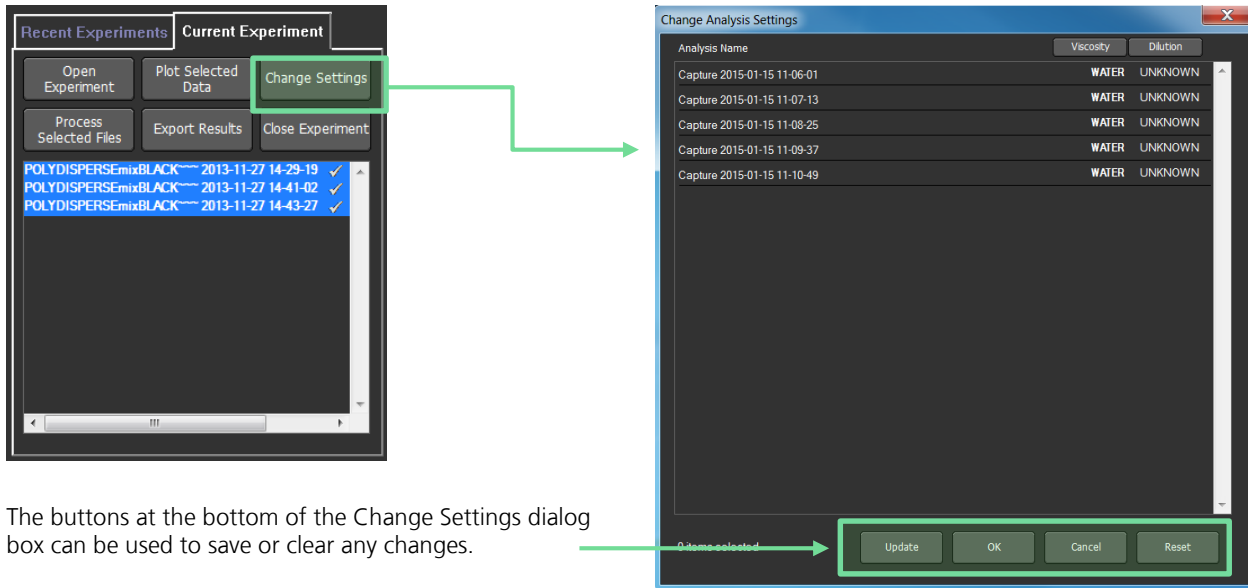
Selected concentration is available in size and diffusion, vs concentration graph views.



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

Viscosity	Dilution
WATER	UNKNOWN
WATER	UNKNOWN
WATER	UNKNOWN
WATER	UNKNOWN
WATER	UNKNOWN
WATER	UNKNOWN
WATER	UNKNOWN
WATER	UNKNOWN
WATER	UNKNOWN
WATER	UNKNOWN

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

Viscosity	Dilution
WATER	UNKNOWN
WATER	UNKNOWN
WATER	UNKNOWN
WATER	UNKNOWN
WATER	0.000
WATER	UNKNOWN
WATER	UNKNOWN
WATER	UNKNOWN
WATER	UNKNOWN
WATER	UNKNOWN

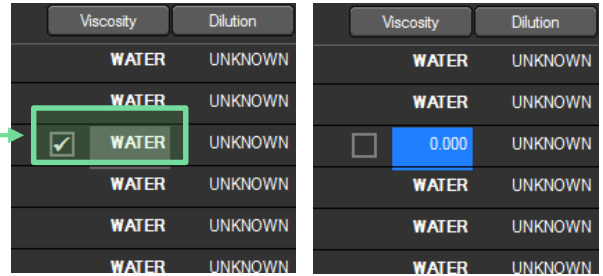
3. Press the Enter key to automatically update all values in the column

Viscosity	Dilution
WATER	1.000000e+009
WATER	1.000000e+009
WATER	1.000000e+009
WATER	1.000000e+009
WATER	1.000000e+009
WATER	1.000000e+009
WATER	1.000000e+009
WATER	1.000000e+009
WATER	1.000000e+009
WATER	1.000000e+009

Viscosity Setting

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

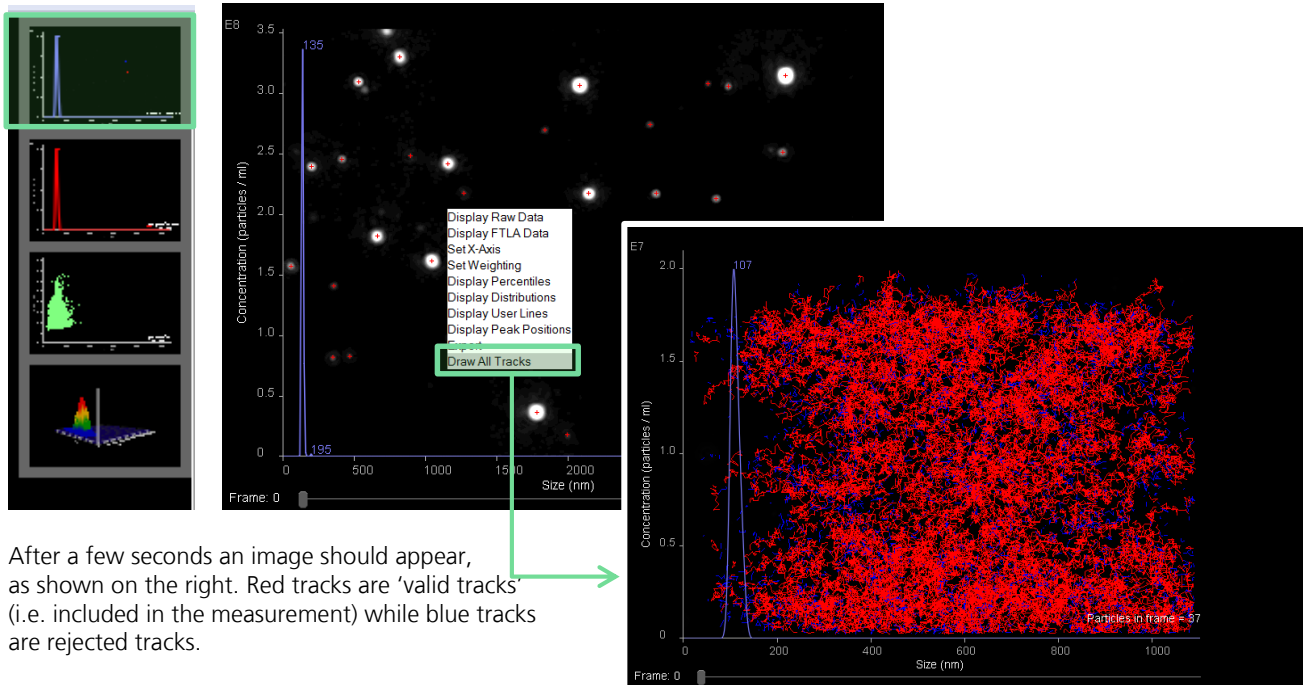
Select the tick box to set the viscosity of the sample for water at the measured temperature. When using other solvents, un-tick the box to manually enter the appropriate viscosity.



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.

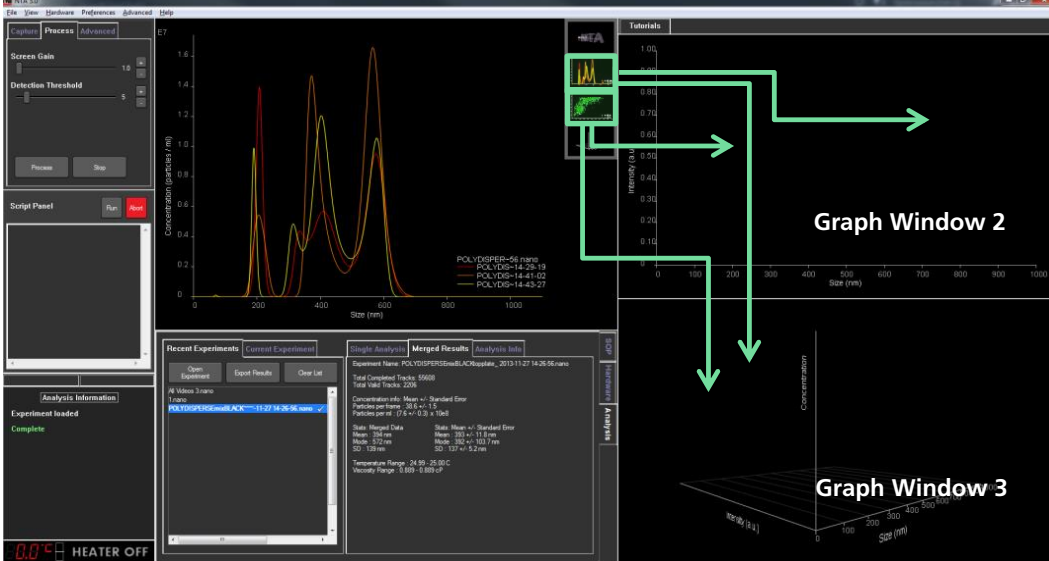


After a few seconds an image should appear, as shown on the right. Red tracks are 'valid tracks' (i.e. included in the measurement) while blue tracks are rejected tracks.

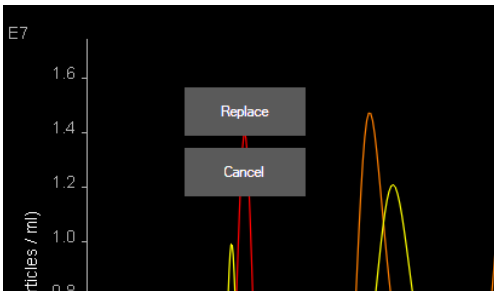
Right click -> Export can be used to export the image to file.

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

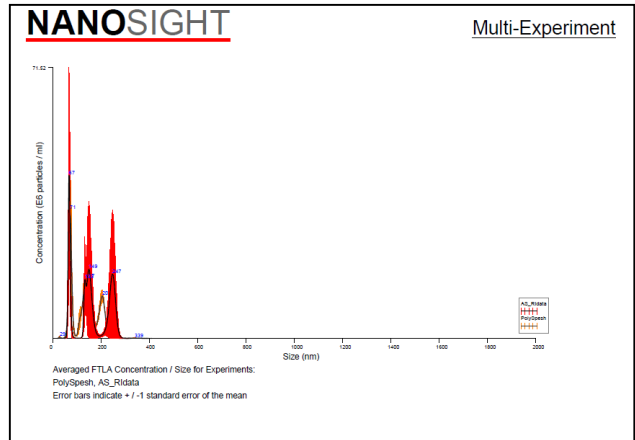
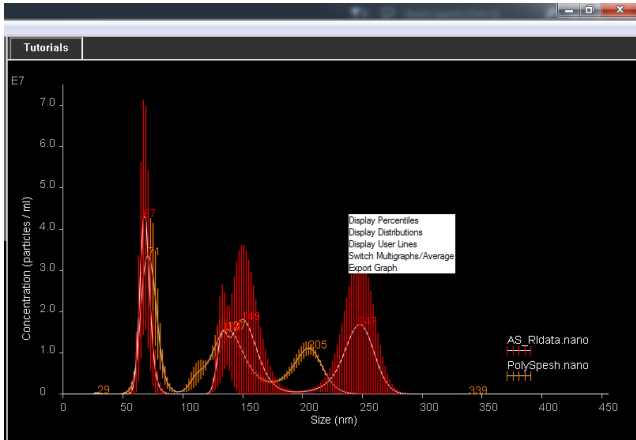
As above, but with **Overlay** button, for when datasets are compatible for overlay. Select *Overlay* to overlay the two graphs on a common axis.

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

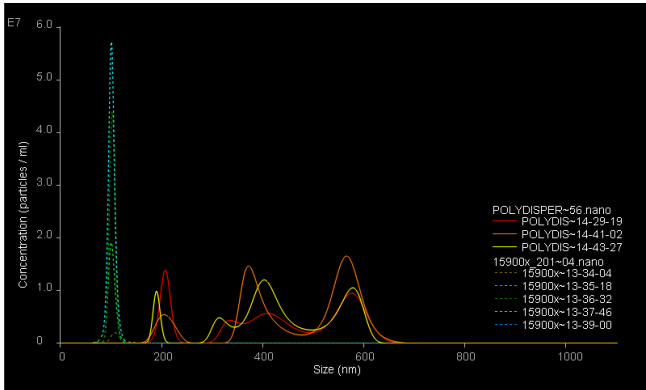
SOFTWARE UPDATE NOTIFICATION

context menu. Graphs will be exported into the same location as the currently loaded experiments.

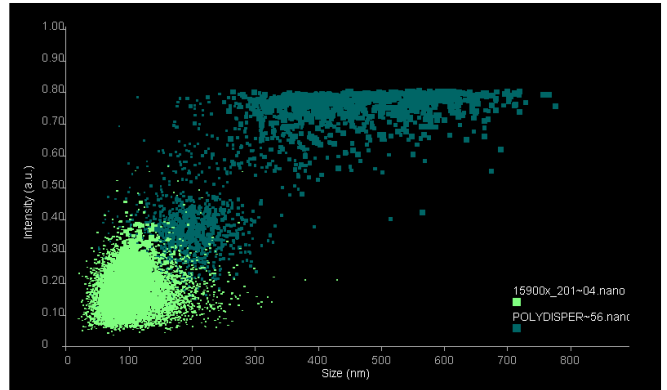


Examples of possible overlaid graph options are shown below:

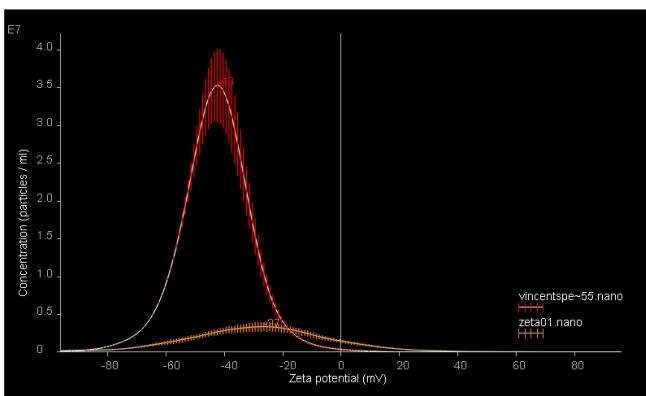
Two sets of size vs concentration multi-plots



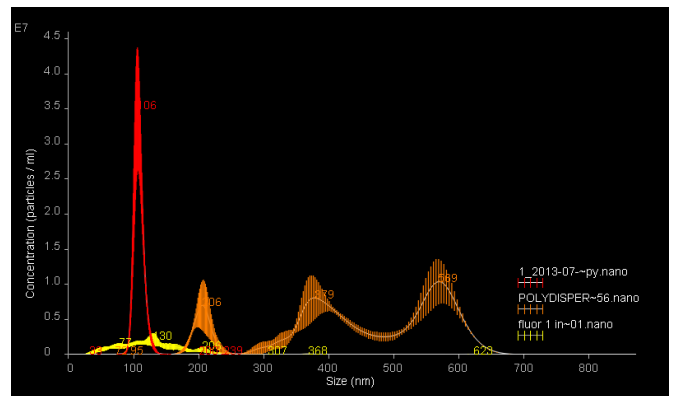
Two sets of size vs intensity scatterplots



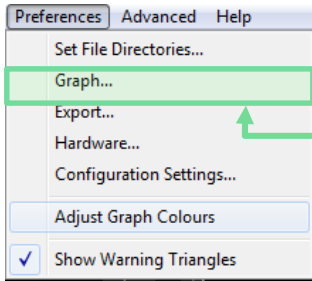
Two sets of zeta vs concentration plots with error bars



Three sets of size vs concentration plots with error bars

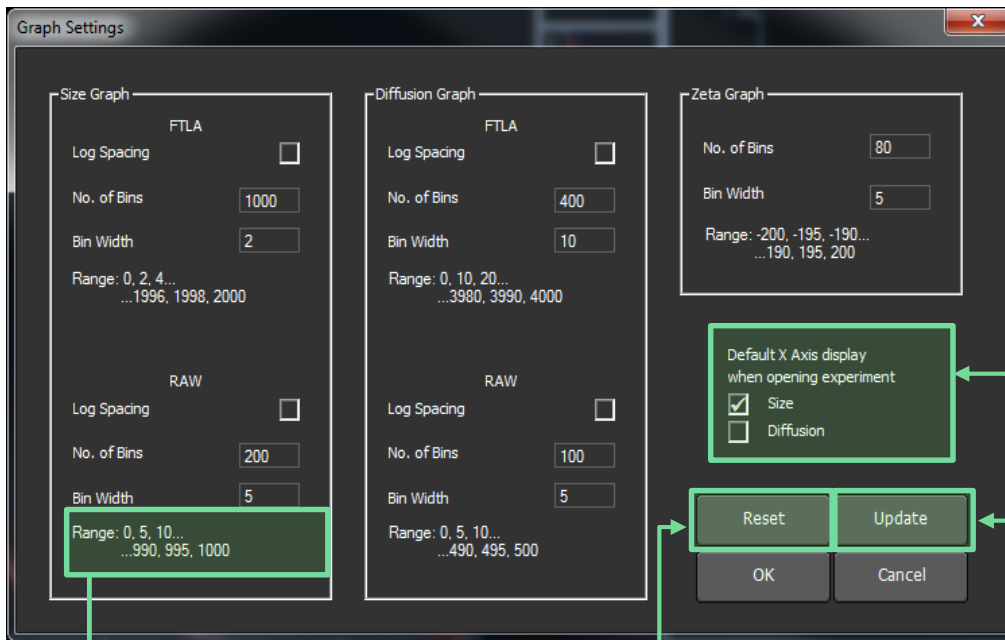


Graph Settings Dialogue



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

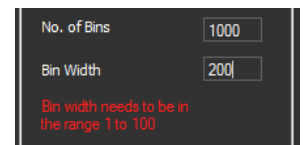
Reset resets all graph settings back to their default values

Update

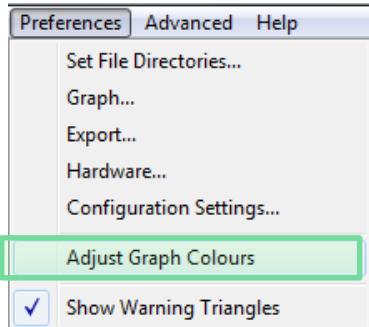
This saves the graph settings and updates any graph currently displayed by the software

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.

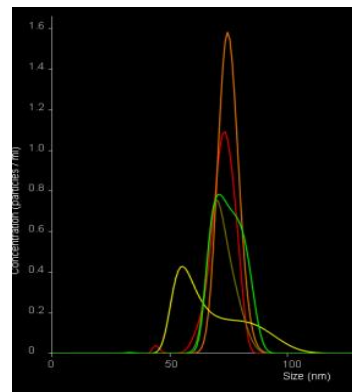
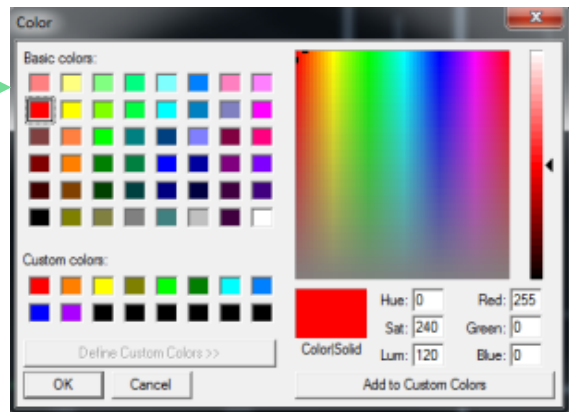
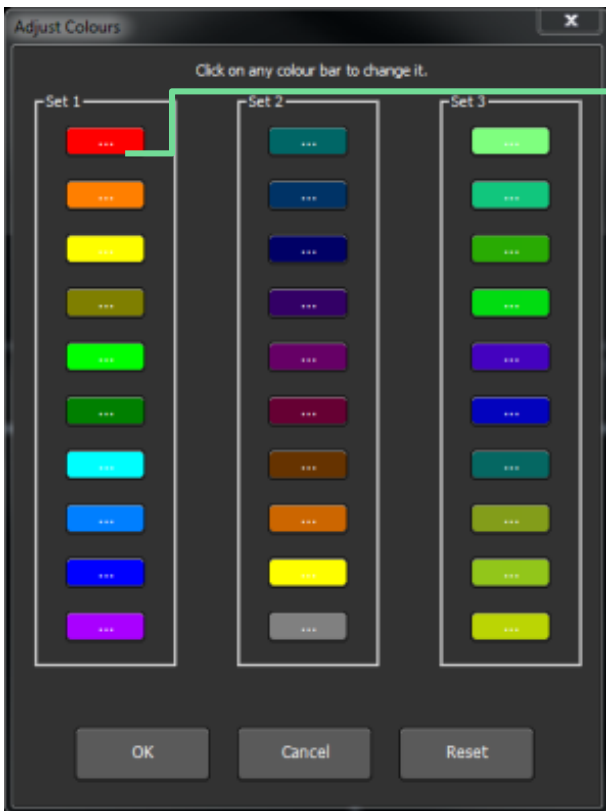
The colour maps shown in the dialogue box for Sets 1, 2 and 3 are used for different plot types within NTA.

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

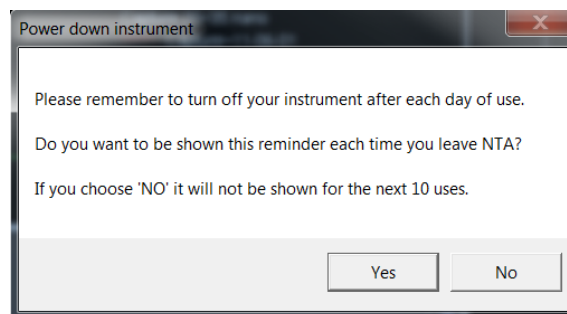
Emptying the Fluidics

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

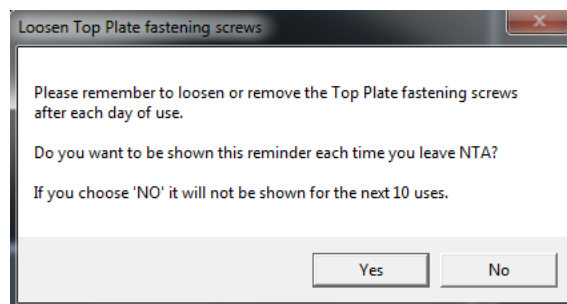
A message will appear when shutting down the software to remind the user to switch off the instrument. The user can click "No" on the prompt window to choose to stop this message appearing for the next 10 uses.



All Systems

Top-plate Screws Reminder

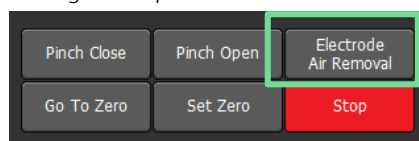
When closing down the software, the user will be reminded to loosen or remove the top plate fastening screws after each day of use. The user can click "No" on the prompt window to choose to stop this message appearing for the next 10 uses.



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

Electrode Air Removal

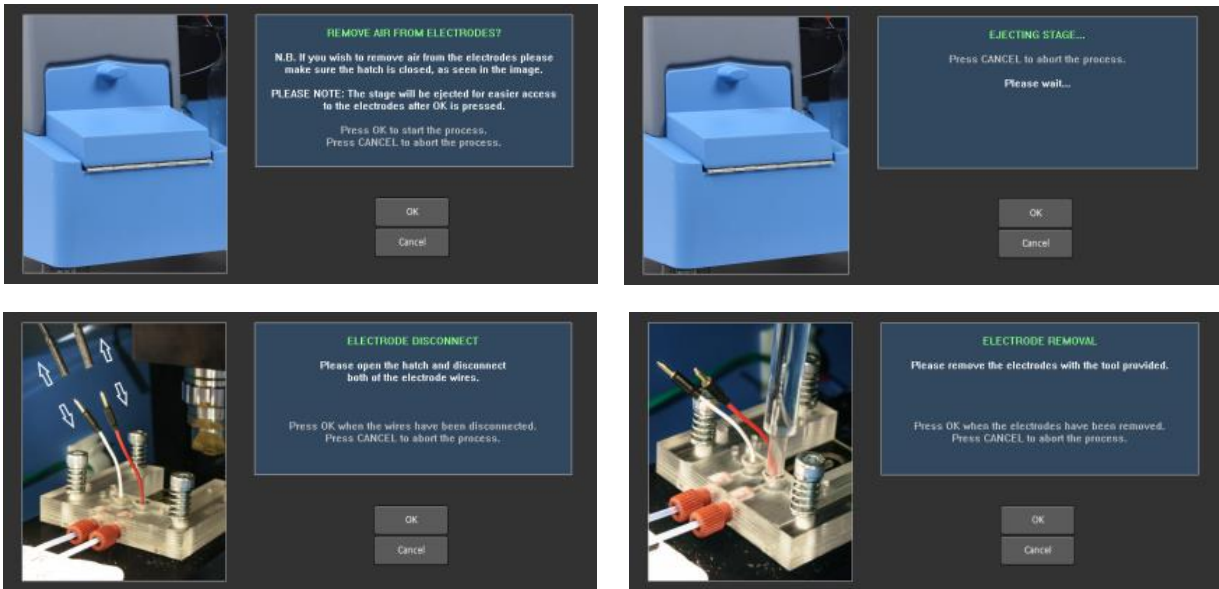
NTA 3.1 includes integrated guidance on the recommended procedure to ensure any air is removed from the flow cell before running a zeta potential measurement.



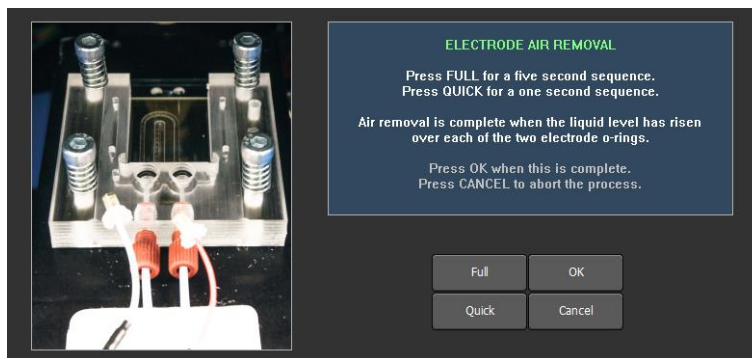
Once the sample has been loaded, click the *Electrode Air Removal* button on the Zeta Hardware tab for easy to follow instructions on this procedure.

SOFTWARE UPDATE NOTIFICATION

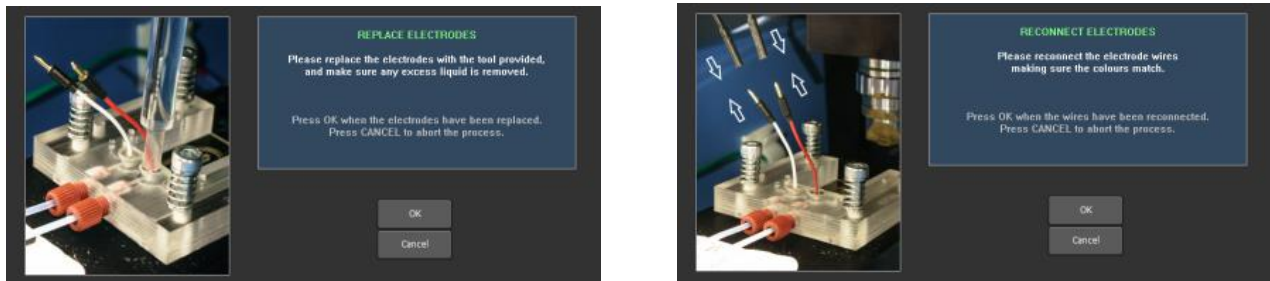
Disconnect and remove the electrodes from the top-plate:

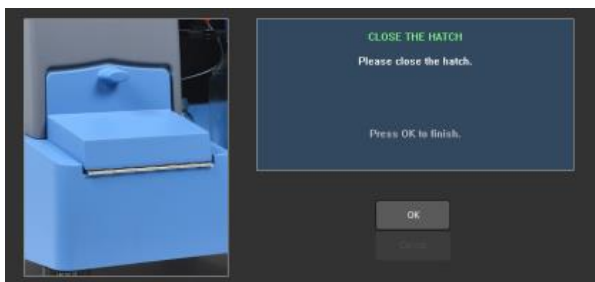


Push some sample through the system to remove any air from the electrode ports:



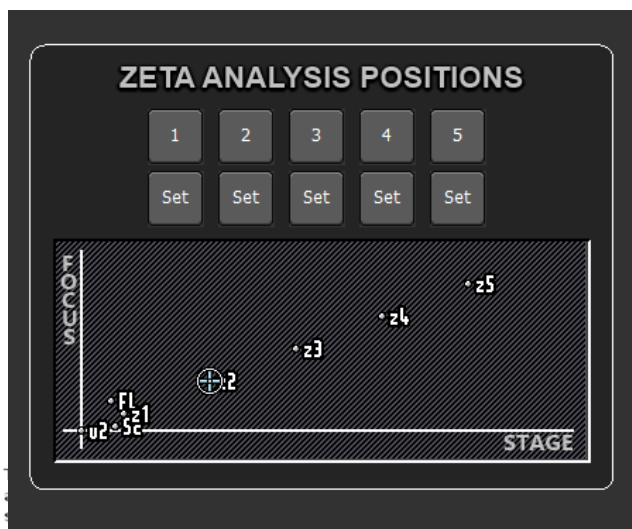
Replace and reconnect the electrodes:





Zeta Measurement Positions

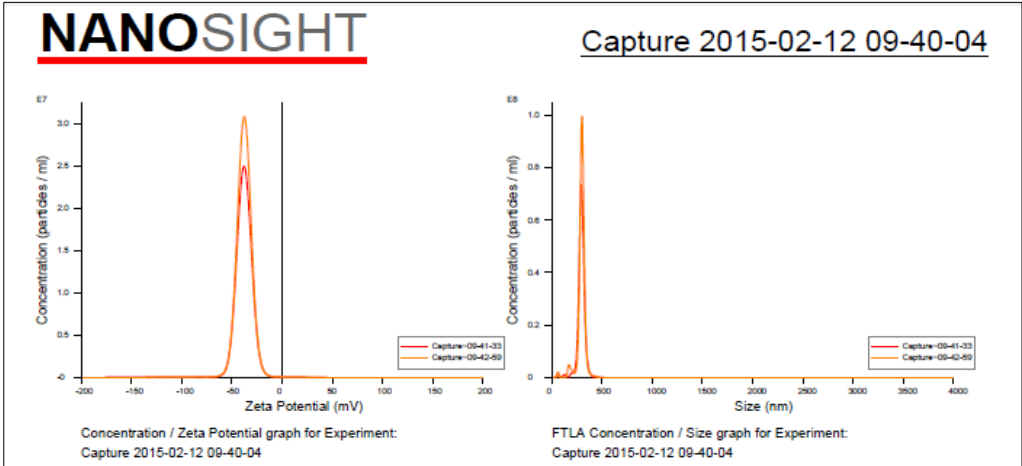
The standard zeta potential experiment uses 5 measurement positions, corresponding to the different depths with the sample chamber. The Zeta Controls tab displays buttons to move to each of these 5 measurement positions, with the current position being highlighted in the graph below.



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:



Included Files

Capture 2015-02-12 09-41-33
 Capture 2015-02-12 09-42-59

Details

NTA Version: NTA 3.1 Build 3.1.38
 Script Used: SOP Zeta Measurement 09-40-04AM 12Feb201~
 Time Captured: 09:40:04 12/02/2015
 Operator: Jo
 Pre-treatment:
 Sample Name:
 Diluent:
 Remarks:

Capture Settings

Camera Type: sCMOS
 Camera Level: 8 (NTA 3.0 Levels)
 Slider Shutter: 317
 Slider Gain: 15
 FPS: 25.0
 Number of Frames: 1499
 Temperature: 25.0 °C
 Viscosity: (Water) 0.9 cP
 Dilution factor: Dilution not recorded

Analysis Settings

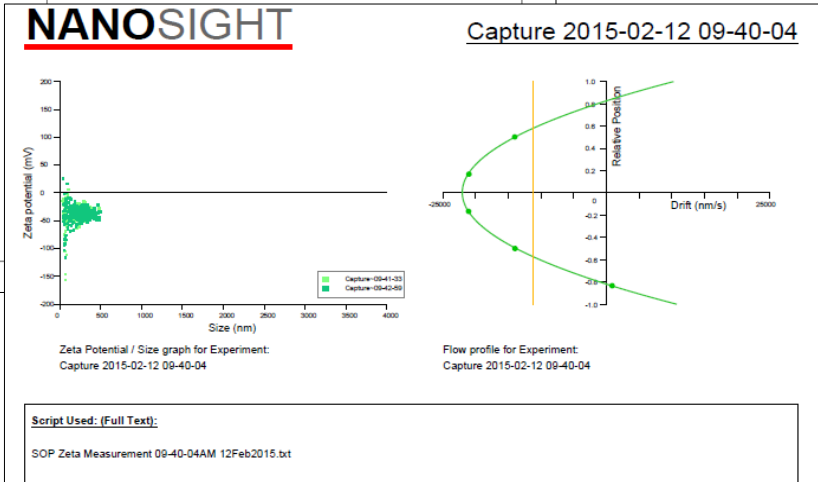
Detect Threshold: 20
 Blur Size: Auto
 Max Jump Distance: Auto: 10.7 pix

Results

Stats: Mean +/- Standard Error
 Mean: 296.2 +/- 0.9 nm
 Mode: 299.6 +/- 2.4 nm
 SD: 38.6 +/- 1.0 nm
 D10: 256.4 +/- 3.4 nm
 D50: 289.6 +/- 1.9 nm
 D90: 321.1 +/- 1.1 nm
 Concentration: 4.88e+008 +/- 3.94e+007 particles/ml
 24.8 +/- 2.0 particles/frame
 29.4 +/- 2.9 centres/frame

Zeta Settings and Results

Parabola fit complete
 Adjusted r-square: 1.00
 Applied Voltage: 24.0 V
 Dielectric Constant: 78.50
 Average Current: 25.73 - 26.86 µA
 Stats: Mean +/- Standard Error
 Mean: -37.6 +/- 0.0 mV
 Mode: -37.7 +/- 0.1 mV
 SD: 7.7 +/- 0.2 mV
 D10: -47.7 +/- 0.1 mV
 D50: -38.6 +/- 0.0 mV
 D90: -29.5 +/- 0.2 mV



Appendix II: Non-standard software update

If you are installing the software on a **blank computer** to run with the NanoSight instrument, you will need to:

- 1) Ensure the PC specifications are appropriate : Windows 7 Ultimate 32 bit, Intel Core i5-4570s Processor (Quad Core, 2.90GHz, 6MB, HD4600 Graphics), 4GB (1x4GB) RAM, 1TB Hard Drive, IEEE 1394 adapter card, Serial port Adapter card, Mouse and one 58cm (23 inch) wide-screen LED monitor. Physical dimensions 360x420x75mm.
- 2) Ensure system settings are copied across. Follow the instructions below for a basic install and then follow directions for backing up and transferring settings below.

If you are running **NTA 2.2 or earlier or for installations on a blank computer**, you should first check that the PC you are upgrading meets the current PC specifications. You can install the new software as above, however additionally:

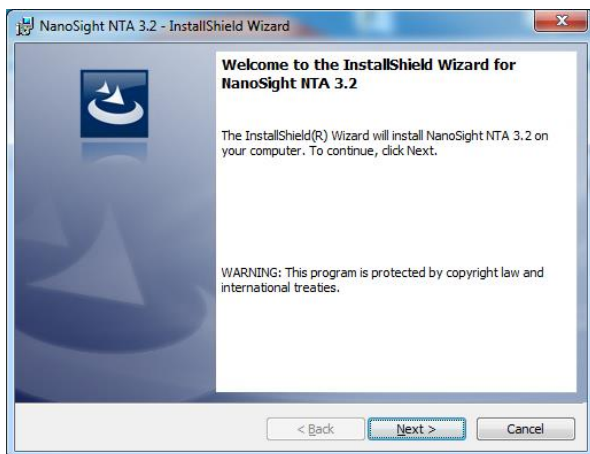
- 1) It is likely you will need to update or install camera drivers (the installation manager will inform you of this during the process). For details of driver upgrades/installation, see below.
- 2) If you have multiple laser modules, you will have been given different calibration values to put in the nano.ini file. This file is no longer used in NTA3.2. In this case refer to the 'Backing up and restoring System settings' section at the end of this document and enter the correct values relating to the relevant laser module and camera.
- 3) If the upgrade appears to all work, but the camera just shows a black screen when you click on 'start' it is likely that your graphics card is unsuitable or the drivers are out-of-date.
- 4) If the upgrade appears to work, but there is an error when a 'capture' command called from 'Sentinel' then it is likely the firmware of the system is out of date. In this case contact helpdesk@Malvern.com for the Hasp firmware upgrade software tool.

Basic Installation

In order to install the software you will need the installer file called 'NanoSight NTA 3.2 Installer.exe'.

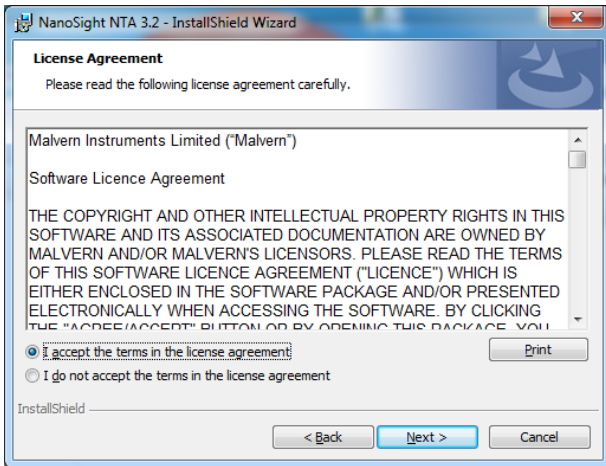
Ensure that sufficient space is available on the computer (approximately 1GB of space is recommended for installation).

If the installer is contained in a zipped file, extract to a convenient location and double click '**NanoSight NTA 3.2 Installer.exe**' to start. The installation Wizard will then run through initial installation of NTA 3.2 presenting the dialog window below.

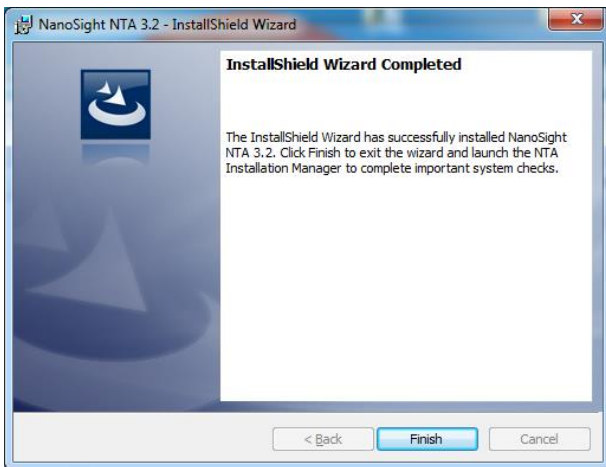


- 1) Select '**Next**', and review and accept the terms of the license agreement

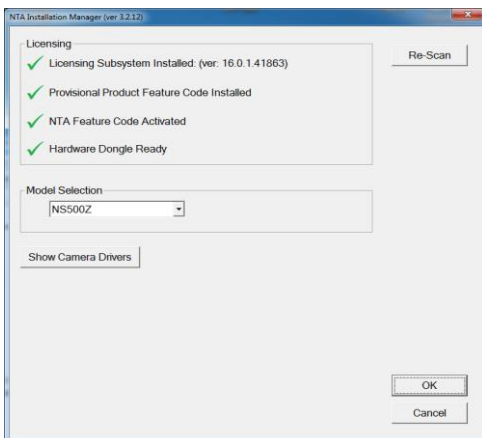
SOFTWARE UPDATE NOTIFICATION



- 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 3.2 software has now been installed on the computer. This will open the NTA Installation Manager to check further details of your individual system.



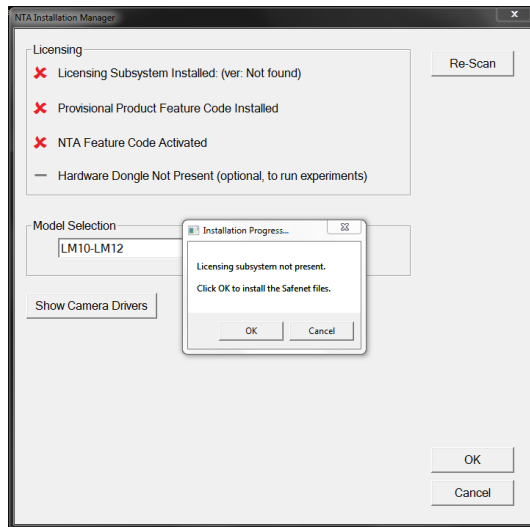
The NTA Installation Manager controls security, model selection and camera drivers. When upgrading from NTA 2.3, NTA 3.0 or NTA 3.1, all licence features should show a green tick with the correct instrument model selected.

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.

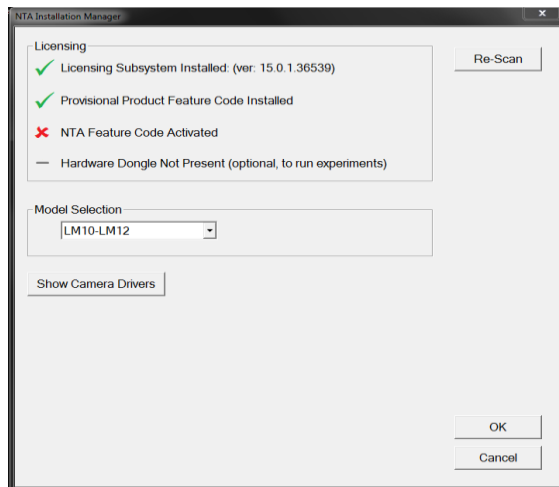
Selecting '**OK**' will close the dialog box and complete the installation of NTA 3.2.

Activation process:

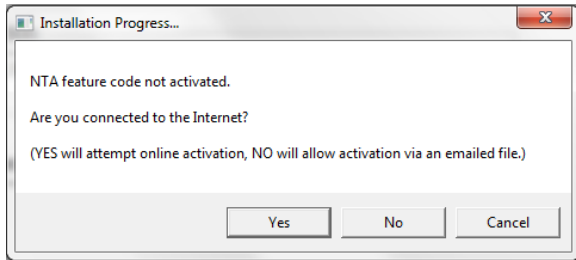
If installing NTA 3.2 software on a new computer, when the NTA Installation Manager begins, the Licensing features will not be ticked, and will have a red cross next to them as shown below. Follow the instructions below to activate NTA.



Selecting '**OK**' will run the NTA license installer '*Sentinel Run-time Environment Installer*'. After completion the '*Licensing Subsystem*' and '*Provisional Product Feature Code*' will be installed.

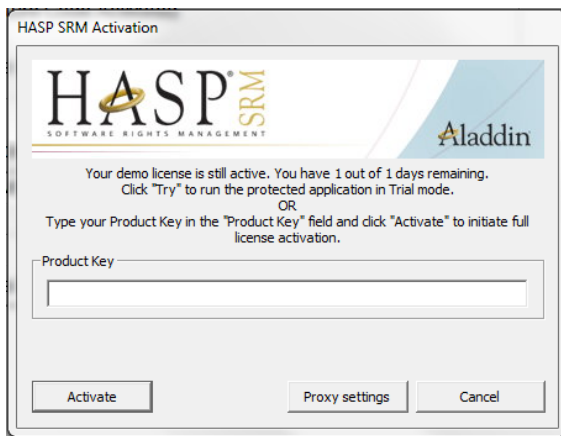


Selecting '**Re-Scan**' again will begin the activation process for the '*NTA Feature Code*'. If the computer is connected to the internet select '**Yes**' for online activation. If the computer is not connected to the internet, select '**No**' and offline activation will be started.



Online Activation:

For online activation an activation code is required. Type the product key into the box and click 'Activate'. This can be found on a printed sheet inside the first page of the system manual delivered with the instrument. If you do not have an activation code contact helpdesk@malvern.com to request a quotation (part number NTA0003).



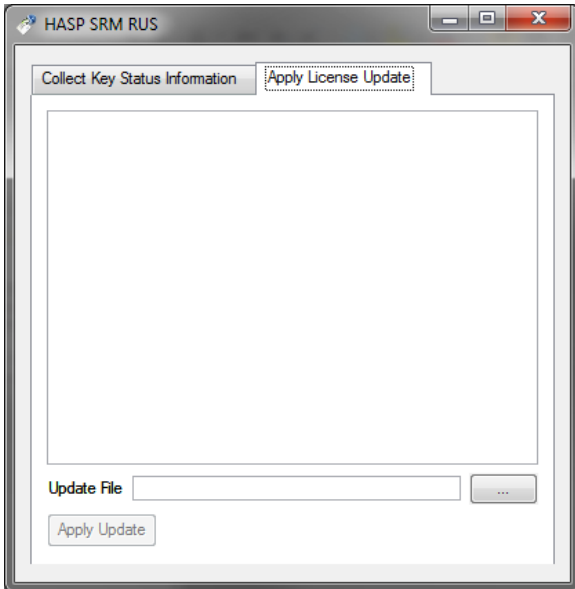
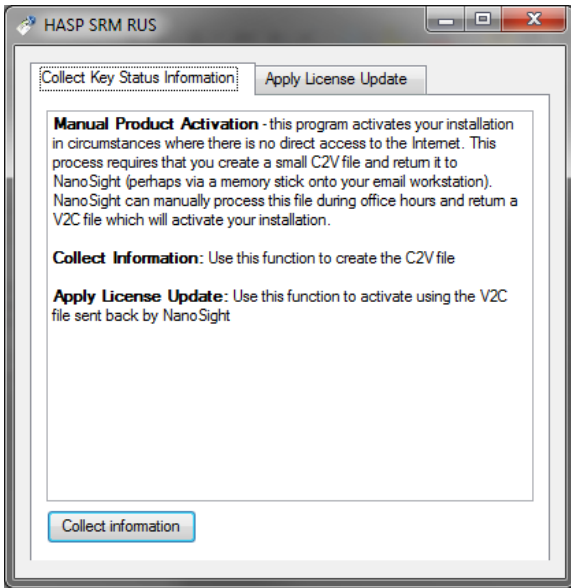
Offline Activation:

Select '**Collect Information**' to generate a c2v file. Save the file to disk using the name of your organisation, this is for our reference.

Transfer this file onto an internet enabled computer and email this file (a *.c2v file) along with an activation code to Malvern at helpdesk@malvern.com. The email should include 'NanoSight key activation' in the subject line and your contact details to allow us to identify you.

A corresponding file will then be generated by Malvern (*.v2c file) which should be transferred back to the machine you wish to activate the NanoSight software on.

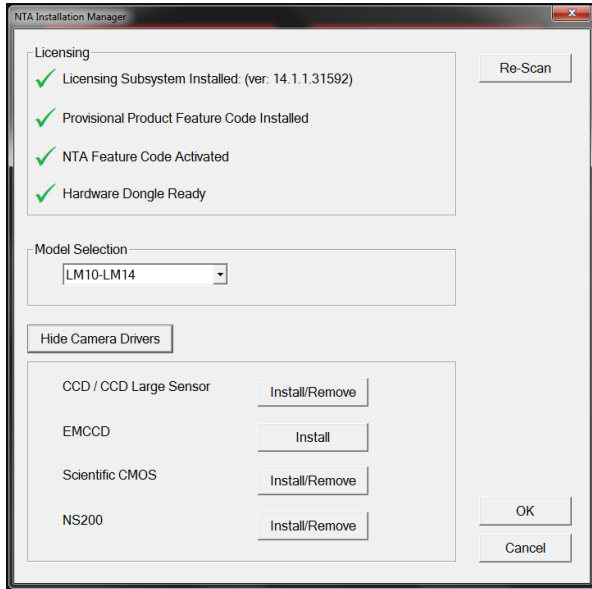
Select the Apply License Update tab and browse to the *.v2c file sent to you and click Open. You will then be informed of a successful activation and be able to start NanoSight NTA 3.2 from the desktop (note the first loading of this may take some time).



Installing camera drivers:

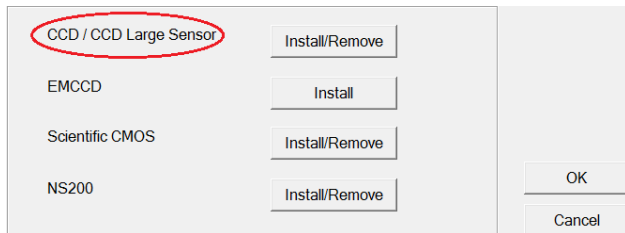
If running the software with NanoSight hardware, the correct camera driver requires installing. This will be for CCD, EMCCD or Scientific CMOS cameras. This is controlled in the 'NTA Installation Manager' window. If you are unsure which camera(s) your system has, please contact the helpdesk, providing the serial number of the instrument.

This is not needed if you are using the software for analysis only.



CCD / CCD Large Sensor (Marlin)

In the camera driver box click on the '**Install/Remove**' box adjacent to 'CCD / CCD Large Sensor'.



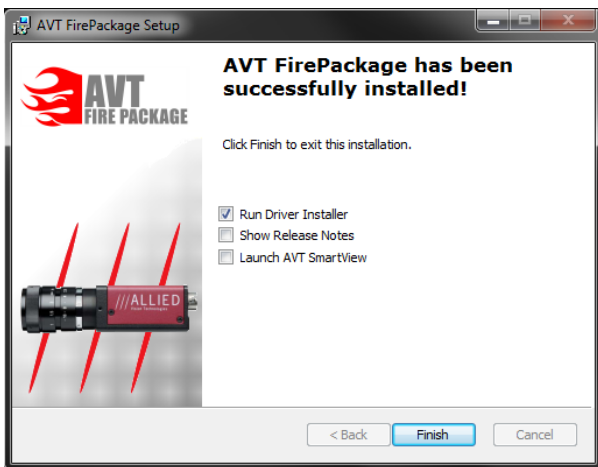
This will open the AVT FirePackage Installation Wizard.

SOFTWARE UPDATE NOTIFICATION



Click '**Next**' and continue the installation keeping all defaults.

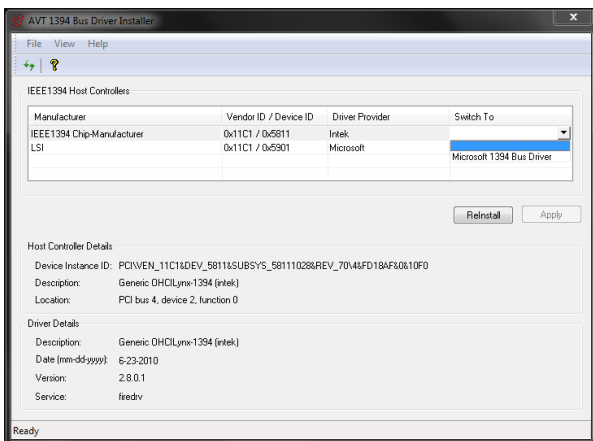
In the final window leave the '**Run Driver Installer**' box ticked before selecting '**Finish**'.



If the **Driver Provider** column is not currently set to '*Intek*', set the Switch To column to '*Intek*' and click '**Apply**'.

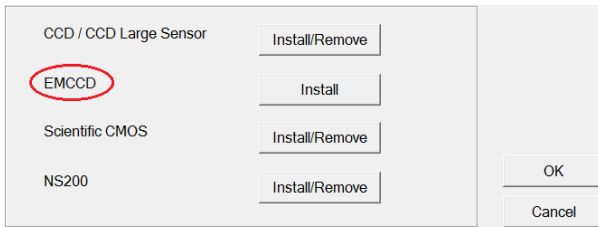
If the **Driver Provider** column is currently set to '*Intek*', click '**ReInstall**'.

After the driver installation has completed you will need to restart the computer.

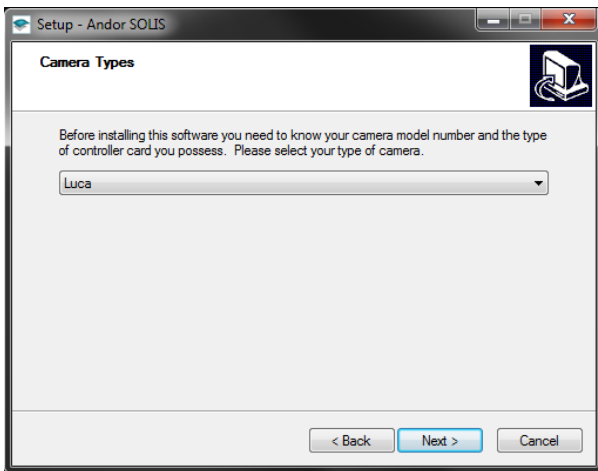


EMCCD (Andor Luca)

In the camera driver box click on the 'Install' button next to EMCCD.



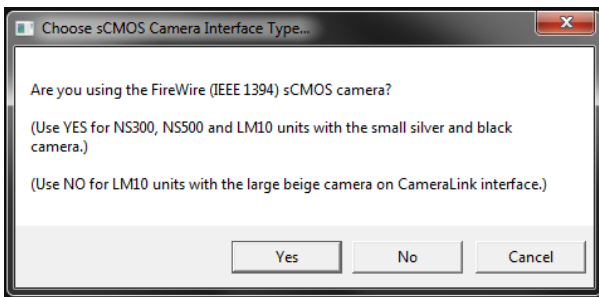
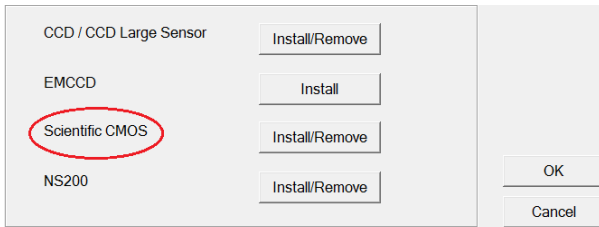
This will initiate the Andor SOLIS setup wizard. Follow the installation process selecting '**Luca**' in the camera type's window.



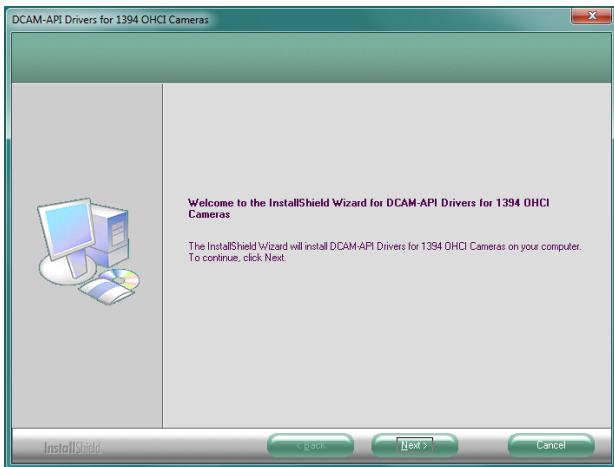
Continue the installation process restarting the computer to complete the installation.

Scientific CMOS (Hamamatsu)

In the camera driver box click the '**Install/Remove**' button adjacent to '*Scientific CMOS*' and select '**Yes**' if the camera described matches the system in the next window.



This will then run the InstallShield Wizard for DCAM-API driver.



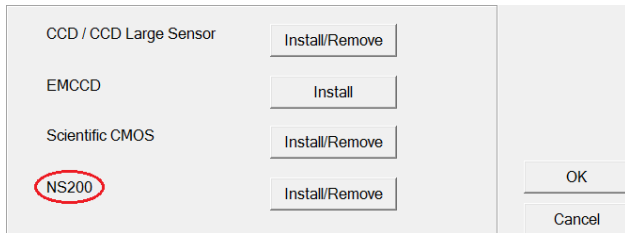
Select '**Next**' and accept the license terms by selecting the appropriate box and clicking '**Next**'. Finally, select '**Install**', it may take a few minutes to install the driver and then select '**Finish**' at the next dialog window to complete the process.

NS200 (Raptor)

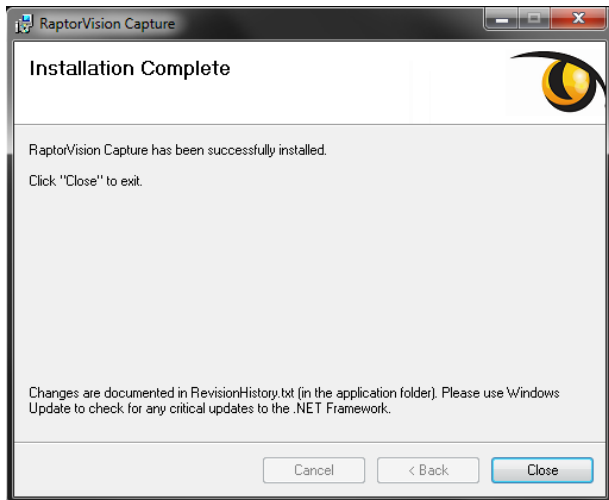
IMPORTANT NOTE – *Ensure the camera is not connected to the computer during installation of the Raptor software.*

In the camera driver box click on the '**Install/Remove**' button adjacent to '*NS200*'. This will start the RaptorVison Capture Setup Wizard.

SOFTWARE UPDATE NOTIFICATION

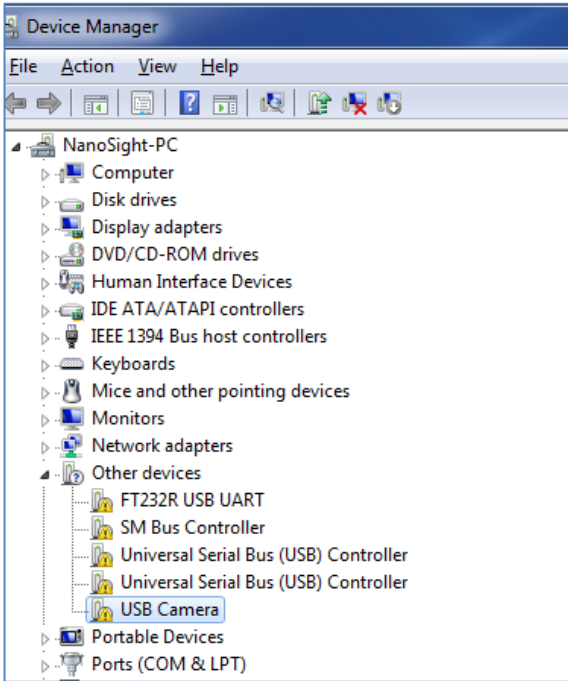


Click '**Next**', and ensure the '*Everyone*' option is ticked in the following window. Continue with the installation process.

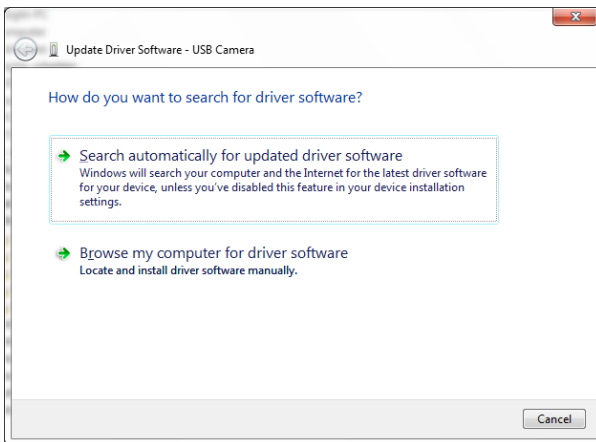


The Raptor camera software (but not the drivers) is now installed, click '**Close**'. It is recommended that you check Windows Update at this point. After installing any updates, restart the computer.

Connect the NS200 to the computer via the USB lead; automatic driver installation will begin and fail. Navigate to device manager, right click on "*USB camera*" and click '**update driver software...**'

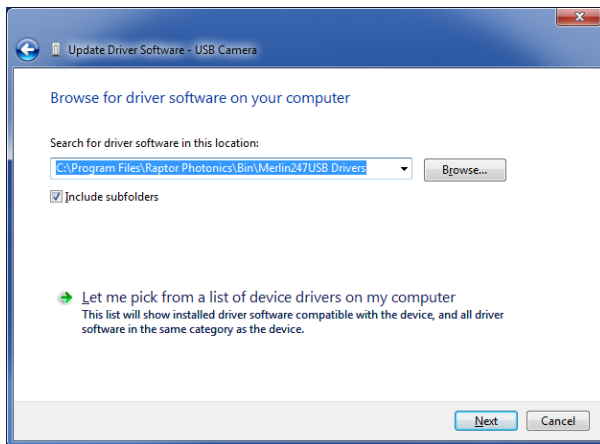


Select '**Browse my computer for driver software**'.

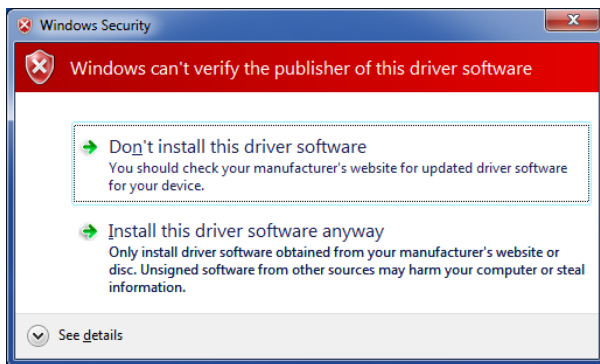


Navigate to '**C:\Program Files\Raptor Photonics\Bin\Merlin247USB Drivers**' to search for drivers and click '**Next**' to begin installation.

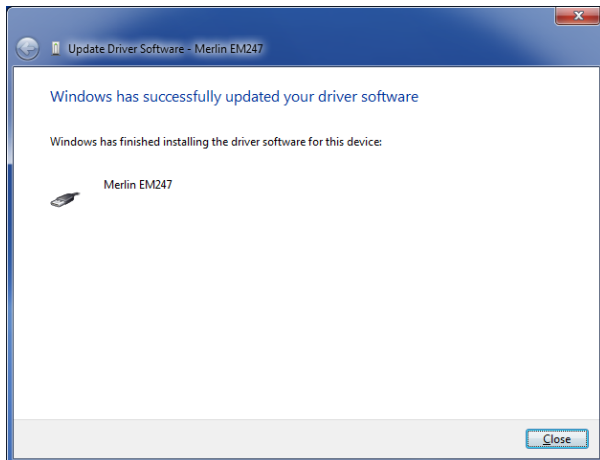
SOFTWARE UPDATE NOTIFICATION



If you see a Windows Security window appear, click '**Install this driver software anyway**'.

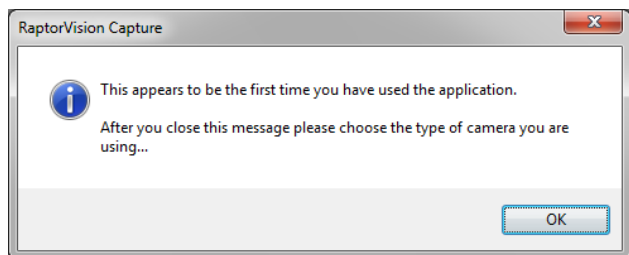


Driver installation is complete, click '**Close**' to exit. **Do not** run the NTA software yet.



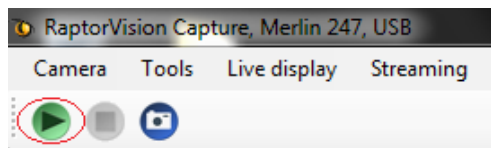
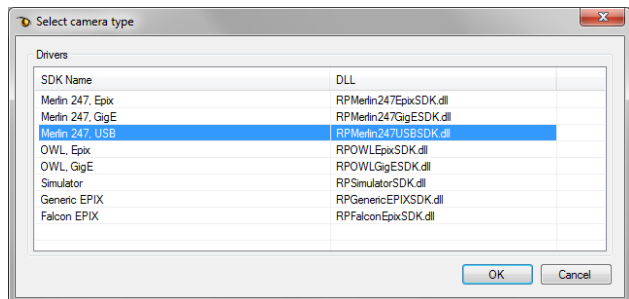
Go to **Start->programs->Raptor Photonics-> Raptor Vision Capture**, the following message should appear:

SOFTWARE UPDATE NOTIFICATION

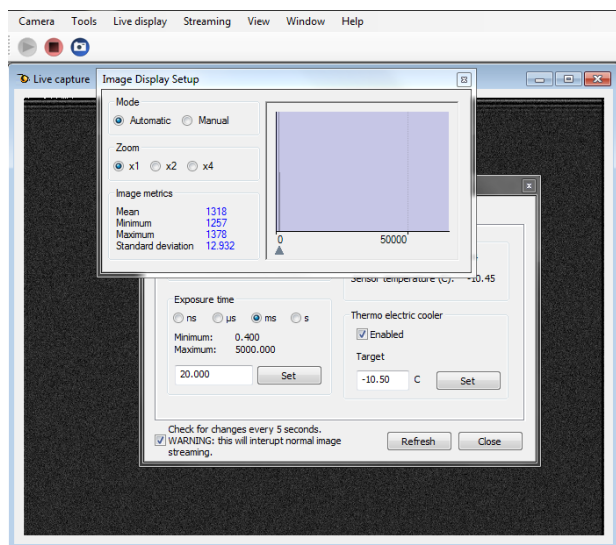


Click 'OK'

Select 'Merlin 247, USB' from the list, click 'OK'



Click the Start button in the top left corner. The follow screen should appear.



This confirms the driver installation. The Raptor Vision Capture program must then be closed before running NTA.

Backing up and transferring system settings

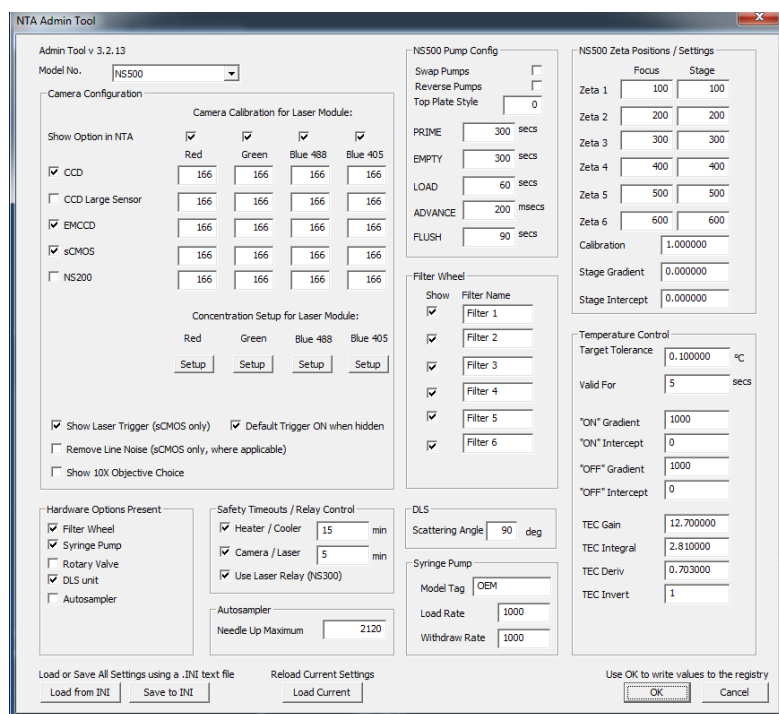
In versions NTA2.3 and before there exists a configuration file nano.ini which will be located in 'my documents'. These text file can be viewed in e.g. Notepad. Within that file there is are the calibration values for the system. Specifically lines starting PixelSize=xxx and PixelSizeEM=yyy (where xxx would refer to the CCD camera calibration value and yyy would refer to either CCD large sensor, EMCCD or sCMOS, dependent on which camera you have).

NTA 3.2 stores and accesses important system settings for the NanoSight instrument using the Windows registry. If the registry becomes corrupt or is deleted, the operating system is reinstalled, or the software is transferred to a new computer, these settings will need to be replaced before using the software with the instrument.

If new hardware is purchased, or your instrument is recalibrated, modifications to these settings may be required.

The NTA 3.2 installation includes a user interface to manage these settings. It is called 'NTA Admin Tool' and can be accessed from the start menu or via Preferences->Configuration Settings... within the NTA 3.2 software. The tool requires administrator rights to run.

The tool will appear like the image below:



If upgrading from NTA2.3 or earlier, or installing on a new system you should either enter or confirm values xxx and yyy identified in the nano.ini file discussed above are entered in the relevant fields above under camera configuration.

To back-up the current settings click **Save to INI** (bottom left) and save the file in a convenient location on disk.

To reload settings from a back-up (e.g. on a new computer or re-installed operating system) click **Load from INI** and locate the backed-up file on disk.

After loading or changing any settings, click **OK** to save the settings. Clicking **Cancel** will not save any changes to current settings.

Should changes be required after new hardware is purchased or recalibration has taken place, Malvern will provide information on the changes required.

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