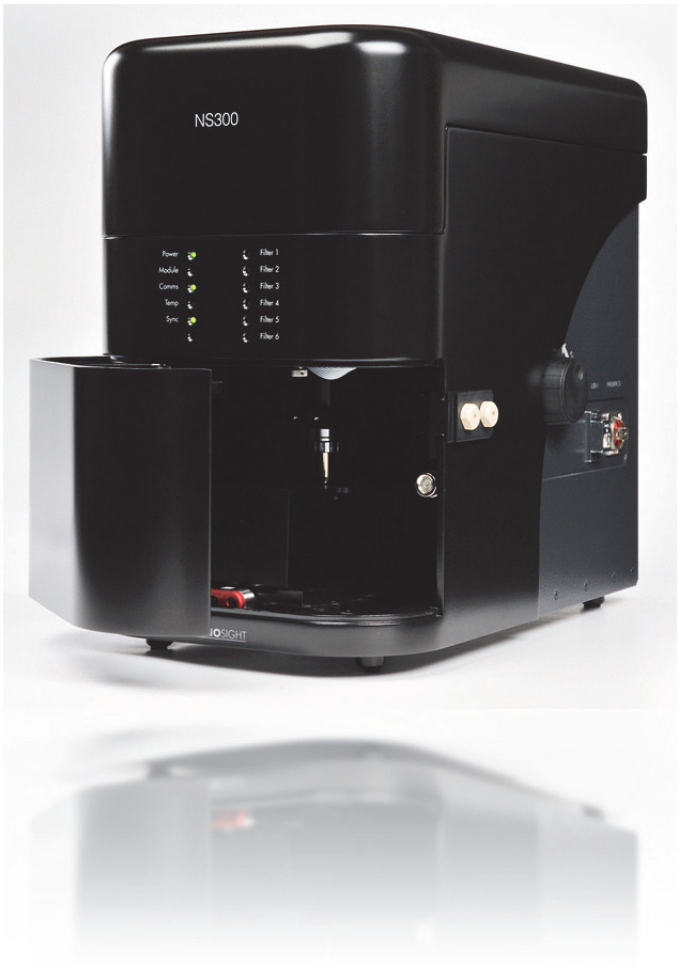
NANOSIGHT NS300
NTA SOFTWARE GUIDE

MAN0545-01-EN-00 May 2014



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NanoSight Software NTA

NTA is Malvern's software NanoSight range of instruments. It allows videos of particles moving under Brownian motion to be captured and analyzed to generate high resolution size and concentration data.

Measurements are run via SOP-type procedures with default options for standard measurements and user defined options for additional flexibility.

Hardware control is integrated into the software for control of temperature, flow rate, focus etc… when appropriate hardware is available.

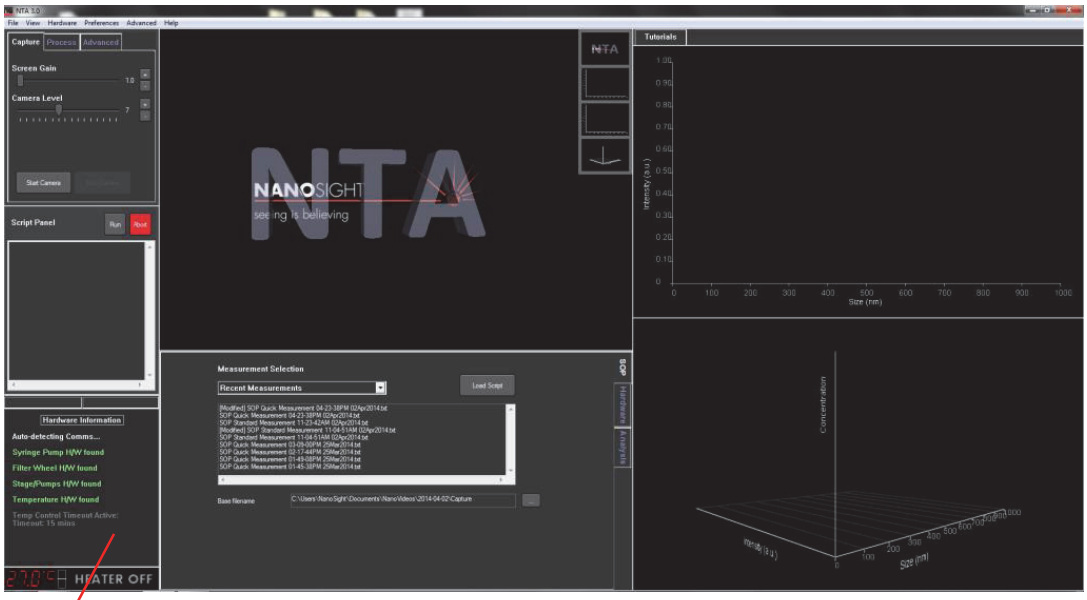
NTA Key Features

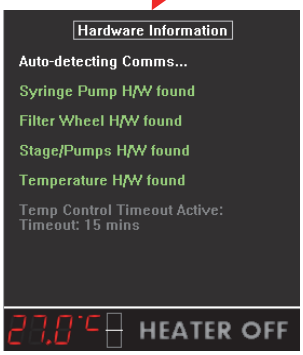
- High resolution particle size distribution algorithm
- Advanced image analysis, particle detection and tracking
- Integrated scripting option for SOP development
- Basic statistical parameter output
- Vibration detection and correction
- Integrated hardware control and communication
- PDF and CSV document export options

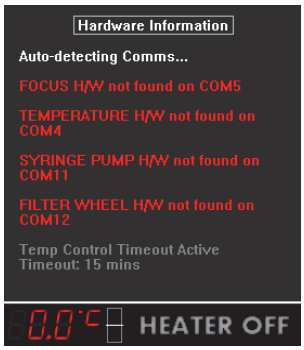
Getting Started

Double click the NanoSight NTA Software Icon on desktop

Typical NTA Home Screen





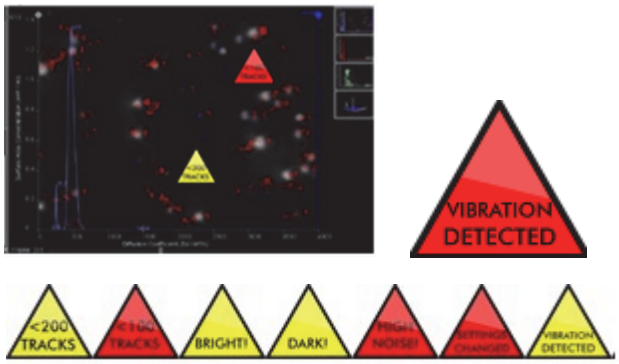


Connection status is detailed in the Hardware Information window. Any hardware attached to the equipment, if available, such as the syringe pump will be automatically detected by the software. Check that the required hardware is detected. If the required hardware is shown as 'not found', check connections and power.

Notes:

- For users new to the NanoSight Instrument and NTA software, we recommend making first measurements using size-calibrated standard particles.

Information Symbols



During processing various information symbols may be displayed, hovering over these will give more information about the warning.

Making a Measurement

This is a two-step process:

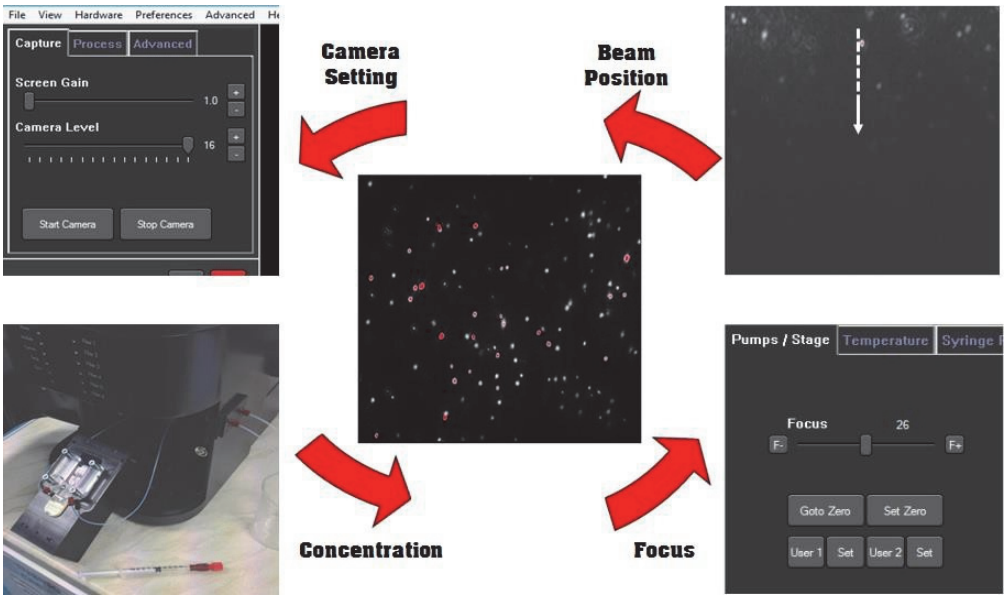
1. Optimize the image
2. Take a measurement.

For advice on loading samples and operation of the hardware go to the NanoSight NS300 operating manual.

1. Optimizing the Image

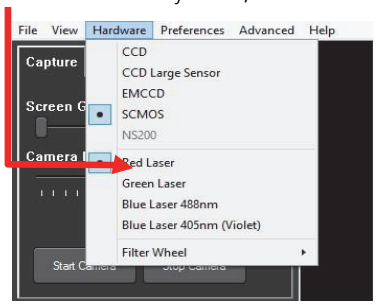
This is an iterative process between Camera Level, Sample Concentration, Beam Position, and Focus.

Different sample types will require different final settings.

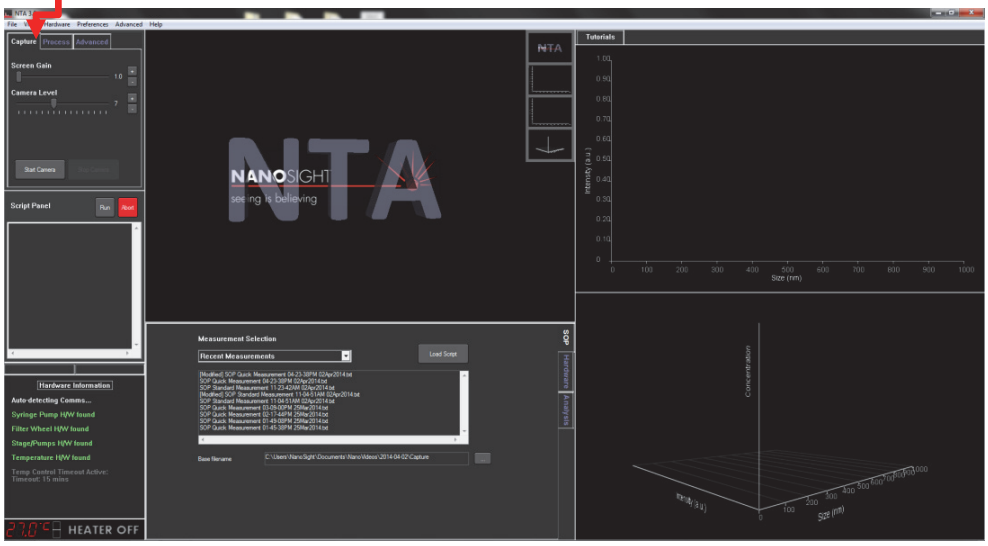


To obtain an initial live image for optimization

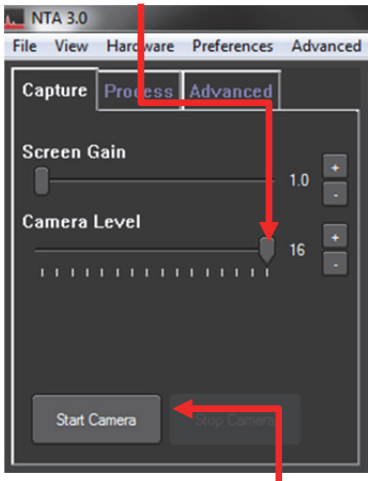
- For multi-laser systems, select the correct laser from the hardware tab at the top of the screen.



- Select **Capture**



- Set **Camera Level** to **Max**



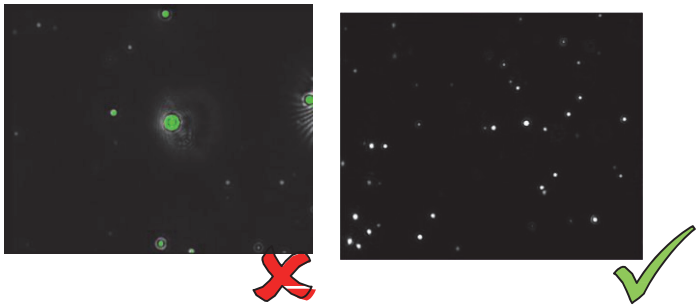
- Click **Start Camera**

Adjusting the Camera Level

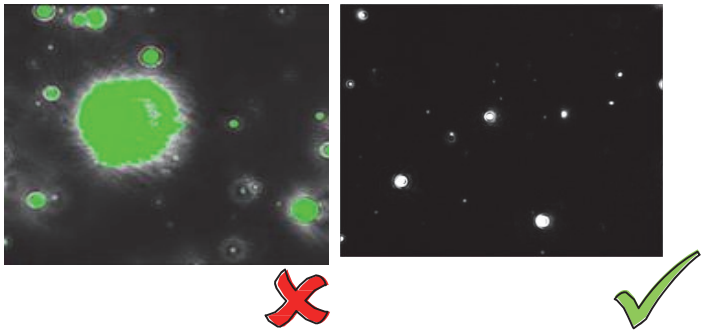
Correctly setting up the camera and sample image prior to capturing the video are essential to achieving valid results.

The software is designed to provide warnings at the extremes of operation, but to optimize the results from a particular sample, the user must ensure that the manual settings are as close to perfect as possible.

- Increase the **Camera Level** until all of the particles in the sample can be seen clearly but no more than 20% are saturated (coloured pixels).
- Poor Monodisperse Sample Good Monodisperse Sample



- Poor Polydisperse Image Good Polydisperse Sample



Concentration

The NanoSight instruments can work with particle concentrations in the range of ~10^7-10^9 particles/ml, which is approximately 20-100 particles in the field of view.

Too high a sample concentration may prevent accurate particle tracking.

Lower concentrations require longer capture and analysis time to produce statistically significant results.

It is possible to use the NanoSight syringe pump to improve results by sampling more particles.

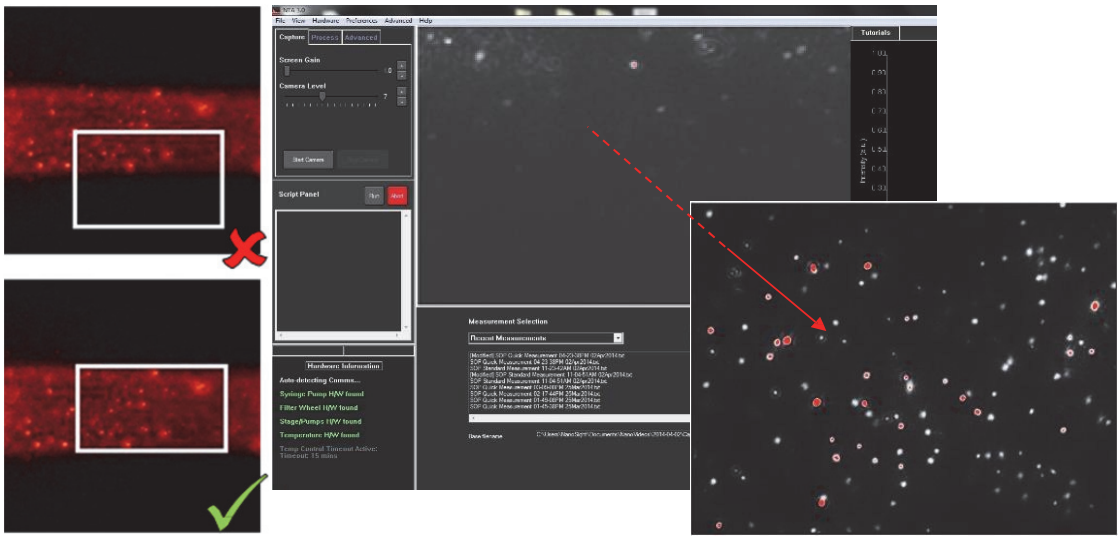
a) Concentration too high 208 particles identified

b) Concentration too low 1 particle identified

c) Ideal concentration 44 particles identified

Laser Beam Position

The illuminated particles need to fill the field of view.

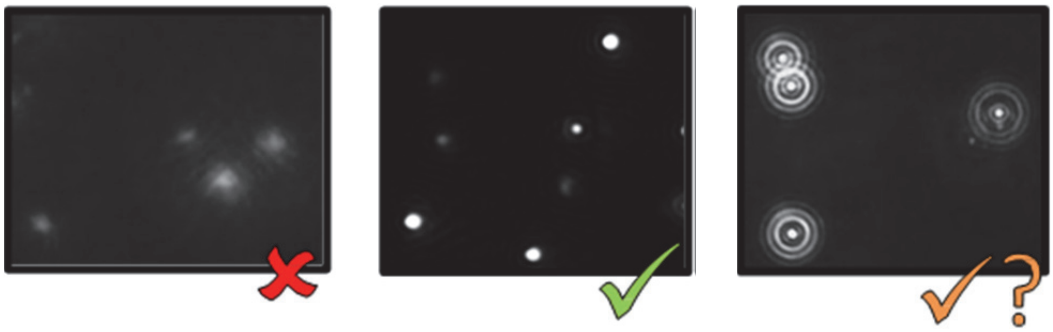


Use left mouse button to drag and center the image.

Image Focus

Initial focus is set with the manual control on the side of the NanoSight NS300, adjust for fine focus within the software.

Because the particles are constantly moving it can be difficult to achieve a uniform perfect spherical focus.



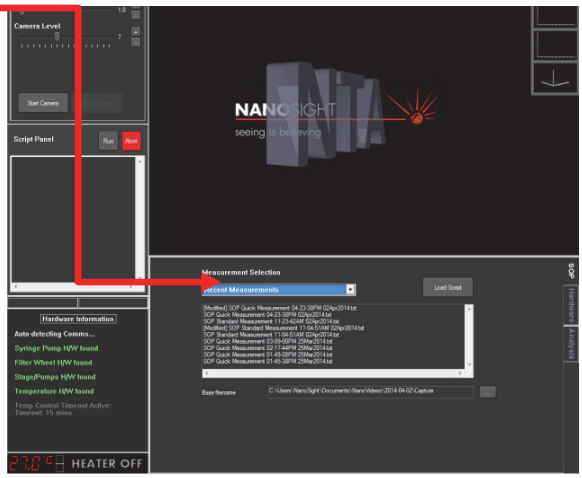
a) b) c)

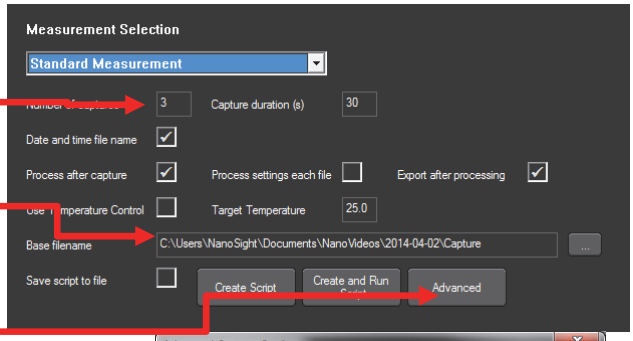
Indistinct particles, as in a) will give inaccurate results.

Ideally, particles should appear as in b) but those in c) will be acceptable if that is the best image that can be achieved.

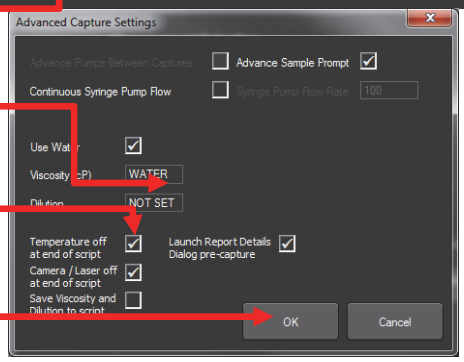
Taking a Measurement

- Select Standard measurement





- **Number of Captures:**
  The default number and length of video captures are suitable for most samples.
- **Base Filename**:
  Select the name and location for the captured video files.
  - For dilution and viscosity settings press **Advanced**.
  - Sample **dilution** factor can be entered. Where the diluent is **not** water, **solvent viscosity** must be entered here.
  - It is recommended that the lower check boxes are left checked to avoid heat build up between readings (**Temperature off..**, **Camera/laser off…**, **Save Viscosity…**).
  - Click **OK**.



- The sample is ready to be measured.
- Click **Create and Run script**.

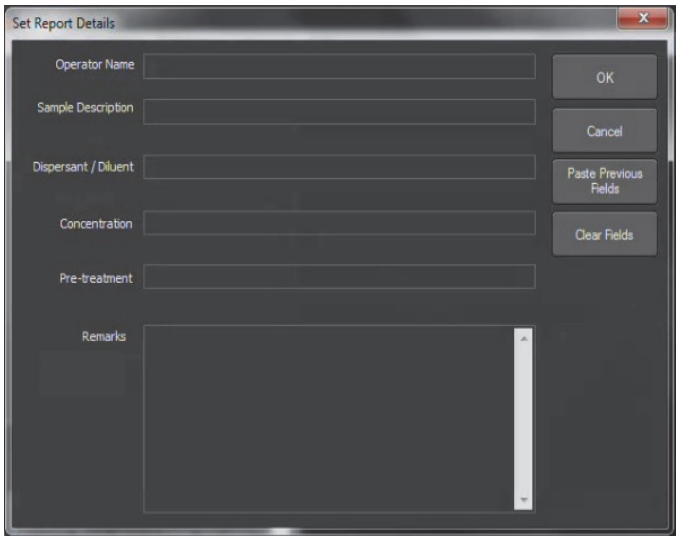
'Scripts' are sets of instructions that control the software for each measurement.

The NTA software contains options for:

- **Standard Measurement**
  Suitable for most size and concentration measurement within instrument specifications.
- **Quick Measurement**
  Creates a single video and analysis.
- **Recent Measurements**
  A list of the last 10 measurements taken allowing the rapid repeat of readings.
- **Zeta Measurement**
  Not applicable for NS300 instruments.

Capture

User prompted for and sample details to be included with the video files and output files (optional).

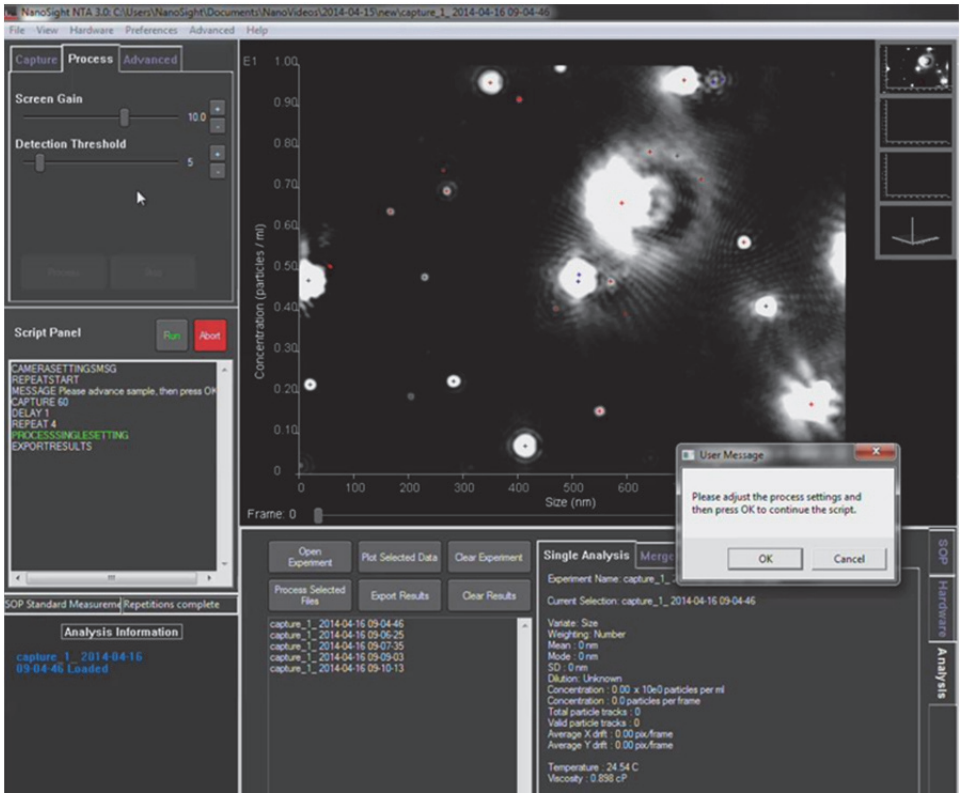


- Click **OK**

The software will prompt for the sample to be advanced (this will be requested at the start of each repeat capture according to the script). The NanoSight Syringe Pump accessory provides the alternative of continuous sample flow.

Following video capture the software defaults to immediate processing.

This screen is now showing the captured video rather than the live sample image.



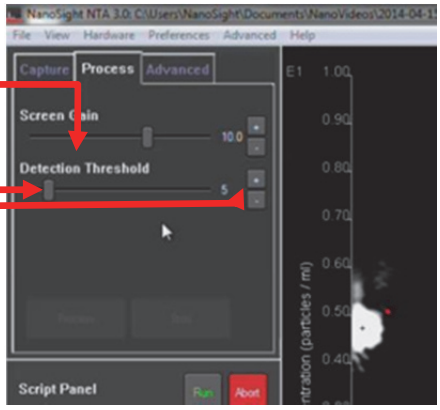
Detection Threshold must now be set before starting video processing.

Detection Threshold

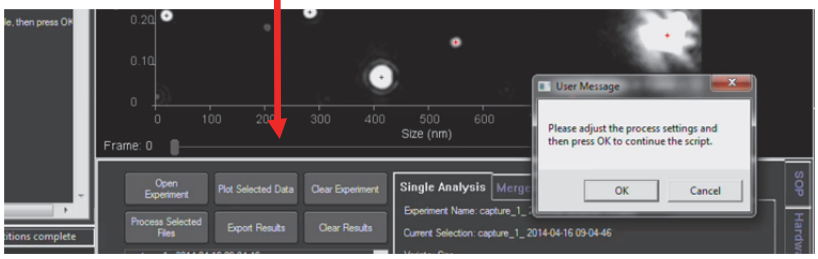
- The Detection Threshold determines the minimum brightness of pixels to be considered for tracking.
- The lower the setting the more centers will be found; however if it is too low, 'noise' can be tracked. If the setting is too high, particles will be excluded.
- For the best analysis, identify the center of each particle by reducing the Detection Threshold to a level to include as many particles as possible and within the following restrictions:
    - In the bottom right of the image is a count of the number of red crosses. This should be between 10 and 100.
    - When considering the image by eye, some of the red crosses may not appear to be distinct particles. Ideally there should be <10 such crosses.
    - There may also be blue crosses on the screen. Ideally there should be <5.
    - IMPORTANT: The detection threshold setting must not be altered between videos on the same sample measurement (e.g. all 5 videos recorded of sample with the same Capture Settings should be analysed with the same detection threshold).

Set **Detection Threshold**

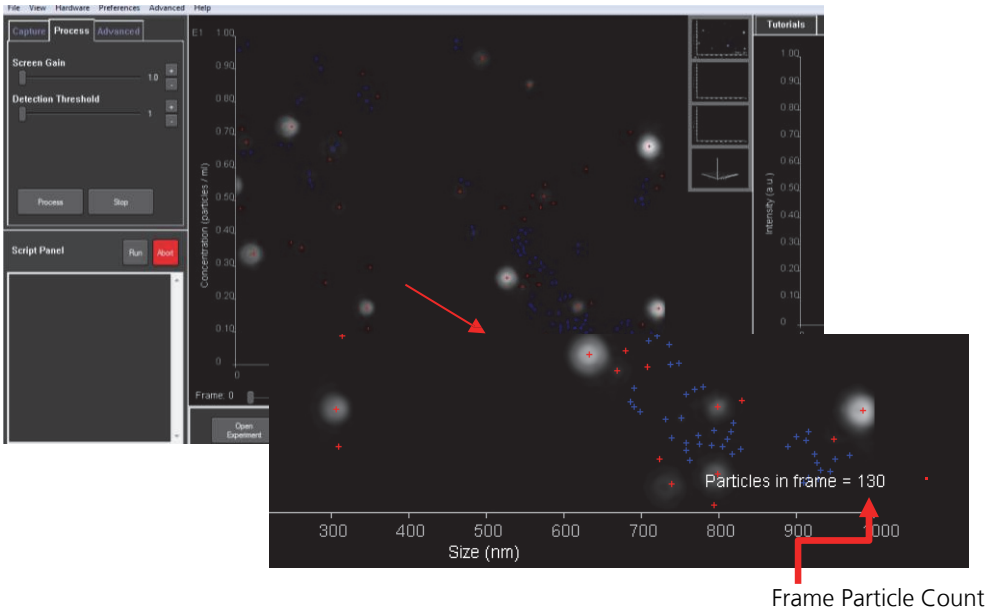
Adjustment is available with **slider** or the **+** or **–** buttons.



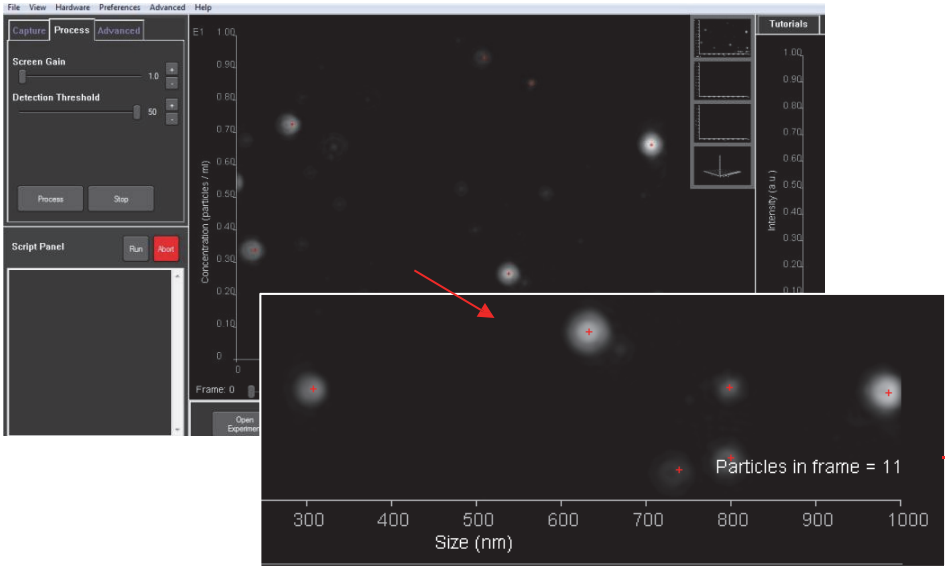
Check the selected setting with multiple frames of the video. By moving the slider under the main screen the image quality in additional frames can be checked.



Low Detection Threshold

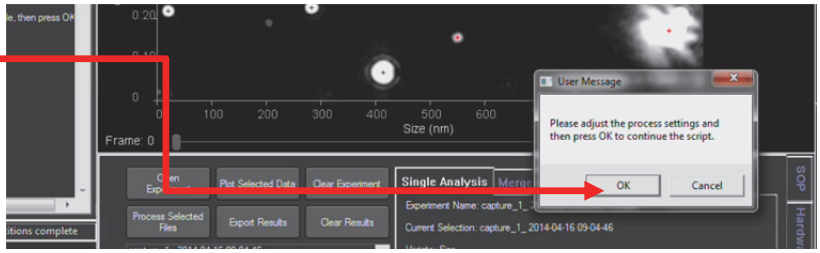


High Detection Threshold



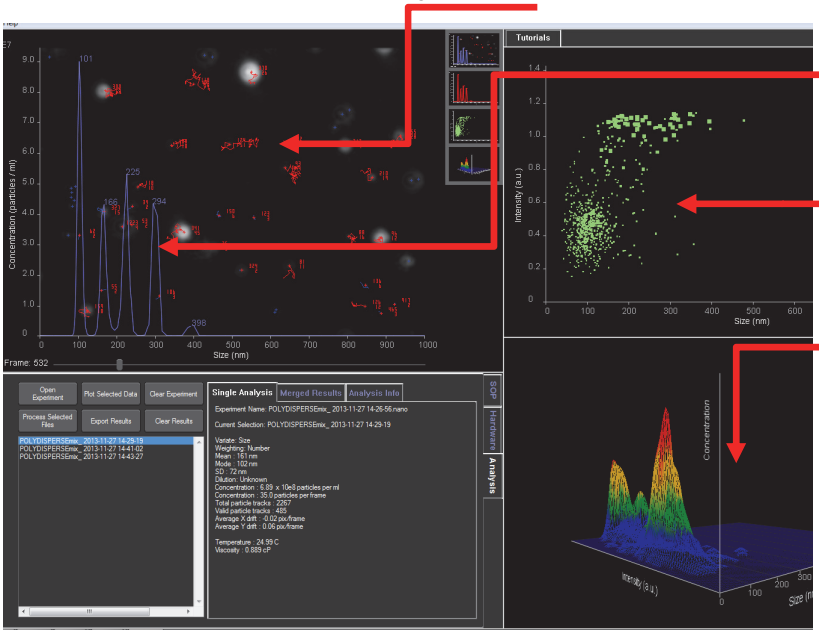
When the particle **Detection Threshold** has been set the measurement process can be started

Press **Ok**



During processing the image will appear to be brighter than during the set-up phase.

As the software processes the video images red 'tracks' appear on the screen depicting the Brownian Motion of the particles.



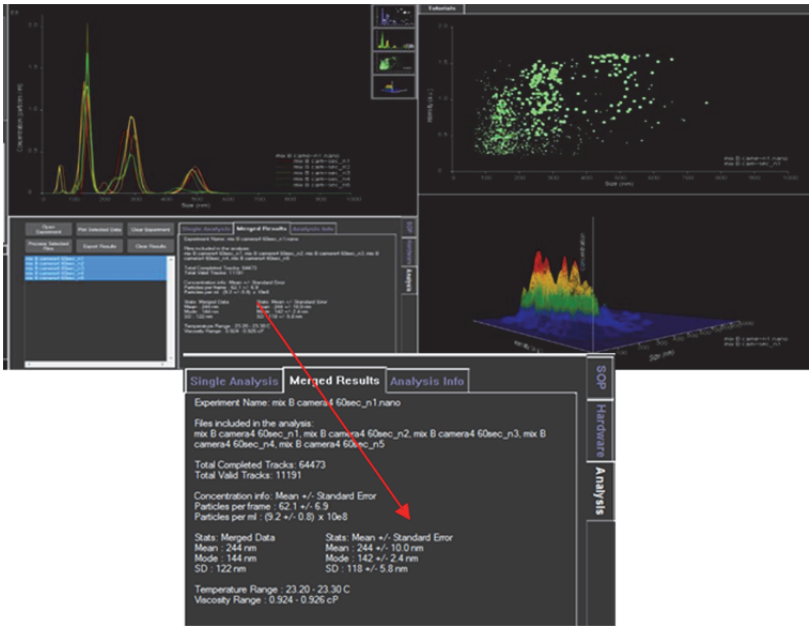
Size (nm) vs Concentration (particles/ml) measurements are shown on the blue (default) graph, overlaying the particle screen as the video(s) are processed.

The same measurements are additionally displayed as a **scatter plot** (Size (nm) vs Intensity (a.u.)), and as a **3D plot** Size (nm) vs Concentration (particles / ml) vs Intensity (a.u.)).

- Any vibration will affect the motion of the particles which can influence sizing accuracy.
- Although the NTA3.0 software compensates for some interference, best results are achieved with zero vibration.

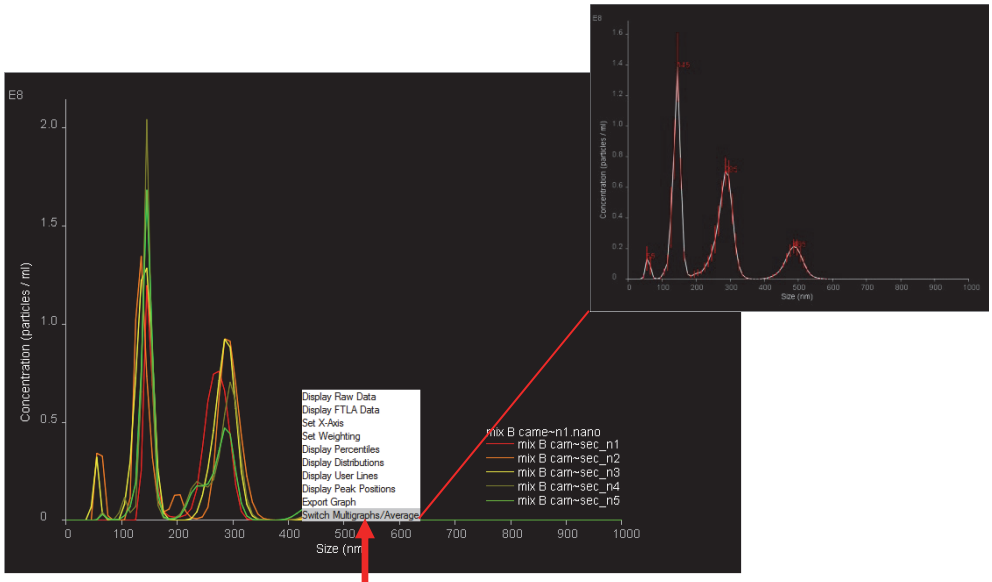
Example: Screen view at the end of processing for n=5 captures from one sample.

Individual size distribution profiles of the 5 captures for the sample are over- plotted.



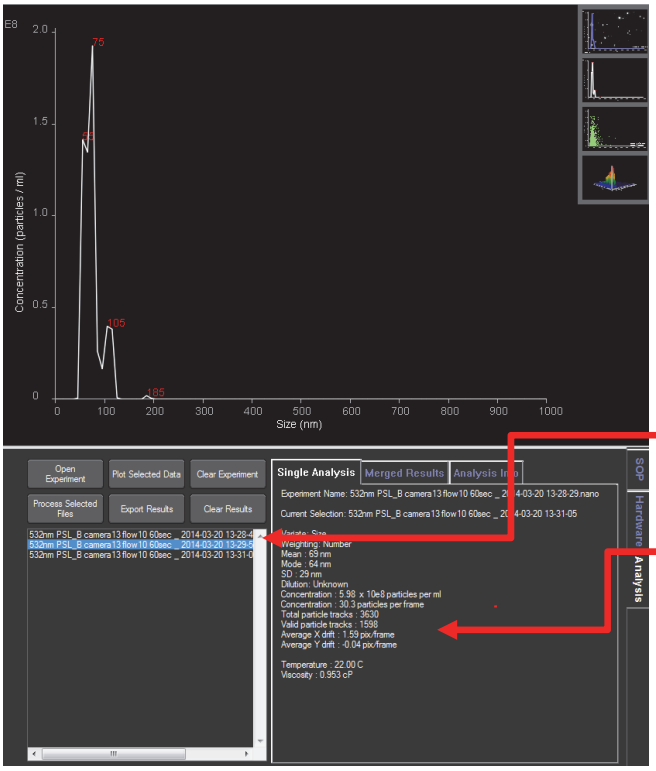
Mean ± SEM for the concentration, mean size, modal size and SD of the sample are shown.

To View the Combined Data Profile ± SEM



Place the cursor in the main graph area, right click the mouse to open a graph display options menu and select **Switch Multigraphs/Average**.

To View the Data for a Single Capture.



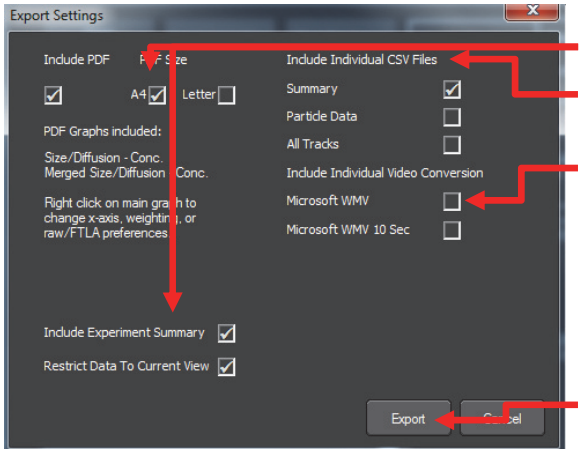
Select File.

Double click the chosen file.

The size distribution data for the chosen capture will be displayed.

Data Export

At the completion of processing the software automatically opens the Export Settings options window.



The defaults are for PDF graphs and batch summaries.

Raw data for further processing can be exported as CSV files

The AVI files used to capture the video data are very large (e.g. a 60 sec video uses ~0.5GB of data).NTA Software gives the option to export the videos as WMV files for customer support, demonstration and presentation purposes, including a 10 sec option.

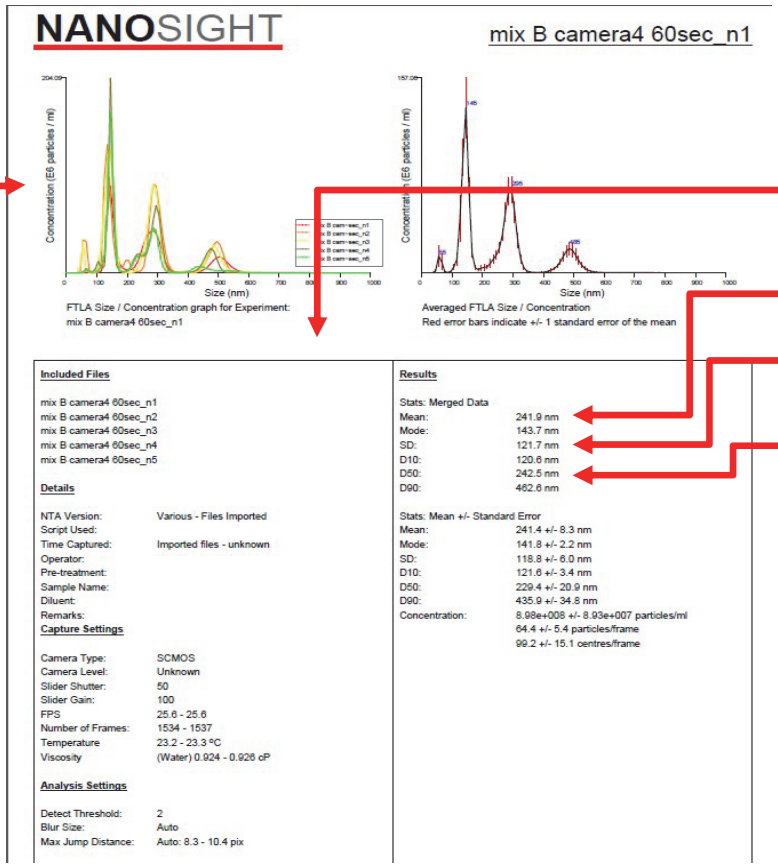
Click **Export**.

PDF Data Export

Example of exported PDF report for n=5 data from one sample;

The size distribution profile data are shown over-plotted

Mean ± SEM data are shown, with the size of the key peaks annotated.



The lower panels include data on the settings used along with a results summary.

Typically the modal particle size is used to describe the sample.

The SD is a measure of the width (spread) of the size distribution profile.

D10, D50 and D90 values indicate percent under-size, for example 50% particles are 229nm or smaller, giving another indication of the spread of particle sizes within the sample.

When vibration has been detected during the measurement, the size reported will be smaller than the true size of the particles in the sample.

CSV file Data Export

	A	B	C	D	E	F	G	H	I
1	NTA Experiment Summary File								
2	Created with NTA 3.0 0058 RC2								
3									
4	[Experiment Details]								
5	Software Build	Various - Files Imported							
6	Experiment Name	mix B camera4 60sec_n1.nano							
7	Sample Name								
8	Operator Name								
9	Time Captured	Imported files - unknown							
10	Pre-treatment								
11	Diluent								
12	Remarks								
13									
14	Filename:	mix B cam	mix B cam	mix B cam	mix B cam	mix B camera4 60sec_n5			
15									
16	[Conditions]								
17	Temperature/C	23.2	23.2	23.2	23.3	23.2			
18	Viscosity/cP	0.926377	0.926377	0.926377	0.924217	0.926377			
19	Camera Type	sCMOS	sCMOS	sCMOS	sCMOS	sCMOS			
20	Camera Level	Unknown	Unknown	Unknown	Unknown	Unknown			
21	Slider Shutter	50	50	50	50	50			
22	Slider Gain	100	100	100	100	100			
23	Shutter/ms	0.989583	0.988027	0.98957	0.98957	0.988412			
24	Camera Histogram Upper Limit	16380	16380	16380	16380	16380			
25	Camera Histogram Lower Limit	130	130	130	130	130			
26	Frame rate/fps	25.6	25.6272	25.6036	25.5794	25.6056			
27	Syringe Pump Speed/AU	Unknown	0	0	0	0			
28									
29	[Settings]								
30	Detection Threshold	5	2	2	2	2			
31	Max Jump Mode	Auto	Auto	Auto	Auto	Auto			
32	Max Jump Distance	7.76191	9.61719	10.3877	8.91149	8.91987			
33	Blur	Auto	Auto	Auto	Auto	Auto			
34	Min Track Length	Auto	Auto	Auto	Auto	Auto			
35	First frame	0	0	0	0	0			
36	Total frames analysed	966	1537	1536	1534	1536			
38	[Results]						Average	Standard Error	
39	Dilution factor (concentrations	Not recor	Not recor	Not recor	Not recor	Not recorded			
40	Concentration (Particles / ml)	8.09E+08	1.09E+09	1.11E+09	8.77E+08	7.05E+08	9.19E+08	7.98E+07	
41	Particles per frame	41.1	75.4	78.3	62.8	53	62.1	6.9	
42	Centres per frame	42.3	131.6	137.9	90	70.8	94.5	18.1	
43	Completed tracks	2683	20577	19169	12342	9702			
44	X-Drift (pix/frame)	0.1	0.1	0.1	0.1	0.1			
45	Y-Drift (pix/frame)	0	0	0	0	0			
46									
47	[Information]								
48	Completed Tracks	OK	OK	OK	OK	OK			
49	Concentration	OK	OK	OK	OK	OK			
50	Video length	OK	OK	OK	OK	OK			
51	Noise level	No	High noise	High noise	High noise	Noise detected			
52	Vibration detected	No	No	No	No	No			
53	Vibration correction applied	No	No	No	No	No			
54	Settings changed?	No	No	No	No	No			
55	Errors	None	None	None	None	None			
56									
57	[Data Included]								
58	Size distribution - Number weighting - With Percentiles								
59									
60	[Size Data]								
61	Analysis Method	FTLA							
62	Weighting	Number							
63	Filename	mix B cam	mix B cam	mix B cam	mix B cam	mix B cam	Average	Standard Error	
64	Mean	274.5	251	237.9	242.5	212.7	243.7	10	
65	Mode	146.5	133.5	141.3	145.1	145.4	142.3	2.4	
66	SD	121.4	132.2	124.5	113.8	98	118	5.8	
67	D10	134.6	112.7	114.1	128.4	124.8	122.9	4.2	
68	D50	255.5	265.7	246.9	233.6	148.3	230	21.1	
69	D90	486.1	476.3	464.8	452	298.8	435.6	34.7	
70	Graph Data								
71	Bin centre (nm)	Concentra	Concentra	Concentra	Concentra	Concentra	Concentra	Standard Error	
72	5	0	0	0	0	0	0	0	
73	15	0	0	0	0	0	0	0	
74	25	0	0	0	0	0	0	0	
75	35	0	0	17.7	0	0	3.5	3.5	
76	45	0	34670.1	6877499	5.4	44.9	1382444	1373780	
77	55	0	34224854	32228637	329166.6	337898.5	13424111	8090780	

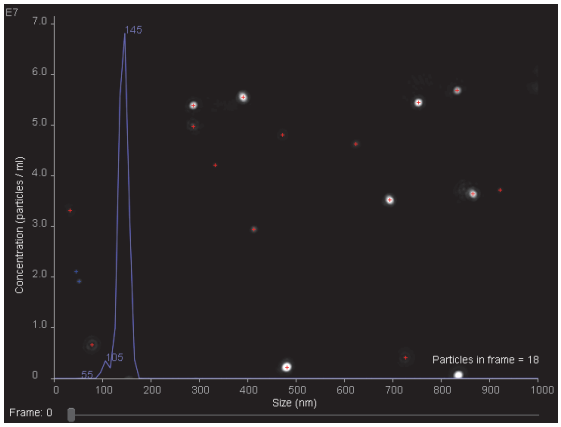
Notes

1. Each column represents a captured file
2. Initial camera settings and capture information
3. Processing settings
4. Concentration and included particles
5. Processing and warnings history
6. Analysis results

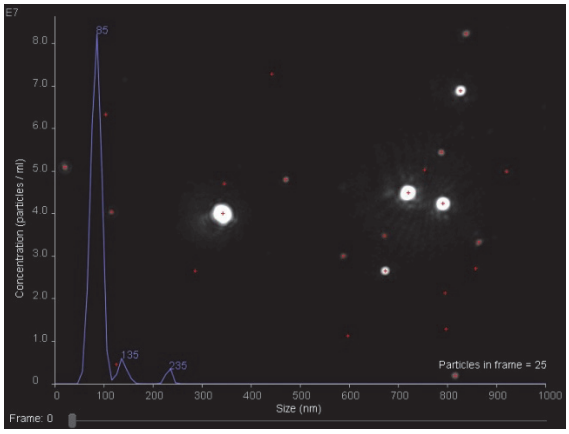
Appendix

Appendix 1: Typical data profiles

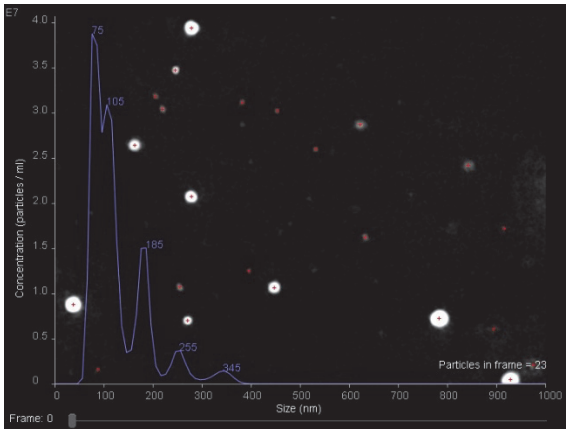
The profile obtained depends upon the type of sample measured.



When the particle size of the sample is more controlled, e.g. size standards, extruded liposomes etc., a narrow size distribution profile with a single peak should be obtained, indicating a mono dispersed sample (see above).



For samples such as extracellular vesicles purified with a sucrose gradient, it is likely that the main peak if the profile is broader with one or more peaks identified (see above).



For polydispersed samples e.g. aggregated protein, a very polydispersed size distribution profile might be expected with many peaks identified, which typically decrease in peak-height as size increases.

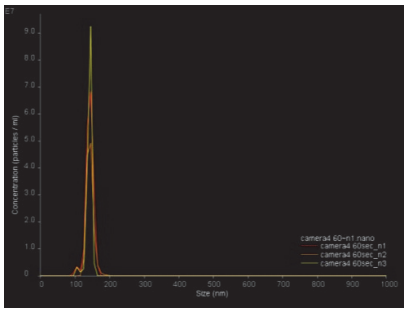
Appendix 2: Guidance for accepting data

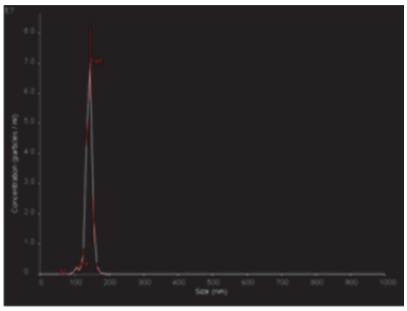
Accepting the data depends on the type of sample you are measuring.

When multiple profiles for the same sample measurement are plotted together, the similarity of the profiles should be in keeping with the level of polydipsersity, providing no sampling errors are present and the sample was measured for long enough,

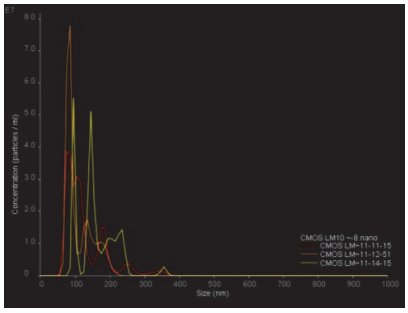
i.e. a monodispersed sample should over-lay closely for the data to be acceptable, whilst a polydisperse sample can have less reproducible profiles and the data may still be acceptable.

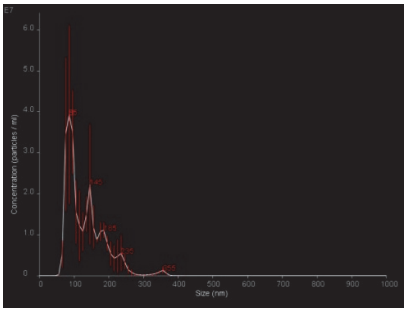
Monodisperse sample





Polydisperse sample





Notes

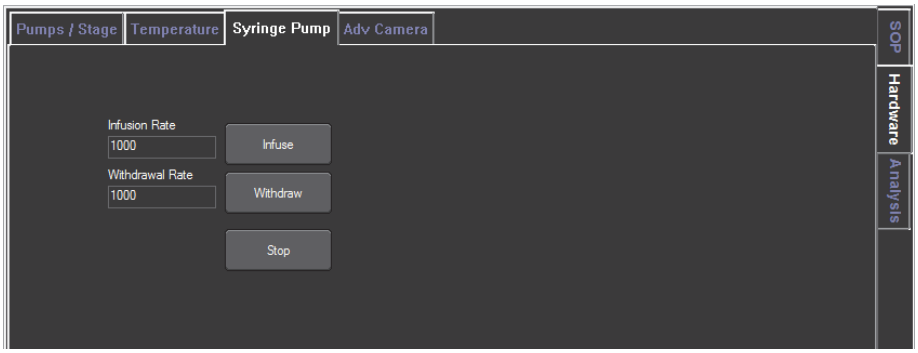
- Samples of the same type should be measured using the same settings for comparison.
- Polydispersed samples will typically need to be captured for longer to generate good data.
- Samples with a naturally low level of particles/ml will need to be captured for longer to generate good data.
- Users are advised to refer to the PDF and CSV data output files to check for warnings that could result in invalid results.

Appendix 3: Fluorescence Measurements and Syringe Pump

- Insert sample as normal

If using a syringe pump:

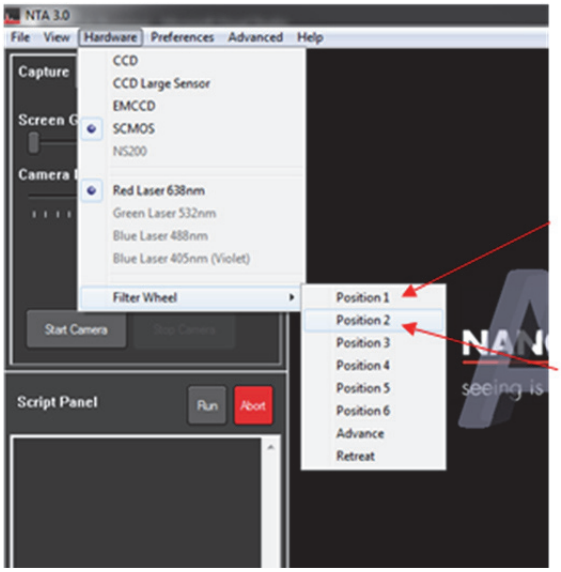
1. Set Infusion Rate to 1000 and click Infuse
2. When flow is established, reduce Infusion, rate to the required level and click 'Infuse' again



Set flow speed

Laser Module Top Plate Style	Recommended Flow Speed
NS300 "O" ring (metal)	50-80
NS300 flow-cell	20-50

Select filter from the Hardware->Filter Wheel menu



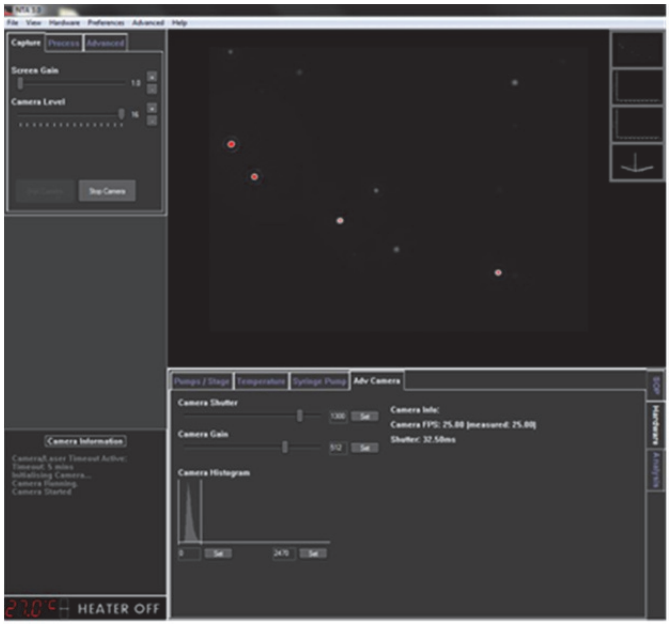
- Position 1: Clear (empty) scatter measure
- Position 2: Fluorescence

Notes

Always follow the flow speeds recommended

Appendix 4: Fluorescence Measurements

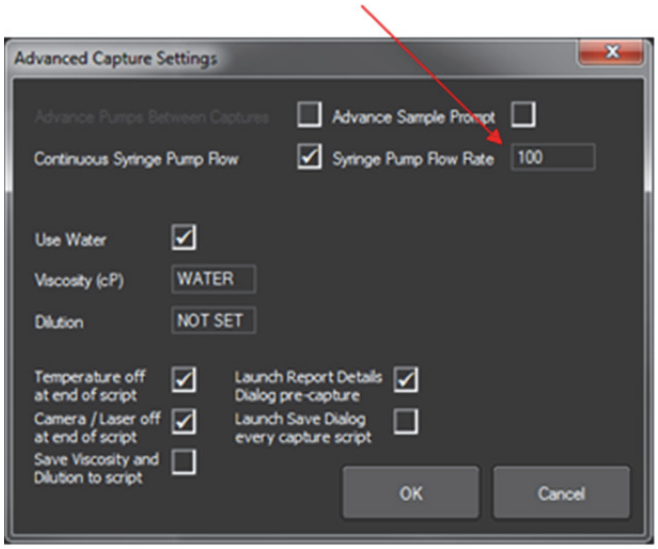
Increase camera level to maximum, and reduce histogram upper limit in the 'Adv Camera' hardware tab, if required



Adjust focus if required

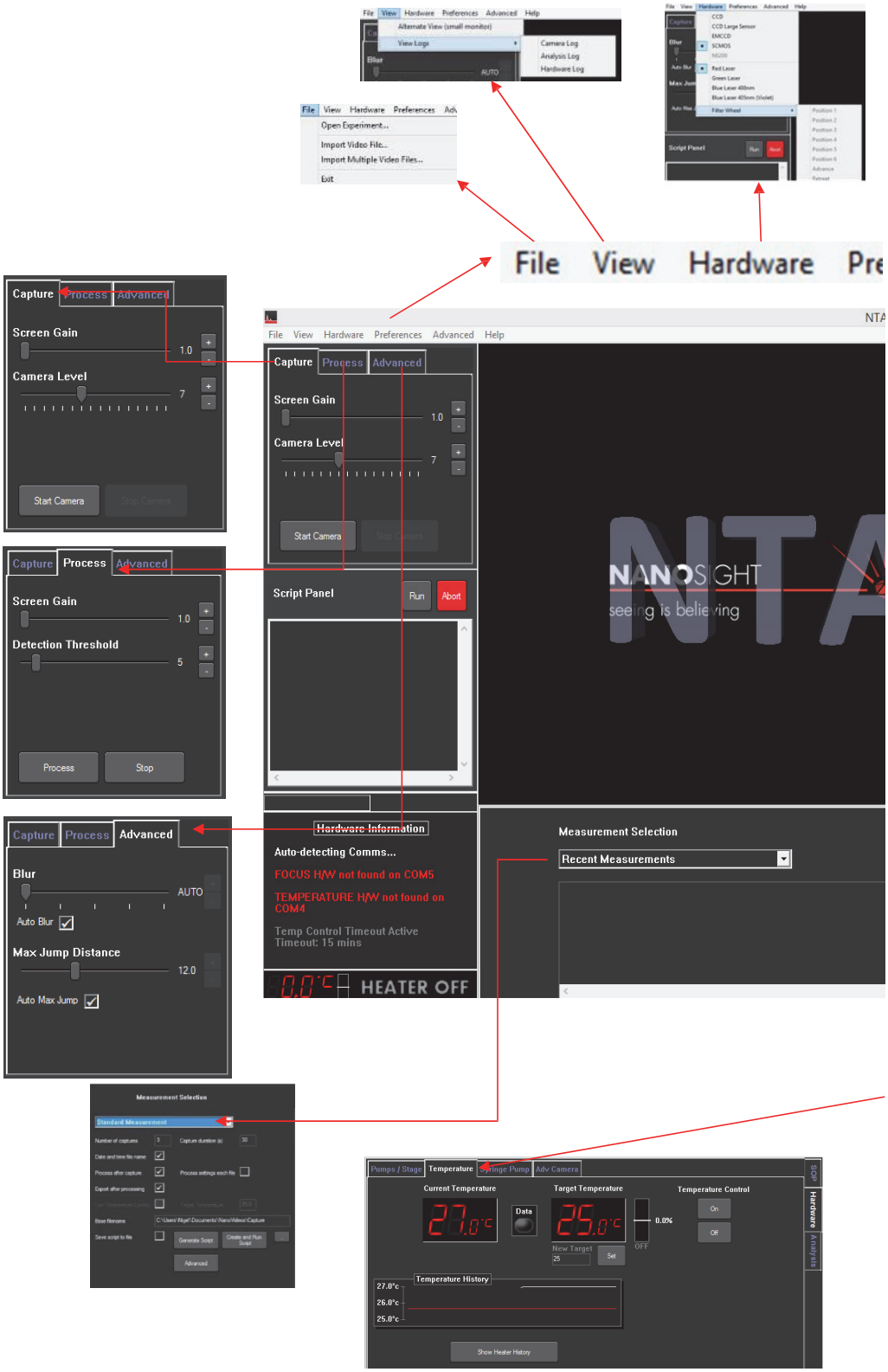
Select and run and 'Standard Measurement' as previously described.

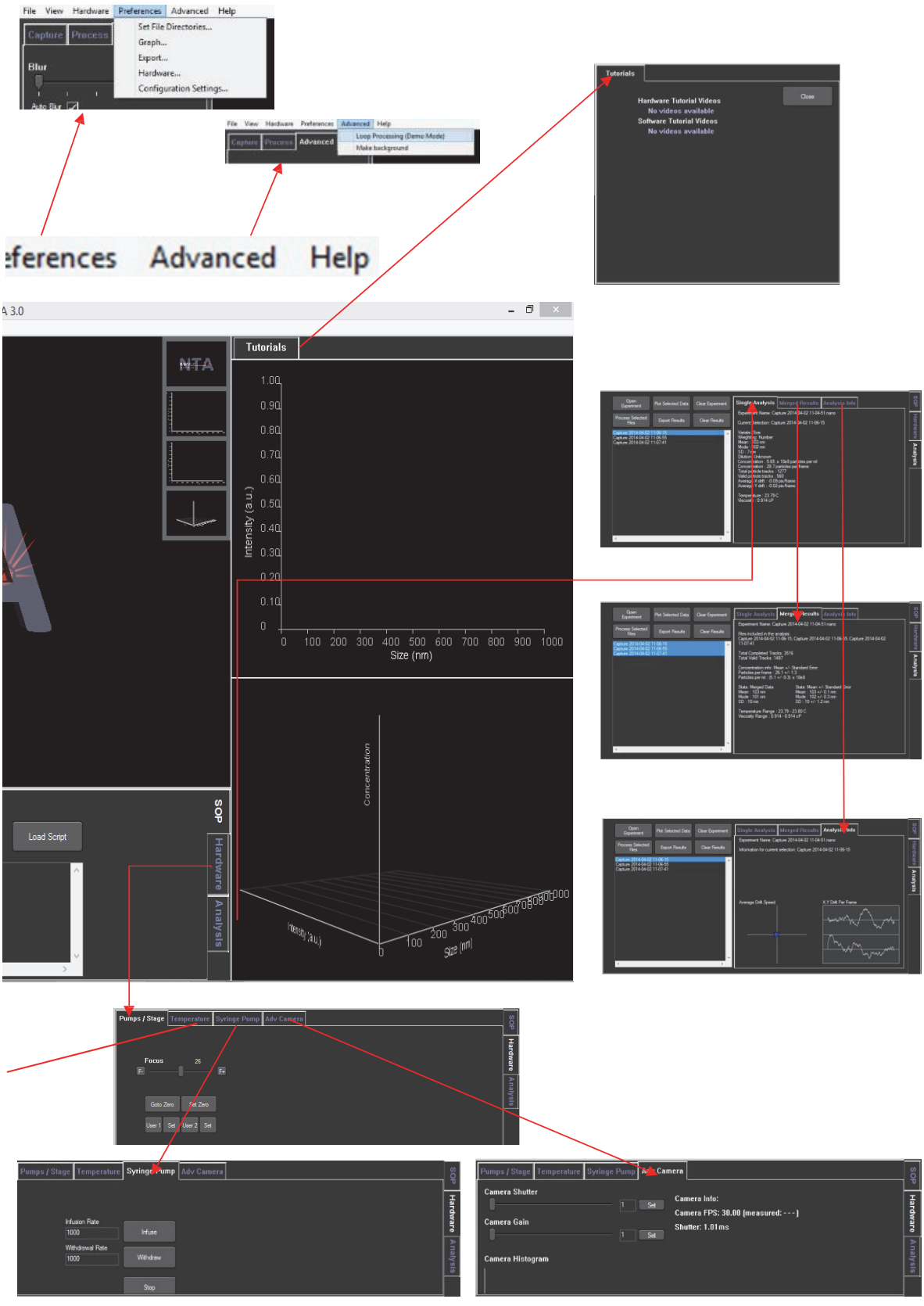
If using the syringe pump, select 'Continuous Syringe Pump Flow rate' and set the required rate in the Advanced capture settings window



Appendix 5: NTA Software

Software Map




File View Hardware Preferences Advanced Help
Capture Process
Blur
Set File Directories...
Graph...
Export...
Hardware...
Configuration Settings...
Loop Processing (Demo Mode)
Make background
Tutorials
Hardware Tutorial Videos
No videos available
Software Tutorial Videos
No videos available
Close
NTA
Intensity (a.u.)
Size (nm)
Concentration
Load Script
SOP
Hardware
Analysis
Pumps / Stage
Temperature
Syringe Pump
Adv Camera
Focus
Goto Zero
Set Zero
Infusion Rate
Withdrawal Rate
Infuse
Withdraw
Stop
Camera Shutter
Camera Gain
Camera Histogram
Camera Info:
Camera FPS: 30.00 (measured: - - -)
Shutter: 1.01ms