

**SOP#: 008**

Date Issued: 8/03/2021

Date Revised: 7/08/2021

**TITLE: NanoSight N300 nano particle analyzer**

**SCOPE: Research Personnel**

**RESPONSIBILITY: BORC staff**

**PURPOSE: To outline the proper procedures for use and maintenance of the NanoSight N300 nano particle analyzer.**

## 1 PURPOSE

This SOP explains how to use NanoSight N300 to analyze the size distribution and concentration of Nano size particles.

## 2 RESPONSIBILITY

It is the responsibility of the BORC staff to ensure that equipment is appropriately cleaned, maintained in good working order, and available for research personnel as requested.

## 3 BEFORE USE N300

3.1 Concentration. The NanoSight instruments can work with particle concentrations in the range of ~107-109 particles/ml, which is approximately 20-100 particles in the field of view.

3.2 Too high a sample concentration may prevent accurate particle tracking. Lower concentrations require longer capture and analysis time to produce statistically significant results.

## 4 PROCEDURES

### 4.1 Open NanoSight NTA

Click the desktop icon or via the start menu (under All Programs > NanoSight).

### 4.2 Load samples using the syringe-pump

We recommend that before proceeding with analysis, a sample of any buffer or diluent is checked to confirm that it doesn't contain any contaminating nanoparticles.

Load a syringe with sample and connect it to the luer fitting on the end of the inlet tubing.

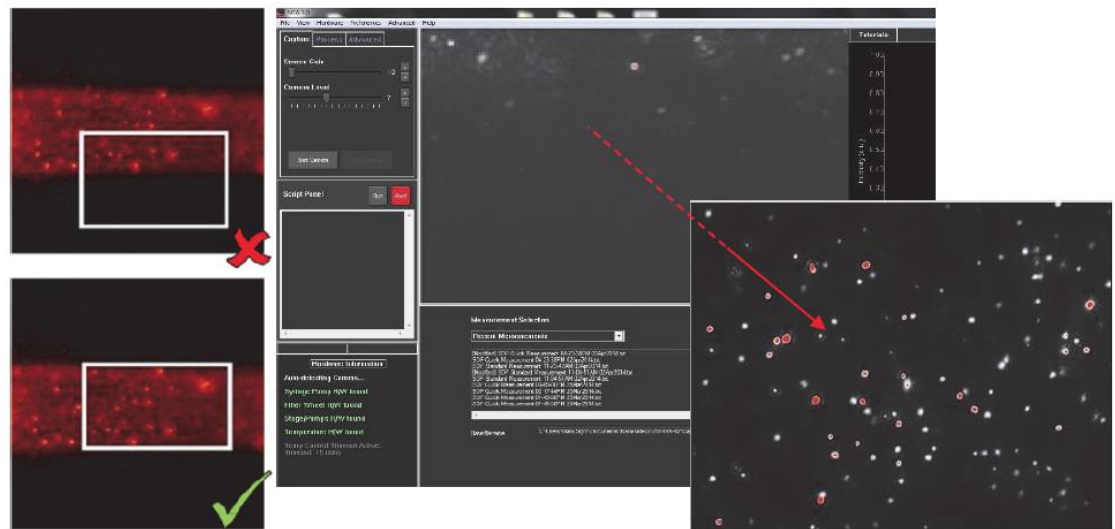
Once liquid is seen emerging in the waste tubing, place the syringe into the syringe pump holder as described in

the NanoSight Syringe Pump Operating Manual

**4.3 Adjust the NS300 to obtain a video.**

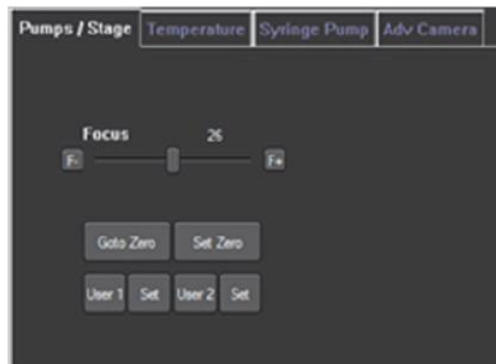
**4.3.1 Laser Beam Position**

The system is designed to have good beam relocation once set-up, although small adjustments may be necessary to optimize the image. If the beam appears not to be central in the field of view on screen, i.e. not filling the top or the bottom of the screen, the image can be adjusted up and down by a small amount. With a view of the particles on the NTA screen in capture mode, left-click the mouse button on the video image. Hold the left mouse button down and drag the image up or down until the laser beam is illuminating particles over the whole field of view.

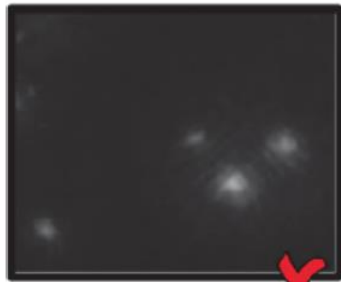


**4.3.2 Image focus**

To adjust the focus in the software, use the focus slider bar in the Hardware Control Panel. The focus can also be adjusted using the mouse wheel. Dragging the slider on the left gives fine adjustments, while using the slider on the right gives coarser adjustments



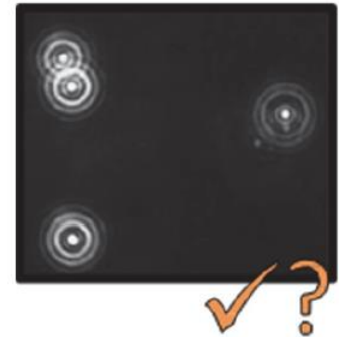
Because the particles are constantly moving it can be difficult to achieve a uniform perfect spherical focus.



a)



b)



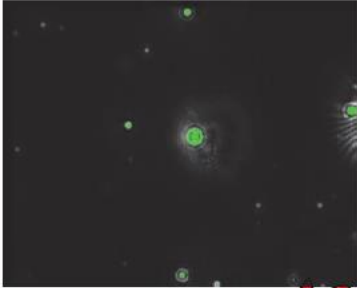

c)

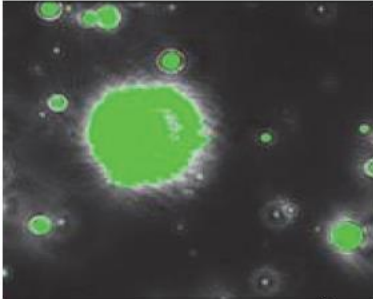
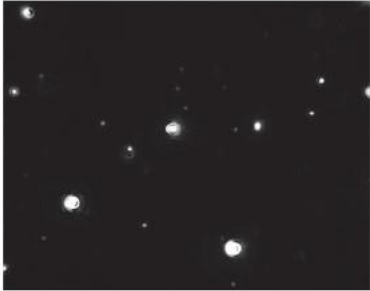
#### 4.3.3 Adjust camera level

Click on Capture in the top left corner of the screen to enter capture mode, the camera will take a few seconds to initialize.

Adjust the camera level so all particles are visible. Increase the Camera Level until all of the particles in the sample can be seen clearly but no more than 20% are saturated (colored pixels).

- Poor Monodisperse Sample      Good Monodisperse Sample



- Poor Polydisperse Image      Good Polydisperse Sample

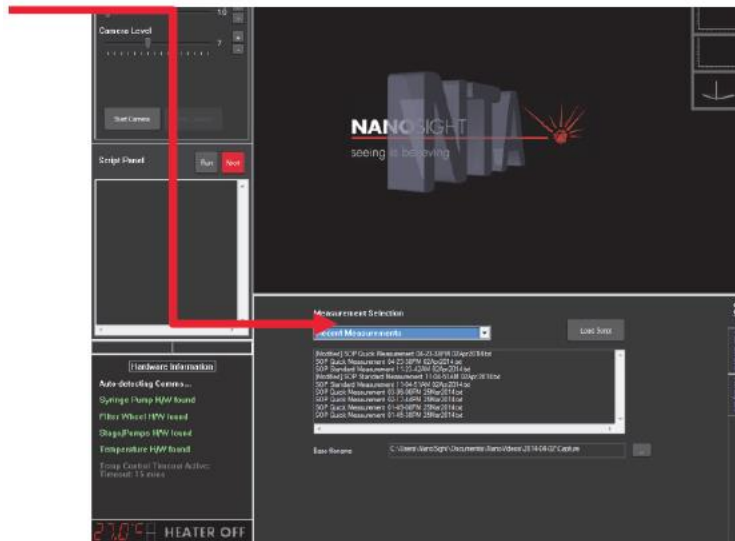



#### 4.4 Capture Settings

Once an image can be seen on the capture screen, fine tune the focus as appropriate. A movie can be recorded using suitable camera settings.

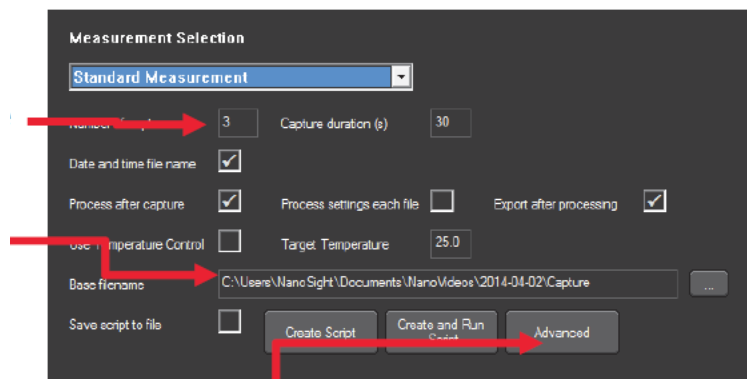
##### 4.4.1 Set measurement parameters

Select standard measurement



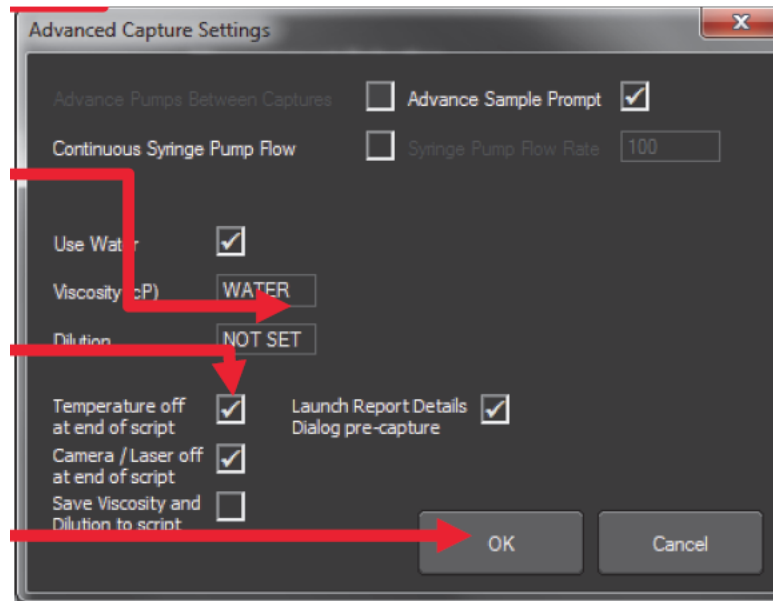
#### 4.4.2 Setup numbers of captures, name and location for the captured video files

The default number and length of video captures are 3 and 30 seconds each, which are suitable for most samples. The default folder used to save the data files is C:\users\NanoSight\documents\NanoVideos\.



#### 4.4.3 Advanced settings for dilution and viscosity

Click Advanced to enter advanced capture settings window. 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 building up between readings (Temperature off..., Camera/laser off..., Save Viscosity...).



Click OK after all settings are done.

#### 4.5 Create and Run Script

'Scripts' are sets of instructions that control the software for each measurement. The NTA software contains options for:

##### 4.5.1 Standard Measurement

Suitable for most size and concentration measurement within instrument specifications.

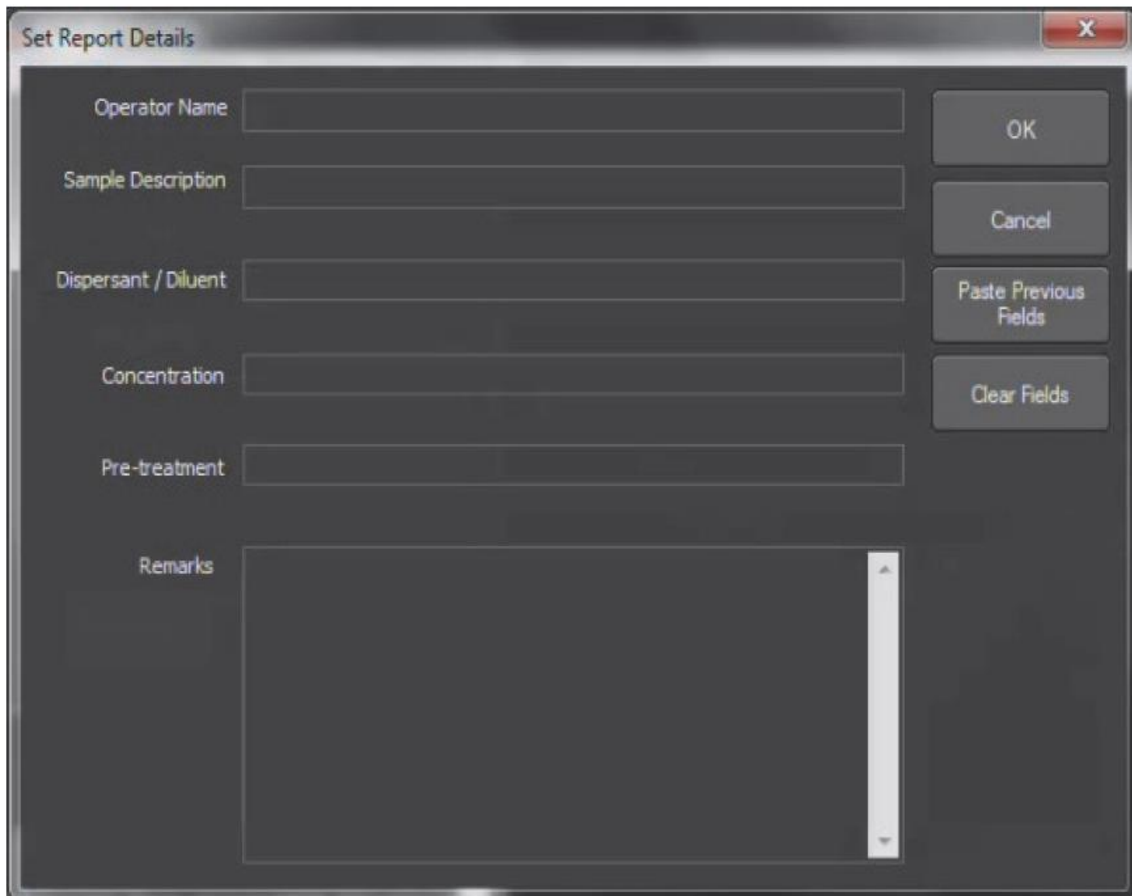
##### 4.5.2 Quick Measurement

Creates a single video and analysis.

##### 4.5.3 Recent Measurements

A list of the last 10 measurements taken allowing the rapid repeat of readings.

After clicking **Create and Run Script**, the User is prompted for and sample details to be included with the video files and output files (optional). Click OK.



Set Report Details

Operator Name

Sample Description

Dispersant / Diluent

Concentration

Pre-treatment

Remarks

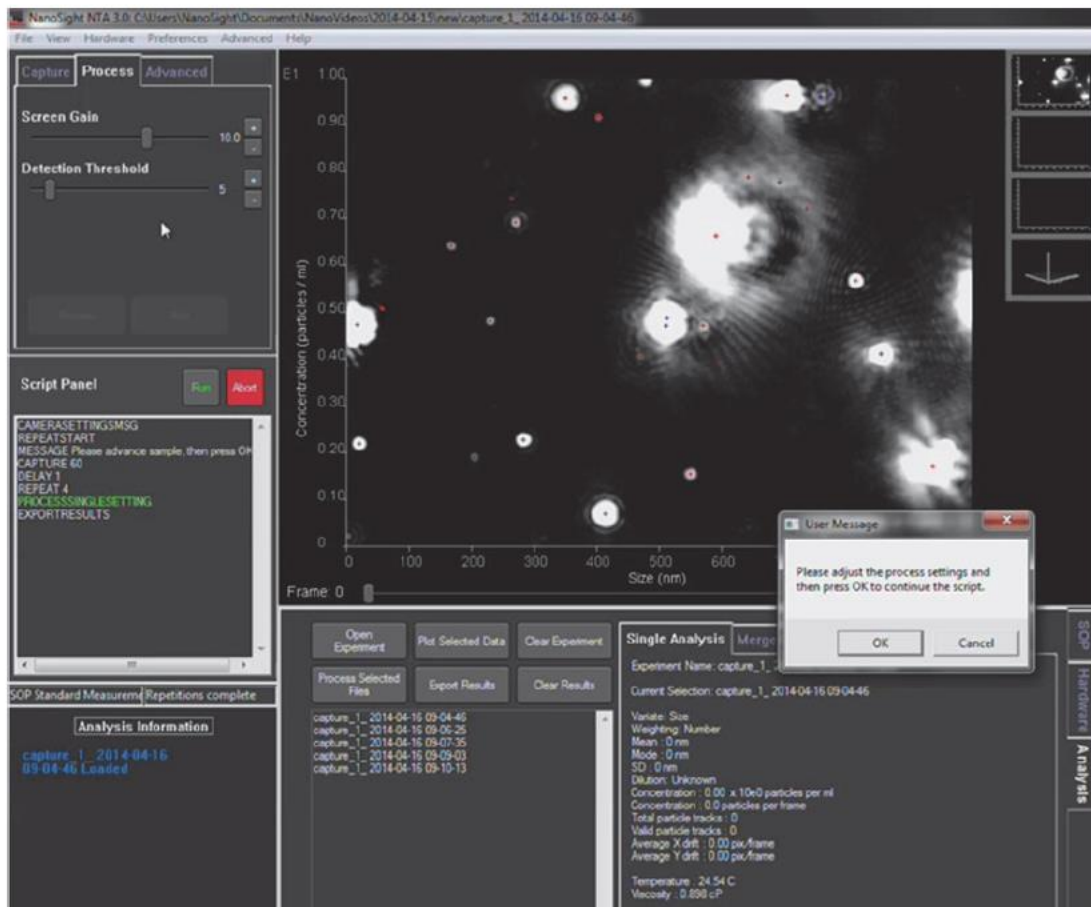
OK

Cancel

Paste Previous Fields

Clear Fields

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). Following video capture the software defaults to immediate processing. This screen is now showing the captured video rather than the live sample image.



#### 4.6 Set Detection Threshold

Detection Threshold must now be set before starting video processing. 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.

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

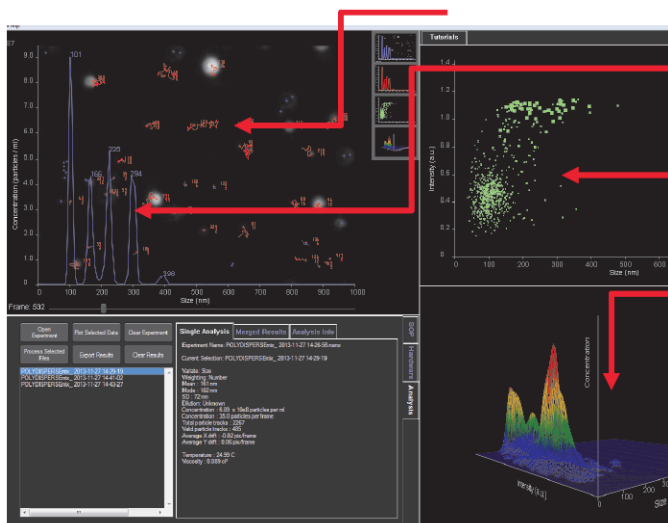


sample with the same Capture Settings should be analysed with the same detection threshold).

#### 4.7 Process the video.

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

#### 4.8 View the processed data of video

**4.8.1. To View the Data for a Single Capture.**

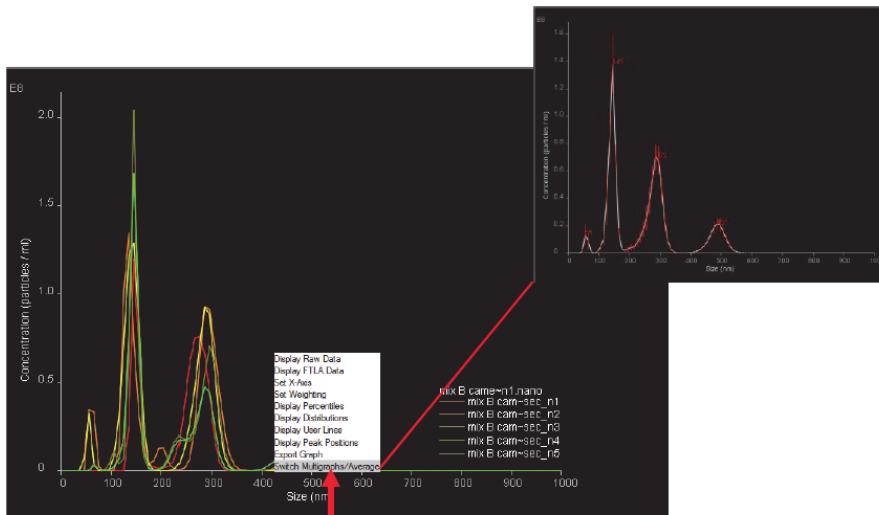


Select File.

Double click the chosen file.

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

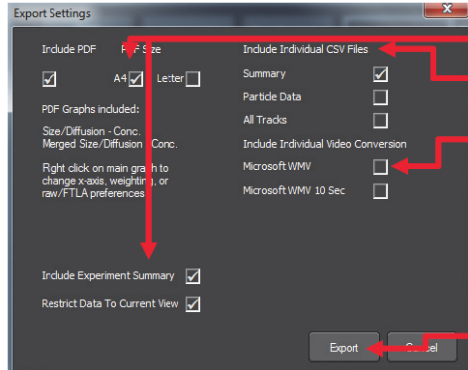
**4.8.2 View the Combined Data Profile ± SEM**



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

**4.9 Data Export**

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

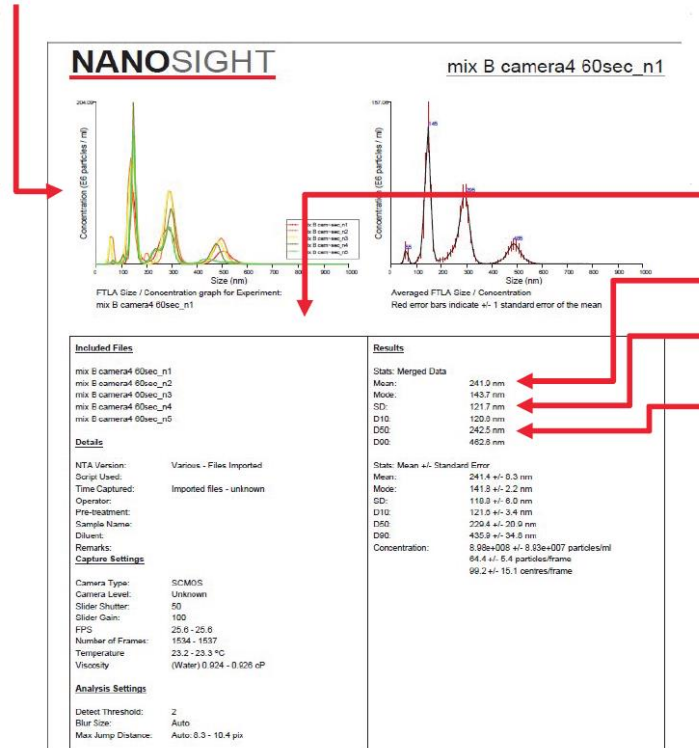


The defaults are for PDF graphs and batch summaries.  
Raw data for further processing can be exported as CSV files  
The AVI files used to capture the video data are very large (e.g. a 60 sec video uses ~0.5GB of data). NTA Software gives the option to export the videos as WMV files for customer support, demonstration and presentation purposes, including a 10 sec option.  
Click **Export**.

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

The size distribution profile data are shown over-plotted

Mean  $\pm$  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.

## 5 MAINTENANCE

The NS300 should always be left clean and dry whenever the system is not in use. The NTA software should be shut down and the NS300 power switch should be turned off.

After use, the system should be flushed through with clean diluent to remove all traces of sample from the tubing and optical surfaces. If using diluent with a high concentration of dissolved solids (i.e. saline solution), flush clean water through the system after use.

After the fluidic system is clean, empty the top-plate by extracting all liquid with a syringe, and then manually disassemble, clean and dry the sample chamber as described in Section 2.2.4.

## **6 REFERENCES**

Refer to the manufacturer's manual for additional information.

**END OF DOCUMENT**