



NANOSIGHT TRAINING COURSE: NANOPARTICLE TRACKING ANALYSIS (NTA) FOR SIZE, CONCENTRATION, AND FLUORESCENCE MEASUREMENTS

Westborough office – February 13-14, 2018

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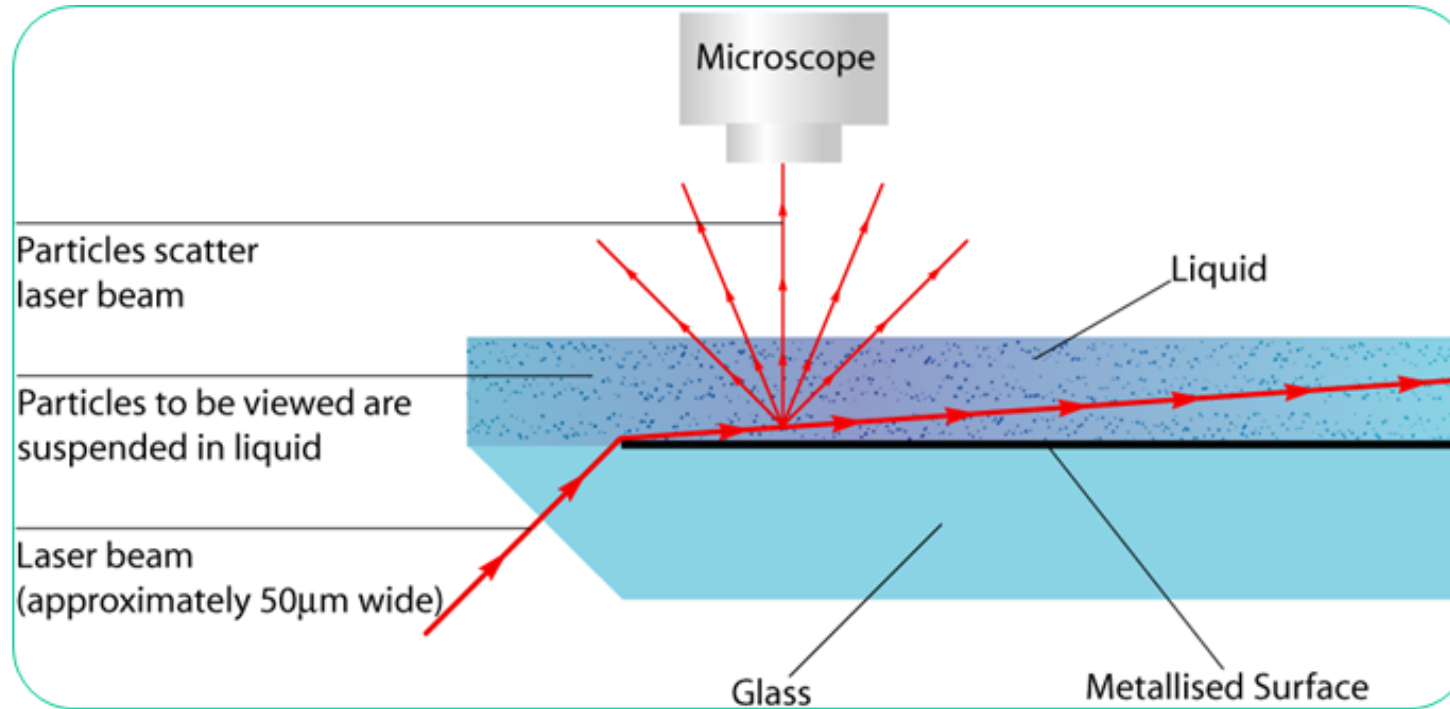
HOW DOES NANOSIGHT WORK?

SEEING IS BELIEVING!



- Light scattered from particles seen moving under Brownian motion
 - *Not a direct image, no shape information*
- Speed of particles varies directly with particle size
- Full analysis within a few minutes

PATENTED OPTICAL ARRANGEMENT

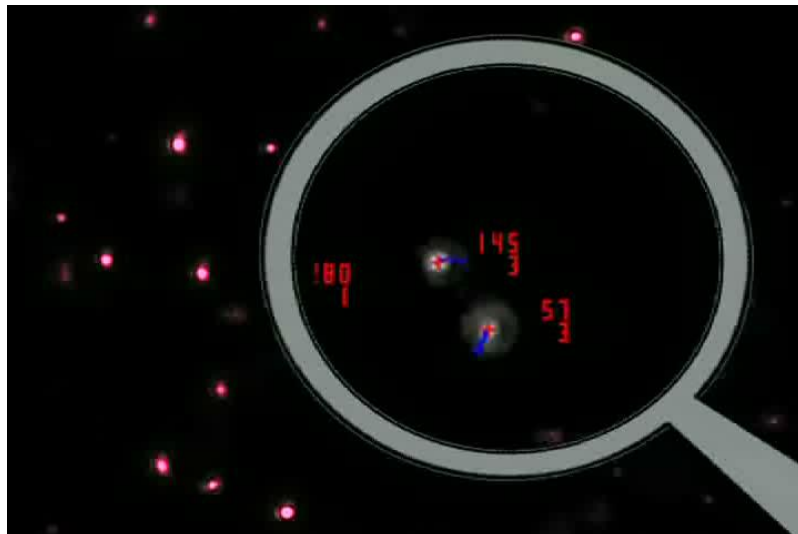


- Shaped laser beam matches microscope depth of focus
 - *Maximum signal to noise*
- Precise Z-dimension for reproducible concentration measurements
- Unparalleled lower detection limit

SIZING: STOKES-EINSTEIN



- Measure particles' mean square displacement (MSD) due to Brownian motion
- Calculated parameter is particle's sphere equivalent hydrodynamic diameter.
- Temperature measured and appropriate viscosity used.
- Absolute method – no calibration required



$$\langle \text{MSD} \rangle = \frac{4K_B T t_s}{3\pi\eta d_h}$$

Stokes-Einstein equation

K_B = Boltzmann Constant

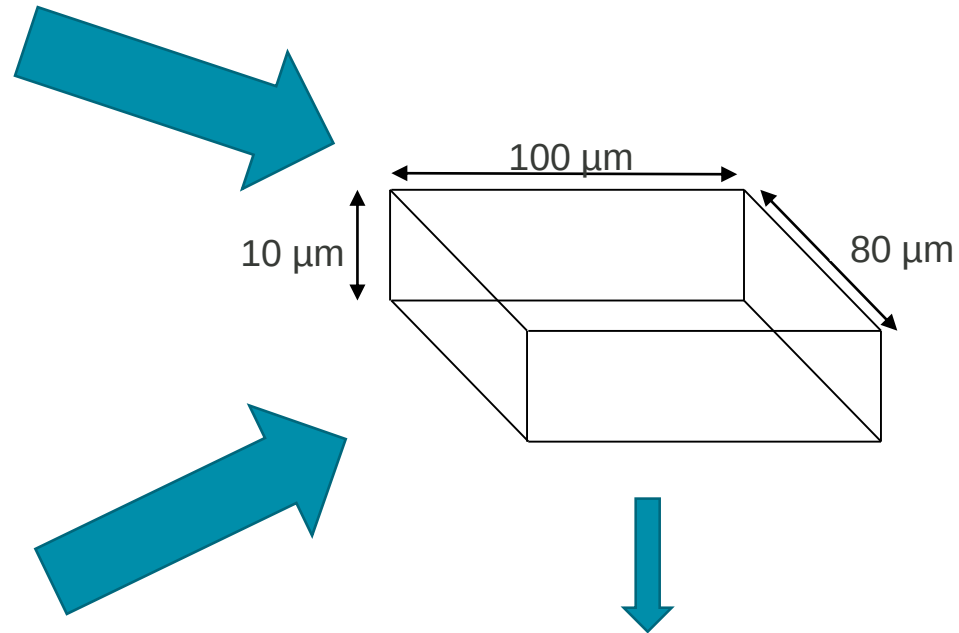
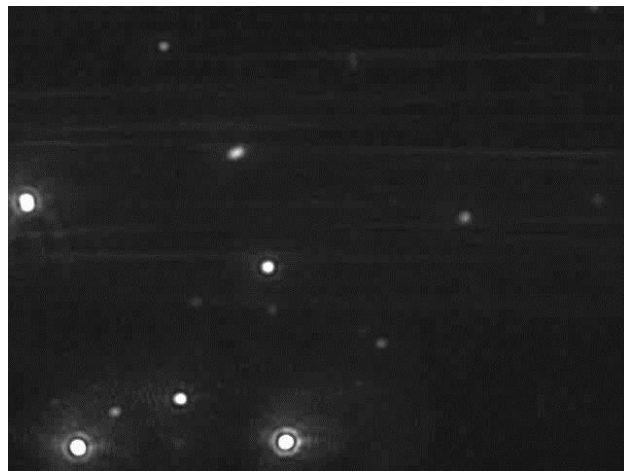
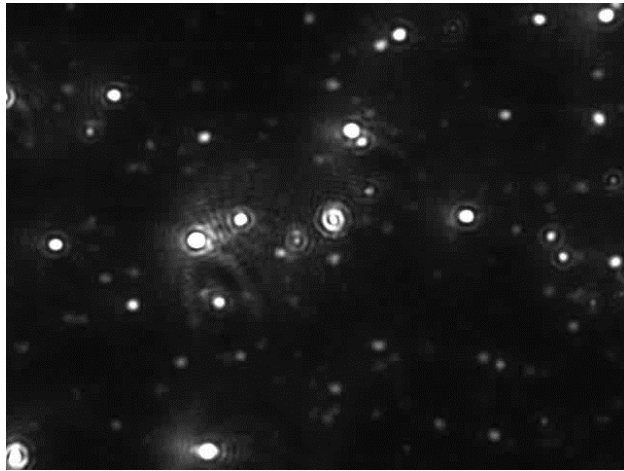
T = temperature

t_s = sampling time

η = viscosity

d_h = hydrodynamic diameter

CONCENTRATION: QUANTIFY PARTICLES IN KNOWN VOLUME



**Absolute number average of
concentration (particles/mL)**

NTA SPECIFICATIONS: SIZE AND CONCENTRATION LIMITS



Size



10 – 40 nm

1000 – 2000 nm

Minimum size limit is related to:

- › Material type
- › Camera Sensitivity

Maximum Size limit is related to:

- › Limited Brownian motion
- › Viscosity of solvent

Concentration



~ 10⁶-10⁷ particles/ mL

~ 10⁹-10¹⁰ particles/ mL

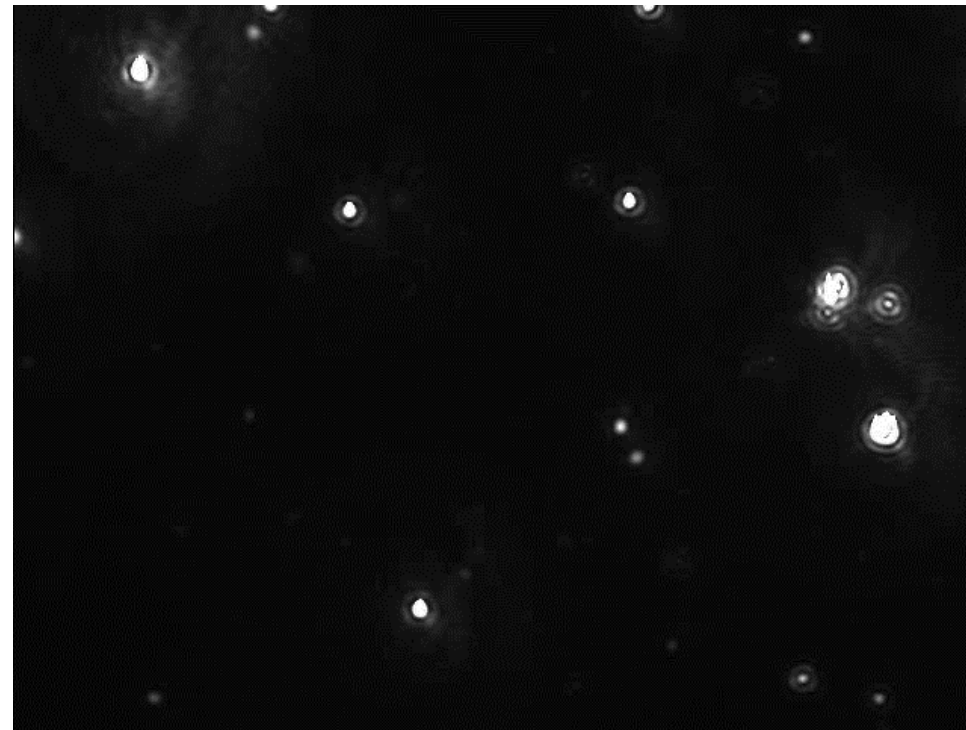
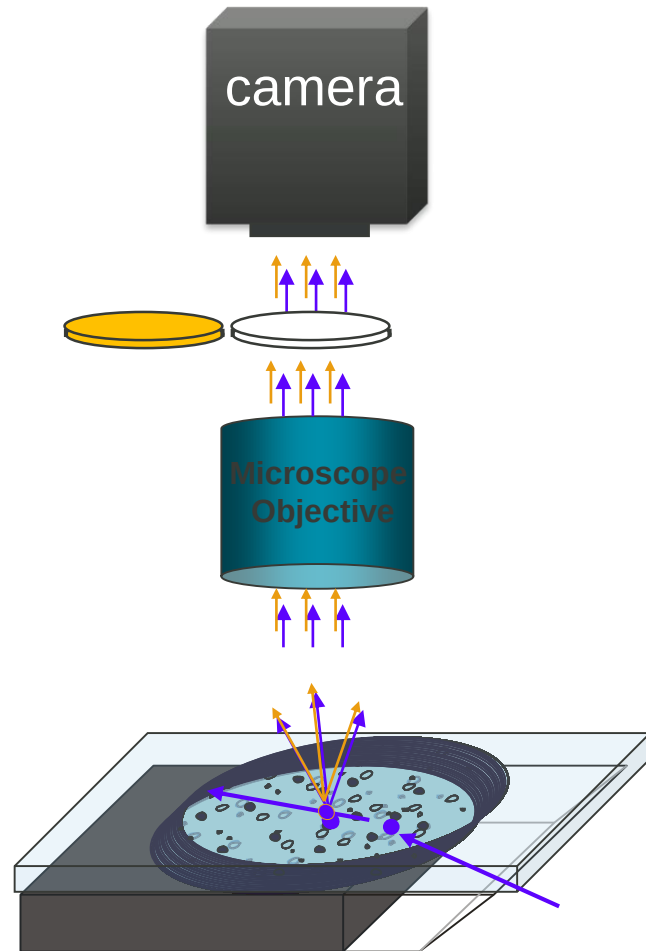
Minimum is related to:

- › Poor statistics (Requiring longer analysis time)

Maximum is related to:

- › Inability to resolve neighboring particles
- › Tracks too short before crossing occurs

FLUORESCENCE MEASUREMENT (OPTIONAL)

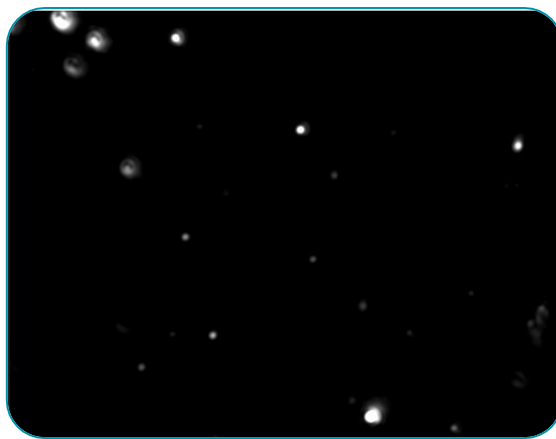


Video of fluorescence process

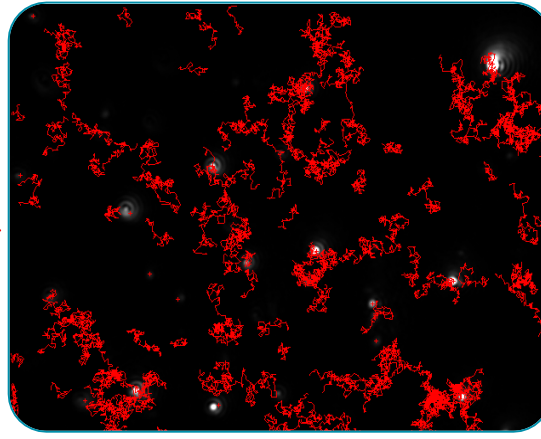
NTA EXPERIMENTAL PROTOCOL



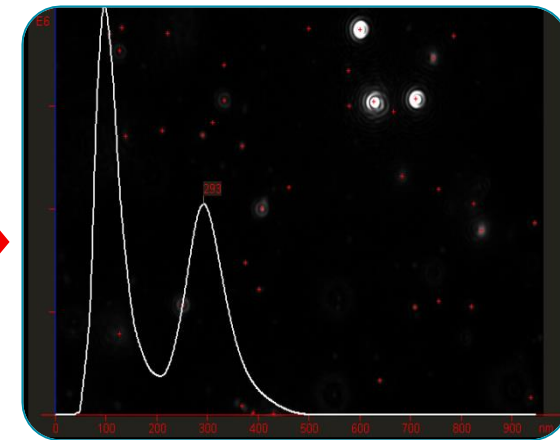
1. Capture: The software captures a movie file of the particles moving under Brownian motion
2. Tracking: The software locates each particle and tracks the mean square displacement of each particle independently.
3. Analysis: Application of the Stokes Einstein equation converts mean square displacement to particle size. The distribution is an accumulation of all the single particle measurements.



Capture (~60 sec)



Tracking

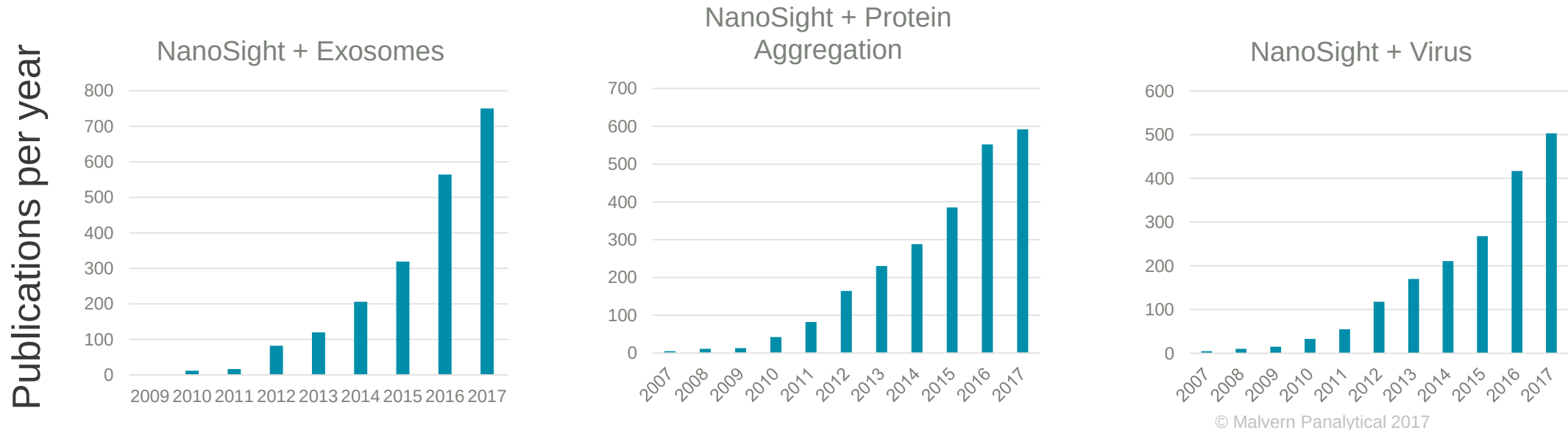


Analysis

INDUSTRY & SCIENTIFIC COMMUNITY ACCEPTANCE

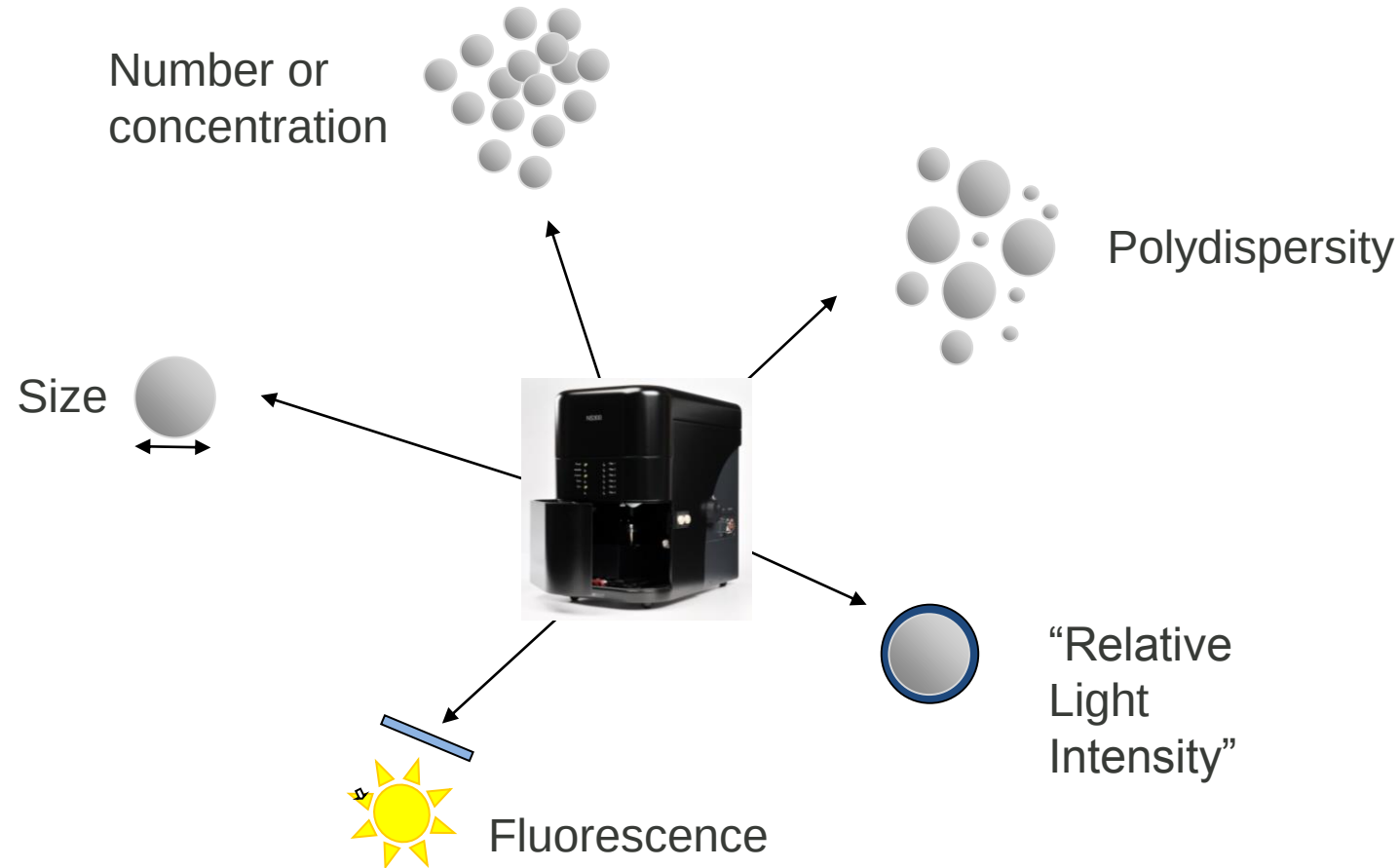


- ASTM E2834-12 Guide on NTA technique
 - *Standard Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Nanoparticle Tracking Analysis (NTA)*
 - URL: <https://www.astm.org/Standards/E2834.htm>
- ISO standard (ISO/DIS 19430.2)
 - *Particle size analysis – Particle tracking analysis (PTA) method*
 - URL: http://www.iso.org/iso/catalogue_detail.htm?csnumber=64890

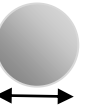


WHAT DOES NANOSIGHT PROVIDE?

PARAMETERS MEASURED BY NTA

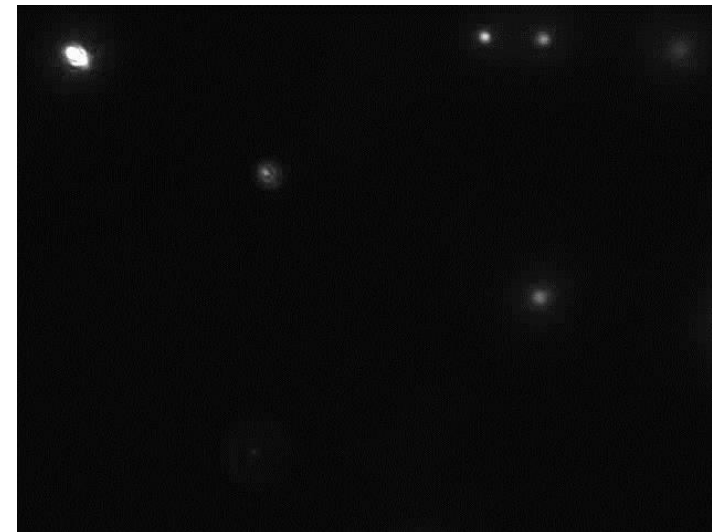
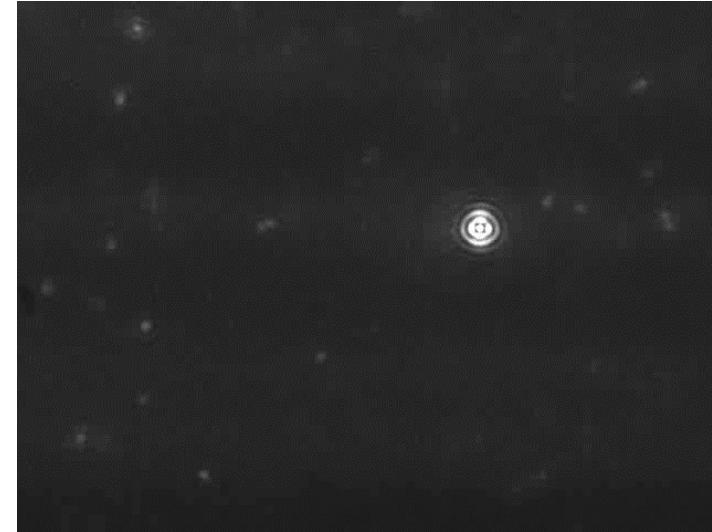
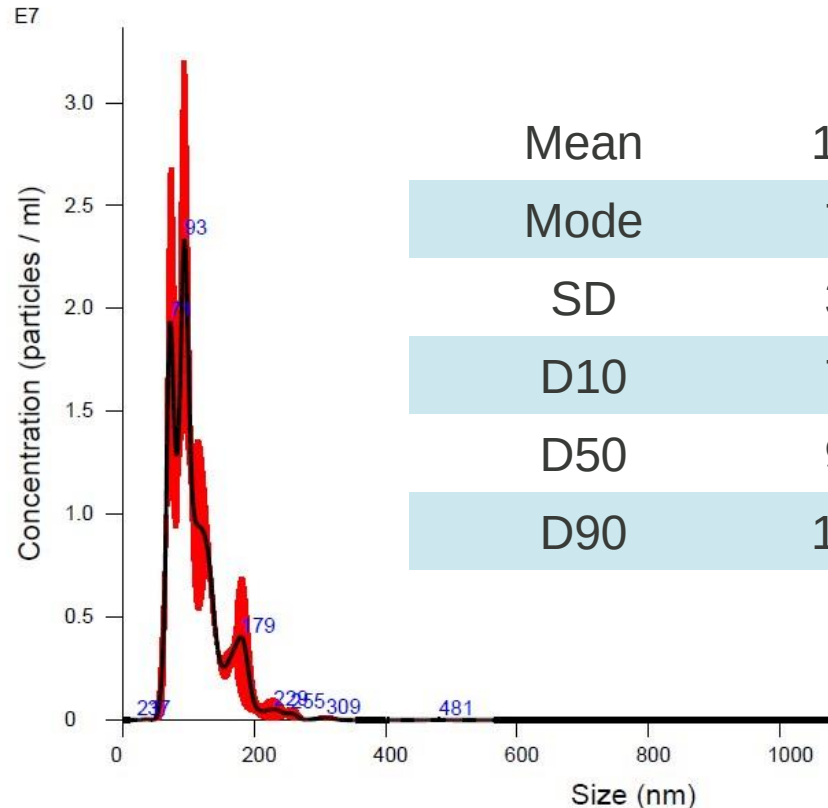


...simultaneously, 'real time', particle-by-particle

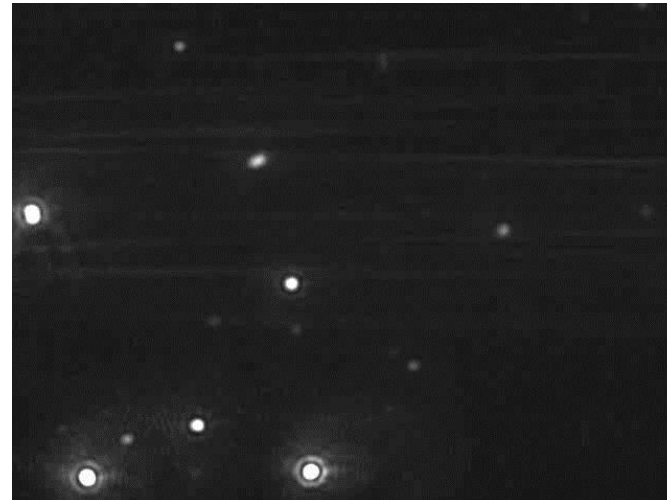
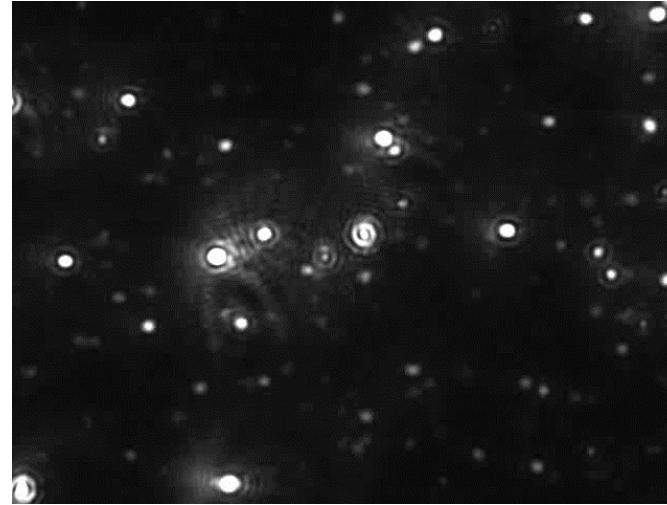
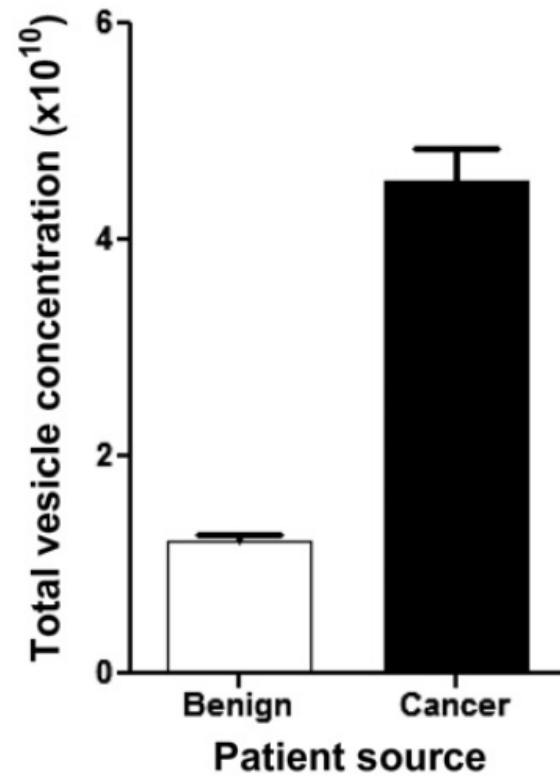


SIZE WITH SINGLE-PARTICLE RESOLUTION

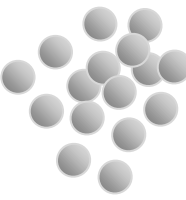
- Mean and Mode Size
- Size Distribution (number, volume, and surface area weighted histogram)
- Polydispersity (SD): D10, D50, D90



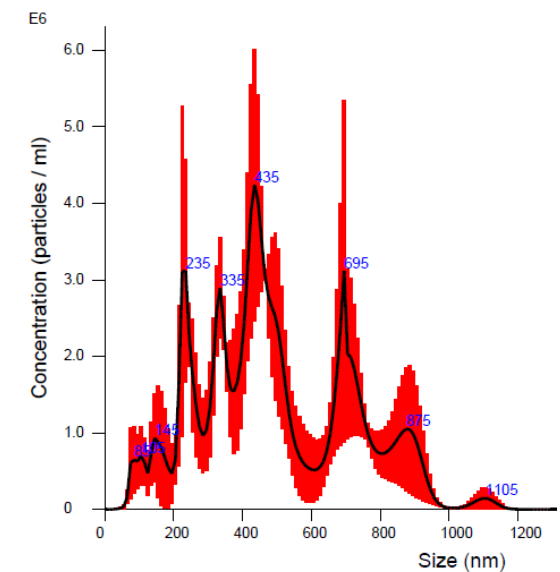
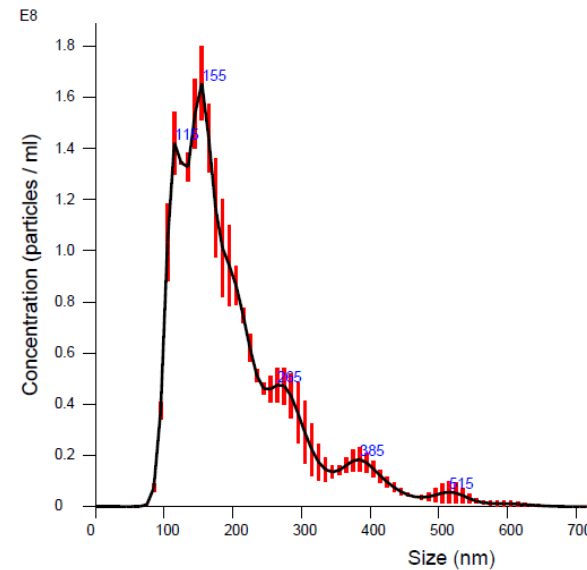
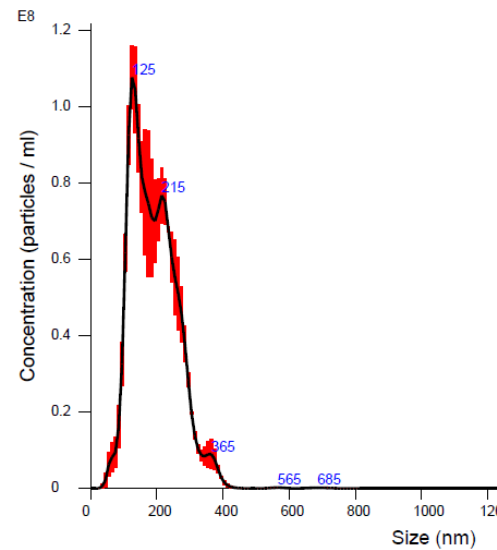
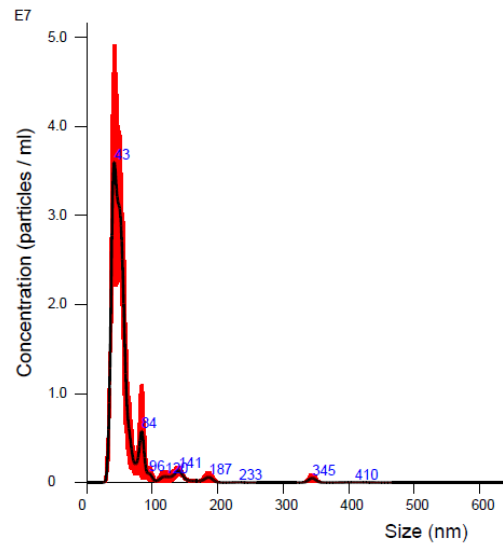
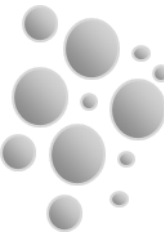
CONCENTRATION



Malvern
Panalytical



POLYDISPERSITY – SHAPE, BREADTH, MODES

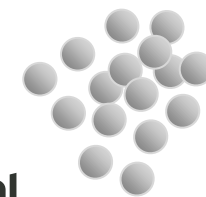


Monodisperse, bimodal, broad, tails, outliers, aggregation...

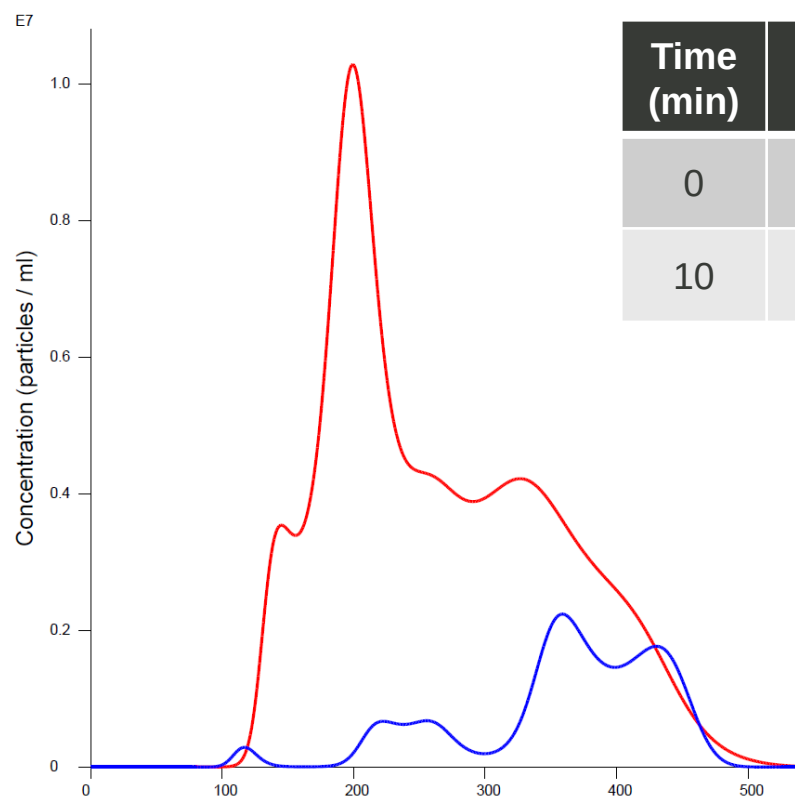
CONCENTRATION – TRACKING AGGREGATION



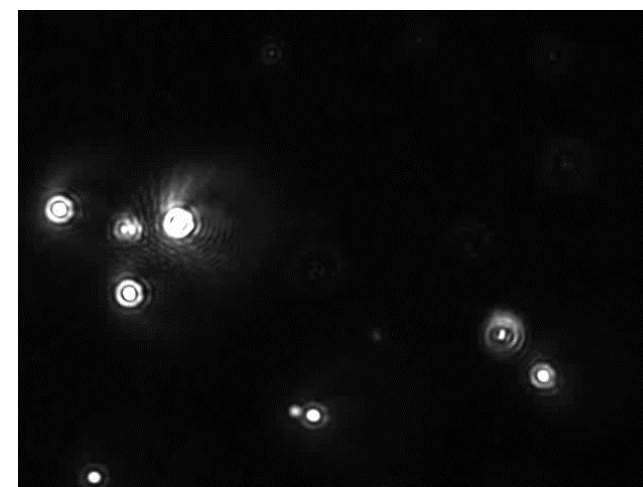
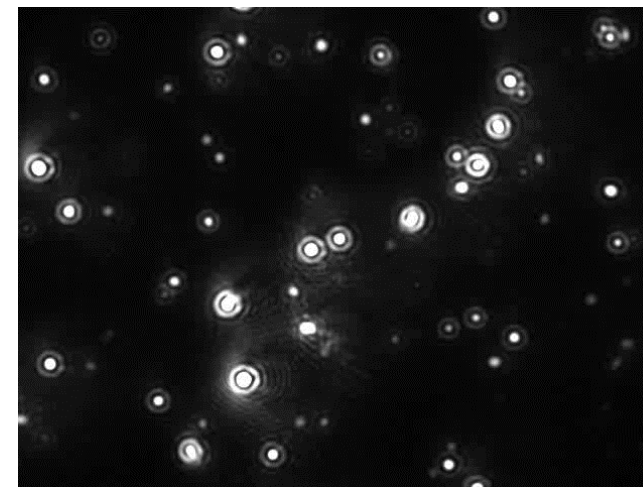
Malvern
Panalytical



- Decreased concentration as aggregate size increases



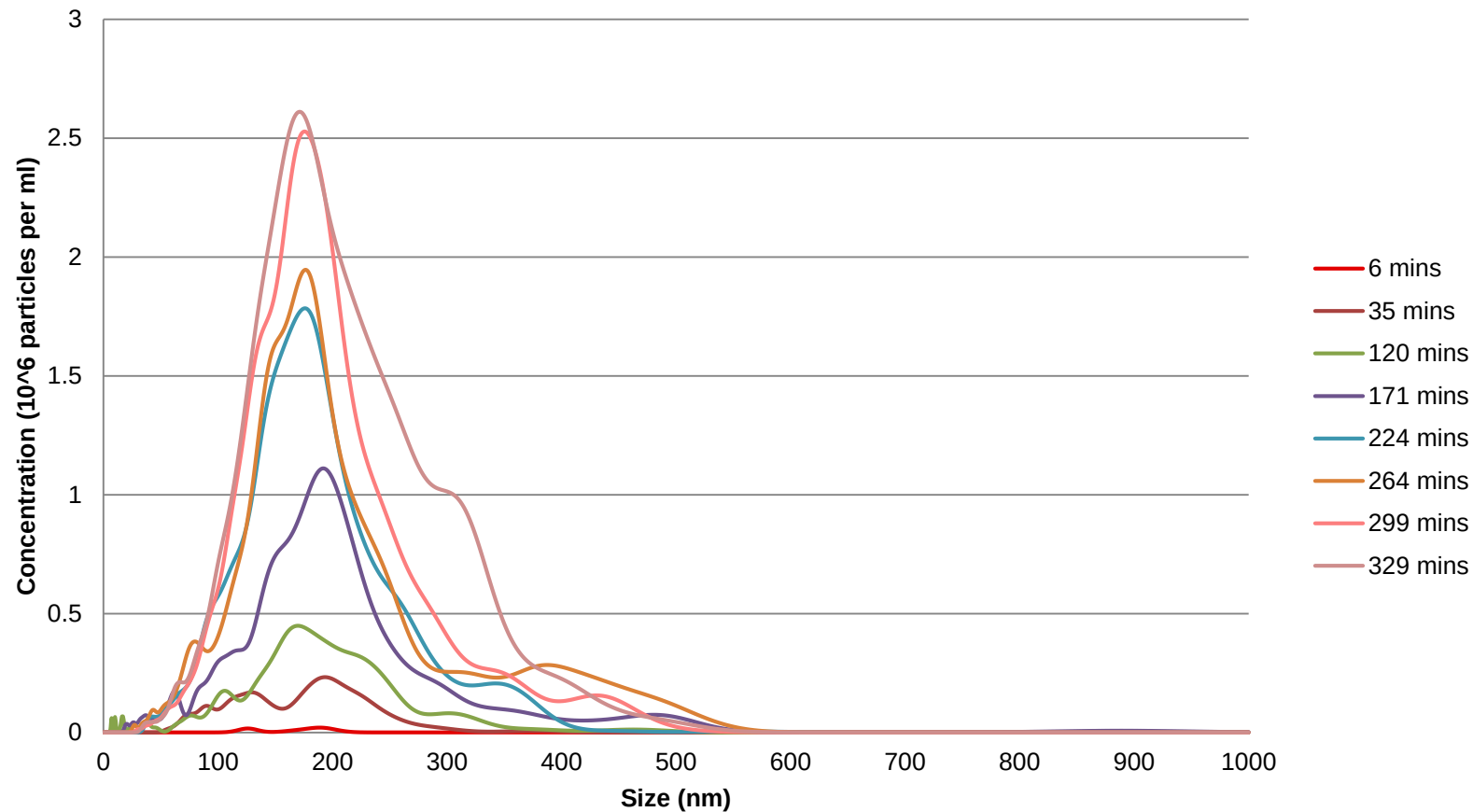
Time (min)	Mean (nm)	Conc (part/mL)
0	266	1.38E+09
10	359	2.91E+08



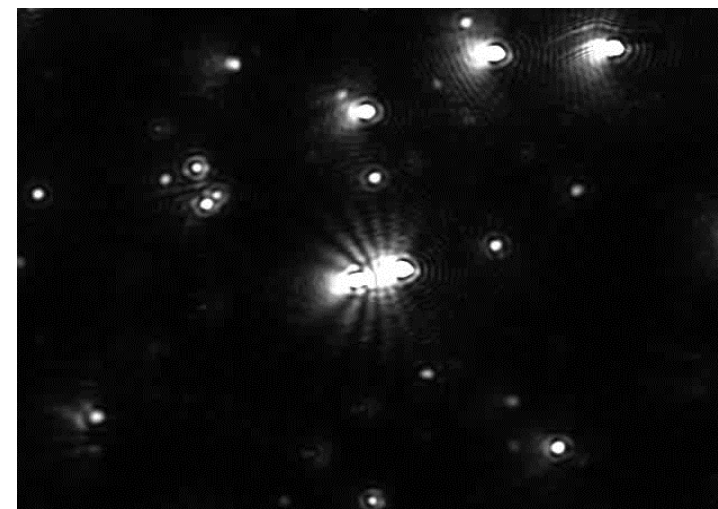
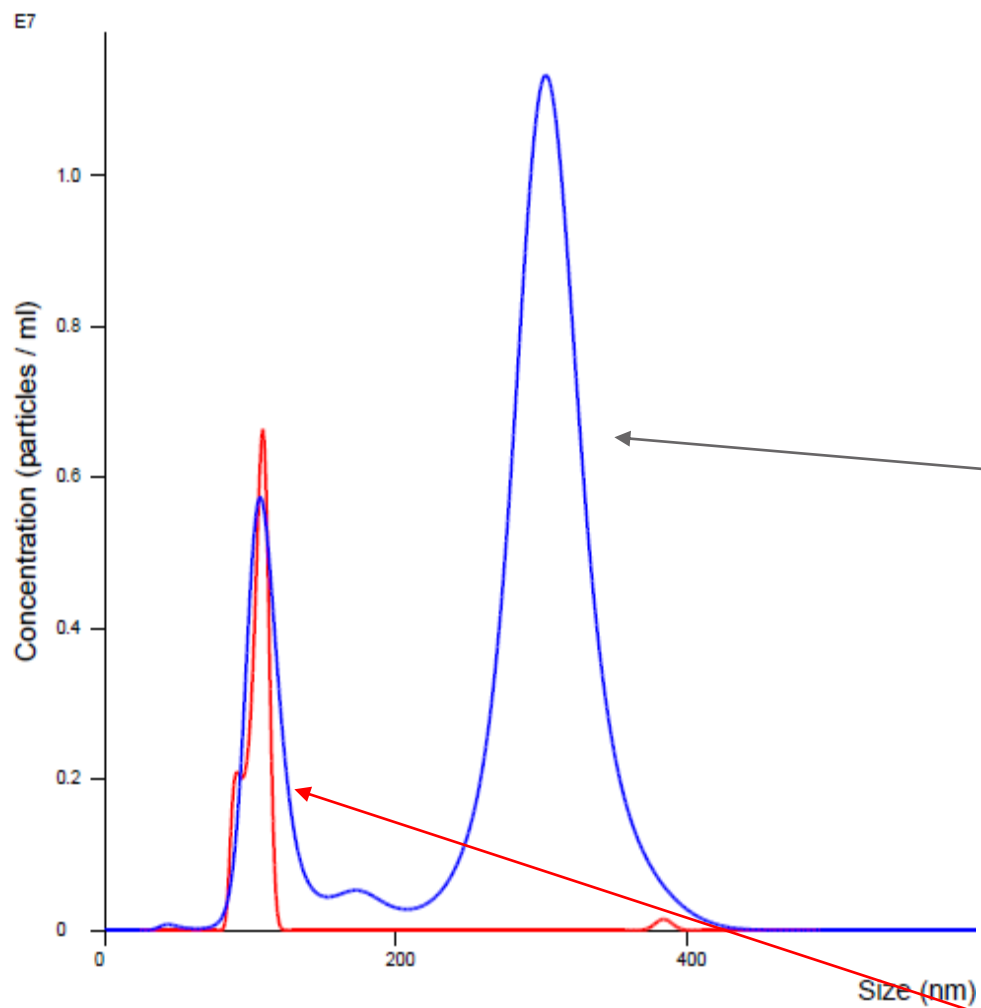
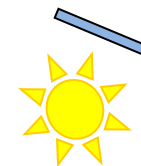
CONCENTRATION – PROTEIN AGGREGATION



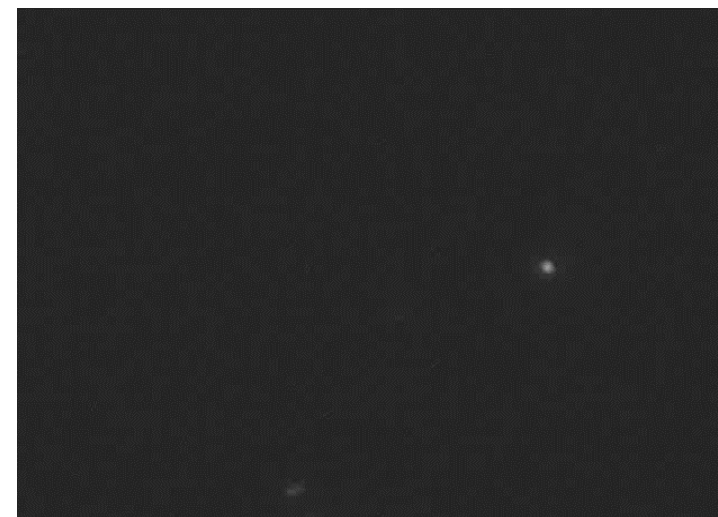
- Heat aggregated IgG shows increasing number of aggregates over time.
- Monomer too small to measure directly.



FLUORESCENCE SPECIATION



All particles (scatter mode)

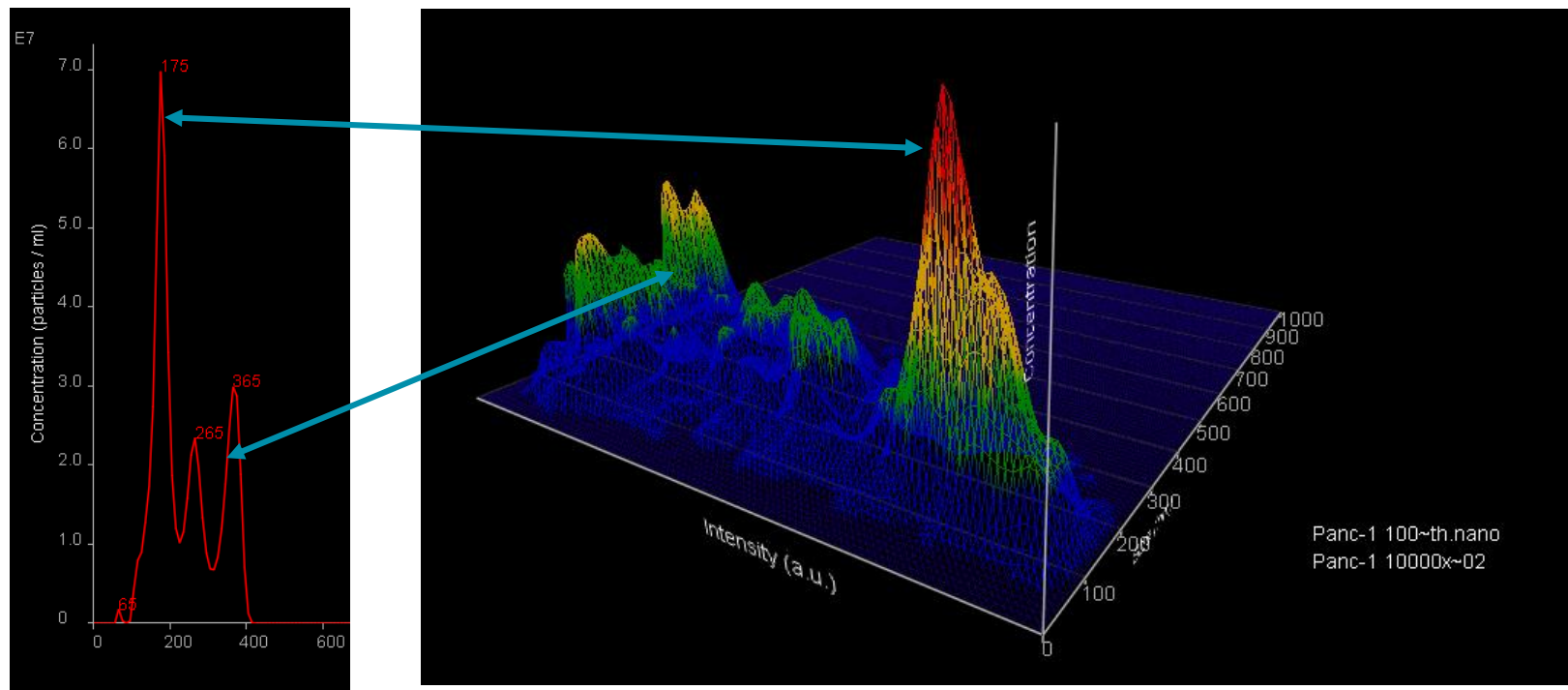


With Fluorescence Filter



RELATIVE LIGHT INTENSITY

- Qualitative confirmation of discrete populations
- Intensity is independent variable from diffusion/size, so acts as internal validation



SOFTWARE MAIN VIEW, NTA 3.2 (NTA 3.3 COMING VERY SOON!)



Analysis Tabs

Camera Level
Detection
Threshold

Script Panel

View and create
all scripting
commands

Analysis Info

Activity Log
information

The screenshot shows the NTA 3.2 software interface. On the left, there are three vertical panels: 'Analysis Tabs' with sliders for Screen Gain, Camera Level, and a checkbox for Auto setup; 'Script Panel' with a text area and Run/Abort buttons; and 'Analysis Info' showing a script abort message. The central 'Primary window' displays a live particle view with the NTA logo. On the right, there are two plots: a '2D Scatter Plot' of Intensity vs. Diameter and a '3D Plot' of Concentration vs. Diameter vs. Intensity. At the bottom, the 'Control Tabs' (SOP, Hardware, Analysis) are visible, along with a 'Measurement Selection' section containing various settings like Number of captures, Capture duration, and Base filename. A red banner at the bottom left reads 'NO TEMPERATURE MODULE'.

Primary window

Live view of particles
Video analysis
View size results

2D Scatter Plot

Size vs. Scattering Intensity

3D Plot

Size vs. Concentration vs Scattering
Intensity

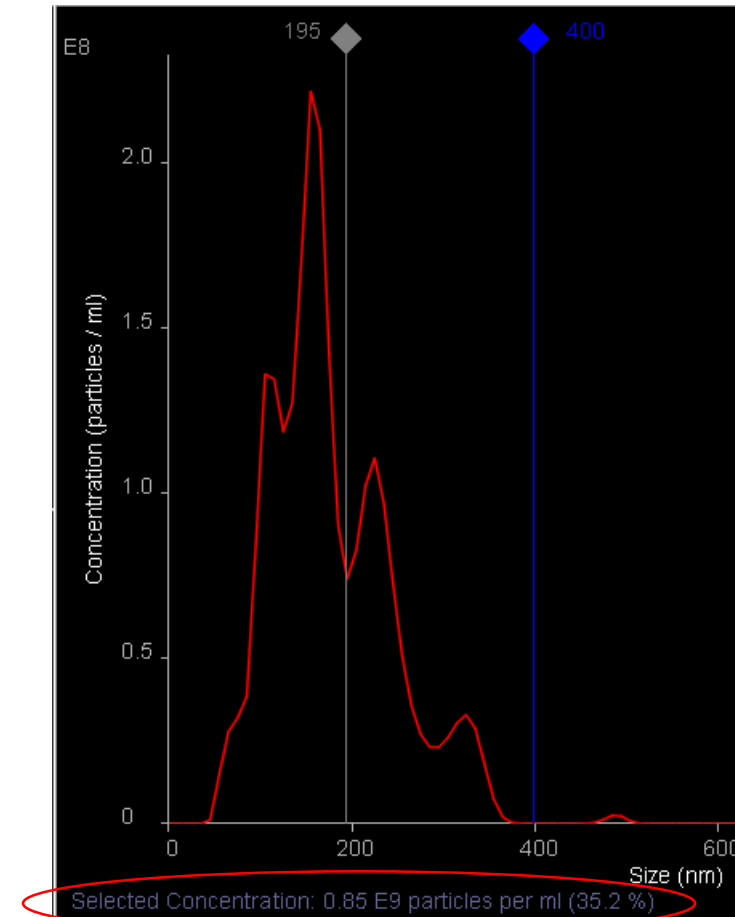
Control Tabs

SOP, Hardware,
Analysis

CONCENTRATION OF SELECTED RANGE

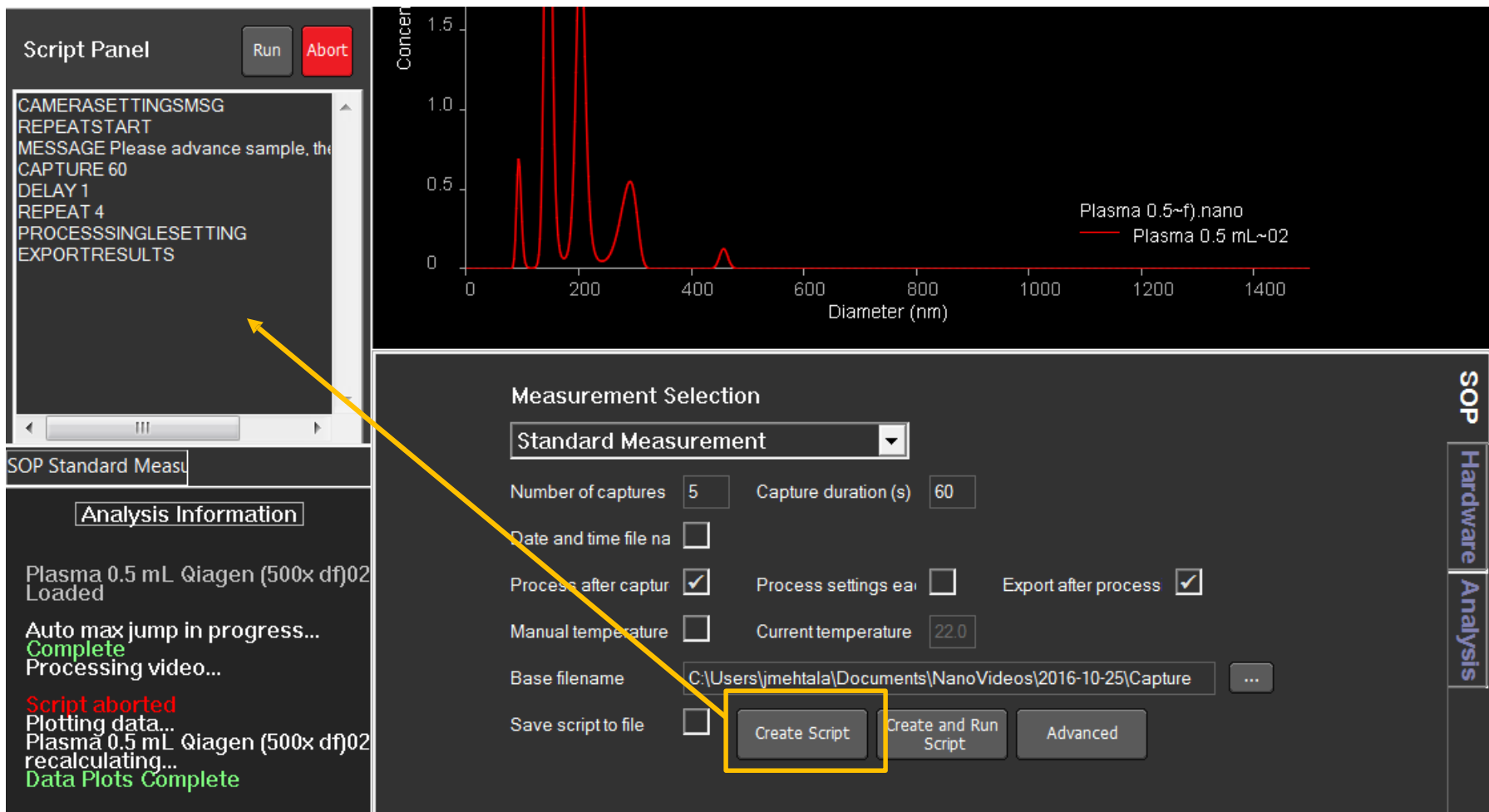


- Concentration can be reported for any subset
- Data is accumulation of individual particle measurements, so any statistical measure can be applied.



Concentration info:
Particles per frame : 122.2
Particles per ml : 2.4 x 10e9

SOP: CREATE SCRIPT



The screenshot displays the Malvern Panalytical software interface. On the left, the 'Script Panel' contains a list of commands: CAMERASETTINGSMSG, REPEATSTART, MESSAGE Please advance sample, the, CAPTURE 60, DELAY 1, REPEAT 4, PROCESSSSINGLESETTING, and EXPORTRESULTS. Below this, the 'SOP Standard Measu' section shows 'Analysis Information' for 'Plasma 0.5 mL Qiagen (500x df)02', indicating 'Auto max jump in progress...', 'Complete', 'Processing video...', 'Script aborted', 'Plotting data...', 'Plasma 0.5 mL Qiagen (500x df)02 recalculating...', and 'Data Plots Complete'. On the right, a size distribution plot shows 'Concen' vs 'Diameter (nm)' with two curves: 'Plasma 0.5~f).nano' (red) and 'Plasma 0.5 mL~02' (black). The 'Measurement Selection' dialog is open, showing 'Standard Measurement' selected. It includes settings for 'Number of captures' (5), 'Capture duration (s)' (60), 'Date and time file na' (unchecked), 'Process after captur' (checked), 'Process settings ea' (unchecked), 'Export after process' (checked), 'Manual temperature' (unchecked), 'Current temperature' (22.0), 'Base filename' (C:\Users\jmehtala\Documents\NanoVideos\2016-10-25\Capture), and 'Save script to file' (unchecked). The 'Create Script' button is highlighted with a yellow box. A yellow arrow points from the 'Script Panel' to the 'Create Script' button. The right sidebar shows 'SOP', 'Hardware', and 'Analysis' tabs.

Script Panel

Run Abort

CAMERASETTINGSMSG
REPEATSTART
MESSAGE Please advance sample, the
CAPTURE 60
DELAY 1
REPEAT 4
PROCESSSSINGLESETTING
EXPORTRESULTS

SOP Standard Measu

Analysis Information

Plasma 0.5 mL Qiagen (500x df)02
Loaded

Auto max jump in progress...
Complete
Processing video...

Script aborted
Plotting data...
Plasma 0.5 mL Qiagen (500x df)02
recalculating...
Data Plots Complete

Measurement Selection

Standard Measurement

Number of captures 5 Capture duration (s) 60

Date and time file na ☐

Process after captur ☒ Process settings ea ☐ Export after process ☒

Manual temperature ☐ Current temperature 22.0

Base filename C:\Users\jmehtala\Documents\NanoVideos\2016-10-25\Capture ...

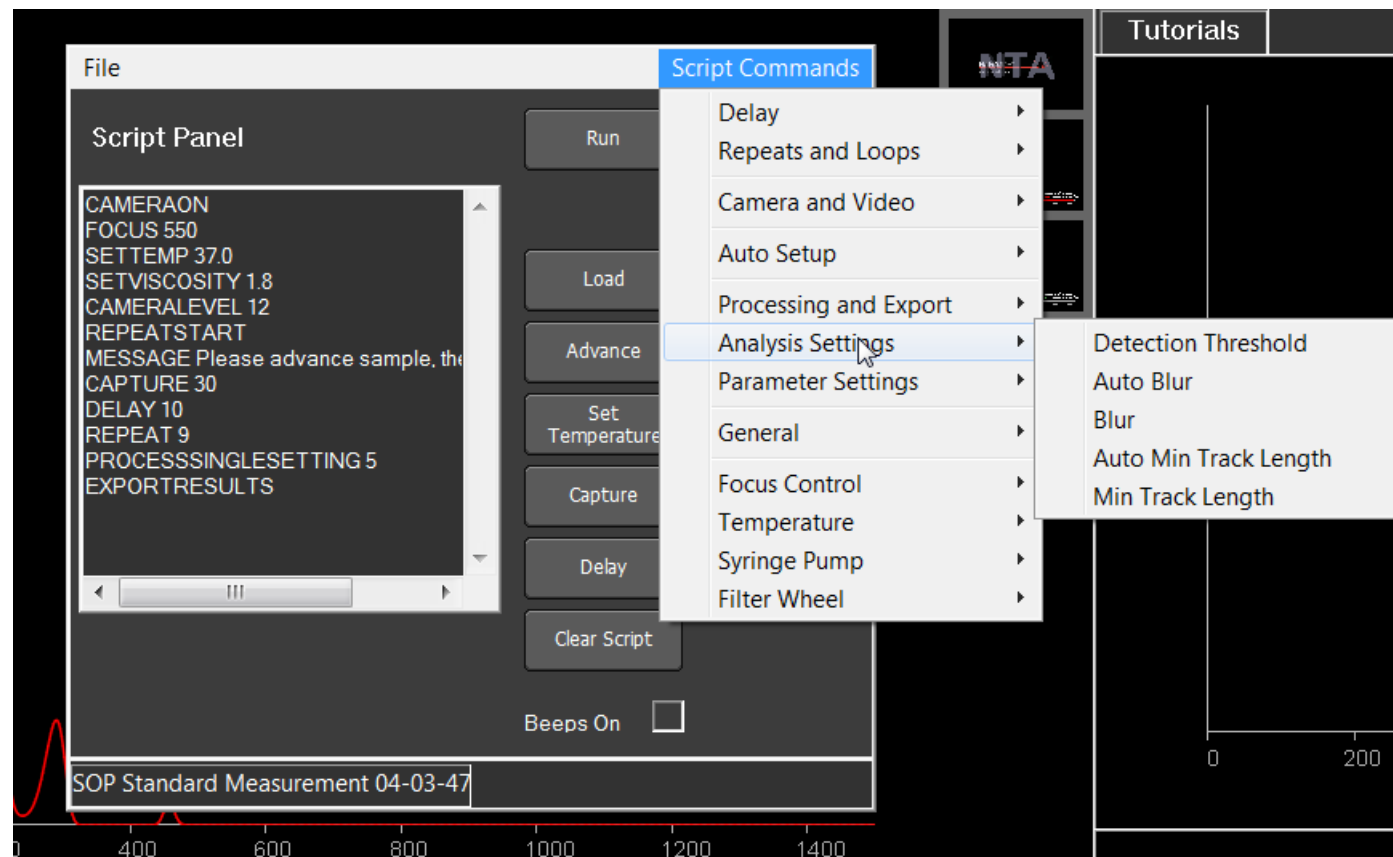
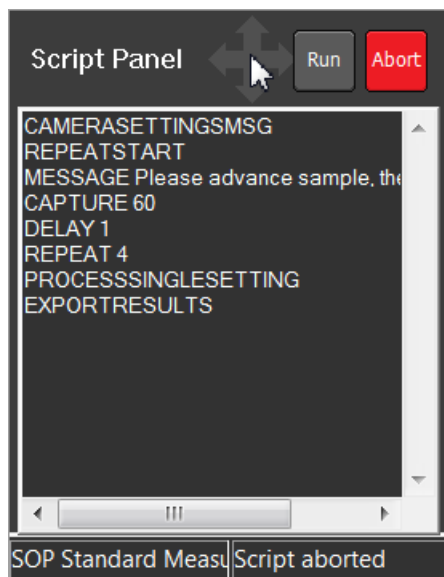
Save script to file ☐ Create Script Create and Run Script Advanced

SOP Hardware Analysis

SCRIPT PANEL: ADVANCED

Hover mouse and drag scripting panel

<http://www.malvern.com/en/support/resource-center/technical-notes/TN151117NTA3-2ScriptFunctions.aspx>

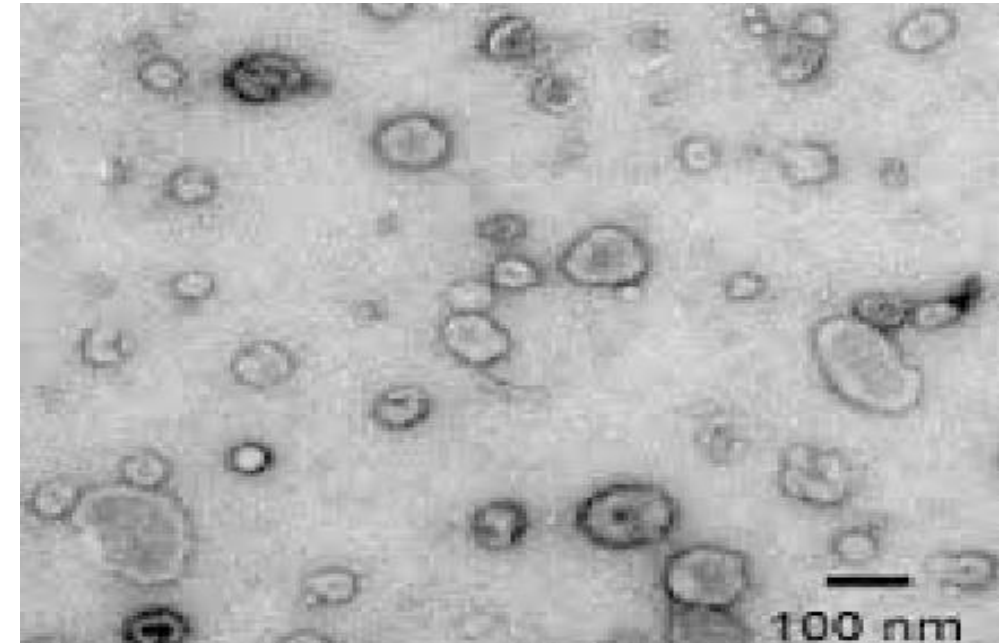


APPLICATION EXAMPLES – LIFE SCIENCE

LIFE SCIENCE EXAMPLES



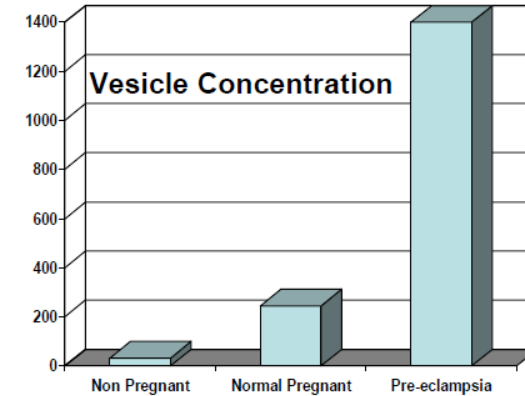
- Exosomes and Microvesicles
 - *Size, count, speciation*
- Therapeutic proteins
 - *To assess formulation stability and aggregation*
 - *Sub-micron particle count (predictor of immunogenic risk)*
- Virus and VLP samples
 - *Rapid titer, aggregation state*
 - *Empty/full (development work underway)*
- Liposomes and other drug delivery vehicles
 - *Size, concentration*
 - *Dosimetry*
 - *Protein Corona*



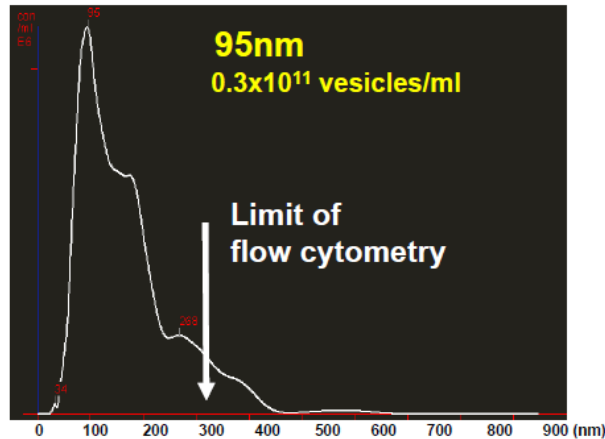
EXOSOME DIAGNOSTICS – PRE-ECLAMPSIA SCREENING



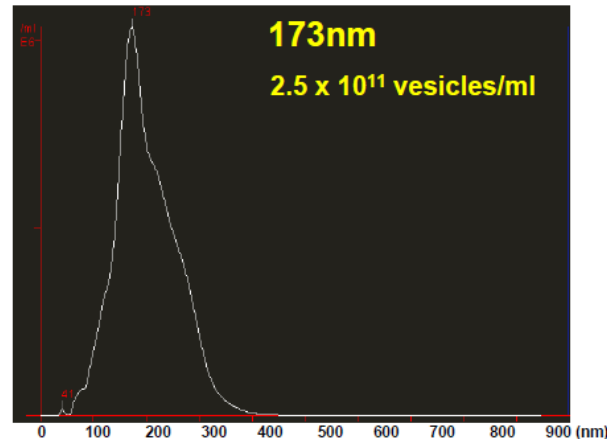
- Pre-eclampsia is a pregnancy disorder which can have severe complications
- Size and concentration of EVs increase in patients exhibiting pre-eclampsia*



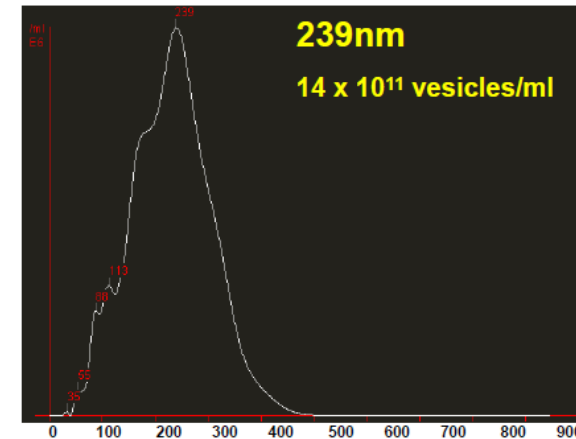
Non Pregnant Plasma



Normal Pregnancy Plasma



Pre-eclampsia Plasma

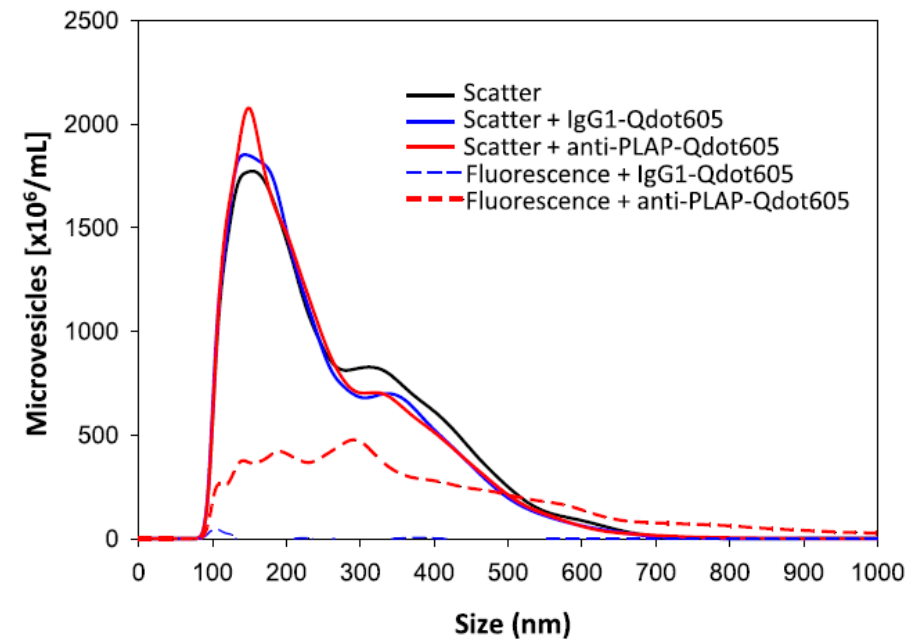


*"Multicolor Flow Cytometry and Nanoparticle Tracking Analysis of Extracellular Vesicles in the Plasma of Normal Pregnant and Pre-eclamptic Women." Dragovic et al. *Biol. Reprod.* **2013**, 89, 151.

FLUORESCENCE EXAMPLE – ANTIBODY-QUANTUM DOTS



- Antibody mediated quantum dot labelling of exosomes.
- Agreement between solid lines show that Ab does not affect size.
- IgG shows no labelling, confirming specificity.
- PLAP is specific target and confirms labelling.
- Proper fluorescence experiments require a number of controls.

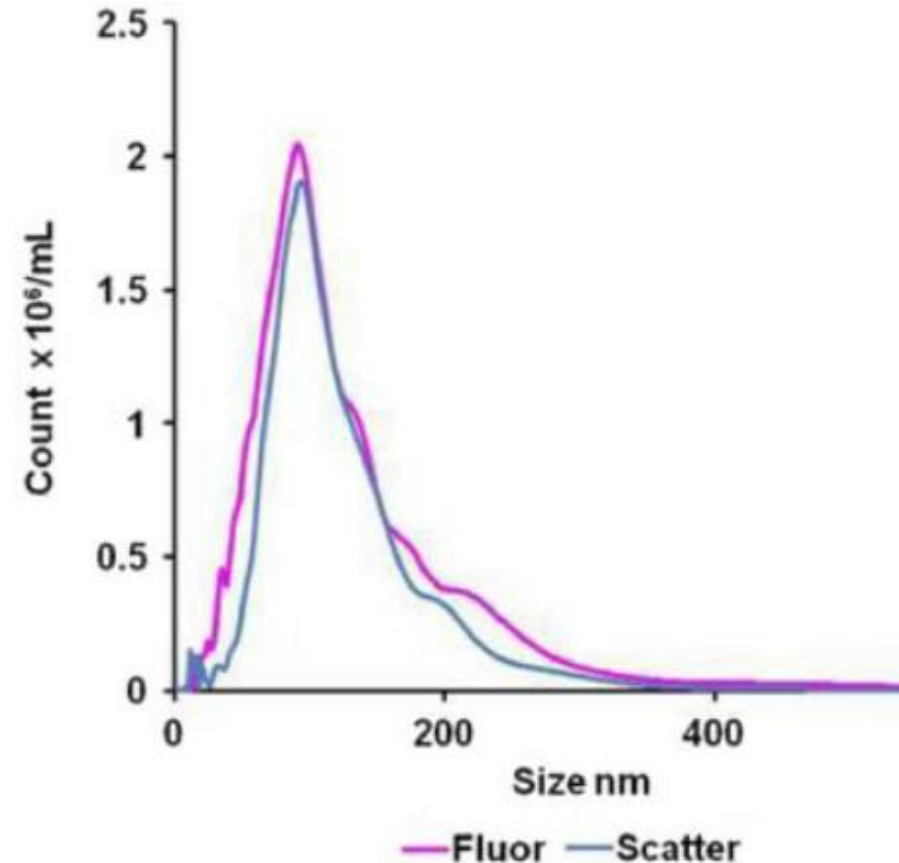


“Isolation of syncytiotrophoblast microvesicles...” Dragovic et al., *Methods* **2015**, 87, 64.

FLUORESCENCE EXAMPLE – MEMBRANE LABELLING



- Membrane dyes will attach to any lipid
- Non-specific but often more thorough labelling.
- Non-specific approach can be used if you are confident in your isolation protocol.



“Extracellular vesicle sizing and enumeration by nanoparticle tracking analysis.” Gardiner et al., JEV, 2013, 2: 19671

RESULT QUALITY

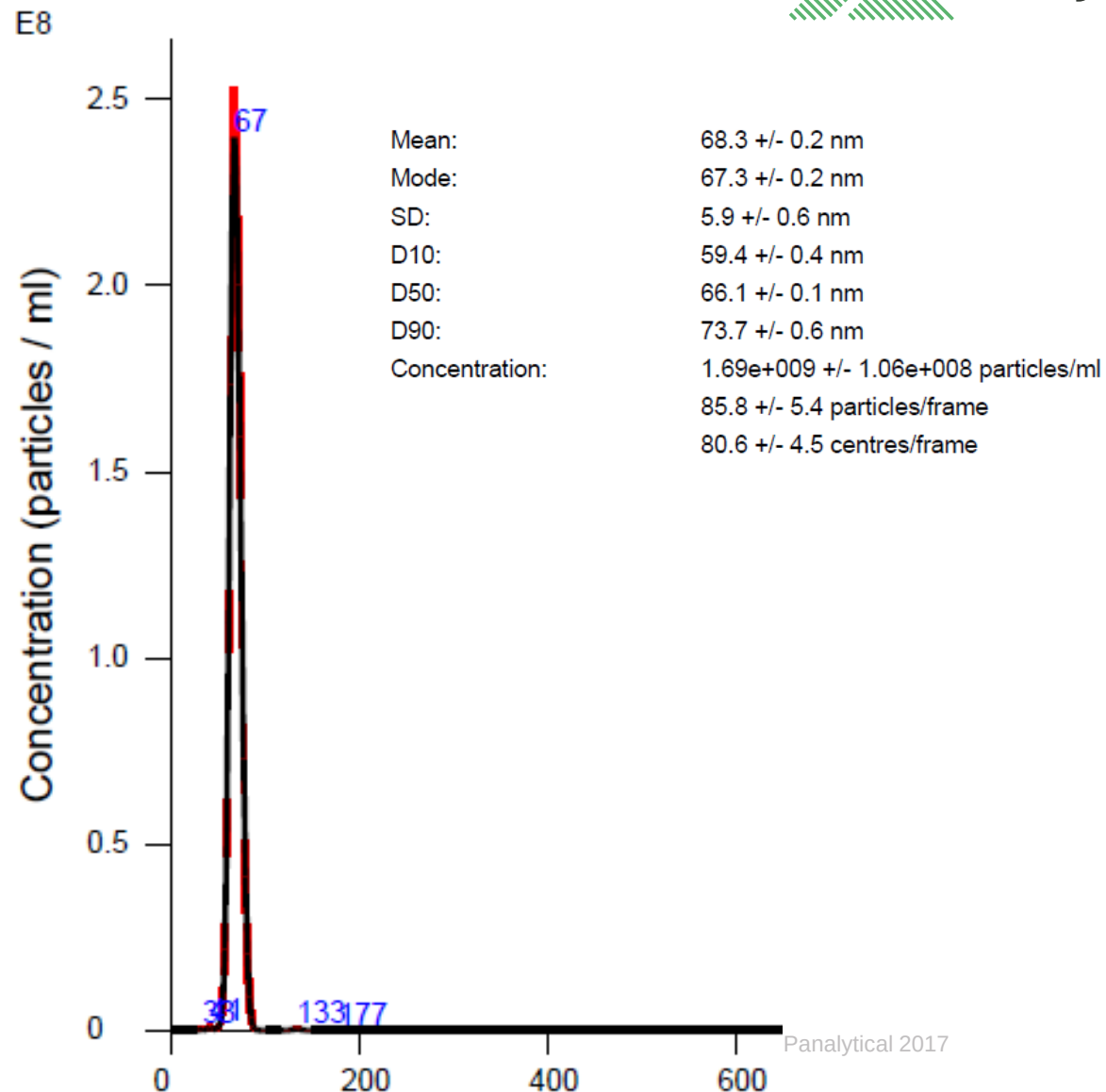
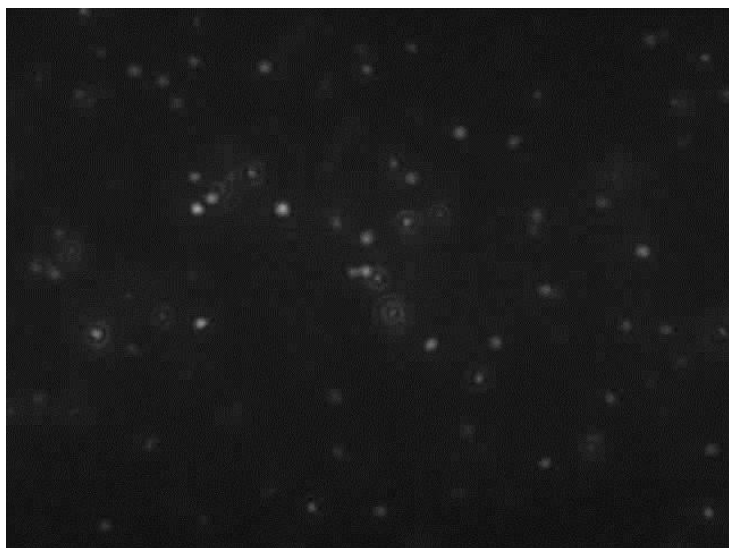


- Key indicator: Particle concentration
 - *Ideal range is 10-100 particles/frame*
- Over 100:
 - *Short tracking and missed tracking*
 - *Measurement will under-report concentration*
 - *Lose peak-to-peak resolving power, peak broadening*
- Under 5:
 - *Poor statistics*
 - *Large error bars*
 - *Still useful to determine order of magnitude concentration changes between samples*

EXAMPLE 1: GOOD QUALITY MONODISPERSE DATA



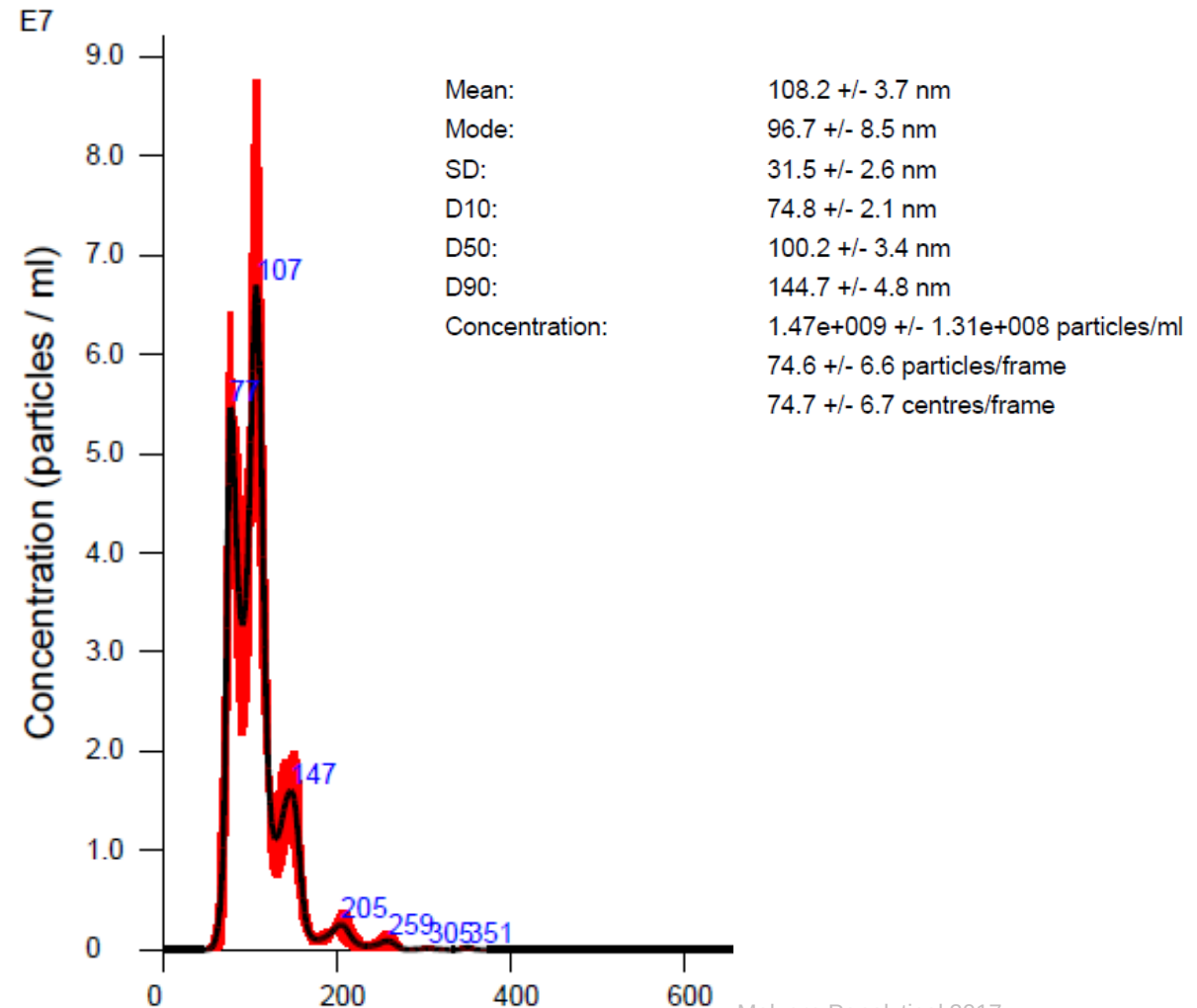
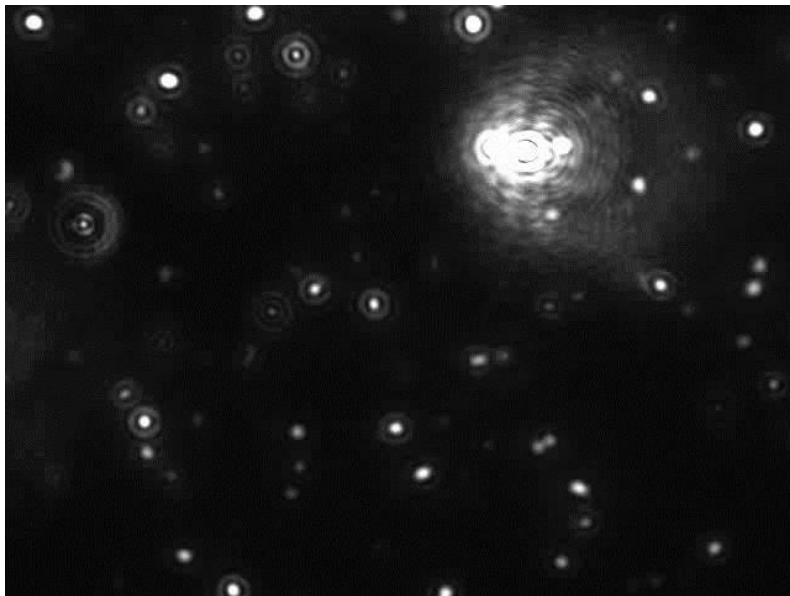
- Monodisperse Liposomes
- Ideal concentration
- Small error bars
- Repeatable concentration
- Recommended: 3 x 60 sec videos



EXAMPLE 2: GOOD QUALITY POLYDISPERSE DATA



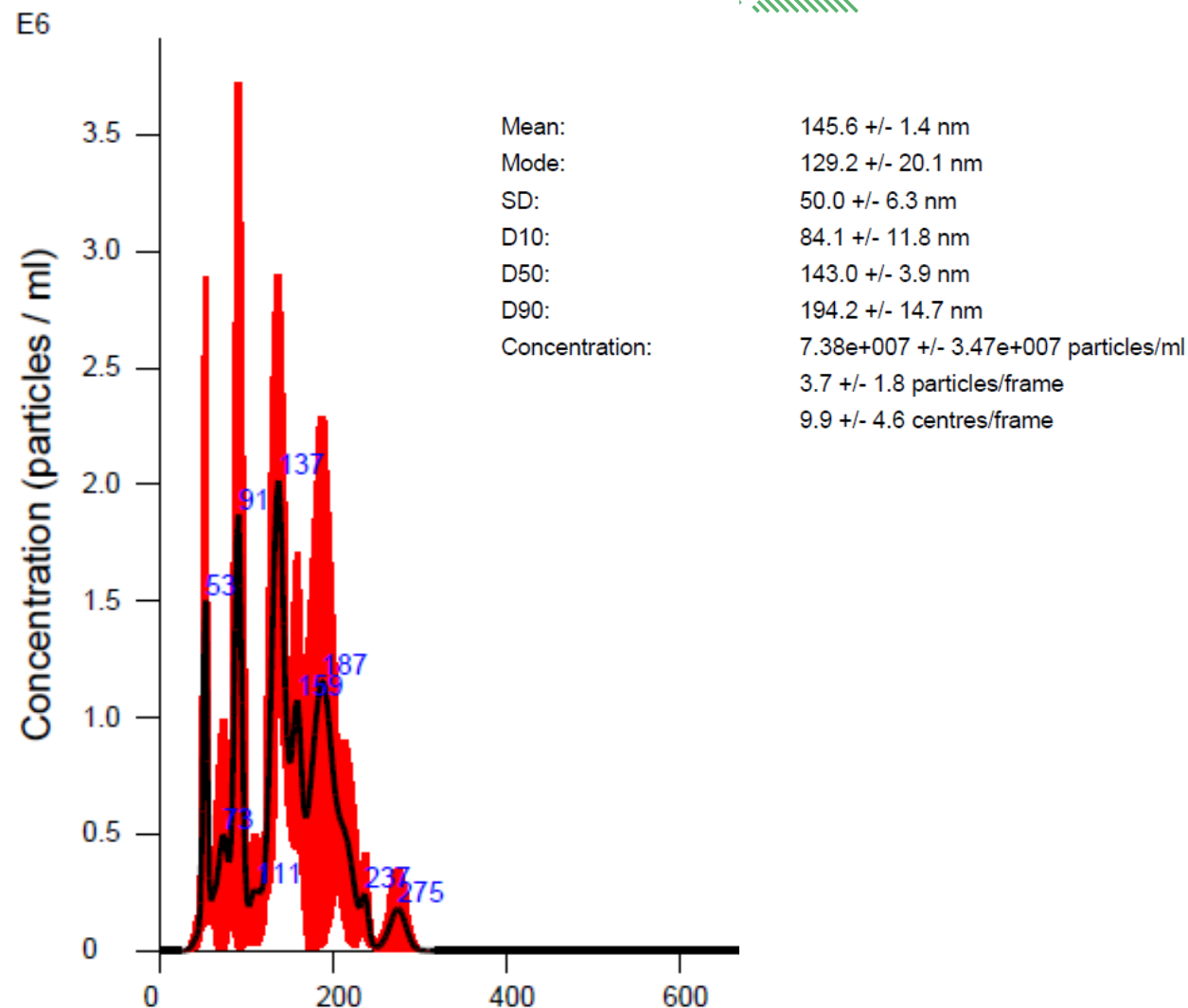
- Aggregated Liposomes
- Ideal concentration
- Small error bars
- Repeatable concentration
- Recommended: 5 x 60 sec videos with flow



EXAMPLE 3: LOW QUALITY POLYDISPERSE DATA



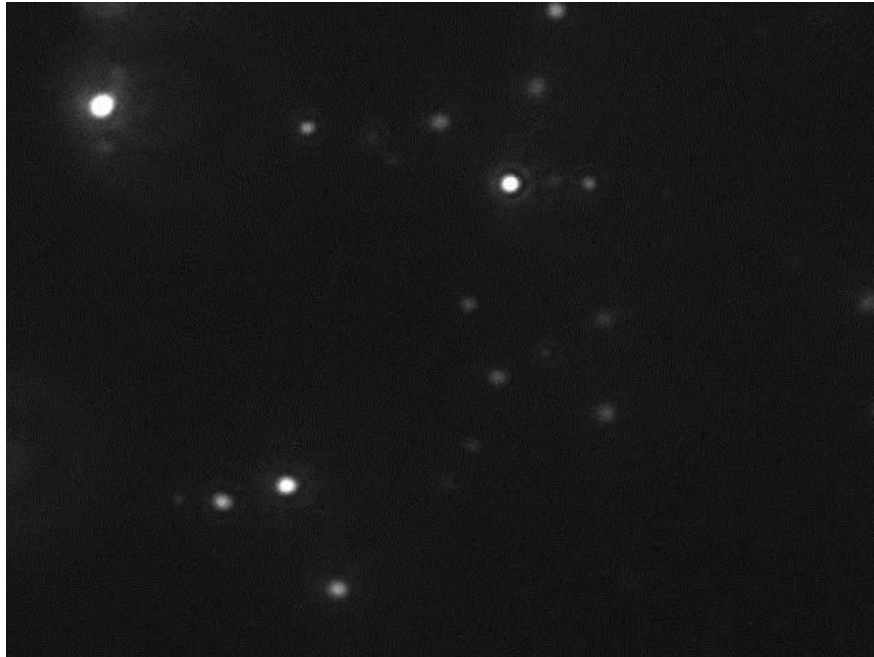
- Polydisperse water treatment sample
- Low concentration
- Results barely above background
- Repeatable concentration
- Recommended: 10+ x 60 sec videos with flow



EXAMPLE 4: PHOTOBLEACHING IN STATIC VS FLOW MODE



Fluorescent Exosomes (Dil)
No Flow



- › Particle count decreases at end of movie.

Fluorescent Exosomes (GFP)
With Flow



- › Particle count is consistent during entire movie

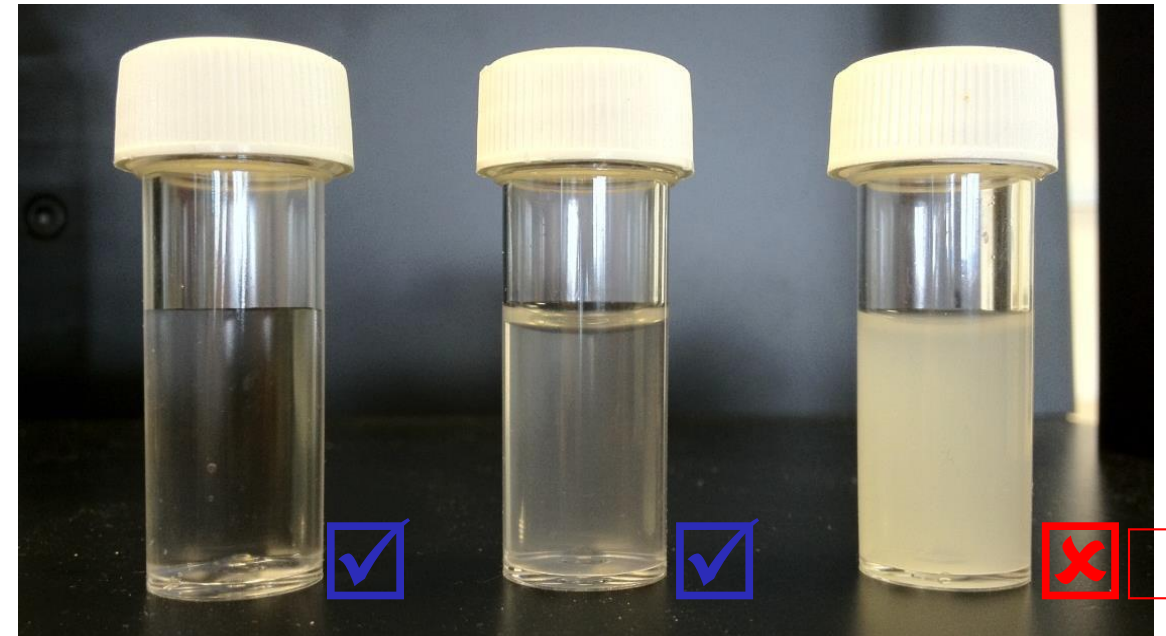
METHOD DEVELOPMENT



- Step 1: Configure the instrument
 - *Choose the top-plate for your sample*
 - *Choose the appropriate laser wavelength*
 - *Choose static or flow mode*
- Step 2: Prepare the instrument and sample
 - *Prime fluidics to check diluent*
 - *Dilute sample to appropriate particle concentration range*
- Step 3: Load the sample
 - *By syringe (NS300 and LM10) or by vial (NS500)*
- Step 4: Set capture settings
 - *Adjust camera position to center of laser beam*
 - *Adjust camera level and focus*
 - *Choose appropriate number and length of movies*
- Step 5: Set analysis settings
 - *Adjust detection threshold*

SAMPLE PREPARATION

- Samples must be in a liquid suspension
- Requirements of sonication, filtration, etc will be sample specific and up to the customer
- Sample should not be visibly too concentrated (opaque or strong tint, see below)
- Ideal concentration: between 10-100 particles on screen (you can take a quick video to confirm)
- Recommendation: For samples of unknown particle concentration, prepare a linear dilution: 10x, 100x, 1000x, 10,000x, etc.
 - *Test more dilute samples first*



PRIME FLUIDICS WITH DILUENT AND CHECK BACKGROUND

- Priming the flow cell

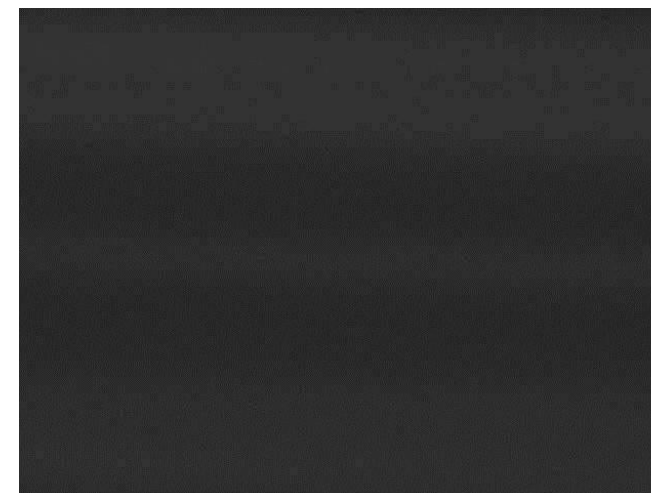


- Diluent background should have fewer than 5 particles/frame

- No filtration

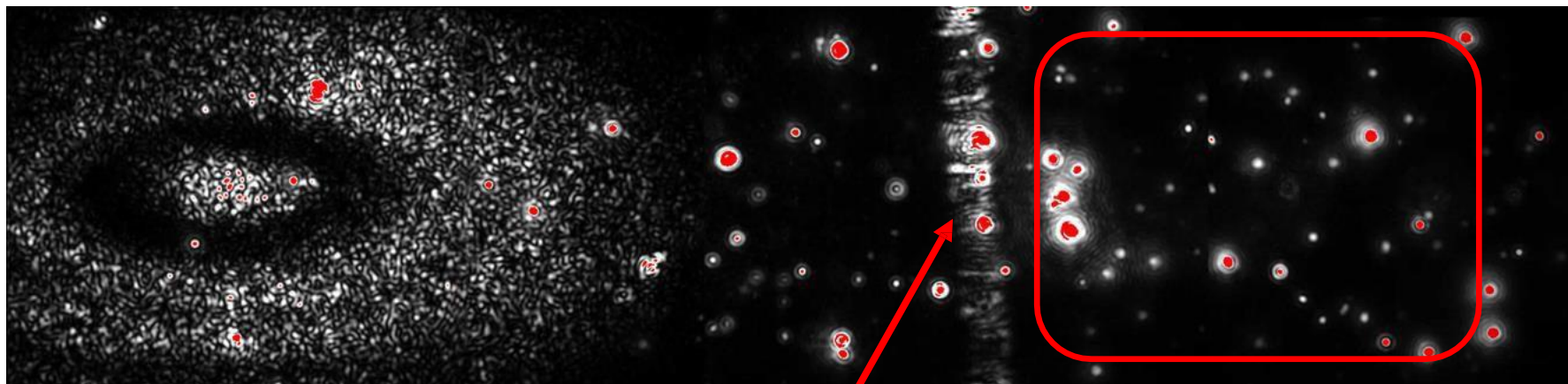


- 0.02 μm syringe filter



LOCATING THE OPTIMAL VIEWING POSITION

- For LM10: Find the thumbprint region

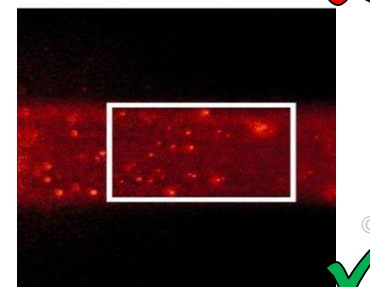
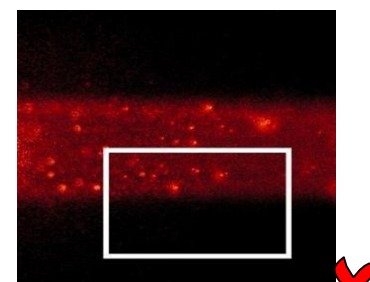
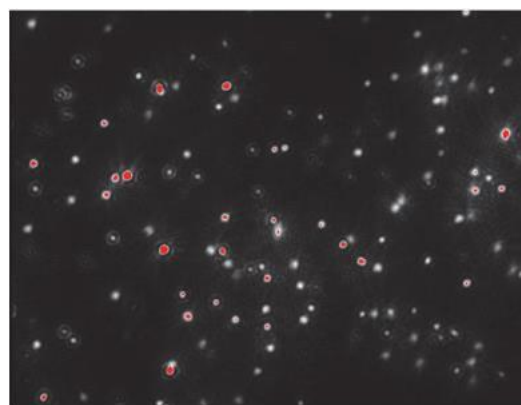


“Thumbprint”

Interface / Chrome line

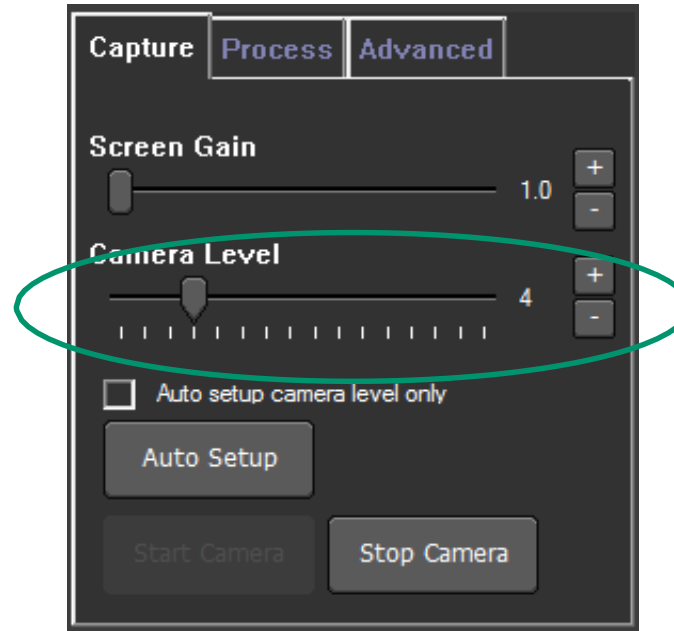
Optimal viewing

- For NS300: Center laser beam in middle of screen

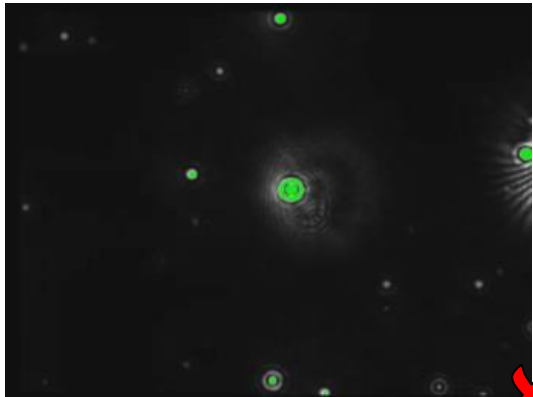


CAMERA SETTINGS

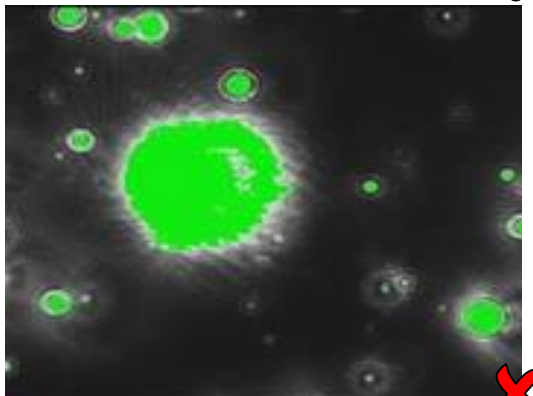
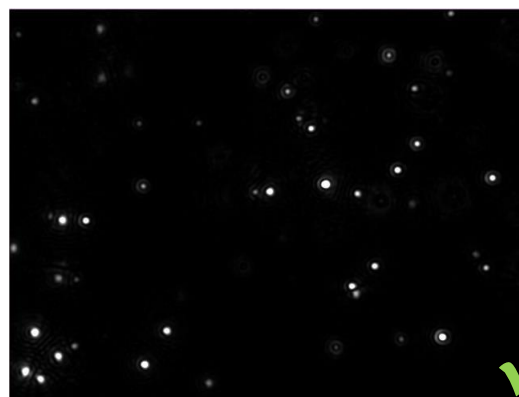
- Adjust Camera Level until all particles in sample can be seen clearly but no more than 20% are saturated (colored pixels)



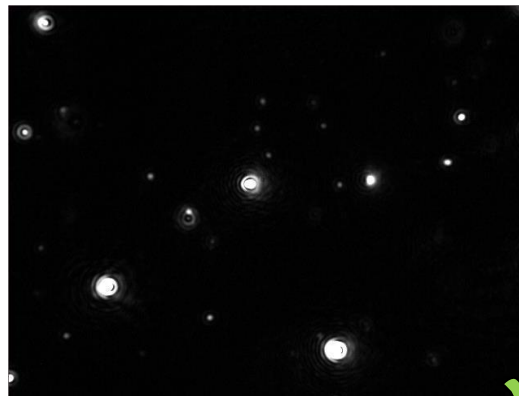
Poor Monodisperse Sample Image



Good Monodisperse Sample Image



Poor Polydisperse Sample Image

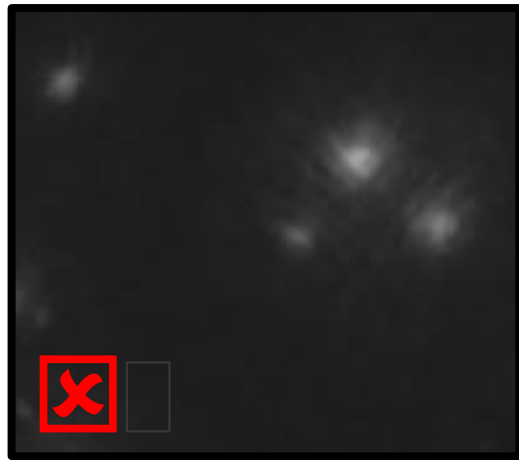


Good Polydisperse Sample Image

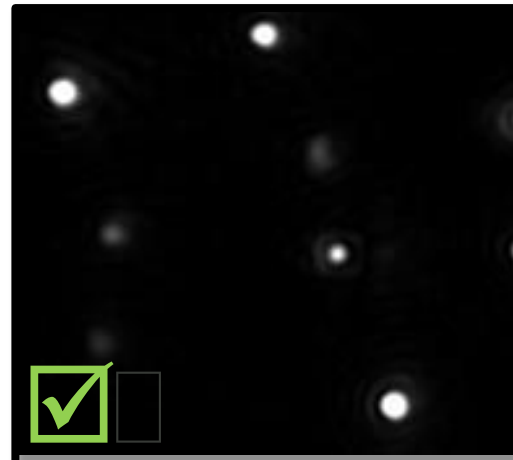
- Protocol:
 - Increase camera level to 16
 - Decrease until dimmest particles are no longer visible
 - Increase camera level again by one or two levels

FOCUS

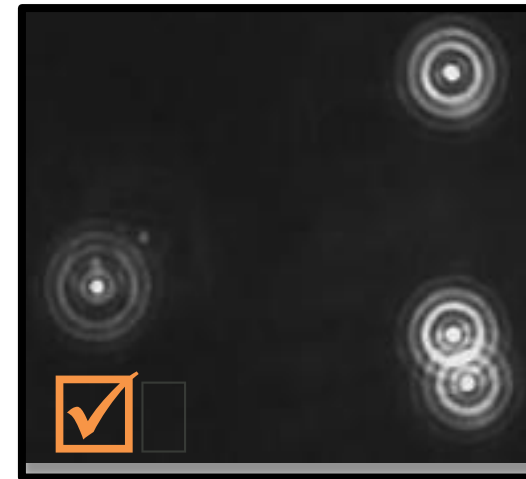
- Adjust focus so that particles appear as smooth circles
- Better to be slightly overfocused (rings) than underfocused (blurred)



Poor Polydisperse Sample Image



Good Polydisperse Sample Image



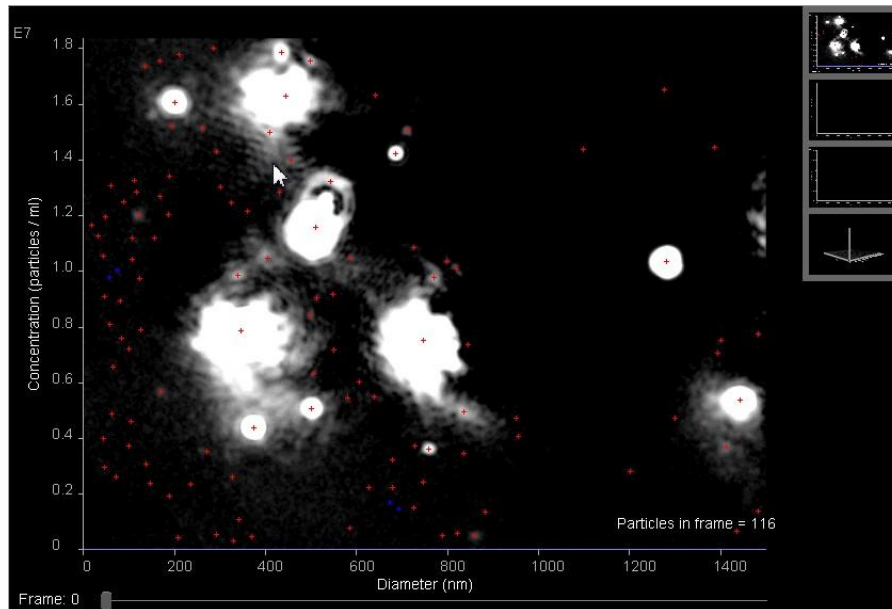
- Alternate between adjusting camera level and focus to ensure focus is fine tuned at the desired camera level

DETECTION THRESHOLD (DT)

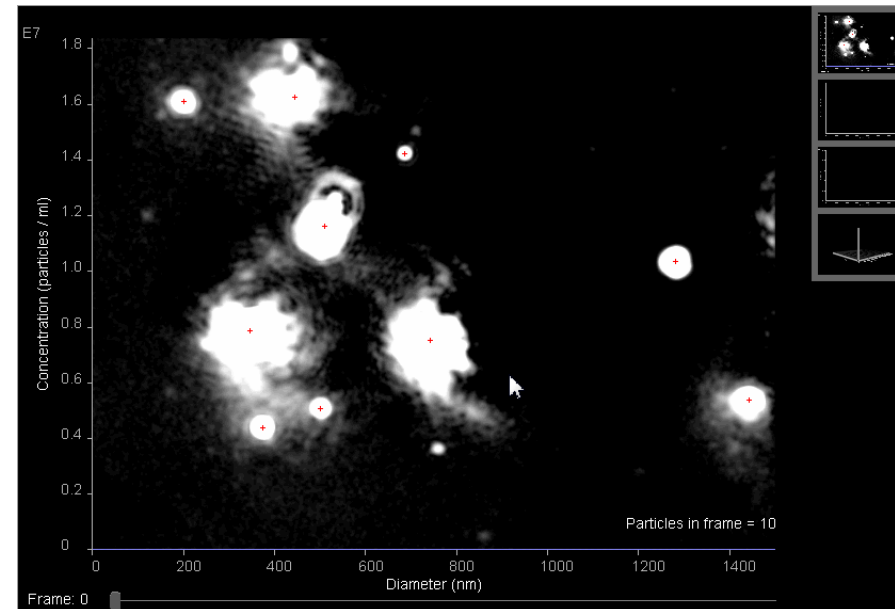


- Determines minimum particle brightness to be tracked
 - *If background is dark and particles are in focus, a detection threshold setting of 5 will work for most samples.*
 - *All visible particles should have a cross on it*
- Red crosses are valid tracks
- Blue crosses are near the threshold
- No more than 5-8 blue crosses / frame

DT 2
(too low)

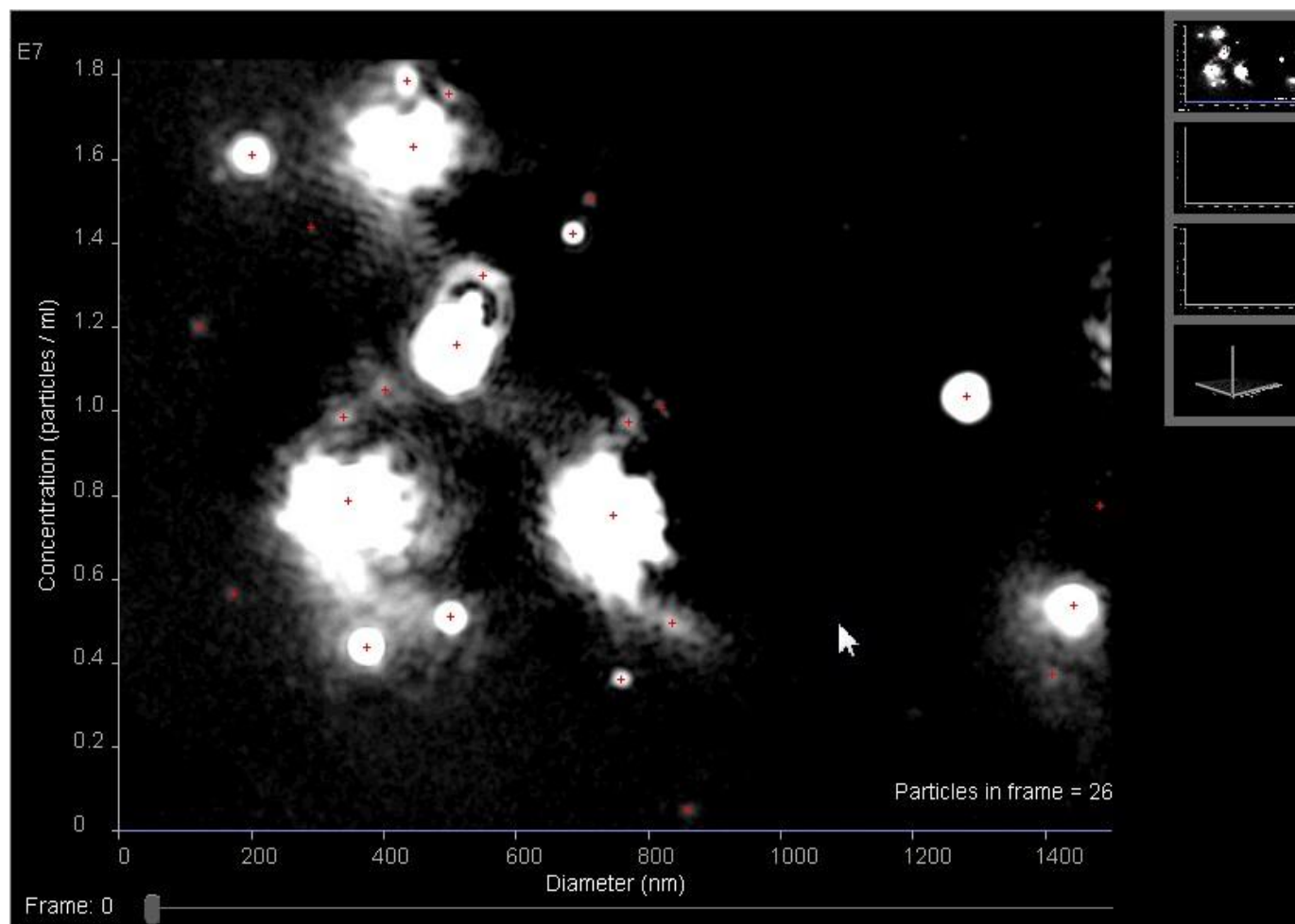


DT 40
(too high)



DETECTION THRESHOLD CONTINUED...

DT 5 (good)

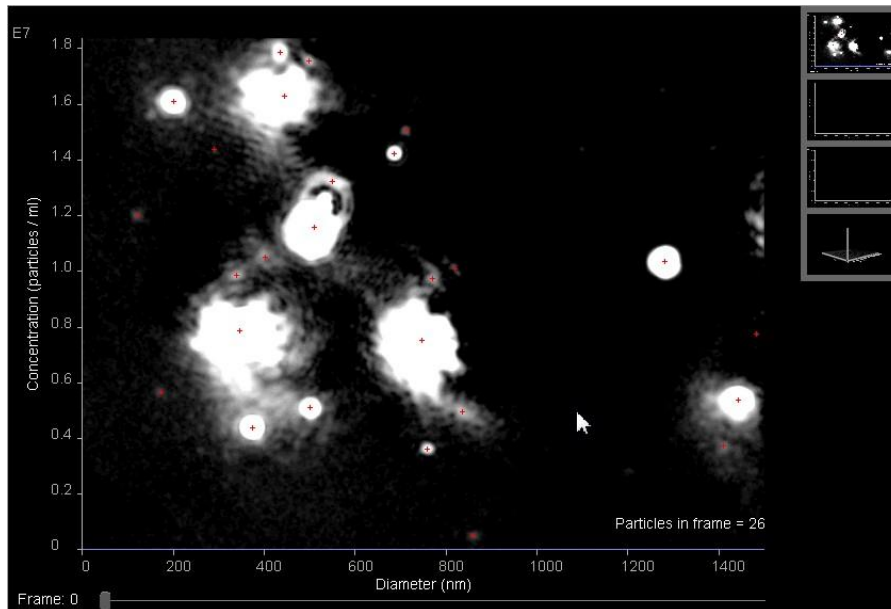


DETECTION THRESHOLD (DT)

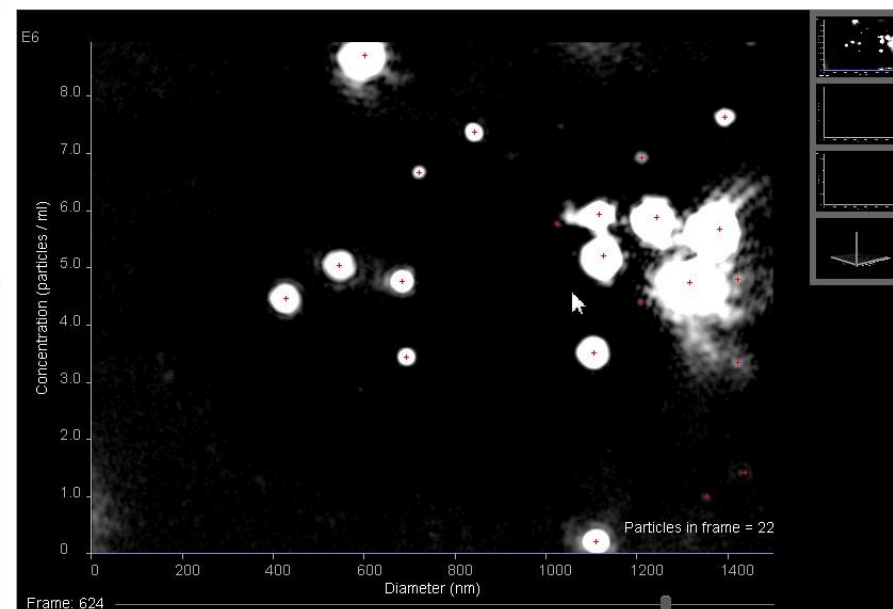


- Drag movie progress bar to inspect different parts of the movie.
- Ensures appropriate DT settings across entire movie.
- Helps to distinguish between noise and a dim particle

DT 5 (Frame 0)



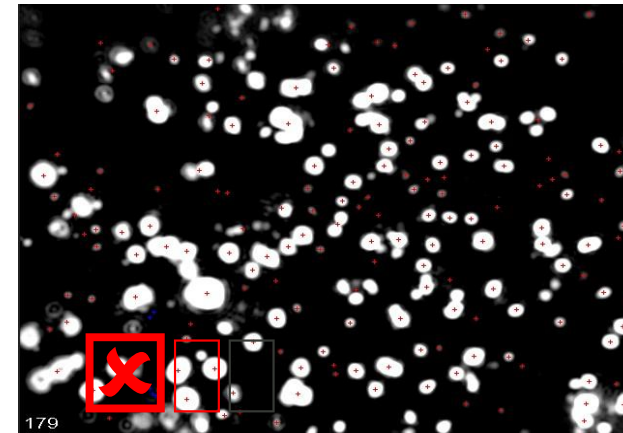
DT 5 (Frame 678)



CONCENTRATION CHECK USING THE NTA SOFTWARE



- Concentration check: $1\text{E}8 - 2\text{E}9$ particles/mL = 10-100 average particles/frame

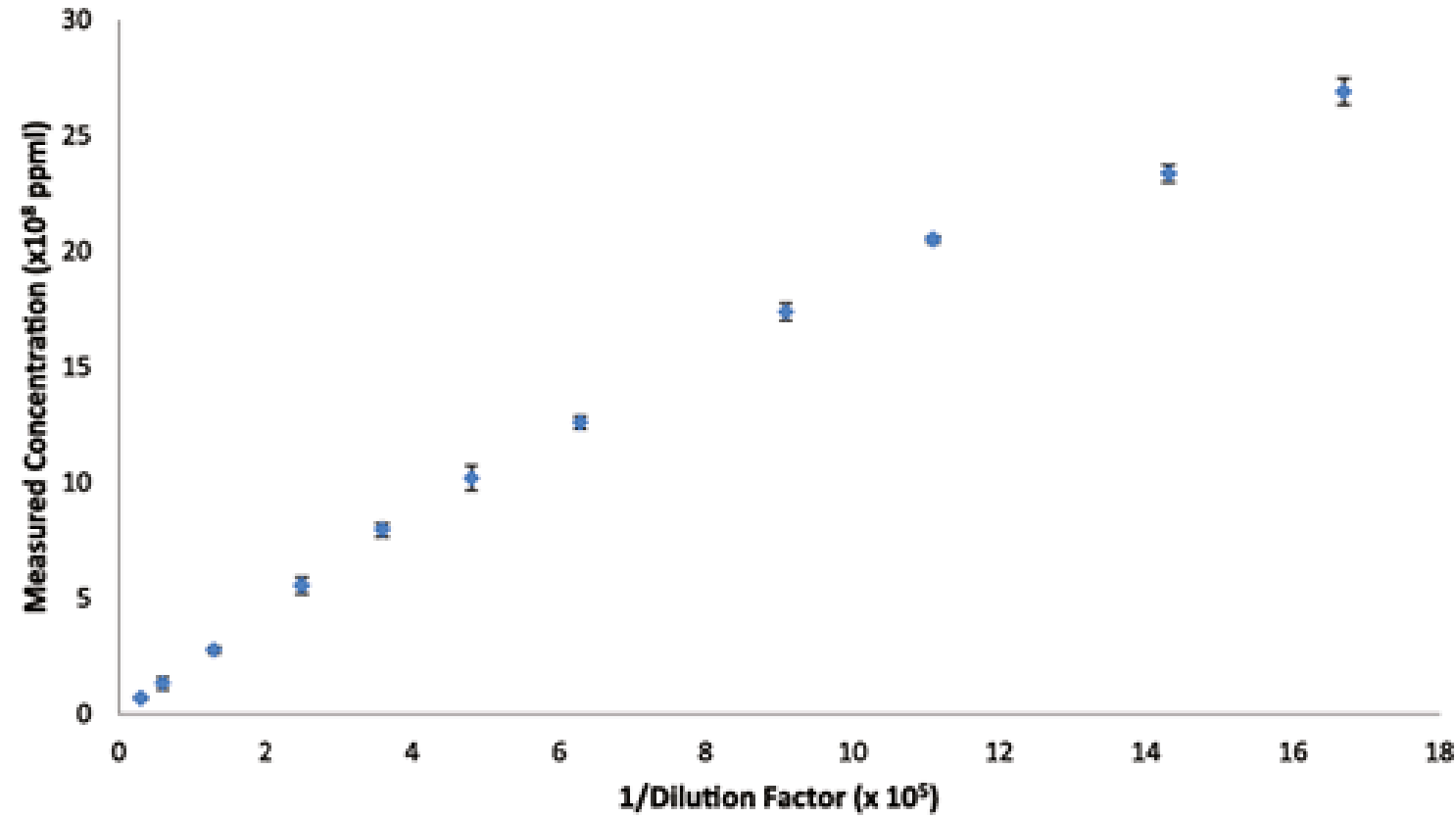


- Too low = poor statistics, require longer movies and/or syringe pump
- Too high = overlapping paths, missed tracks, under-report concentration, lower resolution.

CONCENTRATION CHECK CONTINUED...



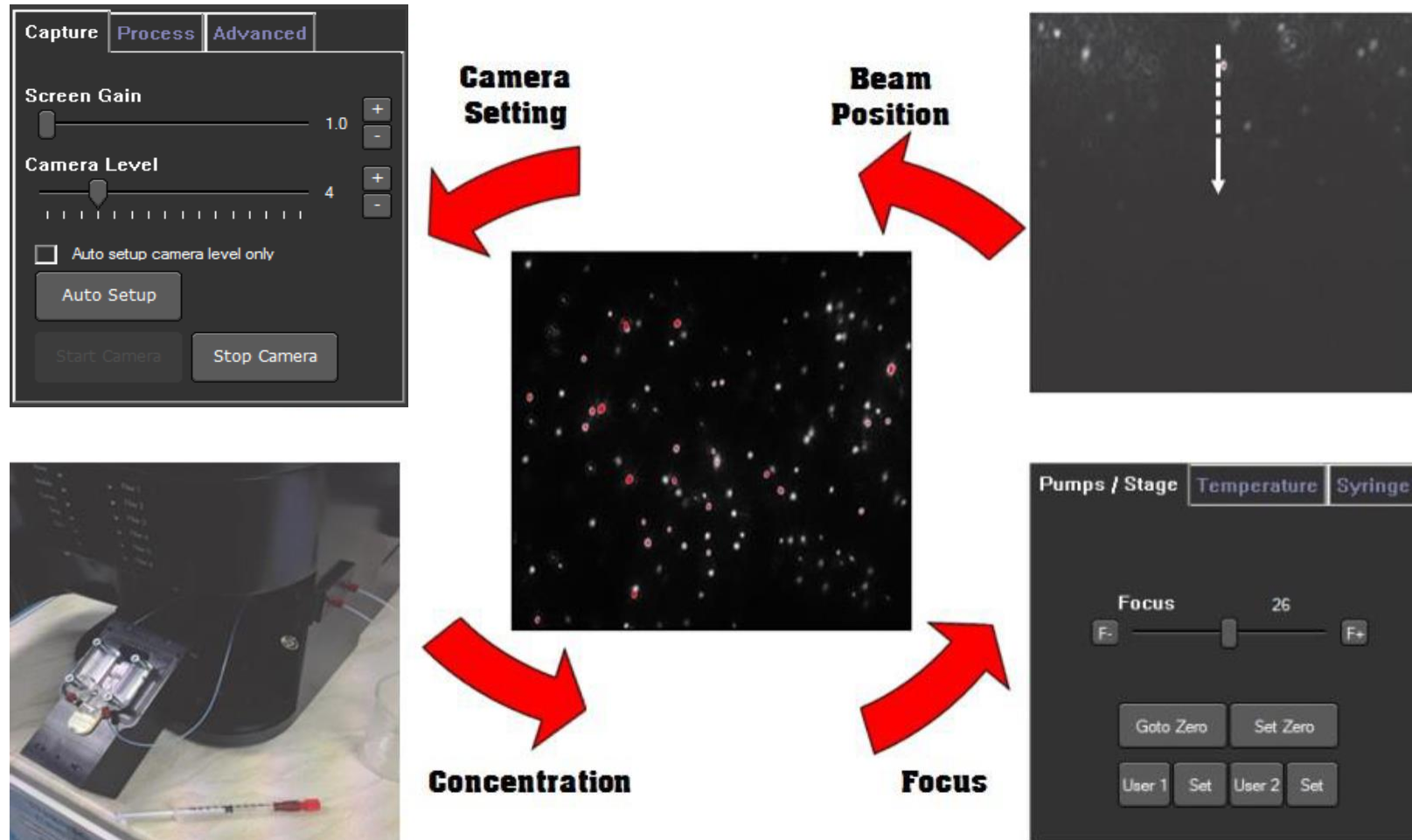
- Under normal conditions, when analyzing optimal concentrations of nanoparticles exhibiting similar optical characteristics, such as a monodisperse polystyrene, concentration accuracies can be as good as $\pm 5\%$ - 10% if the sample is diluted to a suitable concentration range.
- Higher concentrations deviate from this linear relationship



CONCENTRATION CHECK CONTINUED...



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



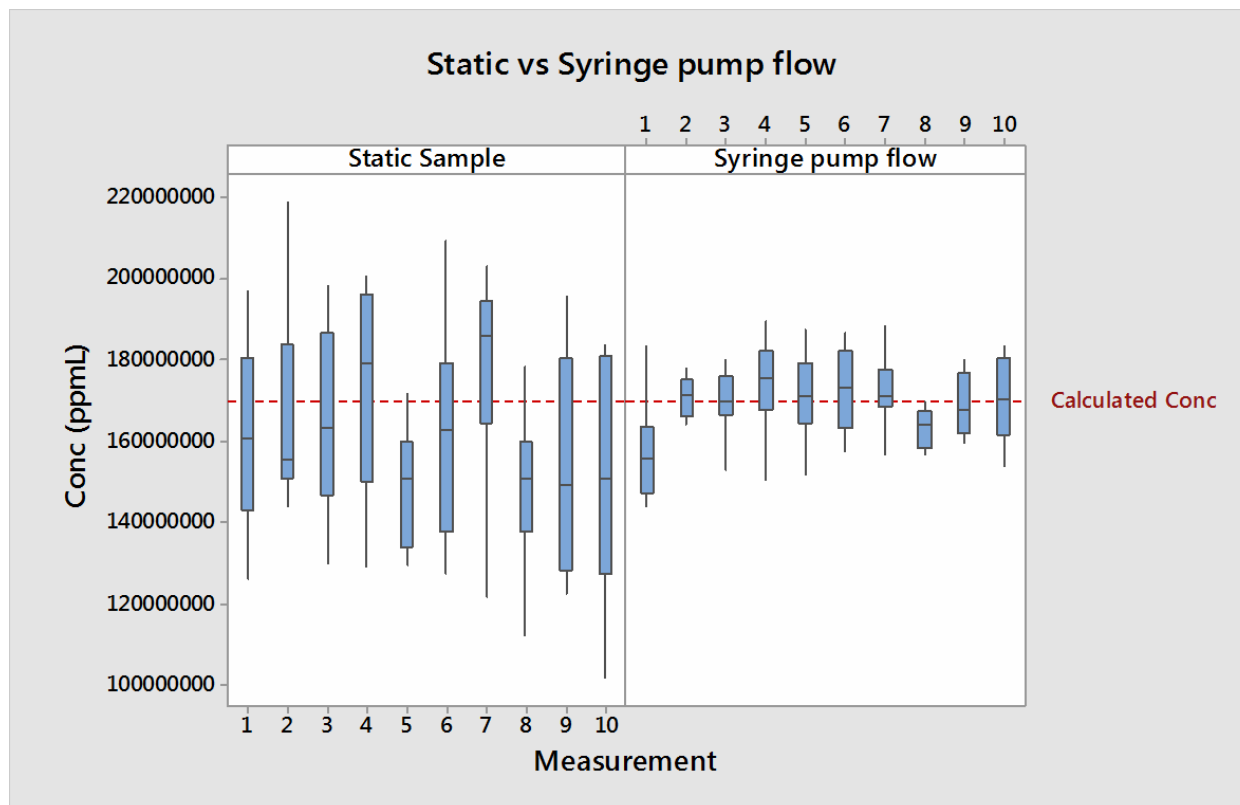
CHOOSING VIDEO LENGTH FOR MEASUREMENT



- Select a measurement duration that will allow sufficient particles to be measured for representative and reproducible results
- Rule of thumb: at least 500 valid tracks per video
- At optimum concentrations, 30 seconds may be sufficient for narrow size distributions, 60 seconds for polydisperse
- Recommended:
 - *3 x 30 sec videos for a quick check of monodisperse samples in appropriate concentration range*
 - *5 x 30-60 sec videos for most samples and better reproducibility. Minimum for making comparisons between samples (standard measurement).*
 - *7 x 60-90 sec videos for dilute and/or very polydisperse samples*

FLOW MODE

- Flow yields more particles analyzed per video length
- Improved reproducibility due to better statistics
- Minimizes photo bleaching in fluorescent mode
- Automates SOP sample advancing



SYRINGE PUMP



- Add-on compatible with all NTA systems
- **Strongly recommended** for all fluorescence work
- Constantly flow allows fresh sample to be continuously introduced to sample chamber
- Recommended flow rates (5-10 seconds for particle to travel across field of view)

Laser Module Top Plate Style	Recommended Flow Rate (AU)
LM10-T14 (LM14)	50-80
LM10 (LM12)	20-50
NS500	20-50

SAMPLE SCRIPT FOR SYRINGE PUMP ANALYSIS



- Step 1: Load sample, adjust camera and focus settings
- Step 2: Advance syringe pump on flow 1000 until particles move, then slow down flow to recommended rates (previous slide)
- Step 3: Run script (can be automatically generated in software):

CAMERASETTINGMSG

SYRINGELOAD 30

DELAY 5

REPEATSTART

CAPTURE 60

DELAY 1

REPEAT 4

SYRINGESTOP

PROCESSSINGLESETTING

EXPORTRESULTS

HIGH CONCENTRATION PROTEIN ANALYSIS



- A 200 mg/mL mAb formulation can be a challenging sample to run on NanoSight
- Sample handling
 - *Proteins can easily foam at air interfaces in syringes*
 - *Avoid introducing bubbles*
 - *Bubble removal more difficult due to high viscosity*
- High scattering background
 - *100 nm PSL can be detected on camera level 8/9 in water*
 - *Scattering background for 200 mg/mL mAb formulation might oversaturate camera on camera level 5/6*
 - *Due to scattering S/N, lower sizing limit for protein aggregates in 200 mg/mL protein solutions might be higher than normal*

HIGH CONCENTRATION PROTEIN ANALYSIS



- Need to know sample viscosity
 - *The hydrodynamic size is inversely proportional to viscosity (see Stokes-Einstein equation)*
 - *NTA software is defaulted to water/PBS temperature curve. Operating viscosity limits: 1-3 cP.*
- Sample dilution may be required
- If aggregates dissociate upon dilution, do they pose immunogenic risk?

$$\langle r_{MSD} \rangle = \frac{4K_B T t_s}{3\pi\eta d_h}$$

Stokes-Einstein equation

K_B = Boltzmann Constant

T = temperature

t_s = sampling time

η = viscosity

d_h = hydrodynamic diameter

HIGH CONCENTRATION PROTEIN ANALYSIS

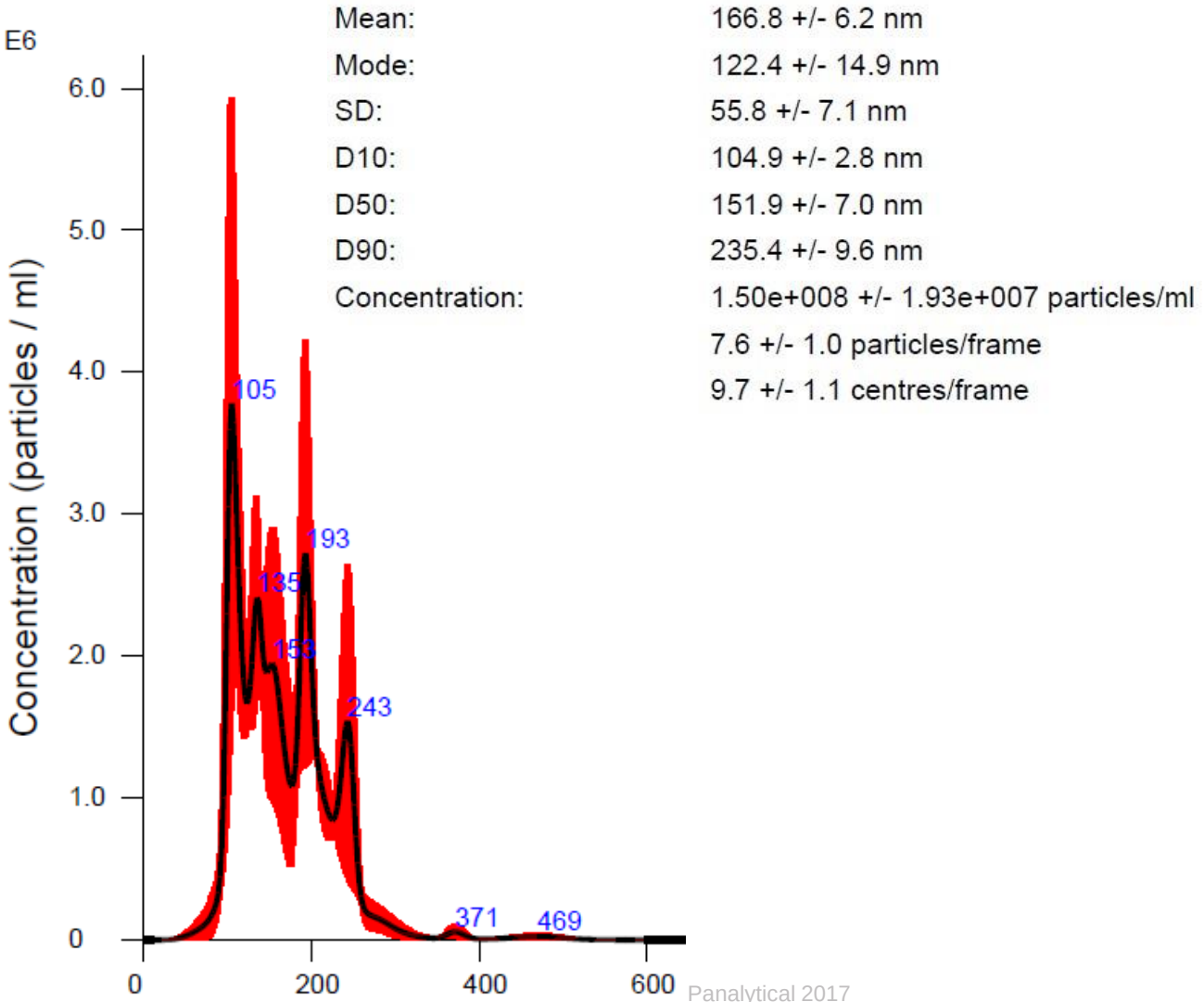
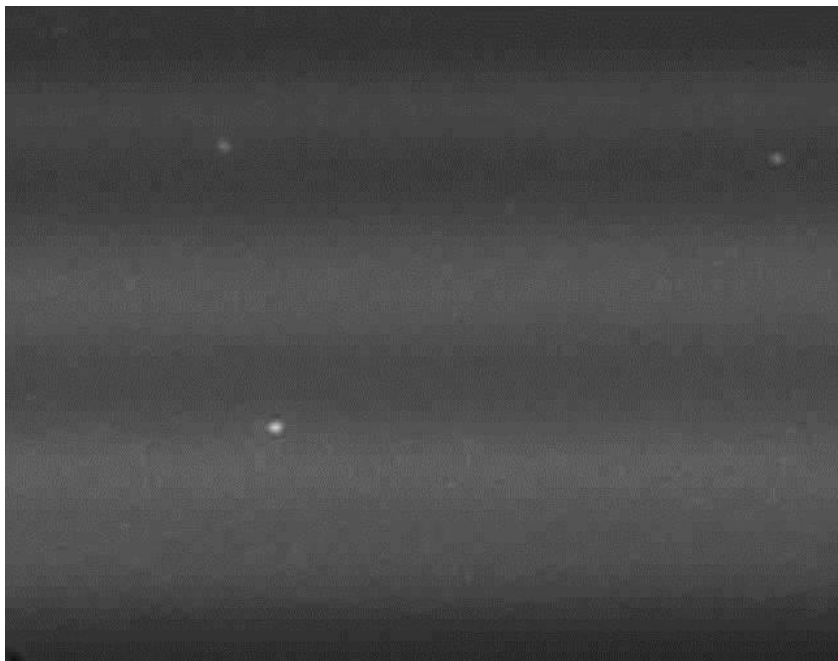


- Additional flushing and cleaning
 - *High concentration protein samples can be difficult to flush out from system.*
 - *May need multiple rounds of flushes with buffer, detergent, buffer, water, air.*
 - *Never introduce proteins directly to water or organic solvents.*
- Flow cell top plate might not be ideal.
 - *Tubing represents larger surface area, more likelihood of sample carryover.*
 - *Flow cell gasket is fragile, may not last as long with aggressive flushing and cleaning required to remove sample*
- O-ring top plate may be preferred
 - *Bypasses fluidics and is more robust, compatible with aggressive cleaning*
 - *Windex, aggressive detergents, organic solvents.*
 - *Can soak overnight in cleaning solution (without O-ring itself)*



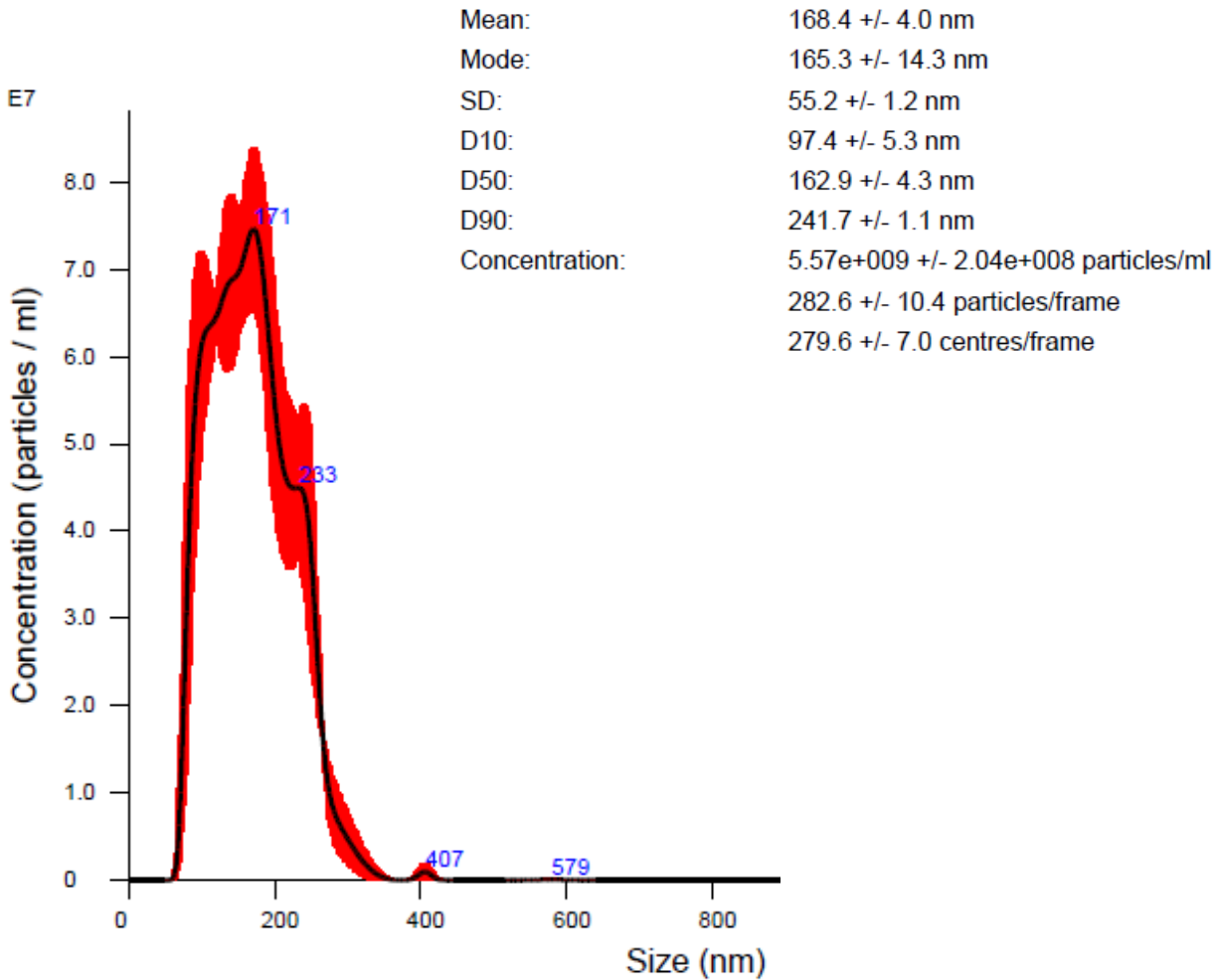
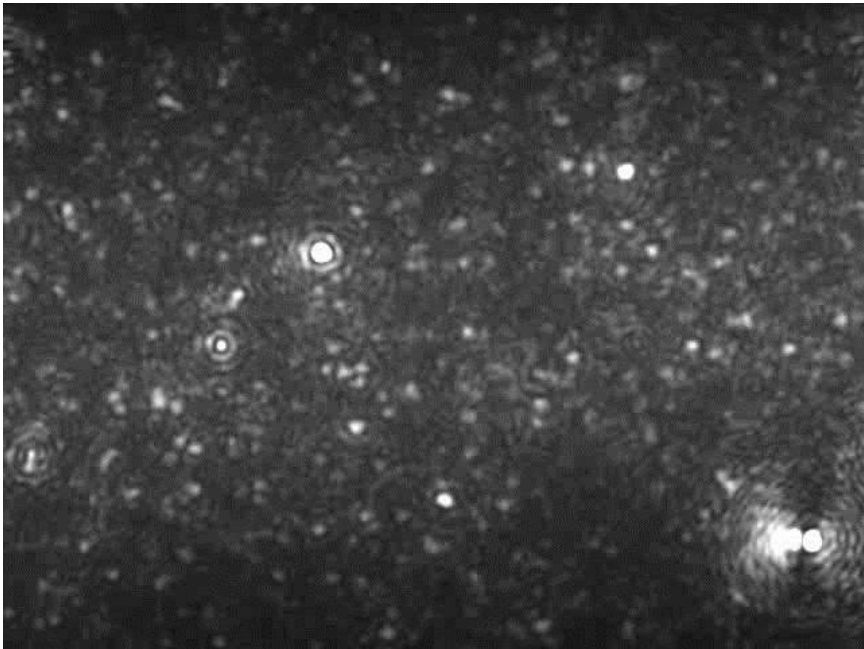
EXAMPLE 5: mAb Protein A

10 mg/mL – good particle count



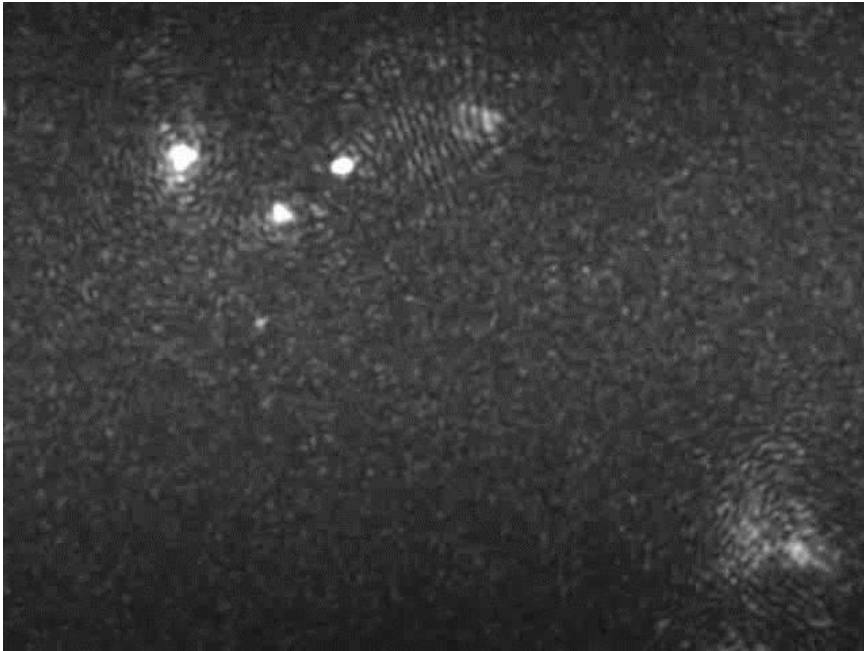
EXAMPLE 6: mAb Protein B

5 mg/mL – high particle count

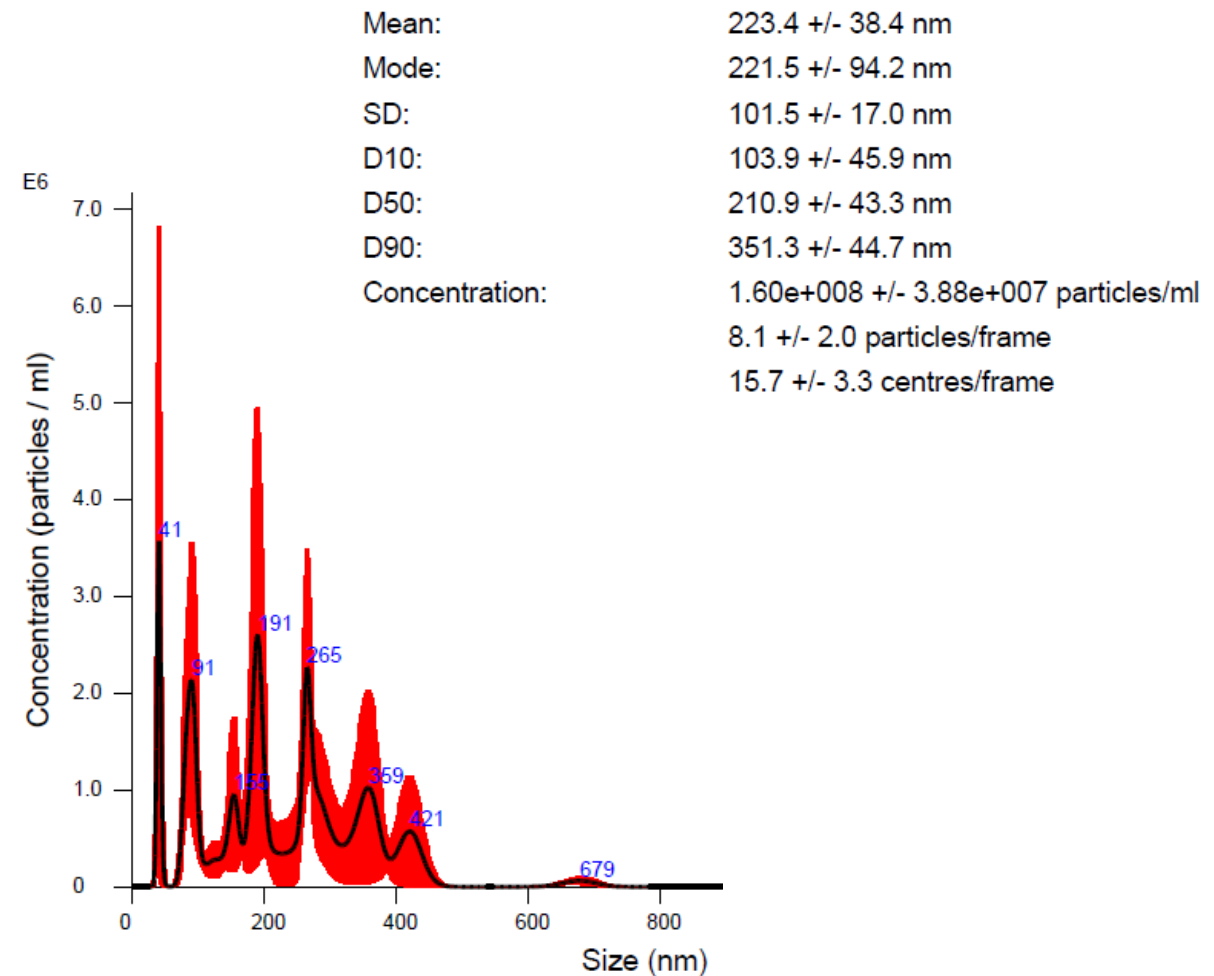


EXAMPLE 7: mAb Protein C

25 mg/mL – low particle count



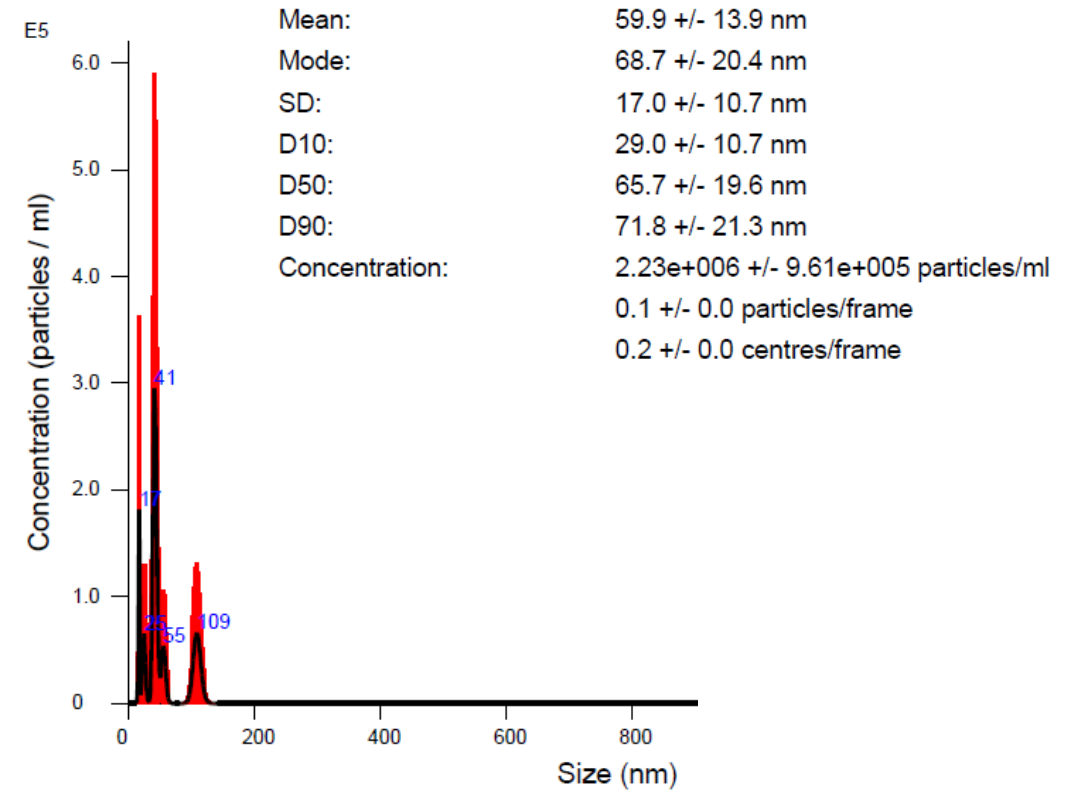
Higher background scattering, only larger particles are visible.



EXAMPLE 8: mAb Protein D



80 mg/mL – low particle count



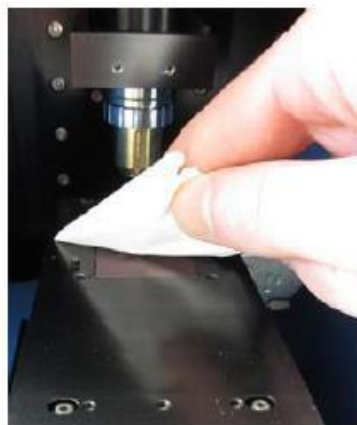
- Very high background signal (camera level 3)
- Low particle count.
- Are particles masked by presence of protein scattering background?
- To test, spike sample with 100 nm, 200 nm, 400 nm PSL.

FLOW-CELL TOP PLATE

Flow-cell top plate



Flush water/diluent.
Extract the liquid in
the sample chamber.

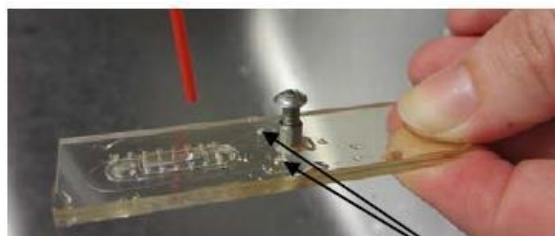


Wipe with **water** or **water** then
ethanol. Dry with **compressed air**.

**Do not pour any liquid over
the laser module.**



Loosen the fixing bolt and slide out the
gasket component.
Rinse with **water**, or **water** than **up to
10% ethanol**.



a) Dry channel thoroughly through the holes on the
top of the gasket component

Dry with compressed air



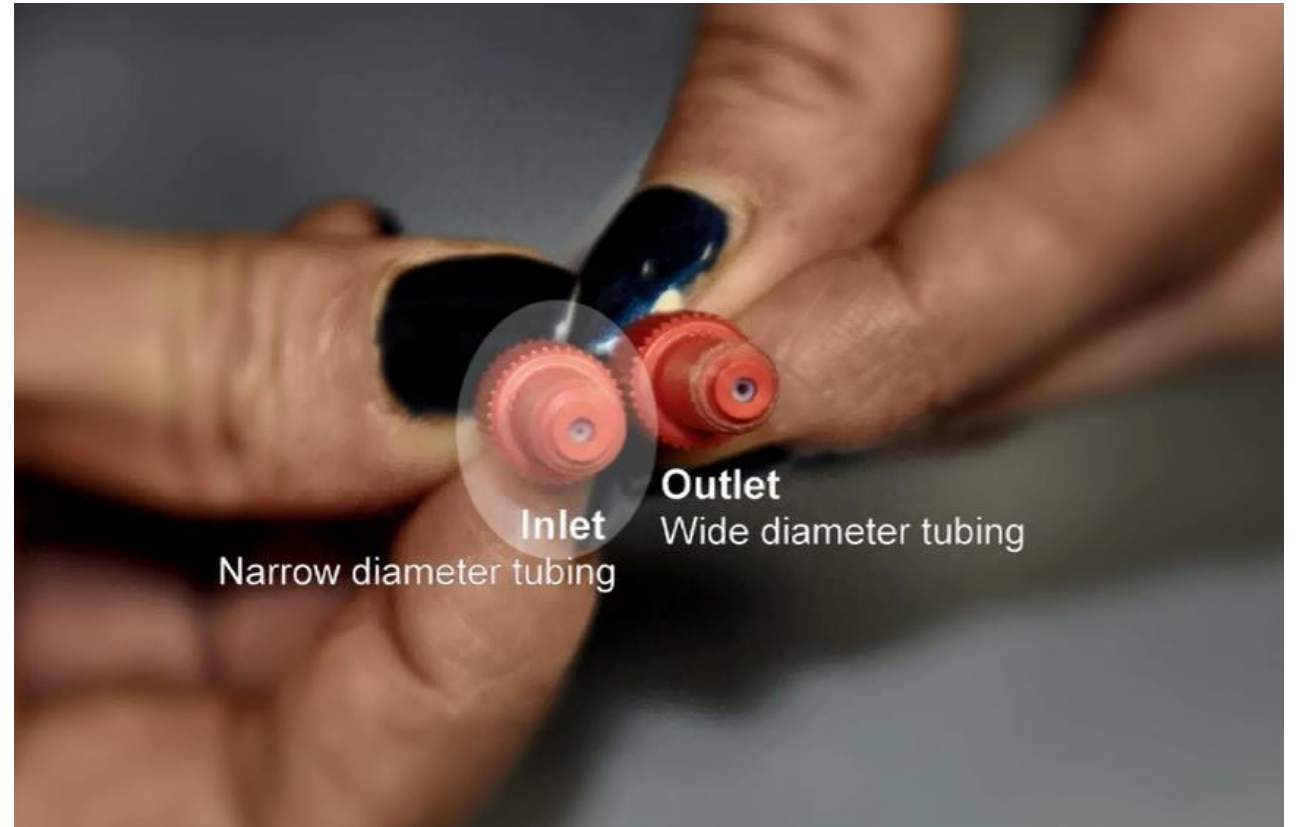
b) Dry underside of the gasket component, keeping the
air duster nozzle away from the seal

© Malvern Panalytical 2017

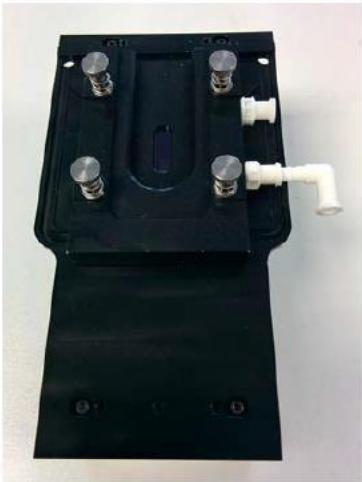
NanoSight NS300 operating manual

CONNECT THE TUBING

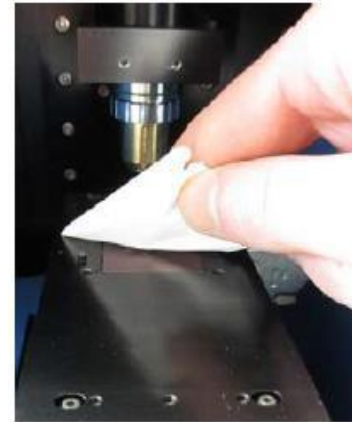
- Prevent back pressure
 - *Inlet tubing = smaller inner diameter (more opaque color)*
 - *Outlet tubing = larger inner diameter (more transparent)*
- First insert connector with thread pointing to the end of the tubing
- Second insert ferrule with wider side pointing to the end of the tubing



O-RING (METAL) TOP PLATE



Wipe with water
or ethanol



Dry with **compressed
air**.



Clean with **water** or **water**
then **ethanol**.



**Do not pour any liquid
over the laser module.**

Rinse the ports and dry with compressed air.

NanoSight NS300 operating manual

EXAMPLE CLEANING PROTOCOL



- At end of experiment
 - *Flush with buffer, PBS, water, then push air through to dry.*
 - *Detergent may be required for high concentration protein samples.*
- For decontamination
 - *Only required for hazardous materials*
 - *Flush with 10-20% bleach or ethanol for 1 minute*
 - *Immediately followed with several mL of water to rinse*
- At the end of day
 - *Once dry, loosen screws ½ turn to loosen gasket pressure on top plate*
 - *No need to disconnect tubing or remove the top plate*
 - *Closed down software then turn off instrument*

WHAT TO AVOID...



- Injecting large air bubbles
 - *Can add background noise and cause sample drift (flow is no longer horizontal)*
 - *Remove laser module, place on end, inject more diluent to remove sample, then reinsert laser module*
- Vibrations
 - *Adds energy into sample, size results will be under-reported*
 - *Improved vibration correction in NTA 3.2*
 - *Use instrument on steady bench (if not possible, use anti-vibration mats)*
 - *Avoid using next to hood fans and centrifuges*
- Avoid non-continuous flow
 - *Some syringes will pulse as they skip forward – apply more grease*
 - *Some surfactants and aqueous solutions can change wetting properties of syringe pump, and cause it to skip forward*
 - *Use 1mL norm-ject syringes*

FOR COMPARING A SERIES OF SAMPLES



- Optimize capture and analysis settings for every sample
 - *If there are significant changes in particle size, polydispersity, or concentration the ideal settings will likely change*
 - *Sufficiently similar samples can be analyzed with the same settings.*

HOW TO DEAL WITH LARGE PARTICLES



- Don't worry, they won't clog the instrument
- If you have huge particles (aggregates, cell debris, etc.)
 - *Multi-micron particles (sub-visible)*
 - *Will oversaturate camera*
 - *Will not be tracked – too large to properly focus on and won't move far enough between frames*
 - *Could mask the presence of smaller particles*
 - *If scattering corona generates a large amount of invalid tracks, consider disregarding that measurement file*
 - *To remove large particles: filter, spin down, or dilute sample if possible*
- Malvern technology for characterizing sub-visible particles:
 - *Archimedes (RMM)*
 - *Morphologi 4, G3, G3-ID (Automated imaging)*
 - *Mastersizer 3000 (Diffraction)*

WHAT TO WATCH FOR...



- A sample that is too concentrated (> 100 particles/frame)
 - *Dilute sample*
 - *Reduce camera level (smaller particles may be missed)*
- A sample that is too dilute (< 5 particles/frame)
 - *Concentrate sample*
 - *Use syringe pump flow mode*
 - *Record longer videos*
- Particles or background that is too bright
 - *Decrease camera level, dilute sample, or use neutral density filter*
- Blurry particles or large diffraction rings
 - *Adjust focus*
- Too many red or blue crosses
 - *Increase detection threshold*
- Particles that photo bleach too quickly
 - *Use syringe pump and increase flow rate or modify labelling conditions*

THANK YOU FOR ATTENTION!

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