Technical Note

How to make Concentration Measurements using NanoSight LM Series Instruments

Last updated 17/06/09

Introduction

NanoSight LM series allow the user to estimate the concentration of the nanoparticles tracked by the system. This technical note explains how to get the best results from the system.

Methodology

The technique has been calibrated internally and was based on measurements on monodispersed latex size calibration particles from Duke Scientific.

The concentration depends on the following factors:

1) Base sample concentration. Samples are frequently too concentrated for analysis directly by the NanoSight technique and will therefore need some dilution before analysis.
2) Dilution. The sample must be diluted accurately, the dilution factor noted and the dilution factor must be suitable (see below).
3) Cleaning method. It is imperative that there is no carry-over of sample and hence a rigorous cleaning procedure must be followed.
4) Capture settings. The camera capture settings of the camera must be suitable.
5) Analysis setting. The analysis settings of the recorded video must be suitable.
6) Time of analysis. The time of analysis must be sufficient.

Initial assessment

It is likely that your sample will need diluting in order to bring it down to a suitable concentration for the NanoSight technique to assess.

Firstly assess your sample by eye. In a standard 5ml clear bottle, if the sample it is not totally transparent it is probably too concentrated. If this is the

case dilute 100x and reassess. When the sample appears transparent or very near transparent you can assess using NTA.

Cleanliness of chamber

It is necessary to check that the sample chamber is clean prior to a measurement. The best way to do this is to check that the solvent which is being used to dilute the sample is totally free from nanoparticles.

To get optimum results, the instrument should be cleaned prior to every concentration analysis. To check the effectiveness of cleaning and the cleanliness of the solvent, regularly inject just the solvent into the sample chamber. Observing close to the thumbprint, which should still be visible due to imperfections in the glass surface (note, this is not due to particles stuck to the glass surface), there should be no particles (identifiable by their motion). If this is not the case then either the solvent is contaminated or cleaning is insufficient. With many solvents it will be hard to remove all nanoparticles and one or two nanoparticles in the field of view is typical. Clearly this will alter the accuracy of the concentration measurement for lower concentrations more significantly than higher concentrations.

Cleaning the instrument with water may not be sufficient as some particles may remain stuck in the chamber or be stuck to the glass. Cleaning instrument with ethanol (or a water-ethanol mix) will help to get reliable results. A common cleaning issue is omitting to flush through the luer ports to remove sample held in ‘dead’ volume within.

Correct dilution factor

In order to back calculate base sample concentration, it is necessary to know the dilution factor that has been applied. A suitable dilution factor depends on base sample concentration and on particle size. Usually it is necessary to make several dilutions before the sample is sufficiently dilute. In general it is better not to dilute by a factor of greater than 200 at any time. If a greater dilution is required, this should be carried out by serial dilution, i.e. diluting an already diluted sample, again.

Correct imaging position

It is necessary to be interrogating the correct position of the laser beam. This should be as close to the ‘thumbprint’ as possible (and not more than three field of views displaced from this point) whilst not observing the significant scattering from the thumbprint itself. The beam should fill or overfill screen (i.e. particles should be illuminated both at the left edge and at the right edge of the laser beam (top and bottom of screen). Focus should be set so that, under low camera capture and gain settings, in focus particles can be seen only in that position (and refocusing to different heights would require an increase in capture settings to see any particles).

Assessing dilution factor

The best method is to assess the image by eye. If either:

1) there are many particles in the image (>120),
2) the particles are observed very close together or
3) the background scatter from particles (i.e. flickering as on an untuned television) is significant.

then the ssample is too concentrated to measure all the particles in the system. If point 1) is hard to assess then it is possible to get NTA to count the number of particles. To do this:

a) record a short (1s) video.
b) Open the video for pre preprocessing.
c) Set suitable analysis settings.
d) In the bottom left of the screen there is a count of the number of potential particles identified. (see figure 1)

For too concentrated samples the analysis will give a concentration lower than it should. This is because as particles come close there is an ambiguity about how to track them. Therefore both particles are discounted by the software. For the same reason the particle size would also tend to be too low.

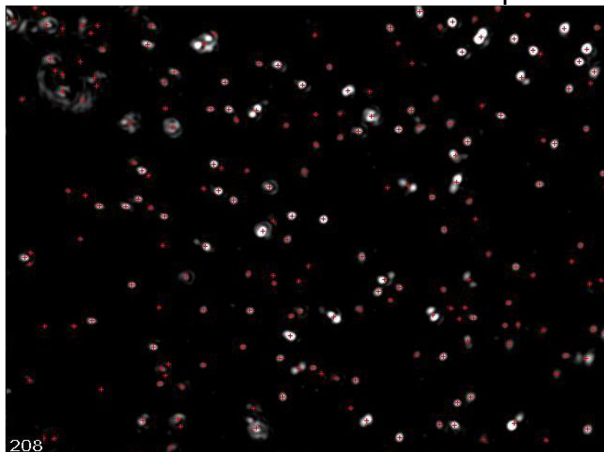


Figure 1. Too high concentration, 208 particles identified.

When there are very few particles (less than 10) then statistically there are not enough particles to track (see figure 2). The sample is too low concentrated and a less dilute sample should be assessed. For very low concentrated (<5*10^7 particles per ml) base samples, analysis will need to be carried out for a very long time to get reasonable results and is not recommended.

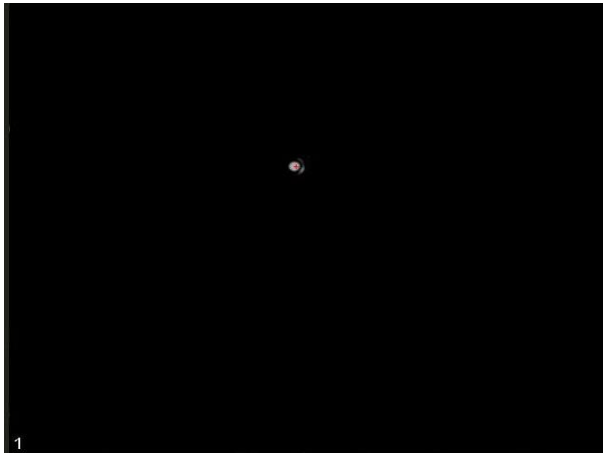


Figure 2.Low concentration, 1 particle identified.

If the number of particles on the screen is approximately about 20-60 this is an ideal concentration to analyse a sample (figure 3).

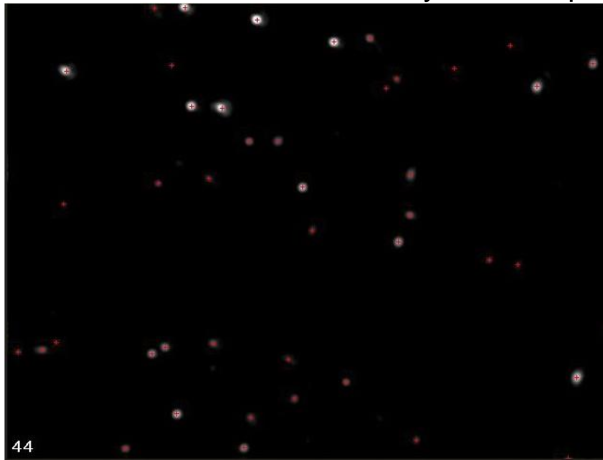


Figure 3.Good concentration, 44 particles identified.

Ideal concentration

Good concentrations readings should be between 1*10^8 and 25*10^8 particles/ml.
However for individual sample this range may differ.

Capture Settings

Gain and Shutter

To see all particles set the shutter and gain to maximum. Slowly reduce shutter keeping an eye on the dimmest particles until they start to disappear then increase the shutter a little. Next carefully decrease gain but only when you see large, saturated blobs. Remember not to lose any particles.

Record time

We recommend recording for 60 sec or longer to get a stable and realistic concentration.

It was found that the concentration decreases with time especially for small particles and highly concentrated samples. The most reliable concentration results were therefore those started being recorded within the first minute after injection. After this time the particles tend to settle down or adhere to the glass. Injecting more sample (about 0.1ml) to refresh the particles in the field of view may help to recover the original concentration. However, this usually works only when sample is left no longer then 30 - 45 minutes.

Analysis Settings

1) Set the Detection Threshold to 192.
2) Set Blur to 5x5.
3) Increase Brightness to zero and Gain to high to see all particles.
4) Slowly reduce Brightness if there is a lot of noise on the background or very saturated particles; keep an eye on the dimmest particles all the time. The aim is to get a black background.
5) Reduce gain until you start loosing particles.
6) Set Detection Threshold to auto.
7) Adjust Gain to get a cross on every particle.
8) If there is a lot of crosses due to large, saturated particles or from diffraction rings you can increase blur, this may also be an indication that the sample is polydispersed and not suitable for analysis.
9) After changing Blur the Brightness and Gain must be set again (step 2).

Generally set up as clean an image as you can without losing particles ensuring centres on all particles.

Additional advice

You may wish to repeat the analysis 2-3 times to make sure your measurement results are repeatable but note the settling issue (see record time).

When you are recording the same sample several times, don’t change capture and analysis setting significantly as it can give different concentration results.

Measurement accuracy

In the absence of suitable traceable standards, the Nanosight device has been calibrated using latex particles from Duke Scientific. These are given as ~1% by weight. This allows a calculation of the stock concentration and this is the basis for our calibration.

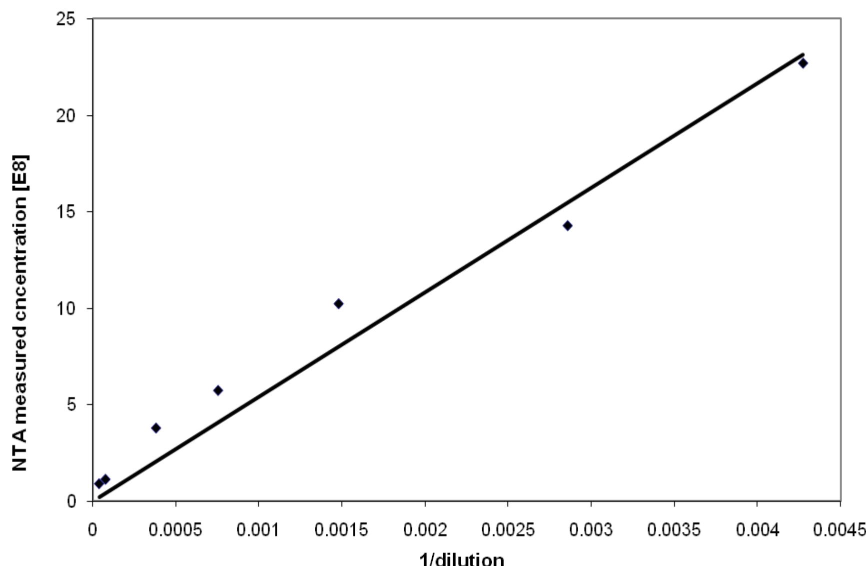
The first dilution for standard latex samples will depend on particle size (clearly smaller standard particles require higher dilution factors than the larger sizes). The second dilution depends on concentration required. Example dilutions for Duke Scientific standard particle size (1%wt) are shown in the table 1 below.

Particle size	1st dilution (in 5ml water)	2nd dilution (in 5ml water)	Total dilution	Concentration
100nm	0.05ml latex	0.01ml latex	50601	~3.9*10^8
200nm	0.1ml latex	0.05ml latex	5151	~4.7*10^8
300nm	0.1 ml latex	0.1 ml latex	2601	~2.6*10^8
400nm	0.2 ml latex	0.1 ml latex	1326	~2.1*10^8

Table 1: Typical dilutions required for polystyrene latex standards.

Typical results for two of these standards are given below (figure 4) to demonstrate the accuracy of the system. The following points can be noted:

1) The measurement is approximately linear over a dilution range of a factor of 10.
2) The largest/average deviations from the proportional linear fit for these samples are 45%/21% for 400nm and 50%/20% for the 200nm particles.
3) The proportional error increases with decreasing concentration and so the larger errors were seen at the greater dilution factors.
4) These measurements are carried out on spherical monodisperse particles, and hence are the most simple to visualise and track.
5) The system has been calibrated to 100nm.



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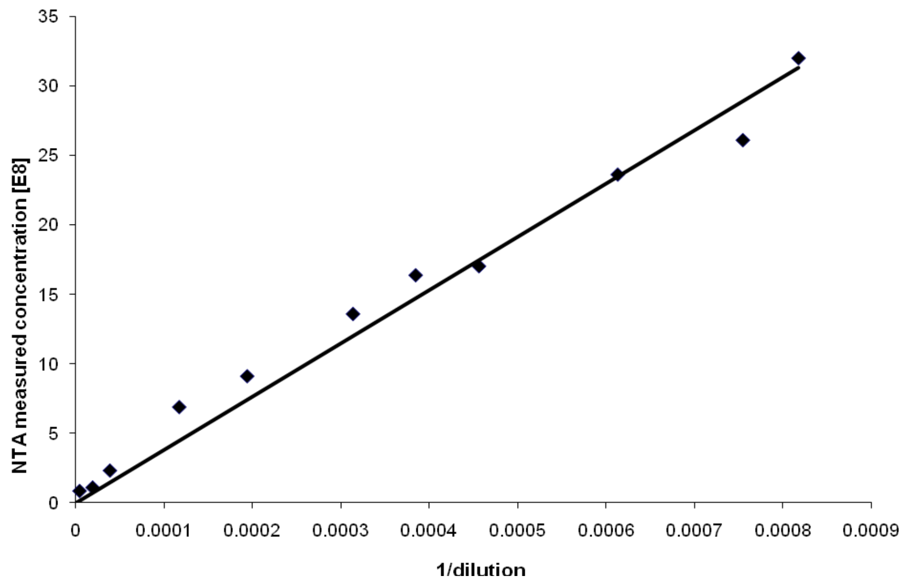

Figure 4. Sample dilution against NTA measured concentration for a) 400nm and b) 200nm Duke Scientific standard particles.