

Analysis of 25 nm functionalized QDs using fluorescence-NTA with ZetaView® QUATT system

While it is uncertain how far the NTA community is able to push the limits of current technology in order to detect the smallest possible nanoparticles, the results of analyzing 25 nm quantum dots functionalized with streptavidin (S-QDs) show that the ZetaView® QUATT system from Particle Metrix is the leader in fluorescence detection capabilities using NTA methods.

Vendor Sample ID: Invitrogen™ Qdot™ 655 streptavidin conjugates

Scatter mode issues with aggregates.

The first phase of these experiments involved setting up a reasonably robust SOP for detecting the 25 nm S-QDs in scatter mode. It quickly became apparent that the number of large particles present was quite substantial, and that the tracking efficiency of building a particle size distribution (PSD) to include S-QDs would be poor, due to an abundant level of scatter noise (results not shown). Instead, the camera settings & tracking parameters were decreased in order to tune out the smaller S-QDs, preferentially measuring the larger ones.

Fluorescence detection yields a clean data set.

The properties of Invitrogen™ Qdot™ 655 streptavidin fit very well using a 405 nm laser & 430 nm high-pass filter from our QUATT system; an SOP was quickly and easily determined by starting with parameters from a scatter SOP for S-QDs, then adjusting the camera, followed by using our low-bleach method to reduce laser exposure to the sample. Bleaching was not an issue, yet data acquisition was still improved with our low bleach function. This SOP is robust, meaning the results did not differ significantly with minor adjustment of tracking parameters.

The PSD from fluorescence-NTA analysis turned out quite clean (i.e., low noise), as compared with the

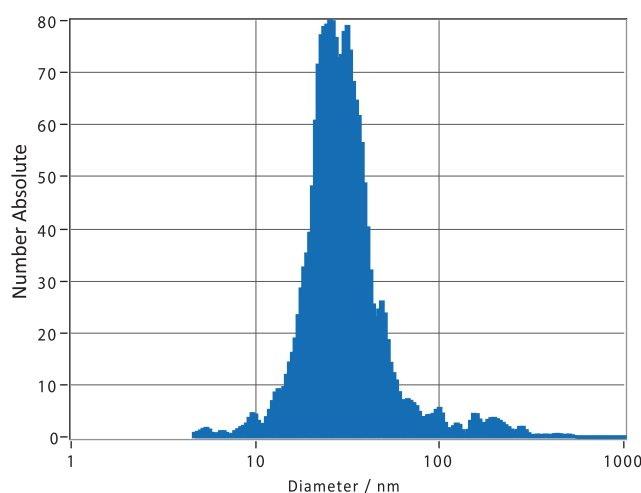
scatter mode analysis, where the larger particles do not appear in fluorescence mode. These factors indicate the two detection modes are measuring two different populations of particles, as shown in an overlay of both PSDs. Clearly, we see different size profiles from these two analyses of one single sample volume. Notably, the number concentration of the larger, non-fluorescent particles in scatter mode is almost as abundant as the S-QDs from f-NTA mode.

Conclusions:

Size and concentration measurements:

1. Polymeric QDs as small as 25 nm can be selectively analyzed with fluorescence NTA detection using the ZetaView QUATT system, effectively filtering out the measurement of larger interfering particles in scatter mode;
2. Low-bleach capabilities can be used even if bleaching is not present, while nonetheless improving data collection;
3. Sometimes, fluorescence detection is the preferred mode over scatter mode, even when the experiment does not involve analysis of biological particles.

Strep-coated QDs, 25.1 & 31.1 nm size modes, 405 nm fluorescence-NTA



Strep-coated QDs, overlay of 405 nm f-NTA (green) & scatter mode data

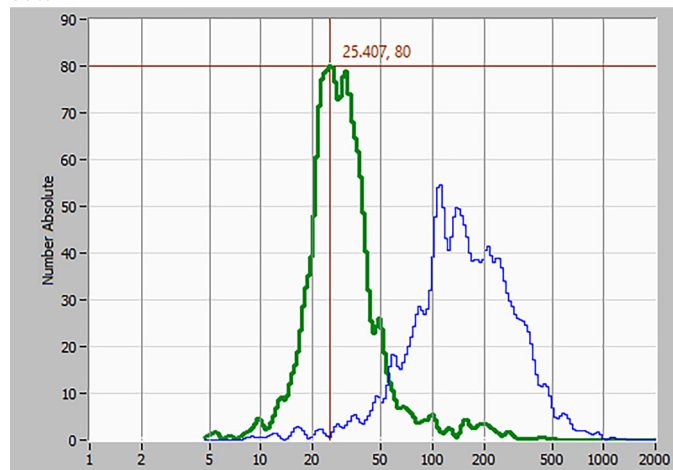
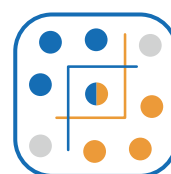
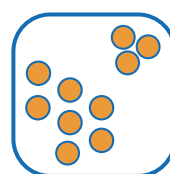
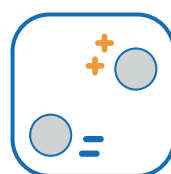
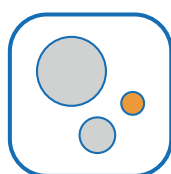
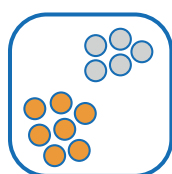


Table of SOPs & Results: 405 nm fluorescence & 488 nm scatter modes

Parameters, Data Result	405 f-NTA	488 Scatter
Detection	fluorescence	scatter
# Cycles	2	2
Dilution	1,000,000x	1,000,000x
Camera Settings:		
Sensitivity	89	82
Shutter	200	125
Frame Rate	60 fps	30
Tracking Parameters:		
Min Brightness	17	20
Max Area	1000 pixels	1000
Min Area	8 pixels	10
Tracelength	7 jumps	7
Low Bleach?	Yes	N/A
Pump Advance?	No	N/A
Data Results:		
Size Mode (s)	25 & 31 nm	110 & 211 nm
X50	28.1 nm	146.3 nm
Span	1,2	1,9
# traced particles	2063	2113
Ave # / frame	165	86
Concentration	5.5x10 ¹³ /ml	2.9x10 ¹³ / ml

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