APPLICATION NOTE

MICROTRAC MRB
PARTICLE CHARACTERIZATION
part of VERDER scientific

Measurement of very small sample volume (2 µl) with Nanotrac Flex

INTRODUCTION

Very often it happens that there is not enough material for analytical questions because the synthesis of the material is very time consuming or very expensive. The Microtrac Dynamic Light Scattering (DLS) instrument Nanotrac Flex is perfectly suitable for measuring the particle size in very low sample volume (Figure 1). One other big advantage is that measurements can be performed at original high concentrations to avoid effects of dilution on the dispersion. This can be used for samples like therapeutic drugs, proteins, polymer solutions and others.

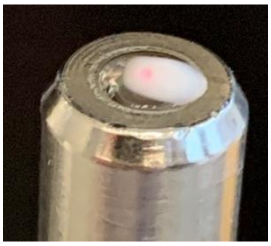


Figure 1: 2µl sample on the tip of the Nanotrac probe

DYNAMIC LIGHT SCATTERING

Microtrac MRB’s NANOTRAC Flex is a highly versatile Dynamic Light Scattering (DLS) analyzer that provides information on particle size, concentration, and molecular weight. The innovative design of the NANOTRAC Flex provides fast measurements with reliable technology, highest precision, and excellent accuracy. The unique external probe design allows to convert nearly every vessel into a measurement cell (Figure 2). It can also measure of samples over a wide concentration range, both monomodal and multimodal dispersions, all without prior knowledge of the particle size distribution.

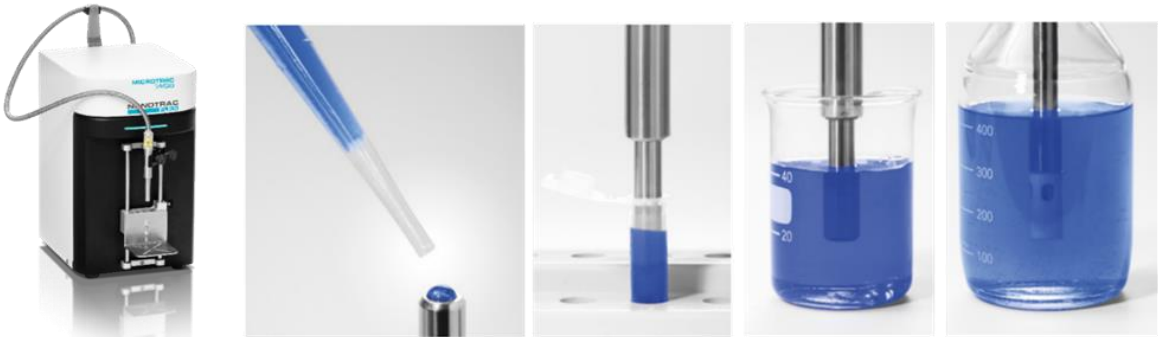


Figure 2: The dip-in probe of the Nanotrac FLEX allows direct measurements in vessel: beaker, bottle, Eppendorf tube, or even a single drop of liquid.

180° DYNAMIC LIGHT SCATTERING

Nanoparticles suspended in a liquid dispersion are subject to Brownian motion, which is a result of random collisions of molecules in the liquid medium. The particles' velocity distribution, averaged over time, approaches a known functional form – their size distribution. Dynamic Light Scattering (DLS) is the technology used to calculate that size distribution, based on the particles' measured velocity distribution.

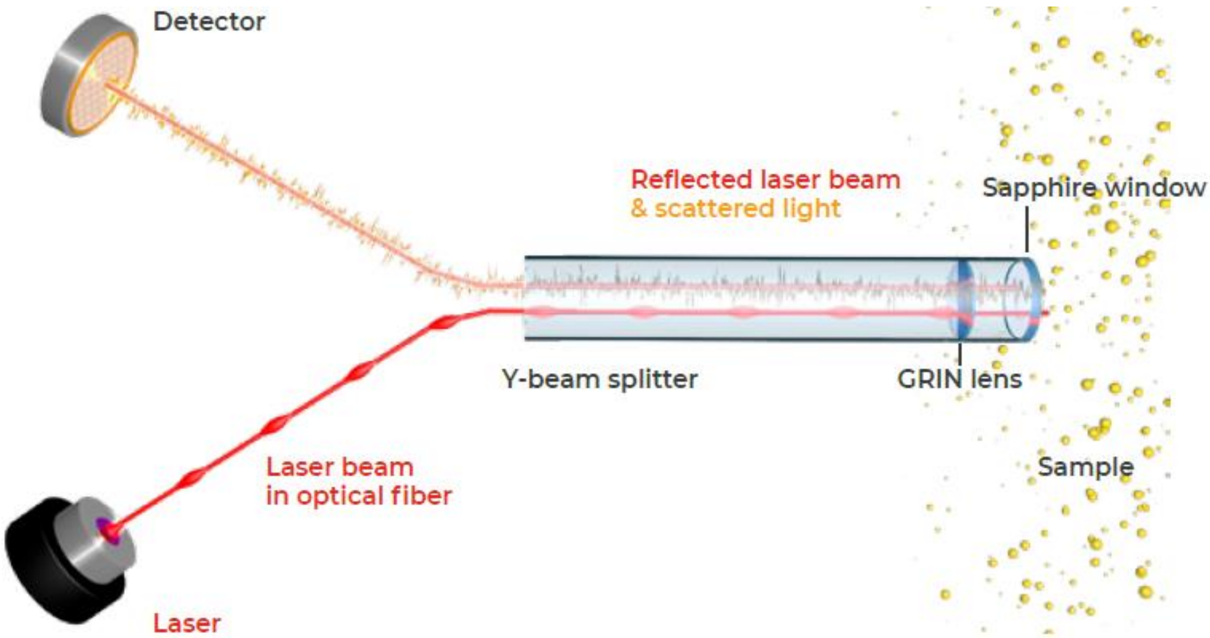


Figure 3: Probe design used in Microtrac DLS analyzers

The optical bench of the Nanotrac Flex is a probe containing an optical fiber coupler with a Y splitter (Figure 3). Laser light is focused on a volume of sample close to the interface of the probe window and the dispersion. The high reflectivity sapphire window reflects a portion of the laser beam back to a photodiode detector. The laser light also penetrates the dispersion and the particles' scattered light reflects at 180 degrees back to the same detector. The scattered light from the sample has a low optical signal relative to the reflected laser beam. The reflected laser beam mixes with the scattered light from the sample, adding the high amplitude of the laser beam to the low amplitude of the raw scatter signal. This Laser Amplified Detection method provides up to 10^6 of times better signal to noise ratio of other DLS methods like Photon Correlation Spectroscopy (PCS) and Nanoparticle Tracking Analysis (NTA), Figure 4.

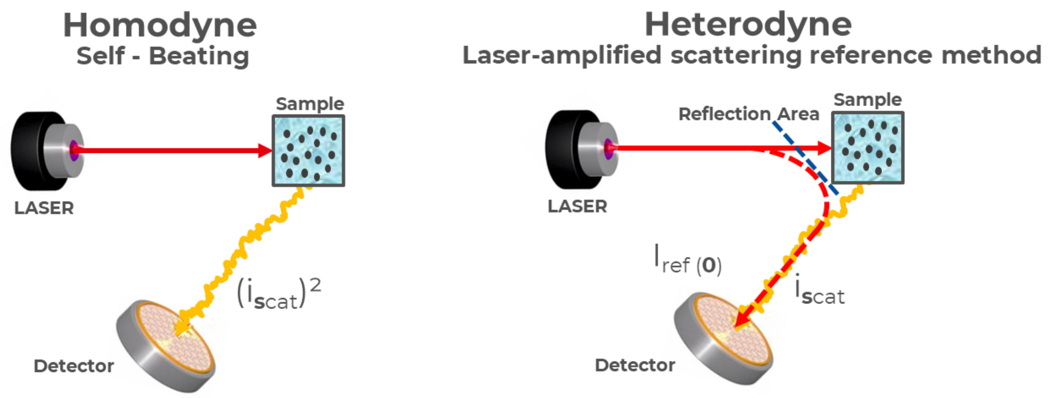


Figure 4: Homodyne and heterodyne approach in DLS. The heterodyne setup used by instruments of the Nanotrac series provides significantly better signal to noise ratio.

A Fast Fourier Transform (FFT) of the laser amplified detection signal results in a linear frequency power spectrum which is then transformed to a logarithmic scale and deconvoluted to give the resulting particle size distribution (Figure 5). Combined with laser amplified detection, this frequency power spectrum provides robust calculation of all types of particle size distributions – narrow, broad, mono- or multi-modal – with no need for a priori information for algorithm fitting as in PCS.

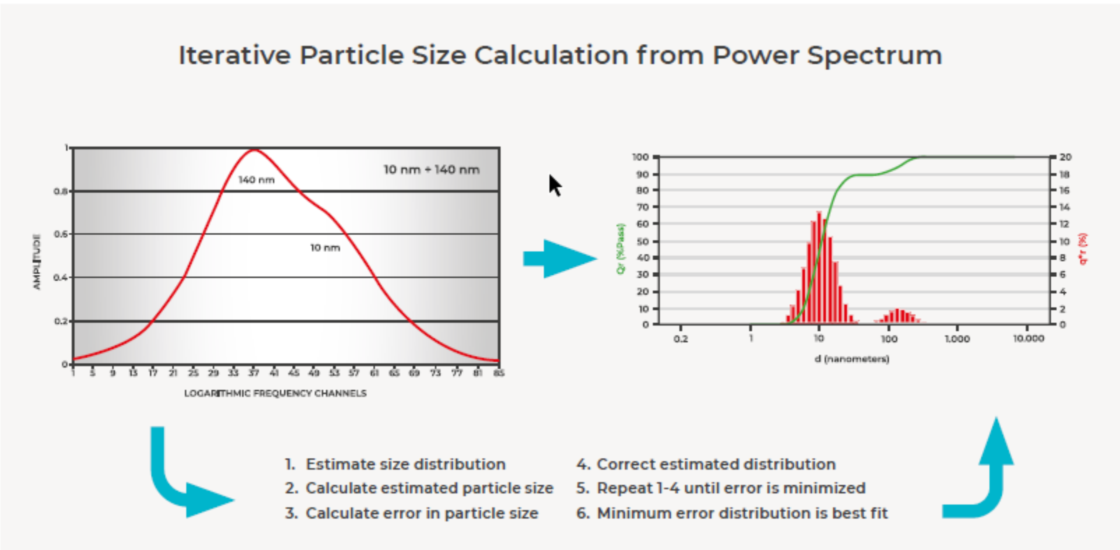


Figure 5: Evaluation of DLS measurement with Fast Fourier Transformation and Frequency-Power-Spectrum method.

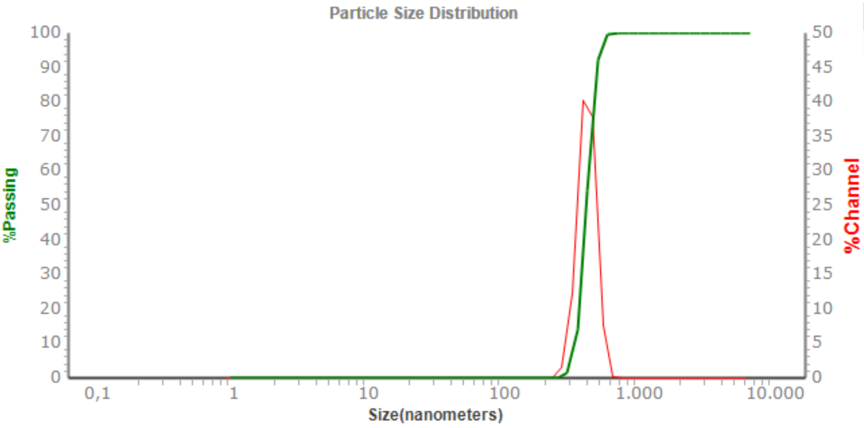
SAMPLE PREPARATION

For testing the minimum volume, a 5 µL drop and a 2 µL drop were used (Figure 6). The probe of the Nanotrac Flex was used upside-down to place the drop with the pipette in the middle of the probe. As a sample a 400 nm polystyrene with a tolerance of ± 10 nm was used in original concentration without dilution. Additionally, the sample was diluted strongly with DI water and a 2 µL was used for the measurement.

Figure 6:The sample is directly applied to the tip of the upside-down probe. From left to right: 5 µl undiluted sample, 2 µl undiluted sample, 2 µm highly diluted sample.

RESULTS

The following graphs show the results of the different measurement of the 400 nm standard with different sample quantity volumes and dilution. As a reference also one sample was measured in the classical way of dipping the probe into the sample.

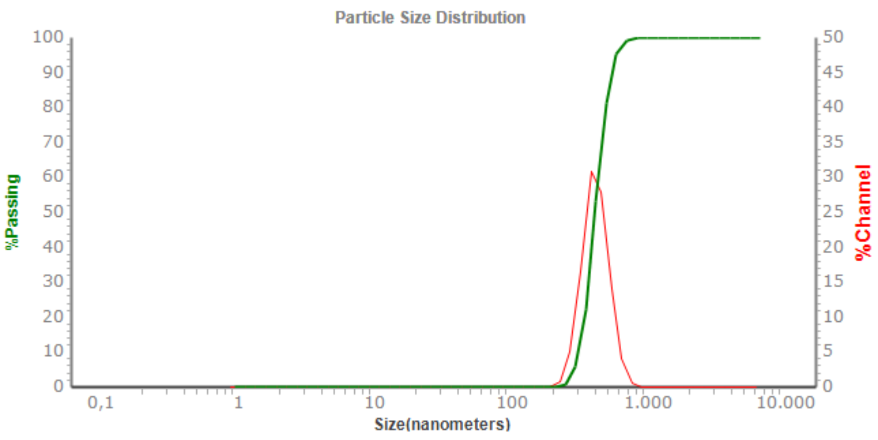


Summary	
Data	Value
MI(nm):	405,0
MN(nm):	380,0
MA(nm):	396,0
CS:	15,14
SD:	57,50
PDI:	0,02021

Percentiles	
%Tile	Size(nm)
10,00	333,0
20,00	355,0
30,00	372,0
40,00	387,0
50,00	402,0
60,00	418,0
70,00	435,0
80,00	455,0
90,00	480,0
95,00	502,0

Peaks Summary		
Dia(nm)	Vol%	Width
402	100	115

400 nm polystyrene undiluted in classical way as reference

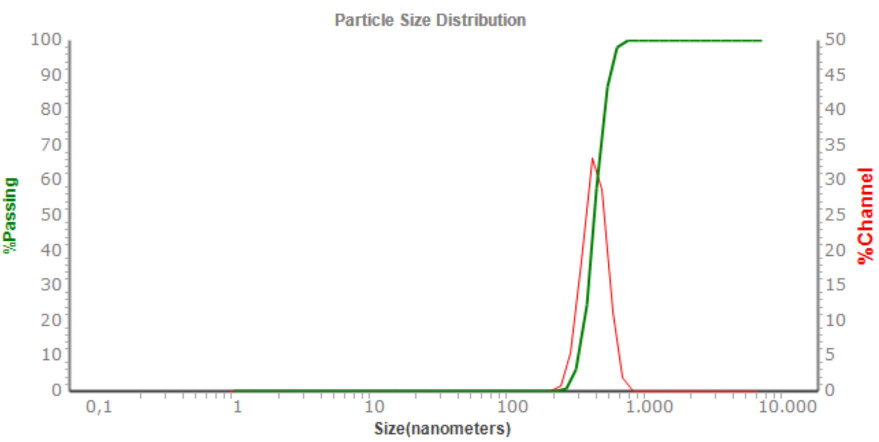


Summary	
Data	Value
MI(nm):	412,0
MN(nm):	361,0
MA(nm):	394,0
CS:	15,22
SD:	86,20
PDI:	0,0461

Percentiles	
%Tile	Size(nm)
10,00	307,0
20,00	338,0
30,00	361,0
40,00	381,0
50,00	402,0
60,00	425,0
70,00	450,0
80,00	482,0
90,00	531,0
95,00	575,0

Peaks Summary		
Dia(nm)	Vol%	Width
402	100	172,4

400 nm polystyrene undiluted 5 µL drop

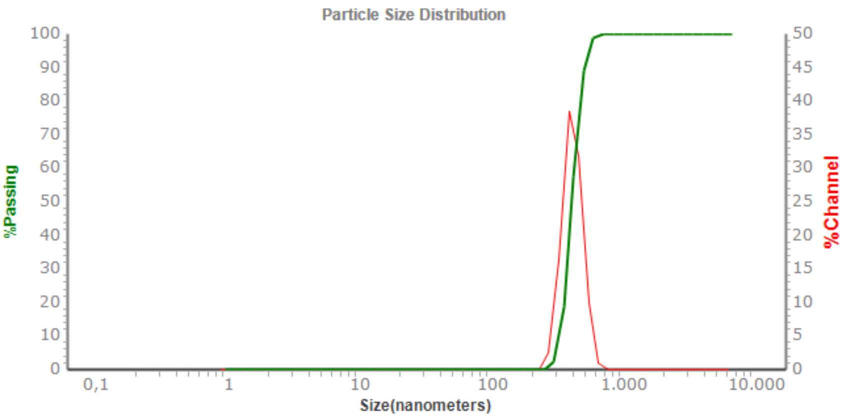


Summary	
Data	Value
MI(nm):	400,0
MN(nm):	357,0
MA(nm):	385,0
CS:	15,59
SD:	77,20
PDI:	0,0373

Percentiles	
%Tile	Size(nm)
10,00	304,0
20,00	333,0
30,00	355,0
40,00	374,0
50,00	393,0
60,00	414,0
70,00	436,0
80,00	463,0
90,00	503,0
95,00	540,0

Peaks Summary		
Dia(nm)	Vol%	Width
393	100	154,4

400 nm polystyrene undiluted 2 µL drop



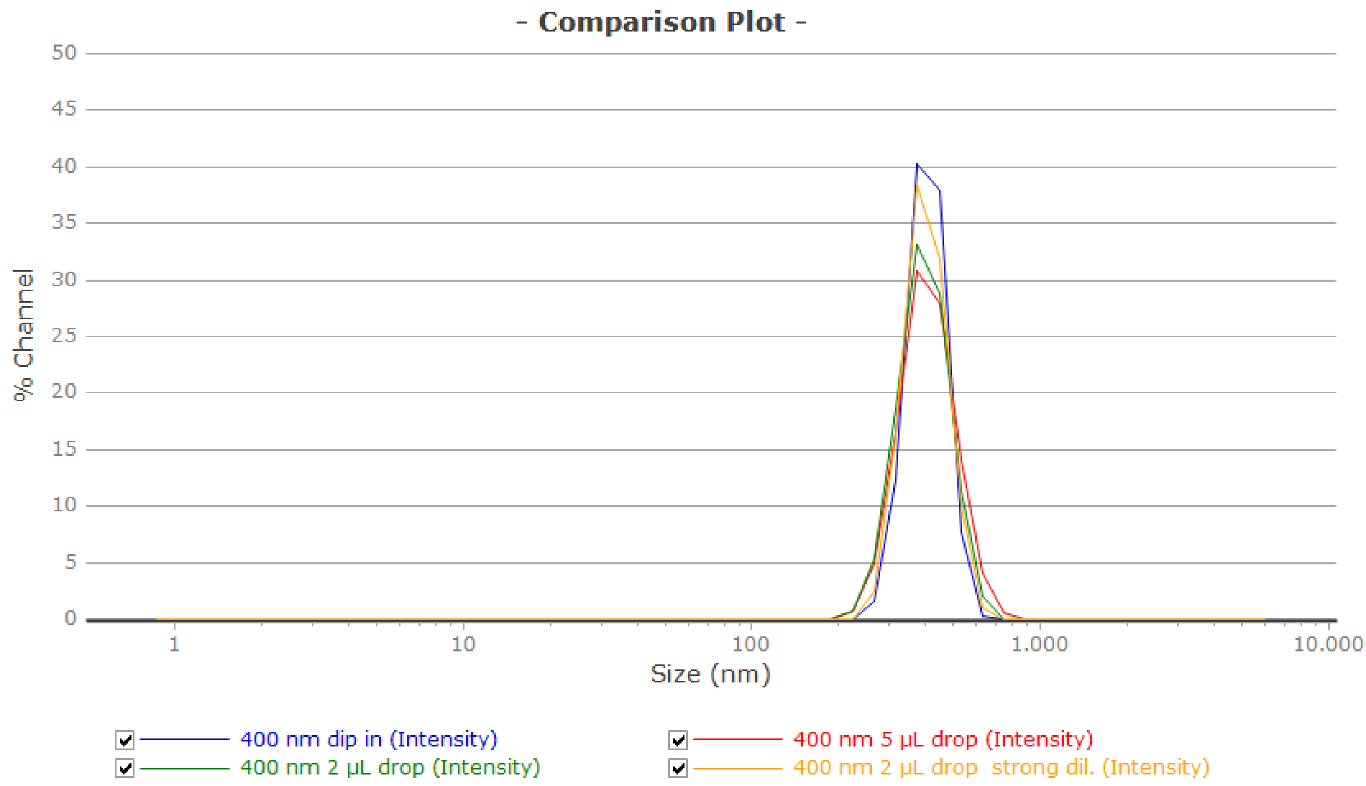
Summary	
Data	Value
MI(nm):	403,0
MN(nm):	372,0
MA(nm):	392,0
CS:	15,31
SD:	65,80
PDI:	0,02671

Percentiles	
%Tile	Size(nm)
10,00	321,0
20,00	346,0
30,00	364,0
40,00	380,0
50,00	396,0
60,00	414,0
70,00	434,0
80,00	458,0
90,00	491,0
95,00	522,0

Peaks Summary		
Dia(nm)	Vol%	Width
396	100	131,6

400 nm polystyrene strong diluted 2 µL drop

All measurements show identical results. They all in the same range which can be seen very clearly in the following compare plot and table of all 4 different measurements.



Sample	MI / nm	MA / nm	D50 / nm	Peak /nm
400 nm Dip in (int)	405,0	396,0	402,0	402
400 nm 5 µL drop (int)	412,0	394,0	402,0	402
400 nm 2 µL drop (int)	400,0	385,0	393,0	393
400 nm 2 µL drop (int) Strong diluted	403,0	392,0	396,0	396

SUMMARY

The Microtrac MRB Nanotrac Flex DLS analyzers have the capability to measure samples up to a very high concentration and very small volumes like 2 µL. This small amount of sample can be very helpful for very expansive or rare samples. The Nanotrac has also the capability to measure diluted samples as shown. In general, the best way to characterize a dispersion will be as close to the real concentration of sample as possible, which DLS can do.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FLEX