Detecting and Number Counting of Single Engineered Nanoparticles by Digital Particle Polymerase Chain Reaction

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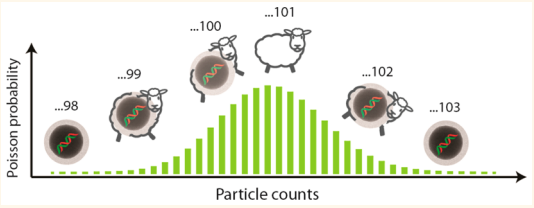
ABSTRACT The concentrations of nanoparticles present in colloidal dispersions are usually measured and given in mass concentration (*e.g.* mg/mL), and number concentrations can only be obtained by making assumptions about nanoparticle size and morphology. Additionally traditional nanoparticle concentration measures are not very sensitive, and only the presence/absence of millions/billions of particles occurring together can be obtained. Here, we describe a method, which not only intrinsically results in number concentrations, but is also sensitive enough to count individual nanoparticles, one by one. To make this possible, the sensitivity of the polymerase chain reaction (PCR) was combined with a binary (=0/1, yes/no) measurement arrangement, binomial statistics and DNA comprising monodisperse silica nanoparticles. With this method, individual tagged particles in the range of 60−250 nm could be detected and counted in drinking water in absolute number, utilizing a standard qPCR device within 1.5 h of measurement time. For comparison, the method was validated with single particle inductively coupled plasma mass spectrometry (sp-ICPMS).



KEYWORDS: particle analytics · encapsulation · silica · DNA · digital PCR · sol−gel processes

The measurement of nanoparticle concentrations down to the level of single particles is increasingly important due to the growing use in consumer goods, industrial applications and medical diagnostics. To assess their potential impact, the development of suitable analytical methods is needed to determine nanoparticle concentrations by designing portable, inexpensive and simple detection tools able to detect unique particles in nonidealized environments. In this context, there is a demand of increasingly more sensitive methods, and some market regulations (*e.g.* European Medical Devices)[1] are asking for proving the presence/absence of individual particles, far beyond the capability of the established nanometrology methods.

Several analytical methods to characterize nanoparticles are already standard research infrastructure in many laboratories, such as dynamic light scattering (DLS) for size determination, zeta-potential for surface charge measurement or the Brunauer−Emmett−Teller (BET) method for surface area estimation.[2] Despite the progress in the field, most particle analysis techniques are limited by particle size and concentration, and often require large and complex instruments. Although electron-microscopy and probe scanning techniques can provide images of single particles, they are based on expensive instrumentation, and particle counting is time-consuming even with modern automated image recognition systems.

In the past decade, several innovative approaches for detecting single nanoparticles have been developed,[3] ranging from mechanical (*e.g.* weighing of biomolecules/nanoparticles by microchannel resonators)[4] to optical detection, fluorescence microscopy,[5] and label-free sensing technologies such as whispering-gallery-mode (WGM) biosensors,[6] and surface plasmon resonance microscopy (SPRM).[7] However, because of their complexity, the use of these techniques is restricted to few laboratories and only applicable to a small set of samples.

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TABLE 1. Standard Biotechnical Detection Methods for Protein/DNA

method	entity measured	limit of detection	measuring duration	effort
enzyme-linked immunosorbent assay (ELISA)	proteins/antigens	1–10 pM[8,11] 10^7 *molecules per drop* (60 μL)	2.5 to 4 h	10000 U.S.$; Only plate reader required
real-time polymerase chain reaction (PCR)	DNA/RNA/viruses/bacteria	<1–10 copies[12,13] *observation per PCR reaction (20–50 μL)*	1–2 h	15000–30000 U.S.$; Standard qPCR machine, benchtop instrument
digital droplet PCR (ddPCR)	DNA/rare mutations	1–10^6 copies, 20 μL split into 20000 droplets[14] *observation of individual events per nl*	1–2 h	~100000 U.S.$; Similar workflow to real-time PCR with additional microfluidic devices

In contrast thereto, medical diagnostics has common access to highly sensitive techniques to detect and monitor biomolecules and bioparticles (cells/viruses/bacteria), close to single molecules (down to 10^{-18} mol/L, see Table 1). These ultralow limits of detection can be achieved by either signal-based or target-based amplifying strategies.[8] While the signal-based amplification detects the increased signal after the binding of the biomolecule to a complex-labeled system (*e.g.* enzyme-linked immunosorbent assay (ELISA)[9]), the target-based amplification is based on the identification and exponential amplification of the target. Due to the fact that the target concentration is increased exponentially, target-based amplification strategies are more sensitive. The most common target-based amplification method, the polymerase chain reaction (PCR), is able to detect few copies of nucleic acids.[8,10] Because of its high sensitivity, selectivity, and versatility of detection, as well as low costs and portability, PCR has been established as the benchmark for nucleic acid detection in different areas, ranging from clinical diagnostics to forensics and is the standard for identification of many important viral and bacterial diseases (*e.g.* HIV).

Attracted by the sensitivity and ease of application of the PCR method, we wanted to exploit this method for the detection of nanoparticles, especially as geometrically (in terms of size), many viruses and nanoparticles are comparable. In our previous work, we have shown that silica particle detection is compatible with DNA, but accessible detection limits have been >10 000 particles per analysis,[15] still far away from the potential PCR analytics has to offer. To reach the goal of detecting single nanoparticles, we had to significantly improve the synthesis of DNA comprising particles to yield individual, nonagglomerated sub-100 nm particles. Additionally to this, we decided for a signal detection scheme based on taking binary measurements for PCR with which, as shown below, individual nanoparticles can be literally counted.

RESULTS AND DISCUSSION

Establishing a precise particle detection tool requires access to particles of excellent homogeneity, both in size and in shape. Previously established methods for the formation of DNA loaded nanoparticles[16–18] resulted in nonspherical agglomerated particles with relatively broad particle size distributions. While the agglomerated particles could be used as tracers in ecological studies, the monitoring of wastewater treatment plants and the barcoding of oils,[19–21] these materials are not fit to provide single nanoparticle based analytics. In this study, we therefore combined DNA deposition on the surface of preformed silica particles *via* a layer-by-layer approach with sol–gel silica formation under acid catalysis (Figure 1) to yield homogeneous particles (see results further below and Supporting Information for particle characterization).

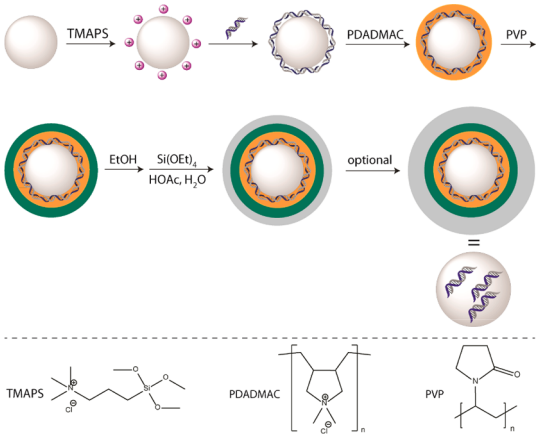

Figure 1. Synthesis of DNA/SiO_2 particles using a layer-by-layer approach. First, DNA was immobilized onto the surface of positively charged silica particles *via* electrostatic interaction with the negatively charged phosphate backbone of the DNA molecules. A cationic polymer, poly(diallyldimethylammonium chloride) (PDADMAC), was then deposited onto the adsorbed DNA. A second polymer layer of poly(vinylpyrrolidone) (PVP) was deposited before growing the outer silica shell by a sol–gel process, using tetraorthosilicate (TEOS) as Si source.

Within the encapsulates, the DNA is protected against harsh environmental conditions, such as high temperature, radicals or UV irradiation.[22] Due to the DNA therein, the particles can be quantified by real-time PCR (= quantitative PCR, qPCR) after the silica shell has been removed by a fluoride comprising buffer (Figure 2, top schematic). To avoid false signals in the PCR from external genomic DNA, we designed DNA amplicons, which do not overlap with known biological

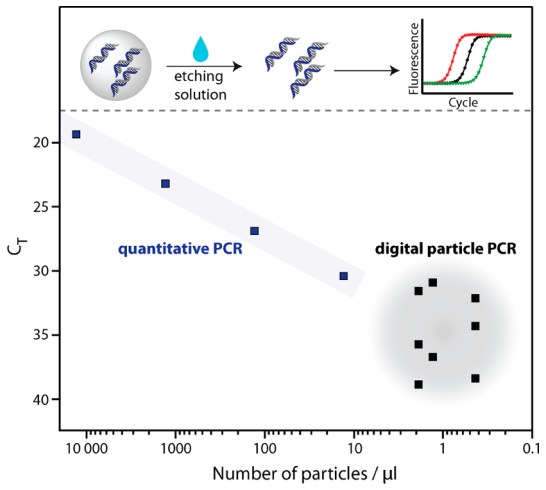

Figure 2. Measuring SiO_2 particles *via* PCR analysis. After the SiO_2 dissolution step with a buffered fluoride solution (F^- = 250 mg/L, pH = 4−5) the released dsDNA can be directly amplified using PCR. In the range of higher DNA/SiO_2 particle concentrations (>10 particles/μL), quantitative information can be obtained by comparison with a standard curve. Below a certain amount of DNA/SiO_2 particles (<10 particles/μL), the C_T values for the same concentration of particles increase in variability. In this range, digital particle PCR can be used to obtain quantitative information.

DNA, and tested them for uniqueness with the Basic Local Alignment Search Tool (BLAST).[23] The use of qPCR gives information on the DNA concentration by analyzing the threshold cycle (C_T), the point on the fluorescence curve at which the signal increases above the background. With the use of a standard curve obtained from samples of known concentration (straight line in Figure 2), the detection of released DNA allows an indirect measurement of silica particle concentrations down to the range of parts per billion (ppb = microgram per liter). However, if samples of even lower particle concentrations are analyzed, the C_T values obtained increase in variability for replicates and only a part of the replicates amplify at all (Figure 2, bottom right).

From a statistical point of view, this is not unexpected: If, for example, the sampled volume for a PCR reaction is 1 μL (as in our case) and the concentration of suspended, randomly distributed particles is around 1 particle/μL, already a random error of +1 or −1 particle per reaction can have a huge impact on the amplification outcome. Nevertheless, a PCR reaction provides information on the particle concentration, as the probability of a reaction containing at least one (leading to a positive amplification signal) or no particle (no amplification) is a function of the particle concentration. Using this insight, we envisioned that a digital quantification method of particle concentration based on binary measurements (amplification and no amplification) obtained by end-point PCR could be used to measure single particle concentrations. A similar digital approach in combination with PCR has already been introduced in 1992 by Sykes *et al.*[24] for the absolute quantification of DNA itself, and was further developed to digital droplet PCR (ddPCR), a technology enabling the detection of rare mutations (with a detection limit of 0.001% mutant fraction for *BRAF* V600E mutation).[14,25] Our method, which we term digital particle PCR (dpPCR), differentiates from ddPCR by the fact that it quantifies particle concentrations (containing DNA) and can be performed with a standard qPCR thermal cycler without additional infrastructure or reagents.

For initial tests, DNA loaded SiO_2 particles with a diameter of 137 nm and a narrow particle size distribution were synthesized (see Figure 1). Particle suspensions with ultralow concentrations were prepared by serial dilution to contain ∼0.4−13 particles/μL (∼10^8-fold dilution). For every concentration, 14 partitions (V = 1 μL) of the particle suspension were distributed to a PCR plate by directly pipetting into 14 wells (Figure 3A). For every concentration chosen, some of the wells contained at least one particle, while others were empty.

A volume of 1.5 μL of a diluted fluoride comprising buffer solution (=buffered oxide etch, BOE; F^- = 250 mg/L, pH = 4−5) was added to each well to dissolve the silica particles and release the DNA. Following the addition of polymerase, deoxynucleoside triphosphates, and buffer, the DNA molecules were thermally amplified *via* PCR to end-point (experimental details are described in Methods and the Supporting Information in Tables S1 and S2). As the DNA concentrations per well and cycle were monitored by Taq-man probe fluorescence in real-time, we were able to directly differentiate wells initially containing particles (= positive partition) or not (negative). The results of this experiment are plotted in terms of positive partitions over total partitions (= P/T) against the relative sample concentrations from the serial dilution (data points in Figure 3B). While negative control samples did not amplify in any of the cases, for practical reasons we chose a cutoff at a C_T of 39 (see Supporting Information for effect of cutoff choice). The cutoff was chosen to exclude false positive signals by dpPCR, since we calculated that above a C_T value of 39 a sample would contain <1 DNA molecule, whereas a single particle contains on average 16 DNA molecules. Even if the DNA loading per particle is unevenly distributed, the chosen cutoff and the developed approach can handle a wide DNA loading variation, which only requires that every particle contains more than one DNA molecule. In case of the 67 nm particles (see detailed discussion below), we further found a highly homogeneous distribution of C_T demonstrating that every particle carries a very comparable amount of DNA.

From the assumption that the particles are uniformly distributed in the solution, it follows that the number of particles in a well is binomial or approximately Poisson distributed. Our measurements cannot be used to

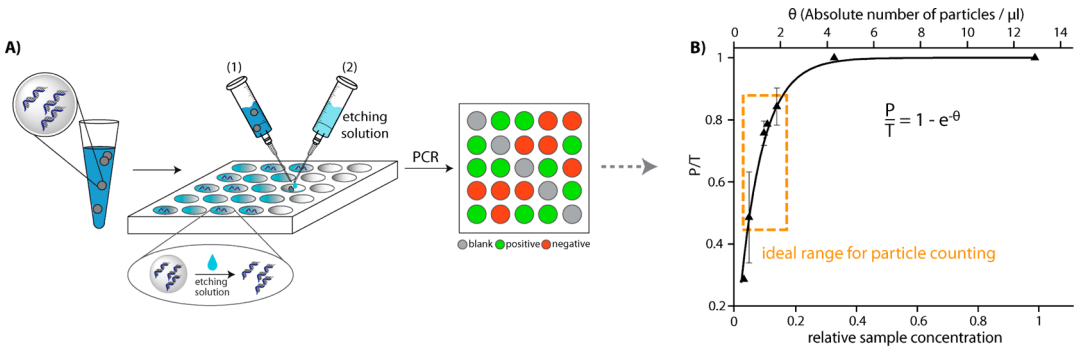

Figure 3. Principle and workflow of digital particle PCR (dpPCR). (A) Partitions (1 μL) of a particle dispersion comprising ~1 particle/μL are transferred to a 96-well PCR plate, and the particles are directly dissolved with a buffered fluoride buffer. Following DNA amplification by PCR and online detection by fluorescence, samples containing one or more tagged particles yield positive results, whereas those without particles result in negative responses. (B) Solutions with nominative relative particle concentrations were processed by dpPCR, resulting in digital signals (P/T = positive over total). By fitting these signals to eq 1, the Poisson distribution of the measurement events is demonstrated (14 positive/negative wells per sample, performed 5 times per concentration for determination of measurement error). The absolute particle number (top axis) is calculated by fitting the relative data to the given equation.

determine the number of particles in a well, but it can be used to determine if a well contains one or more particles. The expected number of positive wells over total wells (= P/T) is equal to the probability that a well contains one or more particles and becomes, in terms of the particle concentration θ (number particles/μL)

$$E\left(\frac{P}{T}\right) = \sum_{k=1}^{\infty} X_\theta(k) = 1 - X_\theta(0) = 1 - e^{-\theta} \quad (1)$$

where $X_\theta(k)$ is the probability of having k particles in one partition, and $X_\theta(0)$ is the probability of an empty partition. By comparing this theoretical result with our experimental findings shown in Figure 3B, we observed a good correlation, which can be expressed by the adjusted R^2 = 0.99 if the experimental data is fitted to the equation above. This demonstrates that the Poisson statistics hold for the experimental procedures performed. This further shows that dpPCR can be used as a tool to count individual particles at very low concentrations, without the need for a calibration curve or a correction factor. These advantages come at the cost of the limited dynamic range of the method. As shown in Figure 3B, a good correlation between the measured signal (= P/T) and particle concentration can only be found in a narrow concentration range of ~0.5−3 particles per partition. This is intuitive, as at higher particle concentrations essentially all wells give positive results, and at much lower particle concentrations essentially all wells are empty (further statistical discussion can be found in the Supporting Information). This is in strong contrast to qPCR, which has an enormous dynamic range of many orders of magnitude, but can only differentiate sample concentrations on a logarithmic scale and always requires a standard curve for absolute quantification. To determine the particle concentration of completely uncharacterized samples, a combination of qPCR and dpPCR is useful.

The obtained absolute numbers of DNA tagged particles measured by digital particle PCR were verified by comparing the detection of the same particle dispersions with an established single particle analysis technique: single-particle inductively coupled plasma mass spectrometry (sp-ICPMS).[26,27] For this, the measurement system was investigated under idealized conditions: Ultrapure water was chosen as matrix, and larger, 252 nm sized particles were used to guarantee that sp-ICPMS can be performed at sufficiently high accuracy. This method measures the silicon content in small droplets by mass spectrometry, and very similar to dpPCR, can discriminate between empty droplets and droplets comprising particles. This method is highly sensitive, enables to discriminate between individual particles of a wide range of compositions and covers a wide concentration range.[26] The detailed operating conditions, the data processing, and evaluation of the sp-ICPMS method are described in the Supporting Information. The ultra-diluted particle samples (initial concentrations 7 μg/μL; diluted >10^8-fold) were measured simultaneously by dpPCR and sp-ICPMS (Figure 4C).

For the dilutions corresponding to ~1 and 0.5 particles/μL, the results from both methods corresponded well to each other: Both dilutions could also be discriminated from each other with statistical significance (t test). For a higher concentration (~2 particles/μL), dpPCR yielded a significantly lower result than sp-ICPMS. This already shows the narrower measurement range of the dpPCR method as discussed above (see Figure 3B). Sensitivity and measurement range of the dpPCR method may be further increased by analyzing more than 14 wells per sample; however, it has to be noted that the reagent cost is not insignificant at ~0.5 USD per PCR well (see discussion on accuracy *versus* number of wells in Supporting Information). Overall, the results obtained by dpPCR could be directly confirmed by sp-ICPMS and showed a similar variability as the more

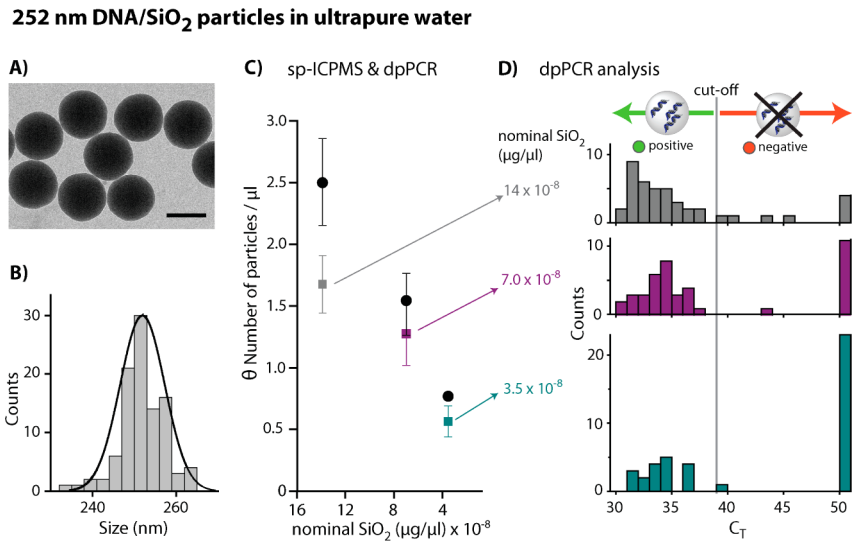

Figure 4. Detection of DNA/SiO_2 particles (d = 252 nm) in ultrapure water. (A) Transmission electron microscopy (TEM) image of DNA/SiO_2 particles; scale bar: 200 nm. (B) Particle size distribution obtained by transmission electron microscopy image analysis. (C) Comparison of sp-ICPMS (black spheres) with dpPCR (colored squares); measurements in triplicate, undiluted sample concentration c_0 = 7 μg/μL. (D) Distribution of C_T values of the individual wells displaying the original signals, from which the data in (C) is calculated.

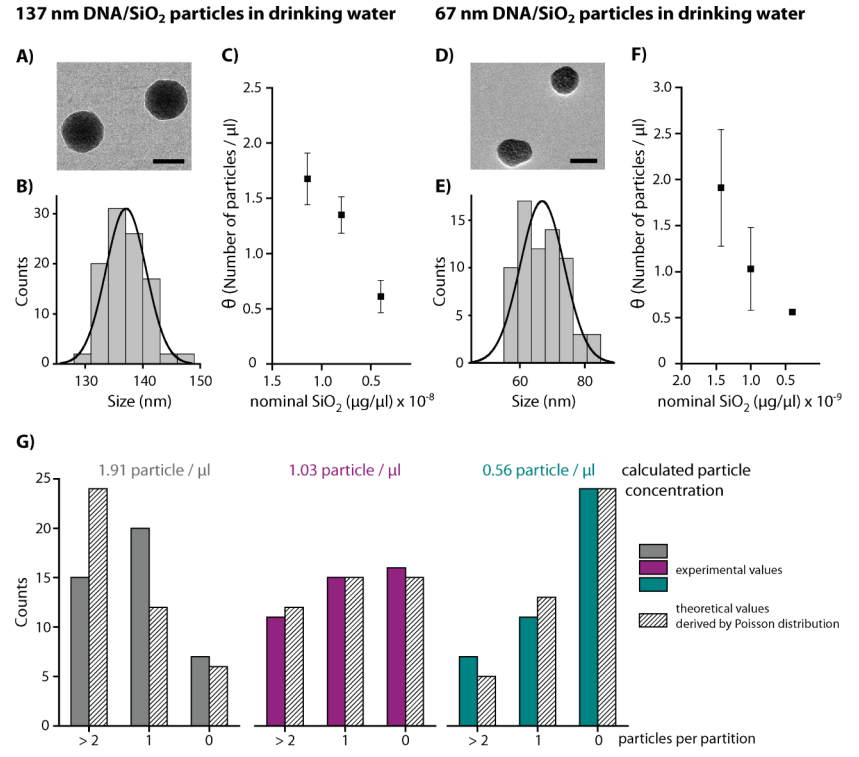

Figure 5. Analysis of nanoparticles in drinking water. (A) TEM image of DNA/SiO_2 particles, scale bar: 100 nm. (B and E) Particle size distribution obtained by TEM image analysis. (C) dpPCR of DNA/SiO_2 (d = 137 nm) particles in drinking water, measurements in triplicate, undiluted sample concentration c_0 = 0.8 μg/μL. (D) TEM image of DNA/SiO_2 particles, scale bar: 50 nm. (F) dpPCR DNA/SiO_2 (d = 67 nm) particles in drinking water, measurements in triplicate, undiluted sample concentration c_0 = 0.02 μg/μL. (G) In case of the 67 nm particles, the distribution of C_T values can be further correlated to the expected presence of 0, 1, and >2 particles per well from Poisson statistics (see Supporting Information for further details).

established method. Although similarly accurate when within its ideal measurement range, dpPCR uses standard infrastructure, is less complex and does not require any calibration or signal correction.

To show the flexibility of the dpPCR method in terms of particle size and independence from dissolved, contaminating silicon, three particle dilutions in the range of 0.5−2 particles/μL were quantified with DNA

TABLE 2. Comparison of particle detection metohds

method	approximate size range	limit of detection	measuring duration	effort
		Standard Particle Detection Methods		
confocal fluorescence microscopy[5]	<10 nm (particles tagged with fluorescent label, quantum dots)	ppb−ppm; *10^2−10^3 particles per μl dried out drop*[a]	hours	50000−150000 U.S.$ large infrastructure skilled operator required
scanning electron microscopy (SEM)	10 to >1000 nm^2 any solid inorganic material	ppb−ppm^2; *10^2−10^3 particles per μL dried out drop*[a]	hours	150000−500000 U.S.$, large infrastructure skilled operator required
atomic force microscopy (AFM)	0.5 to >1000 nm^2 any solid inorganic material	ppb−ppm^2; *10^2-10^3 particles per μL dried out drop*[a]	hours	20000−100000 U.S.$, highly skilled operator required
single-particle inductively coupled plasma mass spectrometry (sp-ICP-MS)	strongly element dependent <10 to >500 nm (*e.g.*, Si > 100−400 nm)[27]	ppt−ppb; *~1−10^3 particles/μL*	minutes to hours	100000−500000 U.S.$, large infrastructure and maintenance (*e.g.*, substantial argon consumption)
		Examples of Recent Techniques for Particle Detection		
microchannel resonators	100 nm Au, 1.5 μm polystyrene nanoparticles[4]	<fg[4]; *~ 10^4 particles/μL*[b]	minutes to hours	microfluidic device, individually designed equipment
whispering-gallery-mode (WGM) sensors[c]	30 to <175 nm (KCl and polystyrene nanoparticles)[d]	1 pg/mm^2 (for biomolecules[6])[e]; *~10^3 particles/mm^2*	minutes	individually designed equipment
surface plasmon resonance microscopy (SPRM)[c]	>100 nm (SiO_2, viruses)[7]	0.2 fg/mm^2 (for viral particles)[7]; *~ 1 particle/mm^2*	minutes	skilled operator required
		This Work		
Digital particle PCR (dpPCR)	>60 nm (DNA tagged silica)	<1 ppt = <1 fg/μL ~ *1 particle per μL*	1.5 h	15000−30000 U.S.$; standard qPCR machine, benchtop instrument

[a] Analysis on dried sample only with evenly distributed nanoparticles. Minimum drop volume 1 μL, minimal 100 particles per sample to avoid misinterpretation of artifacts (dust/aggregation).[28] Statistically robust quantification of particle concentration is very time-consuming, because of the use or very small volumes from dried out liquids. [b] Injected concentrations of particles samples were between 10^7 and 5×10^8 per mL.[4] [c] 2D technology, measure of surface density (particles/mm^2 instead of volumetric particle concentration). [d] Ultrahigh-quality-factor (Q) WGA microresonators were used.[29] The upper size limit of particles is imposed by the wavelength (radius $\ll \lambda$). [e] The throughput capacity in these devices is limited in order to preserve the original sensitivity underlying the characteristics changes of the WGM through the interaction by nanoparticles.[3]

tagged SiO_2 particles of 137 and 67 nm diameter in drinking water. The particle concentration could be quantified successfully without any disturbance by the dissolved silicon and other molecules in drinking water (Figure 5). Under these conditions, the sp-ICPMS method could not be performed as it could not detect the smaller tagged SiO_2 particles, even in ultrapure water, due to background noise, which can be assigned to Si eroded from the ICP torch, memory effects and incomplete particle events. For this reason, the measurement results are best compared with the silica content of the undiluted sample (from gravimetry). Assuming spherical silica particles of a uniform size and a density of 2200 kg/m^3, the theoretical result after 10^8-fold dilution is in the range of the measured value (*ca.* factor 2, which can be explained by uncertainty in particle size measurement, $M_{particle} \propto r^3$). High background concentrations of silicon and other molecules further limit the application of elemental methods for silica particle measurements in environmental samples. For example, drinking water already contains silicic acid at 4 ppm (parts per million, Zurich 2013), making any comparison of dpPCR for silica with alternative methods at lower sample concentrations nearly impossible.

To put these experimental results into perspective, Table 2 summarizes the different types of particle detection methods taking into account the limit of detection and the overall effort of the method. In comparison, the method described here gives rise to nearly unprecedented detection limits for nanoparticles in complex matrices with relatively low infrastructural requirements. An additional advantage is that the method results in an absolute particle number and, therefore, does not require normalization (or background correction).

Two basic requirements are needed for successful application of the dpPCR method in more complex media: (a) the particles have to remain individual particles in the medium. If not, the method still measures the particle concentration (particles per mL), but no longer the primary particle number but the aggregate number. (b) The action of the polymerase during PCR may not be interfered by the sample

medium (after 1:10 dilution in PCR preparation, with Mastermix *etc.*), which is possible for a large array of nonbiological samples. Biological samples (*e.g.*, cell suspensions) may also be compatible with the method after the diluting-out of interfering species.

The method developed here can only detect DNA loaded nanoparticles and is therefore only useful for detecting the flow of artificial silica nanoparticles. Future development to expand the technique to nanoparticles of other composites or particle in various sizes can increase the application range. Investigations of particles comprising TiO_2 or Fe_2O_3 loaded with DNA were already conducted in previous studies and showed compatibility with standard PCR after dissolving the particles.[21,22] The use of sol–gel chemistry further allows to adopt the size of particles by adding layers of silica (as done in the preparation of the 252 nm particles, see Methods). Additionally functionalization of the silica surface can be directly applied by using silanes with different functional groups, such as positive charged ammonium, carboxylic acid, C_6 or polyethylene glycol (PEG). These different sized or functionalized particles could be used for studying and analyzing their behavior in various fluids and mixtures and the resulting particle concentrations could be measured with the method developed here without restriction. While the method can certainly be expanded, the method is not able to measure/detect naturally occurring or accidentally liberated nanoparticle flows. In spite of these constraints, we believe that the dpPCR method is most valuable in understanding/tracing/detecting the behavior of nanoparticles—down to the level of individual particles. Also the method is most suited to test the absolute efficiency of nanoparticle removal techniques (*e.g.* filtration devices). As the method gives absolute particle numbers without normalization, it may further be useful for the calibration/correction of other nanoparticle measurement devices (*i.e.*, certification of calibration dispersions).

CONCLUSIONS

In summary, this study provides a new quantification technique for measuring ultralow concentrations of tagged nanoparticles with a detection limit of <1 particle/μL (*i.e.*, $<1 \times 10^{-9}$ μg/μL silica = <1 ppt; see Supporting Information discussion on detection limit as a function of the amount of measurement points). dpPCR does not require a preceding calibration for quantification and only a standard qPCR machine is needed, which is available in many research facilities and medical laboratories. We have demonstrated that dpPCR is not sensitive to contaminating ions and can also quantify tagged silica particles suspended in drinking water. The sensitivity is based on the indirect measurement of the particles, which requires the use of engineered, DNA tagged particles. Here, we used encapsulated DNA/SiO_2 particles to validate our method, but the same principle can be extended to other particles. The successful incorporation of DNA into additional inorganic nanoparticles has already been shown in previous studies.[21,22] Engineered particles are commonly used as tagging systems in diverse applications ranging from biological detection and imaging techniques, such as cell labeling,[30] cell tracking,[31] and detection of DNA[32] and proteins[33] and several methods have been proposed that use nanoparticles for the amplification of measurement signals.[34,35] With the use of dpPCR, the quantification of such particles on a single particle level is simplified and will further decrease the detection limits of the particulate taggants and any measurement derived thereof.

METHODS

Synthesis of the 137 nm DNA/SiO_2 Particles. DNA-labeled SiO_2 particles were synthesized by optimizing a previous developed procedure of DNA encapsulation into SiO_2.[16,36] To obtain monodisperse encapsulates, a layer-by-layer approach was followed. In a first step, 132 nm silica particles (SiO_2-R-L2902, 50 mg/mL, microparticles GmbH) were functionalized with *N*-trimethoxysilylpropyl-*N*,*N*,*N*-trimethylammonium chloride (TMAPS, 50% in methanol, ABCR) to achieve a positively charged surface, able to adsorb double-stranded DNA. To bind DNA onto the particle surface, 35 μL of functionalized particle suspension (50 mg/mL) was mixed with 1 mL of a 12 μg/mL DNA solution (dsDNA: 5′-CGT GGA AGG TAA CAG CAC CGG TGC GAG CCT AAT GTG CCG TCT CCA CGA ACA CAA GGC TGT CCG ATC GTA TAA TAG GAT TCC GCA ATG GGG TTA GCA AGT GG-3′ (101 bp)). After the particles were washed twice with water, 0.5 mL of 1 mg/mL poly(diallyldimethylammonium chloride) solution (PDADMAC, 20 wt % in H_2O, M_w 20 0000–350 000 g/mol, Sigma-Aldrich) was deposited onto the particles. After 20 min at room temperature, the particles were washed three times and redispersed into 1 mL of 0.1 mg/mL poly(vinylpyrrolidone) solution (PVP, M_w ~ 10 000 g/mol, Sigma-Aldrich) for 20 min at room temperature, followed by two washing cycles in water and one in ethanol. The PVP layer enables the particle transfer into ethanol,[37] which allows the growth of silica *via* acidic catalyzed Stöber reaction (233 μL EtOH, 72 μL H_2O, 22.1 μL tetraethoxysilane (TEOS, ≥99.0% Aldrich), 5 μL of 10 mol/L HOAc).[38] The reaction mixture was stirred (900 rpm) overnight at room temperature and a silica shell with a thickness of 2–4 nm was obtained. The particles were washed twice with ethanol, once with water and redispersed in 1 mL ethanol. To obtain only single particles, the particle solution was centrifuged for 3 min at 1150*g* and 800 μL of the supernatant was collected. The supernatant contained 1.32 mg/mL of monodisperse encapsulated DNA/SiO_2 particles.

Additional SiO_2 Layer: 252 nm DNA/SiO_2 Particles. To obtain an additional SiO_2 layer onto the encapsulated DNA/SiO_2 particles, a standard Stöber reaction was performed. The above synthesized particles were centrifuged for 4 min at 21 100*g* and redispersed in a solution of 4.2 vol % ammonia (25 wt % NH_3 in water) in an ethanol/water mixture (366 μL ethanol/22 μL H_2O). Subsequently, 16.5 μL of TEOS was added and the reaction was stirred for 3 h (900 rpm, room temperature). The obtained particles were washed twice with ethanol, once with water, and stored in ethanol (7 mg/mL).

Synthesis of the 67 nm DNA/SiO_2 Particles. In a first step, 61 nm silica particles (sicastar 43-00-701, 25 mg/mL, micromod

Partikeltechnologie GmbH) were functionalized with *N*-trimethoxysilylpropyl-*N*,*N*,*N*-trimethylammonium chloride (TMAPS, 50% in methanol, ABCR). To bind DNA onto the particle surface, 20 μL of functionalized particle suspension (50 mg/mL) was mixed with 1 mL of a 12.8 μg/mL dsDNA solution (ssDNA (forward): 5′-(thiol modified)-AAA AAA AAA ACA CGA GGT AAA TAT GGG ACG CGT CCG ACC TGG CTC CTG GCG TTC TAC GCC GCC ACG TGT TCG TTA ACT GTT GAT TGG TAG CAC A-3′ (94), ssDNA (reverse): 5′-TGT GCT ACC AAT CAA CAG TTA ACG AAC ACG TGG CGG CGT AGA ACG CCA GGA GCC AGG TCG GAC GCG TCC CAT ATT TAC CTC GTG-3′ (84)). After the particles were washed twice with water, 1 mL of 4 mg/mL poly(diallyldimethylammonium chloride) solution (PDADMAC, 35 wt % in H_2O, M_w < 100 000 g/mol, Sigma-Aldrich) was deposited onto the particles. After 20 min at room temperature, the particles were washed three times and redispersed in a 1 mL of 0.5 mg/mL poly(vinylpyrrolidone) solution (PVP, $M_w \sim$ 10 000 g/mol, Sigma-Aldrich) for 20 min at room temperature, followed by two washing cycles in water and one in ethanol. The particles were redispersed in 275 μL of EtOH and subsequently 6 μL of H_2O, 6 μL of tetraethoxysilane (TEOS, ≥ 99.0% Aldrich) and 3 μL of 10 mol/L HOAc) were added. The reaction mixture was stirred (900 rpm) overnight at room temperature and a silica shell with a thickness of 6 nm was obtained. The particles were washed twice with ethanol, once with water and redispersed in 1 mL ethanol. To obtain only single particles, the particle solution was centrifuged for 7 min at 9390*g* and 500 μL of the supernatant was collected.

DNA Recovery. The dsDNA was recovered from the encapsulated DNA/SiO_2 ($c \leq 1.32$ μg/mL) by using a 1:100 diluted buffered oxide etch solution (BOE, 0.23 g of NH_4FHF (pure, Merck) and 0.19 g of NH_4F (puriss, Sigma-Aldrich) in 10 mL water).

Digital Particle PCR Instrumentation and Workflow. The digital particle PCR (dpPCR) was performed at low concentrations. The particle solutions were diluted in water to a concentration of 0.4−13 particles/μL. One microliter of this solution was directly pipetted into a well of a 96-well PCR plate and dissolved with 1 μL of a 1:100 diluted BOE solution. The other PCR reagents, 2× Taqman PCR Mastermix, $MgCl_2$, primers and probe (both designed and purchased by Microsynth AG, see Table S1 in Supporting Information) were premixed and added to the dissolved particle solution. The composition of all PCR reagents is shown in detail in Supporting Information in Table S2. Each 96-well plate column contained a negative control, corresponding to the diluted BOE solution, to ensure that the BOE does not contain contaminating DNA. The PCR reaction was performed with a Roche LightCycler 96 using the following parameters: 2 min at 50 °C and 10 min at 95 °C for activation, followed by 50 cycles of a two-step thermal profile (15 s at 95 °C, 60 s at 60 °C).

Conflict of Interest: The authors declare no competing financial interest.

Supporting Information Available: The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.5b04429.

Material characterization by TEM; PCR reagent composition and raw data with corresponding cutoff; operating conditions, data processing and evaluation of the sp-ICPMS, discussion of LOD; statistical estimation of particle concentration, including the limit of detection (PDF)

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