DISPERTECH

Whitepaper

# MEASURE SIZE AND SCATTERING INTENSITY OF SMALL NANOPARTICLES IN HOLLOW OPTICAL FIBERS

- **Measure particle sizes down to 20 nm**
- **Scattering intensity gives precise single-particle sizes**
- **Diffusion measurements yields absolute size information**
- **Very long time-traces guaranteed by nano-fluidic system**
- **Single-particle measurements allows identification of sub-populations**

## INTRODUCTION

Particles with dimensions below 100 nm are of relevance in topics such as extracellular-vesicle research, virology, and nanomedicine. However, characterizing them is difficult without access to time-consuming and expensive equipment such as electron microscopes. Small nanoparticles are hard to detect optically, without fluorescent labelling, because they have relatively small scattering cross sections. High noise and background levels make it challenging to analyze scattering data from single-particles reliably.

Nanoparticle tracking analysis (NTA), also known as single-particle tracking (SPT), is a useful technique to measure the size of individual nanoparticles. It is based on recording the positions of the objects over time and on deriving their diffusion coefficient. Thereby, using the Stokes-Einstein relation, it is possible to calculate the diameter of each particle. In conventional flow channels, the number of frames available for particle tracking is limited because they move in and out of the focal plane. A lower number of frames limits the accuracy of the method for sizing applications.

Particles moving in and out of the focal plane result in intensity fluctuations. Moreover, since every particle follows a different trajectory, the recorded average intensity is prone to systematic errors. Since the scattering cross section scales as the sixth power of the diameter, measuring the scattering intensity from a single particle, reliably, is much more sensitive to changes in size than the diffusion coefficient alone.

To overcome the limitations mentioned above, we have developed a novel technique using hollow-core optical fibers. By confining nanoparticles' movement to an essentially 1-dimensional trajectory, we eliminate the background generated by particles outside the measurement plane. Moreover, the confinement guarantees that each particle can be tracked for thousands of frames, providing a better accuracy to diffusion measurements. Additionally, since the illumination profile is homogeneous along our geometry, scattered-intensity data can achieve higher accuracy when measuring sizes.

## NANOCET TECHNOLOGY

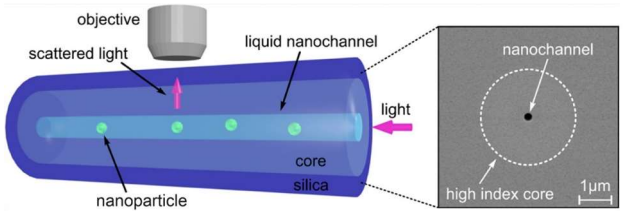


Figure 1: A 633 nm laser is coupled to the fiber, and the light scattered from single-particles is imaged onto a camera through a microscope aligned perpendicularly to the fiber axis.

NanoCET instruments use the scattered light from nanoparticles inside hollow-optical fibers such as the one shown in Fig. 1. The particles' trajectories are imaged using an optical microscope while they diffuse. Both the movement and intensity are used to calculate each nanoparticle's size using a custom software and a self-calibrating procedure.

The custom-designed optical fibers confine the particles to an essentially 1-D movement and guarantee that they are always in the microscope's focal plane. They also guarantee a homogeneous illumination across the field of view. With the current generation of nanoCET devices, a 50 mW, 633 nm laser is automatically coupled into the fiber through a 3-D piezo stage. The nano-channel diameter can be chosen between 200 nm and 700 nm, depending on the application.

To introduce the sample into the channel, a droplet of the suspension of particles (which can be as small as 1 µl) is placed at the end of the fiber opposite the incident laser. Capillary forces pull the liquid into the channel, and the measurement begins. Since the movement of the particles is restricted to a single path, it is enough to record a small area of the camera, typically 16 px × 1900 px. The measurement lasts until the liquid fills the capillary, which typically takes 5 minutes. The amount of data generated in this configuration, even at 1000fps, can be easily handled and analyzed by most computers.

## EXAMPLE APPLICATION: MEASURING SIZE DISTRIBUTION OF 60NM POLYSTYRENE PARTICLES

After depositing a droplet of the sample at the end of the channel, the liquid is pumped inside by capillary forces. While the liquid flows towards the other end of the fiber, each particle appears as a bright spot moving along the camera's horizontal axis. The data generated by the nanoCET device in an entire measurement can be compressed to a 2D image. Each frame is transformed into a 1-dimensional dataset by summing all pixels perpendicular to the optical fiber axis. Each 1-D frame is stacked horizontally, generating an image like the one shown in Fig. 2.

The vertical axis is the position along the fiber, while the horizontal axis is the timestamp of the frames. Each bright-line corresponds to a 60 nm polystyrene particle moving along the fiber center. Since particles are confined and allowed to move along the channel, we can consistently acquire thousands of frames per particle.

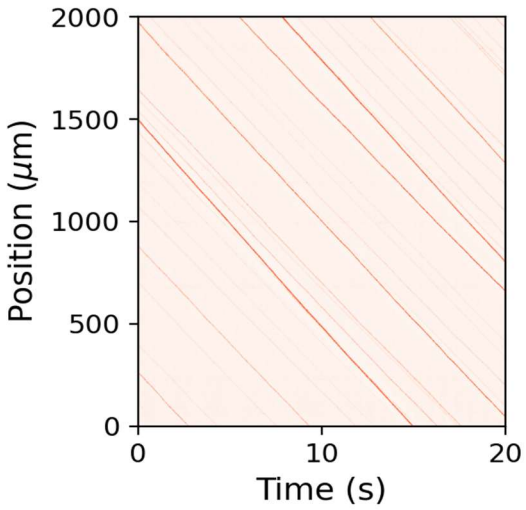


Figure 2: 60 nm polystyrene particles diffusing in a nano-channel, at 200 fps.

A higher number of frames is fundamental to achieving higher accuracy for the diffusion coefficient and the scattering cross section of each particle. In the example shown in Fig. 2, images were acquired at 200 fps. The device can easily perform measurements at 1000 fps if particles are sufficiently bright.

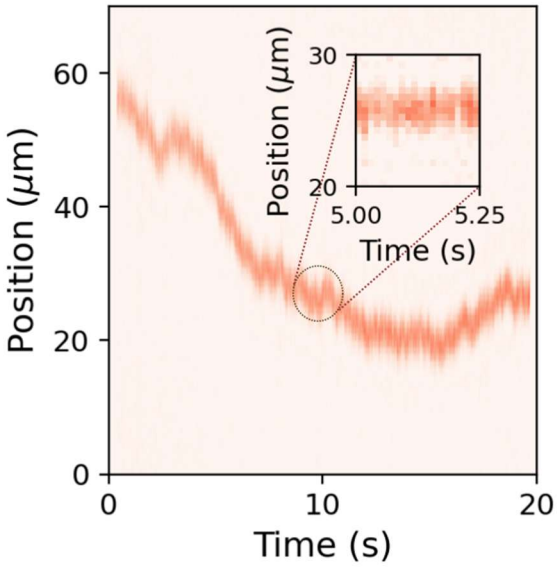


Figure 3: Trace of a 60 nm polystyrene particle after correcting for the fluid drag. The inset shows a detail of the trace where each individual frame and pixel can be recognized.

Our analysis software is based on a modified version of the Crocker-Grier algorithm adapted for one-dimensional data to find each particles' position in every frame. Fig. 3 shows the movement of a single particle once the drag of the fluid has been taken into account. From this example, we can see that the scattered intensity is constant across the entire field of view, and we can accurately calculate the particle's position in over 2000 frames.

We repeat the same procedure for the data of each particle. First, we locate the particles on each frame. By looking at the neighboring pixels in the frames before and after, we can link the positions that belong to the same particle and label them accordingly. Then, we build trajectories of single particles that contain the location, intensity, and timestamp. This information is required to calculate the average intensity of single particles along a trace and their diffusion properties.

As each particle moves along the fiber, we record its intensity. From Rayleigh scattering, expected for the particle in this size range, we know that the cross section scales as the sixth power of the diameter:

$$\sigma_S = \frac{2\pi^5}{3}\frac{d^6}{\lambda^4}\left(\frac{n^2-1}{n^2+2}\right)^2 \quad (1)$$

We can convert the recorded intensity to the scattering cross section, and therefore to the diameter, by calibrating the measurement as we will discuss later. The other parameters in the equation are known: the wavelength of the incident light, and the difference of refractive index of the particles and the medium surrounding them.

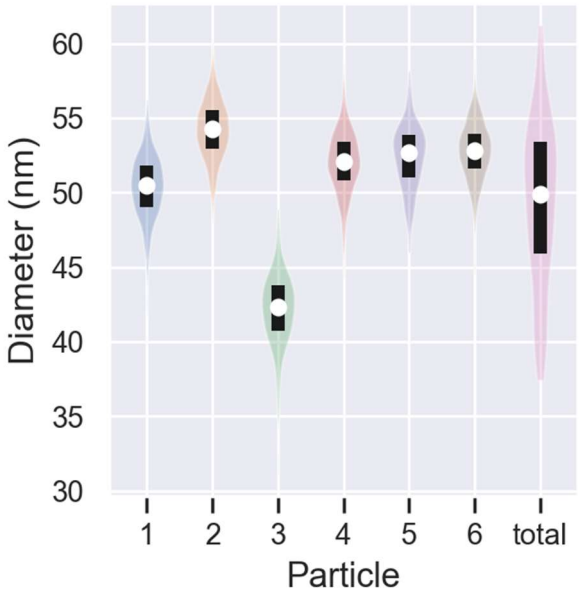


Figure 4: Examples of the uncertainty in size-measurements derived from the scattered intensity of 60nm polystyrene particles.

We can measure the apparent size of each particle in every frame. It will give us a distribution of diameters for individual particles that can be used to estimate the procedure's accuracy. Fig. 4 shows examples of the calculated sizes for various particles. We can see that each particle has a narrow distribution of sizes, much narrower than the sample's overall distribution (rightmost column). The standard deviation for each particle measurement is approximately 4%, or 2.4 nm.

We use the average intensity of all the tracked particles to calculate the sample's distribution of sizes. Fig. 5 shows the histogram after analyzing more than 160 traces. The standard deviation of the distribution is 9.5 nm which is in excellent agreement with the 9.1 nm deviation reported by the manufacturer. Since the error in calculating each particle diameter is much smaller than the coefficient of variation of the sample, we can be confident that there is no broadening due to the method.

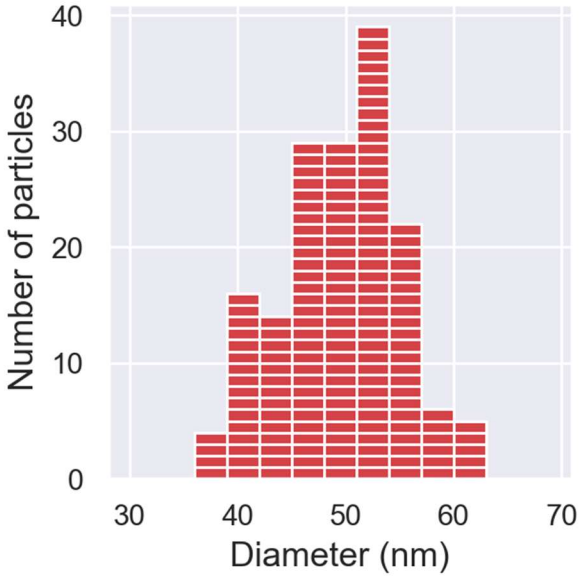


Figure 5: Distribution of sizes in the sample agrees with the specifications of the manufacturer.

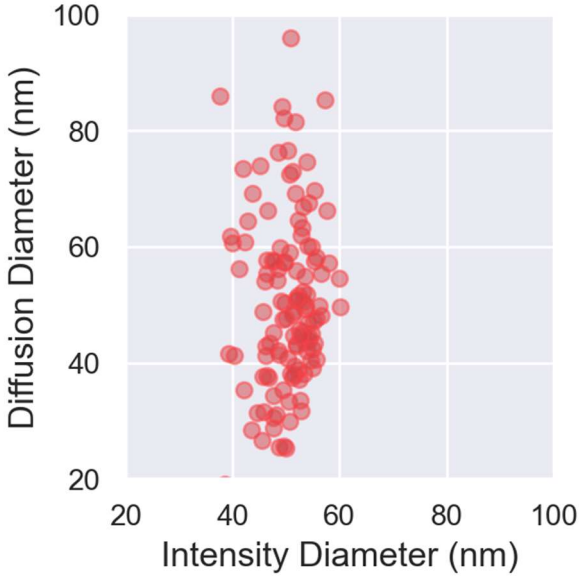


Figure 6: Distribution of diameter and intensities

Alternatively, we can use the particles' location to calculate the diffusion coefficient and derive the diameter from it. This is the standard procedure in an NTA experiment. To calculate the diffusion coefficient, we must retrieve the precise position of each particle at every timestamp. Particles undergoing Brownian motion will show the following relationship between diffusion coefficient (D) and mean-squared displacement ($< x^2 >$):

$$< x^2(\tau) >= 2\,\mathrm{D}\,\tau \tag{2}$$

Where τ is the time-delay between positions. We can calculate the diffusion coefficient using the location information as the one shown in Fig. 3. The last step requires the Stokes-Einstein relationship to calculate the diameter of the particle from its diffusion coefficient:

$$\mathrm{D} = \frac{k_b\,T}{3\pi\eta\,d} \tag{3}$$

The equation requires that the temperature and viscosity are known to be able to retrieve the diameter. In the fibers, the diffusion must be corrected by a hindrance coefficient that is well-modelled or the diameter will be under-estimated. This method's accuracy is mostly limited by the localizition accuracy and the number of frames.

Fig. 6 shows the distribution of diameters calculated from the diffusion coefficient and every particle's average intensity. It is visible that the distribution obtained from the diffusion is much broader than the distribution obtained from the particles' intensity. The power of the measurement through the diffusion coefficient lies in the absence of a calibration requirement. Knowing the temperature and viscosity of the medium is enough to know the size of an object.

Even though the diffusion-based measurements' accuracy is less precise for each individual particle, the distribution's median is still representative assuming a sufficiently mono-disperse sample. To achieve a **self-calibrating** measurement, we used the median size of the particles obtained from diffusion to calculate the normalization factor for the intensity measurements. With this approach, no a-priori knowledge of the sample was assumed.

## CONCLUSIONS

NanoCET devices allow researchers to characterize nanoparticles with an accuracy not possible before. The hollow optical fibers deliver unprecedented signal-to-background ratios, which allows the detection of smaller particles at higher frame rates. These particles can be as small as viruses of few tens of nanometers, or gold spheres as small as 10 nm. The long traces result in precise intensity measurements and reliable diffusion coefficient calculations.

In the example measurement, we have shown how to convert intensity to diameter through the Rayleigh scattering model. It is essential to point out that the intensity can yield other relevant parameters. For example, it can characterize a sample with a mixture of particles of different materials but with the same size. Alternatively, it is possible for shell-like particles to think about the intensity as a measure for mass.

It is possible to obtain self-calibrating measurements by combining the measurement based on the diffusion coefficient and the one based on the scattered intensity.

Even though NTA results are less accurate, their median values represent the sample as a whole. This information is, in turn, used for the intensity-based measurement, making the entire measurement calibration-free and without requiring a-priori knowledge of the sample.

## ABOUT DISPERTECH

Dispertech B.V. develops and markets systems and solutions for single nanoparticle analysis. Dispertech uses advanced nanofluidics and optics to analyze scattered light signals of nanoparticles. The technology enables measurement of both scattering cross section and diffusion coefficient of individual nanoparticles down to 20 nm at the same time and with unprecedented accuracy.

Dispertech focuses on the market of academic and industrial research in the field of life science (such as extracellular vesicles, virology and nanomedicine) and material science.

The technology of Dispertech was developed in 2016 by Dr. Sanli Faez at Leiden University. In 2018 Dr. Faez was awarded an NWO Take-off Fase 1 grant to conduct a technical and commercial feasibility study. At the beginning of 2019, Dispertech B.V was founded and seed capital was provided by Nascent Ventures, a VC focused on advanced instrumentation. In 2020, Dispertech received additional funding as part of NWO Take-off Phase 2 for the commercialization of the technology. The company has its office and lab at the Sciencepark in Amsterdam.

More information: visit www.dispertech.com or send an email to contact@dispertech.com