Nanoparticles Size and Concentration Characterization by Corning® Videodrop

Interferometric Light Microscopy

Application Note

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Introduction

Here we describe the principles of Interferometric Light Microscopy (ILM) technology and how it allows the Corning Videodrop to measure the size and concentration of nanoparticles (NPs) in a microliter droplet solution without labeling and in less than a minute. ILM uses a transmission brightfield microscope used as a homodyne interferometer to detect, count, and track nanoparticles. Counting particles allows to measure the concentration, while tracking their Brownian motion allows to measure individually their hydrodynamical diameter and compute the sample size distribution. ILM is the combination of an optical system and image processing algorithms. We also explain how ILM differentiates itself from other nanoparticles analysis techniques and why it can be particularly relevant for in process quality control measurements, especially for bioproduction applications.

Whatever their nature, biological (virus, extracellular vesicles) or physical (plastic, metallic) particles in solution that are small enough (<400 nm) are constantly submitted to random collisions with the fluid molecules that generate their motion. This random movement of a NP is called Brownian motion and is driven by thermal agitation of surroundings molecules. The smaller the particle, the more sensitive it is to collisions, and the greater is its Brownian motion. This is exemplified by the simulation of a 1-second long 2D displacement of a 400 nm (blue) and a 100 nm (red) NP (Figure 1).

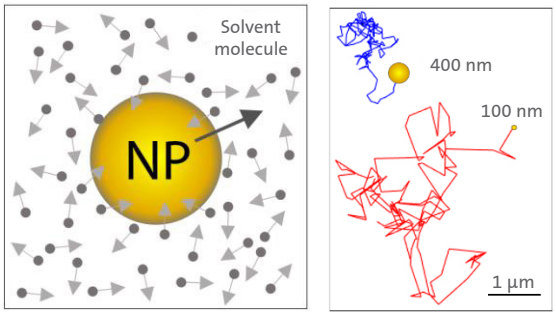

Figure 1. Nanoparticles in solution are constantly moving objects.

Imaging NPs with optical methods is challenging

Optical methods are a natural choice for NP samples characterization. In its simplest form, a visible microscope does not require much sample preparation and is not destructive, while being robust, fast, and easy to use. However, they are not readily adapted to characterize nanoparticles. The diffraction limit does not allow an optical microscope to resolve the details of a structure with a spatial resolution under 200 nm. Objects smaller than this limit are therefore imaged as diffraction limited spots whose sizes do not reflect the real object size.

Secondly, for particles smaller than the illumination wavelength, the amount of light they scatter is proportional to the power six of their radius (Figure 5). Therefore, to decrease 10-fold the minimum detectable object size, a system that detects the scattered intensity must detect signals one million times weaker.

In practice, conventional brightfield microscopes coupled to a standard camera do not have the sensibility to detect the nanoparticles diffraction limited spots that are hidden by different noises, like camera noises.

NPs scatter light

When a NP is illuminated by a light field, optical waves interact with the electrons of the atoms constituting the NP. It induces a dipole that radiates a spherical optical wave if the NP diameter is smaller than the wavelength (Figure 2).

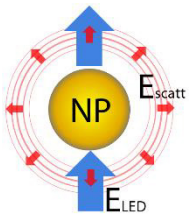

Figure 2. A nanoparticle (NP) illuminated by an incident light E_{LED} (blue) scatters a spherical optical wave E_{scatt} (red).

This phenomenon is called light scattering. The scattered light power is the signature of the NP presence and holds information on the NP physical parameters (Figure 5) as it is proportional to the incident illumination, and their scattering cross section.

The amplitude of the scattered field is tiny compared to incident light which makes it technically challenging to detect. For instance, techniques that also use this scattered signal, such as Nanoparticle Tracking Analysis (NTA) or Dynamic Light Scattering (DLS) collect the light at 90° of the optical path with a darkfield illumination to avoid the illuminating light.

The inventiveness of ILM is to enable the detection of the scattered light directly in the optical path using the simplicity of a conventional brightfield transmission microscope configuration. This is achieved by taking advantage of the physical phenomenon of optical interference that amplifies the weak scattered signal.

Interference is a physical phenomenon in which two optical waves of the same wavelength can interact in certain conditions of coherence. They combine to form interference patterns. The resulting intensity is the sum of the two initial ones plus the intensity of the interference term (Equation 1). When the two interfering fields have different intensities, the intensity of the interference term is bigger than the smaller one.

Videodrop optical system: A brightfield microscope as a homodyne interferometer

The Corning® Videodrop is a custom brightfield microscope designed to detect the NP interference patterns with a single arm whereas conventional interferometers require more complex systems with a second reference arm that are sensitive to vibrations or thermal drifts. Figure 3 describes the Corning Videodrop optical system.

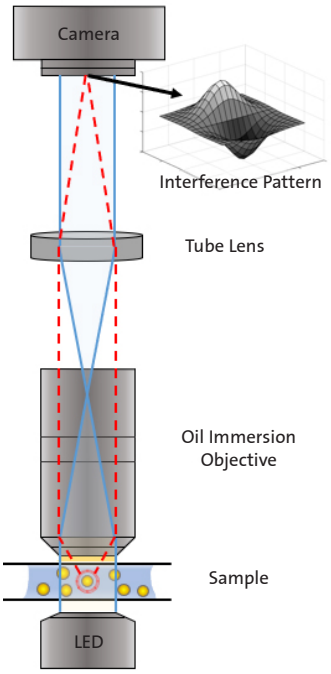

Figure 3. Corning Videodrop optical system

The sample is a 5 µL droplet (placed between two glass slides) containing NPs. The light source, a powerful 2W LED, illuminates in transmission the sample. The NPs scatter a small amount of light. Both the direct LED (blue) and scattered field (red) are collected by the objective and projected on a high dynamic range CMOS camera by the tube lens. On the camera plane, the LED field and the scattered field interfere. A high frame rate movie is recorded in order to be processed.

Those interferences can be described by the following equation giving the intensity I_{cam} measured by the camera.

$$I_{cam} = |E_{LED} + E_{scatt}|^2$$

$$I_{cam} = I_{LED} + I_{scatt} + \underbrace{2\sqrt{I_{LED} \cdot I_{scatt}} \cdot \cos \Phi}_{\widehat{I_{interf}}} \quad (1)$$

With E_{LED}, the LED light field, E_{scatt}, the scattered light field, and the Φ phase shift (due to the difference in propagation paths) in between the two fields on the camera plane.

$$I_{LED} \sim 10^3 \times I_{interf} \sim 10^6 \times I_{scatt}$$

The interference signal is three orders of magnitude larger than the direct scattering signal. Yet the background from the LED illumination is three orders of magnitude larger than the interference patterns of 70 nm beads (see Figure 5 for more details about orders of magnitude). That is why on the intensity recorded by the camera, the NP interference patterns are hidden by the uniform intense background from the LED illumination (see raw image in Figure 4).

A proper background removal is necessary to reveal the interference patterns.

Image processing to remove the LED background

Custom image treatment algorithms are used to subtract the I_{LED} signal from every image of the movie. This is achieved using the fact that I_{interf} is dynamic. The interference patterns are moving as they are generated by NPs undergoing Brownian motion. On the other hand, the LED background is static and can be temporally filtered by subtracting a temporal mean image to each image. Figure 4 illustrates the effect of the background removal.

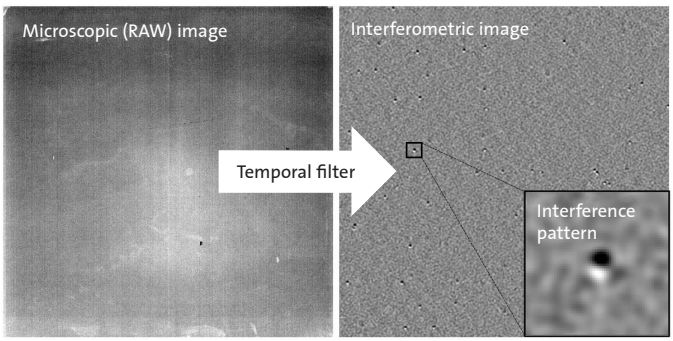

Figure 4. Effect of the image processing to subtract the static LED background from the raw image and to reveal the interference field with its characteristic localized interference patterns shaped like doublets. Each interference pattern corresponds to a NP in the detection volume of the sample.

Each pattern made of a black and a white spot is a localized interference pattern created by a NP and is the signature of its presence and position in the droplet sample.

Further analysis of these interferometric (processed) images allow to compute the NP size and concentration.

The equation (1) describing the interferential phenomenon is fundamental for ILM. Examining the orders of magnitude of its three components gives insight on the sensibility of ILM for NPs detection. We can rewrite it by considering that a fraction f_{col} of the light scattered by the NP is focused on an Airy disk surface S_{Airy}. The amount of light scattered by a particle (of diameter d) is proportional to σ the scattering cross section and the LED field (of wavelength λ). Therefore (1) becomes:

$$I_{cam} = I_{LED} + \underbrace{I_{LED}\frac{\sigma \cdot f_{col}}{S_{Airy}}}_{I_{scatt} \propto d^6} + \underbrace{2I_{LED}\sqrt{\frac{\sigma \cdot f_{col}}{S_{Airy}}} \cdot \cos\Phi}_{I_{interf} \propto d^3} \quad (2)$$

The expression of the scattering cross section in the Rayleigh regime (i.e., when λ >> d) is:

$$\sigma = \left(\frac{2 \cdot \pi^5}{3}\right)\frac{d^6}{\lambda^4}\left(\frac{n_{NP}^2 - n_{water}^2}{n_{NP}^2 + 2 \cdot n_{water}^2}\right)^2$$

And the surface on which this light is focused is $S_{Airy} = \pi \cdot \left(\frac{\lambda}{1.22 \cdot NA}\right)^2$

For a diameter d = 70 nm large spherical bead of polystyrene with an optical index n_{NP} = 1.49 in water (n_{water} = 1.33) with a light source wavelength of λ = 450 nm, using an objective with a numerical aperture NA = 1.25 and a collection fraction f_{col} = 0.22 we have:

$$\frac{I_{scatt}}{I_{LED}} = 2.9 \cdot 10^{-6} \text{ and } \frac{2\sqrt{I_{LED} \cdot I_{scatt}}}{I_{LED}} = 3.4 \cdot 10^{-3}$$

We see in this calculation that the direct scattering is six orders of magnitude smaller than the reference beam, while the interference signal is only around one permille of the reference beam. The interference amplifies the signal by a factor of one thousand.

Figure 5. Scattered light – Orders of magnitudes.

How is NP concentration measured?

The interference patterns are automatically identified on each interferometric image. The number of NPs (nb_{NP}) is counted. By knowing the volume in which the objects are detected one can compute the NP concentration ($C = nb_{NP}/V$). This volume depends on the NP size (see Figure 6 for more details). This dependence has been characterized (intensity calibration) and each nanoparticle can be associated with a detection volume.

The final sample concentration is therefore simply the mean number of detected particles weighted by their detection volume.

$$C_{sample} = \sum_{i\ NP} \frac{1}{V_i}$$

With V_i the detection volume of the i^{th} nanoparticles.

The detection volume is a thin squared layer which dimensions are defined by the microscope and NPs' characteristics. Its width is imposed by the optical magnification (×187.5) and camera sensor dimensions (1 cm^2) . Its thickness depends on the microscope optics, but also on the amount of scattered light. Larger particles scatter more light and produce more intense interference patterns. These patterns are above the camera noise level and can be detected even when a little defocused (i.e., in a larger volume). The typical detection volume is 15 pL (58 x 58 x 5 µm). Figure 6 shows the detection volume as a function particle size (for PS beads).

Due to their constant movement, the NPs present in the detection volume are renewed every 16 s (typical value for 100 nm NP).

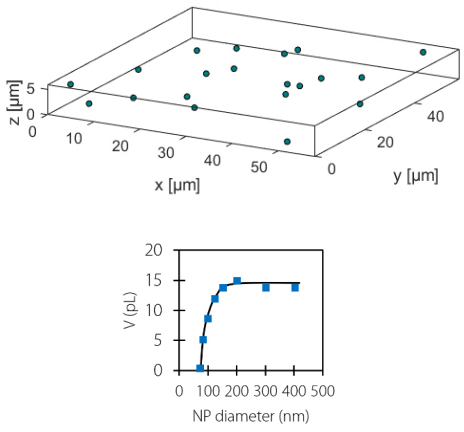

Figure 6. Detection volume.

How is the size distribution measured?

The NPs detected move randomly in the three dimensions of the detection volume (Figure 1). The camera images the 2D projection of these movements through the interference patterns displacements. Interference patterns are used to localize the corresponding NPs on every picture of the movie. The tracks of each NP are reconstructed between all the pictures of the movie as shown in Figure 7.

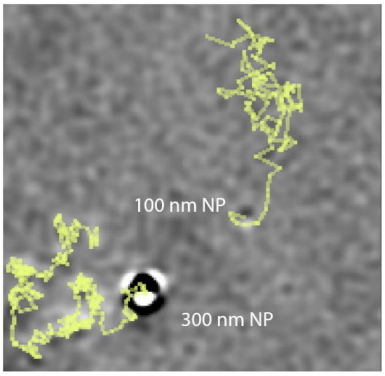

Figure 7. Tracks of two interference patterns (yellow lines) recorded during an acquisition. One particle has a 300 nm diameter and the other a 100 nm diameter.

The NP diffusion is driven by the thermal agitation energy ($k_b \cdot T$) of the surrounding molecules and held back by the mobility of the nanoparticle that decrease with its size and the medium viscosity (μ). Therefore, the diffusion of a nanoparticle is linked to its size, making it possible to measure it through an analysis of its movement.

The mean square displacement ($d^2(\Delta t)$) of a particle in between two successive frames spaced by a time Δt can be experimentally measured and is proportional to the particle diffusion coefficient D:

$$d^2(\Delta t) = 4 \cdot D \cdot \Delta t$$

The measured diffusion coefficient is then used to compute the particle hydrodynamical radius R_h using the Stoked-Einstein relation.

$$R_h = \frac{k_b \cdot T}{6 \cdot \pi \cdot \mu \cdot D}$$

This method allows to measure individually the hydrodynamic radius of each nanoparticle and compute the size distribution of the sample. Figure 8 illustrates size distribution histograms measured with this method.

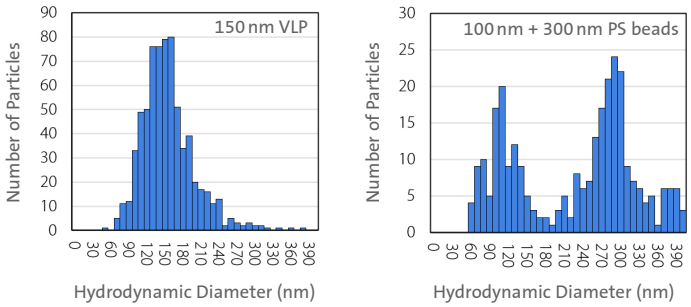

Figure 8. Example of size distribution results obtained with the Corning Videodrop for monodisperse sample of virus-like particles (VLPs) of 150 nm (left) and for a polydisperse mix of 100 nm and 300 nm polystyrene beads (right).

Technical differentiators of ILM

Among other NP optical characterization techniques, ILM is the only commercially available technology to use the interference phenomenon for label-free detection of NP in bulk. The single arm interferometer configuration of the Corning® Videodrop allows to:

- Use a simple setup, without fine-tuning and calibration.
- Work with a 5 µL droplet sample with various viscosities (enabled by the absence of fluidic path).
- Obtain a wider detection dynamic range which enables to image simultaneously NPs with large size differences. Indeed, in the limit of small NPs relatively to λ, the interference signal depends on the particle size to the power 3 whereas it is to the power 6 for scattered intensity (Figure 5). It means that ILM is far less sensitive to sensor saturation by bigger NPs than NTA for instance.
- Visualize microscopic (500 nm to 10 µm) objects and characterize nano-object with a single acquisition. The raw images are indeed optical transmission microscopy images where bigger objects are visible.

Interferometric detection coupled with low coherence LED illumination and image temporal filtering make the whole system particularly robust to external factors. The Corning Videodrop requires low maintenance.

ILM is based on particles tracking that individually contribute to the size distribution measurement. It is well adapted to characterize polydisperse samples, which is challenging when using global methods like DLS that measure the sample as a whole.

Within particles tracking based techniques ILM has an advantage for size measurement accuracy. Because of the interferometric nature of the system, the light beam captured by the camera is very intense. This large quantity of light enables to shorten the exposure time (less blurring) and increase the frame rate of the camera. It leads to better localization of the NPs and longer tracks. The greater those two parameters are the better is the size measurement accuracy.

Measurement features

Size range:

- 50 to 500 nm for metallic particles
- 80 to 500 nm for biological particles

Concentration range: 10^8 to 10^{10} part./mL
Time to result from 40 s.

Conclusions

ILM is a particle tracking based method for size and concentration characterization of various NPs:

- Biological particles such as viruses, extracellular vesicles, or phages
- Metallic particles such as gold or oxide particles
- Silica particles
- Polymeric particles such as Polystyrene beads
- Microemulsions, etc.

It distinguishes from other techniques by using interferometric amplification of the NP scattering signals. Its unique features make it particularly suited for bioproduction quality control application.

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