Abstract

Cell-derived vesicles are spherical sub micrometer particles present in body fluids like blood and play a role in a variety of homeostatic processes. The possibility of vesicles to be used for prognosis and therapy has increased their clinical interest. However, the detection of vesicles is cumbersome because of their small size and heterogeneity. Therefore, novel optical techniques that detect scattering from vesicles are being explored. The amount of light scattered by a vesicle depends on its size and refractive index. Knowledge on the refractive index of vesicles may provide insight in the detection limit of novel techniques and provide a label-free parameter to differentiate vesicles from other particles. However, the refractive index of vesicles smaller than 500 nm has never been measured.

In this work we have developed a procedure to determine the refractive index of vesicles in solution by nanoparticle tracking analysis (NTA). NTA is a technique where particles in suspension are visualised using a dark field microscope and a video sequence is recorded. NTA determines the diameter of a particle by tracking its Brownian motion through the video sequence and also measures the particle’s scattering intensity in every frame.

To find the relation between the diameter, refractive index, and light scattering of a particle, we measured the light scattering from beads of known diameter and refractive index and described the results by Mie theory. Mie calculations were used to make a lookup table to find the refractive index for a measured diameter and scattering intensity of a particle.

As a proof of principle, the refractive index of polystyrene and silica beads is measured with an accuracy of 1.5% and 3.6%, respectively. The refractive index of vesicles from urine is found to range from 1.35 to 1.50 with a peak at 1.35 and shows a decrease with increasing diameter. This agrees with expectations and a possible diameter dependent refractive index caused by the vesicle structure.

For vesicles from plasma, we obtained a distribution that shows an equal frequency of particles with a refractive index between 1.35 and 1.5. It also shows particles with a refractive index greater than 1.5, which suggests the presence of other particles with a relatively high refractive index like lipoproteins.

In conclusion, NTA can be used to assess the refractive index of sub micrometer particles in a liquid. We expect that improvements in the scattering intensity attributed to a particle, the hardware and the measurement procedure may contribute to an increase in the accuracy of the refractive index determination with NTA.