HORIBA Scientific

Application Note

Particle Size Distribution and Concentration of Exosomes

AN218

Introduction

Previously regarded as merely being extracellular "junk," there has been a noticeable growth in research of exosomes due to their recently discovered role in transferring genetic material and intercellular communication. As such, their potential as biomarkers and as means of drug delivery has drawn great interest in the scientific community. However, further developments in the research of exosomes has always been limited by the technology used to analyze them. Therefore, solutions have been sought in order to accurately characterize exosomes via particle size and concentration.

Current existing solutions include methods such as electron microscopy, conventional flow cytometry, and dynamic light scattering. Each method has its own specific drawbacks. For example, electron microscopy requires extensive sample preparation, conventional flow cytometry cannot detect particles much less than 300 nm in size, and dynamic light scattering cannot accurately measure mixtures of microvesicles and exosomes.

One promising method, however, is nanoparticle tracking analysis (NTA). This method provides real-time analysis of nanoparticles by using the Brownian motion of a particle to calculate its particle size and the total concentration of the particles in solution. The main limitation of NTA has been the relatively narrow range of particle sizes that could be analyzed in a sample. Until recently, all commercial NTA systems featured a single laser as a light source. For a given set of parameters (laser power, camera gain, shutter speed, etc.), particles that were larger than optimal would saturate the camera over a wide area, precluding accurate particle tracking. Particles that were smaller than optimal could not be tracked at all.

The ViewSizer 3000 features three simultaneously operating lasers with independently adjustable power and a color camera. In this way, the data from each laser is collected simultaneously as a separate video (color) stream. In addition, the system is designed to ensure only diffusive motion can be captured, eliminating the need for artifact inducing drift correction schemes. Finally, with vigorous stirring between short videos, the suspension is randomly sampled to ensure a representative result.

Figure 1: Image of the ViewSizer® 3000

In this study, we demonstrate the ViewSizer 3000's (Figure 1) capabilities as a next generation NTA based analysis instrument in order to accurately and efficiently measure and characterize exosomes via particle size and concentration.

Materials & Methods

Exosome Samples: Human Preadipocyte (Mesenchymal Stem Cell) Exosomes (100 ug) sample was acquired from ZenBio and was provided as a frozen solution. Upon receipt from ZenBio, the exosome sample was separated into 15ul aliquots in order to avoid constant thawing and re-freezing which would be harmful to the sample. These exosome sample aliquots were stored at -20°C and were allowed to thaw and equilibrate to ambient laboratory temperature prior to analysis. When preparing dilutions for measurement, the exosome sample was diluted using 20mM PBS pH 7.4.

Instrument and Data Collection: Measurements were carried out at ambient laboratory temperature using the ViewSizer 3000. The measurements were recorded with the following parameters: frame rate: 30 frames/sec; exposure: 15 msec; gain: 30; blue laser power: 210 mW; green laser power: 12 mW; and red laser power: 8 mW; temperature control: active, 22°C. 25 short videos were collected with 5 seconds of stirring between each video to ensure completely independent sets of particles in each video.

By using short 10 second videos and ignoring particles that drifted into the video, diffusion related counting errors were completely eliminated. Prior to analyzing the sample, the sample cuvette, insert, and stir bar must be washed with water and ethanol. To verify its cleanliness, approximately 400 microliters of the PBS buffer was placed into the cuvette and the image streamed and observed to confirm that there were few, if any, particles present in the cuvette. Once it had been verified that the cuvette was sufficiently cleaned, the buffer was pipetted out and 400uL of a 1000x dilution of exosome sample was pipetted in. The data was then recorded as per the settings described above.

After recording measurements, the data must be processed and plotted. Prior to doing that, the processing and plotting settings were set to the following: Auto detection override disabled, with a feature diameter of 30 and "Main Chart" volume factor LogBinSilica was selected. This volume factor table accurately accounts for the variation in effective measurement volume with particle scattering power. Once the data was processed, all relevant data and results could be reviewed.

Results

The ViewSizer 3000 system is an advanced instrument for particle counting and sizing. It tracks particle Brownian motion. Distinct from the conventional NTA systems that uses one laser (one wavelength) to illuminate particles in the colloid, the ViewSizer 3000 instrument includes a patented system of three solid-state lasers with wavelengths of 445 nm, 520 nm, and 635 nm. Due to the range of laser powers used, varying the power a wider range of particle sizes in the same sample can be analyzed. The combination of the 3 lasers overcomes the common drawback of conventional NTA which is the failure to size particles accurately in a polydisperse sample.

The particle-size distribution was measured for an exosome sample (1000 X dilution) by tracking the particle's Brownian motion. Results showed a profile with D50 particle diameter of 148 nm and total particle concentration of 5.7 x 10^7 particles/mL (Figure 2). For comparison, the certificate of analysis provided by ZenBio for the exosome sample states a mean particle diameter of 139.1 nm and an undiluted concentration of 7.2 x 10 particles/mL. These measurements were taken by ZenBio via Tunable Resistive Pulse Sensing (TRPS, Izon Science Ltd) using a NP150 nanopore membrane at a 47nm stretch and calibrated with 110 nm PSL at 1.1 x 10^{13} particles/mL.

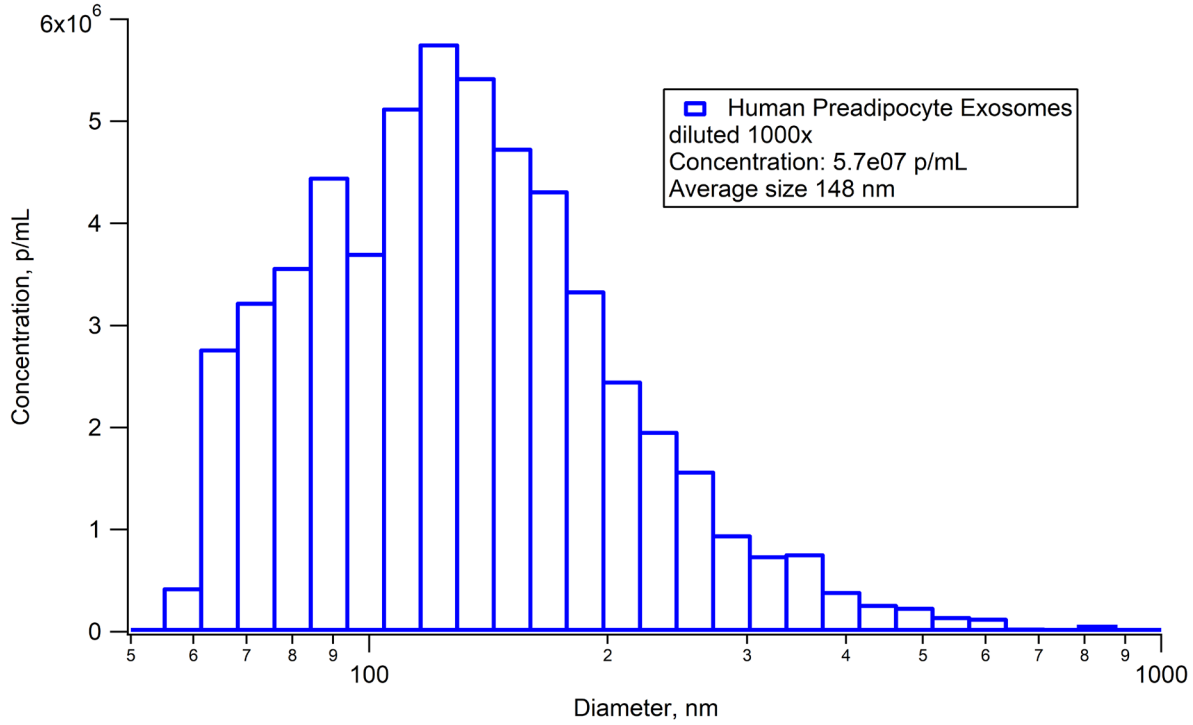

Figure 2: Results reported from the measurement of the 1000x dilution sample of exosomes after processing and plotting shown on the right. Graph depicting the particle size distribution of the sample shown on the left. Particle size by diameter in nm is shown on the x-axis and particle concentration is shown on the y-axis.

Conclusion

From the data collected, it can be observed that exosome measurements by ViewSizer 3000 yielded results close to the method employed by ZenBio, and thereby demonstrate the ViewSizer 3000's unique capabilities with regards to providing real-time size-distribution data of exosomes and direct measurement of concentration.

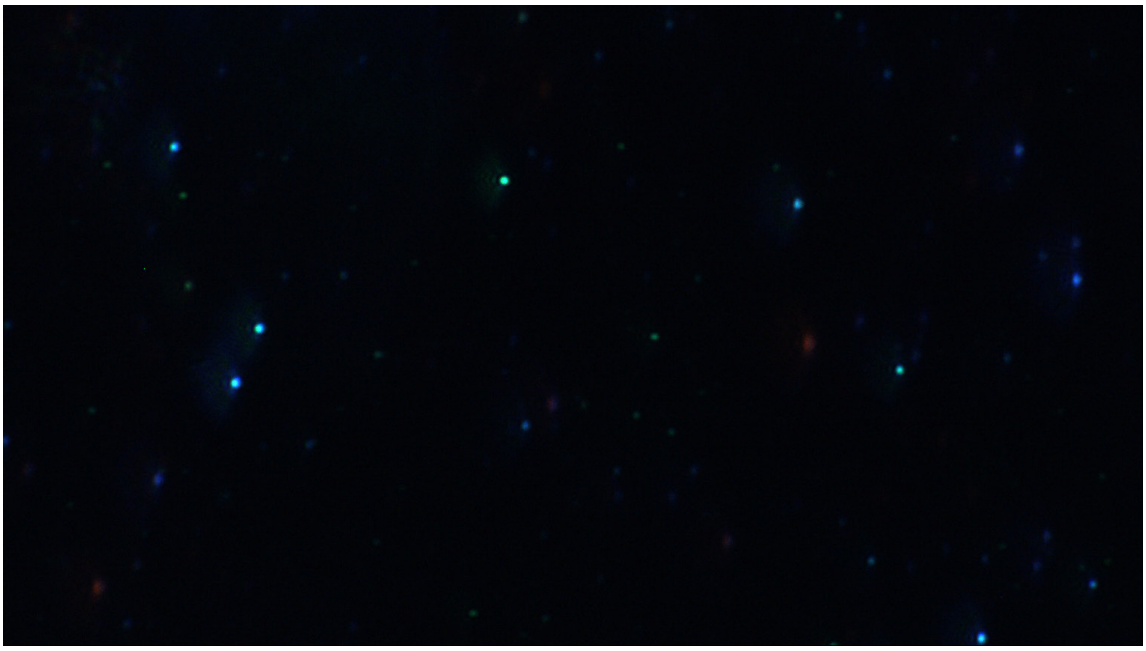

Figure 3: Image of the 1000x dilution sample of exosomes under analysis via NTA. Imaged spots are scattering from individual particles in the sample. Particles were illuminated with blue, green, and red lasers at the settings described above.

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