KINETIC RIVER®

Delaware Flow NanoCytometer®

Where Light Meets Life® Tech Note

with materials from

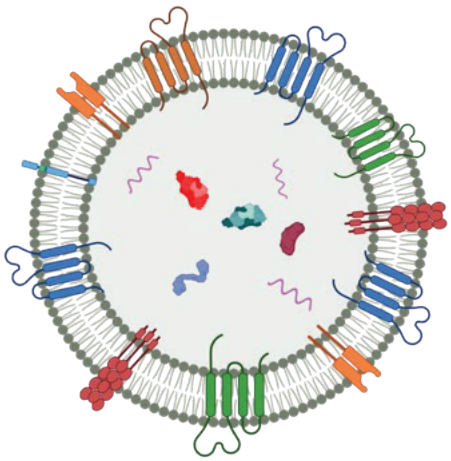
HansaBioMed Life Sciences

Pioneering the Extracellular Vesicles field

Multiparameter Exosome Analysis on the *Delaware* Flow NanoCytometer

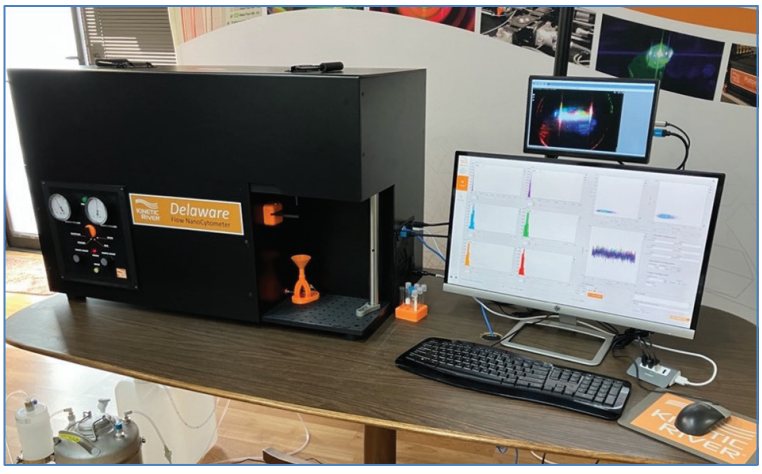
Introduction. Extracellular Vesicles (EVs), such as exosomes and microvesicles, are nanoscale subcellular particles secreted by cells and used for intercellular signaling and transport.

EVs can be characterized in several ways, including: (i) light scattering; (ii) membrane staining; and (iii) antibody binding. Light scattering carries information on the size of the EV; membrane staining can help distinguish EVs from protein aggregates and other background nanoparticles in the sample; and binding with fluorescent antibody conjugates can selectively identify different EVs based on their surface protein expression, such as that of tetraspanins CD9, CD63, or CD81.



EVs consist of a lipid membrane bilayer with surface and transmembrane proteins, and of a luminal space that can, depending on the source of the EV, contain proteins, RNA, or other cellular molecular material.

The *Delaware* Flow NanoCytometer was designed from the ground up as a flow cytometer for analyzing nanoparticles, such as exosomes and other extracellular vesicles (EVs). It combines superior sensitivity (≤ 68 nm liposomes; ≤ 60 nm polystyrene; ≤ 28 nm gold) and resolution (6 nm) with the ability to measure fluorescence on up to six channels, high throughput, and ease of use. The ***Delaware*** Flow NanoCytometer has sufficient sensitivity to detect sub-100-nm EVs on a single-particle basis by light scatter as well as by fluorescence. The five-laser ***Delaware*** configuration offers up to three channels of light scattering and up to six channels of simultaneous fluorescence detection—enough for a membrane stain, the three main tetraspanins, and a cargo-specific marker, with optionally a channel for negative discrimination (such as CD45). Its application is not limited to EVs; it can just as easily measure whole cells and other particles, up to sizes of tens of microns, by simply loading a different settings file.



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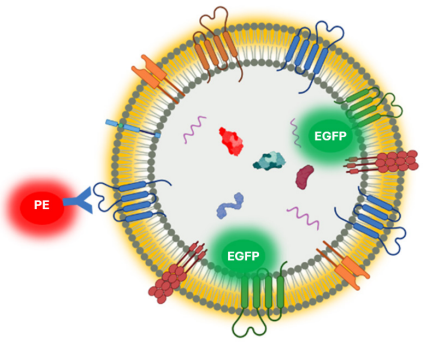
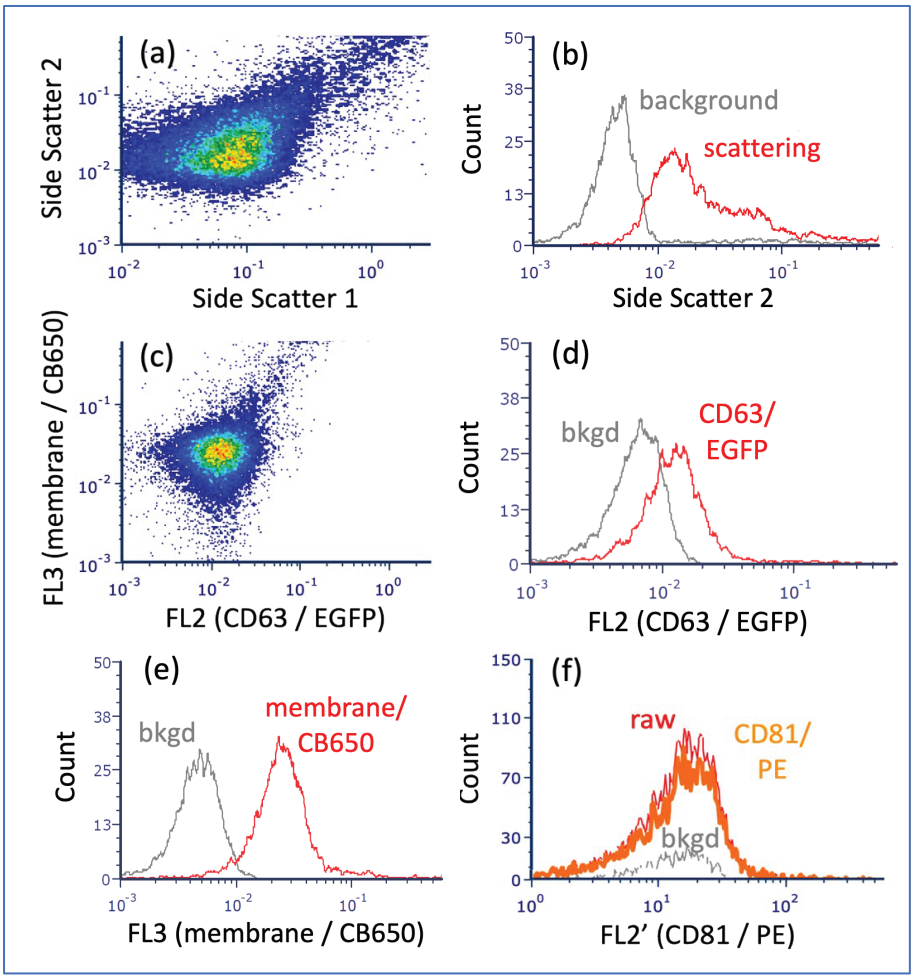
Analysis of EVs on the *Delaware* Flow NanoCytometer. To demonstrate the capabilities of the ***Delaware*** for analysis of EVs, we have performed measurements on the FluoEVs provided by HansaBioMed Life Sciences. FluoEVs are stably labeled HEK293-derived EVs, expressing EGFP as a fusion protein with the EV surface marker CD63. Independent measurements by a ZetaView NTA indicate a mean size for FluoEVs of around 90 nm. The panels (a) and (b) below show the EV population detected on the ***Delaware*** by both UV and visible light scattering.



HansaBioMed FluoEVs were characterized on the ***Delaware*** using EGFP expression (green), fluorescent tetraspanin markers (red), membrane stains (gold), and cargo labels.

Next, membrane staining using a Biotium dye brings out the EVs clearly against background in fluorescence channel 3 (here assigned the spectral band 645–695 nm—panels (c), (e)). Simultaneously, EGFP, genetically engineered to co-express on the CD63 tetraspanin, shows up in fluorescence channel 2 (here assigned the band 500–550 nm—panels (c), (d)). Additionally, staining the EV sampled with phycoerythrin-conjugated CD81 tetraspanin (PE/CD81) produces a distinct signal above background (in fluorescence channel 2, reassigned in panel (f) to the PE band 565–580 nm).

Conclusion. As this Tech Note shows, the ***Delaware*** readily measures EVs simultaneously by light scattering and on multiple channels of fluorescence. This makes it uniquely suited to the demanding needs of exosome biology research and vaccine research and development, as well as quality control in production of nanotherapeutics, pharmaceuticals, and other nanoparticles.



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