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Benefits of imaging flow cytometry for the analysis of nanoparticles in the biological environment.

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Exosomes are natural nanoparticles secreted by different cells and capable of carrying protein markers and genetic information, thus participating in intercellular communication. There are serious reasons to believe that the quantitative and qualitative characteristics of microparticles produced by cells of various tissues in normal and pathological ways can provide significant diagnostic and prognostic information and serve as a biomarker for various diseases, including oncological diseases.

Only recently has the importance of extracellular vesicles as key mediators of intercellular communication been appreciated. Extracellular vesicles are membrane derived structures that include exosomes, microvesicles and apoptotic bodies. Exosomes have been shown to transfer molecules between cells and have the potential to transfer signals between cells. Exosomes are released under normal physiological conditions; however, exosomes are also believed to serve as mediators in the pathogenesis of neurological, vascular, hematological and autoimmune diseases as well as cancer. Quantifying and characterizing exosomes in a reproducible and reliable manner has been difficult due to their small size (50 – 100 nm in diameter). Exosomes analysis can be done using high-magnification microscopy; however, this technique has a very low throughput. Attempts to analyze exosomes using traditional flow cytometers has been hampered by the limit of detection of such small particles and low refractive index. To overcome these limitations we have employed multispectral imaging flow cytometry that has the advantage of combining high throughput flow cytometry with higher sensitivity to small particles and the added benefit of imaging that can provide visual confirmation of particle integrity and characterization. In this study we use multispectral imaging flow cytometry to investigate the interaction of exosomes with white blood cells. Exosomes derived from Jurkat cells will be investigated for their preferential interactions with blood cell subsets by combining immunophenotyping with morphological parameters to measure their binding and internalization.

Exosomes derived from Jurkat cells were labeled with anti-human CD63-AF647 and added to human white blood cells. The cells labeled for immunophenotyping, fixed, and then labeled with anti-human CD63-PE to identify external exosomes. By plotting Internalization vs Bright Detail Similarity we were able to identify 3 populations: Internal Exosomes, External/Internal Exosomes, and Co-localized External Exosomes. Neutrophils, monocytes, and lymphocytes were identified by immunophenotyping; we investigated what % of each blood cell subset was associated with the CD63-AF647 labeled exosomes and whether the exosomes were internalized or external. The monocytes had the highest % of cell associated with CD63-AF647 labeled exosomes at 67%. And in all of the cell types the majority of the cells associated with CD63-AF647 labeled exosomes were either internalized or partially internalized (External/Internal Exosomes population).

The ImageStreamX MkII imaging flow cytometry platform has the quantitative power of large sample sizes common to flow cytometry with the information content of microscopy. This study showed a method to determine if exosomes have been internalized by the different blood cell subsets in an objective, quantitative, and statistically robust manner