

## Review Article

## Flow cytometry what you see matters: Enhanced clinical detection using image-based flow cytometry

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Inflammation

Diet-induced weight gain

## ABSTRACT

Image-based flow cytometry combines the throughput of traditional flow cytometry with the ability to visually confirm findings and collect novel data that would not be possible otherwise. Since image-based flow cytometry borrows measurement parameters and analysis techniques from microscopy, it is possible to collect unique measures (i.e. nuclear translocation, co-localization, cellular synapse, cellular endocytosis, etc.) that would not be possible with traditional flow cytometry. The ability to collect unique outcomes has led many researchers to develop novel assays for the monitoring and detection of a variety of clinical conditions and diseases. In many cases, investigators have innovated and expanded classical assays to provide new insight regarding clinical conditions and chronic disease. Beyond human clinical applications, image-based flow cytometry has been used to monitor marine biology changes, nanoparticles for solar cell production, and particle quality in pharmaceuticals. This review article summarizes work from the major scientists working in the field of image-based flow cytometry.

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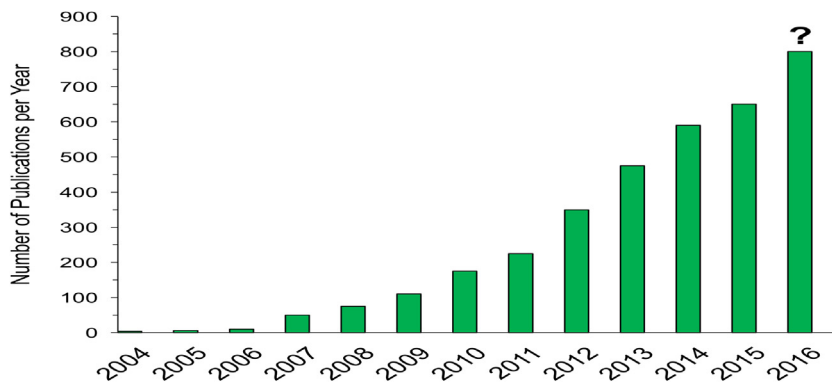
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## 1. Introduction

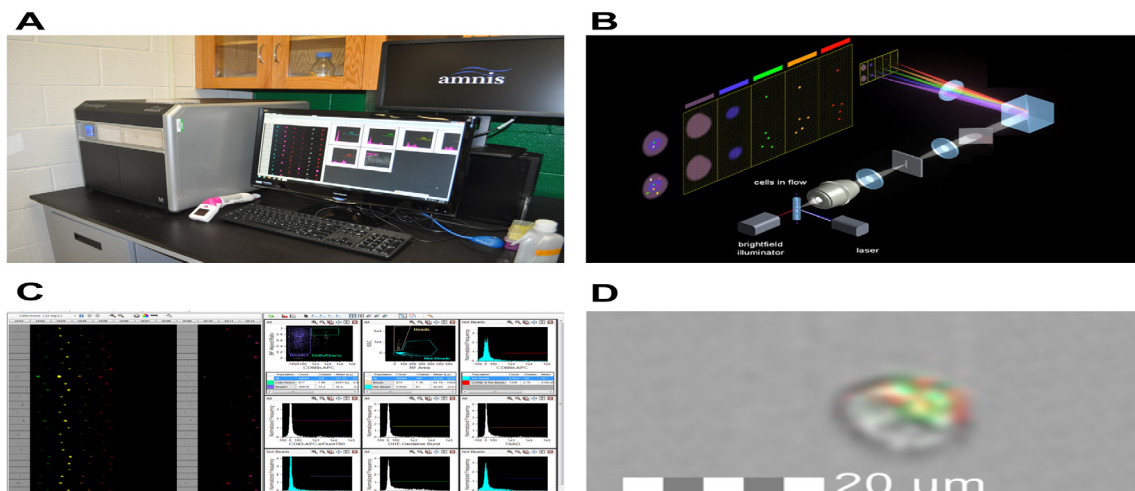
Over the past decade flow cytometry instrumentation and image-processing technology has advanced to the point that high-throughput image-based flow cytometry is available to a greater segment of the scientific community. In particular, significant strides have been made with respect to not only hardware, but software analysis. Advances in both hardware and software have led to an explosion of publishing in the field of image-based

flow cytometry. Based on previous publishing trends (Fig. 1), it is anticipated that more than 800 manuscripts will be published using image-based flow cytometry in 2016 representing a 400% increase over the past decade (see Fig. 2).

The research of many laboratories, including ones highlighted in this review are focused on the development of novel methods and/or analysis techniques [1–15]. This special issue METHODS entitled: *Flow Cytometry What You See Matters: Enhanced Detection using Image-Based Flow Cytometry* includes manuscripts from the



**Fig. 1.** Publishing trends in image-based flow cytometry. Publication numbers were obtained from manufacture sources, internet searches, and PubMed. Over the past decade there has been a substantial increase in the number of studies that employ image-based flow cytometry. The popularity of this technique has lead its use to spread from traditional immunology and cell biology fields to radiation biology, nutrition, and exercise.



**Fig. 2.** Depicts the aspects associated with image-based flow cytometry. The MilliporeSigma Amnis FlowSight in a benchtop instrument capable of detecting up to 12-channels of data on every cell (Panel A). The higher resolution counterpart of the FlowSight is the ImageStreamX Mark II (not shown). The FlowSight and ImageStreamX collect their data using a multi-spectral decomposition method (Panel B) that involves a patented combination of lasers (for fluorescent excitation) and LED illuminators (for brightfield) which transmit the emission signal through a spectral decomposition filter and eventually to a CCD camera where it is collected for subsequent analysis (Panel C). We have provided a represented image of a cell that has undergone phagocytosis (Panel D). In this example image, two unique intracellular signals have been overlaid on brightfield to demonstrate internalization and co-localization of signals. The ability to visually confirm internalization is unique to image-based flow cytometry. The ability to co-localize two intracellular signals and visually identify their location is only possible because of image-based flow cytometry. Image-based flow cytometry has allowed researchers to innovate classic immune system measures to provide additional, novel information about cellular function. In particular, these outcomes can be leveraged to better understand the role of the immune system in the onset and progression of chronic disease.

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