Multicolor Flow Cytometry: The future is now!

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Polychromatic flow cytometry (6 or more colors) is no longer the venue of a few specialized laboratories. With the advent of benchtop instruments capable of 20 parameter measurements, the proliferation of antibody-conjugates available commercially, and the development of sophisticate analysis software, the technology has now become available to much larger community. Unfortunately, it hasn't become any easier to do! There is now a bewildering array of fluorochromes that can be conjugated to antibodies; there are any number of new artefacts arising from multicolor analysis; there are geometrically more possible interactions between reagents that can lead to problems... leading many researchers to ask themselves if it's really worth it to enter this new realm. I will discuss some of the pitfalls and problems that are bound to occur with these types of experiments; as well as some pointers on how to design, implement, and validate multicolor staining protocols. I hope to show that these problems are not only surmountable, but that once you cross into the multicolor world, you will never look back! We have used this technology to interrogate the immune system and its responses to disease (or vaccination) in a depth never-before achieved. Our studies illustrate that the T cell responses are far more complex and rich than we had envisioned, and give us an wide range of potential variables to analyze in our search for the correlate of efficacy in vaccination -- or the correlate of pathogenesis in disease. Finally, I will discuss whether (or how) multicolor flow cytometry can become part of clinical analysis.