Editorial

Everything You Never Wanted to Know About Polarization – But Were Afraid You Might Find Out

Most practitioners of cytometry, whether "flow jocks" or more casual users ("Joe and/or Josie Three-Color"), measure the intensity of scattered light and fluorescence emission in one or more selected wavelength regions, and pay no attention to the degree or direction of polarization of that light. Most of the time, we have gotten away with it. However, the article by Asbury, Uy, and van den Engh in this issue of *Cytometry* provides incontrovertible evidence that the time has come to pay more attention to what may be the biggest skeleton in our cytometric closet.

Because the light emitted by most lasers used as light sources in cytometry is polarized, the light scattered by, and the fluorescence emitted by, cells are also polarized. Scattering and emission occur in many directions, over a large range of angles to the incident beam; the polarized nature of the scattered and emitted light makes the intensity of detected signals more dependent on the angle and direction at which they are detected than would otherwise be the case. Differences from instrument to instrument in optical geometry, and in the polarization response of optical elements such as lenses, dichroics, and filters, may therefore lead to otherwise inexplicable differences in the intensity of signals measured from supposedly identical cells or particles. Further complications may be introduced by the fact that different fluorescent probes exhibit differing degrees of fluorescence polarization, some intrinsic to the molecular structure of the probes, and some dependent on binding to macromolecules and on other environmental characteristics.

The physics of polarization are best appreciated through the use of mathematics, and, while the authors have made their theoretical discussion as clear as possible under the circumstances, the article has its share of trigonometry and a few intimidating double integrals. Read it anyway; there are good data there, and their fit to the theory is what's important. Skim or ignore the math and look at the pictures and the table. The bottom line for Joe and Josie Three-Color is that polarization-related differences in the response of different instruments may interfere with the standardization of quantitative fluorescence measurements, a subject important enough in itself to have occupied an entire issue of *Cytometry* (1998; 33:2). The bottom line for flow jocks is that we need to determine the nature and extent of those differences among instruments, in hopes of reconciling results from existing systems and improving the design of future systems, possibly by making use of the "magic angle". I won't tell you what that is; you'll have to read about it.

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