Application Note

Fluorescence Polarization

Introduction

While the majority of fluorescence microscopists investigate cell structure and function using only the intensity information from spectral emission, an increasing number of scientists are turning their attention to the technique of fluorescence polarization to probe biophysical properties of fluorescent samples. The degree of depolarization in the emission can be used to infer physical properties of the sample. Radiationless energy transfer among fluorophores (e.g., FRET) may also lead to a rapid depolarization of the emission signal. When such effects occur on the order of the timescale of the rate of fluorescent emission, the depolarization can be significant.

If a fluorescent molecule is excited with polarized light, it will emit light of the same polarization assuming the molecule does not rotate during its emission lifetime. Depolarization, or a decrease in light being emitted in the same polarization direction, occurs when the molecule rotates during its emission lifetime. Polarization and anisotropy measurements are often used to estimate the rate and extent of rotational diffusion during the lifetime of the excited state. These, in turn, have been used to quantify biochemical properties such as protein denaturation, protein-ligand association reactions, and the rotational rates of proteins. The anisotropies of fluorophores bound to membranes have been measured in order to estimate the internal viscosities of membranes and the dependency of membrane phase transitions on membrane composition.[1]

Example Application: Anisotropy/FLIM

The Photometrics® DV2™ can be used to simultaneously acquire two-dimensional polarization and lifetime images, which can then be transformed to yield maps of rotational correlation time and fluorescence lifetime.[2] This technique has been labeled time-resolved fluorescence anisotropy imaging (TR-FAIM), an extension of time-domain FLIM. It utilizes linearly polarized laser pulses to excite a sample. The parallel and perpendicular components of the fluorescence emission of the sample are then imaged simultaneously using the DV2 system.

Figure 1 shows the result of applying this technique to the images of several wells of a standard multiple-well plate with rhodamine 6G in methanol, ethylene glycol, trimethylene glycol, and glycerol. The acquired images are processed to recover the rotational correlation times and unperturbed fluorescence lifetimes of the sample. These results are used to determine the local viscosity and refractive index of the probe environment.

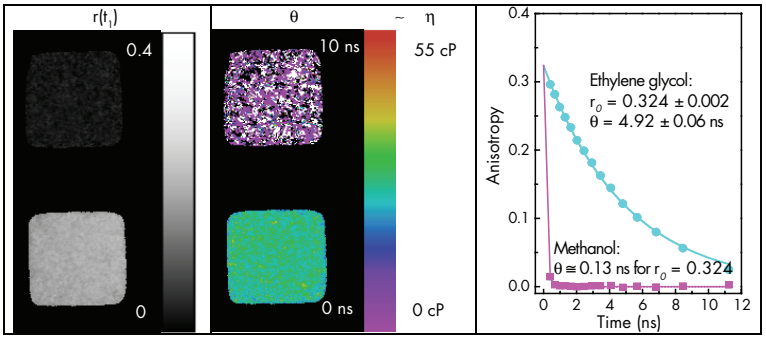

Figure 1. TR-FAIM results of two wells filled with rhodamine 6G in methanol (top) and ethylene glycol (bottom). Left panel: Transient anisotropy 400 ps after the excitation pulse, showing strong anisotropy in ethylene glycol and apparently weak anisotropy in the sample in methanol since it has already decayed almost entirely. Middle panel: Map of the rotational correlation time θ in a pseudocolor scale from 0 ns to 10 ns. The timescale has also been converted directly into a viscosity scale ranging from 0 cP to 55 cP. Right panel: The anisotropy decays averaged over the individual well areas of the (middle panel). Images courtesy of Siegel *et al*.

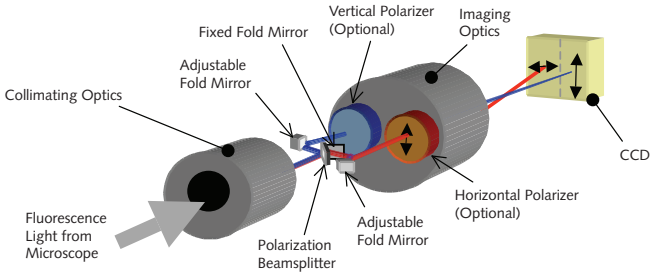

Figure 2. Operation of the DV2 fluorescence polarization imaging system.

System Configuration

The DV2 has been used successfully in many fluorescence imaging applications for microscopy. In most applications, the DV2 is fitted with dichroic filters and spectral emission filters for simultaneous imaging of multiple fluorescent labels; however, the DV2 can also be fitted with a high-efficiency polarization beamsplitter and high-efficiency linear polarizers for polarization imaging of fluorescent samples. This configuration allows simultaneous acquisition of two polarization images: one parallel to the polarization of the source and the other perpendicular to the polarization of the source. These images are used to calculate the two-dimensional polarization/anisotropy of the sample. The operation of this system is shown conceptually in **Figure 2**.

Technique Summary

For anisotropy measurements, a fluorescent sample is excited with polarized light, yielding fluorescent emission from the sample. This simple property of fluorescent samples can be used to probe different biochemical properties of cellular structures. When implemented on a microscope, the technique requires excitation of the sample with vertically polarized light. On the emission side, two separate images need to be acquired. One image is taken with a vertically polarized filter in one emission channel; the second is taken with a horizontally polarized filter in the other emission channel. The first image is $I_{||}$ and the second image is $I_{\perp}$. The two images can then be used to determine the polarization (P) and anisotropy (r) at each pixel in the image.

If both images have been acquired simultaneously, then using the ratio of the images has significant advantages because it normalizes for fluctuations in source intensity, photobleaching, and uneven dye loading.

Citations

1. Lakowicz, J.R., *Principles of Fluorescence Spectroscopy*, (New York: Plenum Press) ch. 5.

2. Siegel, J., Suhling, K., Leveque-Fort, S., Webb, S.E.D., Davis, D.M., Phillips, D., Sabharwal, Y., French, P.M.W. (2003) "Wide-field time-resolved fluorescence anisotropy imaging (TR-FAIM): Imaging the rotational mobility of a fluorophore," *Review of Scientific Instruments* **74,** 1, 182-192.

Resources

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