Fluorescence Polarization Measures Changes In Molecular Complex

by Xavier Amouretti

Fluorescent polarization is calculated from two measurements and is inversely related to the speed of molecular rotation of the labeled complex.

Both high throughput screening (HTS) and life science research laboratories require methods to analyze an ever-increasing amount of samples. HTS laboratories focus on testing large numbers of compounds for their ability to elicit or prevent a defined response, while life science research requires easy-to-use tools to study molecular interactions.

To meet these demands, Fluorescence Polarization (FP) has evolved in both markets. FP is a common homogenous assay technology that does not require a wash step to remove unbound materials, thus reducing overall costs and sources of error. By its nature it is a very powerful tool to monitor biomolecular interactions.

How FP works

FP is based on the observation that fluorescent molecules in solution, when excited by polarized light, will then emit polarized light. The resultant light is emitted at a different plane than the original excitatory light (Figure 1) due to the rotation of the molecule. All molecules naturally rotate in solution, although at different rates. Rotation may be influenced by solution viscosity, absolute temperature, molecular volume and the gas constant. If viscosity and temperature are constant through the sample volume, then molecular volume or molecular weight becomes the key variable associated with rotation speed. Large molecules tend to rotate slowly while small molecules rotate rapidly, as the rate of rotation is inversely proportional to a molecule's size.

Excited by polarized light, rapidly rotating fluorescent molecules will emit polarized light at a different plane than the original excitatory light.

In a typical FP assay, a suitable fluorophore (fluorescent molecule) is bound to a molecule, forming a tracer molecule. As the tracer interacts with other molecules, it may bind to form larger complexes that rotate at a slower rate. This change in rotation speed is identified when the fluorophore is excited with a beam of polarized light. If a molecule remains in the same plane during its excited state, emitted light from the fluorophore will remain polarized. If a molecule rotates out of the plane during the excited state, the emitted light is depolarized.

The emitted light, from the excited fluorophore, is passed through both parallel and perpendicular polarizers (Figure 2). The change of emission intensity from parallel to perpendicular is proportional to the rotational movement of the sample tagged molecule in solution. FP is calculated from the two measurements (parallel and perpendicular) and is inversely related to the speed of molecular rotation of that complex; the value obtained is expressed in millipolarization units (mP or millipee).

All the molecules in solution are not initially aligned. Those that are aligned perpendicular to the polarized light will not absorb light and therefore will not emit light, and those at other orientations will absorb light to varying degrees depending on their orientation. The end result will be emitted light that has a fluorescence of some degree in the perpendicular plane to the excitation polarization filter, and a limiting polar-

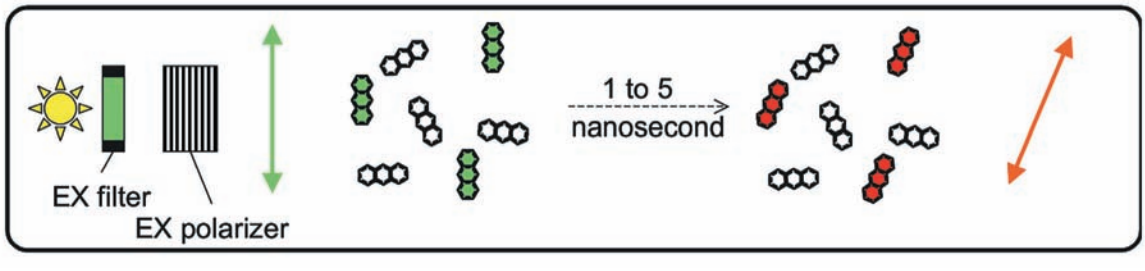

Figure 1. Excitation of fluorescent molecules with polarized light. Molecules in the same plane as the polarized light will be excited. The depolarization of the emitted light results from molecular rotation during the molecule's fluorescence lifetime.

ization of 500 mP. Therefore, polarization values, corrected for background fluorescence, that are greater than 500 mP indicate either the presence of scattered light, or that the instrument is incorrectly calibrated.

Why use fluorescence polarization?

As fluorescence polarization provides direct and immediate measure of the binding of a tagged molecule to a target molecule, it is ideally suited to the investigation of molecular interactions. FP experiments are performed without solid support, allowing true steady state analysis to be performed without removal of material or extra washing steps. As FP measurements do not adulterate samples, they can be reanalyzed after modifications and additions, providing an obvious time and cost-savings. Reactions are very rapid, and the reagents are stable, so the process is highly conducive to automation methods. FP reactions are therefore often faster, and more cost efficient than heterogeneous methods.

FP assays are easy to construct and are unaffected by solution turbidity, addition of dyes, or sample concentration. Only one tracer is required, and crude receptor preparations may be utilized in the process. Finally, FP results are resistant to variations from detection instruments.

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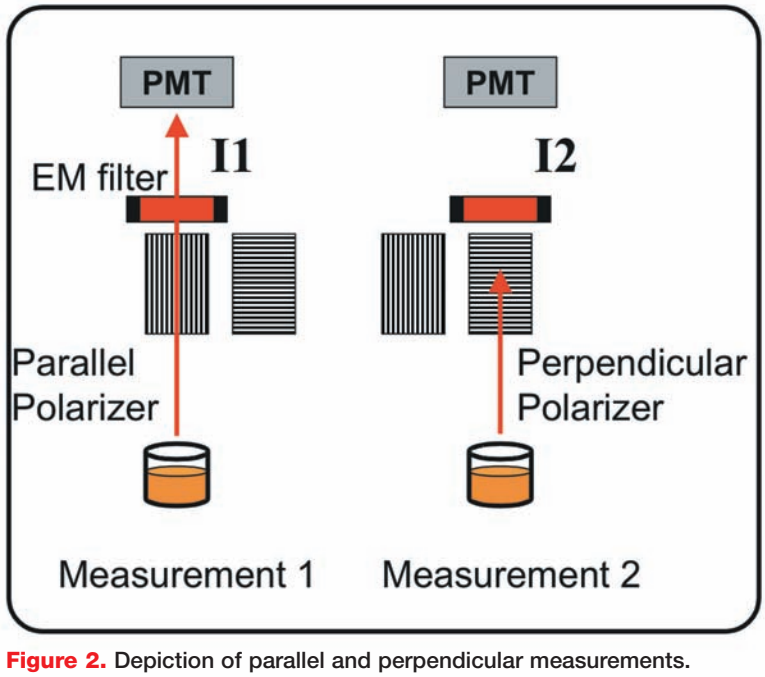

Figure 2. Depiction of parallel and perpendicular measurements. Polarized light is used to excite fluorescent molecules. Emitted light is passed through two different polarizing filters, one parallel and one perpendicular to the plane of the excitatory polarizing filter, and the wavelength-specific signal is measured by a photomultiplier tube.

Figure 3. Synergy 2 multi-detection microplate reader.

When to use fluorescence polarization

As the principle goal of any FP assay is to measure a change in the size of a molecular complex from larger to smaller or vice versa, FP is popularly used to detect changes such as molecular orientation, mobility, binding, and modulating processes between molecules. The most common areas of investigation benefiting from fluorescence polarization include: receptor/ligand binding, protein interactions, nucleic acid/protein binding, antibody/antigen interactions, tyrosine kinase assays and competitive immunoassays.

Additional homogeneous assay technologies exist that also measure changes in the size of a molecular complex such as Fluorescence Resonance Energy Transfer (FRET), but involve a more difficult dual-labeling process.

Summary

Fluorescence polarization offers a number of benefits to researchers in both life sciences and high throughput screening. It is one of the most simple techniques available to study biomolecular interactions. It provides cost and time savings, without sacrificing quality and allows for rapid, reproducible and precise results, regardless of the type or quantity of sample being tested.

About the author

Xavier Amouretti is a Product Manager with BioTek Instruments, Inc. The company's Synergy 2 Multi-Detection Microplate Reader (Figure 3) instrument is commonly used to measure FP assays. Gen5 software automatically calculates polarization and corrects for background interference.

More information is available from:

■ BioTek Instruments, Inc.
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