

Fluorescence Polarization

Fluorescence polarization indirectly measures the speed of rotation of molecules in a solution. A motionless fluorescent molecule that is excited with plane-polarized light will, after a short lag (the 'fluorescence lifetime'), emit light polarized in the same plane. If rotation of the excited molecule occurs during the fluorescence lifetime (usually a few nanoseconds) the emitted light will be depolarized with respect to the plane of the excitation light. Because the speed of rotation is inversely related to molecular size, small fluorescent molecules show a large depolarising effect. However, when a small fluorescent molecule binds to a much larger molecule (e.g. antibody, receptor or bead) the resulting binary complex has a greatly diminished depolarising effect due to the effective increase in molecular size.

Fluorescence polarization (P) is a dimensionless quantity that is calculated thus:

$$P = (I_{\text{par}} - I_{\text{perp}}) / (I_{\text{par}} + I_{\text{perp}})$$

where I_{par} and I_{perp} represent emission intensity when the emission polarizer is in the parallel and perpendicular position respectively in relation to the excitation polarizer. Since all fluorescence plate readers give the values of P automatically it is not necessary to perform these calculations.

The P values are usually displayed as milli-polarization units (mP):

$$P \text{ (mP)} = 1000(I_{\text{par}} - I_{\text{perp}}) / (I_{\text{par}} + I_{\text{perp}})$$

Measured values for P in assay applications typically range from 50 to 400 mP. This measurement range is not as narrow as it might appear, because very precise measurements are readily attainable with modern plate readers.