Anisotropy

Fluorescence continues to be a powerful tool for life science research due to its sensitivity, selectivity, and general usability. Typically, the presence and amount of a fluorescently-tagged analyte is correlated to the intensity of the fluorescence measured. A species that absorbs a fluorescence is considered a ‘fluorophore’.

Fluorescence Polarization

The use of fluorescence polarization (FP) uses a fluorophore's properties to indicate binding state and microenvironment. Polarization is a measurement of a molecule's rotation from its excitation until its emission. Polarization (P) is defined as:

\[
P = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp}
\]

Where \( I_\parallel \) is the emission intensity parallel to the excitation plane and \( I_\perp \) is the intensity perpendicular to the excitation plane.

If the emission is completely maintained in the parallel direction then \( P=+1 \). If the emitted light is totally polarized in the perpendicular direction then \( P=-1 \). In the actual measurement of polarization there is usually an angle between the excitation dipole and the emission dipole within a molecule. For example, even a fixed, non-rotating fluorophore, will have some “intrinsic” depolarization due to this angle.

Anisotropy

Another term representing the degree of polarized emission is anisotropy \( (r) \) which is defined as:

\[
r = \frac{I_\parallel - I_\perp}{I_\parallel + 2I_\perp}
\]

The difference in the way polarization and anisotropy is defined is the presence of a second perpendicular intensity term in the denominator. The definition of anisotropy takes into account the second possible perpendicular emission plane, which is oriented along the axis of propagation. Anisotropy is a more accurate representation of the physical phenomena because it takes into consideration the contribution of all degrees of rotational freedom and reflects
orientations of constrained molecules that can be detected through two-dimensional mapping of polarized fluorescence emission.

**Relating Fluorescence Polarization and Anisotropy**

Given the definition of polarization and anisotropy, one can show a correlation between polarization and anisotropy values.

<table>
<thead>
<tr>
<th>$P$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>0.40</td>
</tr>
<tr>
<td>0.30</td>
<td>0.22</td>
</tr>
<tr>
<td>0.10</td>
<td>0.069</td>
</tr>
</tbody>
</table>

The information content in the polarization function and the anisotropy function is identical and the use of one term or the other is dictated by practical considerations.