Anisotropy Decay Measurements

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Introduction

Knowledge of dynamic fluorescence parameters such as the fluorescence lifetime or the rotational correlation time is crucial for the quantitative interpretation of fluorescence data for quenching, energy transfer or polarization [1]. The lifetime is the average time a fluorophore spends in the excited state before returning to the ground state by emitting a photon. It is calculated as the time when the initial intensity has decayed by a factor of 1/e. Similarly the rotational correlation time is the time at which the initial anisotropy has decayed to 1/e of its original value.

Methods

As there are 2 methods to measure the lifetime there are also two ways to measure the rotational correlation times [2]

Time-domain method (TD)

In time domain one measures the time-dependent intensity decays of the polarized components I_V and I_H with vertical excitation. These polarized decays are then used to calculate the time- dependent anisotropy decay:

$$r(t) = \frac{I_{\parallel}(t) - I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)} \qquad [1]$$

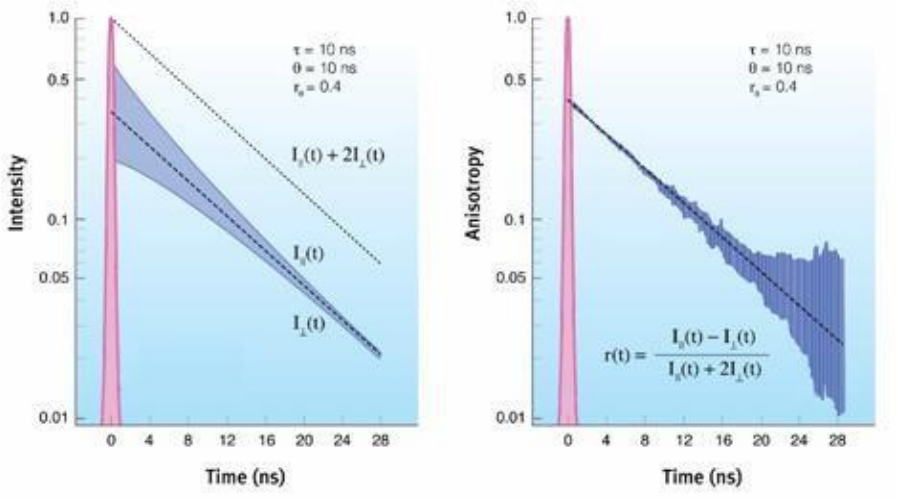

Figure 1. Time-dependent anisotropy decays (left) and calculated anisotropy decay (right). [2]

The anisotropy decay $r(t)$ is then analyzed to determine which model is most consistent with the data.

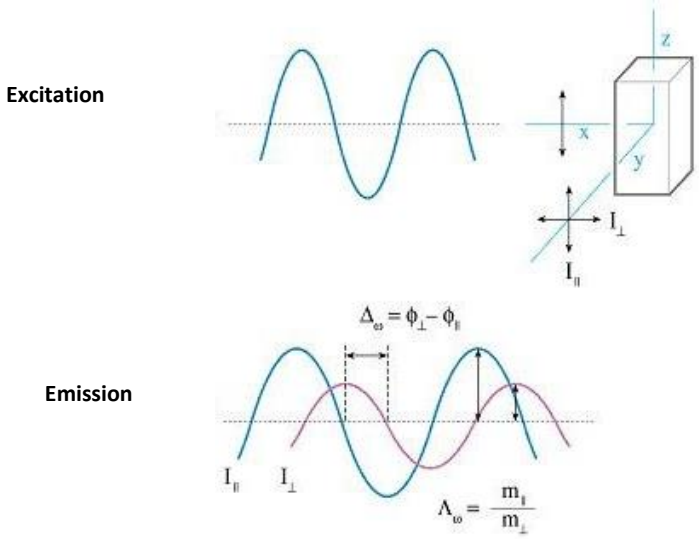

Figure 2. Schematic drawing of measurement of anisotropy decays with FD.

In frequency domain the sample is excited with amplitude-modulated light and the emission is observed at parallel and perpendicular polarizer settings. There are two parameters, which characterize the anisotropy decay in FD:

Differential phase angle between the perpendicular and parallel components of the emission:

$$\Delta\omega = \phi_\perp - \phi_\parallel \qquad [2]$$

and the amplitude ratio between the parallel and perpendicular amplitudes of the modulation:

$$\Lambda_\omega = \frac{m_\parallel}{m_\perp} \qquad [3]$$

The amplitude ratios are often expressed as modulated anisotropy because of its higher information content:

$$r_\omega = \frac{\Lambda_\omega - 1}{\Lambda_\omega + 1} = \frac{m_\parallel - m_\perp}{m_\parallel + 2m_\perp} \qquad [4]$$

The parameter r_ω is the frequency dependent anisotropy and it approaches r_0 at high modulation frequencies while at low modulation frequency it approaches the steady state anisotropy value. The differential phase angles $\Delta\omega$ functions are approximately Lorentzian in shape on the log-frequency scale. Most fluorescent dyes show single correlation times in polar solvent systems even though they are not spherical molecules. This is due to hydrogen bonding between solvent and the fluorescent molecules.

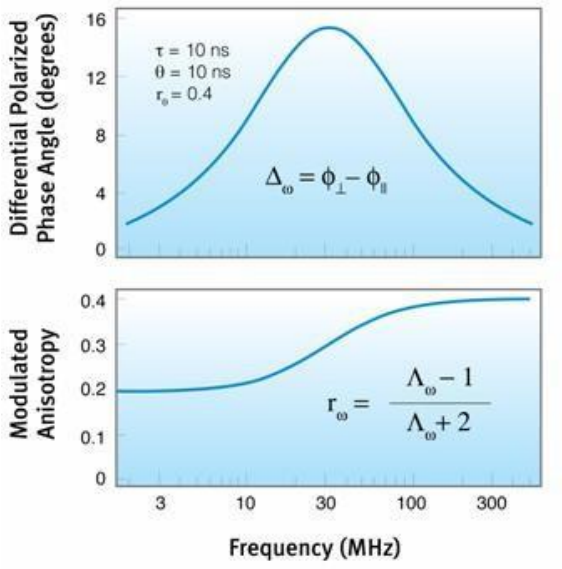

Figure 3. Calculated differential phase and modulated anisotropy curves for τ = 10 ns, θ = 10 ns and r_0 = 0.4 [2]

Measurement Examples

We have measured several anisotropy decays on ChronosFD and found the measurement of time-resolved parameters to be fast and easy. In FD there is no need to measure the lifetime separately as the FD analysis provides information of both – the lifetimes and the rotational correlation times of the investigated system.

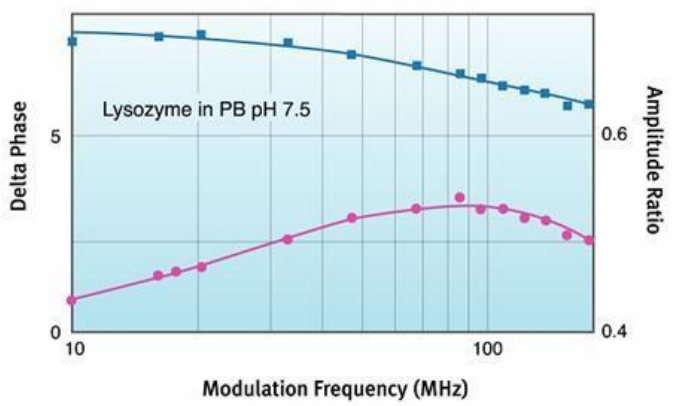

Figure 4. Frequency-domain anisotropy decays (Differential Polarized Phase Angle and Amplitude Ratio) of Lysozyme in phosphate buffer pH 7.5 measured with a 300-nm LED. The emission was collected using a WG320. Calculated values for θ_1 = 0.8 and θ_2 = 6.08, R_0 = 0.17 with τ_1 = 0.7 ns and τ_2 = 2.58 ns, f_1 = 0.26.

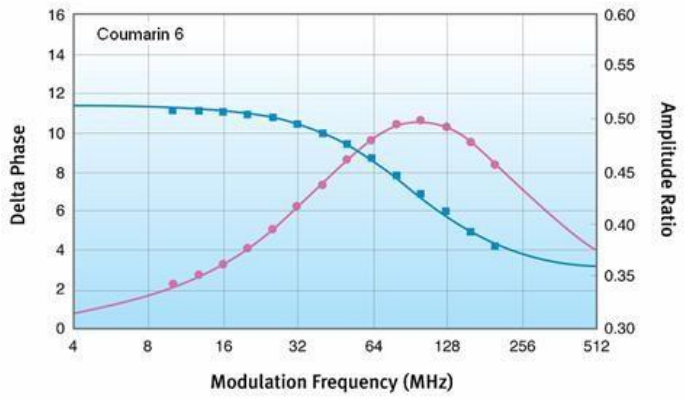

Figure 5. Frequency-domain anisotropy decays (Differential Polarized Phase Angle and Amplitude Ratio) of Coumarin 6 in propylene glycol measured on ChronosFD using a 473-nm laser diode. The emission was collected using a 500-nm long-pass filter. Calculated values for θ = 4.5 ns with R0 = 0.38 and τ = 2.5 ns, T = 27-28°C.

For other examples please go to the measurement examples page. In summary frequency domain measurements provide excellent resolution of complex anisotropy decays and this can be considered one of the strengths of the FD method [3]. One possible reason for this is that FD enables direct measurement of the difference between the polarized components, while in time-domain the polarized components are measured separately and then the anisotropy decay is calculated. The other explanation is that FD uses 2 parameters that are directly measured ($\Delta\omega$ and Λ_ω) and therefore has a better resolution potential [3].

References

1. Jameson, D.M. and Sawyer, W.H.; *Fluorescence anisotropy applied to biomolecular interactions.* Meth. Enzym. 246, 284-300 (1995).
2. Lakowicz, J.R; *Principles of Fluorescence Spectroscopy, 2nd Edition,(1999).* Kluwer Academic/Plenum Publishers, New York.

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