A Little Magic for the Measurements: Magic Angle

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The Tutorial

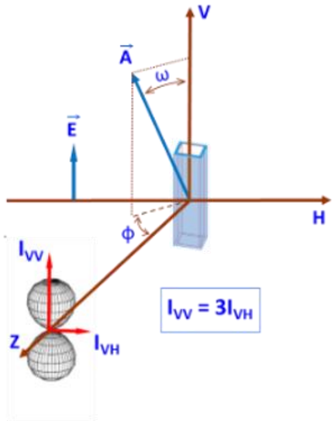
Fluorescence is one of the major tools in many diverse fields, from molecular biology, and chemistry, to material sciences. More than 10% of published peer-reviewed research papers are about fluorescence or use fluorescence techniques. Over the last few decades, both steady-state and time-resolved fluorescence instrumentation have improved significantly, allowing for fast and precise measurements. With advanced electronics and lasers, the time required to collect fluorescence data has shortened to minutes/seconds.

Consequently, such highly advanced tools allow for highly precise fluorescence measurements stimulating researchers to study more detailed effects in biochemical processes. However, we need to realize that experimental conditions and proper instrumentation setups can greatly impact the outcomes of such measurements.

Our goal is to help young researchers in their fluorescence measurements, especially to avoid unnecessary mistakes. This tutorial will focus on the excitation/emission polarization conditions: Magic Angle in steady-state fluorescence and time-resolved measurements as we answer the following questions.

Is there a polarization angle that can give reliable results of the total fluorescence intensity on all machines? Is this angle also reliable when used in Time-Resolved Measurements?

What We Know



$$I_T = I_{VV} + 2I_{VH}$$

Polarization is $\propto \cos^2\phi$

For I_{VV} polarization $\cos^2\phi$

For I_{VH} polarization $\cos^2(90-\phi)$

Is there an ϕ where $I_{VV} = 2I_{VH}$ so that $I_{VV} = I_{VH} = I_{VZ}$?

Yes! When $< \cos^2\phi > = \frac{1}{3}$

$$I_{MA} = \frac{1}{3}(I_{VV} + I_{VH} + I_{VZ})$$

$$= \frac{1}{3}(I_{VV} + 2I_{VH})$$

$$\phi = 54.7°$$

Figure 1. *Coordinate system for square geometry. Top: The distribution of the fluorophore's excited dipoles with a vertically polarized excitation. The shape is a result of photo-selection. The vertical component is 3-times stronger than the horizontal one.*

"Magic" Verification: Steady State

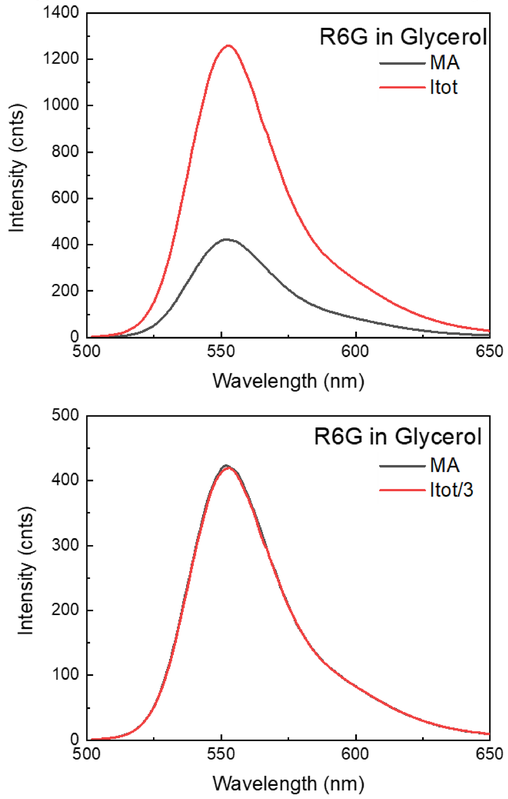


Figure 2. *Top: spectrum measured at MA conditions (black) and the spectrum obtained from total intensity Bottom: Comparison of spectra measured at MA and 1/3 of total intensity*

Conclusion

Magic Angle, 54.7° , is a polarization angle at which both the Steady State and Time Resolved Measurements can accurately result in the true spectral and lifetime values of the samples being measured

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"Magic" Verification: Time- Resolved

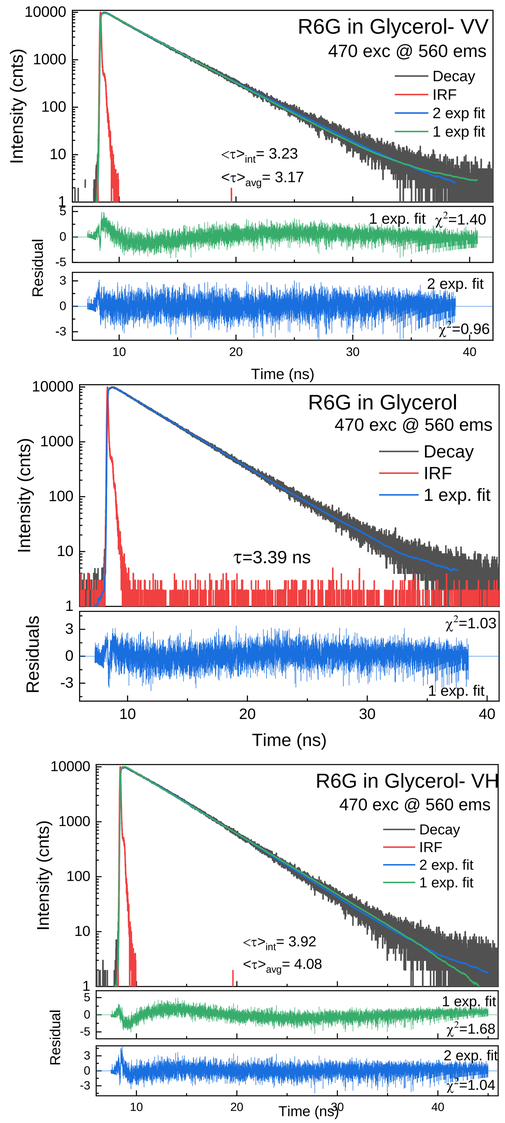

Figure 3. *Fluorescence intensity decays of R6G in Glycerol excited at 470 nm and measured at 560 nm emission. Top: The polarizers were oriented V at the excitation and V at the emission. Middle: measured at 560 nm emission, the peak emission wavelength value. Right: The polarizers were oriented V at the excitation and H at the emission.*

Publication

1. E. Kitchner et al. **2022** Methods Appl. Fluoresc. **10,** 043001. DOI: 10.1088/2050-6120/ac92c5