

Verifying The Polarized Fluorescence Microscopy Model Using Anisotropy

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1 Introduction

Chemists often use the *anisotropy* of a sample to characterize rotational dynamics and the transition moments of molecules. In these notes I will use the polarized fluorescence microscopy model from previous notes to calculate the anisotropy of several fluorophore distributions. I will reproduce known results to verify the model. This work is useful for verifying that our model is correct and for relating our work to published results.

2 Measuring The Anisotropy

The anisotropy, r , of a sample is measured by (1) exciting a sample with a low-NA beam of linearly polarized light, (2) placing a low-NA detection arm orthogonal to the illumination arm, (3) placing a linear polarizer in the detection arm, (4) measuring the intensity with the detection polarizer parallel (I_{\parallel}) and perpendicular (I_{\perp}) to the illumination polarization, (5) calculating the anisotropy using

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}. \quad (1)$$

See Figure 1 for a schematic of the I_{\parallel} and I_{\perp} measurements.

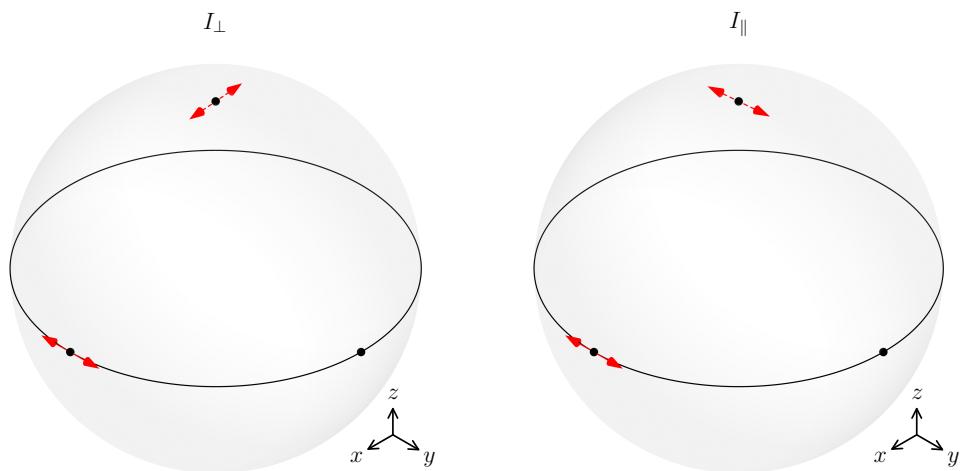


Figure 1: Schematics for anisotropy measurements. Solid (dashed) arrows show the illumination (detection) polarization orientation. $NA_{\text{ill}} = NA_{\text{det}} = 0$.

Lakowicz [1] gives several known anisotropies for special dipole distributions. We assume that the excitation and emission dipole moments are collinear. If the dipole moment orientations are

- completely aligned and parallel to the excitation polarization $\rightarrow r = 1$
- completely aligned and perpendicular to the excitation polarization $\rightarrow r = -0.5$
- uniformly distributed $\rightarrow r = 0.4$.

3 Calculating The Anisotropy With The Polarized Fluorescence Microscopy Model

We can calculate the intensities measured by the experiments in Figure 1 using

$$I \propto \int_{\mathbb{S}^2} d\hat{\mathbf{r}} f(\hat{\mathbf{r}}; \hat{\boldsymbol{\mu}}, \kappa) \eta_{\text{exc}}(\hat{\mathbf{r}}) \eta_{\text{det}}(\hat{\mathbf{r}}) \quad (2)$$

where $f(\hat{\mathbf{r}}; \hat{\boldsymbol{\mu}}, \kappa)$ is the Watson distribution with central orientation $\hat{\boldsymbol{\mu}}$ and concentration parameter κ , η_{exc} is the excitation efficiency of a single fluorophore and η_{det} is the detection efficiency of a single fluorophore. See previous note sets and the paper for the efficiency expressions.

Figures 2 and 3 show the intensities and anisotropy of the experiment in Figure 1 as a function of fluorophore distribution ($\hat{\boldsymbol{\mu}}$ and κ). The results match the special cases mentioned in Lakowicz. When the fluorophores are completely aligned ($\kappa = \infty$) and parallel to the excitation polarization ($\hat{\mathbf{y}}$ -axis) then $r = 1$ (see bottom right sphere in Figure 2 or right plot in Figure 3). When the fluorophores are completely aligned ($\kappa = \infty$) and perpendicular to the excitation polarization ($\hat{\mathbf{x}}$ -axis) then $r = -0.5$ (see bottom right sphere in Figure 2 or right plot in Figure 3). Finally, when the fluorophores are uniformly distributed ($\kappa = 0$) then $r = 0.4$ (see Figure 2 bottom row second from the left or the right plot in Figure 3).

References

- [1] Joseph R. Lakowicz. *Principles of fluorescence spectroscopy*. Second edition. New York : Kluwer Academic/Plenum, 1999.

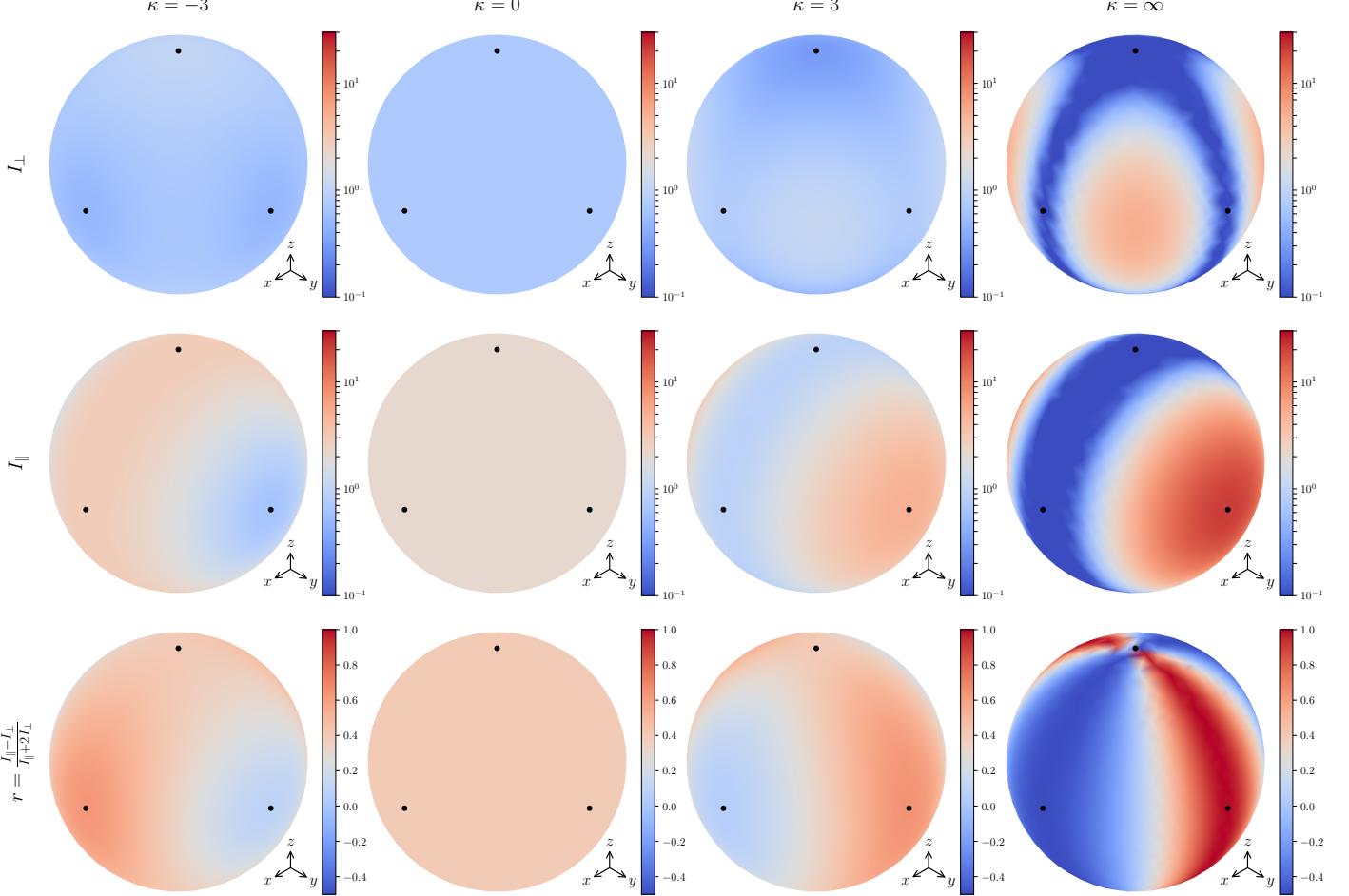


Figure 2: Intensities and anisotropy as a function of fluorophore distribution. **Rows:** 1) I_{\perp} see Figure 1; 2) I_{\parallel} see Figure 1; 3) Anisotropy see Equation 1. **Columns:** Varying concentration parameter of the Watson distribution. Note that $\kappa = \infty$ corresponds to a single fluorophore or a perfectly concentrated ensemble of fluorophores.

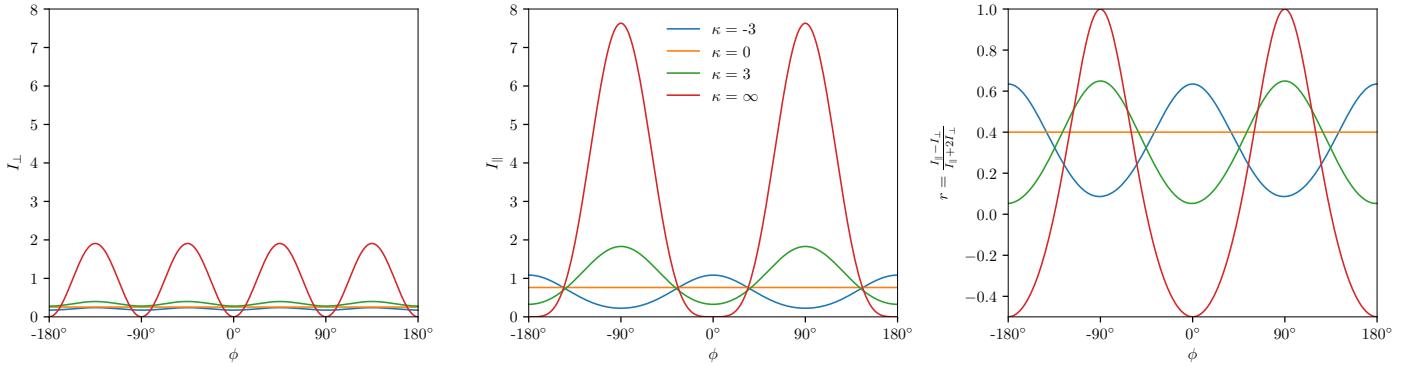


Figure 3: Profiles of the spheres in Figure 2 in the $x - y$ plane. ϕ is the azimuth angle measured from the $+x$ -axis in the $x - y$ plane. **Columns:** 1) I_{\perp} see Figure 1; 2) I_{\parallel} see Figure 1; 3) Anisotropy see Equation 1. **Colors:** Varying concentration parameter of the Watson distribution.