

Excitation and Detection Efficiency of Ensembles of Molecules Under Polarized Illumination

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1 Introduction

In these notes I will calculate the excitation and detection efficiencies of ensembles of molecules as a function of polarizer orientation and microscope geometry. This work extends the results in the 2017-06-14 notes from single molecules to ensembles of molecules.

We start by extending the excitation and detection efficiencies to arbitrary distributions of molecules. Next, we review possible orientation distribution functions that we can expect real ensembles to follow. Finally, we see how orientation distributions affect the intensities measured by microscopes.

2 Excitation and Detection Efficiency for Arbitrary Distributions of Molecules

Previously we showed that the excitation efficiency of a single molecule under incoherent uniform angular illumination is given by

$$\eta_{\text{abs}}^{\text{single}} = D \{ A + B \sin^2 \Theta + C \sin^2 \Theta \cos [2(\Phi - \phi_{\text{exc}})] \} \quad (1)$$

where

$$A = \frac{1}{4} - \frac{3}{8} \cos \alpha + \frac{1}{8} \cos^3 \alpha \quad (2a)$$

$$B = \frac{3}{16} \cos \alpha - \frac{3}{16} \cos^3 \alpha \quad (2b)$$

$$C = \frac{7}{32} - \frac{3}{32} \cos \alpha - \frac{3}{32} \cos^2 \alpha - \frac{1}{32} \cos^3 \alpha \quad (2c)$$

$$D = \frac{4}{3(1 - \cos \alpha)}. \quad (2d)$$

If an ensemble of independent fluorescent molecules is illuminated then the absorption efficiency of the ensemble is

$$\eta_{\text{abs}} = \int_{\mathbb{S}^2} d\hat{\mathbf{r}} f(\hat{\mathbf{r}}) \eta_{\text{abs}}^{\text{single}} \quad (3)$$

where $f(\hat{\mathbf{r}})$ is the orientation probability distribution function of the fluorophores. For equation 3 to be true the fluorophores must absorb light independently—no homo-FRET or coherence effects.

Similarly, the detection efficiency of an ensemble is

$$\eta_{\text{det}} = \int_{\mathbb{S}^2} d\hat{\mathbf{r}} f(\hat{\mathbf{r}}) \eta_{\text{det}}^{\text{single}} \quad (4)$$

which is only true if the fluorophores emit light independently. I haven't given the full expression for $\eta_{\text{det}}^{\text{single}}$ here—see the paper or equation 2 with $D = 1$.

Note that equations 3 and 4 are much more computationally expensive than the single molecule efficiencies that I've implemented previously. The double integrals do not have closed form solutions, so I am using a numerical integration scheme—Gausse-Legendre adaptive quadrature methods implemented in QUADPACK and called from `scipy`. These methods allow us to easily trade speed for accuracy.

3 Axial Distributions On The Sphere

In this section we will review several orientation probability distribution functions.

The **Von Mises-Fisher distribution** is given by

$$f(\hat{\mathbf{r}}; \hat{\boldsymbol{\mu}}, \kappa) = \frac{\sqrt{\kappa}}{(2\pi)^{3/2} I_{1/2}(\kappa)} \exp\{\kappa \hat{\boldsymbol{\mu}}^T \hat{\mathbf{r}}\}$$

where $I_{1/2}$ denotes the modified Bessel function of the first kind. The Von Mises-Fisher distribution is rotationally symmetric about $\hat{\boldsymbol{\mu}}$, but it is not antipodally symmetric. Therefore, the Von Mises-Fisher distribution is not an appropriate choice for modeling axial data.

$$\kappa = 0.1 \quad \kappa = 0.5 \quad \kappa = 1.0 \quad \kappa = 2.0 \quad \kappa = 3.0 \quad \kappa = 5.0 \quad \kappa = 7.0$$



Figure 1: Von Mises-Fisher distributions with constant $\hat{\boldsymbol{\mu}}$ and varying κ .

The **Watson distribution** [1, 2] is given by

$$f(\hat{\mathbf{r}}; \hat{\boldsymbol{\mu}}, \kappa) = \frac{1}{4\pi_1 F_1\left(\frac{1}{2}, \frac{3}{2}, \kappa\right)} \exp\{\kappa (\hat{\boldsymbol{\mu}}^T \hat{\mathbf{r}})^2\}$$

where ${}_1F_1$ denotes a confluent hypergeometric function. The Watson distribution is antipodally symmetric ($f(\hat{\mathbf{r}}) = f(-\hat{\mathbf{r}})$) and rotationally symmetric about $\hat{\boldsymbol{\mu}}$. The parameter κ is a concentration parameter—when κ is large and positive the distribution is concentrated near μ , when κ is large and negative the distribution is concentrated near the great circle orthogonal to $\hat{\boldsymbol{\mu}}$, and when κ is small the distribution is uniform. $\hat{\boldsymbol{\mu}}$ is a unit vector which can be specified using two angles, so the Watson distribution is a 3 parameter distribution.

The Watson distribution is (1) antipodally symmetric, (2) rotationally symmetric about a single axis, and (3) smooth, so we think it is a good distribution to describe distributions of molecules in biological samples. The Watson distribution has also been used as a model for paleomagnetic data (orientations of the magnetic axis in rocks) [3], MRI diffusion tensor imaging data [4], and microphone array directionality data [5]. We will use the Watson distribution as a model for fluorophores for the remaining sections of these notes.

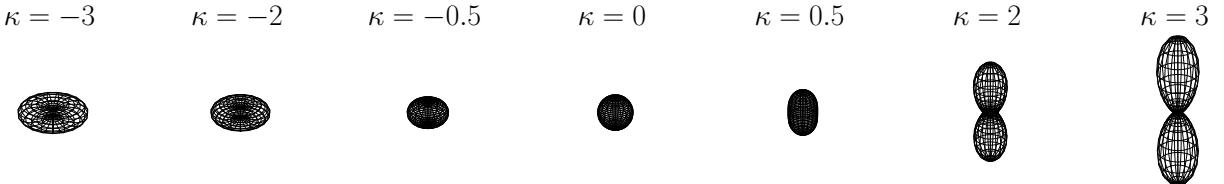


Figure 2: Watson distributions with constant $\hat{\boldsymbol{\mu}}$ and varying κ .

For completeness, the **Bingham distribution** [1, 2] is a generalized version of the Watson distribution that allows for distributions that are not rotationally symmetric about a single axis. The Bingham distribution is

given by

$$f(\hat{\mathbf{r}}; \hat{\boldsymbol{\mu}}, \kappa) = \frac{1}{4\pi_1 F_1 \left(\frac{1}{2}, \frac{3}{2}, \mathbf{K} \right)} \exp\{\hat{\mathbf{r}}^T \mathbf{K} \hat{\mathbf{r}}\} \quad (5)$$

where ${}_1F_1$ is denotes a confluent hypergeometric function with a matrix argument. The matrix \mathbf{K} has three free parameters (two eigenvalues and a choice of principal axis), so the Bingham distribution is a 5 parameter distribution.

4 Forward Model For Watson Distributed Molecules

The detected intensity is proportional to the number of molecules in the ensemble N , the absorption efficiency η_{abs} , and the detection efficiency η_{det}

$$I \propto N \eta_{\text{abs}} \eta_{\text{det}}. \quad (6)$$

Our goal is to estimate the parameters of the ensemble distribution (Θ, Φ, κ) and the number of molecules in the distribution N from the intensity measurements. If we can estimate the number of photons each fluorophore emits (from a datasheet or a separate experiment), then we can estimate N directly. If this information is not available, we will estimate a number that is directly proportional to N .

Figure 3 shows representative examples of intensity measurements collected from Watson-distributed fluorophores. Note how the total efficiency pattern changes as κ changes. For large κ , we recover the case of single fluorophores and there is a concentrated set of orientations with high total efficiency. As κ decreases, the set of orientations with high total efficiency grows until $\kappa = 0$ and the total efficiency is independent of $\hat{\boldsymbol{\mu}}$ (this is because the mean orientation is meaningless for a uniform distribution). When $\kappa < 0$ the total efficiency is high in a ring of orientations.

5 Computation Time Estimates

A function evaluation for the intensity collected from a single fluorophore **currently** takes ~ 0.1 ms.

To calculate the intensity collected from a distribution of fluorophores we need to repeat this calculation for each fluorophore orientation. Empirically, ~ 500 orientations bounds the error on the integral below 1%, so the full calculation takes ~ 50 ms.

We're planning to implement a Fisher scoring algorithm that acts on the data from each voxel to estimate 4 parameters: the orientation distribution (Θ, Φ, κ) and the number of fluorophores N . The algorithm (1) computes the Fisher information matrix at a starting guess which takes 5 function evaluations (a central point and 4 derivative directions) which takes ~ 0.25 s, (2) updates its guess (cheap), then (3) recomputes the Fisher information matrix until convergence. It's difficult to guess the number of iterations we will require for convergence, but let's say we can get it down to 10 iterations (~ 2.5 s).

The data set we took this summer has $\sim 150 \times 150 \times 800 \approx 2 \times 10^7$ useful voxels which will take $\sim 5 \times 10^7$ s = 1.5 y.

There are still plenty of places we can save time. In approximate order of ease:

1. Move the function evaluations from Python to C. This shouldn't take much more than a day, and forums report a 3-10× improvement.
2. Optimize the integration method to reduce the number of function evaluations. Empirically, when κ is small we need fewer than 500 function evaluations for the integration to have a 1% error.
3. Bin voxels ($2 \times 2 \times 2$ binning gives 8× speedup, $3 \times 3 \times 3$ gives 27× speedup).

4. Process voxels in parallel on the GPU (10-1000× speedup)

All of these improvements together will (conservatively) put the reconstruction time at ~ 1 day, and likely much faster.

References

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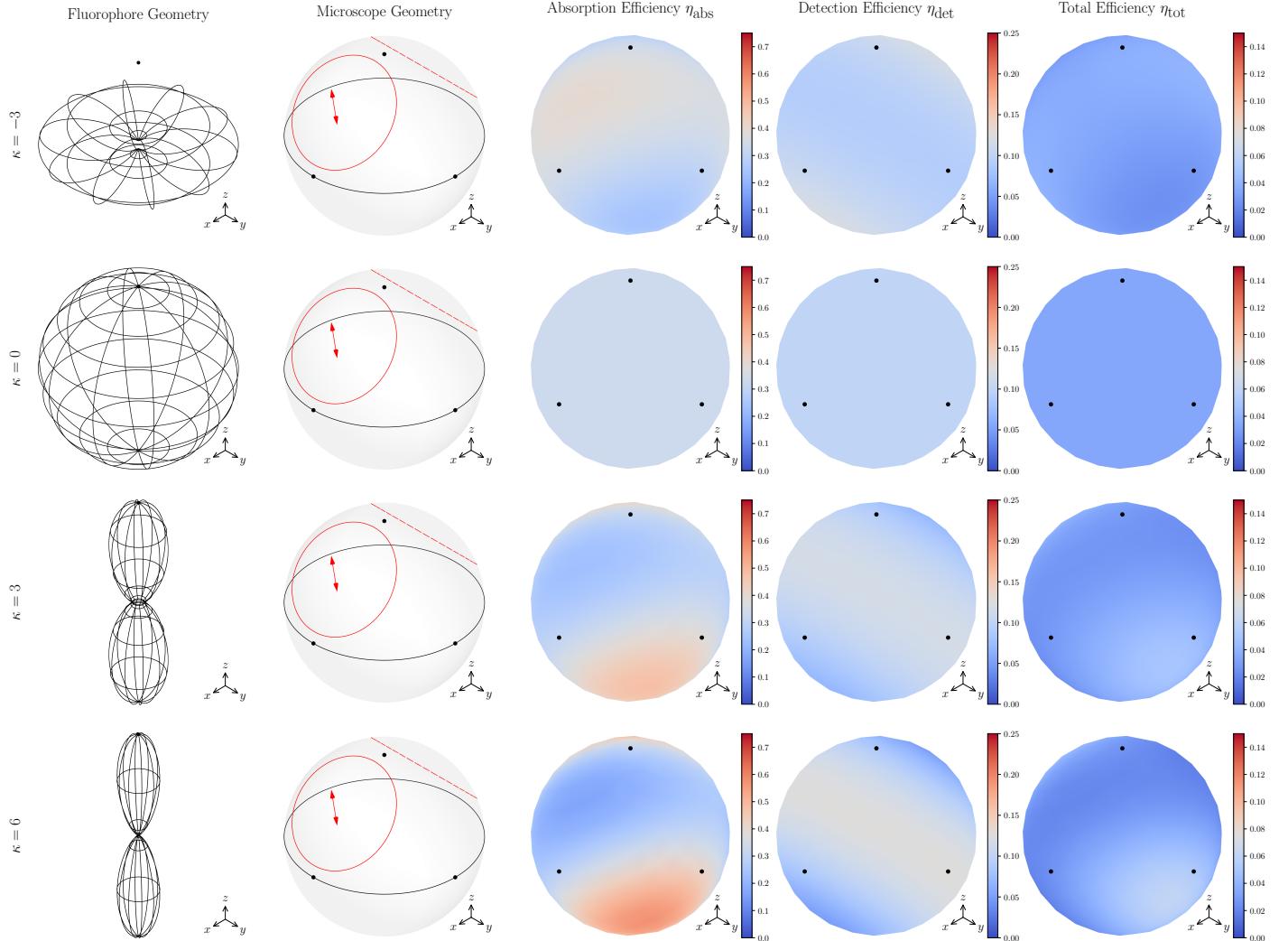


Figure 3: Representative examples of ensemble intensity measurements. Black dots indicate where the Cartesian unit vectors intersect the unit sphere.

Columns left to right: 1) fluorophore distributions with $\hat{\mu} = \hat{z}$ and varying κ ; 2) microscope geometry schematics—all cases use two 0.8 NA objectives in a diSPIM geometry 3) the absorption efficiency as a function of $\hat{\mu}$, see equation 3; 3) the detection efficiency as a function of $\hat{\mu}$, see equation 4; (4) the total efficiency, the product of the absorption and detection efficiencies. **Note:** The last three columns do not use the same scale.