Inconsistency In "Rapid determination of the three-dimensional orientation of single molecules"

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1 Introduction

There is an inconsistency in John T. Fourkas' paper titled "Rapid determination of the three dimensional orientation of single molecules"[1]. In section 2 I describe the inconsistency. In section 3 I follow Fourkas' calculation in detail and correct it. In section 4 I show the results of the corrected calculation and show that the new results are consistent. Finally, in section 5 I discuss how these notes will affect other papers and the applicability of these results.

2 Inconsistency

Equations 4 and 5 of [1] model the fraction of the total intensity from a single fluorophore collected by an objective with a polarizer in the back focal plane as a function of polarizer orientation, dipole orientation (Θ, Φ), and half angle of the light collection cone ($\alpha = \sin^{-1}(\text{NA}/n)$). I expect that as α approaches $\frac{\pi}{2}$ (or a NA approaches n) the fraction of the total power radiated by the dipole that is collected by the lens should approach $\frac{1}{2}$

$$\lim_{\alpha\to\frac{\pi}{2}} \frac{I_0(\Theta,\Phi,\alpha)+I_{90}(\Theta,\Phi,\alpha)}{I_{\text{tot}}(\Theta,\Phi)} = \frac{1}{2}. \tag{1}$$

First, I reproduce equations 4 and 5 from [1]

$$I_0(\Theta,\Phi,\alpha) = I_{\text{tot}}(t,t+\tau)(A + B\sin^2\Theta + C\sin^2\Theta\cos 2\Phi) \tag{2a}$$
$$I_{45}(\Theta,\Phi,\alpha) = I_{\text{tot}}(t,t+\tau)(A + B\sin^2\Theta + C\sin^2\Theta\sin 2\Phi) \tag{2b}$$
$$I_{90}(\Theta,\Phi,\alpha) = I_{\text{tot}}(t,t+\tau)(A + B\sin^2\Theta - C\sin^2\Theta\cos 2\Phi) \tag{2c}$$
$$I_{135}(\Theta,\Phi,\alpha) = I_{\text{tot}}(t,t+\tau)(A + B\sin^2\Theta - C\sin^2\Theta\sin 2\Phi), \tag{2d}$$

and

$$A = \frac{1}{6} - \frac{1}{4}\cos\alpha + \frac{1}{12}\cos^3\alpha \tag{3a}$$
$$B = \frac{1}{8}\cos\alpha - \frac{1}{8}\cos^3\alpha \tag{3b}$$
$$C = \frac{7}{48} - \frac{1}{16}\cos\alpha - \frac{1}{16}\cos^2\alpha - \frac{1}{48}\cos^3\alpha. \tag{3c}$$

Next, I take the limit proposed in equation 1

$$\lim_{\alpha\to\frac{\pi}{2}} \frac{I_0(\Theta,\Phi,\alpha)+I_{90}(\Theta,\Phi,\alpha)}{I_{\text{tot}}(\Theta,\Phi)} = \lim_{\alpha\to\frac{\pi}{2}} 2\left(A(\alpha)+B(\alpha)\sin^2\Theta\right) = 2\left(\frac{1}{6}+0\right) = \frac{1}{3} \neq \frac{1}{2}. \tag{4}$$

Fourkas' model predicts that only $\frac{1}{3}$ of the total power radiated by the fluorophore is collected by an objective with a collection half angle of $\frac{\pi}{2}$.

3 Corrected Calculation

I start by following Fourkas and define the z axis as the optical axis of the objective. I express a directional unit vector $\hat{\mathbf{r}}$ and the fluorescence emission dipole $\hat{\boldsymbol{\mu}}_{\text{em}}$ in spherical coordinates as follows

$$\hat{\mathbf{r}} = \sin\theta\cos\phi\hat{\mathbf{i}} + \sin\theta\sin\phi\hat{\mathbf{j}} + \cos\theta\hat{\mathbf{k}} \tag{5}$$

$$\hat{\boldsymbol{\mu}}_{\text{em}} = \sin\Theta\cos\Phi\hat{\mathbf{i}} + \sin\Theta\sin\Phi\hat{\mathbf{j}} + \cos\Theta\hat{\mathbf{k}}. \tag{6}$$

Next, I calculate the generalized Jones vector $\mathbf{A}$ (GJV or the complex envelope) along a direction $\hat{\mathbf{r}}$ in the far field

$$\hat{\mathbf{A}}_{\text{ff}}(\hat{\mathbf{r}}) = \hat{\mathbf{r}} \times \hat{\boldsymbol{\mu}}_{\text{em}} \times \hat{\mathbf{r}} \tag{7}$$

$$= (\tilde{\mathbf{I}} - \hat{\mathbf{r}}\hat{\mathbf{r}}^{\dagger})\hat{\boldsymbol{\mu}}_{\text{em}} \tag{8}$$

where $\mathbf{I}$ is the identity matrix and † is the adjoint operator. Notice that $(\tilde{\mathbf{I}} - \hat{\mathbf{r}}\hat{\mathbf{r}}^{\dagger})$ is the Green's tensor with the phase excluded. Also notice that $\hat{\mathbf{A}}_{\text{ff}}(\hat{\mathbf{r}})$ is not the electric field—it is the GJV because I have stopped keeping track of the phase. I will follow Fourkas and use equation 7, but I have written equation 8 to relate this work to future work with the Green's tensor.

Next, I model the action of an ideal, infinity corrected, polarization preserving objective on the GJV. The lens rotates the GJV so that it is perpendicular to the z axis at every point on the unit sphere—we can model this by rotating the ray by $-\phi$ about the z axis, by $-\theta$ about the y axis, then by ϕ about z axis. In matrix form the position-dependent rotation matrix is

$$\tilde{\mathbf{R}}(\hat{\mathbf{r}}) = \begin{bmatrix} \cos\theta\cos^2\phi + \sin^2\phi & (\cos\theta - 1)\sin\phi\cos\phi & -\sin\theta\cos\phi \\ (\cos\theta - 1)\sin\phi\cos\phi & \cos\theta\sin^2\phi + \cos^2\phi & -\sin\theta\sin\phi \\ \sin\theta\cos\phi & \sin\theta\sin\phi & \cos\theta \end{bmatrix}, \tag{9}$$

which is identical to the equation 2 in [1].

Next, I express the transmission axis of the polarizer $\hat{\mathbf{P}}$ using

$$\hat{\mathbf{P}} = \cos\phi_{\text{pol}}\hat{\mathbf{i}} + \sin\phi_{\text{pol}}\hat{\mathbf{j}}. \tag{10}$$

I model the action of the polarizer in the back focal plane by taking the dot product of the generalized Jones vector with the polarizer axis $\hat{\mathbf{P}}$ and multiplying by the polarizer axis direction. The GJV in the back focal plane of the objective is given by

$$\mathbf{A}_{\text{bfp}}(\hat{\mathbf{r}}) = \hat{\mathbf{P}}\left[\hat{\mathbf{P}} \cdot \tilde{\mathbf{R}}(\hat{\mathbf{r}})(\hat{\mathbf{r}} \times \hat{\boldsymbol{\mu}}_{\text{em}} \times \hat{\mathbf{r}})\right]. \tag{11}$$

Notice that I am following Fourkas and mapping the GJV directly to the back focal plane without considering the change of coordinates. I will discuss this approximation in section 5.

Next, I model the detection process by squaring the GJV and integrating over the cap of the sphere collected by the objective, Ω. The complete model of the detected intensity is

$$I_{\phi_{\text{pol}}}(\Theta, \Phi) \propto \int_{\Omega} d\hat{\mathbf{r}}\ \left|\hat{\mathbf{P}}\left[\hat{\mathbf{P}} \cdot \tilde{\mathbf{R}}(\hat{\mathbf{r}})(\hat{\mathbf{r}} \times \hat{\boldsymbol{\mu}}_{\text{em}} \times \hat{\mathbf{r}})\right]\right|^2. \tag{12}$$

Next, I calculate the total power emitted by a unit dipole

$$I_{\text{tot}} = \int_{\mathbb{S}^2} d\hat{\mathbf{r}}\ |\hat{\mathbf{r}} \times \hat{\boldsymbol{\mu}}_{\text{em}} \times \hat{\mathbf{r}}|^2 = \int_0^{2\pi} d\phi \int_0^{\pi} d\theta \sin^3\theta = \frac{8\pi}{3} \tag{13}$$

Finally, I calculate the fraction of the total power detected

$$\frac{I_{\phi_{\text{pol}}}(\Theta,\Phi)}{I_{\text{tot}}} = \frac{3}{8\pi}\int_{\Omega} d\hat{\mathbf{r}}\ \left|\hat{\mathbf{P}}\left[\hat{\mathbf{P}}\cdot\tilde{\mathbf{R}}(\hat{\mathbf{r}})(\hat{\mathbf{r}}\times\hat{\boldsymbol{\mu}}_{\text{em}}\times\hat{\mathbf{r}})\right]\right|^2 \tag{14}$$

where the total radiated power I_{tot} was found using equation 12 with no polarizer or objective and by integrating over all directions.

Notice that I normalized using the total radiated intensity. Fourkas incorrectly normalized the GJV before calculating the intensity as follows

$$\frac{I_{\phi_{\text{pol}}}(\Theta,\Phi)}{I_{\text{tot}}} \neq \int_{\Omega} d\hat{\mathbf{r}}\left|\left(\frac{\mathbf{A}_{\phi_{\text{pol}}}}{\mathbf{A}_{\text{tot}}}\right)\right|^2 \tag{15}$$

It's not physically clear what $\mathbf{A}_{\text{tot}}$ represents, but I can make a good guess about how Fourkas calculated it

$$\mathbf{A}_{\text{tot}} = \int_{\mathbb{S}^2} d\hat{\mathbf{r}}(\hat{\mathbf{r}}\times\hat{\boldsymbol{\mu}}_{\text{em}}\times\hat{\mathbf{r}}) = \int_0^{2\pi} d\phi\int_0^{\pi} d\theta\sin\theta = 4\pi \tag{16}$$

Plugging 16 into 15 gives

$$\frac{I_{\phi_{\text{pol}}}(\Theta,\Phi)}{I_{\text{tot}}} \neq \frac{1}{16\pi}\int_{\Omega} d\hat{\mathbf{r}}\ \left|\hat{\mathbf{P}}\left[\hat{\mathbf{P}}\cdot\tilde{\mathbf{R}}(\hat{\mathbf{r}})(\hat{\mathbf{r}}\times\hat{\boldsymbol{\mu}}_{\text{em}}\times\hat{\mathbf{r}})\right]\right|^2 \tag{17}$$

Notice that equation 17 and equation 14 differ by a factor of $\frac{3}{2}$.

4 Results

I evaluated equations 14 and 17 by substituting equations 5, 6, 9, and 10 and simplifying using SymPy, an open-source computer algebra system. Equation 17 yields Fourkas' published expressions. Equation 14 yields Fourkas' expressions multiplied by a factor of $\frac{3}{2}$. We can make this correction by changing the coefficients of A, B, and C to

$$A_{\text{new}} = \frac{1}{4} - \frac{3}{8}\cos\alpha + \frac{1}{8}\cos^3\alpha \tag{18a}$$

$$B_{\text{new}} = \frac{3}{16}\cos\alpha - \frac{3}{16}\cos^3\alpha \tag{18b}$$

$$C_{\text{new}} = \frac{7}{32} - \frac{3}{32}\cos\alpha - \frac{3}{32}\cos^2\alpha - \frac{1}{32}\cos^3\alpha. \tag{18c}$$

Fourkas' published inversion expressions still hold if we use A_{new}, B_{new}, and C_{new}.

Finally, I confirm that taking the limit with the new expressions approaches $\frac{1}{2}$

$$\lim_{\alpha\to\frac{\pi}{2}}\frac{I_0(\Theta,\Phi)+I_{90}(\Theta,\Phi)}{I_{\text{tot}}(\Theta,\Phi)} = \lim_{\alpha\to\frac{\pi}{2}} 2\left(A_{\text{new}} + B_{\text{new}}\sin^2\Theta\right) = 2\left(\frac{1}{4}+0\right) = \frac{1}{2} \tag{19}$$

5 Discussion

Fourkas' expressions underpredict the fraction of the total power collected by an objective with a polarizer by a constant fraction of $\frac{3}{2}$. Therefore, using Fourkas' expressions to find I_{tot} will result in overpredictions of I_{tot}, but the incorrect expressions will not cause any error in the predictions of molecular orientation.

Lu and Bout [2] study the effect of noise on the reconstruction of single fluorophores using Fourkas' expressions. Their main results are unaffected by the correction introduced in these notes, but because these notes predict a higher intensity than Fourkas, Lu and Bout overpredict the effect of noise in their model.

As mentioned after equation 11, Fourkas uses an approximation when he directly maps the GJV in the back focal plane to the GJV in the detector plane. To conserve energy, the power carried by a ray that makes an angle θ with the optical axis needs to be attenuated by a factor of $\sqrt{\cos\theta}$ [3, 4]. This attenuation factor can be ignored under the paraxial approximation ($\cos\theta \approx 1$), which means that all of the results in Fourkas and the correction in these notes only apply to low NA lenses. Analytically extending these results to high NA lenses could be the subject of future work.

As noted in [5], Fourkas' model only considers single molecules in homogeneous environments. With an extension of the Green's tensor (equation 8) [3, 4], we could consider single molecules near glass interfaces.

References

[1] John T. Fourkas. Rapid determination of the three-dimensional orientation of single molecules. *Opt. Lett.*, 26(4):211–213, Feb 2001.

[2] Chun-Yaung Lu and David A Vanden Bout. Analysis of orientational dynamics of single fluorophore trajectories from three-angle polarization experiments. *The Journal of chemical physics*, 128 24:244501, 2008.

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[5] M. Andreas Lieb, James M. Zavislan, and Lukas Novotny. Single-molecule orientations determined by direct emission pattern imaging. *J. Opt. Soc. Am. B*, 21(6):1210–1215, Jun 2004.