

Finding degeneracy in fluorescence orientation microscopes

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Consider a microscope with an angular point spread function $h_i(\hat{\mathbf{s}})$ —we’re assuming that the spatial and angular problems are decoupled. The forward model for this microscope is

$$g_i = \int_{\mathbb{S}^2} d\hat{\mathbf{s}} h_i(\hat{\mathbf{s}}) f(\hat{\mathbf{s}}), \quad i = 1, 2, \dots, N. \quad (1)$$

where $f(\hat{\mathbf{s}})$ is the angular density of fluorophores and i indexes N measurements.

We would like to find the angular degeneracies of this microscope. In other words, what rotations \mathbf{R} can we apply to samples $f(\hat{\mathbf{s}})$ and measure the same data? Mathematically, we need to find the set of matrices \mathbf{R} and functions $f(\hat{\mathbf{s}})$ that satisfy

$$\int_{\mathbb{S}^2} d\hat{\mathbf{s}} h_i(\hat{\mathbf{s}}) f(\hat{\mathbf{s}}) - \int_{\mathbb{S}^2} d\hat{\mathbf{s}} h_i(\hat{\mathbf{s}}) f(\mathbf{R}^{-1}\hat{\mathbf{s}}) = 0, \quad i = 1, 2, \dots, N. \quad (2)$$

We will start by restricting the problem to objects where all of the fluorophores are oriented in the same direction so that $f(\hat{\mathbf{s}}) = \delta(\hat{\mathbf{s}} - \hat{\mathbf{s}}_1)$. In this case we need to find the set of directions $\hat{\mathbf{s}}_1$ and matrices \mathbf{R} that satisfy

$$h_i(\hat{\mathbf{s}}_1) - h_i(\mathbf{R}^{-1}\hat{\mathbf{s}}_1) = 0, \quad i = 1, 2, \dots, N. \quad (3)$$

We have a set of N equations that we need to solve simultaneously for $\hat{\mathbf{s}}_1 \in \mathbb{S}^2$ and $\mathbf{R} \in \mathbb{O}(3)$. Notice that we allow for improper rotations ($\det \mathbf{R} = -1$) by allowing $\mathbf{R} \in \mathbb{O}(3)$ instead of restricting to $\mathbf{R} \in \mathbb{SO}(3)$.

As far as I can tell there is no analytic way to solve this set of equations in the general case. I tried expanding onto spherical and rotational harmonics, but that didn’t help because there’s no easy way to relate a solution in the frequency domain to a solution in the normal domain. Instead, we can find as many solutions as we can “by hand” then check our results numerically. I’ve used optimization packages that can optimize on $\mathbb{S}^2 \times \mathbb{O}(3)$ —my rough plan is to seed an optimization procedure at many place on a grid in $\mathbb{S}^2 \times \mathbb{O}(3)$ and check (not prove!) that we have all of the solutions with those results. Other ideas welcome!

For now let’s solve a simple case “by hand”. If we have a single point detector along the z axis then our angular point spread function is given by

$$h^{(\hat{\mathbf{z}})}(\hat{\mathbf{s}}_1) = 1 - (\hat{\mathbf{s}}_1 \cdot \hat{\mathbf{z}})^2. \quad (4)$$

This is the first time I’m expressing an angular point spread function in vector notation, but it’s easy to check that this reduces to the familiar $h^{(\hat{\mathbf{z}})}(\hat{\mathbf{s}}_1) = \sin^2 \vartheta$ if you choose spherical coordinates so that $\hat{\mathbf{s}}_1 = \cos \varphi \sin \vartheta \hat{\mathbf{x}} + \sin \varphi \sin \vartheta \hat{\mathbf{y}} + \cos \vartheta \hat{\mathbf{z}}$. Plugging Eq. 4 into Eq. 2 gives

$$-(\hat{\mathbf{s}}_1 \cdot \hat{\mathbf{z}})^2 + (\mathbf{R}^{-1}\hat{\mathbf{s}}_1 \cdot \hat{\mathbf{z}})^2 = 0. \quad (5)$$

I can find the following solutions to this equation for all $\hat{\mathbf{s}}_1 \in \mathbb{S}^2$

$$\mathbf{R}_0 = \begin{bmatrix} \cos \theta & -\sin \theta & 0 \\ \sin \theta & \cos \theta & 0 \\ 0 & 0 & 1 \end{bmatrix} \text{ for } 0 \leq \theta < 2\pi, \quad (6)$$

which is an arbitrary rotation about the z axis;

$$\mathbf{R}_1 = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & -1 \end{bmatrix}, \quad (7)$$

which is a mirroring operation about the transverse plane; and

$$\mathbf{R}_2 = \begin{bmatrix} -1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & -1 \end{bmatrix}, \quad (8)$$

which is an inversion operation.

We can identify the solutions \mathbf{R}_0 , \mathbf{R}_1 , and \mathbf{R}_2 as matrix representations of a group. This group is a *Frieze group* with *dihedral symmetry*, an infinite number of possible rotations, and a horizontal reflection axis. Using Schönflies notation we say that $D_{\infty h}$ is the symmetry group of the imaging system.

There are more solutions to the problem—for example a proper rotation that maps $\hat{\mathbf{s}}_1$ to $-\hat{\mathbf{s}}_1$ instead of an improper rotation—but these solutions give the same mappings as the solutions above, so it's okay to ignore them. Alternatively, we could restrict ourselves to finding solutions in $\mathbb{SO}(3)$ to find unique solutions, but this makes the solutions much more difficult to identify.