

Single Molecule Fluorescence Microscopy Update

Talon Chandler

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1 Current Model and Limitations

In the previous note set, we developed the following forward model

$$I_{\text{img}}(\mathbf{r}'') = \left| \mathcal{F}_{3D} \left\{ \tilde{\mathbf{O}}_{\text{obj}} \overset{\leftrightarrow}{\mathbf{G}}_{FF} \overset{\leftrightarrow}{\boldsymbol{\alpha}} \mathbf{E}_{\text{in}} \right\} \right|^2. \quad (1)$$

Rudolph and Shalin identified several limitations of the model:

- The model needlessly tracks phase from start to finish. Rudolph and Shalin correctly pointed out that phase is irrelevant on the illumination side because we measure the intensity over long time periods compared to the period of the electric fields.
- The model only considers monochromatic illumination by a single plane wave and focused laser illumination, not Köhler illumination by a broadband source.
- The model only considers excitation of a single fluorophore.

These notes address these limitations.

1.1 Single Fluorophore, Monochromatic Plane Wave

Consider a monochromatic plane wave with polarization axis \mathbf{E} incident on a single fluorophore with absorption dipole moment axis $\hat{\boldsymbol{\mu}}_{\text{abs}}$ and emission dipole moment $\hat{\boldsymbol{\mu}}_{\text{em}}$. The incident plane wave induces a dipole moment given by

$$\boldsymbol{\mu}_{\text{ind}} \propto \hat{\boldsymbol{\mu}}_{\text{em}} [\hat{\boldsymbol{\mu}}_{\text{abs}} \cdot \mathbf{E}]. \quad (2)$$

1.2 Many Fluorophores, Monochromatic Plane Wave

Now consider N fluorophores in the specimen volume with absorption dipole moments $\hat{\boldsymbol{\mu}}_{\text{abs},i}$, emission dipole moments $\hat{\boldsymbol{\mu}}_{\text{em},i}$, and positions \mathbf{r}_i . We define the *emission dipole moment map* as $\boldsymbol{\mu}_{\text{em}}(\mathbf{r}) \equiv \sum_{i=0}^N \hat{\boldsymbol{\mu}}_{\text{em},i} \delta(\mathbf{r} - \mathbf{r}_i)$. Similarly, we define the *absorption dipole moment map* as $\boldsymbol{\mu}_{\text{abs}}(\mathbf{r}) \equiv \sum_{i=0}^N \hat{\boldsymbol{\mu}}_{\text{abs},i} \delta(\mathbf{r} - \mathbf{r}_i)$. When we illuminate the fluorophores with a monochromatic plane wave we create the *induced dipole map* given by

$$\boldsymbol{\mu}_{\text{ind}}(\mathbf{r}) \propto \boldsymbol{\mu}_{\text{em}}(\mathbf{r}) [\boldsymbol{\mu}_{\text{abs}}(\mathbf{r}) \cdot \mathbf{E}]. \quad (3)$$

1.3 Many Fluorophores, Focused Laser Illumination

Under focused laser illumination the electric field in the specimen volume is position dependent. If $\mathbf{E}(\mathbf{r})$ is the real 3D electric field direction at every point in the specimen volume, then the induced dipole map is given by

$$\boldsymbol{\mu}_{\text{ind}}(\mathbf{r}) \propto \boldsymbol{\mu}_{\text{em}}(\mathbf{r}) [\boldsymbol{\mu}_{\text{abs}}(\mathbf{r}) \cdot \mathbf{E}(\mathbf{r})]. \quad (4)$$

1.4 Many Fluorophores, Broadband Plane Wave

If we illuminate a fluorophore with a broadband plane wave and measure the induced dipole moment over a period of time much longer than the coherence time, then the effective induced dipole moment will be the sum of

the induced dipole moments created by each frequency component. In other words, each frequency component acts independently on the fluorophore to create an induced dipole moment.

If we want to compare illumination sources with different spectra, we will need to consider the *excitation efficiency*, $\eta(\omega)$, of each frequency ω . For a two level system, $\eta(\omega) = 1$ at the resonance frequency and drops to 0 far from the resonance frequency following a Lorentzian function. $\eta(\omega)$ is a more complicated function for real fluorophores. The induced dipole map is given by

$$\boldsymbol{\mu}_{\text{ind}}(\mathbf{r}) \propto \int_{-\infty}^{\infty} d\omega \eta(\omega) \boldsymbol{\mu}_{\text{em}}(\mathbf{r}) [\boldsymbol{\mu}_{\text{abs}}(\mathbf{r}) \cdot \mathbf{E}(\omega)] . \quad (5)$$

1.5 Many Fluorophores, Köhler Illumination

If we illuminate a fluorophore with two plane waves with a random phase difference traveling in different directions and measure the induced dipole over a long time compared with the coherence time of the two waves, the induced dipole is the sum of the induced dipole created by each plane wave independently. In other words, each plane wave creates an induced dipole independently. (Thank you Rudolph and Shalin for steering me in this direction).

Under Köhler illumination with a polarizer in the back focal plane, each paraxial point in the back focal plane of the condenser creates a polarized plane wave that is constant throughout the specimen volume. Therefore, to find the induced dipole moment map, we can integrate over the back focal plane of the condenser

$$\boldsymbol{\mu}_{\text{ind}}(\mathbf{r}) \propto \int_{-\infty}^{\infty} d\omega \int d\mathbf{r}' \eta(\omega) \boldsymbol{\mu}_{\text{em}}(\mathbf{r}) [\boldsymbol{\mu}_{\text{abs}}(\mathbf{r}) \cdot \mathbf{R}_{\hat{\mathbf{s}}}(\mathbf{r}') \mathbf{E}_{\text{bfp}}(\omega)] \quad (6)$$

where \mathbf{r}' is the position in the back focal plane, \mathbf{E}_{bfp} is the electric field direction in the back focal plane set by a linear polarizer, and $\mathbf{R}_{\hat{\mathbf{s}}}(\mathbf{r}')$ is a rotation matrix that rotates the electric field in the back focal plane to the electric field in the specimen volume.

Note that equation 6 depends on the paraxial approximation. Points in the back focal plane that are far from the optical axis do not create perfect plane waves in the specimen volume. We can explore more accurate models for high NA illumination if necessary.

2 Revised Model Summary

On the detection side, the model remains the same. The intensity in the image plane is given by

$$I_{\text{img}}(\mathbf{r}'') \propto \left| \mathcal{F}_{3D} \left\{ \tilde{\mathbf{O}}_{\text{obj}} \overset{\leftrightarrow}{\mathbf{G}}_{FF} \boldsymbol{\mu}_{\text{ind}}(\mathbf{r}) \right\} \right|^2 . \quad (7)$$

Under Köhler illumination the induced dipole moment map is

$$\boldsymbol{\mu}_{\text{ind}}(\mathbf{r}) \propto \int_{-\infty}^{\infty} d\omega \int d\mathbf{r}' \eta(\omega) \boldsymbol{\mu}_{\text{em}}(\mathbf{r}) [\boldsymbol{\mu}_{\text{abs}}(\mathbf{r}) \cdot \mathbf{R}_{\hat{\mathbf{s}}}(\mathbf{r}') \mathbf{E}_{\text{bfp}}(\omega)] \quad (8)$$

Under laser illumination the induced dipole moment is

$$\boldsymbol{\mu}_{\text{ind}}(\mathbf{r}) \propto \boldsymbol{\mu}_{\text{em}}(\mathbf{r}) [\boldsymbol{\mu}_{\text{abs}}(\mathbf{r}) \cdot \mathbf{E}(\mathbf{r})] \quad (9)$$

where $\mathbf{E}(\mathbf{r})$ is the electric field in the specimen volume.

3 Matrix Form of the Model

Rudolph and Shalin suggested simplifying the model by defining a *polarization resolved intensity*, $\mathbf{I}_{\text{in}} = [I_{\text{in}}^x, I_{\text{in}}^y, I_{\text{in}}^z]$ that we could use to create a matrix form of the model. Unfortunately, both the emission and absorption dipole maps appear in equations 8 and 9. This means that even if the emission and absorption dipoles point in the same direction, the induced dipole moments depend on the cross terms of the dipole direction. In other words, the model is linear in $[\mu_x^2, \mu_y^2, \mu_z^2, \mu_x\mu_y, \mu_x\mu_z, \mu_y\mu_z]$, not just $[\mu_x^2, \mu_y^2, \mu_z^2]$. If we define a polarization resolved intensity vector it will need six terms, not three.

Backer and Moerner made a similar simplification on the detection side to speed up their deconvolutions [1].

References

- [1] Adam S. Backer and W. E. Moerner. Extending single-molecule microscopy using optical fourier processing. *The Journal of Physical Chemistry B*, 118(28):8313–8329, 2014. PMID: 24745862.