Update On 3D Orientation Reconstruction

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January 10, 2017

Forward Model

$$g_i = \int_{\mathbb{S}^2} d\hat{\mathbf{r}}\ h_i(\hat{\mathbf{r}})f(\hat{\mathbf{r}})$$

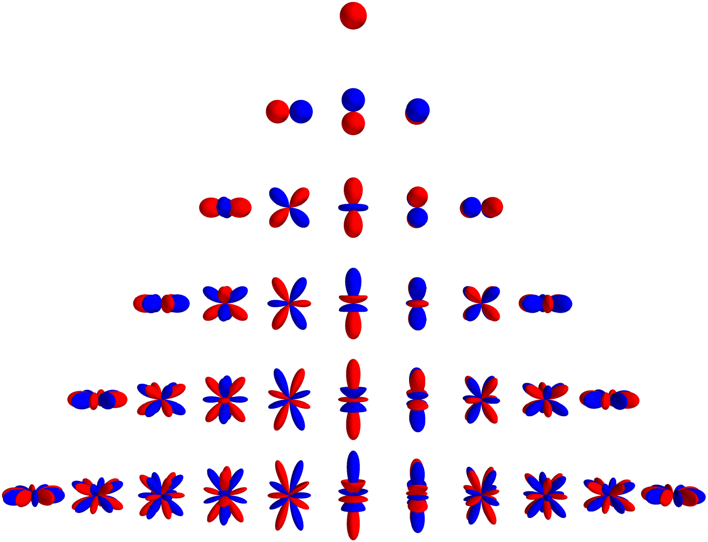
- $g_i \rightarrow$ is the ith intensity measurement
- $h_i(\hat{\mathbf{r}}) \rightarrow$ is the ith point response function
- $f(\hat{\mathbf{r}}) \rightarrow$ is the orientation distribution function

Fourier Transforms

$$\text{Fourier Transform} \rightarrow F(\nu) = \int_{\mathbb{R}} dx\ f(x)e^{-2\pi i x \nu}$$

$$\text{Real Spherical Fourier Transform} \rightarrow F_l^m = \int_{\mathbb{S}^2} d\hat{\mathbf{r}}\ f(\hat{\mathbf{r}}) y_l^m(\hat{\mathbf{r}})$$

Spherical Harmonics $y_l^m(\hat{\mathbf{r}})$



Integral $\to$ Matrix

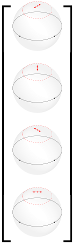
$$g_i = \int_{\mathbb{S}^2} d\hat{\mathbf{r}}\ h_i(\hat{\mathbf{r}}) f(\hat{\mathbf{r}})$$

$$g_i = \mathbf{H}_i^T \mathbf{F}$$

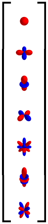
$$\mathbf{g} = \mathbf{\Psi} \mathbf{F}$$

Single-view epi-illumination microscope NA = 0.8

$$\mathbf{g} = \mathbf{\Psi}\mathbf{F}$$



$$= \begin{bmatrix} 0.50 & 0.00 & -0.31 & 0.41 & 0.00 & 0.07 & -0.07 \\ 0.50 & -0.41 & -0.31 & 0.00 & 0.07 & 0.07 & -0.00 \\ 0.50 & -0.00 & -0.31 & -0.41 & 0.00 & 0.07 & 0.07 \\ 0.50 & 0.41 & -0.31 & -0.00 & -0.07 & 0.07 & 0.00 \end{bmatrix}$$



Inverting A Rectangular Matrix

$$\underset{N\times 1}{\mathbf{g}} = \underset{N\times M}{\mathbf{\Psi}}\underset{M\times 1}{\mathbf{F}}$$

Decompose $\mathbf{\Psi}$ into three matrices

$$\underset{N\times 1}{\mathbf{g}} = \underset{N\times N}{\mathbf{U}}\underset{N\times M}{\mathbf{\Sigma}}\underset{M\times M}{\mathbf{V}^T}\underset{M\times 1}{\mathbf{F}}$$

where $\mathbf{U}$ and $\mathbf{V}$ are orthogonal matrices—that is $\mathbf{U}^T = \mathbf{U}^{-1}$ or equivalently $\mathbf{U}^T\mathbf{U} = \mathbf{I}$—and $\mathbf{\Sigma}$ is a diagonal matrix. Solving for $\mathbf{F}$ gives

$$\underset{M\times 1}{\mathbf{F}} = \underset{M\times M}{\mathbf{V}}\underset{M\times N}{\mathbf{\Sigma}^{-1}}\underset{N\times N}{\mathbf{U}^T}\underset{N\times 1}{\mathbf{g}}.$$

But wait! $\mathbf{\Sigma}^{-1}$ doesn't exist. Instead, replace every element of $\mathbf{\Sigma}$ with its inverse (except the zeros), takes its transpose, and call it $\mathbf{\Sigma}^+$

$$\underset{M\times 1}{\mathbf{F}} = \underset{M\times M}{\mathbf{V}}\underset{M\times N}{\mathbf{\Sigma}^{+}}\underset{N\times N}{\mathbf{U}^T}\underset{N\times 1}{\mathbf{g}}.$$

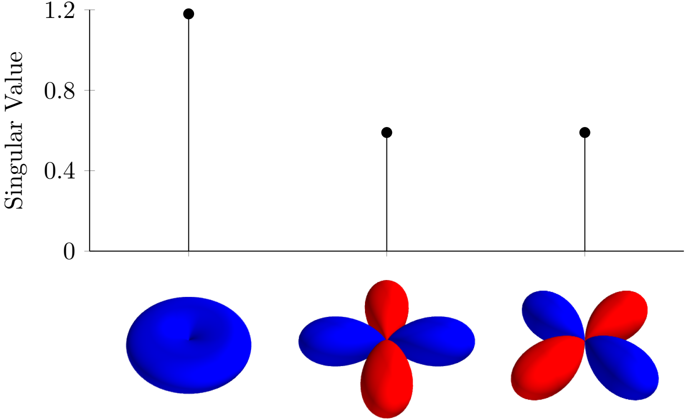
This gives us the closest we can get to an inverse—the pseudo-inverse

$$\underset{M\times 1}{\mathbf{F}} = \underset{M\times N}{\mathbf{\Psi}^+}\underset{N\times 1}{\mathbf{g}}.$$

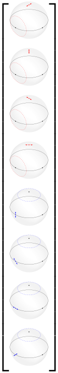
Vocabulary

$$\begin{aligned}
\boldsymbol{\Psi}^{+} &\rightarrow \textbf{pseudo-inverse} \text{ of } \boldsymbol{\Psi} \\
\boldsymbol{\Psi} = \mathbf{U}\boldsymbol{\Sigma}\mathbf{V}^{T} &\rightarrow \textbf{singular-value decomposition} \text{ of } \boldsymbol{\Psi} \\
\text{Diagonal elements of } \boldsymbol{\Sigma} &\rightarrow \textbf{singular values} \text{ of } \boldsymbol{\Psi} \\
\text{Columns of } \mathbf{V} &\rightarrow \textbf{right singular vectors} \text{ of } \boldsymbol{\Psi} \\
\text{Columns of } \mathbf{U} &\rightarrow \textbf{left singular vectors} \text{ of } \boldsymbol{\Psi}
\end{aligned}$$

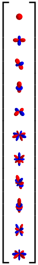
Single-view epi-illumination microscope NA = 0.8



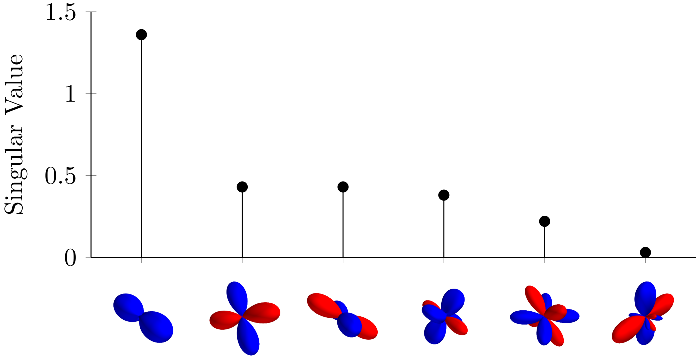
Symmetric diSPIM NA $= 0.8$



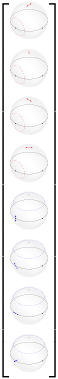
$$= \begin{bmatrix} 0.27 & 0.00 & 0.00 & -0.05 & 0.09 & 0.00 & 0.00 & 0.00 & -0.05 & 0.07 & -0.10 \\ 0.38 & -0.28 & 0.00 & -0.13 & -0.19 & 0.10 & -0.04 & 0.00 & -0.03 & 0.04 & -0.00 \\ 0.50 & -0.00 & 0.00 & -0.20 & -0.48 & 0.00 & -0.00 & 0.00 & -0.02 & -0.00 & 0.10 \\ 0.38 & 0.28 & 0.00 & -0.13 & -0.19 & -0.10 & 0.04 & 0.00 & -0.03 & 0.04 & 0.00 \\ 0.27 & 0.00 & 0.00 & 0.10 & 0.00 & 0.00 & 0.00 & 0.00 & -0.13 & 0.00 & 0.00 \\ 0.38 & 0.00 & 0.28 & -0.10 & -0.21 & 0.00 & 0.00 & -0.10 & -0.03 & 0.04 & 0.00 \\ 0.50 & 0.00 & 0.00 & -0.31 & -0.41 & 0.00 & 0.00 & -0.00 & 0.07 & 0.07 & 0.00 \\ 0.38 & 0.00 & -0.28 & -0.10 & -0.21 & 0.00 & 0.00 & 0.10 & -0.03 & 0.04 & 0.00 \end{bmatrix}$$



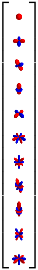
Symmetric diSPIM, NA = 0.8



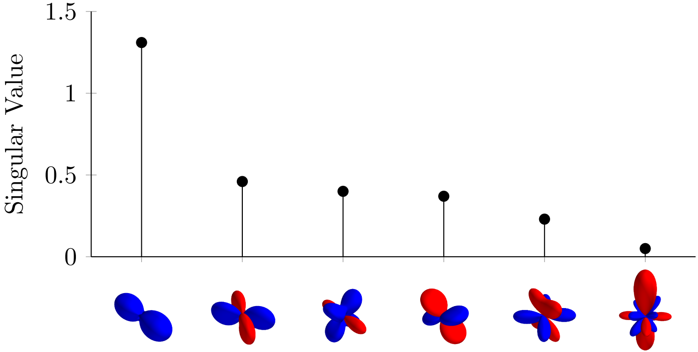
Asymmetric diSPIM, NA = 1.1/0.7



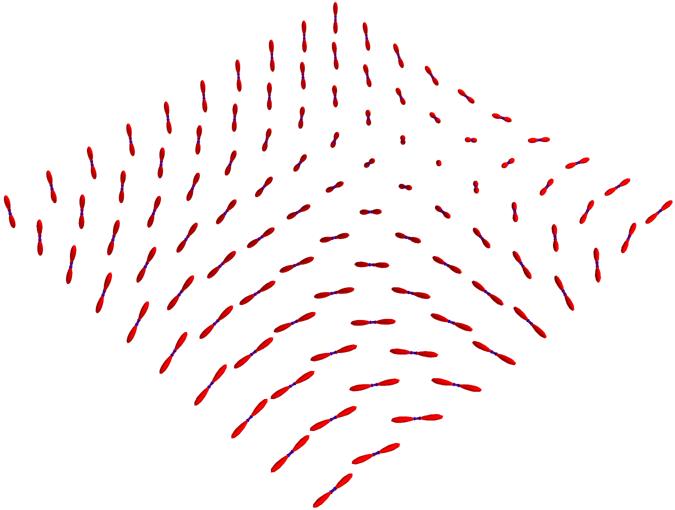
$$
= \begin{bmatrix}
0.28 & 0.00 & 0.00 & -0.05 & 0.09 & 0.00 & 0.00 & 0.00 & -0.06 & 0.08 & -0.11 \\
0.41 & -0.30 & 0.00 & -0.13 & -0.22 & 0.11 & -0.04 & 0.00 & -0.04 & 0.04 & -0.00 \\
0.54 & -0.00 & 0.00 & -0.22 & -0.52 & 0.00 & -0.00 & 0.00 & -0.02 & 0.00 & 0.11 \\
0.41 & 0.30 & 0.00 & -0.13 & -0.22 & -0.11 & 0.04 & 0.00 & -0.04 & 0.04 & 0.00 \\
0.28 & 0.00 & 0.00 & 0.17 & 0.00 & 0.00 & 0.00 & 0.00 & -0.07 & 0.00 & 0.00 \\
0.34 & 0.00 & 0.26 & -0.03 & -0.17 & 0.00 & 0.00 & -0.06 & -0.02 & 0.02 & 0.00 \\
0.41 & 0.00 & 0.00 & -0.23 & -0.33 & 0.00 & 0.00 & -0.00 & 0.04 & 0.04 & 0.00 \\
0.34 & 0.00 & -0.26 & -0.03 & -0.17 & 0.00 & 0.00 & 0.06 & -0.02 & 0.02 & 0.00
\end{bmatrix}
$$



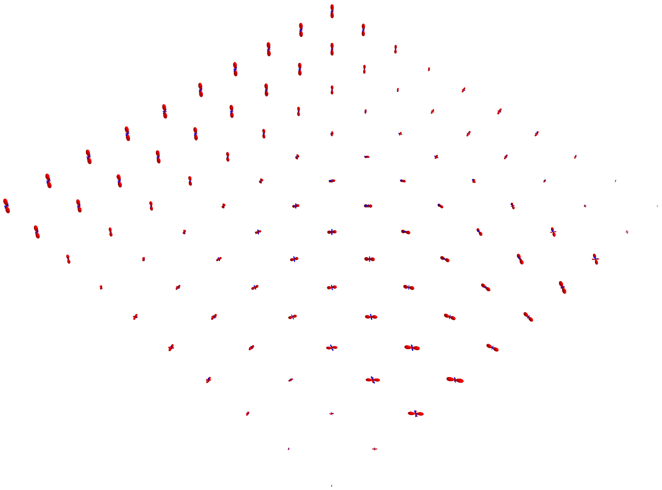
Asymmetric diSPIM NA = 1.1/0.7



Test phantom - single fluorophores approximated by 7 degrees of spherical harmonics



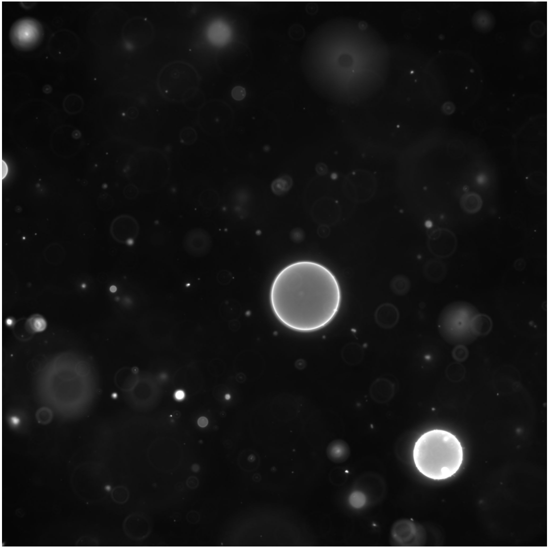
Measured by the asymmetric diSPIM then reconstructed. No priors! Not even positivity ;)



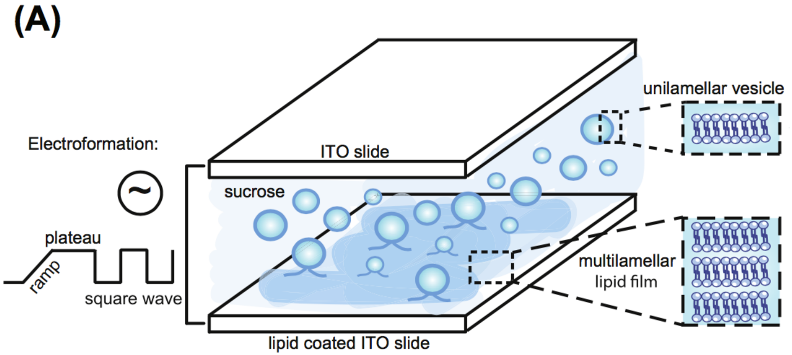
Up Next

- Incorporating priors—positivity, single fluorophore, rotational symmetry
- Math OSA 2-page abstract on Jan 18—I'll include the work in these slides. I'll possibly include priors depending on how quickly that goes.

Giant Unilamallar Vesicles (GUV)
FOV $\approx 150 \times 150\ \mu$m

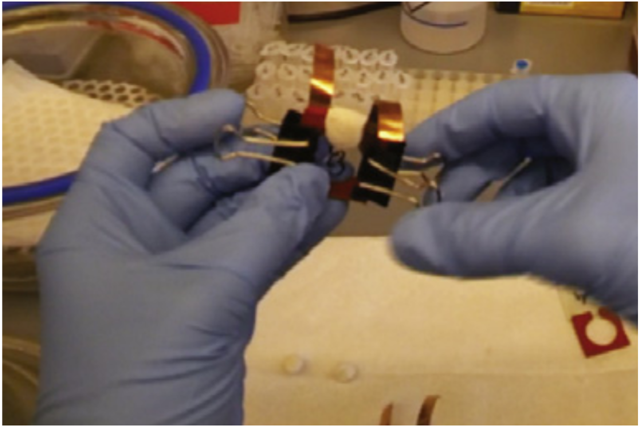


GUV Protocol



Schmid, 2015

GUV Chamber



Schmid, 2015