

Update On 3D Orientation Reconstruction

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Forward Model

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

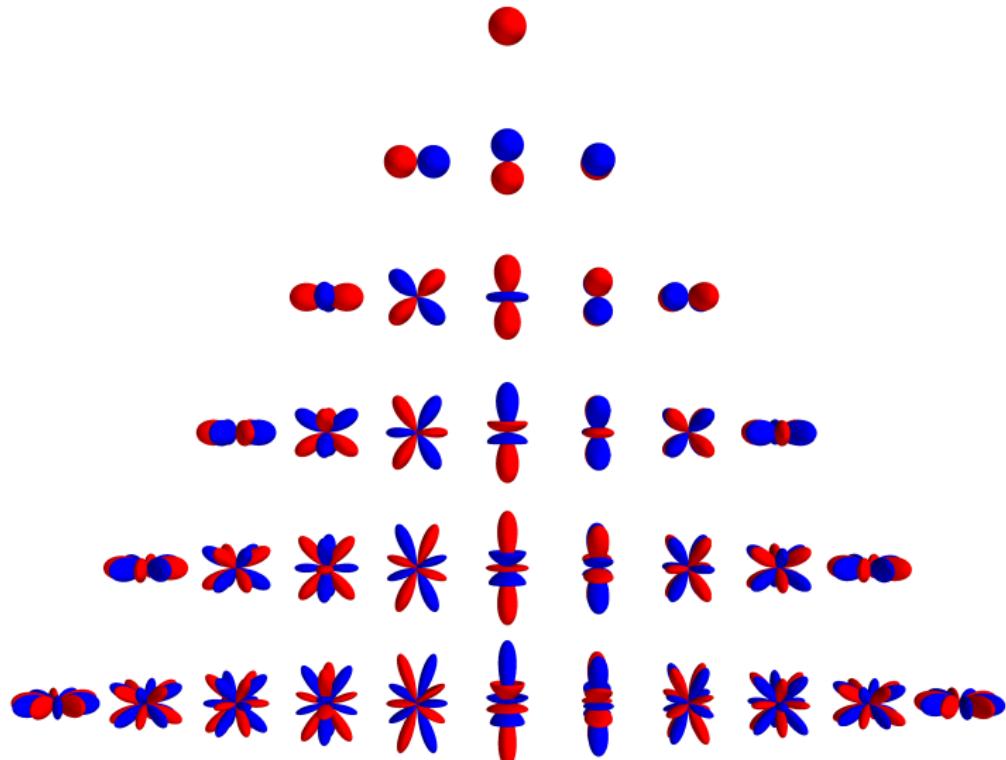
- ▶ $g_i \rightarrow$ is the i th intensity measurement
- ▶ $h_i(\hat{\mathbf{r}}) \rightarrow$ is the i th point response function
- ▶ $f(\hat{\mathbf{r}}) \rightarrow$ is the orientation distribution function

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 → Matrix

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

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

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

Single-view epi-illumination microscope NA = 0.8

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

$$\begin{bmatrix} \text{Image 1} \\ \text{Image 2} \\ \text{Image 3} \\ \text{Image 4} \end{bmatrix} = \begin{bmatrix} 0.50 & 0.00 & -0.31 & 0.41 & 0.00 & 0.07 & -0.07 \\ 0.50 & -0.41 & -0.31 & 0.00 & 0.07 & 0.07 & -0.00 \\ 0.50 & -0.00 & -0.31 & -0.41 & 0.00 & 0.07 & 0.07 \\ 0.50 & 0.41 & -0.31 & -0.00 & -0.07 & 0.07 & 0.00 \end{bmatrix} \begin{bmatrix} \text{Feature 1} \\ \text{Feature 2} \\ \text{Feature 3} \\ \text{Feature 4} \\ \text{Feature 5} \\ \text{Feature 6} \\ \text{Feature 7} \\ \text{Feature 8} \end{bmatrix}$$

Inverting A Rectangular Matrix

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

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

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

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 $\boldsymbol{\Sigma}$ is a diagonal matrix. Solving for \mathbf{F} gives

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

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

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

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

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

Vocabulary

Ψ^+ → pseudo-inverse of Ψ

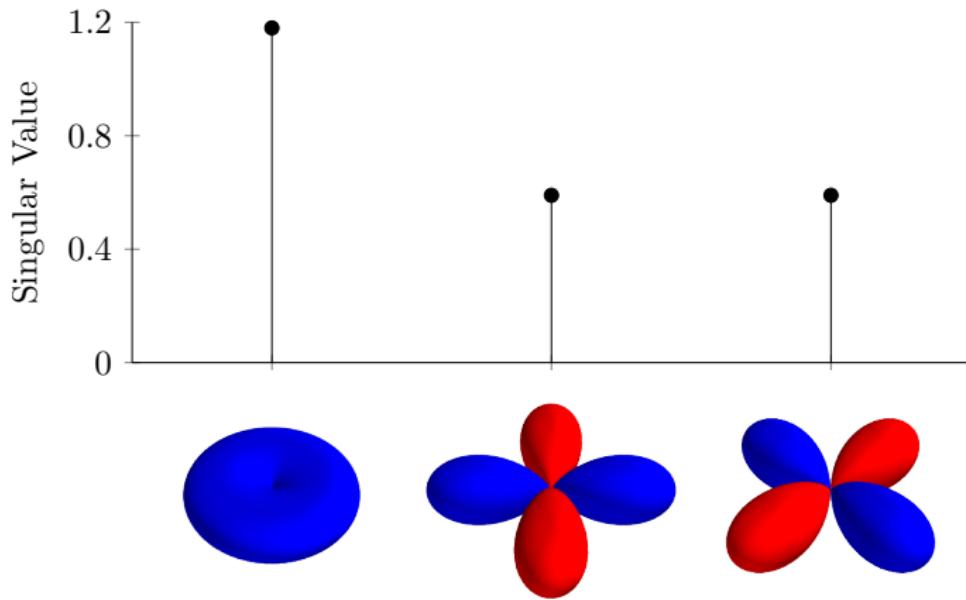
$\Psi = \mathbf{U}\Sigma\mathbf{V}^T$ → singular-value decomposition of Ψ

Diagonal elements of Σ → singular values of Ψ

Columns of \mathbf{V} → right singular vectors of Ψ

Columns of \mathbf{U} → left singular vectors of Ψ

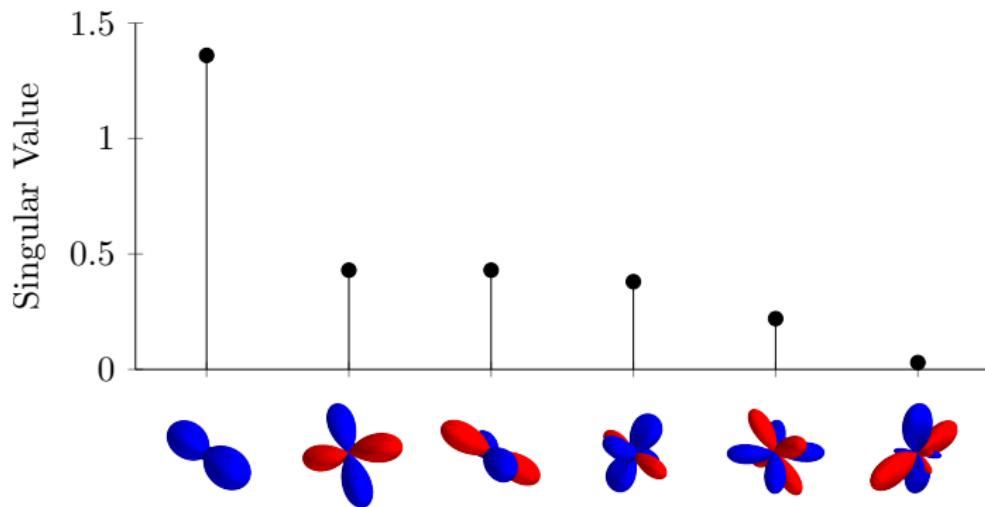
Single-view epi-illumination microscope NA = 0.8



Symmetric diSPIM NA = 0.8

$$\begin{bmatrix} \text{Image 1} \\ \text{Image 2} \\ \text{Image 3} \\ \text{Image 4} \\ \text{Image 5} \\ \text{Image 6} \\ \text{Image 7} \\ \text{Image 8} \\ \text{Image 9} \end{bmatrix} = \begin{bmatrix} 0.27 & 0.00 & 0.00 & -0.05 & 0.09 & 0.00 & 0.00 & 0.00 & -0.05 & 0.07 & -0.10 \\ 0.38 & -0.28 & 0.00 & -0.13 & -0.19 & 0.10 & -0.04 & 0.00 & -0.03 & 0.04 & -0.00 \\ 0.50 & -0.00 & 0.00 & -0.20 & -0.48 & 0.00 & -0.00 & 0.00 & -0.02 & -0.00 & 0.10 \\ 0.38 & 0.28 & 0.00 & -0.13 & -0.19 & -0.10 & 0.04 & 0.00 & -0.03 & 0.04 & 0.00 \\ 0.27 & 0.00 & 0.00 & 0.10 & 0.00 & 0.00 & 0.00 & 0.00 & -0.13 & 0.00 & 0.00 \\ 0.38 & 0.00 & 0.28 & -0.10 & -0.21 & 0.00 & 0.00 & -0.10 & -0.03 & 0.04 & 0.00 \\ 0.50 & 0.00 & 0.00 & -0.31 & -0.41 & 0.00 & 0.00 & -0.00 & 0.07 & 0.07 & 0.00 \\ 0.38 & 0.00 & -0.28 & -0.10 & -0.21 & 0.00 & 0.00 & 0.10 & -0.03 & 0.04 & 0.00 \end{bmatrix} \begin{bmatrix} \text{Target 1} \\ \text{Target 2} \\ \text{Target 3} \\ \text{Target 4} \\ \text{Target 5} \\ \text{Target 6} \\ \text{Target 7} \\ \text{Target 8} \\ \text{Target 9} \end{bmatrix}$$

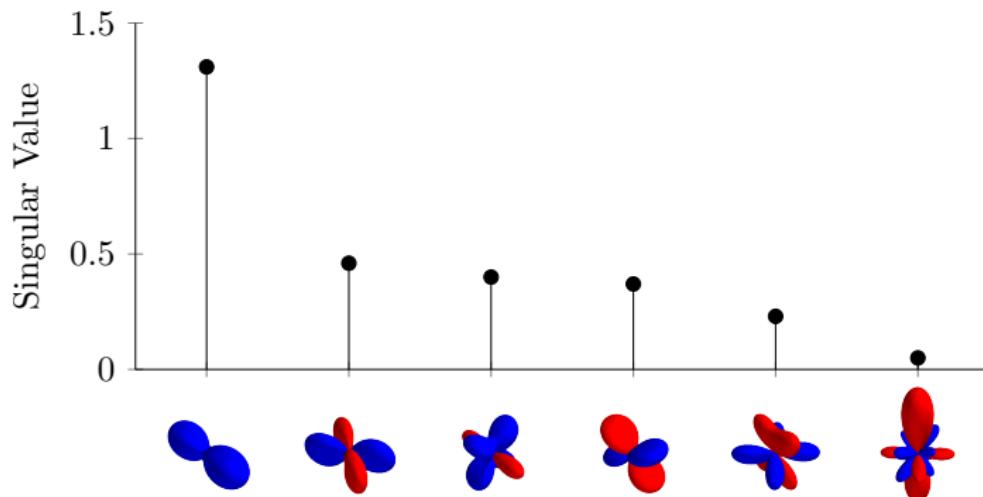
Symmetric diSPIM, NA = 0.8



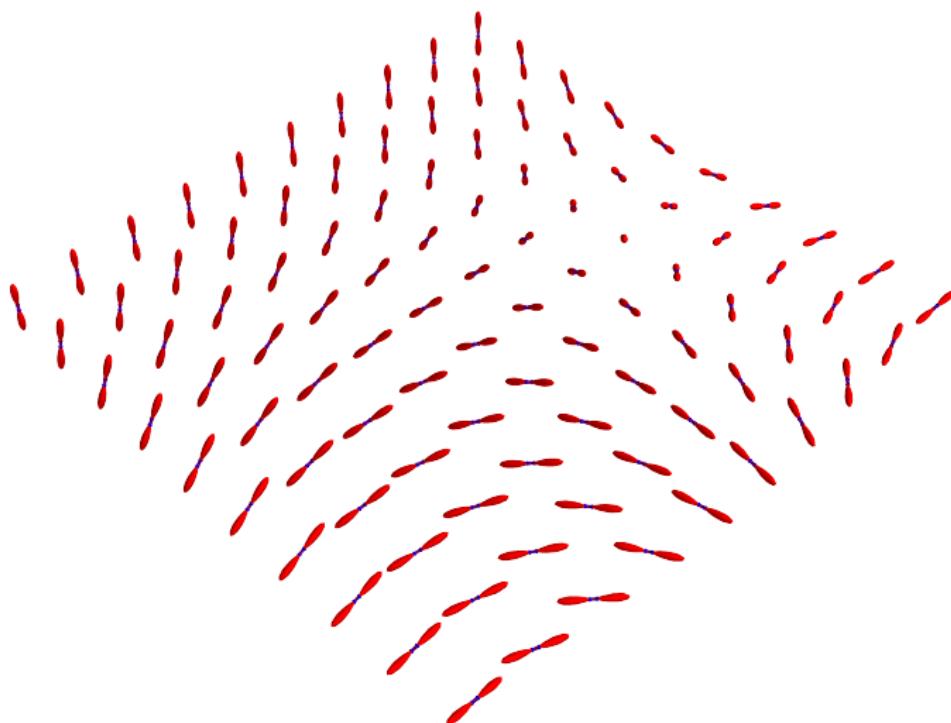
Asymmetric diSPIM, NA = 1.1/0.7

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

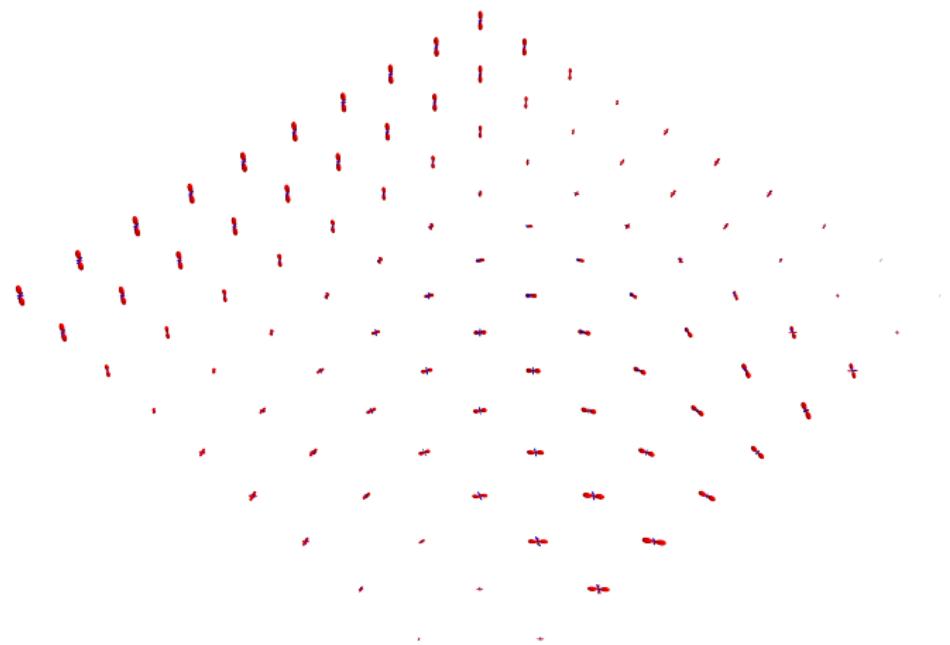
Asymmetric diSPIM NA = 1.1/0.7



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



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

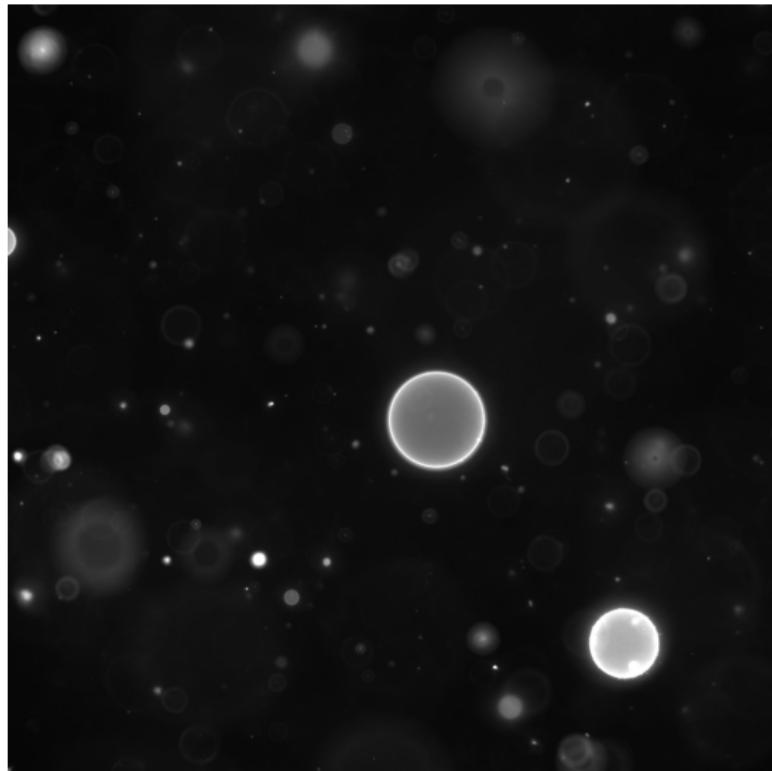


Up Next

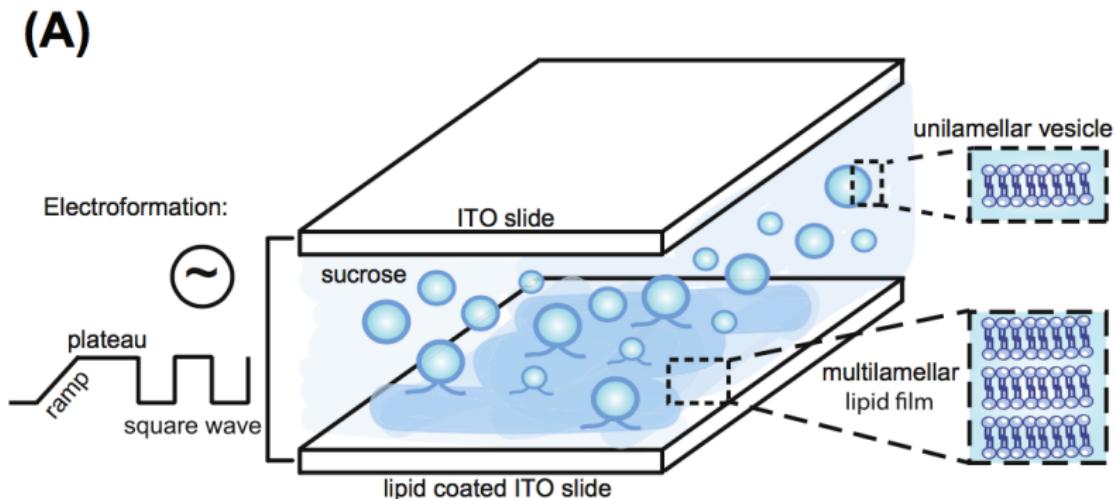
- ▶ Incorporating priors—positivity, single fluorophore, rotational symmetry
- ▶ Math OSA 2-page abstract on Jan 18—I'll include the work in these slides. I'll possibly include priors depending on how quickly that goes.

Giant Unilamellar Vesicles (GUV)

$\text{FOV} \approx 150 \times 150 \mu\text{m}$

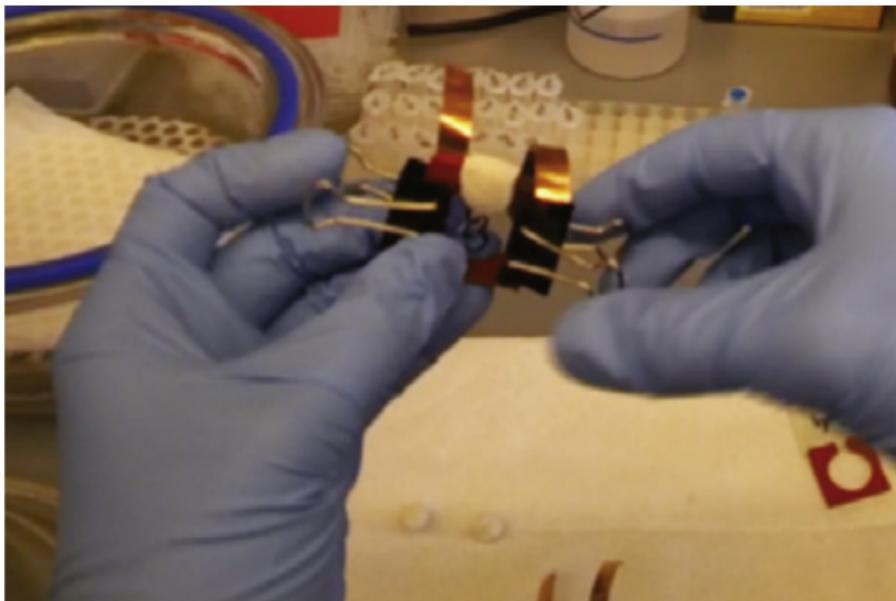


GUV Protocol



Schmid, 2015

GUV Chamber



Schmid, 2015