

Polarized Illumination For Determining Single-Molecule Orientation: Modeling and Design Comparison

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Abstract: Abstract here.

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References and links

1. K. Gallo and G. Assanto, "All-optical diode based on second-harmonic generation in an asymmetric waveguide," *J. Opt. Soc. Am. B* **16**(2), 267–269 (1999).

1. Introduction

Single molecule orientation determination is important because

A variety of techniques have been tested to determine the orientation of single molecules: polarized detection [Fourkas], shape analysis [Moerner], other things from Moerner review.

We propose polarized illumination as an complementary method to polarized detection. We get the speed (no shape analysis required). We don't waste any dose (polarized light throws away light dose).

In this work we make three contributions:

- we develop the polarized illumination forward model for a broad class of microscopes (section 2.X-2.X)
- we develop metrics to compare the performance of polarized illumination microscopes
- we use these metrics to compare microscopes design and make recommendations for polarized illumination microscopes.

2. Methods

2.1. Illumination Model

$$\eta_{\text{det}, \phi_{\text{det}}} = \frac{\int_{\Omega} d\hat{\mathbf{r}} |\hat{\mathbf{P}}_{\text{det}} \cdot \tilde{\mathbf{R}}(\hat{\mathbf{r}})(\hat{\mathbf{r}} \times \hat{\mu}_{\text{em}} \times \hat{\mathbf{r}})|^2}{\int_{\mathbb{S}^2} d\hat{\mathbf{r}} |\hat{\mathbf{r}} \times \hat{\mu}_{\text{em}} \times \hat{\mathbf{r}}|^2} \quad (1)$$

where

$$\hat{\mathbf{r}} = \sin \theta \cos \phi \hat{\mathbf{i}} + \sin \theta \sin \phi \hat{\mathbf{j}} + \cos \theta \hat{\mathbf{k}} \text{ is the dummy integration vector,} \quad (2)$$

$$\hat{\mu}_{\text{em}} = \sin \Theta \cos \Phi \hat{\mathbf{i}} + \sin \Theta \sin \Phi \hat{\mathbf{j}} + \cos \Theta \hat{\mathbf{k}} \text{ is the emission dipole moment,} \quad (3)$$

$$\tilde{\mathbf{R}}(\hat{\mathbf{r}}) = \begin{bmatrix} \cos \theta \cos^2 \phi + \sin^2 \phi & (\cos \theta - 1) \sin \phi \cos \phi & -\sin \theta \cos \phi \\ (\cos \theta - 1) \sin \phi \cos \phi & \cos \theta \sin^2 \phi + \cos^2 \phi & -\sin \theta \sin \phi \\ \sin \theta \cos \phi & \sin \theta \sin \phi & \cos \theta \end{bmatrix} \quad (4)$$

$$\text{is the rotation matrix that models the objective lens,} \quad (5)$$

$$\hat{\mathbf{P}}_{\text{det}} = \cos \phi_{\text{det}} \hat{\mathbf{i}} + \sin \phi_{\text{det}} \hat{\mathbf{j}} \text{ is the pass axis of the polarizer,} \quad (6)$$

$$\Omega = \{(\phi, \theta) | \phi \in (0, 2\pi] \text{ and } \theta \in (0, \alpha]\} \text{ is the cap of the sphere collected by the objective.} \quad (7)$$

$$\eta_{\text{exc}, \phi_{\text{pol}}}(\Theta, \Phi, \alpha) = D \{A + B \sin^2 \Theta + C \sin^2 \Theta \cos [2(\Phi - \phi_{\text{pol}})]\}. \quad (8)$$

$$A = \frac{1}{4} - \frac{3}{8} \cos \alpha + \frac{1}{8} \cos^3 \alpha \quad (9a)$$

$$B = \frac{3}{16} \cos \alpha - \frac{3}{16} \cos^3 \alpha \quad (9b)$$

$$C = \frac{7}{32} - \frac{3}{32} \cos \alpha - \frac{3}{32} \cos^2 \alpha - \frac{1}{32} \cos^3 \alpha \quad (9c)$$

$$D = \frac{4}{3(1 - \cos \alpha)}. \quad (9d)$$

2.2. Detection Model

$$\eta_{\text{det}}(\Theta, \alpha) = 2(A + B \sin^2 \Theta) \quad (10)$$

2.3. Complete Forward Model

$$I = I_{\text{tot}} \eta_{\text{exc}} \eta_{\text{det}} \quad (11)$$

2.4. Microscope Designs

Widefield illumination vs light sheet illumination.

- Light sheet has low illumination. - Restricted to orthogonal detection.

2.5. Evaluation Metrics

$$F = \sum_{i=0}^N \frac{1}{I} \begin{bmatrix} \frac{\partial I}{\partial \Theta} \frac{\partial I}{\partial \Theta} & \frac{\partial I}{\partial \Theta} \frac{\partial I}{\partial \Phi} \\ \frac{\partial I}{\partial \Phi} \frac{\partial I}{\partial \Theta} & \frac{\partial I}{\partial \Phi} \frac{\partial I}{\partial \Phi} \end{bmatrix} \quad (12)$$

$$\sigma_{\Omega} = \frac{\sin \Theta}{\sqrt{\det\{F\}}} \quad (13)$$

We call σ_{Ω} the *solid-angle uncertainty*. We sampled σ_{Ω} on 100,000 approximately equally spaced points on the unit sphere and calculated the *coefficient of variation* c_v of these points where

$$c_v = \frac{\sigma_{\sigma_{\Omega}}}{\mu_{\sigma_{\Omega}}} \quad (14)$$

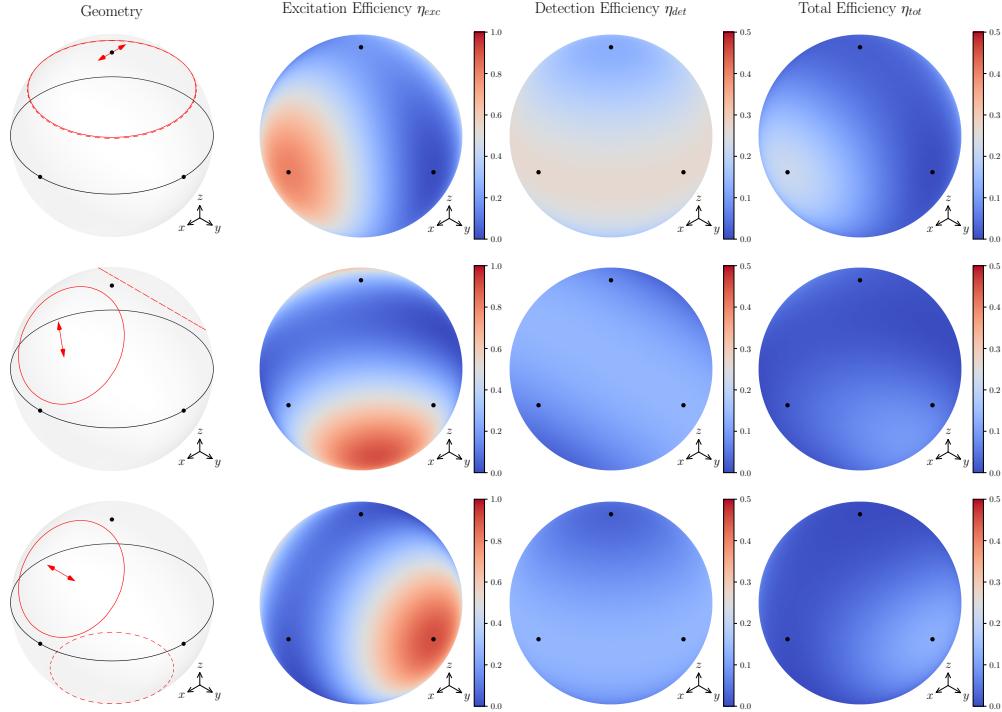


Fig. 1. Representative examples of the forward model for single frame microscopes.

Columns left to right: (1) schematics of single frame microscopes where the solid line encloses the illumination solid angle, the dashed line encloses the detection solid angle, and the arrow indicates the transmission axis of the illumination polarizer; (2) the excitation efficiency see equation XX; (3) the detection efficiency see equation X; (4) the total efficiency see equation X.

Rows top to bottom: (1) epi-illumination ($\text{NA} = 1.1$) with x -polarized light and epi-detection ($\text{NA} = 1.1$); (2) orthogonal illumination ($\text{NA} = 0.8$) and detection ($\text{NA} = 0.8$); (3) oblique illumination ($\text{NA} = 0.8$) and detection ($\text{NA} = 0.8$).

3. Results

3.1. One-Arm Designs

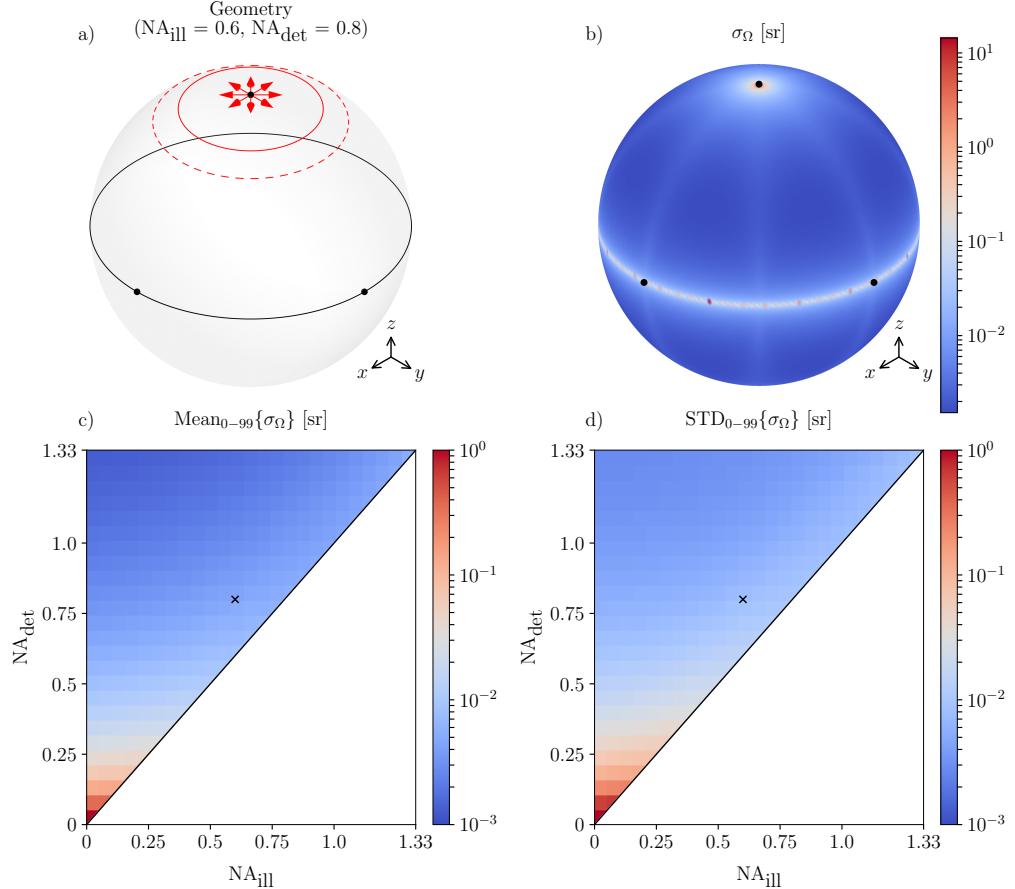


Fig. 2. **Left:** Schematic of a single-arm four-frame epi-illumination microscope with $\text{NA} = 0.8$ and $n = 1.33$. **Right:** Solid angle uncertainty for the same microscope when $I_{\text{tot}} = 1000$ photons. Note the regions of high uncertainty near the equator and poles as noted in XXX.

Can't use c_v because it doesn't converge.
High variance around equator and poles.

3.2. Two-Arm Designs

Widefield vs light sheet.

Widefield NA vs angle/.

Sweep asymmetry for full NA light sheet.

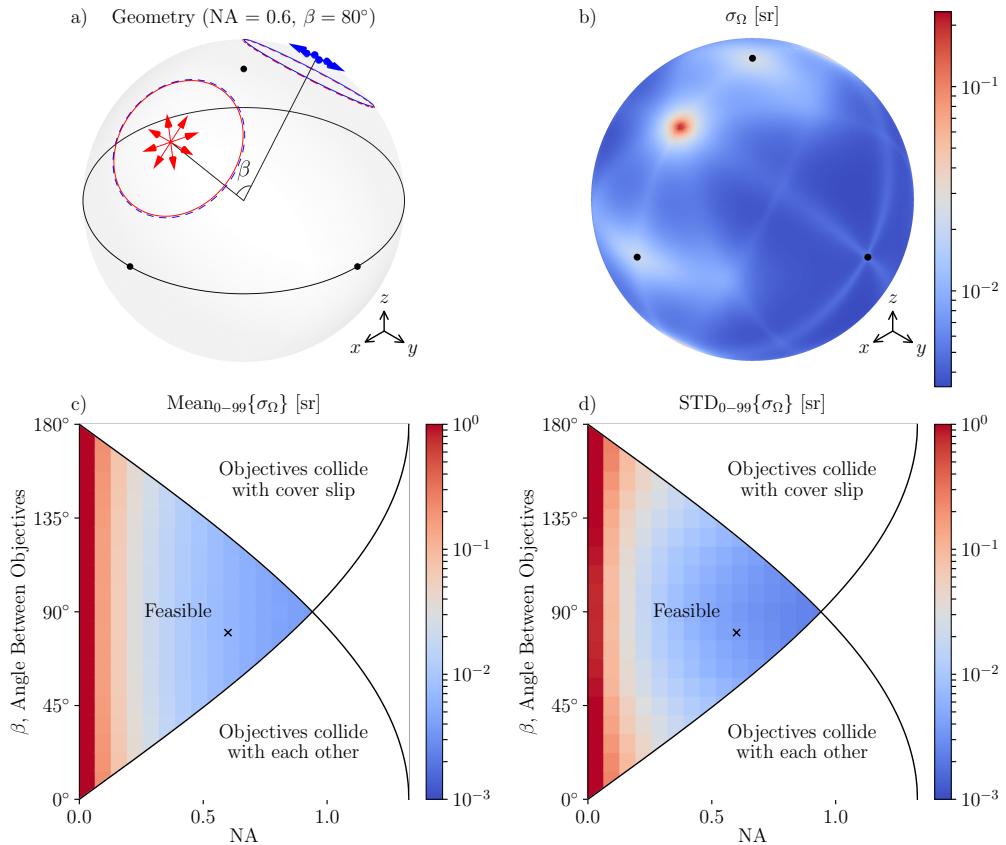


Fig. 3.

3.3. Three-Arm Designs

4. Discussion

5. Conclusion

Funding

TODO

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Disclosures

The authors declare that there are no conflicts of interest related to this article.

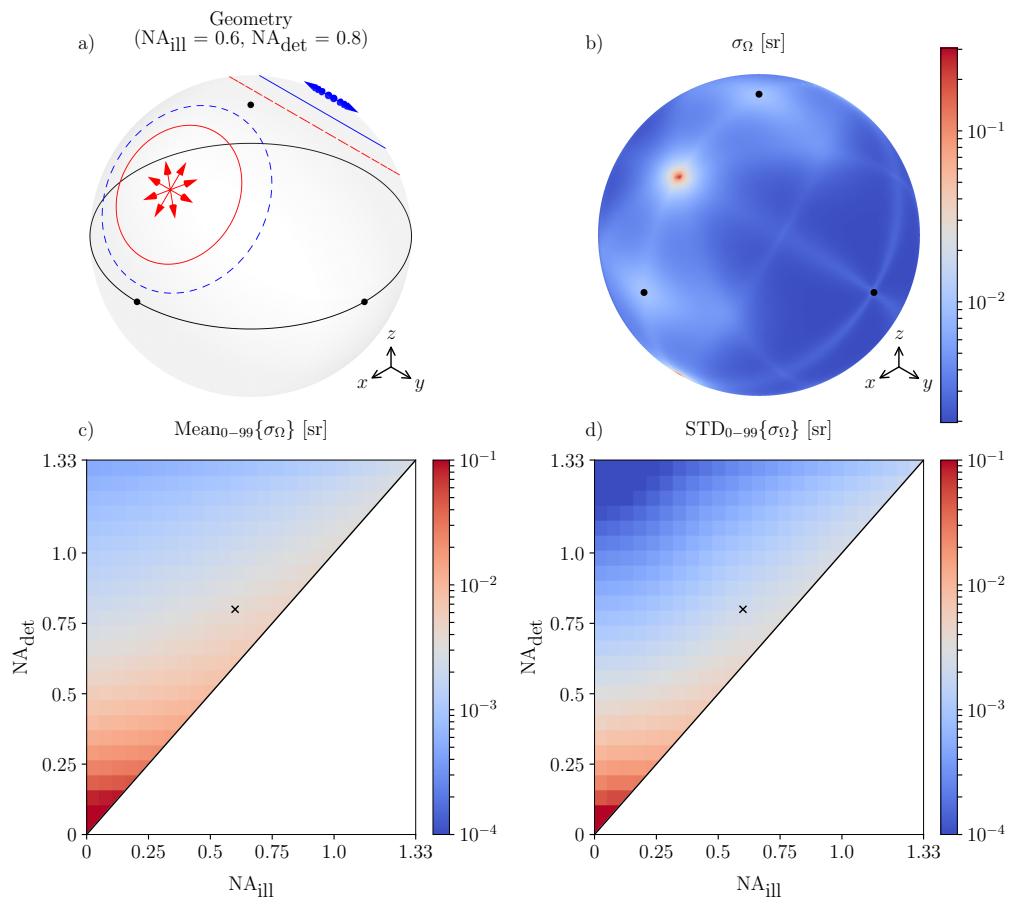


Fig. 4.

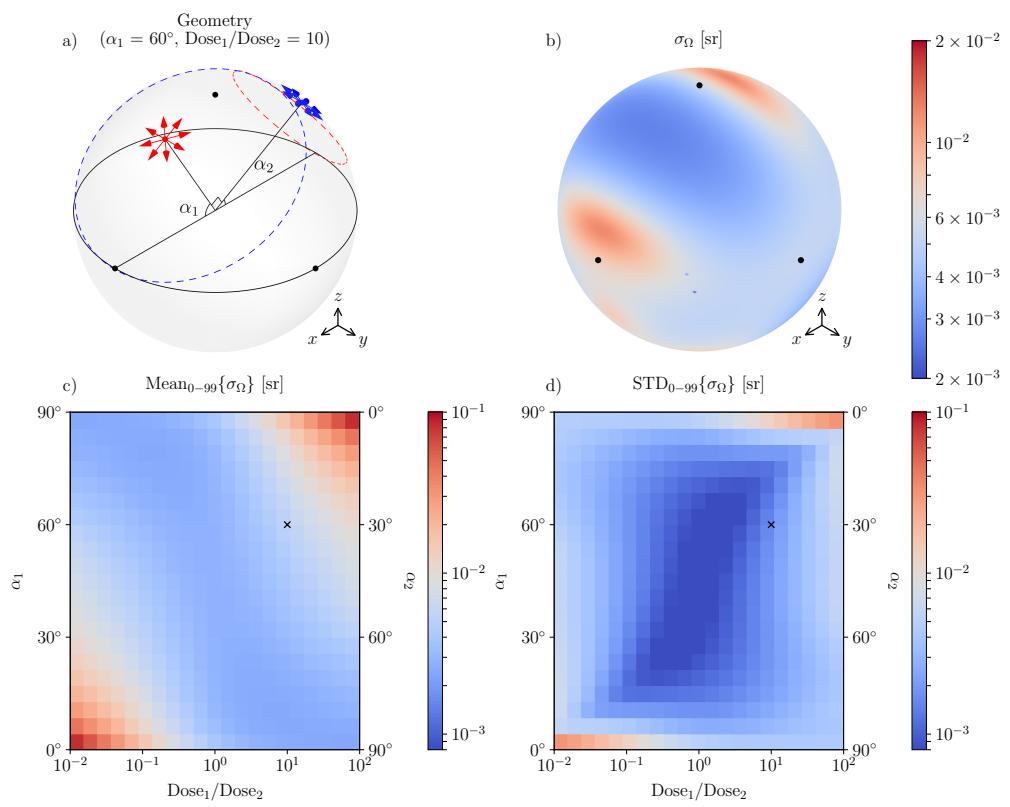


Fig. 5.

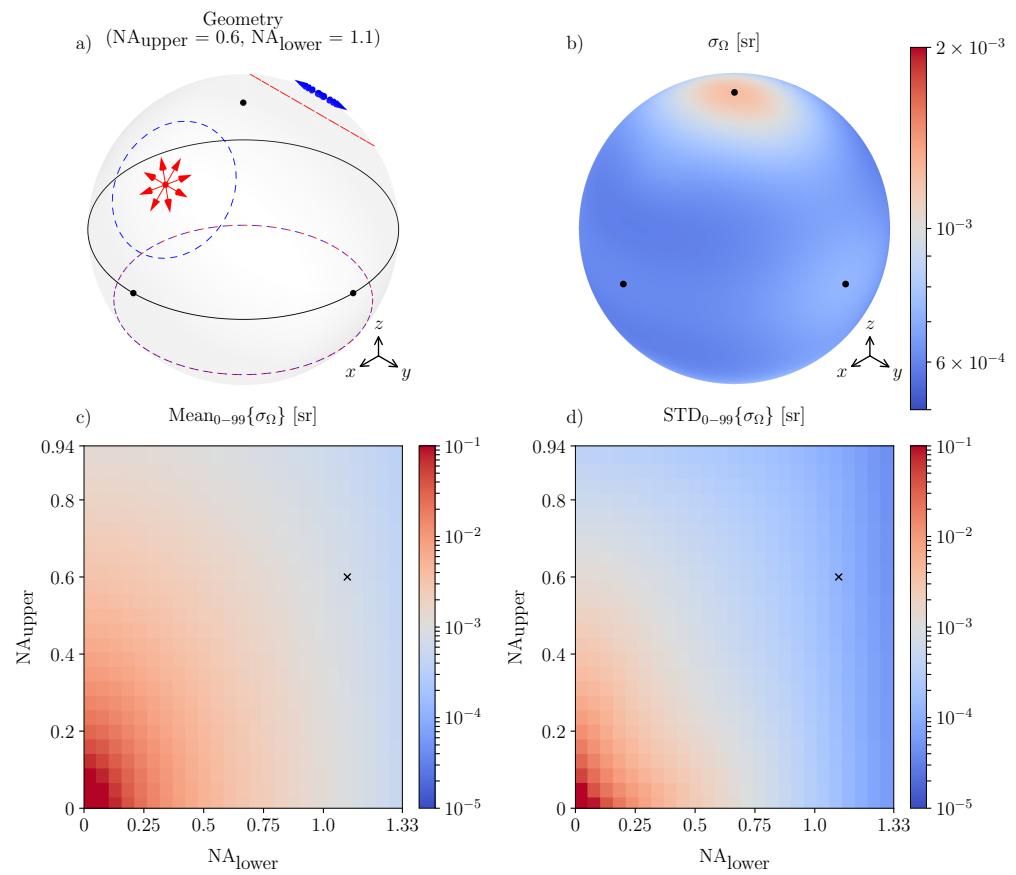


Fig. 6.