

# More Design Studies of Multiview Polarized Illumination and/or Detection Microscopy

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August 31, 2017

## 1 Introduction

In these notes I will use the model and metrics we established in the working paper draft to study three more design questions:

- What is the effect of using fewer than four illumination polarization orientation?
- Do polarized illumination or polarized detection microscopes provide better orientation reconstructions?
- How do microscopes with polarizers on both the illumination and detection paths compare to microscopes with polarizers on only the illumination or detection paths.

## 2 Methods

Figure 1 shows the efficiencies for single measurements taken with polarized illumination and/or detection microscopes. These results were created with Fourkas' expressions on the detection side and my expressions from the paper on the illumination side. Notice that the dashed arrows indicate polarized detectors. Also notice that the detection efficiency range is only 0-0.25 instead of 0-0.5 in the paper because the polarizer on the detector blocks half of the photons.

Unless otherwise stated, all of the designs I will compare in these notes will use  $\text{NA}_{\text{ill}} = 0$  and  $\text{NA}_{\text{det}} = 0.8$ , and all 2-view microscopes will use orthogonal views.

All designs use the same total sample exposure. This means that  $I_{\text{exp}} = 4000/N$  where  $N$  is the number of intensity measurements. Note that  $N$  includes polarized detection orientations as well as polarized illumination orientations.

## 3 Results & Discussion

Table 1 shows the results for microscopes with varying polarization on the detection arm, the illumination arm, and both arms; varying numbers of views; and varying numbers of polarization orientations per view.

### 3.1 Minimum Number of Illumination Polarizations

In the paper draft we only considered microscopes with four illumination polarizations per view. Figure 3 shows a comparison of several polarized illumination microscopes and the top third of Table 1 shows the summary statistics.

We find that for low-NA illumination with a single view there is no advantage to using more than three polarization orientations. Two polarization orientations is too few because the system suffers from an extra symmetry. Four polarization orientations adds no extra information, and in a real system each polarized illumination orientation requires extra switching and data processing time.

We also find that for low-NA illumination with two views that two polarization orientations per view is sufficient for an orientation reconstruction.

Recall that these results use a Poisson noise model. I expect that with a Poisson+Gaussian model that fewer polarization frames would be even more advantageous because each intensity measurement will collect more photons and each measurement would avoid the noise floor.

Figures 5 and 6 show how the number of polarization frames affects the reconstruction when the illumination NA is larger than 0. For large illumination NA, more polarized illumination orientations offers slightly better reconstructions. As the illumination NA decreases, the benefit of increasing the number of polarization orientations vanishes.

### 3.2 Polarized Illumination vs. Polarized Detection

In the paper we only considered polarized illumination microscopes and ignored polarized detection microscopes. Figure 3 shows a comparison of several polarized detection microscopes and the middle third of Table 1 shows the summary statistics.

Polarized illumination microscopes outperform polarized detection microscopes with the same number of frames. The main difference between polarized detection and polarized illumination is the size of the intensity signals for the same sample exposure. Polarized detection signals are smaller than polarized illumination signals because the polarizer blocks half of the photons from the detector, so polarized detection signals suffer from a relative uncertainty.

The preference for polarized illumination would be even stronger if we used a Poisson+Gaussian noise model because larger intensity measurements would contain more information about the fluorophore orientation.

Note that these results only apply if we split the sample exposure equally between intensity measurements. If a lossless beam splitter is available, then adding the beam splitter is a “free” source of information about the fluorophore orientation.

## 4 Polarized Illumination and Detection

Figure 4 shows a comparison of several polarized illumination+detection microscopes, and the bottom third of Table 1 shows the summary statistics.

Polarized illumination outperforms the polarized illumination+detection microscopes. Some of the polarizer orientation combinations do not give much information about the fluorophore orientation, so they are relatively useless measurements. This results shows that more measurements are not always beneficial.

Once again, these results only apply if we split the sample exposure equally between intensity measurements. Adding a lossless beam splitter is still a good choice if you have the option.

Table 1: Comparison of designs in each class of microscope. All solid-angle uncertainty statistics are in steradians.

Polarized Ill. or Det.	Number of Views	Polarization Settings Per View	$n$ -fold Degeneracy	$\text{Max}\{\sigma_\Omega\}$	$\text{Median}\{\sigma_\Omega\}$	$\text{MAD}\{\sigma_\Omega\}$
Illumination	1	2	8	$5.10 \times 10^{00}$	$1.65 \times 10^{-3}$	$3.77 \times 10^{-4}$
		3	4	$5.10 \times 10^{00}$	$1.65 \times 10^{-3}$	$3.79 \times 10^{-4}$
		4	4	$5.10 \times 10^{00}$	$1.65 \times 10^{-3}$	$3.77 \times 10^{-4}$
	2	2	2	$6.23 \times 10^{-3}$	$1.79 \times 10^{-3}$	$1.66 \times 10^{-4}$
		3	2	$1.24 \times 10^{-2}$	$1.79 \times 10^{-3}$	$1.66 \times 10^{-4}$
		4	2	$6.23 \times 10^{-3}$	$1.79 \times 10^{-3}$	$1.65 \times 10^{-4}$
Detection	1	2	8	$9.16 \times 10^{+2}$	$2.62 \times 10^{-3}$	$1.08 \times 10^{-3}$
		3	4	$1.30 \times 10^{+1}$	$2.38 \times 10^{-3}$	$7.62 \times 10^{-4}$
		4	4	$1.30 \times 10^{+1}$	$2.28 \times 10^{-3}$	$6.55 \times 10^{-4}$
	2	2	8	$2.41 \times 10^{+1}$	$3.20 \times 10^{-3}$	$8.01 \times 10^{-4}$
		3	2	$8.02 \times 10^{-2}$	$2.97 \times 10^{-3}$	$7.32 \times 10^{-4}$
		4	2	$8.08 \times 10^{-2}$	$3.06 \times 10^{-3}$	$7.96 \times 10^{-4}$
Both	1	2 ill. $\times$ 2 det. = 4	8	$7.22 \times 10^{00}$	$2.66 \times 10^{-3}$	$6.63 \times 10^{-4}$
		3 ill. $\times$ 3 det. = 9	4	$7.21 \times 10^{00}$	$2.63 \times 10^{-3}$	$6.19 \times 10^{-4}$
		2 ill. $\times$ 4 det. = 8	4	$7.19 \times 10^{00}$	$2.62 \times 10^{-3}$	$6.04 \times 10^{-4}$
	2	2 ill. $\times$ 2 det. = 4	2	$7.04 \times 10^{-3}$	$2.67 \times 10^{-3}$	$3.76 \times 10^{-4}$
		3 ill. $\times$ 3 det. = 9	2	$1.72 \times 10^{-2}$	$2.66 \times 10^{-3}$	$4.84 \times 10^{-4}$
		2 ill. $\times$ 4 det. = 8	2	$7.49 \times 10^{-3}$	$2.70 \times 10^{-3}$	$5.37 \times 10^{-4}$

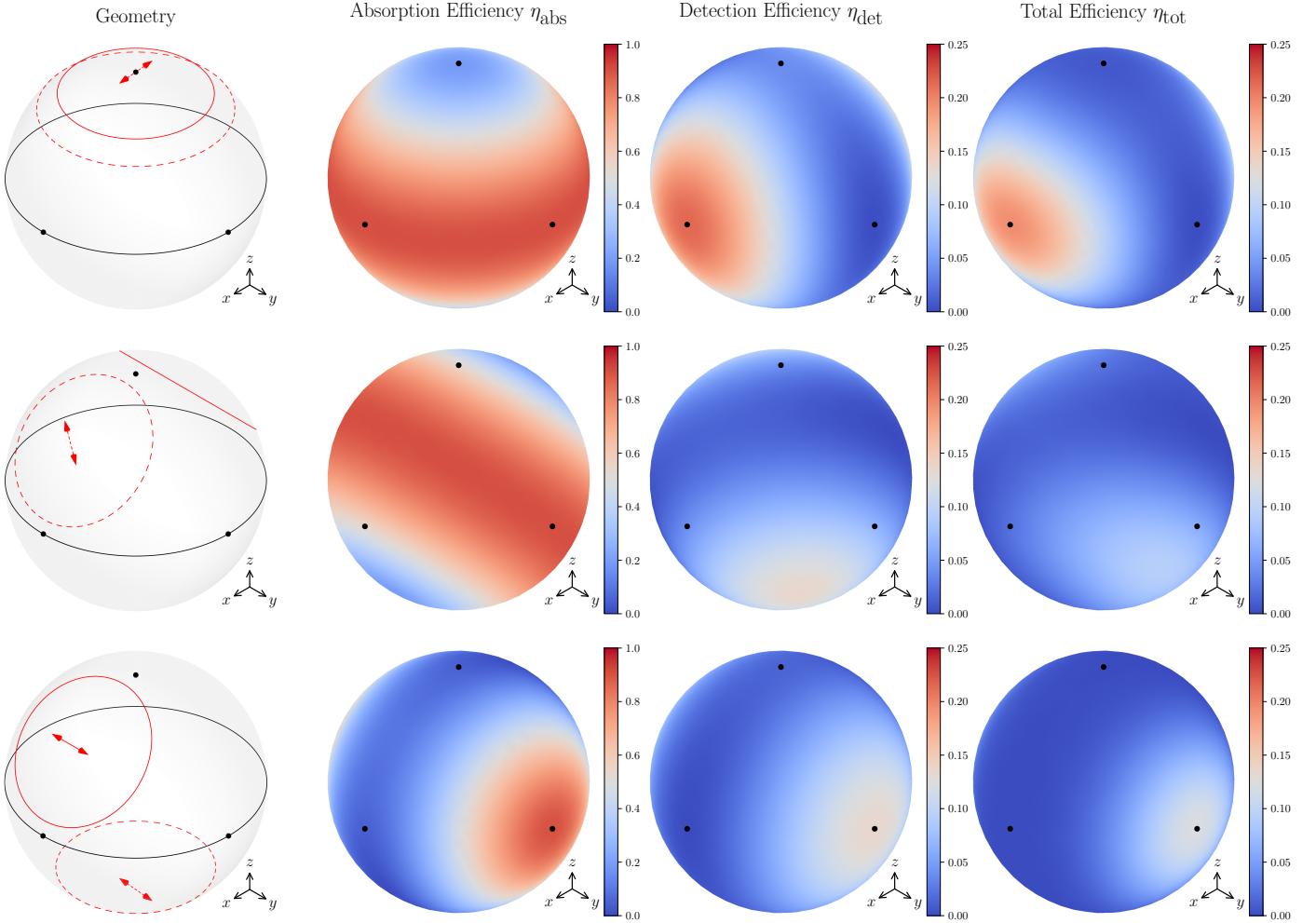


Figure 1: Representative examples of single intensity measurements. Black dots indicate where the Cartesian unit vectors intersect the unit sphere.

**Columns left to right:** 1) schematics where the solid line encloses the illumination solid angle, the dashed line encloses the detection solid angle, the solid arrow indicates the transmission axis of the illumination polarizer, and the dashed arrow indicates the transmission axis of the detection polarizer; 2) the absorption efficiency; 3) the detection efficiency; 4) the total efficiency, the product of the absorption and detection efficiencies.

**Rows top to bottom:** 1) coincident illumination ( $\text{NA} = 0.8$  with  $x$ -polarized light) and detection ( $\text{NA} = 1.1$ ); 2) non-coincident orthogonal illumination ( $\text{NA} = 0.8$ ) and detection ( $\text{NA} = 0.8$ ); 3) non-coincident  $135^\circ$ -separated illumination ( $\text{NA} = 0.8$ ) and detection ( $\text{NA} = 0.8$ ). All simulations use  $n = 1.33$ .

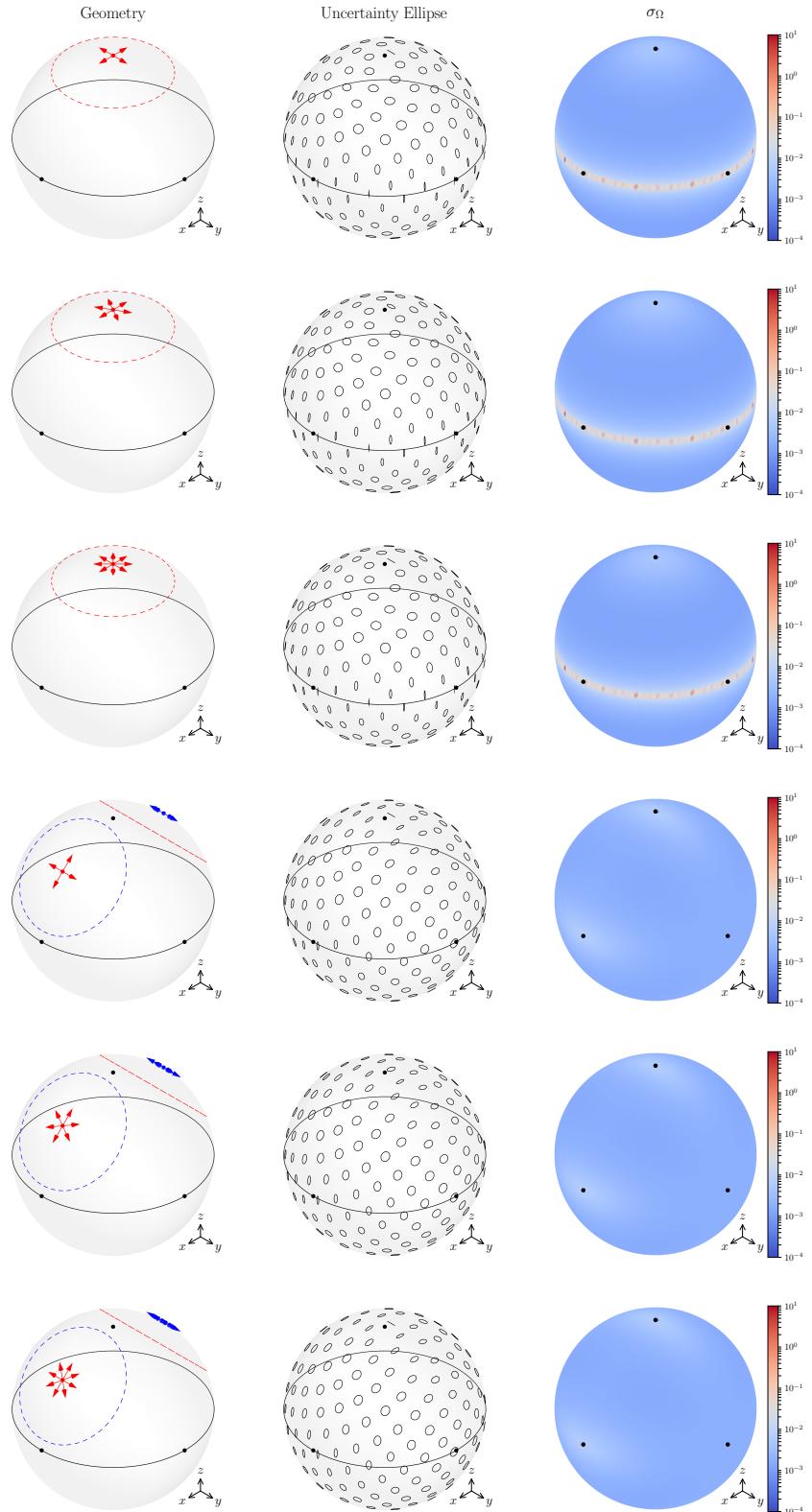


Figure 2: Comparing polarized illumination microscope designs. Each row in this figure corresponds to a row in the top third of Table 1.

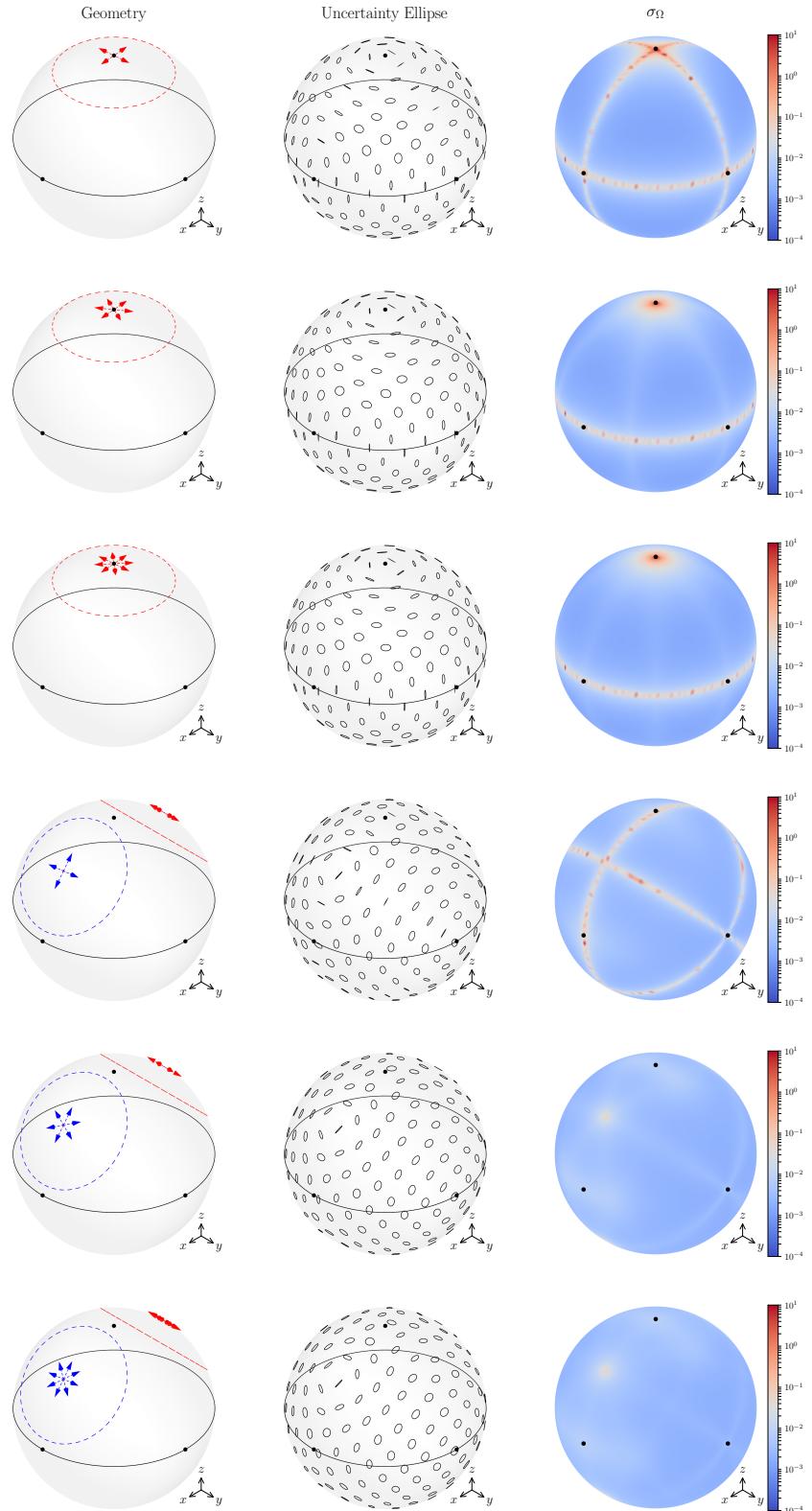


Figure 3: Comparing polarized detection microscope designs. Each row in this figure corresponds to a row in the middle third of Table 1.

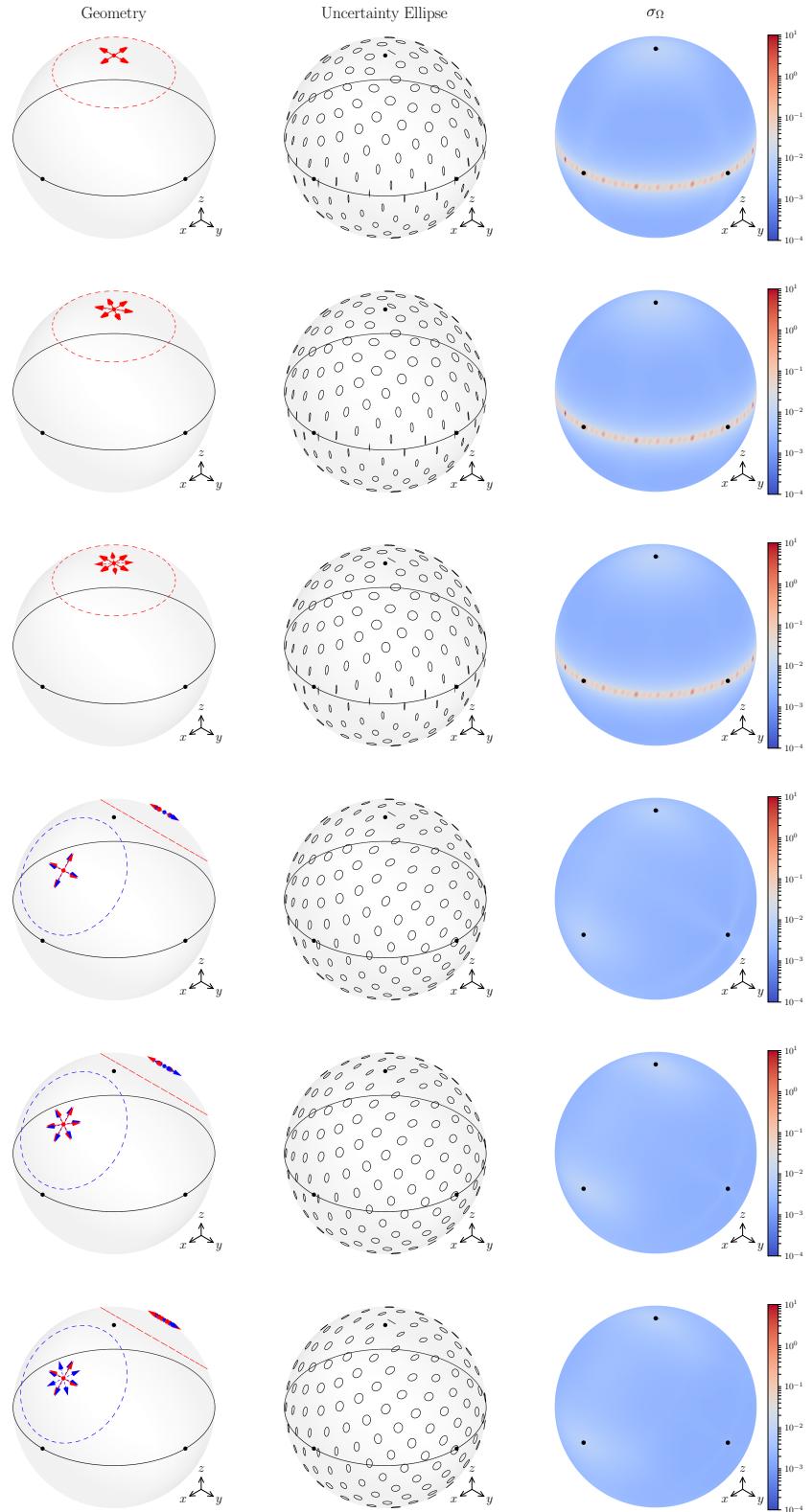


Figure 4: Comparing polarized illumination and detection microscope designs. Each row in this figure corresponds to a row in the bottom third of Table 1. The illumination and detection polarization arrows in the schematic are offset by a few degrees for visualization purposes only—in the simulations the orientations are identical.

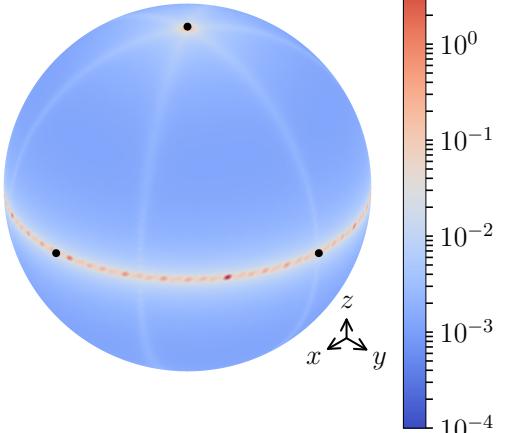
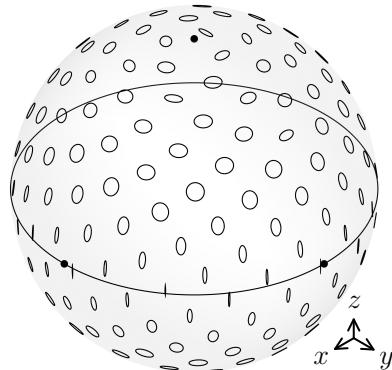
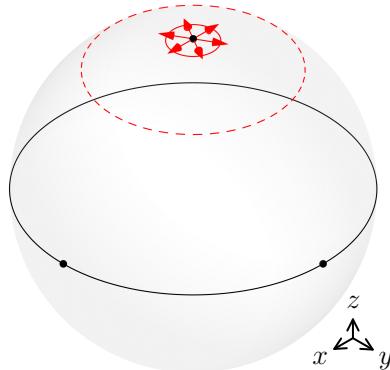
Geometry ( $NA_{ill} = 0.2$ ,  $NA_{det} = 0.8$ )

a) 3 illumination polarizations    b)

Uncertainty Ellipses

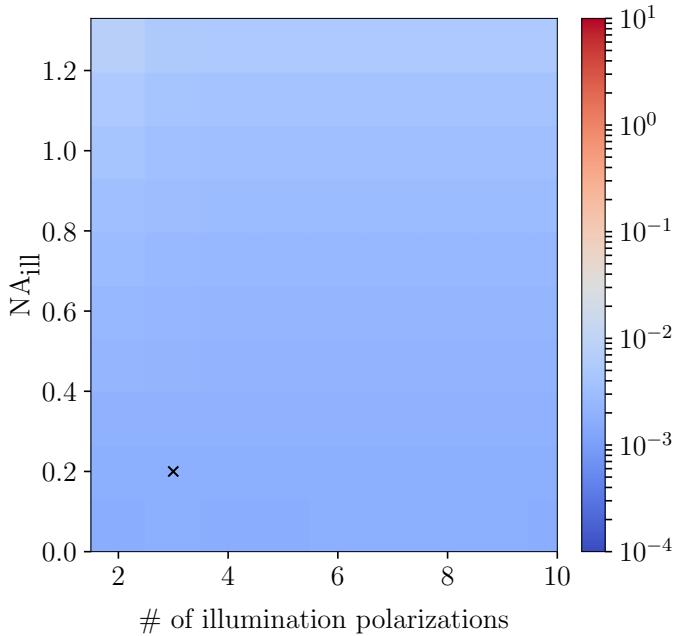
c)

$\sigma_\Omega$  [sr]



d)

Median{ $\sigma_\Omega$ } [sr]



e)

MAD{ $\sigma_\Omega$ } [sr]

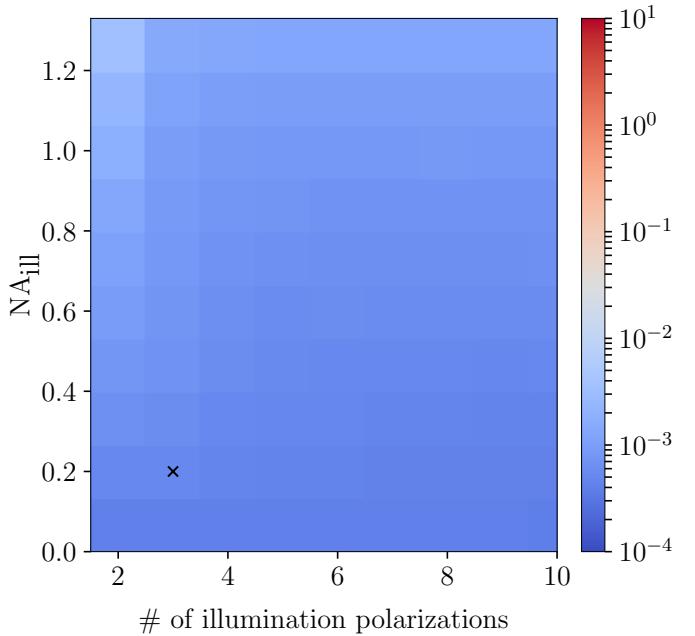
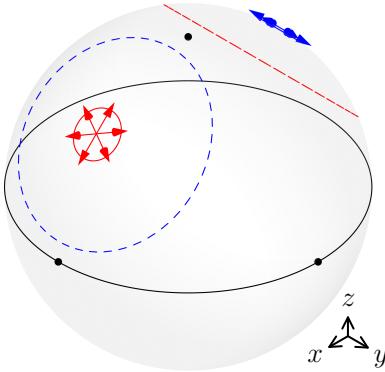
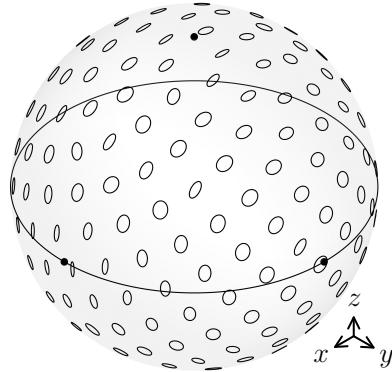


Figure 5: Single-view Köhler illumination microscope with varying number of illumination polarization orientations and illumination NA. b) Solid-angle uncertainty for the microscope in a). c) Median of the solid-angle uncertainty as a function of illumination polarization orientation and illumination NA. d) MAD of the solid-angle uncertainty as a function of illumination polarization orientation and illumination NA. The microscope in a) and b) is indicated by a cross in c) and d).

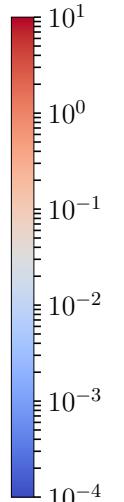
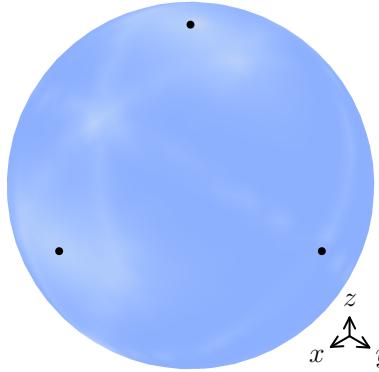
Geometry ( $NA_{ill} = 0.2$ ,  $NA_{det} = 0.8$ )  
 a) 3 illumination polarizations)



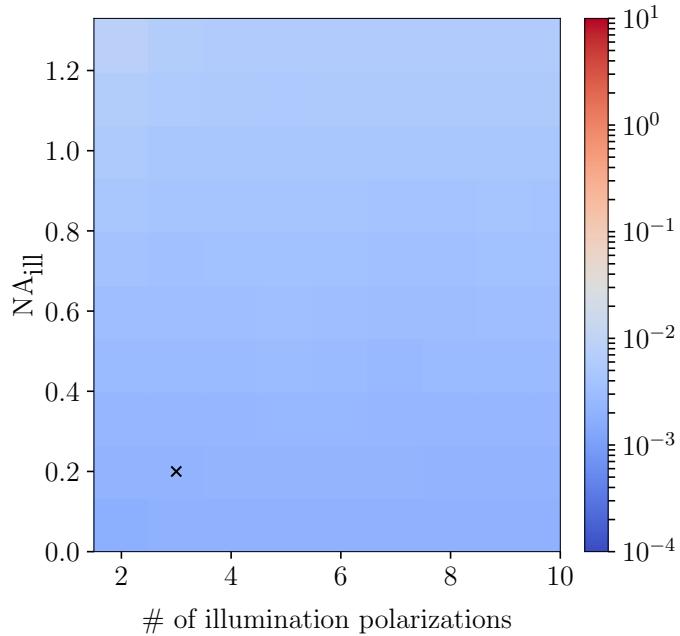
b) Uncertainty Ellipse



c)  $\sigma_\Omega$  [sr]



d) Median{ $\sigma_\Omega$ } [sr]



e) MAD{ $\sigma_\Omega$ } [sr]

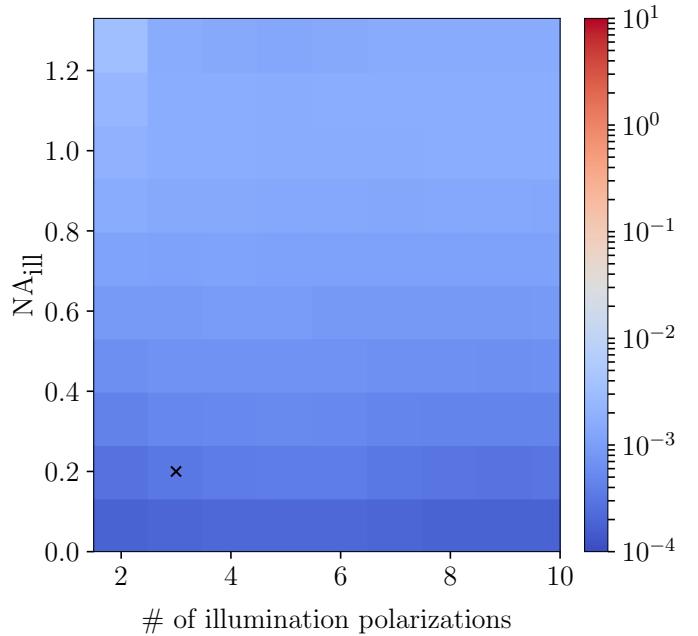


Figure 6: Dual-view symmetric Köhler illumination microscope with varying number of illumination polarization orientations and illumination NA. b) Solid-angle uncertainty for the microscope in a). c) Median of the solid-angle uncertainty as a function of illumination polarization orientation and illumination NA. d) MAD of the solid-angle uncertainty as a function of illumination polarization orientation and illumination NA. The microscope in a) and b) is indicated by a cross in c) and d).