Polarized tilting diSPIM design recommendation

February 21, 2019

Here we recommend a polarized tilting diSPIM design and compare the design to earlier non-tilting designs. These notes summarize two note sets here and here.

Our goal was to find a design that can optimally measure angular distributions of fluorophores given the following requirements: (1) the design must meet practical experimental constraints—we can only recommend diSPIM tilting angles up to 15° and the polarizer settings must be easy to align, (2) the design must measure all fluorophore orientations in 3D without major blind spots, and (3) the design should minimize the number of measurements. In the previous notes we formulated this design problem as an optimization problem. We chose to optimize the condition number of the system matrix—the "invertibility" of the reconstruction problem—subject to the constraints above.

Figures 1–3 summarize three angular designs: our recommended design, the current 3-polarization diSPIM without tilting, and a single-view design. In each **top left** subfigure we show a schematic of the angular design. Each arrow indicates a polarizer orientation and the center of each arrow indicates the excitation optical axis (the tilt angle).

In each **top right** subfigure we show a set of single direction reconstructions with the design. We start by creating a uniformly oriented phantom for each of the 250 directions in the positive octant of the sphere indicated by green dots. Next, we simulate the imaging process, simulate Poisson noise with SNR = 20, reconstruct the phantom, then find the maximum of the reconstructed ODF. We mark each reconstructed maximum with a red dot and connect each phantom/reconstruction pair with a black line. These figures show the "blind spots" of the design—long lines indicate a large angular error.

In each **bottom** subfigure we show the singular value spectrum of the imaging system with the singular functions plotted along the x axis. The singular function are mutually orthogonal functions, so they necessarily have positive (red) and negative (blue) components. The singular value spectrum is a generalized version of the familiar OTF. In an OTF the x axis is always implicitly labeled with complex exponentials, while here the x axis is explicitly labeled with angular functions.

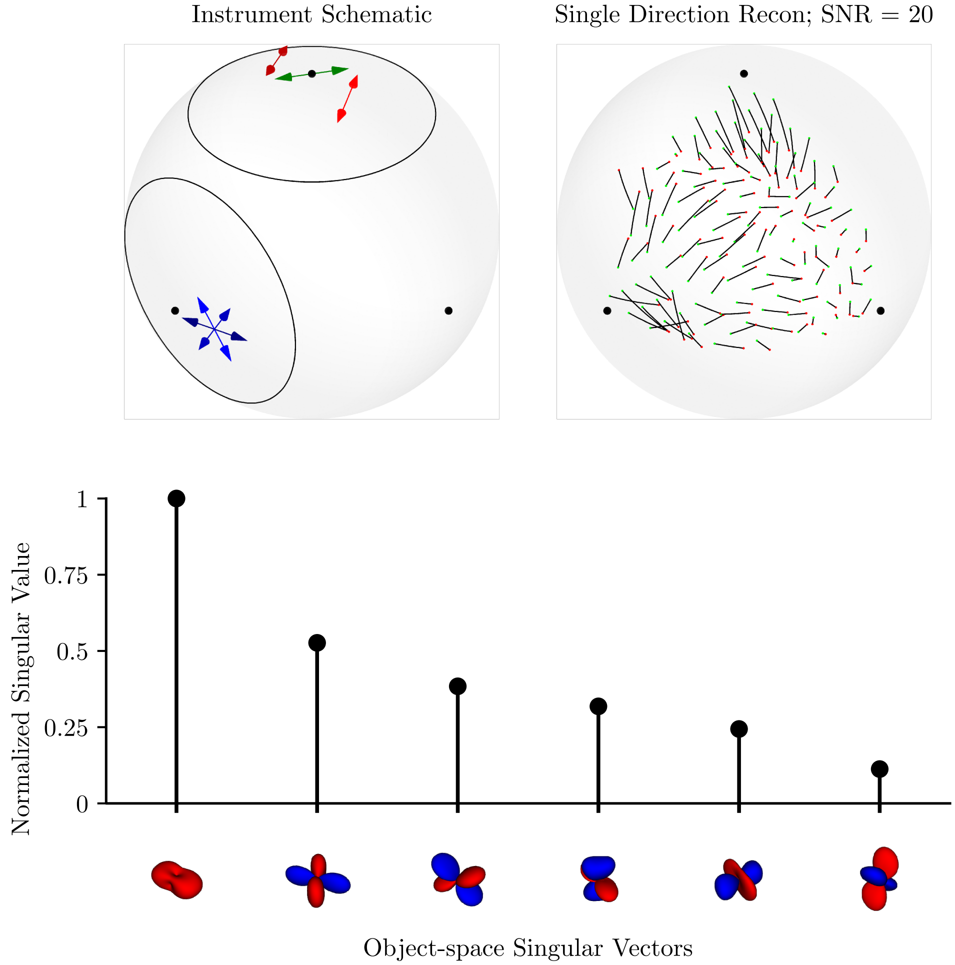

Figure 1: Our recommendation for a 6-measurement polarized tilting diSPIM design. First, collect the blue samples by tilting the light sheet and cycling through the three polarizer settings (0°, 60°, 120°). Next, collect the red samples from the other view by choosing the polarizer setting that points towards the blue excitation axis (60°) then tilting the excitation sheet both directions. Finally, collect the green sample by changing the polarizer setting to 120° and exciting along the optic axis. This design requires 3 equally spaced polarizer settings and 3 equally spaced tilt settings on one arm.

The single direction reconstruction shows that the angular error introduced by this design is $\approx 15°$. Also, the singular value spectrum shows that the last singular value is ≈ 0.15, so it will not be lost in the noise at SNR = 20.

Note that the order of collection (blue, red, green) is not important—any order will work. Also, the design shows 15° tilt angles, but any tilt angle will work. Larger tilt angles are better conditioned, so we recommend using all of the available tilt.

This design has the largest condition number for an instrument with 6 measurements and 3 equally spaced polarization settings. We originally tried optimizing the root sum of squares of the singular values (the Schatten 2-norm) and these optimizations resulted in intuitive symmetric designs—equally spaced polarizers with equally spaced tilts for both views. However, these designs performed poorly because the last singular value was very small and lost in the noise. Optimizing the condition number leads to less intuitive designs with better performance in noisy conditions.

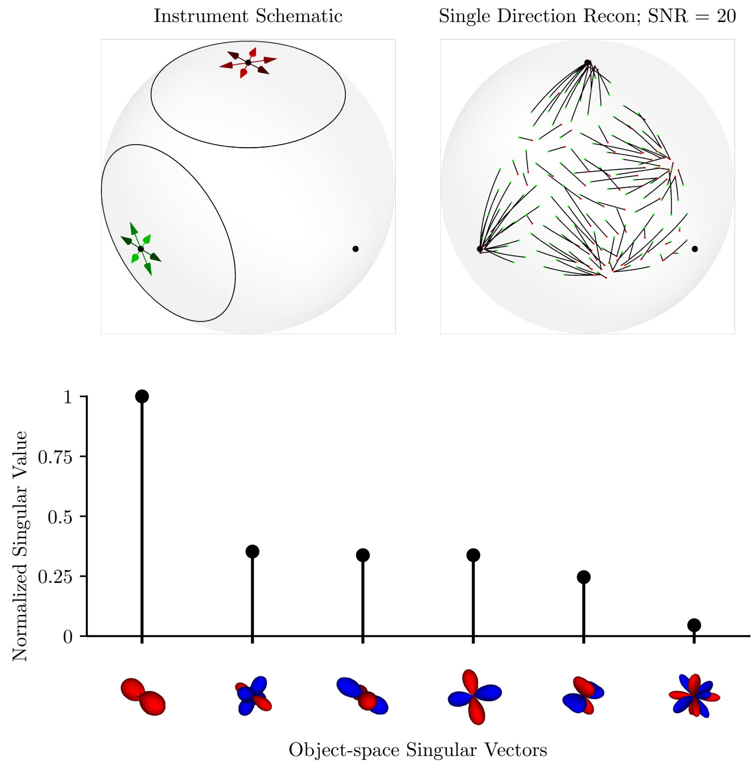

Figure 2: Current 3-polarization diSPIM design without tilting. The current design introduces errors as large as 30° for single-direction reconstructions, and the last singular value is very small so we effectively measure a 5-dimensional space. Furthermore, this design has a blind spot in the $\ell = 2$ band—see previous notes for more details.

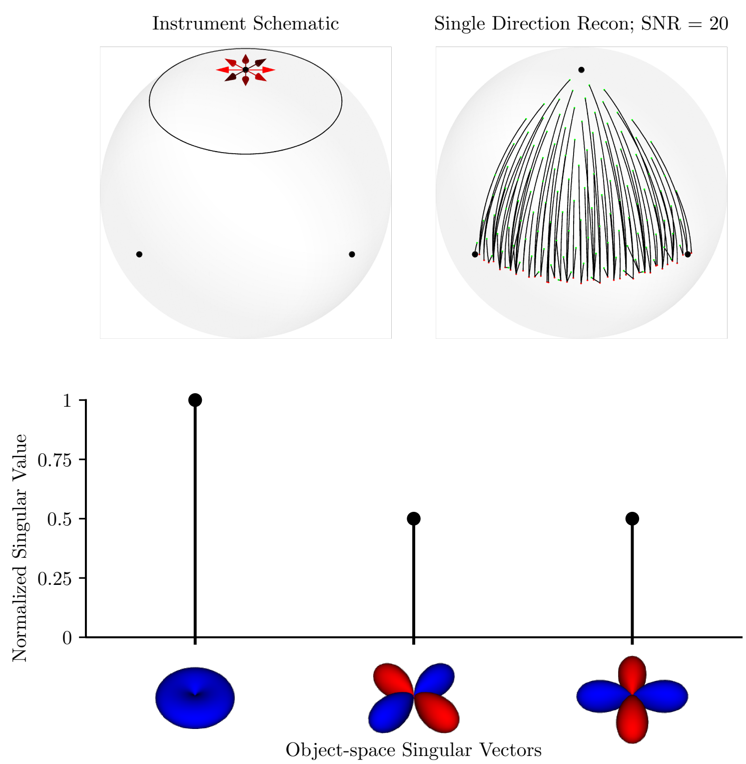

Figure 3: Single-view PolScope design. This design "projects" single-direction reconstructions into the transverse plane of the microscope and measures a 3-dimensional angular space.