Supplemental information for "Twirling of actin by myosins II and V observed via polarized TIRF in a modified gliding assay"

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Contents

A supplemental figures

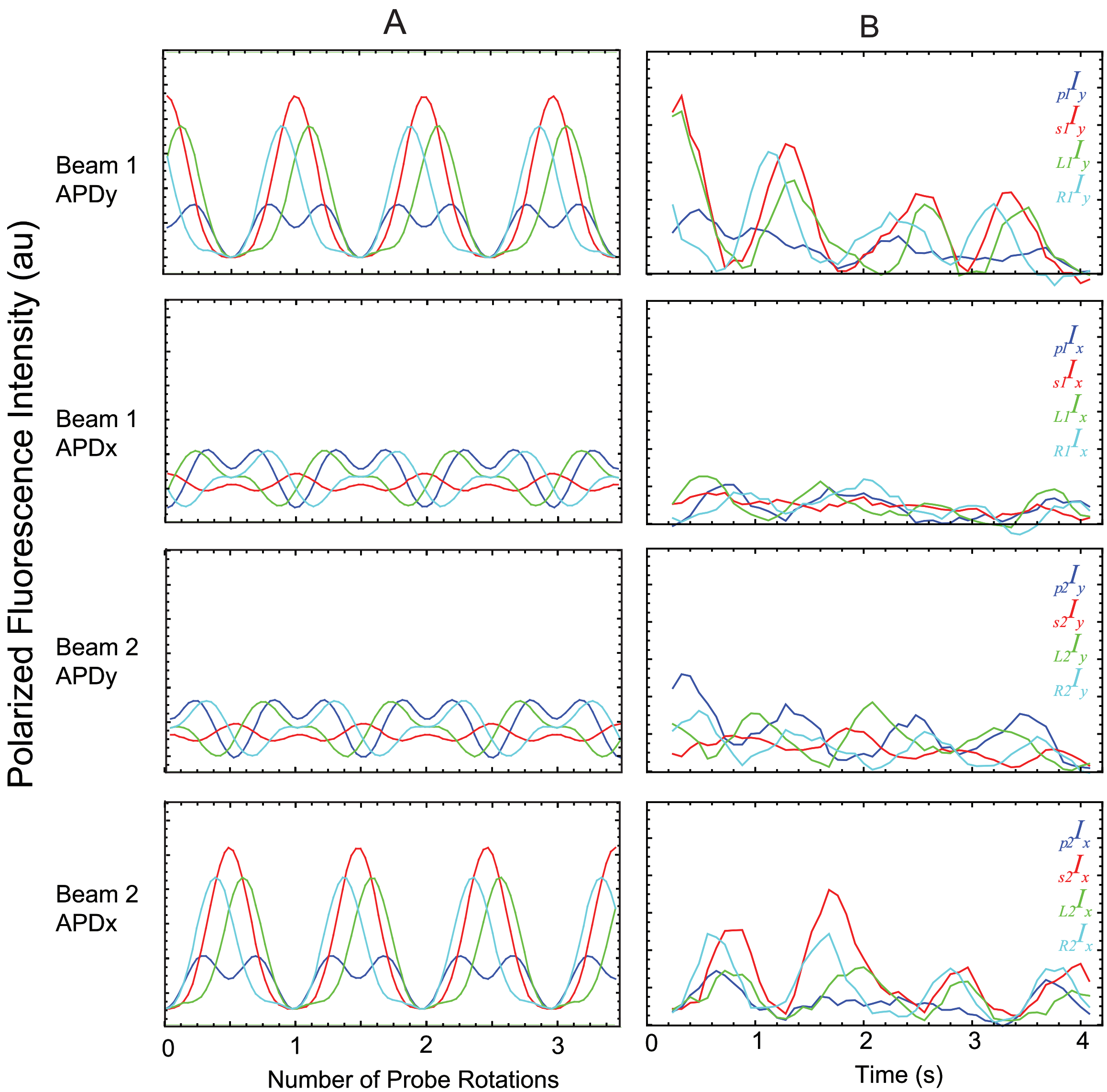

Figure 1: Relative phasing amongst the different polarizations (*colors*) is in good agreement between a simplified simulation (A) and the data from Fig. 2 in the text reproduced in (B). The simulation assumes a uniformly twirling probe undergoing -3.5 rotations (i.e., $\Delta\alpha = -1260°$) with $\beta = 45°$, $\delta = 45°$, and $\phi_{\text{actin}} = -135°$. The simulated trace does not display the decrease in intensity found in the actual data (see Fig. 2 for details and complete maximum likelihood solution).

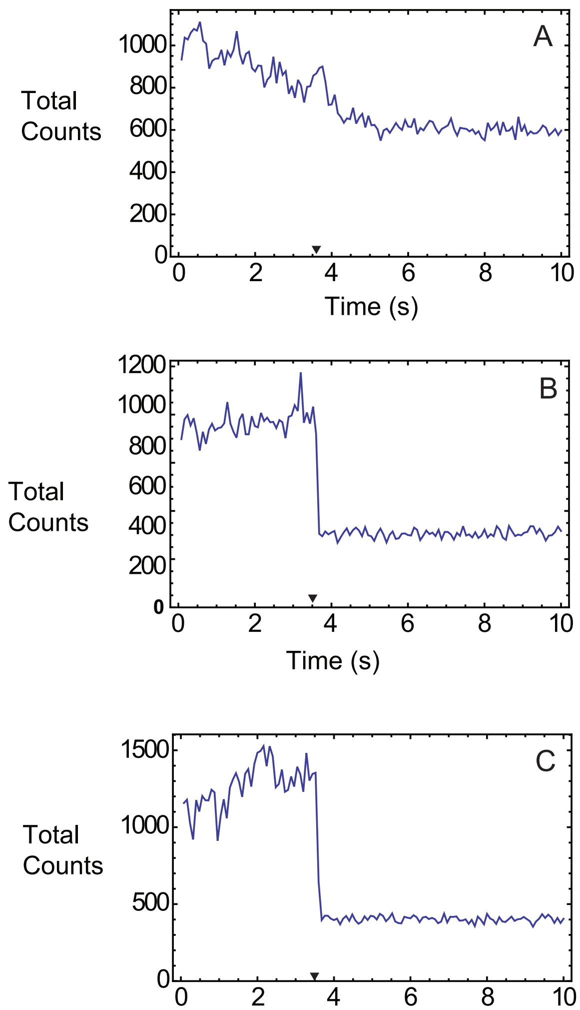

Figure 2: Total intensity for each 80 ms cycle of the 16 raw, uncorrected fluorescent intensities for (A) the molecule shown in Fig. 2 of the main text, (B) a typical molecule with constant intensity prior to bleaching (indicated by ▼), and (C) the molecule shown in supplemental Fig 3. Twirling motion is accompanied by strong undulations in individual polarization intensities (see Fig. 2) that when summed together during a complete polarization cycle are relatively flat, apart from photon noise fluctuations, prior to bleaching. Bleaching to background in a single step is indicative of a single fluorophore recording. Occasionally, molecules decrease in intensity during the recording despite clear twirling motions (e.g., A). Two possible mechanisms for this decrease in signal include the molecules being translocated away from the small spot detected by the APDs, or the filament moving away from the surface (and thus out of the evanescent field).

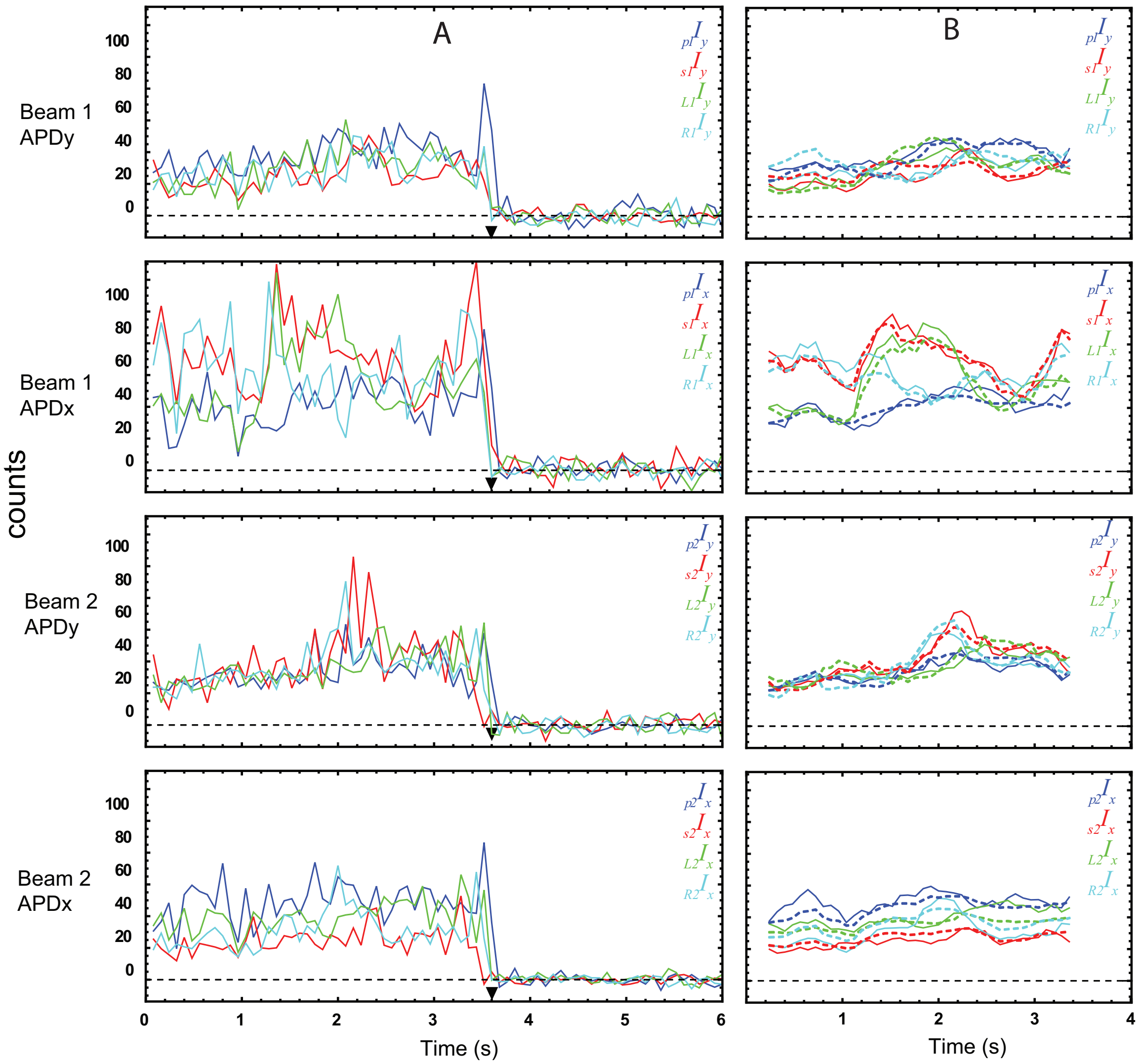

Figure 3: Measured fluorescence polarization intensities (A) for a rejected filament. The agreement between the filtered data (B, *solid*) and the maximum likelihood intensity (B, *dashed*) is good despite its rejection. The initial rejection of 2817 molecules was due to missed recording of the molecule (e.g., recording the background), recording of contamination (unreasonably high intensity or irregular intensity), double molecules, etc. . . These molecules were never analyzed. Of the remaining 711 after the first cut, 567 were rejected because the total rotation was less than 180° or the bleach duration was less than 1.6 s or the magnitude of the correlation coefficient, r, was less than 0.9. Of the remaining 144 molecules after the second cut, 47 were rejected due to abrupt change in β, θ, or ϕ. Abrupt changes did not occur in α (i.e., they didnt twirl one way and then the other nor did they simply stop twirling), but rather there was a large change in some other angle that was inconsistent with a continuously translocating/ twirling filament (see SI Fig. 4).

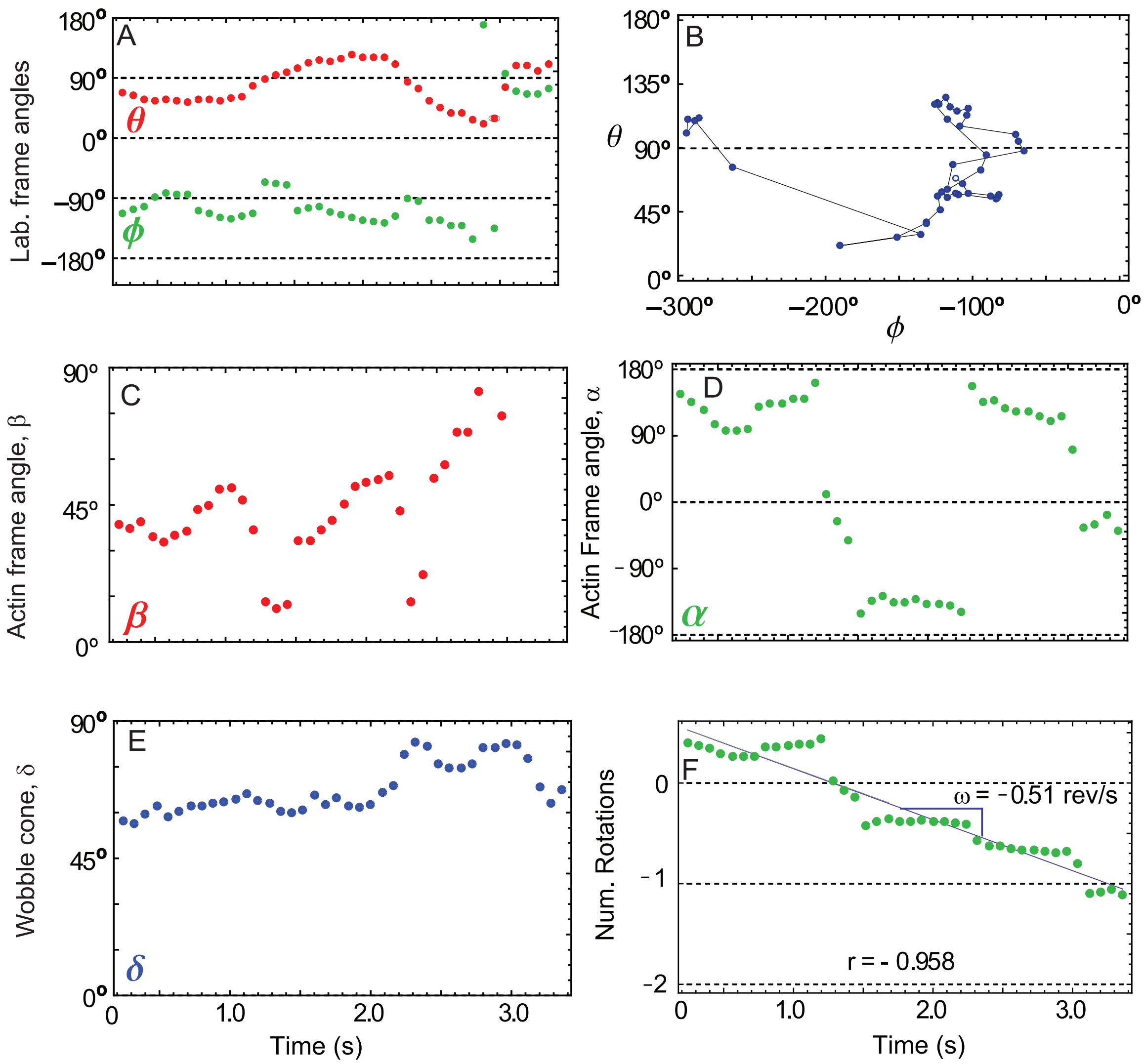

Figure 4: Maximum likelihood results for the recording shown in supplemental Fig 3. Refer to Fig. 3 in the main text for detailed descriptions of the parameters in *A*-*F*. This molecule had a high quality bleach to background (supplemental Fig.2) and $|r| > 0.9$, but was rejected because of the abrupt change in θ and ϕ at ~ 3 s.

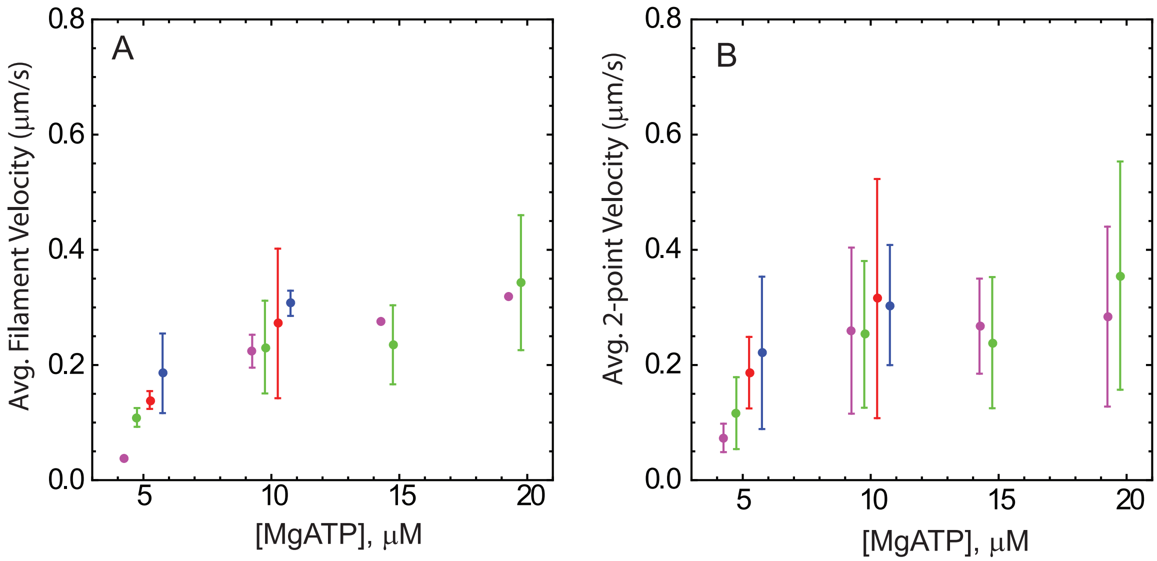

Figure 5: Average filament velocities and standard deviations at 5, 10, 15, and 20 μm MgATP concentrations for the four myosin loading concentrations 0.03 (*magenta*), 0.1 (*green*), 1.0 (*red*), 3.2 mg/ml (*blue*). As discussed in the text, the average in (A) is from 5-7 filaments each recorded for 30 frames, and in (B) the average is of all the two-point velocities obtained for each molecule from the two CCD images just before the polTIRF recording. The two trends are similar, but the standard deviation of the average in (B) is greater than in (A) since each two-point velocity estimate was more variable than the velocity obtained from a longer recording.

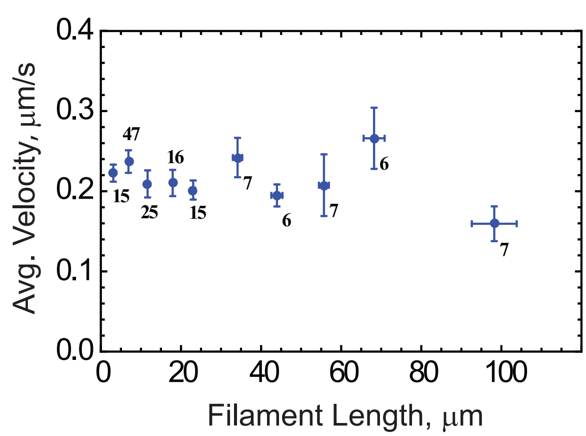

Figure 6: Average filament velocity (including twirling and non-twirling filaments) at myosin loading concentrations of 0.03 and 0.1 mg/μml is independent of filament lengths between 1-100 μm.

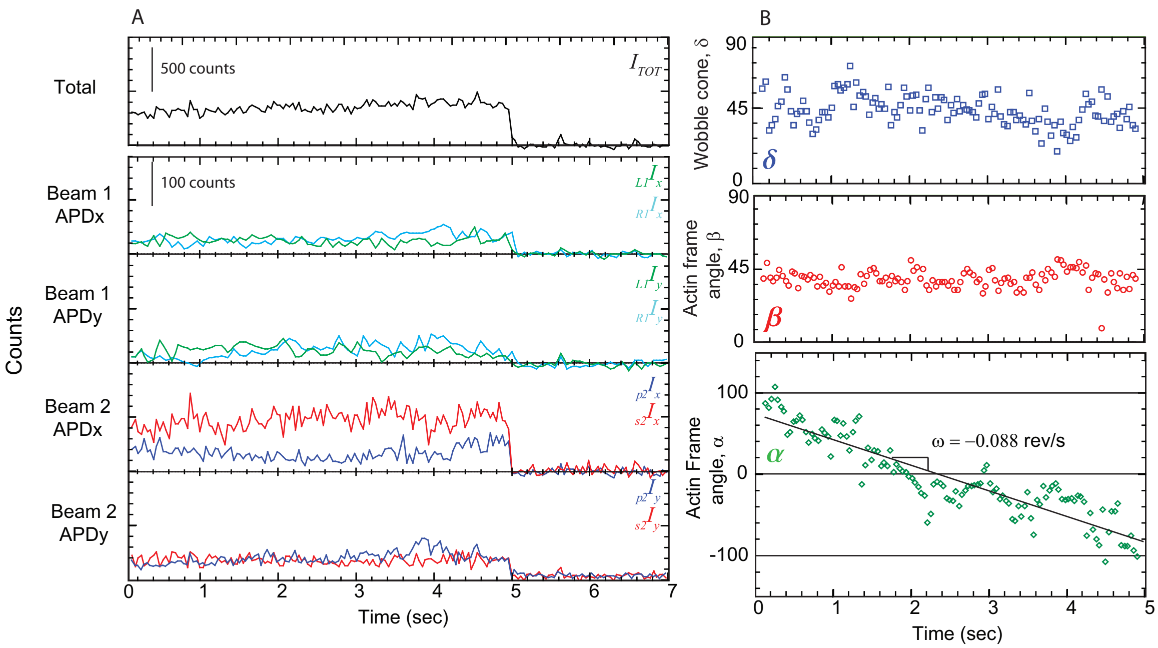

Figure 7: Polarized fluorescence intensities (A) from a rhodamine molecule attached to a twirling actin filament that is being translocated by myosin V. The myosin V twirling data was obtained from 4 incident polarizations: L1 (*green*), R1 (*cyan*), p2 (*blue*), and s2 (*red*), with background intensity for each removed. The simultaneous bleaching of the individual polarized intensities and the total intensity (*black*) at $\sim$ 5 s indicates that all counts were derived from a single molecule. The range of discernible orientation angles was one quarter of a sphere due to reflection symmetries about the y-z plane and the intrinsic dipole symmetry of the probe. Nevertheless, twirling could be determined for filaments restricted to translocating along the x-axis. A maximum likelihood analysis determines the orientation (B) of the probe from the intensities in (A). The linearly decreasing polar angle α (*green*) and relatively constant azimuthal angle β (*red*) indicate a filament twirling with ~ -0.09 rev/s. The direction of filament motion along the + x-axis (i.e., $\phi_{\text{actin}} = 10°$) and the direction of probe rotation indicate left-handed twirling.

B $\pm 45^\circ$ degree polarization terms

In order to determine the $3D$ orientation and rotational ('wobble') motions of a single fluorescent molecule, measured polarized fluorescence intensities are compared to intensities that are calculated from a theoretical model of the molecule. This model approximates the probe as an electromagnetic dipole $\hat{\mu}$ (see main text and Forkey 2005) that absorbs and emits photons polarized preferentially along its dipole axis. This appendix derives the equation for calculating the intensity I of a probe oriented with respect to both the polarization direction of the detector and the linearly polarized electric field of the incident illumination:

$$_{\hat{\varepsilon}}I_{\hat{\alpha}} = \kappa \cdot P_a(\hat{\mu}_a, \hat{\varepsilon}) \cdot P_e(\hat{\mu}_e, \hat{\alpha}) \tag{1}$$

Where I is the number of photons collected from a static dipole $\hat{\mu}$ with probability P of absorbing (a) or emitting (e) photons relative to the incident electric field polarization $(\hat{\varepsilon})$ or analyzer $(\hat{\alpha})$. κ is an overall normalization factor. The complex quantity $(\hat{\varepsilon})$ describes the polarization of the electric field **E**, but not its magnitude E_0:

$$\mathbf{E} = E_0 \hat{\varepsilon} e^{-z/d} e^{-i\omega t} \tag{2}$$

$\hat{\varepsilon}$ can be decomposed into its Cartesian components:

$$\hat{\varepsilon} = [\hat{x}\varepsilon_x e^{-i\delta_x} + \hat{y}\varepsilon_y e^{-i\delta_y} + \hat{z}\varepsilon_z e^{-i\delta_z}] \tag{3}$$

This appendix is concerned with the new features of the model required for the off-axis incident polarizations $(L1, R1, L2, R2)$; thus only the terms describing the absorption probability, P_a, need to be modified:

$$P_a(\hat{\mu}_a, \hat{\varepsilon}) \equiv |\hat{\mu}_a \cdot \hat{\varepsilon}|^2 \tag{4}$$

$$\propto (\varepsilon_x^2\mu_x^2 + \varepsilon_y^2\mu_y^2 + \varepsilon_z^2\mu_z^2 + 2\varepsilon_x\varepsilon_y\mu_x\mu_y \sin(\delta_s - \delta_p) \tag{5}$$

$$2\varepsilon_y\varepsilon_z\mu_y\mu_z \cos(\delta_s - \delta_p))$$

Note that for beam 2, ε_x should be replaced with ε_y and *vice versa* in Eq. (5). Similarly for μ_x and μ_y.

Calculations are performed in the reference frame of the microscope; where z points into the objective and the x-y plane is parallel to the stage with the x and y axes along the direction of propagation of beam 1 and 2, respectively, see Fig 3 in the main text. The orientation (θ, ϕ) of the dipole in this frame is given by $\hat{\mu} = (\sin(\theta)\cos(\phi),\ \sin(\theta)\sin(\phi),\ \cos(\theta))$. The Cartesian components of $\hat{\varepsilon}$ in the microscope frame are determined from the magnitude of the s and p polarizations. The s component of ε is entirely in the $y(x)$

direction for beam 1(2). Due to the shallow glancing angle (θ_i) of the incident beams, the p component of ε is predominantly normal to the reflection plane ($\varepsilon_{p,n} \sim \hat{z}$); however the small tangential component ($\varepsilon_{p,t} \sim \hat{x}$ and $\hat{y}$) in beam 1 and 2, respectively, is also included in the analytical expressions. The cross terms (4^{th} and 5^{th} terms of Eq. (5)) arise when the polarization of the electric field, given by angle ζ, is intermediate between p and s. The components of the evanescent field are given by:

$$\begin{aligned} \varepsilon_{p,t} &= -2\cos(\zeta)\cos(\theta_i)\sin(\delta_p) \\ \varepsilon_{p,n} &= 2\cos(\zeta)\sin(\theta_i)\cos(\delta_p)/\xi^2 \\ \varepsilon_s &= 2\sin(\zeta)\cos(\delta_s) \end{aligned} \tag{6}$$

Where ξ is the ratio of the indices of refraction in the aqueous solution to the higher index slide material at the reflecting surface, and $\zeta = +45°$ and $-45°$ for ε_L and ε_R, respectively.

$$\begin{aligned} \cos^2(\delta_p) &= \frac{\xi^4\cos^2(\theta_i)}{\xi^4\cos^2(\theta_i) + \sin^2(\theta_i) - \xi^2} \\ \sin^2(\delta_p) &= \frac{\sin^2(\theta_i) - \xi^2}{\xi^4\cos^2(\theta_i) + \sin^2(\theta_i) - \xi^2} \\ \cos^2(\delta_s) &= \frac{\cos^2(\theta_i)}{1 - \xi^2} \end{aligned} \tag{7}$$

The previous equations describe a static dipole. As described in more detail in (Forkey 2005), rotational motions of the dipole are separated into a fast and slow time scale. Rapid motions that re-orient the dipole on a time scale faster than the $\sim 4\,\text{ns}$ fluorescent lifetime are assumed to occur within a wobble cone of half-angle δ_f. Rotational motion of the probe on time scales slower than $\sim 4\,\text{ns}$, yet faster than the $10\,\text{ms}$ illumination time are assumed to occur within a wobble cone of half-angle δ. See Appendix E for a complete derivation of these terms, which are used in a Levenberg-Marquardt C code to find the parameters $(\theta, \phi, \delta, \delta_f, \kappa)$ that maximize the likelihood of the measured intensities.

C Calibration factors

Calibration factors are defined for the additional L and R excitation polarizations:

$$\begin{aligned} {}_{p1}I_y &= {}_{p1}I_y^r \\ {}_{p1}I_x &= {}_{p1}I_x^r/C_d \\ {}_{s1}I_y &= {}_{s1}I_y^r/X_1 \end{aligned}$$

$$
\begin{aligned}
{}_{s1}I_x &= {}_{s1}I_x^r/(X_1 \cdot C_d) \\
{}_{p2}I_y &= {}_{p2}I_y^r/X_{12} \\
{}_{p2}I_x &= {}_{p2}I_x^r/(X_{12} \cdot C_d) \\
{}_{s2}I_y &= {}_{s2}I_y^r/(X_2 \cdot X_{12}) \\
{}_{s2}I_x &= {}_{s2}I_x^r/(X_2 \cdot C_d \cdot X_{12}) \\
{}_{L1}I_y &= {}_{L1}I_y^r/X_{L1} \\
{}_{L1}I_x &= {}_{L1}I_x^r/(X_{L1} \cdot C_d) \\
{}_{R1}I_y &= {}_{R1}I_y^r/X_{R1} \\
{}_{R1}I_x &= {}_{R1}I_x^r/(X_{R1} \cdot C_d) \\
{}_{L2}I_y &= {}_{L2}I_y^r/(X_{L2} \cdot X_{12}) \\
{}_{L2}I_x &= {}_{L2}I_x^r/(X_{L2} \cdot C_d \cdot X_{12}) \\
{}_{R2}I_y &= {}_{R2}I_y^r/(X_{R2} \cdot X_{12}) \\
{}_{R2}I_x &= {}_{R2}I_x^r/(X_{R2} \cdot C_d \cdot X_{12})
\end{aligned} \tag{8}
$$

Where C_d and the various X's represent the 8 calibration factors, and the r superscript indicates a raw polarized fluorescence intensity. Calibration data is obtained by recording three background measurements followed by three recordings of a 15 nM solution of Rhodamine B in dimethylformamide that is flowed into the sample chamber (see Forkey 2005 for details). The calibration factors are obtained from a model similar to the one described in Appendix B, but modified to accommodate multiple molecules free in solution by increasing the wobble cone to $\delta = 90°$.

D Twirling Analysis

The path traced out by a probe bound to an actin filament that is twirling about its longitudinal axis is evident in two different representations of the angular motion. First a plot of θ vs. ϕ for one rotation of the probe traces out a circle that is centered at $(90°, \phi_{\text{actin}})$ where ϕ_{actin} is the direction in the x-y plane of the actin filament translocation. The reason that $\theta = 90°$ is because a probe that is attached to a filament, which is uniformly twirling about its axis in the x-y plane, maps out a locus of points that lie along the surface of a cone. Alternatively, the probe orientation can be represented in the (β, α) reference frame of the actin filament (Rosenberg 2005, Beausang 2008), where β is the polar angle with respect to the forward moving end of the actin filament and α is the azimuthal angle around the filament; for details, see Fig. 3 in the main text. In the actin frame a uniformly left-hand twirling filament has a constant β and a decreasing α with a constant rate equal to the angular velocity (ω) of the twirling motion.

The inherent dipole symmetry implies two equally valid solutions to Eq. (1): (θ, ϕ) and $(\theta', \phi') = (180° - \theta, \phi + 180°)$, either of which the numerical solver can obtain. Consequently, we need to determine one set of solutions that describes the trajectory of one end of the dipole. This is relatively straightforward for a uniformly twirling filament because subsequent orientations of the dipole should be nearby previous orientations. Prior to quantifying any twirling motions, we determine the minimum trajectory by choosing the orientation at time t (either $(\theta, \phi)_t$ or $(\theta', \phi')_t$) that is closesr to the orientation at $t-1$ and then repeat for the entire trace. Mathematically, we accomplish this by choosing $\hat{\mu}_t$ that minimizes: $\psi_t = \arccos(\hat{\mu}_t \cdot \hat{\mu}_{t-1})$. We arbitrarily choose the initial probe orientation as the one that points closest to the direction of positive translocation.

In order to reduce the impact of a single spurious point on the entire trajectory, the process is repeated omitting the point with the largest ψ to make sure the path of the minimum trajectory does not change. If $\phi_{t'}$ crosses the hemisphere from $+180°$ to $-180°$ then $360°$ is added to ϕ values with $t > t'$ in order to generate a continuous trajectory (similarly, $360°$ is subtracted for crossings in the opposite direction). Sometimes this automated detection fails and we manually shift the data onto a more confined trajectory in order to undo unrealistically large and sudden angle changes.

In order to transform angles into the actin frame, we rotate the twirling axis of the filament to align with the x-axis (i.e., $\phi' = \phi - \phi_{\text{actin}}$) and apply the transformation:

$$\begin{aligned} \alpha &= \operatorname{arctan2}(\mu_y, \mu_z) \\ \beta &= \arccos(\mu_x) \end{aligned} \tag{9}$$

Where $\alpha = \operatorname{arctan2}(\mu_y, \mu_z)$ is similar to $\alpha = \arctan(\mu_y/\mu_z)$ but with a larger range of unique angles: $-180° < \alpha < 180°$. After this transformation, a uniformly rotating probe will exhibit a constant β and a saw tooth pattern for α, which repeats after every rotation. In order to determine the angular velocity, ω, we fit a line to α after first shifting all α's after each complete right (left) handed rotation by $+(-)360°$. Because ϕ_{actin} is determined from only two CCD frames, we sometimes manually adjust ϕ_{actin} to minimize the error of the fit, usually only within $\pm 30°$ and never by more than $90°$, which would change the handedness of rotation. Due to the probe's dipole symmetry, orientations that are aligned with $\alpha = 0°\text{and}180°$ have identical intensities in all 16 polarizations. These points are manually adjusted by $\pm 180°$ prior to fitting the line to α.

E MathCad supplement

See separate file: "Beausang_AppE (mathcad supplement)".

Bibliography

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Forkey, J. N., M. E. Quinlan, and Y. E. Goldman. 2005. Measurement of single macromolecule orientation by total internal reflection fluorescence polarization microscopy. *Biophysical Journal* 89:1261-1271.

Rosenberg, S. A., M. E. Quinlan, J. N. Forkey, and Y. E. Goldman. 2005. Rotational motions of macro-molecules by single-molecule fluorescence microscopy. *Acc Chem Res* 38:583-593.

polTIRF worksheet for determining (θ, ϕ, δ, κ) from 16 polarization intensities

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Purpose
This MathCAD v. 2001 worksheet calculates the intensities measured from a dipole with 3D orientation (θ,ϕ) that absorbs incident light of specified polarization and then emits fluorescence that is collected by a high NA lens and directed onto two orthogonally polarized photodiodes. Relatively fast (t << 4 ns) and slow (4 ns<<t<<10 ms) rotational motions of the dipole are modelled as wobble cones with 1/2 angle δf and δ, respectively. The intensities are determined to within an overall scale factor κ.

References:
Joseph N. Forkey, Margot E. Quinlan, and Yale E. Goldman. *Measurement of Single Macromolecule Orientation by Total Internal Reflection Fluorescence Polarization Microscopy*, Biophysical Journal, v. 89 August 2005 1261–1271.

Conventions:
1. Coordinate system: Beam 1(2) is predominately in the direction of the x(y) axis, and the microscope objective is in the +z direction
2. Polarizaiton naming convention: eBd where e is the excitation polarization (p,s,L or R), B is the beam (1 or 2), and d is the detector (x,y).
3. For beam 1, L indicates ζ =+45 degrees where positive ζ is defined w.r.t. the z axis (down) toward postive y (direction of propagation of beam 2). R indicates ζ = -45 degrees. For beam 2, the convention is the same except positive ζ is defined from the z axis towards -x. See figure in main text.
4. High index is quartz, low index is water.

Requirements:
1. MathCAD v2001 (or higher)
2. input file of polarized fluorescent intentsities (named 0001d_ib.txt). See below for details.

Variables

peak electric field (in quartz)

$$A_o := 1$$

angle of linear polarization w.r.t. z axis

$\zeta := 47\text{deg}$ 47deg in air results in ~ 45 in the evanescent field.

Index of refraction

$n_1 := 1.46$ (Quartz)

$n_2 := 1.33$ (Water)

Index ratio n2/n1

$$\xi := .911$$

Angle of incidence of beam 1 and 2 on reflecting surface (w.r.t. -z axis)

$$\theta i := 68.5\text{deg}$$

Numerical aperture of lens (here, NA=1.2)

$$\theta_0 = \text{asin}\left(\frac{NA}{1.33}\right)$$

$$\theta_0 := 64\text{deg}$$

s and p phase shifts

$$\delta_p := \text{atan}\left(\frac{\sqrt{\sin(\theta i)^2 - \xi^2}}{\xi^2 \cdot \cos(\theta i)}\right) \qquad \delta_s := \text{atan}\left(\frac{\sqrt{\sin(\theta i)^2 - \xi^2}}{\cos(\theta i)}\right)$$

$$\delta_p = 31.868063\ \text{deg} \qquad \delta_s = 27.290961\ \text{deg}$$

Useful relationships

$$\sin(\delta_p)^2 = \frac{\sin(\theta i)^2 - \xi^2}{\xi^4 \cdot \cos(\theta i)^2 + \sin(\theta i)^2 - \xi^2} \qquad \cos(\delta_p)^2 = \frac{\xi^4 \cdot \cos(\theta i)^2}{\xi^4 \cdot \cos(\theta i)^2 + \sin(\theta i)^2 - \xi^2}$$

$$\sin(\delta_s)^2 = \frac{\sin(\theta i)^2 - \xi^2}{1 - \xi^2} \qquad \cos(\delta_s)^2 = \frac{\cos(\theta i)^2}{1 - \xi^2}$$

$$\sin(\delta_p) = 0.527965 \qquad \cos(\delta_p) = 0.849266$$

$$\sin(\delta_s) = 0.458509 \qquad \cos(\delta_s) = 0.888690$$

Excitation

Evenescent electric field

$$\varepsilon_{pt}(\zeta) := -2\cdot A_o\cdot\cos(\zeta)\cdot\cos(\theta i)\cdot\sqrt{\frac{\sin(\theta i)^2-\xi^2}{\xi^4\cdot\cos(\theta i)^2+\sin(\theta i)^2-\xi^2}}$$

p polarized, tangential to reflecting surface

$$\varepsilon_{pn}(\zeta) := 2\cdot A_o\cdot\cos(\zeta)\cdot\frac{\cos(\theta i)\cdot\sin(\theta i)}{\sqrt{\xi^4\cdot\cos(\theta i)^2+\sin(\theta i)^2-\xi^2}}$$

p polarized, normal to reflecting surface

$$\varepsilon_s(\zeta) := 2\cdot A_o\cdot\sin(\zeta)\cdot\cos(\theta i)\cdot\sqrt{\frac{1}{1-\xi^2}}$$

s polarzied

beam 1

$$E_1 = \begin{bmatrix}\vec{x}\cdot\varepsilon_{pt}(\zeta)\cdot\exp\left[-i\cdot\left(\delta_p+\frac{\pi}{2}\right)\right]\ \ldots\\ +\vec{y}\cdot\varepsilon_s(\zeta)\cdot\exp(-i\cdot\delta_s)\ \ldots\\ +\vec{z}\cdot\varepsilon_{pn}(\zeta)\cdot\exp(-i\cdot\delta_p)\end{bmatrix}\cdot\exp\left(\frac{-z}{d}\right)\cdot\exp\left[-i\cdot\left(\omega\cdot t-n_1\cdot k_0\cdot x\cdot\sin(\theta i)\right)\right]$$

$$\varepsilon_{x1}(\zeta) := \varepsilon_{pt}(\zeta)$$

$$\varepsilon_{y1}(\zeta) := \varepsilon_s(\zeta)$$

$$\varepsilon_{z1}(\zeta) := \varepsilon_{pn}(\zeta)$$

beam 2

$$E_1 = \begin{bmatrix}\vec{x}\cdot\varepsilon_s(\zeta)\cdot\exp\left[-i\cdot(\delta_s)\right]\ \ldots\\ +\vec{y}\cdot\varepsilon_{pt}(\zeta)\cdot\exp\left[-i\cdot\left(\delta_p+\frac{\pi}{2}\right)\right]\ \ldots\\ +\vec{z}\cdot\varepsilon_{pn}(\zeta)\cdot\exp(-i\cdot\delta_p)\end{bmatrix}\cdot\exp\left(\frac{-z}{d}\right)\cdot\exp\left[-i\cdot\left(\omega\cdot t-n_1\cdot k_0\cdot x\cdot\sin(\theta i)\right)\right]$$

$$\varepsilon_{x2}(\zeta) := \varepsilon_s(\zeta)$$

$$\varepsilon_{y2}(\zeta) := \varepsilon_{pt}(\zeta)$$

$$\varepsilon_{z2}(\zeta) := \varepsilon_{pn}(\zeta)$$

16 Polarization angles

$$\begin{matrix} \text{trace \#} \\ \text{polarization} \end{matrix} \begin{pmatrix} 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 & 11 & 12 & 13 & 14 & 15 & 16 \\ p1x & s1x & p1y & s1y & p2x & s2x & p2y & s2y & L1x & R1x & L1y & R1y & L2x & R2x & L2y & R2y \end{pmatrix}$$

For beam1:
p is polarized in the xz plane
s is polarized in the xy plane
L is polarized (mostly) in the yz plane, +ζ degrees from the z axis
R is polarized (mostly) in the yz plane, -ζ degrees from the z axis

For beam2:
p is polarized in the yz plane
s is polarized in the xy plane
L is polarized (mostly) in the xz plane, -ζ degrees from the z axis
R is polarized (mostly) in the xz plane, +ζ degrees from the z axis

$$\zeta := 47\text{deg}$$

Physically, the direction of the E field (looking along the direction of propagation) is:

p1	|	$\zeta_{p1} := 0\text{deg}$	p2	|	$\zeta_{p2} := 0\text{deg}$
s1	_	$\zeta_{s1} := 90\text{deg}$	s2	_	$\zeta_{s2} := 90\text{deg}$
L1	\	$\zeta_{L1} := \zeta$	L1	\	$\zeta_{L2} := -\zeta$
R1	/	$\zeta_{R1} := -\zeta$	R1	/	$\zeta_{R2} := \zeta$

The photons absorbed by a dipole, μ, in the evenescent field is proportional to $|\mu \cdot E|^2$ where μ and E are vectors and E is complex. These details have been suppressed. In what follows, the photons absorbed and emitted by the dipole has been expanded first in the fast wobble cone for absorption (Econst terms) and fast wobble cone for emission (Fconst terms) and then into the slow wobble cones (term1, term2...). Again, the details are suppressed. Instead of expanding in terms of the vector components (EconstX, EconstY, EconstZ) as was done in Forkey et al. 2005 the power is expanded in order parameters: Ecosnt1=$\sin^2(\theta)\cos^2(\phi)$, Econst2=$\cos^2(\theta)$ and Econst3=a constant. Similarly for the absorption (Fconst) terms. L and R polarizations are composed of both s and p and have different exanpsions (Econst4 and Econst5).

Fast Wobble- absorption

fast wobble cone absorption

$\delta a := 24deg$

fast wobble cone emission

$\delta_e := 24deg$

terms after fast wobble cone integration

$$Econst1(\zeta) := \frac{1}{2}\cdot\left(\varepsilon_{x1}(\zeta)^2 - \varepsilon_{y1}(\zeta)^2\right)\cdot\left(\cos(\delta a) + \cos(\delta a)^2\right)$$

$$Econst1bm2(\zeta) := \frac{1}{2}\cdot\left(\varepsilon_{x2}(\zeta)^2 - \varepsilon_{y2}(\zeta)^2\right)\cdot\left(\cos(\delta a) + \cos(\delta a)^2\right)$$

$$Econst2(\zeta) := \frac{1}{2}\cdot\left(\varepsilon_{z1}(\zeta)^2 - \varepsilon_{y1}(\zeta)^2\right)\cdot\left(\cos(\delta a) + \cos(\delta a)^2\right)$$

$$Econst2bm2(\zeta) := \frac{1}{2}\cdot\left(\varepsilon_{z2}(\zeta)^2 - \varepsilon_{y2}(\zeta)^2\right)\cdot\left(\cos(\delta a) + \cos(\delta a)^2\right)$$

$$Econst3(\zeta) := \left[\left[\frac{-1}{6}\cdot\left(\cos(\delta a) + \cos(\delta a)^2\right)\cdot\left(\varepsilon_{x1}(\zeta)^2 + \varepsilon_{z1}(\zeta)^2 - 2\cdot\varepsilon_{y1}(\zeta)^2\right)\right] + \frac{1}{3}\cdot\left(\varepsilon_{x1}(\zeta)^2 + \varepsilon_{y1}(\zeta)^2 + \varepsilon_{z1}(\zeta)^2\right)\right]$$

$$Econst3bm2(\zeta) := \left[\left[\frac{-1}{6}\cdot\left(\cos(\delta a) + \cos(\delta a)^2\right)\cdot\left(\varepsilon_{x2}(\zeta)^2 + \varepsilon_{z2}(\zeta)^2 - 2\cdot\varepsilon_{y2}(\zeta)^2\right)\right] + \frac{1}{3}\cdot\left(\varepsilon_{x2}(\zeta)^2 + \varepsilon_{y2}(\zeta)^2 + \varepsilon_{z2}(\zeta)^2\right)\right]$$

$$Econst4(\zeta) := \varepsilon_{pt}(\zeta)\cdot\varepsilon_s(\zeta)\cdot\sin(\delta_s - \delta_p)\cdot\left(\cos(\delta a)^2 + \cos(\delta a)\right)$$

$\sin(\delta_s - \delta_p) = -0.079801$

$$Econst5(\zeta) := \varepsilon_s(\zeta)\cdot\varepsilon_{pn}(\zeta)\cdot\cos(\delta_s - \delta_p)\cdot\left(\cos(\delta a)^2 + \cos(\delta a)\right)$$

$\cos(\delta_s - \delta_p) = 0.996811$

Generate Econst matrix (index order indicates which polarization).

p1x
s1x
p1y
s1y
p2x
s2x
p2y
s2y
L1x
R1x
L1y
R1y
L2x
R2x
L2y
R2y

$$\mathrm{Econst}_1 := \begin{bmatrix} \frac{1}{2}\cdot\left(\varepsilon_{x1}(\zeta_{p1})^2 - \varepsilon_{y1}(\zeta_{p1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x1}(\zeta_{s1})^2 - \varepsilon_{y1}(\zeta_{s1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x1}(\zeta_{p1})^2 - \varepsilon_{y1}(\zeta_{p1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x1}(\zeta_{s1})^2 - \varepsilon_{y1}(\zeta_{s1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x2}(\zeta_{p2})^2 - \varepsilon_{y2}(\zeta_{p2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x2}(\zeta_{s2})^2 - \varepsilon_{y2}(\zeta_{s2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x2}(\zeta_{p2})^2 - \varepsilon_{y2}(\zeta_{p2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x2}(\zeta_{s2})^2 - \varepsilon_{y2}(\zeta_{s2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x1}(\zeta_{L1})^2 - \varepsilon_{y1}(\zeta_{L1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x1}(\zeta_{R1})^2 - \varepsilon_{y1}(\zeta_{R1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x1}(\zeta_{L1})^2 - \varepsilon_{y1}(\zeta_{L1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x1}(\zeta_{R1})^2 - \varepsilon_{y1}(\zeta_{R1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x2}(\zeta_{L2})^2 - \varepsilon_{y2}(\zeta_{L2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x2}(\zeta_{R2})^2 - \varepsilon_{y2}(\zeta_{R2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x2}(\zeta_{L2})^2 - \varepsilon_{y2}(\zeta_{L2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{x2}(\zeta_{R2})^2 - \varepsilon_{y2}(\zeta_{R2})^2\right) \end{bmatrix}\cdot\left(\cos(\delta a) + \cos(\delta a)^2\right)$$

$$\mathrm{Econst}_2 := \begin{bmatrix} \frac{1}{2}\cdot\left(\varepsilon_{z1}(\zeta_{p1})^2 - \varepsilon_{y1}(\zeta_{p1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z1}(\zeta_{s1})^2 - \varepsilon_{y1}(\zeta_{s1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z1}(\zeta_{p1})^2 - \varepsilon_{y1}(\zeta_{p1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z1}(\zeta_{s1})^2 - \varepsilon_{y1}(\zeta_{s1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z2}(\zeta_{p2})^2 - \varepsilon_{y2}(\zeta_{p2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z2}(\zeta_{s2})^2 - \varepsilon_{y2}(\zeta_{s2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z2}(\zeta_{p2})^2 - \varepsilon_{y2}(\zeta_{p2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z2}(\zeta_{s2})^2 - \varepsilon_{y2}(\zeta_{s2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z1}(\zeta_{L1})^2 - \varepsilon_{y1}(\zeta_{L1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z1}(\zeta_{R1})^2 - \varepsilon_{y1}(\zeta_{R1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z1}(\zeta_{L1})^2 - \varepsilon_{y1}(\zeta_{L1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z1}(\zeta_{R1})^2 - \varepsilon_{y1}(\zeta_{R1})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z2}(\zeta_{L2})^2 - \varepsilon_{y2}(\zeta_{L2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z2}(\zeta_{R2})^2 - \varepsilon_{y2}(\zeta_{R2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z2}(\zeta_{L2})^2 - \varepsilon_{y2}(\zeta_{L2})^2\right) \\ \frac{1}{2}\cdot\left(\varepsilon_{z2}(\zeta_{R2})^2 - \varepsilon_{y2}(\zeta_{R2})^2\right) \end{bmatrix}\cdot\left(\cos(\delta a) + \cos(\delta a)^2\right)$$

$$\mathrm{Econst}_3 := \begin{pmatrix} \mathrm{Econst3}(\zeta_{p1}) \\ \mathrm{Econst3}(\zeta_{s1}) \\ \mathrm{Econst3}(\zeta_{p1}) \\ \mathrm{Econst3}(\zeta_{s1}) \\ \mathrm{Econst3bm2}(\zeta_{p2}) \\ \mathrm{Econst3bm2}(\zeta_{s2}) \\ \mathrm{Econst3bm2}(\zeta_{p2}) \\ \mathrm{Econst3bm2}(\zeta_{s2}) \\ \mathrm{Econst3}(\zeta_{L1}) \\ \mathrm{Econst3}(\zeta_{R1}) \\ \mathrm{Econst3}(\zeta_{L1}) \\ \mathrm{Econst3}(\zeta_{R1}) \\ \mathrm{Econst3bm2}(\zeta_{L2}) \\ \mathrm{Econst3bm2}(\zeta_{R2}) \\ \mathrm{Econst3bm2}(\zeta_{L2}) \\ \mathrm{Econst3bm2}(\zeta_{R2}) \end{pmatrix}$$

combine into matrix

$$
Econst := \left|\begin{array}{l}
\text{for } j \in 1..16 \\
\quad \left|\begin{array}{l}
M_{1,j} \leftarrow Econst1_{j-1} \\
M_{2,j} \leftarrow Econst2_{j-1} \\
M_{3,j} \leftarrow Econst3_{j-1} \\
M_{4,j} \leftarrow Econst4_{j-1} \\
M_{5,j} \leftarrow Econst5_{j-1}
\end{array}\right. \\
M
\end{array}\right.
$$

Econst =

		I_{p1x}	I_{s1x}	I_{p1y}	I_{s1y}	I_{p2x}	I_{s2x}	I_{p2y}	I_{s2y}	I_{L1x}	I_{R1x}	I_{L1y}	I_{R1y}	I_{L2x}
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
0	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
1	0.000000	0.130906	-2.761208	0.130906	-2.761208	-0.130906	2.761208	-0.130906	2.761208	-1.416023	-1.416023	-1.416023	-1.416023	1.416023
2	0.000000	3.169340	-2.761208	3.169340	-2.761208	3.038434	0.000000	3.038434	0.000000	-0.002781	-0.002781	-0.002781	-0.002781	1.413242
3	0.000000	0.158513	2.893831	0.158513	2.893831	0.289419	0.132623	0.289419	0.132623	1.621575	1.621575	1.621575	1.621575	0.205552
4	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.047860	-0.047860	0.047860	-0.047860	-0.047860
5	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	2.941626	-2.941626	2.941626	-2.941626	-2.941626

FAST Wobble - emission

Mixing of otherwise orthogonal polarizations by the high NA objective:
see Axelrod, D. 1989. *Methods in Cell Biol*. 30: 245-270. for details.

$$Ca := (0\ \ 0\ \ 0\ \ 0\ \ 0\ \ 0\ \ 0\ \ 0)^T$$

$$Cb := (0\ \ 0\ \ 0\ \ 0\ \ 0\ \ 0\ \ 0\ \ 0)^T$$

$$Cc := (0\ \ 0\ \ 0\ \ 0\ \ 0\ \ 0\ \ 0\ \ 0)^T$$

$$Ca_1 := \left(\frac{-3}{4}\cdot\pi\cdot\cos(\theta_0) + \frac{7}{12}\cdot\pi - \frac{1}{4}\cdot\pi\cdot\cos(\theta_0)^2 + \frac{5}{12}\cdot\pi\cdot\cos(\theta_0)^3\right)\cdot(1-\cos(\theta_0))^{-1}$$

$$Ca_2 := Ca_1 \qquad Ca_5 := Ca_1 \qquad Ca_6 := Ca_1$$

$$Cb_1 := \left(\frac{7}{6}\cdot\pi - \frac{1}{2}\cdot\pi\cdot\cos(\theta_0) - \frac{1}{6}\cdot\pi\cdot\cos(\theta_0)^3 - \frac{1}{2}\cdot\pi\cdot\cos(\theta_0)^2\right)\cdot(1-\cos(\theta_0))^{-1}$$

$$Cb_2 := Cb_1 \qquad Cb_5 := Cb_1 \qquad Cb_6 := Cb_1$$

$$Cc_1 := \left(\frac{1}{12}\cdot\pi + \frac{1}{4}\cdot\pi\cdot\cos(\theta_0)^2 - \frac{1}{12}\cdot\pi\cdot\cos(\theta_0)^3 - \frac{1}{4}\cdot\pi\cdot\cos(\theta_0)\right)\cdot(1-\cos(\theta_0))^{-1}$$

$$Cc_2 := Cc_1 \qquad Cc_5 := Cc_1 \qquad Cc_6 := Cc_1$$

$$Ca_3 := \left(\frac{-7}{12}\cdot\pi + \frac{7}{12}\cdot\pi\cdot\cos(\theta_0)^3 - \frac{1}{4}\cdot\pi\cdot\cos(\theta_0) + \frac{1}{4}\cdot\pi\cdot\cos(\theta_0)^2\right)\cdot(1-\cos(\theta_0))^{-1}$$

$$Ca_4 := Ca_3 \qquad Ca_7 := Ca_3 \qquad Ca_8 := Ca_3$$

$$Cb_3 := \left(\frac{1}{2}\cdot\pi\cdot\cos(\theta_0)^2 + \frac{1}{2}\cdot\pi\cdot\cos(\theta_0) + \frac{1}{6}\cdot\pi\cdot\cos(\theta_0)^3 - \frac{7}{6}\cdot\pi\right)\cdot(1-\cos(\theta_0))^{-1}$$

$$Cb_4 := Cb_3 \qquad Cb_7 := Cb_3 \qquad Cb_8 := Cb_3$$

$$Cc_3 := \left(\frac{5}{4}\cdot\pi - \frac{1}{4}\cdot\pi\cdot\cos(\theta_0)^2 - \frac{3}{4}\cdot\pi\cdot\cos(\theta_0) - \frac{1}{4}\cdot\pi\cdot\cos(\theta_0)^3\right)\cdot(1-\cos(\theta_0))^{-1}$$

$$Cc_4 := Cc_3 \qquad Cc_7 := Cc_3 \qquad Cc_8 := Cc_3$$

For convenience combine terms in a matrix

$$
\begin{aligned}
Fconst :=\ & \text{for } j \in 1..16 \\
& \quad \text{if } j < 9 \\
& \quad\quad M_{1,j} \leftarrow \frac{1}{2}\cdot Cb_j\cdot\left(\cos(\delta_e)^2 + \cos(\delta_e)\right) \\
& \quad\quad M_{2,j} \leftarrow \frac{1}{2}\cdot Ca_j\cdot\left(\cos(\delta_e)^2 + \cos(\delta_e)\right) \\
& \quad\quad M_{3,j} \leftarrow -\frac{1}{6}\cdot\left(Ca_j + Cb_j\right)\cdot\left(\cos(\delta_e)^2 + \cos(\delta_e)\right) + \frac{1}{3}\cdot\left(Ca_j + Cb_j\right) + Cc_j \\
& \quad \text{if } j > 8 \\
& \quad\quad M_{1,j} \leftarrow \frac{1}{2}\cdot Cb_{j-8}\cdot\left(\cos(\delta_e)^2 + \cos(\delta_e)\right) \\
& \quad\quad M_{2,j} \leftarrow \frac{1}{2}\cdot Ca_{j-8}\cdot\left(\cos(\delta_e)^2 + \cos(\delta_e)\right) \\
& \quad\quad M_{3,j} \leftarrow -\frac{1}{6}\cdot\left(Ca_{j-8} + Cb_{j-8}\right)\cdot\left(\cos(\delta_e)^2 + \cos(\delta_e)\right) + \frac{1}{3}\cdot\left(Ca_{j-8} + Cb_{j-8}\right) + Cc_{j-8} \\
& M
\end{aligned}
$$

$$
Ca = \begin{pmatrix} 0.000000 \\ 1.351516 \\ 1.351516 \\ -3.332417 \\ -3.332417 \\ 1.351516 \\ 1.351516 \\ -3.332417 \\ -3.332417 \end{pmatrix}
\quad
Cb = \begin{pmatrix} 0.000000 \\ 4.683933 \\ 4.683933 \\ -4.683933 \\ -4.683933 \\ 4.683933 \\ 4.683933 \\ -4.683933 \\ -4.683933 \end{pmatrix}
\quad
Cc = \begin{pmatrix} 0.000000 \\ 0.082579 \\ 0.082579 \\ 4.766512 \\ 4.766512 \\ 0.082579 \\ 0.082579 \\ 4.766512 \\ 4.766512 \end{pmatrix}
$$

Fconst =

	0	1	2	3	4	5	6	7	8	9	10	11	12	13
0	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
1	0.000000	4.094017	4.094017	-4.094017	-4.094017	4.094017	4.094017	-4.094017	-4.094017	4.094017	4.094017	-4.094017	-4.094017	4.094017
2	0.000000	1.181300	1.181300	-2.912717	-2.912717	1.181300	1.181300	-2.912717	-2.912717	1.181300	1.181300	-2.912717	-2.912717	1.181300
3	0.000000	0.335956	0.335956	4.429973	4.429973	0.335956	0.335956	4.429973	4.429973	0.335956	0.335956	4.429973	4.429973	0.335956

SLOW WOBBLE terms

$$\theta_{test} := 2.059305 \qquad \phi_{test} := -2.687483 \qquad \delta_{test} := 1.272889 \qquad trace := 16$$

$$\theta_{test} = 117.989485\,deg \qquad \phi_{test} = -153.981433\,deg \qquad \delta_{test} = 72.931167\,deg$$

Term 1

$$term1(\theta,\phi,\delta,i) := \overset{pre1}{\frac{1}{40}\cdot Econst_{1,i}\cdot Fconst_{1,i}}\cdot\left[\left(\cos(\delta)+\cos(\delta)^2\right)\cdot\left[\begin{array}{l}\cos(\delta)^2\cdot\left(-30\cdot\cos(\phi)^2+35\cdot\cos(\theta)^4\cdot\cos(\phi)^4+30\cdot\cos(\theta)^2\cdot\cos(\phi)^2-70\cdot\cos(\theta)^2\cdot\cos(\phi)^4+3+35\cdot\cos(\phi)^4\right)\ ...\\ +-15\cdot\cos(\theta)^4\cdot\cos(\phi)^4-15\cdot\cos(\phi)^4+30\cdot\cos(\phi)^2+30\cdot\cos(\theta)^2\cdot\cos(\phi)^4-30\cdot\cos(\theta)^2\cdot\cos(\phi)^2-7\end{array}\right]+8\right]$$

Term 2

$$term2(\theta,\phi,\delta,i) := -\frac{1}{120}\cdot\left(Econst_{1,i}\cdot Fconst_{2,i}\right)\cdot\left[\left(\cos(\delta)+\cos(\delta)^2\right)\cdot\left[\begin{array}{l}\cos(\delta)^2\cdot\left(-120\cdot\cos(\phi)^2\cdot\cos(\theta)^2+15\cdot\cos(\phi)^2+105\cdot\cos(\phi)^2\cdot\cos(\theta)^4-3+15\cdot\cos(\theta)^2\right)\ ...\\ +-45\cdot\cos(\phi)^2\cdot\cos(\theta)^4-15\cdot\cos(\theta)^2-15\cdot\cos(\phi)^2+7+60\cdot\cos(\phi)^2\cdot\cos(\theta)^2\end{array}\right]-8\right]$$

Term 3

$$term3(\theta,\phi,\delta,i) := -\frac{1}{6}\cdot\left(Econst_{1,i}\cdot Fconst_{3,i}\right)\cdot\left[\left(-3\cdot\cos(\phi)^2+3\cdot\cos(\theta)^2\cdot\cos(\phi)^2+1\right)\cdot\left(\cos(\delta)+\cos(\delta)^2\right)-2\right]$$

Term 4

$$term4(\theta,\phi,\delta,i) := -\frac{1}{120}\cdot\left(Econst_{2,i}\cdot Fconst_{1,i}\right)\cdot\left[\cos(\delta)\cdot(1+\cos(\delta))\cdot\left[\begin{array}{l}\cos(\delta)^2\cdot\left(-120\cdot\cos(\phi)^2\cdot\cos(\theta)^2+15\cdot\cos(\phi)^2+105\cdot\cos(\phi)^2\cdot\cos(\theta)^4-3+15\cdot\cos(\theta)^2\right)\ ...\\ +-45\cdot\cos(\phi)^2\cdot\cos(\theta)^4-15\cdot\cos(\theta)^2-15\cdot\cos(\phi)^2+7+60\cdot\cos(\phi)^2\cdot\cos(\theta)^2\end{array}\right]-8\right]$$

Terr

$$term5(\theta,\phi,\delta,i) := \frac{1}{40}\cdot\left(Econst_{2,i}\cdot Fconst_{2,i}\right)\cdot\left[\left(35\cdot\cos(\theta)^4-30\cdot\cos(\theta)^2+3\right)\cdot\left[\left(\cos(\delta)+\cos(\delta)^2\right)\cdot\cos(\delta)^2\right]+-1\cdot\left(7-30\cdot\cos(\theta)^2+15\cdot\cos(\theta)^4\right)\cdot\left(\cos(\delta)^2+\cos(\delta)\right)+8\right]$$

Term 6

$$term6(\theta,\phi,\delta,i) := \frac{1}{6}\cdot\left(Econst_{2,i}\cdot Fconst_{3,i}\right)\cdot\left[\left(3\cdot\cos(\theta)^2-1\right)\cdot\left(\cos(\delta)^2+\cos(\delta)\right)+2\right]$$

Term 7

$$term7(\theta,\phi,\delta,i) := -\frac{1}{6}\cdot\left(Econst_{3,i}\cdot Fconst_{1,i}\right)\cdot\left[\left(-3\cdot\cos(\phi)^2+3\cdot\cos(\theta)^2\cdot\cos(\phi)^2+1\right)\cdot\left(\cos(\delta)+\cos(\delta)^2\right)-2\right]$$

Term 8

$$term8(\theta,\phi,\delta,i) := \frac{1}{6}\cdot\left(Econst_{3,i}\cdot Fconst_{2,i}\right)\cdot\left[\left(3\cdot\cos(\theta)^2-1\right)\cdot\left(\cos(\delta)^2+\cos(\delta)\right)+2\right]$$

Term 9

$$term9(\theta,\phi,\delta,i) := Econst_{3,i}\cdot Fconst_{3,i}$$

cross terms (xy):

$$\mathrm{term10}(\theta,\phi,\delta,i) := \frac{1}{8}\cdot\left(\mathrm{Econst}_{4,i}\cdot\mathrm{Fconst}_{1,i}\right)\cdot\cos(\phi)\cdot\sin(\phi)\cdot\sin(\theta)^2\cdot\left(\cos(\delta)^2+\cos(\delta)\right)\cdot\left[\cos(\delta)^2\cdot\left(7\cdot\cos(\phi)^2\cdot\sin(\theta)^2-3\right)-3\cdot\cos(\phi)^2\cdot\sin(\theta)^2+3\right]$$

$$\mathrm{term11}(\theta,\phi,\delta,i) := \frac{1}{8}\cdot\left(\mathrm{Econst}_{4,i}\cdot\mathrm{Fconst}_{2,i}\right)\cdot\cos(\phi)\cdot\sin(\phi)\cdot\sin(\theta)^2\cdot\left(\cos(\delta)^2+\cos(\delta)\right)\cdot\left[\cos(\delta)^2\cdot\left(7\cdot\cos(\theta)^2-1\right)-3\cdot\cos(\theta)^2+1\right]$$

$$\mathrm{term12}(\theta,\phi,\delta,i) := \frac{1}{2}\cdot\left(\mathrm{Econst}_{4,i}\cdot\mathrm{Fconst}_{3,i}\right)\cdot\cos(\phi)\cdot\sin(\phi)\cdot\sin(\theta)^2\cdot\left(\cos(\delta)^2+\cos(\delta)\right)$$

beam 1 yz cross terms:

$$\mathrm{term13}(\theta,\phi,\delta,i) := \frac{1}{8}\cdot\left(\mathrm{Econst}_{5,i}\cdot\mathrm{Fconst}_{1,i}\right)\cdot\sin(\theta)\cdot\cos(\theta)\cdot\sin(\phi)\cdot\left(\cos(\delta)^2+\cos(\delta)\right)\cdot\left[\cos(\delta)^2\cdot\left(7\cdot\cos(\phi)^2\cdot\sin(\theta)^2-1\right)-3\cdot\cos(\phi)^2\cdot\sin(\theta)^2+1\right]$$

$$\mathrm{term14}(\theta,\phi,\delta,i) := \frac{1}{8}\cdot\left(\mathrm{Econst}_{5,i}\cdot\mathrm{Fconst}_{2,i}\right)\cdot\sin(\theta)\cdot\cos(\theta)\cdot\sin(\phi)\cdot\left(\cos(\delta)^2+\cos(\delta)\right)\cdot\left[\cos(\delta)^2\cdot\left(7\cdot\cos(\theta)^2-3\right)+3\cdot\sin(\theta)^2\right]$$

$$\mathrm{term15}(\theta,\phi,\delta,i) := \frac{1}{2}\cdot\left(\mathrm{Econst}_{5,i}\cdot\mathrm{Fconst}_{3,i}\right)\cdot\sin(\theta)\cdot\cos(\theta)\cdot\sin(\phi)\cdot\left(\cos(\delta)^2+\cos(\delta)\right)$$

beam 2 'yz' cross terms

$$\mathrm{term16}(\theta,\phi,\delta,i) := \frac{1}{8}\cdot\left(\mathrm{Econst}_{5,i}\cdot\mathrm{Fconst}_{1,i}\right)\cdot\left[\cos(\theta)\cdot\sin(\theta)\cdot\cos(\phi)\cdot\left(\cos(\delta)^2+\cos(\delta)\right)\cdot\left[\cos(\delta)^2\cdot\left(7\cdot\cos(\phi)^2\cdot\sin(\theta)^2-3\right)-3\cdot\left(\cos(\phi)^2\cdot\sin(\theta)^2-1\right)\right]\right]$$

$$\mathrm{term17}(\theta,\phi,\delta,i) := \frac{1}{8}\cdot\left(\mathrm{Econst}_{5,i}\cdot\mathrm{Fconst}_{2,i}\right)\cdot\left[\cos(\theta)\cdot\sin(\theta)\cdot\cos(\phi)\cdot\left(\cos(\delta)^2+\cos(\delta)\right)\cdot\left[\cos(\delta)^2\cdot\left(7\cdot\cos(\theta)^2-3\right)+3\cdot\sin(\theta)^2\right]\right]$$

$$\mathrm{term18}(\theta,\phi,\delta,i) := \frac{1}{2}\cdot\left(\mathrm{Econst}_{5,i}\cdot\mathrm{Fconst}_{3,i}\right)\cdot\left[\sin(\theta)\cdot\cos(\theta)\cdot\cos(\phi)\cdot\left(\cos(\delta)^2+\cos(\delta)\right)\right]$$

for convenience, combine all terms into matrix

```
Allterms(θ, φ, δ) := | for i ∈ 1 .. 16
                     |   if i ≥ 9 ∧ i ≤ 12
                     |     bm2 ← 0
                     |     bm1 ← 1
                     |   if i ≥ 13 ∧ i ≤ 16
                     |     bm2 ← 1
                     |     bm1 ← 0
                     |   N ← (term1(θ,φ,δ,i)  term2(θ,φ,δ,i)  term3(θ,φ,δ,i)  term4(θ,φ,δ,i)  term5(θ,φ,δ,i)  term6(θ,φ,δ,i)  term7(θ,φ,δ,i)  term8(θ,φ,δ,i)  term9(θ,φ,δ
                     |   sum ← 0
                     |   for j ∈ 1 .. 18
                     |     sum ← sum + N_{0, j−1}
                     |     M_{j,i} ← N_{0, j−1}
                     |   M_{0,i} ← sum
                     | M
```

Values for intensities for each trace (sum of terms is in row zero)

$\text{Allterms}(\theta_{test}, \phi_{test}, \delta_{test}) =$

		p1x	s1x	p1y	s1y	p2x	s2x	p2y	s2y	I_{L1x}
	0	1	2	3	4	5	6	7	8	9
0	0.000000	2.425478	1.543189	2.152517	2.670394	2.320238	3.763005	2.202937	1.606874	1.996934
1	0.000000	0.136538	-2.879991	-0.136538	2.879991	-0.136538	2.879991	0.136538	-2.879991	-1.476938
2	0.000000	0.010272	-0.216658	-0.025326	0.534211	-0.010272	0.216658	0.025326	-0.534211	-0.111108
3	0.000000	0.017134	-0.361406	0.225931	-4.765567	-0.017134	0.361406	-0.225931	4.765567	-0.185339
4	0.000000	0.861855	-0.750869	-0.861855	0.750869	0.826257	0.000000	-0.826257	0.000000	-0.000756
5	0.000000	0.703136	-0.612589	-1.733714	1.510455	0.674094	0.000000	-1.662105	0.000000	-0.000617
6	0.000000	0.332062	-0.289301	4.378629	-3.814771	0.318347	0.000000	4.197775	0.000000	-0.000291
7	0.000000	0.252831	4.615695	-0.252831	-4.615695	0.461628	0.211535	-0.461628	-0.211535	2.586432
8	0.000000	0.058397	1.066109	-0.143990	-2.628692	0.106624	0.048859	-0.262902	-0.120472	0.597400
9	0.000000	0.053254	0.972200	0.702210	12.819593	0.097232	0.044555	1.282121	0.587516	0.544778
10	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.003522
11	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000318
12	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000938

Measured Intensity

II is the predicted intensity for a dipole oriented at (θ,φ) with slow wobble cone δ, fast wobble cone = 24 degrees when illuminated/detected by the i'th combination of excitation/detection polarizations.

$$bm1(i) := \left| \begin{array}{l} ans \leftarrow 1 \quad \text{if } i \ge 9 \wedge i \le 12 \\ ans \end{array} \right. \qquad bm2(i) := \left| \begin{array}{l} ans \leftarrow 1 \quad \text{if } i \ge 13 \wedge i \le 16 \\ ans \end{array} \right.$$

$$\begin{aligned} II(\theta,\phi,\delta,i,\kappa) := \kappa\cdot\Big[& term1(\theta,\phi,\delta,i) + term2(\theta,\phi,\delta,i) + term3(\theta,\phi,\delta,i) + term4(\theta,\phi,\delta,i) + term5(\theta,\phi,\delta,i) + term6(\theta,\phi,\delta,i) + term7(\theta,\phi,\delta,i) + term8(\theta,\phi,\delta,i) \ldots \\ & + term9(\theta,\phi,\delta,i) + \big[term10(\theta,\phi,\delta,i) + term11(\theta,\phi,\delta,i) + term12(\theta,\phi,\delta,i) \ldots \\ & \quad + bm1(i)\cdot(term13(\theta,\phi,\delta,i) + term14(\theta,\phi,\delta,i) + term15(\theta,\phi,\delta,i)) \ldots \\ & \quad + bm2(i)\cdot(term16(\theta,\phi,\delta,i) + term17(\theta,\phi,\delta,i) + term18(\theta,\phi,\delta,i)) \big] \Big] \end{aligned}$$

Example:

Read in Data

the provided data file (XXXXx_ib.txt file) contains simulated data for a single fluorophore twirling about the x-axis. The columns contain the traces (p1x, s1x...), with time increasing down. The first row is the average background for each trace (here set to zero in the simulation). In actual data, the background intensity for each polarization must be determined from the recording after the fluorophore bleach. These background intensities should then be subtracted from each intensity leaving the raw signal for analysis. The format for the ib file is:

col 1	col2	col3	col4	col5...
filename	point #	cycle #	p1x	s1x...

Each point (i.e., fluorophore bleach) to be analyzed consists of a background and several measurement cycles.

CWD = "D:\YEGlab\2008_TwirlingPaper\mathCAD supplement\"

ib_file := concat(CWD, "0001d_ib.txt")

mathCAD supplement

ib_file = "D:\YEGlab\2008_TwirlingPaper\mathCAD supplement\0001d_ib.txt"

I_{ib} := READPRN(ib_file)

routine to separate the row of background intensities from the signal intensities in the input (XXXXx_ib.txt) file.

$$RemoveBak(I_{ib}) := \left| \begin{array}{l} i \leftarrow 0 \\ bakVec \leftarrow \left(i \;\; I_{ib_{0,2}} \;\; I_{ib_{0,3}} \;\; I_{ib_{0,4}} \;\; I_{ib_{0,5}} \;\; I_{ib_{0,6}} \;\; I_{ib_{0,7}} \;\; I_{ib_{0,8}} \;\; I_{ib_{0,9}} \;\; I_{ib_{0,10}} \;\; I_{ib_{0,11}} \;\; I_{ib_{0,12}} \;\; I_{ib_{0,13}} \;\; I_{ib_{0,14}} \;\; I_{ib_{0,15}} \;\; I_{ib_{0,16}} \;\; I_{ib_{0,17}} \right) \\ \text{for } i \in 0..rows(I_{ib}) - 2 \\ \quad \text{for } j \in 0..16 \\ \quad\quad M_{i,j} \leftarrow I_{ib_{i+1,j+1}} \\ \begin{pmatrix} bakVec \\ M \end{pmatrix} \end{array} \right.$$

$x_{dat} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 0\rangle}$

cycle number (k)

$t_{dat} := x_{dat_{0,0}} - 1 .. x_{dat(rows(x_{dat})-1),0} - 1$

$kmax := rows(x_{dat}) - 1$

$k := 0 .. rows(x_{dat}) - 1$

$bak_{p1x} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,1}$

$I_{p1x} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 1\rangle}$

$bak_{p2x} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,5}$

$I_{p2x} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 5\rangle}$

$bak_{s1x} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,2}$

$I_{s1x} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 2\rangle}$

$bak_{s2x} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,6}$

$I_{s2x} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 6\rangle}$

$bak_{p1y} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,3}$

$I_{p1y} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 3\rangle}$

$bak_{p2y} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,7}$

$I_{p2y} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 7\rangle}$

$bak_{s1y} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,4}$

$I_{s1y} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 4\rangle}$

$bak_{s2y} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,8}$

$I_{s2y} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 8\rangle}$

$bak_{L1x} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,9}$

$I_{L1x} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 9\rangle}$

$bak_{L2x} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,13}$

$I_{L2x} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 13\rangle}$

$bak_{R1x} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,10}$

$I_{R1x} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 10\rangle}$

$bak_{R2x} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,14}$

$I_{R2x} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 14\rangle}$

$bak_{L1y} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,11}$

$I_{L1y} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 11\rangle}$

$bak_{L2y} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,15}$

$I_{L2y} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 15\rangle}$

$bak_{R1y} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,12}$

$I_{R1y} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 12\rangle}$

$bak_{R2y} := \left(\mathrm{RemoveBak}(I_{ib})_{0,0}\right)_{0,16}$

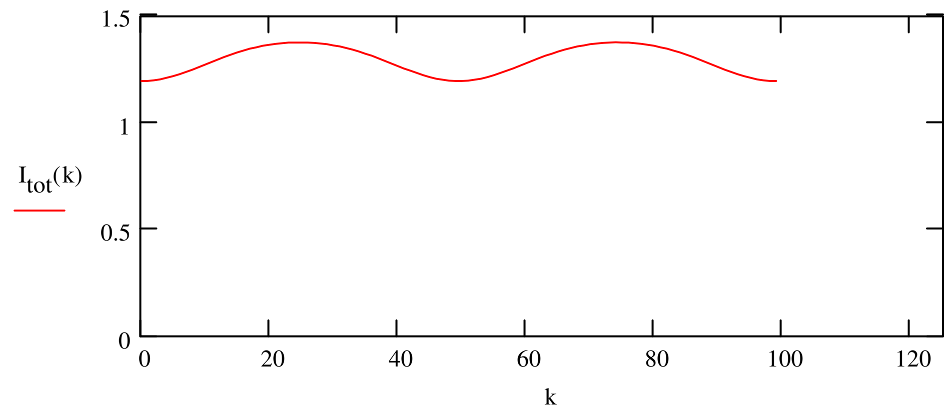
$I_{R2y} := \left(\mathrm{RemoveBak}(I_{ib})_{1,0}\right)^{\langle 16\rangle}$

combine into one matrix

$I_{act}(p) := \left(p\ \ I_{p1x_p}\ \ I_{s1x_p}\ \ I_{p1y_p}\ \ I_{s1y_p}\ \ I_{p2x_p}\ \ I_{s2x_p}\ \ I_{p2y_p}\ \ I_{s2y_p}\ \ I_{L1x_p}\ \ I_{R1x_p}\ \ I_{L1y_p}\ \ I_{R1y_p}\ \ I_{L2x_p}\ \ I_{R2x_p}\ \ I_{L2y_p}\ \ I_{R2y_p}\right)^T$

$I_{tot}(p) := \frac{1}{16} \cdot \sum_{i=1}^{16} I_{act}(p)_i$

Total intensity plot



cycle number (k)

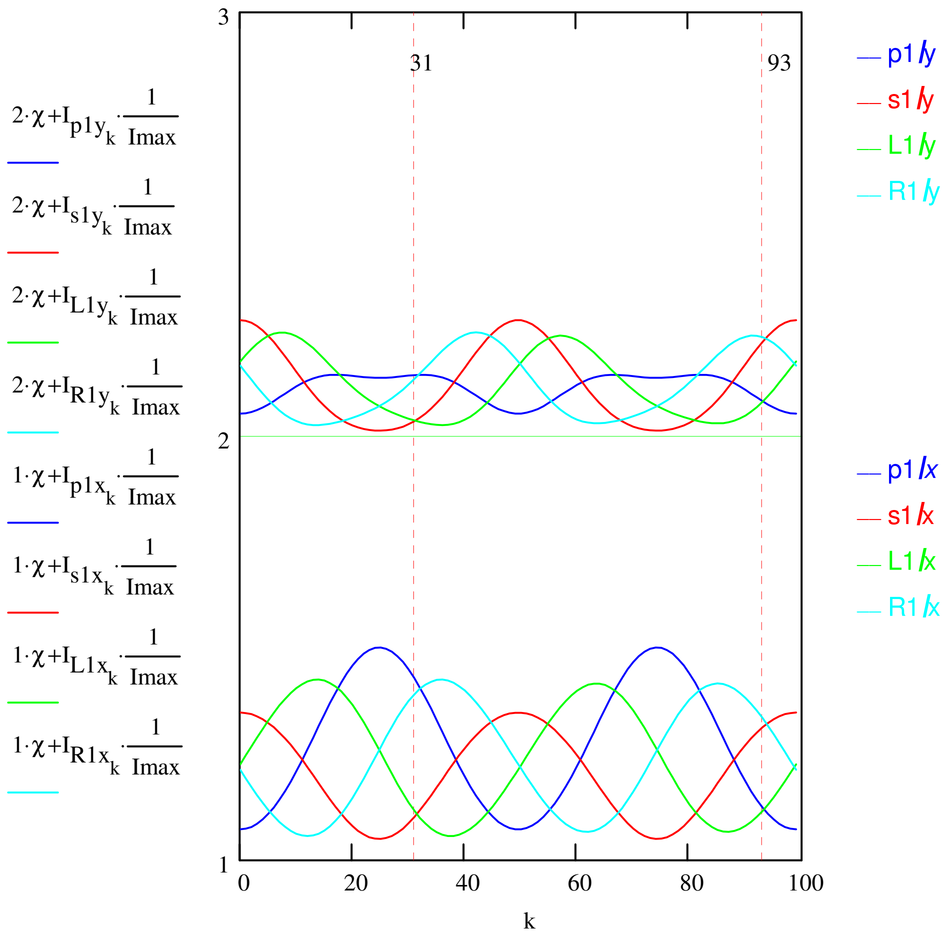
scale factors (for plotting)

$\chi := 1$

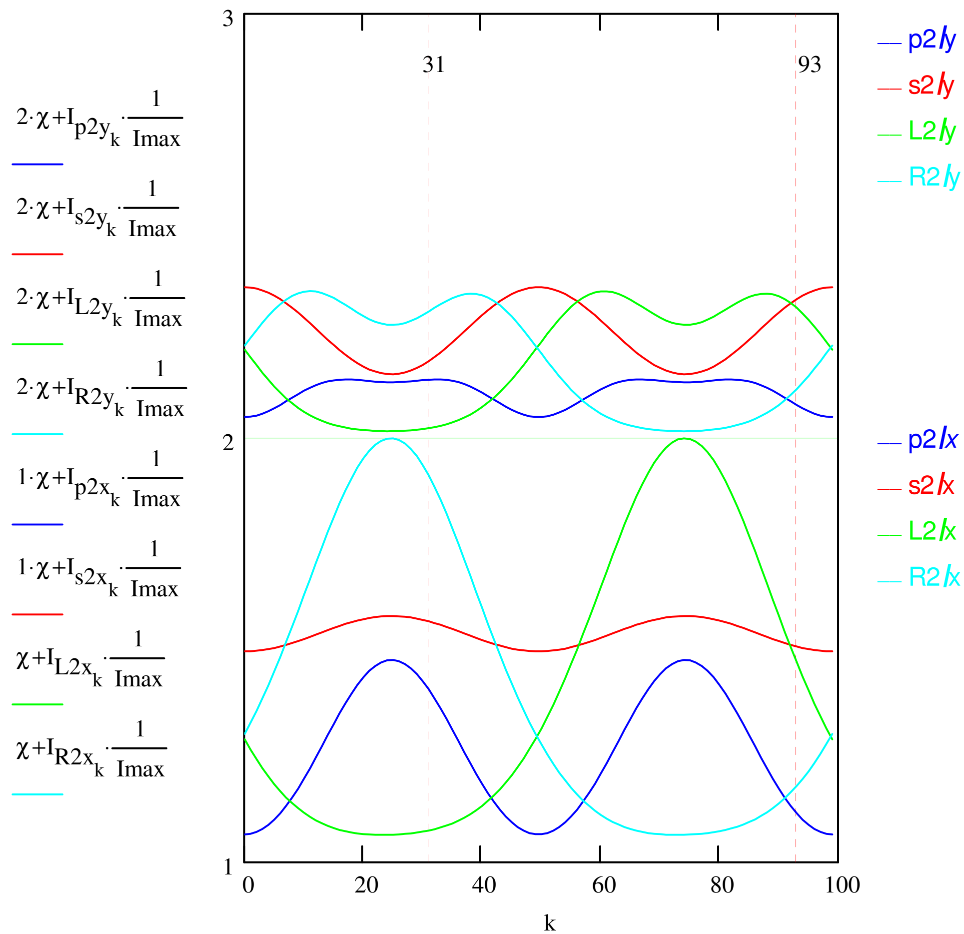
$Imax := \max\left(\mathrm{submatrix}(I_{ib}, 1, kmax, 2, 17)\right)$

$Imax = 5.433000$

Beam 1, APD y (top), APD x (bottom)



Beam 2, APD y (top), APD x (bottom)



Solultion in MathCAD via minimizing the Root mean Square

Guesses

$t := 2$ $\theta' := 80deg$ $\phi' := 35deg$ $\delta' := 15deg$ $\kappa' := 1$

Given (minimize this function)

$$\frac{\left(I_{p1x_t} - II(\theta',\phi',\delta',1,\kappa')\right)^2}{II(\theta',\phi',\delta',1,\kappa')} + \frac{\left(I_{s1x_t} - II(\theta',\phi',\delta',2,\kappa')\right)^2}{II(\theta',\phi',\delta',2,\kappa')} + \frac{\left(I_{p1y_t} - II(\theta',\phi',\delta',3,\kappa')\right)^2}{II(\theta',\phi',\delta',3,\kappa')} + \frac{\left(I_{s1y_t} - II(\theta',\phi',\delta',4,\kappa')\right)^2}{II(\theta',\phi',\delta',4,\kappa')} + \frac{\left(I_{p2x_t} - II(\theta',\phi',\delta',5,\kappa')\right)^2}{II(\theta',\phi',\delta',5,\kappa')} + \frac{\left(I_{s2x_t} - II(\theta',\phi',\delta',6,\kappa')\right)^2}{II(\theta',\phi',\delta',6,\kappa')}$$

$$+ \frac{\left(I_{L1x_t} - II(\theta',\phi',\delta',9,\kappa')\right)^2}{II(\theta',\phi',\delta',9,\kappa')} + \frac{\left(I_{R1x_t} - II(\theta',\phi',\delta',10,\kappa')\right)^2}{II(\theta',\phi',\delta',10,\kappa')} + \frac{\left(I_{L1y_t} - II(\theta',\phi',\delta',11,\kappa')\right)^2}{II(\theta',\phi',\delta',11,\kappa')} + \frac{\left(I_{R1y_t} - II(\theta',\phi',\delta',12,\kappa')\right)^2}{II(\theta',\phi',\delta',12,\kappa')} + \frac{\left(I_{L2x_t} - II(\theta',\phi',\delta',13,\kappa')\right)^2}{II(\theta',\phi',\delta',13,\kappa')} + \frac{\left(I_{R2x_t} - II(\theta',\phi',\delta\right.}{II(\theta',\phi',\delta',14}$$

$\delta' > 0$ $\delta' < \frac{\pi}{2}$

$ans(\theta',\phi',\delta',\kappa',t) := MinErr(\theta',\phi',\delta',\kappa',t)$ $t := k$

Find minimum for each cycle k using the result from k-1 as the new guess at k

$$answer := \left| \begin{array}{l} M_0 \leftarrow ans(80deg, 35deg, 15deg, 1, 0) \\ \text{for } t \in 1..kmax \\ \quad M_t \leftarrow ans\left[(M_{t-1})_0, (M_{t-1})_1, (M_{t-1})_2, (M_{t-1})_3, t\right] \\ M \end{array} \right.$$

<---Enable this if you want to execute solve block

numerical solutions (points on below graphs)

$\Theta_{ans_t} := (answer_t)_{0,0}$ $\Phi_{ans_t} := (answer_t)_{1,0}$ $\delta_{ans_t} := (answer_t)_{2,0}$ $\kappa_{ans_t} := (answer_t)_{3,0}$

$\alpha_t := atan2\left(\sin(\Theta_{ans_t})\cdot\sin(\Phi_{ans_t}), \cos(\Theta_{ans_t})\right)$ $\beta_t := acos\left(\sin(\Theta_{ans_t})\cdot\cos(\Phi_{ans_t})\right)$

$$\chi2_t := \left(I_{p1x_t} - II(\Theta_{ans_t},\Phi_{ans_t},\delta_{ans_t},1,\kappa_{ans_t})\right)^2 + \left(I_{s1x_t} - II(\Theta_{ans_t},\Phi_{ans_t},\delta_{ans_t},2,\kappa_{ans_t})\right)^2 + \left(I_{p1y_t} - II(\Theta_{ans_t},\Phi_{ans_t},\delta_{ans_t},3,\kappa_{ans_t})\right)^2 + \left(I_{s1y_t} - II(\Theta_{ans_t},\Phi_{ans_t},\delta_{ans_t},4,\kappa_{ans_t})\right)$$

$$+ \left(I_{L1x_t} - II(\Theta_{ans_t},\Phi_{ans_t},\delta_{ans_t},9,\kappa_{ans_t})\right)^2 + \left(I_{R1x_t} - II(\Theta_{ans_t},\Phi_{ans_t},\delta_{ans_t},10,\kappa_{ans_t})\right)^2 + \left(I_{L1y_t} - II(\Theta_{ans_t},\Phi_{ans_t},\delta_{ans_t},11,\kappa_{ans_t})\right)^2 + \left(I_{R1y_t} - II(\Theta_{ans_t},\Phi_{ans_t},\delta_{ans_t},12,\right.$$

known solutions (for simulation, dotted lines)

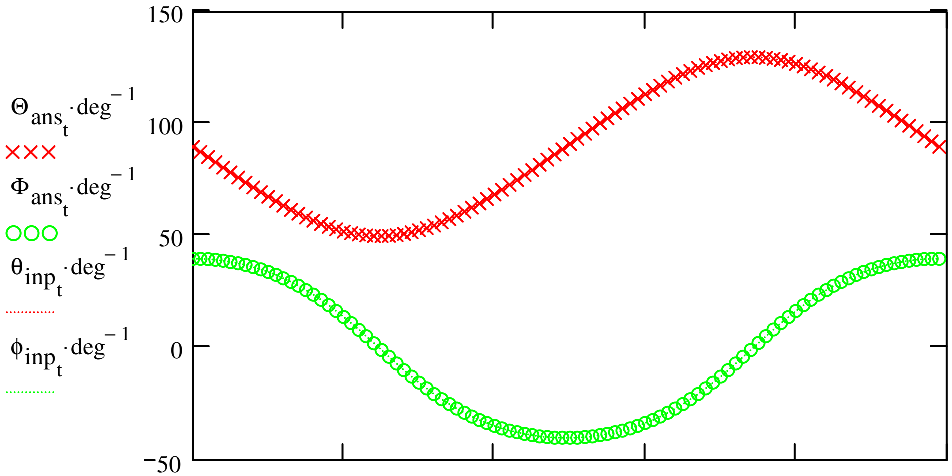
$\alpha_{inp_k} := mod\left[\left(2\cdot\pi\cdot\frac{k}{kmax}\right) + \pi, 2\cdot\pi\right] - \pi$ $\beta_{inp_k} := 40deg$ $\delta_{inp_k} := 10deg$ $\kappa_{inp_k} := (1 - \cos(\theta_0))$

$\theta_{inp_k} := acos\left(\sin(\beta_{inp_k})\cdot\sin(\alpha_{inp_k})\right)$ $\phi_{inp_k} := asin\left(\frac{\sin(\beta_{inp_k})\cdot\cos(\alpha_{inp_k})}{\sin\left(acos\left(\sin(\beta_{inp_k})\cdot\sin(\alpha_{inp_k})\right)\right)}\right)$

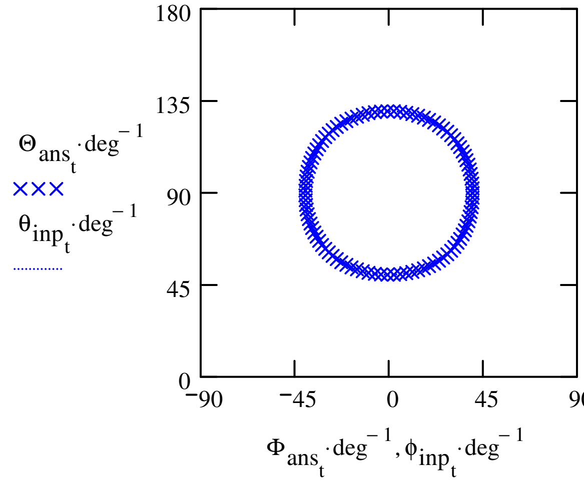
$\chi2_{avg} := \left(\sum_t \chi2_t\right)\cdot\left(\sum_t 1\right)^{-1}$

$\chi2_{avg} = 1.235445 \times 10^{-6}$

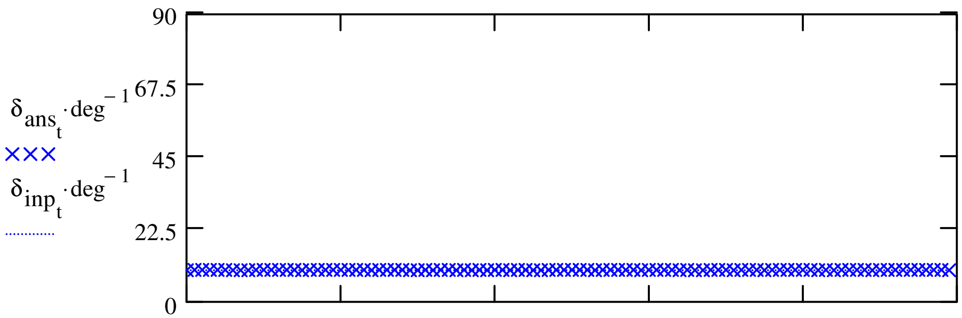
θ
ϕ



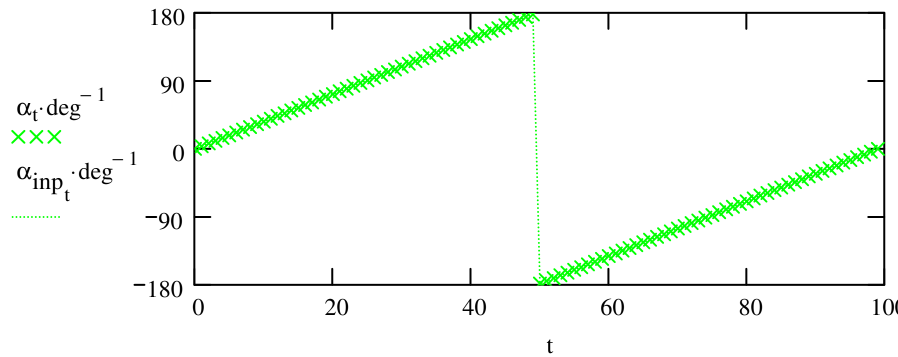
θ vs ϕ



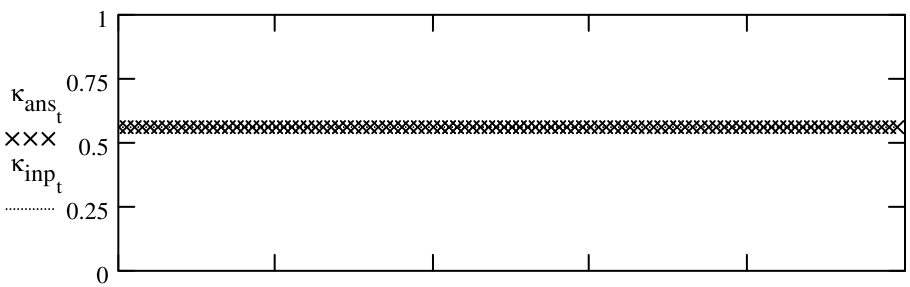
δ



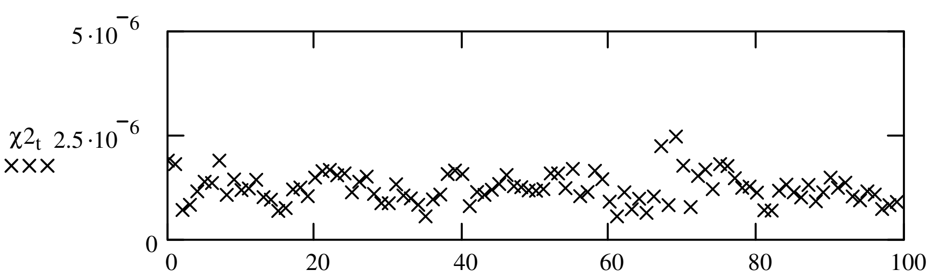
α



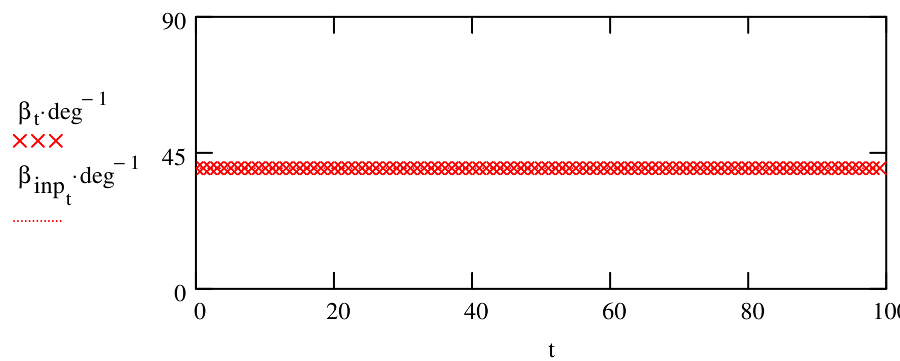
κ



χ2



β



t

maximum likelihood intensities

$$I'_{p1x_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 1, \kappa_{ans_t}\right)$$

$$I'_{s1x_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 2, \kappa_{ans_t}\right)$$

$$I'_{p1y_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 3, \kappa_{ans_t}\right)$$

$$I'_{s1y_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 4, \kappa_{ans_t}\right)$$

$$I'_{L1x_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 9, \kappa_{ans_t}\right)$$

$$I'_{R1x_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 10, \kappa_{ans_t}\right)$$

$$I'_{L1y_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 11, \kappa_{ans_t}\right)$$

$$I'_{R1y_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 12, \kappa_{ans_t}\right)$$

$$I'_{p2x_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 5, \kappa_{ans_t}\right)$$

$$I'_{s2x_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 6, \kappa_{ans_t}\right)$$

$$I'_{p2y_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 7, \kappa_{ans_t}\right)$$

$$I'_{s2y_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 8, \kappa_{ans_t}\right)$$

$$I'_{L2x_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 13, \kappa_{ans_t}\right)$$

$$I'_{R2x_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 14, \kappa_{ans_t}\right)$$

$$I'_{L2y_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 15, \kappa_{ans_t}\right)$$

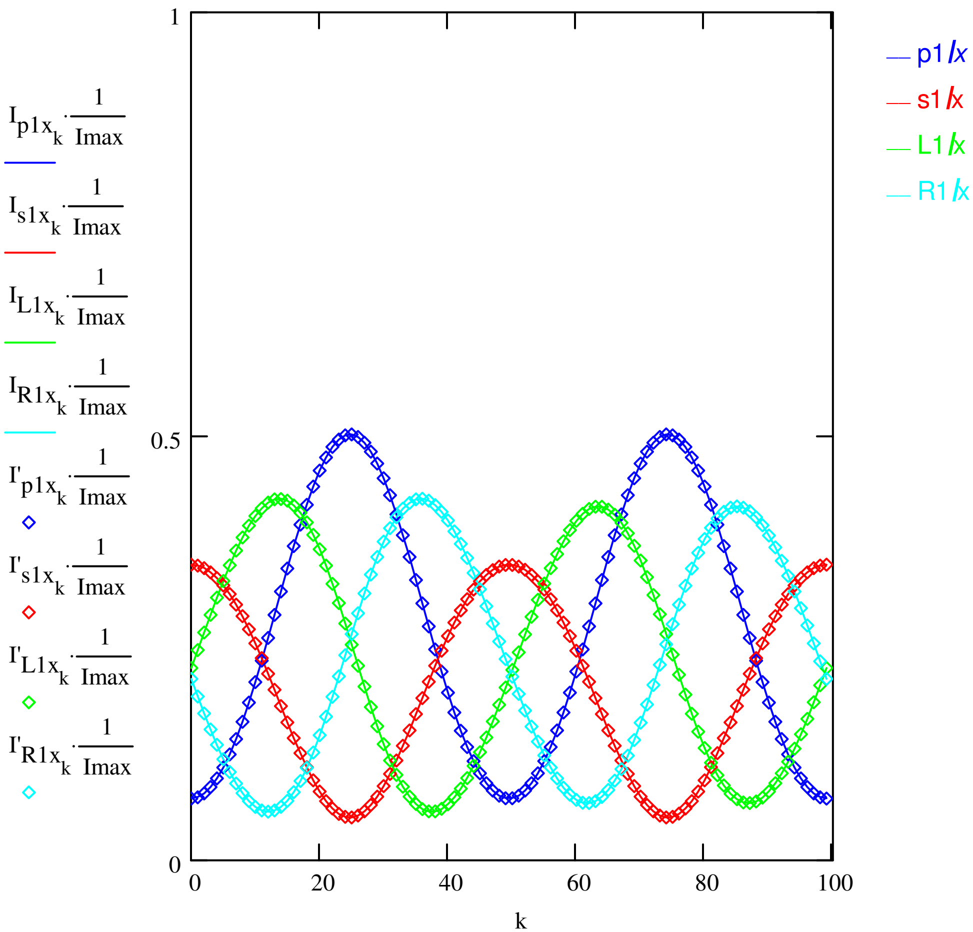
$$I'_{R2y_t} := II\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 16, \kappa_{ans_t}\right)$$

Maximum likelihood Intensities

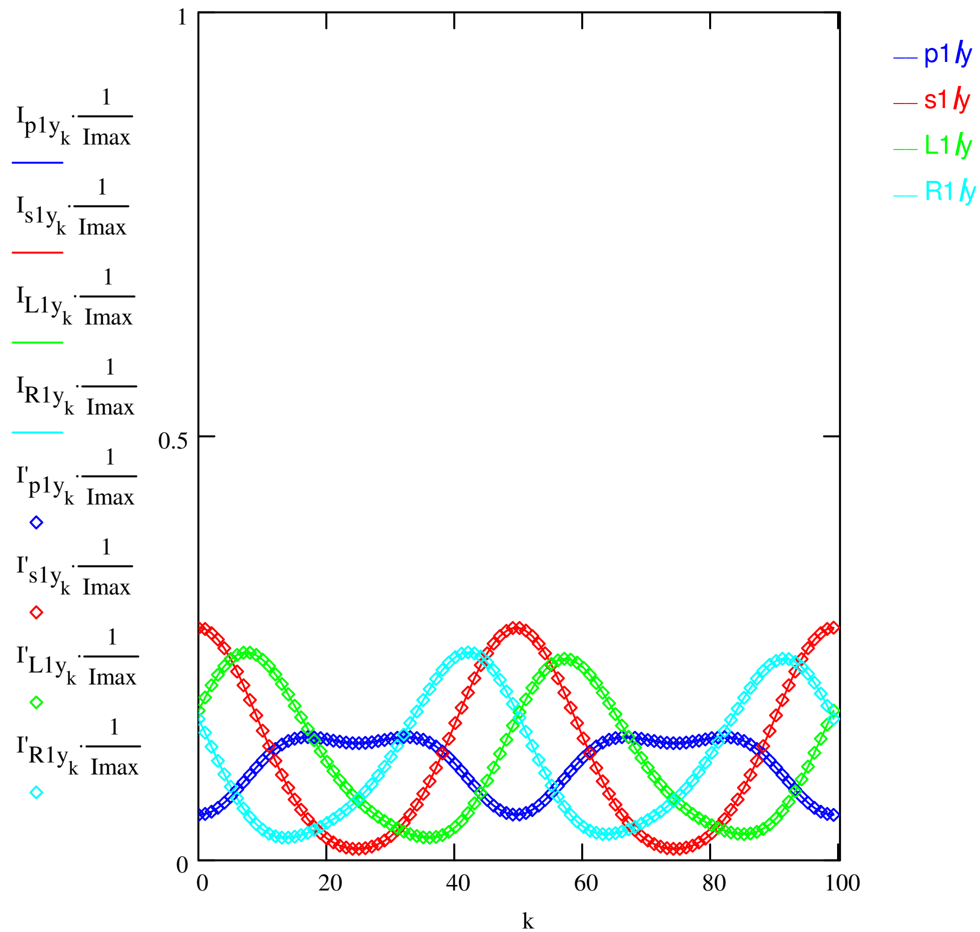
— simulated intensity
◇ maximum likelihood intensity

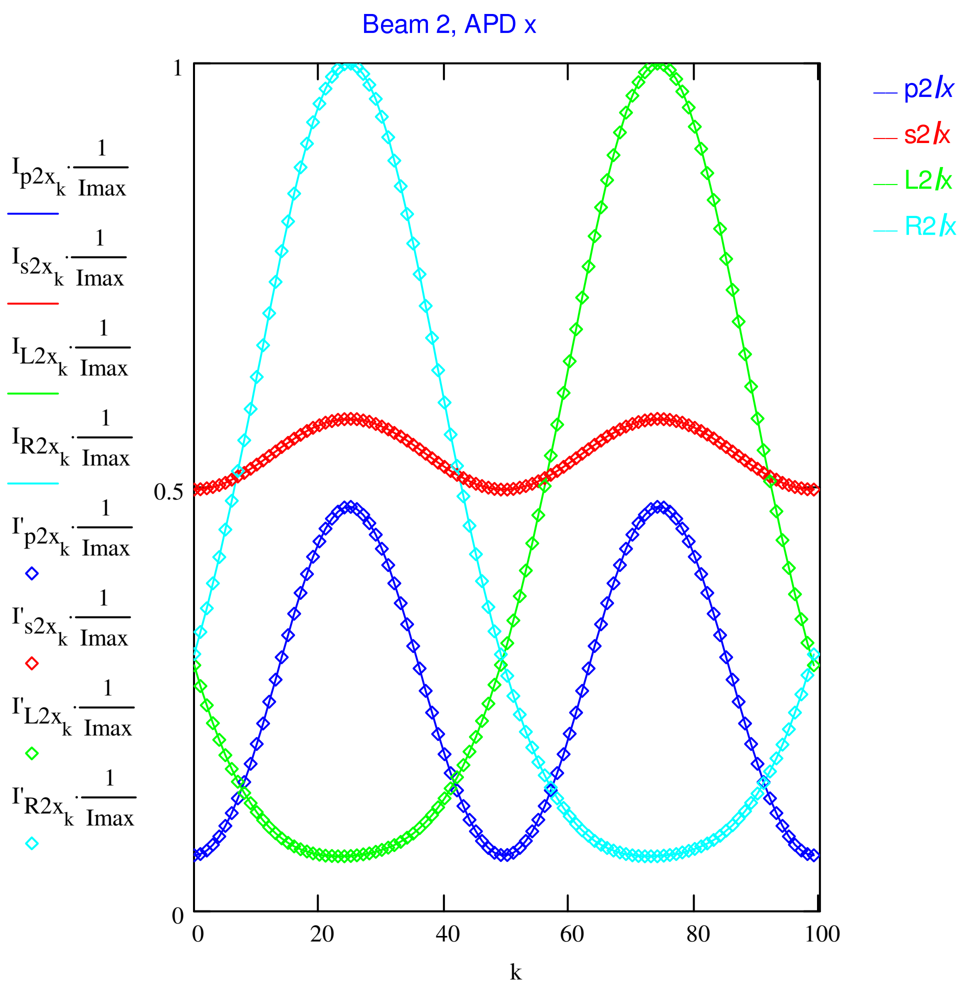
$\theta_0 = 64.000000\ \text{deg} \quad \delta_e = 24.000000\ \text{deg}$

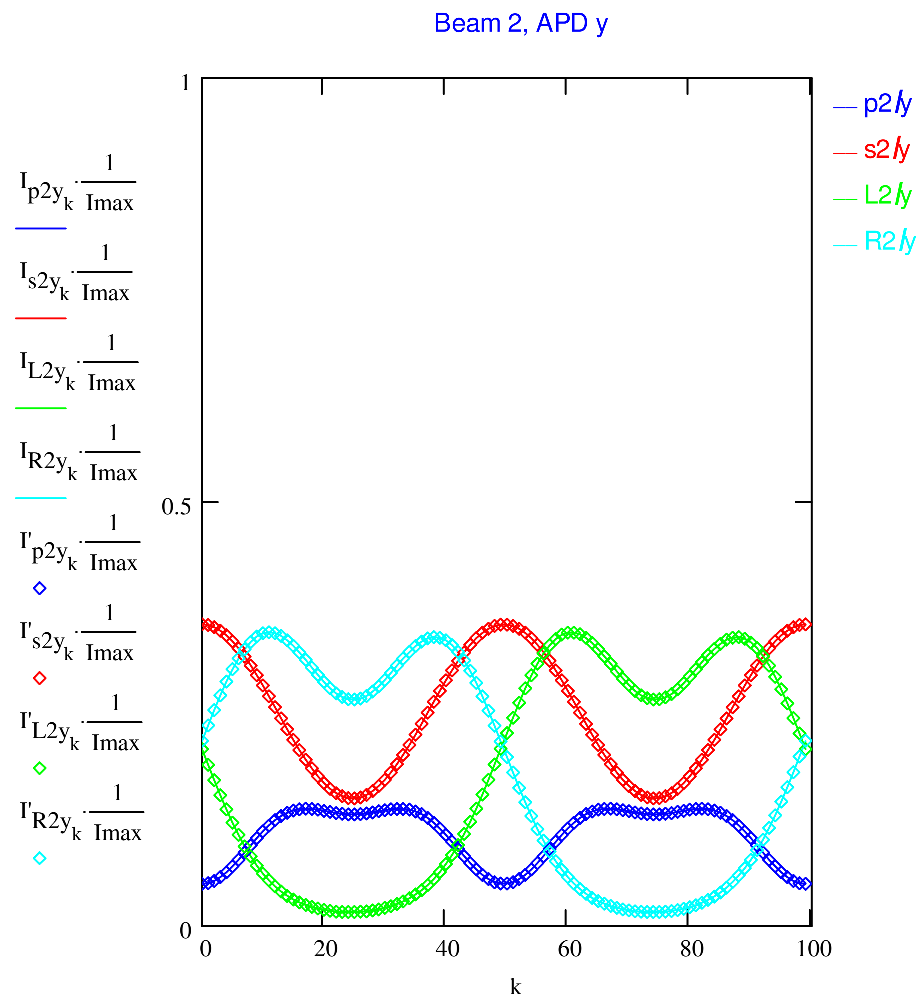
Beam 1, APD x



Beam 1, APD y




Beam 2, APD x
1
0.5
0
0
20
40
60
80
100
k
p2Ix
s2Ix
L2Ix
R2Ix


Beam 2, APD y
1
0.5
0
0
20
40
60
80
100
k
p2Iy
s2Iy
L2Iy
R2Iy

the following pages were cut off during conversion of the mathcad file to a pdf.

$$\text{Econst}_4 := \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ \text{Econst4}(\zeta_{L1}) \\ \text{Econst4}(\zeta_{R1}) \\ \text{Econst4}(\zeta_{L1}) \\ \text{Econst4}(\zeta_{R1}) \\ \text{Econst4}(\zeta_{L2}) \\ \text{Econst4}(\zeta_{R2}) \\ \text{Econst4}(\zeta_{L2}) \\ \text{Econst4}(\zeta_{R2}) \end{pmatrix}$$

$$\text{Econst}_5 := \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ \text{Econst5}(\zeta_{L1}) \\ \text{Econst5}(\zeta_{R1}) \\ \text{Econst5}(\zeta_{L1}) \\ \text{Econst5}(\zeta_{R1}) \\ \text{Econst5}(\zeta_{L2}) \\ \text{Econst5}(\zeta_{R2}) \\ \text{Econst5}(\zeta_{L2}) \\ \text{Econst5}(\zeta_{R2}) \end{pmatrix}$$

I_{R2x}	I_{L2y}	I_{R2y}	
14	15	16	
0.000000	0.000000	0.000000	
1.416023	1.416023	1.416023	Econst1
1.413242	1.413242	1.413242	Econst2
0.205552	0.205552	0.205552	Econst3
0.047860	-0.047860	0.047860	Econst4
2.941626	-2.941626	2.941626	Econst5

14	15	16
0.000000	0.000000	0.000000
4.094017	-4.094017	-4.094017
1.181300	-2.912717	-2.912717
0.335956	4.429973	4.429973

$$\mathrm{term1}\left(\theta_{test},\phi_{test},\delta_{test},\mathrm{trace}\right) = -1.476938$$

$$\mathrm{term2}\left(\theta_{test},\phi_{test},\delta_{test},\mathrm{trace}\right) = -0.273958$$

$$\mathrm{term3}\left(\theta_{test},\phi_{test},\delta_{test},\mathrm{trace}\right) = 2.443913$$

$$\mathrm{term4}\left(\theta_{test},\phi_{test},\delta_{test},\mathrm{trace}\right) = -0.384310$$

$$\mathrm{term5}\left(\theta_{test},\phi_{test},\delta_{test},\mathrm{trace}\right) = -0.773081$$

$$\mathrm{term6}\left(\theta_{test},\phi_{test},\delta_{test},\mathrm{trace}\right) = 1.952476$$

$$\mathrm{term7}\left(\theta_{test},\phi_{test},\delta_{test},\mathrm{trace}\right) = -0.327859$$

$$\mathrm{term8}\left(\theta_{test},\phi_{test},\delta_{test},\mathrm{trace}\right) = -0.186719$$

$$\mathrm{term9}\left(\theta_{test},\phi_{test},\delta_{test},\mathrm{trace}\right) = 0.910592$$

$$\text{term10}\left(\theta_{test}, \phi_{test}, \delta_{test}, \text{trace}\right) = -0.003522$$

$$\text{term11}\left(\theta_{test}, \phi_{test}, \delta_{test}, \text{trace}\right) = -7.848211 \times 10^{-4}$$

$$\text{term12}\left(\theta_{test}, \phi_{test}, \delta_{test}, \text{trace}\right) = 0.012372$$

$$\text{term13}\left(\theta_{test}, \phi_{test}, \delta_{test}, \text{trace}\right) = 0.061875$$

$$\text{term14}\left(\theta_{test}, \phi_{test}, \delta_{test}, \text{trace}\right) = -0.163635$$

$$\text{term15}\left(\theta_{test}, \phi_{test}, \delta_{test}, \text{trace}\right) = 0.449715$$

$$\text{term16}\left(\theta_{test}, \phi_{test}, \delta_{test}, \text{trace}\right) = -0.262278$$

$$\text{term17}\left(\theta_{test}, \phi_{test}, \delta_{test}, \text{trace}\right) = -0.335226$$

$$\text{term18}\left(\theta_{test}, \phi_{test}, \delta_{test}, \text{trace}\right) = 0.921294$$

$$,i) \quad term10(\theta,\phi,\delta,i) \quad term11(\theta,\phi,\delta,i) \quad term12(\theta,\phi,\delta,i) \quad bm1\cdot term13(\theta,\phi,\delta,i) \quad bm1\cdot term14(\theta,\phi,\delta,i) \quad bm1\cdot term15(\theta,\phi,\delta,i) \quad bm2\cdot term16(\theta,\phi,\delta,i) \quad bm2\cdot term17(\theta,\phi,\delta,i) \quad bm2\cdot ter$$

I_{R1x}	L1y	R1y	I_{L2x}	I_{R2x}	L2y	R2y		
10	11	12	13	14	15	16	17	
1.910187	2.785538	2.073498	2.619062	3.564824	1.552261	2.215971		sum
-1.476938	1.476938	1.476938	1.476938	1.476938	-1.476938	-1.476938		term1
-0.111108	0.273958	0.273958	0.111108	0.111108	-0.273958	-0.273958		term 2
-0.185339	-2.443913	-2.443913	0.185339	0.185339	2.443913	2.443913		
-0.000756	0.000756	0.000756	0.384310	0.384310	-0.384310	-0.384310		
-0.000617	0.001521	0.001521	0.313536	0.313536	-0.773081	-0.773081		
-0.000291	-0.003842	-0.003842	0.148070	0.148070	1.952476	1.952476		
2.586432	-2.586432	-2.586432	0.327859	0.327859	-0.327859	-0.327859		
0.597400	-1.473003	-1.473003	0.075727	0.075727	-0.186719	-0.186719		
0.544778	7.183534	7.183534	0.069057	0.069057	0.910592	0.910592		term 9
-0.003522	-0.003522	0.003522	-0.003522	0.003522	0.003522	-0.003522		term 10
-0.000318	-0.000785	0.000785	-0.000318	0.000318	0.000785	-0.000785		
-0.000938	0.012372	-0.012372	-0.000938	0.000938	-0.012372	0.012372		term 15

$$
\begin{aligned}
\mathrm{II}(\theta,\phi,\delta,i,\kappa) := \kappa\cdot\Big[&\mathrm{term1}(\theta,\phi,\delta,i) + \mathrm{term2}(\theta,\phi,\delta,i) + \mathrm{term3}(\theta,\phi,\delta,i) + \mathrm{term4}(\theta,\phi,\delta,i) + \mathrm{term5}(\theta,\phi,\delta,i) + \mathrm{term6}(\theta,\phi,\delta,i) + \mathrm{term7}(\theta,\phi,\delta,i) + \mathrm{term8}(\theta,\phi,\delta,i \\
&+ \mathrm{term9}(\theta,\phi,\delta,i) + \Big[\mathrm{term10}(\theta,\phi,\delta,i) + \mathrm{term11}(\theta,\phi,\delta,i) + \mathrm{term12}(\theta,\phi,\delta,i)\ \ldots \\
&\qquad + \mathrm{bm1}(i)\cdot\left(\mathrm{term13}(\theta,\phi,\delta,i) + \mathrm{term14}(\theta,\phi,\delta,i) + \mathrm{term15}(\theta,\phi,\delta,i)\right)\ \ldots \\
&\qquad + \mathrm{bm2}(i)\cdot\left(\mathrm{term16}(\theta,\phi,\delta,i) + \mathrm{term17}(\theta,\phi,\delta,i) + \mathrm{term18}(\theta,\phi,\delta,i)\right)\Big]
\end{aligned}
$$

$$ \cdots + \frac{\left(I_{p2y_t} - \Pi(\theta', \phi', \delta', 7, \kappa')\right)^2}{\Pi(\theta', \phi', \delta', 7, \kappa')} + \frac{\left(I_{s2y_t} - \Pi(\theta', \phi', \delta', 8, \kappa')\right)^2}{\Pi(\theta', \phi', \delta', 8, \kappa')} \ldots = 0 $$

$$ \cdots 14, \kappa')\Big)^2 \cdots + \frac{\left(I_{L2y_t} - \Pi(\theta', \phi', \delta', 15, \kappa')\right)^2}{\Pi(\theta', \phi', \delta', 15, \kappa')} + \frac{\left(I_{R2y_t} - \Pi(\theta', \phi', \delta', 16, \kappa')\right)^2}{\Pi(\theta', \phi', \delta', 16, \kappa')} $$

$$ \cdots)^2 + \left(I_{p2x_t} - \Pi\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 5, \kappa_{ans_t}\right)\right)^2 + \left(I_{s2x_t} - \Pi\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 6, \kappa_{ans_t}\right)\right)^2 + \left(I_{p2y_t} - \Pi\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 7, \kappa_{ans_t}\right)\right)^2 + \left(I_{s2y_t} - \Pi\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 8, \kappa_{ans_t}\right)\right)^2 \ldots $$

$$ \kappa_{ans_t}\Big)\Big)^2 + \left(I_{L2x_t} - \Pi\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 13, \kappa_{ans_t}\right)\right)^2 + \left(I_{R2x_t} - \Pi\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 14, \kappa_{ans_t}\right)\right)^2 + \left(I_{L2y_t} - \Pi\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 15, \kappa_{ans_t}\right)\right)^2 + \left(I_{R2y_t} - \Pi\left(\Theta_{ans_t}, \Phi_{ans_t}, \delta_{ans_t}, 1\cdots \right.\right. $$

.

$$\text{5}, \kappa_{ans_t}))^2$$