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Bayesian Estimation of Photobleaching Steps with Physical Priors

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Counting photobleach steps lets us infer the number of oligomeric subunits of fluorescently-labelled protein complexes. While ad hoc step-counting algorithms are adequate for low noise imaging with small numbers of steps, noise increases with the number of fluorophores and introduces bias when the intensity trace is filtered to reduce noise. We present a principled Bayesian approach with a prior distribution that incorporates the statistics of photobleaching and that does not require filtering. Our physics-based prior leads to a simple and efficient numerical scheme for maximum a posteriori probability (MAP) estimates of the initial fluorophore number n0. We illustrate how experimental data can be used to calibrate the photophysics. Using simulated data where n0 is known, we show that the bias of our MAP estimate remains minimal as the number of fluorophores increase. We investigate how our errors scale with n0, with the signal-to-noise ratio (SNR), and with the cam- era exposure time t or, equivalently, the illumination intensity. We find that the dimensionless ratio of camera exposure time to the average time to the first bleach step controls the imprecision of the MAP estimation. Many short exposures are recommended with our approach.

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