SPAD Theory

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Basic formalism

The fundamental object is the likelihood $\mathcal{L}(n|N,\zeta)$ of a number of counts in a single frame.

- ζ is the probability of a count from a single fluorophore
- N is a number of fluorophores in the ROI

From the likelihood, we measure a time series n_t of counts and an empirical histogram of counts $X(n)$. All analyses are then derived directly from n_t or $X(n)$

Second order coherence

In practice we calculate the second order coherence as

$$G^{(2)}(m) = \sum_t \mathbb{I}(n_t n_{t+m} \geq 1)$$

for $-m_{min} \leq m \leq +m_{max}$ using periodic boundary conditions (rolling the sequence). This function is generally normalized with respect to its average over m: $g^{(2)}(m) = G^{(2)}(m)/\langle G^{(2)}(m)\rangle_m$

Second order coherence

We can estimate $G^{(2)}(m)$ theoretically for different m

$$G^{(2)}(m=0) \sim \mathrm{Binomial}(N_{\mathrm{frames}}, \sum_{n\geq 2} \mathcal{L}(n|\theta))$$

Note that we have $G^{(2)}(0) = \sum_{n=2}^{\infty} X(n)$. For all other lags m

$$G^{(2)}(m\neq 0) \sim \mathrm{Binomial}(N_{\mathrm{frames}}, (\sum_{n\geq 1} \mathcal{L}(n|\theta))^2)$$

Uncertainty in the zero-lag second order coherence

$G^{(2)}(0)$ is binomially distributed, so estimates of $g^{(2)}(0)$ are stochastic.

$$g^{(2)}(0) = G^{(2)}(0)/\langle G^{(2)}(m)\rangle_m$$

Let $p = \sum_{n\geq 2}\mathcal{L}(n|\theta)$ and $q = (\sum_{n\geq 1}\mathcal{L}(n|\theta))^2$.

$$\langle G^{(2)}(m)\rangle_m = N_{\mathrm{frames}}q$$

The variance of $g^{(2)}(0)$ is then

$$\begin{aligned}\mathrm{Var}(g^{(2)}(0)) &= \frac{\mathrm{Var}(G^{(2)}(0))}{(\langle G^{(2)}(m)\rangle_m)^2} \\ &= \frac{1}{N_{\mathrm{frames}}}\frac{p(1-p)}{q^2}\end{aligned}$$

Uncertainty in the zero-lag second order coherence

I want to plot $\sqrt{\mathrm{Var}(g^{(2)}(0))}$ as a function of N_{frames} for different values of ζ.

Total Detected Counts

The total number of counts n detected in the ROI following a single pulse is the sum of signal and background counts:

$$n = n_{\mathrm{signal}} + n_{\mathrm{background}}$$

The likelihood for the total counts is given by a convolution of Poisson and Binomial distributions:

$$\mathcal{L}(n \mid N, \zeta) = \sum_{i=0}^{\infty} \binom{N}{i} \zeta^i (1-\zeta)^{N-i} \frac{\lambda^{n-i}}{(n-i)!} e^{-\lambda}$$

λ is treated as a hyperparameter

Posterior Estimation via Monte Carlo Integration

We aim to estimate the posterior $p(N = N' \mid n)$ by marginalizing over ζ:

$$p(N = N' \mid n) \propto \int_0^1 \mathcal{L}(n \mid N', \zeta) p(\zeta) \, d\zeta$$

where the likelihood is given by:

$$\mathcal{L}(n \mid N', \zeta) = \prod_{j=1}^{N_{\mathrm{frames}}} p(n_j \mid N', \zeta)$$

To make the likelihood tractable, we employ a log-sum-exponential trick:

$$\mathcal{L}(n \mid N', \zeta) = e^{\left(\sum_j \ell(n_j \mid N', \zeta) + C\right)}$$

where C is an empirically determined constant used for all N'.

Posterior Estimation via Monte Carlo Integration

Monte Carlo integration is then applied to integrate out ζ. We sample ζ values from the Gaussian prior:

$$p(\zeta) = \mathcal{N}(\mu_\zeta, \sigma_\zeta^2)$$

For each sampled ζ_i, we compute the Poisson-Binomial likelihood $\mathcal{L}(n \mid N', \zeta_i)$. These likelihoods are weighted by their prior probabilities $p(\zeta_i)$. Finally, we approximate the integral:

$$p(N = N' \mid n) \approx \frac{1}{M} \sum_{i=1}^{M} \mathcal{L}(n \mid N', \zeta_i) p(\zeta_i)$$

where M is the number of samples drawn from the prior.

Table 1 - Posterior Parameters

Sample	μ_ζ	σ_ζ	λ	Samples	Batches
Qdot655	0.01	0.005	0.008	100	50
ATTO532	0.002	0.001	0.006	100	50

To determine λ, we estimate the total background count per pixel and multiply by d^2/N_{frames}

To determine μ_ζ, we estimate the total counts in the ROI, subtract $\lambda N_{\mathrm{frames}}$ and multiply by $1/N_{\mathrm{frames}}$