Quantum enhanced fluorescence microscopy with a single photon avalanche diode array

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Localization microscopy uses precise localization of isolated fluorescent emitters to produce super-resolved images. The number of fluorescent emitters is a critical piece of information during localization, which cannot be reliably estimated with conventional microscopies. Photon statistics can enable localization in non-sparse scenes by providing information on the number of active fluorescent emitters. This work introduces a model for accurately counting active fluorescent emitters, demonstrated using a single photon avalanche diode (SPAD) array. SPAD cameras, with their high temporal resolution and single photon sensitivity, offer significant advantages for widefield imaging. Integrating photon statistics with conventional super-resolution techniques may enhance bioimaging capabilities, building on previous methods utilizing small detector bundles and laser scanning.

Introduction

Far-field optical microscopy is fundamentally limited by diffraction, with the maximum attainable resolution being limited to approximately half the wavelength of light. Several schemes to beat the diffraction limit have been developed in recent years. Many of these schemes utilize the concept of precise localization of isolated fluorescent emitters which blink over a time series of frames. One inherent problem of these methods is the requirement that fluorescent emitters be isolated, slowing down the acquisition of super-resolved images. To address this, gathering additional information on the number of active emitters by computing photon correlation statistics, enables localization in non-sparse scenes. However, photon correlation statistics indirectly provide information on the number of active fluorescent emitters in the sample. In this work, we introduce a model for precise counting of the number of active fluorescent emitters and demonstrate our method using a single photon avalanche diode (SPAD) array.

Molecular counting with photon statistics has a fairly simple motivation: coincidence of photons at multiple detector elements during high speed imaging provides evidence for the number of emitters present in the imaged region. Combining the ideas of conventional super-resolution approaches, with photon statistics may prove to be a powerful set of methods for bioimaging. Innovations in single photon detection technologies have begun to be integrated into fluorescence microscopes (Forbes 2019). Importantly, single photon detectors such as SPAD cameras have orders of magnitude higher temporal resolutions than standard CMOS cameras, single photon sensitivity, and theoretically zero readout noise. Such properties make these devices highly desirable for widefield imaging applications; however, application of SPAD arrays in imaging have been limited to small bundles of detector elements combined with laser scanning (Israel 2017; Forbes 2019; Tenne 2019).

Results

We consider the case of pulsed excitation where the interval between pulses much longer than the fluorescence lifetime. Upon excitation, a single fluorophore emits at most one photon with a probability ζ.

$$\zeta = \epsilon\eta\sigma_{\text{abs}} j_{\text{exc}} \tag{1}$$

where ϵ is a detection efficiency, η the quantum efficiency of the fluorophore, σ_{abs} the absorption cross-section and the excitation photon flux j_{exc}. Fluorophores were excited using a picosecond 532nm pulsed laser triggered at 500kHz. Emission light was collected using an oil-immersion 100× objective with numerical aperture (NA) 1.4 (Nikon). The emission signal was then filtered to exclude the laser line (Semrock) and projected onto the SPAD512 sensor (Pi Imaging Technologies) using a tube lens. A simplified diagram of the complete system is depicted in (Figure 1a). Each acquisition consists of $N = 5 \times 10^5$ frames, synchronized with each laser pulse, using a 1us exposure per frame (Figure 1b).

For N fluorophores within the region of interest, the number of photons emitted following a single excitation pulse follows a Binomial distribution: $x_{\text{signal}} \sim \text{Binom}(N, \zeta)$. Such behavior is profoundly different from the Poissonian statistics of classical light (Schwartz 2012). Background signal within the region of interest is modeled as shot noise: $x_{\text{background}} \sim \text{Poisson}(\lambda)$, where background photons arrive at single pixel with a rate λ. The total signal is then distributed by the likelihood

$$p(x_j = k \mid N, \zeta) = \sum_{i=0}^{\infty} \binom{N}{i} \zeta^i (1-\zeta)^{N-i} \frac{\lambda^{k-i}}{(k-i)!} e^{-\lambda} \tag{2}$$

The likelihood in (2) can be used to model photon arrivals measured by a SPAD array, as well as investigate properties of the second order coherence function $g^{(2)}(\tau)$

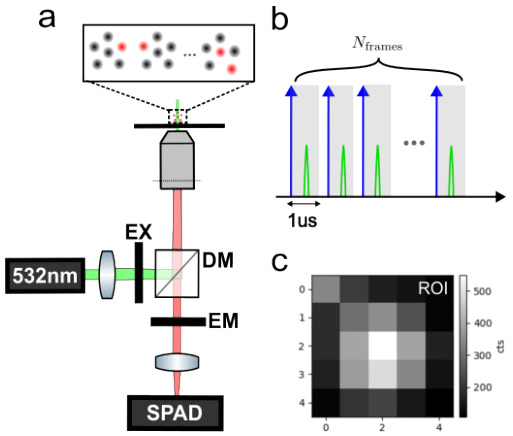

FIG. 1: (a) Conventional widefield microscopy with integrated SPAD array (b) Single photon imaging scheme using 1us exposures containing a picosecond laser pulse (c) Sum of photon counts over a 5x5 region of interest (ROI), taken with $N_{\text{frames}} = 5 \times 10^5$

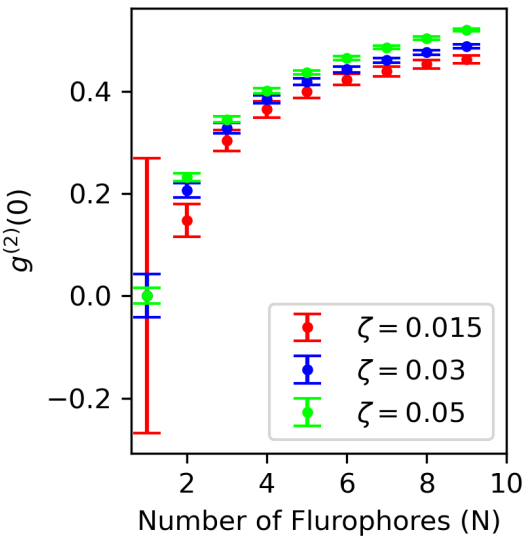

FIG. 2: Zero-lag second order coherence

over a region of interest. For simplicity, we use the zero-lag second order coherence $g^{(2)}(0)$, which provides information on the number of active fluorescent emitters (Israel 2017).

$$g^{(2)}(0) = \frac{G^{(2)}(0) - B}{\langle G^{(2)}(m) \rangle - B} \tag{3}$$

where $B = N_{\text{frames}} \lambda \zeta$ is the expected number of background-signal coincidences in the region of interest. The quantities $G^{(2)}(0)$ represents the number of zero-lag signal-signal coincidences in the region of interest, over N_{frames}

$$G^{(2)}(0) \sim \text{Binomial}(N_{\text{frames}}, \mathbb{P}(\mathbf{x}_t \geq 2))$$
$$\mathbb{P}(\mathbf{x}_t \geq 2) = 1 - (1-\zeta)^n - n\zeta(1-\zeta)^{n-1}$$

The quantity $G^{(2)}(m)$ represents the number of signal-signal coincidences at lag m in the region of interest

$$G^{(2)}(m) \sim \text{Binomial}(N_{\text{frames}}, \mathbb{P}(\mathbf{x}_t \geq 1 \text{ and } \mathbf{x}_{t+m} \geq 1))$$
$$\mathbb{P}(\mathbf{x}_t \geq 1 \text{ and } \mathbf{x}_{t+m} \geq 1)) = (1 - (1-\zeta)^n)^2$$

Sampling from (2), we find a lack of sensitivity in $g^{(2)}(0)$, making inference of the fluorophore number N intractable. We therefore choose to write a posterior distribution on the Binomial parameters used in the likelihood (2) using Bayes rule

$$p(N, \zeta | x) \propto p(x | N, \zeta) p(N) p(\zeta)$$

$p(N)$ is taken to be uniform and $p(\zeta) = \mathcal{N}(\mu_\zeta, \sigma_\zeta)$

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APPENDIX

$$p(N=n|x) \propto \int_0^1 \prod_j p(x_j|n,\zeta)p(\zeta)d\zeta$$

which is estimated via MC integration. Minibatch the data and average the posterior $p(N|x)$ over minibatches of size M

When $N = 1$, we see some unique behavior of this function

$$g^{(2)}(0) = \frac{G^{(2)}(0) - B}{\langle G^{(2)}(m)\rangle - B} = -\frac{B}{N_{\text{frames}}\zeta(\zeta-\lambda)}$$