Statistical Connection of Photon Counting and Photon Averaging

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The spatial distribution and crystalline properties of dosed active pharmaceutical ingredients (APIs) can affect their bioavailability and efficacy. Second harmonic generation (SHG) microscopy as measured by a photomultiplier tube (PMT) has been shown to be effective for imaging of APIs. Data from PMTs are typically analyzed by either simple averaging to acquire a value presumably proportional to the Poisson mean of photons (λ) for bright signals, or by counting the number of signal events per number of trials which directly recovers λ for dim signals. In recent work, the range of photon counting was extended out of the dim signal limit by a statistically derived log transformation. Here, work has been done to explicitly derive λ from signal averaging data, and then to statistically bridge the two strategies into a single technique. Experimentally, the achieved signal/noise was 87% to approaching 100% of the theoretical maximum Poisson signal/noise across the entire dynamic range of the PMT used. Future work will allow multiple simultaneous optical channels of detection to enable quantitative API analyses.

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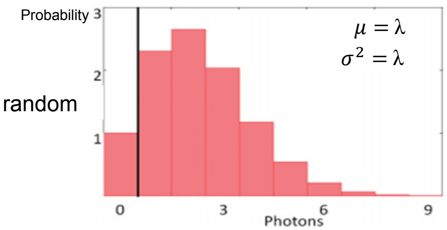
Random Number of Random Numbers

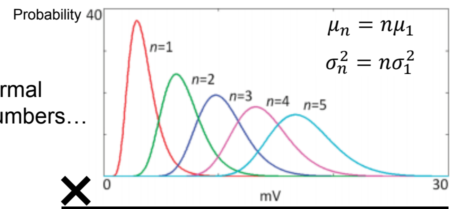
Above: SHG imaging of crystallization of the antibiotic griseofulvin using counting alone. While counting works well for imaging small nucleation sites, the large crystals have saturated the techique. Signal averaging is necessary.

Right: A Poisson random number of second harmonic photons is generated at the sample. When these photons reach the detector, a coherently summed lognormal random amount of voltage is generated per photon. The observed voltage distribution from the detector conforms to a Poisson weighted linear combination of lognormal distributions.

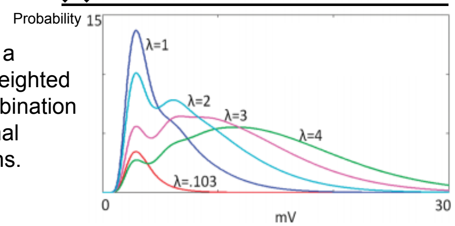
A Poisson random number…



…of lognormal random numbers…



…leads to a Poisson weighted linear combination of lognormal distributions.



Photon Counting

$$p = \sum_{n=1}^{\infty} \frac{\lambda^n e^{-\lambda}}{n!} = 1 - e^{-\lambda}$$

$$\lambda = -\ln(1-p)$$

$$SNR_{count} = \lambda \sqrt{\frac{N}{e^{\lambda} - 1}}$$

Photon Averaging

$$E[f_{PL}] = E[f_P]E[f_L]$$

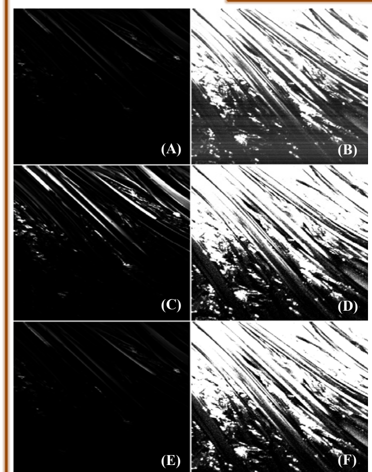
$$\mu_{sample} = \lambda\mu_1 \rightarrow \lambda = \frac{\mu_{sample}}{\mu_1}$$

$$SNR_{ave} = \mu_1 \lambda \sqrt{\frac{N}{\lambda\mu_1^2 + \lambda\sigma_1^2 + \sigma_J^2}}$$

Kissick, D. J.; Muir, R. D.; Simpson, G. J. "Statistical Treatment of Photon/Electron Counting: Extending the Linear Dynamic Range from the Dark Count Rate to Saturation" *Analytical Chemistry* **2010**, *82*, 10129

Muir, R. D.; Kissick, D. J.; Simpson, G. J. "Statistical connection of binomial photon counting and photon averaging in high dynamic range beam-scanning microscopy" *Opt. Express* **2012**, *20*, 10406.

Experimental Results

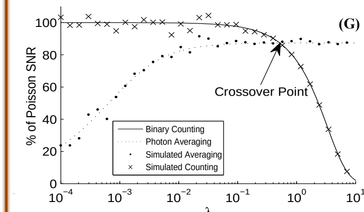


SHG images of crystalline urea. (Left Column) Full contrast image, (Right Column) Contrast adjusted to λ_{max}=0.02.

(A,B) Analysis with photon averaging only. The majority of the image is silhouetted in comparison to the brightest pixels in (A), up to λ=74. The dimmest pixels are evident in (B), but are as prominent as the horizontal streaks and noise in the image.

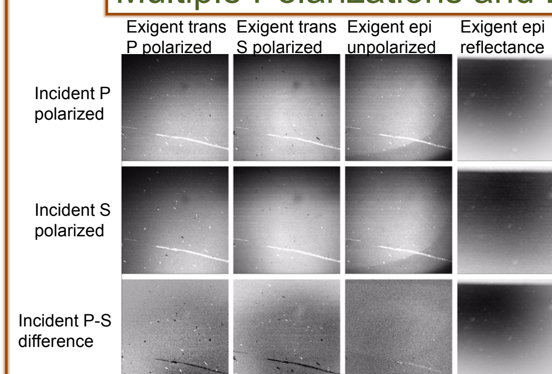
(C,D) Analysis with binomial counting only. The largest recoverable value was λ = 6.23; pixels brighter than this were clipped to this value in (C). The dimmest pixels are easily identifiable in (D), and the instrument noise is not evident.

(E,F) Preferential crossover analysis incorporating photon averaging and binomial counting. Pixels brighter than λ=0.48 were analyzed by photon averaging, so the full upper range of detection is preserved in (E). Pixels dimmer than λ=0.48 were analyzed by binomial counting, so the lower range of detection is preserved in (F).



(G) Signal/noise of photon averaging and binary counting as a ratio of the theoretical maximum SNR (the SNR of the underlying Poisson distribution). SNR at the crossover point is at ~87% of the theoretical limit. μ_1 = 7.2 mV, σ_1 = 4 mV, σ_J = 0.3 mV. Analytical equations are plotted against simulation, where data were simulated per sample by summing a Poisson random amount of lognormal random numbers, with an additional normal random number to represent Johnson noise (between 2 $\times 10^6$ values at low λ to 5000 at high λ).

Multiple Polarizations and Detectors



SHG imaging of cellobiose deposited on z-cut quartz immersed in oil. Four detectors were simultaneously used to capture the data with an Alazar ADC capture card. The 80MHz imaging laser was firing rate doubled to 160MHz, which generates orthogonally interleaved laser shots. Lock-in amplification is replicated in software by simple subtraction of the images generated by incident P and S polarizations.