Photon Counting Histograms and the Point Spread Function

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Outline

- PCH theory
- Measuring the point spread function (PSF)
- Effect of the PSF on the photon counting histogram
- Effect of the PSF on fluorescence correlation spectroscopy
- 2D PCH
How do we get from $I(t)$ to $N$?
Intensity Fluctuations
Pick the Dimers
Aggregates of 10
Now add 2 more degrees of freedom
The Point Spread Function (PSF)

Variation in excitation intensity maps to the width of the photon counting histogram

Note: $\omega_0 = 2\sigma$

$$I_{3DG}(r, z) = \exp\left(-\frac{2r^2}{\omega_0^2} - \frac{2z^2}{z_0^2}\right)$$

$$I_{GL^2}(r, z) = \frac{4\omega_0^4}{\pi^2 \omega^4(z)} \exp\left(-\frac{4r^2}{\omega^2(z)}\right)$$

$$\omega^2(z) = \omega_0^2 \left(1 + \left(\frac{z}{z_R}\right)^2\right)$$

$$z_R = \frac{\pi \omega_0^2}{\lambda}$$
Fixed Particle

Noise is Poisson

\[ Poi(k, \langle k \rangle) = \frac{k^k}{k!} \exp(-\langle k \rangle) \]
Have to sum up the poissonian distributions for all possible positions of the particle within the PSF

\[
p^{(1)}(k) = \frac{1}{V_0} \int_{V_0} Poi\left(k, \varepsilon_{PSF}(\vec{r})\right) d\vec{r}
\]
More Particles

\[ p^{(n)}(k) = p^{(1)}(k) \otimes p^{(n-1)}(k) = \sum_{r=0}^{r=k} p^{(1)}(k-r) \cdot p^{(n-1)}(r) \]

\[ \Pi(k; \varepsilon, N) = \sum_{n} p^{(n)}(k; \varepsilon) \cdot P(n, N) \]
PSF (Arbitrary)

Detector Noise
Digital: Poisson
Analog: ?

Multiple Species

Number Fluctuations: Poisson

PCH
Or
FIDA
Point Spread Function Effects

\[ p^{(1)}(k) = \frac{1}{V_0} \int_{V_0} Poi(k, \varepsilon \text{PSF}(\vec{r})) d\vec{r} \]

This is an equation that will work for ANY PSF shape.
Effect of Concentration

N=1

N=10
Effect of Brightness

\[ \varepsilon = 0.1 \]  

\[ \varepsilon = 1 \]
The Point Spread Function
Raster Scan Measurement
PCH Measurements

Problem: When a histogram is made of the counts in all time bins, particles are randomly distributed throughout space:

Doesn’t give the Point Spread Function itself, but

Gives the Probability Distribution of the PSF
PSF Size Dependence

Number = 1
Brightness = 1

V = 0.25 \text{ um}^3
C = 6.6 \text{ nM}

V = 1.23 \text{ um}^3
C = 1.4 \text{ nM}

V = 0.89 \text{ um}^3
C = 1.9 \text{ nM}
Functional Form Matters for PCH

PSF z-Profile

Intensity vs. z (um)

- 3D Gaussian
- GL Squared

Intensity (Log) vs. z (um)

- 3D Gaussian
- GL Squared

PCH

Probability vs. k (counts per time bin)

- 3D Gaussian
- Gauss-Lorentz Squared
FCS: Diffusion Coefficient

- Highly dependent on $\omega_0$ (radial waist) but much less dependent on $z_0$ (axial waist).
- Why?
FCS: G(0)

• G(0) = \( \gamma/N \):
  – Gamma is the numerical factor which represents the functional form of the point spread function
  – Volume dependence is same as for PCH—just changes N for a constant concentration

• Does not depend on \( z_0/\omega_0 \) ratio

  – \( \gamma = \frac{\int [PSF^2(r)dr]^3}{\int [PSF(r)dr]^3} \)

  – For 3DG: \( \gamma = 2^{3/2}/8 = 0.3536 \)

  – For GL Squared: \( \gamma = 3/4\pi^2 = 0.0760 \)
Shot Noise in FCS

- Shot noise is uncorrelated: only present in first point of autocorrelation
  - Use extrapolated $G(0)$
2 color experiments
Much easier to see the dimers than before!
2D PCH

\[ PCH(\varepsilon_A, \varepsilon_B, N; k_A, k_B) = P_B(\varepsilon_A / \varepsilon, k_A, k) \cdot PCH(\varepsilon, N; k) \]

where,

\[ P_B(x, k, N) = \binom{N}{k} x^k (1 - x)^{N-k} \]

(the binomial distribution)

We can calculate the PCH from the single channel PCH!
(Still have to convolute for multiple species)

Dependent vs. Independent Species

2 independent species:

1 species, fluorescent in both channels:

Mixture of dual color and single color species: