Correction for out-of-focus emission in Fluorescence Fluctuation Spectroscopy; generalization of the algorithms

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Photon counting distribution analysis with correction for out-of-focus emission

PCH theory

In the theory of PCH the photon counting distribution (PCD) from a number of molecules M is a weighted average of single molecule PCD convolved M times

$$p^{(1)}(n,Q,q)=\frac{1}{QV_{ref}}\int_{-\infty}^{\infty} Poi(n,qT\,B(\mathbf{r}))d\mathbf{r} \quad (1) \qquad P(n,N,q)=\sum_{M=0}^{\infty} p^{(M)}(n,Q,q)\,Poi(M,QN) \quad (2)$$

$B(r)$ is approximated by Gaussian or Gauss-Lorenzian approximations.

PCH with out-of-focus correction theory

Perroud and Huang (Perroud et al. 2003, Huang et al. 2004) introduced additional fitting parameters defined as a relative difference between integrals of the actual brightness profile and its Gaussian approximation $F_k=(\chi_k-\chi_{Gk})/\chi_{Gk}$. $p^{(1)}(n)$ takes the form

$$p^{(1)}(n,Q,q)=\frac{1+F_2}{(1+F_1)^2}\left[p_G^{(1)}(n,Q,q)+\frac{1}{n!QV_G}\sum_{k=n}^{\infty}\frac{(-1)^{k-n}(qT)^k F_k\chi_{Gk}}{(k-n)!}\right] \quad (3)$$

Photon counting generation function

Generation function (GF) (Kask et al. 1999) $G(\xi)=\sum_{n=0}^{\infty}\xi^n P(n)$ of photon counts can be written in the form

$$G(\xi)=\exp\left(\lambda T(\xi-1)+\sum_i N_i\int_V\left(e^{(\xi-1)q_iTB(\mathbf{r})}-1\right)d\mathbf{r}\right) \quad (4)$$

Photon counting GF with correction for out-of focus emission

Expanding the exponent under integral into a Taylor series and taking into account $F_k=(\chi_k-\chi_{Gk})/\chi_{Gk}$ that can be defined for arbitrary $B(r)$, one obtains

$$G(\xi)=\exp\left\{\lambda T(\xi-1)+\sum_i N_i\sum_{k=1}^{\infty}\frac{(\xi-1)^k q_i^k T^k(1+F_k)\chi_{Gk}}{k!}\right\} \quad (5) \qquad G(\xi)=G_{B(r)}(\xi)G_{Corr}(\xi)G_{BG}(\xi)$$

$$G_{B(r)}(\xi)=\exp\left\{\sum_i N_i\int_V\left(e^{(\xi-1)q_iTB(\mathbf{r})}-1\right)d\mathbf{r}\right\} \quad G_{Corr}(\xi)=\exp\left\{\sum_i N_i\sum_{k=1}^{\infty}\frac{(\xi-1)^k q_i^k T^k F_k\chi_{Gk}}{k!}\right\} \quad G_{BG}(\xi)=\exp\{\lambda T(\xi-1)\}$$

Generalization of algorithms

One molecule GF can be written in the form

$$G^{(1)}(\xi)=\frac{1}{QV_{ref}}\int_V e^{(\xi-1)qTB(\mathbf{r})}d\mathbf{r}$$

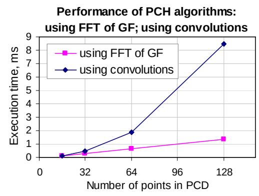
Eq. 1 can be derived by differentiating of the GF

$$p^{(1)}(n)=\left.\frac{d^nG^{(1)}(\xi)}{n!\,d\xi^n}\right|_{\xi=0}\Rightarrow\frac{1}{QV_{ref}}\int_V Poi(n,qTB(\mathbf{r}))d\mathbf{r}.$$

Eq. 3 can be derived from the GF after Taylor expansion of the exponent under integral

$$p^{(1)}(n)=\left.\frac{d^nG^{(1)}(\xi)}{n!\,d\xi^n}\right|_{\xi=0}\Rightarrow\frac{1+F_2}{(1+F_1)^2}\left[\frac{1}{n!QV_G}\sum_{k=n}^{\infty}\frac{(-1)^{k-n}(qT)^k(1+F_k)\chi_{Gk}}{(k-n)!}\right]$$

- Eq. 5 can be used in the PCH algorithm instead of Eq 1, 2. The advantage is that using GF the resulting PCD can be obtained through the FFT (in the same way as in FIDA), which is much faster than traditional convolutions.



- GF approach does not require introducing Q parameter, which must be arbitrary defined in Eq. 1, 3.

Application of corrections for out-of-focus emission in FCA

Substituting Eq 4 into $K_k=\left.\frac{d^k\ln G(\xi)}{d\xi^k}\right|_{\xi=1}$, $k=1,2,\ldots$ yields

$$K_1=\lambda T+(1+F_1)\chi_{G1}\sum_i N_iq_iT \qquad K_k=(1+F_k)\chi_{Gk}\sum_i N_iq_i^kT^k \quad (6)$$

After normalization to the effective volume we arrive at

$$\frac{\chi_{Gk}}{V_{eff}}=\frac{\chi_{Gk}(1+F_2)}{V_{G\,eff}(1+F_1)^2}=\frac{\gamma_2\gamma_k(1+F_2)}{(1+F_1)^2}$$

For the first order correction Eq. 5 becomes

$$K_1=\lambda T+\frac{1}{2\sqrt{2}(1+F_1)}\sum_i N_iq_iT$$

$$K_k=\frac{1}{(2k)^{3/2}(1+F_1)^2}\sum_i N_iq_i^kT^k,\quad k=2,3,\ldots$$

and for the second one

$$K_1=\lambda T+\frac{(1+F_2)}{2\sqrt{2}(1+F_1)}\sum_i N_iq_iT$$

$$K_2=\frac{(1+F_2)^2}{8(1+F_1)^2}\sum_i N_iq_i^2T^2$$

$$K_k=\frac{(1+F_2)}{(2k)^{3/2}(1+F_1)^2}\sum_i N_iq_i^kT^k,\quad k=3,4,\ldots$$

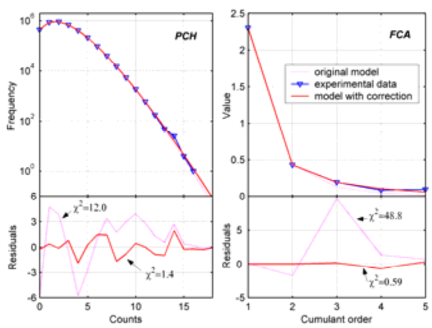
One component model

Sample: Alexa 488 dye

Measurements time: 30 sec

Number of collected photons: 8.6E+6

Both PCH and FCA analysis with out-of-focus correction were performed.



Parameter	PCH with corrections	FCA with corrections
q	103800 [95330; 112300]	102100 [87400; 116600]
N	12.29 [12.15; 12.44]	12.28 [12.05; 12.56]
F	0.57 [0.45; 0.70]	0.55 [0.32; 073]

- Estimations of parameters are almost the same for both methods that proofs applicability of corrections to FCA
- Application of corrections improves fit quality

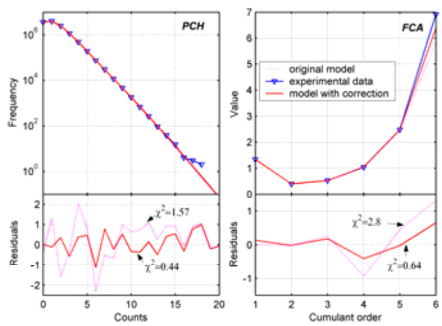
Two components model

Sample: mixture Alexa 488 dye with Floorescein

Measurements time: 120 sec

Number of collected photons: 16E+6

Both PCH and FCA analysis with out-of focus correction were performed.



Parameter	PCH with corrections	FCA with corrections
q_1	78740 [54030; 101800]	82040 [54040; 101900]
q_2	331500 [314500; 352200]	340600 [296900; 390800]
N_1	6.15 [5.42; 7.70]	6.11 [5.55; 7.59]
N_2	0.64 [0.57; 0.72]	0.58 [0.34; 0.98]
F	0.86 [0.59; 1.03]	0.86 (fixed)

- Both methods successfully resolve the mixture
- Applied corrections allow to get correct concentration ratio

Conclusions

1) We generalized the algorithms for out-of-focus correction in FFS. Our theory is based on the generation functions concept and show that FIDA, PCH and PCH with corrections are mathematically equivalent methods because they all can be directly derived from the photon counting generation function. The methods differ only in applied brightness profile approximations and details of numerical algorithms.
2) We developed FIDA-like algorithm for the PCH with out-of-focus correction and tested its performance.
3) We applied corrections for out-of-focus emission in FCA and demonstrated improved results of the analysis. We can conclude that FCA is an appropriate method for the analysis of FFS data alongside with PCH and FIDA.