Fluorescence Correlation Spectroscopy (FCS) Technical Manual

Appendix II

ISS®
1602 Newton Drive
Champaign, Illinois 61822

TEL: (217) 359-8681
FAX: (217) 359-7879

Fourth Edition;
Sep 2000; Printed in USA

Third Edition;
June 2000; Printed in USA

Second Edition;
Oct 1999; Printed in USA

First Edition;
December 1998; Printed in USA

ISS Inc.

MAILING ADDRESS: P.O. BOX 6930
CHAMPAIGN, ILLINOIS 61826-6930; U.S.A.

Shipping Address: 1602 Newton Drive
Champaign, Illinois 61822; U.S.A.

Telephone: (217) 359-8681
FAX: (217) 359-7879
E-mail address: Iss@iss.com (marketing)
Service@iss.com (technical support & service)

NOTICE

ISS Inc. reserves the right to make any changes or improvement to its products without prior notice. ISS assumes no responsibility for any errors that may appear in this document. All of the documentation has been carefully written to cover most of the questions that may arise during normal use. For additional assistance, a Product Specialist is available during normal business hours, or contact the Technical and Support Department at the following E-mail address: service@iss.com.

Table of Contents

Appendix II. Fitting Procedures

The Marquardt-Levenberg algorithm is utilized for fitting the following data:

In autocorrelation analysis-

a. Concentration
b. Diffusion constant
c. Flow rate
d. Excitation beam waist(2D and 3D)
e. Excitation beam length(3D)
f. Two component global fitting

In histogram analysis-

a. Counts per second per molecule(cpsm)
b. Number of molecules
c. Excitation beam waist(2D and 3D)
d. Excitation beam length(3D)
e. Two component global fitting

Additionally, it would be desirable to be able to fit data to built-in equations entered by the user.

Goal
The first part of this document contains a physical background of FCS (Fluorescent Correlation Spectroscopy) and the derivation of the Z_0, W_0, D, N parameters.

The second part contains the derivation of PCH(Photon Counting Histogram) N and E parameters.

The third part of this document contains the ISS analysis program fitting procedure.

1. Correlation Equation Models

Overview
FCS is a method for investigating molecular dynamics. The fluorescent beads or molecules are homogeneously distributed in the sample container in a rest condition. The excitation light source focus into the sample container to form a focus spot. Whenever the fluorescent beads or molecules move into the focus spot, they absorb the energy and emit fluorescent light. Then PMT (Photo Multiplier Tube) or APD (Avalanche Photo Detector) are used to detect the fluorescent light. The detected analog signal from PMT or APD is then converted to TTL digital signal (photon counts) by discriminator. The photon counts are stored as the raw data in a sampling time ΔT. Thus, the detected fluorescence fluctuation $F(t)$ as a function of time t ($t = i\Delta T$, $i = 0$ to $M-1$, M is the raw data size) is measured. The average of $F(t)$ is denoted by

$$< F >=< F(t) >= \sum_{i=0}^{M-1} \frac{F(i\Delta T)}{M}$$

(1)

Where ΔT is the sampling time
M is the raw data size.

The normalized autocorrelation function $G(\tau)$ of temporal fluctuations in the measured fluorescence $F(t)$ is given by

$$G(\tau) = \frac{< \delta F(t) \delta F(t-\tau) >}{< F >^2}$$

(2)

Where $\delta F(t) = F(t) - < F >$

(3)

Using Eq. (3) in Eq. (2), we find that

$$G(\tau) = \frac{< (F(t) - < F >)(F(t-\tau) - < F >) >}{< F >^2}$$
$$= \frac{< F(t)F(t-\tau) > - < F(t) < F >> - << F > F(t-\tau) > + < F >^2}{< F >^2}$$
$$= \frac{< F(t)F(t-\tau) > - < F >^2 - < F >^2 + < F >^2}{< F >^2} = \frac{< F(t)F(t-\tau) > - < F >^2}{< F >^2}$$
$$= \frac{< F(t)F(t-\tau) >}{< F >^2} - 1$$

(4)

For $\tau = i\Delta T$,

$$G(i\Delta T) = \frac{< F(t)F(t-i\Delta T) >}{< F >^2} - 1 = \frac{\left[\frac{\sum_{j=i+1}^{M} n_j n_{j-i}}{M-i} \right]}{\left(\frac{\sum_{j=1}^{M} n_j}{M} \right)^2} - 1$$

(5)

Where $< F(t)F(t-i\Delta T) >= \sum_{j=i+1}^{M} \frac{F(j\Delta T)F(j\Delta T - i\Delta T)}{(M-i)} = \frac{\sum_{j=i+1}^{M} n_j n_{j-i}}{M-i}$

(6)

$G(i\Delta T)$ is the autocorrelation function.
M is the total data point acquired.
n_j is the obtained values.
ΔT is the sampling time (typically ranges from 1μs to 1ms).

If only one fluorescent chemical species is present in the sample region,

$$F(t) = kQ\int E(r)C(r,t)dr \tag{7}$$

Where k is a constant
Q is the product of the absorptivity, fluorescence quantum efficiency, and experimental fluorescence collection efficiency of the fluorescent molecules
$E(r)$ is the spatial intensity profile of the excitation light
$C(r,t)$ is the number density at position r and time t

Then, one can write Eq. (3) as follows:

$$\delta F(t) = F(t) - \langle F \rangle = kQ\int E(r)\delta C(r,t)dr \tag{8}$$

Where $\delta C(r,t) = C(r,t) - \langle C \rangle$

By substituting Eq. (8) into Eq. (2), we can get:

$$G(\tau) = \frac{\iint E(r)E(r') \langle \delta C(r,t)\delta C(r',t+\tau) \rangle drdr'}{(\langle C \rangle \int E(r)dr)^2} \tag{9}$$

For the 3D Gaussian model, with one photon excitation,

$$E(r) = E(x,y,z) = E_0 \exp\left(-\frac{2(x^2+y^2)}{w_0{}^2} - \frac{2z^2}{z_0{}^2} \right) \tag{10}$$

Where w_0 is the beam waist
z_0 is the length of the beam in z axis

For the 3D Gaussian-Lorentzian model, with one photon excitation,

$$E(r) = E(x,y,z) = \frac{2E_0 w_0{}^2}{\pi w^2(z)} \exp\left(\frac{-2(x^2+y^2)}{w^2(z)} \right) \tag{11}$$

Where $w^2(z) = w_0{}^2 \left(1 + \left(\frac{z}{z_R} \right)^2 \right)$, and $z_R = \frac{\pi w_0{}^2}{\lambda}$

(12)

For Brownian (translational) diffusion only, the fluctuation $\delta C(r,t)$ will have characteristic behavior governed by the diffusion equation:

$$\frac{\partial \delta C(r,t)}{\partial t} = D\nabla^2 \delta C(r,t)$$

(13)

For simplicity, a solution for Eq (13) can be verified is:

$$\delta C(r,t) = \frac{<C>}{\sqrt{4\pi Dt}} \exp\left(-\frac{r^2}{4Dt}\right)$$

(14)

By assuming that the sample is stationary[1],

$$<\delta C(r,t)\delta C(r',t+\tau)> = \frac{<C>}{\left(\sqrt{4\pi D\tau}\right)^3} \exp\left(-\frac{(r-r')^2}{4D\tau}\right)$$

(15)

Equation (15) is the probability of finding a diffusive molecule at position *r* 'and time $t+\tau$, given that the molecule was at position *r* at time *t*.

a. 3D Gaussian Model with one photon excitation

Substituting Eq. (15) and Eq. (10) into Eq. (9), and with the fact that

$$\int_{-\infty}^{\infty} \exp(-ax^2)dx = \sqrt{\frac{\pi}{a}}$$

(16)

For one photon excitation, we get the denumerator part of Eq. (9) as follow:

$$(<C> \int E(r)dr)^2 = \left(<C> E_0 \iiint \exp\left(-\frac{2(x^2+y^2)}{w_0{}^2} - \frac{2z^2}{z_0{}^2}\right) dxdydz \right)^2$$

$$= \left(<C> E_0 \left(\sqrt{\frac{\pi w_0{}^2}{2}}\right)\left(\sqrt{\frac{\pi w_0{}^2}{2}}\right)\left(\sqrt{\frac{\pi z_0{}^2}{2}}\right) \right)^2$$

$$= \frac{<C>^2 \; E_0{}^2 \pi^3 w_0{}^4 z_0{}^2}{2^3}$$

(17)

and the numerator part of Eq. (9) as follow:

$$\iint E(r)E(r') <\delta C(r,t)\delta C(r',t+\tau)> drdr'$$

$$= \iiint\iiint E_0{}^2 \exp\left(-\frac{2(x^2+y^2)}{w_0{}^2} - \frac{2z^2}{z_0{}^2}\right) \exp\left(-\frac{2(x'^2+y'^2)}{w_0{}^2} - \frac{2z'^2}{z_0{}^2}\right)$$

$$\frac{<C>}{\left(\sqrt{4\pi D\tau}\right)^3} \exp\left(-\frac{(x-x')^2+(y-y')^2+(z-z')^2}{4D\tau}\right) dxdydzdx'dy'dz'$$

$$= E_0{}^2 \frac{<C>}{\left(\sqrt{4\pi Dt}\right)^3} \iint \exp\left(-\frac{2x^2}{w_0{}^2}\right)\exp\left(-\frac{2x'^2}{w_0{}^2}\right)\exp\left(-\frac{x^2-2xx'+x'^2}{4D\tau}\right)dxdx'$$

$$\iint \exp\left(-\frac{2y^2}{w_0{}^2}\right)\exp\left(-\frac{2y'^2}{w_0{}^2}\right)\exp\left(-\frac{y^2-2yy'+y'^2}{4D\tau}\right)dydy'$$

$$\iint \exp\left(-\frac{2z^2}{z_0{}^2}\right)\exp\left(-\frac{2z'^2}{z_0{}^2}\right)\exp\left(-\frac{z^2-2zz'+z'^2}{4D\tau}\right)dzdz' = E_0{}^2 \frac{<C>}{\left(\sqrt{4\pi D\tau}\right)^3} XYZ$$

(18)

Where,

$$X = \iint \exp\left(-\frac{2x^2}{w_0{}^2}\right)\exp\left(-\frac{2x'^2}{w_0{}^2}\right)\exp\left(-\frac{x^2-2xx'+x'^2}{4D\tau}\right)dxdx'$$

$$= \int \exp\left(-\frac{2x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int \exp\left(-\frac{2x^2}{w_0{}^2}\right)\exp\left(-\frac{x^2-2xx'}{4D\tau}\right)dx\right]dx'$$

$$= \int \exp\left(-\frac{2x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int \exp\left(-\frac{2x^2}{w_0{}^2}-\frac{x^2}{4D\tau}\right)\exp\left(\frac{2xx'}{4D\tau}\right)dx\right]dx'$$

$$= \int \exp\left(-\frac{2x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int \exp\left(-\frac{8D\tau+w_0{}^2}{4D\tau w_0{}^2}x^2+\frac{2x'}{4D\tau}x\right)dx\right]dx' \qquad set \quad A=\frac{8D\tau}{w_0{}^2}+1$$

$$= \int \exp\left(-\frac{2x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int \exp\left(-\frac{A}{4D\tau}\left(x^2-\frac{2x'}{A}x\right)\right)dx\right]dx'$$

$$= \int \exp\left(-\frac{2x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\exp\left(\frac{x'^2}{4D\tau A}\right)\int \exp\left(-\frac{A}{4D\tau}\left(x-\frac{x'}{A}\right)^2\right)d(x-\frac{x'}{A})\right]dx'$$

$$= \int \exp\left(-\frac{2x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\sqrt{\frac{4\pi D\tau}{A}}\exp\left(\frac{x'^2}{4D\tau A}\right)\right]dx'$$

$$= \sqrt{\frac{4\pi D\tau}{A}}\int \exp\left(-(\frac{2}{w_0{}^2}+\frac{1}{4D\tau}-\frac{1}{4D\tau A})x'^2\right)dx' = \sqrt{\frac{4\pi D\tau}{A}}\int \exp\left(-(\frac{A^2-1}{4D\tau A})x'^2\right)dx'$$

$$= \sqrt{\frac{4\pi D\tau}{A}}\sqrt{\frac{4\pi D\tau A}{A^2-1}} = \frac{4\pi D\tau}{\sqrt{\left(\frac{8D\tau}{w_0{}^2}\right)\left(\frac{8D\tau}{w_0{}^2}+2\right)}} = \frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{4D\tau}{w_0{}^2}}\right)}$$

(19)

Similarly,

$$Y = \iint \exp\left(-\frac{2y^2}{w_0^{\ 2}}\right)\exp\left(-\frac{2y'^2}{w_0^{\ 2}}\right)\exp\left(-\frac{y^2 - 2yy' + y'^2}{4D\tau}\right)dydy' = \frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{4D\tau}{w_0^{\ 2}}}\right)}$$

(20)

and

$$Z = \iint \exp\left(-\frac{2z^2}{z_0^{\ 2}}\right)\exp\left(-\frac{2z'^2}{z_0^{\ 2}}\right)\exp\left(-\frac{z^2 - 2zz' + z'^2}{4D\tau}\right)dzdz' = \frac{z_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{4D\tau}{z_0^{\ 2}}}\right)}$$

(21)

Substituting Eq. (19), Eq. (20) and Eq. (21) into Eq. (18). Then, Substituting Eq. (17) and Eq. (18) into Eq. (9), we get:

$$G(\tau) = \frac{E_0^{\ 2}\frac{<C>}{\left(\sqrt{4\pi D\tau}\right)^3}\frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{4D\tau}{w_0^{\ 2}}}\right)}\frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{4D\tau}{w_0^{\ 2}}}\right)}\frac{z_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{4D\tau}{z_0^{\ 2}}}\right)}}{\frac{<C>^2 E_0^{\ 2}\pi^3 w_0^{\ 4} z_0^{\ 2}}{2^3}}$$

That is,

$$G(\tau) = \frac{1}{\pi\sqrt{\pi}w_0^{\ 2}z_0 <C>}\left(1+\frac{4D\tau}{w_0^{\ 2}}\right)^{-1}\left(1+\frac{4D\tau}{z_0^{\ 2}}\right)^{-\frac{1}{2}}$$

(22)

Providing the fact that, the excitation volume is as follow:

$$V = \iiint \exp\left(-\frac{2(x^2+y^2)}{w_0^{\ 2}} - \frac{2z^2}{z_0^{\ 2}}\right)dxdydz = \left(\sqrt{\frac{\pi w_0^{\ 2}}{2}}\right)\left(\sqrt{\frac{\pi w_0^{\ 2}}{2}}\right)\left(\sqrt{\frac{\pi z_0^{\ 2}}{2}}\right) = \frac{\pi\sqrt{\pi}w_0^{\ 2}z_0}{2\sqrt{2}}$$

(23)

Also, $<N> = <C>V$, We get

$$G(\tau) = \frac{1}{2\sqrt{2}\frac{\pi\sqrt{\pi}w_0^{\ 2}z_0}{2\sqrt{2}}<C>}\left(1+\frac{4D\tau}{w_0^{\ 2}}\right)^{-1}\left(1+\frac{4D\tau}{z_0^{\ 2}}\right)^{-\frac{1}{2}} = \frac{1}{2\sqrt{2}<N>}\left(1+\frac{4D\tau}{w_0^{\ 2}}\right)^{-1}\left(1+\frac{4D\tau}{z_0^{\ 2}}\right)^{-\frac{1}{2}}$$

$$= G(0)\left(1+\frac{4D\tau}{w_0^{\ 2}}\right)^{-1}\left(1+\frac{4D\tau}{z_0^{\ 2}}\right)^{-\frac{1}{2}}$$

(24)

The number of molecules and the concentration can be calculated as follow:

$$< N > = \frac{1}{2\sqrt{2}G(0)} \quad , and \quad < C > = \frac{1}{\pi\sqrt{\pi}w_0^{\ 2}z_0 G(0)}$$

(25)

b. 3D Gaussian Model with two photon excitation

For two photon excitation, we get the denumerator part of Eq. (9) as follow:

$$(< C > \int E(r)dr)^2 = \left(< C > E_0 \iiint \exp\left(-\frac{4(x^2+y^2)}{w_0^{\ 2}} - \frac{4z^2}{z_0^{\ 2}} \right) dxdydz \right)^2$$

$$= \left(< C > E_0 \left(\sqrt{\frac{\pi w_0^{\ 2}}{4}} \right)\left(\sqrt{\frac{\pi w_0^{\ 2}}{4}} \right)\left(\sqrt{\frac{\pi z_0^{\ 2}}{4}} \right) \right)^2 = \frac{< C >^2 \ E_0^{\ 2}\pi^3 w_0^{\ 4} z_0^{\ 2}}{4^3}$$

(26)

and the numerator part of Eq. (9) as follow:

$$\iint E(r)E(r') < \delta C(r,t)\delta C(r',t+\tau) > drdr'$$

$$= \iiint\iiint E_0^{\ 2} \exp\left(-\frac{4(x^2+y^2)}{w_0^{\ 2}} - \frac{4z^2}{z_0^{\ 2}} \right) \exp\left(-\frac{4(x'^2+y'^2)}{w_0^{\ 2}} - \frac{4z'^2}{z_0^{\ 2}} \right)$$

$$\frac{< C >}{\left(\sqrt{4\pi D\tau}\right)^3} \exp\left(-\frac{(x-x')^2+(y-y')^2+(z-z')^2}{4D\tau} \right) dxdydzdx'dy'dz'$$

$$= E_0^{\ 2} \frac{< C >}{\left(\sqrt{4\pi Dt}\right)^3} \iint \exp\left(-\frac{4x^2}{w_0^{\ 2}} \right) \exp\left(-\frac{4x'^2}{w_0^{\ 2}} \right) \exp\left(-\frac{x^2-2xx'+x'^2}{4D\tau} \right) dxdx'$$

$$\iint \exp\left(-\frac{4y^2}{w_0^{\ 2}} \right) \exp\left(-\frac{4y'^2}{w_0^{\ 2}} \right) \exp\left(-\frac{y^2-2yy'+y'^2}{4D\tau} \right) dydy'$$

$$\iint \exp\left(-\frac{4z^2}{z_0^{\ 2}} \right) \exp\left(-\frac{4z'^2}{z_0^{\ 2}} \right) \exp\left(-\frac{z^2-2zz'+z'^2}{4D\tau} \right) dzdz' = E_0^{\ 2} \frac{< C >}{\left(\sqrt{4\pi D\tau}\right)^3} XYZ$$

(27)

Where, $X = \iint \exp\left(-\frac{4x^2}{w_0^{\ 2}} \right) \exp\left(-\frac{4x'^2}{w_0^{\ 2}} \right) \exp\left(-\frac{x^2-2xx'+x'^2}{4D\tau} \right) dxdx'$

$$= \int \exp\left(-\frac{4x'^2}{w_0^{\ 2}} \right) \exp\left(-\frac{x'^2}{4D\tau} \right) \left[\int \exp\left(-\frac{4x^2}{w_0^{\ 2}} \right) \exp\left(-\frac{x^2-2xx'}{4D\tau} \right) dx \right] dx'$$

$$= \int \exp\left(-\frac{4x'^2}{w_0^{\ 2}} \right) \exp\left(-\frac{x'^2}{4D\tau} \right) \left[\int \exp\left(-\frac{4x^2}{w_0^{\ 2}} - \frac{x^2}{4D\tau} \right) \exp\left(\frac{2xx'}{4D\tau} \right) dx \right] dx'$$

$$= \int \exp\left(-\frac{4x'^2}{w_0^{\ 2}} \right) \exp\left(-\frac{x'^2}{4D\tau} \right) \left[\int \exp\left(-\frac{16D\tau + w_0^{\ 2}}{4D\tau w_0^{\ 2}} x^2 + \frac{2x'}{4D\tau} x \right) dx \right] dx' \qquad set \quad A = \frac{16D\tau}{w_0^{\ 2}} + 1$$

$$
\begin{aligned}
&= \int \exp\left(-\frac{4x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int \exp\left(-\frac{A}{4D\tau}\left(x^2 - \frac{2x'}{A}x\right)\right)dx\right]dx' \\
&= \int \exp\left(-\frac{4x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\exp\left(\frac{x'^2}{4D\tau A}\right)\int \exp\left(-\frac{A}{4D\tau}\left(x - \frac{x'}{A}\right)^2\right)d(x - \frac{x'}{A})\right]dx' \\
&= \int \exp\left(-\frac{4x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\sqrt{\frac{4\pi D\tau}{A}}\exp\left(\frac{x'^2}{4D\tau A}\right)\right]dx' \\
&= \sqrt{\frac{4\pi D\tau}{A}}\int \exp\left(-(\frac{4}{w_0{}^2} + \frac{1}{4D\tau} - \frac{1}{4D\tau A})x'^2\right)dx' = \sqrt{\frac{4\pi D\tau}{A}}\int \exp\left(-(\frac{A^2-1}{4D\tau A})x'^2\right)dx' \\
&= \sqrt{\frac{4\pi D\tau}{A}}\sqrt{\frac{4\pi D\tau A}{A^2-1}} = \frac{4\pi D\tau}{\sqrt{\left(\frac{16D\tau}{w_0{}^2}\right)\left(\frac{16D\tau}{w_0{}^2}+2\right)}} = \frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{8D\tau}{w_0{}^2}}\right)\left(\sqrt{2}\right)}
\end{aligned}
\tag{28}
$$

Similarly,

$$
Y = \iint \exp\left(-\frac{4y^2}{w_0{}^2}\right)\exp\left(-\frac{4y'^2}{w_0{}^2}\right)\exp\left(-\frac{y^2 - 2yy' + y'^2}{4D\tau}\right)dydy' = \frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{8D\tau}{w_0{}^2}}\right)\left(\sqrt{2}\right)}
\tag{29}
$$

and

$$
Z = \iint \exp\left(-\frac{4z^2}{z_0{}^2}\right)\exp\left(-\frac{4z'^2}{z_0{}^2}\right)\exp\left(-\frac{z^2 - 2zz' + z'^2}{4D\tau}\right)dzdz' = \frac{z_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{8D\tau}{z_0{}^2}}\right)\left(\sqrt{2}\right)}
\tag{30}
$$

Substituting Eq. (28), Eq. (29) and Eq. (30) into Eq. (27). Then, Substituting Eq. (26) and Eq. (27) into Eq. (9), we get:

$$
G(\tau) = \frac{E_0{}^2 \frac{<C>}{\left(\sqrt{4\pi D\tau}\right)^3} \frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{8D\tau}{w_0{}^2}}\right)\left(\sqrt{2}\right)} \frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{8D\tau}{w_0{}^2}}\right)\left(\sqrt{2}\right)} \frac{z_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{8D\tau}{z_0{}^2}}\right)\left(\sqrt{2}\right)}}{\frac{<C>^2\ E_0{}^2\pi^3 w_0{}^4 z_0{}^2}{4^3}}
$$

That is,

$$
G(\tau) = \frac{2\sqrt{2}}{\pi\sqrt{\pi}w_0{}^2 z_0 <C>}\left(1+\frac{8D\tau}{w_0{}^2}\right)^{-1}\left(1+\frac{8D\tau}{z_0{}^2}\right)^{-\frac{1}{2}}
\tag{31}
$$

Providing the fact that, the excitation volume is as follow:

$$V = \iiint \exp\left(-\frac{4(x^2+y^2)}{{w_0}^2} - \frac{4z^2}{{z_0}^2}\right) dxdydz = \left(\sqrt{\frac{\pi {w_0}^2}{4}}\right)\left(\sqrt{\frac{\pi {w_0}^2}{4}}\right)\left(\sqrt{\frac{\pi {z_0}^2}{4}}\right) = \frac{\pi\sqrt{\pi}{w_0}^2 z_0}{2^3}$$

(32)

and $<N>=<C>V$, We have

$$G(\tau) = \frac{2\sqrt{2}}{\pi\sqrt{\pi}{w_0}^2 z_0 <C>}\left(1+\frac{8D\tau}{{w_0}^2}\right)^{-1}\left(1+\frac{8D\tau}{{z_0}^2}\right)^{-\frac{1}{2}} = \frac{2\sqrt{2}}{2^3\frac{\pi\sqrt{\pi}{w_0}^2 z_0}{2^3}<C>}\left(1+\frac{8D\tau}{{w_0}^2}\right)^{-1}\left(1+\frac{8D\tau}{{z_0}^2}\right)^{-\frac{1}{2}}$$

$$= \frac{1}{2\sqrt{2}<N>}\left(1+\frac{8D\tau}{{w_0}^2}\right)^{-1}\left(1+\frac{8D\tau}{{z_0}^2}\right)^{-\frac{1}{2}} = G(0)\left(1+\frac{8D\tau}{{w_0}^2}\right)^{-1}\left(1+\frac{8D\tau}{{z_0}^2}\right)^{-\frac{1}{2}}$$

(33)

The number of molecules and the concentration can be calculated as follow:

$$<N> = \frac{1}{2\sqrt{2}G(0)} \quad ,and \quad <C> = \frac{2\sqrt{2}}{\pi\sqrt{\pi}{w_0}^2 z_0 G(0)}$$

(34)

c. 2D Gaussian Model with one photon excitation

For one photon excitation, we get the denumerator part of Eq. (9) as follow:

$$(<\rho>\int E(r)dr)^2 = \left(<\rho> E_0 \iint \exp\left(-\frac{2(x^2+y^2)}{{w_0}^2}\right)dxdy\right)^2 = \left(<\rho> E_0\left(\sqrt{\frac{\pi {w_0}^2}{2}}\right)\left(\sqrt{\frac{\pi {w_0}^2}{2}}\right)\right)^2$$

$$= \frac{<\rho>^2\ {E_0}^2\pi^2{w_0}^4}{2^2}$$

(35)

and the numerator part of Eq. (9) as follow:

$$\iint E(r)E(r')<\delta\rho(r,t)\delta\rho(r',t+\tau)>drdr'$$

$$= \iint\iint {E_0}^2 \exp\left(-\frac{2(x^2+y^2)}{{w_0}^2}\right)\exp\left(-\frac{2(x'^2+y'^2)}{{w_0}^2}\right)\frac{<\rho>}{\left(\sqrt{4\pi D\tau}\right)^2}\exp\left(-\frac{(x-x')^2+(y-y')^2}{4D\tau}\right)dxdydx'dy'$$

$$= {E_0}^2\frac{<\rho>}{\left(\sqrt{4\pi Dt}\right)^2}\iint \exp\left(-\frac{2x^2}{{w_0}^2}\right)\exp\left(-\frac{2x'^2}{{w_0}^2}\right)\exp\left(-\frac{x^2-2xx'+x'^2}{4D\tau}\right)dxdx'$$

$$\iint \exp\left(-\frac{2y^2}{{w_0}^2}\right)\exp\left(-\frac{2y'^2}{{w_0}^2}\right)\exp\left(-\frac{y^2-2yy'+y'^2}{4D\tau}\right)dydy' = {E_0}^2\frac{<\rho>}{\left(\sqrt{4\pi D\tau}\right)^2}XY$$

(36)

Where,

$$X = \iint \exp\left(-\frac{2x^2}{w_0^{\ 2}}\right)\exp\left(-\frac{2x'^2}{w_0^{\ 2}}\right)\exp\left(-\frac{x^2-2xx'+x'^2}{4D\tau}\right)dxdx'$$

$$= \int \exp\left(-\frac{2x'^2}{w_0^{\ 2}}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int \exp\left(-\frac{2x^2}{w_0^{\ 2}}\right)\exp\left(-\frac{x^2-2xx'}{4D\tau}\right)dx\right]dx'$$

$$= \int \exp\left(-\frac{2x'^2}{w_0^{\ 2}}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int \exp\left(-\frac{2x^2}{w_0^{\ 2}}-\frac{x^2}{4D\tau}\right)\exp\left(\frac{2xx'}{4D\tau}\right)dx\right]dx'$$

$$= \int \exp\left(-\frac{2x'^2}{w_0^{\ 2}}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int \exp\left(-\frac{8D\tau+w_0^{\ 2}}{4D\tau w_0^{\ 2}}x^2+\frac{2x'}{4D\tau}x\right)dx\right]dx' \qquad set \quad A=\frac{8D\tau}{w_0^{\ 2}}+1$$

$$= \int \exp\left(-\frac{2x'^2}{w_0^{\ 2}}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int \exp\left(-\frac{A}{4D\tau}\left(x^2-\frac{2x'}{A}x\right)\right)dx\right]dx'$$

$$= \int \exp\left(-\frac{2x'^2}{w_0^{\ 2}}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\exp\left(\frac{x'^2}{4D\tau A}\right)\int \exp\left(-\frac{A}{4D\tau}\left(x-\frac{x'}{A}\right)^2\right)d(x-\frac{x'}{A})\right]dx'$$

$$= \int \exp\left(-\frac{2x'^2}{w_0^{\ 2}}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\sqrt{\frac{4\pi D\tau}{A}}\exp\left(\frac{x'^2}{4D\tau A}\right)\right]dx'$$

$$= \sqrt{\frac{4\pi D\tau}{A}}\int \exp\left(-(\frac{2}{w_0^{\ 2}}+\frac{1}{4D\tau}-\frac{1}{4D\tau A})x'^2\right)dx' = \sqrt{\frac{4\pi D\tau}{A}}\int \exp\left(-(\frac{A^2-1}{4D\tau A})x'^2\right)dx'$$

$$= \sqrt{\frac{4\pi D\tau}{A}}\sqrt{\frac{4\pi D\tau A}{A^2-1}} = \frac{4\pi D\tau}{\sqrt{\left(\frac{8D\tau}{w_0^{\ 2}}\right)\left(\frac{8D\tau}{w_0^{\ 2}}+2\right)}} = \frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{4D\tau}{w_0^{\ 2}}}\right)}$$

(37)

Similarly,

$$Y = \iint \exp\left(-\frac{2y^2}{w_0^{\ 2}}\right)\exp\left(-\frac{2y'^2}{w_0^{\ 2}}\right)\exp\left(-\frac{y^2-2yy'+y'^2}{4D\tau}\right)dydy' = \frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{4D\tau}{w_0^{\ 2}}}\right)}$$

(38)

Substituting Eq. (38) and Eq. (37) into Eq. (36). Then, Substituting Eq. (36) and Eq. (35) into Eq.(9), we get:

$$G(\tau) = \frac{E_0^{\ 2}\frac{<\rho>}{\left(\sqrt{4\pi D\tau}\right)^2}\frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{4D\tau}{w_0^{\ 2}}}\right)}\frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{4D\tau}{w_0^{\ 2}}}\right)}}{\frac{<\rho>^2\ E_0^{\ 2}\pi^2 w_0^{\ 4}}{2^2}}$$

That is,

$$G(\tau)=\frac{1}{\pi w_0{}^2<\rho>}\left(1+\frac{4D\tau}{w_0{}^2}\right)^{-1}$$

(39)

Providing the fact that, the excitation volume is as follow:

$$A=\iint\exp\left(-\frac{2(x^2+y^2)}{w_0{}^2}\right)dxdy=\left(\sqrt{\frac{\pi w_0{}^2}{2}}\right)\left(\sqrt{\frac{\pi w_0{}^2}{2}}\right)=\frac{\pi w_0{}^2}{2}$$

(40)

and $<N>=<\rho>A$, We have

$$G(\tau)=\frac{1}{\pi w_0{}^2<\rho>}\left(1+\frac{4D\tau}{w_0{}^2}\right)^{-1}=\frac{1}{2\frac{\pi w_0{}^2}{2}<\rho>}\left(1+\frac{4D\tau}{w_0{}^2}\right)^{-1}$$

$$=\frac{1}{2<N>}\left(1+\frac{4D\tau}{w_0{}^2}\right)^{-1}=G(0)\left(1+\frac{4D\tau}{w_0{}^2}\right)^{-1}$$

(41)

The number of molecules and surface number density can be calculated as follow:

$$<N>=\frac{1}{2G(0)}\quad ,and\quad <\rho>=\frac{1}{\pi w_0{}^2G(0)}$$

(42)

d. 2D Gaussian Model with two photon excitation

For two photon excitation, we get the denumerator part of Eq. (9) as follow:

$$(<\rho>\int E(r)dr)^2=\left(<C>E_0\iint\exp\left(-\frac{4(x^2+y^2)}{w_0{}^2}\right)dxdy\right)^2=\left(<\rho>E_0\left(\sqrt{\frac{\pi w_0{}^2}{4}}\right)\left(\sqrt{\frac{\pi w_0{}^2}{4}}\right)\right)^2$$

$$=\frac{<\rho>^2\ E_0{}^2\pi^2w_0{}^4}{4^2}$$

(43)

and the numerator part of Eq. (9) as follow:

$$\iint E(r)E(r')<\delta\rho(r,t)\delta\rho(r',t+\tau)>drdr'$$

$$=\iint E_0{}^2\exp\left(-\frac{4(x^2+y^2)}{w_0{}^2}\right)\exp\left(-\frac{4(x'^2+y'^2)}{w_0{}^2}\right)$$

$$\frac{<\rho>}{\left(\sqrt{4\pi D\tau}\right)^2}\exp\left(-\frac{(x-x')^2+(y-y')^2}{4D\tau}\right)dxdydx'dy'$$

$$= E_0{}^2 \frac{<\rho>}{\left(\sqrt{4\pi Dt}\right)^3} \iint \exp\left(-\frac{4x^2}{w_0{}^2}\right)\exp\left(-\frac{4x'^2}{w_0{}^2}\right)\exp\left(-\frac{x^2-2xx'+x'^2}{4D\tau}\right)dxdx'$$
$$\iint \exp\left(-\frac{4y^2}{w_0{}^2}\right)\exp\left(-\frac{4y'^2}{w_0{}^2}\right)\exp\left(-\frac{y^2-2yy'+y'^2}{4D\tau}\right)dydy' = E_0{}^2 \frac{<\rho>}{\left(\sqrt{4\pi D\tau}\right)^2} XY$$

(44)

Where,

$$X = \iint \exp\left(-\frac{4x^2}{w_0{}^2}\right)\exp\left(-\frac{4x'^2}{w_0{}^2}\right)\exp\left(-\frac{x^2-2xx'+x'^2}{4D\tau}\right)dxdx'$$
$$= \int \exp\left(-\frac{4x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int \exp\left(-\frac{4x^2}{w_0{}^2}\right)\exp\left(-\frac{x^2-2xx'}{4D\tau}\right)dx\right]dx'$$
$$= \int \exp\left(-\frac{4x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int \exp\left(-\frac{4x^2}{w_0{}^2}-\frac{x^2}{4D\tau}\right)\exp\left(\frac{2xx'}{4D\tau}\right)dx\right]dx'$$
$$= \int \exp\left(-\frac{4x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int \exp\left(-\frac{16D\tau+w_0{}^2}{4D\tau w_0{}^2}x^2+\frac{2x'}{4D\tau}x\right)dx\right]dx' \qquad set \quad A = \frac{16D\tau}{w_0{}^2}+1$$
$$= \int \exp\left(-\frac{4x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int \exp\left(-\frac{A}{4D\tau}\left(x^2-\frac{2x'}{A}x\right)\right)dx\right]dx'$$
$$= \int \exp\left(-\frac{4x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\exp\left(\frac{x'^2}{4D\tau A}\right)\int \exp\left(-\frac{A}{4D\tau}\left(x-\frac{x'}{A}\right)^2\right)d(x-\frac{x'}{A})\right]dx'$$
$$= \int \exp\left(-\frac{4x'^2}{w_0{}^2}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\sqrt{\frac{4\pi D\tau}{A}}\exp\left(\frac{x'^2}{4D\tau A}\right)\right]dx'$$
$$= \sqrt{\frac{4\pi D\tau}{A}}\int \exp\left(-(\frac{4}{w_0{}^2}+\frac{1}{4D\tau}-\frac{1}{4D\tau A})x'^2\right)dx' = \sqrt{\frac{4\pi D\tau}{A}}\int \exp\left(-(\frac{A^2-1}{4D\tau A})x'^2\right)dx'$$
$$= \sqrt{\frac{4\pi D\tau}{A}}\sqrt{\frac{4\pi D\tau A}{A^2-1}} = \frac{4\pi D\tau}{\sqrt{\left(\frac{16D\tau}{w_0{}^2}\right)\left(\frac{16D\tau}{w_0{}^2}+2\right)}} = \frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{8D\tau}{w_0{}^2}}\right)\left(\sqrt{2}\right)}$$

(45)

Similarly,

$$Y = \iint \exp\left(-\frac{4y^2}{w_0{}^2}\right)\exp\left(-\frac{4y'^2}{w_0{}^2}\right)\exp\left(-\frac{y^2-2yy'+y'^2}{4D\tau}\right)dydy' = \frac{w_0\pi\sqrt{D\tau}}{\left(\sqrt{1+\frac{8D\tau}{w_0{}^2}}\right)\left(\sqrt{2}\right)}$$

(46)

Substituting Eq. (46) and Eq. (45) into Eq. (44). Then, Substituting Eq. (44) and Eq. (43) into Eq. (9), we get:

$$G(\tau) = \frac{E_0^{\ 2} \dfrac{<\rho>}{\left(\sqrt{4\pi D\tau}\right)^2} \dfrac{w_0 \pi \sqrt{D\tau}}{\left(\sqrt{1+\dfrac{8D\tau}{w_0^{\ 2}}}\right)\left(\sqrt{2}\right)} \dfrac{w_0 \pi \sqrt{D\tau}}{\left(\sqrt{1+\dfrac{8D\tau}{w_0^{\ 2}}}\right)\left(\sqrt{2}\right)}}{\dfrac{<\rho>^2 \ E_0^{\ 2} \pi^2 w_0^{\ 4}}{4^2}}$$

That is,

$$G(\tau) = \frac{2}{\pi w_0^{\ 2} <\rho>} \left(1 + \frac{8D\tau}{w_0^{\ 2}}\right)^{-1} \qquad (47)$$

Providing the fact that, the excitation volume is as follow:

$$A = \iint \exp\left(-\frac{4(x^2+y^2)}{w_0^{\ 2}}\right) dxdy = \left(\sqrt{\frac{\pi w_0^{\ 2}}{4}}\right)\left(\sqrt{\frac{\pi w_0^{\ 2}}{4}}\right) = \frac{\pi w_0^{\ 2}}{2^2} \qquad (48)$$

and $<N> = <\rho> A$, We have

$$G(\tau) = \frac{2}{\pi w_0^{\ 2} <\rho>} \left(1 + \frac{8D\tau}{w_0^{\ 2}}\right)^{-1} = \frac{2}{2^2 \dfrac{\pi w_0^{\ 2}}{2^2} <\rho>} \left(1 + \frac{8D\tau}{w_0^{\ 2}}\right)^{-1}$$

$$= \frac{1}{2<N>} \left(1 + \frac{8D\tau}{w_0^{\ 2}}\right)^{-1} = G(0)\left(1 + \frac{8D\tau}{w_0^{\ 2}}\right)^{-1} \qquad (49)$$

The number of molecules and the surface number can be calculated as follow:

$$<N> = \frac{1}{2G(0)} \quad , and \quad <\rho> = \frac{2}{\pi w_0^{\ 2} G(0)} \qquad (50)$$

e. Gaussian-Lorentzian Model

Substituting Eq. (15) and Eq. (11) into Eq. (9), and use the fact of Eq (16)

We get the denumerator part of Eq. (9) as follow:

$$(<C>\int E(r)^2 dr)^2 = \left(\frac{4<C>E_0{}^2 w_0{}^4}{\pi^2} \iiint \frac{\exp\left(-\frac{4(x^2+y^2)}{w^2(z)}\right)}{w^4(z)} dxdydz \right)^2$$

$$= \left(\frac{4<C>E_0{}^2 w_0{}^4}{\pi^2} \int \frac{1}{w^4(z)} \iint \exp\left(-\frac{4(x^2+y^2)}{w^2(z)}\right) dxdydz \right)^2$$

$$= \left(\frac{4<C>E_0{}^2 w_0{}^4}{\pi^2} \int \frac{\left(\sqrt{\frac{\pi w^2(z)}{4}}\right)\left(\sqrt{\frac{\pi w^2(z)}{4}}\right)}{w^4(z)} dz \right)^2$$

$$= \left(\frac{<C>E_0{}^2 w_0{}^4}{\pi} \int \frac{1}{w^2(z)} dz \right)^2 = \left(\frac{<C>E_0{}^2 w_0{}^4}{\pi} \int_{-\infty}^{\infty} \frac{1}{w_0{}^2 (1+\left(\frac{z}{z_R}\right)^2)} dz \right)^2 \qquad , Let \quad \tan\theta = \frac{z}{z_R}$$

$$= \left(\frac{<C>E_0{}^2 w_0{}^4}{\pi} \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \frac{z_R}{w_0{}^2} d\theta \right)^2 = \left(<C>E_0{}^2 w_0{}^2 z_R\right)^2 \qquad , where \quad z_R = \frac{\pi w_0{}^2}{\lambda}$$

$$= \left(\frac{<C>E_0{}^2 w_0{}^4 \pi}{\lambda} \right)^2 = \frac{<C>^2 \; E_0{}^4 w_0{}^8 \pi^2}{\lambda^2} \tag{51}$$

and the numerator part of Eq. (9) as follow:

$$\iint E(r)^2 E(r')^2 <\delta C(r,t)\delta C(r',t+\tau)> drdr'$$

$$= \iiint\iiint \frac{4E_0{}^2 w_0{}^4}{\pi^2 w^4(z)} \exp\left(-\frac{4(x^2+y^2)}{w(z)^2}\right) \frac{4E_0{}^2 w_0{}^4}{\pi^2 w^4(z')} \exp\left(-\frac{2(x'^2+y'^2)}{w(z')^2}\right)$$
$$\frac{<C>}{\left(\sqrt{4\pi D\tau}\right)^3} \exp\left(-\frac{(x-x')^2+(y-y')^2+(z-z')^2}{4D\tau}\right) dxdydzdx'dy'dz'$$

$$= \frac{16E_0{}^4 w_0{}^8 <C>}{\pi^4 \left(\sqrt{4\pi Dt}\right)^3}$$
$$\iint \left[\begin{array}{l} \iint \exp\left(-\frac{4x^2}{w^2(z)}\right) \exp\left(-\frac{4x'^2}{w^2(z')}\right) \exp\left(-\frac{x^2-2xx'+x'^2}{4D\tau}\right) dxdx' \\ \iint \exp\left(-\frac{4y^2}{w^2(z)}\right) \exp\left(-\frac{4y'^2}{w^2(z')}\right) \exp\left(-\frac{y^2-2yy'+y'^2}{4D\tau}\right) dydy' \end{array} \right] \frac{\exp\left(-\frac{z^2-2zz'+z'^2}{4D\tau}\right)}{w^4(z)w^4(z')} dzdz'$$

$$=\frac{16E_0{}^4 w_0{}^8 <C>}{\pi^4\left(\sqrt{4\pi Dt}\right)^3}\iint XY\frac{\exp\left(-\frac{z^2-2zz'+z'^2}{4D\tau}\right)}{w^4(z)w^4(z')}dzdz'$$

(52)

Where,

$$X=\iint\exp\left(-\frac{4x^2}{w^2(z)}\right)\exp\left(-\frac{4x'^2}{w^2(z')}\right)\exp\left(-\frac{x^2-2xx'+x'^2}{4D\tau}\right)dxdx'$$

$$=\int\exp\left(-\frac{4x'^2}{w^2(z')}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int\exp\left(-\frac{4x^2}{w^2(z)}\right)\exp\left(-\frac{x^2-2xx'}{4D\tau}\right)dx\right]dx'$$

$$=\int\exp\left(-\frac{4x'^2}{w^2(z')}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int\exp\left(-\frac{4x^2}{w^2(z)}-\frac{x^2}{4D\tau}\right)\exp\left(\frac{2xx'}{4D\tau}\right)dx\right]dx'$$

$$=\int\exp\left(-\frac{4x'^2}{w^2(z')}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int\exp\left(-\frac{16D\tau+w^2(z)}{4D\tau w^2(z)}x^2+\frac{2x'}{4D\tau}x\right)dx\right]dx' \qquad set \quad A=\frac{16D\tau}{w^2(z)}+1$$

$$=\int\exp\left(-\frac{4x'^2}{w^2(z')}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\int\exp\left(-\frac{A}{4D\tau}\left(x^2-\frac{2x'}{A}x\right)\right)dx\right]dx'$$

$$=\int\exp\left(-\frac{4x'^2}{w^2(z')}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\exp\left(\frac{x'^2}{4D\tau A}\right)\int\exp\left(-\frac{A}{4D\tau}\left(x-\frac{x'}{A}\right)^2\right)d(x-\frac{x'}{A})\right]dx'$$

$$=\int\exp\left(-\frac{4x'^2}{w^2(z')}\right)\exp\left(-\frac{x'^2}{4D\tau}\right)\left[\sqrt{\frac{4\pi D\tau}{A}}\exp\left(\frac{x'^2}{4D\tau A}\right)\right]dx'$$

$$=\sqrt{\frac{4\pi D\tau}{A}}\int\exp\left(-(\frac{4}{w^2(z')}+\frac{1}{4D\tau}-\frac{1}{4D\tau A})x'^2\right)dx' \qquad set \quad A'=\frac{16D\tau}{w^2(z')}+1$$

$$=\sqrt{\frac{4\pi D\tau}{A}}\int\exp\left(-(\frac{AA'-1}{4D\tau A})x'^2\right)dx'=\sqrt{\frac{4\pi D\tau}{A}}\sqrt{\frac{4\pi D\tau A}{AA'-1}}=\frac{4\pi D\tau}{\sqrt{\left(\frac{16D\tau}{w^2(z)}+1\right)\left(\frac{16D\tau}{w^2(z')}+1\right)-1}}$$

$$=\frac{4\pi D\tau w(z)w(z')}{\sqrt{\left(16D\tau+w^2(z)\right)\left(16D\tau+w^2(z')\right)-w^2(z)w^2(z')}}=\frac{\pi\sqrt{D\tau}w(z)w(z')}{\sqrt{\left(16D\tau+w^2(z)+w^2(z')\right)}}$$

(53)

Similarly,

$$Y=\iint\exp\left(-\frac{4y^2}{w^2(z)}\right)\exp\left(-\frac{4y'^2}{w^2(z')}\right)\exp\left(-\frac{y^2-2yy'+y'^2}{4D\tau}\right)dydy'$$

$$=\frac{\pi\sqrt{D\tau}w(z)w(z')}{\sqrt{\left(16D\tau+w^2(z)+w^2(z')\right)}}$$

(54)

Substituting Eq. (54) and Eq. (53) into Eq. (52), we have

$$\iint E(r)^2 E(r')^2 < \delta C(r,t)\delta C(r',t+\tau) > drdr'$$

$$= \frac{16E_0{}^4 w_0{}^8 < C >}{\pi^4\left(\sqrt{4\pi Dt}\right)^3}\iint XY \frac{\exp\left(-\frac{z^2 - 2zz' + z'^2}{4D\tau}\right)}{w^4(z)w^4(z')} dzdz'$$

$$= \frac{16E_0{}^4 w_0{}^8 < C >}{\pi^4\left(\sqrt{4\pi Dt}\right)^3}\iint \frac{\pi^2 D\tau w^2(z)w^2(z')}{\left(16D\tau + w^2(z) + w^2(z')\right)} \frac{\exp\left(-\frac{z^2 - 2zz' + z'^2}{4D\tau}\right)}{w^4(z)w^4(z')} dzdz'$$

$$= \frac{4E_0{}^4 w_0{}^8 < C >}{\pi^3\sqrt{4\pi Dt}}\iint \frac{\exp\left(-\frac{z^2 - 2zz' + z'^2}{4D\tau}\right)}{\left(16D\tau + w^2(z) + w^2(z')\right)w^2(z)w^2(z')} dzdz', w^2(z) = w_0{}^2\left(1 + \left(\frac{z}{z_R}\right)^2\right), z_R = \frac{\pi w_0{}^2}{\lambda}$$

$$= \frac{4E_0{}^4 w_0{}^8 < C >}{\pi^3\sqrt{4\pi Dt}}\iint \frac{\exp\left(-\frac{z^2 - 2zz' + z'^2}{4D\tau}\right)}{\left(16D\tau + w_0{}^2\left[2 + \frac{z^2 + z'^2}{z_R{}^2}\right]\right)w_0{}^2\left(1 + \left[\frac{z}{z_R}\right]^2\right)w_0{}^2\left(1 + \left[\frac{z'}{z_R}\right]^2\right)} dzdz'$$

$$= \frac{4E_0{}^4 w_0{}^4 < C >}{\pi^3\sqrt{4\pi Dt}}\iint \frac{\exp\left(-\frac{z^2 - 2zz' + z'^2}{4D\tau}\right)}{\left(16D\tau + w_0{}^2\left[2 + \frac{z^2 + z'^2}{z_R{}^2}\right]\right)\left(1 + \frac{z^2 + z'^2}{z_R{}^2} + \frac{z^2 z'^2}{z_R{}^4}\right)} dzdz'$$

Set $z = bz_R \sin\theta$ and $z' = bz_R \sin\theta$, That is, $b^2 = \frac{z^2 + z'^2}{z_R{}^2}$ and $dzdz' = bz_R{}^2 dbd\theta$

$$= \frac{4E_0{}^4 w_0{}^4 < C >}{\pi^3\sqrt{4\pi Dt}}\iint \frac{z_R{}^2 b\exp\left(-\frac{z_R{}^2 b^2}{4D\tau}\right)\exp\left(\frac{z_R{}^2 b^2 \sin\theta\cos\theta}{2D\tau}\right)}{\left(16D\tau + w_0{}^2\left[2 + b^2\right]\right)\left(1 + b^2 + \frac{b^2 z_R{}^2(\sin^2\theta)b^2 z_R{}^2(\cos^2\theta)}{z_R{}^4}\right)} dbd\theta$$

Also, $\sin 2\theta = 2\sin\theta\cos\theta$

$$= \frac{4E_0{}^4 w_0{}^4 < C >}{\pi^3\sqrt{4\pi Dt}}\int_0^\infty \frac{z_R{}^2 b \exp\left(-\frac{z_R{}^2 b^2}{4D\tau}\right)}{\left(16D\tau + w_0{}^2\left[2+b^2\right]\right)} \frac{1}{2}\int_0^{2\pi} \frac{\exp\left(\frac{z_R{}^2 b^2 \sin 2\theta}{4D\tau}\right)}{\left(1+b^2+\frac{b^4\sin^2 2\theta}{4}\right)} d2\theta db$$

$$= \frac{2E_0{}^4 w_0{}^2 < C >}{\pi^3\sqrt{4\pi Dt}}\int_0^\infty \frac{z_R{}^2 b \exp\left(-\frac{z_R{}^2 b^2}{4D\tau}\right)}{\left(\frac{16D\tau}{w_0{}^2}+2+b^2\right)} T db$$

(55)

Where

$$T = \int_0^{4\pi} \frac{\exp\left(\frac{z_R{}^2 b^2 \sin\theta}{4D\tau}\right)}{\left(1+b^2+\frac{b^4\sin^2\theta}{4}\right)} d\theta \quad ,Set \quad z = e^{i\theta}, \quad dz = ie^{i\theta}d\theta = izd\theta \quad \Rightarrow \quad d\theta = \frac{dz}{iz};$$

Also $z = e^{i\theta} = \cos\theta + i\sin\theta, \quad z^{-1} = e^{-i\theta} = \cos\theta - i\sin\theta \quad \Rightarrow \quad \sin\theta = \frac{e^{i\theta}-e^{-i\theta}}{2i} = \frac{z-z^{-1}}{2i};$

$$= 2\oint_C \frac{\exp\left(\frac{z_R{}^2 b^2}{4D\tau}\left(\frac{z-z^{-1}}{2i}\right)\right)}{\left(1+b^2+\frac{b^4}{4}\left(\frac{z-z^{-1}}{2i}\right)^2\right)}\frac{dz}{iz} = 2\oint_C \frac{\exp\left(\frac{z_R{}^2 b^2}{4D\tau}\left(\frac{z-z^{-1}}{2i}\right)\right)}{iz\left(\left(1+b^2\right)-\frac{b^4}{16}\left(z^2-2+z^{-2}\right)\right)}dz$$

$$= 2\oint_C \frac{z\exp\left(\frac{z_R{}^2 b^2}{4D\tau}\left(\frac{z^2-1}{2iz}\right)\right)}{i\left(z^2\left(1+b^2\right)-\frac{b^4}{16}\left(z^4-2z^2+1\right)\right)}dz = 2\oint_C \frac{z\exp\left(\frac{z_R{}^2 b^2}{4D\tau}\left(\frac{z^2-1}{2iz}\right)\right)}{i\left(\left[z\sqrt{1+b^2}\right]^2-\left[\frac{b^2}{4}\left(z^2-1\right)\right]^2\right)}dz$$

$$= 2\oint_C \frac{z\exp\left(\frac{z_R{}^2 b^2}{4D\tau}\left(\frac{z^2-1}{2iz}\right)\right)}{i\left(z\sqrt{1+b^2}+\frac{b^2}{4}\left(z^2-1\right)\right)\left(z\sqrt{1+b^2}-\frac{b^2}{4}\left(z^2-1\right)\right)}dz$$

$$= -2\oint_C \frac{z\exp\left(\frac{z_R{}^2 b^2}{4D\tau}\left(\frac{z^2-1}{2iz}\right)\right)}{i\left(\frac{b^2}{4}\right)^2\left(z^2+\frac{4\sqrt{1+b^2}}{b^2}z-1\right)\left(z^2-\frac{4\sqrt{1+b^2}}{b^2}z-1\right)}dz$$

$$= \frac{-32}{b^4} \oint_C \frac{z \exp\left(\frac{{z_R}^2 b^2}{4D\tau} \left(\frac{z^2 - 1}{2iz} \right) \right)}{i \left(z^2 + \frac{4\sqrt{1+b^2}}{b^2} z - 1 \right) \left(z^2 - \frac{4\sqrt{1+b^2}}{b^2} z - 1 \right)} dz \quad , Set \quad a = \frac{2\sqrt{1+b^2}}{b^2};$$

$$= \frac{-32}{ib^4} \oint_C \frac{z \exp\left(\frac{{z_R}^2 b^2}{4D\tau} \left(\frac{z^2 - 1}{2iz} \right) \right)}{(z^2 + 2az - 1)(z^2 - 2az - 1)} dz$$

(56)

The roots of z will be $z = -a \pm \sqrt{a^2 + 1}$ *and* $z = a \pm \sqrt{a^2 + 1}$

Only $z = -a + \sqrt{a^2 + 1}$ *and* $z = a - \sqrt{a^2 + 1}$ lie in the circle of $e^{i\theta}$

Consider $z^2 + 2az - 1 = 0$; that is, $z^2 - 1 = -2az$;

Residue at $z = -a + \sqrt{a^2 + 1}$ is

$$\mathrm{Re}\, s1 = \lim_{z \to -a + \sqrt{a^2+1}} \left(z + a - \sqrt{a^2 + 1} \right) \frac{z \exp\left(\frac{{z_R}^2 b^2}{4D\tau} \left(\frac{z^2 - 1}{2iz} \right) \right)}{(z^2 + 2az - 1)(z^2 - 2az - 1)}$$

$$= \lim_{z \to -a + \sqrt{a^2+1}} \frac{z \exp\left(\frac{{z_R}^2 b^2}{4D\tau} \left(\frac{z^2 - 1}{2iz} \right) \right)}{\left(z + a + \sqrt{a^2 + 1} \right)(z^2 - 2az - 1)} = \lim_{z \to -a + \sqrt{a^2+1}} \frac{z \exp\left(\frac{{z_R}^2 b^2}{4D\tau} \left(\frac{-2az}{2iz} \right) \right)}{\left(z + a + \sqrt{a^2 + 1} \right)(-4az)}$$

$$= \frac{\exp\left(\frac{{z_R}^2 b^2}{4D\tau} \left(\frac{-a}{i} \right) \right)}{\left(2\sqrt{a^2 + 1} \right)(-4a)} = \frac{-\exp\left(\frac{{z_R}^2 b^2}{4D\tau} \left(\frac{-a}{i} \right) \right)}{\left(8a\sqrt{a^2 + 1} \right)} = \frac{-\exp\left(a \frac{{z_R}^2 b^2}{4D\tau} i \right)}{\left(8a\sqrt{a^2 + 1} \right)}$$

(57)

Consider $z^2 - 2az - 1 = 0$; that is, $z^2 - 1 = 2az$;

Residue at $z = a - \sqrt{a^2 + 1}$ is

$$\mathrm{Re}\, s2 = \lim_{z \to a - \sqrt{a^2+1}} \left(z - a + \sqrt{a^2 + 1} \right) \frac{z \exp\left(\frac{{z_R}^2 b^2}{4D\tau} \left(\frac{z^2 - 1}{2iz} \right) \right)}{(z^2 + 2az - 1)(z^2 - 2az - 1)} = \lim_{z \to a - \sqrt{a^2+1}} \frac{z \exp\left(\frac{{z_R}^2 b^2}{4D\tau} \left(\frac{z^2 - 1}{2iz} \right) \right)}{\left(z - a - \sqrt{a^2 + 1} \right)(z^2 - 2az - 1)}$$

$$= \lim_{z \to a - \sqrt{a^2+1}} \frac{z \exp\left(\frac{{z_R}^2 b^2}{4D\tau} \left(\frac{2az}{2iz} \right) \right)}{\left(z - a - \sqrt{a^2 + 1} \right)(4az)} = \frac{\exp\left(\frac{{z_R}^2 b^2}{4D\tau} \left(\frac{a}{i} \right) \right)}{\left(-2\sqrt{a^2 + 1} \right)(4a)} = \frac{-\exp\left(\frac{{z_R}^2 b^2}{4D\tau} \left(\frac{a}{i} \right) \right)}{\left(8a\sqrt{a^2 + 1} \right)} = \frac{-\exp\left(-a \frac{{z_R}^2 b^2}{4D\tau} i \right)}{\left(8a\sqrt{a^2 + 1} \right)}$$

(58)

Use the result of Eq. (58) and Eq. (57) into Eq. (56), We have

$$T=\frac{-32}{ib^4}\oint_C \frac{z\exp\left(\frac{{z_R}^2b^2}{4D\tau}\left(\frac{z^2-1}{2iz}\right)\right)}{\left(z^2+2az-1\right)\left(z^2-2az-1\right)}dz=\frac{-32}{ib^4}(2\pi i)(\operatorname{Re}s1+\operatorname{Re}s2)$$

$$=\frac{-32}{ib^4}(2\pi i)\frac{-1}{\left(8a\sqrt{a^2+1}\right)}\left(\exp\left(a\frac{{z_R}^2b^2}{4D\tau}i\right)+\exp\left(-a\frac{{z_R}^2b^2}{4D\tau}i\right)\right)$$

$$=\frac{16\pi}{b^4\left(a\sqrt{a^2+1}\right)}\frac{\exp\left(a\frac{{z_R}^2b^2}{4D\tau}i\right)+\exp\left(-a\frac{{z_R}^2b^2}{4D\tau}i\right)}{2},Set\ \theta=a\frac{{z_R}^2b^2}{4D\tau}=\frac{2\sqrt{1+b^2}}{b^2}\frac{{z_R}^2b^2}{4D\tau}=\frac{{z_R}^2\sqrt{1+b^2}}{2D\tau}$$

and with the fact that $\cos\theta=\frac{e^{i\theta}+e^{-i\theta}}{2}$

$$=\frac{16\pi}{b^4\left(a\sqrt{a^2+1}\right)}\cos\left(\frac{{z_R}^2\sqrt{1+b^2}}{2D\tau}\right)=\frac{16\pi}{b^4\left(\frac{2\sqrt{1+b^2}}{b^2}\sqrt{\frac{4\left(1+b^2\right)}{b^4}+1}\right)}\cos\left(\frac{{z_R}^2\sqrt{1+b^2}}{2D\tau}\right)$$

$$=\frac{16\pi}{b^4\left(\frac{2\sqrt{1+b^2}}{b^2}\sqrt{\frac{\left(b^2+2\right)^2}{b^4}}\right)}\cos\left(\frac{{z_R}^2\sqrt{1+b^2}}{2D\tau}\right)=\frac{8\pi}{\sqrt{1+b^2}\left(b^2+2\right)}\cos\left(\frac{{z_R}^2\sqrt{1+b^2}}{2D\tau}\right)$$

(59)

Substitute Eq. (59) into Eq. (55), We have

$$\iint E(r)^2E(r')^2<\delta C(r,t)\delta C(r',t+\tau)>drdr'$$

$$=\frac{2{E_0}^4{w_0}^2<C>}{\pi^3\sqrt{4\pi Dt}}\int_0^\infty\frac{{z_R}^2b\exp\left(-\frac{{z_R}^2b^2}{4D\tau}\right)}{\left(\frac{16D\tau}{{w_0}^2}+2+b^2\right)}\frac{8\pi}{\sqrt{1+b^2}\left(b^2+2\right)}\cos\left(\frac{{z_R}^2\sqrt{1+b^2}}{2D\tau}\right)db$$

$$=\frac{16{E_0}^4{w_0}^2<C>}{\pi^2\sqrt{4\pi Dt}}\left(\frac{\pi{w_0}^2}{\lambda}\right)^2\int_0^\infty\frac{b\exp\left(-\frac{{z_R}^2b^2}{4D\tau}\right)}{\left(\frac{16D\tau}{{w_0}^2}+2+b^2\right)}\frac{1}{\sqrt{1+b^2}\left(b^2+2\right)}\cos\left(\frac{{z_R}^2\sqrt{1+b^2}}{2D\tau}\right)db$$

(60)

Divide Eq. (60) by Eq. (51), We have

$G(\tau)$

$$= \frac{\lambda^2}{<C>^2\ E_0{}^4 w_0{}^8 \pi^2} \frac{16 E_0{}^4 w_0{}^2 <C>}{\pi^2 \sqrt{4\pi D t}} \left(\frac{\pi w_0{}^2}{\lambda}\right)^2 \int_0^\infty \frac{b \exp\left(-\frac{z_R{}^2 b^2}{4D\tau}\right)}{\left(\frac{16D\tau}{w_0{}^2} + 2 + b^2\right)} \frac{1}{\sqrt{1+b^2}\left(b^2+2\right)} \cos\left(\frac{z_R{}^2\sqrt{1+b^2}}{2D\tau}\right) db$$

$$= \frac{16}{<C> w_0{}^2 \pi^2 \sqrt{4\pi D t}} \int_0^\infty \frac{b \exp\left(-\frac{z_R{}^2 b^2}{4D\tau}\right)}{\left(\frac{16D\tau}{w_0{}^2} + 2 + b^2\right)} \frac{1}{\sqrt{1+b^2}\left(b^2+2\right)} \cos\left(\frac{z_R{}^2\sqrt{1+b^2}}{2D\tau}\right) db$$

(61)

By Defining $<C> = \frac{N}{\left(\frac{w_0}{2}\right)^2 \left(\frac{z_R}{2}\right)}$, We have

$$G(\tau) = \frac{2w_0}{\lambda \pi N \sqrt{4\pi D t}} \int_0^\infty \frac{b \exp\left(-\frac{z_R{}^2 b^2}{4D\tau}\right)}{\left(\frac{16D\tau}{w_0{}^2} + 2 + b^2\right)} \frac{1}{\sqrt{1+b^2}\left(b^2+2\right)} \cos\left(\frac{z_R{}^2\sqrt{1+b^2}}{2D\tau}\right) db$$

(62)

2. Histogram Equation Models

The probability p(k) to observe k photoelectron events depends on the statistical properties of the light reaching the detector:

$$p(k)=\int_0^\infty \frac{(\eta_I I_D)e^{-\eta_I I_D}}{k!}p(I_D)dI_D=\int_0^\infty Poi(k,\eta_I I_D)p(I_D)dI_D$$

(63)

The fluorescence intensity I_D at the detector for a fluorophore at position $\vec{r}_0$ is given by the PSF, $\overline{PSF}(\vec{r}_0)$ and the excitation intensity at the center of the PSF I_0^n. For two photon excitation n=2, while for normal excitation n=1. The coefficient η is the detection efficiency. The coefficient β contains the excitation probability, the fluorescence quantum yield and all of the instrument dependent factors, such as the transmittance of the microscope optics.

$$I_D=I_0^n\beta\overline{PSF}(\vec{r}_0)$$

(64)

$p(I_D)$ is the energy distribution, $p(k)$ is the Poisson transformation of the energy distribution $p(I_D)$.

The energy distribution is as follow:

$$p(I_D)=\int\delta\left(I_D-I_0^n\beta\overline{PSF}(\vec{r})\right)p(\vec{r})d\vec{r}$$

(65)

where $p(\vec{r})=\begin{cases}\frac{1}{V_0}, & for\ \vec{r}\in V_0\\ 0\ , & for\ \vec{r}\notin V_0\end{cases}$, assume the particle can be found with equal probability at any position within the volume V_0

Insert Eq. (65) and Eq. (64) into Eq. (63) we get as follow:

$$
\begin{aligned}
p(k) &= \int_0^\infty \frac{(\eta_I I_D)e^{-\eta_I I_D}}{k!} p(I_D)dI_D = \int_0^\infty Poi(k,\eta_I I_D)p(I_D)dI_D \\
&= \int_0^\infty Poi(k,\eta_I I_D)\int \delta\left(I_D - I_0^n \beta \overline{PSF}(\vec{r})\right) p(\vec{r})d\vec{r}dI_D \\
&= \int\int_0^\infty Poi(k,\eta_I I_D)\delta\left(I_D - I_0^n \beta \overline{PSF}(\vec{r})\right) dI_D\, p(\vec{r})d\vec{r} \\
&= \int Poi(k,\eta_I I_0^n \beta \overline{PSF}(\vec{r}))p(\vec{r})d\vec{r} = \int Poi(k,\varepsilon \overline{PSF}(\vec{r}))p(\vec{r})d\vec{r} \\
&= p^{(1)}(k;V_0,\varepsilon) \\
&= \frac{1}{V_0}\int_{V_0} Poi(k,\varepsilon \overline{PSF}(\vec{r}))d\vec{r}
\end{aligned}
$$

(66)

Where, $\varepsilon = \eta_I I_0^n \beta$. Above Eq. (66) is the fundamental equation to determine the PCH $p^{(1)}(k;V_0,\varepsilon)$ for a single molecule.

a. Homogeneous Model

The Homogeneous point spread function is as follow:

$$
\overline{PSF}_{Homogeneous}(\rho,z) = 1
$$

(67)

Insert Eq. (67) into Eq. (66), we have:

$$
\begin{aligned}
p^{(1)}_{Homogeneous}(k;V_0,\varepsilon) &= \frac{1}{V_0}\int_{V_0} Poi(k,\varepsilon \overline{PSF}_{Homogeneous}(\vec{r}))d\vec{r} \\
&= \frac{1}{V_0}\iiint_{V_0} Poi(k,\varepsilon \overline{PSF}_{Homogeneous}(\rho,z))\rho d\rho d\theta dz \\
&= \frac{1}{V_0}\iiint_{V_0} Poi(k,\varepsilon)\rho d\rho d\theta dz = \frac{1}{V_0}\iiint_{V_0} \frac{(\varepsilon)^k}{k!}e^{-\varepsilon}\rho d\rho d\theta dz \\
&= \frac{1}{V_0}\frac{(\varepsilon)^k}{k!}e^{-\varepsilon}\iiint_{V_0}\rho d\rho d\theta dz = \frac{(\varepsilon)^k}{k!}e^{-\varepsilon}
\end{aligned}
$$

(68)

For a probability function $f(x)$, the Laplace transform and Poisson transform are as follow:

$$
F(s) = L\{f(x)\} = \int f(x)e^{-sx}dx
$$

(69)

$$P(n) = P\{f(x)\} = \int f(x)\frac{x^n e^{-x}}{n!}dx$$

(70)

Also, the The Laplace transform of the probability function $f(x)$ is also its moment generating function:

$$F(s) = L\{f(x)\} = \int f(x)e^{-sx}dx = \int f(x)(1 - sx + \frac{s^2}{2!}x^2 + \frac{s^3}{3!}x^3 ...)dx$$
$$= \int f(x) - xf(x)s + x^2 f(x)\frac{s^2}{2!} + x^3 f(x)\frac{s^3}{3!}...dx$$
$$= 1 - E(X)s + E(X^2)\frac{s^2}{2!} - E(X^3)\frac{s^3}{3!} + ...$$

(71)

That is,

$$E(X^n) = (-1)^n \left.\frac{\partial^n F(s)}{\partial s^n}\right|_{s=0}$$

(72)

Also,

$$E(X^n) - E(X^{n+1})s + E(X^{n+2})\frac{s^2}{2!} - E(X^{n+3})\frac{s^3}{3!} ... = (-1)^n \left.\frac{\partial^n F(s)}{\partial s^n}\right|_{s=1}$$

(73)

Therefore, derive Eq. (70) further with Eq. (73), we have:

$$P(n) = P\{f(x)\} = \int f(x)\frac{x^n e^{-x}}{n!}dx = \frac{1}{n!}\int x^n f(x)e^{-x}dx = \frac{1}{n!}\int x^n f(x)(1 - x + \frac{x^2}{2!} - \frac{x^3}{3!}...)dx$$
$$= \frac{1}{n!}\int x^n f(x) - x^{n+1} f(x) + \frac{x^{n+2}}{2!} f(x) - \frac{x^{n+3}}{3!} f(x)...dx$$
$$= \frac{1}{n!}\left.\left(E(X^n) - E(X^{n+1})s + E(X^{n+2})\frac{s}{2!} - E(X^{n+3})\frac{s}{3!} + ...\right)\right|_{s=1}$$
$$= \frac{(-1)^n}{n!}\left.\frac{\partial^n F(s)}{\partial s^n}\right|_{s=1}$$

(74)

In short, we can calculate $P(n)\big|_{n=1}$, then find out $F(s)$. With the information of $F(s)$, then we know $p(n)$ with n other than 1.

Here is what we do for Eq. (68) with $k=1$:

$$p^{(1)}_{Homogeneous}(1;V_0,\varepsilon)=\frac{\varepsilon^k}{k!}e^{-\varepsilon}=\varepsilon e^{-\varepsilon}=(-1)\frac{\partial F(s)}{\partial s}\bigg|_{s=1}$$

(75)

With the fact that, the zeroth moment of a probability distribution is equal to one. Also, from Eq. (72) :

$$E(X^0)=(-1)^0\frac{\partial^0 F(s)}{\partial s^0}\bigg|_{s=0}=F(0)=1$$

(76)

One of the solution for $F(s)$ in Eq. (75) is as follow:

$$F(s)=e^{-\varepsilon s}+C$$

(77)

From Eq. (76) and Eq. (77), we can find $C=0$ as follow:

$$F(s)\big|_{s=0}=e^{-\varepsilon s}\big|_{s=0}+C=1+C=1+0=1$$

(78)

With information of C from Eq. (78), the Eq. (77) can be re-written as follow:

$$F(s)=e^{-\varepsilon s}$$

(79)

With information of Laplace transform $F(s)$ from Eq. (79) and the relationship between photon counting histogram and $F(s)$ in Eq. (74), we have:

$$p^{(1)}_{Homogeneous}(k;V_0,\varepsilon)=\frac{(-1)^k}{k!}\frac{\partial^k F(s)}{\partial s^k}\bigg|_{s=1}=\frac{(-1)^k}{k!}(-1)^k\varepsilon^k e^{-\varepsilon s}\bigg|_{s=1}=\frac{\varepsilon^k}{k!}e^{-\varepsilon}$$, for $k\geq 0$.

(80)

For N independent particles, the properties will be:

$$p_{Homogeneous}^{(N)}(k;V_0,\varepsilon)=\frac{(-1)^k}{k!}\frac{\partial^k F^N(s)}{\partial s^k}\bigg|_{s=1}=\frac{(-1)^k}{k!}(-1)^k(N\varepsilon)^k e^{-N\varepsilon s}\bigg|_{s=1}=\frac{(N\varepsilon)^k}{k!}e^{-N\varepsilon}$$

(81)

The PCH for a homogeneous distributed brightness is experessed as

$$\prod\left(k;\overline{N},\varepsilon\right)=\sum_{N=0}^{\infty}p(N)\,p(k|N)=\sum_{N=0}^{\infty}p(N)\,p_{Homogeneous}^{(N)}(k;V_0,\varepsilon)$$
$$=\sum_{N=0}^{\infty}\frac{\left(\overline{N}\right)^N}{N!}e^{-\overline{N}}\frac{(N\varepsilon)^k}{k!}e^{-N\varepsilon}$$

(82)

where $p(N)$ is the Poissonian distribution of the number of molecules with the mean value $\overline{N}$, $p(k|N)$ is the conditional distribution of the number of photon counts, provided there are N molecules inside the confocal volume, which is also a Poissonian distribution with the mean value $N\varepsilon$.

Instead of evaluating Eq. (82), we can use the moment generating function to determine the PCH as following relationship:

$$Q(s)=\sum_{k=0}^{\infty}\prod\left(k;\overline{N},\varepsilon\right)e^{-sk}=\sum_{k=0}^{\infty}\sum_{N=0}^{\infty}\frac{\left(\overline{N}\right)^N}{N!}e^{-\overline{N}}\frac{(N\varepsilon)^k}{k!}e^{-N\varepsilon}e^{-sk}$$
$$=e^{-\overline{N}}\sum_{N=0}^{\infty}\frac{\left(\overline{N}\right)^N}{N!}e^{-N\varepsilon}\sum_{k=0}^{\infty}\frac{(N\varepsilon)^k}{k!}e^{-sk}=e^{-\overline{N}}\sum_{N=0}^{\infty}\frac{\left(\overline{N}\right)^N}{N!}e^{-N\varepsilon}\sum_{k=0}^{\infty}\frac{\left(N\varepsilon e^{-s}\right)^k}{k!}$$
$$=e^{-\overline{N}}\sum_{N=0}^{\infty}\frac{\left(\overline{N}\right)^N}{N!}e^{-N\varepsilon}e^{N\varepsilon e^{-s}}=e^{-\overline{N}}\sum_{N=0}^{\infty}\frac{\left(\overline{N}e^{-\varepsilon}e^{\varepsilon e^{-s}}\right)^N}{N!}=e^{-\overline{N}}\sum_{N=0}^{\infty}\frac{\left(\overline{N}e^{-\varepsilon(1-e^{-s})}\right)^N}{N!}$$
$$=e^{-\overline{N}}e^{\overline{N}e^{-\varepsilon(1-e^{-s})}}=e^{(e^{-\varepsilon(1-e^{-s})}-1)\overline{N}}$$

by assuming that, $1-e^{-s}=1-\left(1-s+\frac{s^2}{2!}-\frac{s^3}{3!}...\right)\cong 1-(1-s)=s$

$$=e^{(e^{-s\varepsilon}-1)\overline{N}}$$

(83)

Also,

$$Q(s)=\sum_{k=0}^{\infty}\prod\left(k;\overline{N},\varepsilon\right)e^{-sk}=\sum_{k=0}^{\infty}\prod\left(k;\overline{N},\varepsilon\right)\left(1-ks+\frac{k^2}{2!}s^2-\frac{k^3}{3!}s^3...\right)$$
$$=\sum_{k=0}^{\infty}\left(\prod\left(k;\overline{N},\varepsilon\right)-k\prod\left(k;\overline{N},\varepsilon\right)s+\frac{k^2}{2!}\prod\left(k;\overline{N},\varepsilon\right)s^2-\frac{k^3}{3!}\prod\left(k;\overline{N},\varepsilon\right)s^3...\right)$$

(84)

Therefore,

$$\prod\left(k;\overline{N},\varepsilon\right)=\frac{(-1)^k}{k!}\frac{\partial^k}{\partial s^k}Q(s)$$

(85)

b. Gaussian-Lorentzian Model

For the two photon excitation, the Gaussian-Lorentzian point spread function is as follow:

$$\overline{PSF}_{2GL}(\rho,z)=\frac{4w_0{}^4}{\pi^2 w^4(z)}\exp\left(-\frac{4(\rho^2)}{w^2(z)}\right)$$

(86)

Where $w^2(z)=w_0{}^2\left(1+\left(\frac{z}{z_R}\right)^2\right)$, and $z_R=\frac{\pi w_0{}^2}{\lambda}$

(87)

Insert Eq. (86) and Eq. (87) into Eq. (66), we have:

$$p_{2GL}^{(1)}(k;V_0,\varepsilon)=\frac{1}{V_0}\int_{V_0}Poi(k,\varepsilon\overline{PSF}_{2GL}(\vec{r}))d\vec{r}=\frac{1}{V_0}\int_{-\infty}^{\infty}\int_0^{2\pi}\int_0^{\infty}Poi(k,\varepsilon\overline{PSF}_{2GL}(\rho,z))\rho d\rho d\theta dz$$

$$=\frac{1}{V_0}\int_{-\infty}^{\infty}\int_0^{2\pi}\int_0^{\infty}Poi(k,\varepsilon\frac{4w_0{}^4}{\pi^2 w^4(z)}\exp\left(-\frac{4(\rho^2)}{w^2(z)}\right))\rho d\rho d\theta dz$$

$$=\frac{1}{V_0}\int_{-\infty}^{\infty}\int_0^{2\pi}\int_0^{\infty}\frac{\left(\varepsilon\frac{4w_0{}^4}{\pi^2 w^4(z)}\exp\left(-\frac{4(\rho^2)}{w^2(z)}\right)\right)^k}{k!}e^{-\varepsilon\frac{4w_0{}^4}{\pi^2 w^4(z)}\exp\left(-\frac{4(\rho^2)}{w^2(z)}\right)}\rho d\rho d\theta dz$$

Let $t=\varepsilon\frac{4w_0{}^4}{\pi^2 w^4(z)}\exp\left(-\frac{4(\rho^2)}{w^2(z)}\right)$,

Then $dt=\varepsilon\frac{4w_0{}^4}{\pi^2 w^4(z)}\exp\left(-\frac{4(\rho^2)}{w^2(z)}\right)\left(\frac{-8\rho}{w^2(z)}\right)d\rho$, and $\rho=\Big|_0^{\infty}$, $t=\Big|_{\varepsilon\frac{4w_0{}^4}{\pi^2 w^4(z)}}^{0}$

$$= \frac{1}{V_0}\int_{-\infty}^{\infty}\int_0^{2\pi}\int_0^{\infty}\frac{\left(\varepsilon\frac{4w_0^{\ 4}}{\pi^2 w^4(z)}\exp\left(-\frac{4(\rho^2)}{w^2(z)}\right)\right)^k}{k!}e^{-\varepsilon\frac{4w_0^{\ 4}}{\pi^2 w^4(z)}\exp\left(-\frac{4(\rho^2)}{w^2(z)}\right)}\rho d\rho d\theta dz$$

$$= \frac{2\pi}{V_0 k!}\int_{-\infty}^{\infty}\int_0^{\varepsilon\frac{4w_0^{\ 4}}{\pi^2 w^4(z)}} t^{k-1}e^{-t}\left(\frac{w^2(z)}{8}\right)dtdz = \frac{\pi}{4V_0 k!}\int_{-\infty}^{\infty} w^2(z)\int_0^{\varepsilon\frac{4w_0^{\ 4}}{\pi^2 w^4(z)}} t^{k-1}e^{-t}dtdz$$

$$= \frac{\pi}{4V_0 k!}\int_{-\infty}^{\infty} w^2(z)\gamma(k,\varepsilon\frac{4w_0^{\ 4}}{\pi^2 w^4(z)})dz = \frac{\pi}{4V_0 k!}\int_{-\infty}^{\infty} w_0^{\ 2}\left(1+\left(\frac{\lambda z}{\pi w_0^{\ 2}}\right)^2\right)\gamma(k,\varepsilon\frac{4w_0^{\ 4}}{\pi^2 w_0^{\ 4}\left(1+\left(\frac{\lambda z}{\pi w_0^{\ 2}}\right)^2\right)^2})dz$$

Let $x = \frac{\lambda z}{\pi w_0^{\ 2}}$; then $dx = \frac{\lambda}{\pi w_0^{\ 2}}dz$

$$= \frac{\pi^2 w_0^{\ 4}}{4\lambda V_0 k!}\int_{-\infty}^{\infty}\left(1+x^2\right)\gamma(k,\frac{4\varepsilon}{\pi^2\left(1+x^2\right)^2})dx$$

$$= \frac{\pi^2 w_0^{\ 4}}{2\lambda V_0 k!}\int_0^{\infty}\left(1+x^2\right)\gamma(k,\frac{4\varepsilon}{\pi^2\left(1+x^2\right)^2})dx$$

(88)

For a probability function $f(x)$, the Laplace transform and Poisson transform are as follow:

$$F(s) = L\{f(x)\} = \int f(x)e^{-sx}dx$$

(89)

$$P(n) = P\{f(x)\} = \int f(x)\frac{x^n e^{-x}}{n!}dx$$

(90)

Also, the Laplace transform of the probability function $f(x)$ is also its moment generating function:

$$F(s) = L\{f(x)\} = \int f(x)e^{-sx}dx = \int f(x)(1 - sx + \frac{s^2}{2!}x^2 + \frac{s^3}{3!}x^3 ...)dx$$

$$= \int f(x) - xf(x)s + x^2 f(x)\frac{s^2}{2!} + x^3 f(x)\frac{s^3}{3!}...dx$$

$$= 1 - E(X)s + E(X^2)\frac{s^2}{2!} - E(X^3)\frac{s^3}{3!} + ...$$

(91)

That is,

$$E(X^n) = (-1)^n \left. \frac{\partial^n F(s)}{\partial s^n} \right|_{s=0}$$

(92)

Also,

$$E(X^n) - E(X^{n+1})s + E(X^{n+2})\frac{s^2}{2!} - E(X^{n+3})\frac{s^3}{3!} \ldots = (-1)^n \left. \frac{\partial^n F(s)}{\partial s^n} \right|_{s=1}$$

(93)

Therefore, derive Eq. (90) further with Eq. (93), we have:

$$P(n) = P\{f(x)\} = \int f(x)\frac{x^n e^{-x}}{n!}dx = \frac{1}{n!}\int x^n f(x) e^{-x} dx = \frac{1}{n!}\int x^n f(x)(1 - x + \frac{x^2}{2!} - \frac{x^3}{3!} \ldots)dx$$

$$= \frac{1}{n!}\int x^n f(x) - x^{n+1} f(x) + \frac{x^{n+2}}{2!} f(x) - \frac{x^{n+3}}{3!} f(x) \ldots dx$$

$$= \frac{1}{n!}\left.\left(E(X^n) - E(X^{n+1})s + E(X^{n+2})\frac{s^2}{2!} - E(X^{n+3})\frac{s^3}{3!} + \ldots \right)\right|_{s=1}$$

$$= \frac{(-1)^n}{n!} \left. \frac{\partial^n F(s)}{\partial s^n} \right|_{s=1}$$

(94)

In short, we can calculate $P(n)|_{n=1}$, then find out $F(s)$. With the information of $F(s)$, then we know $p(n)$ with n other than 1.

Here is what we do for Eq. (88) with $k = 1$:

$$p_{2GL}^{(1)}(1; V_0, \varepsilon) = \frac{\pi^2 w_0^{\ 4}}{2\lambda V_0}\int_0^\infty (1 + x^2)\gamma(1, \frac{4\varepsilon}{\pi^2(1+x^2)^2})dx = (-1)\left.\frac{\partial F(s)}{\partial s}\right|_{s=1}$$

$$= \frac{\pi^2 w_0^{\ 4}}{2\lambda V_0}\int_0^\infty (1 + x^2)\int_0^{\frac{4\varepsilon}{\pi^2(1+x^2)^2}} e^{-t} dt dx = \frac{\pi^2 w_0^{\ 4}}{2\lambda V_0}\int_0^\infty (1 + x^2)\left(-e^{-t}\Big|_0^{\frac{4\varepsilon}{\pi^2(1+x^2)^2}} \right)dx$$

$$= \frac{\pi^2 w_0^{\ 4}}{2\lambda V_0}\int_0^\infty (1 + x^2)\left(1 - e^{-\frac{4\varepsilon}{\pi^2(1+x^2)^2}} \right)dx = \frac{\pi^2 w_0^{\ 4}}{2\lambda V_0}\int_0^\infty (1 + x^2)\left(1 - \sum_{k=0}^{\infty}\frac{1}{k!}\left(-\frac{4\varepsilon}{\pi^2(1+x^2)^2} \right)^k \right)dx$$

$$= -\frac{\pi^2 w_0^{\;4}}{2\lambda V_0}\int_0^\infty \left(1+x^2\right)\sum_{k=1}^{\infty}\frac{1}{k!}\left(-\frac{4\varepsilon}{\pi^2}\right)^k\left(\frac{1}{\left(1+x^2\right)^{2k}}\right)dx$$

$$= -\frac{\pi^2 w_0^{\;4}}{2\lambda V_0}\sum_{k=1}^{\infty}\frac{1}{k!}\left(-\frac{4\varepsilon}{\pi^2}\right)^k\int_0^\infty\left(\frac{1}{\left(1+x^2\right)^{2k-1}}\right)dx$$

Let $x=\tan\theta$, $dx=\sec^2\theta d\theta$, while $x\big|_0^\infty$ and $\theta\big|_0^{\frac{\pi}{2}}$

$$= -\frac{\pi^2 w_0^{\;4}}{2\lambda V_0}\sum_{k=1}^{\infty}\frac{1}{k!}\left(-\frac{4\varepsilon}{\pi^2}\right)^k\int_0^{\frac{\pi}{2}}\frac{\sec^2\theta}{\left(\sec^2\theta\right)^{2k-1}}d\theta = \frac{\pi^2 w_0^{\;4}}{2\lambda V_0}\sum_{k=1}^{\infty}\frac{1}{k!}\left(-\frac{4\varepsilon}{\pi^2}\right)^k\int_0^{\frac{\pi}{2}}\left(\cos^4\theta\right)^{k-1}d\theta$$

Let $k'=k-1$;

$$= -\frac{\pi^2 w_0^{\;4}}{2\lambda V_0}\sum_{k'=0}^{\infty}\frac{1}{(k'+1)!}\left(-\frac{4\varepsilon}{\pi^2}\right)^{k'+1}\int_0^{\frac{\pi}{2}}\left(\cos^4\theta\right)^{k'}d\theta$$

$$= \frac{\pi^2 w_0^{\;4}}{2\lambda V_0}\left(\frac{4\varepsilon}{\pi^2}\right)\sum_{k'=0}^{\infty}\frac{1}{(k'+1)}\frac{1}{k'!}\left(-\frac{4\varepsilon}{\pi^2}\right)^{k'}\int_0^{\frac{\pi}{2}}\left(\cos^4\theta\right)^{k'}d\theta$$

Providing the fact that $\int_0^{\frac{\pi}{2}}\cos^p\theta d\theta = \frac{1}{2}\frac{3}{4}\frac{5}{6}\frac{7}{8}\ldots\frac{(p-1)}{p}\frac{\pi}{2}$, with even p.

$$= \frac{2\varepsilon w_0^{\;4}}{\lambda V_0}\left(\frac{\pi}{2}\right)\left(1+\frac{1}{2}\frac{1}{1!}\left(-\frac{4\varepsilon}{\pi^2}\right)\frac{1}{2}\frac{3}{4}+\frac{1}{3}\frac{1}{2!}\left(-\frac{4\varepsilon}{\pi^2}\right)^2\frac{1}{2}\frac{3}{4}\frac{5}{6}\frac{7}{8}+\ldots\right)$$

$$= \frac{2\varepsilon w_0^{\;4}}{\lambda V_0}\left(\frac{\pi}{2}\right)\left(1+\frac{1}{2}\frac{1}{1!}\left(-\frac{4}{\pi^2}s\varepsilon\right)\frac{1}{2}\frac{3}{4}+\frac{1}{3}\frac{1}{2!}\left(-\frac{4}{\pi^2}s\varepsilon\right)^2\frac{1}{2}\frac{3}{4}\frac{5}{6}\frac{7}{8}+\ldots\right)\Bigg|_{s=1}$$

$$= \frac{2\varepsilon w_0^{\;4}}{\lambda V_0}\left(\frac{\pi}{2}\right)\left(1+\frac{1}{2}\frac{1}{1!}\left(-\frac{4}{\pi^2}s\varepsilon\right)\frac{\frac{1}{4}}{\frac{2}{4}}\frac{\frac{3}{4}}{\frac{4}{4}}+\frac{1}{3}\frac{1}{2!}\left(-\frac{4}{\pi^2}s\varepsilon\right)^2\frac{\frac{1}{4}}{\frac{2}{4}}\frac{\frac{3}{4}}{\frac{4}{4}}\frac{\frac{5}{4}}{\frac{6}{4}}\frac{\frac{7}{4}}{\frac{8}{4}}+\ldots\right)\Bigg|_{s=1}$$

$$= \frac{2\varepsilon w_0^{\;4}}{\lambda V_0}\left(\frac{\pi}{2}\right)\left(1+\frac{1}{1!}\left(-\frac{4}{\pi^2}s\varepsilon\right)\frac{\frac{1}{4}}{\frac{1}{2}}\frac{\frac{3}{4}}{1}\frac{1}{2}+\frac{1}{2!}\left(-\frac{4}{\pi^2}s\varepsilon\right)^2\frac{\frac{1}{4}}{\frac{1}{2}}\frac{\frac{3}{4}}{1}\frac{\frac{5}{4}}{\frac{3}{2}}\frac{\frac{7}{4}}{2}\frac{1}{3}+\ldots\right)\Bigg|_{s=1}$$

$$= \frac{\varepsilon\pi w_0^{\;4}}{\lambda V_0}\sum_{k=0}^{\infty}\frac{\left(\frac{1}{4}\right)_k\left(\frac{3}{4}\right)_k}{\left(\frac{1}{2}\right)_k(2)_k}\frac{\left(-\frac{4}{\pi^2}s\varepsilon\right)^k}{k!}\Bigg|_{s=1} = \frac{\varepsilon\pi w_0^{\;4}}{\lambda V_0}\,{}_2F_2\left[\begin{matrix}1/4,3/4\\1/2,2\end{matrix}\middle|-\frac{4}{\pi^2}s\varepsilon\right]\Bigg|_{s=1}$$

$$= \varepsilon r\left({}_2F_2\left[\begin{matrix}1/4,3/4\\1/2,2\end{matrix}\middle|-\frac{4}{\pi^2}s\varepsilon\right]\right)\Bigg|_{s=1}$$

with $V_{PSF} = w_0^{\;2}z_R = w_0^{\;2}\frac{\pi w_0^{\;2}}{\lambda} = \frac{\pi w_0^{\;4}}{\lambda}$, and $r=\frac{V_{PSF}}{V_0}$

(95)

The generalized hypergeometric function ${}_pF_q$ is defined as:

$$ {}_pF_q(\alpha_1,\alpha_2,...\alpha_p;\rho_1,\rho_2,...,\rho_q;z) = {}_pF_q\left(\begin{matrix}\alpha_1,\alpha_2,...\alpha_p \\ \rho_1,\rho_2,...,\rho_q\end{matrix}\middle| z\right) = \sum_{k=0}^{\infty}\frac{\prod_{h=1}^{p}(\alpha_h)_k}{\prod_{h=1}^{q}(\rho_h)_k}\frac{z^k}{k!} $$

(96)

With the fact that, the zeroth moment of a probability distribution is equal to one. Also, from Eq. (92) :

$$ E(X^0) = (-1)^0\left.\frac{\partial^0 F(s)}{\partial s^0}\right|_{s=0} = F(0) = 1 $$

(97)

For short of Eq. (95) :

$$ p_{2GL}^{(1)}(1;V_0,\varepsilon) = \varepsilon r\left({}_2F_2\left[\begin{matrix}1/4,3/4 \\ 1/2,2\end{matrix}\middle| -\frac{4}{\pi^2}s\varepsilon\right]\right)\Bigg|_{s=1} = (-1)\left.\frac{\partial F(s)}{\partial s}\right|_{s=1} $$

(98)

From the information of Eq. (97) and (98), we have:

$$ (-1)\frac{\partial F(s)}{\partial s} = \varepsilon r\left({}_2F_2\left[\begin{matrix}1/4,3/4 \\ 1/2,2\end{matrix}\middle| -\frac{4}{\pi^2}s\varepsilon\right]\right) = \varepsilon r\sum_{k=0}^{\infty}\frac{\left(\frac{1}{4}\right)_k\left(\frac{3}{4}\right)_k}{\left(\frac{1}{2}\right)_k(2)_k}\frac{\left(-\frac{4}{\pi^2}s\varepsilon\right)^k}{k!} $$

(99)

Integrate Eq. (99) on both side:

$$ F(s) = -\varepsilon r\sum_{k=0}^{\infty}\frac{\left(\frac{1}{4}\right)_k\left(\frac{3}{4}\right)_k}{\left(\frac{1}{2}\right)_k(2)_k}\frac{\left(-\frac{4}{\pi^2}s\varepsilon\right)^k\frac{s}{k+1}}{k!} + C $$

$$ = r\left(\frac{\pi^2}{4}\right)\sum_{k=0}^{\infty}\frac{\left(\frac{1}{4}\right)_k\left(\frac{3}{4}\right)_k}{\left(\frac{1}{2}\right)_k(2)_k}\frac{\left(-\frac{4}{\pi^2}s\varepsilon\right)^k\left(-\frac{4}{\pi^2}\right)\frac{s\varepsilon}{k+1}}{k!} + C = r\left(\frac{\pi^2}{4}\right)\sum_{k=0}^{\infty}\frac{\left(\frac{1}{4}\right)_k\left(\frac{3}{4}\right)_k}{\left(\frac{1}{2}\right)_k(2)_k}\frac{\left(-\frac{4}{\pi^2}s\varepsilon\right)^{k+1}}{(k+1)!} + C $$

$$= r\left(\frac{\pi^2}{4}\right)\left(-\frac{8}{3}\right)\sum_{k=0}^{\infty}\frac{\left(-\frac{3}{4}\right)\left(-\frac{1}{4}\right)}{\left(-\frac{1}{2}\right)(1)}\frac{\left(\frac{1}{4}\right)_k\left(\frac{3}{4}\right)_k}{\left(\frac{1}{2}\right)_k(2)_k}\frac{\left(-\frac{4}{\pi^2}s\varepsilon\right)^{k+1}}{(k+1)!}+C$$

$$= r\left(\frac{\pi^2}{4}\right)\left(-\frac{8}{3}\right)\sum_{k=0}^{\infty}\frac{\left(-\frac{3}{4}\right)_{k+1}\left(-\frac{1}{4}\right)_{k+1}}{\left(-\frac{1}{2}\right)_{k+1}(1)_{k+1}}\frac{\left(-\frac{4}{\pi^2}s\varepsilon\right)^{k+1}}{(k+1)!}+C$$

Let $k' = k+1$

$$= r\left(\frac{\pi^2}{4}\right)\left(-\frac{8}{3}\right)\sum_{k'=1}^{\infty}\frac{\left(-\frac{3}{4}\right)_{k'}\left(-\frac{1}{4}\right)_{k'}}{\left(-\frac{1}{2}\right)_{k'}(1)_{k'}}\frac{\left(-\frac{4}{\pi^2}s\varepsilon\right)^{k'}}{(k')!}+C$$

$$= r\left(\frac{\pi^2}{4}\right)\left(-\frac{8}{3}\right)\sum_{k'=0}^{\infty}\frac{\left(-\frac{3}{4}\right)_{k'}\left(-\frac{1}{4}\right)_{k'}}{\left(-\frac{1}{2}\right)_{k'}(1)_{k'}}\frac{\left(-\frac{4}{\pi^2}s\varepsilon\right)^{k'}}{(k')!}+r\left(\frac{\pi^2}{4}\right)\left(\frac{8}{3}\right)+C$$

$$= r\left(\frac{\pi^2}{4}\right)\left(-\frac{8}{3}\right)\left({}_2F_2\left[\begin{matrix}-3/4,-1/4\\ -1/2,1\end{matrix}\middle|-\frac{4}{\pi^2}s\varepsilon\right]\right)+r\left(\frac{\pi^2}{4}\right)\left(\frac{8}{3}\right)+C$$

(100)

From Eq. (97) and Eq. (100), we can find C as follow:

$$F(s)\big|_{s=0} = r\left(\frac{\pi^2}{4}\right)\left(-\frac{8}{3}\right)\left({}_2F_2\left[\begin{matrix}-3/4,-1/4\\ -1/2,1\end{matrix}\middle|-\frac{4}{\pi^2}s\varepsilon\right]\right)\Bigg|_{s=0}+r\left(\frac{\pi^2}{4}\right)\left(\frac{8}{3}\right)+C = C = 1$$

(101)

With information of C from Eq. (101), the Eq. (100) can be re-written as follow:

$$F(s) = r\left(\frac{\pi^2}{4}\right)\left(-\frac{8}{3}\right)\left({}_2F_2\left[\begin{matrix}-3/4,-1/4\\ -1/2,1\end{matrix}\middle|-\frac{4}{\pi^2}s\varepsilon\right]\right)+r\left(\frac{\pi^2}{4}\right)\left(\frac{8}{3}\right)+1$$

$$= 1 + r\left(\frac{\pi^2}{4}\right)\left(\frac{8}{3}\right)\left\{1-{}_2F_2\left[\begin{matrix}-3/4,-1/4\\ -1/2,1\end{matrix}\middle|-\frac{4}{\pi^2}s\varepsilon\right]\right\}$$

(102)

With information of Laplace transform $F(s)$ from Eq. (102) and the relationship between photon counting histogram and $F(s)$ in Eq. (94), we have:

$$p_{2GL}^{(1)}(k;V_0,\varepsilon)=\frac{(-1)^k}{k!}\frac{\partial^k F(s)}{\partial s^k}\bigg|_{s=1}\text{, for } k=0\text{,}$$

$$=p_{2GL}^{(1)}(0;V_0,\varepsilon)=F(s)\big|_{s=1}=1+r\left(\frac{\pi^2}{4}\right)\left(\frac{8}{3}\right)\left\{1-{}_2F_2\left[\begin{matrix}-3/4,-1/4\\-1/2,1\end{matrix}\middle|-\frac{4}{\pi^2}\varepsilon\right]\right\}$$

(103)

and,

$$p_{2GL}^{(1)}(k;V_0,\varepsilon)=\frac{(-1)^k}{k!}\frac{\partial^k F(s)}{\partial s^k}\bigg|_{s=1}\text{, for } k\geq 1\text{,}$$

$$=-\frac{(-1)^k}{k!}r\left(\frac{\pi^2}{4}\right)\left(\frac{8}{3}\right)\frac{\partial^k}{\partial s^k}{}_2F_2\left[\begin{matrix}-3/4,-1/4\\-1/2,1\end{matrix}\middle|-\frac{4}{\pi^2}s\varepsilon\right]\bigg|_{s=1}$$

$$=-\frac{(-1)^k}{k!}r\left(\frac{\pi^2}{4}\right)\left(\frac{8}{3}\right)\frac{\partial^k}{\partial s^k}\sum_{n=0}^{\infty}\frac{\left(-\frac{3}{4}\right)_n\left(-\frac{1}{4}\right)_n}{\left(-\frac{1}{2}\right)_n(1)_n}\frac{\left(-\frac{4}{\pi^2}s\varepsilon\right)^n}{n!}\Bigg|_{s=1}$$

$$=-\frac{(-1)^k}{k!}r\left(\frac{\pi^2}{4}\right)\left(\frac{8}{3}\right)\frac{\left(-\frac{3}{4}\right)_k\left(-\frac{1}{4}\right)_k}{\left(-\frac{1}{2}\right)_k(1)_k}\sum_{n=k}^{\infty}\frac{\left(-\frac{3}{4}+k\right)_{n-k}\left(-\frac{1}{4}+k\right)_{n-k}}{\left(-\frac{1}{2}+k\right)_{n-k}(1+k)_{n-k}}\frac{\left(-\frac{4}{\pi^2}\varepsilon\right)^n\frac{n!(s)^{n-k}}{(n-k)!}}{n!}\Bigg|_{s=1}$$

$$=-\frac{(-1)^k}{k!}r\left(\frac{\pi^2}{4}\right)\left(\frac{8}{3}\right)\left(-\frac{4}{\pi^2}\varepsilon\right)^k\frac{\left(-\frac{3}{4}\right)_k\left(-\frac{1}{4}\right)_k}{\left(-\frac{1}{2}\right)_k(1)_k}\sum_{n=k}^{\infty}\frac{\left(-\frac{3}{4}+k\right)_{n-k}\left(-\frac{1}{4}+k\right)_{n-k}}{\left(-\frac{1}{2}+k\right)_{n-k}(1+k)_{n-k}}\frac{\left(-\frac{4}{\pi^2}\varepsilon\right)^{n-k}(s)^{n-k}}{(n-k)!}\Bigg|_{s=1}$$

Let $n'=n-k$

$$=-\frac{\varepsilon^k}{k!}r\left(\frac{8}{3}\right)\left(\frac{4}{\pi^2}\right)^{k-1}\frac{\left(-\frac{3}{4}\right)_k\left(-\frac{1}{4}\right)_k}{\left(-\frac{1}{2}\right)_k(1)_k}\sum_{n'=0}^{\infty}\frac{\left(-\frac{3}{4}+k\right)_{n'}\left(-\frac{1}{4}+k\right)_{n'}}{\left(-\frac{1}{2}+k\right)_{n'}(1+k)_{n'}}\frac{\left(-\frac{4}{\pi^2}s\varepsilon\right)^{n'}}{(n')!}\Bigg|_{s=1}$$

$$=-\left(\frac{2}{\pi}\right)^{2(k-1)}\frac{\varepsilon^k}{k!}r\left(\frac{8}{3}\right)\frac{\left(-\frac{3}{4}\right)_k\left(-\frac{1}{4}\right)_k}{\left(-\frac{1}{2}\right)_k(1)_k}\left({}_2F_2\left[\begin{matrix}-3/4+k,-1/4+k\\-1/2+k,1+k\end{matrix}\middle|-\frac{4}{\pi^2}s\varepsilon\right]\right)\Bigg|_{s=1}$$

$$=-\left(\frac{2}{\pi}\right)^{2(k-1)}\frac{\varepsilon^k}{k!}r\left(\frac{8}{3}\right)\frac{\left(-\frac{3}{4}\right)_k\left(-\frac{1}{4}\right)_k}{\left(-\frac{1}{2}\right)_k(1)_k}\left({}_2F_2\left[\begin{matrix}-3/4+k,-1/4+k\\-1/2+k,1+k\end{matrix}\middle|-\frac{4}{\pi^2}\varepsilon\right]\right)$$

(104)

For N independent particles, the properties will be:

$$\left.\frac{\partial^k}{\partial s^k}Y\right|_{s=1} = \frac{\partial^k}{\partial s^k}\,{}_2F_2\left[\left.\begin{matrix}-3/4,-1/4\\-1/2,1\end{matrix}\right|-\frac{4}{\pi^2}s\varepsilon\right]\Bigg|_{s=1}$$

$$=\left(-\frac{4}{\pi^2}\varepsilon\right)^k\frac{\left(-\frac{3}{4}\right)_k\left(-\frac{1}{4}\right)_k}{\left(-\frac{1}{2}\right)_k(1)_k}\left({}_2F_2\left[\left.\begin{matrix}-3/4+k,-1/4+k\\-1/2+k,1+k\end{matrix}\right|-\frac{4}{\pi^2}s\varepsilon\right]\right)\Bigg|_{s=1}$$

Let $Y_k={}_2F_2\left[\left.\begin{matrix}-3/4+k,-1/4+k\\-1/2+k,1+k\end{matrix}\right|-\frac{4}{\pi^2}s\varepsilon\right]$, and $a_k=\left(-\frac{4}{\pi^2}\right)^k\frac{\left(-\frac{3}{4}\right)_k\left(-\frac{1}{4}\right)_k}{\left(-\frac{1}{2}\right)_k(1)_k}$

$$=a_k\varepsilon^kY_k\Big|_{s=1}$$

(105)

For Y^N, we use polynomial expansion as follow:

$$Y^N={}_2F_2\left[\left.\begin{matrix}-3/4,-1/4\\-1/2,1\end{matrix}\right|-\frac{4}{\pi^2}s\varepsilon\right]^N=\left(\sum_{n=0}^{\infty}\frac{\left(-\frac{3}{4}\right)_n\left(-\frac{1}{4}\right)_n}{\left(-\frac{1}{2}\right)_n(1)_n}\frac{\left(-\frac{4}{\pi^2}s\varepsilon\right)^n}{n!}\right)^N$$

$$=\left(1+\sum_{n=1}^{\infty}a_n\varepsilon^ks^n\right)^N$$

(106)

where, $a_n=\frac{\left(-\frac{3}{4}\right)_n\left(-\frac{1}{4}\right)_n}{\left(-\frac{1}{2}\right)_n(1)_n}\frac{\left(-\frac{4}{\pi^2}\right)^n}{n!}$

(107)

Use Eq. (106) for $(1-Y)^N$, we have:

$$(1-Y)^N=\left(-\sum_{n=1}^{\infty}a_n\varepsilon^ks^n\right)^N=(-1)^N\left(a_1\varepsilon s+a_2\varepsilon^2s^2+\ldots\right)^N$$

(108)

Therefore, the probability of k photon counts with N particles and particle brightness ε is as follow:

For $k = 0$;

$$p_{2GL}^{(N)}(k;V_0,\varepsilon) = \frac{(-1)^k}{k!}\frac{\partial^k F^N(s)}{\partial s^k}\bigg|_{s=1}$$

$$= p_{2GL}^{(N)}(0;V_0,\varepsilon) = F^N(s)\big|_{s=1}$$

$$= \left(1 + r\left(\frac{\pi^2}{4}\right)\left(\frac{8}{3}\right)\left\{1 - {}_2F_2\left[\begin{matrix}-3/4,-1/4\\ -1/2,1\end{matrix}\middle| -\frac{4}{\pi^2}s\varepsilon\right]\right\}\right)^N\Bigg|_{s=1} = \left(1 + r\left(\frac{2\pi^2}{3}\right)(1-Y)\right)^N\Bigg|_{s=1}$$

$$= \left(1 + r\left(\frac{2\pi^2}{3}\right)\left(-\sum_{n=1}^{\infty} a_n \varepsilon^k s^n\right)\right)^N\Bigg|_{s=1} = \left(1 + \sum_{n=1}^{\infty} - r\left(\frac{2\pi^2}{3}\right)a_n \varepsilon^k s^n\right)^N\Bigg|_{s=1}$$

Let $b_n = -r\left(\frac{2\pi^2}{3}\right)a_n$

$$= \left(1 + \sum_{n=1}^{\infty} b_n \varepsilon^k s^n\right)^N\Bigg|_{s=1} = \left(1 + b_1\varepsilon s + b_2\varepsilon^2 s^2 + \dots\right)^N\Big|_{s=1}$$

Let $c_{m,N}$ be the m th coefficient of polynomial expansion of power of N

$$= \left(1 + c_{1,N}\varepsilon s + c_{2,N}\varepsilon^2 s^2 + \dots\right)\Big|_{s=1} = \left(1 + c_{1,N}\varepsilon + c_{2,N}\varepsilon^2 + \dots\right)$$

(109)

For $k \geq 1$;

$$p_{2GL}^{(N)}(k;V_0,\varepsilon) = \frac{(-1)^k}{k!}\frac{\partial^k F^N(s)}{\partial s^k}\bigg|_{s=1}$$

$$= \frac{(-1)^k}{k!}\frac{\partial^k}{\partial s^k}\left(1 + r\left(\frac{2\pi^2}{3}\right)(1-Y)\right)^N\Bigg|_{s=1}$$

with same definition as in Eq. (109)

$$= \frac{(-1)^k}{k!}\frac{\partial^k}{\partial s^k}\left(1 + c_{1,N}\varepsilon s + c_{2,N}\varepsilon^2 s^2 + \dots\right)\Big|_{s=1}$$

$$= \frac{(-1)^k}{k!}\left(k!c_{k,N}\varepsilon^k + \frac{(k+1)!}{1!}c_{k+1,N}\varepsilon^{k+1}s + \frac{(k+2)!}{2!}c_{k+2,N}\varepsilon^{k+2}s^2 + \dots\right)\Bigg|_{s=1}$$

$$= \frac{(-1)^k}{k!}\left(k!c_{k,N}\varepsilon^k + \frac{(k+1)!}{1!}c_{k+1,N}\varepsilon^{k+1} + \frac{(k+2)!}{2!}c_{k+2,N}\varepsilon^{k+2} + \dots\right)$$

(110)

Finally, the PCH for a Gaussian-Lorentzian brightness profile is as follow:

For $k=0$;

$$\prod\left(k;\overline{N},\varepsilon\right)=\sum_{N=0}^{\infty}p(N)p(k|N)=\sum_{N=0}^{\infty}p(N)p_{2GL}^{(N)}(k;V_0,\varepsilon)$$

$$=\prod\left(0;\overline{N},\varepsilon\right)=\sum_{N=0}^{\infty}p(N)p(0|N)=\sum_{N=0}^{\infty}p(N)p_{2GL}^{(N)}(0;V_0,\varepsilon)$$

with same definition of $c_{m,N}$ as in Eq. (109)

$$=\sum_{n=0}^{\infty}\frac{\left(\overline{N}\right)^n}{n!}e^{-\overline{N}}\left(1+r\left(\frac{2\pi^2}{3}\right)(1-Y)\right)^n\Bigg|_{s=1}=\sum_{n=0}^{\infty}\frac{\left(\overline{N}\right)^n}{n!}e^{-\overline{N}}\left(1+c_{1,n}\varepsilon+c_{2,n}\varepsilon^2+\ldots\right)$$

$$=\sum_{n=0}^{\infty}\frac{\left(\overline{N}\right)^n}{n!}e^{-\overline{N}}\left(1+\sum_{m=1}^{\infty}c_{m,n}\varepsilon^m\right)$$

(111)

For $k\geq 1$;

$$\prod\left(k;\overline{N},\varepsilon\right)=\sum_{N=0}^{\infty}p(N)p(k|N)=\sum_{N=0}^{\infty}p(N)p_{2GL}^{(N)}(k;V_0,\varepsilon)$$

with same definition of $c_{m,N}$ as in Eq. (109)

$$=\sum_{n=0}^{\infty}\frac{\left(\overline{N}\right)^n}{n!}e^{-\overline{N}}\frac{(-1)^k}{k!}\left(k!c_{k,n}\varepsilon^k+\frac{(k+1)!}{1!}c_{k+1,n}\varepsilon^{k+1}+\frac{(k+2)!}{2!}c_{k+2,n}\varepsilon^{k+2}+\ldots\right)$$

$$=\sum_{n=0}^{\infty}\frac{\left(\overline{N}\right)^n}{n!}e^{-\overline{N}}\frac{(-1)^k}{k!}\sum_{m=0}^{\infty}\frac{(k+m)!}{m!}c_{k+m,n}\varepsilon^{k+m}$$

(112)

where $p(N)$ is the Poissonian distribution of the number of molecules with the mean value $\overline{N}$, $p(k|N)$ is the conditional distribution of the number of photon counts, provided there are N molecules inside the confocal volume, which is also a Poissonian distribution with the Gaussian-Lorentzian intensity profile.

3. Data Fitting

a. Gaussian Model

The Model to be fitted is

$$y = y(x;\mathbf{a})$$

(113)

and the χ^2 merit function is

$$\chi^2(\mathbf{a}) = \sum_{i=1}^{N}\left[\frac{y_i - y(x_i;\mathbf{a})}{\sigma_i}\right]^2$$

(114)

By defining

$$\beta_k \equiv -\frac{1}{2}\frac{\partial\chi^2}{\partial a_k} = \sum_{i=1}^{N}\frac{\left[y_i - y(x_i;\mathbf{a})\right]}{\sigma_i^{\ 2}}\frac{\partial y(x_i;\mathbf{a})}{\partial a_k} \qquad k = 1,2,\dots,M$$

(115)

and

$$\alpha_{kl} \equiv \frac{1}{2}\frac{\partial^2\chi^2}{\partial a_k \partial a_l} = \sum_{i=1}^{N}\frac{1}{\sigma_i^{\ 2}}\left[\frac{\partial y(x_i;\mathbf{a})}{\partial a_k}\frac{\partial y(x_i;\mathbf{a})}{\partial a_l} - \left[y_i - y(x_i;\mathbf{a})\right]\frac{\partial^2 y(x_i;\mathbf{a})}{\partial a_k \partial a_l}\right] \approx \sum_{i=1}^{N}\frac{1}{\sigma_i^{\ 2}}\left[\frac{\partial y(x_i;\mathbf{a})}{\partial a_k}\frac{\partial y(x_i;\mathbf{a})}{\partial a_l}\right]$$

(116)

We can relate the first derivative β_k and second derivative α_k as follow:

$$\sum_{l=1}^{M}\alpha_{kl}\delta a_l = \beta_k$$

(117)

The recipe is as follows:

Step 1. Compute $\chi^2(\mathbf{a})$.

Step 2. Pick a modest value for λ, for example λ=0.001.

Step 3. Solve the linear equations $\sum_{l=1}^{M}\alpha'_{kl}\delta a_l = \beta_k$ for $\delta\mathbf{a}$ and evaluate $\chi^2(\mathbf{a}+\delta\mathbf{a})$,

where $\alpha'_{jj} \equiv \alpha_{jj}(1+\lambda) \qquad and \qquad \alpha'_{jk} \equiv \alpha_{kj} \qquad (j \neq k)$.

Step 4. If $\chi^2(\mathbf{a}+\delta\mathbf{a}) \geq \chi^2(\mathbf{a})$, increase λ by a factor of 10 and go back to Step 3.

Step 5. If $\chi^2(\mathbf{a}+\delta\mathbf{a}) < \chi^2(\mathbf{a})$, decrease λ by a factor of 10, update the trial solution $\mathbf{a} \leftarrow \mathbf{a}+\delta\mathbf{a}$ and go back to Step 3.

Stop iterating on the first or second occasion that χ^2 decreases by a negligible amount like10^{-3}.

Set λ=0 and compute the matrix $[\mathbf{C}] \equiv [\alpha]^{-1}$,which is the estimated covariance matrix of the standard errors in the fitted parameters **a**.

In 3D Gaussian and one photon excitation, We can rewrite Eq. (22) as follow:

$$G(\tau) = \frac{1}{\pi\sqrt{\pi} < C > (\omega_0{}^2 + 4D\tau)\sqrt{z_0{}^2 + 4D\tau}}$$

(118)

Where

$$< C >= \frac{1}{\pi\sqrt{\pi}\omega_0{}^2 z_0 G(0)}$$

(119)

For up to three components, we have

$$G(\tau) = \left(\frac{1}{\pi\sqrt{\pi} < C >}\right) \times \left(\frac{f_1}{(\omega_0{}^2 + 4D_1\tau)\sqrt{z_0{}^2 + 4D_1\tau}} + \frac{f_2}{(\omega_0{}^2 + 4D_2\tau)\sqrt{z_0{}^2 + 4D_2\tau}} + \frac{f_3}{(\omega_0{}^2 + 4D_3\tau)\sqrt{z_0{}^2 + 4D_3\tau}}\right)$$

(120)

Consider the flow in addition to the diffusion of the sample, we have

$$G(\tau) = \left(\frac{1}{\pi\sqrt{\pi} < C >}\right) \times \left(\frac{f_1}{(\omega_0{}^2 + 4D_1\tau)\sqrt{z_0{}^2 + 4D_1\tau}} \exp\left[\frac{-(V_f\tau)^3}{(\omega_0{}^2 + 4D_1\tau)\sqrt{z_0{}^2 + 4D_1\tau}}\right] + \frac{f_2}{(\omega_0{}^2 + 4D_2\tau)\sqrt{z_0{}^2 + 4D_2\tau}} \exp\left[\frac{-(V_f\tau)^3}{(\omega_0{}^2 + 4D_2\tau)\sqrt{z_0{}^2 + 4D_2\tau}}\right] + \frac{f_3}{(\omega_0{}^2 + 4D_3\tau)\sqrt{z_0{}^2 + 4D_3\tau}} \exp\left[\frac{-(V_f\tau)^3}{(\omega_0{}^2 + 4D_3\tau)\sqrt{z_0{}^2 + 4D_3\tau}}\right]\right)$$

(121)

Define

$$K = \frac{1}{\pi\sqrt{\pi} < C >}$$

$$I_1 = \frac{1}{(\omega_0{}^2 + 4D_1\tau)\sqrt{z_0{}^2 + 4D_1\tau}}$$

$$E_1 = \exp\left[-(V_f\tau)^3 I_1\right]$$

$$\frac{\partial I_1}{\partial D_1} = -2I_1\left[\frac{2\tau}{\omega_0{}^2 + 4D_1\tau} + \frac{\tau}{z_0{}^2 + 4D_1\tau}\right]$$

$$\frac{\partial I_1}{\partial \omega_0} = -2I_1\left[\frac{\omega_0}{\omega_0{}^2 + 4D_1\tau}\right]$$

$$\frac{\partial I_1}{\partial z_0} = -I_1\left[\frac{z_0}{z_0{}^2 + 4D_1\tau}\right]$$

$$\frac{\partial E_1}{\partial D_1} = -E_1\left[(V_1\tau)^3 \frac{\partial I_1}{\partial D_1}\right]$$

$$\frac{\partial E_1}{\partial \omega_0} = -E_1\left[(V_1\tau)^3 \frac{\partial I_1}{\partial \omega_0}\right]$$

$$\frac{\partial E_1}{\partial z_0} = -E_1\left[(V_1\tau)^3 \frac{\partial I_1}{\partial z_0}\right]$$

$$\frac{\partial E_1}{\partial V_1} = -E_1\left[3(V_1\tau)^2 \tau I_1\right]$$

Similar definition to I_2, I_3, E_2, E_3 and we have:

$$G(\tau) = K \times \left(f_1 I_1 E_1 + f_2 I_2 E_2 + f_3 I_3 E_3\right)$$

The derivative equations needed are as follow:

$$\frac{\partial G(\tau)}{\partial < C >} = -\frac{K}{< C >} \times \left(f_1 I_1 E_1 + f_2 I_2 E_2 + f_3 I_3 E_3\right)$$

$$\frac{\partial G(\tau)}{\partial \omega_0} = K \times \left[f_1\left(\frac{\partial I_1}{\partial \omega_0} E_1 + I_1 \frac{\partial E_1}{\partial \omega_0}\right) + f_2\left(\frac{\partial I_2}{\partial \omega_0} E_2 + I_2 \frac{\partial E_2}{\partial \omega_0}\right) + f_3\left(\frac{\partial I_3}{\partial \omega_0} E_3 + I_3 \frac{\partial E_3}{\partial \omega_0}\right)\right]$$

$$\frac{\partial G(\tau)}{\partial z_0} = K \times \left[f_1\left(\frac{\partial I_1}{\partial z_0} E_1 + I_1 \frac{\partial E_1}{\partial z_0}\right) + f_2\left(\frac{\partial I_2}{\partial z_0} E_2 + I_2 \frac{\partial E_2}{\partial z_0}\right) + f_3\left(\frac{\partial I_3}{\partial z_0} E_3 + I_3 \frac{\partial E_3}{\partial z_0}\right)\right]$$

$$\frac{\partial G(\tau)}{\partial f_1} = K \times I_1 E_1$$

$$\frac{\partial G(\tau)}{\partial D_1} = K \times f_1\left(\frac{\partial I_1}{\partial D_1}E_1 + I_1\frac{\partial E_1}{\partial D_1}\right)$$

$$\frac{\partial G(\tau)}{\partial V_1} = K \times f_1 I_1\frac{\partial E_1}{\partial V_1}$$

$$\frac{\partial G(\tau)}{\partial f_2} == K \times I_2 E_2$$

$$\frac{\partial G(\tau)}{\partial D_2} = K \times f_2\left(\frac{\partial I_2}{\partial D_2}E_2 + I_2\frac{\partial E_2}{\partial D_2}\right)$$

$$\frac{\partial G(\tau)}{\partial V_2} = K \times f_2 I_2\frac{\partial E_2}{\partial V_2}$$

$$\frac{\partial G(\tau)}{\partial f_3} == K \times I_3 E_3$$

$$\frac{\partial G(\tau)}{\partial D_3} = K \times f_3\left(\frac{\partial I_3}{\partial D_3}E_3 + I_3\frac{\partial E_3}{\partial D_3}\right)$$

$$\frac{\partial G(\tau)}{\partial V_3} = K \times f_3 I_3\frac{\partial E_3}{\partial V_3}$$

b. Gaussian-Lorentzian Model

Omitted.

c. PCH(Photon Counting Histogram) Analysis, Unity Model

- **For one component:**

$$\prod(k;\overline{N},\varepsilon) = \frac{(-1)^k}{k!}\frac{d^k}{ds^k}Q(s)\bigg|_{s=1} ; \quad where \quad Q(s) = e^{(e^{-s\varepsilon}-1)\overline{N}}$$

For illustration, $k = 0 \ to \ 5$:

$$\prod(0;\overline{N},\varepsilon) = Q(s)\big|_{s=1} = e^{(e^{-\varepsilon}-1)\overline{N}} = Q(1)$$

(122)

$$\prod(1;\overline{N},\varepsilon) = \frac{-1}{1}\frac{d}{ds}Q(s)\big|_{s=1} = -(-\varepsilon\overline{N}e^{-s\varepsilon})Q(s)\big|_{s=1} = (\varepsilon\overline{N}e^{-\varepsilon})Q(1)$$

$$\prod(2;\overline{N},\varepsilon) = \frac{1}{2!}\frac{d^2}{ds^2}Q(s)\Big|_{s=1} = \frac{1}{2}\frac{d}{ds}\left((-\varepsilon\overline{N}e^{-s\varepsilon})Q(s)\right)\Big|_{s=1} = \frac{1}{2}\left(\frac{d(-\varepsilon\overline{N}e^{-s\varepsilon})}{ds}Q(s) + (-\varepsilon\overline{N}e^{-s\varepsilon})\frac{dQ(s)}{ds}\right)\Bigg|_{s=1}$$

$$= \frac{1}{2}\left((\varepsilon^2\overline{N}e^{-s\varepsilon}) + (-\varepsilon\overline{N}e^{-s\varepsilon})^2\right)Q(s)\Big|_{s=1} = \frac{1}{2}\left((\varepsilon\overline{N})^2 e^{-2\varepsilon} + \varepsilon^2\overline{N}e^{-s\varepsilon}\right)Q(1)$$

$$\prod(3;\overline{N},\varepsilon) = \frac{-1}{3!}\frac{d^3}{ds^3}Q(s)\Big|_{s=1} = \frac{-1}{3!}\frac{d}{ds}\left(\left((\varepsilon^2\overline{N}e^{-s\varepsilon}) + (-\varepsilon\overline{N}e^{-s\varepsilon})^2\right)Q(s)\right)\Big|_{s=1}$$

$$= \frac{-1}{3!}\left(\frac{d\left((\varepsilon^2\overline{N}e^{-s\varepsilon}) + (-\varepsilon\overline{N}e^{-s\varepsilon})^2\right)}{ds}Q(s) + \left((\varepsilon^2\overline{N}e^{-s\varepsilon}) + (-\varepsilon\overline{N}e^{-s\varepsilon})^2\right)\frac{dQ(s)}{ds}\right)\Bigg|_{s=1}$$

$$= \frac{-1}{3!}\left((-\varepsilon^3\overline{N}e^{-s\varepsilon} - 2\varepsilon^3\overline{N}^2 e^{-2s\varepsilon}) + \left((\varepsilon^2\overline{N}e^{-s\varepsilon}) + (-\varepsilon\overline{N}e^{-s\varepsilon})^2\right)(-\varepsilon\overline{N}e^{-s\varepsilon})\right)Q(s)\Big|_{s=1}$$

$$= \frac{-1}{3!}\left(-\varepsilon^3\overline{N}e^{-s\varepsilon} - 2\varepsilon^3\overline{N}^2 e^{-2s\varepsilon} - \varepsilon^3\overline{N}^2 e^{-2s\varepsilon} + (-\varepsilon\overline{N}e^{-s\varepsilon})^3\right)Q(s)\Big|_{s=1}$$

$$= \frac{1}{3!}\left((\varepsilon\overline{N})^3 e^{-3\varepsilon} + 3\varepsilon^3\overline{N}^2 e^{-2\varepsilon} + \varepsilon^3\overline{N}e^{-\varepsilon}\right)Q(1)$$

$$\prod(4;\overline{N},\varepsilon) = \frac{1}{4!}\frac{d^4}{ds^4}Q(s)\Big|_{s=1} = \frac{1}{4!}\frac{d}{ds}\left(\left(-\varepsilon^3\overline{N}e^{-s\varepsilon} - 3\varepsilon^3\overline{N}^2 e^{-2s\varepsilon} + (-\varepsilon\overline{N}e^{-s\varepsilon})^3\right)Q(s)\right)\Big|_{s=1}$$

$$= \frac{1}{4!}\left(\begin{array}{l}\dfrac{d\left(-\varepsilon^3\overline{N}e^{-s\varepsilon} - 3\varepsilon^3\overline{N}^2 e^{-2s\varepsilon} + (-\varepsilon\overline{N}e^{-s\varepsilon})^3\right)}{ds}Q(s) + \\ \left(-\varepsilon^3\overline{N}e^{-s\varepsilon} - 3\varepsilon^3\overline{N}^2 e^{-2s\varepsilon} + (-\varepsilon\overline{N}e^{-s\varepsilon})^3\right)\dfrac{dQ(s)}{ds}\end{array}\right)\Bigg|_{s=1}$$

$$= \frac{1}{4!}\left(\begin{array}{l}(\varepsilon^4\overline{N}e^{-s\varepsilon} + 6\varepsilon^4\overline{N}^2 e^{-2s\varepsilon} + 3\varepsilon^4\overline{N}^3 e^{-3s\varepsilon}) + \\ \left(-\varepsilon^3\overline{N}e^{-s\varepsilon} - 3\varepsilon^3\overline{N}^2 e^{-2s\varepsilon} + (-\varepsilon\overline{N}e^{-s\varepsilon})^3\right)(-\varepsilon\overline{N}e^{-s\varepsilon})\end{array}\right)Q(s)\Bigg|_{s=1}$$

$$= \frac{1}{4!}\left(\varepsilon^4\overline{N}e^{-s\varepsilon} + 7\varepsilon^4\overline{N}^2 e^{-2s\varepsilon} + 6\varepsilon^4\overline{N}^3 e^{-3s\varepsilon} + (-\varepsilon\overline{N}e^{-s\varepsilon})^4\right)Q(s)\Big|_{s=1}$$

$$= \frac{1}{4!}\left((\varepsilon\overline{N})^4 e^{-4\varepsilon} + 6\varepsilon^4\overline{N}^3 e^{-3\varepsilon} + 7\varepsilon^4\overline{N}^2 e^{-2\varepsilon} + \varepsilon^4\overline{N}e^{-\varepsilon}\right)Q(1)$$

$$\prod(5;\overline{N},\varepsilon) = \frac{-1}{5!}\frac{d^4}{ds^4}Q(s)\Big|_{s=1} = \frac{-1}{5!}\frac{d}{ds}\left(\left(\varepsilon^4\overline{N}e^{-s\varepsilon} + 7\varepsilon^4\overline{N}^2 e^{-2s\varepsilon} + 6\varepsilon^4\overline{N}^3 e^{-3s\varepsilon} + (-\varepsilon\overline{N}e^{-s\varepsilon})^4\right)Q(s)\right)\Big|_{s=1}$$

$$= \frac{-1}{5!}\left(\begin{array}{l}\dfrac{d\left(\varepsilon^4\overline{N}e^{-s\varepsilon} + 7\varepsilon^4\overline{N}^2 e^{-2s\varepsilon} + 6\varepsilon^4\overline{N}^3 e^{-3s\varepsilon} + (-\varepsilon\overline{N}e^{-s\varepsilon})^4\right)}{ds}Q(s) + \\ \left(\varepsilon^4\overline{N}e^{-s\varepsilon} + 7\varepsilon^4\overline{N}^2 e^{-2s\varepsilon} + 6\varepsilon^4\overline{N}^3 e^{-3s\varepsilon} + (-\varepsilon\overline{N}e^{-s\varepsilon})^4\right)\dfrac{dQ(s)}{ds}\end{array}\right)\Bigg|_{s=1}$$

$$= \frac{-1}{5!}\left(\begin{pmatrix}-\varepsilon^5\overline{N}e^{-s\varepsilon} - 14\varepsilon^5\overline{N}^2e^{-2s\varepsilon} - 18\varepsilon^5\overline{N}^3e^{-3s\varepsilon} - 4\varepsilon^5\overline{N}^4e^{-4s\varepsilon}\end{pmatrix} + \\ \begin{pmatrix}\varepsilon^4\overline{N}e^{-s\varepsilon} + 7\varepsilon^4\overline{N}^2e^{-2s\varepsilon} + 6\varepsilon^4\overline{N}^3e^{-3s\varepsilon} + (-\varepsilon\overline{N}e^{-s\varepsilon})^4\end{pmatrix}(-\varepsilon\overline{N}e^{-s\varepsilon})\right)Q(s)\Bigg|_{s=1}$$

$$= \frac{-1}{5!}\left(-\varepsilon^5\overline{N}e^{-s\varepsilon} - 15\varepsilon^5\overline{N}^2e^{-2s\varepsilon} - 25\varepsilon^5\overline{N}^3e^{-3s\varepsilon} - 10\varepsilon^5\overline{N}^4e^{-4s\varepsilon} + (-\varepsilon\overline{N}e^{-s\varepsilon})^5\right)Q(s)\Big|_{s=1}$$

$$= \frac{1}{5!}\left((\varepsilon\overline{N})^5e^{-5\varepsilon} + 10\varepsilon^5\overline{N}^4e^{-4\varepsilon} + 25\varepsilon^5\overline{N}^3e^{-3\varepsilon} + 15\varepsilon^5\overline{N}^2e^{-2\varepsilon} + \varepsilon^5\overline{N}e^{-\varepsilon}\right)Q(1)$$

Derivation $Q(1)$ by parameter $\overline{N}$ and ε will be

$$\frac{\partial Q(1)}{\partial\overline{N}} = (e^{-\varepsilon}-1)e^{(e^{-\varepsilon}-1)\overline{N}} = (e^{-\varepsilon}-1)Q(1)$$

and

$$\frac{\partial Q(1)}{\partial\varepsilon} = -\overline{N}e^{-\varepsilon}e^{(e^{-\varepsilon}-1)\overline{N}} = -\overline{N}e^{-\varepsilon}Q(1)$$

For $k \geq 1$;

$$\prod(k;\overline{N},\varepsilon) = \frac{1}{k!}\sum_{i=1}^{k} a(k,i)\varepsilon^k\overline{N}^i e^{-i\varepsilon}Q(1)$$

(123)

where

$a(k,i) = (k-1)a(k-1,i-1) + a(k-1,i)$, with $a(k,1) = 1$ and $a(k,k) = 1$

Derivation of parameter $\overline{N}$:

$$\frac{\partial\prod(0;\overline{N},\varepsilon)}{\partial\overline{N}} = \frac{\partial}{\partial\overline{N}}e^{(e^{-\varepsilon}-1)\overline{N}} = (e^{-\varepsilon}-1)e^{(e^{-\varepsilon}-1)\overline{N}} = (e^{-\varepsilon}-1)Q(1)$$

(124)

For $k \geq 1$;

$$\frac{\partial\pi(k;\overline{N},\varepsilon)}{\partial\overline{N}} = \frac{1}{k!}\frac{\partial}{\partial\overline{N}}\sum_{i=1}^{k} a(k,i)\varepsilon^k\overline{N}^i e^{-i\varepsilon}Q(1)$$

$$= \frac{1}{k!}\sum_{i=1}^{k} a(k,i)\varepsilon^k e^{-i\varepsilon}\left(\frac{d\overline{N}^i}{d\overline{N}}Q(1) + \overline{N}^i\frac{\partial Q(1)}{\partial\overline{N}}\right)$$

$$= \frac{1}{k!}\sum_{i=1}^{k} a(k,i)\varepsilon^k e^{-i\varepsilon}\left(\frac{i\overline{N}^i}{\overline{N}} + \overline{N}^i(e^{-\varepsilon}-1)\right)Q(1)$$

$$= \frac{1}{k!}\sum_{i=1}^{k} a(k,i)\varepsilon^k\overline{N}^i e^{-i\varepsilon}\left(\frac{i}{\overline{N}} + e^{-\varepsilon}-1\right)Q(1)$$

(125)

Derivation of parameter ε:

$$\frac{\partial \prod(0;\overline{N},\varepsilon)}{\partial \varepsilon} = \frac{\partial}{\partial \varepsilon} e^{(e^{-\varepsilon}-1)\overline{N}} = (-\overline{N}e^{-\varepsilon})e^{(e^{-\varepsilon}-1)\overline{N}} = (-\overline{N}e^{-\varepsilon})Q(1)$$

(126)

For $k \geq 1$;

$$\frac{\partial \prod(k;\overline{N},\varepsilon)}{\partial \varepsilon} = \frac{1}{k!}\frac{\partial}{\partial \varepsilon}\sum_{i=1}^{k} a(k,i)\varepsilon^k \overline{N}^i e^{-i\varepsilon}Q(1)$$

$$= \frac{1}{k!}\sum_{i=1}^{k} a(k,i)\overline{N}^i \left(\frac{d\left(\varepsilon^k e^{-i\varepsilon}\right)}{d\varepsilon}Q(1) + \varepsilon^k e^{-i\varepsilon}\frac{\partial Q(1)}{\partial \overline{N}}\right)$$

$$= \frac{1}{k!}\sum_{i=1}^{k} a(k,i)\overline{N}^i \left(\left(\frac{k\varepsilon^k e^{-i\varepsilon}}{\varepsilon} - i\varepsilon^k e^{-i\varepsilon}\right) + \varepsilon^k e^{-i\varepsilon}(-\overline{N}e^{-\varepsilon})\right)Q(1)$$

$$= \frac{1}{k!}\sum_{i=1}^{k} a(k,i)\varepsilon^k \overline{N}^i e^{-i\varepsilon}\left(\frac{k}{\varepsilon} - \overline{N}e^{-\varepsilon} - i\right)Q(1)$$

(127)

- **For two component:**

$$\prod(j;\overline{N}_1,\varepsilon_1,\overline{N}_2,\varepsilon_2) = \prod(k_1;\overline{N}_1,\varepsilon_1)\otimes\prod(k_2;\overline{N}_2,\varepsilon_2) = \sum_{l=0}^{j}\prod(l;\overline{N}_1,\varepsilon_1)\prod(j-l;\overline{N}_2,\varepsilon_2)$$

Derivation of parameter $\overline{N}_1$, ε_1, and $\overline{N}_2$, ε_2:

$$\frac{\partial}{\partial \overline{N}_1}\prod(j;\overline{N}_1,\varepsilon_1,\overline{N}_2,\varepsilon_2) = \sum_{l=0}^{j}\frac{\partial \prod(l;\overline{N}_1,\varepsilon_1)}{\partial \overline{N}_1}\prod(j-l;\overline{N}_2,\varepsilon_2)$$

$$\frac{\partial}{\partial \varepsilon_1}\prod(j;\overline{N}_1,\varepsilon_1,\overline{N}_2,\varepsilon_2) = \sum_{l=0}^{j}\frac{\partial \prod(l;\overline{N}_1,\varepsilon_1)}{\partial \varepsilon_1}\prod(j-l;\overline{N}_2,\varepsilon_2)$$

$$\frac{\partial}{\partial \overline{N}_2}\prod(j;\overline{N}_1,\varepsilon_1,\overline{N}_2,\varepsilon_2) = \sum_{l=0}^{j}\prod(i;\overline{N}_1,\varepsilon_1)\frac{\partial \prod(j-l;\overline{N}_2,\varepsilon_2)}{\partial \overline{N}_2}$$

$$\frac{\partial}{\partial \varepsilon_2}\prod(j;\overline{N}_1,\varepsilon_1,\overline{N}_2,\varepsilon_2) = \sum_{l=0}^{j}\prod(l;\overline{N}_1,\varepsilon_1)\frac{\partial \prod(j-l;\overline{N}_2,\varepsilon_2)}{\partial \varepsilon_2}$$

d. PCH(Photon Counting Histogram) Analysis, Gaussian-Lorentzian Model

- **For one component:**

For $k = 0$;

$$\prod\left(0;\overline{N},\varepsilon\right) = \sum_{N=0}^{\infty} p(N)\,p(0|N) = \sum_{N=0}^{\infty} p(N)\,p_{2GL}^{(N)}(0;V_0,\varepsilon)$$
$$= \sum_{n=0}^{\infty} \frac{\left(\overline{N}\right)^n}{n!} e^{-\overline{N}} \left(1 + \sum_{m=1}^{\infty} c_{m,n}\varepsilon^m\right)$$

(128)

For $k \geq 1$;

$$\prod\left(k;\overline{N},\varepsilon\right) = \sum_{N=0}^{\infty} p(N)\,p(k|N) = \sum_{N=0}^{\infty} p(N)\,p_{2GL}^{(N)}(k;V_0,\varepsilon)$$
$$= \sum_{n=0}^{\infty} \frac{\left(\overline{N}\right)^n}{n!} e^{-\overline{N}}\, \frac{(-1)^k}{k!} \sum_{m=0}^{\infty} \frac{(k+m)!}{m!} c_{k+m,n}\varepsilon^{k+m}$$

(129)

Derivation of parameter $\overline{N}$:

For $k = 0$;

$$\frac{\partial \prod(0;\overline{N},\varepsilon)}{\partial \overline{N}} = \frac{\partial}{\partial \overline{N}} \sum_{n=0}^{\infty} \frac{\left(\overline{N}\right)^n}{n!} e^{-\overline{N}} \left(1 + \sum_{m=1}^{\infty} c_{m,n}\varepsilon^m\right) = \sum_{n=0}^{\infty} \frac{d}{d\overline{N}} \left(\frac{\left(\overline{N}\right)^n}{n!} e^{-\overline{N}}\right)\left(1 + \sum_{m=1}^{\infty} c_{m,n}\varepsilon^m\right)$$
$$= \sum_{n=0}^{\infty} \left(\frac{n\left(\overline{N}\right)^{n-1}}{n!} e^{-\overline{N}} - \frac{\left(\overline{N}\right)^n}{n!} e^{-\overline{N}}\right)\left(1 + \sum_{m=1}^{\infty} c_{m,n}\varepsilon^m\right) = \sum_{n=0}^{\infty} \left(n - \overline{N}\right)\left(\frac{\left(\overline{N}\right)^{n-1}}{n!} e^{-\overline{N}}\right)\left(1 + \sum_{m=1}^{\infty} c_{m,n}\varepsilon^m\right)$$

(130)

For $k \geq 1$;

$$\frac{\partial \prod(k;\overline{N},\varepsilon)}{\partial \overline{N}} = \frac{\partial}{\partial \overline{N}} \sum_{n=0}^{\infty} \frac{\left(\overline{N}\right)^n}{n!} e^{-\overline{N}}\, \frac{(-1)^k}{k!} \sum_{m=0}^{\infty} \frac{(k+m)!}{m!} c_{k+m,n}\varepsilon^{k+m}$$
$$= \sum_{n=0}^{\infty} \frac{d}{d\overline{N}} \left(\frac{\left(\overline{N}\right)^n}{n!} e^{-\overline{N}}\right) \frac{(-1)^k}{k!} \sum_{m=0}^{\infty} \frac{(k+m)!}{m!} c_{k+m,n}\varepsilon^{k+m}$$
$$= \sum_{n=0}^{\infty} \left(n - \overline{N}\right)\left(\frac{\left(\overline{N}\right)^{n-1}}{n!} e^{-\overline{N}}\right) \frac{(-1)^k}{k!} \sum_{m=0}^{\infty} \frac{(k+m)!}{m!} c_{k+m,n}\varepsilon^{k+m}$$

(131)

Derivation of parameter ε:

For $k=0$;

$$\frac{\partial\prod(0;\overline{N},\varepsilon)}{\partial\varepsilon}=\frac{\partial}{\partial\varepsilon}\sum_{n=0}^{\infty}\frac{(\overline{N})^n}{n!}e^{-\overline{N}}\left(1+\sum_{m=1}^{\infty}c_{m,n}\varepsilon^m\right)=\sum_{n=0}^{\infty}\left(\frac{(\overline{N})^n}{n!}e^{-\overline{N}}\right)\frac{d}{d\varepsilon}\left(1+\sum_{m=1}^{\infty}c_{m,n}\varepsilon^m\right)$$

$$=\sum_{n=0}^{\infty}\left(\frac{(\overline{N})^n}{n!}e^{-\overline{N}}\right)\left(\sum_{m=1}^{\infty}c_{m,n}m\varepsilon^{m-1}\right)$$

(132)

For K>1;

$$\frac{\partial\prod(k;\overline{N},\varepsilon)}{\partial\varepsilon}=\frac{d}{d\varepsilon}\sum_{n=0}^{\infty}\frac{(\overline{N})^n}{n!}e^{-\overline{N}}\frac{(-1)^k}{k!}\sum_{m=0}^{\infty}\frac{(k+m)!}{m!}c_{k+m,n}\varepsilon^{k+m}$$

$$=\sum_{n=0}^{\infty}\frac{(\overline{N})^n}{n!}e^{-\overline{N}}\frac{(-1)^k}{k!}\sum_{m=0}^{\infty}\frac{(k+m)!}{m!}c_{k+m,n}(k+m)\varepsilon^{k+m-1}$$

(133)

- **For two component:**

$$\prod(j;\overline{N}_1,\varepsilon_1,\overline{N}_2,\varepsilon_2)=\prod(k_1;\overline{N}_1,\varepsilon_1)\otimes\prod(k_2;\overline{N}_2,\varepsilon_2)=\sum_{l=0}^{j}\prod(l;\overline{N}_1,\varepsilon_1)\prod(j-l;\overline{N}_2,\varepsilon_2)$$

Derivation of parameter $\overline{N}_1$, ε_1, and $\overline{N}_2$, ε_2:

$$\frac{\partial}{\partial\overline{N}_1}\prod(j;\overline{N}_1,\varepsilon_1,\overline{N}_2,\varepsilon_2)=\sum_{l=0}^{j}\frac{\partial\prod(l;\overline{N}_1,\varepsilon_1)}{\partial\overline{N}_1}\prod(j-l;\overline{N}_2,\varepsilon_2)$$

$$\frac{\partial}{\partial\varepsilon_1}\prod(j;\overline{N}_1,\varepsilon_1,\overline{N}_2,\varepsilon_2)=\sum_{l=0}^{j}\frac{\partial\prod(l;\overline{N}_1,\varepsilon_1)}{\partial\varepsilon_1}\prod(j-l;\overline{N}_2,\varepsilon_2)$$

$$\frac{\partial}{\partial\overline{N}_2}\prod(j;\overline{N}_1,\varepsilon_1,\overline{N}_2,\varepsilon_2)=\sum_{l=0}^{j}\prod(i;\overline{N}_1,\varepsilon_1)\frac{\partial\prod(j-l;\overline{N}_2,\varepsilon_2)}{\partial\overline{N}_2}$$

$$\frac{\partial}{\partial\varepsilon_2}\prod(j;\overline{N}_1,\varepsilon_1,\overline{N}_2,\varepsilon_2)=\sum_{l=0}^{j}\prod(l;\overline{N}_1,\varepsilon_1)\frac{\partial\prod(j-l;\overline{N}_2,\varepsilon_2)}{\partial\varepsilon_2}$$

e. Cumulant Analysis

$$<\phi>=<k>=m_1$$
$$<\phi^2>=<k(k-1)>=<k^2-k>=<k^2>-<k>=m_2-m1$$
$$<\phi^3>=<k(k-1)(k-2)>=<k^3-3k^2+2k>=<k^3>-3<k^2>+2<k>=m_3-3m_2+2m_1$$
$$<\phi^4>=<k(k-1)(k-2)(k-3)>=<k^4-6k^3+11k^2-6k>=<k^4>-6<k^3>+11<k^2>-6<k>$$
$$=m_4-6m_3+11m_2-6m_1$$

$$<\Delta k^2>=<(k-<k>)^2>=<k^2-2k<k>+<k>^2>=<k^2>-<k>^2=\sigma^2=m_2-m_1{}^2$$
$$<\Delta k^3>=<(k-<k>)^3>=<k^3-3k^2<k>+3k<k>^2-<k>^3>=<k^3>-3<k^2><k>+2<k>^3$$
$$=m_3-3m_2m_1+2m_1{}^3$$
$$<\Delta k^4>=<(k-<k>)^4>=<k^4-4k^3<k>+6k^2<k>^2-4k<k>^3+<k>^4>$$
$$=<k^4>-4<k^3><k>+6<k^2><k>^2-3<k>^4=m_4-4m_3m_1+6m_2m_1{}^2-3m_1{}^4$$

$$<\Delta\phi^2>=<(\phi-<\phi>)^2>=<\phi^2-2\phi<\phi>+<\phi>^2>=<\phi^2>-<\phi>^2=m_2-m_1-m_1{}^2$$
$$=<\Delta k^2>-<k>$$
$$<\Delta\phi^3>=<(\phi-<\phi>)^3>=<\phi^3-3\phi^2<\phi>+3\phi<\phi>^2-<\phi>^3>$$
$$=<\phi^3>-3<\phi^2><\phi>+3<\phi><\phi>^2-<\phi>^3$$
$$=m_3-3m_2+2m_1-3(m_2-m_1)m_1+3m_1{}^3-m_1{}^3=m_3-3m_2+2m_1-3m_2m_1+3m_1{}^2+2m_1{}^3$$
$$=(m_3-3m_2m_1+2m_1{}^3)-3(m_2-m_1{}^2)+2m_1=<\Delta k^3>-3<\Delta k^2>+2<k>$$
$$<\Delta\phi^4>=<(\phi-<\phi>)^4>=<\phi^4-4\phi^3<\phi>+6\phi^2<\phi>^2-4\phi<\phi>^3+<\phi>^4>$$
$$=<\phi^4>-4<\phi^3><\phi>+6<\phi^2><\phi>^2-4<\phi><\phi>^3+<\phi>^4$$
$$=(m_4-6m_3+11m_2-6m_1)-4(m_3-3m_2+2m_1)m_1+6(m_2-m_1)m_1{}^2-3m_1{}^4$$
$$=(m_4-6m_3+11m_2-6m_1)-4m_3m_1+12m_2m_1-8m_1{}^2+(6m_2m_1{}^2-6m_1{}^3)-3m_1{}^4$$
$$=(m_4-4m_3m_1+6m_2m_1{}^2-3m_1{}^4)-6(m_3-3m_2m_1+2m_1{}^3)+11(m_2-m_1{}^2)-6m_1$$
$$-6m_2m_1+6m_1{}^3+3m_1{}^2$$
$$=<\Delta k^4>-6<\Delta k^3>+11<\Delta k^2>-6<k>-6<k><\Delta k^2>+3<k>^2$$

Moment Generating Function:

$$\Phi(s)=\int_{-\infty}^{\infty}f(x)e^{sx}dx$$

$$m_n=\left.\frac{d^n\Phi(s)}{ds^n}\right|_{s=0}=\left.\int_{-\infty}^{\infty}f(x)\frac{d^ne^{sx}}{ds^n}dx\right|_{s=0}$$

For illustration,

$$m_0=\Phi(0)=\int_{-\infty}^{\infty}f(x)dx=1$$

$$m_1 = \left.\frac{d\Phi(s)}{ds}\right|_{s=0} = \left.\int_{-\infty}^{\infty} f(x) x e^{sx} dx\right|_{s=0} = \int_{-\infty}^{\infty} x f(x) dx = < x >$$

$$m_2 = \left.\frac{d^2\Phi(s)}{ds^2}\right|_{s=0} = \left.\int_{-\infty}^{\infty} f(x) x^2 e^{sx} dx\right|_{s=0} = \int_{-\infty}^{\infty} x^2 f(x) dx = < x^2 >$$

Cumulant Generating Function:
$\Psi(s) = \ln \Phi(s)$ or $\Phi(s) = e^{\Psi(s)}$

$$\lambda_n = \left.\frac{d^n\Psi(s)}{ds^n}\right|_{s=0}$$

For illustration, the zero order cumulant is:
$\lambda_0 = \Psi(0) = \ln \Phi(0) = \ln 1 = 0$

The first order Cumulant is:

$$\left.\frac{d\Phi(s)}{ds}\right|_{s=0} = \left.\frac{d\Psi(s)}{ds} e^{\Psi(s)}\right|_{s=0} = \left.\frac{d\Psi(s)}{ds}\right|_{s=0} e^{\Psi(0)} = \left.\frac{d\Psi(s)}{ds}\right|_{s=0} = \lambda_1 = m_1$$

The second order Cumulant is:

$$\left.\frac{d^2\Phi(s)}{ds^2}\right|_{s=0} = \left.\left(\frac{d^2\Psi(s)}{ds^2} + \left(\frac{d\Psi(s)}{ds}\right)^2\right) e^{\Psi(s)}\right|_{s=0} = \left.\frac{d^2\Psi(s)}{ds^2}\right|_{s=0} + m_1^{\ 2} = m_2$$

Thus, $\lambda_2 = \left.\frac{d^2\Psi(s)}{ds^2}\right|_{s=0} = m_2 - m_1^{\ 2} = \sigma^2$

The third order Cumulant is:

$$\left.\frac{d^3\Phi(s)}{ds^3}\right|_{s=0} = \left.\left(\frac{d^3\Psi(s)}{ds^3} + 3\frac{d^2\Psi(s)}{ds^2}\frac{d\Psi(s)}{ds} + \left(\frac{d\Psi(s)}{ds}\right)^3\right) e^{\Psi(s)}\right|_{s=0}$$

$$= \left.\frac{d^3\Psi(s)}{ds^3}\right|_{s=0} + 3(m_2 - m_1^{\ 2}) m_1 + m_1^{\ 3} = m_3$$

Thus, $\lambda_3 = \left.\frac{d^3\Psi(s)}{ds^3}\right|_{s=0} = m_3 - 3(m_2 - m_1^{\ 2}) m_1 - m_1^{\ 3} = m_3 - 3m_1 m_2 + 2m_1^{\ 3}$

With the same procedure, The forth order Cumulant is:

$$\lambda_4 = \left.\frac{d^4\Psi(s)}{ds^4}\right|_{s=0} = m_4 - 4m_1 m_3 + 12 m_1^{\ 2} m_2 - 6 m_1^{\ 4} - 3 m_2^{\ 2}$$

For the cumulants we use,

$q_1 = <\phi> = m_1 = <k>$

$q_2 = <\Delta\phi^2> = m_2 - m_1 - m_1^{\ 2} = <\Delta k^2> - <k>$

$q_3 = <\Delta\phi^3> = (m_3 - 3m_2 m_1 + 2m_1^{\ 3}) - 3(m_2 - m_1^{\ 2}) + 2m_1 = <\Delta k^3> -3<\Delta k^2> +2<k>$

$$q_4 = <\Delta\phi^4> - 3 <\Delta\phi^2>^2$$
$$= (m_4 - 4m_3m_1 + 6m_2{m_1}^2 - 3{m_1}^4) - 6(m_3 - 3m_2m_1 + 2{m_1}^3) + 11(m_2 - {m_1}^2) - 6m_1$$
$$- 6m_2m_1 + 6m_1^3 + 3m_1^2 - 3(<\Delta k^2> - <k>)^2$$
$$= <\Delta k^4> - 6 <\Delta k^3> + 11 <\Delta k^2> - 6 <k> - 6 <k><\Delta k^2> + 3 <k>^2$$
$$- 3 <\Delta k^2>^2 + 6 <\Delta k^2><k> - 3 <k>^2$$
$$= <\Delta k^4> - 6 <\Delta k^3> + 11 <\Delta k^2> - 6 <k> - 3 <\Delta k^2>^2$$

- **For one component:**

$$q_1 = \varepsilon_1 n_1$$
$$q_2 = \chi_2 {\varepsilon_1}^2 n_1$$

Where, $\chi_2 = \frac{x_1}{x_2} = \frac{3}{4\pi^2}$

- **For two component:**

$$q_1 = \varepsilon_1 n_1 + \varepsilon_2 n_2$$
$$q_2 = \chi_2 ({\varepsilon_1}^2 n_1 + {\varepsilon_2}^2 n_2)$$
$$q_3 = \chi_3 ({\varepsilon_1}^3 n_1 + {\varepsilon_2}^3 n_2)$$
$$q_4 = \chi_4 ({\varepsilon_1}^4 n_1 + {\varepsilon_2}^4 n_2)$$

Where

$$\chi_2 = \frac{x_2}{x_1} = \frac{3}{4\pi^2}$$
$$\chi_3 = \frac{x_3}{x_1} = \frac{35}{24\pi^4}$$
$$\chi_4 = \frac{x_4}{x_1} = \frac{231}{64\pi^6}$$

References

1. Nancy L. Thompson. **Topics in Fluorescence Spectroscopy, Volume 1: Techniques**, *edited by Joseph R. Lakowicz. Plenum Press, New York, 1991.*

2. D. Magde, E.L. Elson, W.W. Webb. **Thermodynamic Fluctuations in a Reacting System – Measurement by Fluorescence Correlation Spectroscopy.** *Phys. Rev. Lett., 29, pp. 705-708(1972).*

3. E.L. Elson, D. Magde. **Fluorescence Correlation Spectroscopy. I. Conceptual Basis and Theory.** *Biopolymers 13, pp. 1-27 (1974).*

4. D. Magde, E.L. Elson, W.W. Webb**.** **Fluorescence Correlation Spectroscopy. II. An Experimental Realization.** *Biopolymers 13, pp. 29-61 (1974).*

5. D. Magde, W. Webb, E. Elson. **Fluorescence Correlation Spectroscopy. III. Uniform Translation and Laminar Flow.** *Biopolymers 17, pp. 361-376 (1978).*

6. M. Ehrenberg, R. Rigler. **Rotational Brownian Motion and Fluorescence Intensity Fluctuations.** *Chem. Phys. 4, pp. 390-401 (1974).*

7. D. Magde. **Chemical Kinetics and Fluorescence Correlation Spectroscopy.** *Quart. Rev. Biophys. 9, pp. 35-47 (1976).*

8. M.B. Weissman, H. Schindler, G. Feher. **Determination of Molecular Weights by Fluctuation Spectroscopy: Application to DNA.** *Proc. Natl. Acad. Sci. USA 73, pp. 2776-2780 (1976).*

9. R.D. Icenogle, E.L. Elson. **Fluorescence Correlation Spectroscopy and Photobleaching Recovery of Multiple Binding Reactions. I. Theory and Fcs measurements.** *Biopolymers 22, pp. 1919-1948 (1983).*

10. R.D. Icenogle, E.L. Elson. **Fluorescence Correlation Spectroscopy and Photobleaching Recovery of Multiple Binding Reactions. II. FPR and FCS measurements at low and high DNA concentrations.** *Biopolymers 22, pp. 1949-1966 (1983).*

11. E.L. Elson. **Fluorescence Correlation Spectroscopy and Photobleaching Recovery.** *Annu. Rev. Phys. Chem. 36, pp. 379-406 (1985).*

12. A.G. Palmer, N.L. Thompson. **Molecular Aggregation Characterized by High-Order Autocorrelation in Fluorescence Correlation Spectroscopy.** *Biophys. J. 52, pp. 257-270 (1987).*

13. J. Schneider, J. Ricka, T. Binkert. **Improved Fluorescence Correlation Analysis for Precise Measurements of Correlation Functions.** *Rev. Sci. Instrum. 59, pp. 588-590 (1988).*

14. J. Ricka, T. Binkert**.** **Direct Measurement of a Distinct Correlation Function by Fluorescence Cross Correlation.** *Phys. Rev. A 39, pp. 2646-2652 (1989).*

15. A.G. Palmer, N.L. Thompson**.** **High-order Fluorescence Fluctuation Analysis of Model Protein Clusters.** *Proc. Natl. Acad. Sci. USA 86, pp. 6148-6152 (1989).*

16. J. Widengren, U. Mets, R. Rigler**.** **Fluorescence Correlation Spectroscopy of Triplet States in Solution: A Theoretical and Experimental Study.** *J. Phys. Chem. 99, pp. 13368-13379 (1995).*

17. Enrico Gratton and Martin J. vandeVen. **Handbook of Biological Confocal Microscopy,** *edited by James B. Pawley. Plenum Press, New York, 1995.*

18. K.M. Berland, P.T. So, E. Gratton. **Two-Photon Flurescence Correlation Spectroscopy: Method and Application to the Intracellular Environment.** *Biophys. J. 68, pp. 694-701, (1995).*

19. K. Startchev, J. Zhang, C. Buffle**. Applications of Fluorescence Correlation Spectroscopy: particles size effect.** *J. Colloid and Interface Science 203, pp. 189-196 (1998).*