Cumulant-based formulation of higher-order fluorescence correlation spectroscopy

Farshad Abdollah-Nia*

Department of Physics, Colorado State University, Fort Collins, Colorado 80523

Abstract

Extended derivations regarding the cumulant-based formulation of higher-order fluorescence correlation spectroscopy (FCS) are presented. First, we review multivariate cumulants and their relation to photon counting distributions in single and multi-detector experiments. Then we derive the factorized correlation functions which describe system of diffusing and reacting molecules using two independent approaches. Finally, we calculate the variance of these correlation functions up to the fourth order.

Contents

1 Introduction 3

2 Mathematical introduction and notation 3

2.1 (Central) moments and cumulants 4

2.2 Factorial moments and factorial cumulants 8

3 The relation between fluorescence intensity and photon counting distributions 12

3.1 Two-time correlations 16

3.1.1 Single-Detector Experiment 16

3.1.2 Multi-Detector Experiment 16

4 Modeling correlations for diffusing and reacting molecules 17

4.1 Palmer-Thompson approach 20

4.2 Mehta-Hall approach 32

4.3 Normalized higher order correlations 34

5 Variance of correlations 36

5.1 Order (1,1) 36

5.2 Order (2,1) 39

5.3 Order (2,2) 43

A Variance and covariance of a function 48

B Some sampling moments 49

C A tensor notation rule 52

D Two-state transition factors 52

*fabdo@rams.colostate.edu
1 Introduction

Fluorescence correlation spectroscopy (FCS) is a powerful technique for a time-resolved analysis of reaction, diffusion, and flow properties of individual fluorescent molecules moving through a laser-illuminated probe region[4, 7, 16]. Conventional FCS is based on second-order correlation functions only, hence insufficient for measuring the parameters which describe molecular reactions or mixtures[1, 19] such as species populations and brightness values and the kinetic rate constants. Higher-order correlation functions can provide the necessary information for a complete measurement of the underlying reaction or mixture parameters. Previous work to define higher-order correlations based on higher moments of the fluorescence signal lead to complex expressions for mixtures of diffusing molecules[11, 13, 12], thus no extension of such approach to include reactions has been proposed. More recently, a formulation based on the cumulants of the fluorescence intensity or the factorial cumulants of the detected photon counts has been published, which results in correlation functions incorporating reaction and diffusion properties in a simple factorized form[9]. To experimentally apply the technique to the study of fast molecular kinetics, difficulties due to shot noise and detector artifacts have been recently overcome[1, 2].

Theoretically, the formulation of correlation functions based on multi-variate cumulants utilizes a variety of concepts not commonly covered in the available literature on FCS. A detailed description of the theoretical basis and derivations can make the underlying work for some earlier publications[1, 2, 9] more accessible. This document has been produced with such purpose in mind, and presents no further results and findings.

The material in this document appears in developmental order. That is, some introductory content is presented first and a continuous forward flow of reading is assumed. However, informed reader may skip to the desired section. Effort has been made to facilitate independent reading of this document by including the necessary preliminary information, derivations, and relations.

2 Mathematical introduction and notation

In this section we review the definitions, notation, and relations between multivariate moments, central moments, cumulants, and their factorial counterparts. For a more thorough treatment, the reader may refer to [3, 15].

In what follows, we use \( \mu_{i_1, i_2, \ldots, i_l} \) to denote \( \mu_{i_1, i_2, \ldots, i_l, 0, \ldots, 0} \) and use \( \kappa_{i_1, i_2, \ldots, i_l} \) to denote \( \kappa_{i_1, i_2, \ldots, i_l, 0, \ldots, 0} \). Also, we will use the differential operators

\[
D_i = \frac{\partial}{\partial i}
\]

\[
D'_i = \frac{\partial^r}{\partial r^i}
\]

We will occasionally exchange the order of product and summation in the following way:

\[
\prod_{i=1}^{k} \sum_{j_i=0}^{r_i} f(r_i, j_i) = [f(r_1, 0) + \ldots + f(r_1, r_1)] \ldots [f(r_k, 0) + \ldots + f(r_k, r_k)]
\]

\[
= \sum_{j_1=0}^{r_1} \ldots \sum_{j_k=0}^{r_k} f(r_1, j_1) f(r_2, j_2) \ldots f(r_k, j_k)
\]

\[
= \sum_{j_1=0}^{r_1} \ldots \sum_{j_k=0}^{r_k} \prod_{i=1}^{k} f(r_i, j_i)
\]

For simplicity, we assume the moments, etc. exist and the expansions converge.
2.1 (Central) moments and cumulants

Let $\vec{X} = (X_1, X_2, \ldots, X_k)$ be a multivariate random vector. For $\vec{r} = (r_1, r_2, \ldots, r_k)$ the $\vec{r}$th moment of $\vec{X}$ is defined as

$$\mu_{\vec{r}} = E[\prod_{i=1}^{k} X_i^{r_i}]$$

where $E$ denotes the expectation operator. In particular, $\mu_{\vec{u}_i} = E[X_i]$ where $\vec{u}_i = (0, \ldots, 0, 1, 0, \ldots, 0)$ has only the $i$th element equal to 1.

The $\vec{r}$th central moment of $\vec{X}$ is defined as

$$\mu_{\vec{r}} = E[\prod_{i=1}^{k} (X_i - E[X_i])^{r_i}]$$

We denote the moment generating function of $\vec{X}$ by

$$M'((\vec{t}) = E[e^{\vec{t} \cdot \vec{X}}]$$

and the central moment generating function of $\vec{X}$ by

$$M((\vec{t}) = E[e^{\vec{t} \cdot (\vec{X} - \vec{\theta})}]$$

where

$$\vec{\theta} = (E[X_1], E[X_2], \ldots, E[X_k])$$

and $\mu_{\vec{r}}$ is the $\vec{r}$th central moment (or moment about the mean) of $\vec{X}$.

The cumulant generating function of $\vec{X}$ is defined as

$$K((\vec{t}) = \ln M'((\vec{t})$$

which also defines $\kappa_{\vec{r}}$, the $\vec{r}$th cumulant of $\vec{X}$, through multiple differentiation of $K((\vec{t})$

$$\kappa_{\vec{r}} = [D_{t_1}^{r_1} \ldots D_{t_k}^{r_k} K((\vec{t}))]_{\vec{t} = \vec{0}}$$

One can proceed to substitute the expansions (6) and (4) into relation (5), or into $M'((\vec{t}) = \exp(K((\vec{t})$, then expand the ln or the exp function and set the terms of equal order in $\prod_{i=1}^{k} t_i^{r_i}$ equal to each other to obtain the relationship between the cumulants $\kappa_{\vec{r}}$ and the moments $\mu_{\vec{r}}$. Then setting all the first order moments $\mu_{\vec{u}_i}$ equal to zero in the resultant expressions and dropping the primes will yield the relations between the
cumulants $\kappa_{\vec{r}}$ and the central moments $\mu_{\vec{r}}$. Alternatively, one can write, using (3),

$$M(\vec{t}) = e^{-\vec{t} \cdot \vec{\theta}} E[e^{\vec{t} \cdot \vec{X}}]$$

$$= e^{-\vec{t} \cdot \vec{\theta}} M'(\vec{t})$$

$$= e^{-\vec{t} \cdot \vec{\theta}} e^{K(\vec{t})}$$

$$= \exp[K(\vec{t}) - \vec{t} \cdot \vec{\theta}]$$

and expand to obtain the relations between $\kappa_{\vec{r}}$ and $\mu_{\vec{r}}$. The central moments, and therefore $M(\vec{t})$, do not depend on the mean values $\mu'_{\vec{u}_i} = \theta_i$. In other words, $\mu'_{\vec{u}_i} = 0$, thus terms of individual $t_i$ do not appear in the expansion of $M(\vec{t})$. Thus (7) immediately shows that the first cumulant of each $X_i$ is equal to its mean,

$$\kappa_{\vec{u}_i} = \mu'_{\vec{u}_i} = E[X_i]$$

Also, all higher order central moments are independent of mean in the sense that they are invariant under translation of origin. Therefore (7) shows that all higher order cumulants are also independent of mean, i.e. invariant under change of origin.

Cumulants share the described invariance property with central moments. However, cumulants also have another property which makes them unique: Each cumulant of a sum of independent random variables is equal to the sum of the corresponding cumulants of those random variables. To see this, consider two independent random vectors $\vec{X}$ and $\vec{Y}$. Directly from definition (2) we have for the moment generating function of their sum, $\vec{X} + \vec{Y}$,

$$M_{\vec{X} + \vec{Y}}(\vec{t}) = E[e^{\vec{t} \cdot (\vec{X} + \vec{Y})}]$$

$$= E[e^{\vec{t} \cdot \vec{X}} e^{\vec{t} \cdot \vec{Y}}]$$

$$= E[e^{\vec{t} \cdot \vec{X}}] E[e^{\vec{t} \cdot \vec{Y}}]$$

$$= M_{\vec{X}}(\vec{t}) M_{\vec{Y}}(\vec{t})$$

Now from (5)

$$K_{\vec{X} + \vec{Y}}(\vec{t}) = \ln[M_{\vec{X} + \vec{Y}}(\vec{t})]$$

$$= \ln[M_{\vec{X}}(\vec{t}) M_{\vec{Y}}(\vec{t})]$$

$$= \ln[M_{\vec{X}}(\vec{t})] + \ln[M_{\vec{Y}}(\vec{t})]$$

$$= K_{\vec{X}}(\vec{t}) + K_{\vec{Y}}(\vec{t})$$

Expanding and setting the coefficients equal we get:

$$\kappa_{\vec{r}}(\vec{X} + \vec{Y}) = \kappa_{\vec{r}}(\vec{X}) + \kappa_{\vec{r}}(\vec{Y})$$

This property makes cumulants particularly useful in the study of single molecules in solution, where each molecule can be considered as an independent emitter of photons. Fluorescence Cumulant Analysis (FCA) [10, 18] and Higher Order Fluorescence Correlation Spectroscopy [9] are examples of such applications.

To obtain relations between multivariate cumulants and central moments, it will be more convenient to use recursive formulas rather than the expansion method mentioned above. Such formulas are obtained through successive differentiation of the relevant moment generating function, as presented by Balakrishnan et al [3]. For $1 \leq l \leq k$ let us find the central moment $\mu_{r_1, r_2, \ldots, r_l}$ in terms of the cumulants $\kappa_{i_1, i_2, \ldots, i_l}$ of order $(r_1, r_2, \ldots, r_l)$ and less (i.e. $i_m \leq r_m$ for all $m$), and the central moments $\mu_{i_1, i_2, \ldots, i_l}$ of order $(r_1, r_2, \ldots, r_l - 1)$
and less:

\[ \mu_{r_1, r_2, \ldots, r_l} = \left[ D_{i_1}^{r_1} D_{i_2}^{r_2} \ldots D_{i_l}^{r_l} e^{K(\vec{i}) - \vec{\theta} \cdot \vec{d}}} \right]_{\vec{r} = 0} \]

\[ = \left[ D_{i_1}^{r_1} D_{i_2}^{r_2} \ldots D_{i_l}^{r_l-1} \{ D_{i_l} K(\vec{i}) - \vec{r} \cdot \vec{d} \} e^{K(\vec{i}) - \vec{\theta} \cdot \vec{d}}} \right]_{\vec{r} = 0} \]

\[ = \left[ D_{i_1}^{r_1} D_{i_2}^{r_2} \ldots D_{i_l}^{r_l-1} \{ D_{i_l} K(\vec{i}) - \theta_l \} e^{K(\vec{i}) - \vec{\theta} \cdot \vec{d}}} \right]_{\vec{r} = 0} \]

\[ = \left[ D_{i_1}^{r_1} D_{i_2}^{r_2} \ldots D_{i_l}^{r_l-1} \{ D_{i_l} K(\vec{i}) \} e^{K(\vec{i}) - \vec{\theta} \cdot \vec{d}}} \right]_{\vec{r} = 0} \]

\[ - E[X_l] \left[ D_{i_1}^{r_1} D_{i_2}^{r_2} \ldots D_{i_l}^{r_l-1} e^{K(\vec{i}) - \vec{\theta} \cdot \vec{d}}} \right]_{\vec{r} = \vec{0}} \quad (9) \]

It is easy to see for multiple derivatives of a product

\[ D_{i_1}^{r_1} \left[ f(\vec{i}) g(\vec{i}) \right] = \sum_{i_0=0}^{r_1} \binom{r_1}{i_0} [D_{i_0}^0 f(\vec{i})] [D_{i_1-i_0}^{r_1-i_0} g(\vec{i})] \]

From there,

\[ D_{i_1}^{r_1} \ldots D_{i_l}^{r_l} \left[ f(\vec{i}) g(\vec{i}) \right] = \sum_{i_0=0}^{r_1} \ldots \sum_{i_l=0}^{r_l} \binom{r_1}{i_0} \ldots \binom{r_l}{i_l} \times [D_{i_0}^0 \ldots D_{i_l}^{r_l} f(\vec{i})] [D_{i_1-i_0}^{r_1-i_0} \ldots D_{i_l-i_l}^{r_l-i_l} g(\vec{i})] \]

Applying this to (9) with \( f(\vec{i}) = e^{K(\vec{i}) - \vec{\theta} \cdot \vec{d}} = M(\vec{i}) \) and \( g(\vec{i}) = D_l K(\vec{i}) \) we obtain

\[ \mu_{r_1, r_2, \ldots, r_l} = \sum_{i_0=0}^{r_1} \ldots \sum_{i_l=0}^{r_l} \binom{r_1}{i_0} \ldots \binom{r_l}{i_l} \times \left\{ [D_{i_0}^0 \ldots D_{i_l}^{r_l} M(\vec{i})] [D_{i_1-i_0}^{r_1-i_0} \ldots D_{i_l-i_l}^{r_l-i_l} K(\vec{i})] \right\}_{\vec{r} = 0} \]

\[ - E[X_l] \left[ D_{i_1}^{r_1} D_{i_2}^{r_2} \ldots D_{i_l}^{r_l-1} e^{K(\vec{i}) - \vec{\theta} \cdot \vec{d}}} \right]_{\vec{r} = \vec{0}} \]

\[ = \sum_{i_0=0}^{r_1} \ldots \sum_{i_l=0}^{r_l} \binom{r_1}{i_0} \ldots \binom{r_l}{i_l} \kappa_{r_1-i_0, \ldots, r_l-i_l \mu_{i_0, \ldots, i_l}} \]

\[ - E[X_l] \mu_{r_1, r_2, \ldots, r_l-1} \quad (10) \]

We write the first few relations up to a total order of four. For a univariate distribution, we have directly from definition (1):

\[ \mu_0 = 1 \]

\[ \mu_1 = 0 \]

Then from (5)

\[ \kappa_0 = \ln \mu_0 = 0 \]

The recursive formula (10) now generates the rest:

\[ \mu_1 = \kappa_1 - E[X] \]

which equals zero, thus

\[ \kappa_1 = E[X] \]

Again from (10),

\[ \mu_2 = \kappa_2 \mu_0 + \kappa_1 \mu_1 - E[X] \mu_1 \]

\[ = \kappa_2 \]

6
\[ \mu_3 = \kappa_3 \mu_0 + \kappa_2 \mu_1 + \kappa_1 \mu_2 - \operatorname{E}[X] \mu_2 = \kappa_3 \]

And for \( m \geq 4 \):

\[ \mu_m = \kappa_m + \sum_{i=2}^{m-2} \binom{m-1}{i} \kappa_{m-i} \mu_i \quad (m \geq 4) \]

For example,

\[ \mu_4 = \kappa_4 + 3 \kappa_2 \mu_2 = \kappa_4 + 3 \kappa_2^2 \]

conversely,

\[ \kappa_4 = \mu_4 - 3 \mu_2^2 \]

and so forth.

For a bivariate distribution, we have directly from the definitions, (1):

\[ \mu_{0,0} = 1 \]
\[ \mu_{1,0} = \mu_{0,1} = 0 \]

and from (5):

\[ \kappa_{0,0} = \ln \mu_{0,0} = 0 \]

The recursive formula (10) now yields

\[ \mu_{0,1} = \kappa_{0,1} \mu_{0,0} - \operatorname{E}[X] \mu_{0,0} \]

which vanishes, thus

\[ \kappa_{0,1} = \operatorname{E}[X_2] \]

\[ \kappa_{1,0} \] also follows the univariate relation:

\[ \kappa_{1,0} = \operatorname{E}[X_1] \]

As evident from basic definitions, \( \kappa_{m,0}, \mu_{m,0}, \kappa_{0,m} \) and \( \mu_{0,m} \) are identical to univariate cases and follow their relations:

\[ \kappa_{2,0} = \mu_{2,0} \]
\[ \kappa_{3,0} = \mu_{3,0} \]
\[ \kappa_{4,0} = \mu_{4,0} - 3 \mu_2^2 \]
\[ \kappa_{0,2} = \mu_{0,2} \]
\[ \kappa_{0,3} = \mu_{0,3} \]
\[ \kappa_{0,4} = \mu_{0,4} - 3 \mu_0^2 \]

and so forth.

A useful symmetry property also follows from the definitions: exchanging the subscripts in any valid relation between \( \kappa \)s and \( \mu \)s will produce a valid relation. Thus we can find the expression for \( \kappa_{n,m} \) (or \( \mu_{n,m} \)) from the that of \( \kappa_{m,n} \) (or \( \mu_{m,n} \)).

Once again our recursive formula (10) can be applied:

\[ \mu_{1,1} = \kappa_{1,1} \mu_{0,0} + \kappa_{0,1} \mu_{1,1} - \operatorname{E}[X] \mu_{1,0} = \kappa_{1,1} \]
\[ \mu_{2,1} = \kappa_{2,1} \mu_{0,0} + \kappa_{1,1} \mu_{1,0} + \kappa_{0,1} \mu_{2,0} - \operatorname{E}[X] \mu_{2,0} = \kappa_{2,1} \]
By symmetry,  
\[ \mu_{1,2} = \kappa_{1,2} \]

In general, for \( m + n \geq 4 \),
\[ \mu_{m,n} = \kappa_{m,n} + \sum_{i=0}^{m} \sum_{j=0}^{n} \binom{m}{i} \binom{n-1}{j} \kappa_{m-i,n-j}\mu_{i,j} \quad (m+n \geq 4) \]

For example,
\[ \mu_{2,2} = \kappa_{2,2} + 2\kappa_{1,1}\mu_{1,1} + \kappa_{0,2}\mu_{2,0} \]
\[ = \kappa_{2,2} + 2\kappa_{1,1}^2 + \kappa_{0,2}\kappa_{2,0} \]

Conversely,
\[ \kappa_{2,2} = \mu_{2,2} - 2\mu_{1,1}^2 - \mu_{0,2}\mu_{2,0} \]

As other examples,
\[ \mu_{3,1} = \kappa_{3,1} + 3\kappa_{1,1}\mu_{2,0} \]
\[ = \kappa_{3,1} + 3\kappa_{1,1}\kappa_{2,0} \]
\[ \mu_{1,3} = \kappa_{1,3} + \kappa_{1,1}\mu_{0,2} + 2\kappa_{0,2}\mu_{1,1} \]
\[ = \kappa_{1,3} + 3\kappa_{1,1}\kappa_{0,2} \]

which could also be obtained by swapping the subscripts. Conversely,
\[ \kappa_{3,1} = \mu_{3,1} - 3\mu_{1,1}\mu_{2,0} \]
\[ \kappa_{1,3} = \mu_{1,3} - 3\mu_{1,1}\mu_{0,2} \]

and so forth.

### 2.2 Factorial moments and factorial cumulants

The tools developed in this section are particularly useful for discreet random variables. In what follows, suppose \( X_i \) can take only non-negative integer values \( \{0, 1, \ldots\} \).

The \( r \)th (descending) factorial moment of \( \bar{X} \) is defined as
\[ \mu^{(r)}_r = E\left[ \prod_{i=1}^{k} X_i^{[r,i]} \right] \]

where
\[ x^{[r]} = x(x-1)(x-2)\ldots(x-r+1) \]
\[ = \frac{x!}{(x-r)!} \]
\[ = \sum_{j=1}^{r} s(r,j)x^j \]

denotes the \( r \)th (falling) factorial power of \( x \). Because \( x \in \{0, 1, \ldots\} \), \( x^{[r]} = 0 \) for \( r > x \). The constants \( s(r,j) \) are called the Stirling numbers of the first kind. Conversely,
\[ x^r = \sum_{j=1}^{r} S(r,j)x^j \]
defines the Stirling numbers of the second kind, \( S(r, j) \).

Thus we have

\[
\mu'_{[r_1, \ldots, r_k]} = E\left[ \prod_{i=1}^{k} r_i \right] = E\left[ \sum_{j_1=1}^{r_1} \cdots \sum_{j_k=1}^{r_k} \prod_{i=1}^{k} s(r_i, j_i) \right] = \sum_{j_1=1}^{r_1} \cdots \sum_{j_k=1}^{r_k} s(r_1, j_1) \cdots s(r_k, j_k) \mu'_{[j_1, \ldots, j_k]} \tag{11}
\]

which gives factorial moments in terms of moments. Similarly,

\[
\mu'_{[r_1, \ldots, r_k]} = \sum_{j_1=1}^{r_1} \cdots \sum_{j_k=1}^{r_k} S(r_1, j_1) \cdots S(r_k, j_k) \mu'_{[j_1, \ldots, j_k]} \tag{12}
\]

giving moments in terms of factorial moments.

The **probability generating function** of \( \vec{X} \) is defined as

\[
\Omega(\vec{t}) = E\left[ \prod_{i=1}^{k} t_i X_i \right] \tag{13}
\]

\[
= \sum_{x_1=0}^{\infty} \cdots \sum_{x_k=0}^{\infty} p_{\vec{x}} \prod_{i=1}^{k} t_i^{x_i}
\]

where

\[
p_{\vec{x}} = \Pr(\vec{X} = \vec{x}) = \left[ D_{x_1}^{r_1} \cdots D_{x_k}^{r_k} \Omega(\vec{t}) \right]_{\vec{t}=0}
\]

is the probability that \( \vec{X} \) takes the value \( \vec{x} = (x_1, x_2, \ldots, x_k) \).

Now we define \( \Phi(\vec{t}) \) through a change of variable in the probability generating function, \( \Omega(\vec{t}) \):

\[
\Phi(\vec{t}) = \Omega(\vec{1} + \vec{t}) \tag{14}
\]

\[
= \sum_{x_1=0}^{\infty} \cdots \sum_{x_k=0}^{\infty} p_{\vec{x}} \prod_{i=1}^{k} (1 + t_i)^{x_i}
\]

\[
= \sum_{x_1=0}^{\infty} \cdots \sum_{x_k=0}^{\infty} p_{\vec{x}} \prod_{i=1}^{k} x_i^{r_i} t_i^{x_i}
\]

\[
= \sum_{x_1=0}^{\infty} \cdots \sum_{x_k=0}^{\infty} p_{\vec{x}} \prod_{i=1}^{k} x_i^{r_i} t_i^{x_i}
\]

\[
= \sum_{x_1=0}^{\infty} \cdots \sum_{x_k=0}^{\infty} p_{\vec{x}} \sum_{r_1=0}^{x_1} \cdots \sum_{r_k=0}^{x_k} \prod_{i=1}^{k} x_i^{[r_i]} t_i^{x_i}
\]

\[
= \sum_{x_1=0}^{\infty} \cdots \sum_{x_k=0}^{\infty} p_{\vec{x}} \sum_{r_1=0}^{x_1} \cdots \sum_{r_k=0}^{x_k} \prod_{i=1}^{k} x_i^{[r_i]} t_i^{x_i} \frac{r_i}{r_i!}
\]
Since $x_i^{[r_i]} = 0$ for $r_i > x_i$, 
\[
= \sum_{r_1=0}^{\infty} \cdots \sum_{r_k=0}^{\infty} p_{r_1} \cdots p_{r_k} \prod_{j=1}^{k} x_j^{[r_j]} t_i^{r_i} / r_i!
\]
Now we can switch the summation order
\[
= \sum_{r_1=0}^{\infty} \cdots \sum_{r_k=0}^{\infty} \left\{ \sum_{x_1=0}^{\infty} \cdots \sum_{x_k=0}^{\infty} p_{x_1} \cdots p_{x_k} \prod_{j=1}^{k} x_j^{[r_j]} \right\} \prod_{i=1}^{k} t_i^{r_i} / r_i!
\]
\[
= \sum_{r_1=0}^{\infty} \cdots \sum_{r_k=0}^{\infty} \mu_{r_1}^{[r]} \prod_{i=1}^{k} t_i^{r_i} / r_i!
\]
Therefore, $\Phi(\vec{t}) = \Omega(\vec{1} + \vec{t})$ is in fact the factorial moment generating function of $\vec{X}$.

The factorial cumulant generating function of $\vec{X}$ is then defined as
\[
\Psi(\vec{t}) = \ln \Phi(\vec{t})
\]
\[
= \sum_{r_1=0}^{\infty} \cdots \sum_{r_k=0}^{\infty} \kappa_{r_1}^{[r]} \prod_{i=1}^{k} t_i^{r_i} / r_i!
\]
which also defines $\kappa_{r_1}^{[r]}$, the $r_1$th factorial cumulant of $\vec{X}$, through multiple differentiation of $\Psi(\vec{t})$:
\[
\kappa_{r_1}^{[r]} = \left[ D_{t_1}^{r_1} \cdots D_{t_k}^{r_k} \Psi(\vec{t}) \right]_{\vec{t} = \vec{0}}
\]
As with ordinary moments and cumulants, one can proceed to expand $\Psi(\vec{t})$ and $\ln \Phi(\vec{t})$ in (15) and compare term by term to obtain the relations between $\kappa_{r_1}^{[r]}$ and $\mu_{r_1}^{[r]}$. This procedure is identical to that of finding the relations between $\kappa_\vec{r}$ and $\mu_\vec{r}$, noting the similarity between (15) and $K(\vec{t}) = \ln M'(\vec{t})$. Therefore the relations between factorial moments and factorial cumulants are formally similar to those between moments and cumulants, obtained in section 2.1.

It remains to determine the relations between cumulants and factorial cumulants. To this purpose, we first examine the relation between the moment generating function, $M'(\vec{t})$, and the factorial moment generating function, $\Phi(\vec{t})$, of $\vec{X}$. Using the change of variables $\vec{s}(\vec{t}) = (e^{t_1}, \ldots, e^{t_k})$ in (13) and comparing with (2) we can write
\[
\Omega(\vec{s}(\vec{t})) = \mathbb{E}\left[ \prod_{i=1}^{k} e^{t_i X_i} \right] = \mathbb{E}\left[ e^{\vec{s} \vec{X}} \right] = M'(\vec{t})
\]
On the other hand, (14) gives $\Omega(\vec{s}(\vec{t})) = \Phi\left( \vec{s}(\vec{t}) - \vec{1} \right)$, thus we have
\[
M'(\vec{t}) = \Phi\left( \vec{s}(\vec{t}) - \vec{1} \right)
\]
Taking the natural logarithm of both sides we also have
\[
K(\vec{t}) = \Psi\left( \vec{s}(\vec{t}) - \vec{1} \right)
\]
as the relation between the cumulant generating function, $K(\vec{t})$, and the factorial cumulant generating function, $\Psi(\vec{t})$, of $\vec{X}$. Expanding both sides of (16) and equating terms of equal order in $\prod t_i^{r_i}$ yields the
relations between moments and factorial moments, which were previously found in (11) and (12) through direct use of definitions. Noting the similarity between (16) and (17), formally similar relations are then obtained through expansion of (17) between cumulants and factorial cumulants:

\[
\kappa_{[r]} = \sum_{j_1=1}^{r_1} \cdots \sum_{j_k=1}^{r_k} s(r_1, j_1) \cdots s(r_k, j_k) \kappa_j
\]

\[
\kappa_\nu = \sum_{j_1=1}^{r_1} \cdots \sum_{j_k=1}^{r_k} S(r_1, j_1) \cdots S(r_k, j_k) \kappa_{[j]}
\]

It will be instructive to actually expand (16) and equate the corresponding coefficients of \( \prod t_i^{e_i} \) on the two sides. Expanding the right hand side gives:

\[
M'(\vec{t}) = \Phi \left( \vec{s}(\vec{t}) - \vec{1} \right)
\]

\[
= \sum_{j_1=0}^{\infty} \cdots \sum_{j_k=0}^{\infty} \mu'_{[j]} \prod_{i=1}^{k} \left( e^{t_i} - 1 \right)^{j_i} \frac{j_i!}{j_i!}
\]

\[
= \sum_{j_1=0}^{\infty} \cdots \sum_{j_k=0}^{\infty} \mu'_{[j]} \prod_{i=1}^{k} \left( j_i \right) \frac{(-1)^{j_i-n_i} e^{n_i t_i}}{j_i!}
\]

\[
= \sum_{j_1=0}^{\infty} \cdots \sum_{j_k=0}^{\infty} \mu'_{[j]} \prod_{i=1}^{k} \frac{(-1)^{j_i-n_i}}{n_i!(j_i-n_i)!} \sum_{r_i=0}^{\infty} \frac{n_i^{e_i} t_i^{e_i}}{r_i!}
\]

while the left hand side is equal to

\[
= \sum_{r_1=0}^{\infty} \cdots \sum_{r_k=0}^{\infty} \mu'_\nu \prod_{i=1}^{k} \frac{t_i^{e_i}}{r_i!}
\]

Therefore,

\[
\mu'_\nu = \sum_{j_1=0}^{\infty} \cdots \sum_{j_k=0}^{\infty} \mu'_{[j]} \prod_{i=1}^{k} \frac{(-1)^{j_i-n_i} n_i^{e_i}}{n_i!(j_i-n_i)!}
\]

(18)

Let us rewrite (12) as:

\[
\mu'_{[\nu]} = \sum_{j_1=1}^{r_1} \cdots \sum_{j_k=1}^{r_k} \mu'_{[j]} \prod_{l=1}^{k} S(r_l, j_l)
\]

Since \( S(r, j) = 0 \) for \( j > r \) and for \( j = 0 \)

\[
= \sum_{j_1=0}^{\infty} \cdots \sum_{j_k=0}^{\infty} \mu'_{[j]} \prod_{l=1}^{k} S(r_l, j_l)
\]

Comparing with (18) we get

\[
S(r, j) = \sum_{n=0}^{j} \frac{(-1)^{j-n} n^r}{n!(j-n)!}
\]
an explicit expression for the Stirling numbers of the second kind.

We finish this section by tabulating the relations between cumulants and factorial cumulants up to 4th order for future reference.

\[
\begin{align*}
\kappa_1 &= \kappa_1 \\
\kappa_2 &= \kappa_2 - \kappa_1 \\
\kappa_3 &= \kappa_3 - 3\kappa_2 + 2\kappa_1 \\
\kappa_4 &= \kappa_4 - 6\kappa_3 + 11\kappa_2 - 6\kappa_1 \\
\kappa_{[1,1]} &= \kappa_{1,1} \\
\kappa_{[1,2]} &= \kappa_{1,2} - \kappa_{1,2} \\
\kappa_{[1,3]} &= \kappa_{1,3} - 3\kappa_{1,2} + 2\kappa_{1,1} \\
\kappa_{[2,2]} &= \kappa_{2,2} - \kappa_{2,1} - \kappa_{1,2} + \kappa_{1,1}
\end{align*}
\]

Conversely

\[
\begin{align*}
\kappa_2 &= \kappa_2 + \kappa_1 \\
\kappa_3 &= \kappa_3 + 3\kappa_2 + \kappa_1 \\
\kappa_4 &= \kappa_4 + 6\kappa_3 + 7\kappa_2 + \kappa_1 \\
\kappa_{1,2} &= \kappa_{[1,2]} + \kappa_{1,1} \\
\kappa_{1,3} &= \kappa_{[1,3]} + 3\kappa_{[1,2]} + \kappa_{[1,1]} \\
\kappa_{2,2} &= \kappa_{[2,2]} + \kappa_{[2,1]} + \kappa_{[1,2]} + \kappa_{[1,1]}
\end{align*}
\]

\[\text{(19)}\]

3 The relation between fluorescence intensity and photon counting distributions

Consider a fluorescence detection experiment involving \(k\) channels and denote the fluorescence light intensity in channel \(i\) at time \(t\) by \(I_i(t)\). Typically, different channels consist of different detectors and/or signals at different lag times.

Let us for the time being limit our discussion to a single channel \(i\) and drop the subscript \(i\) in the relevant quantities. Consider a time interval (bin) of size \(T\) and set the origin at its beginning: \([0, T]\). Absorbing all efficiency parameters into \(I_i\), or assuming perfect efficiency, the probability of detecting a photon in the infinitesimal interval \([t_j, t_j + dt_j]\) is given by \(I_i(t_j)dt_j\). The probability of detecting no photons in the interval \([t_{j-1}, t_j]\) is given by the product of the probabilities of detecting no photons in each infinitesimal interval \(\delta t'\) in that interval:

\[
\lim_{\delta t' \to 0} \prod_{\delta t' \epsilon [t_{j-1}, t_j]} (1 - I(t')\delta t') = \lim_{\delta t' \to 0} \prod_{\delta t' \epsilon [t_{j-1}, t_j]} \exp[-I(t')\delta t']
\]

\[
= \exp \left[ - \int_{t_{j-1}}^{t_j} I(t')dt' \right]
\]

Let us also define \(t_0 = 0\) and \(t_{n+1} = T\). For \(j = 1, 2, \ldots, n\) the probability of detecting exactly \(n\) photons at \(n\) infinitesimal intervals \([t_j, t_j + dt_j]\) where \(0 \leq t_1 < \cdots < t_n < T\) is given by

\[
\left\{ \prod_{j=1}^{n+1} \exp \left[ - \int_{t_{j-1}}^{t_j} I(t')dt_j' \right] \right\} \left\{ \prod_{j=1}^{n} I(t_j)dt_j \right\}
\]

\[
= \exp \left[ - \int_{0}^{T} I(t')dt' \right] \prod_{j=1}^{n} I(t_j)dt_j
\]
Thus we obtain Mandel’s formula:

\[ P(n; T|I) = \exp \left[ - \int_0^T I(t') dt' \right] \int_0^T dt_1 \int_0^{t_1} dt_2 \cdots \int_0^{t_{n-1}} dt_n I(t_1)I(t_2)\ldots I(t_n) \]

\[ = \exp \left[ - \int_0^T I(t') dt' \right] \int_0^T dt_n \int_0^{t_n} \cdots \int_0^{t_2} dt_1 I(t_1)I(t_2)\ldots I(t_n) \]

The integrand \( I(t_1)I(t_2)\ldots I(t_n) \) is symmetric: it has the same value at any permutation of the \( t_j \)s. Any permutation of \( t_j \)s in \( 0 < t_1 < \cdots < t_n < T \) covers a separate block of the parameter space, and the integration yields the same value over each block. The union of all such blocks, or permutations of the \( t_j \)s, covers the entire span of \( 0 < t_j < T \) for all \( j \). There are \( n! \) blocks, therefore we have

\[ P(n; T|I) = \exp \left[ - \int_0^T I(t') dt' \right] \int_0^T \frac{1}{n!} \int_0^T dt_1 \int_0^{t_1} dt_2 \cdots \int_0^{t_{n-1}} dt_n I(t_1)I(t_2)\ldots I(t_n) \]

\[ = \frac{1}{n!} \left[ \int_0^T I(t) dt \right]^n \exp \left[ - \int_0^T I(t') dt' \right] \quad (20) \]

Now we take the fluctuations of \( I(t) \) into account. Let \( P(I)dI \) denote the probability that the fluorescence intensity takes a value within an infinitesimal variation \( dI \) around \( I(t) \) defined over \([0, T]\). Assuming a stationary process, binning a long experimental time and averaging a quantity over all such bins, as done in an FCS experiment, is equivalent to averaging that quantity over an ensemble of \( I(t) \) (ergodicity). The probability of detecting \( n \) photons in each bin is therefore

\[ P(n; T) = \int P(n; T|I)P(I)dI \quad (21) \]

where \( P(n; T|I) \) is given by (20). A more useful random variable is the integrated intensity within the bin time \( T \), defined as

\[ W = \int_0^T I(t) dt \quad (22) \]

The probability of detecting \( n \) photons in the bin, (21), can be written as

\[ P(n; T) = \int P(n; T|W)P(W)dW \]

where, by (20),

\[ P(n; T|W) = \frac{W^n}{n!}e^{-W} = \text{Poi}(n, W) \]

Thus we obtain Mandel’s formula[8]:

\[ P(n; T) = \int \text{Poi}(n, W)P(W)dW \]

We can now consider the case of multiple channels, and a common bin size \( T \). The fluorescence intensities \( I_i(t) \) at different channels are not necessarily independent. Neither are the integrated intensities, \( W_i = \)
\[ \int_0^T I_i(t)dt. \] However, given particular \( W_1, W_2, \ldots, W_k \) the occurrence probabilities of photons in distinct channels are, by definition, independent:

\[
P(n_1, n_2, \ldots, n_k; T|W_1, W_2, \ldots, W_k) = \prod_{i=1}^{k} P(n_i; T|W_i)
\]

\[
= \prod_{i=1}^{k} \text{Poi}(n_i, W_i)
\]

"Distinct" channels in practice can be bins (overlapping with no cross-talk, or non-overlapping) in independent detector signals, or non-overlapping bins in a single detector signal. Denoting the joint probability of integrated intensities taking the values \( W_1, W_2, \ldots, W_k \) by \( P(W_1, W_2, \ldots, W_k) \) and integrating (23) we obtain for the joint probability of detecting \( n_i \) photons in channel \( i \):

\[
P(n_1, n_2, \ldots, n_k; T) = \int \int \ldots \int P(W_1, W_2, \ldots, W_k) \prod_{i=1}^{k} \text{Poi}(n_i, W_i)dW_i
\]

which is the multivariate form of Mandel's formula.

To abbreviate notation, we define \( \vec{W} = (W_1, W_2, \ldots, W_k) \) and \( \vec{n} = (n_1, n_2, \ldots, n_k) \) using boldface symbols in our general treatment of multiple channels, to be distinguished from vectors introduced later specific to two-time correlation experiments.

Let us now examine the relations between moments (or cumulants) of \( \vec{W} \) and moments (or cumulants) of \( \vec{n} \). The probability generating function of \( \vec{n} \) is

\[
\Omega_{\vec{n}}(\vec{t}) = \sum_{\vec{n}} P(\vec{n}; T) \prod_{i=1}^{k} t_i^{n_i}
\]

where the shorthand notation

\[
\sum_{\vec{n}} = \sum_{n_1=0}^{\infty} \cdots \sum_{n_k=0}^{\infty}
\]

has been used. The factorial moment generating function of \( \vec{n} \) is

\[
\Phi_{\vec{n}}(\vec{t}) = \Omega_{\vec{n}}(\vec{t} + \vec{1})
\]

\[
= \sum_{\vec{n}} P(\vec{n}; T) \prod_{i=1}^{k} (t_i + 1)^{n_i}
\]
using Mandel's formula, (24),

\[
\begin{align*}
&= \sum_{\mathbf{n}} \left\{ \int_{\mathbf{W}} P(\mathbf{W}) d^k W \prod_{i=1}^{k} \frac{W_{n_i}}{n_i!} e^{-W_i} \right\} \prod_{i=1}^{k} (t_i + 1)^{n_i} \\
&= \int_{\mathbf{W}} P(\mathbf{W}) d^k W \left\{ \prod_{i=1}^{k} e^{-W_i} \right\} \sum_{\mathbf{n}} \prod_{i=1}^{k} \frac{W_{n_i}}{n_i!} (t_i + 1)^{n_i} \\
&= \int_{\mathbf{W}} P(\mathbf{W}) d^k W \left\{ \prod_{i=1}^{k} e^{-W_i} \right\} \prod_{i=1}^{k} \sum_{n_i} \frac{(W_i t_i + W_i)^{n_i}}{n_i!} \\
&= \int_{\mathbf{W}} P(\mathbf{W}) d^k W \left\{ \prod_{i=1}^{k} e^{-W_i} \right\} \prod_{i=1}^{k} e^{W_i t_i + W_i} \\
&= \int_{\mathbf{W}} P(\mathbf{W}) d^k W \prod_{i=1}^{k} e^{W_i t_i} \\
&= \mathbb{E}[e^{\mathbf{W} \cdot \mathbf{t}}] \\
&= M_{\mathbf{W}}'(\mathbf{t})
\end{align*}
\]

the moment generating function of $\mathbf{W}$. This shows that the factorial moments of $\mathbf{n}$ are equal to the (ordinary) moments of $\mathbf{W}$.

Taking the natural logarithm of (25) we also obtain:

\[
\Psi_{\mathbf{n}}(\mathbf{t}) = K_{\mathbf{W}}(\mathbf{t})
\]

That is, the factorial cumulants of $\mathbf{n}$ are equal to the (ordinary) cumulants of $\mathbf{W}$.

Alternatively, we can calculate the factorial moments of $\mathbf{n}$ directly:

\[
\mu^{[r]}(\mathbf{n}) = \sum_{\mathbf{n}} P(\mathbf{n}) \prod_{i=1}^{k} n_i^{[r_i]}
\]

and since $n^{[r]} = 0$ for $n < r$

\[
\begin{align*}
&= \sum_{\mathbf{n} \geq \mathbf{r}} \left\{ \int_{\mathbf{W}} P(\mathbf{W}) d^k W \prod_{i=1}^{k} \frac{W_{n_i}}{n_i!} e^{-W_i} \right\} \prod_{i=1}^{k} \frac{n_i!}{(n_i - r_i)!} \\
&= \int_{\mathbf{W}} P(\mathbf{W}) d^k W \left\{ \prod_{i=1}^{k} e^{-W_i} \right\} \prod_{i=1}^{k} \sum_{n_i=r_i}^{\infty} \frac{W_{n_i}^{n_i}}{(n_i - r_i)!} \\
&= \int_{\mathbf{W}} P(\mathbf{W}) d^k W \left\{ \prod_{i=1}^{k} e^{-W_i} \right\} \prod_{i=1}^{k} W_{r_i}^{r_i} e^{W_i} \\
&= \int_{\mathbf{W}} P(\mathbf{W}) d^k W \prod_{i=1}^{k} W_{r_i}^{r_i} \\
&= \mu^{[r]}(\mathbf{W})
\end{align*}
\]

Then we can argue that the f.m.g.f. of $\mathbf{n}$ is equal to m.g.f. of $\mathbf{W}$. From there, (26) and the equality of the factorial cumulants of $\mathbf{n}$ with the corresponding cumulants of $\mathbf{W}$ follow:

\[
\kappa^{[r]}(\mathbf{n}) = \kappa^{[r]}(\mathbf{W})
\]
3.1 Two-time correlations*

We can now use Equation (27) to describe the correlation functions between two lag times, 0 and $t$. A number of methods have been proposed due to the experimental limitations which arise from detector artifacts, namely dead-time, after-pulsing, and cross-talk. The effects of these artifacts in higher-order correlations extend to all time scales, beyond the better-known effects in second-order FCS which occur at short lag times only. Multi-detector and/or “sub-binning” approaches have been proposed to overcome these issues in higher-order FCS. These methods have been described in another article[1] along with the advantages and disadvantages of each method.

In brief, a single-detector method with no modification usually suffers most severely from detector artifacts. Earlier work tried to estimate and account for these artifacts[9, 5, 17], however, the extra modeling, approximations, and calibrations can make such analysis more complicated and less versatile. Using two or more detectors may avoid dead-time and after-pulsing artifacts, however, cross-talk between the detectors may become an issue if not experimentally prevented. Sub-binning refers to using smaller, non-overlapping intervals (sub-bins) within the larger sampling intervals (bins). The independent sub-bins virtually convert a single-detector experiment to a multi-detector one with no cross-talk issue. In a true multi-detector experiment, sub-binning also helps avoid the cross-talk artifact. Therefore, sub-binning yields artifact-free results in both single-detector and multi-detector experiments.[1]

The discussion below assumes no sub-binning. For sub-binning we can employ the multi-detector formulation without sub-binning; no separate formulation is needed.

3.1.1 Single-Detector Experiment

When only one detector is used in the experiment (without sub-binning), we can denote the fluorescence light intensity arriving at the detection point at zero lag time by $I(0)$ and at lag time $t$ by $I(t)$. Take $W(0)$ and $W(t)$ to denote the corresponding integrated intensities over some binning time $T$, that is

$$W(t) = \int_t^{t+T} I(t') dt'$$

Also, $n(0)$ and $n(t)$ denote the number of photons detected in the corresponding bins. The random vectors $\vec{I} = [I(0), I(t)]$, $\vec{W} = [W(0), W(t)]$, and $\vec{n}_{1d} = [n(0), n(t)]$ are then defined accordingly. Relation (27) then directly yields

$$\kappa_{[p,q]} [n(0), n(t)] = \kappa_{p,q} [W(0), W(t)]$$

(28)

3.1.2 Multi-Detector Experiment

We can use $n_d$ independent detectors (without sub-binning) to obtain correlations of order $(p, q)$ with $p, q \leq n_d$. We assume that the beamsplitters and the detection efficiencies are adjusted such that the light intensity is equal for all detectors at any moment. Then take $I(0)$ to denote the fluorescence light intensity arriving at any detector at lag time zero, and $I(t)$ to denote that intensity at lag time $t$. Correspondingly, $W(0)$ and $W(t)$ are defined by integration over a sampling interval (bin) of size $T$, as in the single-detector case. The random vectors $\vec{I} = [I(0), I(t)]$ and $\vec{W} = [W(0), W(t)]$ are also defined accordingly. The photon count $n_i(t)$ in the $i$th detector during a bin is a distinct random variable for each detector, $i$. Therefore we define the vector

$$\vec{n} = [n_1(0), n_2(0), \ldots, n_p(0), n_1(t), n_2(t), \ldots, n_q(t)]$$

(29)

Relation (27) directly yields

$$\kappa_{[p+q]} (\vec{n}) = \kappa_{p+q} [W(0), \ldots, W(0), W(t), \ldots, W(t)]$$

$$= \kappa_{p,q} [W(0), W(t)]$$

$$= \kappa_{p,q} (\vec{W})$$

(30)

*The content of this section is available in reference [1] and is brought here for the sake of continuity.
where $\vec{1}_{p+q} = (1, 1, \ldots, 1)$ has $p + q$ elements.

In particular, for the case of two detectors named A and B we have:

$$\kappa_{[1,1,1]} [n_A(0), n_B(0), n_A(t)] = \kappa_{2,1} [W(0), W(t)]$$

$$\kappa_{[1,1,1]} [n_A(0), n_A(t), n_B(t)] = \kappa_{1,2} [W(0), W(t)]$$

$$\kappa_{[1,1,1,1]} [n_A(0), n_B(0), n_A(t), n_B(t)] = \kappa_{2,2} [W(0), W(t)]$$

## 4 Modeling correlations for diffusing and reacting molecules

In this section we derive the relations describing higher order fluorescence correlations for molecules of a single-species which simultaneously diffuse and undergo reaction between multiple states. We assume the molecules have the same diffusion constant in all the reaction states. This multi-state system reduces to a multi-species non-interacting system when the reaction rates are set to zero, with identical diffusion constant for all species assumed. A mixture of reacting and non-reacting species can also be described by setting only a subset of the reaction rates equal to zero.

Palmer and Thompson [11] defined higher-order correlations using higher-order moments of intensity. For mixtures of diffusing molecules, the moment-based definition of correlation functions leads to complex expressions that depend on lower-order correlation functions. No such expression has been proposed to include reactions of diffusing molecules due to the increased complexity of formulation. Later, Melnykov and Hall[9], following the approach developed by Müller in the study of Fluorescence Cumulant Analysis [10], presented a definition of higher-order correlations based on higher-order cumulants. In their derivation, Melnykov and Hall used the additive property of cumulants to arrive at simple factorized expressions for the cumulant-based higher-order correlation functions describing systems of diffusing molecules with reactions.

In comparison to moments, the computation of cumulants from the experimental photon stream is only slightly more complicated: moments are first computed, then converted to cumulants using tabulated relations. However, with the cumulant-based formulation, the theoretical relations which describe systems of diffusing and reacting molecules are much simpler than with the moment-based formulation. Most importantly, with the cumulant-based formulation, the expressions factorize into pure reaction and diffusion parts for systems with independent reaction and diffusion processes. This allows for the experimental removal of any dependence on the molecular detection function (MDF, defined as the combination of laser intensity distribution, collection point-spread function, and pinhole aperture[14]) and on the diffusion constant by using a reference measurement[2].

In this section, before presenting the derivation reported by Melnykov and Hall, we present an alternative derivation starting from simpler premises and use a reverse reasoning process: we start from the explicit integrals following the definition of higher-order moments, (33), and find conversion relations by only demanding simple final expressions which are factorized into reaction and diffusion parts, (60), without assuming any knowledge about cumulants, their properties, and their relation to moments. Only then, we show that such conversion relations are in general equivalent to the well-known conversion relations between moments and cumulants. We label this approach the Palmer-Thompson approach because the explicit expression of integrals using Dirac and Kronecker delta functions was inspired by the work of those authors. On the other hand, Melnykov and Hall used the well-known additive property of cumulants to directly derive the simple factorized expressions for a multi-particle system based on the moments for a single particle. While the approach by Melnykov and Hall is more concise and elegant, the Palmer-Thompson approach is more elaborate and instructive therefore it is discussed first. The two derivations are formally independent and the reader may skip to the second approach if desired (after the introductory discussion below).

Some preliminary discussion comes first. Take the random vector $\vec{W} = [W(0), W(t)]$, where, following (22),

$$W(t) = \int_t^{t+T} I(t')dt'$$
The general dependence of non-correlated higher moments of $W(t)$ on the bin size, $T$, has been studied by Wu and Müller [18] for non-interacting diffusing molecules through the introduction of “binning functions”.

In this report, however, we limit our attention to the case of small bin sizes. For a short bin size $T$ over which the variations of intensity can be neglected, we have

$$W(t) \approx TI(t)$$

$$\mu'_{m,n}[W(0), W(t)] \approx T^{m+n}\mu'_{m,n}[I(0), I(t)]$$

(31)

which becomes exact in the limit $T \rightarrow 0$.

Absorbing any detection efficiency factors into $I$, we can write

$$I(t) = \sum_s Q_s \int_V L(\vec{r})C_s(\vec{r}, t) d^3r$$

(32)

where $L(\vec{r})$ is the laser illumination profile normalized to its peak value, $J$ is the number of molecular states, $Q_s$ is the brightness of state $s$ at illumination peak in counts per unit time per molecule, $C_s$ is the concentration of the particles in state $s$ at position $\vec{r}$ and time $t$, and $V$ is an integration volume that includes the illuminated region. $V$ can be taken to be the entire sample volume containing a fixed number of molecules, $M$.

Therefore we have, in the limit $T \rightarrow 0$,

$$\mu'_{m,n}[I(0), I(t)]$$

$$= \langle I^m(0)I^n(t) \rangle$$

$$= \sum_{s_1=1}^J \cdots \sum_{s_{m+n}=1}^J Q_{s_1} \cdots Q_{s_{m+n}} \int \cdots \int d^3r_1 \cdots d^3r_{m+n}$$

$$\times L(\vec{r}_1) \cdots L(\vec{r}_{m+n})G'_{m,n}(s_1, \ldots, s_{m+n}; \vec{r}_1, \ldots, \vec{r}_{m+n}; t)$$

(33)

where

$$G'_{m,n}(s_1, \ldots, s_{m+n}; \vec{r}_1, \ldots, \vec{r}_{m+n}; t)$$

$$= \langle C_{s_1}(\vec{r}_1, 0) \ldots C_{s_m}(\vec{r}_m, 0)C_{s_{m+1}}(\vec{r}_{m+1}, t) \ldots C_{s_{m+n}}(\vec{r}_{m+n}, t) \rangle$$

(34)

The concentration of particles in state $s$ at position $\vec{r}$ at time $t$ is given by

$$C_s(\vec{r}, t) = \sum_{j=1}^M \delta(s, s_j(t))\delta(\vec{r} - \vec{r}_j(t))$$

(35)

where $s_j(t)$ and $\vec{r}_j(t)$ are the state and position of the $j$th particle at time $t$, respectively. $\delta(s, s')$ and $\delta(\vec{r} - \vec{r}')$ denote the Kronecker and the Dirac delta functions, respectively. $M$ is the total number of molecules in the large sample volume $V$. We will later take the limit $M, V \rightarrow \infty$.

Assuming a stationary (ergodic) system, the expected value of concentration of particles in state $s$ at position $\vec{r}$ can be obtained by averaging over $s_j(t)$ and $\vec{r}_j(t)$ as they vary over time:

$$\langle C_s \rangle = \sum_{j=1}^M \langle \delta(s, s_j(t))\delta(\vec{r} - \vec{r}_j(t)) \rangle_{\vec{r}_j, s_j}$$

where

$$\langle \delta(s, s_j(t))\delta(\vec{r} - \vec{r}_j(t)) \rangle = \sum_{s_j=1}^J P(s_j)\delta(s, s_j) \int_V d^3r_j P(\vec{r}_j)\delta(\vec{r} - \vec{r}_j)$$

(36)

$$= P(s)/V$$

(37)
Here, \( P(s) \) denotes the probability that a given particle is in state \( s \) and

\[
P(\vec{r}) = \frac{1}{V}
\]

is a uniform probability density that the particle is found anywhere in the solution. Denoting the expected number of particles in state \( s \) by

\[
M_s = P(s)M
\]

we get

\[
\langle C_s \rangle = \frac{M_s}{V}
\]  
(38)

Also, assuming ergodicity,

\[
\langle C_s \rangle = \lim_{V \to \infty} \frac{1}{V} \int_V C_s(\vec{r}, t) d^3r
\]

Taking the expectation of (32) and using (38), the mean detection count rate is found to be

\[
\langle I \rangle = \sum_{s=1}^{J} Q_s N_s
\]  
(39)

where we have defined

\[
N_s = \frac{V_{MDF}}{V} M_s
\]  
(40)

and

\[
V_{MDF} = \int_V L(\vec{r}) d^3r
\]  
(41)

In the limit \( V \to \infty \), \( V_{MDF} \) approaches the volume of the molecular detection function (observation volume, or probe region), and \( N_s \) approaches the average number of molecules in state \( s \) in the observation volume.

Consider a single particle, for example the \( j \)th one. We define and evaluate

\[
U(1)(s_1, s_2; \vec{r}_1, \vec{r}_2; t) = \langle \delta(s_1, s_j(0))\delta(\vec{r}_1 - \vec{r}_j(0))\delta(s_2, s_j(t))\delta(\vec{r}_2 - \vec{r}_j(t)) \rangle
\]  
(42)

For brevity, take \( s = s_j(0), \vec{r} = \vec{r}_j(0), s' = s_j(t), \) and \( \vec{r}' = \vec{r}_j(t) \). Then

\[
\langle \delta(s_1, s)\delta(\vec{r}_1 - \vec{r})\delta(s_2, s')\delta(\vec{r}_2 - \vec{r}') \rangle
\]

\[
= \sum_{s=1}^{J} \sum_{s'=1}^{J} \delta(s_1, s)\delta(s_2, s')P(s, s'; t) \int_V \int_V \delta(\vec{r}_1 - \vec{r})\delta(\vec{r}_2 - \vec{r}') P(\vec{r}, \vec{r}'; t) d^3r d^3r'
\]

\[
= P(s_1, s_2; t)P(\vec{r}_1, \vec{r}_2; t)
\]

where \( P(s, s'; t)P(\vec{r}, \vec{r}'; t) \) denotes the joint probability that the particle is at state \( s \) and position \( \vec{r} \) at time 0, and at state \( s' \) and position \( \vec{r}' \) at time \( t \). It can be expressed in terms of conditional probabilities,

\[
P(s_1, s_2; t) = P(s_2|s_1; t)P(s_1),
\]

\[
P(\vec{r}_1, \vec{r}_2; t) = P(\vec{r}_2|\vec{r}_1; t)P(\vec{r}_1)
\]

where \( P(s_2|s_1; t) \) denotes the probability that the particle is found in state \( s_2 \) at time \( t \) given it was in state \( s_1 \) at time 0, and is obtained by solving linear rate equations, Appendix D:

\[
P(s_2|s_1; t) = Z_{s_2,s_1}(t)
\]

19
Similarly, the probability density function of the particle being at position $\vec{r}_2$ at time $t$ given it was at position $\vec{r}_1$ at time 0 is [obtained from solving the diffusion equation]

$$P(\vec{r}_2|\vec{r}_1; t) = \frac{\exp\left[-|\vec{r}_2 - \vec{r}_1|^2/4Dt\right]}{(4\pi Dt)^{3/2}}$$

Therefore for a single molecule we obtain

$$U^{(1)}(s_1, s_2, \vec{r}_1, \vec{r}_2; t) = \frac{P(s_1)}{V} Z_{s_2, s_1}(t) \frac{\exp\left[-|\vec{r}_1 - \vec{r}_2|^2/4Dt\right]}{(4\pi Dt)^{3/2}}$$  \hspace{1cm} (43)

Next, it will be helpful to define and evaluate

$$U(s_1, s_2, \vec{r}_1, \vec{r}_2; t) = \sum_{j=1}^{M} \langle \delta[s_1, s_j(0)]\delta[\vec{r}_1 - \vec{r}_j(0)]\delta[s_2, s_j(t)]\delta[\vec{r}_2 - \vec{r}_j(t)] \rangle$$  \hspace{1cm} (44)

Immediately from (42), (43), and (38) we get:

$$U(s_1, s_2, \vec{r}_1, \vec{r}_2; t) = MU^{(1)}(s_1, s_2, \vec{r}_1, \vec{r}_2; t)$$

$$= \langle C_{s_1} \rangle \sum_{j=1}^{M} \frac{\exp\left[-|\vec{r}_1 - \vec{r}_2|^2/4Dt\right]}{(4\pi Dt)^{3/2}}$$  \hspace{1cm} (45)

Setting $t = 0$ we have:

$$U(s_1, s_2, \vec{r}_1, \vec{r}_2; 0) = \langle C_{s_1} \rangle \delta(s_1, s_2) \delta(\vec{r}_1 - \vec{r}_2)$$

### 4.1 Palmer-Thompson approach

This method follows the work of Palmer and Thompson [11] where they directly evaluate $G'_{m,n}$. We have modified the notation to incorporate multiple states, and will show that this method is equivalent to the cumulant-based formulation.

Using (35) we have

$$G'_{m,n}(s_1, \ldots, s_{m+n}, \vec{r}_1, \ldots, \vec{r}_{m+n}; t)$$

$$= \sum_{i_1=1}^{M} \sum_{i_2=1}^{M} \cdots \sum_{i_{m+n}=1}^{M} \langle \delta[s_1, s_{i_1}(0)]\delta[\vec{r}_1 - \vec{r}_{i_1}(0)] \cdots \delta[s_m, s_{i_m}(0)]\delta[\vec{r}_m - \vec{r}_{i_m}(0)] \rangle$$

$$\times \langle \delta[s_{m+1}, s_{i_{m+1}}(t)]\delta[\vec{r}_{m+1} - \vec{r}_{i_{m+1}}(t)] \cdots \delta[s_{m+n}, s_{i_{m+n}}(t)]\delta[\vec{r}_{m+n} - \vec{r}_{i_{m+n}}(t)] \rangle$$

We break the summation according to the following cases:

- When two or more particle indices are different (at any lag time) the expectation operator of product of independent random variables factorizes. As a special case, when all particles at both lag times are different:

  $$\sum_{i_1=1}^{M} \sum_{i_{m+n}=1}^{M} \langle \delta[s_1, s_{i_1}(0)]\delta[\vec{r}_1 - \vec{r}_{i_1}(0)] \rangle \times \cdots \times \langle \delta[s_m, s_{i_m}(0)]\delta[\vec{r}_m - \vec{r}_{i_m}(0)] \rangle \times \cdots \times \langle \delta[s_{m+n}, s_{i_{m+n}}(t)]\delta[\vec{r}_{m+n} - \vec{r}_{i_{m+n}}(t)] \rangle$$

  $$= \langle C_{s_1} \rangle \cdots \langle C_{s_{m+n}} \rangle$$
To justify this approximation, notice that according to (37) each term in the sum is of order $V^{-(m+n)}$. The summation on the left has exactly $M(M-1) \ldots (M-m-n)$ terms, and the right hand side, written as products of sums and expanded, has exactly $M^{m+n}$ terms. The sum of the extra terms on the right hand side (pertaining to two or more equal indices) is then of order $1/V$. In the thermodynamic limit $V, M \to \infty$ these extra terms become negligible and the relation becomes exact.

- When two or more particle indices are the same at the same lag time, the expectation operator factorizes into products of delta functions and a single concentration. As a special case, when all particle indices at lag time 0 are identical:

$$\sum_{j=1}^{M} \langle \delta[s_1, s_j(0)]\delta[r_1 - r_j(0)] \ldots \delta[s_m, s_j(0)]\delta[r_m - r_j(0)] \rangle$$

$$= \delta(s_1, s_2)\delta(r_1 - r_2) \ldots \delta(s_1, s_m)\delta(r_1 - r_m) \sum_{j=1}^{M} \langle \delta[s_1, s_j(0)]\delta[r_1 - r_j(0)] \rangle$$

$$= \delta(s_1, s_2)\delta(r_1 - r_2) \ldots \delta(s_1, s_m)\delta(r_1 - r_m) \langle C_{s_1} \rangle$$

This can be justified by writing the expectation operator in the explicit form of (36) and using the change of variables $\vec{y} = \vec{r}_1 - \vec{r}_j$ for the spatial part. The discrete part is nonzero only when all indices are identical.

- When two or more particle indices are the same at different lag times, the expectation operator factorizes into products of delta functions and a single propagator. As a special case, when all indices at lag time 0 and $t$ are identical:

$$\sum_{j=1}^{M} \langle \delta[s_1, s_j(0)]\delta[r_1 - r_j(0)] \ldots \delta[s_m, s_j(0)]\delta[r_m - r_j(0)] \rangle$$

$$\times \langle \delta[s_{m+1}, s_j(t)]\delta[r_{m+1} - r_j(t)] \ldots \delta[s_{m+n}, s_j(t)]\delta[r_{m+n} - r_j(t)] \rangle$$

$$= \delta(s_1, s_2)\delta(r_1 - r_2) \ldots \delta(s_1, s_m)\delta(r_1 - r_m) \times \delta(s_{m+1}, s_{m+2})\delta(r_{m+1} - r_{m+2}) \ldots \delta(s_{m+n}, s_{m+n})\delta(r_{m+n} - r_{m+n})$$

$$\times U(s_1, s_{m+1}, r_1, r_{m+1}; t)$$

(47)

where $U(s_1, s_{m+1}, r_1, r_{m+1}; t)$ is defined in (44).

Let us start with calculating the simpler cases of $G'_{m,0}$ and $G'_{0,n}$. We have by definition (34)

$$G'_{1,0}(s_1, s_2) = \langle C_{s_1}(r_1, 0) \rangle = \langle C_{s_1} \rangle$$

$$G'_{0,1}(s_1, s_2) = \langle C_{s_1}(r_1, t) \rangle = \langle C_{s_1} \rangle$$

For moments of order $(2,0)$, the summation in $G'_{20}$ runs over two particle indices. Therefore, it can be broken into two cases where the two indices are either equal or not equal:

$$G'_{2,0}(s_1, s_2, r_1, r_2)$$

$$= \sum_{j=1}^{M} \sum_{k=1}^{M} \langle \delta[s_1, s_j(0)]\delta[r_1 - r_j(0)]\delta[s_2, s_k(0)]\delta[r_2 - r_k(0)] \rangle$$

$$= \sum_{j=1}^{M} \langle \delta[s_1, s_j(0)]\delta[r_1 - r_j(0)] \rangle \langle \delta[s_2, s_j(0)]\delta[r_2 - r_j(0)] \rangle + \sum_{j=1}^{M} \sum_{k=1}^{M} \langle \delta[s_1, s_j(0)]\delta[r_1 - r_j(0)] \rangle \langle \delta[s_2, s_k(0)]\delta[r_2 - r_k(0)] \rangle$$

$$= \langle C_{s_1} \rangle \delta(s_1, s_2)\delta(r_1 - r_2) + \langle C_{s_1} \rangle \langle C_{s_2} \rangle$$
A similar result is obtained for $G'_{0,2}(s_1, s_2, \vec{r}_1, \vec{r}_2)$, in a similar fashion.

For correlation of order $(1,1)$, the summation in $G'_{11}$ is also over two particle indices only, and it can be broken in a similar way:

$$
G'_{1,1}(s_1, s_2, \vec{r}_1, \vec{r}_2; t) = \sum_{j=1}^{M} \sum_{k=1}^{M} \langle \delta[s_1, s_j(0)] \delta[\vec{r}_1 - \vec{r}_j(0)] \delta[s_2, s_k(t)] \delta[\vec{r}_2 - \vec{r}_k(t)] \rangle
$$

$$
= \sum_{j=1}^{M} \langle \delta[s_1, s_j(0)] \delta[\vec{r}_1 - \vec{r}_j(0)] \delta[s_2, s_j(t)] \delta[\vec{r}_2 - \vec{r}_j(t)] \rangle
$$

$$
+ \sum_{j=1}^{M} \sum_{k=1}^{M} \langle \delta[s_1, s_j(0)] \delta[\vec{r}_1 - \vec{r}_j(0)] \rangle \langle \delta[s_2, s_k(t)] \delta[\vec{r}_2 - \vec{r}_k(t)] \rangle
$$

$$
= U(s_1, s_2, \vec{r}_1, \vec{r}_2; t) + \langle C_{s_1} \rangle \langle C_{s_2} \rangle \tag{48}
$$

For order $(2,1)$, there are three particle indices:

$$
G'_{2,1}(s_1, s_2, s_3, \vec{r}_1, \vec{r}_2, \vec{r}_3; t) = \sum_{j=1}^{M} \sum_{k=1}^{M} \sum_{l=1}^{M} \langle \delta[s_1, s_j(0)] \delta[\vec{r}_1 - \vec{r}_j(0)] \delta[s_2, s_k(0)] \delta[\vec{r}_2 - \vec{r}_k(0)] \delta[s_3, s_l(t)] \delta[\vec{r}_3 - \vec{r}_l(t)] \rangle
$$
and we break the sum into five categories:

\[
= \sum_{j=1}^{M} (\delta[s_1, s_j(0)]\delta[\tilde{r}_1 - \tilde{r}_j(0)]\delta[s_2, s_j(0)]\delta[\tilde{r}_2 - \tilde{r}_j(0)] \\
\times \delta[s_3, s_j(t)]\delta[\tilde{r}_3 - \tilde{r}_j(t)]) \\
+ \sum_{j=1}^{M} \sum_{k=1}^{M} (\delta[s_1, s_j(0)]\delta[\tilde{r}_1 - \tilde{r}_j(0)]\delta[s_2, s_j(0)]\delta[\tilde{r}_2 - \tilde{r}_j(0)]) \\
\times \delta[s_3, s_k(t)]\delta[\tilde{r}_3 - \tilde{r}_k(t)]) \\
+ \sum_{j=1}^{M} \sum_{k=1}^{M} (\delta[s_1, s_j(0)]\delta[\tilde{r}_1 - \tilde{r}_j(0)]\delta[s_3, s_j(t)]\delta[\tilde{r}_3 - \tilde{r}_j(t)]) \\
\times \delta[s_2, s_k(0)]\delta[\tilde{r}_2 - \tilde{r}_k(0)]) \\
+ \sum_{j=1}^{M} \sum_{k=1}^{M} (\delta[s_1, s_j(0)]\delta[\tilde{r}_2 - \tilde{r}_j(0)]\delta[s_3, s_j(t)]\delta[\tilde{r}_3 - \tilde{r}_j(t)]) \\
\times \delta[s_2, s_k(t)]\delta[\tilde{r}_2 - \tilde{r}_k(0)]) \\
+ \sum_{j=1}^{M} \sum_{k=1}^{M} \sum_{l=1}^{M} \sum_{m=1}^{M} (\delta[s_1, s_j(0)]\delta[\tilde{r}_1 - \tilde{r}_j(0)]\delta[s_2, s_k(0)]\delta[\tilde{r}_2 - \tilde{r}_k(0)]) \\
\times \delta[s_3, s_l(t)]\delta[\tilde{r}_3 - \tilde{r}_l(t)]\delta[s_4, s_m(t)]\delta[\tilde{r}_4 - \tilde{r}_m(t)]) \\
\times (\text{no two indices equal}) \\
= \delta(s_1, s_2)\delta(\tilde{r}_1 - \tilde{r}_2)U(s_1, s_3, \tilde{r}_1, \tilde{r}_3; t) \\
+ \langle C_{s_1} \rangle \langle C_{s_1} \rangle \delta(s_1, s_2)\delta(\tilde{r}_1 - \tilde{r}_2) \\
+ \langle C_{s_1} \rangle U(s_1, s_3, \tilde{r}_1, \tilde{r}_3; t) \\
+ \langle C_{s_1} \rangle U(s_2, s_3, \tilde{r}_2, \tilde{r}_3; t) \\
+ \langle C_{s_1} \rangle \langle C_{s_1} \rangle \langle C_{s_1} \rangle \langle C_{s_1} \rangle \\
(49)
\]

The categorization of the terms in the summation above corresponds to partitioning of a set of 3 items. A partition of a set \(A\) is defined as a set of nonempty, pairwise disjoint subsets of \(A\) whose union is \(A\). There is a one-to-one correspondence between the 5 terms in (49) and the 5 partitions of a set of 3 items shown in Figure 1, left.

For order \((2, 2)\) we have:

\[
G_{2,2}'(s_1, s_2, s_3, r_1, r_2, r_3, r_4; t) \\
= \sum_{j=1}^{M} \sum_{k=1}^{M} \sum_{l=1}^{M} \sum_{m=1}^{M} (\delta[s_1, s_j(0)]\delta[\tilde{r}_1 - \tilde{r}_j(0)]\delta[s_2, s_k(0)]\delta[\tilde{r}_2 - \tilde{r}_k(0)]) \\
\times \delta[s_3, s_l(t)]\delta[\tilde{r}_3 - \tilde{r}_l(t)]\delta[s_4, s_m(t)]\delta[\tilde{r}_4 - \tilde{r}_m(t)]) \\
\times (\text{no two indices equal}) \\
\]

Once again, we break the summation depending on which particle indices are equal. This will correspond to partitioning a set of 4 elements as shown in Figure 1, right. If all indices are equal, corresponding to the first row in the figure, we have a single term

\[
\sum_{j=1}^{M} (\delta[s_1, s_j(0)]\delta[\tilde{r}_1 - \tilde{r}_j(0)]\delta[s_2, s_j(0)]\delta[\tilde{r}_2 - \tilde{r}_j(0)]) \\
\times \delta[s_3, s_j(t)]\delta[\tilde{r}_3 - \tilde{r}_j(t)]\delta[s_4, s_j(t)]\delta[\tilde{r}_4 - \tilde{r}_j(t)]) \\
\]

23
Corresponding to the second row in the figure, we have four terms of the form

$$
\sum_{j=1}^{M} \sum_{k=1}^{M} \sum_{j \neq k} \langle \delta[s_1, s_j(0)]\delta[\vec{r}_1 - \vec{r}_j(0)]\delta[s_2, s_j(0)]\delta[\vec{r}_2 - \vec{r}_j(0)] \\
\times \delta[s_3, s_j(t)]\delta[\vec{r}_3 - \vec{r}_j(t)] \rangle \langle \delta[s_4, s_k(t)]\delta[\vec{r}_4 - \vec{r}_k(t)] \rangle
$$

Corresponding to the third row, we have two terms of the form

$$
\sum_{j=1}^{M} \sum_{k=1}^{M} \sum_{l=1}^{M} \langle \delta[s_1, s_j(0)]\delta[\vec{r}_1 - \vec{r}_j(0)]\delta[s_2, s_j(0)]\delta[\vec{r}_2 - \vec{r}_j(0)] \\
\times \delta[s_3, s_k(t)]\delta[\vec{r}_3 - \vec{r}_k(t)] \rangle \langle \delta[s_4, s_l(t)]\delta[\vec{r}_4 - \vec{r}_l(t)] \rangle
$$

and four terms of the form

$$
\sum_{j=1}^{M} \sum_{k=1}^{M} \sum_{l=1}^{M} \langle \delta[s_1, s_j(0)]\delta[\vec{r}_1 - \vec{r}_j(0)]\delta[s_3, s_j(t)]\delta[\vec{r}_3 - \vec{r}_j(t)] \\
\times \delta[s_2, s_k(0)]\delta[\vec{r}_2 - \vec{r}_k(0)] \rangle \langle \delta[s_4, s_l(t)]\delta[\vec{r}_4 - \vec{r}_l(t)] \rangle
$$
Corresponding to the fourth row we have a term of the form
\[
\sum_{j=1}^{M} \sum_{k=1}^{M} \sum_{j \neq k} \langle \delta[s_1, s_j(0)] \delta[\vec{r}_1 - \vec{r}_j(0)] \delta[s_2, s_j(0)] \delta[\vec{r}_2 - \vec{r}_j(0)] \rangle 
\times \langle \delta[s_3, s_k(t)] \delta[\vec{r}_3 - \vec{r}_k(t)] \delta[s_4, s_k(t)] \delta[\vec{r}_4 - \vec{r}_k(t)] \rangle
\]
and two terms of the form
\[
\sum_{j=1}^{M} \sum_{k=1}^{M} \sum_{j \neq k} \langle \delta[s_1, s_j(0)] \delta[\vec{r}_1 - \vec{r}_j(0)] \delta[s_3, s_j(t)] \delta[\vec{r}_3 - \vec{r}_j(t)] \rangle 
\times \langle \delta[s_2, s_k(0)] \delta[\vec{r}_2 - \vec{r}_k(0)] \delta[s_4, s_k(t)] \delta[\vec{r}_4 - \vec{r}_k(t)] \rangle
\]
Finally, corresponding to the last row we have a single term
\[
\sum_{j=1}^{M} \sum_{k=1}^{M} \sum_{l=1}^{M} \sum_{m=1}^{M} \sum_{(\text{no two indices equal})} \langle \delta[s_1, s_j(0)] \delta[\vec{r}_1 - \vec{r}_j(0)] \rangle \langle \delta[s_2, s_k(0)] \delta[\vec{r}_2 - \vec{r}_k(0)] \rangle \langle \delta[s_3, s_l(t)] \delta[\vec{r}_3 - \vec{r}_l(t)] \rangle \langle \delta[s_4, s_m(t)] \delta[\vec{r}_4 - \vec{r}_m(t)] \rangle
\]
Putting all these terms together, we get, in the order presented above,
\[
\mathcal{G}_{2,2}^\prime(s_1, s_2, s_3, s_4, \vec{r}_1, \vec{r}_2, \vec{r}_3, \vec{r}_4; t) \\
= \delta(s_1, s_2) \delta(\vec{r}_1 - \vec{r}_2) \delta(s_3, s_4) \delta(\vec{r}_3 - \vec{r}_4) U(s_1, s_3, \vec{r}_1, \vec{r}_3; t) \\
+ \langle C_{s_1} \rangle \delta(s_1, s_2) \delta(\vec{r}_1 - \vec{r}_2) U(s_1, s_3, \vec{r}_1, \vec{r}_3; t) \\
+ \langle C_{s_3} \rangle \delta(s_1, s_2) \delta(\vec{r}_1 - \vec{r}_2) U(s_1, s_4, \vec{r}_1, \vec{r}_4; t) \\
+ \langle C_{s_1} \rangle \delta(s_3, s_4) \delta(\vec{r}_3 - \vec{r}_4) U(s_2, s_3, \vec{r}_2, \vec{r}_3; t) \\
+ \langle C_{s_2} \rangle \delta(s_3, s_4) \delta(\vec{r}_3 - \vec{r}_4) U(s_1, s_3, \vec{r}_1, \vec{r}_3; t) \\
+ \langle C_{s_1} \rangle \langle C_{s_2} \rangle \langle C_{s_3} \rangle \delta(s_1, s_2) \delta(\vec{r}_1 - \vec{r}_2) \\
+ \langle C_{s_1} \rangle \langle C_{s_2} \rangle \langle C_{s_3} \rangle \delta(s_3, s_4) \delta(\vec{r}_3 - \vec{r}_4) \\
+ \langle C_{s_2} \rangle \langle C_{s_1} \rangle \langle C_{s_4} \rangle U(s_1, s_3, \vec{r}_1, \vec{r}_3; t) \\
+ \langle C_{s_2} \rangle \langle C_{s_4} \rangle U(s_1, s_3, \vec{r}_1, \vec{r}_3; t) \\
+ \langle C_{s_1} \rangle \langle C_{s_4} \rangle U(s_2, s_3, \vec{r}_2, \vec{r}_3; t) \\
+ \langle C_{s_1} \rangle \langle C_{s_4} \rangle U(s_1, s_3, \vec{r}_1, \vec{r}_3; t) \\
+ \langle C_{s_2} \rangle \langle C_{s_3} \rangle U(s_1, s_3, \vec{r}_1, \vec{r}_3; t) \\
+ \langle C_{s_1} \rangle \langle C_{s_3} \rangle \delta(s_1, s_2) \delta(\vec{r}_1 - \vec{r}_2) \delta(s_3, s_4) \delta(\vec{r}_3 - \vec{r}_4) \\
+ U(s_1, s_3, \vec{r}_1, \vec{r}_3; t) U(s_2, s_4, \vec{r}_2, \vec{r}_4; t) \\
+ U(s_1, s_4, \vec{r}_1, \vec{r}_4; t) U(s_2, s_3, \vec{r}_2, \vec{r}_3; t) \\
+ \langle C_{s_1} \rangle \langle C_{s_2} \rangle \langle C_{s_3} \rangle \langle C_{s_4} \rangle
\]
(50)

It is now time to revisit the relations between the moments and the cumulants of distribution. To better illustrate the point, we start with a univariate distribution $X$. Following the notation developed in section 2.1, we have from the relation between the moment generating function and the cumulant generating
function of $X$:

$$
\sum_{r=0}^{\infty} \mu_r \frac{t^r}{r!} = \exp \left( \sum_{p=0}^{\infty} \kappa_p \frac{t^p}{p!} \right)
= \prod_{p=0}^{\infty} \exp \left( \kappa_p \frac{t^p}{p!} \right)
= \prod_{p=0}^{\infty} \sum_{\pi_p} \frac{1}{\pi_p!} \left( \kappa_p \frac{t^p}{p!} \right)^{\pi_p}
$$

Multiplying both sides by $m!$ and picking out the terms in the exponential expansions which, when multiplied together, give a power of $t^m$ we have

$$
\mu_m = \sum \left( \frac{\kappa_{p_1}}{p_1!} \right)^{\pi_{p_1}} \left( \frac{\kappa_{p_2}}{p_2!} \right)^{\pi_{p_2}} \cdots \left( \frac{\kappa_{p_i}}{p_i!} \right)^{\pi_{p_i}} \frac{m!}{\pi_{p_1!} \pi_{p_2!} \cdots \pi_{p_i!}}
$$

In the summation, each term corresponds to a set of distinct integers $\{p_1, p_2, \ldots, p_i\}$ and their associated multiplicities $\{\pi_{p_1}, \pi_{p_2}, \ldots, \pi_{p_i}\}$ such that $p_1 \pi_{p_1} + p_2 \pi_{p_2} + \cdots + p_i \pi_{p_i} = m$

and the summation runs over all such sets. The summation can be limited to $p_i \geq 1$ and $\pi_{p_i} \geq 1$, because $\kappa_0 = 0$, and zero multiplicities $\pi_{p_i} = 0$ are not significant. Each term then represents an “integer partition” of the number $m$ into $\sum_{i=1}^{t} \pi_{p_i}$ positive integer summands.

Now consider a partition of a set of $m$ distinct objects, as shown in Figure 2, left, that includes $\pi_{p_1}$ blocks of size $p_1$, $\pi_{p_2}$ blocks of size $p_2$, ..., and $\pi_{p_i}$ blocks of size $p_i$. The number of all possible partitions with those given blocks and their sizes is exactly equal to the coefficient of $\kappa_{p_1}^{\pi_{p_1}} \kappa_{p_2}^{\pi_{p_2}} \cdots \kappa_{p_i}^{\pi_{p_i}}$ in (51):

$$
\frac{1}{\pi_{p_1!} \pi_{p_2!} \cdots \pi_{p_i!}} \cdot \frac{m!}{(p_1!)^{\pi_{p_1}} (p_2!)^{\pi_{p_2}} \cdots (p_i!)^{\pi_{p_i}}}
$$

There is a parallel situation in the case of a bivariate distribution $\vec{X} = (X_1, X_2)$. Once again, we have from the relation between the moment generating function and the cumulant generating function of $\vec{X}$:

$$
\sum_{m=0}^{\infty} \sum_{n=0}^{\infty} \mu_{m,n} \frac{t^m t^n}{m! n!} = \exp \left( \sum_{p=0}^{\infty} \sum_{q=0}^{\infty} \kappa_{p,q} \frac{t^p t^q}{p! q!} \right)
= \prod_{p=0}^{\infty} \prod_{q=0}^{\infty} \exp \left( \kappa_{p,q} \frac{t^p t^q}{p! q!} \right)
= \prod_{p=0}^{\infty} \prod_{q=0}^{\infty} \sum_{\pi_{p,q}} \frac{1}{\pi_{p,q}!} \left( \kappa_{p,q} \frac{t^p t^q}{p! q!} \right)^{\pi_{p,q}}
$$
Figure 2: Example partition blocks of a set of $m$ elements (left) and those of a set of $m + n$ elements divided into two subsets (right).
Multiplying both sides by $m!n!$ and picking out the terms in the exponential expansions which, when multiplied together, give a power of $t_i^n t_j^l$ we get

$$
\mu'_{m,n} = \sum \frac{(\kappa_{p_1,q_1})}{p_1 q_1 !} \frac{\pi_{p_1,q_1}}{p_1 q_1 !} \frac{\pi_{p_2,q_2}}{p_2 q_2 !} \cdots \frac{\pi_{p_i,q_i}}{p_i q_i !} \frac{m!}{\pi_{p_1,q_1}! \pi_{p_2,q_2}! \cdots \pi_{p_i,q_i}!} \quad (52)
$$

Each term in the summation corresponds to a set of distinct ordered pairs \{(\pi_{p_1,q_1}, \pi_{p_2,q_2}, \ldots, \pi_{p_i,q_i})\} with multiplicities \{\pi_{p_1,q_1}, \pi_{p_2,q_2}, \ldots, \pi_{p_i,q_i}\} subject to the conditions

$$
p_1 \pi_{p_1,q_1} + p_2 \pi_{p_2,q_2} + \ldots + p_i \pi_{p_i,q_i} = m
$$

and

$$
q_1 \pi_{p_1,q_1} + q_2 \pi_{p_2,q_2} + \ldots + q_i \pi_{p_i,q_i} = n
$$

and the summation runs over all such sets. The summation can be limited to \((p_i + q_i) \geq 1\) (while either \(p_i\) or \(q_i\) can be zero), and nonzero multiplicities \(\pi_{p_i,q_i} \geq 1\). This will correspond to integer partitioning the ordered pair of positive integers \((m,n)\) into \(\sum_{i=1}^{\pi_{p_i,q_i}}\) ordered pairs of positive integers. By ordered, we mean \((p_i,q_i)\) and \((q_i,p_i)\) are different pairs.

Now consider a partition of a set of \(n\) distinct elements, as shown in Figure 2, right. The set, denoted by \(S\), is divided into two complementary subsets \(L\) and \(R\) of sizes \(m\) and \(n\) respectively. Each block of an arbitrary partition of \(S\) consists of \(p_i\) elements in \(L\) and \(q_i\) elements in \(R\), and there are \(\pi_{p_i,q_i}\) such blocks. Notice that the a block of size \((p_i,q_i)\) is considered to have a different size than a block of size \((q_i,p_i)\). We claim that the total number of partitions with a given set of blocks and their sizes, that is a given integer partition of \((m,n)\), is equal to the coefficient of \(\kappa_{p_1,q_1}^{\pi_{p_1,q_1}} \kappa_{p_2,q_2}^{\pi_{p_2,q_2}} \ldots \kappa_{p_i,q_i}^{\pi_{p_i,q_i}}\) in (52):

$$
\frac{1}{\pi_{p_1,q_1}! \pi_{p_2,q_2}! \cdots \pi_{p_i,q_i}!} \frac{m!}{(p_1!)^{\pi_{p_1,q_1}} (p_2!)^{\pi_{p_2,q_2}} \cdots (p_i!)^{\pi_{p_i,q_i}}} \frac{n!}{(q_1!)^{\pi_{p_1,q_1}} (q_2!)^{\pi_{p_2,q_2}} \cdots (q_i!)^{\pi_{p_i,q_i}}} \quad (55)
$$

To see this, we argue that only by permuting the members of \(L\) within \(L\) and the members of \(R\) within \(R\), all the set partitions of \(S\) relevant to the given integer partition of \((m,n)\) are generated. Consider exchanging a member of \(L\) with a member of \(R\). If the exchange happens within the same block, no new partition is created. Therefore it is not counted. If the exchange happens between two distinct blocks, the sizes of the blocks have changed by definition and the resultant set partition of \(S\) is counted under a different integer partition of \((m,n)\). Coefficient (55) then gives the number of all the set partitions relevant to a given number partition, by arguments similar to the univariate case. Each and every partition of \(S\) is constructed by a partition of \((m,n)\) together with a permutation of the members of \(L\) and \(R\) within their home subsets.

As a result, there is a one-to-one correspondence between the partitions of a set of \(m+n\) distinct elements and the products of bivariate cumulants appearing in the expansion of \(\mu'_{m,n}\). For each partition there is a product term in the expansion, and for each block of size \((p_i,q_i)\) in the partition there is a \(\kappa_{p_i,q_i}\) in the product term.

On the other hand, we saw that the partitions of a set of \(m+n\) distinct elements corresponds uniquely to the terms appearing in the expansion of \(\mathcal{G}'_{m,n}(s_1, \ldots, s_{m+n}; r_1, \ldots, r'_{m+n}; t)\). Each term in the expansion corresponds to a partition of the set. In each term, groups of equal particle indices, corresponding to the blocks of the partition, break the summation into products of some factors; each factor being of the form:

$$
\mathcal{F}_{0,1}(s_1, r_1) = \mathcal{F}_{0,1}(s_1, r_1) = \langle C_{s_1} \rangle
$$

$$
\mathcal{F}_{2,0}(s_1, s_2, r_1, r_2) = \mathcal{F}_{0,2}(s_1, s_2, r_1, r_2) = \langle C_{s_1} \rangle \delta(s_1, s_2) \delta(r_1 - r_2)
$$

$$
\mathcal{F}_{1,1}(s_1, s_2, r_1, r_2; t) = U(s_1, s_2, r_1, r_2; t)
$$

$$
\mathcal{F}_{2,1}(s_1, s_2, s_3, r_1, r_2, r_3; t) = \delta(s_1, s_2) \delta(r_1 - r_2) U(s_1, s_3, r_1, r_2, r_3; t)
$$

$$
\mathcal{F}_{1,2}(s_1, s_2, s_3, r_1, r_2, r_3; t) = \delta(s_2, s_3) \delta(r_2 - r_3) U(s_1, s_2, r_1, r_2, r_3; t)
$$

$$
\mathcal{F}_{2,2}(s_1, s_2, s_3, r_1, r_2, r_3, r_4; t) = \delta(s_1, s_2) \delta(r_1 - r_2)
$$

$$
\times \delta(s_3, s_4) \delta(r_3 - r_4) U(s_1, s_3, r_1, r_2, r_3; t)
$$

28
and in general, for \( m, n \geq 1 \),
\[
\mathcal{F}_{m,n}(s_1, \ldots, s_{m+n}, \vec{r}_1, \ldots, \vec{r}_{m+n}; t)
\]
\[
= \delta(s_1, s_2)\delta(\vec{r}_1 - \vec{r}_2) \cdots \delta(s_m, s_m)\delta(\vec{r}_1 - \vec{r}_m)
\]
\[
\times \delta(s_{m+1}, s_{m+2})\delta(\vec{r}_{m+1} - \vec{r}_{m+2}) \cdots \delta(s_{m+n}, s_{m+n})\delta(\vec{r}_{m+1} - \vec{r}_{m+n})
\]
\[
\times U(s_1, s_{m+1}, \vec{r}_1, \vec{r}_{m+1}; t)
\]
(56)

and, for \( m, n, n' \geq 1 \),
\[
\mathcal{F}_{m,0}(s_1, \ldots, s_m, \vec{r}_1, \ldots, \vec{r}_m) = \mathcal{F}_{0,m}(s_1, \ldots, s_m, \vec{r}_1, \ldots, \vec{r}_m)
\]
\[
= \mathcal{F}_{n,n'}(s_1, \ldots, s_m, \vec{r}_1, \ldots, \vec{r}_m; 0)
\]
\( (n + n' = m) \)

For example, we write the first few \( \mathcal{G}'_{m,n} \) in terms of \( \mathcal{F}_{p,q} \):

\( \mathcal{G}'_{1,0}(s_1, \vec{r}_1) = \mathcal{F}_{1,0}(s_1, \vec{r}_1) \)

\( \mathcal{G}'_{2,0}(s_1, s_2, \vec{r}_1, \vec{r}_2) = \mathcal{F}_{2,0}(s_1, s_2, \vec{r}_1, \vec{r}_2) + \mathcal{F}_{1,0}(s_1, \vec{r}_1)\mathcal{F}_{1,0}(s_2, \vec{r}_2) \)

and

\( \mathcal{G}'_{1,1}(s_1, s_2, \vec{r}_1, \vec{r}_2; t) = \mathcal{F}_{1,1}(s_1, s_2, \vec{r}_1, \vec{r}_2; t) + \mathcal{F}_{1,0}(s_1, \vec{r}_1)\mathcal{F}_{0,1}(s_2, \vec{r}_2) \)

\( \mathcal{G}'_{2,1}(s_1, s_2, s_3, \vec{r}_1, \vec{r}_2, \vec{r}_3; t) = \mathcal{F}_{2,1}(s_1, s_2, s_3, \vec{r}_1, \vec{r}_2, \vec{r}_3; t)
\]
\[
+ \mathcal{F}_{2,0}(s_1, s_2, \vec{r}_1, \vec{r}_2)\mathcal{F}_{0,1}(s_3, \vec{r}_3)
\]
\[
+ \mathcal{F}_{1,0}(s_2, \vec{r}_2)\mathcal{F}_{1,1}(s_1, s_3, \vec{r}_1, \vec{r}_3; t)
\]
\[
+ \mathcal{F}_{1,0}(s_1, \vec{r}_1)\mathcal{F}_{1,1}(s_2, s_3, \vec{r}_2, \vec{r}_3; t)
\]
\[
+ \mathcal{F}_{0,1}(s_3, \vec{r}_3)\mathcal{F}_{0,1}(s_4, \vec{r}_4)\mathcal{F}_{2,0}(s_1, s_2, \vec{r}_1, \vec{r}_2)
\]
\[
+ \mathcal{F}_{1,0}(s_1, \vec{r}_1)\mathcal{F}_{0,1}(s_2, \vec{r}_2)\mathcal{F}_{0,2}(s_3, s_4, \vec{r}_3, \vec{r}_4)
\]
\[
+ \mathcal{F}_{1,0}(s_2, \vec{r}_2)\mathcal{F}_{0,1}(s_4, \vec{r}_4)\mathcal{F}_{1,1}(s_1, s_3, \vec{r}_1, \vec{r}_3; t)
\]
\[
+ \mathcal{F}_{1,0}(s_1, \vec{r}_1)\mathcal{F}_{0,1}(s_3, \vec{r}_3)\mathcal{F}_{1,1}(s_2, s_4, \vec{r}_2, \vec{r}_4; t)
\]
\[
+ \mathcal{F}_{1,0}(s_2, \vec{r}_2)\mathcal{F}_{0,1}(s_4, \vec{r}_4)\mathcal{F}_{1,1}(s_1, s_4, \vec{r}_1, \vec{r}_4; t)
\]
\[
+ \mathcal{F}_{2,0}(s_1, s_2, \vec{r}_1, \vec{r}_2)\mathcal{F}_{0,2}(s_3, s_4, \vec{r}_3, \vec{r}_4)
\]
\[
+ \mathcal{F}_{1,1}(s_1, s_3, \vec{r}_1, \vec{r}_3; t)\mathcal{F}_{1,1}(s_2, s_4, \vec{r}_2, \vec{r}_4; t)
\]
\[
+ \mathcal{F}_{1,1}(s_1, s_4, \vec{r}_1, \vec{r}_4; t)\mathcal{F}_{1,1}(s_2, s_3, \vec{r}_2, \vec{r}_3; t)
\]
\[
+ \mathcal{F}_{1,0}(s_1, \vec{r}_1)\mathcal{F}_{0,1}(s_2, \vec{r}_2)\mathcal{F}_{0,1}(s_3, \vec{r}_3)\mathcal{F}_{0,1}(s_4, \vec{r}_4)
\]
\( (59) \)

The functions \( \mathcal{G}'_{m,n} \) and \( \mathcal{F}_{m,n} \) are not yet in the form of bivariate moments and cumulants, respectively. They are in fact \( (m + n) \)-variate moments and cumulants of order \( (1, 1, \ldots, 1) \) of the local concentration
variables on which they depend. However, consider summation and integration of both sides of the following form; and define

\[ G'_{m,n}(t) = \sum_{s_1=1}^{j} \ldots \sum_{s_{m+n}=1}^{j} Q_{s_1} \ldots Q_{s_{m+n}} \int \ldots \int d^3r_1 \ldots d^3r_{m+n} \]

\[ \times L(r_1) \ldots L(r_{m+n}) G'_{m,n}(s_1, \ldots, s_{m+n}, r_1, \ldots, r_{m+n}; t) \]

\[ F_{m,n}(t) = \sum_{s_1=1}^{j} \ldots \sum_{s_{m+n}=1}^{j} Q_{s_1} \ldots Q_{s_{m+n}} \int \ldots \int d^3r_1 \ldots d^3r_{m+n} \]

\[ \times L(r_1) \ldots L(r_{m+n}) F_{m,n}(s_1, \ldots, s_{m+n}, r_1, \ldots, r_{m+n}; t) \]  \hspace{1cm} (60)

Since each \( F_{p,q} \) in each term of the expansion of \( G'_{m,n} \) corresponds to a separate block of variables, the integral of their product factorizes into the product of integrals, \( F_{p_1,q_1} F_{p_2,q_2} \ldots F_{p_{m+n},q_{m+n}} \). Each product term corresponds to an integer partition of the number \( m+n \), and the number of occurrences of such terms is equal to the number of partitions consisting of those block sizes, of a set of \( m+n \) distinct objects. All such partitions are constructed by permuting the two groups of \( m \) and \( n \) particle indices among themselves, and all such permutations yield the same integration result. Therefore, the relations between \( G'_{m,n} \) and \( F_{m,n} \) are exactly those between moments and cumulants of a bivariate distribution. Finally, noting that \( G'_{m,n} \) is indeed the \((m,n)\)th moment of the bivariate distribution \( \bar{I} = (I(0), I(t)) \) (see (33)),

\[ G'_{m,n}(t) = \langle I^m(0)I^n(t) \rangle = \mu'_{m,n}[I(0), I(t)] \]

we conclude that \( F_{m,n} \) are indeed the cumulants of \( \bar{I} \):

\[ F_{m,n}(t) = \kappa_{m,n}[I(0), I(t)] \]  \hspace{1cm} (61)

We can simply write the first few moments of \( \bar{I} \) in terms of its cumulants using (59):

\[ G'_{1,0} = F_{1,0} \]
\[ G'_{2,0} = F_{2,0} + F_{1,0}^2 \]
\[ G'_{1,1} = F_{1,1}(t) + F_{1,0}F_{0,1} \]
\[ G'_{2,1}(t) = F_{2,1}(t) + 2F_{2,0}F_{0,1} + 2F_{1,0}F_{1,1}(t) + F_{1,0}^2F_{0,1} \]
\[ G'_{2,2}(t) = F_{2,2}(t) + 2F_{2,1}F_{1,1}(t) + 2F_{1,0}F_{2,1}(t) + F_{0,1}^2F_{2,0} + 2F_{1,0}F_{2,1} + 2F_{1,1}(t) + 4F_{1,0}F_{1,1}(t) + F_{2,0}F_{0,2} + 2F_{1,1}(t)F_{0,1} \]  \hspace{1cm} (62)

The advantage of cumulant functions, \( F_{m,n} \), is that the integrals yield simple analytical forms, and the advantage of \( G'_{m,n} \) functions is that they are simply related to observable intensities. Since the collection of all nonzero cumulants also gives a complete description of the distribution, we can primarily define higher order correlations based on cumulants. To experimentally compute cumulants based on the observed moments of intensity, the inverse relations can be found from (62):

\[ F_{1,0} = G'_{1,0} \]
\[ F_{2,0} = G'_{2,0} - G'_{1,0}G'_{0,1} \]
\[ F_{1,1}(t) = G'_{1,1}(t) - G'_{1,0}G'_{0,1} \]
\[ F_{2,1}(t) = G'_{2,1}(t) - G'_{2,0}G'_{0,1} - 2G'_{1,0}G'_{1,1}(t) + 2G'_{1,0}G'_{0,1} \]
\[ F_{2,2}(t) = G'_{2,2}(t) - 2G'_{2,0}G'_{2,1}(t) - 2G'_{1,0}G'_{2,1}(t) + 2G'_{0,1}G'_{2,0} + 2G'_{1,0}G'_{0,2} + 8G'_{1,0}G'_{0,1}G'_{1,1}(t) - G'_{2,0}G'_{0,2} - 2G'_{1,0}G'_{1,1}(t) - 6G'_{1,0}G'_{0,1} \]  \hspace{1cm} (63)

Notice that e.g. \( F_{12} \) can be found by exchanging the subscripts in the relation for \( F_{21} \), and similarly for \( G'_{12} \) and \( G'_{21} \). As a general rule, the coefficients in (63) are obtained from the coefficients in (62) multiplied by \((-1)^{\rho-1}(\rho-1)!\) where \( \rho \) is the total number of blocks in the partition (To see this, compare (51) and (68)).

Conversion to central moments

The relations involving central moments rather than moments are significantly simpler to write and handle. We define

\[ G_{m,n}(s_1, \ldots, s_{m+n}, r_1, \ldots, r_{m+n}; t) = \langle \delta C_{s_1}(r_1, 0) \cdots \delta C_{s_m}(r_m, 0) \delta C_{s_{m+1}}(r_{m+1}, t) \cdots \delta C_{s_{m+n}}(r_{m+n}, t) \rangle \]

where

\[ \delta C_s(r, t) = C_s(r, t) - \langle C_s \rangle \]

We first explain the method suggested by Palmer and Thompson, then explain an easier method of shifting the origin. They write

\[
G'_{1,1}(s_1, s_2, r_1, r_2; t) = \langle C_{s_1}(r_1, 0)C_{s_2}(r_2, t) \rangle \\
= \langle [\delta C_{s_1}(r_1, 0) + \langle C_{s_1} \rangle][\delta C_{s_2}(r_2, t) + \langle C_{s_2} \rangle] \rangle \\
= \langle \delta C_{s_1}(r_1, 0)\delta C_{s_2}(r_2, t) \rangle + \langle C_{s_1} \rangle \langle C_{s_2} \rangle \\
= G_{1,1}(s_1, s_2, r_1, r_2; t) + F_{1,0}(s_1, r_1)F_{0,1}(s_2, r_2)
\]

Comparing with (57) we obtain

\[
G_{1,1}(s_1, s_2, r_1, r_2; t) = F_{1,1}(s_1, s_2, r_1, r_2; t)
\]

Similarly,

\[
G_{2,0}(s_1, s_2, r_1, r_2) = F_{2,0}(s_1, s_2, r_1, r_2)
\]

For order (2, 1) we have

\[
G'_{2,1}(s_1, s_2, s_3, r_1, r_2, r_3; t) = \langle C_{s_1}(r_1, 0)C_{s_2}(r_2, 0)C_{s_3}(r_3, t) \rangle \\
= \langle \delta C_{s_1}(r_1, 0)\delta C_{s_2}(r_2, 0)\delta C_{s_3}(r_3, t) \rangle \\
+ \langle C_{s_1} \rangle \langle \delta C_{s_2}(r_2, 0) \rangle \langle C_{s_3} \rangle \\
+ \langle C_{s_2} \rangle \langle \delta C_{s_1}(r_1, 0) \rangle \langle C_{s_3} \rangle \\
+ \langle C_{s_3} \rangle \langle \delta C_{s_2}(r_2, 0) \rangle \langle C_{s_1} \rangle \\
+ \langle C_{s_3} \rangle \langle C_{s_2} \rangle \langle C_{s_3} \rangle \\
= G_{2,1}(s_1, s_2, s_3, r_1, r_2, r_3; t) \\
+ F_{0,1}(s_3, r_3)G_{2,0}(s_1, s_2, r_1, r_2) \\
+ F_{1,0}(s_2, r_2)G_{1,1}(s_1, s_3, r_1, r_3; t) \\
+ F_{1,0}(s_1, r_1)G_{1,1}(s_2, s_3, r_2, r_3; t) \\
+ F_{1,0}(s_1, r_1)F_{1,0}(s_2, r_2)F_{0,1}(s_3, r_3)
\]

Then substituting the already calculated \(G_{2,0}\) and \(G_{1,1}\) with \(F_{2,0}\) and \(F_{1,1}\) respectively and comparing with (68) we obtain

\[
G_{2,1}(s_1, s_2, s_3, r_1, r_2, r_3; t) = F_{2,1}(s_1, s_2, s_3, r_1, r_2, r_3; t)
\]

and the procedure can continue to all higher orders.

However, one can see that this procedure is equivalent to shifting the origin such that

\[
\langle C_s \rangle = F_{1,0}(s, r) = F_{0,1}(s, r) \\
= G'_{1,0}(s, r) = G_{0,1}(s, r) \\
= 0
\]

31
for all $s$, also

$$\langle I(t) \rangle = F_{1,0} = F_{0,1}$$

$$= G_{1,0}' = G_{0,1}'$$

$$= 0$$

Therefore, the relations for central moments instead of moments are obtained simply by dropping the terms that contain any $\langle C_s \rangle$ (any $s$) in (48)–(50), the terms that contain any $F_{1,0}$ or $F_{0,1}$ in (57)–(59), the terms that contain any $F_{1,0}$ or $F_{0,1}$ in (62), and the terms that contain any $G_{1,0}'$ or $G_{0,1}'$ in (63), and removing the primes on any remaining $G$ and $G$ letters. The latter two sets of relations then become the known relations between the central moments and the cumulants of the bivariate distribution $\bar{I} = (I(0), I(t))$:

$$G_{1,0}' = F_{1,0}$$

$$G_{2,0}' = F_{2,0}$$

$$G_{1,1}(t) = F_{1,1}(t)$$

$$G_{2,1}(t) = F_{2,1}(t)$$

$$G_{2,2}(t) = F_{2,2}(t) + 2F_{1,1}(t) + F_{2,0}F_{0,2}$$

and

$$F_{2,2}(t) = G_{2,2}(t) - 2G_{1,1}(t) - G_{2,0}G_{0,2}$$

### 4.2 Melnykov-Hall approach

This derivation exploits the additive property of cumulants. It was first presented by Melnykov and Hall [9] to derive higher order correlations, following the work of Müller [10] in the context of Fluorescence Cumulant Analysis. We review the derivation in the context of multi-state reacting and diffusing particles.

For a single particle we have (similar to (47) but without summation)

$$G_{m,n}(s_1, \ldots, s_{m+n}, \bar{r}_1, \ldots, \bar{r}_{m+n}; t)$$

$$= \delta(s_1, s_2)\delta(\bar{r}_1 - \bar{r}_2) \ldots \delta(s_l, s_m)\delta(\bar{r}_l - \bar{r}_m)$$

$$\times \delta(s_{m+1}, s_{m+2})\delta(\bar{r}_{m+1} - \bar{r}_{m+2}) \ldots \delta(s_{m+n}, s_{m+n})\delta(\bar{r}_{m+n} - \bar{r}_{m+n})$$

$$\times U^{(1)}(s_1, s_{m+1}, \bar{r}_1, \bar{r}_{m+1}; t)$$

where $U^{(1)}$ is given by (43). Substituting this into (33) we obtain, after summation and integration of delta functions, the $(m, n)$th moment of the intensity vector $\bar{I}^{(1)} = [I^{(1)}(0), I^{(1)}(t)]$ for a single particle:

$$\mu_{m,n}^{(1)}[\bar{I}^{(1)}] = \gamma_{m+n}X_{m,n}^{(1)}(t)Y_{m,n}(t)$$

where we have defined:

$$\gamma_k = \frac{\int_V L^k(\bar{r})d^3r}{\int_V L(\bar{r})d^3r}$$

(66)

the single-molecule reaction factor:

$$X_{m,n}^{(1)}(t) = \sum_{s=1}^{J} \sum_{s'=1}^{J} \frac{V_{MDF}^s}{V^s} P(s)Q^n_{s'} Q^n_{s} Z_{s',s}(t)$$

and the spatial factor:

$$Y_{m,n}(t) = \frac{1}{\gamma_{m+n}V_{MDF}} \int_V \int_V L^m(\bar{r})L^n(\bar{r}') \exp\left[-|\bar{r} - \bar{r}'|^2/4Dt\right] \frac{d^3r d^3r'}{(4\pi Dt)^{3/2}}$$

(67)

The volume of the molecular detection function, $V_{MDF}$, is defined in (41).
Now consider the relation between the cumulant generating function and the moment generating function of a bivariate distribution (see (5)):

\[ \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} \kappa_{m,n} \frac{t^m t^n}{m! n!} = \ln \left( \sum_{p=0}^{\infty} \sum_{q=0}^{\infty} \mu'_{p,q} \frac{t^p t^q}{p! q!} \right) \]

\[ = \sum_{\rho=1}^{\infty} \frac{(-1)^{\rho-1}}{\rho} \left( \sum_{p=0}^{\infty} \sum_{q=0}^{\infty} \mu'_{p,q} \frac{t^p t^q}{p! q!} \right)^\rho - 1 \]

\[ = \sum_{\rho=1}^{\infty} \frac{(-1)^{\rho+1}}{\rho} \left( \sum_{p=0}^{\infty} \sum_{q=0}^{\infty} \mu'_{p,q} \frac{t^p t^q}{p! q!} \right)^\rho \]

where we have used \( \mu'_{0,0} = 1 \). Through multinomial expansion of the last expression and equating the powers of \( t^m t^n \) on the two sides, we obtain:

\[ \kappa_{m,n} = \sum \left( \frac{\mu'_{p_1 q_1}}{p_1! q_1!} \right)^{p_1 \pi_{p_1,q_1}} \left( \frac{\mu'_{p_2 q_2}}{p_2! q_2!} \right)^{p_2 \pi_{p_2,q_2}} \ldots \left( \frac{\mu'_{p_{\rho} q_{\rho}}}{p_{\rho}! q_{\rho}!} \right)^{p_{\rho} \pi_{p_{\rho},q_{\rho}}} \frac{(-1)^{\rho-1}(\rho-1)! m! n!}{\pi_{p_1,q_1} \pi_{p_2,q_2} \ldots \pi_{p_{\rho},q_{\rho}}} \]

(68)

where the summation is subject to the conditions (53) and (54), corresponding to integer partitioning of the ordered pair \((m, n)\) into blocks of size \((p_i, q_i)\) and multiplicity \(\pi_{p_i,q_i}\), and \(\rho\) is the number of blocks in each partition:

\[ \rho = \sum_{i=1}^{l} \pi_{p_i,q_i} \]

This shows that for the intensity vector \( \vec{I}^{(1)} = [I^{(1)}(0), I^{(1)}(t)] \) arising from one particle, we have

\[ \kappa_{m,n}[\vec{I}^{(1)}] = \mu_{m,n}[\vec{I}^{(1)}] + f(\mu'_{p,q}[\vec{I}^{(1)}]) \]

where \( f(\mu'_{p,q}) \) denotes a linear combination of the moments of lower order. Now we use the additive property of cumulants, (8), to write the cumulant of the sum of \( M \) independent intensity vectors, \( \vec{I} = \sum_{j=1}^{M} \vec{I}^{(1)} \), arising from \( M \) independent molecules in the sample volume \( V \):

\[ \kappa_{m,n}(\vec{I}) = M \kappa_{m,n}[\vec{I}^{(1)}] \]

\[ = M \mu_{m,n}[\vec{I}^{(1)}] + M f(\mu'_{p,q}[\vec{I}^{(1)}]) \]

In the thermodynamic limit \( M, V \to \infty \), this becomes

\[ \kappa_{m,n}(\vec{I}) = M \mu_{m,n}[\vec{I}^{(1)}] \]

(69)

which, upon substitution from (65), yields

\[ \kappa_{m,n}(\vec{I}) = \gamma_{m+n} X_{m,n}(t) Y_{m,n}(t) \]

(70)

and, using (31),

\[ \kappa_{m,n}(\vec{W}) \approx T^{m+n} \gamma_{m+n} X_{m,n}(t) Y_{m,n}(t) \]

(71)

where we have defined

\[ X_{m,n}(t) = \sum_{s=1}^{J} \sum_{s'=1}^{J} N_s Q_s^m Q_{s'}^n Z_{s,s'}(t) \]

(72)

and

\[ N_s = \frac{V_{MDF}}{V} P(s) M \]
(also defined in (40)) is the expected number of molecules in state $s$ in the observation volume.

The connection of this approach to that inspired by Palmer and Thompson can be seen by comparing the key relation (69) with

$$ F_{m,n} = MG_{m,n}'(1) $$

which follows from (56), (64), and (45).

### 4.3 Normalized higher order correlations

Given the simpler analytical form of cumulants to study a system of diffusing molecules in solution, we define normalized higher order correlations, with

$$ \vec{W} = [W(0), W(t)] $$

as

$$ g_{m,n}(t) = \frac{\kappa_{m,n}({\vec{W}})}{\kappa_{m,0}(W)\kappa_{0,n}(W)} $$

which, in a multi-detector experiment (and/or sub-binning approach) with $\vec{n}$ defined in (29), becomes

$$ g_{m,n}(t) = \frac{\kappa_{{\vec{n}}_{m+n}}(\vec{n})}{\kappa_{\vec{n}_m,\vec{n}_n}(\vec{n})} $$

and in a single-detector experiment, with $\vec{n}_{1d} = [n(0), n(t)]$, becomes, as obtained by Melnykov and Hall[9],

$$ g_{m,n}(t) = \frac{\kappa_{[m,n]}(\vec{n}_{1d})}{\kappa_{[m,0]}(\vec{n}_{1d})\kappa_{[0,n]}(\vec{n}_{1d})} $$

The two forms follow directly from (28) and (30):

$$ \kappa_{{\vec{n}}_{m+n}}(\vec{n}) = \kappa_{[m,n]}(\vec{n}_{1d}) = \kappa_{m,n}(\vec{W}) $$

Using (71) in the limit $T \to 0$, we obtain the simple analytical form

$$ g_{m,n}(t) = \gamma_{m,n} R_{m,n}(t) Y_{m,n}(t) $$

for the normalized correlation functions, where

$$ \gamma_{m,n} = \frac{\gamma_{m+n}}{\gamma_{m}\gamma_{n}} $$

$\gamma_k$ is defined in (66),

$$ R_{m,n}(t) = \frac{X_{m,n}(t)}{X_{m,0}X_{0,n}} $$

and

$$ Y_{m,n}(t) $$

is defined in (67).

In practice, one of the parameters can be found from the mean channel count rate, (39):

$$ \langle I \rangle = \sum_{s=1}^{J} Q_s N_s $$

*The content of this section is available in references [1, 2] and is brought here for the sake of continuity.
Without loss of generality, it can be taken to be the first brightness level \( Q_1 \). Thus a set of \( \{ N_1, \ldots, N_J \} \) and \( \{ Q_2, \ldots, Q_J \} \) remain to be determined, as well as the reaction relaxation times which result from \( Z_{s', s}(t) \).

Alternatively, we can define
\[
N = \sum_{s=1}^{J} N_s
\]
as the total number of molecules in the probe region regardless of their state. Then we have
\[
R_{m,n}(t) = \frac{1}{N} \frac{\sum_{s=1}^{J} \sum_{s'=1}^{J} K_s q_s^n q_s'^n Z_{s', s}(t)}{\left( \sum_{s=1}^{J} x_s q_s^n \right) \left( \sum_{s'=1}^{J} x_{s'} q_{s'}^n \right)}
\]
where
\[
K_s = \frac{N_s}{N_1}
\]
and
\[
q_s = \frac{Q_s}{Q_1}
\]
are the concentration (equilibrium constant) and brightness of state \( s \) relative to state 1. Obviously, \( q_1 = 1 \) and \( K_1 = 1 \). Thus the number of independent parameters has not changed: \( \{ N, K_2, \ldots, K_J \} \) and \( \{ q_2, \ldots, q_J \} \) (again, plus the relaxation times). In practice, the rate constants constructing \( Z(t) \) may be more desirable to find. In that case, the system is inversely solved to find the rate constants using the relations that link \( K_i \) and relaxation times to the rate constants, and possibly the detailed balance relations.

Here, the usefulness of the factorized form of (76) for obtaining information on multi-state reactions becomes evident. The factors \( \gamma_{m,n} \) and \( Y_{m,n}(t) \) depend only on the illumination profile and the diffusion constant. Therefore, if the reaction parameters, including rates, relative concentrations, and the relative brightness values are of interest only, then higher order correlations \( g_{m,n}^{\text{(ref)}}(t) \) from a “reference” sample with identical diffusional properties can be used to extract the relative reaction function
\[
R_{m,n}^{\text{(rel)}}(t) = \frac{g_{m,n}(t)}{g_{m,n}^{\text{(ref)}}(t)} = \frac{R_{m,n}(t)}{R_{m,n}^{\text{(ref)}}(t)}
\]
This eliminates the need to characterize the illumination profile and calibrate the beam shape and diffusion parameters, greatly simplifying the technique and making it more accurate. In practice, the reference sample can consist of non-reacting molecules, or reacting molecules labeled such as to remains in a single brightness state. In this case, we simply have \( R_{m,n}^{\text{(ref)}}(t) = 1/N^{\text{(ref)}} \) and we get
\[
R_{m,n}^{\text{(rel)}}(t) = \frac{1}{N^{\text{(ref)}}} \frac{\sum_{s=1}^{J} \sum_{s'=1}^{J} x_s q_s^n q_s'^n Z_{s', s}(t)}{\left( \sum_{s=1}^{J} x_s q_s^n \right) \left( \sum_{s'=1}^{J} x_{s'} q_{s'}^n \right)}
\]
where
\[
N^{\text{(rel)}} = \frac{N}{N^{\text{(ref)}}} = \frac{\langle C \rangle}{\langle C \rangle^{\text{(ref)}}}
\]
The ratio of the concentration of the sample of interest (“test” sample) to that of the reference sample, \( N^{\text{(rel)}} \), can be obtained either as a fitting parameter in higher-order FCS, or, if possible, through independent techniques such as UV-Vis to reduce the number of higher order correlations required. The values of the absolute parameters \( N, N^{\text{(ref)}}, N_s \) and \( Q_s \) are usually of no general interest since they depend on the experimental setup. However, they can be determined using \( N^{\text{(ref)}} \), measured for example by second-order FCS, and the mean detector count, \( \langle I \rangle \).
5 Variance of correlations

In a multi-detector (and/or sub-binning) approach we have

\[ g_{m,n}(t) = \frac{\kappa_{\mathbf{1}_{m+n}}(\mathbf{n})}{\kappa_{\mathbf{1}_{m}}(\mathbf{n})\kappa_{\mathbf{1}_{n}}(\mathbf{i}_n)} \]

in a population, and

\[ \tilde{g}_{m,n}(t) = \frac{k_{\mathbf{1}_{m+n}}(\mathbf{n})}{k_{\mathbf{1}_{m}}(\mathbf{n})k_{\mathbf{1}_{n}}(\mathbf{i}_n)} \]

in a sample.

Using (101), the sampling variance of \( \tilde{g}_{m,n} \) is related to sampling moments of \( k \)-statistics:

\[
\text{var}(\tilde{g}_{m,n}) = \tilde{g}_{m,n}^2 \left\{ \frac{\text{var}(k_{\mathbf{1}_{m+n}})}{\kappa^2_{\mathbf{1}_{m+n}}} + \frac{\text{var}(k_{\mathbf{1}_{m}})}{\kappa^2_{\mathbf{1}_{m}}} + \frac{\text{var}(k_{\mathbf{1}_{n}})}{\kappa^2_{\mathbf{1}_{n}}} - 2\frac{\text{cov}(k_{\mathbf{1}_{m+n}}, k_{\mathbf{1}_{m}})}{\kappa_{\mathbf{1}_{m+n}}\kappa_{\mathbf{1}_{m}}} \right. \\
-2\frac{\text{cov}(k_{\mathbf{1}_{m+n}}, k_{\mathbf{1}_{n}})}{\kappa_{\mathbf{1}_{m+n}}\kappa_{\mathbf{1}_{n}}} - 2\frac{\text{cov}(k_{\mathbf{1}_{m}}, k_{\mathbf{1}_{n}})}{\kappa_{\mathbf{1}_{m}}\kappa_{\mathbf{1}_{n}}} + 2\frac{\text{cov}(k_{\mathbf{1}_{m}}, k_{\mathbf{1}_{n}})}{\kappa_{\mathbf{1}_{m}}\kappa_{\mathbf{1}_{n}}} \right\} \tag{78}
\]

Unless specified otherwise, the cumulants and \( k \)-statistics are of random vector \( \mathbf{n} \) (the multi-channel photon count) defined as

\[ \mathbf{n} = [n_1(0), n_2(0), \ldots, n_p(0), n_1(t), n_2(t), \ldots, n_q(t)] \]

The signal-to-noise ratio is defined as

\[ \text{SNR}_{m,n} = \sqrt{\frac{\text{var}(\tilde{g}_{m,n})}{\text{var}(\tilde{g}_{m,n})}} \]

5.1 Order (1,1)

To calculate the sampling variance of

\[ \tilde{g}_{11} = \frac{k_{11}}{k_{10}k_{01}} \]
equation (78) becomes

\[
\text{var}(\tilde{g}_{11}) = \tilde{g}_{11}^2 \left\{ \frac{\text{var}(k_{11})}{\kappa^2_{11}} + \frac{\text{var}(k_{10})}{\kappa^2_{10}} + \frac{\text{var}(k_{01})}{\kappa^2_{01}} - 2\frac{\text{cov}(k_{11}, k_{10})}{\kappa_{11}\kappa_{10}} \right. \\
-2\frac{\text{cov}(k_{11}, k_{01})}{\kappa_{11}\kappa_{01}} + 2\frac{\text{cov}(k_{10}, k_{01})}{\kappa_{10}\kappa_{01}} \right\} \tag{79}
\]
We need to find

\[
\begin{align*}
\text{var}(k_{11}) &= \kappa \begin{pmatrix} 1 & 1 \\ 1 & 1 \end{pmatrix} \\
\text{var}(k_{10}) &= \kappa \begin{pmatrix} 1 & 1 \\ 0 & 0 \end{pmatrix} \\
\text{var}(k_{01}) &= \kappa \begin{pmatrix} 0 & 0 \\ 1 & 1 \end{pmatrix} \\
\text{cov}(k_{11}, k_{10}) &= \kappa \begin{pmatrix} 1 & 1 \\ 1 & 0 \end{pmatrix} \\
\text{cov}(k_{11}, k_{01}) &= \kappa \begin{pmatrix} 1 & 0 \\ 1 & 1 \end{pmatrix} \\
\text{cov}(k_{10}, k_{01}) &= \kappa \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}
\end{align*}
\]

By definition

\[k_1 = m'_1\]

and as shown in (103)

\[\text{var}(m'_r) = \frac{1}{n}(\mu'_{2r} - \mu'_{r}^2)\]

Thus

\[\text{var}(k_1) = \text{var}(m'_1) = \frac{\mu_2}{n} = \frac{\kappa_2}{n}\]

Now we can add a zero subscript to both sides:

\[
\begin{align*}
\text{var}(k_{10}) &= \frac{\kappa_{20}}{n} \\
\text{var}(k_{01}) &= \frac{\kappa_{02}}{n}
\end{align*}
\]

(80)

Here we will use the formulae written in “tensor notation” by Kaplan[6] (also listed in [15]). In tensor notation,

\[
\begin{align*}
\kappa(i, j) &= \kappa \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} \\
\kappa(i, kl) &= \kappa \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}
\end{align*}
\]

and so forth.

As a simple formula in this notation (see (109)),

\[\kappa(i, j) = \frac{\kappa_{ij}}{n}\]

which means

\[\kappa \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} = \frac{\kappa_{11}}{n}\]

(81)

As another useful formula (see (110)):

\[\kappa(i, kl) = \frac{\kappa_{ikl}}{n}\]

meaning

\[\kappa \begin{pmatrix} 1 & 0 \\ 0 & 1 \\ 0 & 1 \end{pmatrix} = \frac{\kappa_{111}}{n}\]
Merging (adding and replacing) the first two rows (i.e. the first two variates are identical) we get
\[ \kappa \begin{pmatrix} 1 & 1 \\ 1 & 0 \end{pmatrix} = \kappa \begin{pmatrix} 1 & 1 \\ 0 & 1 \end{pmatrix} = \frac{\kappa_{21}}{n} \] (82)

Multivariate cumulants are symmetric functions, thus we can exchange the matrix columns.
In a similar way we have:
\[ \kappa(ij,k) = \frac{\kappa_{ijk}}{n} \]
which means
\[ \kappa \begin{pmatrix} 1 & 0 \\ 1 & 0 \\ 0 & 1 \end{pmatrix} = \frac{\kappa_{111}}{n} \] (83)

Merging the last two rows
\[ \kappa \begin{pmatrix} 1 & 0 \\ 1 & 1 \\ 1 & 1 \end{pmatrix} = \kappa \begin{pmatrix} 0 & 1 \\ 1 & 1 \end{pmatrix} = \frac{\kappa_{12}}{n} \] (84)

We could also obtain this by exchanging the subscripts in (82), however, the intermediate relation will be useful (83) later.

Kaplan’s Equation (3) reads:
\[ \kappa(ab,ij) = \frac{1}{n}\kappa_{abij} + \frac{1}{n-1}\sum_{k=1}^{2}\kappa_{ai}\kappa_{bj} \]

In the summation, \(a\) and \(b\) indicate whichever of the first two subscripts that are nonzero. \(i\) and \(j\) indicate whichever of the last two subscripts that are nonzero. All other subscripts in each \(\kappa\) must be zero. \(a, b, i\) and \(j\) must be non-identical. The summation superscript 2 indicates that there are two distinct terms of such kind. Thus we have:
\[ \kappa \begin{pmatrix} 1 & 0 \\ 1 & 0 \\ 0 & 1 \\ 0 & 1 \end{pmatrix} = \frac{1}{n}\kappa_{1111} + \frac{1}{n-1}(\kappa_{1010}\kappa_{0101} + \kappa_{0110}\kappa_{1001}) \] (85)

Merging row 1 with row 3, and row 2 with row 4, we get
\[ \kappa \begin{pmatrix} 1 & 1 \\ 1 & 1 \end{pmatrix} = \frac{1}{n}\kappa_{22} + \frac{1}{n-1}(\kappa_{20}\kappa_{02} + \kappa_{11}^2) \] (86)

The right hand side is in terms of cumulants of \(\bar{n}\) (the multi-channel photon count) which have not been calculated, while the cumulants of \(\bar{W} = [W(0), W(t)]\) (the underlying integrated intensity) have been calculated in (71):
\[ \kappa_{m,n}(\bar{W}) = T^{m+n}\gamma_{m+n}X_{m,n}(t)Y_{m,n}(t) \] (87)

To simplify matters, we only consider the shot-noise-dominant limit \(t \to 0\) in which \(Y_{m,n}(0) = 1\) and
\[ X_{m,n}(0) = \sum_{s=1}^{J} N_s Q_s^{m+n} \]
and to further simplify, we only consider a single species, thus:
\[ X_{m,n}(0) = NQ^{m+n} \]

Therefore (87) becomes
\[ \kappa_{m,n}(\bar{W}) = \gamma_{m+n}N\lambda^{m+n} \] (88)

38
where \( \lambda := QT \) is the average number of photons per bin. Here, we assume the shot-noise-dominant regime, that is
\[
\lambda \ll 1
\]

On the other hand, factorial cumulants of \( \bar{n} \) are equal to cumulants of \( \bar{W} \), by (27):
\[
\kappa_{[\vec{r}]}(\bar{n}) = \kappa_{m,n}(\bar{W})
\]
where \( \vec{r} \) can have elements greater than 1, and \((m, n)\) is constructed by merging the elements of \( \vec{r} \) that are at identical lag times (i.e. identical \( W(t) \)). Therefore, to calculate the cumulants of \( \bar{n} \), as in (86), we express them in terms of factorial cumulants of \( \bar{n} \). The conversion relations are formally similar to those between moments and factorial moments, and are given in (19). Using those, and (86) in the limit \( n \gg 1 \), we write
\[
\frac{\text{var}(k_{11})}{\kappa_{11}^2} = \frac{1}{n} \left( 1 + \frac{\kappa_{20}\kappa_{02}}{\kappa_{11}^2} + \frac{\kappa_{22}}{\kappa_{11}^2} \right) = \frac{1}{n} \left( 1 + \frac{(\kappa_{[20]} + \kappa_{[10]})(\kappa_{[02]} + \kappa_{[01]})}{\kappa_{11}^2} + \frac{\kappa_{[20]} + \kappa_{[21]} + \kappa_{[12]} + \kappa_{[11]}}{\kappa_{11}^2} \right)
\]
then using (88) and (89)
\[
= \frac{1}{n} \left( 2 + 2 \frac{\gamma_2 \gamma_1}{\gamma_2^2 \lambda} + \frac{\gamma_4}{\gamma_2^2 \lambda^2} + \frac{\gamma_4}{\gamma_2 N} + 2 \frac{\gamma_3}{\gamma_2 N \lambda} + \frac{1}{\gamma_2 N \lambda^2} \right)
= \frac{1}{n} \left( \frac{N + \gamma_2}{\gamma_2^2 N \lambda^2} \right) [1 + O(\lambda)]
\]
having used \( \gamma_1 = 1 \).

Following a similar approach, one can show that the order difference between the denominator and the numerator does not exceed 1 in any other term in (79) when written in the form of factorial cumulants, thus they can all be neglected, and the SNR becomes
\[
\text{SNR}_{11} \approx \sqrt{n} \gamma_2 QT \sqrt{\frac{N}{N + \gamma_2}}
\]

### 5.2 Order (2,1)

For the sampling variance of
\[
\tilde{g}_{21} = \frac{k_{111}}{k_{110} k_{001}}
\]
we need to calculate

\[
\begin{align*}
\text{var}(k_{111}) &= \kappa \begin{pmatrix}
1 & 1 \\
1 & 1 \\
1 & 1
\end{pmatrix} \\
\text{var}(k_{110}) &= \kappa \begin{pmatrix}
1 & 1 \\
1 & 1 \\
0 & 0
\end{pmatrix} \\
\text{var}(k_{001}) &= \kappa \begin{pmatrix}
0 & 0 \\
0 & 0 \\
1 & 1
\end{pmatrix} \\
\text{cov}(k_{111}, k_{110}) &= \kappa \begin{pmatrix}
1 & 1 \\
1 & 1 \\
1 & 0
\end{pmatrix} \\
\text{cov}(k_{111}, k_{001}) &= \kappa \begin{pmatrix}
1 & 0 \\
1 & 0 \\
1 & 1
\end{pmatrix} \\
\text{cov}(k_{110}, k_{001}) &= \kappa \begin{pmatrix}
1 & 0 \\
1 & 0 \\
0 & 1
\end{pmatrix}
\end{align*}
\]

Extending the univariate case by adding a neutral 0 subscript, as in (80),

\[
\text{var}(k_{001}) = \frac{\kappa_{002}}{n} = \frac{\kappa_{0[01]}}{n} + \mathcal{O}(\lambda^2)
\]  

(91)

and extending the bivariate case (86)

\[
\begin{align*}
\text{var}(\kappa_{110}) &= \frac{1}{n} \kappa_{220} + \frac{1}{n-1} (\kappa_{200} \kappa_{020} + \kappa_{110}^2) \\
&= \frac{1}{n} \kappa_{[110]} + \frac{1}{n-1} \kappa_{[100]} \kappa_{[010]} + \mathcal{O}(\lambda^3)
\end{align*}
\]

(92)

where we have kept the lowest-order term(s) in conversion to factorial cumulants for later use.

Using the rule of Appendix C again we have:

\[
\kappa(abc, i) = \frac{\kappa_{abc i}}{n}
\]

meaning

\[
\kappa \begin{pmatrix}
1 & 0 \\
1 & 0 \\
1 & 0
\end{pmatrix} = \frac{\kappa_{1111}}{n}
\]

Merging the last two rows:

\[
\kappa \begin{pmatrix}
1 & 0 \\
1 & 0 \\
1 & 1
\end{pmatrix} = \frac{\kappa_{112}}{n} = \frac{1}{n} \kappa_{[111]} + \mathcal{O}(\lambda^4)
\]  

(93)
And, $\text{cov}(k_{110}, k_{001})$ is already calculated in (83).

Kaplan’s Equation (4) reads:

$$\kappa(ab, ijk) = \frac{1}{n} \kappa_{abijk} + \frac{1}{n-1} \sum_6 \kappa_{ai} \kappa_{bjk}$$

which is a shorthand notation for

$$\kappa \left( \begin{array}{cccc} 1 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 0 & 1 & 1 & 0 \\ 0 & 1 & 0 & 1 \\ \end{array} \right) = \frac{\kappa_{11111}}{n} + \frac{1}{n-1} \sum \left( \begin{array}{c} \kappa_{10100} \kappa_{10011} \\ \kappa_{10010} \kappa_{10101} \\ \kappa_{10001} \kappa_{10110} \\ \kappa_{01100} \kappa_{10011} \\ \kappa_{01010} \kappa_{10101} \\ \kappa_{01001} \kappa_{10110} \\ \end{array} \right)$$

where the matrix in front of the summation lists the summed terms. To obtain $\text{cov}(k_{110}, k_{111})$, we merge the rows according to a “subscript identicality pattern” of the form $\{1, 2, 1, 2, 3\}$, which means the first and the third rows (subscripts) are merged together and used as the new first row, the second and the fourth rows are merged together to form the new second row, and the fifth row forms the new third row as is. This yields

$$\kappa \left( \begin{array}{cccc} 1 & 1 & 0 & 0 \\ 1 & 1 & 1 & 0 \\ 0 & 1 & 1 & 0 \\ 0 & 1 & 0 & 1 \\ \end{array} \right) = \frac{\kappa_{221}}{n} + \frac{1}{n-1} \sum \left( \begin{array}{c} \kappa_{209} \kappa_{021} \\ \kappa_{110} \kappa_{111} \\ \kappa_{100} \kappa_{120} \\ \kappa_{110} \kappa_{111} \\ \kappa_{020} \kappa_{201} \\ \kappa_{011} \kappa_{210} \\ \end{array} \right)$$

$$= \frac{\kappa_{[111]}}{n} + \frac{1}{n-1} \left( \kappa_{[100]} \kappa_{[011]} + \kappa_{[010]} \kappa_{[101]} \right) + O(\lambda^4) \quad (95)$$

Upon conversion to factorial cumulants, a $\kappa$ with a subscript of, say, 021 breaks down to $[021]$ plus $[011]$, and we care about the lowest-order terms in $\lambda$ only.

Using Kaplan’s Equation (7):

$$\kappa(abc, ijk) = \frac{\kappa_{abcijk}}{n} + \frac{1}{n-1} \left( \sum_9 \kappa_{ai} \kappa_{bcjk} + \sum_9 \kappa_{abi} \kappa_{cijk} \right)$$

$$+ \frac{n}{(n-1)(n-2)} \sum_6 \kappa_{ai} \kappa_{bjk} \kappa_{cjk}$$

which becomes

$$\kappa \left( \begin{array}{cccc} 1 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 \\ 0 & 1 & 1 & 0 \\ 0 & 1 & 0 & 1 \\ \end{array} \right) = \frac{\kappa_{111111}}{n} + \frac{1}{n-1} \sum \left( \begin{array}{c} \kappa_{100100} \kappa_{101101} \\ \vdots \\ \kappa_{00100} \kappa_{110110} \\ \end{array} \right)$$

$$+ \frac{1}{n-1} \sum \left( \begin{array}{c} \kappa_{110100} \kappa_{001011} \\ \vdots \\ \kappa_{011001} \kappa_{100110} \\ \end{array} \right)$$

$$+ \frac{n}{(n-1)(n-2)} \sum \left( \begin{array}{c} \kappa_{100100} \kappa_{010010} \kappa_{001001} \\ \vdots \\ \kappa_{010001} \kappa_{010010} \kappa_{001100} \\ \end{array} \right)$$

41
The procedure can be computerized to produce all terms of a particular form. The program is included in Supporting Information.

Using the identicality pattern \{123123\}, then converting to factorial cumulants and keeping the lowest-order terms:

\[
\kappa \left( \begin{array}{ccc}
1 & 1 & 1 \\
1 & 1 & 1 \\
1 & 1 & 1
\end{array} \right) = \frac{\kappa_{222}}{n} + \frac{1}{n-1} \sum_{i=0}^{9} \left( \begin{array}{c}
\kappa_{200}\kappa_{022} \\
\vdots \\
\kappa_{002}\kappa_{220}
\end{array} \right) + \frac{1}{n-1} \sum_{i=0}^{9} \left( \begin{array}{c}
\kappa_{210}\kappa_{012} \\
\vdots \\
\kappa_{012}\kappa_{210}
\end{array} \right)
\]

\[
+ \frac{n}{(n-1)(n-2)} \sum_{i=0}^{6} \left( \begin{array}{c}
\kappa_{200}\kappa_{020}\kappa_{002} \\
\vdots \\
\kappa_{101}\kappa_{020}\kappa_{101}
\end{array} \right) \tag{96}
\]

\[
= \frac{\kappa_{[111]}}{n} + \frac{1}{n-1} \left( \kappa_{[100]}\kappa_{[011]} + \kappa_{[010]}\kappa_{[101]} + \kappa_{[001]}\kappa_{[110]} \right)
\]

\[
+ \frac{n}{(n-1)(n-2)} \kappa_{[100]}\kappa_{[010]}\kappa_{[001]} + O(\lambda^4) \tag{97}
\]

We are now ready to evaluate the following terms:

From (91):

\[
\frac{\text{var}(k_{001})}{\kappa_{001}^2} = \frac{O(\lambda)}{O(\lambda^2)} = O(\lambda^{-1})
\]

From (92):

\[
\frac{\text{var}(k_{110})}{\kappa_{110}^2} = \frac{O(\lambda^2)}{O(\lambda^4)} = O(\lambda^{-2})
\]

From (97):

\[
\frac{\text{var}(k_{111})}{\kappa_{111}^2} = \frac{O(\lambda^3)}{O(\lambda^6)} = O(\lambda^{-3})
\]

From (93):

\[
\frac{\text{cov}(k_{111},k_{001})}{\kappa_{111}\kappa_{001}} = \frac{O(\lambda^3)}{O(\lambda^4)} = O(\lambda^{-1})
\]

From (95):

\[
\frac{\text{cov}(k_{111},k_{110})}{\kappa_{111}\kappa_{110}} = \frac{O(\lambda^3)}{O(\lambda^5)} = O(\lambda^{-2})
\]

From (83):

\[
\frac{\text{cov}(k_{110},k_{001})}{\kappa_{110}\kappa_{001}} = \frac{O(\lambda^3)}{O(\lambda^3)} = O(\lambda^0)
\]

Therefore the largest order in \(\frac{1}{\lambda}\) is the term \(\frac{\text{var}(k_{111})}{\kappa_{111}^2}\) which dominates the relative uncertainty. This was expected, as the \(k\)-statistics of higher order have larger relative uncertainties in the shot-noise-dominated regime. Thus, using (97)

\[
\frac{\text{var}(k_{111})}{\kappa_{111}^2} = \frac{1}{\lambda^4} \left( \gamma_3 N \lambda^3 + 3 \gamma_2 N^2 \lambda^3 + \gamma_1 N^3 \lambda^3 \right) + O(\lambda^4)
\]

\[
\approx \frac{1}{n \gamma_3^2 \lambda^3} \left( \frac{\gamma_3 + 3 \gamma_2 N + N^2}{N} \right)
\]

Finally,

\[
\text{SNR}_{21} \approx \sqrt{n} \gamma_3 (QT)^{3/2} \sqrt{\frac{N}{\gamma_3 + 3 \gamma_2 N + N^2}} \tag{98}
\]
Comparing with the single-detector result reported by Melnykov and Hall \[9\],

$$\text{SNR}^{(1d)}_{21} \approx \sqrt{n\gamma_3} (QT)^{3/2} \frac{N}{2\gamma_3 + 6\gamma_2 N + 2N^2}$$

we see that the presence of two independent channels contributes positively by a factor of $\sqrt{2}$. This is intuitive by considering the fact that if $x, x_1, x_2, \ldots, x_p$ are independent random variables with identical distribution, we have

$$\frac{\delta(x^p)}{x^p} = \frac{\delta x}{x}$$

$$\frac{\delta(x_1x_2 \ldots x_p)}{x_1x_2 \ldots x_p} = \sqrt{p} \frac{\delta x}{x}$$

However, it should also be noted that splitting the beam among $m$ detectors is equivalent to reducing the brightness $Q$ by a factor of $m$. The SNR has a power of $(QT)^{1/2}$ for each higher order. Also, sub-binning reduces the effective bin size, $T$. Combining multiple ways to select sub-bins partially makes up for the loss. Therefore, comparing the overall single-detector and multi-detector SNR requires more detailed analysis for each order. An optimally large bin size usually ensures sufficient SNR in each method.

Before we finish this section, we point out that the single-detector $\text{SNR}^{(1d)}_{21}$ can be obtained from

$$\frac{\text{var}(k_{[21]})}{k_{[21]}^2}$$

where the random variables are $\tilde{n}_{1d} = [n(0), n(t)]$. The denominator is the same as in multi-detector case. To calculate the numerator, we use

$$k_{[21]} = k_{21} - k_{11}$$

which yields

$$\text{var}(k_{[21]}) = \text{var}(k_{21}) + \text{var}(k_{11}) - 2\text{cov}(k_{21}, k_{11})$$

We can obtain $\text{var}(k_{21})$ by merging the first two subscripts in the result for $\text{var}(k_{111})$ (Equation (96)). Similarly, $\text{cov}(k_{21}, k_{11})$ is obtained by merging the last two subscripts in $\text{cov}(k_{111}, k_{110})$ (Equation (94)) and swapping the subscripts. The result then has to be converted back to factorial cumulants and the lowest-order terms kept to obtain $\text{SNR}^{(1d)}_{21}$.

### 5.3 Order (2,2)

Now for the sampling variance of

$$\tilde{g}_{22} = \frac{\kappa_{1111}}{\kappa_{1100}\kappa_{0011}}$$

we need to calculate

$$\text{var}(k_{1111})$$

$$\text{var}(k_{1100})$$

$$\text{var}(k_{0011})$$

$$\text{cov}(k_{1111}, k_{1100})$$

$$\text{cov}(k_{1111}, k_{0011})$$

$$\text{cov}(k_{1100}, k_{0011})$$

Simply by rewriting (92) with a neutral 4th subscript, we have

$$\text{var}(k_{1100}) = \frac{1}{n} \kappa_{2000} + \frac{1}{n-1} (\kappa_{2000}\kappa_{0200} + \kappa_{1100}^2)$$

$$= \frac{1}{n} \kappa_{[1100]} + \frac{1}{n-1} \kappa_{[1000]\kappa_{0100]} + O(\lambda^3)$$

43
and \( \text{var}(k_{0011}) \) is obtained by swapping the first two and last two indices.

Also, \( \text{var}(k_{1100}, k_{0011}) \) is already given in (85), which has the same factorial form.

We invoke Kaplan’s Equation (5)

\[
\kappa(ab,ijkl) = \frac{\kappa_{abijkl}}{n} + \frac{1}{n-1} \left( \sum_{i=0}^{8} \kappa_{aiK_{bjkl}} + \sum_{i=0}^{6} \kappa_{aijK_{bkl}} \right)
\]

which yields

\[
\text{cov}(k_{110000}, k_{001111}) = \frac{\kappa_{111111}}{n} + \frac{1}{n-1} \sum_{i=0}^{8} \left( \begin{array}{c} \kappa_{101000K_{010111}} \\ \cdots \\ \kappa_{00100K_{101110}} \end{array} \right) \\
+ \frac{1}{n-1} \sum_{i=0}^{6} \left( \begin{array}{c} \kappa_{101100K_{010011}} \\ \cdots \\ \kappa_{10001K_{011100}} \end{array} \right)
\]

With subscript identicality pattern \( \{1, 2, 1, 2, 3, 4\} \) we get

\[
\text{cov}(k_{1100}, k_{1111}) = \frac{\kappa_{2211}}{n} + \frac{1}{n-1} \left( \sum_{i=0}^{8} \left( \begin{array}{c} \kappa_{2000K_{0211}} \\ \cdots \\ \kappa_{0101K_{2110}} \end{array} \right) + \sum_{i=0}^{6} \left( \begin{array}{c} \kappa_{2100K_{0111}} \\ \cdots \\ \kappa_{1011K_{1200}} \end{array} \right) \right)
\]

\[
= \frac{\kappa_{1111}}{n} + \frac{1}{n-1} \left( \kappa_{1[1000]K_{0111}K_{0111]} + \kappa_{0[100]K_{1011]} \right) \\
+ \frac{1}{n-1} \left( \kappa_{[1010]\kappa_{0101}K_{1001}K_{0110]} + \kappa_{[1001]\kappa_{0110]}} \right) + \mathcal{O}(\lambda^5)
\]

Similarly, \( \text{cov}(k_{1111}, k_{0011}) \) is of order \( \mathcal{O}(\lambda^4) \).

To calculate \( \text{var}(k_{1111}) \) we use Kaplan’s Equation (10), (with a correction in the number of terms of \( \kappa_{abK_{cdjkl}} \)):

\[
\kappa(abcd,ijkl) = \frac{\kappa_{abcdijkl}}{n} + \sum_{i=0}^{16} \frac{\kappa_{aiK_{bcedjkl}}}{n-1} + \sum_{i=0}^{24} \frac{\kappa_{abK_{cdejkl}}}{n-1} + \ldots
\]
This directly yields

\[
\text{cov}(k_{1110000}, k_{00001111}) = \frac{k_{11111111}}{n} \\
+ \frac{1}{n-1} \sum_{i=1}^{16} \begin{pmatrix}
K_{10001000}k_{11101111} \\
\vdots \\
K_{00010001}k_{11101110}
\end{pmatrix} \\
+ \frac{1}{n-1} \sum_{i=1}^{24} \begin{pmatrix}
K_{11001000}k_{00110111} \\
\vdots \\
K_{00110001}k_{11001110}
\end{pmatrix} \\
+ \frac{n}{(n-1)(n-2)} \sum_{i=1}^{72} \begin{pmatrix}
K_{10001000}k_{01000100}k_{00110011} \\
\vdots \\
K_{00100001}k_{00010010}k_{11001100}
\end{pmatrix} \\
+ \frac{1}{n-1} \sum_{i=1}^{16} \begin{pmatrix}
K_{10001110}k_{01110001} \\
\vdots \\
K_{00010111}k_{11101000}
\end{pmatrix} \\
+ \frac{1}{n-1} \sum_{i=1}^{18} \begin{pmatrix}
K_{11001100}k_{00110011} \\
\vdots \\
K_{10010011}k_{01011100}
\end{pmatrix} \\
+ \frac{n}{(n-1)(n-2)} \sum_{i=1}^{144} \begin{pmatrix}
K_{10010000}k_{01100100}k_{00010011} \\
\vdots \\
K_{00010001}k_{01100010}k_{10001100}
\end{pmatrix} \\
+ \frac{n(n+1)}{(n-1)(n-2)(n-3)} \sum_{i=1}^{24} \begin{pmatrix}
K_{10001000}k_{01000100}k_{00100010}k_{00010001} \\
\vdots \\
K_{10000001}k_{01000010}k_{00100100}k_{00011000}
\end{pmatrix}
\]
With the identality pattern \{1, 2, 3, 4, 1, 2, 3, 4\},

\[
\begin{align*}
\text{var}(k_{1111}) &= \frac{k_{2222}}{n} + \frac{1}{n-1} \sum_{i=1}^{16} \begin{pmatrix}
\kappa_{2000}\kappa_{0222} \\
\vdots \\
\kappa_{0002}\kappa_{2220}
\end{pmatrix} \\
& \quad + \frac{1}{n-1} \sum_{i=1}^{24} \begin{pmatrix}
\kappa_{2110}\kappa_{0122} \\
\vdots \\
\kappa_{0012}\kappa_{2210}
\end{pmatrix} \\
& \quad + \frac{n}{(n-1)(n-2)} \sum_{i=1}^{72} \begin{pmatrix}
\kappa_{2000}\kappa_{0200}\kappa_{0022} \\
\vdots \\
\kappa_{0011}\kappa_{0011}\kappa_{2200}
\end{pmatrix} \\
& \quad + \frac{1}{n-1} \sum_{i=1}^{16} \begin{pmatrix}
\kappa_{2110}\kappa_{0112} \\
\kappa_{0112}\kappa_{2110}
\end{pmatrix} + \frac{1}{n-1} \sum_{i=1}^{18} \begin{pmatrix}
\kappa_{2200}\kappa_{0022} \\
\vdots \\
\kappa_{1012}\kappa_{1210}
\end{pmatrix} \\
& \quad + \frac{n}{(n-1)(n-2)} \sum_{i=1}^{144} \begin{pmatrix}
\kappa_{2000}\kappa_{0210}\kappa_{0012} \\
\vdots \\
\kappa_{0002}\kappa_{1012}\kappa_{2100}
\end{pmatrix} \\
& \quad + \frac{n(n+1)}{(n-1)(n-2)(n-3)} \sum_{i=1}^{24} \begin{pmatrix}
\kappa_{2000}\kappa_{0200}\kappa_{0020}\kappa_{0002} \\
\vdots \\
\kappa_{1001}\kappa_{0110}\kappa_{0110}\kappa_{1001}
\end{pmatrix}
\end{align*}
\]

Converting to factorial cumulants and keeping the lowest-order terms

\[
\begin{align*}
\text{var}(k_{1111}) &= \frac{k_{[1111]}}{n} + \frac{1}{n-1} (k_{[1000]}k_{[0111]} + k_{[0100]}k_{[1011]} + k_{[0010]}k_{[1101]}) \\
& \quad + k_{[0001]}k_{[1110]} + O(\lambda^5) + \frac{1}{n-1} O(\lambda^5) \\
& \quad + \frac{n}{(n-1)(n-2)} (k_{[1000]}k_{[0100]}k_{[0011]} + k_{[1000]}k_{[0010]}k_{[0101]}) \\
& \quad + k_{[1000]}k_{[0001]}k_{[1100]} + k_{[0100]}k_{[0010]}k_{[1011]} + k_{[0100]}k_{[0001]}k_{[1010]} \\
& \quad + k_{[0010]}k_{[0001]}k_{[1100]} + O(\lambda^5) + \frac{1}{n-1} O(\lambda^6) \\
& \quad + \frac{1}{n-1} (k_{[1100]}k_{[0011]} + k_{[1010]}k_{[0101]} + k_{[1001]}k_{[0011]} + O(\lambda^5)) \\
& \quad + \frac{n}{(n-1)(n-2)} O(\lambda^5) + \\
& \quad + \frac{n(n+1)}{(n-1)(n-2)(n-3)} (k_{[1000]}k_{[0100]}k_{[0010]}k_{[0001]} + O(\lambda^5))
\end{align*}
\]

Assuming \(n \gg 1\) and using (88),

\[
\text{var}(k_{1111}) \approx \frac{1}{n} (\gamma_4 N \lambda^4 + 4 \gamma_1 \gamma_3 N^2 \lambda^4 + 6 \gamma_1^2 \gamma_2 N^3 \lambda^4 + 3 \gamma_2^2 N^2 \lambda^4 + \gamma_1 N^4 \lambda^4) + O(\lambda^5)
\]

\[\text{(99)}\]

Now we can verify,

\[
\frac{\text{var}(k_{1100})}{k_{1100}^2} = \frac{\text{var}(k_{0011})}{k_{0011}^2} = \frac{O(\lambda^2)}{O(\lambda^4)} = O(\lambda^{-2})
\]
\[
\frac{\text{var}(k_{1111})}{\kappa_{1111}^2} = \mathcal{O}(\lambda^4) = \mathcal{O}(\lambda^{-4})
\]
\[
\frac{\text{cov}(k_{1100}, k_{0011})}{\kappa_{1100}\kappa_{0011}} = \mathcal{O}(\lambda^4) = \mathcal{O}(\lambda^0)
\]
\[
\frac{\text{cov}(k_{1100}, k_{1111})}{\kappa_{1100}\kappa_{1111}} = \text{cov}(k_{1111}, k_{0011}) = \mathcal{O}(\lambda^4) = \mathcal{O}(\lambda^2)
\]

Therefore, \(\frac{\text{var}(k_{1111})}{\kappa_{1111}^2}\) has the lowest order in \(\lambda^{-1}\) as expected.

Using (99),

\[
\frac{\text{var}(k_{1111})}{\kappa_{1111}^2} = \frac{\text{var}(k_{1111})}{(\gamma_4 N \lambda^4)^2}
\approx \frac{1}{n\gamma_4^2\lambda^4} \left( \frac{\gamma_4 + 4\gamma_3 N + 6\gamma_2 N^2 + 3\gamma_2^2 N + N^3}{N} \right)
\]

Therefore,

\[
\text{SNR}_{22} \approx \sqrt{n}\gamma_4 (QT)^2 \sqrt{\frac{N}{\gamma_4 + (4\gamma_3 + 3\gamma_2^2)N + 6\gamma_2 N^2 + N^3}} \quad (100)
\]

(multi-detector case).

The SNR results, (90), (98), and (100), are consistent with

\[
\begin{cases}
\text{SNR}_{m,n} \approx \sqrt{n}\gamma_m^{1/2} (QT)^{(m+n)/2} / \sqrt{N} & \text{for } N \ll 1 \\
\text{SNR}_{m,n} \approx \sqrt{n}\gamma_m^{1/2} (QT)^{(m+n)/2} N^{1-(m+n)/2} & \text{for } N \gg 1
\end{cases}
\]

reported by Melnykov and Hall (they have not reported \(\text{SNR}^{(14)}(22)\) specifically, and we have not calculated it yet either; it is, as one might expect, a tedious task). “In the high-concentration limit, a fluorescence signal becomes Gaussian and therefore correlations other than \(g_{11}\) tend to 0. When \(m = n = 1\) SNR is independent of the number of molecules in the high concentration limit and is proportional to \(N\) in the low concentration limit.” In our current report and experiments, the highest order studied is \(2, 2\) which has our limiting uncertainty. Therefore, assuming a Gaussian illumination profile which yields

\[
\gamma_m = m^{-3/2}
\]

we estimate the optimal value of \(N\) that maximizes (100) to be \(N \approx 0.22\), with half maxima at \((0.016, 1.9)\), corresponding to correlation amplitudes of

\[
g_{11}(0) = \frac{\gamma_2}{N} = \frac{1}{N_{\text{FCS}}} \approx 1.6 \quad (0.19, 22)
\]

or \(N_{\text{FCS}} = N/\gamma_2 \approx 0.62\) \(0.046, 5.4\) \((N_{\text{FCS}}\) is a more common definition of the number of molecules in the probe region in conventional FCS.) The numbers in parentheses indicate the \(N\) values at half maxima of \(\text{SNR}_{22}\).

To examine the validity of the assumption \(QT \ll 1\) at large lag times, consider the lag time \(t \approx 1\) ms and \(T = 0.1t = 100\) \(\mu s\), with \(N \approx 1\) and channel count rate \(R \approx 10\) kHz. We then have \(Q = R/N \approx 10\) kHz then \(QT \approx 1\). But this is about the limit in our experiments. As long as \(QT \gg 1\) is not true, and \(N \approx 1\), the truncated order above gives the right order of magnitude.

In our program implementation, we estimate \(QT\) using \(QT = \kappa_{10}/N = \kappa_{01}/N\), with \(\kappa_{10}\) (ideally, \(= \kappa_{01}\)) being the mean photon count per bin, and \(N\) calculated using the inverse of \(g_{11}(0)\):

\[
N = \frac{\gamma_2}{g_{11}(0)} = \frac{\gamma_2\kappa_{10}\kappa_{01}}{\kappa_{11}(0)} = \frac{\gamma_2\kappa_{10}\kappa_{01}}{\kappa_{20}}
\]

This result assumes not reaction. For reactions, further analysis is needed.
A Variance and covariance of a function

The following derivation is described in [15]. Suppose for \( x_1, x_2, \ldots, x_k \) we have

\[
\begin{align*}
E(x_i) &= \theta_i \\
\text{var}(x_i) &= \mathcal{O}(n^{-r}) \\
\text{cov}(x_i, x_j) &= \mathcal{O}(n^{-r})
\end{align*}
\]

(usually \( r = 1 \)) then \( x_i \to \theta_i \) as \( n \to \infty \). For example, \( x_i \) can be statistics.

Consider

\[
f(x) := f(x_1, x_2, \ldots, x_k)
\]

\[
f'_i(\theta) := \left. \frac{\partial f(x)}{\partial x_i} \right|_{\theta_1, \theta_2, \ldots, \theta_k}
\]

Through Taylor expansion we have

\[
f(x) = f(\theta) + \sum_{i=1}^{k} f'_i(\theta)(x_i - \theta_i) + \mathcal{O}(n^{-r})
\]

Since \( E(x_i) = \theta_i \) we have

\[
E[f(x)] = f(\theta) + \mathcal{O}(n^{-r})
\]

Now

\[
\text{var}[f(x)] = E \left[ \{ f(x) - E[f(x)] \}^2 \right] = E \left[ \left( \sum_{i=1}^{k} f'_i(\theta)(x_i - \theta_i) \right)^2 \right] + \mathcal{O}(n^{-r})
\]

assuming not all \( f'_i(\theta) = 0 \)

\[
= \sum_{i=1}^{k} \{ f'_i(\theta) \}^2 E[(x_i - \theta_i)^2] + \sum_{i=1}^{k} \sum_{j=1}^{k} f'_i(\theta)f'_j(\theta)E[(x_i - \theta_i)(x_j - \theta_j)] + \mathcal{O}(n^{-r})
\]

\[
= \sum_{i=1}^{k} \{ f'_i(\theta) \}^2 \text{var}(x_i) + \sum_{i=1}^{k} \sum_{j=1}^{k} f'_i(\theta)f'_j(\theta)\text{cov}(x_i, x_j) + \mathcal{O}(n^{-r}) \tag{101}
\]

Permutation is allowed in the second summation.

In particular, if \( f \) is a linear function of random variables: \( f(x_1, \ldots, x_k) = \sum a_ix_i \) then

\[
\text{var}[f(x)] = \sum a_i^2 \text{var}(x_i) + \sum_{i \neq j} a_ia_j \text{cov}(x_i, x_j) \tag{exact}
\]

Similarly, one can show

\[
\text{cov}[f(x), g(x)] = \sum_{i=1}^{k} f'_i(\theta)g'_i(\theta)\text{var}(x_i) + \sum_{i=1}^{k} \sum_{j=1}^{k} f'_i(\theta)g'_j(\theta)\text{cov}(x_i, x_j) + \mathcal{O}(n^{-r})
\]
B Some sampling moments

There are three types of moments:

- Population moments (moments of the population), such as $\mu'_r, \mu_r$, etc.
- Sample moments (moments of the sample), such as $m'_r, m_r$, etc.
- Sampling moments (moments of the sampling distribution), such as $E(m'_r), \text{var}(m'_r), E(m_r), \text{var}(m_r)$, etc.

Similarly we have population cumulants, sample cumulants, and sampling cumulants.

Specifically, the $r$th moment is $\mu'_r$, the $r$th moment statistic is

$$m'_r = \frac{1}{n} \sum_{j=1}^{n} x'_j$$

and the $r$th central moment statistic (moment about the sample mean) is

$$m_r = \frac{1}{n} \sum_{j=1}^{n} (x_j - m'_1)^r$$

Here, we aim to find to the first few sampling moments. The material is adopted from [15].

The expectation of $m'_r$ is

$$E(m'_r) = \frac{1}{n} \sum E(x^r) = E(x^r) = \mu'_r$$

The sampling variance of $m'_r$ is

$$\text{var}(m'_r) = E \left[ \left( m'_r - E(m'_r) \right)^2 \right]$$

$$= E \left( \left( \frac{1}{n} \sum x^r - \mu'_r \right)^2 \right)$$

$$= \frac{1}{n^2} E \left[ \left( \sum x^r \right)^2 - \mu^2_r \right]$$

$$= \frac{1}{n^2} E \left[ \sum x^{2r} + \sum x_j x_k^r \right] - \mu^2_r$$

The second summation has $n(n - 1)$ terms with $x_j$ and $x_k$ independent

$$= \frac{1}{n^2} \{ n\mu^2_{2r} + n(n - 1)\mu^2_r \} - \mu^2_r$$

$$= \frac{1}{n}(\mu^2_{2r} - \mu^2_r)$$

which is an exact result.
The expectation of \( m^2 \) is

\[
E(m^2) = E \left[ \frac{\sum x^2}{n} - \left( \frac{\sum x}{n} \right)^2 \right] = E \left[ \frac{\sum x^2}{n} - \frac{1}{n^2} \left( \sum x^2 + \sum_{i \neq j} x_i x_j \right) \right]
\]

Since \( x_i \) and \( x_j \) are independent, \( E(x_i x_j) = \mu^2 \) and

\[
E \left( \sum_{i \neq j} x_i x_j \right) = n(n-1)\mu^2
\]

we get

\[
E(m^2) = (\frac{1}{n} - \frac{1}{n^2})n\mu^2 - \frac{1}{n^2}n(n-1)\mu^2
= \frac{n-1}{n}(\mu^2 - \mu^2)
= \frac{n-1}{n} \mu^2
\]

Thus, asymptotically,

\[
E(m^2) = \mu^2
\]

To calculate the sampling variance of \( m^2 \), we shall generalize this approach for higher moments. Recall e.g.

\[
\sum x_i^2 x_j x_l x_k \quad (\text{all suffixes different})
\]

has \( n(n-1)(n-2)(n-3) \) terms. To simplify the notation, we define the following variants of symmetric functions. A symmetric function \( t(x_1, \ldots, x_n) \) remains unchanged if we permute the \( x \).

- **Augmented symmetric functions:** (all subscripts different)
  
  \[
  [p_1^{\pi_1} p_2^{\pi_2} \ldots p_s^{\pi_s}] = \sum_{\pi_1} x_{i_1}^{p_{\pi_1}} x_{j_2}^{p_{\pi_2}} \ldots x_{l_{\pi_s}}^{p_{\pi_s}}
  \]

  E.g.
  
  \[
  \sum x_i^2 x_j x_l x_k = [1^223]
  \]
  
  \[
  \sum x_i^2 x_j^2 x_l^2 = [2^3]
  \]

- **Monomial symmetric functions:**
  
  \[
  (p_1^{\pi_1} p_2^{\pi_2} \ldots p_s^{\pi_s}) = \frac{[p_1^{\pi_1} p_2^{\pi_2} \ldots p_s^{\pi_s}]}{\pi_1! \pi_2! \ldots \pi_s!}
  \]

  \[
  \]

- **Unitary functions** (no two indices equal):
  
  \[
  a_r = (1^r) = \frac{\sum x_i x_j \ldots x_l}{r!}
  \]

- **One-part functions or power sums:**
  
  \[
  s_r = (r) = \sum x^r = [r]
  \]
From (104)
\[
E[p_{1}^{n_{1}}p_{2}^{n_{2}}\ldots p_{s}^{n_{s}}] = n(n-1)\ldots(n-\rho+1)\mu_{1}'^{n_{1}}\mu_{2}'^{n_{2}}\ldots\mu_{s}'^{n_{s}}
\]  
(105)
where \( \rho = \sum_{i=1}^{s} \pi_{i} \) and \( p := \sum_{i=1}^{s} p_{i} \pi_{i} \) is the “weight” of the symmetric function.

For example, the sample variance (102) for \( r = 2 \) becomes
\[
m_{2} = \frac{\sum x^{2}}{n} - \left( \frac{\sum x}{n} \right)^{2}
\]
\[
= \frac{(2)}{n} - \frac{(1)^{2}}{n^{2}}
\]

We have,
\[
(2) = [2] \quad \text{since} \ 1! = 1
\]
\[
(1)^{2} = [2] + [1^{2}] \quad \text{since} \ \sum x^{2} = \sum x^{2} + \sum_{i \neq j} x_{i}x_{j}
\]
(or from table). Hence,
\[
m_{2} = \frac{[2]}{n} - \frac{[2] + [1^{2}]}{n^{2}}
\]
\[
= \frac{n-1}{n^{2}}[2] - \frac{1}{n^{2}}[1^{2}]
\]  
(106)

Now using (105)
\[
E(m_{2}) = \frac{n-1}{n^{2}}n\mu_{2}' - \frac{1}{n^{2}}n(n-1)\mu_{1}'^{2}
\]
\[
= \frac{n-1}{n}(\mu_{2}' - \mu_{1}'^{2})
\]
\[
= \frac{n-1}{n}\mu_{2}
\]  
(107)

For a shortcut to calculate such expectation values, notice that statistic \( m_{2} \) is independent of the origin. Therefore, we can take the population mean to be zero: \( \mu_{1}' = 0 \). Then ignore any [] containing a unit and (106) immediately gives (107).

Similarly,
\[
m_{2}^{2} = \left[ \frac{(2)}{n} - \frac{(1)^{2}}{n^{2}} \right]^{2}
\]
\[
= \left( \frac{2}{n} \right)^{2} - \frac{2(2)(1)^{2}}{n^{3}} + \frac{(1)^{4}}{n^{4}}
\]

From table or directly
\[
(2)^{2} = [4] + [2^{2}] \quad \text{since} \ \sum x^{2} = \sum x^{2} + \sum_{i \neq j} x_{i}^{2}x_{j}^{2}
\]
\[
(2)(1)^{2} = [4] + 2[31] + [2^{2}] + [21^{2}]
\]
since
\[
\left( \sum x^{3} \right)^{2} = \sum x^{4} + 2\sum x_{i}^{3}x_{j} + \sum x_{i}^{2}x_{j}^{2} + \sum x_{i}^{2}x_{j}x_{k} + \sum x_{i}x_{j}x_{k}x_{l}
\]
and
\[
(1)^{4} = [4] + 4[31] + 3[2^{2}] + 6[21^{2}] + [1^{4}]
\]  
because
\[
\left( \sum x \right)^{4} = \binom{4}{4} \sum x^{4} + \binom{4}{3} \sum x_{i}^{3}x_{j} + \frac{1}{2}\binom{4}{2} \sum x_{i}^{2}x_{j}^{2} + \binom{4}{2} \sum x_{i}^{2}x_{j}x_{k} + \sum x_{i}x_{j}x_{k}x_{l}
\]
Ignoring [ ]’s containing a unit:

\[
E(m_2^2) = E\left[\frac{[4] + [2^2]}{n^2} - 2\frac{[4] + [2^2]}{n^3} + \frac{[4] + 3[2^2]}{n^4}\right]
\]

\[
= E\left[\frac{(n^2 - 2n + 1)}{n^4} [4] + \frac{(n^2 - 2n + 3)}{n^4} [2^2]\right]
\]

and using (105)

\[
= \frac{(n - 1)^2}{n^3} \mu_4 + \frac{(n - 1)(n^2 - 2n + 3)}{n^3} \mu_2^2
\]

Using this and (107) we get

\[
\text{var}(m_2) = E(m_2^2) - (E(m_2))^2
\]

\[
= \frac{(n - 1)^2}{n^3} \mu_4 - \frac{(n - 1)(n - 3)}{n^3} \mu_2^2
\]

\[
\approx \frac{1}{n} (\mu_4 - \mu_2^2)
\]

where \(\approx\) indicates asymptotic equality at large \(n\).

Also, if in (108) we put \(\kappa_4 = \mu_4 - 3\mu_2^2\) and \(\kappa_2 = \mu_2\) and \(k_2 = m_2\)

\[
\text{var}(k_2) = \left(\frac{n - 1}{n}\right)^2 \left(\frac{\kappa_4}{n} + \frac{2}{n-1} \kappa_2^2\right)
\]

\[
\text{C Tensor notation rule}
\]

In Kaplan’s formulae in tensor notation for cumulants of \(k\)-statistics[6], to insert a single subscript, we affix the subscript in every possible position and divide by \(n\). For example, given

\[
\kappa(ab, ijk) = \frac{\kappa_{abijk}}{n} + \sum_6 \kappa_{ai}\kappa_{bjk} \frac{1}{n-1}
\]

we can construct

\[
\kappa(ab, ijk, p) = \frac{\kappa_{abijkp}}{n^2} + \frac{\sum_6 \kappa_{asp}\kappa_{bjkp} + \sum_6 \kappa_{ai}\kappa_{bjkp}}{n(n-1)}
\]

Other examples:

\[
\kappa(i, j) = \frac{\kappa_{ij}}{n}
\]

\[
\kappa(i, kl) = \frac{\kappa_{ikl}}{n}
\]

\[
\kappa(i, j, kl) = \frac{\kappa_{ijkl}}{n^2}
\]

\[
\text{D Two-state transition factors}
\]

Consider a fluorescent particle alternating between two states:

\[
\text{state 1} \xleftrightarrow[k_f}{k_b} \text{state 2}
\]

where “state 1” is usually the brighter (unfolded) state, “state 2” is usually the darker (folded) state, and \(k_f\) and \(k_b\) are the forward and backward (reverse) rates respectively. Denote the probability that the particle
is found in state 1 at time time $t$ with $P_1(t)$, and similarly for state 2. The following equations describe the reaction:

$$\frac{dP_1(t)}{dt} = -k_i P_1(t) + k_b P_2(t)$$
$$P_2(t) = 1 - P_1(t)$$

The solutions are

$$P_1(t) = \frac{1 + [(1 + k)P_1(0) - 1]e^{-t/t_R}}{1 + k}$$
$$P_2(t) = \frac{k - [k - (1 + k)P_2(0)]e^{-t/t_R}}{1 + k}$$

(111)

where $P_1(0)$ and $P_2(0)$ are the initial probabilities at time 0. We have defined

$$k = \frac{k_i}{k_b} = \frac{N_2}{N_1}$$

with $N_i$ being the number of molecules in state $i$ in the ensemble, and

$$t_R = (k_i + k_b)^{-1}$$

as the overall reaction time constant. Also, defining

$$P(i) = P_i(\infty) = \frac{N_i}{N_1 + N_2}$$

as the probability of finding the particle in state $i$ independent of initial conditions, we have

$$k = \frac{P(2)}{P(1)}$$

The transition factor $Z_{s_2,s_1}(t)$, denoting the probability that the particle is found in state $s_2$ at time $t$ given it was in state $s_1$ at time 0, can be found by setting the initial probabilities in (111) equal to 1 or 0:

$$Z_{1,1}(t) = \frac{1 + ke^{-t/t_R}}{1 + k}$$
$$Z_{1,2}(t) = \frac{1 - e^{-t/t_R}}{1 + k}$$
$$Z_{2,1}(t) = \frac{k(1 - e^{-t/t_R})}{1 + k}$$
$$Z_{2,2}(t) = \frac{k + e^{-t/t_R}}{1 + k}$$

References

[1] Farshad Abdollah-Nia, Martin P. Gelfand, and Alan Van Orden. Artifact-free and detection-profile-independent higher-order fluorescence correlation spectroscopy for microsecond-resolved kinetics. 1. multidetector and sub-binning approach. The Journal of Physical Chemistry B, 121(11):2373–2387, 2017. PMID: 28230994.

[2] Farshad Abdollah-Nia, Martin P. Gelfand, and Alan Van Orden. Artifact-free and detection-profile-independent higher-order fluorescence correlation spectroscopy for microsecond-resolved kinetics. 2. mixtures and reactions. The Journal of Physical Chemistry B, 121(11):2388–2399, 2017. PMID: 28182427.
[3] N Balakrishnan, Norman L Johnson, and Samuel Kotz. A note on relationships between moments, central moments and cumulants from multivariate distributions. *Statistics & probability letters*, 39(1):49–54, 1998.

[4] Elliot L Elson and Douglas Magde. Fluorescence correlation spectroscopy. i. conceptual basis and theory. *Biopolymers*, 13(1):1–27, 1974.

[5] Lindsey N Hillesheim and Joachim D Müller. The dual-color photon counting histogram with non-ideal photodetectors. *Biophysical Journal*, 89(5):3491–3507, 2005.

[6] E. L. Kaplan. Tensor notation and the sampling cumulants of k-statistics. *Biometrika*, 39(3/4):319–323, 1952.

[7] Oleg Krichevsky and Grégoire Bonnet. Fluorescence correlation spectroscopy: the technique and its applications. *Reports on Progress in Physics*, 65(2):251, 2002.

[8] L Mandel. Fluctuations of photon beams and their correlations. *Proceedings of the Physical Society*, 72(6):1037, 1958.

[9] Artem V Melyukov and Kathleen B Hall. Revival of high-order fluorescence correlation analysis: generalized theory and biochemical applications. *The Journal of Physical Chemistry B*, 113(47):15629–15638, 2009.

[10] Joachim D Müller. Cumulant analysis in fluorescence fluctuation spectroscopy. *Biophysical Journal*, 86(6):3981–3992, 2004.

[11] A.G. Palmer and N.L. Thompson. Molecular aggregation characterized by high order autocorrelation in fluorescence correlation spectroscopy. *Biophysical Journal*, 52(2):257 – 270, 1987.

[12] Arthur G Palmer and Nancy L Thompson. High-order fluorescence fluctuation analysis of model protein clusters. *Proceedings of the National Academy of Sciences*, 86(16):6148–6152, 1989.

[13] Arthur G Palmer III and Nancy L Thompson. Intensity dependence of high-order autocorrelation functions in fluorescence correlation spectroscopy. *Review of Scientific Instruments*, 60(4):624–633, 1989.

[14] Perry G. Schiro, Christopher L. Kuyper, and Daniel T. Chiu. Continuous-flow single-molecule ce with high detection efficiency. *ELECTROPHORESIS*, 28(14):2430–2438, 7 2007.

[15] Alan Stuart and Keith Ord. *Kendall’s advanced theory of statistics*, volume 1. Arnold, a member of the Hoddder Headline Group, 4th edition, 1994.

[16] Alan Van Orden, Keir Fogarty, and Jaemyeong Jung. Fluorescence fluctuation spectroscopy: a coming of age story. *Applied spectroscopy*, 58(5):122A–137A, 2004.

[17] Bin Wu, Yan Chen, and Joachim D Müller. Dual-color time-integrated fluorescence cumulant analysis. *Biophysical Journal*, 91(7):2687–2698, 2006.

[18] Bin Wu and Joachim D Müller. Time-integrated fluorescence cumulant analysis in fluorescence fluctuation spectroscopy. *Biophysical Journal*, 89(4):2721–2735, 2005.

[19] Zhenqin Wu, Huimin Bi, Sichen Pan, Lingyi Meng, and Xin Sheng Zhao. Determination of equilibrium constant and relative brightness in fluorescence correlation spectroscopy by considering third-order correlations. *The Journal of Physical Chemistry B*, 120(45):11674–11682, 2016. PMID: 27775360.