Ben Sauerwine
Biophysics Homework 3

7. <u>Fluorescence Correlation Spectroscopy</u>
A laser beam illuminates a spot of local intensity $I(\vec{r})$ in a solution. Fluorescently labeled molecules entering this spot are excited and emit light that is subsequently detected. Within a small time window Δt, the detected fluorescence coming out of the spot is given by $n(t) = \Delta t Q \int d^3 r I(\vec{r}) C(\vec{r},t)$, where $C(\vec{r},t)$ is the space and time-dependent density of labeled molecules and Q is a constant determined by experimental details. Let us write $C(\vec{r},t) = \overline{C} + \delta C(\vec{r},t)$, where $\overline{C}$ is the average density and $\delta C(\vec{r},t)$ the fluctuating component, and similarly $n(t) = \overline{n} + \delta n(t)$. We will now see that one can determine the diffusion constant of the labeled molecules from the normalized time-autocorrelation function for the detected light, defined by:

$$G(t) := \frac{\langle \delta n(0) \delta n(t) \rangle}{\overline{n}^2}.$$

Here and below $\langle \ldots \rangle$ denotes a statistical ensemble average.

1. **Find expressions (i.e., integrals) for**
   (i) The average light intensity $\overline{n}$

For some experiment time T, this average is given by:

$$\overline{n} = \frac{1}{T}\int_0^T n(t)dt$$

$$= \Delta t Q \int d^3 r I(\vec{r}) \left[\frac{1}{T}\int_0^T dt C(\vec{r},t) \right]$$

$$= \Delta t Q \int d^3 r I(\vec{r}) \left[\overline{C} + \frac{1}{T}\int_0^T dt \delta C(\vec{r},t) \right]$$

$$= \Delta t Q \overline{C} \int d^3 r I(\vec{r}) + \Delta t Q \int d^3 r I(\vec{r}) \left[\frac{1}{T}\int_0^T dt \delta C(\vec{r},t) \right]$$

Now if I assume that the fluctuating component averages to zero at each position over the time of the experiment, I have

$$\overline{n} = \Delta t Q \overline{C} \int d^3 r I(\vec{r})$$

   (ii) Its fluctuating component $\delta n(t)$

Under the assumption taken above

$$\delta n(t) = n(t) - \bar{n}$$
$$= \Delta t Q \int d^3 r I(\vec{r})\left[\overline{C} + \delta C(\vec{r},t)\right] - \bar{n}$$
$$= \Delta t Q \int d^3 r I(\vec{r}) \delta C(\vec{r},t)$$

2. **Show that** $G(t) = \dfrac{(Q\Delta t)^2}{\bar{n}^2} \int d^3 r \int d^3 r' I(\vec{r}) I(\vec{r}') \langle \delta C(\vec{r},0) \delta C(\vec{r},t) \rangle$

$$G(t) := \frac{\langle \delta n(0) \delta n(t) \rangle}{\bar{n}^2}$$
$$= \frac{\left\langle \Delta t Q \int d^3 r I(\vec{r}) \delta C(\vec{r},0) \Delta t Q \int d^3 r' I(\vec{r}') \delta C(\vec{r}',t) \right\rangle}{\bar{n}^2}$$
$$= \frac{(\Delta t Q)^2}{\bar{n}^2} \left\langle \int d^3 r I(\vec{r}) \delta C(\vec{r},0) \int d^3 r' I(\vec{r}') \delta C(\vec{r}',t) \right\rangle$$
$$= \frac{(\Delta t Q)^2}{\bar{n}^2} \int d^3 r \int d^3 r' I(\vec{r}) I(\vec{r}') \langle \delta C(\vec{r},0) \delta C(\vec{r}',t) \rangle$$

The final step above is justified since the neither the intensities nor the coordinates take part in the statistical ensemble, so I have factored them out.

3. **We are interested in a system where the molecules diffuse freely. If so,** $C(\vec{r},t)$ **-- and hence** $\delta C(\vec{r},t)$ **-- follows the diffusion equation** $\dfrac{\partial}{\partial t} \delta C(\vec{r},t) = D \Delta \delta C(\vec{r},t)$. **Using the following convention for a pair of 3D Fourier transforms:**

$$\tilde{f}(\vec{k}) = \frac{1}{(2\pi)^{3/2}} \int d^3 r f(\vec{r}) e^{i\vec{k}\cdot\vec{r}} \quad \textbf{and} \quad f(\vec{r}) = \frac{1}{(2\pi)^{3/2}} \int d^3 k \tilde{f}(\vec{k}) e^{-i\vec{k}\cdot\vec{r}}$$

derive the differential equation satisfied by $\delta\tilde{C}(\vec{k},t)$ **and show that it is solved by** $\delta\tilde{C}(\vec{k},t) = \delta\tilde{C}(\vec{k},0) e^{-Dk^2 t}$.

$$\frac{\partial}{\partial t} \delta C(\vec{r},t) = D \Delta \delta C(\vec{r},t)$$
$$\frac{\partial}{\partial t}\left[\frac{1}{(2\pi)^{3/2}} \int d^3 k \delta\tilde{C}(\vec{k},t) e^{-i\vec{k}\cdot\vec{r}}\right] = D\Delta\left[\frac{1}{(2\pi)^{3/2}} \int d^3 k \delta\tilde{C}(\vec{k},t) e^{-i\vec{k}\cdot\vec{r}}\right]$$
$$\left[\frac{1}{(2\pi)^{3/2}} \int d^3 k \left[\frac{\partial}{\partial t} \delta\tilde{C}(\vec{k},t)\right] e^{-i\vec{k}\cdot\vec{r}}\right] = \left[\frac{1}{(2\pi)^{3/2}} \int d^3 k \left[-Dk^2 \delta\tilde{C}(\vec{k},t)\right] e^{-i\vec{k}\cdot\vec{r}}\right]$$

By comparison, then, I have:

$$\frac{\partial}{\partial t}\delta\tilde{C}(\vec{k},t) = -Dk^2\delta\tilde{C}(\vec{k},t)$$

This is a first-order differential equation whose solution is clearly

$$\delta\tilde{C}(\vec{k},t) = \delta\tilde{C}(\vec{k},0)e^{-Dk^2t}$$

4. **By following the sequence "Fourier Transform $\delta C(\vec{r},t)$, Use Point 3, Fourier Transform Back", show that**

$$\langle \delta C(\vec{r},0)\delta C(\vec{r}\,',t)\rangle = \frac{\overline{C}}{(2\pi)^3}\int d^3k e^{-Dk^2t} e^{i\vec{k}\cdot(\vec{r}-\vec{r}\,')}$$

To prove this, use the fact that $\langle \delta C(\vec{r},0)\delta C(\vec{r}\,'',0)\rangle = \overline{C}\,\delta^3(\vec{r}-\vec{r}\,'')$. This means that density fluctuations at different positions are uncorrelated and that the local variance is proportional to the mean (i.e., the distribution is "Poissonian").

$$\langle \delta C(\vec{r},0)\delta C(\vec{r}\,',t)\rangle = \left\langle \frac{1}{(2\pi)^{3/2}}\int d^3k\,\delta\tilde{C}(\vec{k},0)e^{-i\vec{k}\cdot\vec{r}} \frac{1}{(2\pi)^{3/2}}\int d^3k'\,\delta\tilde{C}(\vec{k}',t)e^{-i\vec{k}'\cdot\vec{r}\,'} \right\rangle$$

$$= \left\langle \frac{1}{(2\pi)^{3/2}}\int d^3k\,\delta\tilde{C}(\vec{k},0)e^{-i\vec{k}\cdot\vec{r}} \frac{1}{(2\pi)^{3/2}}\int d^3k'\,\delta\tilde{C}(\vec{k}',0)e^{-Dk'^2t}e^{-i\vec{k}'\cdot\vec{r}\,'} \right\rangle$$

$$= \frac{1}{(2\pi)^3}\int d^3k e^{-i\vec{k}\cdot\vec{r}}\int d^3k' e^{-Dk'^2t}e^{-i\vec{k}'\cdot\vec{r}\,'}\left\langle \delta\tilde{C}(\vec{k},0)\delta\tilde{C}(\vec{k}',0)\right\rangle$$

Pressing on through the mess of integrals, I now Fourier transform back:

$$= \frac{1}{(2\pi)^6}\int d^3k e^{-i\vec{k}\cdot\vec{r}}\int d^3k' e^{-Dk'^2t}e^{-i\vec{k}'\cdot\vec{r}\,'}\int d^3r'' e^{i\vec{k}\cdot\vec{r}\,''}\int d^3r''' e^{i\vec{k}'\cdot\vec{r}\,'''}\left\langle \delta\tilde{C}(\vec{r}\,'',0)\delta\tilde{C}(\vec{r}\,''',0)\right\rangle$$

Now using the fact that $\langle \delta C(\vec{r}\,'',0)\delta C(\vec{r}\,''',0)\rangle = \overline{C}\,\delta^3(\vec{r}\,''-\vec{r}\,''')$,

$$= \frac{\overline{C}}{(2\pi)^6}\int d^3k e^{-i\vec{k}\cdot\vec{r}}\int d^3k' e^{-Dk'^2t}e^{-i\vec{k}'\cdot\vec{r}\,'}\int d^3r'' e^{i\vec{k}\cdot\vec{r}\,''}\int d^3r''' e^{i\vec{k}'\cdot\vec{r}\,'''}\delta^3(\vec{r}\,''-\vec{r}\,''')$$

$$= \frac{\overline{C}}{(2\pi)^6}\int d^3k e^{-i\vec{k}\cdot\vec{r}}\int d^3k' e^{-Dk'^2t}e^{-i\vec{k}'\cdot\vec{r}\,'}\int d^3r'' e^{i\vec{k}\cdot\vec{r}\,''}e^{i\vec{k}'\cdot\vec{r}\,''}$$

$$= \frac{\overline{C}}{(2\pi)^6}\int d^3r''\int d^3k e^{i\vec{k}\cdot(\vec{r}\,''-\vec{r})}\int d^3k' e^{-Dk'^2t}e^{i\vec{k}'\cdot(\vec{r}\,''-\vec{r}\,')}$$

Next I need to use the fact that

$$(2\pi)^3 \delta^3(\vec{r}''-\vec{r}) = \int d^3k e^{i\vec{k}\cdot(\vec{r}''-\vec{r})}$$

The above follows from the orthogonality of the functions making up the Fourier transform and the definitions above. This gives me:

$$\langle \delta C(\vec{r},0)\delta C(\vec{r}',t)\rangle = \frac{\overline{C}}{(2\pi)^6}\int d^3r''(2\pi)^3\delta^3(\vec{r}-\vec{r}'')\int d^3k' e^{-Dk'^2t}e^{i\vec{k}'\cdot(\vec{r}''-\vec{r}')}$$

$$= \frac{\overline{C}}{(2\pi)^3}\int d^3k' e^{-Dk'^2t}e^{i\vec{k}'\cdot(\vec{r}-\vec{r}')}$$

5. **Inserting your last result into the equation obtained in point 2, prove that** $G(t) = \frac{(Q\Delta t)^2}{\overline{n}^2}\overline{C}\int d^3k e^{-Dk^2t}e^{i\vec{k}\cdot(\vec{r}-\vec{r}')}$

$$G(t) = \frac{(Q\Delta t)^2}{\overline{n}^2}\overline{C}\int d^3r\int d^3r' I(\vec{r})I(\vec{r}')\frac{1}{(2\pi)^3}\int d^3k e^{-Dk^2t}e^{i\vec{k}\cdot(\vec{r}-\vec{r}')}$$

$$= \frac{(Q\Delta t)^2}{\overline{n}^2}\overline{C}\int d^3k e^{-Dk^2t}\left[\frac{1}{(2\pi)^{3/2}}\int d^3r I(\vec{r})e^{i\vec{k}\cdot\vec{r}}\right]\left[\frac{1}{(2\pi)^{3/2}}\int d^3r' I(\vec{r}')e^{-i\vec{k}\cdot\vec{r}'}\right]$$

$$= \frac{(Q\Delta t)^2}{\overline{n}^2}\overline{C}\int d^3k e^{-Dk^2t}I(\vec{k})I(\vec{k})^*$$

$$= \frac{(Q\Delta t)^2}{\overline{n}^2}\overline{C}\int d^3k e^{-Dk^2t}\left|I(\vec{k})\right|^2$$

6. **If the beam-spot** $I(\vec{r})$ **is Gaussian, with width** σ_x**,** σ_y **and** σ_z **in the three coordinate directions, prove the final formula**

$$\frac{1}{G(t)} = V_{eff}\overline{C}\sqrt{1+\frac{t}{\tau_x}}\sqrt{1+\frac{t}{\tau_y}}\sqrt{1+\frac{t}{\tau_z}}$$

with the effective beam spot volume $V_{eff} = 8\pi^{3/2}\sigma_x\sigma_y\sigma_z$ **and the characteristic times** $\tau_i = \frac{\sigma_i^2}{D}$**. Without proof, you may use the fact that the (1D) Fourier transform of a normalized Gaussian** $g(x,\sigma)$ **of width** σ **is given by** $\sigma^{-1}g(k,\sigma^{-1})$**.**

Let

$$I(\vec{r},\sigma_x,\sigma_y,\sigma_z) = \bar{I} g(\vec{r}\cdot\hat{i},\sigma_x) g(\vec{r}\cdot\hat{j},\sigma_y) g(\vec{r}\cdot\hat{k},\sigma_z)$$

$$g(r,\sigma_r) = \frac{1}{\sigma_r\sqrt{2\pi}} e^{-\frac{r^2}{2\sigma_r^2}}$$

Then,

$$G(t) = \frac{(Q\Delta t)^2}{\bar{n}^2}\bar{C}\int d^3k e^{-Dk^2t}\left[\frac{1}{(2\pi)^{3/2}}\int d^3r I(\vec{r},\sigma_x,\sigma_y,\sigma_z) e^{i\vec{k}\cdot\vec{r}}\right]\left[\frac{1}{(2\pi)^{3/2}}\int d^3r' I(\vec{r},\sigma_x,\sigma_y,\sigma_z) e^{-i\vec{k}\cdot\vec{r}'}\right]$$

$$= \frac{(Q\Delta t)^2}{\bar{n}^2}\left(\frac{1}{\sigma_x\sigma_y\sigma_z}\right)^2 \bar{C}\int d^3k e^{-Dk^2t} I(\vec{k},\sigma_x^{-1},\sigma_y^{-1},\sigma_z^{-1}) I(\vec{k},\sigma_x^{-1},\sigma_y^{-1},\sigma_z^{-1})^*$$

$$= \frac{(Q\Delta t)^2}{\bar{n}^2}\bar{C}\int d^3k \bar{I}^2 \prod_{r\in x,y,z}\frac{1}{2\pi} e^{-Dk_r^2t} e^{-k_r^2\sigma_r^2}$$

$$= \frac{(Q\Delta t)^2}{\bar{n}^2}\bar{C}\bar{I}^2 \frac{1}{(8\pi)^{3/2}}\int d^3k \prod_{r\in x,y,z}\frac{1}{\sqrt{2\pi}} e^{-k_r^2\frac{2(tD+\sigma_r^2)}{2}}$$

$$= \frac{(Q\Delta t)^2}{\bar{n}^2}\bar{C}\bar{I}^2 \frac{1}{(8\pi)^{3/2}}\prod_{r\in x,y,z}\frac{1}{\sqrt{2(tD+\sigma_r^2)}}$$

$$= \frac{(Q\Delta t)^2}{\bar{n}^2}\frac{\bar{C}\bar{I}^2}{8\pi^{3/2}}\frac{1}{\sigma_x\sigma_y\sigma_z}\prod_{r\in x,y,z}\frac{1}{\sqrt{\left(\frac{t}{\tau_r}+1\right)}}$$

I will need to use the fact from part (1i) that $\bar{n} = \Delta t Q\bar{C}\bar{I}$

$$G(t) = \frac{1}{\bar{C}}\frac{1}{8\pi^{3/2}}\frac{1}{\sigma_x\sigma_y\sigma_z}\prod_{r\in x,y,z}\frac{1}{\sqrt{\left(\frac{t}{\tau_r}+1\right)}} = \frac{1}{\bar{C}V_{eff}}\prod_{r\in x,y,z}\frac{1}{\sqrt{\left(\frac{t}{\tau_r}+1\right)}}$$

$$\frac{1}{G(t)} = \bar{C}V_{eff}\prod_{r\in x,y,z}\sqrt{\left(\frac{t}{\tau_r}+1\right)}$$

QED

8. **<u>Cylindrical (or "wormlike") micelles</u>**

In class we have shown that the (scaled) volume fraction $\tilde{\phi}_N$ of N-aggregates for cylindrical micelles follows the exponential distribution $\tilde{\phi}_N \approx \tilde{\phi}_1^{\,N}$ (where $\tilde{x} = xe^{\alpha}$). The overall scaled lipid volume fraction was given by $\tilde{\phi} = \sum_{N=1}^{\infty} N\tilde{\phi}_N$.

1. **Prove that $\sum_{N=1}^{\infty} \tilde{\phi}_N = \frac{\tilde{\phi}_1}{1-\tilde{\phi}_1}$ and $\tilde{\phi} = \frac{\tilde{\phi}_1}{\left(1-\tilde{\phi}_1\right)^2}$.**

Using $\tilde{\phi}_N = \tilde{\phi}_1^{\,N}$, I take:

$$\sum_{N=1}^{\infty} \tilde{\phi}_N = \sum_{N=1}^{\infty} \tilde{\phi}_1^{\,N}$$

$$\sum_{N=1}^{\infty} \tilde{\phi}_1^{\,N} = \tilde{\phi} + \sum_{N=2}^{\infty} \tilde{\phi}_1^{\,N} = \tilde{\phi} + \tilde{\phi}_1 \sum_{N=2}^{\infty} \tilde{\phi}_1^{\,N-1} = \tilde{\phi} + \tilde{\phi}_1 \sum_{N=1}^{\infty} \tilde{\phi}_1^{\,N}$$

$$Define \quad X \equiv \sum_{N=1}^{\infty} \tilde{\phi}_1^{\,N} \quad for \quad clarity$$

$$X \equiv \sum_{N=1}^{\infty} \tilde{\phi}_1^{\,N} = \tilde{\phi} + \tilde{\phi}_1 \sum_{N=1}^{\infty} \tilde{\phi}_1^{\,N} = \tilde{\phi} + \tilde{\phi}_1 X$$

$$X = \tilde{\phi} + \tilde{\phi}_1 X$$

$$X = \frac{\tilde{\phi}}{1-\tilde{\phi}_1}$$

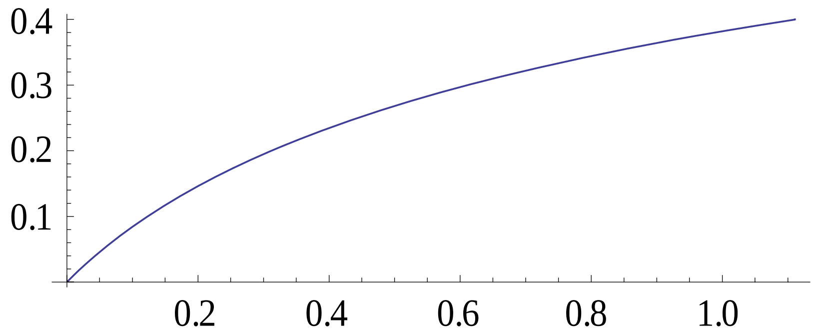
Next,

$$\sum_{N=1}^{\infty} N\tilde{\phi}_N \approx \sum_{N=1}^{\infty} N\tilde{\phi}_1^{\,N} = \sum_{N=1}^{\infty} \tilde{\phi}_1 \frac{\partial}{\partial \tilde{\phi}_1} \tilde{\phi}_1^{\,N} = \tilde{\phi}_1 \frac{\partial}{\partial \tilde{\phi}_1} \sum_{N=1}^{\infty} \tilde{\phi}_1^{\,N}$$

$$= \tilde{\phi}_1 \frac{\partial}{\partial \tilde{\phi}_1} \left[\frac{\tilde{\phi}_1}{1-\tilde{\phi}_1} \right] = \frac{\tilde{\phi}_1}{\left(1-\tilde{\phi}_1\right)^2}$$

2. **Sketch $\tilde{\phi}_1$ as a function of $\tilde{\phi}$. Show that for $\tilde{\phi} << 1$ we have $\tilde{\phi}_1 \approx \tilde{\phi}$ and for $\tilde{\phi} >> 1$, $\tilde{\phi}_1 \approx 1 - \frac{1}{\sqrt{\tilde{\phi}}}$.**

Plotting $\tilde{\phi}_1$ as a function of $\tilde{\phi}$ with $\tilde{\phi} = \frac{\tilde{\phi}_1}{(1-\tilde{\phi}_1)^2}$, I have:



Taking the Taylor series at small $\tilde{\phi}_1$ (and thus also small $\tilde{\phi}$ from my sketch),

$$\tilde{\phi} = \left.\frac{\tilde{\phi}_1}{(1-\tilde{\phi}_1)^2}\right|_{\tilde{\phi}_1 \approx 0} = 0 + \left[\left.\frac{\partial}{\partial \tilde{\phi}_1}\frac{\tilde{\phi}_1}{(1-\tilde{\phi}_1)^2}\right|\right]_{\tilde{\phi}_1 = 0} \tilde{\phi}_1 + O(\tilde{\phi}_1{}^2)$$

$$= 0 + \tilde{\phi}_1 + O(\tilde{\phi}_1{}^2)$$

$$\approx \tilde{\phi}_1$$

And so I see that when $\tilde{\phi} << 1$ we have $\tilde{\phi}_1 \approx \tilde{\phi}$.

Now consider the case when $\tilde{\phi} >> 1$. Manipulating the normal relationship, I get:

$$\tilde{\phi} = \frac{\tilde{\phi}_1}{(1-\tilde{\phi}_1)^2}$$

$$(1-\tilde{\phi}_1)^2 \tilde{\phi} = \tilde{\phi}_1$$

$$0 = \tilde{\phi}_1{}^2 \tilde{\phi} - (2\tilde{\phi} + 1)\tilde{\phi}_1 + \tilde{\phi}$$

Using the Quadratic formula and taking my limit, I have:

$$\tilde{\phi}_1 = \frac{2\tilde{\phi} + 1 \pm \sqrt{(2\tilde{\phi}+1)^2 - 4\tilde{\phi}^2}}{2\tilde{\phi}} = \frac{2\tilde{\phi} + 1 \pm \sqrt{4\tilde{\phi}+1}}{2\tilde{\phi}} \approx 1 \pm \frac{1}{\sqrt{\tilde{\phi}}}$$

Since the fraction of 1-aggregats cannot possibly be more than one, only the minus solution makes sense so:

$$\tilde{\phi}_1 \approx 1 - \frac{1}{\sqrt{\tilde{\phi}}}$$

3. Show that for $\tilde{\phi} >> 1$ the aggregate size $\langle N \rangle = \dfrac{\sum_N N\phi_N}{\sum_N \phi_N}$ grows with density like $\langle N \rangle \approx \sqrt{\tilde{\phi}}$.

$$\langle N \rangle = \frac{\sum_N N\phi_N}{\sum_N \phi_N} = \frac{\sum_N N\tilde{\phi}_N}{\sum_N \tilde{\phi}_N} = \frac{\dfrac{\tilde{\phi}_1}{\left(1-\tilde{\phi}_1\right)^2}}{\dfrac{\tilde{\phi}_1}{1-\tilde{\phi}_1}} = \frac{1}{1-\tilde{\phi}_1} \approx \frac{1}{1-\left(1-\dfrac{1}{\sqrt{\tilde{\phi}}}\right)} = \sqrt{\tilde{\phi}}$$