5. APPLICATIONS

Diffusion is used in a number of different contexts in science and technology. In this chapter we will discuss a few different examples, but the list is, of course, much longer.

We will discuss:

- FRAP
- The Black-Scholes option pricing model
- Tissue optics

5.1 Fluorescence Recovery After Photobleaching (FRAP)

Fluorescence Recovery After Photobleaching – or simply FRAP – is an optical technique used to study lateral diffusion.
FRAP is popular in biological studies e.g. of cell membrane diffusion:

Source of information on FRAP:

- Wikipedia
- Lubelski and Klafter
  Biophys. J. 94, 4646 (2008)
  [Biophys. J. 96, 2055 (2009)]
- Axelrod et al. Biophys. J. 16, 1055 (1976)

The technique can be used to study both ordinary and anomalous diffusion.
In the latter case one talks about fractional FRAP, and the underlying process is described in terms of the CTRW.
(it can also, by some authors, be called anomalous FRAP).

FRAP requires that one may tag the diffusing molecules/particles by a fluorescent tag.

Fluorescence, we recall, is a non-linear optical effect where absorbed light is emitted at a wavelength different from the illuminating wavelength.

The experimental setup consists of:

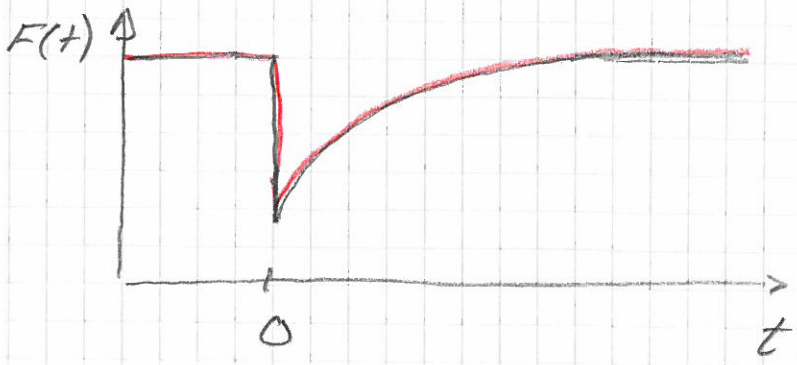
- an optical microscope
- a light source
- a number of fluorescent probes.

A typical experiment is performed in the following way:

* The moving molecules "are made" fluorescent (by tagging)
* A background image is recorded at the fluorescent wavelength.
* The microscope objective is focused onto a small viewable area

* The fluorescent molecules in this area are bleached, i.e. made non-fluorescent.
[This can be done by e.g. a highly intensive laser pulse]

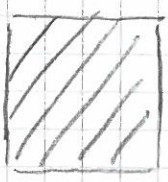
* A consegence of the bleaching is that the fluorescence $F(t)$ goes down, but with time (since the fluorescent molecules are moving) will return to the level befor the bleaching.



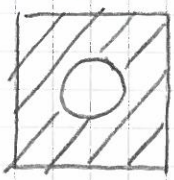
* How $F(t)$ recovers tells about the diffusion constant D of the molecules.
[more about this later]

A Cartoon overview of FRAP

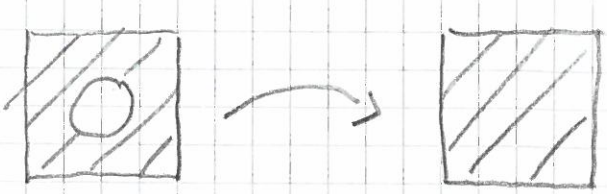
* Background



* Bleaching

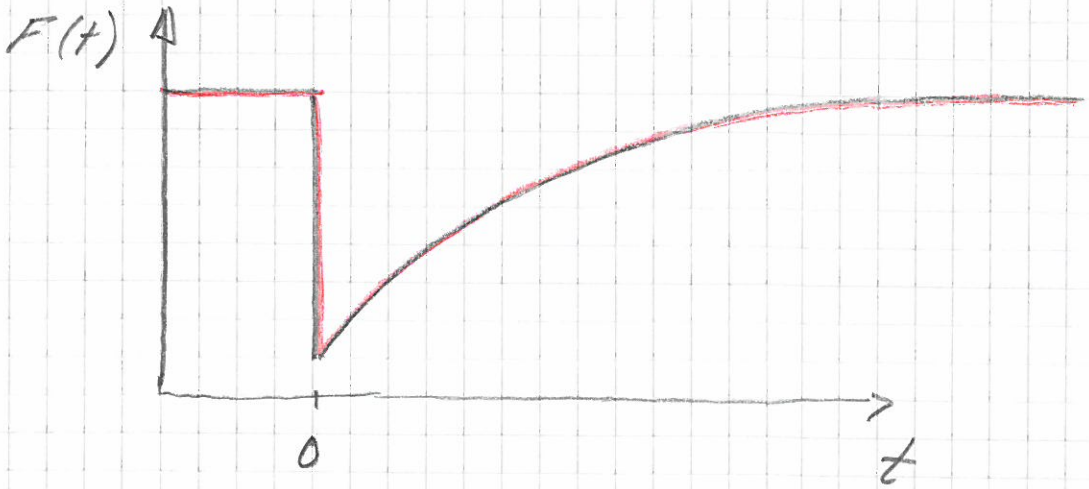


* Recovery



* Resulting FRAP curve:

Bleaching at $t=0$



Q: From the measured FRAP curve, how can one determine the diffusion constant?

A: Let us assume for simplisity that we already know that we are dealing with ordinary diffusion.

Then we know that the spreading goes like

$$\langle x^2(t) \rangle = 4Dt$$

If the size of the bleached spot is w (the diameter) then one would expect

$$D = \frac{\langle x^2(t) \rangle}{4t} = \frac{w^2}{4t}$$

for some typical time-scale for the recovery. Such a time could be, e.g., the time it takes to recover half of the needed fluorescence $t = t_{1/2}$, i.e.

$$D = \frac{w^2}{4t_{1/2}}$$

The above derivation was heuristic, and formally correct derivation is more complicated. However, the result for D still remains the same for normal Diffusion.

For fractional/anomalous FRAP, please consult the paper (handed out)

Biophys. J. 94, 4646 (2008)

The content of this paper is considered part of the class, so you better study its content.

5.2 The Black-Scholes Option Pricing Model

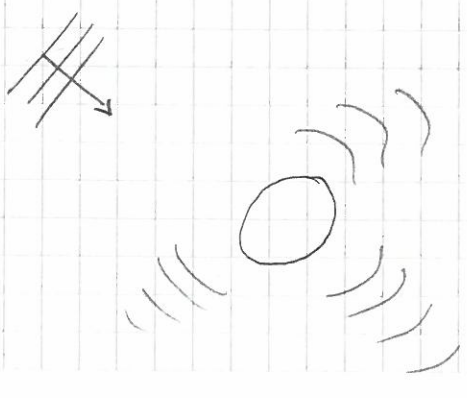
See handed out material

5.3 Tissue Optics

Tissue is an example of what is called a random media, and it consists of numerous scattering centers.

The optical properties of such media have been studied for a number of years, and it is still an active research field with application within many branches of science and technology. In its most general form, wave-scattering in inhomogeneous media is quite challenging, and closed form analytic solutions do not exist (partly due to the random or disordered character of the media involved). This has lead to the use of numerous approximations, some of which we will discuss later in this section.

Lets start with the much simpler problem of the scattering (of light) from a single spherical particle



Even this simplified problem is far from trivial. For instance, elastic scattering, for which the energy of the incident photons is not converted, can be grouped into at least the following groups:

* Rayleigh scattering
  - appropriate for particles small compared to the wavelength

$$I(\theta) \sim \frac{1+\cos^2\theta}{\lambda^4} \sim \underline{\lambda^{-4}}$$

* Mie theory
  - this theory is rather complicated, but exact for a spherical particle. It is needed when the size of the particles is comparable to the wavelength, and it reduces to the Rayleigh result for small particles

$$I(\theta) \sim \lambda^{-1/2} - \lambda^{-2/5}$$

typically when $R \sim \lambda$

* Geometrical optics
  - here the wavelength essentially is zero so GO is appropriate only when particle sizes are huge relative the wavelength ($R \gg \lambda$)

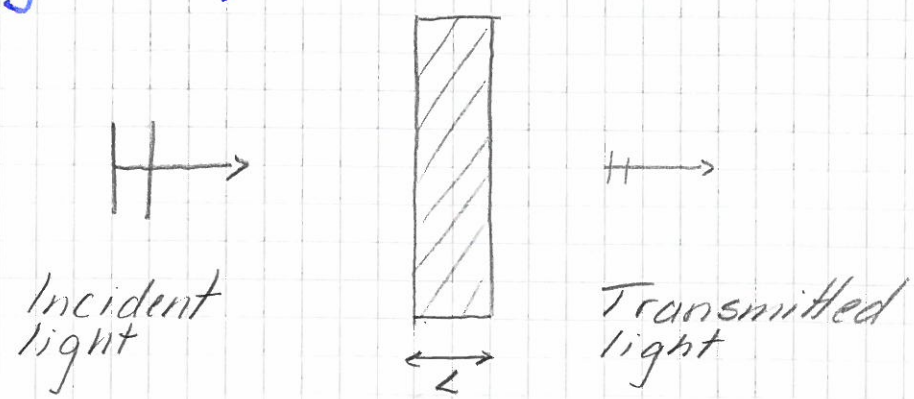
$$I(\theta) \sim \lambda^{\circ}$$

— ∽ —

For a random media the scattering particles do not have the same size. Instead they typically have some size distribution, and therefore a combination of the above types of scattering is observed.

Another issue that is important in random media optics is the concept of absorption.

Consider the following disodered medium geometry:



If the disodered medium is absorbing then the intensity, I, in the <u>forward</u> direction is reduced by two mechanisms:

- absorption
- scattering

The property of the medium depends quite a bit on which one of them that dominates. (or not).

Media that show <u>both</u> mechanisms are called <u>turbid media</u>. Biological tissue is normally considered as a turbid media.

The intensity in the forward direction after propagating a distance z can be described by (Lambert's law)

$$I(z) = I_0 e^{-\mu z}$$

where z is the distance from the "entering" surface, and I_0 is the intensity of the incident light.

In writing this eq. we have introduced the attenuation coefficient, α. Its inverse, is known as the mean-free path of the photons in the medium involved:

$$\ell = \frac{1}{\alpha}.$$

In a turbid medium one has

$$\alpha = \alpha_a + \alpha_s$$

where α_s is the scattering coef., and α_a is the absorption coef.

A quantity used to say something about the main attenuation mechanism is the optical albedo, a, defined as:

$$a = \frac{\alpha_s}{\alpha} = \frac{\alpha_s}{\alpha_a + \alpha_s} = \left[1 + \frac{\alpha_a}{\alpha_s}\right]^{-1}$$

$$a = \begin{cases} 0 & \text{exclusively absorption} \\ 1/2 & \alpha_a = \alpha_s : \text{equal strength} \\ 1 & \text{exclusively scattering} \end{cases}$$

Moreover :

$a \ll 1$: absorption dominates

$a \simeq 1$: scattering — " —

Note: Experimental observation

It is an experimental fact, established in the late 1980's, that in most biological tissue the photons are preferably scattered in the forward direction.

These findings are not consistent with neighter Rayleigh nor Mie scattering.

Hence neither of them can completely describe scattering in tissue.

This experimental fact has lead to the introduction of a probability function, $p(\theta)$, for photons being scattered an angle θ (from the incident direction).

This pdf is used to fit experimental data.

5.3.1 Photon Transport Theory

Two possibilities:

* Analytic approaches
  - Based on the Maxwell equations, and is therefor a fundamental approach.
  - However, its applicability is somewhat limited due to the complexity of the disordered media.

* Transport theory
  - Heuristic in character. (less fundamental)
  - Photon transport is addressed without referring to the Maxwell equation.
  - wide range of applications
  - easier than analytic approaches to use in practice.

In what follows we will consider (photon) transport theory.

In transport theory (for photons) the fundamental quantity is the radiance $J(\vec{r},\vec{s})$ measured in units of $W/m^2 sr$.

It denotes the power flux density in a spesific direction $\vec{s}$ within a unit solid angle $d\Omega$

Radiance is related to intensity $I(\vec{r})$ via

$$I(\vec{r}) = \int_{4\pi} d\Omega \; J(\vec{r},\vec{s})$$

or

$$J(\vec{r},\vec{s}) = I(\vec{r})\,\delta(\Omega - \Omega_s)$$

where Ω_s is the solid angle element associated with the vector $\vec{s}$.

The governing differential equation for radiance is so-called radiative transport equation (RTE)

$$\frac{dI(\vec{r},\vec{s})}{ds} = -\kappa I(\vec{r},\vec{s}) + \frac{\sigma_s}{4\pi}\int_{4\pi} p(\vec{s},\vec{s}')\, I(\vec{r},\vec{s}')\, d\Omega'$$

where $p(\vec{s},\vec{s}')$ is the phase function of a photon to be scattered from direction $\vec{s}'$ to $\vec{s}$.

If the scattering is symmetric about the optical axis:

$$p(\vec{s},\vec{s}') = p(\theta).$$

Interpretation of RTE:

1. The LHS gives the change in radiance over a length ds
2. The RHS consists of what radiance is removed from direction s due to <u>both</u> absorption and scattering plus what is scattered <u>into</u> direction $\vec{s}$ from other direction.
3. LHS = RHS

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It is very common in the study of wave-scattering from disodered system to divide quantities into <u>coherent</u> (in phase) and <u>incoherent</u> (out of phase) or <u>diffuse</u>.

This also applies to the radiance, and one writes:

$$J = J_c + J_d$$

The coherent component of the radiance, J_c, is reduced due to attenuation only, since the scattering into direction $\vec{s}$ from other directions typically will not be in phase (and is therefor not coherent.)

Hence, the RTE for the coherent component becomes:

$$\frac{dJ_c}{ds} = -\kappa J_c$$

with solution (assuming constant attenuation)*

$$J_c = I_0 \delta(\Omega - \Omega_s) e^{-\kappa s}$$

Hence, the main remaining challenge for RT is to calculate the incoherent/diffusive component.

In general this is not easy, and various approximations are (or can be) used.

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* Note that $\kappa s = s/\ell$ is known as the optical depth d. In general it is defined as

$$d = \int_0^s \kappa \, ds'$$

We will now look at some of the most common approximations:

A First-Order Scattering

In this approximation the diffuse component is considered small so that

$$J = J_c + J_d \simeq J_c$$

with the solution given earlier. Multiple scattering is neglected and hence for this approximation to hold one needs at least

$$a \ll 1/2$$

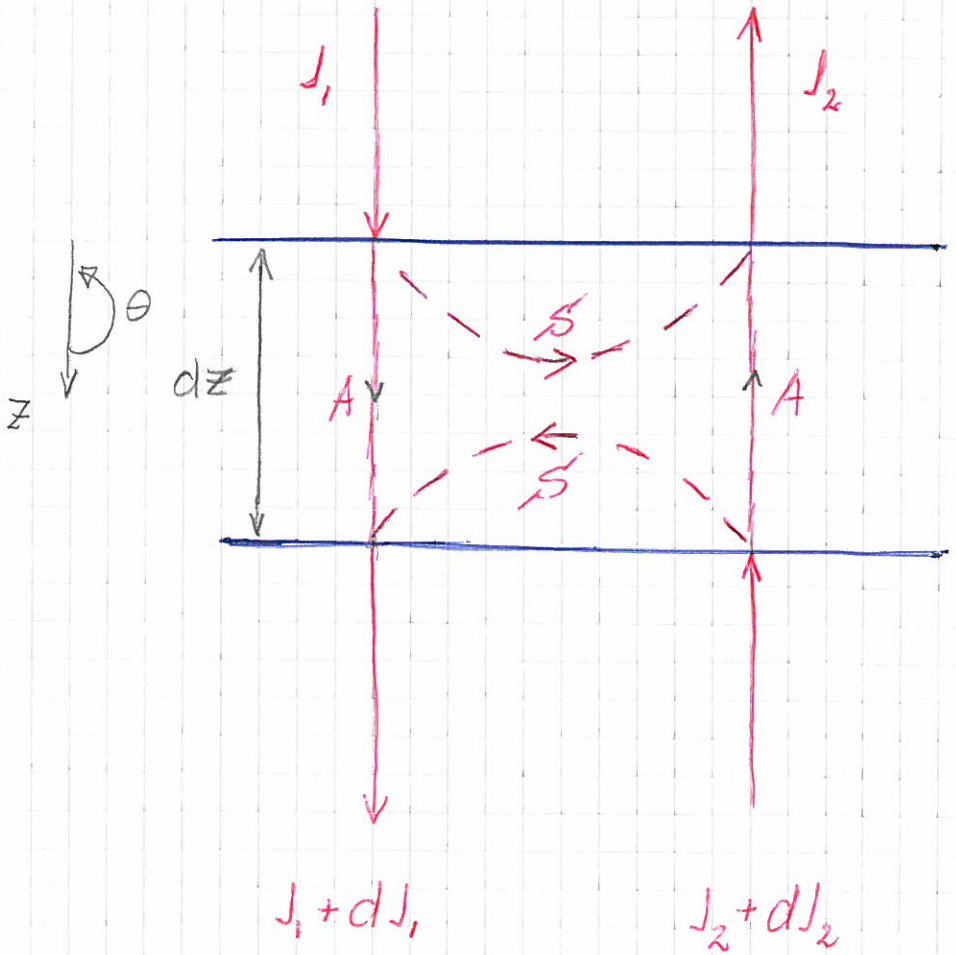
$$d \ll 1$$

B Kubelka-Munk Theory (KM) (1931)

KM-theory takes the other extreme and does the following approximation

$$J = J_c + J_d \simeq J_d$$

The theory assumes plane geometry and up and down going fluxes.



The coupled set of first order DE is:
(for this model) (look at $J_1 + dJ_1 - J_1 = dJ_1$)

$$\frac{dJ_1}{dz} = -S J_1 - A J_1 + S J_2$$

$$\frac{dJ_2}{dz} = S J_1 - S J_2 - A J_2$$

$$\frac{d}{dz}\begin{pmatrix} J_1 \\ J_2 \end{pmatrix} = \begin{pmatrix} -S-A & S \\ S & -S-A \end{pmatrix}\begin{pmatrix} J_1 \\ J_2 \end{pmatrix}$$

The coefficients A and S are the Kubelka-Munk coeffisients defined for the absorption and scattering of diffuse radiation, respectively.

The above system of DE are solved by assuming a trail solution of the form

$$\vec{J}(z) = \vec{v} e^{\lambda z}$$

This leads to conclusion that $\vec{v}$ and λ have to be eigenvectors and egenvalues of the system-matrix.

Thus the transport equation, within the KM approximation, is readily solved.

The most severe problem with the KM theory is the description of phenomenological coefficients, A and S, in terms of K_a and K_s, i.e. in terms of absorption and scattering (attenuation) coefficients.

Within some approximations, it can be deduced (not repeated here) that

$$A = 2K_a$$
$$S = K_s$$

Therefor, the KM-theory can be solved to predict the diffuse fluxes J_d.

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KM-theory is a special case of a more general theory known as <u>many flux theory</u>. Here more than just forward and backward propagating fluxes are considered, and the transport equation is converted into matrix DE by considering the radiance at many discrete angles.

C Diffusion Approximation

For albedos $a \gg 1/2$, i.e. if scattering overwhelms absorption, the diffusive part of

$$I(\vec{r}) = \int d\Omega \; J(\vec{r}, \vec{s})$$

tends to be almost isotropic.

According to Ishimaru (1989), one may expand the diffuse radiance J_d in a series by

$$J_d = \frac{1}{4\pi} \left(I_d + 3 \vec{F}_d \cdot \vec{s} + \ldots \right)$$

where I_d is the diffuse intensity, and vector flux $\vec{F}_d$ is determined by

$$\vec{F}_d(\vec{r}) = \int_{4\pi} d\Omega \; J_d(\vec{r}, \vec{s}) \, \vec{s} .$$

The first two terms of the above expansion of J_d constitute the diffusion approximation. This approximation is valid if $a \gg 1/2$, i.e. the medium is strongly scattering.

Moreover, it can be shown that the diffuse intensity I_d itself satisfy the <u>diffusion equation</u>:

$$(\nabla^2 - \varkappa^2)\, I_d(\vec{r}) = -Q(\vec{r})$$

where $\varkappa^2$ is the diffusion parameter, and Q represents the source of scattered photons.

This equation suggests that for diffuse light one can introduce an effective diffusion lenght, ℓ_{eff}, and a corresponding effective attenuation coefficient

$$\alpha_{eff} = \frac{1}{\ell_{eff}}.$$

Within the diffusion approximation one thus has for the (coherent and diffuse) intensity

$$I = I_c + I_d$$

$$= C_1 e^{-\alpha z} + C_2 e^{-\alpha_{eff} z}$$

where

$$C_1 + C_2 = I_0.$$

D Monte Carlo Simulations

A numerical approach to solving the radiative transport equation:

$$\frac{dJ(\vec{r},\vec{s})}{ds} = -\alpha J(\vec{r},\vec{s}) + \frac{\alpha_s}{4\pi}\int d\Omega' p(\vec{s},\vec{s}') J(\vec{r},\vec{s}')$$

is based on what is called Monte Carlo (MC) simulations. MC simulations are <u>statistial</u> approaches to the above transport equation.

The MC method traces a large number of individual photons through the scattering medium. These photons undergo random walks due to scattering (and can be absorbed with given probability).

The goal of the MC method, is to calculate statistical averages of the photon fluxes in the various directions leaving the system.

Since the accuracy of results based on statistics is proportional to $1/\sqrt{N}$, where N is the number of photons used to calculate the averages, a large number of photons has to be taken into account to give accurate results.

Therefor, the MC method is rather time consuming. However, it can easily be adapted to take advantage of todays powerful parallell computers which results in much lower execution time.

The MC method was first propose back in the late 1940's by Metropolis and Ulam (1949) Today it has advanced to a very powerful tool which is quite general and hence used in many disciplines.

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The principle idea of MC simulations applied to tissue optics is to follow the optical paths of individual photons as they propagate through the tissue.

The distance between two (consequtive) collisions is drawn from log-arithmic distribution (generated in the computer) Absorption is implemented by associating a weight with each photon and permanently reducing this weight during propagation. If scattering is to occure, a new direction of propagation is chosen according to a given phase function and another random number (i.e. a new random direction).

The main steps of MC simulations for scattering and absorptions are :

- source photon generation.
- pathway generation
- absorption
- elimination
- detection

We will now briefly comment on each of these points

① Source photon generation

Source photons are generated at the surface of the considered medium, and with a spatial and angular distribution reflecting the light source used (e.g. a Gaussian laser beam)

② Path-way generation

After generating a photon, the distance to the first collision is determined. Absorbing and scattering particles are assumed to be randomly distributed in the medium. Mean free path is $1/\rho\sigma_s$ where ρ is the particle density, and σ_s the scattering cross section.

③ Absorption

Absorption is taken into account by a photon weight factor. On generation each photon is associated with weight 1. As the photon propagates, its weight is reduced towards 0. (by a factor $\exp(-\kappa_t \ell)$ where ℓ is the distance between the the last collision)

④ Elimination

This is the mechanism for removing photons from the simulation. If the present value of the weight of a photon is less than a cut-off value, say W_c, then the photon is removed, or eliminated.

⑤ Detection

After repeated steps 1-4 for a sufficiently large number of photons, a map of pathways is calculated and available in the computer.

Hence, statistical statements about angular distributions and fraction of absorbed photons can be made for those of them escaping from the considered medium.

5.3.2 Comments

This concludes our brief discussion of tissue optics. Even though we did not have time, nor the intension, to give a thorough discussion of the topic, I still hope that you have obtained a "feel" of the issue and its challenges.

Notice in particular that many of the issues discussed in this class on transport theory, it being stochastic theory or diffusion, enters naturally into the theory on tissue optics.