Universität Leipzig

Fakultät für Physik und Geowissenschaften

Institute for Experimental Physics I

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Biophysics Lab Course

Film Balance: Energy Transfer in Thin Layers (FB)

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1 Introduction

Using a technique developed by Langmuir and Blodgett, units of molecular size can be built. Hereby, one produces a monomolecular layer on a water surface and transfers this to a solid substrate. In this way, multilayers can be built, which can consist of different substances. So, one can specifically influence the physical properties of such multi-layer system.

In this experiment the distance dependence of the Förster-Perrin's energy transfer will be studied from a donor to an acceptor molecule. Thus, a layer system will be made consisting of cyanine dyes and fatty acids. The fluorescence intensity will be measured depending on different intervals of the acceptor, whereby the distance is varied by the number of fatty acid layers.

In this manual, the theoretical basis of the Förster -Perrin's energy transfer, the film balance technique and the preparation of Langmuir Blodgett (LB) multi-layers is described, as well as the exact task and the practical realization of the experiment.

Get your own thoughts on the following key points:

- energy transfer: dipole moment, transition dipole, absorption/fluorescence spectroscopy, Einstein coefficients, Jablonski term scheme, Frank-Condon factors etc.

- LB: surface tension, amphiphiles, p-V-diagram (isotherms), two-dimensional gas, etc.

2 Basics

2.1 Monolayers on water surface

Molecules composed of a hydrophilic part (usually a charged or polar headgroup) and a hydrophobic part (one or more aliphatic hydrocarbons fuel chains) are referred as amphiphiles. For sufficiently large chain length (more than 14 carbon atoms), the water solubility of such substances is negligible. Solving such molecules in a volatile solvent (e.g. chloroform) and drop this liquid on a water surface, the

amphiphiles spontaneously form a monolayer. This monolayer reduces the surface tension γ_0 of water (at room temperature $\gamma_0 \sim 72.4$ mNm^{-1}). The amount of this reduction is not proportional to the surface density of the molecules, but it is depend on the phase state of the layer. The difference in surface tension γ of the film-covered surface to the surface tension γ_0 of pure water versus air is as lateral pressure:

$$\pi = \gamma_0 - \gamma \tag{1}$$

Plotting at constant temperature the lateral pressure versus the molecular area A, one obtain an isotherm similar to the pV-diagrams of three-dimensional systems.
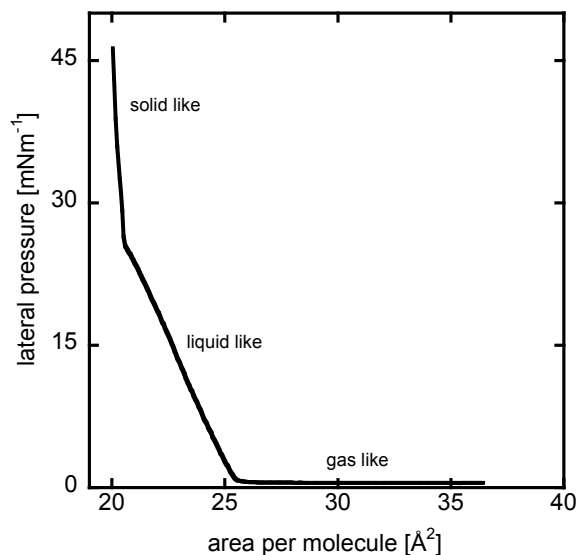

Figure 1: Schematic π/A isotherm of a monolayer

The regions with different slopes of the isotherms can assign different phase states. Thus, one can differentiate between a gas analog, liquid analog and a solid analog phase (see fig. 1). Kinks and regions with zero slope of the isotherm account for a discontinuity or divergence of the compressibility and thus are an indication of a phase transition in the monolayer. The compressibility is defined as:

$$\kappa = -\frac{1}{A}\frac{\delta A}{\delta \pi} \tag{2}$$

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2.2 The film balance

A film balance consists essentially of a Teflon trough, moving Teflon barriers, employed to change the size of the surface, and a measuring system for the lateral pressure (fig. 2). Usually, the lateral pressure is measured by a Wilhelmy system. Thereby, the force is measured with a leaf of filter paper, which is easily immersed in the subphase. This force is proportional to the surface tension. The required counterforce is generated by a spring (previously, the counterforce were generated by weights on a balance beam, hence the name "film balance").
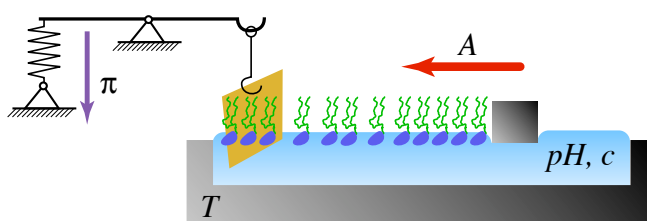

Figure 2: Sketch of a film balance with a Wilhelmy pressure measurement system

2.3 Assembly of multi-layer systems with the LB technique

Here, a solid substrate (e.g. a cleaned glass slide) is slowly submerged through a monolayer of amphiphile molecules, located on a water surface, in vertical direction (see fig. 3).

By repeated in and out dipping of the substrate, any numbers of monolayers can be overlapped (at least in principle). The procedure is schematically illustrated in fig. 4).

It should be noted, systems in which the last layer is hydrophilic are not stable - they must always be kept under water. By transferring the layer on the substrate a loss of substance occurs, which can be compensated by a reduction of the surface by means of the barriers. This is achieved by a constant system pressure. The transfer of amphiphiles can be quantified due to the relation between the decreasing area and the area of the substrate.
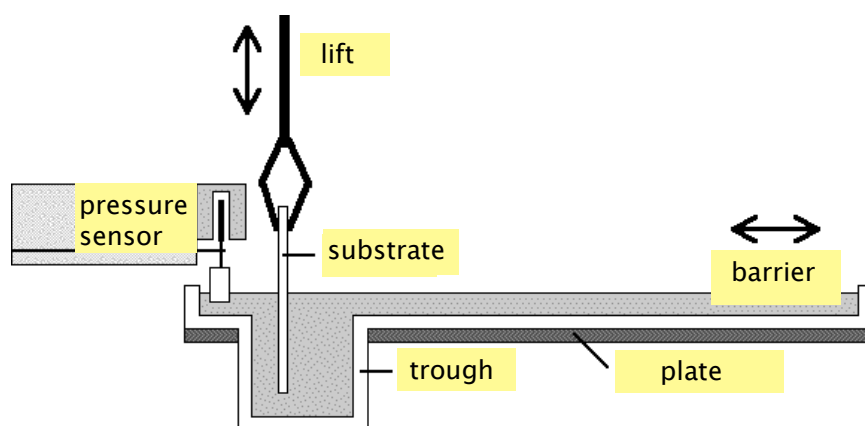
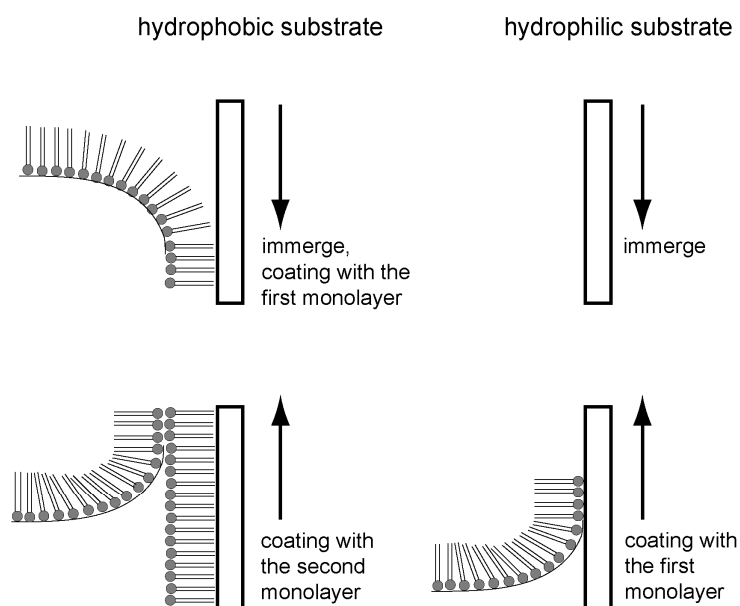**Figure 3:** Scheme of a film balance for preparation of LB multi-layers

hydrophobic substrate   hydrophilic substrate

immerge,
coating with the
first monolayer

immerge

coating with
the second
monolayer

coating with
the first
monolayer

Figure 4: Development of multi-layers by LB technique

2.4 The Förster-Perrin's energy transfer

We consider a system of two fluorescent dye molecules, whereby the emission band of the donor and the absorption band of the acceptor overlap. Exciting this system with light with the wavelength of the absorption maximum of the donor, at large distance of the acceptor, only the fluorescence of the donor can be observed (i.e. much larger than 100 Å). If we reduce this distance, the emission of the donor decreases and the acceptor fluorescence appears. At very short distance, only the

acceptor emission can be observed and the donor is completely extinguished. Obviously, the excitation energy is passed from the donor to the acceptor. This process is commonly referred as energy transfer or Förster energy transfer.
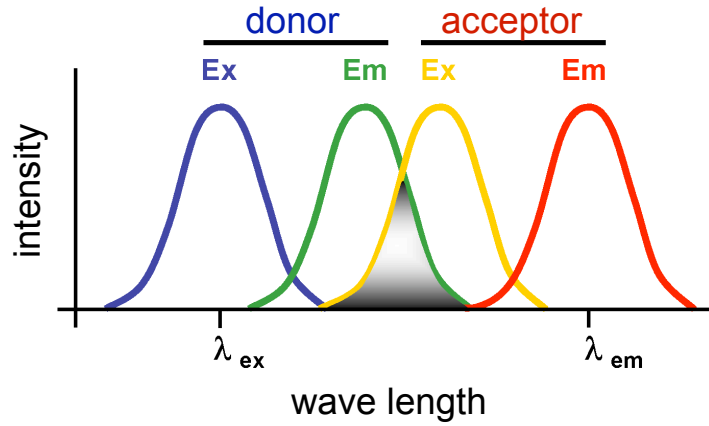

Figure 5: Schematic spectra of a donor-acceptor pair

This energy transfer is nonradiative and classically explained by the interaction of two dipoles. Quantum-mechanically, this energy transfer follows from the weak coupling of two nearly degenerate systems. It is characteristic for the weak coupling, that the adsorption spectra of the molecules are not or very weakly changed due to this interaction.

We consider the following energy diagram for the quantum mechanical derivation of the transfer rate by Förster: If the donor molecule exited by light of frequency ν', it passes very quickly to the vibrational ground state of the first excited electronic state $\mid D_b >$ (tortuous arrow in fig. 6). The emission energy of D and the absorption energy of A coincide, then by the weak coupling the following response occurs:

$$k_T$$

$$\mid D_b A_a > \Longleftrightarrow \mid D_a A_b > \tag{3}$$

$$k_{-T}$$

Thus, we have the excited state of molecule (b) and the ground state of molecule (a) integrated in a total wave function. However, there are both the state $\mid D_a >$ and $\mid A_b >$ in an excited vibrational state, so that the fast relaxation in the vibrational
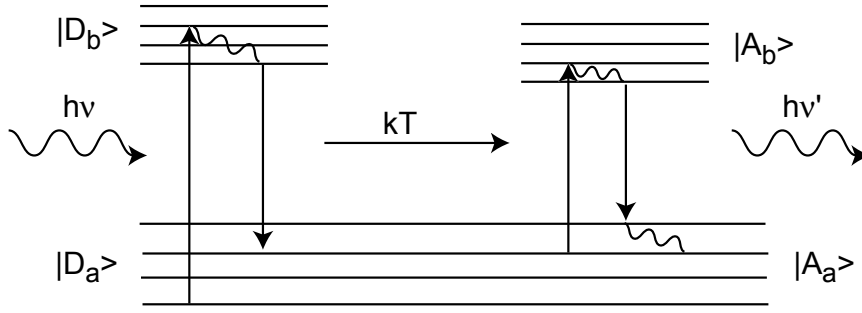
Figure 6: Energy diagram for Förster transfer

ground state of the back process k_T is practically impossible, even is k_T very effective.

The transfer rate k_T can be calculated employing Fermi's "golden rule":

$$k_T = \frac{1}{\hbar^2} \int_0^\infty |< D_b A_a \mid V \mid D_a A_b >|^2 \, \rho_D(\nu)\rho_A(\nu)d\nu \qquad (4)$$

The term $\rho_D(\nu)\rho_A(\nu)$ denotes the number of energy states in the interval ν, $\nu + d\nu$ and includes the Frank-Condon factors and the occupation probability of the vibrational states. V describes the interaction. Thus, we only consider the dipole term and get:

$$V = \frac{\mu_D \mu_A}{R_{AD}^3} - 3\,\frac{(\mu_D R_{AD})(\mu_A R_{AD})}{R_{AD}^5} = \kappa \frac{\mid \mu_D \mid\mid \mu_A \mid}{R_{AD}^3} \qquad (5)$$

and

$$\kappa = cos(\angle(\mu_D, \mu_A)) - 3\,cos(\angle(\mu_D, R_{AD}))cos(\angle(\mu_A, R_{AD})). \qquad (6)$$

$\mu = \sum_{i=1}^N q_i r_i$ refers to the respective dipole moment and R_{AD} the distance between donor and acceptor.

Since μ_D is only depend on the electron coordinates of the donor and μ_A on those of the acceptor, equ. 6 can be written as:

$$k_T = \frac{\kappa^2}{R_{AD}^6}\frac{1}{\hbar^2} \int_0^\infty |< D_a \mid \mu_D \mid D_b >|^2 |< A_b \mid \mu_A \mid A_a >|^2 \, \rho_D(\nu)\rho_A(\nu)d\nu \quad (7)$$

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The expressions $|<D_a \mid \mu_D \mid D_b>|^2$ and $|<A_b \mid \mu_A \mid A_a>|^2$ are transition dipole moments and can be calculated from the Einstein coefficients. In particular, in case of the acceptor there is stimulated absorption:

$$B_{ab} = \frac{2\pi}{3\hbar^2} |<A_b \mid \mu_A \mid A_a>|^2 \tag{8}$$

This coefficient can be expressed by the decadal extinction coefficient in the absorption spectrum:

$$B_{ab} = \frac{ln10 \ c \ \epsilon(\nu)}{h\nu \ L'} \tag{9}$$

where c is the speed of light in the solvent and L' the number of molecules in mmol. This results in:

$$|<A_b \mid \mu_A \mid A_a>|^2 = \frac{3 \ ln10 \ c \ \hbar}{4\pi^2 \ L'} \frac{\epsilon(\nu)}{\nu} \tag{10}$$

Similarly, the relation for the spontaneous emission (=fluorescence) of the donor can be derived. It generally applies for this Einstein coefficient:

$$A_{ba} = \frac{8\pi \ h\nu^3}{c^3} B_{ab} = \frac{32\pi^2}{3c^3\hbar}\nu^3 \ |<D_a \mid \mu_D \mid D_b>|^2 \tag{11}$$

Furthermore, it yields:

$$A_{ba} = \frac{1}{\tau_D} \tag{12}$$

whereby τ_D characterizes the natural life time of the state $\mid D_b >$. So we obtain:

$$|<D_a \mid \mu_D \mid D_b>|^2 = \frac{3c^3\hbar}{32\pi^3 \ \tau_D} \frac{1}{\nu^3} \tag{13}$$

The transfer rate finally yields from equ. 10 and 13:

$$k_T = \frac{\kappa^2}{R_{AD}^6} \frac{1}{\tau_D} \frac{9 \ ln10 \ c^4}{128\pi^5 \ L' \ n^4} \int_0^\infty \frac{\epsilon(\nu)f(\nu)}{\nu^4}d\nu \tag{14}$$

Thus, we have introduced the refractive index n of the surrounding medium and we have considered the density of states $\rho_D(\nu)$ and $\rho_A(\nu)$ in $\epsilon(\nu)$. $f(\nu)$ is the

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normalized probability for the fluorescence emission in the interval $\nu, \nu + d\nu$, i.e. it yields:

$$\int_0^\infty f(\nu)d\nu = 1 \tag{15}$$

The transfer rate is proportional to the overlap of the emission spectrum of D with the absorption spectra, weighted by $1/\nu^4$ and is vice versa proportional to the sixth power of the distance. By the way, the equation does not include Planck's constant and can be derived purely classical, too (see Försterling/Kuhn). In abbreviated form it usually writes:

$$k_T = \frac{1}{\tau_D} \left(\frac{R_0}{R_{AD}} \right)^6 \tag{16}$$

R_0 is called the characteristic transfer radius which is for the most systems in a range of 10 - 100 Å.

2.5 Energy transfer in a system of monolayers

So far, we have only investigated the energy transfer from a donor to an acceptor. In the following, we want to study a flat monolayer of donor molecules opposite to an acceptor layer with the distance d. In this case, the energy of the donor passes to many acceptors and the whole transfer rate is given by:

$$K_T = \sum_{i=1}^\infty k_T(i) = C \sum_{i=1}^\infty \frac{\kappa_i^2}{R_{A_i D}} \tag{17}$$

Here, C contains all constants and the integral of equ.14, i numbers the acceptor molecules.

Assuming the acceptor molecules are homogeneously distributed in the layer, so we can introduce the surface density σ and replace the sum by an integral over the entire surface:

$$K_T = C\sigma \int \frac{\kappa^2}{r^6} df \tag{18}$$

For the calculation of κ we have to know how the dipole moments of molecules are orientated in the layer. In our experiment, the mixture of cyanine dyes with arachidic acid has a ration of 1:10 and we can make the following assumption:

- all transition dipole moments are in plane, i.e. perpendicular to the surface normal

- location and direction of the individual transition dipole moments in the plane are uniformly distributed

With these assumptions, we have for a layer spacing d:

$$K_T = \frac{3\pi}{16} \, C \, \sigma \, \frac{1}{d^4} \tag{19}$$

An excited donor molecule can be deactivated by fluorescence emission (k_F), nonradiative processes (k_S) or energy transfer (k_T). The decay rate without energy transfer is $k = k_F + k_S$ and the corresponding lifetime is then $\tau = 1/(k_F + k_S)$. Denoting I_∞ as the fluorescence intensity of D in case there is no acceptor present and I_d at acceptor presence at distance d, we obtain the ratio of both from the ratio of the transfer rates to:

$$\frac{I_d}{I_\infty} = \frac{k_F + k_S}{k_F + k_S + k_T} = \frac{1}{1 + \tau K_T} \tag{20}$$

Now we define a critical distance d_0 at which the energy transfer and the fluorescence are equal probable, $1/\tau dt = k_T dt \Rightarrow \tau k_T = 1$:

$$\tau = \frac{16}{3\pi\sigma C} \, d_0^{\,4} \tag{21}$$

Finally, we obtain:

$$\frac{I_d}{I_\infty} = \frac{1}{1 + \left(\frac{d_0}{d}\right)^4} \tag{22}$$

According to this formula, the critical distance d_0 can easily be experimentally determined by measuring the fluorescence emission of the donor at different layer distances of the acceptor.

An exact calculation of the distance d_0 is theoretically possible, but it is very complex and contains many parameters which are experimentally difficult to access. It counts:

$$d_0{}^4 = \frac{3\pi}{16} \frac{9 \, ln10 \, c^4}{128\pi^5 \, L' \, n^4} \frac{\tau_S}{\tau_d} \sigma \int_0^\infty \frac{\epsilon(\nu)f(\nu)}{\nu^4} \, d\nu \qquad (23)$$

2.6 Tasks

1. measure an isotherm of arachidic acid on pure Millipore water; determine the phase transition pressure and space requirement per molecule at a lateral pressure about 30 mNm^{-1}; discuss the error sources (area per molecule by x-ray 19.8 Å^2); additionally, calculate the weight and the volume of the spreaded arachidic acid monolayer

2. determine from at least one of the obtained isotherms the transfer ratio in the LB-transfer; estimate how much the average molecular surface (closely packed) increases in the mixture dye/arachidic acid versus pure arachidic acid and compare with the experimentally obtained value

3. make a substrate with a multi-layer system for the energy transfer measurement; with the data from the fluorescence spectrometer one obtain the fluorescence intensity of the donor; relate the intensities to the layers and calculate the relation I_d/I_∞

4. provide from other groups more I_d/I_∞ values for other d values; from this, calculate the Förster distance d_0 by the diagram $log((I_\infty/I_d) - 1)$ versus $log(d)$; which value results from the slope of the line and what you expect for this purpose from the theory;

   enter the values I_d/I_∞ versus d and sketch the theoretical curve progression; for the determination of d_M use the molecule length, calculated by Tanford; briefly illustrate, why the disappearance of the donor emission cannot be explained due to re-absorption by the acceptor

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3 Practical procedure

3.1 Substances

The dye molecules, used for the energy transfer, are depicted in fig. 7. These carbocyanines are amphiphilic due to their octadecyl chains and form spontaneously monolayers. In order to avoid the aggregation of the dye molecules, they have to be mixed with a fatty acid (arachidic acid) in a molar ratio of 1:10. Both dyes are solved in chloroform in a concentration of 10^{-4}M, the concentration of the arachidic acid is 10^{-3}M.
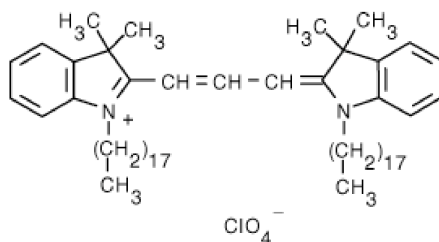
Donor:
DiOC$_{18}$(3)3,3'-dioctadecyloxacarbocyanine perchlorat
(DiO); M=881 gmol^{-1}
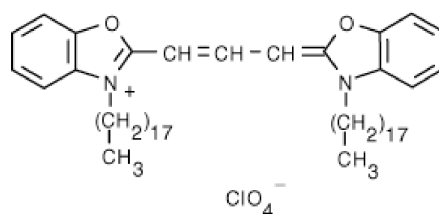
Acceptor:
DiIC$_{18}$(3)1,1'dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine
perchlorate (DiI); M=934 gmol^{-1}

fatty acid:
arachidic acid; CH$_3$-(CH$_2$)$_n$-COOH; n=18; M=312 gmol^{-1}

Figure 7: Used substances

As subphase we will use pure water, cleaned in a Millipore unit. After the cleaning, the water is free of ions and biological active substances and has a specific electrical resistance of 18.2 MΩcm.

3.2 Spreading the film-forming substances

The trough is filled with subphase (Millipore water), one to two millimeters above the edge. Due to the hydrophobic property of Teflon, the water forms a meniscus.

For spreading, the dissolved substance is pulled into a μl-syringe and slowly dropped onto the water surface. The solvent evaporate and a monolayer remains. Since a monolayer requires a very small amount of substance (10^{16} molecules), utmost cleanliness is necessary!

For cleaning the syringe and avoiding a dilution of the sample, the following procedure is recommended:

- rinse the syringe with chloroform, at least 3x

- rinse the syringe with the sample, 1x

- pull the sample into the syringe

- spreading

- rinse the syringe with chloroform, at least 3x

The volume of the sample, which has to be spreaded, can be calculated from the molecular data (fig. 7), the concentration of the solution and the trough area. Usually, one spreads a volume that the most close molecular packing is reached at approximately the half trough area (area of arachidic acid is about $20\mathring{A}$).

3.3 Transferring films of solid substrate

The transfer will carry out with the NIMA alternate layer Langmuir Blodgett trough, as solid substrate we use glass slides. Before using, the glass slides have to be cleaned thoroughly. First the glass slides are wiped with acetone and then cleaned with the RCA method ($H_2O:H_2O_2:NH_3$=5:1:1, at 70°C for 15 minutes). Thereafter, the glass slides are hydrophile and can be stored under water.

The glass slides are attached on the dipper (do not touch with hands, use tweezers and gloves, only touch the upper part) and lowered. Following, the monolayer

is prepared and will be compressed to a lateral pressure of 30 mNm^{-1}. At this pressure the monolayer is in the condensed phase, the molecules are in a most close packing. The transfer will be carried out at this pressure. At the transfer we have a loss of substance in the monolayer. This will be compensated by the constant pressure mode, which can be realized by a reduction of the surface by means of the barriers. Consequently, the barriers should continuously decrease the surface during the transfer. If the barriers stop or open again, no transfer happens or even the layer peels away. In this case, the slides are not clean and have to be cleaned again. For the preparation of multi-layers, the monolayer is changed several times, during which the substrate must keep under water. Here is an important source of error for the energy transfer measurements. If the previous layer is not completely removed, the following layer will be transferred with the remains of the previous layer. Therefore, the entire monolayer has to be very carefully sucked up, except the part below the dipper.

3.4   Buildup of the multi-layer system

For measuring the energy transfer, two samples are prepared as shown in fig. 8. The fluorescence intensity of the donor (I_∞) and whose at presence of the acceptor in distance d (I_d) will be measured.

Here, the preparation of the layer system is described:

- coating both glass slides with 3 layers of arachidic acid

- aspirating the monolayer, rinsing with water, spreading the arachidic acid/dye mixture (donor), dipping the glass slides

- aspirating the monolayer, rinsing with water, spreading the arachidic acid, pulling out the glass slides, take one of the slides

- the remaining slide n-fold dipping and pulling out (it gives n=1, 2, 3 ... double layer)

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- aspirating the monolayer, rinsing with water, spreading the arachidic acid/dye mixture (acceptor), pull out the glass slides

- aspirating the monolayer, rinsing with water, spreading the arachidic acid, coating two layers as protecting layer
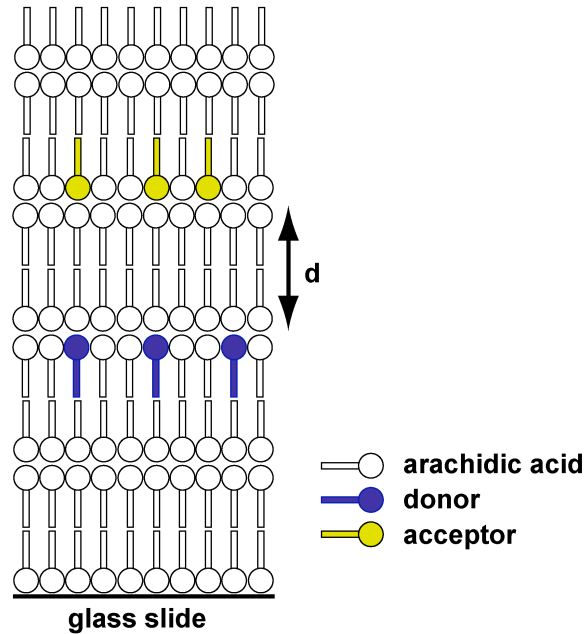

Figure 8: Multi-layer system

3.5 Fluorescence measurements

The coated glass substrates are measured with the FS9000CDT steady-state fluorescence spectrometer from Edinburgh Instruments (schematic overview fig. 9). The excitation source is a xenon high pressure lamp which has to be cooled during operation. Excitation and emission are realized via grating monochromators and the emission signal is detected with a photomultiplier. Further explanation to the spectrometer is given in the manual.
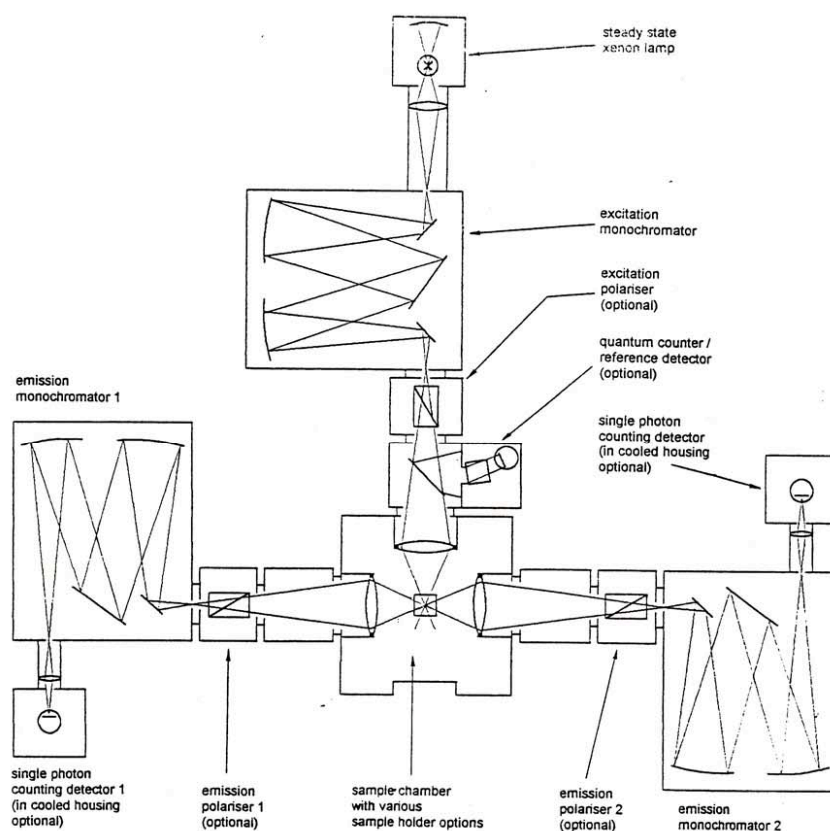
Figure 9: Optical layout of the spectrometer FS900CDT

3.6 Experimental parameters

Film balance

- maximum position

- minimum position

- width

Substances

- arachidic acid, c = 0.78 mg / ml

- Donor DiO, c = 0.99 mg / ml

- Acceptor DiI, c = 0.92 mg / ml

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