Chapter

Special Cuvettes for Spectrofluorimeters

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Abstract

Special cuvettes that dramatically increase the fluorescence intensity of diluted solutions and suspensions of isolated biomembranes and living cells are described. (1) Mirror cuvettes have a reflecting aluminum or silver layer, putting on their three external sides, having a slot-hole window at frontal side center. (2) Mirror microcuvettes have two reflecting sides. (3) Total internal reflection cuvettes have lateral reflecting side and two triangular prisms, attached outside; the cuvette works effectively with a parallel excitation light beam. (4) In cuvette with diagonal quartz plate, very small quantity of a sample is attached in the form of a thin layer. (5) Mirror cuvette with diagonal plate. All described cuvettes can be applied for measurements of spectra excitation and emission, excited-state lifetime, and polarization degree. Also, they can be used for photo-bleaching of biological chromophores.

Keywords: cuvettes, fluorescence, spectroscopy, excitation, emission, lifetime, polarization degree, photo-bleaching

1. Introduction

Fluorescence spectroscopy is one of the most high-sensitive methods, which allow to detect very low concentrations of substances (μM, nM, and even pM) and to distinguish one substance from another [1–5]. Fluorescence spectroscopy is used for studying the functioning of living cells [6–8]. It is intended also for detecting various dyes and substances in cells and membranes [6–9].

Fluorescence spectroscopy gives valuable information on spatial organization and behavior of biological structures and about appearance of intermolecular complexes, for example, DNA with proteins, enzymes with substrates, etc. It is applied in studies of conformation properties, mobility, and other properties of macromolecules, biological membranes, and living cells [6–9].

It is an extremely informative method, since it allows us to receive the data on the distances, orientations, and intermolecular interactions. An advantage of fluorescent spectroscopy is the absence of damage of a sample in the course of research. Also, it is possible to work with native biological preparations.

The measured intensity of fluorescence of a biological sample is determined by its optical density, lifetime of the excited state, fluorescence quantum yield, and effective collection of emission beam.

Fluorescence spectra can be detected by special techniques—spectrofluorimeters. In such devices, a scanning of excitation wavelength by the first monochromator (placed before a cuvette with a testified sample) leads to changes in the intensity of fluorescence according to the form of the absorption spectrum; that is, form and

position of the excitation spectrum should coincide with the absorption spectrum. At fixed excitation wavelength, a scanning of the second monochromator (placed after a cuvette with a sample) gives the emission spectrum (named also as spectrum of fluorescence or emission).

Various fluorescence techniques are widely used to increase the sensitivity of the method [1–6]. One of the approaches to enhance the sensitivity of fluorescence analysis is the increase of the length of optical path of an exciting light in a sample. Usually, it is reached by consecutive reflections between concave mirrors, located in a common spectrofluorimeter near the cuvette [5] filled with a studied solution or suspension. However, only two passes of stimulating light in such systems take place, and also losses of light at media borders exist. Therefore, the gain (amplification factor) G is <3. Besides, parasitic reflections at borders and mirrors lead to penetration of exciting light into registration channel that leads to impossibility of correct measurements of weak fluorescence.

That is why special cuvettes which dramatically increase the fluorescence intensity of diluted solutions and suspensions of isolated biomembranes or living cells were invented [7, 8]. They will be described below.

2. Methods and results

2.1 Multipass cuvettes

To overcome the mentioned specified difficulties, the multipass cuvettes (**Figure 1**), allowing to raise G up to 4–10 times and to remove parasitic reflections, can be used [7, 8]. Such cuvettes allow to lower the concentration of substances and to receive an intensive fluorescence signal.

Mirror cuvettes are intended for measuring the fluorescence of weakly absorbing solutions and can be used to register the excitation and emission spectra in UV and visible region and lifetimes. The cuvettes provide for many-fold increase of fluorescence intensity due to multiple passage of exciting light through the solution being tested and due to additional fluorescence collected.

The stimulating light in multipass cuvettes is almost wholly absorbed by a solution or suspension, and, thus, there are almost no parasitic reflections at cuvette sides (as against usual cuvettes with external concave mirrors, all reflections here are useful). Penetration of stimulating light into registration channel, therefore, sharply decreases. It is especially essential at low intensities of emission

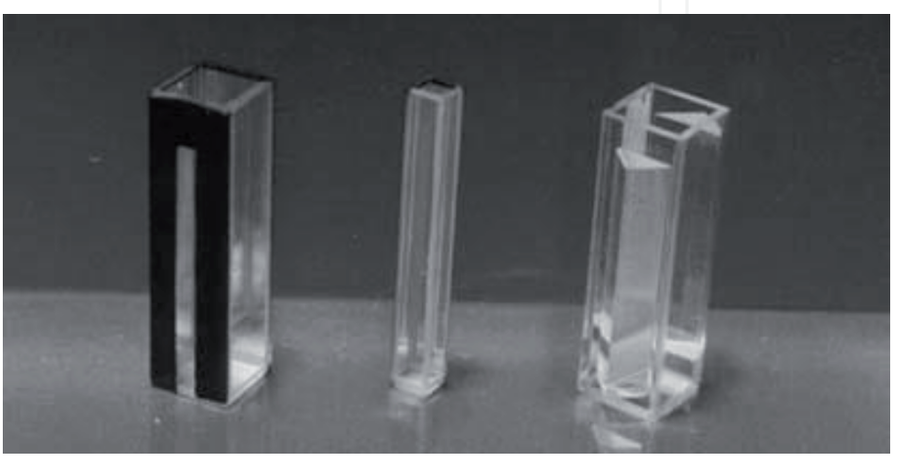

Figure 1.
Mirror cuvette, mirror microcuvette, and TIR cuvette.

of light-scattered suspensions of cells or membranes. Amplification of fluorescence intensity from a sample in multipass cuvettes allows also to raise the accuracy of measurements of fluorescence polarization degree and lifetime of excited state.

Mirror cuvettes represent themselves as a quartz 1-cm cell with a reflecting aluminum or silver layer, putting on their three external sides (outside protection is enamel), having a slot-hole window at frontal side center—for entrance of stimulating light (**Figure 2**). Mirror cuvettes may be applied in serial spectrofluorimeters without changes in their design. The cuvette can be used for recording emission or excitation spectra and for determining the excited-state lifetime of weakly absorbing solutions and suspensions.

Mirror microcuvettes are a quartz 0.4-cm cuvette with a reflecting layer, putting on their two external sides. This cuvette allows work with small volumes (0.15–0.2 ml) of solutions or suspensions. Mirror microcuvettes may be applied in spectrofluorimeters, using a special holder, which has a standard external size of 12.5 × 12.5 × 40 mm.

Total internal reflection (TIR) cuvettes contain a lateral reflecting side and two triangular prisms elements, attached outside to two transparent sides (**Figure 2**). A parallel excited light beam enters sideways in frontal side and, passing through solution (or diluted suspension) and back prism, undergoes total internal reflections at quartz/air borders and comes back, making 3–5 times passes through solution or suspension, having very low optical density (as rule, not more 0.01). The TIR cuvette works effectively only with a parallel excitation light beam, especially at laser excitation. It requires application a special holder, displacing the cuvette relating to exciting beam to the best position.

The gain (G) of fluorescence intensity in multipass cuvettes (in comparison with standard single-pass cuvettes) is determined by the expression:

$$G = \left(1 + \rho\, T + \rho^2\, T^2 + \ldots \rho^n\, T^n\right)\,(1 + \rho). \tag{1}$$

Here ρ is the reflection factor of a mirror or prism, T is the light transmission of a solution or suspension at the excitation band at one beam pass, and n is the number of passes. The first member of the equation describes amplification of fluorescence intensity due to repeated passage of stimulating light, and the second one takes into account additional collection of emission due to the lateral reflecting wall. The more T (i.e., the less optical density), the bigger G, aspiring, however, to

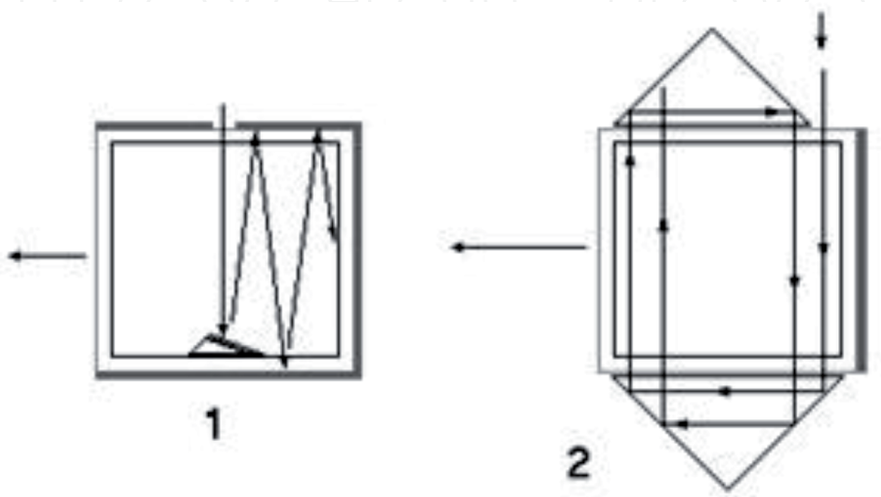

Figure 2.
Scheme of Mirror cuvette (1) and TIR cuvette (2); top view. Arrows show the direction of light beams. Mirror layers are shaded.

some limit. Really, the *G* value for the 1-cm mirror cuvette with aluminum covering reaches 4–7 (at UV region) and with silver covering reaches 6–9 (at visible region). For TIR cuvette, *G* reaches 10, if excitation is done by laser beam.

2.2 Spectral measurements

Emission spectra of 10-μM solution of ANS dye in ethanol in standard and mirror cuvettes are given in **Figure 3**. The intensity of fluorescence in mirror cuvettes is sometimes higher than that in the standard one. The form of the emission spectrum does not change.

Table 1 summarizes the calculated and experimental gain G, obtained for various samples (diluted solutions and suspensions) in different cuvettes.

2.3 Excited-state lifetime measurements

If an object is excited by short-time light flare, which has a duration less than the time of emission transition of molecules from S_1 into S_0, it is possible, using a stroboscopic multichannel detector or phase detector, to register the decay emission kinetics in nanosecond timescale.

In the presence of only one kind of radiating molecules, decay is described as [1–6]:

$$dN\,(t) = -A\,N\,(t)\,dt. \tag{2}$$

Here dN is the loss of the number of excited molecules during time t, A is Einstein's factor for spontaneous transitions from S_1 to S_0, and N is the number of molecules at S_1 level. The integration gives:

$$N\,(t) = -N\,exp\,(-A\,t). \tag{3}$$

Decay has exponential character. The decrease of decay intensity in *exp* times (~ in 2.7 times) is named as *lifetime* (τ) of the excited state. The lifetime is a quantitative characteristic of the average duration of existence of molecules in the excited state. For instance, perylene, consisting of five condensed aromatic rings, has τ ~ 5 ns, and pyrene, consisting of the same four aromatic rings, has τ more than 300 ns. Molecules of one kind in different conditions have various lifetimes. For example, NADH in water has τ = 0.5 ns, but in the mitochondrial NADH dehydrogenase 2 ns [9]. Generally speaking, τ does not depend on concentration of fluorescent molecules, if they do not form own aggregates.

Since the fluorescence intensity of a sample in multipass cuvette strongly increases, the excited-state lifetime measurements become more accurate.

Figure 4 demonstrates the lifetime distributions of tryptophan fluorescence of cod parvalbumin solution measured in a standard cuvette and in a multipass one. Cod parvalbumin was dissolved in a 25 mM of Tris-HCl and 1 mM of $CaCl_2$ with pH of 8.2; the absorbance at 295 nm was less than 0.1; excitation wavelength was 295 nm, and emission was detected at 320 nm; and monochromator slits were 5 nm. The UV excitation was made with the Orsay synchrotron (France).

Lifetime distributions were measured through correlation fluorescence spectroscopy. The 0.03 ns band, obtained with standard cuvettes (**Figure 4**, left), is an artifact, caused by a big noise of small fluorescence signal and also by some penetration of the exciting light into registering channel. It is prevented if multipass cuvettes are used (**Figure 4**, right).

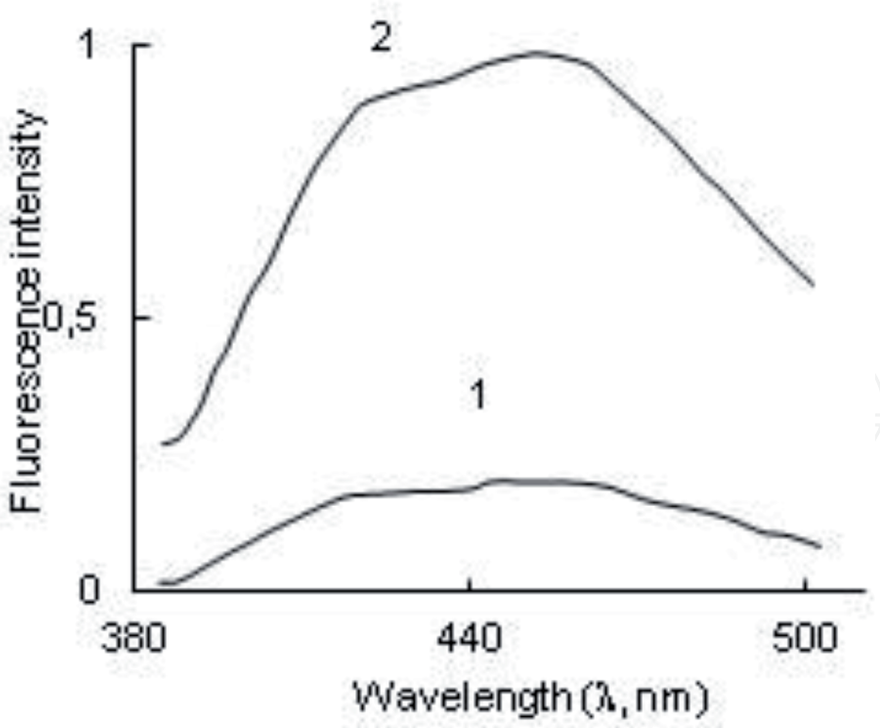

Figure 3.
Fluorescence spectrum of ANS (in ethanol) in standard cuvette (1) and in mirror cuvette (2). Excitation was 360 nm; slits were 5 nm. Spectrofluorimeter was "Perkin Elmer MPF-44B."

Cuvette	$G_{theor.}$	$G_{exper.}$
Standard 1-cm cuvette	1	1
Cuvette and two outer concave mirrors	3.3	3
Mirror cuvette with aluminum coating	9	6
Mirror cuvette with silver coating (excepting UV region)	17	8
Total internal reflection cuvette	20	10

Table 1.
Gain of fluorescence intensity, obtained with various cuvettes.

In the case of subnanosecond lifetimes, the measurements should be made using multipass cuvettes in all channels—for studied sample and for reference blank sample.

Table 2 presents the lifetime components of fluorescence of ribonuclease T_1, parvalbumin, and phospholipase A_2. Excitation was 295 nm (slits were 5 nm). RNAase T1 was dissolved in the 100 mM acetic buffer (pH 5.5). The calcium form of parvalbumin was used in the 25 mM Tris-HCl buffer with 1 mM $CaCl_2$ (pH 8.2). Pork phospholipase A_2 was in the 100 mM acetic buffer (pH 5.8) or in 90% glycerol. Detection of emission at 310 and 320 nm was done in mirror cuvettes to increase the intensity. Emission at other wavelengths was measured in standard 1-cm quartz cuvettes.

Multipass cuvettes are especially useful in phase modulation measurements of lifetimes, when, because of narrow slits of entrance monochromator and modulator, the intensity of exciting beam is too small. These cuvettes allow to raise the accuracy of phase modulation measurements. For example, a solution of ANS in

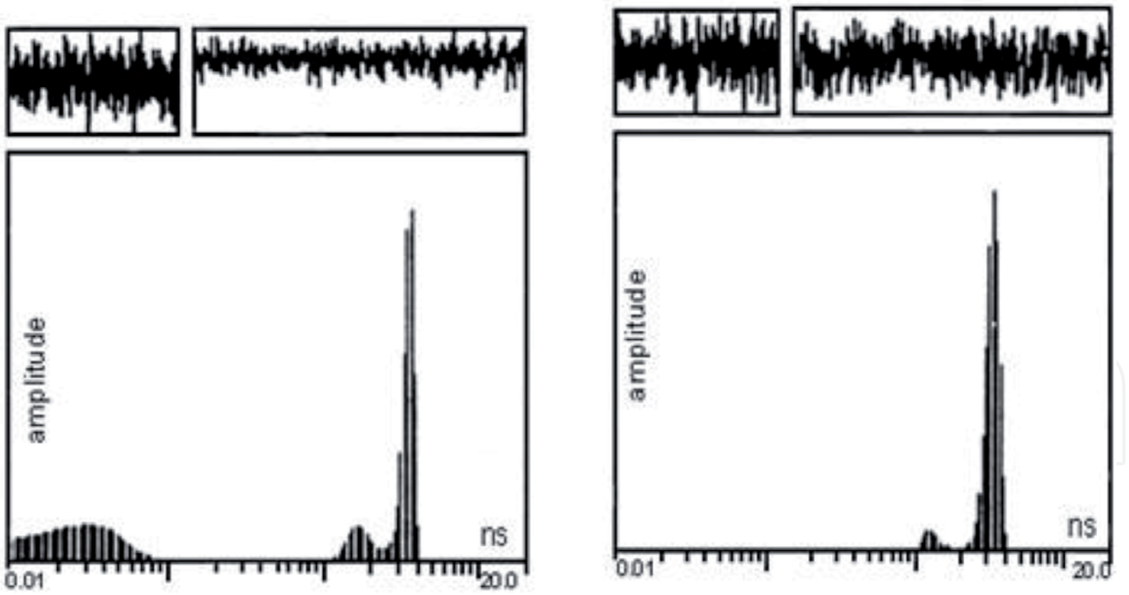

Figure 4.
Lifetime distributions of tryptophan fluorescence of cod parvalbumin in a standard 1-cm quartz cuvette (left figure) and in a multipass one (right figure).

Protein	λ_{em} (nm)	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	a_1	a_2	a_3	τ (ns)
Ribonuclease	310	—	—	4	—	—	1	4
	320	—	—	4	—	—	1	4
	340	—	—	4	—	—	1	4
	375	—	—	4	—	—	1	4
Parvalbumin	310	—	1.1	3.4	—	0.15	0.85	2.7
	320	—	1.3	3.3	—	0.07	0.93	3.2
	340	—	1.7	3.3	—	0.05	0.95	3.3
	375	—	—	3.4	—	—	1	3.4
Phospholipase	320	0.6	2.1	5.3	0.71	0.24	0.05	1.2
	350	0.7	2.7	7.2	0.63	0.32	0.05	1.7
	385	0.7	2.7	6.5	0.61	0.33	0.06	1.7
Phospholipase in glycerol	320	0.4	2.1	5.6	0.4	0.43	0.17	2
	350	0.8	2.7	6.3	0.19	0.54	0.27	3.3
	385	—	—	4	—	—	1	4

Table 2.
Lifetimes and amplitudes of tryptophan emission of ribonuclease T_1, parvalbumin, and phospholipase A_2.

ethanol in standard cuvettes gives fluctuations in lifetime > ± 0.5 ns (lifetime was 7.7 ns; it was measured by "SLM-4800" at frequency of 30 MHz; excitation was 370 nm; excitation slits were 1 nm), but in multipass cuvettes (at the same conditions), fluctuations were < ±0.1 ns.

Multipassing does not bring appreciable contribution in measurements of nanosecond lifetimes, since several passes of the beam lead to artifact delay only about of 0.1 ns. In the case of subnanosecond lifetimes, it is necessary to do measurements with differential mode, using multipass cuvettes in all channels and entering an amendment for artifact delay.

3. Polarization degree of fluorescence

Molecules of many organic substances consist of a flat chromophore and various groups attached to it. Their structures are optically anisotropic. Therefore, emission of motionless molecules is polarized (even at the nonpolarized excitation). The greatest contribution to the total emission is brought by those molecules, in which chromophores are located in a perpendicularly position to the excitation light flow. It means that even at a chaotic arrangement of molecules, for example, in suspensions of membranes or cells, the emission is partially polarized. The intensity of fluorescence in some direction can be presented as a sum of two light flows, F_{II} and $F_{\perp}$, polarized at right angle.

If to illuminate an object by linearly polarized light, the fluorescence becomes even more polarized. For example, it is possible to direct the light beam from a lamp on a sample through the polarizer (polarizing prism) and to put the analyzer (the same second element) sideways of the sample, passing through which the emission will get on the photomultiplier. The intensity of emission, measured at identical orientations of polarizer and analyzer, is named as "parallel" component. The intensity, registered at orientations, crossed under 90 degrees between the polarizer and the analyzer, is the "perpendicular" component. The degree of polarization at excitation by linearly polarized light is expressed as [6]:

$$P = (F\| - F\perp)/(F\| + F\perp). \quad (4)$$

Fluorescence polarization degree (P) of various samples in standard cuvettes and mirror cuvettes is presented in **Table 3**. A very small decrease of P in mirror cuvettes is due to depolarization of exciting light at reflections on mirror sides. This small artifact can be easily eliminated as an addition of constant value 0.005.

3.1 Photo-bleaching

Multipass cuvettes may be useful not only for fluorescence analysis but also for many-fold intensification of photo-bleaching or laser flash photolysis of chromophores or dyes in solutions or suspensions.

The energy of UV quantum, absorbed by a cell or biomembrane, is spent mainly for fluctuations, so - on instant strong local heating [7, 8].

If such local heating is too high, it can result to denaturation of proteins and their aggregation. For instance, the influence of UV irradiation on intensity of tryptophan fluorescence of α-crystalline (it is a protein from bovine eye lens) is shown in **Figure 5**. A solution of the protein, hermetically closed and thermostated at 10°C (here, thermo-aggregation is possible to be neglected) in mirror microcuvette, was continuously irradiated for 120 min with UV light of a 450-W xenon lamp through holographic monochromator, positioned at 280 nm with slits of 16 nm. The intensity of tryptophan fluorescence (excitation was 295 nm; emission was 340 nm; emission slits were 4 nm) was quickly measured during a course of irradiation. The observed decrease in fluorescence of α-crystalline, caused by photoinduced denaturation, is due mainly to the increase of light-scattering during aggregation. Light-scattering can be measured from optical density at 310 nm of the sample in a standard compartment of spectrophotometer or, by the other way, from intensity of scattering light, detected from the sample by the photomultiplier of a spectrofluorimeter under a right angle. In the second case, both monochromators have to be established at an identical wavelength of 310 nm (at slits = 1 nm).

Sample (solution or suspension)	P in standard cuvette	P in mirror cuvette
Tryptophan residues of sarcoplasmic reticulum (ex. 295 nm, em. 320 nm)	0.36	0.355
Tryptophan residues of mitochondrial suspension	0.29	0.285
ANS in bovine albumin	0.28	0.275
7-Aminoactinomycin in DNA	0.31	0.305

Table 3.
Fluorescence polarization degree of various samples.

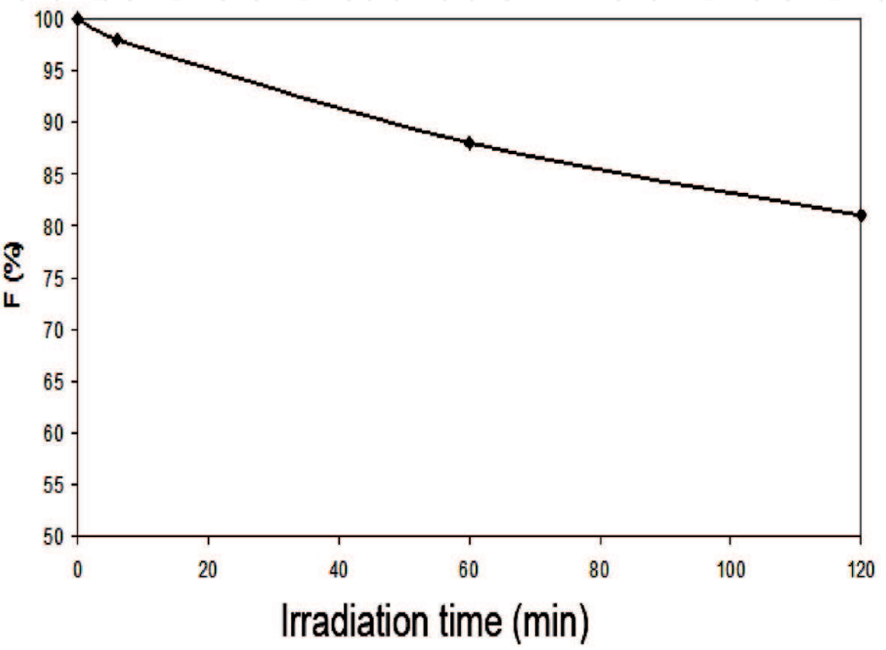

Figure 5.
Influence of UV radiation on the intensity of tryptophan fluorescence of α-crystalline (0.7 mg/ml) during photoaggregation of the protein at 10°C. irradiation was at ~280 nm in mirror microcuvette.

Fluorescent kinetics of photo-bleaching of flavin mononucleotide (FMN) in aqueous solution and in diluted suspension of proto-mitochondria [9] after illumination by blue light of the mercury lamp SVD-120A in a mirror cuvette is given in **Figure 6**.

Photo-bleaching is induced by photo-destruction of FMN, namely, by oxidation of its triplet state by molecular oxygen. The absorption band of FMN, detected at 450 nm, was "burns out" [7, 9].

3.2 Cuvette with diagonal plate

Cuvette with transparent diagonal quartz plate can be applied for many-fold increase of fluorescence signal and for many-time using of a single sample, adsorbed on the plate. A schematic diagram of a cuvette with the diagonal transparent quartz plate is shown in **Figure 7**. The sample in the form of a smear or film is located on the side of the plate facing the exciting light.

A sample of cells or isolated organelles is attached on the surface of a transparent quartz plate, which is placed inside a standard cuvette at a right angle to the exciting light. A sample has to be as a smear or film. The cuvette should be filled by water (if cells cannot be desorbed from the plate) or hydrophobic solvent like hexane (if cells are not tightly fixed on the plate).

A transparent quartz plate is used for many-fold increase of the sensitivity of the fluorescence analysis. The sample (matter of inquiry) is placed exactly at the center of the surface of the plate. The size of the attached smear (film of organelles or cells or other samples) should correspond to the light spot, formed by the exciting lens (**Figure 7**).

To get the best results, the object under study (cells, cellular organelles, or nuclear DNA, etc.) is applied to the surface of the plate in the form of a thin smear (with drying from water within 4–5 min). Fluorescent dyes, dissolved in a solution, are deposited as microdroplets (1–4 μl) to the sample. After that, the sample is dried for 1–2 min. The plate with the dried sample is placed into the cuvette filled by liquid, which weakly interacted with sample, for example, hexane, perfluorodecalin or, in opposite case, for water-insoluble samples, by isotonic aqueous medium or water.

In all experiments, the plate was placed along a diagonal of the cuvette, as shown in **Figure 7**, to exclude the penetration of the reflected artifact light into the recording emission channel. Virtually, any films of samples (stained cells or

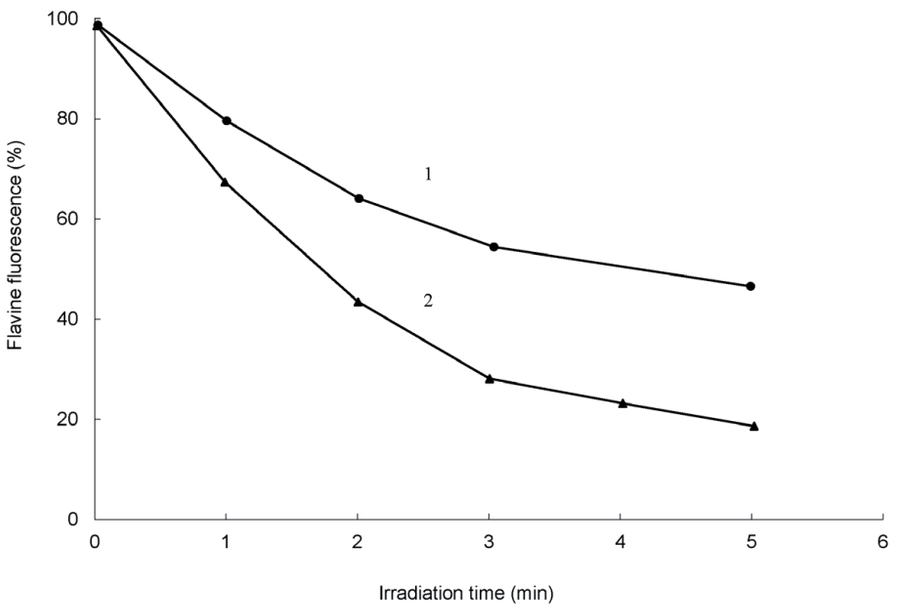

Figure 6.
Dependence of photodestruction of proto-mitochondrial flavins (1) and free FMN (2) at the time of blue irradiation. It was measured by fall in the intensity of flavin fluorescence, appeared in the burning band at 450 nm, and detected as decrease in fluorescence intensity at 525 nm.

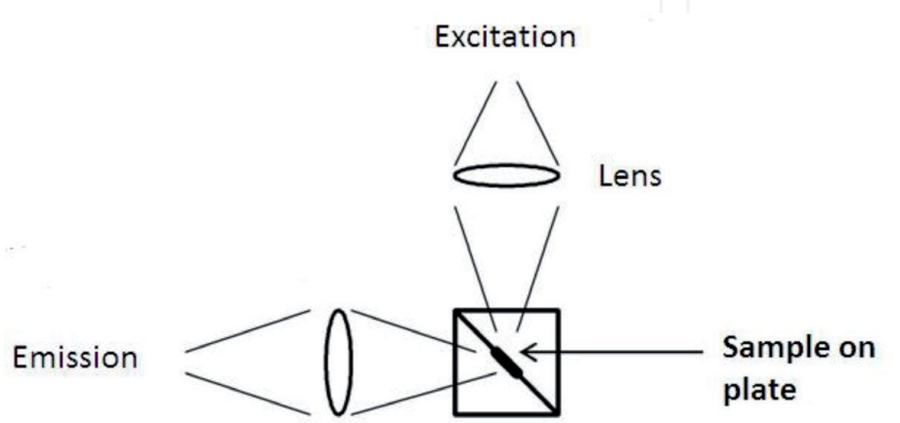

Figure 7.
Cuvette with the diagonal transparent quartz plate (top view). The sample in the form of a smear or film is located on the side of the plate facing the exciting light.

organelles, etc.) applied on the surface of the plate produce fluorescence signals that are several times higher than signals yielded by the same quantity of the studied substance in the solution filling the cuvette volume, although the sample on the plate is usually hypochromized (the extinction factor of chromophores is substantially decreased due to the sieve and screening effects).

High intensity of fluorescence of the layer on the plate arises due to several reasons. First, the whole studied substance is concentrated in the small square of layer, but, while being in a solution, it is distributed over the whole volume of the cuvette. Second, the sample is attached on the plate at the same place, where the focus of both lenses is located (the area of the sample is equal to that of the light spot). Third, the excited molecules of the sample in the condensed phase are less subjected to deactivation (quenching) than in solutions.

The cuvette with transparent diagonal quartz plate can be applied for chemical treatments of a sample. For instance, **Table 4** presents the data on formation of pyrene excimers (excitation was 335 nm, and emission was 393 nm for monomers and 470 nm for excimers) before and after extraction of mitochondrial lipids by acetone from mitochondrial smear. Also, quenching of tryptophan fluorescence (excitation was 286 nm, and emission was 335 nm) of mitochondrial smear by pyrene (2.4 μM) is shown.

During the oxidation of succinate in respiratory chain of mitochondria, a consumption of oxygen, accompanied by increased τ_m and F_m of pyrene, takes place (**Table 5**) [8]. At exhaustion of oxygen, luminescence of pyrene leaves on a plateau. Mitochondria were 0.8 mg of protein per ml in the incubation solution: 10 mM Tris-HCl, 10 mM phosphate, 50 mM KCl, 150 mM sucrose, and pH = 7.5. Pyrene was 1 μM. Excitation was 336 nm, and emission was 393 nm (monomers) and 480 nm (excimers); slits were 2 nm.

In anaerobic conditions, the lifetime of pyrene monomers in mitochondria equals 157 ns (**Table 5**). In without-oxygen organic solvents, τ of pyrene is much more; for example, τ in cyclohexane is 370 ns.

The highly sensitive sensor was designed to measure the molecular oxygen content in solutions and diluted suspensions, placed in a quartz cuvette, which contains the diagonal plate with a pyrene, protected by a Teflon film (**Figure 8**). The sensor can be used in any standard spectrofluorimeter. Unlike analogs, this oxygen sensor is not inertial.

3.3 Mirror cuvette with diagonal plate

An additional multiple increase in the fluorescent signal is attained by the use of special mirror cuvette with diagonal plate. Such cuvette (**Figure 9**) is very useful when the emission intensity from a sample is too low.

In all experiments, the cuvette with the plate was filled with a solvent, the refractive index of which is close to that of quartz. The filling with a solvent is intended to (i) eliminate spurious light reflections at boundaries, (ii) reliably prevent the fluorescence channel from the incoming exciting light, (iii) prevent

Mitochondria	**Quenching (%)**	***Fe/Fm***
Native	27	0.47
Without lipids	23	0.1

Table 4.
Quenching of tryptophan fluorescence of mitochondrial smear by pyrene and pyrene monomer/excimer ratio before and after extraction of lipids from mitochondrial smear.

Oxygen (μM)	*Fm*	*Fe*	*Fe/Fm* τ_m (ns)
130	95	11.5	0.12105
~1	150	23	0.15157

Table 5.
Fluorescence of pyrene in membranes of mitochondria in aerobic and anaerobic conditions.

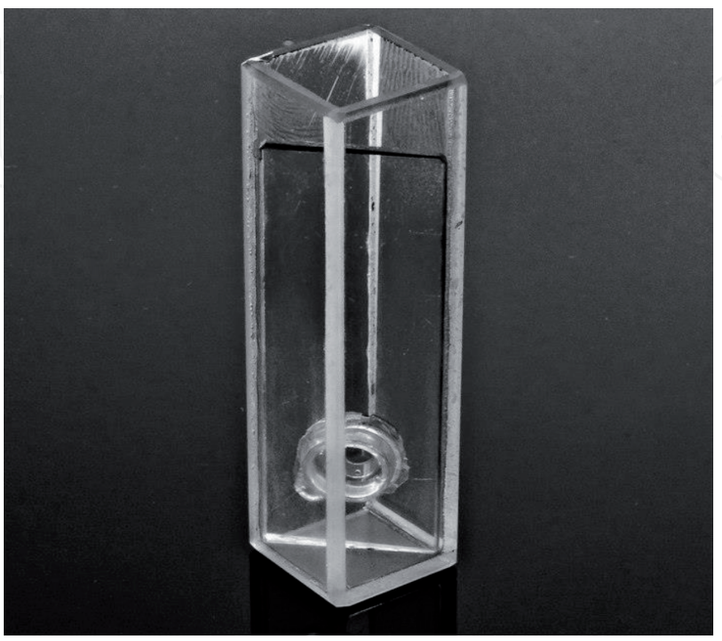

Figure 8.
Cuvette with the diagonal transparent quartz plate with plastic microcontainer. The sample—suspension of cells or solution of pyrene (oxygen sensor).

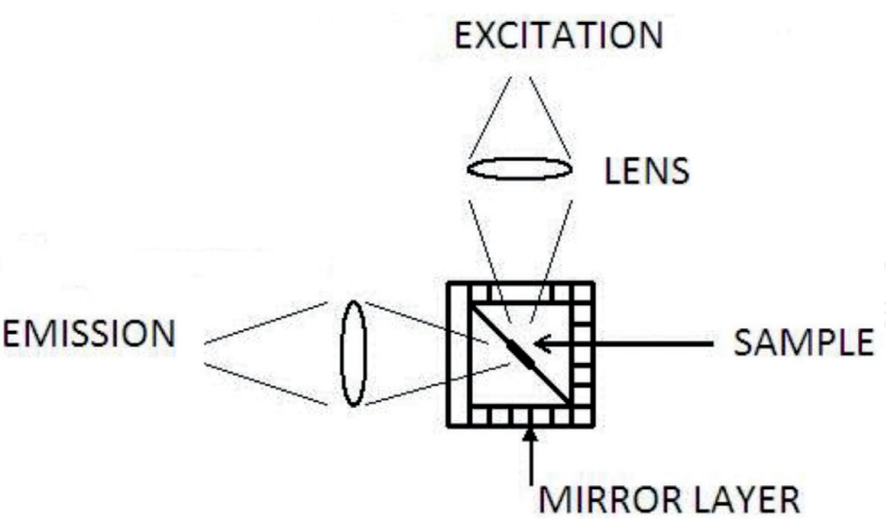

Figure 9.
Schematic diagram of mirror cuvette with the diagonal transparent quartz plate (top view). The sample in the form of a smear or film is located on the side of the plate facing the exciting light.

the sample from strong overheating by the exciting light, and (iv) allow measurement of the kinetic processes on the sample surface. The matter is that molecules of the studied substance are sufficiently mobile on the sample surface contacting the solution and make it possible to observe the kinetics of chemical and biochemical reactions using fluorescence spectroscopy methods.

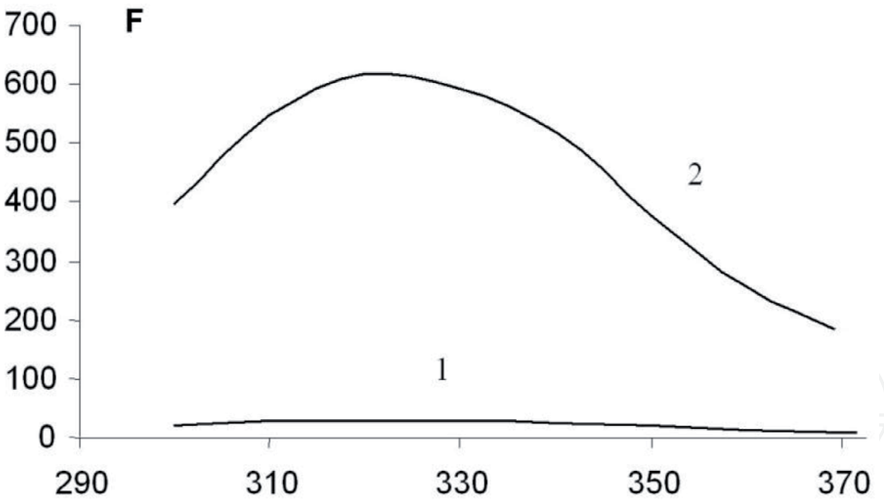

Figure 10.
Spectrum of the tryptophan fluorescence of mitochondrial proteins: (1) aqueous solution in the standard cuvette and (2) sample film on the surface of the quartz plate in the mirror cuvette.

Sample	Exciting wavelength (nm)	Maximum of fluorescence spectrum (nm)	Fluorescence intensity of		
			Dissolved sample in mirror cuvette	Sample layer on the plate in standard cuvette	Sample layer on the plate in mirror cuvette
Rhodamine B	540	570	3.5	4.1	13.8
Pyrene	335	390	3.3	3.8	11.9
Tryptophan	280	350	3.1	3.5	10.1
7-AAMD	550	660	3.6	3.8	12.9
7-AAMD in DNA	570	630	3.7	4.2	14.8
Mitochondria	286	340	3.4	4.7	15.2
Leucocytes	450	525	3.1	3.8	11.1

Note: concentrations <1 μM; fluorescence intensity of the dissolved sample in the standard cuvette was assumed to be 1; 7-AAMD is 7-aminoactinomycin D.

Table 6.
Fluorescence parameters and intensities (gain, G) of various samples on the plate, diagonally cutting in quartz 1-cm cuvette.

The use of the plate ensures another possibility; namely, the film sample on it can be used repeatedly, placing the plate (after rinsing) in the required medium. For example, a thin layer of mitochondria (from rat liver) safely lies on the plate even at a long (1–2 h) period of being in the isotonic water phase (the adhesion and stability of a thick layer are rather worse). In this case, the initial activity of enzymes is not lost. In the inert perfluorodecalin, this layer remains unchanged for many hours.

Figure 10 shows, for example, spectra of the tryptophan fluorescence of proteins of mitochondrial diluted suspension in the standard cuvette (curve *1*) and in the form of a film on the quartz plate, diagonally cutting into the mirror cuvette (curve *2*). This mirror cuvette ensures a higher fluorescence signal from the sample on the surface of the plate than that from the solution in the cuvette volume. The 1-cm mirror cuvette usually increases the output signal from the dissolved sample by a factor of 3.1–3.7 as compared with the standard cuvette. It additionally increases the signal by a factor of 3.5–4.7 for a sample layer on the plate,

as compared with the standard cuvette with a diagonal plate. As a result, the total gain of the recorded fluorescence signal in the mirror cuvette with a diagonal plate proves to be at least an order of magnitude higher than that of the standard cuvette filled with a solution of the same substance (**Table 6**).

Similar results also were observed in experiments with dyes and aromatic hydrocarbons. **Table 6** summarizes relative fluorescence values of some substances, when they are placed on the plate's surface (comparing to the solution of a sample in the cuvette volume).

4. Conclusions

A number of special cuvettes which dramatically increase the fluorescence intensity of diluted solutions and suspensions are tested. All described cuvettes can be applied for measurements of spectra excitation and emission, excited-state lifetime, and polarization degree. These cuvettes enable a multiple increase in the sensitivity of fluorimetric measurements with common spectrofluorimeters. It is also possible to study, in particular, the kinetics of phenomena on the sample surface, to detect small concentrations of substances, e.g., slight quantities of proteins and DNA in solutions, and this information can be used for biomedical analyses, in crime detection, etc. Also, in principle, the described cuvettes could be applied for photo-bleaching experiments.

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