# TO THE PROBLEMS OF FLUORESCENCE EXCITATION SPECTRUMS

## ОБ СПЕКТРАХ ВОЗБУЖДЕНИИ ФЛУЮРЕСЦЕНЦИИ

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**Abstract:** *The present paper is dedicated to constructing a phase fluorometer on a phase screen model. The numerical correlation method are applied to evaluate fluorescent signals. The phase shift between the reference signal and the fluorescence signal are studied as the function of statistic moments of random phase screen.*
**KEYWORDS:** *FLUORESCENCE, SPECTRUM, PHASE SCREEN, LASER, RANDOM*

## 1. Introduction

Fluorescence spectroscopy represents the effective method of studying dynamic processes in solutions. This method greatly applied in biology, medicine, material studies and nanotechnology, and of course, in ecology. The parameters of fluorescence spectroscopy, like: radiation spectrum, life duration, quantum yield, fluorescence anisotropy – represent the sensitive functions of the processes, which depend on the life duration of excited condition. The molecules can participate in these processes which are in 10 nm distance from fluorofore at the moment of excitation.

All the molecular system is characterized with the own fluorescence in more or less intensity. Polysaturated, condensed and of course, aromatic and polyaromatic compounds are well fluorescencing. Hetero atomic or electron-domain systems should be particularly mentioned. Special organic and non-organic substances are fluorescencing perfectly, which are used in making displays, monitors, photodiodes and lasers. Natural compounds are fluorescencing in comparatively less quality. From natural admixtures which are fluorescencing well, should be mentioned natural paints, some amino acids (Tryptophan and Tyrosine) and the protein containing them, cofactors (NADH), vitamins (riboflavin). The own fluorescence is the basis of discovering and identifying natural admixtures and oil products.

It is well-known that *impulsive* and *phase-modulated (harmonic)* methods of fluorescence analyses of liquid media, each method has its advantages in certain direction. For instance, in impulsive method the sample is radiated with short light impulses and measured the influence of fluorescence intensity upon time. In modern spectroscopy, the source of radiation is impulsive lasers of Picoseconds. In periodically repeated impulses, the fluorescence attenuation is registered via either *stroboscopic method* or *photon counting method*.

## 2. Problem discussion

There are two direct methods of measuring the damping velocity of the fluorescence, so there are phase and impulsive fluorimeters. It is noteworthy that the impulsive fluorimeters - represent simple tools to define life expectancy of fluorescence. The sample is lit with the impulsive light source. The duration of the impulse is lower than the fluorescence life expectancy. Damping of fluorescence is registered on the oscillograph or on the personal computer via the electronics consisting of or analog-digital converters. The principle of measurement is similar to the principle of phosphorescence and slow fluorescence measurement (with several milliseconds of life expectancy).

However, of course, on the frequencies that correspond to the angular damping velocity of fluorescence, it is impossible to create exciting light impulses. For this purpose, two methods are used. In one of the methods, the photomultiplier works in impulsive mode, the time of impulsive mode of which is lower than the life expectancy of fluorescence. The impulsive source and the photomultiplier are turned on with a frequency of several thousand rounds in a second, but with some delay. Fluorescence curves are taken by measuring the time of delay between excitation and registration. The enhanced signal from the photomultiplier connects to the phasemeter's first channel with the coaxial cable. The impulse source of light used during this method should have less duration than the fluorence signal fading time, so it is easy to use hydrogen impulse lamps.

In the second method, photomultiplier works in impulsive mode and has high sensitivity. In this case, the lifespan of light impulses is several times higher than the lifespan of fluorescence. Photomultiplier signal is registered through the detector and can be visualized. For normal intensity, which is enough to get a photographic image, it is necessary to repeat this operation several times. **Birke, King** and **Murno** [1] used the strobing oscillographs and recorded the curves on the self-recorder.

In the grant projects GNSF/ST08/5-451, FR/152/9-240/14 our team has managed to modernize the laser spectrofluorometer. As a light source we used high-sensitivity impulsive nitrogen laser NL-100 (frequency 0-25 Hz) with which strobing of monochromator and sensitive photomultiplier was carried out. In order to reduce the "electronic noise" caused by the relaxation processes, we used a multichannel analyzer that is compatible with computer-compatible electronic devices such as ADC (analog-digital conversion), DAC (digital analog conversion) and electronic circuits [2,3].

Thus, the phase fluorometer allows us to determine the duration of the received signal through the phase screen with a well-known modulation signal. It is important to know the polarization of the source in defining the phase screen regulations (viscosity, temperature, structure):

$$I = \left(\frac{\partial \mu}{\partial \rho}\right)_{T,X}^2 \cdot \left(\frac{\partial \rho}{\partial p}\right)_{S,X}^2 \cdot (\bar{\Delta}p)_{T,X}^2 + \left(\frac{\partial \mu}{\partial \rho}\right)_{T,X}^2 \cdot \left(\frac{\partial \rho}{\partial s}\right)_{P,X} \cdot (\bar{\Delta}s)^2 + \quad (1)$$
$$+ \left(\frac{\partial \mu}{\partial x}\right)_{P,T} \cdot (\bar{\Delta}x)^2,$$

where $\mu$ - a refraction index, $\rho$ - density, $p$ -pressure, $T$ - temperature, $S$ -entropy, $x$ - molar concentration of a solution.

Schematically, the photon radiation and the absorption is expressed with **Iablonski diagram** [4]. The energy of electronic transition is equal to the energy difference between the starting energy level and the final level. Therefore, the transition energy:

$$E = h\vartheta, \quad (2)$$

where $h = 6.62 \cdot 10^{-34} \, j \cdot sec$ – plank constant, $\vartheta$ -light frequency.

Emission occurs from a population of *n* excited fluorophores with intensity *I:*

$$I = nE. \quad (3)$$

The energy absorbed by a fluorophore is more important than the energy of an emitted photon. For absorption and emission energy we have:

$$E_a = \frac{h\vartheta}{\lambda_a}, \quad E_{em} = \frac{h\vartheta}{\lambda_{em}}, \quad E_a < E_{em}, \quad \lambda_a > \lambda_{em}, \quad (4)$$

where $\lambda_a$ and $\lambda_{em}$ are wavelengths absorption and emission spectra peaks.

Since not all the molecules present at the excited states will participate in the fluorescence process, a quantum yield $\phi_f$ exists:

$$\phi_f = \frac{I_f}{I_a}, \qquad (5)$$

where $I_f$ is fluorescence intensity, $I_a$ - intensity of absorption. In fact $\phi_f < 1$ therefore:

$$I_a = I_0 - I_f . \qquad (6)$$

Fluorescence excitation in phase fluorometers occurs with a light beam that is modulated by high frequency. Fluorescence phase, i.e., the degree of modulation was compared to the phase of source. In the case of exponentially fading fluorescence, the shift $\Psi$ between the fluorescent radiation and excitation light phases is depicted with the expression: $\omega\tau = tg(\Psi)$, where $\tau$ is fluorescence life expectancy, and $\omega$ is an angular velocity of the modulation. The signal from the source and the fluorescent signal excited on the sample hit the detector that registers the phase shifts among these signals. For example, **Bailey** and **Rolefson** measured the phase shifts between these two signals, whereas the two output signals coincide with the phase, the detector registered a minimum signal at the output. There are other methods of measuring the ψ phase shifts based on the determination of the quantity of these two signals. It is obvious that the longer is the life expectancy of fluorescence, the smaller is the size of its modulation. The quality of modulation ( $m_s$ ) in the light beam and fluorescence signal modulation quality ( $m_f$ ) is associated with the ψ phase shifts with the ratio of:

$$\frac{m_f}{m_s} = \cos(\Psi) . \qquad (7)$$

The phase shifts measured by these two methods coincide with each other with great accuracy in the case of exponentially fading fluorescence.The distinction is observed only in case of non-exponential fading of fluorescence. One of the main drawbacks of the phase fluorometer in principle is the difficulty in the interpretation of the results which arise in case of non-exponential fading of the fluorescence, but Birks, Dyson and Munro have used this method even in case of non-exponential fading of the fluorescence. In the first phase fluorometers, polarizing exciting light and Kerr effect were used for light beam modulation. In this case, the high-frequency field acts on the fluid or crystal electronic-optical effect. Then, for light modulation, the light diffraction was studied on the ultrasonic waves in the fluid that formed the quartz crystal. The light beam with the constant intensity falls onto the diffraction grating. Among the easiest methods a high-frequency pipe is used, in which the modular light is obtained directly. A relatively convenient method is to use a hydrogen lamp that is powered by a modulated signal.

With the method offered by us, the lifespan of fluorescence can be determined indirectly, from the attempts of damping fluorescence. For the first time this method was proposed by **Eisenthal** [5], which described the indirect methods of measuring fluorescent radiation ( $10^{-11}$ sec). The impulse of polarized laser light with doubled frequency is partially absorbed by the sample, the absorbed light from the special reflective plate is still going back to the sample, but with modified polarization and some delay. The time of delay is determined by the distance between the sample and the reflective plate. The experiment indicates the dependence of the anisotropy of the impulse on the time of delay. The velocity of the disappearance of anisotropy in small viscosity solutions is measured by the rotation of molecules, and in the viscous solutions – by the lifespan of the excited condition.

### 3. Objectives and Research Methodology

Signal source quality is one of the most important parameters, including advanced radar systems. The smaller the phase noise, the greater the spatial distinguishability, and the more accurate the measurement results, including from the moving and turbulent objects. In order to measure the phase noise of the signal source in impulsive mode, it is necessary to operate a very complex system that consists of a number of components, such as phase detectors, Fourier analyzers, and high-quality sources of the reference signal. To ensure accuracy, signal sources have a very high quality. If the quality of the source signal is not provided, then the correlation theory can be used, which is based on correlation of two different source signals. In our case, we get these two sources by directing the reference signal through the cuvette (fig.1).
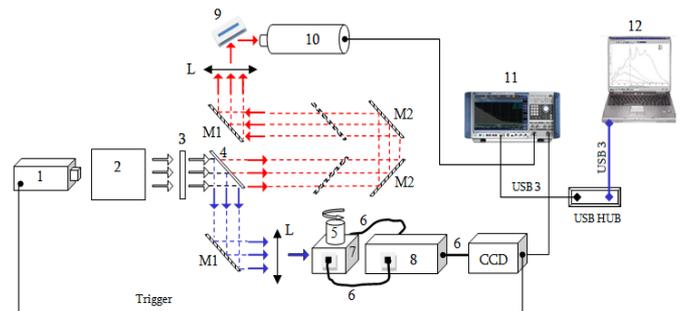


**Fig. 1.** *Principal scheme of phase fluorometer. 1- light souser, 2 – optical modulator; 3- optical filter, 4- light spliter; 5 – cuvete with rotate system; 6 – optical fiber; 7 – cuvete holder; 8 – correlator of coherence beams; 9 - scattering plate; 10 - photomultiplier; 11 – phase meter; 12 - computer; M1 – stationary minor; M2 - moving mirror; L - converging lens; CCD - monochromator.*

By 90 degree from the base signal, by using optical fiber waveguide we receive partially coherent waves. Extinguishing the noise of the source and the components of the survey system can be realized by averaging the noise in two different measurement tracts. Of course this complicates the measuring system, but it makes it possible to achieve significant improvements in the sensitivity of the detected signal. Using the intercorrelation theory increasing the sensitivity to phase noise can be expressed with the following expression: $\Delta L = 5\log(n)$, where $n$ is the number of correlations. For instance, the averaged increase of the number of correlation 10 times provides the reduction of phase noise in measuring system by 5 dB. At the same time, phase noise is a pivotal parameter not only for radar use but also for tasks that takes into account especially precise measurements. In technology, high-precision measurements of phase noise are used to measure high-sensitive generator parameters such as thermostatic quartz generators, generators with dielectric resonator, synthesizers and so on.

The iterative methods have been developed and widely applied in the tasks of synthesis of diffractive optical elements [6]. The main advantage of these methods is that Iteratical algorithms are more accurate than other algorithms. On the other hand, the realization of the algorithm of synthesis of optical elements on the computer requires significant computing costs. The disadvantage of these algorithms is the use of approximation to describe the laser beam distribution. Within the framework of the project, the algorithm will be developed, which will be realized on the optical-electronic scheme. The main characteristic of this algorithm is that it operates at the hardware level and through it we can manage the high-sensitive nitrogen laser NL-100 and monochromator. The monochromator gives a picture in the Fourier plane. This scheme allows to use the laser with any initial distribution of intensity. The algorithm will allow us to select the phase function to allocate laser intensity in order to minimize the errors when forming a diffractive picture of the output signal, and therefore we assume that its use can increase the functioning of the phase spectrofluorometer.

The Einstein-Fokker-Kolmogorov's equation enables us to calculate the angular and linear inclination from the source direction [7]. So, while propagating waves in the chaotic inhomogeneous media, the absorption of the energy of electromagnetic waves significantly influences on the statistical characteristics of the scattered radiation. The absorption may cause the deformation of the power of radiation spectrum while discussing the asymmetric

problem [8]. The salvation of such type problems has scientific and practical importance. Nowadays, there is not a universal analytical method for solving such problems and therefore, we can get the information on the scattered radiation by applying the figural methods or from the experiment itself.

It turned out that partial spatial coherence of the source reduces the scintillation noise. The function of Gaussian partial coherent source is studied in the work [9]. In conditions of the weak and strong turbulence the analytical expression of correlation function of the space-part coherent intensity was obtained in the work [10], where the general principle of Hugens-Frannel and the general solution of Relay-Zommerfeld were used. In the work [11] Ritov's method is used, in conditions of weak turbidity atmosphere.

Let's say, on the $z = 0$ plane the primary $s_0$ field statistics are given, i.e. its moments (coherence function) are given. It is necessary to find out how these functions are changing away from the $z = 0$ plane, if on the way the field is transformed (for example, the wave is going through a diaphragm, lens or other).

Formally this task is solved easily: If it is known how the determined (totally coherent) wave changes, then it is enough to make an ensemble averaging of the determined solution of $s_0$ field. But this method, as a rule, reduced to difficultly calculating integrals. For example, when calculating the relative fluctuations of intensities $\sigma = <I^2 - <I^2>>/<I^2>$ (the same as the "Scintillation index") it is necessary to calculate the eight-fold integral, which is practically impossible to be calculated even in the case of simplified model. The simplified marginal model implies that a flat wave $e^{ikz}$ falls on the layer, directly on the other side of the screen $s_0 = \exp(ikz + i\Psi)$, where $\Psi(x, y)$ is the random phase. Knowledge of phase statistics on the screen determines the field statistics on $z = 0$ flatness. The "system" which "transforms" the field out of the screen is just a free space in the given case. As a result of diffraction, the wave, which passes through the chaotic phase screen, is experiencing fluctuations. However, the intensity of the phase screen is permanent (Fig. 2).
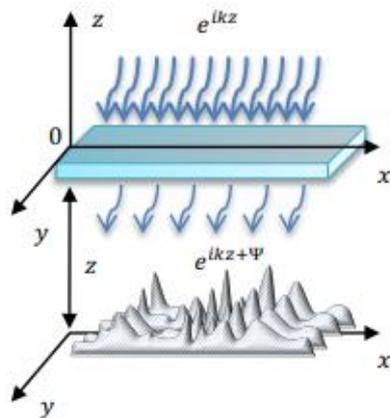


**Fig 2.** *Distribution of wave $I(x)$ intensity via random phase screen*

Suppose that the statistics of the incident laser signal on the detector is known ( $s(t)$ ). If the probability distribution of the signal on the detector is also given by W (s), then the average value of the signal at the output of the detector is determined by:

$$\langle f(s) \rangle = \int_{-\infty}^{+\infty} f(s)W(s)ds = \lim_{T \to \infty} \frac{1}{T} \int_{-T/2}^{T/2} f\big(s(t)\big)dt,$$

$$W(f) = W(s)\frac{ds}{df}, \qquad (8)$$

where $W(f)$ - is the distribution of the signal at the output of the detector. The signal power spectrum or auto-correlation function cannot provide complete information about the process of scattering, but these values are subject to experimental measurement, therefore, the theoretical and experimental measurement of these parameters may become the basis for creating an adequate theoretical model. From this perspective, the first and second order statistical moments are presented as experimental measurable physical values on the basis of the correlation functions of the scattered signal and intensity:

$$g^{(1)}(\tau) = \frac{\langle s^+(0)s^-(\tau) \rangle}{\langle I \rangle^2}, \quad g^{(2)}(\tau) = \frac{\langle I(0)I(\tau) \rangle}{\langle I \rangle^2}, \qquad (9)$$

Where, $I(t) = s^+(t)s^-(t)$ - represents a magnitude that is recorded by a detector. In the quantum-mechanical point of view, we can say that the detector, which works on photon registration (through photo multiplier and photodiode), records this value. $s^+(t)$ and $s^-(t)$ represents the signal components that correspond to positive and negative frequencies and are determined by the Fourier series:

$$s(t) = \sum_{\omega > 0} a_\omega^* e^{-i\omega t} + \sum_{\omega \geq 0} a_\omega e^{-i\omega t} = s^+(t) + s^-(t),$$

$$a_\omega = \frac{1}{T} \int_{-T/2}^{T/2} s(t)e^{-i\omega t} = a_{-\omega}^*. \qquad (10)$$

It is noteworthy that from $g^{(1)}(\tau)$ and $g^{(2)}(\tau)$ statistical moments only the second order statistical moment $g^{(2)}(\tau)$ is to be measured on the detector. That's why, the matter of finding the connection between $g^{(2)}(\tau)$ and $g^{(1)}(\tau)$ statistical moments is an urgent issue. This connection was found for statistically independent random variables on the basis of probability theory and was brought to the expression that is a well-known ratio and which allows the first-range spectral characteristics to be calculated by the simplest spectral characteristics of the second order:

$$g^{(2)}(\tau) = <I(t)I(t+\tau)> = <I>^2 \left(1 + \left|g^{(1)}(\tau)\right|^2\right). \qquad (11)$$

Similar calculations were conducted for a specific task when the scattered field:

$$s^+(t) = s_0 \exp\left[-i\big(\omega_0 t + \varphi_0(t)\big)\right] + f(t)s_1 \exp\left[-i\big(\omega_1 t + \varphi_1(t)\big)\right]$$

and primary source field were radiated from the same point of space and the intensity correlation function was calculated for Lorenz: $g_L^{(1)}(\tau) = \exp(-2\alpha\tau)$ and Gaussian: $g_G^{(1)}(\tau) = \exp(-\beta^2\tau^2)$ forms:

$$g^{(2)}(t;T) = 1 + \frac{sh^2(\alpha\tau)}{(\alpha\tau)^2}\exp(-2\alpha\tau), \quad \tau > T, \qquad (12)$$

$$g^{(2)}(t;T) = 1 + \frac{\exp\left[-\beta^2(\tau^2 + T^2)\right]}{\beta^2 T^2}sh(2\beta^2\tau T) +$$

$$+ \frac{1}{\beta T^2}\left\{(\tau + T)erf\left[\beta(\tau + T)\right] + \right. \qquad (13)$$

$$\left. + (\tau - T)erf\left[\beta(\tau + T)\right] - 2\tau erf(\beta\tau)\right\}.$$

On Fig. 3 is shown the distribution of the signal at the input and output of the square detector.

Based on the first and second orders of the laser radiation scattered from the random phase screen, can be received:

$$<I(\theta;t)> = \pi^2 W_0^2 |E_0|^2 \times$$

$$\times \int_0^\infty dr\, r\, J_0(kr\sin\theta)\exp\left\{\left[-\bar{\phi}^2(1 - \rho(r))\right] - \frac{r^2}{W_0^2}\right\}. \qquad (14)$$

$$\frac{<I^2>}{<I>^2} = 2 - \frac{2l^2}{W_0^2} + \frac{l^2\bar{\phi}^2}{4W_0^2}\exp\left[\frac{k^2l^2\sin^2\theta}{4\bar{\phi}^2}\right]. \qquad (15)$$

when $l/W_0 \to 0$, $<I^2>/<I>^2 = 2$, that is characterized to a Gaussian statistics. When $l << W_0$ the distribution is significantly different from Gaussian and the existence of $\bar{\phi}^2$ circumstance, which is quite large in terms of access. Finally, when $l << W_0$ the second order moment may be more than 2 if $\bar{\phi}^2$ is a big enough

value. Moreover, this effect will increase even more by increasing $\theta$ .
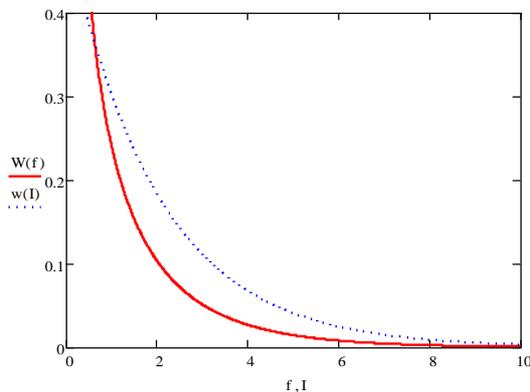


**Fig. 3.** *Distribution of Gaussian signal at the input and output of square detector.*

| № | Simple | Parameters of Fluorescence excitation spectrum | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Excitation by UV light (390 nm) | | | Excitation by Red light (660 nm) | | | Excitation by Green light (470 nm) | | |
| | | BASE | Power Spectral Density - PSD | Width | BASE | Power Spectral Density - PSD | Width | BASE | Power Spectral Density - PSD | Width |
| 1 | Sea water | 1837.4 | 134137.79 | 13.51 | 2069.2 | 1736774.15 | 26.56 | 2001.00 | 2168830.8 | 33.02 |
| 2 | Vinegar | 1766.8 | 10888.11 | 33.5 | 2176.4 | 1485608.45 | 25.35 | 2168.6 | 1649046.07 | 33.31 |
| 3 | Mineral water | 1887.6 | 17618.48 | 18.62 | 2149.4 | 112147.34 | 28.67 | 1940.00 | 442933.54 | 31.33 |
| 4 | Milk | 1968.8 | 77010.83 | 14.23 | 2005 | 1830624.19 | 27.15 | | | |
| 5 | Distillate | 1883.2 | 16268.35 | 18.98 | 2138.4 | 26896.18 | 36.51 | 2071.8 | 57543.31 | 38.45 |
| 6 | Drink water | 1833 | 13478.5 | 21.17 | 2203.8 | 98305.21 | 28.96 | | | |

*Table 1.*

On Table 1 present some parameters of fluorescence excitation spectrum for simples such as: sea water, vinegar, mineral water, milk, distillate and drink water, when excitation leads by phase modulated light with different wave length (UV light – 390 nm; Red light – 660 nm; Green light – 470 nm). The parameters: Base – is intensity from zero stage still base stage of spectrum; PSD – is power spectral density light under the excitation spectrum; Width – is half width of the spectrum.
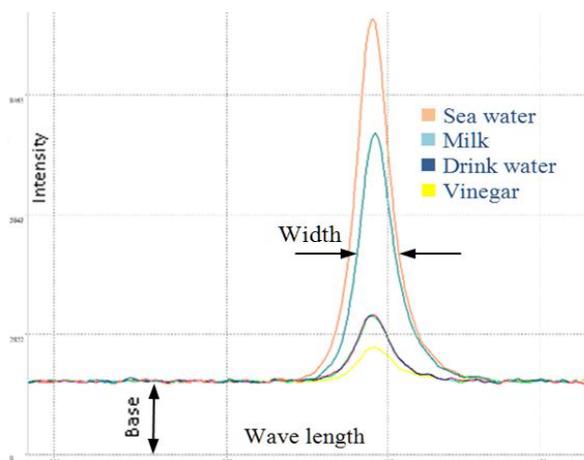


**Fig.4.** *Excitation fluorescence spectrum of phase modulated UV light ( $\lambda = 390$ nm) of different simples*

Fig. 4. shows excitation fluorescence spectrum of phase modulated UV light via different simples, such as: sea water, milk, drink water and vinegar.

### 4. Conclusion

Measurements and experimental works were made to identify following values: the spectrum intensity of the fluorescence signal radiated from fluorofol, quantum yield, fluorescence decay time. Database was created for Hexane, distillate, drinking water, sea water, for the liquid crystals used in monitors and displays, etc. The environment, in general case, was represented by an optically solid "phase screen". The autocorrelator was managed with "sketch" written in special programming language (which represents a C clone). Signal/noise ratio exceeded 10000:1 value. The first and second order statistical moments of the correlative function of the laser radiation intensity scattered from the optically solid, random "phase screen" for Gaussian and non-Gaussian statistics were obtained.

Unfortunately, technical problems limit the creation of the tools through which it will be possible to verify modulation frequencies. As a rule, the number of modulation frequencies is limited with two or three fixed values. Only in certain favorable conditions, for fixed values of modulation frequencies, we can get fluorescence attenuation components in heterogenic system. Phase-modulated method is faster with milliseconds than photon counting method.

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