Registration of fluorescence in biomolecular solutions using dynamic pin photodiode

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Abstract. The application of a dynamic pin-photodiode as a recording device of the fluorescence radiation is considered. An experimental setup for registration of luminescence signals from biomolecules excited by laser radiation was developed. Rhodamine 6G and chlorophyll were investigated.

1. Introduction
Fluorescent diagnostic methods are becoming more and more popular in clinical practice [1]. For example, in medical research special fluorescent dyes introduced into the body and distributed to tissues according to their properties are used [2]. The prospects of development of such testing methods are in their high sensitivity and speed of analysis of signals. There are various new areas of fluorescence research and technology based on the migration of excitation energy in biological material and fluorescent probes, such as FRET and plasmon resonance sensors. In addition, by using a combination of different fluorescence methods it is possible to study the movement of cell structures, to measure temporal kinetics of fluorescence imaging with temporal selection and spectral resolution for detection of molecular interactions, and to study the fluorescence spectra, from which the information on fluorescent molecules, their concentration, conformation, and interaction within cells and tissues (aggregation) can be obtained [3].

To register fluorescent signals, sufficiently sensitive hardware systems should be used [4]. Registered signals typically consist of not only the fluorophore fluorescence, but also background fluorescence of solvents and scattering from turbid solutions: Rayleigh or Raman scattering. The measurement of the fluorescence intensity can be influenced by the polarization of emitted light [5]. Therefore, to obtain reliable spectral data, it is necessary to take into account all types of registered fluorescence and scattering signals and to modify some spectrofluorimeter units.

In our work we suggest a new modification of the spectrofluorimeter: a dynamic pin-photodiode (DPD) with an insulated gate is used as a registration element of the device (instead of a standard photomultiplier).

2. Techniques
Standard devices for registration of fluorescence typically have the following disadvantages. Ordinary vacuum photomultipliers have slow speeds, high dark currents and high noises; detectors of THz radiation are expensive, a thermistor type of detectors of microwave radiation has no high enough sensitivity, and conventional semiconductor photodetectors have a high noise level. Recently a new device based on a pin photodiode [6] with an insulated gate operating in the dynamic mode has appeared. This device is promising for application in systems of registration of weak radiation from
activated biomolecular solutions. A pin-photodiode contains two strongly p- and n-doped regions separated by a weakly doped (or undoped) region (shown in Figure 1,a).

![PIN diode schematic](image)

**Figure. 1.** PIN diode in static and dynamic modes: (a) a schematic structure of a PIN diode; (b) PIN diode dark I-V curve; (c) applied voltage; (d) current-time dependence for DPD. Triggering time $t$ depends on the light intensity [6].

In the static regime, the diode is kept at a fixed reverse bias (point A in Figure 1,b), where the dark current is very low. Under illumination, the device current increases proportionally to the number of absorbed photons.

In our work we used the dynamic photodiode where the applied voltage was switched, as shown in Figure 1,c, from the reverse to a forward regime (from point A to point B in Figure 1,b). The applied forward bias induced a high forward current after a time delay (Figure 1,d). The measured photocurrent of the same device used in the static mode of the photodiode was up to 18 nA at maximum light intensity. At the same time, the output current of DPD was 0.8 mA, which was more than four orders of magnitude higher than the photocurrent. The magnitude of the dc current was controlled only by the value of the applied dc voltage and did not depend on the light intensity. In contrast, the delay time was a function of the absorbed light power.

The physics of DPD can be explained as follows. When the reverse bias is applied, the excess carriers are removed from the depleted region. After switching to the forward bias, the charge in the depleted regions adjacent to n+ and p+ creates potential barriers, which strongly suppress the dc current. In the end, hole and electron currents from the p+ and n+ regions reduce these potential barriers and the dc current starts to flow.

A sharp increase in the measured current is due to a positive feedback mechanism: holes coming from contact p+ reduce the potential barrier near the contact n+ and increase electronic injection, which, in turn, lowers the potential barrier near the contact p+ and increases the current holes. In the presence of incident light, barriers still exist after switching the voltage, but the induced photocarriers accelerate the reduction of the barrier and reduce the switching time. A simple device without any optimization has a delay time of 100 ps. A short light pulse considerably reduces the switching time (triggering time) of the device. In our work the modifications of the pin structure with the delay time of the nanosecond–millisecond range was used [6]. The dark triggering time of the device (self-triggering time) is about 0.6 µs.

The DPD has several important features: it has a high output signal, which is normally several orders of magnitude higher than that of a standard pin diode; the output signal can be digitalized without any analog-to-digital converter [6].
The model of the diode with the dimensions of the actual device was created in the program Comsol. The simulation provided an opportunity to obtain the total charge \( Q_{\text{tot}} \) needed to calculate the intensity of the radiation incident on the diode. \( Q_{\text{tot}} \) is the total charge of current carriers which are necessary for neutralization of volume charge under the gate in the depletion mode region.

To evaluate the value of \( Q_{\text{tot}} \), we should calculate the charge in the depletion mode region. The charge may be calculated as an area integral of carrier concentration in the presence of a positive gate bias and without voltage. Figure 2 presents the model of the hole concentration distribution. The area integral was calculated in the area marked by the rectangle in the upper left corner. This area corresponds to the depletion mode region where probabilities of photon absorption and electron-hole generation are the highest.

![Figure 2. Distribution of hole concentration.](image)

After calculating the total charge \( Q_{\text{tot}} \) of the media, the intensity was calculated from the following formulas [7]:

\[
\frac{1}{T_{\text{trig}}} = \frac{1}{N_{\text{tot}}} (n_{\text{phot}} + n_{\text{dark}}) = \frac{q}{Q_{\text{tot}}} \left( \frac{A \times \text{QE} \times \lambda}{\hbar c} \right) I_{\text{light}} + n_{\text{dark}}
\]

\[
I_{\text{light}} = \frac{Q_{\text{tot}} \times h \times c}{A \times \text{QE} \times \lambda^2 \left( \frac{1}{T_{\text{trig}}} - \frac{1}{T_{\text{strig}}} \right)}
\]

here \( n_{\text{phot}} \) is the number of integrated photogenerated charges per second \([s^{-1}]\); \( n_{\text{dark}} \) is the dark carrier generation \([s^{-1}]\); \( A \) is the active area \([m^2]\) (for our device \(9 \times 12 m^2\)); \( T_{\text{trig}} \) is the triggering time \([s]\); \( T_{\text{strig}} \) is the self-triggering time \([s]\); \( \text{QE} \lambda \) is the quantum efficiency for a different wavelength; \( N_{\text{tot}} \) is the number of carriers needed for triggering.
3. Experimental setup
A scheme of the experimental setup is shown in Figure 3. Light from the laser sources with wavelengths $\lambda_1 = 405$ nm and $\lambda_2 = 532$ nm passes through the prism and is reflected from the side with the sample in the total reflection regime.

The total internal reflection fluorescence (TIRF) is an optical technique that provides for the excitation of fluorophores in an extremely thin axial region (“optical section”). The method is based on the following principle: when excitation light is totally internally reflected in a transparent solid (e.g., coverglass) at its interface with a liquid, an electromagnetic field (the evanescent wave) is generated in the liquid at the solid-liquid interface and is the same frequency as the excitation light. Since the intensity of the evanescent wave exponentially decays with the distance from the surface of the solid, only fluorescent molecules in a layer a few hundred nanometers thick are efficiently excited.

![Figure 3](image)

*Figure 3. 1 — laser, 2 — prism TIR, 3 — sample, 4 — pin diode, 5 — computer.*

The fluorescence intensity of biomolecules was recorded by a computer using a dynamic pin diode placed directly under the sample at a distance of 0.1 m. Due to this design using a prism-total internal reflection, the main beam was not registered by the device, only the fluorescence signal through the filter was received by the diode, then the output signal was entered to the computer to record the triggering time.

4. Samples
Rhodamine 6G and chlorophyll were used as test objects. It was necessary to conduct experiments on the registration of fluorescence in different wavelength ranges to test the sensitivity of the recording instrument.

![Figure 4](image)

*Figure 4. Fluorescence of chlorophyll (a) and rhodamine 6G (b).*
Rhodamine 6G
Rhodamine 6G derivatives are molecules extensively used as a fluorescent labeling reagent due to their excellent photophysical properties, such as absorption and emission extended to the visible region, high fluorescence quantum yield, and high absorption coefficient [8]. Rhodamine 6G has the lasing range at 555 to 585 nm with a maximum at 566 nm [9]. It is often used as a tracer dye in water to determine the rate and direction of flow and transport. Rhodamine 6G dyes are extensively used in biotechnology applications, such as fluorescence microscopy, flow cytometry, fluorescence correlation spectroscopy and enzyme-linked immunosorbent assay. Rhodamine 6G is soluble in water, methanol and ethanol [8].

Chlorophyll
Chlorophyll is the major light-harvesting pigment in photosynthesis. As known, photosynthesis is a powerful indicator of the internal state of the green cells and the amount of chlorophyll shows the ecological status of water objects and natural reservoirs. The fluorescence spectrum of chlorophyll is in the range from 600 to 700 nm.

5. Results
Test measurements on the prism without a sample and on the prism with water as a sample were performed. An object in a volume of 0.1 ml was placed on the bottom face of the prism to form a thin layer. The type of the excitation laser, the concentration of the investigated objects and calculated fluorescence intensity are summarized in Table 1.

| Object               | Concentration | Type of laser (λ, nm) | Intensity, mW/mm² |
|----------------------|---------------|-----------------------|-------------------|
| Clean prism          | —             | 405                   | 0.01              |
|                      |               | 532                   | 0.00              |
| Distilled water      | 100 %         | 405                   | 0.09              |
|                      |               | 532                   | 0.06              |
| Chlorophyll          | 25 % (dissolved in spirit) | 405                   | 11.98             |
|                      |               | 532                   | 0.08              |
| Rhodamine 6G         | 2 % (dissolved in water) | 405                   | 12.81             |

It may be noted that there is a low scattering signal from water (this effect may be caused by the presence of a small number of dust particles in the water). The highest luminescence intensity is exhibited by molecules of chlorophyll (in case of excitation by 405 nm laser) and rhodamine 6G (in case of excitation by 532 nm laser). The signal from rhodamine is not observed on wavelength 405 nm as it is out of lasing range of rhodamine 6G. Dynamic pin-photodiode demonstrated high sensitivity and measurements will be continued in our further works.

6. Conclusions
The preliminary results of the study of fluorescence by a dynamic pin photodiode were obtained. A high sensitivity of the suggested device is shown. In our further studies more measurements of fluorescence from different biological solutions will be conducted.

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