Optica Applicata, Vol. XXXIV, No. 1, 2004

Application of the fluorescent fiber sensor for tumor cells quantification

ELŻBIETA M. BEREŚ-PAWLIK[1], FILIP DYBAŁA[2], WOJCIECH MICHALSKI[1], DANUTA DUŚ[3]

[1] Institute of Telecommunications and Acoustics, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

[2] Department of Physics, Warsaw University of Technology, ul. Koszykowa 75, 00-662 Warszawa, Poland

[3] Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (PAN), ul. Weigla 12, 53-114 Wrocław, Poland

In order to effectively treat or destroy tumor cells we should first detect and localize them. The paper presents a specially designed and constructed fluorescent fiber sensor of a very small size, meant for detecting live tumor cells. The sensor was used to measure the luminescent signal's intensity coming from optically excited cells stained with propidium iodide. The intensity of the luminescent signal is proportional to the amount of pathologically changed cells. The obtained results confirm that it is possible to conduct definite and respectable measurements of luminescent signal's intensity by means of the sensor, and at the same time these results allow us to identify tumor cells. The constructed sensor can be applied to the examination of tumor cells grown *in vitro* or taken from a living organism and examined in laboratory conditions. Because of the small size of the fiber probe it is also possible to use the sensor for detecting tumor cells inside the human body.

Keywords: fluorescent fiber sensor, examination of tumor changes.

1. Introduction

For the last few years, the methods using lasers to detect and destroy tumor cells have been developed intensely all over the world. Research is conducted towards elaborating noninvasive methods of detecting early tumor changes and destruction of cancerous cells. The most widespread and recently quickly developing, also in Poland, is the method of selective destruction of biological materials (among others cancerous cells) called photodynamic therapy (PDT). It is based on photochemical reactions with the use of sensitizers activated with laser light. The processes of photodynamic oxidization of biological materials require appropriate dyes and a light source with appropriate

power and wavelength for inducing the reaction [1]. Pathologically changed cells are selectively destroyed as a result of light-activated chemical reactions.

The paper presents a specially designed and fluorescent fiber sensor of a very small size and the results of tests conducted to detect tumor cells by means of this sensor [2].

2. Measurement setup

The crucial element of the measurement setup is the fiber optic sensor of an external, direct, fluorescent sensor. The sensor consists of three arms constituting the paths for optical signals (Fig. 1). One of the arms is a transmitting path built of a single fiber, through which the light exciting luminescence is conducted. The second arm is a receiving path composed of six fibers. Step-index (200/300 μm) fibers were used to build the sensor. Both arms are connected with a measuring probe, in which the transmitting and the receiving fibers were linked together. The fiber providing exciting light was situated in the middle of a circle made up of fibers receiving the sample response. The proposed sensor configuration ensured a good detection of luminescence signal.

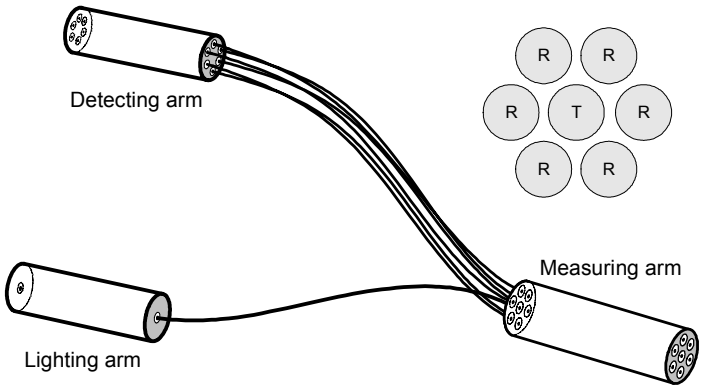

Fig. 1. Fiber sensor probe.

The block scheme of the measurement setup with the fiber sensor is presented in Fig. 2. To excite luminescence, an argon laser working continuously at selected wavelengths and having a regulated output beam power was used. The phase sensitive detection scheme was applied to measure a very low level of the luminescent signal. To enable the phase sensitive detection, we modulated mechanically the light emitted by the argon laser. The examined object was thus excited by rectangular light impulses and in consequence the received luminescent signal also had an impulse character. The photodetector located at the sensor output transformed the optical signal into voltage impulses. The filter placed in front of the ptotodetector blocked exciting light and transmitted only the luminescent light. By means of a homodyne nanovoltmeter the

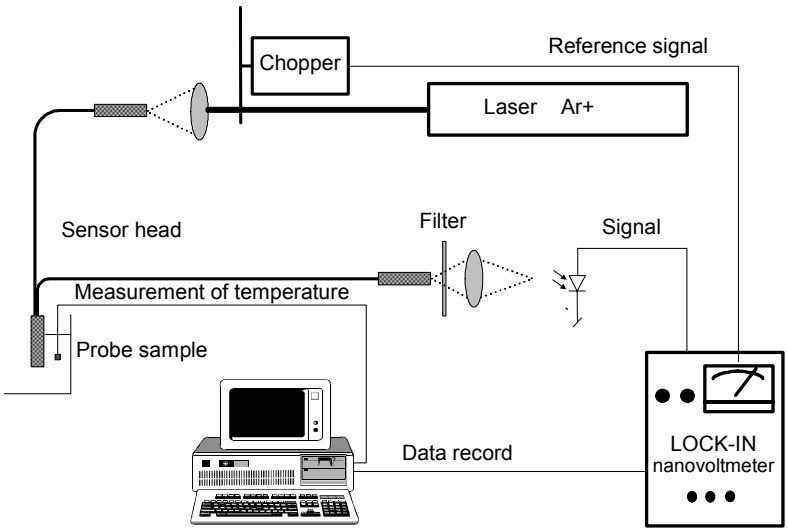

Fig. 2. Measurement setup block scheme.

first harmonic of these impulses was measured. The effective value of this harmonic was proportional to the measured luminescence signal. Laser light, apart from luminescence excitements, also causes the warming up of the examined matter. Therefore the temperature of the examined sample was monitored and recorded alongside luminescence. Both electric signals were computer-recorded.

We observed a luminescence signal in the range of 650 nm wavelength. The induced luminescent signal depended on the distance from the examined sample, in accordance with the theory described in the papers [3] and [4], where theoretical considerations of such sensors were presented. The measurement setup enabled such an experiment. The maximum intensity of luminescence was measured for given measurement conditions, with the placement of the measurement probe towards the examined sample being changed by means of a precision table in order to obtain a maximum value of luminescence.

3. Dye-testing of the measurement setup

There was a number of luminescence measurements, including the investigation of the laser power's impact on the applied dye's luminescence intensity. The dye solution was put in a quartz vessel. For each definite level of the laser beam power changes in luminescence intensity and temperature were measured in the time function. Figure 3 shows the example results obtained from these measurements by means of a 514 nm long laser beam. The continuous line depicts the luminescent signal intensity normalized up to its maximum value (marked as 100%), obtained in particular measurement series. The intermittent line shows temperature changes in the examined sample while illuminated with laser light.

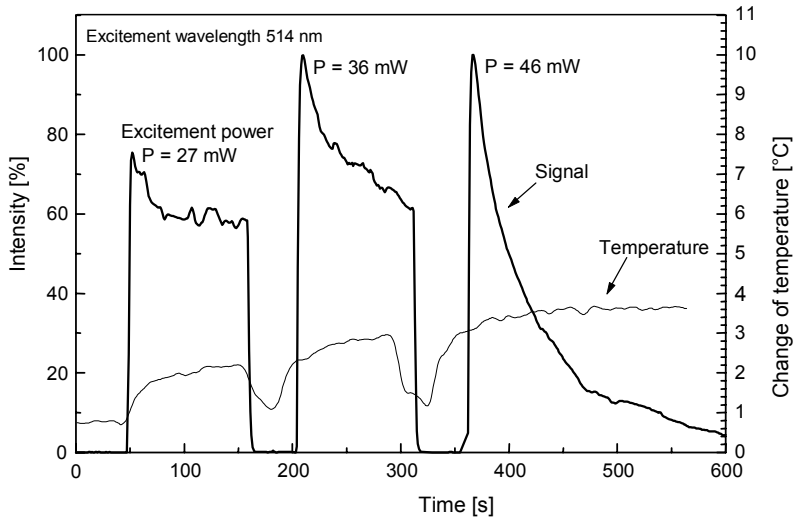

Fig. 3. Dye luminescence intensity characteristics in excitation power function.

What can be concluded from the graphs presented is that using too high intensities of the laser beam exciting luminescence is not desirable, because in this process blanching can be observed.

There was a number of tests conducted with the luminescence exciting laser power and the concentration of propidium iodide dissolved in a physiological salt solution

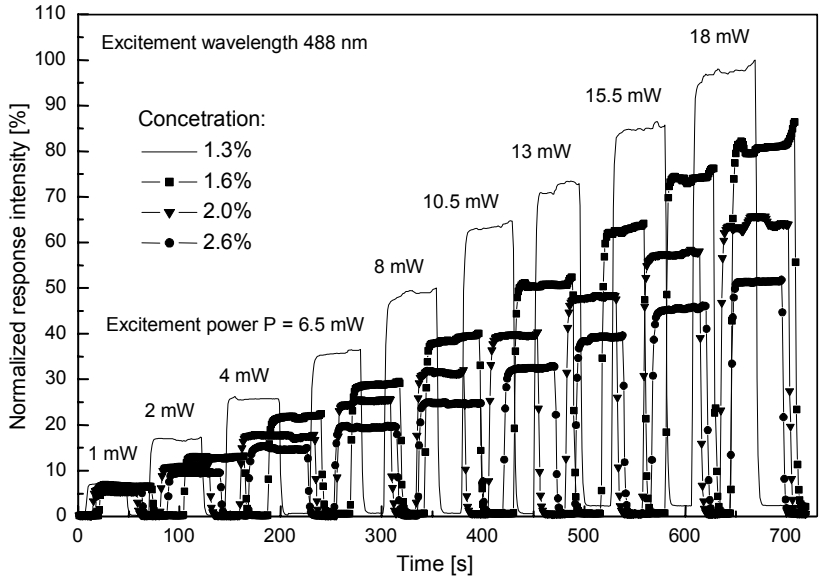

Fig. 4. Luminescence intensity for different dye concentrations and different laser exciting powers.

especially suited to obtain an optimal luminescent response from the examined samples. What is presented in Fig. 4 is the result of examining such ratios with the use of a 488 nm wave argon laser as the exciting source.

The series of curves shown above correspond to different concentrations of propidium iodide used as markers, equal to 1.3%, 1.65%, 2.0% and 2.6%, respectively. Luminescence intensity illustrated as column heights reflects different powers of the exciting signal, expressed in absolute units beside each column. The receiving of the luminescence signal for particular concentrations was done within a few dozens of seconds, for each exciting power of the laser line. The graph shows that using a lower dye concentration is more advantageous and gives a higher rate of the luminescent signal.

4. Results of human tumor cells luminescence examinations

The measurement method was verified on specially processed tumor cells, prepared by specialists working in a laboratory of the Polish Academy of Sciences (PAN). The test was conducted on tumor cells of human breast cancer T47 D, grown and preserved in a 70% ethanol solution in the temperature of 20 °C in the period of 24 hours. These cells were rinsed twice in a PBS (phosphate buffered saline) solution and next incubated in 37 °C for 30 min with 100 U RNAse/ml (Sigma). Cells processed this way were dyed with a 50 μg/ml of propidium iodide (Sigma) solution used for quantitative marking of the DNA in the cells.

The cells for testing were grown in 24-well tissue culture plates vessels. The cells grew in horizontal directions to finally cover the entire surface of the wells bottom with a monolayer. Having grown into the monolayer, they started growing up in vertical directions. Each of the six columns included vessels with slightly different, but approximate within a given column, numbers of marked cells. The columns differed from each other in the degree to which the growing cells covered the vessel's bottom. In the first column the cells in four vessels were made to cover around 1/2 of

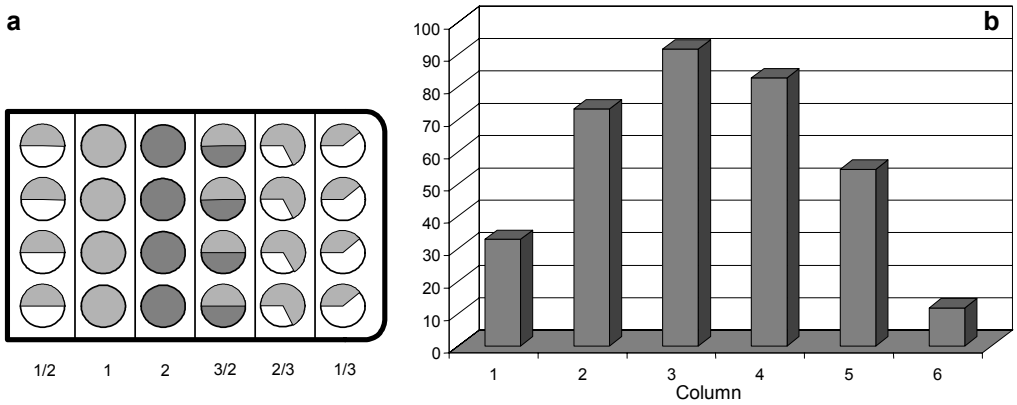

Fig. 5. Distribution of tumor cells (**a**) and test results (**b**).

the bottom surface, in the second column the cells built a single layer on the bottom's entire surface, in the third column there was an approximate double layer, one above the other, in the fourth – 1.5 layers were generated, in the fifth – the cells covered around 2/3 of a single layer, and in the sixth – around 1/3 of a single layer. The luminescence intensity of the grown tumor cells was measured. The result is presented schematically in Fig. 5 in the column diagram showing the luminescent signal average values obtained as the result of testing particular columns. The marked cells were illuminated with an argon laser with the wavelength of 488 nm and the power of 5 mW.

The distribution of the marked tumor cells' measured luminescent signal intensity in particular columns corresponds precisely to their quantity. This proves the high sensitivity of the measurement method in question and the correct construction of the sensor. It is possible to conduct by means of this sensor definite and repeatable measurements of the luminescent signal intensity, proportional to the number of tumor cells marked with fluorochromes.

4. Conclusions

The presented results of measurements done by means of the described luminescent sensor confirm that it can be used to determine the number of tumor cells. The constructed sensor can be used in the luminescence measurements of cells grown *in vitro* or taken from a living organism and examined *in vitro* in laboratory conditions. The sensor's advantages are undoubtedly its simple construction and the small size of the fiber probe.

References

[1] GRACZYK A., SOBCZYŃSKA J., STANOWSKI E., *Zastosowanie laserów do fotodynamicznej metody rozpoznawania i leczenia nowotworów*, X Szkoła Optoelektroniki Zegrze k/Warszawy, 1995, pp.141–170, (in Polish).
[2] DYBAŁA F., M.Sc. Thesis, Wrocław University of Technology, Wrocław, Poland, 2000 (in Polish).
[3] HE GANG, FRANC W. CUOMO, J. Lightwave Technol. **9** (1991), 545.
[4] *Ibidem,* p. 1618.

Received September 1, 2003
in revised form February 2, 2004