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Determination of oxygen saturation and photosensitizer accumulation in the tumor with the help of LED- and laser diode-based irradiation sources and fiber-optics probes

Abstract. *In this paper the possibility of monitoring oxygenation of the tumor tissue through the registration LED technology in photodynamic therapy. The method is applied in the wavelength range, where the spectral difference between oxygenated and deoxygenated hemoglobin is rather high. This method will also help assess the effectiveness of PDT, the level of vascular damage and the degree of the tumor oxygenation.*

Streszczenie*. W artykule przedstawiono możliwość monitorowania stopnia natlenienia tkanki nowotworowej poprzez wykorzystanie źródeł światła LED (light-emitting diodes) w terapii fotodynamicznej (PTD). Metoda może być wykorzystana w takim zakresie długości fal, w którym różnica spektralna pomiędzy hemoglobiną dotlenioną i niedotlenioną jest wysoka. Zaproponowana metoda może być również wykorzystana w ocenie skuteczność terapii PTD, ocenie poziom uszkodzenia naczyń oraz stopienia natlenienia nowotworu. (****Wyznaczanie saturacji tlenem oraz akumulacji fotouczulacza w nowotworze przy użyciu sondy światłowodowej oraz źródeł promieniowania wykorzystujących diody LED oraz diody laserowe****).*

Keywords: photodynamic therapy, oxygen saturation, CCD spectrometer, diffuse reflectance spectroscopy, signal fluorescence
Słowa kluczowe: terapia fotodynamiczna, nasycenie tlenem, spektrometr CCD, spektroskopia odbicia rozproszonego, sygnał rozproszenia i fluorescencji.

1. Introduction

The photodynamic therapy (PDT) procedure involves administration of a photoactive substance – a photosensitizer (PS), which selectively accumulates in malignant tissues, followed by light (laser) irradiation at a wavelength that corresponds to PS absorption characteristics [1, 2]. PS can interact with molecular oxygen, transforming absorbed light energy into singlet oxygen which damages cancer cells due to its strong oxidant activity. Therefore, the effectiveness of PDT treatment depends on molecular oxygen saturation level of irradiated tissues.

In clinical settings tumor is usually exposed to laser radiation continuously and laser beam does not move for the duration of procedure. Upon tumor light exposure molecular oxygen is rapidly depleted because of its interaction with PS followed by singlet oxygen formation [3, 4]. Replenishing of molecular oxygen (and PS) in malignant tissue depends on the blood circulation in this area and requires a certain time during which further tumor irradiation is meaningless and leads to PS photodestruction without achieving the desired effect. Therefore, application of modulated radiation will provide two advatages: minimizing the rate of PS photodestructin and allowing molecular oxygen to re-saturate in the treated tissue [5].

Utilizing a feedback from the treated area to guide the laser beam makes it possible to stop laser irradiation when the oxygen concentration drops and turn laser on automatically upon sufficient concentrations in the zone of exposure [6]. Monitoring of oxygen in the tissues and corresponding correction of irradiation will increase the the PDT effectiveness.

For example, in Fig. 1 show the possibility interaction of diagnostic system (1) laser device for PDT (2) of the biological object (3).

One of method to evaluate the hemoglobin oxygen saturation and relative hemoglobin concentration in tissue from diffuse reflectance spectra in the red and infrared wavelength range is put forward in this paper. The method is applied in the wavelength range, where the spectral difference between oxygenated and deoxygenated hemoglobin is rather high. In this method use of the fiber-optics probes makes possible monitoring the oxygen saturation in surface and intra tumors. proposed here application of CCD spectrometer with accessible interface, high speed and sensitivity for detection of reflected signal, allows real-time estimate dynamics of tumor oxygenation.

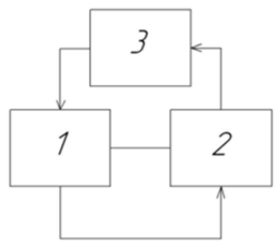

Fig.1 Scheme relation a system for treatment and diagnostics to combine a biological object

2. Materials and methods

Level of saturation (blood oxygen saturation SaO_2) is usually defined as fraction of oxyhemoglobin (HbO_2) relative to total hemoglobin ($Hb+HbO_2$) (1):

$$(1)\qquad SaO_2 = \left(\frac{[HbO_2]}{[HbO_2]+[Hb]}\right)100$$

Oxigen levels can be monitored using non-envasive pulse oximeter sensor. The typical pulse oximetry sensor contains two LED's that emits red (660 nm) and IR light (940 nm) into a tissue bed [8, 9]. Photodetector measures the changing absorbance at each of the wavelengths, allowing it to determine the absorbances due to HbO_2 in the pulsing arterial blood alone, excluding venous blood, skin, bone, muscle, fat, These oximeters are designed to monitor adults and children when saturation ranges from 99 to 70% which is phisilogycal level sufficient to ensure the normal functioning of the body. However, in case of oxygen saturation falling below the threshold (65-5%) ordinary oximeters are not sufficiently accurate and sensitive. It is important to choose proper wavelength for measuring the reflected signals with lower oxygen saturation in treated tissues .

Under low oxygen saturation there is a very significant mismatch between the product of the absorption and scattering coefficients of the 660 nm near red and 890 nm infrared light, with the near red light being more strongly absorbed and scattered (Fig. 2). This very significant absorption and scattering mismatch results in very different tissue being probed by the near red and infrared light which significantly deminishes the accuracy of the arterial oxygen saturation calculation.

Therefore, it is important that there was no big difference between depths of penetration of light from two radiation sources selected for determining the level of saturation. Using wavelengths near 730 nm and 890 nm for lower tissue oxygen saturation should balance absorption and scattering characteristics [10, 11]. As cit an be noticed in Fig. 1, since near the 730 nm extinction and scattering coefficients match closely the 890 nm extinction and scattering coefficients. In addition, 730 nm wavelenght yields smaller variation of the extinction and scattering coefficients as a function of oxygen saturation as compared to 660 nm, thus resulting in more accurate oxygen saturation estimates over a wider range of saturations. If we choose these wavelengths, we minimize the initial difference background signals and have a chance to measure the difference signals of even a small variations in the level of oxyhemoglobin.

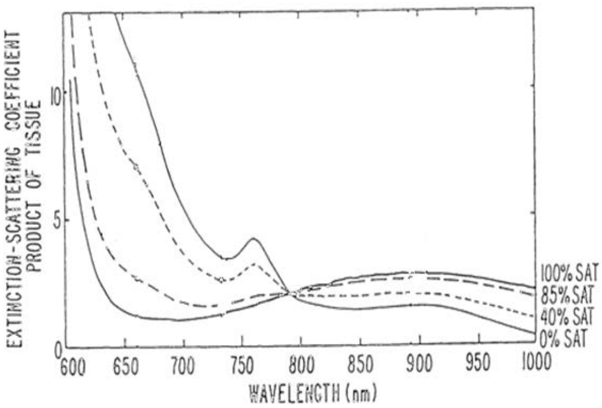

Fig.1. Absorption x scattering (Absorption - times - scattering) coefficients in biologycal tissues of light wavelenghts 600-1000 nm [11]

3. Results and discussion

To in order to test out proposed method we built d a system for treatment and diagnostic by LED (LED-light emiting diode), lasers and conducted a series of experiments. Scheme of the experimental setup for the fluorescence and oxigen saturation measurements is shown in Fig. 2.

To render the PDT method of tumor treatment, the semiconductor laser "Lika-Surgion M" ("Fotonika Plus", Ukraine) was applied. Tumors were irradiated using continuous setting, wavelength (λ = 660 nm), power of light (0,1-3 W). The device can modulate the laser radiation with different duty cycle signal, thereby adjusting intervals of the pulse pause depending on level of molecular oxygen and FS.

For fluorescence excitation laser radiation (λ = 405 nm), power (50 mW) was used in this pilot device "Lika- Khirurg M”. System for measuring the fluorescence of tumor tissue based on exciting 405 nm laser and fluorescence detector (CCD spectrometer Ocean Optics USB4000), allowed to monitor the dynamic as well as detect peak t accumulation of PS in tissues (670 nm). Using this effect we can precisely determine the tumor location, PS concentration in the area and thus monitor the PDT effectiveness. In addition, highly sensitive spectrometer was used to outline borders of malignant tissue.

To determine the level of saturation LEDs with wavelengths of 740 and 860 nm were selected. These wavelength also allow diagnosis in the process of primary radiation with a wavelength of 660 nm. As a result, we get two spectral lines in feedback loop signals that are not overlapping. The use of LEDs was justified by low power requirements of diagnostic radiation – from 1 to 0.8 mW. Radiation from LEDs (1) focuses on the distal of optical fiber which irradiated biological object. Moreover, the radiation from the first pair of LEDs 740 and 860 nm is aimed at the tumor, and another pair - at the area of healthy tissue. This is done to in order to have a threashold, to compare the signal levels between healthy and tumor tissue as a result of comparing the level of oxygenation of tissues. Lights emmited from the LED passes through the tissue and, undergoing reflection and scattering reachesthe receiving fiber of spectrometer (4).

PMMA optical fibers with internal diameter 500 microns were used. Receiving and irradiating fibers were positioned at a short distance from surface of treated area (1 mm) to eliminate interferences with its optical properties. However, since the distance between the optical fiber is more than 3 mm (in our case, from 4 to 8 mm) frenel light reflection from tissues not recorded (Figure 3) [7].

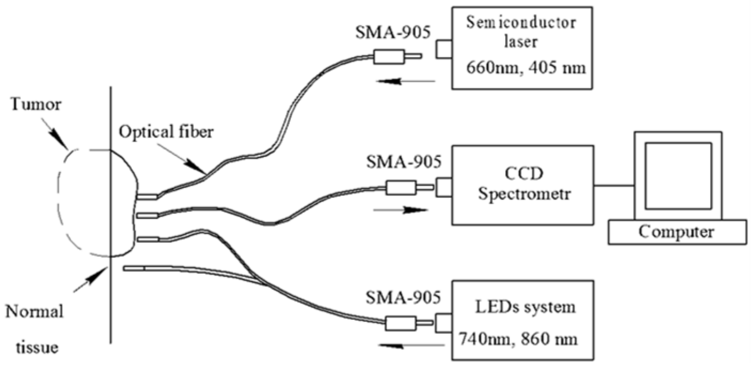

Fig.2. Scheme of the experimental setup for the fluorescence and oxigen saturation measurements

The separation between the fibers (L, Figure 3) can also prevent direct (without going through the fabric) getting radiation from transmission to reception fibers. The receiving fiber-optics probes are send a light directly on the spectrometer, „Ocean Optics USB 4000", USA), which is guided by computer via USB-interface. From receiving fiber the light gets directly on the spectrometer, „Ocean Optics USB 4000", USA), which is guided by computer via USB-interface.

Charge-coupled device (CCD) spectrometers were used to measure intensity of the fluorescence and oxigen signals. Capabilities of spectrometric technique based on CCD elements allow us to register the dynamics of PS accumulation and monitor its fluorescence level.

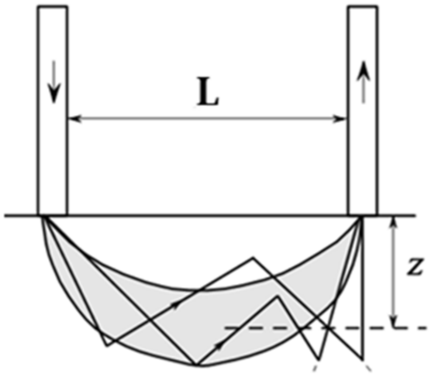

Fig.3. Scheme of the optical fibers in the object [7]

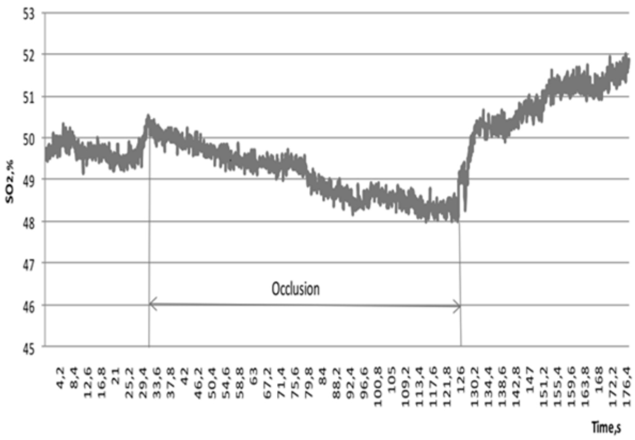

Fig. 4 The dynamics of the degree of oxygenation before and after occlusion

The main component of the spectrometer is CCD matrix consisting of separate light-sensitive elements that can carry electric charge. Light falling on the line of these elements causes charge distribution that match the given signal. The signal is then digitally displayed on computer monitor. Compared to the monochromator, the device gives instantaneous response throughout the whole spectral range, so there is no need to scan the spectrum to measure intensity at certain wavelengths.

Advantages of CDD spectrometer include:

- measurement of signal intensity simultaneously in a wide wavelength range;
- the process of obtaining and analog-to-digital signal processing take place within one CCD structure;
- software and high resolution helps to further process the results;
- fiber optic connector SMA- 905.

After a series of measurements spectrometer Ocean Optics USB4000 (USA) was acknowledged as the best choice because of its sensitivity, wide range wavelengths, compact design and user friendly software.

To determine whether the chosen method reflects the levelof oxygen saturation in the biological tissue we studied relative degree of oxygenation and hemoglobin concentration in the upper outer surface of the phalanx thumb artery occlusion, which was achieved in the braid (binder) clamping arm. The figures are experimentally measured diffuse reflectance spectra. It is seen that the shape of the spectral curve depends on the degree of oxygenation (Fig. 4). Proximity (The similarity of the) experimental results expected us to confirm the use of this algorithm to determine the level of oxygenation in the selected area.

4. Conclusions

The possibility of monitoring oxygenation of the tumor tissue through the registration LED technology. Using this system is planned to evaluate the recovery rate of molecular oxygen and determine the optimal time for re-irradiation zone tumors. This method will also help assess the effectiveness of PDT, the level of vascular damage and the degree of the tumor oxygenation.

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