Optical Techniques for Monitoring Tumour Response to Photodynamic Therapy

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Abstract:

Photodynamic therapy is a relatively new method used in malign tumour detection and treatment. The evaluation of the tumour response to this treatment can be made by optical techniques. In the paper below three optical techniques for tumour detection are presented: steady-state, time-resolved and frequency domain. In the steady- state measurements the light irradiation and detection can be either continuous or pulsed but the length of the pulse is long compared to the propagation time of the photons. The reflected light will consist of two parts: the spectral reflection and the diffuse reflectance, but the only one discussed here is the diffuse reflectance and there are two possible entities that can be measured: the total diffuse reflectance or the local diffuse reflectance. Another technique is the time-resolved one, where the transmitted or diffusely-reflected light from a femto- or picoseconds laser pulse is recorded as a function of time and a mathematical model is used to assess the optical properties. In the frequency-domain technique the optical properties are determined from the measurements of phase shift and demodulation of the detected light. These optical methods are non-invasive and nontoxic and present much interest among scientists and physicians as alternative technique for diagnosis and monitoring of cancer treatment.

Keywords: photodynamic therapy, cancer, optical techniques, steady-state technique, time-resolved technique and frequency domain technique.

1. Introduction

One of the biggest problems in medical world for many years is cancer, the second mortality cause in the world. In Romania, there is an accelerated increase of malign tumours number in the last decade and it represents the second dying cause after cardiovascular disease. The powerful impact of cancer on society reflects not only in the high mortality rate (13-14% of total deceased) but in the number of new cases registered (45000 every year) and the living affected persons (about 200000). Thus treating cancer has become a national priority in medical world.

Till now only a few cancer treatments (surgery, radiotherapy and chemotherapy) are used in hospitals but they are considered to be only curative since the sources and the evolution mechanisms of cancer tumours are not clear yet.

Research in new cancer therapy are in progress all around the world, all of them are trying to elucidate the evolution mechanisms of malign cells in order to obtain a more efficient cure and one of these methods is photodynamic therapy.

Photodynamic therapy consists of administrating a photosensitive substance in the tissue (that is selectively retained in tumours) and determines the malign tissue destruction when illuminating it with a certain wavelength. The photosensitive substance is activated in this

way and its excitation energy is transferred to molecular oxygen pushing it into a singlet state. In this excited state it has a powerful action on biological molecules leading to a selective necrosis of malign lesions. Beside the fact that it does not involve surgery or ionizing radiation, it is relatively pain free requiring minimal sedation and there are minimal side effects.

Although they are still experimental, the results of photodynamic therapy in the last years are remarkable and the method is already applied in several countries in the treatment of urinary apparatus tumours (Canada 1993), of oesophagus obstructing tumours (USA 1996), of oesophagus and lung advanced tumours (France and Holland 1997), of severe lung cancer (Germany 1997), of severe esophagus, lungs, gastric and cervical cancers (Japan 1997), of bronchi cancer (USA 2000), of Barret esophagus (USA 2003), etc.

Photodynamic therapy efficiency depends on several factors like the photosensitive substance, its preparation, administration and concentration, the type of light used for tumour irradiation and its parameters (wavelength, time exposure, pulse duration, pulse frequency, etc.) but also the methods used to evaluate the biologic response [1]. Until now there were a lot of studies aimed at analyzing these factors.

The biological response of the tissues to photodynamic therapy is not completely developed yet and is limited to nuclear methods (*radio tracing of the photo-sensitizer* [2]) and optical methods (*reflectance spectroscopy* [3] *and fluorescence spectroscopy* [4]).

This paper contains a short description of spectral reflection and diffuse reflectance that can be used to determine the photo sensitizer concentration at tumour level.

Diffuse reflectance can be measured by *steady-state techniques* based on space measurements using either continuous or pulsed light if the pulses are longer than the propagation time of the photon and *time resolved measurements* in time or frequency domain using femto- or picoseconds laser pulses (much shorter than the propagation time of photons which is typically of the order of nanoseconds.)

Low energy photons in the near-infrared wavelength region are used in cancer investigation methods such as diaphanography, transillumination and optical mammography. The use of visible light for transillumination of the breast was first suggested in 1928 by Ewing and Adair from the Breast Clinic Memorial Hospital in New York. One year later, Cuttler from the same hospital reports the first results. He used a strong illumination and made direct observation in a darkened room but the method was impractical and the diagnostic limited because blood and tumours appeared opaque and only fat was found to be translucent. From the 1950's to the 1970's a series of technique improvements were performed using filtered lamps with higher intensities at useful wavelengths. The technique was called diaphanography or diaphonoscopy.

The rapid development of optics and electronics during the last decades increased the chances to find new methods for tumour detection. The time-gated viewing technique only the first photon arriving in a short time windows are detected because they are considered to have the shortest path through the tissue and thus they are the least scattered. As a result one can get more information about the spatial localization of an embedded optical inhomogeneity. Time-gate viewing was first proposed by Maarek and his collaborators but the first in vivo results were presented by Berg and his team. They obtained a linear scan over a hand that showed a clear demarcation of the bones.

Recently some homographic systems based on time-resolved detection have been constructed. Delphy and co. at the University College, London developed a system for imaging of the female breast and neonatal head called MONSTIR (Multi Channel Opto-

electronic Near-infrared System). Chance and his collaborators at the University of Pennsylvania constructed another TCPC system and there is even a commercial system constructed by Imaging Diagnostics Systems Inc.

Gratton and his collective obtained the first in vivo images using a frequency domain system in 1993. Laser pulses from a cavity dumped dye laser (690 nm) were incident on a hand and diffusely transmitted light was detected with a cross-correlation technique. The data were presented in three images representing maps of the intensity phase and modulation in each pixel. The authors claimed that the bones and blood vessel could be imaged with a spatial resolution of the order of a millimetre.

In 1999, a group of researchers at the University of Pennsylvania have created the first three-dimensional optical images of human breast cancer in patients based on tissue fluorescence. Fluorescence diffuse **optical tomography**, or FDOT, relies on the presence of fluorophore molecules in tissue that re-radiate fluorescent light after illumination by excitation light of a different colour. The reconstructed images demonstrated significant tumour contrast compared to typical endogenous optical contrast in breast.

Several years later, in 2007, Alper Corlu and collaborators [15] present the first three-dimensional (3D) in vivo images of human breast cancer based on fluorescence diffuse optical tomography (FDOT). Fluorescence excitation and detection are accomplished in the soft-compression, parallel-plane, transmission geometry using laser sources at 786 nm and spectrally filtered CCD detection.

In 2008 a group from the University of California [14] presents simultaneous measurement of enhancement kinetics of an optical and a magnetic resonance (MR) contrast agent in a small animal breast tumour model using a combined MR-diffuse optical tomographic (MR-DOT) imaging system.

The research continues all over the world; physicians and scientists combine different methods (optical techniques, magnetic resonance, ultrasounds) in order to find non-invasive methods of detection and treatment of cancer tumours.

A good understanding of photodynamic therapy efficiency, advantages and disadvantages presumes a clear image of the physical phenomena involved in it and of the different methods of measuring, data collecting and analysis. Basically there are two main techniques: the steady-state ones (for spatially measurements using long pulses of light) and the time-resolved ones that use short laser pulses.

2. Steady-State Techniques

Basically these techniques consist of irradiating the tissue with continuous or long pulses of light and getting all the information about the tissue and the photo sensitizer concentration from reflected light analysis. The reflected light can be analyzed either by *spectral reflection* or *diffuse reflectance*. The spectral reflection is the result of refractive indices differences between the tissue and the surrounding medium on the surface texture but it will not be discussed here.

In order to determine the photo sensitizer concentration inside the tissue, which can give the physician valuable information about the location and the size of the tumour, one must first evaluate the optical properties of biological media. The measurements can be done in two different ways measuring the total diffuse reflectance or the local one.

2.1. Total and Local Diffuse Reflectance

This method consists of sending a continuous light beam through an optical fibre into the tissue and collecting the reflected ones thorough an optical fibre system (Figure no.1).

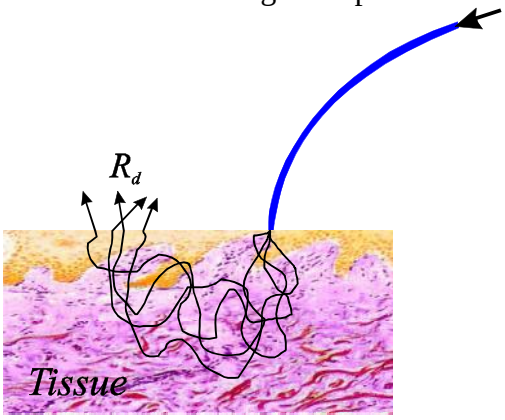


Figure no. 1: Total diffuse reflectance in steady states

The propagation of light in tissues, and hence the diffuse reflectance, is dominated in the red and near infrared region. The light flux rates in the tissues depend upon three quantities: the absorption coefficient (μ_a), the scattering coefficient (μ_s) and the anisotropy parameter (g).

To simplify the light flux we need to make some assumptions. The first one is that a flux due to a highly forwarded peaked scattering can be equated to a flux due to a different set of optical coefficients in which the scattering is isotropic. Thus one can use the transport scattering coefficient

$$\mu_s' = (1-g)\mu_s \qquad 1.$$

Which is smaller than μ_s indicating that a number $\left((1-g)^{-1}\right)$ of no-isotropic scattering events can be seen as one single isotropic scattering event. Now one can use two coefficients (μ_a and μ_s') instead of three for the light flux.

The second assumption is that for the scattered dominated photon propagation the flux rate $\Phi(\rho)$ satisfies the steady-state diffusion equation:

$$-D\nabla^2\Phi(\rho) + \mu_a(\rho) = S(\rho) \qquad 2.$$

Where D is the diffusion coefficient and $S(\rho)$ is the photon source.

Since the fibre diameter is much shorter than the distance ρ from the source to the detector one can neglect the laser and the detector dimensions.

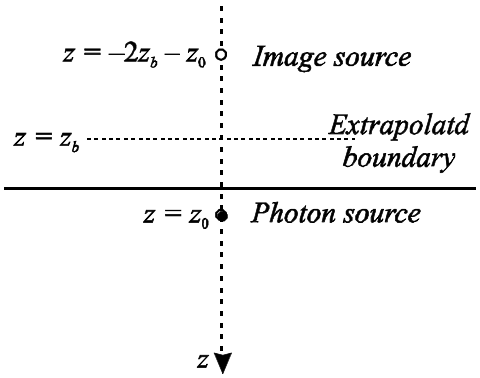
When illuminating the tissue with fibre or a small light spot the most common approximation is to put an isotropic point-source at the depth $z_0 = 1/\mu_s'$. This is possible because on an average all the photons have a scattering mean path length down in the tissue and the calculations are simplified by the assumption of an isotropic source. The problem that remains is how to define the boundary conditions and, in order to get a good approximation, to use an extrapolated boundary.

For a semi-infinite medium the boundary condition used is that there should be no photon current back into the tissue from external medium taking a mismatch of refractive indices between the tissue and surrounding medium into account. This condition is satisfied by

setting the flux equal to zero on an extrapolated boundary at $z_b = 2AD$ above the tissue surface (Figure no. 2). The factor *A* is related to the internal condition and can be derivate from Fresnel reflection coefficients or through the empirical approach.

$$A = (1 + r_d)/(1 - r_d) \quad 3.$$

Figure no. 2. Source and image source positions for a semi-infinite geometry



The flux due to point-source inside the semi-infinite medium can be forced to zero at an extrapolated boundary plane by introducing a negative "image source" of photons. The flux from a single source inside the semi-infinite medium will be given as the sum of the flux from the source and from its image calculated in an infinite medium [7], [9].

The simplest way to calculate the total reflectance for a semi-infinite medium is assuming that the incident and backscattered light are exponentially attenuated [10], [11].

Finally, the total diffuse reflectance of an infinite homogeneous semi-infinite medium is:

$$R_d \approx e^{-A\delta\mu_a} = e^{-\frac{A}{\sqrt{3(1+N')}}} \quad 4.$$

Where $N' = \frac{\mu_s'}{\mu_a}$.

In conclusion, if R_d is measured one can calculate N':

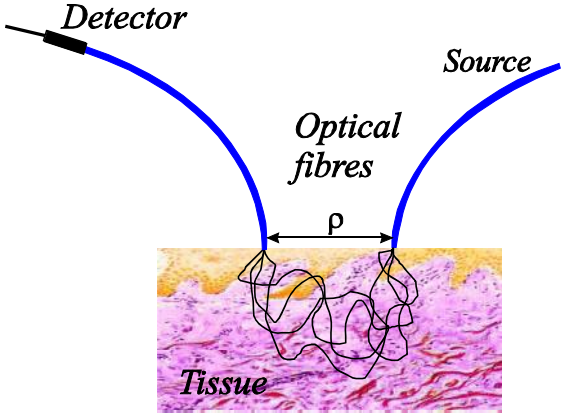
$$N' = \frac{A}{3 \ln R_d} - 1 \quad 5.$$

Which gives us valuable information about scattering and absorption properties of the tissue?

2.2. Local Diffuse Reflectance

The local diffuse reflectance consists in sending a radiation beam through an optical fibre into the tissue and collecting the reflected radiation with another fibre located at a distance ρ from the source [7], [9].

Figure no.3: Local diffuse reflectance schematic setup



For distances longer than the effective depth δ, the diffuse reflectance is:

$$R(\rho) \approx \frac{1}{\rho^m} e^{-\mu_{eff}\rho} \qquad 6.$$

Where m is a constant which has been cited as 2 or 3 depending on the model used and the distances covered. Diffusion theory and Monte Carlo simulation predict a value $m=2$ for good results on tissues so we can assume

$$R(\rho) \approx \rho^{-2} e^{-\mu_{eff}\rho} \qquad 7.$$

And we have effective attenuation coefficient

$$\mu_{eff} = \frac{-\ln[\rho^2 R(\rho)]}{\rho} \qquad 8.$$

This method allows us to quantify the photosensitive substance concentration in cancer tumour by measuring the local diffuse reflectance.

In practice this method consists of determining the local diffuse reflectance of the tumour tissue induced by the presence of the photosensitive substance. The local diffuse reflectance is measured before the photo sensitizer administration ($R_0(r)$) and after it ($R(r)$) and the result is the relative diffuse reflectance $R_0(r)$ / $R(r)$. Usually the results are presented in terms of absorbance

$$A(r,\lambda) = \lg \frac{R_0(r,\lambda)}{R(r,\lambda)} \qquad 9.$$

Optical absorbance of common tissue due to clinical concentration of photosensitive substance is usually much smaller than the absorbance due to the tissue itself [4], [8] and the local diffuse reflectance variations are usually small so one can write

$$A(r,\lambda) = -0.434\ \mu_{ap} \frac{d \ln R(r,\lambda)}{d\mu_{ap}} \qquad 10.$$

Where μ_{ap} is the absorption coefficient of the photo sensitizer.

3. Time Resolved Techniques

When light interacts with the tissue, some of it can be reflected off the surface while the rest is transported inside where it is either absorbed or scattered. The scattering and absorption properties of the tissue are strongly wavelength dependent [6]. The wavelength

region between approximately 650 nm (where the blood absorption falls off) and approximately 1300 nm (where water absorption starts) is called the *optical window* where the tissue absorption is rather low and the scattering is very high. There are two basic geometries for investigation of photon migration in tissue: transmittance (or trans-illumination) and reflectance (or backscattering).

If an ultra-short laser pulse is sent into the tissue at one surface through an optical fibre and it is collected in a point on the opposite tissue surface (trans-illumination) or on the same surface (reflectance) then the optical properties can be derived from the shape of the detected light pulse.

One can calculate the absorption coefficient and the reduced scattering coefficient of the tissue by studying the time dispersion curve (intensity versus time) from diffuse reflectance measurements.

The diffusion equation is an approximation of the transport equation and is valid far from sources and boundaries if the absorption coefficient is much smaller than the scattering coefficient (in the optical window):

$$\frac{1}{c'}\frac{d}{dt}\phi(r,t) - D\nabla^2\phi(r,t) + \mu_a\phi(r,t) = S(r,t) \qquad 11.$$

where $c' = c_0 / n$ is the speed of light in the tissue, n is the refractive index (1,37 ÷ 1,45 for soft tissues in the red/near infrared region), $\phi(r,t)$ is the energy flux rate in point r at the time t, D is the diffusion constant and $S(r, t)$ is the source energy in W/m^3.

$$R(r,t) = \left(4\pi Dc'\right)^{-3/2} z_0 t^{-5/2} \exp\left(-\mu_a c' t\right) \exp\left[-\frac{\left(r^2 + z^2{}_0\right)}{4Dc't}\right] \qquad 12.$$

Where $z_0 = 1/\mu_s$.

So we obtain the absorption coefficient as the final slope of the local reflectance curve:

$$\mu_a = -\frac{1}{c'}\lim_{t\to\infty}\frac{\partial \ln R(r,t)}{\partial t} \qquad 13.$$

The transport scattering coefficient can be calculated from the time to maximum of the reflectance curve. A requirement is that the detection point is located a large distance from the source and the scattering dominates over the absorption i.e. near infrared light is used.

There are two detection techniques for time-resolved measurements presented below: *the single photon counting* and *the streak-camera recording*.

3.1. Time-Correlated Single Photon Counting

Time-correlated single photon counting (TCSPC) is a very sensitive technique based on the detection of single photons and it is used, for example, to determine the life-time of excited states of free atoms and for fluorescence life-time measurements of molecules. In order to increase the signal-to-noise ratio, one can increase the acquisition time. The idea of the method is to measure the delay between two events e.g. an excitation laser pulse and the detection of a fluorescence photon and repeat the procedure for many times to obtain a statistical distribution of the time delay. The light pulse used may be a short (pico- or femtoseconds) laser pulse from a high-repetition-rate laser e.g. a Ti:Saphire laser emitting femtosecond pulses at typically 75 MHz or a pulsed diode laser. The detection of the light can be performed using a photon counting detector i.e. a detector that generates

one electric pulse for every photon it detects. The detected pulse and another reference pulse generated for each laser pulse are led to a time-to-amplitude converter (TAC). The output from the TAC is a voltage pulse with amplitude proportional to the time between the two input signals. This voltage is sent to a multi-channel analyzer (MCA) which converts the analogue signal to a digital one and adds one count to the channel corresponding to the voltage (a time difference between the two pulses). To obtain a correct statistical representation one has to make sure the probability of detecting more than one photon per laser pulse is negligible.

A drawback of this technique is the long acquisition time needed to get a good signal to noise ratio. The relatively low count rate can make the sampling time considerable especially when photon propagation in thick multiple-scattering objects is studied.

3.2. The Streak-Camera

The streak-camera has a better time resolution compared to TCSPC but is very expensive and has a limited dynamic range being able to detect only very weak signals. The principle behind the camera is similar to that of an oscilloscope only it displays the temporal variations of optical rather than electronic signals. The light pulse to be measured is focused onto the adjustable entrance slit of the streak-camera. The slit is then imaged onto a photocathode. When a photon hits the photocathode an electron is emitted and accelerated by a very high voltage to a phosphorus screen. A rapidly rising voltage synchronized with the incident light pulse is applied transversely across the direction of acceleration to deflect the electron. The deflected electron beam is amplified in a micro channel plate before hitting the screen. An image is created on the screen where one axis corresponds to the entrance slit and the other to time. Finally this image is captured by a CCD camera and controlled by a computer.

The output from a streak camera is an image of the entrance slit, a one-dimension image, as a function of time. This dimension can be used as a wavelength axis for simultaneous time resolved measurements over a large wavelength region. Another possibility is to use this axis to study the spatial variations over a line and through a single scan in transverse direction a full image can be obtained. One major disadvantage o streak-cameras is their high costs.

4. Frequency Domain Techniques

All the measurements described in the time techniques have their equivalents in the frequency domain which can be reached through the Fourier transform.

The absorption and reduced scattering coefficients can be calculated from the measurements of the phase shift and demodulation of the detected light.

The advantage of frequency domain measurements is that they are not expensive compared to time-resolved systems.

5. White light generation

An alternative possibility to dye laser used in time-resolved absorption is to create a sub-picoseconds white light pulses through self-phase modulation of the refractive index in water. Self-phase modulation is the frequency broadening of the optical pulse due to the nonlinear port of the refractive index of the medium.

The rapid change in intensity will lead to a spectral broadening of the transmitted pulse. Figure 4 presents a Gaussian pulse and its variations in instantaneous frequency when n_2 is positive.

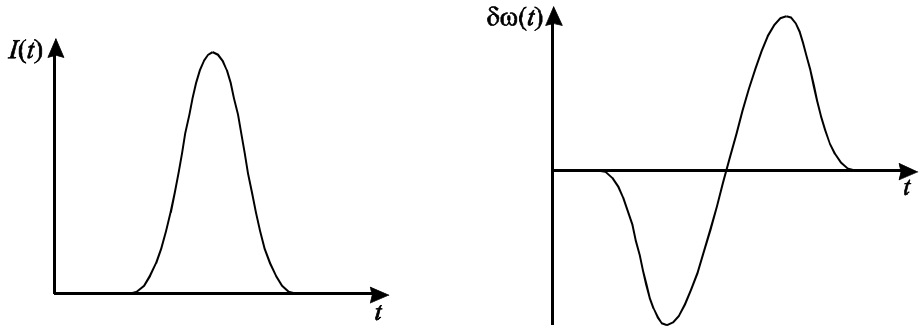


Fig. 4: Time dependence of the incident pulse and the change in instantaneous frequency of the transmitted pulse

The white light femtosecond pulses systems are used to record the in vivo **spectra** of the tumour-seeking agent (photosensitive substance) since the shape of the tumour tissue is clearly different from the healthy tissue shape.

6. Conclusions

Optical methods shortly presented here are a real alternative to X-ray tumour investigation. Although the continuous wave technique does not seem to be superior to ordinary X-ray mammography for tumour detection so far because its poor spatial resolution (especially if the lesions were located more than 2 cm below the surface) and is also limited by the difficulty on distinguish between the malignant and benign lesions it still gives us the chance to avoid the ionizing radiations.

Better results seem to be gained with time-resolved and frequency domain techniques although the data interpretation is a little more difficult as they are made from different maps containing the absorption transport and scattering coefficients of the tissues or intensity phase and modulation in each pixel. Either way some industrial companies have constructed systems based on time-resolved techniques suggesting the increased interest in this domain.

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