The optical properties and spectral features of malignant skin melanocytes in the terahertz frequency range

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Abstract. The samples of cells of mice’s melanocytes have been investigated. Their optical properties and spectral features were investigated by terahertz time-domain spectroscopy (TDS) in transmission mode. It was found that the optical properties of oncological melanocytes and normal cells are different and oncological cells have spectral features of absorption coefficient so it can be concluded that it is easy to discriminate mice’s oncological skin melanocytes by using THz TDS.

1. Introduction

Present day cancer diseases are the most important problem of medicine which requires effective methods of diagnostic and treatment. During the last decade percentage of people who had undergone of different kind of cancer was increased by 15.2 % in Russia [1]. Over the past three decades, more people all over the world have had skin cancer than all other cancers combined [2]. The most dangerous form of skin cancer is melanoma. Fortunately, melanoma is not the most often occurred of the skin cancer, because it caused the most deaths. Cells of melanoma, melanocytes, are multiply rapidly and form malignant tumors. If melanoma was diagnosed and treated early, it is almost always curable, but if it is not, the cancer can advance and spread to other parts of the body, where it becomes hard to treat and can be fatal.

Nowadays there are many techniques for investigation of skin tissues but the most traditional are in vitro. In case of melanoma if specialist would use in vitro technique it could cause most rapidly multiplying of melanocytes and sooner death accordingly. In vivo techniques include such methods as sonography, magnetic resonance tomography, magnetic resonance microscopy, Raman spectroscopy and so on. All these techniques were compared in case of frequency range, penetration depth and resolution and as the most relevance technique was chosen terahertz spectroscopy.

The main advantage of THz radiation over other techniques is that the characteristic energies of molecule’s rotational and vibrational motions lay in THz frequency region, so many chemical and biological molecules can be identified by their characteristic resonant peaks. Also THz radiation is non-ionizing and might be applied in vivo diagnostics and therapy that is the big advantage for biological structures, because there are not appeared any destruction during the procedure [3-5].

The aims of this study was to investigate the optical properties of oncological skin melanocytes at THz frequency range, to research the spectral features of oncological skin melanocytes at THz frequency range and to provide invasive diagnostics of oncological skin melanocytes in terahertz frequency range from 0.1 to 1.5 THz.
2. **Description of the sample preparation and experimental setup**

Subject of study was a monolayer of cells of mice’s melanocytes Clone M-3, which were taken from Bank of Cell Cultures of the Institute of Cytology of the RAS (Saint-Petersburg). The length of such cell varied from 20 µm to 30 µm while width is about 15 µm. The cell thickness was determined by using confocal scanning microscope Zeiss LSM-710 - 8 – 10 µm (figure 1).

![Figure 1. Photo of oncological cells of mice’s melanocytes taken by confocal scanning microscope Zeiss LSM-710.](image)

The research was performed by the THz time domain (TD) spectrometer in the transmission mode (figure 2). The characteristics of the spectrometer are following: the spectral resolution of 15GHz, the sensitivity of lock-in amplifier of 1mV, the lock-in amplifier time constant of 1s. The THz broadband pulsed radiation had the following parameters: the spectral range of 0.1–1.5THz, the pulse duration of 2.7ps, the average power of 30 mW. THz radiation was generated by femtosecond laser (Yb:KYW) irradiation of undoped indium arsenide crystal. The femtosecond laser parameters are following: the wavelength of 1040nm, the pulse duration of 120fs, the pulse repetition rate of 75MHz, the power of 1W. The sample is fixed between two THz lenses in there focuses so the THz radiation may transmit through it. The spectra in frequency domain were obtained by the Fourier transform of the measured time domain waveforms of the reference sample and the biological object.
2.1 Method of optical properties extraction

In case of our study samples were appeared as a thin film, so it was impossible to calculate the optical properties by common equations (1–6).

\[
n_{\text{eff}} = 1 + \frac{(\varphi_{\text{eff}}(f) - \varphi_{\text{ref}}(f)) \cdot c}{2 \cdot \pi \cdot f \cdot d}
\]

\[
\hat{\eta}_{\text{eff}} = n_{\text{eff}} + j \cdot k_{\text{eff}}
\]

\[
n_{\text{eff}} = \frac{n_s \cdot d_s + n_{\text{sub}} \cdot d_{\text{sub}}}{(d_{\text{sub}} + d_s)}
\]

\[
k_{\text{eff}} = -\frac{c}{2 \pi f d} \ln \frac{E_{\text{eff}}(\nu)}{E_{\text{ref}}(\nu)}
\]

\[
\hat{n}_s = n_s + j \cdot k_s
\]

\[
k_s = k_{\text{eff}}(d_{\text{sub}} + d_s) - k_{\text{sub}} \cdot d_{\text{sub}}
\]

\[n_{\text{eff}}, n_s \] — real part of refractive indices of effective medium and sample accordingly, \(k_{\text{eff}}, k_s\) — imagine part of refractive indices of effective medium and sample accordingly, \(\varphi_{\text{eff}}, \varphi_{\text{ref}}\) — phases of object signal and reference signal accordingly, \(f\) — frequency, \(d\) — object thickness, \(E_{\text{eff}}, E_{\text{ref}}\) — amplitudes of object signal and reference signal accordingly.

For this research different methods of calculation of optical properties were compared and the most reasonable and efficient had been chosen. Five models of effective medium were considered - effective medium method [6], Maxwell – Garnett method [7], Bruggeman method [8], Landau-Lifshitz-Looyenga method [9], and method of thin films [10]. There are some limitations for each of these methods except the last one. The method of thin films is more accurate in obtaining the optical properties because it based not on correlation of dielectric permittivity of all components of the structure but on calculation of conductivity of thin film, which has to be investigated [11]. The samples usually are divided into an object under study and its substrate. The point of the method of thin films is that it allows obtaining the optical properties of object which cannot be investigated without substrate. For this paper a monolayer of mice’s skin melanocytes on plastic substrate was investigated. As it was impossible to investigate cells itself, in experiments the effective structure of cells and plastic were investigated.

The calculation of complex film conductivity, in this case conductivity of monolayer of cells, was obtained accordingly to equation (7):

\[
\hat{\sigma}(f) = \frac{[(\hat{n}_{\text{sub}}(f)+1)\hat{E}_0(f)\hat{n}_{\text{sub}}(f)-1]}{z_0 E(f)}
\]
where $f$ — frequency, $n_{\text{sub}}$ — complex refractive index of substrate, $\tilde{E}_0(f)$ and $\tilde{E}(f)$ — complex electric field amplitude of THz wave transmitted through the substrate and the effective medium accordingly, $Z_0 = 377 \ \Omega$ — impedance of vacuum.

Then the complex permittivity of film was calculated as in equations (8):

$$\varepsilon_f(f) = 1 + i \frac{\varepsilon_f(f)}{2\pi f \varepsilon_0 t_f},$$

(8)

where $\varepsilon_0$ — dielectric constant, $t_f$ — thickness of sample;

then refractive index of film as in equations (9):

$$n = \sqrt{\varepsilon_f(f)},$$

(9)

then absorption coefficient of film as in equations (10):

$$\alpha_s = \frac{4\pi k_s}{\lambda},$$

(10)

where $k_s$ — imaginary part of refractive index of sample, $\lambda$ — wavelength;

and finally optical penetration depth into film was calculated as in equations (11):

$$\delta = \frac{1}{\alpha_s}.$$  

(11)

3. Results and Discussions

In our work the optical properties and spectral features of cells of mice’s melanocytes were obtained. Averaging was made by seven measurements. Observed refractive index of oncological skin melanocytes varies from 3.0 to 3.25 at the frequency range 0.1 – 1.0 THz and has the maximum at the 0.34 THz. The real part of permittivity increases from 6.5 to 9.5 and then doesn’t change at the frequency range from 0.35 to 0.75 THz and equals to 9 (figures 3 – 4).

**Figure 3.** The dispersion of complex refractive index of oncological skin melanocytes in frequency range of 0.1 – 1.0 THz.

**Figure 4.** The dispersion of complex dielectric permittivity of oncological skin melanocytes in frequency range of 0.1 – 1.0 THz.
The optical penetration depth into sample has the maximum at frequency range from 0.5 to 0.75 THz with the central peak at the frequency of 0.63 THz and equals to 80 µm (figure 5).

![Figure 5](image)

**Figure 5.** The dispersion of optical penetration depth into oncological skin melanocytes in frequency range of 0.1 – 1.0 THz.

The spectral features included the dispersion of absorption coefficient of oncological skin melanocytes was calculated, it changes from 125 to 225 cm\(^{-1}\) at the frequency range 0.1 – 1.0 THz. Also the maximum of absorption coefficient was revealed at the frequency of 0.4 THz, which may become a biomarker for determination of malignancy of skin melanocytes (figure 6). In the paper [11] normal and oncological tissues was observed (figure 7) and the comparison the results of dispersion of absorption coefficient in frequency range in paper [11] and the results of present work shows that the plots looks the same and only distinction between these two plots is intensity of the peaks. It may be explained by the thickness of investigated samples.

![Figure 6](image)

**Figure 6.** The dispersion of absorption coefficient of THz pulse of oncological skin melanocytes in frequency range of 0.1 – 1.0 THz.

![Figure 7](image)

**Figure 7.** The dispersion of absorption coefficient of THz pulse of oncological and normal tissues in frequency range of 0.1 – 1.0 THz [11].

4. **Conclusions**

The unique optical and spectral features for oncological melanocytes was determined, so it can be concluded that it is easy to diagnose such objects in THz frequency range of 0.1–2.0THz by their dispersions of refractive index which is varies from 3.0 to 3.25 and their absorption coefficient which
changes from 125 to 225 cm\(^{-1}\) at the frequency range 0.1 – 1.0 THz and have maximum at the frequency of 0.4 THz.

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