Analysis of Spectral Characteristics of Normal Fibroblasts and Fibroblasts Cultured with Cancer Cells in Terahertz Frequency Range

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Abstract— We propose a new method of cancer diagnostics based on identification of characteristic cancer spectral lines in the frequency range of 0.1–2 THz. Using terahertz spectral database of normal and pathological human tissues we could monitor the slightest changes caused by the appearance of cancer at the early stages.

1. INTRODUCTION

Nowadays despite the decreasing impact of cancer diseases [1], this problem remains relevant and it is still necessary to perform accurate diagnostics of cancer tumors at the early stages with high reliability. Invasive methods such as biopsy are predominant among various methods of cancer diagnostics [2, 3]. It means that a piece of human tissue with suspected tumor is removed, and if cancer cells are not found in this piece, the procedure should be repeated. Invasive methods are gradually being replaced by non-invasive terahertz (THz) imaging and tomography methods, which are more reliable, but do not allow unique identification of malignant tumor, and the results must be confirmed by invasive methods. Therefore it is essential to develop an independent and accurate methodology for cellular composition analysis of malignant tumors using a vast variety of heterogeneous cell cultures.

In our research we use non-invasive terahertz pulsed spectroscopy method, which has great advantages in comparison with other methods, including short time of excitation (it takes about 3–5 minutes to analyze a section of the sample) and other characteristics associated with the properties of THz radiation. There is no doubt about the statement that THz radiation is nowadays the most promising technique and favorably compares with other types of radiation. It should be mentioned that unlike microwave radiation THz radiation is not ionizing but it has sufficient penetration depth [4].

2. EXPERIMENTAL SETUP

The group of experiments was carried out using the reflection and transmission modes of the universal pulsed broadband THz spectrometer [5]. The generator of THz radiation is based on the InAs crystal in the magnetic system, bombarded with pulses of femtosecond laser FL-1. Following the passage through a teflon filter THz radiation hits the sample, which is fixed at the focal plane perpendicularly to the beam by a triaxial object table. The setup of THz spectrometer is characterized by the following parameters: the pulse power of 1 W, the pulse duration of 2.7 ps, the chopper frequency of 670 Hz, the spectral range from 0.2 to 2.0 THz, the average THz radiation power of 30 mW.

3. OBJECT UNDER THE STUDY

The research was conducted on the following cell cultures: primary human fibroblasts, transplantable lines A549 (human lung carcinoma cells) and COLO 320 HSR (sigmoid colon carcinoma cells); cell cultivation was carried out according to the recommendations of the "Bank of cell cultures" Institute of Cytology RAS. To perform the experiments cells were subcultured into wells of a 6 well flat-bottomed polystyrene plate ("Sarstedt", Germany) and incubated until a confluent monolayer was formed. To investigate the spectral characteristics of heterogeneous cell cultures two different cell types were added to one of the plate's well. Immediately prior to the measurement the biological medium was completely removed. All types of cell cultures were presented in four repeats.

The following Figure 3 demonstrates the sizes of normal fibroblasts in three dimensions.

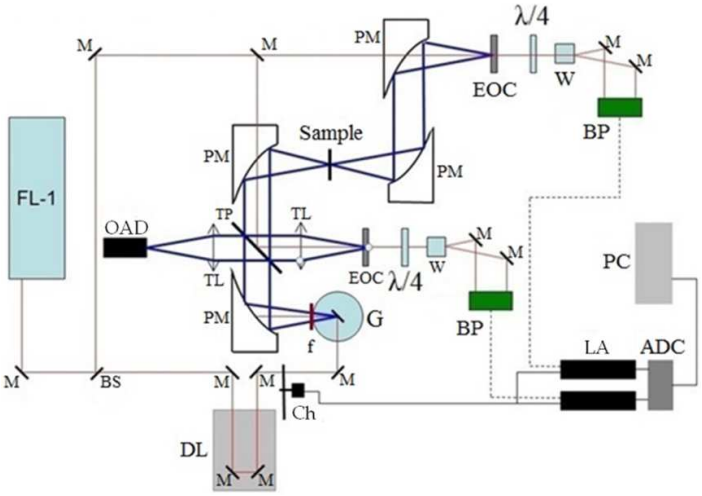
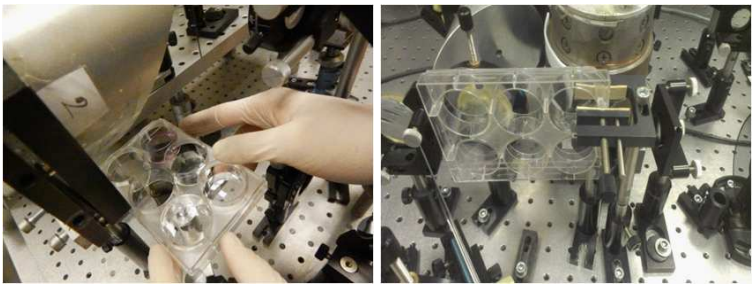


Figure 1: Scheme of the universal pulsed broadband THz spectrometer. FL-1 — infrared laser, M — mirrors, BS — beam splitter, DL — optical delay line, G — generator of THz radiation based on the crystal InAs, PM — parabolic mirrors, TL — lenses for THz radiation, f — teflon filter, TP — translucent silicon plate, Ch — chopper, EOC — electro optical crystal of CdTe, W — Wollaston prism, BP — balanced photodiodes, LA — lock-in amplifier, ADC — analog-to-digital converter, PC — personal computer, OAD — opto-acoustic detector (Golay cell).



(a) (b)

Figure 2: Photo of (a) reflection mode and (b) transmission mode of the universal pulsed broadband THz spectrometer.

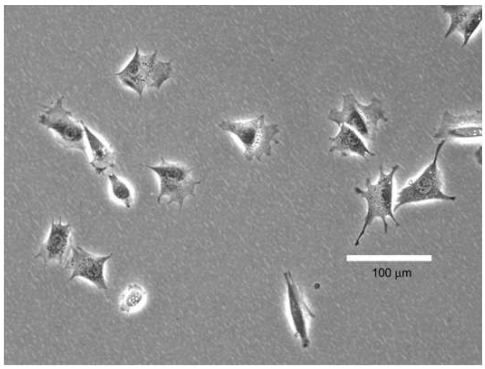


Figure 3: Normal fibroblast cells in-vivo [6].

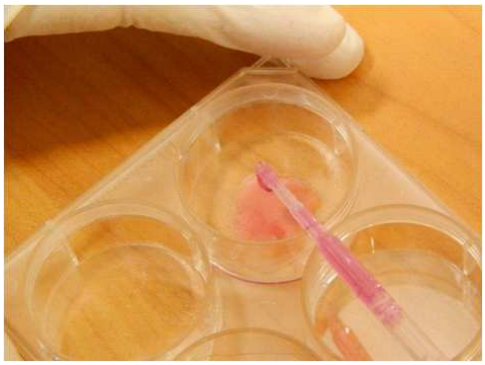


Figure 4: Sample preparation before the experiment.

As per this image the length and width seem to be $\sim$ 30–50 µm (the area should be roughly around 900 μm^2). The third dimension (thickness) can be assumed to be around 3–7 µm [6].

The following Figure 4 illustrates the process of samples preparation before the experiments: Physiological fluid which supports cultured cells must be removed using a special instrument to avoid the absorption of THz radiation by water. The duration of the experiment should not exceed 5 minutes due to the cells death in the absence of liquid.

4. RESULTS AND CONCLUSIONS

Based on the intensity of the reflection and transmission peaks we could make a conclusion about normal/abnormal state of the test cells.

Before conducting the main research the spectrum of the polystyrene material was obtained and analyzed in order to normalize the spectra of investigated samples (Figure 5).

The reflection and transmission spectra of normal fibroblasts, cancer cells, and fibroblasts cultured with pathological cells (A549, COLO 320 HSR) were obtained using standard THz pulsed spectroscopy technique in the frequency range of 0.2–2 THz.

The normalized and averaged reflection spectra of fibroblasts are presented in the Figure 6. It should be mentioned that spectra averaging was performed for the measurements of two groups of identical cells. In case of the fibroblasts with A549 cells one high intensity reflection peak at the frequency of 1.43 THz remains unchanged, the peaks at the frequencies of 1.69 and 1.77 decrease, the peaks at other frequencies are suppressed. In case of the fibroblasts with COLO 320 HSR cells the intensity of reflection peaks at the frequencies of 1.11, 1.33 and 1.43 THz decreases and one case of increase was registered at 1.77 THz.

The transmission spectra of fibroblasts were obtained and processed in a similar manner, and are presented in the Figure 7. In this part of the study we examine the case when fibroblasts were cultured with cancer cells A549. As was expected in this case two transmission peaks appear at the frequencies of 0.92 and 1.93 THz, the high intensity peak at the frequency of 1.52 THz disappears.

The key aspect of non-invasive diagnostics of human integumentary tissues diseases is a possibility to determine different cell types in the examined area. This is especially important in the diagnostics of tumors of various genesis. Appearance of the characteristic peaks, which are specific to certain cells, in the reflection and transmission spectra of the skin samples can be an early marker of malignant transformation.

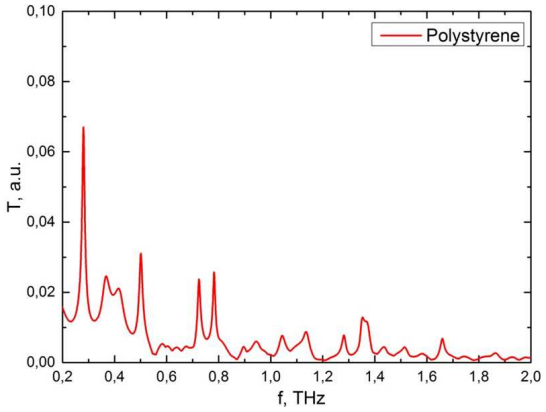


Figure 5: Transmission spectrum of polystyrene substrate without any cells.

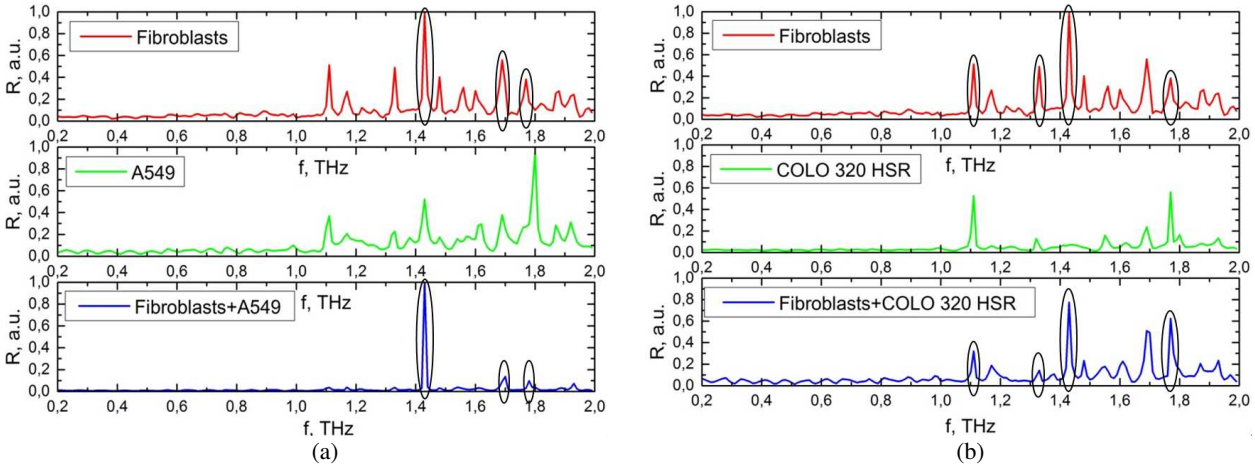


Figure 6: Reflection spectra of normal fibroblasts and fibroblasts cultured together with (a) A549 cancer cells, (b) COLO 320 HSR cancer cells.

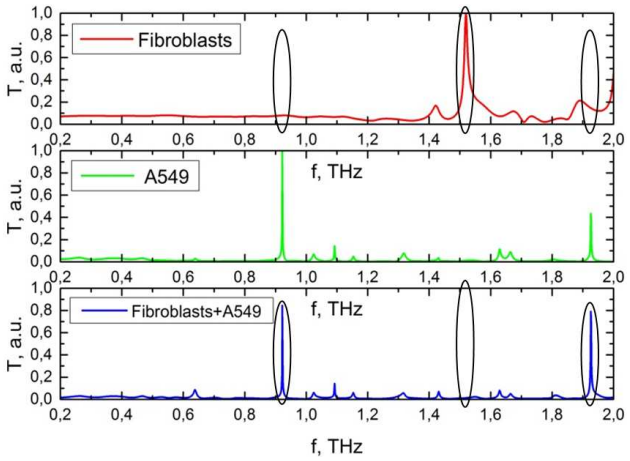


Figure 7: Transmission spectra of normal fibroblasts and fibroblasts cultured together with A549 cancer cells.

Thus, in this paper we propose a new method of diagnostics based on identification of characteristic cancer spectral lines, which allows monitoring of the appearance of cancer at the early stages.

ACKNOWLEDGMENT

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