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THz-SPECTROSCOPY OF BRAIN CANCER AND NORMAL CELL LINES USING CONTRAST NANO-PARTICLES

BY

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Abstract. The paper deals with the spectral investigations performed on a variety of significant biological samples to understand the interaction between THz radiation and biological systems at the molecular level, based on the differences in the water content and of the structural variations of the normal and neoplasia structures with different THz absorption rates. The operational stages of the spectroscopy methodology in the THz field consist of: preparing the samples to be included in the measuring cell, fixing the measuring cell, calibrating the device, performing measurements and interpreting the results. The most relevant optical phenomena in the THz domain must be based in particular on the absorbance phenomena, respectively by evaluating the absorbance difference between the activated with contrast agent and inactivated biological environment, and respectively between normal and cancerous tissue activated with contrast agents. The optimal domain recommended for molecular imaging in the THz domain is about 1.05,…,1.15 THz, at which the absorbance is approx. 6.5 times higher in tumor cells comparing to normal cell when using contrast agents.

Keywords: THz spectroscopy; contrast nanoparticles; brain cancer detection; optical absorbance and transmittance.

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1. Introduction

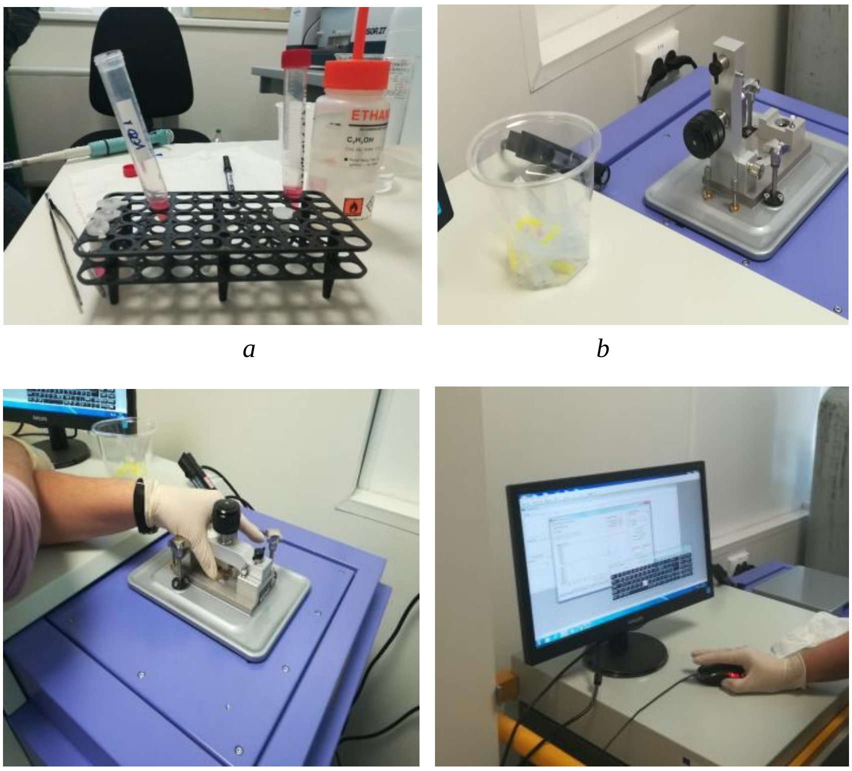
Cancer is one of the most widespread diseases today, with a significant impact on quality of life, health budgets and a great emotional impact on families. Due to their silent nature, rapid and nonspecific spread in the body with metastasis formation, due to differentiated reactivity to oncolytic therapy or to the ability to suppress immune response, neoplastic formations are normally discovered in an advanced phase of carcinogenesis. Recently, Terahertz radiation has attracted attention due to its detection capability, while also displaying non-invasive and non-ionizing properties. The design of terahertz tools has led to impressive discoveries in THz biomedical research, i.e. the medical imaging devices based on such sources have already entered the market (Yang *et al.*, 2016). Current research literature describes spectral investigations performed on a variety of significant biological samples to understand the interaction between THz radiation and biological systems at the molecular level, based on specific resonance processes of the electronic, vibrational and rotational behaviours of complex biological molecules. Based on the differences in the water content and due to the structural variations of the tissues, the normal and neoplastic structures clearly show a different THz absorption, as cancerous tissue contains more interstitial water due to abundant vascularization or edema, as already demonstrated by PET, MRI (Yu *et al.*, 2012; Oh *et al.*, 2012; Han *et al.*, 2017). The purpose of the paper was to investigate the action of contrast agents for THz imaging, carried out on normal and tumoral brain cells, as in vitro models. A major problem in THz imaging is related to the interaction of the wave energy with the substance, essentially within the biological environment to be analysed. Even if the sample parameters are predefined correctly, and the software package is used efficiently with all its facilities, there are clear limitations regarding the way of evaluating the THz methodology (transmission and reflection respectively) vs. the frequency used in the THz domain.

2. Experimental Conditions and Equipment

The imaging tests in the THz domain were performed with a last generation TDS-spectroscopy equipment in the THz domain, upon cellular samples as follows: phosphate saline buffer (without cells, for reference); normal cells; normal cells coated with contrast agents (Fe_3O_4-nanoparticles coated with carboxymethylcellulose); tumoral cells; tumoral cells coated with contrast agent (Mihai *et al.*, 2018).

The culture of neuronal cells is particularly challenging since mature neurons do not undergo cell division. One way to overcome this is to establish secondary cell lines that are derived from neuronal tumors and have become immortalized, having the advantage of being able to be grown fairly easily in cell culture to give unlimited cell numbers as well as minimizing variability between cultures. Differentiated commercial cells are morphologically similar to primary neurons and show increased expression of neuron-specific markers. The laboratory samples were based on commercial Vero cell lines (normal cells) and Brain tumoral cell lines – ECACC (Sigma Aldrich).

The operational stages of the spectroscopy methodology in the THz field consist of: preparing the samples to be included in the measuring cell, fixing the measuring cell, calibrating the device and performing the measurements, Fig. 1.

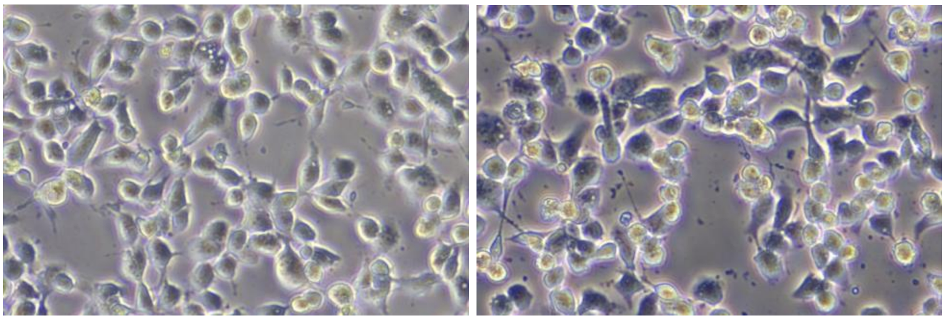


a *b*

c *d*

Fig. 1 – Operational stages of the spectroscopy in the THz field:
a – sample preparation; *b* – fixing the measuring cell; *c* – calibration of the device;
d – performing the measurements

It was previously noticed that the rate of phagocytosis of contrast agents is usually higher for cancer cells, and this is an important advantage for in-vivo imaging technology, but, for the purpose of the herein described in-vitro spectroscopy technique, the same concentration of contrast agents was used for both normal and tumoral cells (Ciobanu *et al.*, 2018). Both cell types were seeded into 12 well plates (80.000 cells/well), allowed to grow overnight in a CO_2 incubator, at 37°C. After the medium was discarded and the cells were washed, the medium was supplemented with the contrast agent: Fe_3O_4-nanoparticles coated with carboxymethylcellulose, with the dilutions of 1:100 in order to check the cells viability by MTT assay. After 24 hours, the plates were investigated with a phase contrast microscopy (Nikon TS2 inverted microscope, 200x magnification), Fig. 2, in order to evaluate the impact of nanoparticles on cells. Accordingly, no significant changes on cell morphology or density were registered.



a *b*

Fig. 2 – *a* – Morphology of normal cells; *b* – cells treated with contrast agent.

3. Experimental Results and Discussion

The analysis of the absorbance of biological samples in the THz domain, as depending on the wavelength (micrometers), led to the following observations: the optical activation with contrast nanoparticles can be observed in normal cells, with a maximum in the frequency area of 1 THz, but without showing a significant difference in absorbance compared to inactivated cells; the THz analysis vs. frequency, without the presence of contrast nanoparticles, leads to relevant absorbance effects in both normal and tumoral cells, especially at higher frequencies, but without being able to state that the effect is selective enough for a clear detection of tumoral tissue; the THz analysis vs. frequency in the presence of contrast nanoparticles leads to relevant absorbance effects mainly for the tumoral cells, especially at higher frequencies.

In Figs. 3 and 4 the absorbance difference for both normal and tumoral cells with and without contrast agent is briefly presented. It is obvious that the optical effect induced by the contrast agent for both normal and tumoral cells becomes relevant after 250 micrometer wavelength, at which both normal and tumoral cells are optically activated simultaneously by the contrast agent. In this domain, the absorbance may become approx. 6.5 times higher in tumoral cells comparing to normal cells, and the optical discrimination between the two types of cells is relevant enough for using such a method for bio-medical analysis.

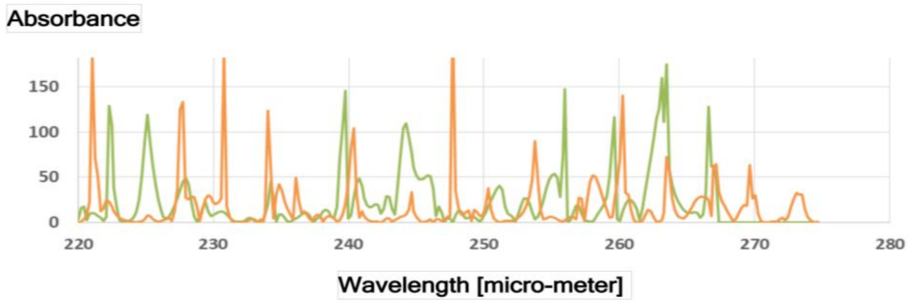


Fig. 3 – Absorbance difference for normal cells with (orange) and without contrast agent (green).

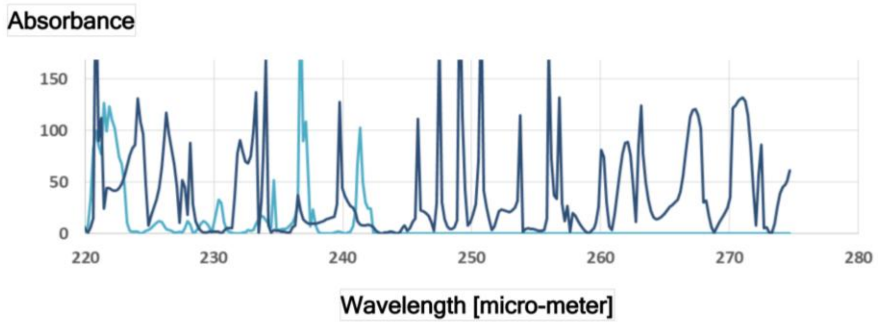


Fig. 4 – Absorbance difference for tumoral cells with (dark blue) and without contrast agent (light blue).

The analysis of the transmission of biological samples in the THz domain, as depending on the wavelength (micrometers), led to the observations that the optical activation with contrast nanoparticles is recorded in the normal cells, with a maximum also in the frequency area of 1 THz, but without showing a significant difference of absorbance against inactivated cells; on the other hand, the exposure analysis vs. frequency, without the presence of contrast nanoparticles, does not lead to relevant optical transmission effects, neither in normal cells, nor in tumoral cells; only the THz analysis vs. frequency in the presence of nanoparticles lead to clear optical transmittance effects, making observable mainly the tumoral cells, Figs. 5 and 6.

Analysis of the biological samples reflectance in the THz domain, depending on the wavelength (micrometers) emphasised that: the influence of the nanoparticles is relevant, as it leads to a distinct reflectivity with respect to the environment on which they were dispersed; the activation effect of nanoparticles is observed especially in normal cells, but without showing a significant difference of reflectance with respect to the inactivated cells; the analysis vs. frequency and the presence of contrast nanoparticles do not lead to relevant optical reflectance effects in tumoral cells, in order to result in their clear optical detection.

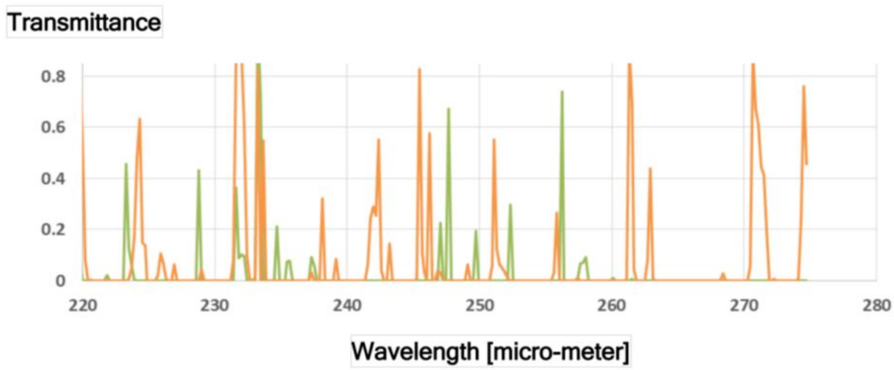


Fig. 5 – Transmittance difference for normal cells with (orange) and without contrast agent (green).

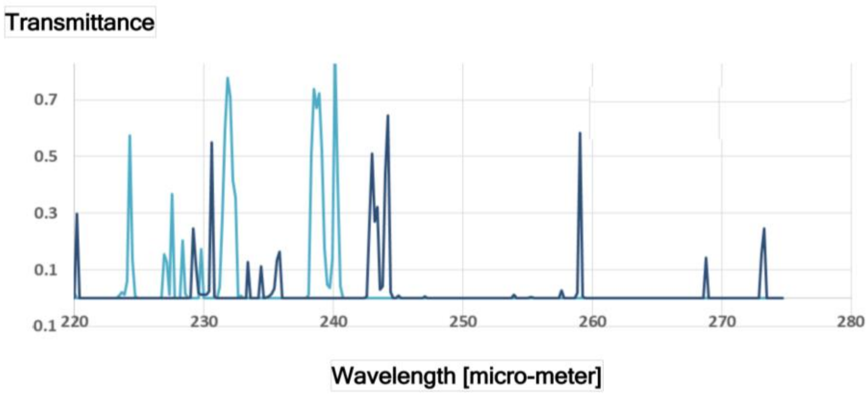
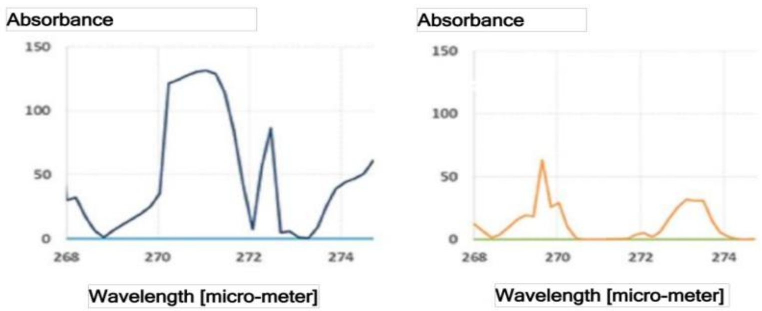


Fig. 6 – Transmittance difference for tumoral cells with (dark blue) and without contrast agent (light blue).

Based on the spectral results, it is assumed that the most relevant optical phenomena in the THz domain must be based in particular on the absorbance phenomena, respectively by evaluating the absorbance difference between the activated with contrast agent and inactivated biological environment, and respectively between normal and cancerous tissue activated with contrast agent. The optimal domain recommended for molecular imaging in the THz domain using as contrast agent Fe_3O_4 nanoparticle composites with carboxymethyl-cellulose, based on the difference in absorbance, is about 1.05,...,1.15 THz, at which normal and tumoral cells are activated simultaneously by the contrast agent. It should be mentioned that if working at a fixed frequency, e.g. 1.1 THz, only tumoral cells activated with contrast agent are optically active, due to the specific optical activity of Fe_3O_4 – carboxymethylcellulose composite. It is also found that at the frequency of 1 THz, the magnitude of the source and the energy are higher, which may lead to the idea that most of the energy will be reflected in the interaction with the analysed material. At higher frequencies, *e.g*. 2 THz, where the magnitude is smaller, it is recommended to analyse also the absorption in the material, because the reflection phenomenon is less relevant. The frequencies of 3 THz and higher do not seem to be relevant for medical imaging, an aspect that is also noted in the analysis of the optical properties of the functionalized contrast agents (gadolinium oxide and superparamagnetic iron oxide composites, based on carboxymethylcellulose), (Ciobanu *et al.*, 2018; Schreiner & Mihai, 2019), where the maximum effect is found in the range of 1,...,2 THz.

Hence, the optimal domain recommended for molecular imaging in the THz domain using as contrast agent Fe_3O_4 nanoparticle composites with carboxymethylcellulose, based on the difference in absorbance, is about 1.05,...,1.15 THz (corresponding to 270,...,272 micrometers), at which normal and tumoral cells are activated simultaneously by the contrast agent, and the absorbance is much higher in tumor cells comparing to normal cells, Fig. 7.



a *b*

Fig. 7 – *a* – ptimal THz domain recommended; *b* – normal cells for tumor brain cells imaging.

4. Conclusions

Based on experimental studies, it is assumed that the development of image processing software to support THz imaging for early detection of brain cancer must be versatile in the frequency range and adaptable to all measurement variants in the TDS-THz methodology (transmission or reflection), to emphasize, with the highest precision and selectivity, the differences in volume and interfaces between normal and cancerous tissue.

The most relevant optical phenomena in the THz domain must be based in particular on the absorbance phenomena, respectively by evaluating the absorbance difference between the activated with contrast agent and inactivated biological environment, and respectively between normal and cancerous tissue activated with contrast agent.

The optimal domain recommended for molecular imaging in the THz domain, by using Fe_3O_4 composites as contrast agent, is about 1.05,...,1.15 THz, due to a clearly higher absorbance of waves in tumor cells comparing to normal cells.

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SPECTROSCOPIA ÎN DOMNIUL THz A LINIILOR CELULARE NORMALE ŞI TUMORALE UTILIZÂND AGENŢI NANOSTRUCTURAŢI DE CONTRAST

(Rezumat)

Lucrarea analizează o serie de investigaţii spectrale in domeniul THz, efectuate pe o varietate de probe biologice semnificative pentru a înţelege interacţiunea dintre radiaţiile THz şi sistemele biologice la nivel molecular, pe baza diferenţelor în

conţinutul de apă şi a variaţiilor morfologice ale structurilor normale şi neoplazice, care prezintă absorbţie diferită in domeniul THz. Etapele operaţionale ale metodologiei spectroscopiei în domeniul THz constau în: pregătirea eşantioanelor pentru a fi incluse în celula de măsurare, fixarea celulei de măsurare, calibrarea dispozitivului şi efectuarea şi interpretarea măsurătorilor. Cele mai relevante fenomene optice din domeniul THz trebuie să se bazeze în special pe fenomenele de absorbţie, respectiv prin evaluarea diferenţei de absorbţie între mediul biologic activat cu agent de contrast şi mediu biologic inactivat şi, respectiv, între ţesutul normal şi canceros activat cu agenţi de contrast. Domeniul optim recomandat pentru imagistica moleculară în domeniul THz este cel situat aproximativ între 1,05-1,15 THz, la care absorbanţa este de aproximativ 6,5 ori mai mare în celulele tumorale comparativ cu celulele normale, atunci când se utilizează agenţi de contrast.