# Development of high-resolution spectroscopy methods for medical studying the pathology tissues of ENT organs

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The chemical composition of the thermal decomposition products of primary metabolites (large biological molecules) specific to the tissues of tumors of the ear, throat and nose organs was studied using high-resolution terahertz spectroscopy using samples of relatively healthy mucous membranes and neoplasms such as papillomas of the tonsils and larynx and cholesteatomas of the middle ear. A number of compounds of the family of organic acids, aldehydes, nitriles, and other organic compounds have been identified, the absorption lines of which are absent in the spectra of relatively healthy tissues. The potential is shown the possibility of using high-resolution terahertz spectroscopy to analyze the metabolic composition of neoplastic tissues.

**Keywords:** high-resolution spectroscopy, terahertz range, metabolites, ENT-organs.

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Metabolic approaches to diagnosing various diseases and pathologies are now being developed actively throughout the world. Chromatographic methods, which are often combined with mass spectrometry, and spectroscopic methods are the most important of the physicochemical techniques that are used to identify metabolite markers of pathologies in biological samples (gas or liquid). However, tissue examination is limited to histological or chemical analysis. Neoplasms (both benign and malignant) hold a special place among the pathologies of ear, nose, and throat (ENT) organ tissues that are critically important to be diagnosed at the early stages. One of the latest statistical reviews of 20 million cancer cases from the GLOBOCAN database diagnosed in 2022 revealed that laryngeal, nasopharyngeal, and oropharynx cancer accounted for 188 960 (0.9 %), 120 416 (0.6 %), and 106 316 cases (0.5 %), respectively [1]. Although tumors of the ENT organs are relatively rare compared to lung, prostate, and breast cancer, they often present considerable difficulties in diagnosis and treatment. Despite significant advances in surgery, oncology, and radiotherapy, the prognosis for patients often remains poor.

Modern clinical methods for detection of tumors in the ENT organs include the following: nasal endoscopy, which uses a thin tube with a light and camera to examine the insides of the nose, and biopsy, which involves taking a sample of cells from the nose or sinuses. The latter may be done during nasal endoscopy with sampling instruments inserted into the tube or with the use of a thin needle for cell collection introduced directly into the region of interest. The obtained samples are sent to a laboratory for testing. In addition, imaging tests, which are used for tumors of the nose and sinuses, may be performed. These include X-ray imaging and scanning, such as computerized tomography, magnetic resonance imaging, and positron emission tomography [2,3]. Digital diaphanoscopy and fluorescence imaging are of note among the imaging methods applied in studies of pathologies of ENT tissues. Digital diaphanoscopy relies on the use of low-intensity visible and near infrared radiation for illumination of the sinuses and subsequent visualization of the light scattering pattern. Machine learning methods are used to process the generated digital images, which may help identify and diagnose diseases [4]. Fluorescence imaging [5] is based on the effect of variation of concentration of intrinsic tissue (e. g., mucosal tissue) fluorophores that is observed in the case of pathological changes. Such fluorophores include, among others, collagen, nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD), and porphyrin, which are present in human oral tissues and the concentration of which increases (in comparison with dysplasia and the reference (normal) group) with the progression of oral mucosal lesions in the case of, e. g., oral squamous cell carcinoma [6]. These methods are non-invasive, allow one to identify differences between healthy and pathologically altered tissues, and may be used both individually and in combination with each other and with spectroscopic methods.

Promising strategies for refining the diagnosis, prognosis, and treatment include the identification of biological markers, such as genetic abnormalities, epigenetic variations, and changes in protein structures and other biological molecules corresponding to a certain type of tumor, based on molecular and genetic studies.

The authors of review [7] have categorized the types of tumors of the paranasal sinuses (adenoid cystic carcinoma, squamous cell carcinoma, intestinal and non-intestinal adenocarcinomas, olfactory neuroblastoma, multiphenotypic

sinonasal carcinoma associated with human papillomavirus, mucosal melanoma, sinonasal undifferentiated carcinoma, SWI/SNF-deficient sinonasal carcinoma, NUT carcinoma, teratocarcinosarcoma, sinonasal neuroendocrine carcinoma, chondrosarcoma, and undifferentiated small round cell sarcomas). Characteristic changes at the genetic level (mutations and genetic abnormalities) have been identified for each type. The changes for certain tumor types (e.g., IDH1/2 mutations for chondrosarcoma) are associated with longer survival and open to therapeutic targeted strategies. Review [8], which is concerned with the identification of characteristic biomarkers of three main histological samples of sinonasal tumors (squamous cell carcinoma, intestinal-type adenocarcinoma, and olfactory neuroblastoma), is focused on immunohistochemical markers, such as tumor cell-specific protein (Ki-67), intracellular protein inhibitor of cyclin-dependent kinases (p21, p27), transcription factor protein regulating the cell cycle (p53), signal proteins (vascular endothelial growth factor), etc. This review highlighted the patterns of variation (intensification or suppression) of expression of the corresponding protein in the presence of malignant tumors or in the case of degeneration of a benign neoplasm into a malignant one.

Spectroscopic approaches (specifically, gas spectroscopy based on non-stationary effects of the terahertz (THz) frequency range, which offers high resolution and sensitivity) allow one to identify the composition of products of thermolysis of tissues and provide important data on the differences in chemical composition between the products of thermolysis of healthy and pathologically altered tissues (i.e., the products of thermolysis of large biological molecules). The present study is focused on examining the chemical composition of products of thermolysis of primary metabolites for tissues of tumors of the ear, throat, and nose via high-resolution THz spectroscopy. Samples of relatively healthy mucous membranes and such neoplasms as papillomas of the tonsils and larynx and cholesteatomas of the middle ear were used as examples.

Five papillomas of the tonsils and larynx and five cholesteatomas of the middle ear were chosen as nosological units for research and identification of secondary metabolites of pathologically altered tissues. The study included ten patients aged 5–17 who were hospitalized at the Children's Municipal Clinical Hospital No. 1 (Nizhny Novgorod) in 2024. The gender distribution was as follows: three boys and seven girls. All test samples were obtained using minimally invasive procedures within the scope of necessary surgical treatment in compliance with current standards of the Ministry of Health of the Russian Federation. All patients underwent a set of diagnostic studies to establish a clinical diagnosis and justify certain surgical intervention. In order to verify the diagnosis, the removed tissue (with a completed form of referral for intravital pathological and anatomical examination of biopsy (surgical) material) was subjected to a histomorphological examination.

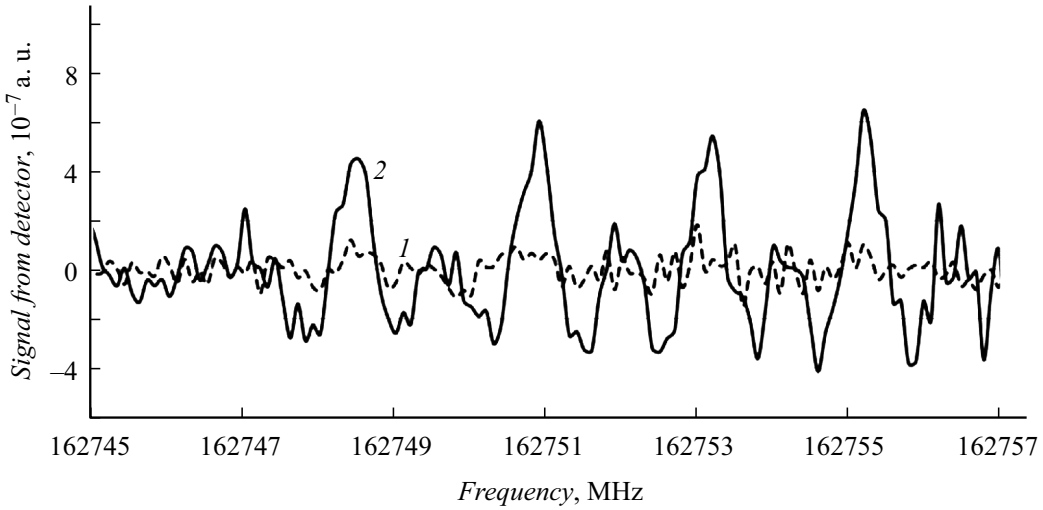
Spectroscopic measurements were carried out using a proprietary high-resolution THz spectrometer with phase switching of radiation acting on gas that operates within the range of $118-175$ GHz [9]. Its sensitivity determined based on the recorded absorption coefficient at a cell length of 1 m falls within the range from $10^{-8}$ to $5 \cdot 10^{-9}$ cm$^{-1}$. This sensitivity corresponds to the minimum detectable absorption coefficient (lines are observable above the noise level). If a substance has sufficiently strong absorption lines within this range (with an integrated intensity at the level of $10^{-3}-10^{-4}$ nm$^2 \cdot$ MHz), it is detectable at concentrations on the order of $5-100$ ppm in a multicomponent gas mixture, where the concentration of components may be very low. If a compound (e.g., hydrogen sulfide) has stronger lines within this range, it may be identified at even lower concentrations. We performed no quantitative assessments and focused on qualitative analysis of the composition of products of thermolysis of the samples. The advantage of the mentioned spectrometer is the possibility of wide-range studies and, consequently, the possibility of identifying the rotational spectrum lines of all polar substances in a mixture with spectral lines of the required integrated intensity within the operating range. Since the procedure of sample preparation and methods for studying the composition of mixtures of products of tissue thermolysis were refined and detailed in [10], we provide only a brief description of them here. The tissue samples under study were heated, and a mixture of thermolysis products was introduced into a pre-evacuated measuring cell. The recorded spectral lines and available on-line spectroscopic databases [11,12] were used to identify substances that are the products of thermolysis of larger biological molecules, which differ in composition between healthy and pathologically altered tissues. In order to perform a qualitative assessment and comparison of the chemical compositions of samples, the number of absorption lines was counted for each identified substance. With the measurement conditions remaining unaltered, this approach allows one to identify qualitative similarities and differences between the samples. Samples of papillomas of the tonsils and larynx and cholesteatomas of the middle ear were the examined tumor tissues. The composition of thermolysis products was compared with the composition of relatively healthy mucous membranes.

Several substances were identified in the process of analyzing the composition of papillomas of the tonsils and larynx (five samples) and cholesteatomas of the middle ear (five samples) and comparing them with relatively healthy nasal mucosa (five samples). The average numbers of observed lines are listed in the table.

The absorption lines of methanol, propanediol, acetaldehyde, pyrrole, and ethylene sulfide were identified in the spectra of samples of relatively healthy mucous membranes and tumor tissues. The relative concentration of these substances in the products of thermolysis of neoplastic tissue samples is higher than the one determined for relatively healthy tissues. In addition, the lines of a number of organic acids, aldehydes, nitriles, and other organic compounds identified in tumor samples were lacking in the spectra of relatively healthy

Chemical composition of products of sample thermolysis ($N$ — average number of absorption lines in the spectra of different samples, P — papilloma, C — cholesteatoma, and H — relatively healthy nasal mucosa)

| Substance | $N$ | | | Substance | $N$ | | |
|---|---|---|---|---|---|---|---|
| | P | C | H | | P | C | H |
| Acetone | 15 | 3 | 0 | Methylbutyronitrile | 17 | 18 | 0 |
| Hydroxyacetone | 6 | 9 | 0 | Pentadienenitrile | 31 | 24 | 0 |
| Dihydroxyacetone | 3 | 6 | 0 | Aminopropionitrile | 6 | 10 | 0 |
| Acetic acid | 11 | 9 | 0 | Acrylonitrile | 8 | 7 | 0 |
| Isocyanic acid | 6 | 12 | 0 | Aminoacetonitrile | 5 | 4 | 0 |
| Methanol | 14 | 7 | 8 | Methanethiol | 53 | 51 | 6 |
| Propanediol | 68 | 34 | 3 | Glycine | 3 | 6 | 0 |
| Alaninol | 13 | 6 | 0 | Alanine | 5 | 8 | 0 |
| Glycerol | 9 | 2 | 0 | Urea | 7 | 11 | 0 |
| Methyl formate | 5 | 9 | 0 | Ethylene glycol | 12 | 5 | 0 |
| Acetaldehyde | 57 | 76 | 39 | Pyrrole | 10 | 13 | 8 |
| Propanal | 10 | 9 | 1 | Pyridine | 9 | 17 | 0 |
| Benzaldehyde | 9 | 9 | 0 | Cyanoxirane | 3 | 3 | 0 |
| Glycolaldehyde | 6 | 13 | 2 | Glycinamide | 9 | 4 | 0 |
| Malondialdehyde | 3 | 3 | 0 | Glycolamide | 3 | 5 | 0 |
| Acetonitrile | 11 | 19 | 1 | Cyanoethynylbenzene | 10 | 14 | 0 |
| Propionitrile | 21 | 38 | 0 | Cyanovinylacetylene | 5 | 3 | 0 |
| Butyronitrile | 16 | 20 | 2 | Carbonyl sulfide | 6 | 11 | 2 |
| Pentanenitrile | 16 | 13 | 1 | Sulfur dioxide | 12 | 13 | 1 |
| Benzonitrile | 5 | 7 | 2 | Ethylene sulfide | 3 | 4 | 3 |




Section of recorded spectra of tissue thermolysis products for samples (*1* (relatively healthy mucosa) and *2* (pharyngeal papilloma) with pyridine absorption lines. The central frequencies in the papilloma sample are $f_{exp} = 162\,748.5$ MHz ($f_{theor} = 162\,748.4937$ MHz), $f_{exp} = 162\,750.9$ MHz ($f_{theor} = 162\,750.8999$ MHz), $f_{exp} = 162\,753.2$ MHz ($f_{theor1} = 162\,753.1032$ MHz, $f_{theor2} = 162\,753.4681$ MHz), $f_{exp} = 162\,755.2$ MHz ($f_{theor} = 162\,755.242$ MHz). $f_{exp}$ — experimentally measured central frequency of the absorption line; $f_{theor}$ — reference central frequency of the absorption line [9].

tissues. A section of recorded spectra of tissue thermolysis products for two samples (relatively healthy mucosa and pharyngeal papilloma) is shown in the figure. Pyridine is used here as an example to highlight the difference in chemical composition of thermolysis products between the pharyngeal papilloma and relatively healthy mucosa samples.

The presented approach enables qualitative determination of the composition of tissue thermolysis products and is (potentially) comparable in sensitivity to a number of substances with the method combining gas chromatography and mass spectrometry; however, literature data on the study of ENT tissues with the latter method are lacking.

Identification of similar substances in tumor tissues and differences between tumors of various types (and neoplastic and healthy unchanged tissues) may be the first step toward determining the overall metabolic profile of tissues characteristic of the neoplastic process and the metabolic profiles of tissue thermolysis products corresponding to specific pathologies. Thus, the potential applicability of high-resolution THz spectroscopy in the analysis of the metabolic composition of neoplastic tissues was demonstrated.

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### Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed voluntary consent was obtained from lawful guardians of each study participant.

### Conflict of interest

The authors declare that they have no conflict of interest.

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