Methods of spectral analysis of exhaled air suitable for routine diagnostics of diseases of the respiratory system

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Diseases of the respiratory system are among the leading causes of death and disability worldwide. Most respiratory disorders are substantially underdiagnosed, with the diagnosis typically delayed until the condition is advanced. Early detection should provide more opportunities to prevent deterioration and lead to reduction of the societal burden of the disease. Exhaled air monitoring is a promising non-invasive and non-expensive procedure for early diagnosis of respiratory diseases. In the present paper, instrumental methods that can provide real-time information on the chemical composition of exhaled air are reviewed and compared in terms of their suitability for routine clinical use.

Keywords: respiratory diseases, early detection, exhaled air monitoring, non-invasive diagnostics

1. Introduction

Diseases of the respiratory system are widespread and the mortality from *various bronchopulmonary conditions* has been steadily increasing over the past few decades. According to the data of the World Health Organization (WHO), cancer of the trachea, bronchus and lung caused 1.6 million deaths in 2012 vs. 1.2 million cases in 2000. Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide (3.1 million cases in 2012). Unfortunately, respiratory diseases are not usually diagnosed until they are clinically apparent and moderately advanced. Early detection has the potential to decrease disease severity and reduce disease burden and mortality.

The monitoring of volatile metabolites-markers present in exhaled *air* has recently become of great interest in the diagnosis and treatment of many respiratory conditions. The advantages of such approach are non-invasiveness, convenience, low cost of measurement procedure and suitability for continuous monitoring. In contrast to blood tests, the analysis of metabolites in exhaled air does not require labor-intensive pre-analytical sample processing. As the concentrations of a volatile compound in the exhaled *air* is directly related to its blood concentration, exhaled air sampling has been proposed as an attractive noninvasive alternative to blood sampling [1].

2. Metabolites in the exhaled air

The detailed analysis of volatile organic compounds (VOCs) in exhaled air, saliva, blood, milk, skin secretions, urine, faeces for healthy people was carried out in [2]. Only 12 VOCs (0.7% of the total number) were found to be present in all the bodily fluids and breath, namely acetaldehyde, 2-propanone (acetone), benzaldehyde, 1-butanol, 2-butanone, hexanal, heptanal, octanal, pentanal, benzene, styrene, toluene. Seven of these compounds possess a carbonyl group, and the latter three are smoking-derived substances.

Pathological processes in lungs change the content of metabolites in the exhaled breath, including:

– inorganic volatile compounds, e.g., carbon dioxide, oxygen, and nitric oxide;

– non-volatile compounds measured in exhaled breath condensate (EBC), e.g., isoprostanes, cytokines, leukotrienes and hydrogen peroxide;

– VOCs of different types, e.g., saturated hydrocarbons (ethane, pentane, aldehydes), unsaturated hydrocarbons (isoprene), oxygen containing (acetone), sulphur containing (ethyl mercaptane, dimethylsulfide) and nitrogen containing (dimethylamine, ammonia). The most commonly

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identified VOCs are isoprene, acetone, ethanol, methanol, other alcohols and alkanes [3].

2.1. Bronchial asthma

Exacerbation of bronchial asthma (BA) is accompanied by an increase in exhaled nitric oxide (NO) levels. Excess NO is produced by activation of inducible NO-synthase during inflammation. Significant correlation between exhaled NO and bronchial responsiveness and between exhaled NO and sputum inflammatory cells (eosinophils) was found in [4]. An increase of NO in the exhaled breath of patients with a very early form of BA which was not detected by regular clinical tests (normal lung function, negative bronholitin test, etc.) was reported in [5].

Exhaled carbon monoxide (eCO) is elevated in non-smoking asthmatics, the levels correlating, to some extent, with the severity of the disease [6]. However, the difference in eCO between normal and asthmatic subjects, however, is much less than the difference in exhaled NO [7].

Elevated levels of exhaled pentane are present during acute asthma exacerbations that are reduced to the normal value during recovery. Exhaled ethane levels are also higher in patients with mild steroid-naïve asthma compared with steroid-treated patients and normal subjects [7].

In [8], the levels of nitric oxide and carbon monoxide in exhaled breath of 40 severe BA patients in the process of anti-inflammatory therapy were measured using chemiluminescence NO/NO_2 gas analyzer N310 and CO analyzer K-100 (Optec, Inc.). The average age of the patients was 49.3 years and the average disease period was 13.2 years. An improvement in clinical and functional parameters following therapy was accompanied by decreased levels of exhaled NO and CO, possibly due to effective suppression of bronchial inflammation.

2.2. Chronic obstructive pulmonary disease (COPD)

The change in the exhaled air composition of patients with COPD is caused by systemic inflammation involving several tissues and organs. C-reactive protein (CRP) [9], fibrinogen, IL-6, IL-8, tumor necrosis factor alpha (TNFa) [10], and leukotriene B4 – LTB4 [9] have all been reported as markers of systemic inflammation and as indicators of severity of COPD.

Paredi et al. reported differences in exhaled ethane levels of COPD patients in comparison with its levels in healthy people and steroid-treated COPD patients [11]. The classifier based on thirteen VOCs allowed one to distinguish COPD patients from healthy people in 100% of cases; 100% sensitivity and 81% specificity was achieved using only six VOCs [12]. The result of classification did not depend on smoking status and use of inhaled corticosteroids.

Basanta et al. identified the profile of VOCs that allowed one to distinguish patients with COPD from asymptomatic smokers with 88% sensitivity and 81% specificity [13].

2.3. Infectious diseases of the lungs

Pulmonary macrophages provide defense against respiratory infections by initiating anti-infective inflammation. One of the mechanisms involved in this process is phagocytosis of the pathogen, which induces the release of cytokines. Therefore, one could expect that the host contribution to the VOCs detected in breath following live pathogen infection will not be the same than those produced by the host after exposure to cell lysates [14].

During respiratory tract infections, ammonia concentration levels in exhaled breath are strongly increased and their monitoring allows for differentiation between bacterial and viral infection in a number of lung diseases [15].

2.4. Tuberculosis

Pulmonary tuberculosis (PTB) can change the composition of VOCs in the exhaled air due to the mycobacteria metabolism and oxidative stress that accompanies the infectious processes in the body [16]. Mycobacterium can induce reactive oxygen species (ROS) production by activating phagocytes, and although an important part of the host defense against mycobacteria, enhanced ROS generation may promote tissue injury and inflammation. Lipid peroxidation (LPO), a general mechanism of tissue damage by free radicals is known to be responsible for cell damage and may induce many pathological events [17]. Volatile hydrocarbons (ethane, propane, butane, pentane) have been advocated as non-specific markers of free-radical induced LPO in humans. These volatiles have been detected in oxidized fatty acid systems (oleic, linolenic and arachidonic acids).

PTB is a strong stimulator of the respiratory "burst" due to formation of the active forms of oxygen and intermediate nitrogen compounds. Kwiatkpwska et al. measured concentrations of hydrogen peroxide in exhaled air condensate of patients with active form of PTB before and after treatment [18]. The level of hydrogen peroxide in the exhaled air condensate from patients with PTB was significantly higher than in healthy smokers and non-smokers. Two months of treatment reduced the level of peroxide of PTB patients to the healthy smoker's level.

Phillips et al. [19] found 130 VOCs in the exhaled air of PTB patients, the most abundant being 1-methylnaphthalene, 3-heptanon, methylcyclododecane, 2,2,4,6,6-pentamethylheptane,1-methyl-4-(1-methylethyl) petrol and 1.4-dimethylcyclohexane.

2.5. Lung cancer (LC)

Analysis of volatile markers in the exhaled air of LC patients and healthy volunteers using the method of mass-spectrometry was performed in [20]. It was shown that us-

ing 15 of the identified markers, the two groups could be distinguished with 71% sensitivity; increasing the number of markers to 21 yielded 80% sensitivity and 100% specificity. The markers included alcohols, aldehydes, ketones and hydrocarbons.

A marked and consistent increase of the concentration of 30 VOCs in the exhaled air of 193 LC patients was observed, including isopropyl alcohol, 2,3-hexandione, camphor, benzophenone, derivatives tetroxane, benzene, anthracene, benzoic acid, furan, esters [19]. It was hypothesized that activation of lethal cytochrome p450 mixed oxidases may lead to lung cancer while independently altering the catabolism of VOCs. No consistent difference between smokers and non-smokers was found.

Of increasing interest for exhaled air analysis is endothelin-1 (ET-1)—a growth factor that is involved in the start and progression of tumors including lung cancer. Breath condensate of patients with non-small cell LC (NSCLC) was reported to contain significantly higher levels of endothelin-1 (ET-1) compared to healthy controls [21]. A significant reduction of ET-1 level was found after surgical removal of the tumor, with and without adjuvant chemotherapy.

A comparative analysis of 68 VOCs concentrations in the exhaled air of 43 patients with NSCLC and 41 healthy volunteers was carried out using gas chromatography (GC)/mass spectrometry (MS) [22]. Significant differences were found between the breath of the subjects with or without lung cancer. 1-Butanol and 3-hydroxy-2-butanone were considered as potential biomarkers in the breath for lung cancer. VOCs levels were not significantly different between the early- and late-stage lung cancer patients

The method of GC-MS with discriminant analysis of the data was used for selecting 22 VOCs (from the 67 registered ones) that allowed one to distinguish between LC patients from healthy persons with 100% sensitivity and 81% specificity of [23]. This set of VOCs included 3-methyloctane, 3-methylnonane, isoprene, cyclohexane, heptanal, hexanal and derivatives of heptane, decane, benzene. The increase of their concentration was partly related to oxidative stress. No correlation between above VOCs concentrations and the stage of disease and the factor of smoking was found.

The multinomial logistic regression method was used to study the quality of classification by profiling 13 VOCs, including isoprene, 2-methylpentan, pentane, ethylenbenzol, xylene, trimethylbenzol, toluene, benzene, heptane, decane, styrene, octane, pentamethylheptane [24]. It was revealed that the profile of these VOCs is able to correctly identify about 80% of the LC patients. Level of 2-methylpentane was higher in NSCLC patients than in COPD and control patients. After surgical treatment, a consistent decrease of isoprene and decane levels was found.

GC-MS method was used for building up the classifier based on 21 VOCs, which provided 80% sensitivity and 100% specificity in diagnostics [25]. Markers of smoking (acetonitrile and benzene) and other potentially exogenous substances (2-propanol, 1,1-difluoroethane, acetyl bromide, ethylbenzene, ethanol, isobutane, diethyl ether, etc.) were not considered. No consistent decrease in the concentrations of isoprene, acetone and methanol in the exhaled air of LC patients was revealed, whereas the concentration of other markers (2-butanone, benzaldehyde, 2,3-butandione, 2-butanone, 1-propanone, acetophenone, cyclopenten, tetramethylcarbamid, butylacetate etc.) increased compared to the control group.

112 potential markers of lung cancer in the exhaled air were registered during the last ten years [26]. Among them: 36 hydrocarbons (e.g., 2-methyl-propane and 5-methyl-tridecan), 7 alcohols, 8 aldehydes (e.g., pentanal, hexanal, octanal, nounal), two acids, 12 ketones (e.g., 6-methyl-5-hepten-2-one), 12 aromatic compounds (e.g., a mixture of benzophenone), two heterocycles, two nitriles, 5 terpenes (e.g., trans-caryophyllene), 9 ethers, one sulfide, two halogenated compounds, and 15 other chemical compounds.

3. Sampling procedure

In addition to identifying the most specific biomarkers, the development of analysis methods and instrumentation, as well as unification of the sampling procedure become increasingly important. Equipment for exhaled air testing varies widely, but the basic principles are the same. All systems have a source of test gas (bag-in-box, spirometer, compressed gas cylinder), a method for measuring inhaled and exhaled volume over time (spirometers with kymographs, pneumotachometers near the mouthpiece or near a bag-in-box), and gas analyzers (single-sample analyzers or continuous high-speed analyzers) [27].

Exhaled air includes a portion of dead space air—the air from the nasopharynx, trachea, bronchi, where no gaseous exchange between inhaled air and blood takes place, and alveolar air originating from the lower airways where gaseous exchange between blood and breath takes place. Therefore, the concentration of the endogenous compounds that are of interest for diagnostics is relatively high in alveolar air compared to dead space air.

Exhaled air can be sampled in two ways: mixed expiratory sampling and end-tidal sampling. Mixed expiratory sampling entails collecting total breath, including the air contained in the upper airways which experiences no gas exchange with blood. End-tidal sampling involves the collection of only end-tidal air, which contains most of the chemical information on blood composition. End-tidal sampling (collecting breath only at the end of exhalation) has proven successful, because samples are less likely to be diluted by mixing with dead space volume (inspired air not taking place in gas exchange) and ambient air [28].

Exogenous compounds present in the breath are one of the main sources of noise affecting the analysis results. An age-old question is how to discriminate between compounds

of the endogenous (i.e. produced inside the body by physiological or pathological metabolism) or exogenous origin [28].

The need for standardization in sampling has been growing with the development in the field of breath research. Modern sampling devices for analysis of the exhaled air have to meet a number of requirements [29, 30].

For measuring NO concentration, the following factors are critical for reproducibility of results:

i) Exclusion of nasal NO. Closure of the velopharyngeal aperture during exhalation is one way to minimize nasal NO leakage. This can be achieved by resistance to exhalation. It has been estimated [31] that resistance to exhalation should be at least 5 cm of water column. At the same time, pressures greater than 20 cm of water column can be uncomfortable for the patient and should be avoided.

ii) Standardization of exhalation flow rate. Exhaled NO plateau values vary considerably with exhalation flow rate. Low flow rates (<0.1 L/s) amplify the measured NO concentrations. Flow rate of 0.05 L/s was found to be a reasonable compromise between measurement sensitivity and patient comfort [31].

Performance standards for equipment for single-breath determination of carbon monoxide uptake in the lungs were defined in [27]. The volume-measurement accuracy should be the same as that determined by ATS/ERS for spirometry, that is, ±3%, regardless of gas mixture, direction of gas flow (e.g. inhaled or exhaled), or pulsatile flow pattern. Gas-analyzer accuracy is important in some circumstances, such as measuring CO "back pressure" (the exhaled fraction of CO when no CO has been inhaled).

In calculating the diffusing capacity of the lungs for CO (DL_{CO}), only the ratios of the alveolar to inhaled CO and tracer gas are needed. Thus, the analyzers must primarily be able to produce an output for measured exhaled CO and tracer gas that is a linear extrapolation between the inhaled (test gas) concentrations and zero (no CO or tracer gas present in the analyzers).

Since the measured DL_{CO} is very sensitive to errors in relative gas concentration, nonlinearity for the analyzers should not exceed 0.5% of full scale (i.e., once the analyzer has been adjusted to zero, with no test gas present and scaled to full scale using test gas concentrations, system nonlinearity in measurements of known dilutions of test gas should be no more than 0.5% of full scale).

If CO_2 and/or H_2O interfere with the gas analyzer performance, their effect can be minimized by two approaches. One is to remove CO_2 and/or H_2O from the test gases before they pass through the gas analyzer. The second remedy for CO_2 and/or H_2O analyzer interference is to characterize the effect of these gases on analyzer output aside, and then adjust the output of the analyzers for the presence of the interfering gas species.

Exhaled air is saturated with water vapor that often interferes with the measurement of the analyzed volatile components. Water vapor condenses on cool surfaces potentially leading to the partial transfer of the volatile components from gas to liquid thereby distorting the measurement result. Due to recent technological advancements, the exhaled breath analysis has moved beyond measuring VOCs in the gas phase only into the measurement of semi-volatiles and dissolved compounds in aerosolized droplets in exhaled breath condensate (EBC) and in exhaled breath vapor (EBV). Aerosolized droplets in EBC can be captured by a variety of methods and analyzed for a wide range of biomarkers, such as metabolic end products, proteins, cytokines, and chemokines, with expanding possibilities. EBV sampling can detect additional compounds not detected in EBC and may provide greater sensitivity as a sampling method, expanding the spectrum of breath sampling [29].

Instrumental methods of profiling volatile organic compounds in exhaled breath suitable for routine tests

Gas chromatography is the "gold standard" for analysis of trace quantities of substances, especially organics in gas mixtures of biological origin. However, this method is too complicated to be used in routine clinical practice.

Electrochemical sensors can monitor changes in electrical properties caused by chemical reactions with a specific gas. Electrochemical sensors are mainly used for detection of gaseous compounds, such as O_2, CO, CO_2, NO, NO_2, H_2S, SO_2, HCN, with concentrations ranging from 0.1 to 100 ppm. Very small sample volumes can be analyzed. The main disadvantages of such sensors are low selectivity, especially for complex gas mixtures, and a short life time of the sensing element.

Devices consisting of a set of sensors, each of which corresponds to a particular substance or group of substances (so called "electronic nose" technology or "e-nose") hold great promise for monitoring exhaled breath. One example of "e-nose" is "Cyranose 320" that relies on a 32-channel carbon-black polymer composite chemiresistor array [32]. These instruments are relatively simple, noninvasive, and transportable tools that potentially allow diagnosis of various human diseases in hospitals and clinical setting [32–36]. The drawbacks of the existing "e-nose"-based instrumental methods are essentially the same as for the individual sensors, i.e. low selectivity and short service life.

To overcome these drawbacks, new types of sensors and sensor coatings are being developed, such as, for example, chemical sensors that change their color when a certain VOC appears (colorimetric sensors), or change the frequency of quartz resonator, etc. [32]. Various devices for selective presampling are applied. Decreasing the number of sensors required for accurate breath monitoring can potentially reduce the costs of clinical testing. Selective sensing elements based on silicon or gold nanostructures hold promise for the development of portable gas analyzers [37–39].

The combination of "e-nose" technology with absorption-based optical sensing devices can potentially increase

the accuracy of VOC detection in exhaled breath and improve its cost effectiveness [40].

The ability of laser absorption spectroscopy (LAS) technique significantly depends on the spectral tuning range of the used lasing source and the profile of spectral sensitivity of photodetector. Typical VOCs have absorption bands in the ranges of 2–5 and 7–11 μm [41].

Photoacoustic spectroscopy (PAS), and cavity-enhanced resonant photoacoustic spectroscopy in particular, is one of the most sensitive methods of trace gas monitoring, [40]. Photoacoustic spectroscopy has a very low detection limit (ppb–ppt levels) and sufficient selectivity. There is no need for pre-concentration and a small volume sample (several ml) is sufficient for analysis. Moreover, photoacoustic spectroscopy techniques may allow for real-time breath monitoring. Both laser-based photoacoustic detection and intracavity laser absorption spectroscopy have the advantage of tracing gases locally and can therefore be used in laboratory studies. The advantage of laser photoacoustics is that it is background free: it does not rely on a decrease of the transmitted light but on an increase from the zero baseline [42].

Photoacoustic spectroscopy-based gas analyzer of ethylene was designed and reported in [43, 44]. The length of the intracavity acoustic cell was 100 mm, and the average power inside the cavity of the CO_2 laser was 100 W. The analyzer allowed measurements of ethylene down to 6 pptv.

Photoacoustic spectroscopy gas analyzer with tunable CO_2 laser, made by Special technologies Ltd., was used for measuring spectral characteristics of exhaled air from four groups of patients [45]: control group—healthy participants, group 2—patients with bronchopulmonary diseases (COPD, asthma, pneumonia), group 3—patients with other diseases (coronary heart disease, gastric ulcer, duodenal ulcer), group 4—patients with tuberculosis.

The comparison of measured spectra of exhaled air from participants from group under study S was carried out in terms of Mahalanobis distance in relation to the reference group S_0. Let the feature vectors of the participants from the groups S and S_0 be y_j, $j=\overline{1,N_S}$ and x_i, $i=\overline{1,N_{S_0}}$, respectively. Here, N_S and N_{S_0} are the total quantities of the feature vectors which correspond to all participant in the group. So, the average square of the Mahalanobis distance can be defined as

$$I_{S_0}(y_j)=\frac{1}{2mN_{S_0}}\sum_{i=1}^{N_{S_0}} d_M^2(y_j,x_i),$$

where $d_M(\mathbf{x},\mathbf{y})=\sqrt{(\mathbf{x}-\mathbf{y})^{\mathrm{T}}C^{-1}(\mathbf{x}-\mathbf{y})}$ is the Mahalanobis distance, C is the covariance matrix of the features of participants from the reference group S_0, and M is the dimension of the feature space.

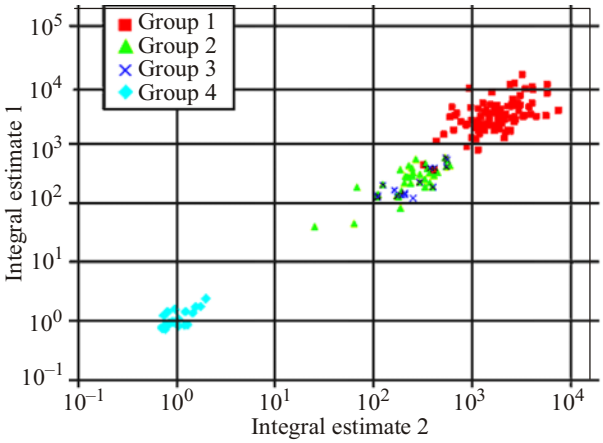

Fig. 1. Distribution of point estimates of absorption spectra of exhaled air. Group 1—healthy participants (control group), group 2—patients with bronchopulmonary diseases (COPD, asthma, pneumonia), group 3—other diseases (coronary heart disease, gastric ulcer, duodenal ulcer), group 4—patients with tuberculosis.

In Fig. 1 the set of absorption coefficients of exhaled air from tuberculosis patients is used as feature vectors x_i of the reference group S_0, the absorption coefficients of exhaled air from other participant are used as feature vectors y_j of the group S. The average square of the Mahalanobis distances of exhaled air absorption spectrum in the 10P and 10R spectral bands of CO_2 laser generation for participants are marked in figure 1 as integral estimations 1 and 2, respectively. This parameter describes the specific difference of the object under study in the feature space relatively to the the reference group in terms of square of the Mahalanobis distance averaged over all objects from the reference group and divided by the the dimension of the feature space.

Table 1. Summary of the patients studied in [46]

Characteristics of group members	Group A		Group B		Group C		Group D
Number, persons	10		10		10		10
Age, years	from 31 to 68		from 35 to 65		from 18 to 28		from 27 to 54
Sex	Male						
Main disease	Lung cancer		COPD		Pneumonia		No
Comorbidities	Yes		Yes		Yes		No
Absence of smoking in the anamnesis	45% Yes	55% No	50% Yes	50% No	60% Yes	40% No	No

Table 2. Sensitivity and specificity of the SVM-method for pairwise classification of all study groups

Pairwise classification	Sensitivity, %	Specificity, %
Group A–Group D	100	63.75–67.5*
Group B–Group D	95–98.75*	92.5–93.75*
Group C–Group D	63.75–68.75*	100
Group A–Group Â	100	97.5–98.75*
Group A–Group C	100	90
Group B–Group C	95–100*	62.5–63.75*

* Results vary depending on the kernel function of SVM-method.

The specificity of the exhaled breath of patients with bronchopulmonary diseases in the spectral range 9.2–10.8 μm was analyzed in [46] using laser spectroscopy and chemometrics methods. The studied groups of patients are shown in Table 1.

The Support Vector Machine (SVM) was used for data classification. SVM classification included the training stage, so the data from each group were randomly divided into two equal sets, one of which was used for training and the other—for classification. Classification was carried out in pairs. The results are shown in Table 2.

In 2013, Special technologies, Ltd. developed a specialized laser photoacoustic spectrometer LaserBreeze based on dual optical parametric oscillator (OPO) [47, 48].

In the dual OPO, we used two types of nonlinear elements: a periodically poled lithium niobate structure (PPLN) and a mercury thiogallate crystal $HgGa_2S_4$ (HGS). Nd:YLF laser (10 ns, 0.5–1.5 kHz, 1.5 mJ) was used as a pump source. The linewidth of the developed OPOs was 3–4 cm^{-1}. The average power of the OPO based on PPLN and HGS was 20 mW (1700 Hz) and 9 mW (900 Hz), respectively. A double channel resonant photo-acoustic cell was used for recording the absorption spectra of the gaseous samples.

Table 3. Technical characteristics of the spectrometer *LaserBreeze*

Parameter	Value
Source of radiation	Optical parametric oscillator
Spectral tuning range	2.5–10.7 μm
Detection limit	≤1 ppb
Number of detected substances	≥20
Relative error in concentration of biomarkers	≤30%
Accuracy and selectivity of biomarkers detection	≥95%
Maximal sampling gas volume	50 cm^3
Maximal registration time of a single biomarker	3 s
Maximal registration time of 10 biomarkers	2 min

The spectrometer LaserBreeze allows measuring the concentration of at least 20 different gaseous biomarkers whose concentration in the exhaled breath is related to the stage of several bronchial and pulmonary diseases (bronchial asthma, acute bronchitis, pneumonia, COPD). The LaserBreeze gas analyzer is described in detail in [47]. Technical characteristics of the spectrometer are represented in Table 3.

Typical absorption spectra measured by LaserBreeze are shown in the Fig. 2.

The analysis of exhaled breath spectra of patients with different diseases allows one to set up a system of classifi-

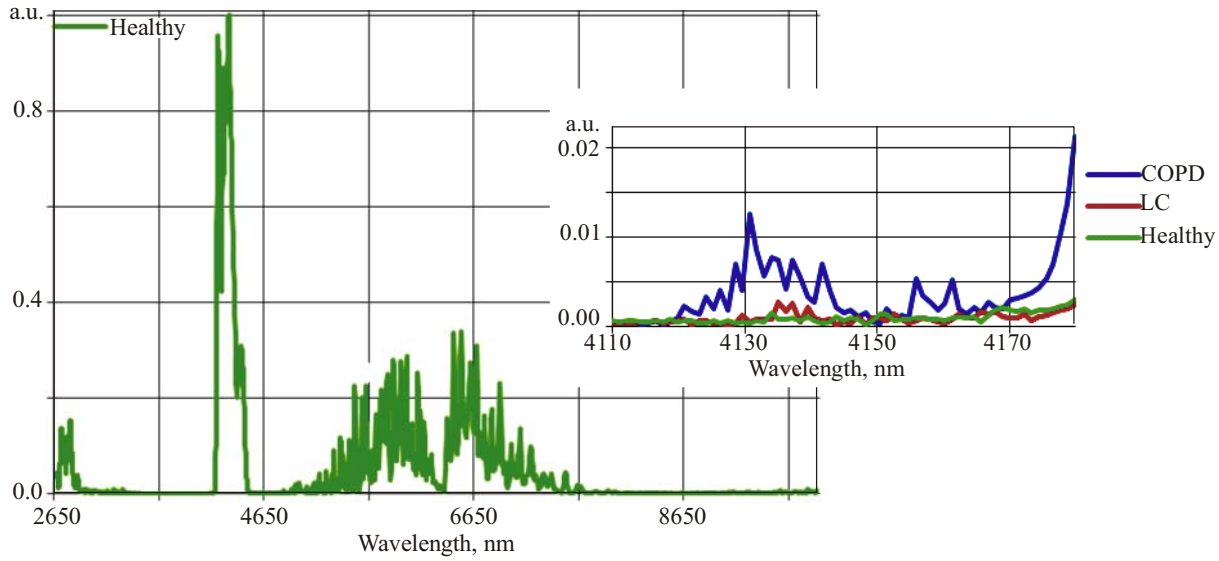

Fig. 2. A typical absorption spectrum of exhaled air of a healthy patient in the range of 2.65 to 9.69 μm, and the enlargement of the range 4.11–4.18 μm with superimposed spectra of exhaled air of COPD and LC and patients (insert).

Table 4. SIMCA classification of the absorption spectra of exhaled air of COPD patients and healthy volunteers in the range of 2.59 to 2.817 μm [41]

Number of samples of the absorption spectra scans		Average classification accuracy*, %
training stage	testing stage	
12	77	96.00
18	71	93.31
24	65	86.15
30	59	71.19

* The average value was calculated using 12 different variants of the scans set in the training stage.

cation rules for diagnostics. Such an approach using the LaserBreeze Spectrometer was reported in [41]. The study involved 11 healthy non-smoking volunteers (control group) and 7 COPD patients (target group). In Table 4, examples of SIMCA (soft independent modeling of class analogy) classification [49] using the profiles of the absorption spectra of breath samples in the range of 2.59 to 2.817 ìm are presented. SIMCA classification procedure included two stages: the training stage, using a set of samples with known class membership, and the testing stage. The results in Table 4 clearly demonstrate that the analysis of the IR absorption spectra of exhaled breath allows for a reasonably accurate discrimination of COPD patients from the control group.

4. Summary

Exhaled air analysis is a promising tool for identifying the profiles of endogenous metabolites and express diagnostics of a number of diseases.

Approaches to diagnostics of bronchopulmonary diseases based on monitoring volatile metabolites-markers in the exhaled air are being intensively developed. The advantages of exhaled air analysis are non-invasiveness, convenience, low cost and suitability for continuous monitoring.

Various analytical methods can be used for measuring the volatile metabolites contents in exhaled air. Gas chromatography is the "gold standard" for analysis of trace quantities of substances, especially organics in gas mixtures of biological origin. However, this method is too complicated to be used in routine clinical practice.

Laser Absorption Spectroscopy (LAS), electrochemical gas sensors and "e-nose" technology (a set of sensors) are most easy to use. From a practical viewpoint, the "e-nose" technology is very perspective, but gas sensors are often much less sensitive and are prone to drift [50]. Implementation of "e-nose" into routine practice will depend on improvement of technical characteristics of contact sensors.

The capability of the LAS technique as an exhaled air analysis method strongly depends on the spectral tuning range of the lasing source. In this respect, the recently developed laser photoacoustic spectrometer LaserBreeze based on a dual optical parametric oscillator with extra-wide spectral tuning range has considerable potential for early detection of bronchopulmonary diseases.

LAS has excellent metrological characteristics, however compared to chemical sensors is more complicated from the technical point of view. A breakthrough in this area can probably be achieved by combining the LAS and "e-nose' approaches.

On the whole, the future of breath air analysis requires the development of cost-effective and informative measurement equipment, standardization of sampling, identification of biomakers with very specific profiles, as well as the development of new effective methods of data analysis and classification.

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