Invited Paper

Noninvasive express diagnostics of pulmonary diseases based on control of patient's gas emission using methods of IR and terahertz laser spectroscopy

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ABSTRACT

Pulmonary diseases diagnostics always occupies one of the key positions in medicine practices. A large variety of high technology methods are used today, but none of them cannot be used for early screening of pulmonary diseases.

We discuss abilities of methods of IR and terahertz laser spectroscopy for noninvasive express diagnostics of pulmonary diseases on a base of analysis of absorption spectra of patient's gas emission, in particular, exhaled air.

Experience in the field of approaches to experimental data analysis and hard-ware realization of gas analyzers for medical applications is also discussed.

Keywords: IR and terahertz spectroscopy, exhaled air, pulmonary diseases

1. INTRODUCTION

Development of the technical basis and application of laser photo-acoustic spectroscopy (LPAS) method for registration of different chemical compounds in spectral range from 2 to 11 μm were studied. For today we have significant background in this direction, including:

1. High sensitivity photo-acoustic detectors design (patented in Russia);
2. Special CO_2-lasers in spectral range from 9,2 to 11,5 μm;
3. Optical parametric oscillator design for measurements in spectral range from 2 to 15 μm;
4. Experimental and serial samples of devices designed for studying atmospheric impurities, as well as monitoring of exhaled air (LGA-2, ILPA, SF_6 LaserGasTest, LaserBreeze);
5. Electronics for measurement process automation and analysis of measurements results;
6. Data processing and presentation program;
7. Original spectral data base for use in spectral data processing;
8. Biomedical experiments on exhaled air monitoring in groups of healthy and sick people;
9. Medical diagnostic method based on concentration level measurement of gaseous biomarkers in referent spectral points.
10. Primary positive results allowing to reliable prediction of different diseases.

So, for today we have technical and methodology base for systematic study of patient's state based on exhaled air analysis using LPAS method in the mid-IR (from 2.5 to 11 μm).

The next step in LPAS method development is spectrum spreading to terahertz range for gas analysis. Now, we start new project (contract No. 14.512.11.0021, 07.03.2013) with Ministry of education and science of Russian Federation within

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Federal program “Research and development in priority directions of Scientific and Technical complex progress in Russia 2007 - 2013”): Gas analyzer model for organic molecules detection using state-of-the-art THz laser source.

In fact, spectroscopic method for the qualitative and quantitative analysis of composition can be classified as follows: by type of source; by type of detector; by method of concentration analysis.

We chose Photo-Acoustic (PA) method based on photo-acoustic effect. PA effect consists in acoustic wave formation in a sample when interacting with pulse or pulse-periodic radiation in the wide spectral range (from UV to THz). Energy of electromagnetic radiation is transformed to heat energy in PA detector. This leads to pressure changing in the sample. That is equal to generation of acoustic waves. Direct registration of acoustic signal realized by piezoelectric transducer (for liquid and solid samples) or by microphones (for gases).

Main features of LPAS method are:

- High spectral resolution, which depends on bandwidth of laser source;
- High sensitivity (minimal measured concentration – 1 ppt);
- Small volume and length of detector's cell;
- Useful signal is proportional to laser power;
- Spectral range: from UV to THz.

Nowadays, LPAS is one of the more sensitive methods of gas analysis (sensitivity up to 1 ppt), especially when using laser sources and resonance photo-acoustic detectors[1,2]. LPAS has advantages in the detection of the gases which have absorption lines overlap with the laser emission lines (for example CO or CO_2 laser). LPAS allows studying absorption spectra of chemical compounds from diatomic molecules up to aliphatic and aromatic carbons in the gas, liquid and solid states.

On the other hand, LPAS has some disadvantages: limited number of analytic lines, interference and overlapping of the absorption lines with those of disturbing gases.

Moreover, PA signal depends on water vapor concentration. Water vapor concentration can change vibration relaxation of studying molecule and nitrogen, operating as buffer of rotational energy. For example, limit for methane detection in the dry air using LPAS is 10 ppb, in pure nitrogen 1 ppb, and with appearance of water – 100 ppb[3].

2. GAS ANALYZER AND SENSORS BASED ON CO_2-LASERS (9,2 – 11,5 μm): ILPA-1 & LGA-2

Special technologies, Ltd. in participation with Institute of laser physics and Institute of atmospheric optics developed laser gas analyzers based on LPAS method[4]: ILPA-1 (2005) and LGA-2 (2007). These gas analyzers were constructed on bases of waveguide, tunable CO_2 lasers and resonance photo-acoustic detectors (PAD). Design philosophy was following: ILPA-1 (figure 1a) had intracavity, whereas LGA-2 (figure 1b) had extracavity detector position. Difference in element base consists in modification of laser sources and type of microphones used in the detectors.

Laser used in ILPA-1 allows measuring main isotope of Carbone dioxide molecule with tuning range from 9,2 to 10,8 μm. This gas analyzer was purposed for exhaled air analysis, registration and measuring low concentration of air pollutions.

Gas analyzer LGA-2 is a modification of ILPA-1 with extracavity detector.

Figure 1. Gas analyzers with intracavity (a) and extracavity (b) detector position.

As a particular solution SF_6 LaserGasTest leak detector (figure 2) was designed[5] for the following applications:

- High-voltage switch-gear manufacturing (quality control, factory tests, etc.);
- High-voltage switch-gear service (in-field tests and maintenance of installed equipment);
- Gas-filled RF waveguides leakage control;
- Air-tightness control of complex, distributed pneumo- or hydraulic pipe systems.

Figure 2. Photo of SF_6 LaserGasTest

Unified technical solutions designed while developing SF_6 LaserGasTes can be used for creation universal platform for gas analyzers for different applications.

3. GAS ANALYZER BASED ON UNIQUE OPTICAL PARAMETRIC OSCILLATOR (2,5-11 μm)

Nowadays medicine science has rather well determined principles of metabolic processes in human organism. While studying gaseous samples from patients with different diseases, some specific chemicals were found. Their presence or changing of their concentration was described in a number of articles. Such chemical compounds were named bio-markers.

Gas analyzer “LaserBreeze” creation is supported by Ministry of education and science of Russian Federation within Federal program “Research and development in priority directions of Scientific and Technical complex progress in Russia 2007 - 2013”.

Photo-acoustic spectroscopy method allows determining concentrations of different gases in human’s exhaled air with very good precision and selectivity (figure 3, 4).

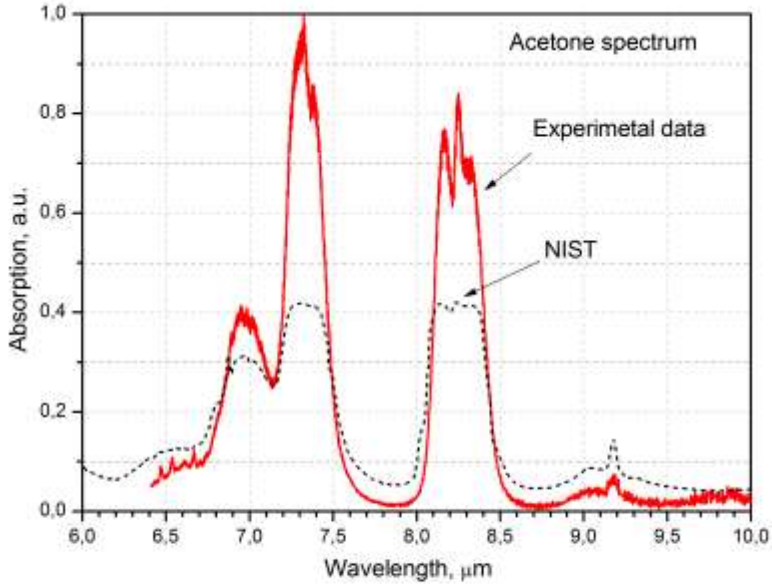

Figure 3. Acetone absorption spectra.

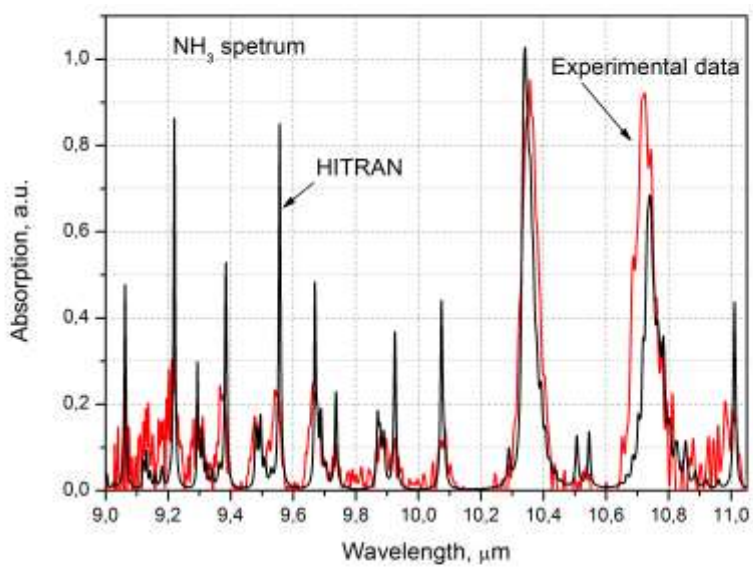

Figure 4. Ammonia absorption spectra.

Gas analyzer “LaserBreeze” could be used not only for multicomponent gas analysis, but also for screening tests, treatment effectiveness evaluation, and differential diagnostics[5].

Very perspective results of clinical research were obtained in Siberian State Medical University (Tomsk). Developing of new diagnostic method is in progress.

The advantages of “LaserBreeze” are: noninvasive, no penetrating radiation, real-time operation, no staff special requirements, sensitivity up to 1 ppb, tunable from 2.5 to 11 µm, volume of gaseous sample required for analysis – not more than 50 cm^3.

Photo of gas analyzer “LazerBreeze” is represented on the figure 5.

Figure 5. Photo of "LaserBreeze"

4. THZ GAS ANALYZER (80 – 1000 μm)

Exploitation of terahertz spectral range opens new perspective in complex molecules analysis. In difference from IR range where characteristic frequencies of localized oscillations of single chemical bonds in molecules are located, spectral response of molecular systems in THz range provides information about molecular motion corresponding to a molecule itself and its oscillations and rotation. Such motions are intrinsic for polypeptide, polysaccharide, and polynucleotide chains in complex biopolymers. Molecules spectra in THz range has intensive absorption lines, which characterize investigated materials definitely.

But we should remember that some of air components are serious obstacle for analysis of gaseous samples in the real conditions, for example: in the exhaled air at sensitivity limit of PA detectors.

On the figure 6 absorption spectra of some light molecules in the range 2-4 μm is shown. This spectrum is conditioned by both absorption of individual gaseous components (1% H_2O or 1% CO_2 or 1 ppm N_2O or another small gaseous component) and total absorption of this gases, calculated using HITRAN with resolution equal 2 cm^{-1}.

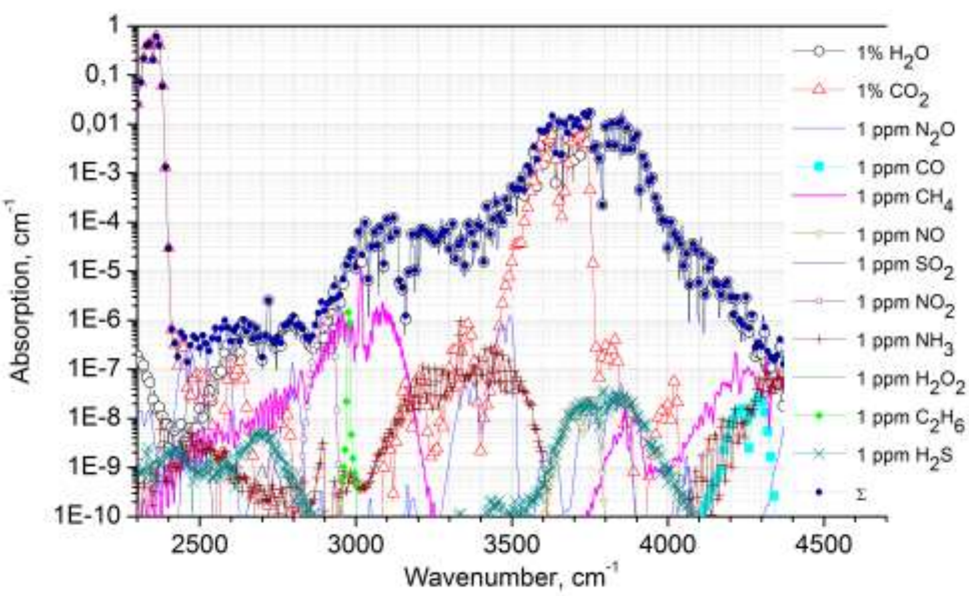

Figure 6. Absorption spectra in spectral range 2-4 μm

So, only in spectral range 2400-2600 and 4300-4500 cm^{-1} contribution of minor constituents to total absorption is visible. Also, for exhaled air we got following possible volume concentration for: CO_2 – 5 ppm, NO – 0.5 ppb, NH_3 – 1 ppb, HCN – 300 ppt, Acetylene – 0.7 ppb.

Absorption of carbon dioxide makes no appreciable contribution when THz optical radiation is using. But, water vapor and oxygen absorption can be significant. For example, exhaled air contents about 16% of oxygen. On the figure 6 you can observe absorption spectra for terahertz range with resolution 0.001 cm^{-1}.

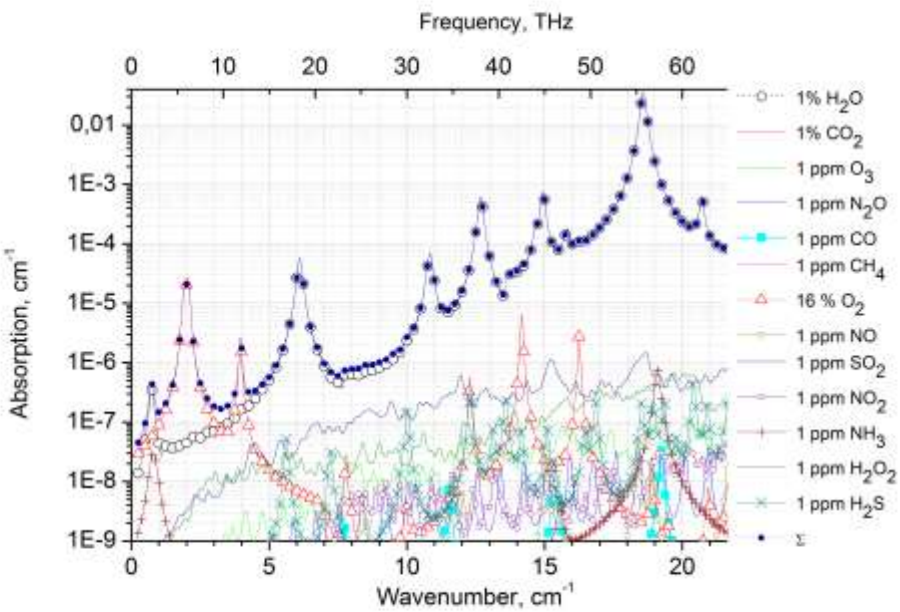

Figure 7. Absorption spectra in spectral range 2-4 μm

It is shown, that condition for determination of minor components' concentration is worse, due to 1% H_2O and 16% O_2 absorption which more than order of magnitude exceeds contribution of these gases. To reduce the concentration of disturbing gases it is necessary to make sample preparation, but it leads to the additional costs and also can change concentration of the investigating gases in the analyzed probe.

Thus, IR range is more appropriate for controlling the presence of light molecules in the exhaled air. THz range is quite attractive especially for strong absorption lines that are characteristic of a number of biological macromolecules if absorption in IR range is not informative.

Among biological macromolecules the biomarker of inflammation in diseases of the broncho-pulmonary system is proteolytic enzyme elastase, which is secreted by neutrophils migrating into the area of inflammation. Elastase can damage the connective tissue of the lungs, destroy the alveolar wall, that leads to reduction of the lung volume and to the development of respiratory failure. Activation of elastase activity is compensated when pneumonia by increase in of its inhibitor – alpha-1-proteinase inhibitor. In chronic inflammation, in the case of obstructive lung disease, excessive activation of elastase is promoted by growing deficit of alpha-1-proteinase inhibitor. The imbalance of the "elastase-inhibitor" system may be the basis of neoplastic cell transformation and the development of lung tumors. Biomarker of neoangiogenesis in tumor tissue is said protein VEGF, the concentration of which increases in the presence of a tumor in the body. These biomarkers are of relatively low molecular weight in the range of 30-40 kDa (atomic mass unit, it is also daltons - Da) and are determined in blood serum and other biological fluids: saliva, induced sputum, broncho-pulmonary lavage. Since there is the relationship between measures of sputum and gas composition exhaled air, the study of the spectrum of these new potential biomarkers in the terahertz frequency region may have important clinical and diagnostic value for the diagnosis of lung diseases. Such markers may potentially be more specific for the lung tissue damage than the low molecular weight biomolecules, such as NO, CO_2, NH_3, whose content in the exhaled air varies from quite general reactions to the inflammatory process.

Within Federal program "Research and development in priority directions of Scientific and Technical complex progress in Russia 2007 - 2013" (contract 14.512.11.0021) Special technologies Ltd. together with Siberian state medical university, Institute of Laser Physics SB RAS and Institute of Atmospheric Optics SB RAS carry out scientific work for development and creation mobile THz systems for non-invasive diagnostic of bronho-pulmonary diseases with use of exhaled air analysis.

THz laser with optical pump from tunable CO_2 laser is used as the basis. Laser block diagram is shown on a figure 8. Pump laser radiation with the matching lens L is input to THz module, providing excitation of the active medium of the laser. To eliminate the influence from pump laser radiation on the results of measurements is used spectral filter. This spectral filter is output window of the module. With help of the beam splitter part of the THz radiation is directed to the reference detector, which can be PAD or bolometer or Golay detector (for weak generation lines).

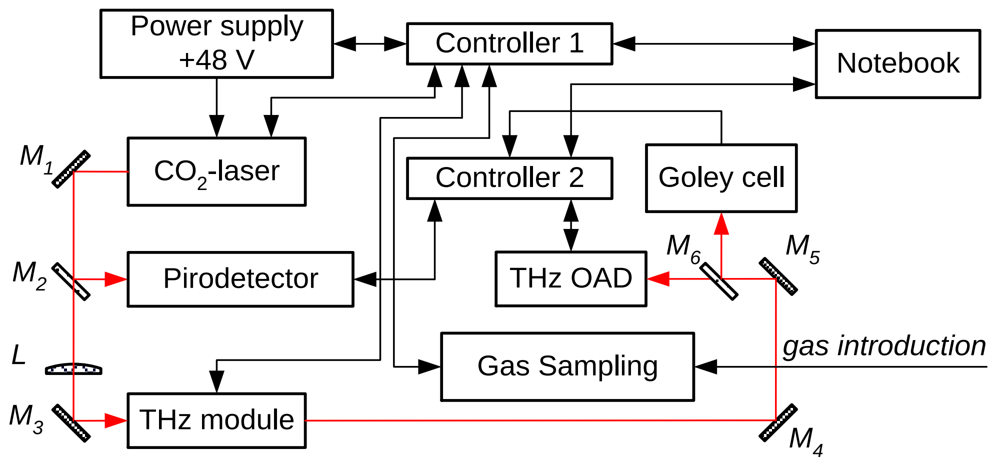

Figure 8. THz Laser block diagram. M1, M3, M4, M5 – deflecting mirrors, M2, M6 – beam splitters, L - focusing lens.

This design provides automatic tuning along the lines of the THz radiation in the range from 80 to 1000 μm. Maximum power THz radiation is about 100 mW. To detect the level of absorption in THz region we use special PAD, adapted to the characteristics of the THz region.

For registration of radiation absorption the laser beam of THz laser is directed to the resonant PAD, which is filled with the sample of analyzed air, previously taken from the patient. PAD has a window with low absorption coefficient in the THz range. Additional detector for beam attenuation recording can be placed at the output of the PAD. Generated acoustic waves are recorded by microphone when amplitude-modulated laser beam absorbed in the gas in the PAD volume. THz module is shown on figure 9.

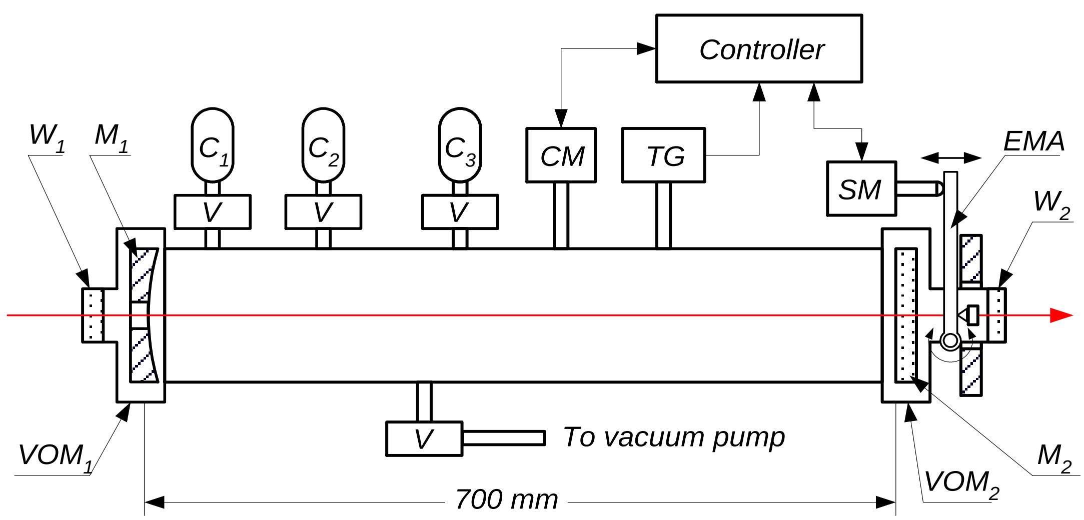

Figure 9. THz module. W_1 – ZnSe window, W_2 – TPX window, V – vacuum valve, C_1 – C_3 – chambers with a gain medium, VOM_1, VOM_2 – vacuum optical mounts, M_1, M_2 – laser cavity mirrors, CM – capacitance manometer, TG – thermocouple gauge, SM – stepper motor, EMA – electro-mechanical actuator.

Optical elements of the resonator are mounted at the ends of the cylinder, and allow inputting into cavity radiation of CO_2 laser and output THz radiation. Encapsulated adjuster/positioner used for adjustment of the cavity mirrors.

Electromechanical actuator is used to adjust the cavity length for choosing a particular generation line.

For filling the vacuum cavity with vapor of working mixture special containers with corresponding chemicals (CH_3OH, R32, R40, R41 and other) are connected to the cavity. These connections are managed by adjustable valves. Pressure control is provided by the associated detector. By the same procedure, if it is necessary, the buffer gas can be added to the cavity. Some other devices like vacuum post, nitrogen trap are connected to the cavity.

ZnSe input window of the THz module allows inputting pump laser radiation inside the vacuum chamber of THz resonator. THz resonator module consists of two mirrors. The total reflector with high reflection coefficient in the mid-IR and THz has coupling aperture, intended for input radiation of the pumping laser. The second mirror is selective. It has high reflectivity for laser pumping. THz radiation removes through coupling aperture. High-purity silicon is used as the mirror substrate.

Proposed approach has a number of differences from other implementations of optical THz spectroscopy. First of all, it should be noted that this system has high conversion efficiency of the laser pump into the THz range (>0,1%) and high absolute value of the power in THz (hundreds of mW) in comparison with other methods, that reduces the sensitivity requirements of THz radiation detectors and, in particular, allows using pyrodetector as a reference.

Another important factor is the narrow THz bandwidth due to the physical features of the laser with optical pumping. This may be critical in the case of detecting real gaseous markers with inevitable presence of water vapor. Since the laser bandwidth with optical pumping may be substantially less than 1 cm^{-1} (as opposed to other sources in this spectral range) referring to water absorption lines can be used, that substantially improves the detection sensitivity threshold.

In addition, such systems are very compact and permit to creation of mobile applications for the laboratory diagnostics of human diseases.

Another feature of our approach is use of three-dimensional PAD that allows examination of gaseous samples. Until now, this method had been studied by us with use of near and mid-IR laser sources, and, as mentioned above, it had been proved to be effective in determining different gases. Development of THz range will significantly expand the diagnostic capabilities of photo-acoustic gas analysis and opens up new possibilities for the study of biological tissue samples in this spectral region.

5. DEVELOPMENT OF TECHNIQUES FOR MEDICAL DIAGNOSTICS

Voluntary patients were investigated using medical devices LGA-2 and ILPA-1. In the study of bio-markers following samples were taken:

1. Exhaled air.

2. Smear from the inner surfaces of the cheeks.

3. Smear from the surface of the tonsils.

4. Smear plaque from the teeth. This trial was going on possibilities: because many patients - older people, some teeth were missing completely.

5. Smear from the surface of the axilla.

6. Smear from the surface of the forearm.

All samples were taken before meals or 30-40 minutes after it. The patients involved in smoking tobacco did not smoke before sampling at least 30 minutes. Prior to sampling the patients rinsed mouth under running water. The study does not imply special cleaning or washing the surface under study[6-8].

There were four groups of patients with traditionally confirmed diagnoses. First group - healthy, second - different pulmonary diseases (COPD, asthma, pneumonia), third - hepatitis, fourth group - tuberculosis. Integral values distribution shows well-determined clusters corresponding to the groups of patients. Test results are given on the figure 10.

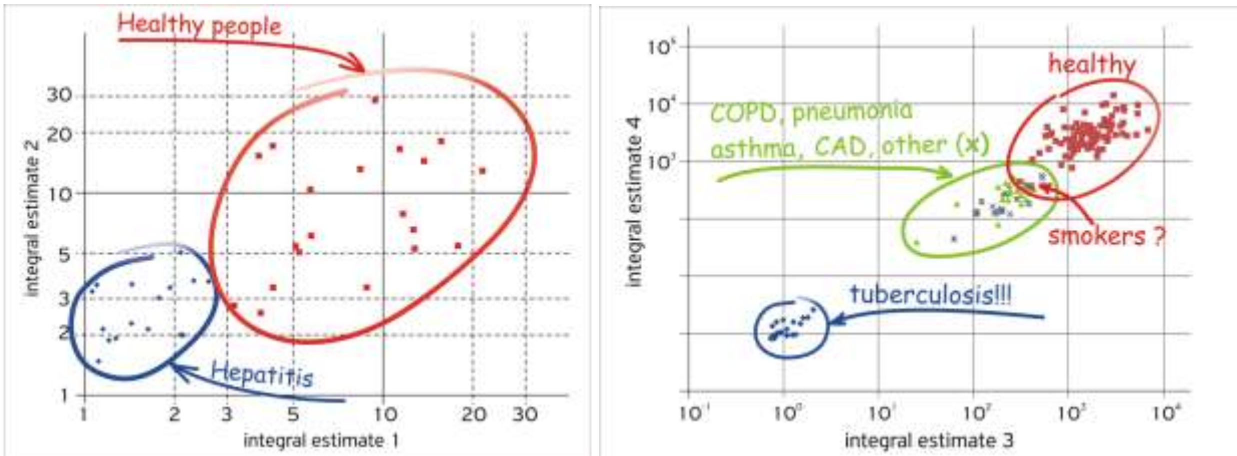

Figure 10.

The figure shows that patients with COPD, lung cancer patients, smokers and non-smokers healthy volunteers divided sufficiently well.

Selecting the most informative spectral ranges from the point of view of separation of the target group, the comparison group and the control group was performed using method of principal components included in the analytical part of the program ViDa Expert 1.0. Processing of the spectral data using the Method of principal component is one of the main ways to reduce the dimensionality of the data without losing so important information[9].

Several most well described bio-markers and corresponding diseases are shown in table 1:

Molecule biomarker	The range of concentrations	The range of concentrations,	Molecule biomarker	The range of concentrations, µm
NH_3	0-1 ppm	250-3000 ppm	Lung cancer Asthma	8,0-10,1; 10,2-12,0
H_2O_2	2-10 ppm	1000 ppm	COPD Asthma Tuberculosis	2,7; 7,2; 7,9; 11,3
NO	1-20 ppb	15-50 ppb	COPD Asthma	5,2-6,2; 7,7
N_2O	1-20 ppb	15-50 ppb	COPD Asthma	4,4-4,7; 7,5-8,0
CO	1-10 ppm	13,5 - 20 ppm	COPD Asthma	4,6-4,8
C_5H_8	50-120 ppb	300-1000 ppb	Lung cancer Alcoholic intoxication	3,2-3,5; 6,1-6,4; 10,5-11,5
C_2H_6	0-10 ppb	50-100 ppb	Lung cancer Asthma	3,2-3,5
C_5H_{12}	0-10 ppb	50-100 ppb	Lung cancer Asthma	3,3-3,5
CH_3-C(O)-CH_3	0,4-1,0 ppm	0,8-5 ppm	Diabetes Lung cancer	5,8-6,0; 7,0-7,6; 8,0-8,5

6. CONCLUSION

We believe our approach to be very perspective for screening studies of population for revealing different diseases. Ongoing research and development not only in the field of medicine and health care, but also in general gas analysis, allow our team successful implementation of such devices as:

• Laser gas analyzer LGA-2

• Laser gas analyzer ILPA-1

• Leak detector SF_6 LaserGasTest

• Laser photo-acoustic medical gas analyzer LaserBreeze

The need for further R&D in the field of gas analysis of various substances in wide spectral range is confirmed by interest towards the above mentioned devices.

Today we can say with certainty about the interest in the recently developed LaserBreeze not only from Russian scientific institutions, but also from a number of foreign companies. LaserBreeze can be used in research institutions, medical and biological schools for precision quantitative study of multi-component gaseous biological samples.

Development of scientific and technical bases for use of terahertz radiation will provide progress in this area.

Working in THz range will significantly expand the diagnostic capabilities of photo-acoustic gas analysis and opens up new possibilities for studying biological tissue samples in this spectral region.

7. ACKNOWLEDGMENTS

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