MEASUREMENTS OF CH_4, $^{12}CO_2$, $^{13}CO_2$ AND H_2S CONCENTRATIONS FOR MEDICAL SCREENING DIAGNOSTICS

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THE SCREENING APPROACH

◆ Human body is a complicated optimized system. Any disease will remove it from equilibrium and can be detected.

Example: temperature. All humans have equilibrium temperature 36.6^{o}C. Temperature change by 1^{o}C (0.3 %) is a disease signature. The same is true for trace molecules concentration in blood or exhaled breath. In equilibrium they have certain value. Disease could shift this equilibrium leading to significant change of trace molecules concentration in blood or exhaled breath.

◆ Most important tasks for screening of exhaled breath are to determine markers that can be used for medical diagnostics, find their “normal” values and define dependences of these values on experimental conditions (exercise stress, breath holding, measurements before/after meals etc.) and patient parameters (age, sex, weight etc.). This requires investigation of a large number of patients with the help of statistical data processing.

POTENTIAL BIOMARKERS

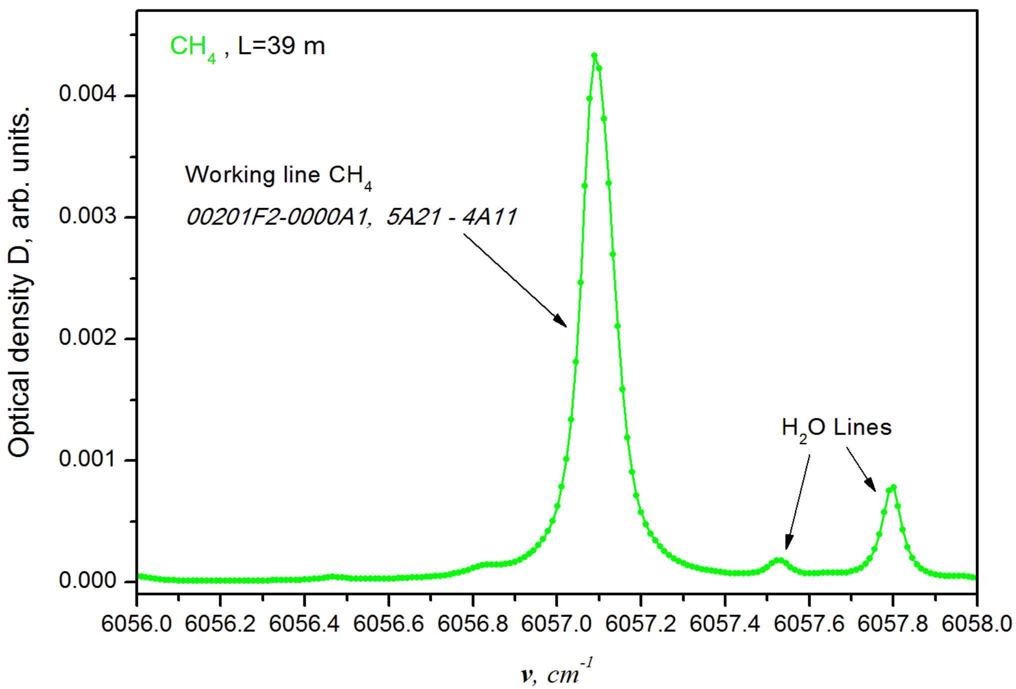
◆ To date, more than *1,000* compounds have been identified to be present in exhaled human breath. Their concentrations range from ppb to ppt levels. Approximately, *35* of the identified compounds in the exhaled breath have been established as potential biomarkers for particular diseases and metabolic disorders [3].

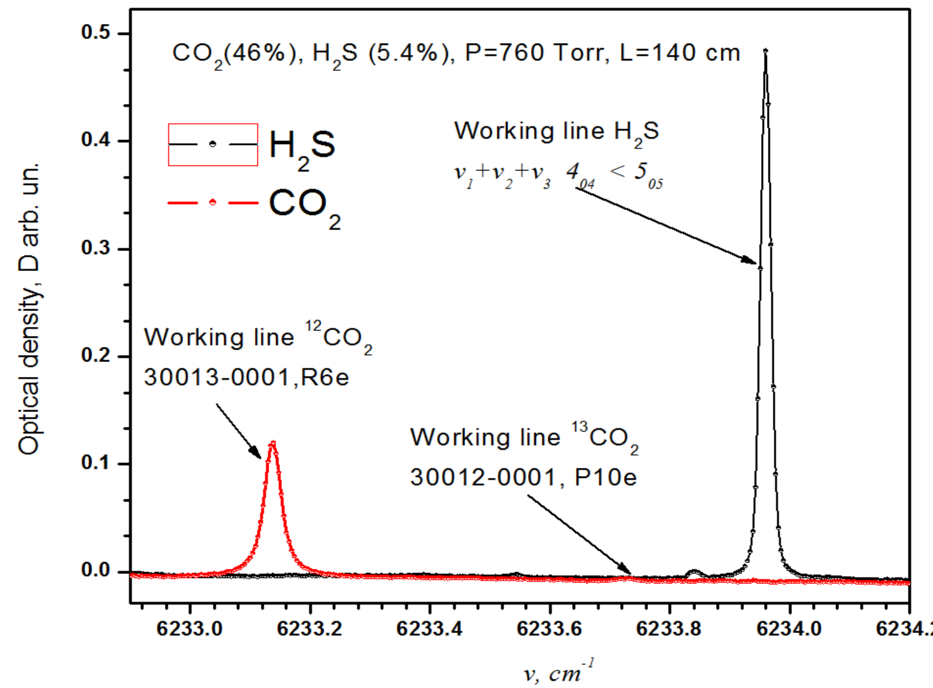
◆ In present work we have focused on:

Name	**Concentration in human breath [4,5]**	**Metabolic Disorders / Diseases**
Carbon dioxides(CO_2)	3 %	The main product of gas exchange. Oxidative stress, helicobacter pylori infection
Hydrogen sulfide (H_2S)	-	Marker of the putrefaction products in the respiratory and gastrointestinal tract
Methane (CH4)	4-6 ppm	The product of bacterial activity. Inflammation in respiratory and gastrointestinal tract. Intestinal problems, colonic fermentation

SPECTRAL RANGES

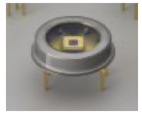
- We have selected two spectral ranges: near 1,65 μm (CH_4) and near 1,60 μm (CO_2 and H_2S).
- Experimental spectra:





TDL SPECTROMETER

- PD – Hamamatsu Photonics InGaAs photodiodes, ø 1 mm, $D^* = 1.5\cdot10^{12}$ $cm\cdot Hz^{1/2}/W$



- DL – NTT Electronics diode lasers

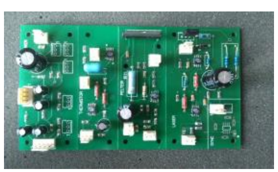
DFB, λ=1.65 μm and λ=1.60 μm

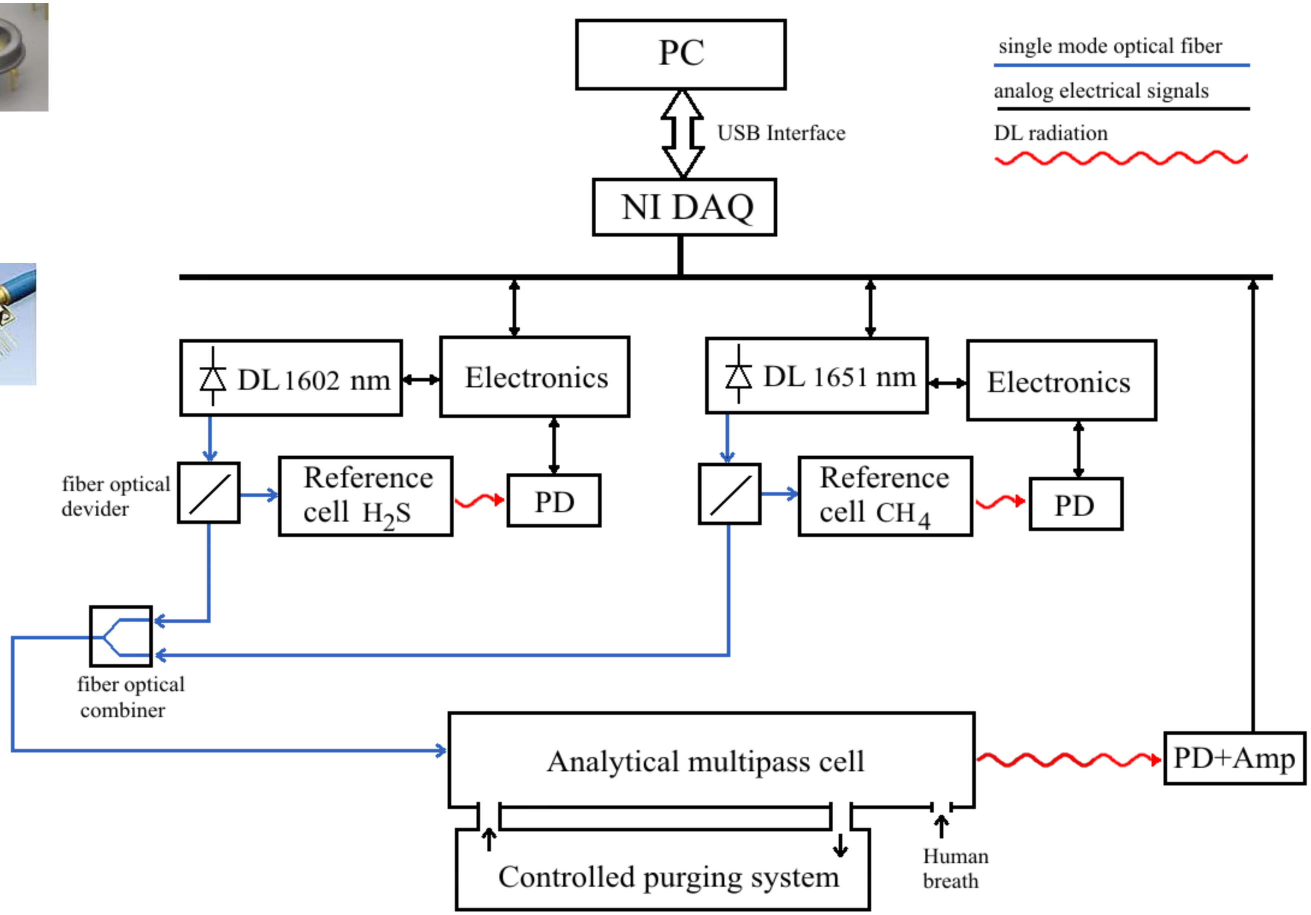
fiber single mode, ø 1.3 μm

P=15 mW. Pulse duration 1-2 ms

- NI DAQ – National instruments multifunctional DAQ board NI USB 6289

- Electronics – DL current and temperature control board, amplifiers

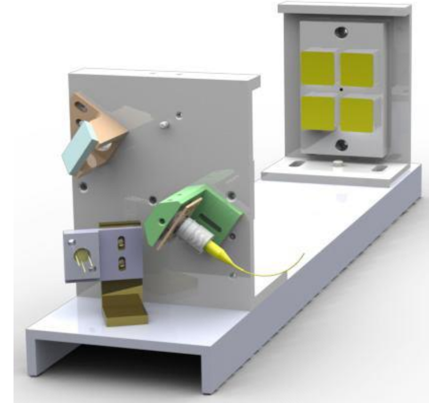




TDL spectrometer block-scheme

TDL SPECTROMETER

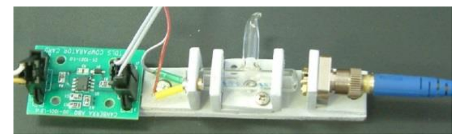
◆Analytical cell - ”Chernin” matrix multipass optical system, single mode fiber input into cell, mirror, and PD to detect DL light from cell. Length 25 cm (between mirrors), optical path L=39 m, physical volume 0,0025 m^3



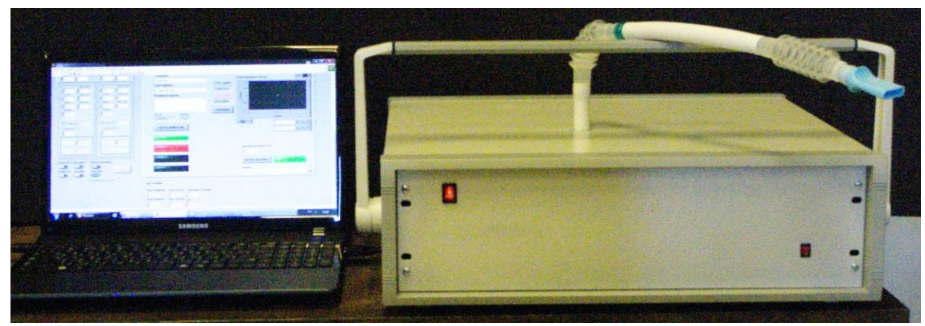
◆Reference cells – 2 glass cells with reference gas.

1 - H_2S L=25 cm, P_{H2S} =60 Torr in 1 Atm N_2;

2 - CH_4 L=4 cm, P_{CH4}=56 Torr in 1 Atm N_2



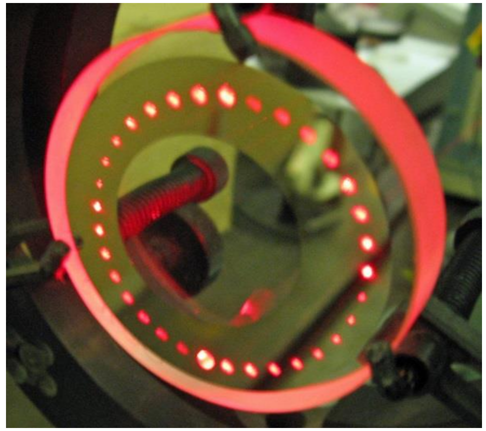
TDL spectrometer - laboratory design

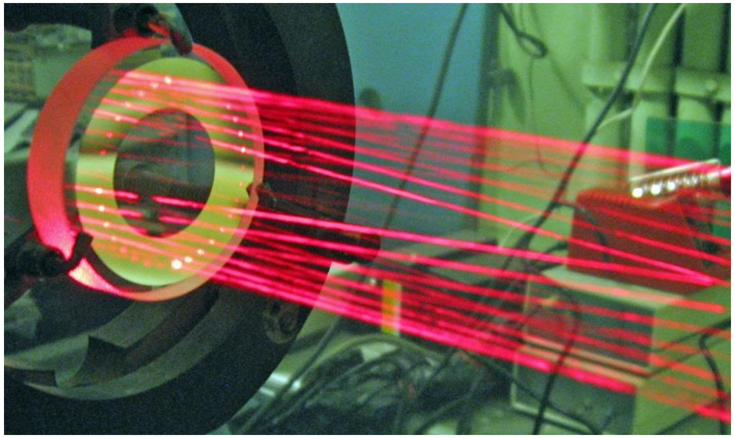


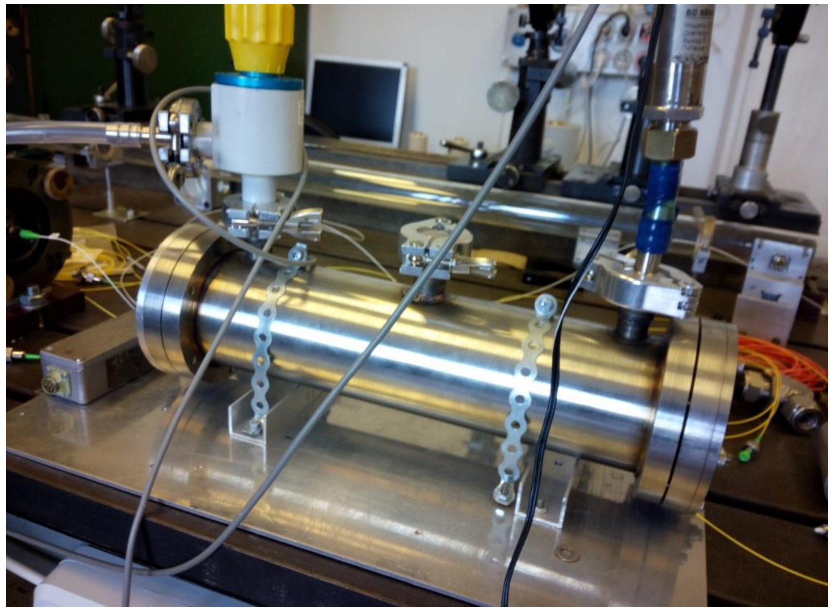
TDL spectrometer - modern design

THE NEW VERSION ANALYTICAL CELL

◆Analytical cell - ”Herriott” multipass optical system, single mode fiber input into cell, mirror, and PD to detect DL light from cell. Length 30 cm (between mirrors), optical path L=25 m, physical volume 2.6 L





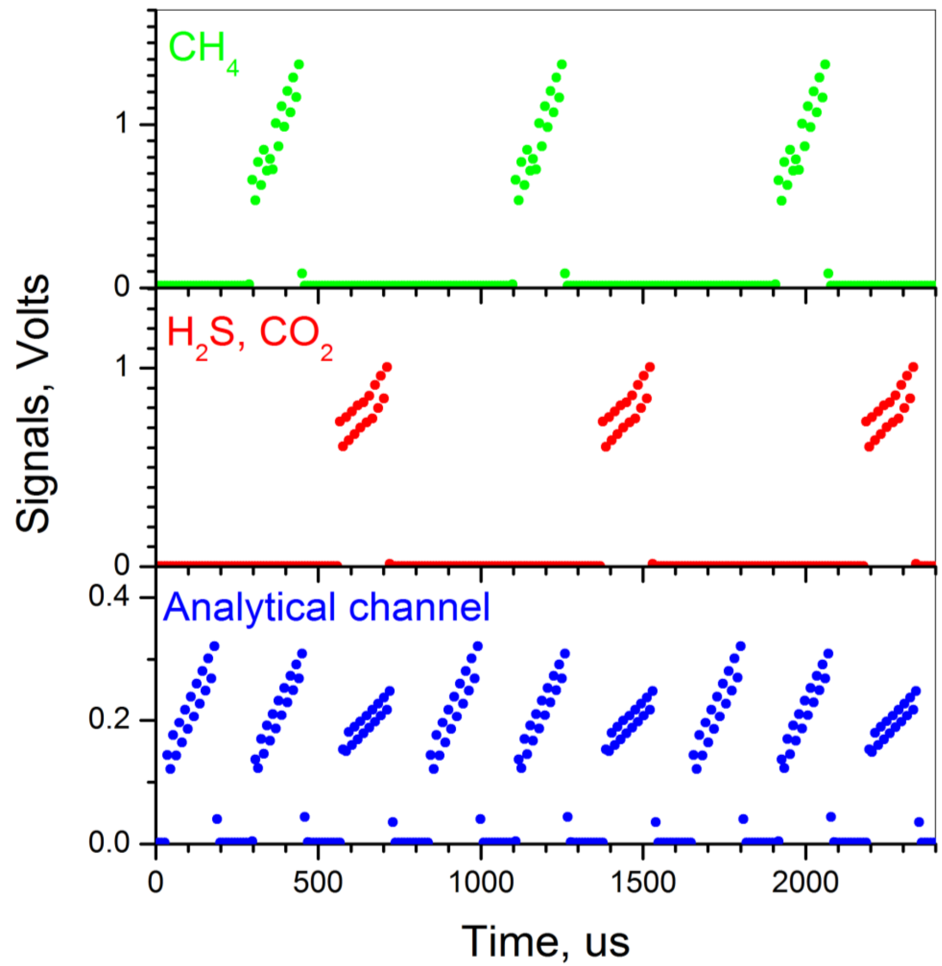


MULTICHANNEL DETECTION

◆To provide selectivity of multiple molecular samples detection, a time multiplexing operation regime is used.

Each laser operates at different time. Two upper graphs show signals recorded in two different reference laser channels. DL radiation from these three channels was combined in one single-mode fiber. Lower graph presents signal in analytical channel.

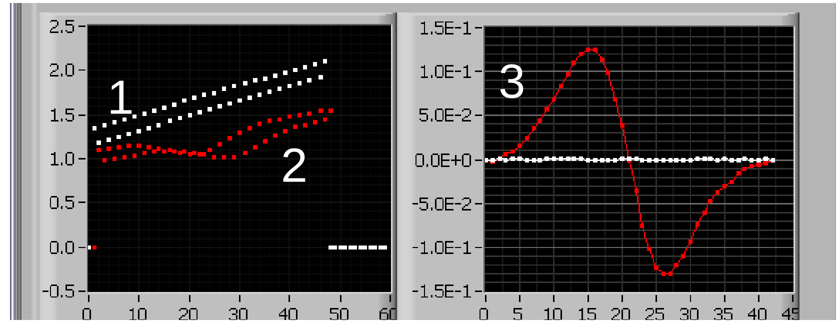
H_2S and CO_2 are recorded by one 1,6 μm DL.

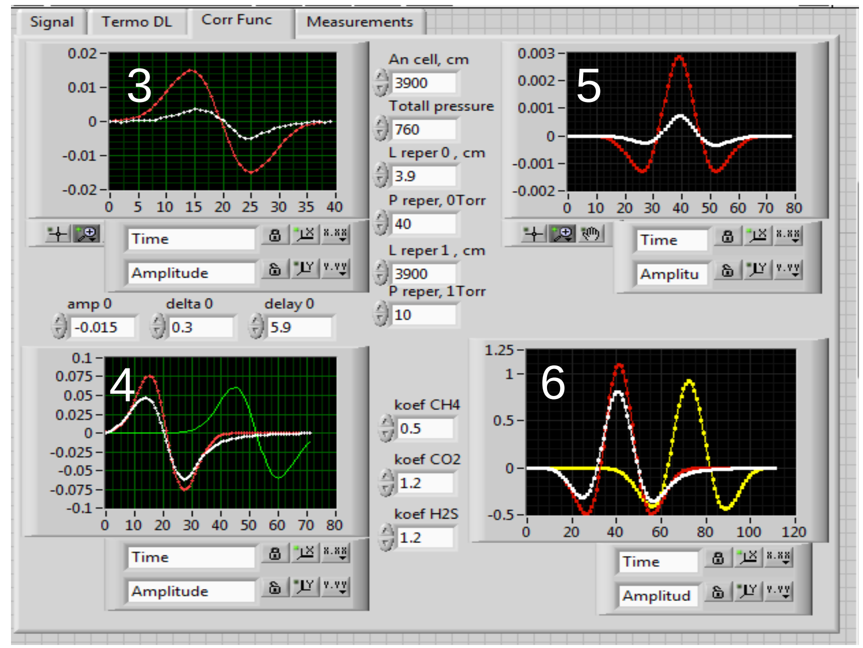


SOFTWARE

- Software for DL spectrometer is based on NI LabView 2014.

DL is excited by trapezoidal current pulse with modulation (1). In presence of molecular absorption two lines can be observed due to modulation (2). Using these data computer calculates signal looking like line first derivative (3) and filters it by correlation with a model function (4). Reference signal used for DL frequency scan stabilization (5) and analytical – for concentration calculations (6).





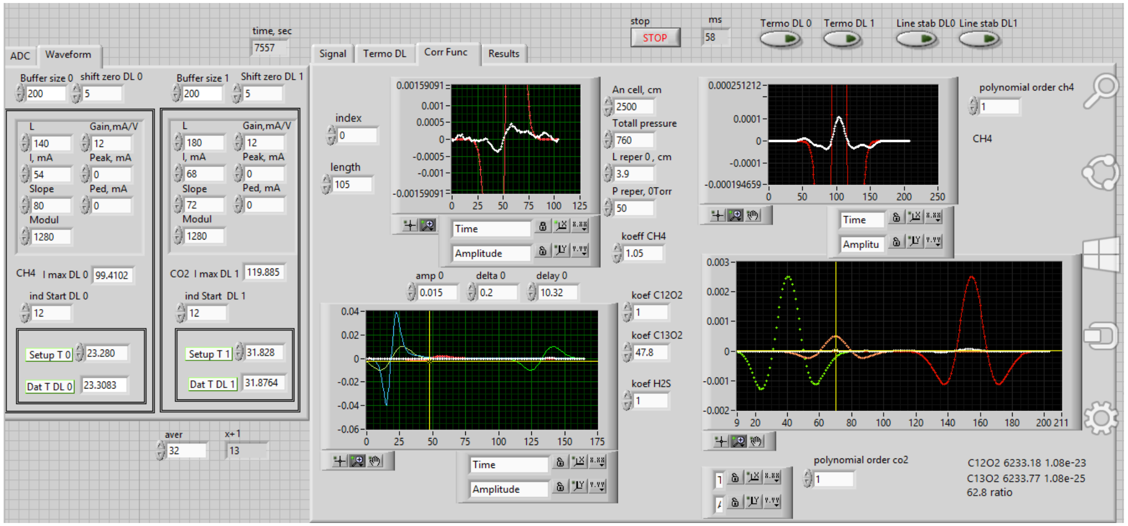
- Concentrations:

$$C = \frac{\alpha \cdot P_r \cdot L_r}{P_a \cdot L_a} \cdot 10^9, [ppb]$$

$$C_{xy} = \alpha \cdot C_{xx}$$

P_r – reference pressure, L_r – reference optical path, P_a - pressure in analytical cell, L_a – optical path in analytical cell. C_{xy} - cross correlation of model function and signal first derivative (white on 6), C_{xx} – autocorrelation of reference model function (red on 6).

SOFTWARE CONTINUE



◆ Left window - simultaneous correlation processing signal from DL. Linear regression for obtain concentration CH_4, $^{12}CO_2$, $^{13}CO_2$, and H_2S.

◆ Right window - Display visualization results of real time measurements of biomarkers concentration (CH_4, $^{12}CO_2$, $^{13}CO_2$, and H_2S).

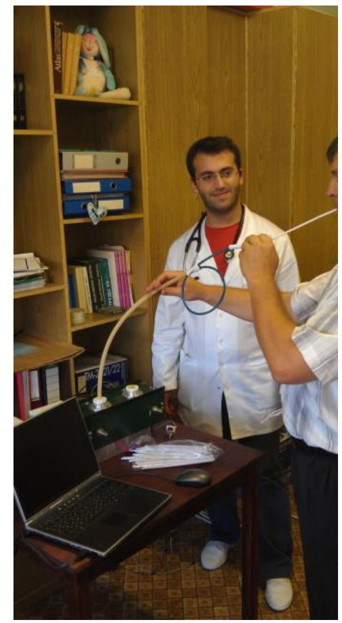
CLINICAL TESTS

- TDL-spectrometer has been tested in Moscow City Clinical Hospital N 12. Exhaled breath of 80 patients with known diagnoses have been studied. 14 patients have been tested before and after meals.

- Studied disease: diabetes mellitus, gastritis, bronchitis, pneumonia, chronic obstructive pulmonary disease, cirrhosis, chronic renal failure, hypertonic disease, coronary artery disease.

- Duration of test procedure <1min

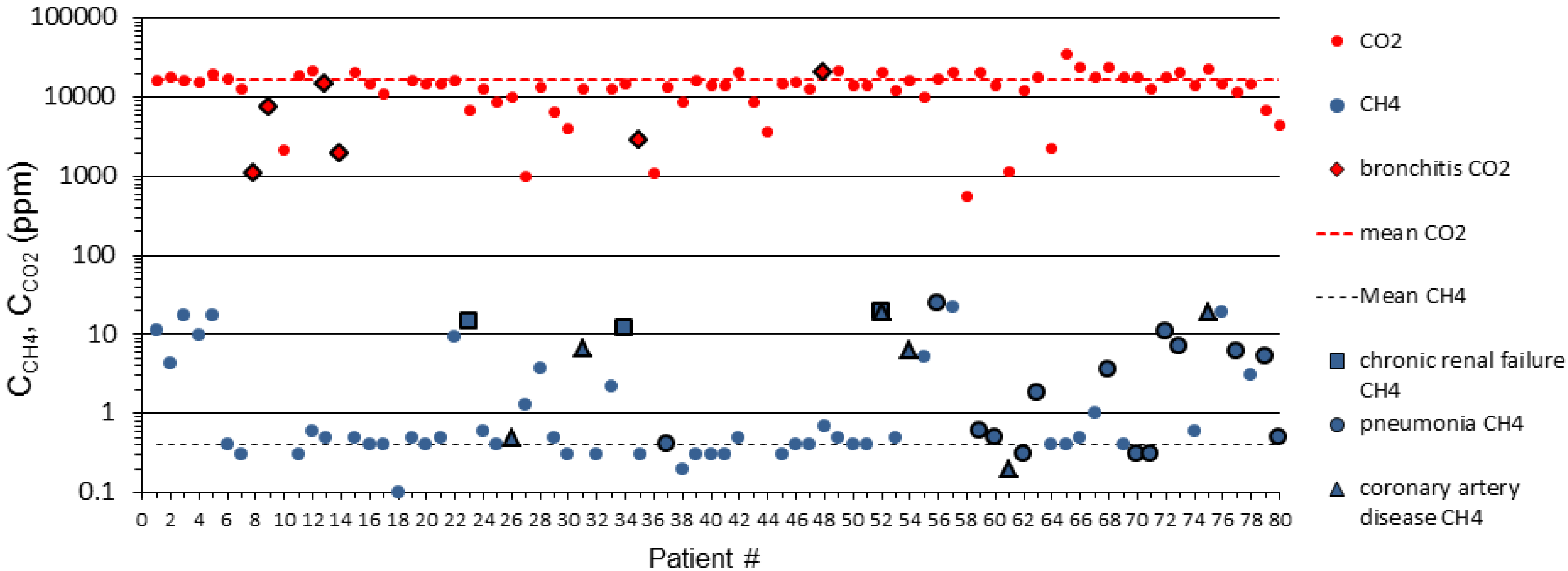
- Moscow City Clinical Hospital N12



- Breath test procedure

CH_4 AND CO_2 CONCENTRATIONS

◆ CH_4 and CO_2 concentrations in exhaled breath for 80 patients.

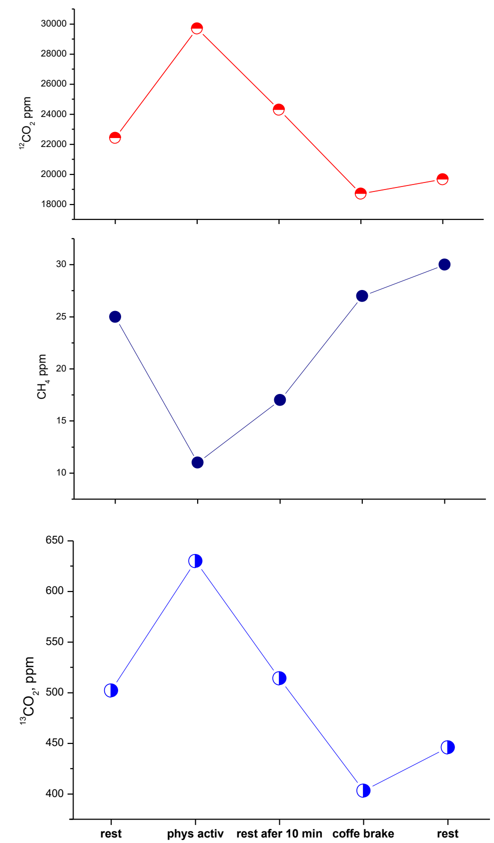


◆ For most patients CO_2 and CH_4 concentrations tend to grouping near constant value (~16000 ppm and 2.5 ppm respectively). For some patients one can see deviations from these values by orders of magnitude. Some typical disease are also presented on this graph

A COMPREHENSIVE ANALYSIS OF GASEOUS METABOLITES EXHALED AIR BY THE TDLS METHOD WITH DIFFERENT PHYSIOLOGICAL STATES OF A HUMAN.

Illustration of the dynamics of exhaled gases under different conditions (patient 72 years old, 10.06.2015 time from 13.00 to 14.30)

Parameters	Исх.показат. калибровки Baseline calibration	State of rest [13.00 hh.mm.]	Physical activity [13.10 hh.mm.]	State of rest 10 minutes. [13.20 hh.mm.]	After 30 min. after meals [14.15 hh.mm.]
CO_2 (ppm)	495	16480	22150	15400	17930
$^{13}CO_2$ (ppm)	34	165	185	154	317
CH_4 (ppm)	2,5	14	6,4	14	21
H_2S (ppm)	0	0	0	0	0
Respiratory rate (breath/min)	-	18	28	20	18
Blood pressure (mm Hg.CT.)	-	140/80	150/80	140/80	130/80
Heart rate (beats/min)	-	86	115	90	89
SpO_2 (%)	-	97,00%	97,00%	98,00%	98,00%
Blood glucose (mmol/l)	-	4,4	4,1	3,9	5,9



Dynamics of changes in concentrations of biomarkers at different physical conditions

RESULTS

◆ The impact of physical load factors significantly changes the composition of exhaled breath biomarkers (see graphs and tables). The most significantly physical loading influenced on such parameters as CO_2 and CH_4 concentrations, as well as heart rate, respiratory rate, SpO_2, blood glucose.

◆ The initial increase in the content of $^{12}CO_2$ indicates hidden or apparent abnormalities of gas exchange in lung function or reduced basal metabolism. This may be an indirect marker of latent respiratory diseases, hypothyroidism, and other pathological processes.

◆ At a physical exercise stress a level of CH_4 in the exhaled air is substantially reduced which results from "burning" of methane due to the acceleration of metabolic reactions.

◆ Concentration of ^{13}CO is clearly correlated with the functional state of gastric digestion (it reacts to the reception of food, the availability of functional disorders of the gastrointestinal tract), what is confirmed by numerous clinical studies of urease breath test.

- At the moment CH_4, CO_2 and H_2S concentrations for 80 patients have been studied. 14 patients were tested before and after meals.

- For some patients with chronic renal failure, coronary artery disease, and pneumonia a high (about an order of magnitude from mean) concentration of CH_4 was discovered. For some patients with bronchitis we discovered abnormally low concentration of CO_2 (also about an order of magnitude).

- For most patients H_2S concentrations was bellow 1 ppm. For some patients (most patients with hypertonic disease and diabetes mellitus) high H_2S concentration have been found (1-5 ppm). However, these data demonstrate large variations and we haven't discovered any trend to "normal" value.

- The main conclusions from the results of preliminary clinical testing of the gas analysis of exhaled air by the method of the diode laser spectroscopy (DLS) are: 1) the DLS method meets the requirements of innovative screening technologies to identify latent pathological processes and functional abnormalities in humans. 2) deviations of gaseous metabolites content in the exhaled air at different physical exercise stress allow to recognize the risk of latent diseases and to assess the degree of functional disorders. 3) Constructive improvement of TDLS-device allowed to simplify the procedure for examination of patients, to provide high mobility of the device and to enhance the safety of its use in mass screening in medical and physiological research.

LINKS

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4. K.L.Moskalenko, A.I.Nadezhdinskii, et all, Tunable diode lasers application for fully automated absolute measurements of CO and CO2 concentrations in human breath, Proc.SPIE, 2205, 440-447, 1994

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