

# Diode laser based photoacoustic gas measuring instruments intended for medical research

Anna Szabó<sup>\*a</sup>, Árpád Mohácsi<sup>a</sup>, Péter Novák<sup>b</sup>, Daniela Aladzic<sup>b</sup>, Kinga Turzó<sup>c</sup>, Zoltán Rakonczay<sup>c</sup>,  
Gábor Erős<sup>d</sup>, Mihály Boros<sup>d</sup>, Katalin Nagy<sup>b</sup>, Gábor Szabó<sup>a</sup>

<sup>a</sup>Department of Optics and Quantum Electronics, University of Szeged, Dóm tér 9., Szeged 6720, Hungary

<sup>b</sup>Department of Oral Surgery, University of Szeged, Tisza L. krt. 64., Szeged 6720, Hungary

<sup>c</sup>Department of Prosthodontics and Oral Biology, University of Szeged, Tisza L. krt. 64., Szeged 6720, Hungary

<sup>d</sup>School of Medicine Institute of Surgical Research, University of Szeged, Pécsi u. 6., 6720 Szeged, Hungary

## ABSTRACT

Analysis of breath and gases emanated from skin can be used for early and non-invasive diagnosis of various kinds of diseases. Two portable, compact, photoacoustic spectroscopy based trace gas sensors were developed for the detection of methane emanated from skin and ammonia emanated from oral cavity. The light sources were distributed feedback diode lasers emitting at the absorption lines of ammonia and methane, at 1.53  $\mu\text{m}$  and 1.65  $\mu\text{m}$ , respectively. Photoacoustic method ensures high selectivity, therefore cross-sensitivity was negligible even with large amount of water vapor and carbon dioxide in the gas sample. In case of ammonia a preconcentration unit was used to achieve lower minimum detectable concentration. Gas sample from the oral cavity was drawn through a glass tube to the preconcentration unit that chemically bonded ammonia and released it when heated. The minimum detectable concentration of ammonia was 10 ppb for gas sample volume of 250  $\text{cm}^3$ . For methane minimum detectable concentration of 0.25 ppm was found with 12 s integration time, and it was proved to be adequate for the detection of methane emanated from human skin and from mice. Instruments measuring methane and ammonia are currently installed at two medical research laboratories at University of Szeged and tested as instruments for non-invasive clinical trials. The aim of the measurements is to determine correlations between diseases or metabolic processes and emanated gases.

**Keywords:** ammonia, methane, near infrared, non-invasive, oral cavity, photoacoustic spectroscopy, medical research

## 1. INTRODUCTION

The idea that body fluids (including blood, urine) and tissues can be collected and analyzed to yield information for diagnosis of disease states or to monitor disease progression has had high importance in health care and research for hundreds of years. Nevertheless, there is a continuous interest in implementing novel non-invasive, real-time, easy-to-use and reliable diagnostic tools for common clinical practice. Breath and gases emanating from skin has been examined as a promising method in recent times, since the measurement of low levels of trace gas components is attainable [1]. Detailed qualitative studies were only feasible as a result of highly selective and sensitive detection (from parts-per-million to parts-per-billion) of gas molecules by gas chromatography, mass or optical spectrometers. As far as breath analysis is concerned, despite the large number of publications and the potential in diagnostic [2, 3], it has not gained wider use for several reasons including the lack of proper instruments, standardization. Correspondingly to breath analysis an increasing number of research into correlations of gases emanating from skin [4, 5] and oral cavity [6] with metabolic processes and diseases have been reported.

\*aszabo@titan.physx.u-szeged.hu; phone 0662544285

Present paper describes two gas measuring instruments developed for clinical applications. The systems are based on photoacoustic (PA) spectroscopy and their light sources are diode lasers operating in the near infrared range. These light sources provide high selectivity, excellent modulation capabilities, ten thousand of hours of operation, high stability and relatively low cost. PA spectroscopy is a subclass of optical absorption spectroscopy and it measures optical absorption indirectly via the conversion of absorbed light energy into acoustic waves due to the thermal expansion of absorbing gas samples. The main advantages of diode laser based PA spectroscopy are high sensitivity, zero-background, concentration measurement over a broad dynamic range and in-situ measurements can be made due to the portable design.

Instruments measuring methane and ammonia gases currently operate at the research laboratories of the Faculties of Medicine and Dentistry of the University of Szeged. The instruments have been specially developed for non-invasive clinical trials of physiologic processes and investigating oral health condition (including halitosis).

One of them measures the methane emission of living animals. Methane production of living organisms may play a role in certain physiologic processes and also may serve as an indicator of different pathologies.

In addition, the other detector has been developed for measuring ammonia from the oral cavity. Halitosis (oral malodor) affects a large part of the population and may cause major social and psychological problems. It is caused by microbial putrefaction within the oral cavity, and volatile sulfur compounds (VSCs), including hydrogen sulfide, methyl mercaptan and dimethyl sulfide are considered the major gases associated with halitosis. These components are the most extensively studied odorous compounds [7, 8]. It is well known that volatile fraction above putrefied saliva contains ammonia; therefore, it can be another component to diagnose oral health/hygiene in clinical dentistry [9]. The aim of the present study was to determine the correlation between ammonia and VSCs in the oral cavity. A commercially available gas chromatograph (OralChroma™ from ABIMEDICAL Corporation, Japan) was used to detect VSCs and an instrument based on PA spectroscopy for measuring ammonia was operated in consecutively.

Preliminary results indicate that gas concentration measurements are well reproducible and devices can operate reliably.

## **2. CHARACTERISTIC FEATURES OF THE INSTRUMENTS**

A PA spectroscopy based gas measuring arrangement generally consists of four main parts: a light source, a measuring cell with a pressure sensor, a controlling and processing electronic unit and gas handling system. The light sources of present devices are distributed feedback diode lasers. Their wavelengths can be tuned to an absorption line of the gas component by adjusting its temperature and current. The modulation required for the PA signal generation is provided by tuning the laser wavelength by its current between a value corresponding to the peak of an absorption line and a value corresponding to the wing of that line (wavelength modulation). The laser beam is directed through a PA cell where the generation of the PA signal takes place. The cell regularly contains a resonator that amplifies the sound generated by the PA effect if the modulation frequency of the laser is equal to a resonance frequency of the resonator. The signal is detected by an electret microphone and then an electronic unit amplifies and processes it. Furthermore, that electronic device (Videoton Holding Plc.) provides overall system control and measurement automatization [10]. It consists of a laser driver and temperature controller, a microphone amplifier, a data processing unit, and several input and output ports.

### **2.1 Methane measuring device**

The light source of the methane measuring instrument has an emitting wavelength of  $1650 \pm 1$  nm with an output power of 15 mW (from NTT Electronics). The laser beam is directed through a dual-pass PA cell (i. e. the output window is substituted by a gold coated mirror). The PA cell contains a cylindrical resonator (length is 3 cm and diameter is 0.43 cm), both were made of stainless steel. The first longitudinal mode of the resonator is excited by adjusting the modulation frequency of the laser to 5200 Hz. An electret microphone (Knowles, EK-3028) is attached at half length of the resonator because pressure antinode of the first longitudinal mode (PA signal maximum) exists there. The PA cell is temperature-stabilized at 40 °C to deflect inaccuracies deriving from the temperature dependence of the PA signal which principally originates from the temperature dependence of the microphone sensitivity and the resonance frequency of the resonator. Gas from the sampling chamber is drawn through the PA cell by a membrane pump and the gas flow rate can be controlled by a mass-flow controller. The complete PA measuring system (excluding the sampling chamber) is built into a portable, 19"4U instrument box.

A sampling chamber was prepared to accumulate methane emanating from a mouse. It was made of glass (dimensions: 4 cm x 5 cm x 9 cm), besides, it includes a metal grid ensuring that virtually the whole body surface of the animal is uncovered. It was found that approximately 10 minutes is required to achieve constant methane concentration in the chamber.

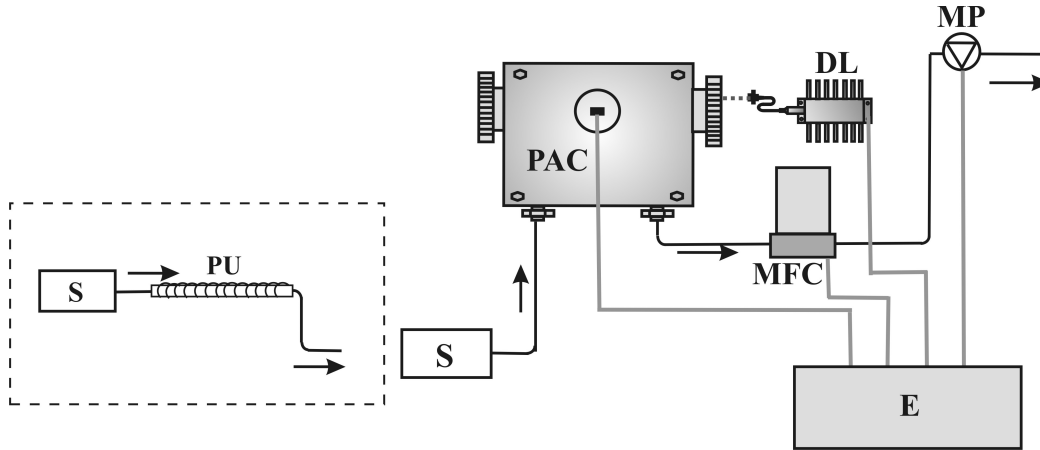


Figure 1. Schematic view of the photoacoustic instruments. Gas samples (S) are drawn by a membrane pump (MP) into the photoacoustic cell (PAC) where the signal is generated by a diode laser (DL). Gas flow rate can be adjusted with a mass-flow controller (MFC). The electronic unit (E) provides system control and data processing. Dashed line indicates that a pre-concentration unit (PU) is attached to the sampling unit (S) in case of the ammonia measuring instrument.

Cross sensitivity for common components of breath and ambient air were examined and no measurable instrument response was found for several vol. % of carbon dioxide or water vapor.

The system was calibrated by measuring the PA signal of various gas mixtures prepared from a cylinder containing 1 vol. % CH<sub>4</sub> in synthetic air and from humidified synthetic air, using mass-flow controllers. It is considered to be a linear relationship over 4-5 orders of magnitude between the PA signal and the concentration of the absorbing component [11], as Fig. 2 shows our results are consistent with that. Linear regression of data leads to the following equation ( $n = 7$ ,  $R^2 = 0.9999$ ):

$$S = 53 + 11.6 \cdot c,$$

where  $S$  is the PA signal in  $\mu\text{V}$  and  $c$  is the concentration of methane in ppm.

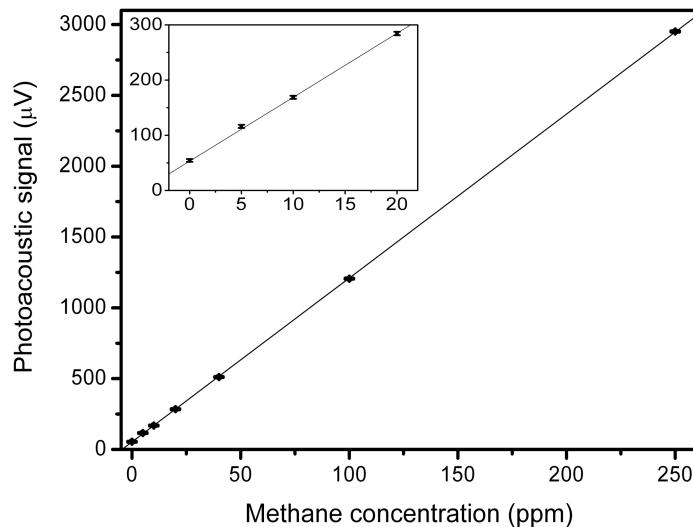


Figure 2. Photoacoustic signal as a function of the methane concentration. Solid line indicates linear regression of data. Error bars show three times the standard deviation of data. For clarity the inset shows low methane concentrations ( $n = 7$ ,  $R^2 = 0.9999$ ).

Minimum detectable concentration is calculated as three times the standard deviation of the measured signal of zero methane concentration ( $3.0 \mu\text{V}$ ), divided by the sensitivity which is the slope of the calibration line. Minimum detectable

concentration was found to be 0.25 ppm with an integration time of 12 seconds. Nevertheless, lower minimum detectable concentration value can be achieved by increasing the integration time.

## 2.2 Ammonia measuring instrument

Main parts of the ammonia measuring system are similar to those of the methane detector. The light source is a diode laser (from Furukawa Inc.) tuned to an ammonia absorption line around 1532 nm, with an output power of 40 mW. The PA cell (dual-pass) and the resonator were made of glass in order to minimize the adverse effects of adsorption-desorption processes on the wall, furthermore, the temperature of the cell is kept constant at 50 °C. Moreover, no sampling bag or chamber are required, gas directly flows to the detection unit. First longitudinal mode of the resonator is excited, therefore the electret microphone is attached to the resonator at half length.

Nevertheless, preconcentration unit is implemented in order to improve measurement accuracy. Gas sample from the oral cavity streams to a preconcentration unit which is a vanadium pentoxide coated glass tube that chemically bonds ammonia and releases it when heated (thermodesorption). The ammonia content of 250 cm<sup>3</sup> gas sample is concentrated in 10-15 cm<sup>3</sup> in each measurement cycle. Consequently, minimum detectable concentration of the system can be improved by one order of magnitude. Details of the utilized preconcentration unit were reported by Pogany et al. [12].

Gas from the oral cavity is drawn into the preconcentration tube through a glass pipe coated with sodium hydroxide solution by a membrane pump. Then the preconcentration unit is heated (around 300 °C) and the ammonia is released from the vanadium pentoxide and streams through the PA cell with a low flow rate. As ammonia releases, it causes a peak in the PA signal and the concentration of ammonia is calculated from the area under that curve [12]. After the total amount of ammonia is released, the preconcentration unit is cooled by a ventilator. Time periods of the measurement cycle are optimized; sampling, heating and cooling times are 15 s, 173 s and 90 s, respectively. Obviously by increasing the sampling time the volume of the sample gas and consequently minimum detectable concentration rise. Ammonia concentration from 0.5 ppb to several ppm can be detected by the alteration of sampling time, furthermore, without preconcentration unit even several vol. % of ammonia can be measured. However, a set of measurements were performed to assess ammonia in the oral cavity and a sampling time of 15 s was found to be optimal considering the dynamic range and sensitivity of the device.

Sodium hydroxide coating was found to enhance measurement reproducibility because wall effect of sampling tube on base coated surfaces significantly decreases.

The instrument was calibrated by measuring the PA signal of various gas mixtures prepared from a cylinder containing 5 ppm NH<sub>3</sub> in synthetic air and from synthetic air, using mass-flow controllers. Minimum detectable concentration was found to be approximately 10 ppb with a dynamic range of 0-2000 ppb for a sampled gas volume of 250 cm<sup>3</sup> (details of the calculation are described elsewhere [12]). Negligible cross sensitivity was found for common components of breath including carbon dioxide and water vapor.

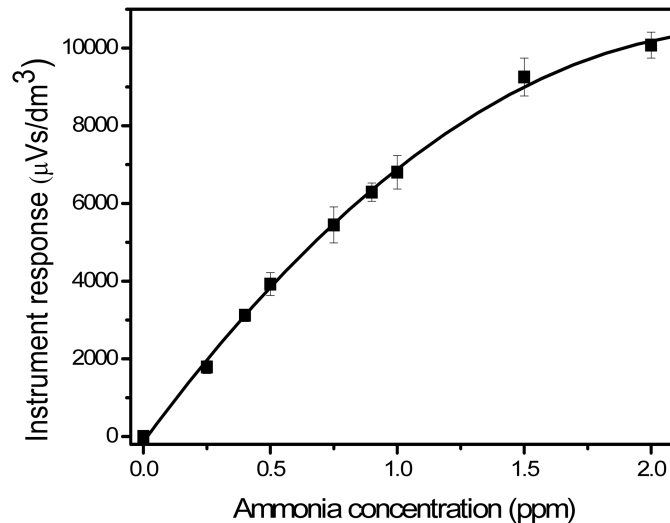


Figure 3. Calibration curve of the ammonia measuring instrument for a gas sample volume of 250 cm<sup>3</sup>. Error bars show the standard deviation of data. Solid line indicates a second-order polynomial fit to data.

The response of the instrument is determined by the PA signal and the characteristics of the preconcentration tube (Fig. 3). Due to the saturation of the preconcentration unit the response of the instrument is not linear function of the ammonia concentration, however, it can be well approximated by a second-order polynomial.

### 3. APPLICATIONS IN MEDICAL RESEARCH

#### 3.1 Methane emission of mice

SKH-1 hairless male mice were used for the experiments (body weight: 34-38 g). The animals were anesthetized with a mixture of ketamine and xylazine. Mice were then placed into the glass container (volume: 180 cm<sup>3</sup>) and the enclosure was not completely gastight in order to replenish sampled gas volume with ambient air. 10 minutes were left for the accumulation of the emitted methane in the container. Gas from the container was subsequently drawn into the PA chamber via a tube made of stainless steel. Measurement periods took 10 minutes. Prior to placing the animals into the container the methane concentration of the air was determined and, as baseline, was used for the calculation of methane emission of the animals.

Mice were randomly allocated into 3 groups (n = 8, each group). Group 1 served as control. The animals of group 2 received antibiotic treatment (Normix) in order to eradicate intestinal bacterial flora which highly contributes to methane production. In group 3 a generalized inflammation was induced by administration of endotoxin (ETX).

Fig. 4a shows the methane emission of a control mouse. For 10 minutes the gas sampling chamber was open and methane concentration of the ambient air (baseline) was measured to be 1.6 ppm. Vertical dashed line represents when the mouse was put into the chamber. Then a significant enhancement of the methane concentration was found and approximately after 10 minutes the methane concentration reached a maximum of 1.9 ppm. Thereafter, the mouse was removed from the container, consequently methane concentration decreased. Methane emission of the mouse was calculated as the difference of the increased and the baseline methane concentrations and was found to be 0.3 ppm.

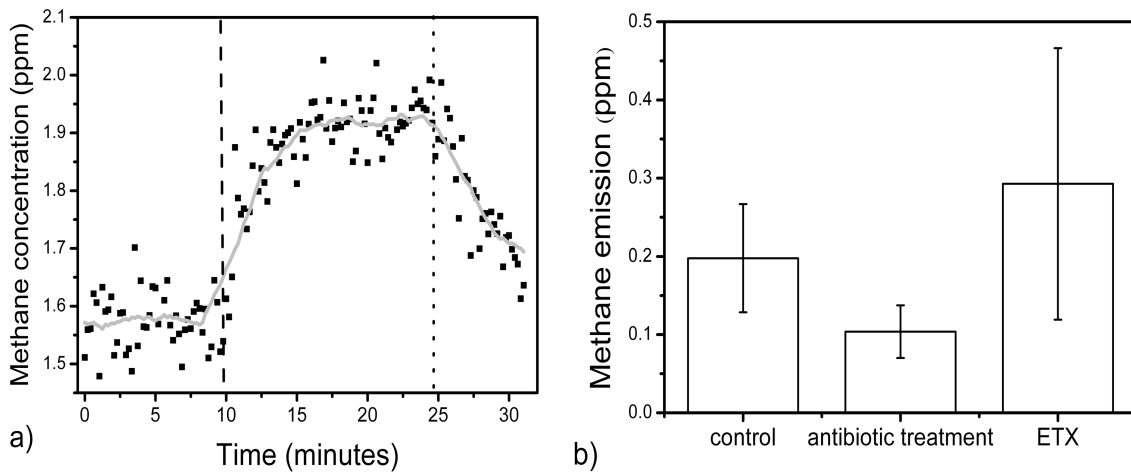


Figure 4. a) Methane emission measurement of an untreated mouse by the photoacoustic instrument. First, a baseline was measured, dashed and dotted lines indicate time when the mouse was placed into and removed from the sampling chamber, respectively. Solid gray line shows moving average over 20 points. b) Mean methane emission of three groups of mice. Control group consisted of untreated mice, another group received antibiotic treatment and ETX indicates the third group in which generalized inflammation was induced by administration of endotoxin. Error bars represent standard deviation of data.

The methane emission in healthy, untreated mice was found to be approximately 0.2 ppm. Elimination of intestinal bacteria led to a considerable decrease in methane emission, however, it remained measurable. Inflammatory condition was accompanied by a significant increase in methane production (Fig. 4b). The results suggest that methane emitted by living organisms originates not only from bacterial activity but also eukaryotic cells are able to produce this gas. Furthermore, methane emission may be an indicator of inflammatory disorders and hereby its detection may contribute to the diagnosis of such pathologies.

### 3.2 Measuring ammonia from the oral cavity

Our preliminary study measuring the concentrations of ammonia and volatile sulfur compounds in halitosis (particularly hydrogen sulfide) comprised 12 women with a mean age of 30 years. All volunteers were free of dental caries and periodontal disease. Treatment with antibiotics in the past three months, and the consumption of onions, garlic, spicy foods and alcohol the day before the examination were exclusion criteria. The volunteers were asked to refrain from eating, tooth cleaning, oral rinsing solution or drinking (except water) for at least 2 hours before testing. The PA system and the OralChroma™ were operating consecutively to detect ammonia and volatile sulfur compounds (hydrogen sulfide, methyl mercaptan and dimethyl sulfide). Three consecutive measurements were performed for each person with the instruments.

Preliminary results are presented in Fig. 5. A significant correlation was found between ammonia and hydrogen sulfide concentrations. Furthermore, correlation analysis was performed with the Pearson correlation coefficient and it was found to be  $r = 0.906$  ( $p = 0.01$ ). These results are in correspondence with the findings of Amano et al. [9] that ammonia can be considered as another component to diagnose oral health/hygiene in clinical dentistry.

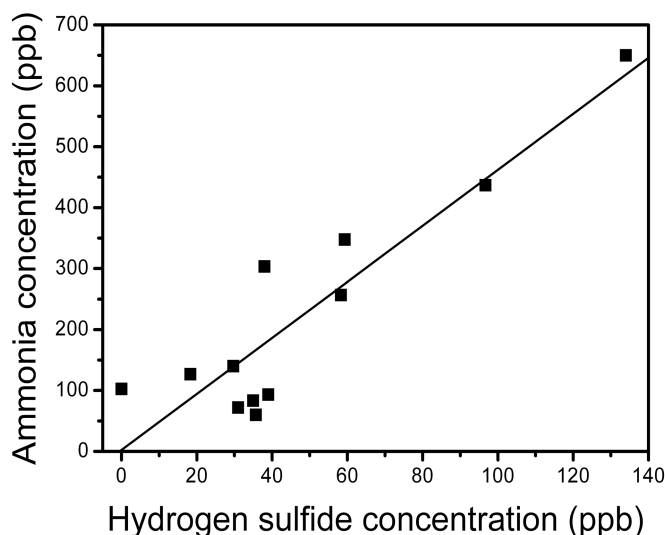


Figure 5. Correlation between hydrogen sulfide and ammonia in halitosis measurements in a healthy women population. Solid line represents linear regression of data ( $n = 12$ ,  $R^2 = 0.821$ ).

## 4. SUMMARY

The PA spectroscopy based methane and ammonia measuring instruments are well applicable for clinical in-vivo gas monitoring. The devices are portable, easy-to-use, can operate without chemicals, as well as have high-stability and reproducibility. The utilization of near-infrared diode lasers provides long lifetime and makes the systems cost-effective. Measurement protocols were established in order to improve reproducibility. Direct gas sampling with inert gas handling systems (stainless steel and glass) were utilized in order to minimize errors deriving from the use of sampling bags and from adsorption-desorption processes.

In consideration of the promising preliminary results the devices currently operate at long-term clinical trials at laboratories of the Faculties of Medicine and Dentistry of the University of Szeged. The aim of the measurements is to determine correlations between diseases or metabolic processes and emanated gases.

## ACKNOWLEDGEMENTS

The authors are grateful for the support of TÁMOP 4.2.1/B-09/1/KONV-2010-005, TÁMOP-4.2.2-08/1-2008-0013 and TÁMOP-4.2.2-08/1-2008-0001 projects of the Hungarian Ministry of Education.

The publication is supported by the European Union and co-funded by the European Social Fund. Project title: "Broadening the knowledge base and supporting the long term professional sustainability of the Research University

## REFERENCES

- [1] Risby, T. H. and Solga, S. F., “Current status of clinical breath analysis,” *Appl. Phys. B* 85, 421-426 (2006).
- [2] Narasimhan, L. R., Goodman, W. and Patel C. K. N., “Correlation of breath ammonia with blood urea nitrogen and creatinine during hemodialysis,” *Proc. Natl. Acad. Sci.* 98, 4617-4621 (2001).
- [3] Lewicki, R., Kosterev, A. A., Thomazy, D. M., Risby, T. H., Solga, S., Schwartz, T. B., Tittel, F. K., “Real time ammonia detection in exhaled human breath using a distributed feedback quantum cascade laser based sensor ” *Proc. SPIE* 7945, 50K-2 (2011).
- [4] Nose, K., Nunome, Y., Kondo, T., Araki, S. and Tsuda, T., “Identification of gas emanated from human skin: methane, ethylene, and ethane,” *Anal. Sci.* 21, 625-628 (2005).
- [5] Tsuda, T., Ohkuwa, T. and Itoh, H., “Findings of skin gases and their possibilities in healthcare monitoring,” [*Gas Biology Research in Clinical Practice*], S. Karger AG, Basel, 125-132 (2011).
- [6] Scully, C., el-Maaytah, M., Porter, SR. and Greenman, J., “Breath odor: etiopathogenesis, assessment and management,” *Eur. J. Oral Sci.* 105, 287-293 (1997).
- [7] Kleinberg, I. and Westbay, G., “Oral malodor” *Crit. Rev. Oral Biol. Med.* 1, 247-59 (1990).
- [8] van den Broek, A. M., Feenstra, L. and de Baat, C., “A review of the current literature on aetiology and measurement methods of halitosis,” *J. Dent.* 35, 627-635 (2007).
- [9] Amano, A., Yoshida, Y., Oho, T. and Koga, T., “Monitoring ammonia to assess halitosis,” *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 94, 692-696 (2002).
- [10] Bozóki, Z., Pogány, A. and Szabó, G., “Photoacoustic instruments for practical applications: present, potentials, and future challenges,” *Appl. Spectrosc. Rev.* 46, 1-37 (2011).
- [11] Sigrist, M. W., “Trace gas monitoring by laser-photoacoustic spectroscopy,” *Infrared Phys. Techn.* 36, 415-425 (1995).
- [12] Pogány, A., Mohácsi, Á., Varga, A., Bozóki, Z., Galbács, Z., Horváth, L. and Szabó, G., “A compact ammonia detector with sub-ppb accuracy using near-infrared photoacoustic spectroscopy and preconcentration sampling,” *Environ. Sci. Technol.* 43(3), 826-830 (2009).