

Real time ammonia detection in exhaled human breath using a distributed feedback quantum cascade laser based sensor

Rafał Lewicki^{*1}, Anatoliy A. Kosterev¹, David M. Thomazy¹, Terence H. Risby², Steven Solga³, Timothy B. Schwartz³, and Frank K. Tittel¹

¹Department of Electrical and Computer Engineering, Rice University, Houston, Texas 77005

²Bloomberg School of Public Health, The Johns Hopkins University, Baltimore, MD 21205

³St. Luke's Hospital, Bethlehem, PA 18015

ABSTRACT

A continuous wave, thermoelectrically cooled, distributed feedback quantum cascade laser (DFB-QCL) based sensor platform for the quantitative detection of ammonia (NH₃) concentrations present in exhaled human breath is reported. The NH₃ concentration measurements are performed with a 2f wavelength modulation quartz enhanced photoacoustic spectroscopy (QEPAS) technique, which is very well suited for real time breath analysis, due to the fast gas exchange inside a compact QEPAS gas cell. An air-cooled DFB-QCL was designed to target the interference-free NH₃ absorption line located at 967.35 cm⁻¹ ($\lambda \sim 10.34 \mu\text{m}$). The laser is operated at 17.5 °C, emitting ~ 24 mW of optical power at the selected wavelength. A 1σ minimum detectable concentration of ammonia for the line-locked NH₃ sensor is ~ 6 ppb with 1 sec time resolution. The NH₃ sensor, packaged in a 12"x14"x10" housing, is currently installed at a medical breath research center in Bethlehem, PA and tested as an instrument for non-invasive verification of liver and kidney disorders based on human breath samples.

Keywords: Quartz enhanced photoacoustic spectroscopy, wavelength modulation, distributed feedback quantum cascade laser, trace gas ammonia detection.

1. INTRODUCTION

Exhaled breath is a mixture of more than a thousand molecules, some of which are present at parts per billion (ppb) or even parts per trillion (ppt) concentration levels [1]. These molecules provide a unique breath profile of the health condition and have endogenous and exogenous origins. The sources of endogenous molecules are normal and abnormal physiological processes, whereas the sources of exogenous molecules are: inspiratory air, ingested food and beverages, or any exogenous molecule that has entered the body by other routes (e.g. dermal absorption) [2]. The concentrations of some of the exhaled molecules can be used as biomarkers for the identification and monitoring of human diseases or wellness states [3]. Therefore, a breath test is becoming increasingly important as a non-invasive procedure for clinical diagnostics [4]. The potential of the breath in medicine was first recognized in ancient times by Hippocrates, considered to be the father of medicine, who suggested that a specific breath odor might reflect a certain disease. The earliest recorded studies of the human breath, related to the role of oxygen in combustion and respiration, were performed more than 200 years ago by Lavoisier and Laplace [4-5].

Currently, the standard analytical chemistry instrumentation for the exhaled human breath analysis is based on gas chromatography and its various detection methods such as flame ionization detection [6-7], mass spectrometry [8], ion mobility spectrometry [9], or selected ion flow tube mass spectrometry [10]. In recent years, several laser based breath analyzers, capable of sensitive and real time detection of molecular species in breath samples, were demonstrated with different laser spectroscopic techniques [11-13]. Promising results from previous studies have confirmed that optical sensors have a considerable potential for monitoring of exhaled breath [14]. In this paper, a novel quantum cascade laser based optical breath sensor for ammonia detection will be described. By monitoring ammonia

* Rafał.Lewicki@rice.edu; phone 1 713 348-2614; fax 1 713 348-5686; www.ece.rice.edu/lasersci/

concentration levels in exhaled breath, a fast, non-invasive diagnostic method for treatment of human subjects with liver and kidney disorders is feasible.

2. AMMONIA BREATH SENSOR ARCHITECTURE

The block diagram of the ammonia breath sensor architecture is depicted in Fig. 1. The optical sensor uses a quartz enhanced photoacoustic spectroscopy (QEPAS) technique [15-16] that employs an ultra-small piezoelectric quartz tuning fork (QTF) as an acoustic transducer. A high resonance frequency of the QTF (in vacuum $f=2^{15}$ Hz~32.7 kHz) results in immunity to low frequency environmental acoustic noise for QEPAS measurements. In addition, QEPAS possesses a large dynamic range of 9 orders of magnitude of the acoustic signal, and its noise is limited by fundamental Johnson thermal noise of the QTF. The signal to noise ratio for QEPAS based trace gas detection measurements can be improved up to 30 times when the QTF is enhanced with two metal tubes acting as a microresonator. The optimum inner diameter and the length of each tube (for a ~32.8 kHz sound wave the tube length estimated to be between $\lambda/4$ and $\lambda/2$ of the acoustic wavelength) were experimentally determined to be 0.6 mm and 4.4 mm, respectively [17]. Moreover, the QEPAS based trace gas sensor is capable of ultra-sensitive trace gas detection and is suitable for real time breath measurements, due to the fast gas exchange inside a compact QEPAS gas cell, which acts as an absorption detection module (ADM). The total volume of a typical QEPAS ADM is ~3.8 cm³. It can be made even smaller in an alternative cell design, because, the volume of the analyzed gas sample is limited by the dimensions of the QTF and the acoustic microresonator tubes to ~3 mm³.

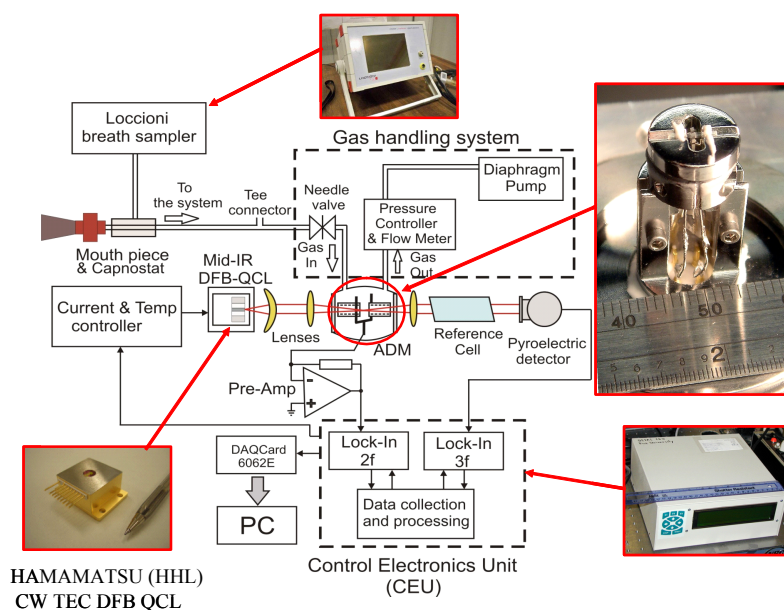


Fig. 1. Block diagram of ammonia breath sensor architecture.

The NH₃ breath sensor employs a continuous wave (CW), room temperature (RT), distributed feedback quantum cascade laser (DFB-QCL) in a High Head Load (HHL) package from Hamamatsu (www.hamamatsu.com). The RT operated DFB-QCL uses only air cooling, produced by a small electronics fan and a heatsink attached to the back of the laser. A 4mm diameter ZnSe aspheric lens, with a working distance of 0.65 mm and clear aperture of 3.6 mm, was used to collimate the laser beam. In order to pass the beam through the microresonator, consisting of two 0.6 mm inner diameter and 4.4 mm in length metal tubes, a 0.5" diameter and 1.17" focal length ZnSe plano convex lens was employed. The laser power was found to be 22mW after being transmitted through the microresonator. This is ~90% of the initial power of the DFB-QCL operated at 17.5 °C. The photoacoustic signal is detected by the QTF and is amplified by a low noise transimpedance preamplifier and delivered to a control electronics unit (CEU) for further data processing.

A 10 cm long reference cell, filled with 0.2% of NH_3 in N_2 at 130 Torr, and a pyroelectric detector were installed after the ADM module to lock the laser frequency to the center of the selected NH_3 absorption line.

A commercially available breath sampler (Loccioni, Italy) was employed to monitor and maintain the pressure of the exhaled breath within an acceptable range and to measure the breath CO_2 concentration level. After a human subject breathes into a mouth piece, the collected breath gas sample simultaneously enters the Loccioni breath sampler and the optical breath sensor. Inside the sensor the collected breath passes through the ADM, a pressure controller (MKS Instruments) followed by a mass-flow meter (see www.mksinst.com) and is then released into the atmosphere by means of a compact oil free diaphragm pump (KNF model UN816.3 KTP). The flow rate through the NH_3 sensor was fixed by a needle valve to 220 ml/min and the pressure value was set and controlled at 130 Torr. When the breath sample is acquired, the breath CO_2 concentration profile, airway pressure, and the NH_3 concentration profile are simultaneously displayed in graphical forms on the Loccioni breath sampler display. At the same time the NH_3 breath profile and the maximum breath NH_3 concentration level in ppbv acquired by NH_3 breath sensor are plotted in real-time on a laptop screen. In addition, the ADM, needle valve, and the mouth piece together with the Loccioni breath sampler pipe line were heated to $\geq 38^\circ\text{C}$ to avoid NH_3 adsorption on the various component surfaces of the sensor as well as to prevent from condensation of the water vapor inside the NH_3 sensor.

The NH_3 breath sensor system consists of 6 main components, which includes the NH_3 optical sensor enclosed in a 14" x 10" x 12" box, the ILX Lightwave QCL controller, the CEU, the Loccioni breath sampler, a laptop and a small power supply (Acopian) for powering self adhesive heaters of the ADM and the heating cord around the needle valve.

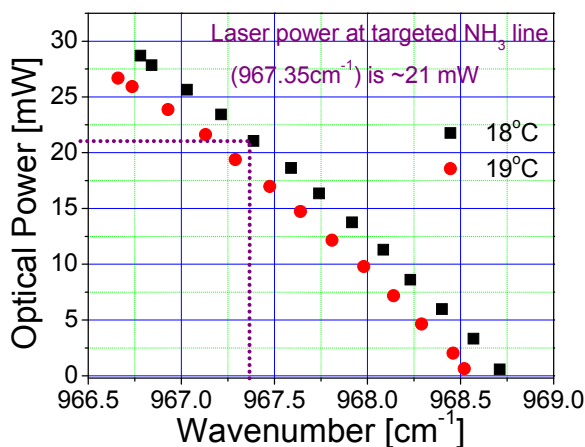


Fig. 2 Optical power and current tuning of the CW DFB-QCL operated at two different quasi-room temperatures.

The Hamamatsu CW RT DFB-QCL was designed to emit radiation at $10.34 \mu\text{m}$ wavelength, within the ν_2 fundamental absorption band of ammonia. The laser can be operated from 0 to 50°C with a spectral shift over temperature of $0.1 \text{ cm}^{-1}/^\circ\text{C}$. For the QCL operating at 18°C , single mode operation between 966.7 and 968.7 cm^{-1} was achieved resulting in spectral shift of $0.017 \text{ cm}^{-1}/\text{mA}$. The optical power and current tuning characteristics of the CW DFB-QCL operated at two different quasi-room temperatures is illustrated in Fig. 2. Two free from H_2O , CO_2 and methanol interferences potential NH_3 absorption lines of the similar intensity can be detected at 967.35 cm^{-1} and 965.35 cm^{-1} within the available DFB-QCL tuning range. The NH_3 absorption line located at 967.35 cm^{-1} can be accessed with laser operating temperature set to 17.5°C , resulting in laser optical power of $> 20 \text{ mW}$. To access the NH_3 line located at 965.35 cm^{-1} , a much higher laser operating temperature of $\sim 30^\circ\text{C}$ is needed, which results in a significant decrease of the DFB-QCL optical output power to $\sim 5 \text{ mW}$. Therefore when using the QEPAS technique, where the detected signal scales linearly with optical power, the optimum selection for the NH_3 absorption line position is at 967.35 cm^{-1} . The HITRAN simulated spectra at 130 Torr indicating NH_3 absorption lines together with the CO_2 and H_2O absorption lines in the $\sim 966 \text{ cm}^{-1}$ spectral region are illustrated in Fig. 3

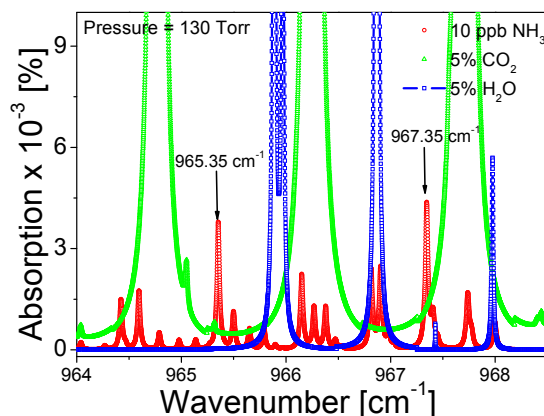


Fig. 3 HITRAN simulated spectra at 130 Torr indicating two potential NH_3 absorption lines of interest for exhaled breath measurements.

In order to verify if the wing of a neighboring CO_2 line centered at 967.71 cm^{-1} can interfere with the selected NH_3 absorption line, two 2f wavelength modulation (WM) scans were performed within spectral range of interest. The initial 2.5 ppm NH_3 in an N_2 mixture was flushed through the system and a 2f QEPAS spectrum for ammonia was recorded by Labview software. Then the same measurement was repeated for 5% CO_2 in N_2 and 1% water vapor delivered to the system. The combined results of this test are illustrated in Fig. 4. From Fig 4 it is clear that no overlap between 967.35 cm^{-1} NH_3 and the adjacent CO_2 absorption line is present for 2f WM technique. In addition when a CO_2 measurement is taken, the signal related to remaining ammonia in the system is also detected by the QTF. This effect of NH_3 molecules sticking to the wall can be eliminated by increasing the temperature of the system to $> 38^\circ\text{C}$, keeping the tubing length as short as possible, and maintaining the flow inside the sensor at reasonably fast level $> 200 \text{ ml/min}$.

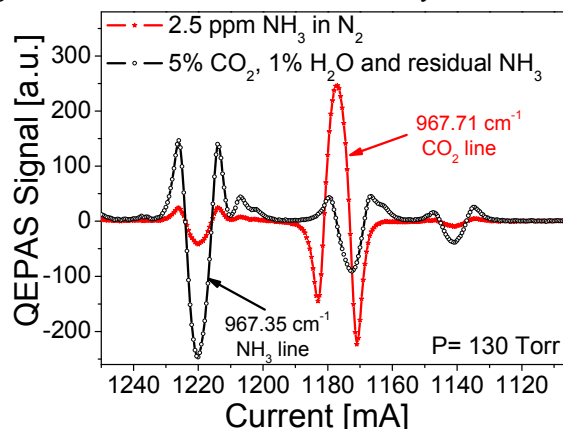


Fig. 4 2f wavelength modulation QEPAS signals for a 2.5 ppm NH_3 in N_2 mixture and 5% CO_2 in N_2 and 1% H_2O mixture recorded within the $967\text{--}968 \text{ cm}^{-1}$ spectral region.

3. EXPERIMENTAL RESULTS FOR DFB-QCL BASED NH_3 GAS SENSOR

Sensitive detection of ammonia at low parts per billion (ppb) concentration levels was performed with a 2f WM technique, which is intrinsically a zero background technique. An optimum NH_3 sensor pressure value of 130 Torr and modulation depth of 15mA were experimentally determined in order to maximize the QEPAS signal. The 1σ minimum detectable concentration of ammonia for a calibrated mixture of 5ppm NH_3 balance N_2 is achieved at $\sim 8 \text{ ppb}$ when the laser frequency is current tuned over the 967.35 cm^{-1} NH_3 absorption line (see black plot in Fig. 5a). However, for the line scanning method, additional noise related to the current frequency tuning process can be introduced into the system. In addition each scan lasts ~ 2 minutes which is too long for some applications where a fast system response is required. For a background free sensor system, where no significant baseline drift is observed, a more convenient method is to

acquire data only at one selected laser frequency corresponding to the peak of the investigated NH_3 absorption line. Therefore, a line-locking scheme was implemented to always keep the laser frequency at a fixed position. A 3f pyroelectric detector signal with a zero crossing point exactly at the maximum of the 2f WM QEPAS signal was used as a reference for the line-locking technique. A 3f signal was also demonstrated in Fig. 5a as a red plot. An example of continuous measurements after locking a laser frequency to the 967.35 cm^{-1} NH_3 absorption line is demonstrated in Fig. 5b. In this experiment the analyzed gas medium was alternately switched between two cylinders; an ultra high purity N_2 and certified 5ppm NH_3 in N_2 . The 1σ minimum detectable concentration of ammonia for a line locked laser frequency was achieved at ~ 6 ppb. The data points for all measurements demonstrated in Fig. 5 were acquired with a 1 sec time resolution.

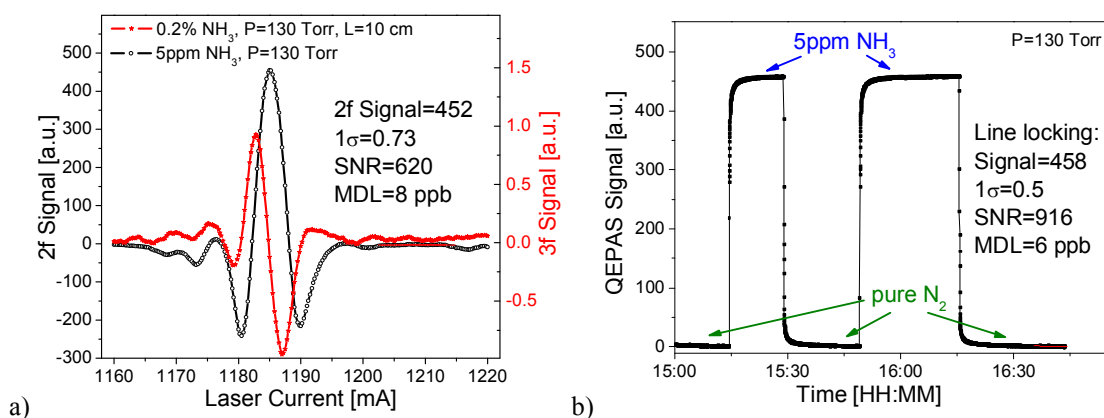


Fig. 5 a) 2f QEPAS signal (black) and reference channel 3f signal (red) when DFB-QCL is tuned across the 967.35 cm^{-1} line; b) 2f QEPAS signal when laser was locked to the 967.35 cm^{-1} line.

A similar detection limit of ~ 6 ppb (1σ) was obtained after diluting a calibrated mixture of 5ppm NH_3 in N_2 to a level of ~ 160 ppb (see Fig. 6). This proves a linear response of the QCL based QEPAS based sensor platform. The results of dilution process, showing both targeted and measured NH_3 concentration values in ppb, were demonstrated in the insert of Fig. 6. A higher discrepancy between the targeted and measured NH_3 concentrations were observed with an increasing dilution ratio most likely resulting from the precision of the gas dilution system during the dilution process.

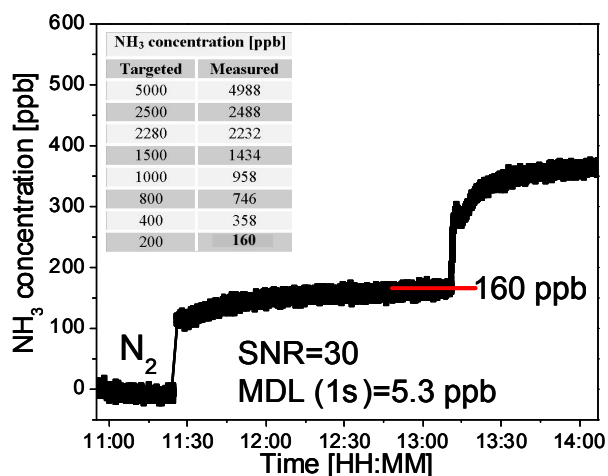


Fig. 6 Dilution process of the 5ppm NH_3 reference concentration.

4. REAL TIME HUMAN BREATH DATA

An NH_3 sensor system for the real time monitoring of ammonia concentration levels in exhaled breath was designed to collect breath samples multiple times with ~ 3 min intervals between each sample. These intervals are needed to remove the remaining ammonia out of the system. An example of the breath ammonia profile was demonstrated in Fig. 7a, where the authentic ammonia concentration level, representing both a normal and an abnormal physiological body processes, is at ~ 450 ppb (at the plateau point). The first part of the breath profile shown in Fig 7a, associated with highest detected NH_3 concentration of ~ 650 ppb, reflects the ammonia concentration in the oral cavity. This concentration is mostly related to oral bacterial processes of ingested food and beverages (cite). For each NH_3 breath measurement, the NH_3 profile is displayed in the real time on the laptop and on the Loccioni breath analyzer screen and then saved and stored on a laptop and the Loccioni memory stick for later data processing.

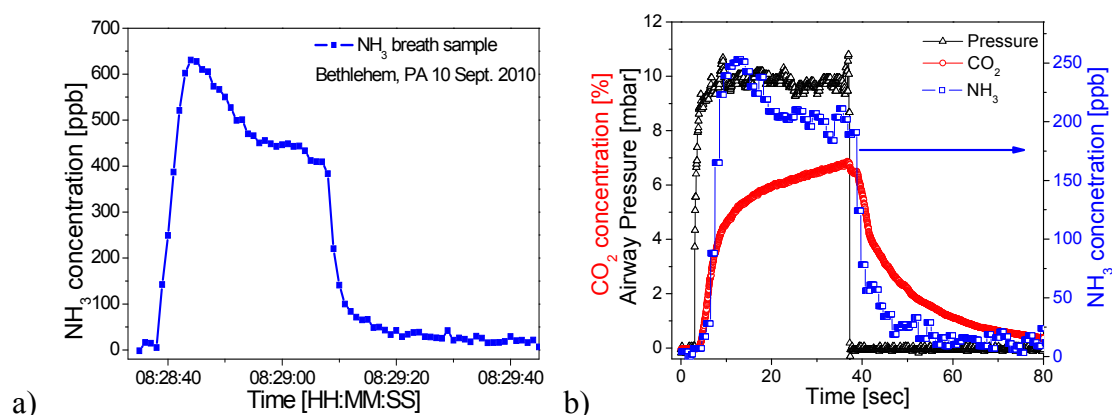


Fig. 7a) An example of the breath ammonia profile, taken at the Bethlehem, PA clinical testing site; b) CO_2 concentration [%], airway pressure [mbar] and NH_3 concentration [ppb] profiles of the same single breath exhalation.

Single breath exhalation profiles for CO_2 concentration [%], airway pressure [mbar] and NH_3 concentration [ppb] are depicted in Fig. 7b. It is clearly visible that no significant delay between airway pressure and breath ammonia profile is observed, which confirms that the NH_3 sensor has an extremely fast response, as desired. In addition, after the breath sampling process is completed, fast ammonia decay from the system is also observed.

5. SUMMARY

Monitoring of ammonia concentration in exhaled breath using laser spectroscopy techniques provides a fast, non-invasive diagnostic method for human subjects with liver and kidney disorders. The achieved minimum detectable concentration of NH_3 (1σ) with DFB-QCL based QEPAS sensor was 6 ppbv with a 1 sec time resolution. This sensitivity is sufficient for detecting exhaled breath ammonia concentrations, which are estimated to be between 0 and 1 ppm in healthy humans. The fast sensor response time was obtained by shortening the length of the breath sampler tube and by keeping the metal components of the sensor at 38°C to minimize ammonia adsorption effects. By using a commercial breath sampler (Loccioni, Italy) with a built-in capnograph device, the CO_2 concentration measurements are performed independently. In addition, the breath sampler helps to standardize the breath collection process by monitoring and maintaining the pressure of exhaled breath within a certain acceptable range. Laser spectroscopy with a mid-infrared, room temperature, continuous wave, high performance DFB QCL is a promising analytical approach for real time breath analysis and the quantification of breath metabolites. Moreover the QEPAS sensor technology is a robust technology for the development of sensitive, compact sensor systems that might be used in a doctor's office for non-invasive verification of a human subject's medical condition.

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