

Real time detection of exhaled human breath using quantum cascade laser based sensor technology

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ABSTRACT

The development and performance of a cw, TE-cooled DFB quantum cascade laser based sensor for quantitative measurements of ammonia (NH₃) and nitric oxide (NO) concentrations present in exhaled breath will be reported. Human breath contains ~ 500 different chemical species, usually at ultra low concentration levels, which can serve as biomarkers for the identification and monitoring of human diseases or wellness states. By monitoring NH₃ concentration levels in exhaled breath a fast, non-invasive diagnostic method for treatment of patients with liver and kidney disorders, is feasible. The NH₃ concentration measurements were performed with a 2f wavelength modulation quartz enhanced photoacoustic spectroscopy (QEPAS) technique, which is suitable for real time breath measurements, due to the fast gas exchange inside a compact QEPAS gas cell. A Hamamatsu air-cooled high heat load (HHL) packaged CW DFB-QCL is operated at 17.5°C, targeting the optimum interference free NH₃ absorption line at 967.35 cm⁻¹ ($\lambda \sim 10.34 \mu\text{m}$), with ~ 20 mW of optical power. The sensor architecture includes a reference cell, filled with a 2000 ppmv NH₃ :N₂ mixture at 130 Torr, which is used for absorption line-locking. A minimum detection limit (1 σ) for the line locked NH₃ sensor is ~ 6 ppbv (with a 1 σ ; 1 sec time resolution of the control electronics). This NH₃ sensor was installed in late 2010 and is being clinically tested at St. Luke's Hospital in Bethlehem, PA.

Keywords: Quartz enhanced photoacoustic spectroscopy, quantum cascade lasers, exhaled breath analysis, trace gas detection, ammonia, nitric oxide

1. INTRODUCTION

Laser based detection, quantification and monitoring of ammonia (NH₃) and nitric oxide (NO) at ppbv concentration levels has made an important impact in medical diagnostics and public health in recent years. To date, more than 500 different compounds contained in human breath, some of which are present at parts per billion (ppb) or even parts per trillion (ppt) concentration levels have been identified [1]. These molecules have both endogenous and exogenous origins and provide information about physiological processes occurring in the body as well as environment related

ingestion or absorption of contaminants. 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. While the presence and concentration of many of these molecules are poorly understood, many “biomarker” molecules have been correlated to specific diseases and metabolic processes. Such correlations can result in noninvasive methods of health screening for a wide variety of medical conditions.

Therefore, breath analysis is becoming increasingly important as a non-invasive procedure for clinical diagnostics [2-4]. Development of trace gas sensor technologies is a key factor in the advancement of breath analysis. Small, affordable, easy-to use, sensitive and accurate techniques are needed for detection of breath compounds. Several methods of trace molecular detection have been applied to breath analysis, including optical detection [5-7], mass spectrometry [8-9], and electronic “noses” [10]. Analytical instrumentation involving mass spectrometry with or without prior separation by gas chromatography is the most commonly used method to quantify exhaled molecules. Currently, atmospheric pressure ionization mass spectrometry (API-MS) and selected ion flow tube mass spectrometry (SIFTMS) are the most frequently used methods for direct breath analysis. In recent years, the development and commercial availability of new mid-infrared semiconductor laser sources has led to mid-infrared laser-based trace gas sensors. In particular, single mode, room temperature, CW and pulsed quantum cascade lasers (QCLs) and tunable external-cavity (EC) QCL have become very attractive for mid-infrared trace gas sensing techniques on account of their ability to provide single frequency, narrow line width, high powers at mid-IR wavelengths (4 to 12 μm) [11,12].

New infrared semiconductor laser based trace gas sensor technology now offers the feasibility of compact (hand held), reliable, non-aliasing, user friendly, autonomous and low-cost devices without sacrificing high detection ranges from ppmv, to sub-ppbv levels depending on the specific biomarker gas species and the detection method employed [13, 14]. Quartz enhanced photoacoustic spectroscopy (QEPAS) is particularly suited for applications where continuous monitoring of targeted exhaled gases with sensitivity, selectivity and fast response are required, such as in critical care and operating room settings, due to the fast gas exchange inside the compact QEPAS gas cell, which acts as an absorption detection module. Exhaled breath QEPAS sensors have already demonstrated. For example, an exhaled NH_3 sensor is currently installed at the medical breath research center in Bethlehem, PA and is being evaluated as an instrument for non-invasive verification of liver and kidney disorders based on human breath samples [15].

Quartz-enhanced photoacoustic spectroscopy (QEPAS) is a gas-sensing technique that allows performing sensitive measurement of trace gases in gas samples of a few mm^3 in volume [16]. The QEPAS technique employs a quartz tuning fork (QTF) as a sharply resonant acoustic transducer, instead of a broadband electric microphone used in conventional photoacoustic spectroscopy (PAS). The QTF is a piezo-electric element, capable of detecting weak acoustic waves generated when the modulated optical radiation interacts with a trace gas, and converts the QTF deformation into an electrical signal (due to charge separation on the electrodes of the QTF). This signal is then processed by an ultra-low noise transimpedance preamplifier mounted close to the QTF to generate an output that is proportional to trace gas concentration. In order to further enhance the QEPAS signal, a micro-resonator (mR) is added to the QEPAS sensor architecture. The typical spectrophone or absorption detection module (ADM) includes a QTF and a mR, consisting of two metallic or glass tubes [17-19]. The QTF is positioned between the tubes to probe the acoustic waves excited in the gas contained inside the mR. For a typical QCL beam, short mR tubes with a larger inner diameter

are advantageous in facilitating optical alignment of the QCL excitation beam with the mR and the QTF.

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 [16-17] 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 mR. 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 mR tubes to ~3 mm³.

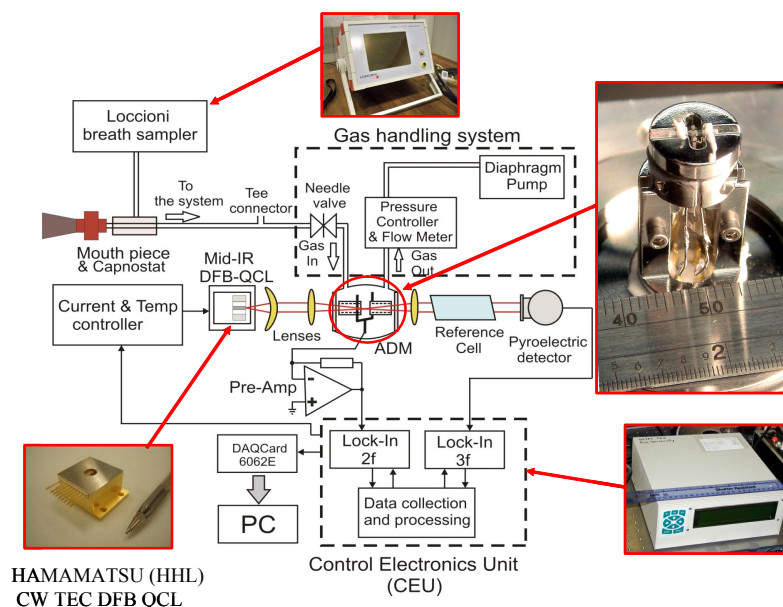


Fig. 1. Block diagram of ammonia breath sensor architecture.

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](http://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 $\sim 90\%$ 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.

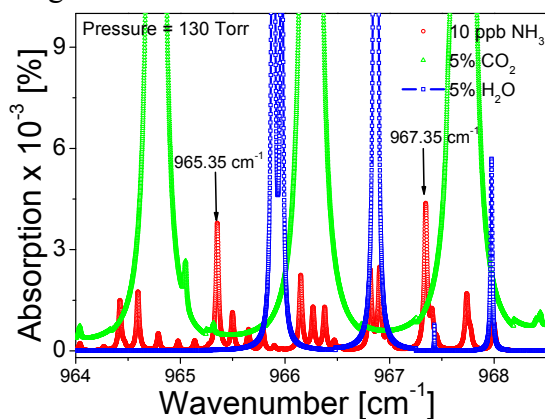


Fig. 2. HITRAN simulated spectra at 130 Torr indicating two potential NH_3 absorption lines of interest for exhaled breath measurements.

The Hamamatsu CW RT DFB-QCL was designed to emit radiation at 10.34 μm 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 cm^{-1}/mA . 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 $^\circ\text{C}$, resulting in laser optical power of > 20 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 ~ 5 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 ~ 966 cm^{-1} spectral region are illustrated in Fig. 2.

3. OPTIMIZATION OF EC-QCL BASED QEPAS NITRIC OXIDE DETECTION

A schematic of the QEPAS sensor for NO detection is depicted in Fig. 4. The sensor platform is based on a $2f$ wavelength-modulation spectroscopy (WMS) based QEPAS-detection approach. A widely tunable CW EC-QCL (Daylight Solutions, Inc., model 21052-MHF) served as the excitation source for generating the QEPAS signal. The EC-QCL frequency tuning was achieved by applying a voltage to the piezoelectric translator that controls a diffraction grating angle. A continuous spectral tuning range from 1763 to 1949 cm^{-1} was obtained at a QCL operating temperature of 16.5 $^\circ\text{C}$ and a maximum QCL current of 450 mA. The diameter of collimated EC-QCL output beam was ~ 3 mm. Two 25 mm focal length plano-convex Ge lenses with broadband antireflection (AR) coating were used to focus the laser radiation through the mR and the prongs of QTF and to re-collimate the laser beam upon exiting the ADM. A beam diameter of ~ 20 μm at the center of the prongs of QTF was obtained from a theoretical calculation using the above-mentioned two lenses. Since previous results showed that the optimal pressure range for NO detection is from 150 and 250 Torr [18], an acoustic mR consisting of two hypodermic tubes, each 3.9 mm long with 0.84 mm inner diameter, was mounted on both side of the QT.. These mR tube parameters result in an optical power transmission of 99% through the mR and the QTF. A beam splitter was inserted, which directs a portion of the EC-QCL radiation to a reference channel consisting of 10 cm long reference cell, filled with a 0.5%

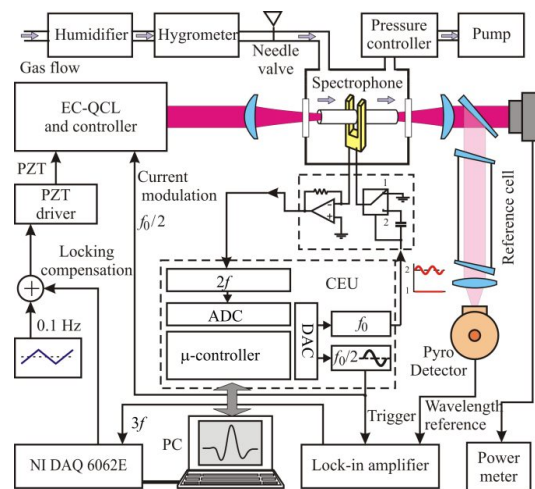


Fig. 3. Schematic of an EC-QCL based NO sensor system.

NO in N₂ and a pyroelectric detector (InfraTec, LIE-332f-63). The beam splitter allowed most of the laser radiation to pass and provides convenient monitoring of the EC-QCL output with a power meter (Ophir, 3A-SH). The QTF was connected to an ultra-low noise transimpedance amplifier with a 10 M Ω feedback resistor that converted the current generated by QTF into a voltage. This voltage was directed to a custom made control electronics unit (CEU), which was employed to realize three functions: 1. to measure three QTF parameters, i.e. the resonant frequency f_0 , quality factor Q and resistance R of the QTF, 2. to modulate the laser current at the frequency $f = f_0/2$, and 3. to measure the $2f$ harmonic component generated by the QTF. The CEU converted the measured current to QEPAS signal counts (1 cnt = 6.67×10^{-16} A). A notebook PC computer communicating with the CEU via a RS232 serial port collected the $2f$ harmonic data. The QTF and mR were enclosed inside a gas enclosure with AR coated ZnSe windows. A pressure controller (MKS Instruments, Inc., Type 640) and an oil free vacuum pump were placed downstream to control and maintain the NO sensor system pressure. A Nafion humidifier (PermaPure) and a hygrometer (DewMaster, EdgeTech) were connected to the upstream side of the ADM gas cell to add water vapor to the gas mixture and monitor the water content, respectively. A needle valve was employed to set the gas flow to a constant rate of 100 scc/min.

The QEPAS based NO concentration measurements were carried out in two operational modes. In a scan mode a 0.1 Hz triangular wave with amplitude of 25.5 V_{pp} was applied to the piezoelectric translator of the EC-QCL resulting in mode-hop-free QCL frequency tuning over the targeted absorption line. In a locked mode the QCL frequency was initially set to the center of the absorption line. The $3f$ component from the pyroelectric detector was measured by a lock-in amplifier (Stanford

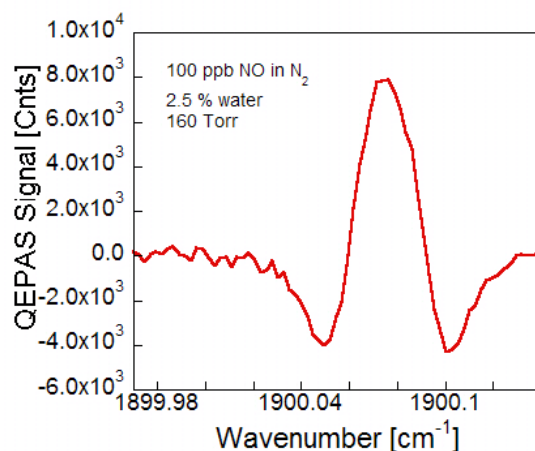


Fig. 4. A QEPAS spectrum of the NO absorption doublet at 1900.08 cm^{-1} . The QEPAS signal is recorded in terms of internal CEU units, where $1 \text{ cnt} = 6.67 \times 10^{-16} \text{ A}$.

Research systems, SR830), subsequently regulated by a Labview-software based proportional-integral-derivative (PID) controller, and finally directed to an electronic adder. The PID controller compensates any laser frequency shift by generating a correction signal to keep the laser frequency always at the center of the absorption line. The electronic adder combined the PID compensation signal and the DC voltage setting the QCL frequency to the center of absorption line, and then directed feed-back to the PZT of the EC-QCL.

The H₂O and CO₂ interference-free NO doublet absorption lines centered at 1900.08 cm^{-1} were found to be optimal for QEPAS based NO detection. A spectral scan of a 100 ppb NO sample at 160 Torr is depicted in Fig. 4. In this scan, there was no background subtraction performed. Compared to

Ref [10], no fringe-like interference pattern was observed in Fig. 4, which is the result of using mR tubes with optimal dimensions. The two lines in the doublet are so close that they are unresolved even at low pressure conditions. To optimize NO QEPAS detection performance, both the gas pressure and the wavelength modulation depth must be appropriately chosen.

4. 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 achieved minimum detection limit (1σ) for a line locked CW TEC DFB QCL NO sensor operating at 1900.075 cm^{-1} ($\lambda \sim 5.263 \text{ }\mu\text{m}$) and room temperature (no water cooling) is ~ 6 ppbv with a 1 sec update time by a custom built control QCL compatible control electronics unit. This sensitivity is sufficient for detecting exhaled breath nitric oxide concentrations, which are estimated to be between 0 and 100 ppb in healthy humans. The QEPAS sensor technology has been demonstrated to be a robust technology for the development of sensitive, compact sensor systems that can be used in a doctor's office for noninvasive verification of a human subject's medical condition.

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