

TOPICAL REVIEW

Recent advances of laser-spectroscopy-based techniques for applications in breath analysis

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Abstract

Laser absorption spectroscopy (LAS) in the mid-infrared region offers a promising new effective technique for the quantitative analysis of trace gases in human breath. LAS enables sensitive, selective detection, quantification and monitoring in real time, of gases present in breath. This review summarizes some of the recent advances in LAS based on semiconductor lasers and optical detection techniques for clinically relevant exhaled gas analysis in breath, specifically such molecular biomarkers as nitric oxide, ammonia, carbon monoxide, ethane, carbonyl sulfide, formaldehyde and acetone.

(Some figures in this article are in colour only in the electronic version)

Introduction

Analyzing compounds in breath for clinical diagnosis and therapeutic management is a burgeoning field with great potential. Breath analysis may in time be incorporated into routine clinical care as blood tests are used today—for disease screening, diagnosis, prognosis, activity (inflammatory status) and response to therapy.

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. Ideally, exhaled molecules can be quantified in real-time using a hand-held device [1].

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 (SIFT-MS) are the most frequently used methods for direct breath analysis [1]. New infrared semiconductor laser based trace gas sensor technology 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 [2, 3]. LAS 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. LAS does not require consumable products or an operator. Single-molecule exhaled breath LAS sensors are already commercially available. For example, an exhaled nitric oxide sensor (Breathmeter) is available from Ekip Technologies, Inc., and an exhaled ammonia sensor (Nephrolux) is available from Pranalytica, Inc. At present, LAS performs best with small molecules and is not amenable to characterization of a large number of molecules, such as in screening for bio-markers of diseases

and cancers. However, recent advances in broadly tunable laser sources using quantum cascade lasers suggest that LAS can be an effective tool for breath profiling (quantification of multiple exhaled molecules including broadband absorbing species) in the future [4].

Laser absorption spectroscopy

The mid-infrared spectral range is ideal for tunable laser absorption spectroscopy (LAS) since most molecular gases possess strong, characteristic fundamental rotational–vibrational lines. High-resolution LAS can resolve absorption features of targeted molecules and selectively access optimal spectral lines at low (≤ 100 Torr) pressure without interference from CO_2 and H_2O to achieve high levels of trace gas detection sensitivity and specificity. Avoiding CO_2 and H_2O interferences is particularly important in the development of biomedical gas sensors for breath analysis.

Laser absorption spectroscopy operates on the principle that the amount of light absorbed by a sample is related to the concentration of the target species in the sample. Light of known intensity is directed through a gas sample cell and the amount of light transmitted through the sample cell is measured by a detector. If we assume incident light intensity, $I_0(x = 0, \lambda)$, and transmitted light intensity, $I(x, \lambda)$, the Beer's law relates the transmitted light to the incident light and the absorption coefficient of the sample, $\alpha(\lambda)$, as

$$I(x, \lambda) = I_0 e^{-\alpha(\lambda)x} \quad (1.1)$$

where λ is the wavelength and x is the path length. Concentration is determined from the absorption coefficient. Each gas has an absorption line at a unique wavelength, preferably free of interference of other gases in the sample cell. Equation (1.1) shows that the ability of a LAS-based sensor to detect a specific concentration depends on the path length through the absorbing medium.

The strongest molecular rotational–vibrational transitions, which are desired to perform ultra-sensitive concentration measurement, are in the mid-infrared (mid-IR) spectral region. The usefulness of laser spectroscopy in this region is limited by the availability of reliable, tunable, continuous wave, infrared laser sources. The most practical sources include lead salt diode lasers, coherent sources based on difference frequency generation (DFG), optical parametric oscillators (OPOs), tunable solid-state lasers, quantum and interband cascade lasers. Sensors which utilize lead salt diode lasers are typically large in size and require cryogenic cooling because such lasers operate at temperatures of < 90 K. DFG-based spectroscopic sources (especially bulk and waveguide periodically poled lithium niobate [PPLN] based) have recently been shown to be reliable and compact [5].

The recent advances of quantum cascade (QC) and interband cascade (IC) lasers fabricated by band structure engineering offer an attractive new source option for mid-infrared absorption spectroscopy with ultra-high resolution and sensitivity [6]. The most technologically developed mid-infrared QC laser source to date is based on type-I intersubband

transitions in InGaAs/InAlAs heterostructures [7–9]. More recently, interband cascade lasers (ICLs) based on type-II interband transition have been reported in the 3–5 μm region [10–13].

LAS-based techniques

Obtaining detection sensitivities at ppb or sub-ppbv levels either requires long effective optical pathlengths or suppression of laser and optical noise. Long optical path lengths (≥ 30 m), typically realized in multipass absorption cells, are used [14–17]. As an example, for nitric oxide detection, a sensitivity of $0.7 \text{ ppb Hz}^{-1/2}$ can be achieved using a multipass cell with a 36 m path and a 0.3 l volume (Aerodyne Research, Inc.—Model AMAC 36) [17].

Another detection technique is based on the absorption that occurs in a high finesse cavity, which can be determined from the rate of decay of light intensity that occurs inside the cavity and is known as cavity ringdown spectroscopy (CRDS). CRDS has been successfully applied to measure NO concentration at ppbv levels [6, 18, 19] (for a review, see [20]). The advantage over direct absorption spectroscopy results from (i) the intrinsic insensitivity of the CRDS technique to light source intensity fluctuations, and (ii) the extremely long effective path lengths (many kilometers) that can be realized in stable high finesse optical cavities. In CRDS, laser light is resonantly coupled into a high- Q optical cavity that consists of two ultra-low-loss dielectric mirrors (~ 250 ppm or better). After the incident laser light is interrupted, the intensity of light in the cavity decays exponentially. The ringdown time τ for a two-mirror cavity is given by

$$\tau = \frac{1}{c} \frac{1}{\alpha l - \ln(R_1 R_2)^{1/2}} \quad (1.2)$$

where l is the cavity length, c is the speed of light, R_1 and R_2 are the cavity mirror reflectivities, and α is the absorption coefficient of the medium inside the cavity. Thus, by measuring the decay constant, the absorption by a sample present in the cavity can be determined.

Another technique, which employs a high-finesse optical cavity and is simpler to implement than CRDS, is integrated cavity output spectroscopy (ICOS). This technique is based upon the excitation of a dense spectrum of transverse cavity modes and time averaging the cavity output. This scheme relies on the accidental coincidence of the frequencies associated with excitation laser source and the ICOS cavity. By external dithering of one of the ICOS mirrors the number of excited ICOS cavity modes can be increased [21, 22]. Less critical alignment of the exciting laser beam with respect to the ICOS cavity can be realized with an off-axis ICOS optical geometry, which provides an increased spectral density of cavity modes, and thereby minimizing noise in the resulting absorption spectra [23–25]. The off-axis ICOS geometry preserves the cavity path length enhancement F/π (where F is the cavity finesse) as well as producing a dense mode spectrum. This interaction between the exciting laser and the ICOS cavity reduces the associated amplitude noise. Moreover, all transverse TEM_{mn} modes contribute

to detection of the intracavity trace gas absorber, and the off-axis ICOS measurement technique is more insensitive to vibrations and misalignments than CRDS and on-axis ICOS. All three forms of cavity-enhanced absorption spectroscopy require sufficient excitation power at levels >1 mW because of reduced transmission through a high finesse optical cavity.

Nitric oxide

The presence of endogenous nitric oxide (NO) in exhaled breath of humans and animals was first reported in 1991 [26]. Since then it has become increasingly apparent that measurements of exhaled NO constitute a new way to monitor the inflammatory status in respiratory disorders, such as asthma and other pulmonary conditions. Exhaled nitric oxide as a measure of inflammation is suggested as providing the best combination of disease evaluation and practical implementation for improved asthma outcomes [27]. Exhaled nitric oxide has been successfully employed in chronic asthma treatment monitoring to reduce the dose of inhaled corticosteroids, which have serious side effects, without compromising asthma control [28]. In treating asthma, which affects 19 million Americans, exhaled NO may soon be incorporated into routine clinical care [29].

Nitric oxide is the only exhaled biomarker for which breath collection guidelines have been published. The American Thoracic Society and the European Respiratory Society published an updated Joint Statement [30] in 2005 with recommendations for standardized procedures for the online and offline measurement of exhaled lower airway and nasal NO. Exhaled NO concentrations from the lower respiratory tract exhibit significant expiratory flow rate dependence [31]. Because of this, exhaled NO is commonly collected using a single breath maneuver at a constant exhalation flow rate. Exhaled NO sharply rises and reaches a plateau, which can have a positive, negative or near-zero slope. The plateau concentration is defined as the average concentration over a 3 s window in the plateau region [30]. The plateau level is reported as the exhaled NO value. Measurements of the NO plateau at multiple flow rates allow for determination of the origin of NO (proximal or distal lung) by estimating the flow-independent exchange parameters [32]. The American Thoracic Society recommends that an NO detection system has a sensitivity of 1 ppb, and response time of <0.5 s. Exhaled NO analyzers based on the chemiluminescent technique [33] are commercially available. Competing current technologies such as LAS, electrochemical [34], and resonance-enhanced multiphoton ionization coupled with time-of-flight mass spectrometry [35] may provide lower initial and operating costs, smaller size and less frequent calibration.

Several groups have reported NO analyzers using LAS. Nelson *et al* [36] reported measurements of nitric oxide in air with a detection limit of less than 1 nmol mol^{-1} (<1 ppbv) using a thermoelectrically cooled quantum cascade laser operated in a pulsed mode at $5.26 \text{ }\mu\text{m}$ (1897 cm^{-1}) and coupled to a 210 m path length multiple-pass absorption cell at a reduced pressure (50 Torr). The sensitivity of the system was

enhanced by normalizing pulse-to-pulse intensity variations with temporal gating on a single HgCdTe detector. A detection precision of $0.12 \text{ ppb Hz}^{-1/2}$ was achieved with a liquid-nitrogen (LN_2)-cooled detector. This detection precision corresponds to an absorbance precision of $1 \times 10^{-5} \text{ Hz}^{-1/2}$ or an absorbance precision per unit path length of $5 \times 10^{-10} \text{ cm}^{-1} \text{ Hz}^{-1/2}$. More recently, a sensitivity of $0.7 \text{ ppb Hz}^{-1/2}$ was reported using a cw TEC-cooled QCL and multipass cell with a 36 m path and a 0.3 l volume [17].

A spectroscopic gas sensor for nitric oxide detection based on a CRDS technique achieved a 0.7 ppb standard error for NO in N_2 for a data collection time of 8 s [18]. In this experiment, a cw LN_2 -cooled distributed-feedback (DFB) QC laser operating at $5.2 \text{ }\mu\text{m}$ was used as a tunable single-frequency mid-infrared light source. Both laser-frequency tuning and abrupt interruptions of the laser radiation were performed by manipulation of the laser current. A single ringdown event sensitivity was achieved to an absorption of $2.2 \times 10^{-8} \text{ cm}^{-1}$.

A gas analyzer based on off-axis integrated cavity output spectroscopy (ICOS) and a cw LN_2 -cooled QCL also operating at $\sim 5.2 \text{ }\mu\text{m}$ was developed by our group to measure NO concentrations in exhaled human breath [37]. A compact ICOS sample cell (length = 5.3 cm , volume $\sim 80 \text{ cm}^3$), which was suitable for on-line and off-line measurements during a single breath cycle, was designed and evaluated. Using a combination of wavelength modulation technique and ICOS resulted in a noise-equivalent (signal-to-noise ratio of 1) sensitivity of 2 ppbv of NO for a 15 s data acquisition and integration time.

Subsequently, a nitric oxide sensor based on off-axis ICOS and a thermoelectrically cooled, cw DFB QCL laser operating at $5.45 \text{ }\mu\text{m}$ (1835 cm^{-1}) combined with a wavelength-modulation technique was developed to determine NO concentrations at the sub-ppbv levels [38, 39]. The sensor as shown in figure 1 employed a 50 cm long high-finesse optical cavity that provided an effective path length of 700 m. A noise equivalent minimum detection limit (MDL) of 0.7 ppbv with a 1 s integration time was achieved. A wavelength-modulated signal for a calibrated NO concentration of 23.7 ppbv was fitted by a pre-acquired reference spectrum using a general linear fit procedure [40] (shown in figure 2).

In [41] a thermoelectrically cooled, continuous-wave, QC laser operating between 1847 and 1854 cm^{-1} was used in combination with wavelength modulation spectroscopy to achieve sub-ppbv level for NO. The P7.5 doublet of NO centered around 1850.18 cm^{-1} was used for concentration measurements. Using an astigmatic multiple-pass absorption cell with an optical path length of 76 m and a total acquisition time of 30 s, a detection limit of 0.2 ppbv was achieved. The corresponding minimal detectable absorption was $8.8 \times 10^{-9} \text{ cm}^{-1} \text{ Hz}^{-1/2}$.

Recently, the optical performance of a NO/ CO_2 sensor employing integrated cavity output spectroscopy (ICOS) with a $5.45 \text{ }\mu\text{m}$ TEC cw DFB QCL [39] and a LN_2 -cooled cw DFB QCL operating at $5.22 \text{ }\mu\text{m}$ capable of real-time simultaneous NO and CO_2 measurements in a single breath

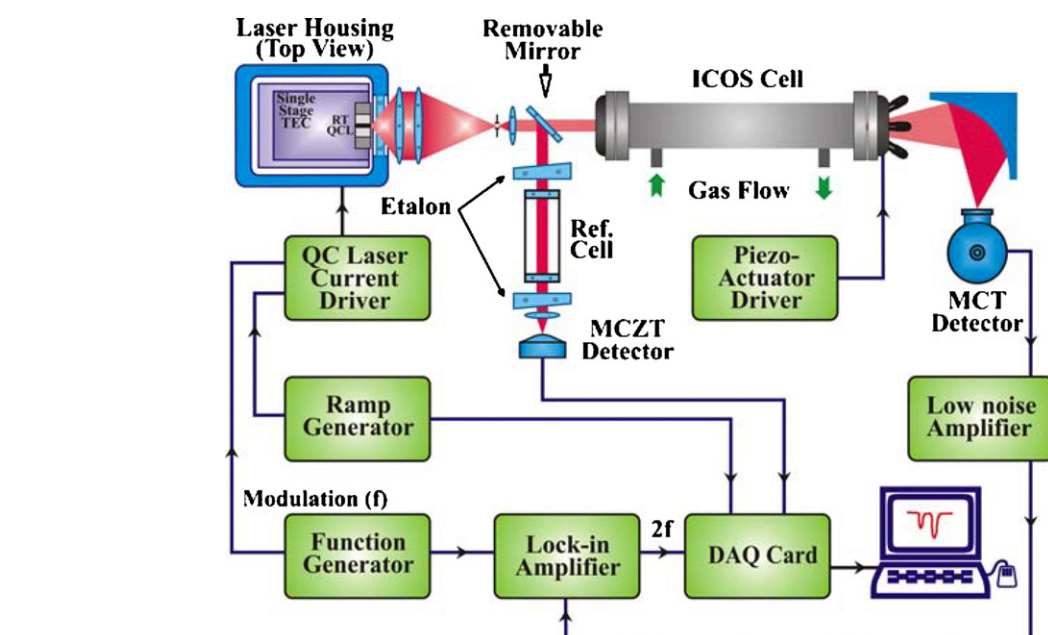


Figure 1. CW-TEC-DFB QC laser based nitric oxide off axis-integrated cavity output spectroscopy [39].

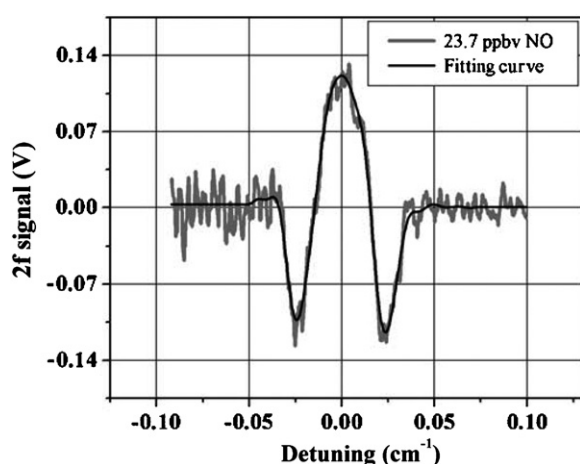


Figure 2. 2f OA-ICOS based NO absorption signal [39].

cycle was reported by our group [42]. A NO noise-equivalent concentration of 0.4 ppb within a 1 s integration time was achieved. The off-axis ICOS sensor performance was compared to a chemiluminescent NO analyzer and a non-dispersive infrared (NDIR) CO₂ absorption capnograph for offline and online human exhaled breath measurements. The off axis ICOS sensor measurements were in good agreement with the data acquired with the two commercial gas analyzers. Figure 3 depicts online nitric oxide concentrations at 3 l min⁻¹ exhalation as a function of time for the ICOS sensor and chemiluminescent analyzer. Fifteen exhaled breaths were measured online with (a) ICOS and (b) chemiluminescence each with 2.4 s averaging/computation time. The NO plateau regions for ICOS and chemiluminescence were in good agreement, with all data points within 1 standard deviation of the 15 measurements. The ICOS measurements had a larger standard deviation for each averaged concentration.

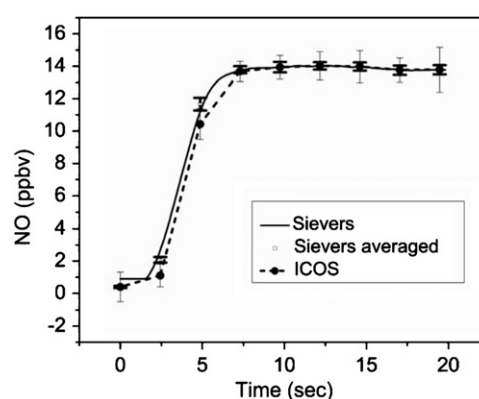


Figure 3. Online nitric oxide concentrations at 3 l min⁻¹ exhalation as a function of time. Fifteen exhaled breaths were measured online with (a) ICOS and (b) chemiluminescence each with 2.4 s averaging/computation time. Solid line represents Sievers data (16 samples s⁻¹) smoothed with a 50 point first-order Savitzky–Golay routine; squares with large cap error bars represent Sievers data after 2.4 s averaging; circles with dashed line and small cap error bars represent ICOS data after 2.4 s averaging and computation; error bars are ± 1 standard deviation [42].

In 2005 concentration measurements of NO for two N isotopes (¹⁴N and ¹⁵N) simultaneously using a CO-laser at $\lambda = 5 \mu\text{m}$ and continuous-wave ring-down detection scheme have been reported [43]. A linear ring-down cavity (length = 0.5 m) with high reflective mirrors ($R = 99.99\%$) was used to achieve a noise-equivalent absorption coefficient of $3 \times 10^{-10} \text{ cm}^{-1} \text{ Hz}^{-1/2}$. This corresponds to a noise-equivalent concentration of 800 parts per trillion (ppt) for ¹⁴NO and 40 ppt for ¹⁵NO in 1 s averaging time. The detection of the ¹⁵NO is unique to this system and allows ¹⁵NO to be used as a tracer molecule in biochemical processes. A precursor molecule containing the ¹⁵N tracer can be introduced into a physiological system, such as the gastrointestinal tract or

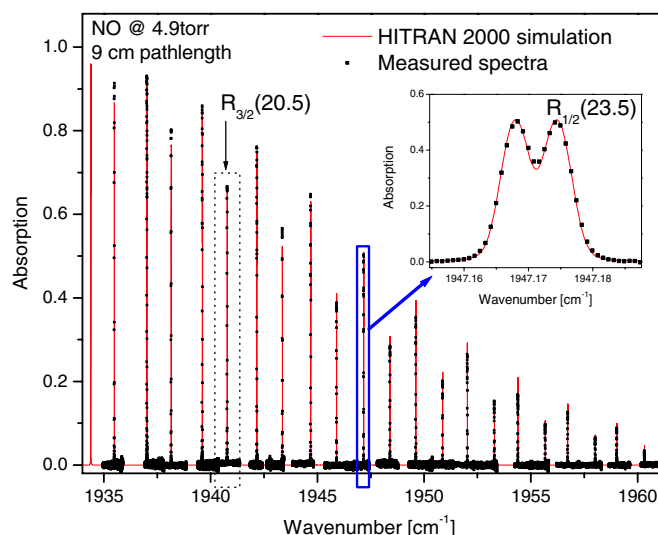


Figure 4. Nitric oxide absorption spectra measured at different EC-QCL grating angles. The narrow laser linewidth allows a spectral resolution of $<0.001 \text{ cm}^{-1}$ [4].

lungs, and the elimination of ^{15}NO in exhaled breath can be measured.

Better selection of an optimum absorption line for sensitive NO measurement as well as simultaneous multi-species detection become feasible with widely tunable external cavity (EC) QCLs. Our first EC-QCL with a thermoelectrically cooled QCL gain medium fabricated using a bound-to-continuum design and operating in continuous wave at $\sim 5.2 \mu\text{m}$ was reported in 2005 [4]. This EC-QCL exhibited a coarse single mode tuning over 35 cm^{-1} and a continuous mode-hop free fine tuning range of $\sim 1.2 \text{ cm}^{-1}$. A direct absorption NO spectrum measured with this EC-QCL is shown in figure 4. The EC-QCL employs a piezo-activated cavity mode tracking system for mode-hop free operation suitable for high-resolution spectroscopic applications (see the inset in figure 4) and multiple species trace-gas detection. Recently a redesigned EC-QCL architecture as depicted in figure 5 was developed. Using a new gain chip it was possible to

extend the EC-QCL tuning range to 155 cm^{-1} from ~ 1825 to 1980 cm^{-1} , which covers almost the entire P, Q and R branches of the fundamental NO vibrational band at $5.2 \mu\text{m}$ with a maximum available optical power of $\sim 11 \text{ mW}$ as depicted in figure 6 [44].

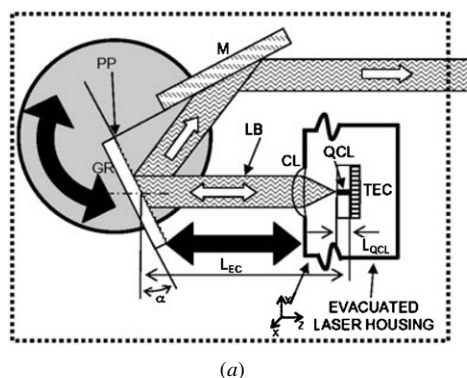
Ammonia

Ammonia (NH_3), a product of urease hydrolysis of urea to ammonia and carbamate, is one of the key steps in the nitrogen cycle. In a recent longitudinal study of 30 healthy volunteers, exhaled ammonia concentrations averaged 0.83 ppm and ranged from 0.25 to 2.9 ppm [45]. Exhaled NH_3 has been shown to increase steadily in the fasting state [46]. Exhaled NH_3 is a potential non-invasive marker of liver [47] and kidney function as well as peptic ulcer disease, and these clinical applications need to be assessed in human clinical studies.

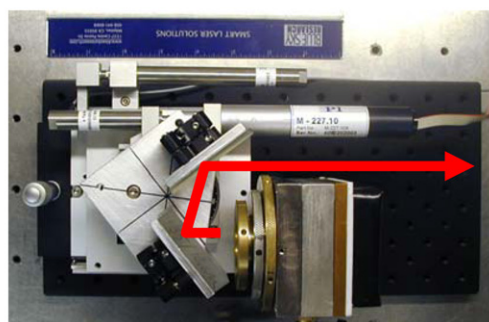
A compact, transportable ammonia sensor based on a thermoelectrically cooled pulsed QC-DFB laser operating at $\sim 10 \mu\text{m}$ was reported to have a sensitivity of better than 0.3 ppmv was achieved with a 1 m optical pathlength [48]. The laser was scanned over two absorption lines of the NH_3 fundamental ν_2 band. This sensor was successfully applied to continuous long-term monitoring of NH_3 concentration levels in the range of 1 to 10 ppmv in bioreactor vent gases at the NASA Johnson Space Center, Houston, TX.

More recently, a gas analyzer based on a pulsed, thermoelectrically-cooled mid-IR quantum cascade laser operating near 970 cm^{-1} using CRDS was developed for the detection of exhaled NH_3 [49]. A sensitivity of ~ 50 parts per billion with a 20 s time resolution was achieved for NH_3 detection from human exhaled breath.

Quartz-enhanced photoacoustic spectroscopy (QEPAS) first introduced in 2002 [50] has a high potential for development of a sensitive miniature breath gas analyzers. Ammonia detection with a MDL of 0.65 ppm (with 1 s lock-in time constant) was demonstrated with a QEPAS sensor employing a $1.53 \mu\text{m}$ telecommunication diode laser delivering 38 mW of optical power [51]. The sensitivity of



(a)



(b)

Figure 5. (a) Schematic diagram of the EC-QCL spectroscopic source and associated measurement system. (QCL—quantum cascade laser; TEC—thermoelectric cooler; CL—collimating lens; LB—laser beam; GR—diffraction grating; PP—pivot point of the rotational movement; M—mirror (mounted on the same platform with GR)). (b) Photograph of EC-QCL assembly [44].

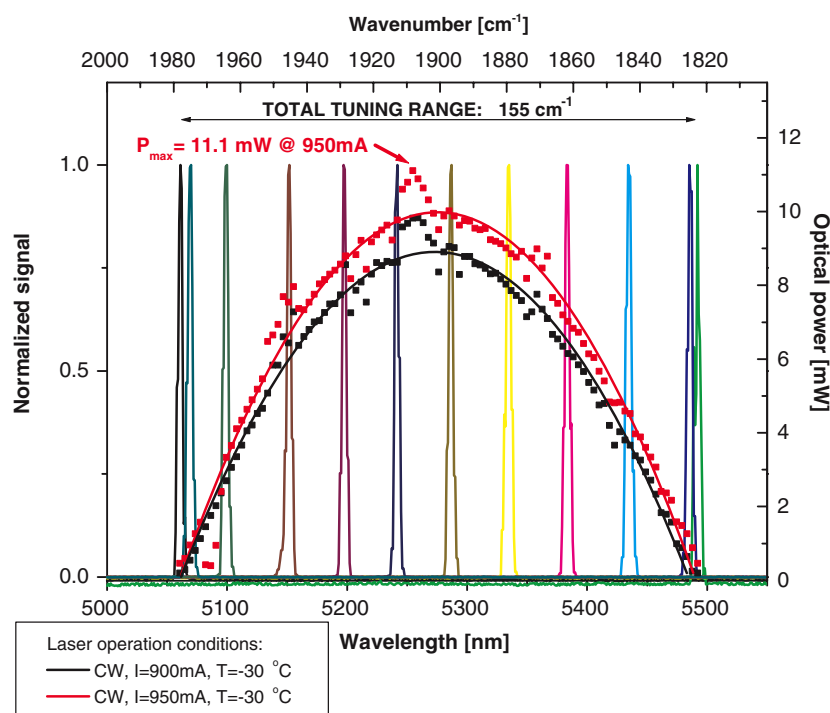


Figure 6. Laser frequency tuning range and corresponding output power of a 5.2 μm EC-QCL [44].

the sensor can be significantly improved (up to ~ 200 times) by targeting the strongest ammonia absorption lines from the fundamental vibrational band in mid-IR ($\sim 10 \mu\text{m}$) as well as by using a redesigned absorption detection module. Recently we have evaluated a thermoelectrically-cooled, distributed feedback QCL that can deliver up to 150 mW of optical power at 9.53 μm , which will be applied to both a LAS and QEPAS [52] based sensor technique. Preliminary calculations indicate that QEPAS detection employing such a QCL will result in a MDL of ammonia concentration at the level of ~ 3 ppb for 1s lock-in time constant.

Carbon monoxide

Carbon monoxide (CO) is a gas that is both formed endogenously and inhaled from the environment. Exhaled CO as a biomarker to assess different diseases (cardiovascular, diabetes, and nephritis) was first reported by Nikberg *et al* in 1972 [53]. Over the last 20 years exhaled CO has been used in smoking cessation, to monitor bilirubin production, including hyperbilirubinemia in newborns, and in the assessment of the lung diffusion capacity. Exhaled CO levels, which are of interest for breath analysis, range from 1 to 5 ppm in healthy subjects.

A pulsed, thermoelectrically cooled distributed feedback QC laser operating at 4.6 μm [54] achieved a noise-equivalent detection limit of 12 ppb with a 2.5 min integration time using a pathlength of 102 cm and the selected CO absorption line at 2158.3 cm^{-1} . The laser frequency could be tuned over a 0.41 cm^{-1} region encompassing the R(3) absorption line at 2158.3 cm^{-1} with appropriate settings of the QC laser temperature and current. This transition is free from

interference of H_2O and CO_2 , which are abundant in exhaled breath. Absolute frequency assignment was performed by comparison of experimental absorption spectra of CO and N_2O with the HITRAN 2000 database [55].

Recently, a thermoelectrically cooled, distributed-feedback pulsed QC laser operating between 2176 and 2183 cm^{-1} was used to measure CO in human breath [56]. The QC laser emission overlaps with the strong R(8)1 CO ro-vibrational transition at 2176.2835 cm^{-1} . A minimal detectable absorption of $1.2 \times 10^{-5} \text{ cm}^{-1}$ was achieved at an acquisition rate of 3 Hz by utilizing the frequency chirp associated with a QCL with long laser pulses. With short laser pulses and slow frequency scanning a minimal detectable absorption $8.2 \times 10^{-7} \text{ cm}^{-1}$ was achieved with a data acquisition time of 60 s. The breath measurements were performed without frequency scanning, yielding a minimum detectable absorption of $7.2 \times 10^{-6} \text{ cm}^{-1}$, corresponding to a detection limit of 175 ppbv using a 0.2 s integration time.

Ethane

Saturated hydrocarbons, such as ethane and pentane, are generated from ω -3 and ω -6 fatty acids, respectively. In contrast to the predominantly airway source of exhaled NO, hydrocarbons are representative of blood-borne concentrations through gas exchange in the blood/breath interface in the lungs. Exhaled concentrations of ethane may be used to monitor the degree of oxidative damage in the body [57]. Initial clinical studies have suggested the use of exhaled ethane in inflammatory lung diseases such as asthma and chronic obstructive pulmonary disease (for a review, see [58]). Exhaled ethane may also correlate with disease status of non-lung inflammatory diseases such as rheumatoid arthritis and

inflammatory bowel disease. Trace-gas sensors suitable for breath ethane analysis should be capable of concentration measurements at 100 ppt levels.

A detection limit of the order of 100 ppt ethane in human breath was achieved by Dahnke *et al* using a 5 s integration time using a high-finesse ring-down cavity and a LN₂-cooled, tunable CO-overtone sideband laser in the 3 μm region [59]. Quantitative time-resolved breath ethane measurements were made during single exhalations [60]. The ethane alveolar plateau was characterized in three subjects. Additionally, the ethane washout process was described after inhalation of ethane. This work was the first to demonstrate ppb-level time-resolved exhaled ethane measurements. Although the number of subjects was small ($n = 3$), the work showed that an ethane sensor with sufficient detection sensitivity and time resolution for real-time exhaled ethane measurements can be realized. Subsequently the same group reported an optical parametric oscillator combined with cavity leak-out spectroscopy to achieve a minimum detectable absorption coefficient of $1.6 \times 10^{-10} \text{ cm}^{-1} \text{ Hz}^{-1/2}$, corresponding to an ethane detection limit of 6 parts per trillion/Hz^{1/2} [61]. Further, frequency-tuning capabilities enabled multi-gas analysis with simultaneous monitoring of ethane, methane and water vapor in human breath.

A LN₂-cooled mid-IR lead-salt laser and Herriott cell were employed by Skeldon *et al* to achieve an ethane detection sensitivity of 70 ppt with a 0.7 s response time [62]. The sensor was used to measure exhaled ethane from animals and humans in several pilot studies [63] including a small lung cancer study of 52 randomly selected patients at a respiratory clinic [64]. Twelve patients were subsequently diagnosed with lung cancer. Twelve control subjects age-matched to the lung cancer group were taken from a larger control group of healthy adults. After correcting for the ambient background, ethane in the control group ranged from 0 to 10.54 ppb (median of 1.9 ppb), lung cancer patients ranged from 0 to 7.6 ppb (median of 0.7 ppb), and non-lung cancer patients presenting for an investigation of respiratory disease ranged from 0 to 25 ppb (median 1.45 ppb). The laser-based ethane sensor proved to be an effective tool for accurate and rapid sample analysis, although there was no significant difference in exhaled ethane among any of the subject groups.

Recently an optical sensor designed to target two trace gases using two separate LN₂ cooled interband cascade lasers (ICLs) in the spectral regions of 3.3 μm (ethane) and 3.6 μm (formaldehyde) was demonstrated by our group (see figure 7). The sensor based on 100 m astigmatic Herriott multi-pass cell utilized a wavelength modulation technique at two different modulation frequencies for ethane and formaldehyde concentration measurements. The sensor exhibited a minimum detection limit of $3.6 \times 10^{-5} \text{ Hz}^{-1/2}$, which corresponds to an ethane concentration of 150 pptv measured with a 1 s integration time. The sensor was evaluated for dual gas sensing using a custom mixture of 79 ppbv ethane and 330 ppbv formaldehyde (balance N₂). During the simultaneous dual trace-gas detection presented in figure 8, the sensor showed a linear response to progressive dilution of both gases and no cross-talk between the channels [65, 66].

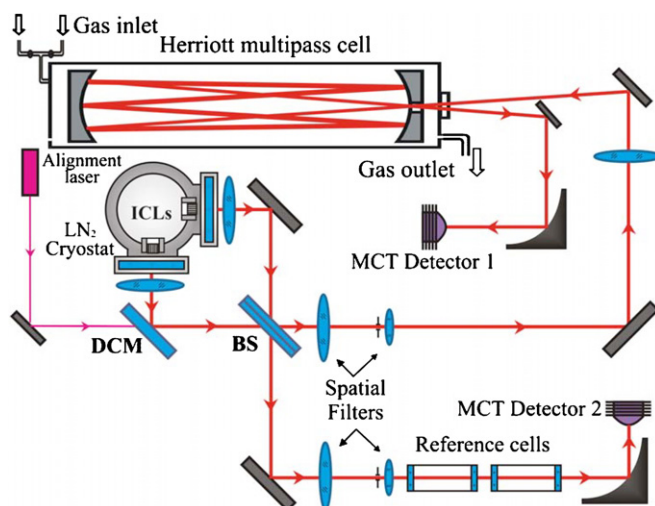


Figure 7. Schematic diagram of a dual ICL based trace gas sensor for simultaneous ethane and formaldehyde concentration measurements [65, 66].

Formaldehyde

Exhaled formaldehyde detection may be useful in monitoring exposure to formaldehyde in the environment, which according to American Conference of Governmental Industrial Hygienists (ACGIH) should not exceed 300 ppb at any time. Exhaled formaldehyde may also be used as a screening test for primary or metastatic cancer. Exhaled formaldehyde has been studied in a mouse model of breast cancer and in six human subjects [67]. Exhaled formaldehyde concentrations from three subjects without breast cancer were 0.3–0.6 ppm and from three subjects with breast cancer were 0.45–1.2 ppm.

The sensor depicted in figure 7 provided a minimum detection limit for formaldehyde (1σ) of ~ 3.5 ppbv with a 1 s integration time. By appropriate selection of the laser sources in both optical channels, the sensor can be reconfigured to target a number of trace-gas species, which possess absorption features within the tuning range of two ICLs or QCLs. Simultaneous multispecies detection of molecules which are involved in closely related bio-chemical processes enables studies of a complex process dynamics.

Formaldehyde detection was also demonstrated using off-axis ICOS with an ICL at 3.53 μm [68]. A 12 mW continuous-wave, mid-infrared, distributed feedback ICL was used to quantify H₂CO in gas mixtures containing ≈ 1 –25 ppmv of H₂CO. Analysis of the spectral measurements indicated that a H₂CO concentration of 150 ppbv produces a spectrum with a signal-to-noise ratio of 3 for a data acquisition time of 3 s.

Carbonyl sulfide

Carbonyl sulfide is a relatively unstudied exhaled gas. The physiological origins of exhaled OCS can include the oxidative metabolism of carbon disulfide [69] and the incomplete metabolism of sulfur-containing essential systems, including methionine [70]. Exhaled OCS has been measured in normal subjects [71, 72], lung transplant recipients [73, 74],

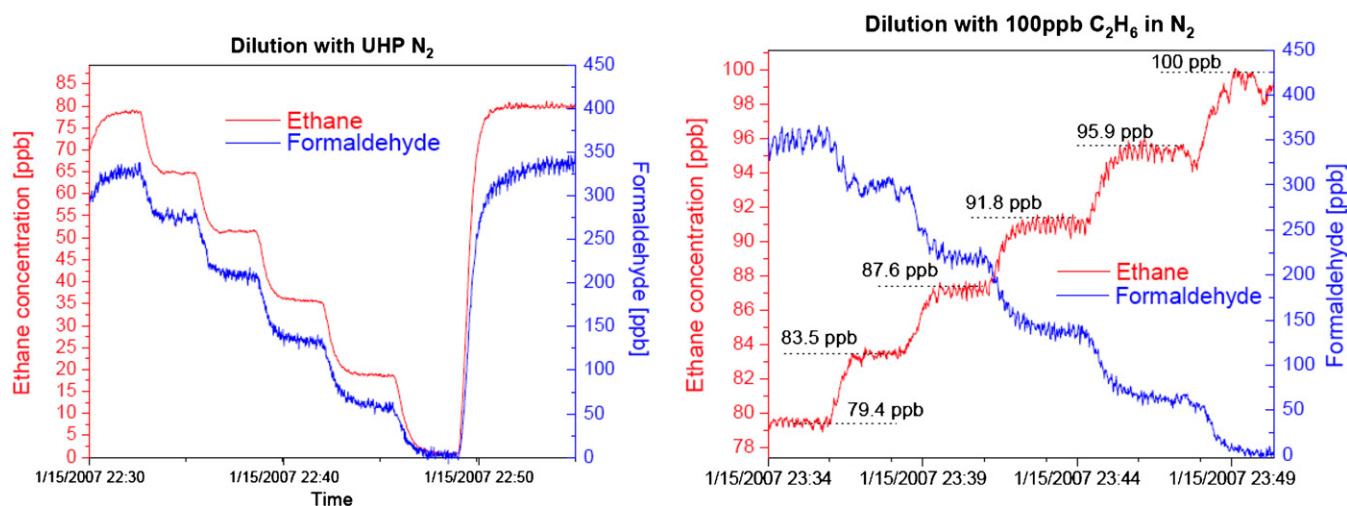


Figure 8. Response of the two-channel optical sensor to a custom mixture of 79 ppbv ethane and 330 ppbv formaldehyde (balance N_2) successively diluted by an ultra high purity (UHP) N_2 (left panel) and by certified mixture of 100 ppb of ethane in N_2 (right panel) [65, 66].

and patients with cystic fibrosis [72]. In a recent study [71], exhaled OCS in normal subjects ranged from ~ 100 –to 300 pptv.

A transportable system employing a thermoelectrically cooled pulsed QCL operating at $4.85\ \mu\text{m}$ coupled to a compact 36 m multipass absorption cell measured OCS with a MDL (1σ) of 1.2 ppb (for 100 averaged 400 point frequency scans acquired within $\sim 0.4\ \text{s}$) [74]. The availability of a neighboring CO_2 line within the tuning range of the QCL allowed ventilation monitoring simultaneously with an OCS measurement and could be used to normalize the resulting OCS concentrations. Application of a pulsed QCL allows utilization of a single HgCdTe detector followed by a time resolved data acquisition system sensor system for simultaneous measurement of the reference and sample beam signals, which is used to minimize pulse-to-pulse fluctuations of the laser radiation and thus significantly improves a signal-to-noise ratio of the OCS sensor.

Ultra-high sensitivity measurements of an exhaled OCS using laboratory setup based on CRDS and a continuous wave, CO laser at $5\ \mu\text{m}$ were also reported by Halmer *et al* [72]. A Fabry–Perot ringdown cavity (0.5 m in length) with $R \geq 99.99\%$ mirrors was used to achieve a noise-equivalent absorption coefficient of $7 \times 10^{-11}\ \text{cm}^{-1}\ \text{Hz}^{-1/2}$. The system was used for real-time OCS monitoring in ambient air and breath samples at ppt levels.

Acetone

Acetone (propanone) is formed by decarboxylation of acetoacetate, which derives from lipolysis or lipid peroxidation. Acetone is a ketone body and is oxidized via the Krebs cycle in peripheral tissue. Ketone bodies in blood (including acetoacetate and β -hydroxybutyrate) are increased in subjects who are in a fasting state. A very significant application of exhaled acetone is in monitoring blood glucose concentration in patients with diabetes mellitus.

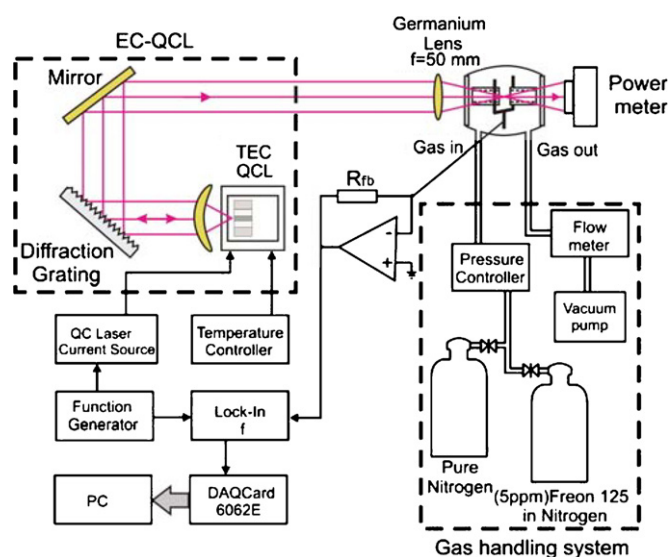


Figure 9. Schematic diagram for a QEPAS sensor platform with CW EC-QCL operating at $8.6\ \mu\text{m}$ as a spectroscopic source [80].

The prevalence of diabetes for all age-groups worldwide was estimated to be 3% and is a major cost of healthcare [75]. Exhaled acetone concentrations increase in patients with uncontrolled diabetes mellitus (levels of 300 ppb to 1 ppm) [76] and have been shown to correlate well with blood glucose concentration in these patients [77].

To detect and monitor diabetes requires acetone detection sensitivities of $\sim 100\ \text{ppb}$. The acetone is a relatively complex molecule and its absorption spectrum contains only broadband ro-vibrationally unresolved features. Measurements of such compounds using laser spectroscopic methods are usually limited due to limited wavelength tunability of available laser sources. To address this requirement, we have used the same EC-QCL architecture developed for a NO sensor operating at $5.2\ \mu\text{m}$ as described above in the NO section after replacing the grating with one blazed for the longer

Table 1. Representative human breath biomarkers.

Compound	Concentration	Physiological basis/pathology indication
Acetaldehyde	ppb	Ethanol metabolism
Acetone	ppm	Decarboxylation of acetoacetate, diabetes
Ammonia	ppb	protein metabolism, liver and renal disease
Carbon dioxide	%	Product of respiration, <i>Helicobacter pylori</i>
Carbon disulfide	ppb	Gut bacteria, schizophrenia
Carbon monoxide	ppm	Production catalyzed by <i>heme oxygenase</i>
Carbonyl sulfide	ppb	Gut bacteria, liver disease
Ethane	ppb	Lipid peroxidation and oxidative stress
Ethanol	ppb	Gut bacteria
Ethylene	ppb	Lipid peroxidation, oxidative stress, cancer
Hydrocarbons	ppb	Lipid peroxidation/metabolism
Hydrogen	ppm	Gut bacteria
Isoprene	ppb	Cholesterol biosynthesis
Methane	ppm	Gut bacteria
Methanethiol	ppb	Methionine metabolism
Methanol	ppb	Metabolism of fruit
Methylamine	ppb	Protein metabolism
Nitric oxide	ppb	Production catalyzed by <i>nitric oxide synthase</i>
Oxygen	%	Required for normal respiration
Pentane	ppb	Lipid peroxidation, oxidative stress
Water	%	Product of respiration

wavelengths in order to optimize EC QCL performance. A MOCVD grown buried heterostructure Fabry–Perot QCL gain chip operating at $8.4\ \mu\text{m}$ in cw mode with thermoelectric cooling was used [9]. The resulting EC-QCL source has a single mode laser frequency tuning range of $\sim 180\ \text{cm}^{-1}$ from 1110 to $1288\ \text{cm}^{-1}$ with a maximum cw output power of $\sim 50\ \text{mW}$ [44].

The $8.4\ \mu\text{m}$ EC-QCL spectroscopic source was integrated with a quartz-enhanced photoacoustic spectroscopic (QEPAS) [52, 78, 79] sensor platform (see figure 9) to detect and quantify broadband absorbers possessing strong absorption features in the accessible spectral region, such as acetone at $\sim 1210\ \text{cm}^{-1}$ and Freon 125 (C_2HF_5 , a convenient safe simulant for toxic chemical and biological agents). In the case of broadband absorbers, the QEPAS signal is generated by modulating the amplitude of the laser radiation with 100% modulation depth and a 50% duty cycle at the resonance frequency of the quartz tuning fork (QTF) used as the acoustic transducer in QEPAS. To demonstrate the capability of this EC-QCL based AM-QEPAS sensor to perform multi-species detection, a spectrum of a mixture of acetone and Freon 125 in N_2 as a buffer gas was acquired. Measured concentrations of acetone at the level of 47.2 ppm and Freon 125 at the level of 4.4 ppm could be retrieved in the data post-processing. The corresponding spectra are shown in figure 10. The system response to the acetone absorption was determined for a single spectral point at the laser frequency ($1217.7\ \text{cm}^{-1}$) corresponding to the maximum absorption coefficient of acetone within the tuning range using a calibration mixture of 8 ppm acetone in N_2 . For a 1 s lock-in time constant and an effective optical power of 6.8 mW a MDL (1σ) of ~ 520 ppb was obtained. At this stage this sensor can be applied to offline breath analysis, in which high measurement speeds are not required. With a 1 min. lock-in time constant the system is capable of acetone detection with a MDL of ~ 70 ppb [80].

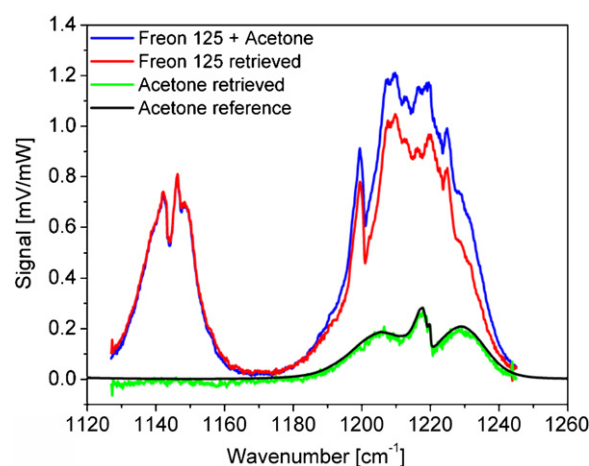


Figure 10. Optical power normalized photoacoustic signal of Freon 125 and acetone mixture (blue line) plotted together with a retrieved component spectra of Freon 125 (red line) and acetone (green line). The calculated acetone spectrum was fitted by a reference spectrum of acetone from the PNNL spectroscopic database shown as a black line [80].

Detection of large organic molecules

The feasibility for sensing small concentrations of large organic molecules in the vapor phase was examined by Fisher *et al* [81] who investigated the potential of mid-infrared photoacoustic spectroscopy for the detection of volatile doping agents (banned substances used by some athletes). Spectra of different doping classes (stimulants, anabolic agents, diuretics and beta blockers) were obtained with an optical parametric oscillator based photoacoustic spectrometer. The sample preparation time will determine whether or not laser spectroscopy is faster than gas chromatography or liquid chromatography with mass spectrometry. Further analysis using urine-like samples containing any doping substances

should provide further insight into the sample preparation needed. The advantage of photoacoustic technique described is that only a single scan over the entire wavelength range is needed for the detection whereas for conventional investigations the samples need to be analyzed separately for each class of substances. Since only pure doping substances were studied, the issue of selectivity needs to be investigated in the presence of interfering gases present in urine or exhaled breath. Furthermore the sensitivity of the photoacoustic sensor for each molecule needs to be characterized.

Summary

Laser absorption spectroscopy (LAS) and quartz enhanced spectroscopy (QEPAS) have been demonstrated to play an important role in the future of exhaled breath analysis. The key attributes of these techniques are sensitivity, selectivity, fast response, ease-of-use, size and low operating cost which make LAS and QEPAS competitive technologies for a number of exhaled bio-markers such as nitric oxide, ammonia, ethane, carbon monoxide, carbon dioxide, acetone and formaldehyde. As many as 400 different molecules in breath, many with well-defined biochemical pathways have been reported in the literature. Table 1 depicts a representative list of these biomarkers. Several investigators have reported sensors with adequate sensitivity for these molecules in ambient air or in cursory exhaled breath samples as an important first step. In many cases, further studies are needed to characterize the ability of these sensors to adequately determine real-time breath concentrations in terms of time resolution (brief data acquisition time and sample cell volume small enough to allow for changes in concentration on the 0.5 s time scale) and selectivity (interfering gases in exhaled breath in normal and disease states). After this characterization, sensors are ready for animal and human clinical investigation, such as the study of exhaled ethane in lung cancer patients reported by Skeldon and colleagues [64]. The use of laser-based sensors in clinical applications is a critical development in the field and demonstrates the utility and relevance of LAS in exhaled breath analysis. Further advances in efficient mid-infrared sources and related technologies will improve detection sensitivities and enable multiple trace gas species detection. At this time commercially available laser-based sensors are limited to the detection of exhaled nitric oxide and ammonia.

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