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Laser-based methods for sensitive trace gas detection

Proefschrift

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Preface

Before bringing you to the results of the last four years of my PhD, I would like to thank people who accompanied me along the way. Nowadays, research field is always an interdisciplinary and multicultural environment. While working in it and being a part of it, in my opinion, one can gain extremely useful experience as well as lots of opportunities for self-development. I’m very glad and proud that I was given a chance to meet the people I’ve worked with. Without all of you guys this work would have never been done!

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Last but not least I would like to thank my parents Sergey and Luidmila Marchenko and my wife Yana for their unconditional trust in me over these years and their support. I dedicate this thesis to you as a sign of my respect and deep gratitude.

Denys Marchenko
01-08-2014
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Chapter 1

Introduction

1.1 Trace gas detection

Sensitive detection and quantification of the trace gases in a gaseous mixture is of great importance for various applications in areas such as atmospheric chemistry [1], environmental research [1–6] and physics [1, 2, 7–10]. Apart from that, there is a great demand for sensitive, fast and precise detection schemes that could be applied for biomedical studies [2, 6, 9–16] where ppmv (1 : 10$^6$, part-per-million by volume), ppbv (1 : 10$^9$, part-per-billion by volume) or even pptv (1 : 10$^{12}$, part-per-trillion by volume) levels of detected concentrations are required. Trace gas detection has important advantages: it is safe, fast and reliable, which makes it also suitable for the clinical studies involving the analysis of Volatile Organic Compounds (VOCs). Therefore, a range of strict requirements should be satisfied while developing a suitable trace gas detector. However, depending on the application area, these requirements may vary. Some research investigations stand in need of a very high sensitivity for a specific compound, whereas others necessitate broadband detection features. Nevertheless, there are some parameters desirable for every trace gas sensor. These are high time resolution, robustness, selectivity, stability and no need for sample preparation. Ideally, these typical features should be combined in a single sensor capable of a long-term continuous operation.

In general, there is a variety of established trace gas detection methods including chemiluminescence [14, 17], gas chromatography [18], electronic nose
Chapter 1: Introduction

[14, 19, 20] and proton transfer reaction based mass spectrometry (PTR-MS) [18]. There is no dispute that every approach has its own points in favour of and against. For instance, mass spectrometry is considered to demonstrate good performance in terms of selectivity and sensitivity but at the same time it is usually massive and expensive [18]. Laser-based absorption spectroscopy meets many of the requirements for sensitive trace gas detection and is currently being utilized in various research areas [1, 2, 13]. The narrow linewidth of the laser sources provides a high spectral power density. Typical linewidth of a continuous wave laser is small compared to the spectral width of the absorption feature of interest. Selectivity is also considered to be one of the strongest points of this technique. Scanning over the absorption feature profile helps to distinguish it from the interfering compounds in a complex gas mixture. In the mid-infrared part of the spectrum (2-20 µm) molecular gases exhibit their unique absorption properties. This region is often referred to as 'the fingerprint region' and represents a favourable area to access ro-vibrational transitions of many specific molecules. So, the identity can often be confirmed by comparing the experimental spectra with those theoretically calculated from a database such as HITRAN [21]. Demonstration of possible experimental approaches for the sensitive trace gas detection (including breath analysis) based on laser absorption spectroscopy in the mid- and near-IR wavelength region is the main purpose of this thesis.

1.2 Breath analysis

Breath analysis is a well established research area [11, 22–27] and is one of the target applications for laser-based absorption spectroscopy. It is a non-invasive, totally painless way of monitoring human health and presents minimal risk for a patient. A breath exhalation can be performed by anybody regardless his age or physical condition. This might be important for those patients who need to control their physiological parameters on a daily basis [28]. Due to the great potential of breath analysis, it has become in great demand for the clinical diagnostics [29].

Breath composition represents a complex mixture of inorganic gases (e.g., CO₂, CO), Volatile Organic Compounds (VOCs) (e.g., acetone, ethane) and other non-volatile composites [22, 30–34]. Useful information can be obtained while studying exhaled breath composition, since some compounds might be considered as indicators playing an important role in physiological and biological path-
ways in a human body. Therefore, absence or presence in higher concentrations of certain gaseous molecules in exhaled breath may indicate a metabolic disorder or a disease. The production of new VOCs may occur as well as a consequence of viral, bacterial or fungal inflammation.

At the moment, there is a range of compounds attributed to certain diseases or metabolic disorders. For instance, high concentrations of nitric oxide (NO) in exhaled breath are related to asthma [35], ethane (C$_2$H$_6$) – to lipid peroxidation and oxidative stress [36], acetone (C$_3$H$_6$O) – to uncontrolled diabetes [37], methane (CH$_4$) – to colonic fermentation [38], etc. However, the biochemical background of many of the processes up to now remains unclear and required further investigation.

So, there is a need in robust and efficient approaches for sensitive and selective detection of gaseous compounds in exhaled breath. This thesis introduces a number of such laser-based absorption spectroscopy methods for the investigation of clinically relevant molecules in mid- and near-IR (such as nitric oxide, acetone, acetylene, and hydrogen cyanide). It can be seen as a step on the way to the development of a new generation portable devices for sensitive trace gas detection both in the clinic and in the field.

### 1.3 Outline of this thesis

A brief introduction to the field of trace gas sensing and breath analysis is given in Chapter 1. Here, the importance of the monitoring and investigation of the exhaled breath compounds is briefly described as well as the requirements applicable for the development of trace gas sensors.

Chapter 2 introduces the concept of Quantum Cascade Lasers (QCLs), a recently developed laser source possessing such distinct features as high quantum efficiency, narrow linewidth, relatively high output power, availability in a broad wavelength region (3.4-24 μm, AlInAs/GaInAs and TeraHertz – 60-250 μm, Al-GaAs/GaAs) and room temperature continuous wave operation. These characteristics make the QCL a promising device for the various scientific applications. Furthermore, a description of the detection methods is given; these approaches include Wavelength Modulation Spectroscopy (WMS), Faraday Rotation Spectroscopy (FRS) and Integrated Cavity Output Spectroscopy (ICOS). The results for the detection of Nitric Oxide (NO) based on FRS technique are also discussed.
Chapter 3 provides a deeper investigation on exhaled and biogenic NO, describing its origin, flow dependency and sampling technique. For simultaneous detection of NO and CO\(_2\) in exhaled human air a sensor based on a cw-QCL in combination with integrated cavity output spectroscopy has been developed, providing detection sensitivity of 0.7 parts-per-billion by volume of NO (acquisition time: 1 s). The sensor provides stable continuous operation over more than 2 days. Comparison is made with the standard chemiluminescence NO analyzer and a commercial CO\(_2\) breath sampler. The QCL-based sensor is tested on healthy subjects at various exhalation flow rates (15, 50, 100 and 300 ml/s) for both online and offline sampling procedures as well as on asthmatic children (offline sampling). Possibilities for measurements of biogenic NO in vivo, i.e. NO originating from human skin tissue, are also demonstrated.

Chapter 4 describes a custom-made continuous wave external cavity quantum cascade laser (EC-QCL) in the Littrow configuration suitable for the detection of molecular gases with broadband absorption features. The laser has an overall tuning range of 230 cm\(^{-1}\) and operates between 1,150 and 1,380 cm\(^{-1}\) (7.25-8.69 \(\mu\)m) producing \(\sim\)1 mW of output power at 243 K. In combination with a multi-pass cell the developed EC-QCL allows fast and robust detection of volatile organic compounds in breath. An absorption profile of 1 ppmv acetone is recorded within 3 s acquisition time covering the wavelength region of \(\sim\)65 cm\(^{-1}\). Noise Equivalent Absorption Sensitivity (NEAS) for the EC-QCL-based spectrometer is estimated at 8.6\(\cdot\)10\(^{-8}\) cm\(^{-1}\)\(\cdot\)Hz\(^{-1/2}\). Possibilities for the online measurements of acetone in breath are demonstrated.

Chapter 5 represents an exploratory study on a prototype of the mid-infrared External Ring Cavity Quantum Cascade Laser (mid-IR ERC-QCL). The QC lasers with 0-, 3- and 7- degrees angled facets use a gain medium centered at 7.72 \(\mu\)m (1295 cm\(^{-1}\)) at room temperature and were placed inside a compact X-shape resonator. Preliminary characterization of the ERC-QCL is performed and some key features of the prototype are studied. Potential to suppress Spatial Hole Burning (SHB) effect is investigated, making ERC-QCL suitable for the active mode-locking in the infrared wavelength region.

Chapter 6 reports on the development of a compact near-infrared Distributed Bragg Reflector (DBR) laser-based spectrometer employing Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS). The spectrometer is capable of simultaneous detection of acetylene (C\(_2\)H\(_2\)) and CO\(_2\) at 1529.2 nm as well as hydrogen
cyanide (HCN) at 1533.5 nm. The detection limits of 8 ppbv for C$_2$H$_2$ and 80 ppbv for HCN are achieved for the acquisition time of 1 sec. The setup has been tested for online measurements of C$_2$H$_2$ in exhaled breath of a smoking subject while HCN has been measured resulting from the metabolism of *Pseudomonas aeruginosa* bacteria *in vitro*.

**References**


References


References


Chapter 2

Quantum cascade laser based absorption spectroscopy in the mid-IR wavelength region

2.1 Quantum cascade laser concept

Addressing the ro-vibrational transitions of the molecules in the mid-IR spectral region with high sensitivity requires advanced sources for these wavelengths. Nowadays, one of the most commonly used laser types is the Quantum Cascade Laser (QCL). The first operating device was reported by Jerome Faist and Federico Capasso at AT&T Bell Laboratories in Murray Hill, N.J., in 1994 [1]. These lasers take advantage of the intersubband transitions rather than electron-hole recombination for the coherent light generation. The composition of the quantum heterostructure is created by Molecular Beam Epitaxial growth (MBE) of semiconductor layers that enables adjusting of the emission wavelength of the laser via the band structure engineering. This allows almost any emission wavelength in the mid-IR (3.4-24 μm, AlInAs/GaInAs) and TeraHertz (60-250 μm, AlGaAs/GaAs) wavelength region by changing the feature size (the layer thickness) rather than the entire materials system (in contrast to conventional diode lasers). Typical features of QCLs in the mid-IR are broad wavelength coverage in the mid-IR, relatively high optical power (Watt-levels at cryogenic tempera-
Chapter 2: Quantum cascade laser based absorption spectroscopy in the mid-IR wavelength region

tures), narrow linewidth (down to tens of kHz), reliability and long-term stability. Consequently, quantum cascade lasers are flexible and very attractive for various applications including trace gas analysis, medical diagnostics, environmental monitoring, military and law enforcement. Complete information on the QCLs, its properties and characteristics can be found in [2].

**Figure 2.1:** Typical schematic representation of the QCL structure. The black line represents the potential energy structure of the conduction band in the quantum cascade laser. Electrons (depicted in blue) are pumped to a given energy level of one quantum well (3). They relax to a lower energy level in another well (2) releasing a photon (red). Then, non-radiative transition occurs (process 2-1) to the low-energy state and electrons tunnel into the next injector region where the process is repeated resulting in a "cascade effect". Defining the structure of the quantum potential wells enables selection of the intended emission wavelength. The figure is reproduced from [1].

Fig. 2.1 shows the potential energy structure of the conduction band in the quantum cascade laser. After pumping to a given energy level of one quantum well, the electrons then undergo the relaxation to lower energy level in another quantum well releasing a photon. Then non-radiative transition occurs to the low-energy state and electrons tunnel into the next injector region where the process is repeated, resulting in a "cascade effect". Quantum engineering of the quantum potential wells structure makes it possible to select the desired emission wavelength.

The QCL power conversion efficiency is typically of the order of a few tens of percent. However, devices with efficiencies around 50% have recently been
demonstrated [3, 4], although only for cryogenic conditions. Continuously operating room-temperature devices [5] are normally limited to moderate output power levels in the range of tens of mWs, whereas higher output powers are easily accessible with cryogenic temperatures. However, even at room temperature, Watt-level peak powers are nowadays possible when using short pump pulses. Typical linewidth of the QCL is rather large – from hundreds of kHz to a few MHz, and can also be affected by the noise level of the current source [6]. However, it has been shown that the linewidth can be enormously reduced even to the Hz-level by fast frequency control [7, 8]. Recently, phase-locking of QCLs has also been achieved [9].

In most QCLs used in present work a quantum well is embedded in a waveguide with Distributed Feedback (DFB). Here, the resonator with a gain medium consists of a periodic structure which acts as a distributed reflector in the wavelength range of laser operation. Despite the fact that there are multiple axial modes in the resonator, only one mode is favored over others due to losses. Apart from DFB QCLs, there are also external-cavity quantum cascade lasers (EC-QCLs), where a wavelength tuning element such as a diffraction grating is incorporated inside the resonator. This particular laser design will be discussed in details in Chapter 4 of this thesis.

2.2 Sensitive molecular absorption spectroscopy in mid-IR

Laser-based absorption spectroscopy employs the absorption of spectral lines, in order to investigate the line strength or the quantity of molecules of interest. These spectral lines are transitions between the energy levels of the molecule which can originate from an electronic, rotational or vibrational state of the molecule. Electronic transitions involve more energy per photon and therefore, are mainly investigated by visible light sources or at the energies corresponding to the UV. Mid-IR radiation can be utilized to probe ro-vibrational transitions. The interaction of the laser emitted light and the absorption feature of interest can be described by Beer-Lambert law:

\[ I(\nu) = I_0(\nu) \cdot e^{-\sigma \cdot N \cdot l} \]  

(2.1)

Here \( I_0(\nu) \) is the transmitted light without any absorbent present, \( \nu \) - frequency of the emitted laser radiation (in cm\(^{-1}\)), \( l \) - absorption path length (in
cm), $N$ - number of absorbing molecules per cm$^3$ (depends on the temperature and pressure), $\sigma$ - absorption cross section in cm$^2$. Absorption at a specific wavelength ($\alpha(\nu)$) can be calculated from:

$$\alpha(\nu) = \sigma \cdot g(\nu)$$  \hspace{1cm} (2.2)

Where $g(\nu)$ - absorption line shape.

In its turn, the absorption line shape is affected by line-broadening processes. The homogeneous broadening of the absorption profile is caused by the pressure inside the absorption cell. This line shape can be described by the Lorentzian line profile:

$$g_L(\nu) = \frac{1}{\pi} \cdot \frac{2\Delta\nu_p}{2(\nu - \nu_0)^2 + \Delta\nu_p^2}$$  \hspace{1cm} (2.3)

Here $\nu_0$ - frequency at the line center in cm$^{-1}$, $\Delta\nu_p$ - the pressure broadened full width half maximum (FWHM) of the line in cm$^{-1}$.

Apart from pressure broadening, the absorption line shape is affected by Doppler broadening caused by a wide range of velocities and directions of the molecules at which they move in the sample with respect to the incident laser beam. This dependency is described by a Gaussian:

$$g_D(\nu) = \frac{2}{\Delta\nu_d} \cdot \sqrt{\frac{ln2}{\pi}} \cdot \exp(-\frac{[2(\nu - \nu_0)]^2}{\Delta\nu_d^2} \cdot ln2)$$  \hspace{1cm} (2.4)

where the Doppler FWHM is given by:

$$\Delta\nu_d = \nu_0 \sqrt{\frac{8 \cdot k \cdot T \cdot ln2}{M \cdot c^2}}$$  \hspace{1cm} (2.5)

Here $T$ - absolute temperature (in Kelvin), $M$ - molecular mass (in a.m.u.), $\nu_0$ - the transition frequency (in cm$^{-1}$).

However, the Lorentzian linewidth increases with pressure, while the Doppler linewidth remains constant with pressure changes. Hence, the Lorentzian is used in order to fit the absorption line shape at higher pressures (typically 200 mbar), whereas the Gaussian lineshape is employed for lower pressures (< 50 mbar). The experiments described in this thesis are performed at the intermediate pressure range (50-200 mbar) where the line shape is described by the numerical approximation of the Voigt profile:
2.2. Sensitive molecular absorption spectroscopy in mid-IR

\[ g_v(u) = \int_0^\infty G(u')L(u - u') du' \] (2.6)

The Voigt profile is an experimentally observed line shape and is a convolution of the Lorentzian \( L \) and the Gaussian \( G \) profiles.

### 2.2.1 Direct Absorption Spectroscopy

Direct Absorption Spectroscopy (DAS) is the most straightforward and fundamental method for trace gas detection. It is based on the transmission reduction through the absorption cell in presence of the absorbing features according to the Beer-Lambert law (Equation 2.1). The principal set up in DAS is depicted in Fig. 2.2. By scanning the laser wavelength an absorption profile can be obtained and further corrected for the background.

![Figure 2.2: Typical detection schematic in Direct Absorption Spectroscopy (DAS). The light intensity from the laser source (A) drops on passing through the absorption cell (B) containing the absorbing species and is detected by a photodiode (C) and retrieved (D).](image)

In DAS a small change of a signal is measured on top of a large background. In order to correct for this, the transmitted light intensity without absorbent \( I_0 \) can be determined in the experiment from a zero absorption reference spectrum. However, any noise induced by the source or the optical system will influence the detection capabilities. Despite the simplicity of DAS, its sensitivity is often limited to an absorbance of \( \approx 10^{-3} \) Hz\(^{-1/2} \) [10], but in some cases reaching \( \approx 10^{-6} \) Hz\(^{-1/2} \) [11, 12]. Therefore, more sophisticated alternative techniques are often applied in order to improve the sensitivity of the setup.
Chapter 2: Quantum cascade laser based absorption spectroscopy in the mid-IR wavelength region

### 2.2.2 Wavelength Modulation Spectroscopy

Wavelength Modulation Spectroscopy (WMS) is an example of a sensitivity enhancing method [13, 14]. However, in WMS the concentration cannot be directly deduced as in DAS. In general, the modulation spectroscopy approach can be divided in two similar concepts. The first one is WMS, where the modulation frequency of the laser wavelength is much smaller as compared to the absorption linewidth of the feature. Usually detection is performed at the modulation frequency \(1f\) or at its higher harmonics \(2f, 3f, \text{etc.}\). Typically, a sinusoidal modulation is applied at the frequencies ranging from a few kHz up to a few MHz. It is well known that the electronic noise at low frequencies decreases (so called \(1/f\) noise). So, the sensitivity can be increased by applying a high modulation frequencies, that allows phase sensitive detection by means of a lock-in amplifier. The second concept is Frequency Modulation Spectroscopy (FMS) that employs modulation of the phase of the light at the frequencies comparable or higher than the absorption linewidth under investigation ranging from 100 MHz up to a few GHz [15–17]. As a result, a pair of sidebands separated from the carrier by the modulation frequency appears, giving rise to a so-called FM-triplet. The signal at the modulation frequency is therefore, a sum of the beat signals of the carrier with each of the two sidebands [18].

Assuming that the output laser frequency is being modulated at a frequency \(\omega_m\) with an amplitude \(\delta \omega\) around its center frequency \(\omega_L\), the instantaneous frequency can be calculated as follows:

\[
\omega = \omega_L + \delta \omega \cdot \cos(\omega_m t) \tag{2.7}
\]

The laser radiation intensity transmitted through the absorption cell can then be expressed as a Fourier series expansion:

\[
I(\omega_L, t) = \sum_{n=0}^{\infty} A_n(\omega_L) \cdot \cos(n\omega_m t), \tag{2.8}
\]

Here \(A_n(\omega_L) (n \geq 0)\) - the individual harmonic components, measured with a lock-in amplifier. At low absorption \((\sigma Nl \ll 1)\), \(A_n(\omega_L)\) can be rewritten as follows:

\[
A_n(\omega_L) = \frac{2I_0 NL}{\pi} \int_0^{\pi} -\sigma(\omega_L + \delta \omega \cdot \cos(\theta)) \cdot \cos(n\theta) d\theta. \tag{2.9}
\]
2.2. Sensitive molecular absorption spectroscopy in mid-IR

Figure 2.3: The principle of the wavelength modulation spectroscopy. Targeting a molecular transition with a laser frequency modulated at $\omega_m$ results in a partial conversion of the wavelength modulation into the amplitude modulation. The first and second harmonics of the WMS signal are proportional to the first and second derivatives of $\sigma(\omega)$, respectively. The figure is reproduced from [19].

Fig. 2.3 represents the case when the modulation amplitude $\delta \omega$ is much smaller in comparison with the absorption linewidth. Each harmonic component in a Taylor series is proportional to the derivative of the $\sigma(\omega)$, which in its turn is proportional to the absorption signal of the species inside the absorption cell.

2.2.3 Faraday Rotation Spectroscopy

Most of the QCL-based NO sensors are employing sources emitting in the 5.2-5.3 $\mu$m, matching the strongest NO absorption band [20]. However, the presence of water (H$_2$O) and carbon dioxide (CO$_2$) in this region disturbs the spectroscopic measurements of the NO concentrations. Therefore, it is essential to eliminate the influence of these interferences, especially when plant or breath samples are analyzed. From this point of view, Faraday Rotational Spectroscopy (FRS) seems to be the most favorable among the spectroscopic techniques, because it is not sensitive to the diamagnetic molecules such as H$_2$O or CO$_2$. The technique, first described in the 1980s [21], is a powerful and versatile method for quantitative and selective detection of paramagnetic molecules, such NO or O$_2$. For NO sensing with FRS, the 5.33 $\mu$m region is commonly used [22–24].

Section 2.2.3 is based on:
The principal experimental set up in FRS consists of an absorption cell with a polarizer at each end. An AC magnetic modulation field is applied to the sample parallel to the laser-beam direction. If a paramagnetic species (such as NO molecule) is present inside the magnetic field the absorption lines split in the simplest case into two components (Fig. 2.4). These components absorb right-hand circularly and left-hand circularly polarized light, respectively. Correlated with this splitting of the absorption lines is a splitting of the corresponding dispersion curves leading to different propagation velocities of the two polarized components (Fig. 2.5). An originally linearly polarized light beam propagating through the sample is equivalent to a beam composed of two circularly polarized components with equal amplitudes, no phase difference but opposite signs. On passing through the sample these two components will propagate with different velocities yielding a phase shift between them.

\[ \theta_F = (n^+ - n^-) \cdot B_0 \cdot l \]  

(2.10)

Here \((n^+ - n^-)\) - difference of refractive indices of left and right hand circularly polarized light, \(B_0\) - magnetic field strength, \(l\) - absorption path length.
Experimental setup and results

The experimental set up in FRS employing a multi-pass cell is shown in Fig. 2.6. The QCL (Alpes Lasers, Switzerland) is thermoelectrically cooled down to -25 °C and generates about 1 mW output power. The current of the QCL is modulated by a 20 Hz triangular signal to scan the Q(5/2) NO transition at 5.33 µm (1875.73 cm⁻¹). The light from the QCL is sent to the astigmatic multi pass cell (AMAC-76, Aerodyne, USA) of 0.5 liters volume and 80 mbar inside pressure. The effective path length inside the cell is equal to 76 m. The cell is placed inside a 25 cm long solenoid producing a modulated magnetic field of 100 G at the frequency of 100 Hz.

**Figure 2.6:** Schematic representation of the multi-pass cell based FRS spectrometer for NO detection at 1875.73 cm⁻¹. The QCL beam is sent to the astigmatic multi-pass cell placed inside a 25 cm long solenoid producing modulated magnetic field of 100 G at 100 Hz. Two Rochon polarizers (MgF₂ prisms, extinction ratio 10⁻⁵) are placed before the cell (polarizer) and after it (analyzer). The beam is focused on a photovoltaic room temperature infrared detector and the output signal is demodulated by a lock-in amplifier and analyzed by a LabVIEW program.

Since the electronic noise is frequency dependent, we have increased the amplitude of the magnetic field at high frequencies. For this, a RLC circuit with a resonant frequency of 6 kHz has been implemented. The QCL beam passes through two Rochon polarizers (MgF₂ prisms, extinction ratio 10⁻⁵, Bernhard Halle Nachfolger GmbH, Germany). The first polarizer is placed before the multi-pass cell and filters out unwanted directions of light polarization, while the second polarizer (analyzer) is placed after the cell almost crossed to the first one.
The angle between the two polarizers is adjusted in order to optimize the Signal-to-Noise Ratio (SNR) of the signal which is a trade-off between laser noise and electronic noise of the detection system. The beam is focused on a photovoltaic room temperature infrared detector (PV-5, D=9·10^8 cm·Hz\(^{1/2}\)/W, \(\tau\)=20 ns, Vigo System, Poland). The output signal is demodulated by a lock-in amplifier (EG&G Princeton Applied Research 5209, USA), acquired via an acquisition card (National Instruments BNC-210, USA) and analyzed by a LabVIEW program.

**Figure 2.7:** The 2f FRS signal for a 76 m path length at 80 mbar pressure of 1 ppmv of NO in N\(_2\) at 1875.73 cm\(^{-1}\); simulated FRS signal (dashed line) based on HITRAN 2008 spectral database and measured data under the same conditions (solid line).

Fig. 2.7 shows a HITRAN simulated FRS signal for 1 ppmv concentration of NO in N\(_2\) for 76 m path length and pressure of 80 mbar and a measured Faraday rotational signal under the same conditions at 1875.73 cm\(^{-1}\) (average of 20 scans during 1 s).

In Fig. 2.8 the Allan variance plot showing the noise level of the detected signal as function of the integration time of the FRS sampling is displayed. The detection limit was 9 ppbv of NO in N\(_2\) for 1 s averaging and the minimum detection limit corresponded to 2.3 ppbv for an averaging time of 16 s. Unfortunately, after the first experiments the laser stopped operating and therefore, no further investigation was possible.

Recently, the development of high performance FR-spectrometers has been
2.2. Sensitive molecular absorption spectroscopy in mid-IR

Figure 2.8: Allan variance for the FRS set up. The sensitivity of 9 ppbv is obtained within 1 second acquisition time. A minimum detection limit of 2.3 ppbv is achieved with 16 s integration time.

reported [22, 25]. The ultimate sensitivity levels achieved in these experiments are 4.7 and 4.5 ppbv for 1 s averaging time, respectively. In comparison, there are several factors affecting the sensitivity of our present set up. Firstly, the NO absorption strength at the wavelength of the available QCL is twice as low as the most sensitive transition for FRS (1875.81 cm$^{-1}$) used elsewhere [22–24]. The power of the QCL is only 1 mW as compared to those reported in literature: 3 mW and 60 mW, respectively [22, 25]. In addition, the detectivity (D) of the detectors used in these studies is 2 orders of magnitude higher than in our experiment. Therefore, by using a more suitable (wavelength) QCL and detector for FRS, the predicted minimum detection limit can be improved by factor of 10 at least, corresponding to a sub-ppbv detection level for 1 s acquisition time.

2.2.4 Integrated Cavity Output Spectroscopy

One of the techniques employing a high finesse cavity to enhance sensitivity is Integrated Cavity Output Spectroscopy (ICOS) also known as Cavity Enhanced Absorption Spectroscopy (CEAS). It has been developed by two independent groups [26, 27] and takes advantage of the integration of the transmitted intensity
Chapter 2: Quantum cascade laser based absorption spectroscopy in the mid-IR wavelength region

through the high finesse cavity in order to obtain absorption spectra. Nowadays, it is increasingly used by many research groups because of its simple alignment procedure since no active locking frequency schemes are required. Moreover, long absorption path length inside the cell provides a very good detection sensitivity improvement, and robustness during the trace gas sensing routines.

The amplitude of the $E$-field transmitted through an absorption cell can be calculated as follows [28]:

$$E(t) = \frac{E_0 e^{i\omega t} t_1 t_2 e^{-\alpha L/2}}{1 - r_1 r_2 e^{-\alpha L} e^{i\delta}}, \quad (2.11)$$

Here $E_0$ and $E(t)$ - the incident and transmitted electrical fields, $t_1$, $t_2$, $r_1$, and $r_2$ - transmission and reflection coefficients for each mirror, $\omega$ - frequency of the light, $\alpha$ - absorption coefficient (in cm$^{-1}$), $L$ - cavity length and $\delta$ - phase shift acquired by the wave in a round trip inside the resonator. For the identical pair of mirrors the transmitted intensity through the cavity can be calculated as follows:

$$\frac{I_t}{I_0} = \left| \frac{E(t)^2}{E_0^2} \right| = \frac{T^2 e^{-\alpha L}}{1 + R^2 e^{-2\alpha L} - 2 Re^{-\alpha L} \cos \delta}, \quad (2.12)$$

Where $R = r_2$, $T = t_2$ and $R + T = 1$. Or in a different way:

$$\frac{I_t}{I_0} = \frac{1}{(1 - Re^{-\alpha L})^2} \cdot \frac{1}{1 + F \sin^2 (\delta/2)}, \quad (2.13)$$

where $1/(1 + F \sin^2 (\delta/2))$ - the Airy function used in multiple-beam interference with the coefficient of finesse: $F = 4R/(1 - R)^2$. In the case of optimal transmission $\delta = 2\pi m$ with $m$ an integer, and the previous equation can be rewritten as follows:

$$\frac{I_t}{I_0} = \frac{(1 - R)^2 e^{-\alpha L}}{(1 - Re^{-\alpha L})^2}. \quad (2.14)$$

The Off-Axis ICOS (OA-ICOS) has been first proposed and implemented in near-IR. This method provides increased spectral density of cavity modes and minimizes the noise in the resulting absorption spectra [30–32]. In OA-ICOS the laser beam is directed at an angle with respect to the cavity axis. The laser beam undergoes multiple reflections before the re-entrant condition is satisfied and is
coupled to a large number of the cavity modes. Fig. 2.9 represents a schematic comparison between on-axis and OA-ICOS arrangements. Effectively increasing the optical path length inside the absorption cell, OA-ICOS is lowering the Free Spectral Range (FSR) of the cavity resulting in a dense mode structure [29, 30]. If the OA-ICOS alignment is successful, the laser linewidth is broad with respect to the cavity FRS but narrow with respect to the absorption feature.

However, potential drawbacks of the ICOS technique are low transmission power (that enables utilizing predominantly high-power laser sources) and its sensitivity to laser power fluctuations.

It should be mentioned though, that apart from the detection methods described above and used in this work, there is a number of other sensitive laser-based techniques successfully applied for the trace gas sensing. Table 2.1 provides an overview of the best sensitivities (per Hz$^{-1/2}$) for given molecular species achieved by using the laser-based molecular absorption spectroscopic techniques in the IR spectral range. Among others, the sensitivities for Quartz Enhanced Photoacoustic Spectroscopy (QEPAS), Cavity Ring-Down Spectroscopy (CRDS),
and Noise-Immune Cavity-Enhanced Optical Heterodyne Molecular Spectroscopy (NICE-OHMS) are given.

**Table 2.1:** Comparison of the best sensitivities achieved by using the laser-based molecular absorption spectroscopic techniques in the IR spectral range.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Wavenumber, cm(^{-1})</th>
<th>Species</th>
<th>Sensitivity, Hz(^{-1/2})</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS</td>
<td>1660, 1664, 1764.9</td>
<td>HONO, NO(_2), CH(_2)O</td>
<td>up to 10(^{-6})</td>
<td>[11, 12]</td>
</tr>
<tr>
<td>WMS</td>
<td>1850.18</td>
<td>NO</td>
<td>(\sim 10^{-5} - 10^{-6})</td>
<td>[33]</td>
</tr>
<tr>
<td>OA-ICOS</td>
<td>6369.43, 2012.2</td>
<td>CO, H(_2)O</td>
<td>(\sim 10^{-9} - 10^{-10})</td>
<td>[29, 34, 35]</td>
</tr>
<tr>
<td></td>
<td>1483.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QEPAS</td>
<td>948.62</td>
<td>SF(_6)</td>
<td>(\sim 10^{-10})</td>
<td>[36]</td>
</tr>
<tr>
<td>CRDS</td>
<td>9398.49, 2038.95</td>
<td>CO(_2), OCS</td>
<td>(\sim 10^{-11})</td>
<td>[37, 38]</td>
</tr>
<tr>
<td>NICE-OHMS</td>
<td>9398.49</td>
<td>C(_2)H(_2)</td>
<td>(\sim 10^{-13})</td>
<td>[39]</td>
</tr>
</tbody>
</table>

**References**


Quantum cascade laser-based sensor for detection of exhaled and biogenic nitric oxide

Abstract
A sensor based on a continuous wave quantum cascade laser, operating near 5.2 μm, in combination with integrated cavity output spectroscopy has been developed for simultaneous measurement of nitric oxide (NO) and carbon dioxide (CO₂) traces in exhaled air. A detection limit of 0.7 parts-per-billion by volume of NO (acquisition time: 1 s) is achieved. Comparison is made with the standard chemiluminescence NO analyzer and commercial CO₂ breath sampler. The QCL-based sensor is tested on healthy subjects at various exhalation flow rates (15, 50, 100 and 300 ml/s) for both online and offline sampling procedures as well as on asthmatic children (offline sampling). Possibilities for measurements of biogenic NO in vivo, i.e. NO coming from human skin tissue, are also demonstrated.

This chapter is based on:
3.1 Introduction

Biomarkers are indicators for particular biological states. They are currently being investigated in many fields of research, including medicine, medical biology and biochemistry. The markers can be specific cells, molecules, genes, gene products, enzymes, or hormones. In particular, this study focuses on molecules in exhaled breath, which may be considered to investigate an organ’s disease state, or to monitor how the body responds to a particular medical treatment. Monitoring gases in exhaled breath is a complicated task due in part to their different concentrations, ranging from ppmv (part per million by volume =1:10^6) to ppbv (part per billion by volume =1:10^9) or even pptv (part per trillion by volume =1:10^12). One of the most appealing and hence studied molecules for medical applications is nitric oxide (NO) which is known as an indicator of airway inflammation in humans [1]. In addition, NO is also a signalling molecule and a physiological messenger in mammalian cells, involved in various important bio-chemical pathways. Exhaled NO (exNO) has been shown to be a potentially useful biomarker in the diagnostics of asthma and other respiratory diseases [2–4]. Despite many research efforts, exNO is not routinely used for clinical diagnoses. One of the reasons for this is contradictory results reported in the early 90’s, before exNO concentration was known to be flow dependent. Since then, measurements of exNO have been performed at multiple flow rates, which, along with modeling of the production and transport of NO through the lungs, can provide specific information concerning treatment and diagnosis [5].

At present, several commercial sensors are available to monitor NO for medical purposes, making use of chemiluminescence, electrochemical technology and more recently, laser-based techniques [6–9]. Chemiluminescent sensors are considered to be the “gold standard”, since they provide both accuracy and precision. However, these analyzers are costly to both acquire and run and require technical expertise for calibration. This has limited their routine use. Electrochemical sensors are convenient and can be easily developed into portable hand-held analyzers. However, the limited sensitivity of these devices is preventing their expansion. Recent advances of Quantum Cascade Laser (QCL) technologies have offered new opportunities for mid-InfraRed (mid-IR) gas sensors (3–24 µm). QCLs are now increasingly used for high resolution spectroscopy because of their narrow linewidth (for distributed feedback QCLs), continuous wave (CW) operation mode and relatively high power even at room temperature [10, 11]. Several approaches for NO sensing have been reported and successfully implemented, namely absorption spectroscopy utilizing a multipass cell [12–14], Faraday rotation spectroscopy [15, 16], photoacoustic spectroscopy [17, 18], and integrated cavity output spectroscopy [19–21] or cavity ring-down spectroscopy [22, 23] using high finesse cavities.

In this paper, the capabilities of a cw-QCL-based sensor employing integrated cavity output spectroscopy will be demonstrated for both online and offline detection of NO and carbon dioxide (CO₂) during single exhalations of human breath. Performance of the
3.2. Exhaled nitric oxide

3.2.1 Origin of NO

Endogenous NO is derived from L-arginine by the enzyme NO Synthase (NOS). NOS itself has at least three isoforms. Among these, two are expressed and activated by the increase in intracellular calcium concentration. Neuronal NOS (NOS1 or nNOS) is predominately expressed in neurons and endothelial NOS (NOS3 or eNOS) predominately in endothelial cells. Inducible NOS (NOS2 or iNOS) has a much greater level of activity and is independent of calcium concentration. NOS2 is induced by inflammatory cytokines, endotoxins and viral infections. Besides, it may show increased expression in inflammatory diseases. The cellular source of NO in the airway is unknown and may include airway epithelial cells. However, exhaled NO is not a direct measure of NOS activity in the lower respiratory tract since NO is produced and consumed by other reactions. NO in exhaled air may also be derived from nitrite protonation to form nitrous acid, which releases NO with acidification [24].

The levels of NO derived from the upper respiratory region (0.2-1 ppmv) [25] and sinuses (1-30 ppmv) [26] are almost two orders of magnitude higher than exhaled NO measured in the lower respiratory area (1-9 ppbv) [27]. However, the source of NO in the lower respiratory area is a mix of concentrations derived from airway and alveolar epithelial cells, which express both NOS3 and NOS1.

Some researchers suggest that NO is derived from the airways rather than from alveoli [28], since simultaneous measurements of expired CO₂ and NO show that eNO precedes the peak value of end-tidal CO₂. Therefore, exNO most likely results from epithelial sources rather than from endothelial.

3.2.2 Measurements of NO concentration

Nitric oxide is the only exhaled trace gas biomarker for which breath collection guidelines have been published. The American Society and the European Respiratory Society published an updated Joint Statement with recommendations for standardized procedures for the online and offline measurements of exhaled lower airway and nasal NO [29]. Exhaled NO concentrations from the lower respiratory tract exhibit significant expiratory flow rate dependence [30]. This flow dependence is a characteristic of a diffusion-based
process for the NO transfer from airway wall to lumen. It can be explained by the different alveolar contribution of NO to the airway depending on the exhalation rate, therefore reducing (at higher rates) or increasing (at lower rates) the amount of NO transferred. Due to this flow dependence, the use of constant expiratory flow rates is commonly used in standardized sampling techniques. Various factors affecting exNO concentration should also be taken into account, including age (exNO increases with age e.g. in children), breath collection procedure, habits (smoking reduces airway NO), condition (airway inflammation increases NO), etc.

The advantages of online sampling are obvious; alternatively, offline breath sampling is to be considered when analysing large numbers of samples, which is the case for clinical studies. For offline measurement, a new custom built hand-held breath collection device has been developed (Fig. 3.1). The breath collection device was designed according to the guidelines of the American Thoracic Society (ATS) and European Respiratory Society (ERS) for the standardized collection of exNO.

**Figure 3.1:** Custom made hand-held breath sampler. (A) Mouth piece. (B) Pressure meter with 3 LEDs helping the person to maintain constant exhalation flow. (C) Fluoroplastic tube (PFA). (D) Teflon piece. By changing the diameter of the hole, different flow rates can be assessed, keeping the mouth pressure of 10 mbar. (E) Mylar balloon. (F) Discard bag.

The sampler is simple, extremely user-friendly, and can be used by nurses and unspecialized medical personnel. It consists of a mouthpiece, a pressure meter, a Teflon piece for flow resistance and a sampling bag. Additionally, a discard bag can be considered to remove the first part of the breath – dead space. A constant exhalation flow rate was maintained by monitoring the mouth pressure. For the breath collection, patients were asked to take a deep breath and perform one single exhalation into the mouthpiece. Three LEDs were giving feedback to the patient to maintain a constant mouth pressure, which was set to 10 mbar overpressure, enough to close the soft palate and to avoid the need for
3.2. Exhaled nitric oxide

a nose clip. The pressure range indicated by the LEDs was set by the pressure meter to ±5%. Breaths samples can be collected at various constant exhalation flows from each subject by easily changing the resistance piece. All exhalation flows (15, 50, 100 and 250 mL/s) are performed at the same mouth pressure of 10 mbar. The calibration of the sampler is made with a mass flow meter (Brooks Instrument, USA) ranging from 0 to 30 slpm (standard liter per minute).

3.2.3 Flow dependency of NO

In 1997, it was discovered that the concentration of exNO was highly dependent on the exhalation flow rate [30], varying significantly from other endogenous gases detected in exhaled air.

Early research established a strong inverse relationship between the exNO concentration and the exhalation flow rates [30–32] (Fig. 3.2). Measurements of the exNO were done with the QCL-based sensor described in Section 3.4.1.

Figure 3.2: Exhaled NO plateau concentrations over various exhalation flows. Exhaled NO concentration depends strongly on the exhalation flow and increases significantly at flow <50 ml/s.

In order to explain these observations, a two-compartment model of the lungs was

Section 3.2.3 is adapted from:
Chapter 3: Quantum cascade laser-based sensor for detection of exhaled and biogenic nitric oxide

introduced by several research groups [32–35]. This model is capable of a suitable explanation of many unique features in NO exchange dynamics, in particular the dependence in the exhalation flow rate. It describes exhaled NO arising from two compartments using three flow-independent exchange parameters: one describing the alveolar region (the steady-state NO alveolar concentration), and two describing the airway region (airway NO diffusing capacity and airway wall NO concentration). With the use of these three parameters, the two-compartment model can then predict the exhaled concentration at any desired exhalation flow rate. It is important to note that the two-compartment model does not consider the nasal compartment and the significant NO production in the sinuses [36]. Thus special precautions to close the soft palate during exhalation must be taken in order to avoid nasal contamination when model predictions are compared to the experimental data.

\[ J_{aw NO} = J'_{aw NO} - D_{aw NO} \cdot C_{NO} \]

Fig. 3.3: Schematic of 2-compartment model used to describe nitric oxide (NO) exchange dynamics. See details in the text. The figure is reproduced from [59].

Fig. 3.3 depicts the basic features of the model. The alveolar NO concentration is the balance between NO produced locally and diffusing away. During an exhalation of more than 10 s [32, 37–40] the concentration in the alveolar region, \( C_{ANO} \), reaches a steady state concentration. During the exhalation the gas flow is mixed with NO diffusing from the airway wall. The amount of NO into the air from the airways per unit time is referred to as the flux of NO from the airways, \( J_{aw NO} \) (pl/s), and is expressed as a linear function of the airway NO concentration, \( C_{NO} \), by the following expression [32]:

\[ J_{aw NO} = J'_{aw NO} - D_{aw NO} \cdot C_{NO} \]  \hspace{1cm} (3.1)

\( J'_{aw NO} \) is the maximum flux of NO from the airway tissue, which is approximately
equal to the airway compartment flux if $C_{NO}$ was zero. $D_{awNO}$ is defined as the airway NO diffusing capacity from the airway tissue to the gas phase and vice versa. The two-compartment model can be used to predict the exhaled concentration of NO, $C_{ENO}$ at any constant exhalation flow ($V_E$) by using the following exponential expression [32–35]:

$$C_{ENO} = C_{awNO} + (C_{A NO} - C_{awNO}) \cdot exp\left(-\frac{D_{awNO}}{V_E}\right)$$

(3.2)

### 3.3 Exhaled carbon dioxide

Generated in tissue by aerobic and anaerobic metabolism, carbon dioxide (CO$_2$) is transported in blood to the alveolar region of the lungs and eliminated during the exhalation. One of the currently used ways of monitoring the respiratory CO$_2$ concentration is capnography. This dependence is represented by time-concentration curve. Capnography is used for the inhaled and exhaled CO$_2$ concentrations monitoring and provides information concerning the CO$_2$ production, pulmonary perfusion, alveolar ventilation, respiratory patterns, and elimination of CO$_2$ from the breathing circuit [41]. Besides, capnography is a commonly used way of the ventilatory status monitoring of patients in the operating room representing a reliable and fast method.

A capnogram is an exhaled CO$_2$ waveform consisting of 3 phases (depicted in Fig. 3.4). Phase I represents the initial stage of expiration. Gas sampled during the initial phase of expiration occupies the anatomic dead space and is normally devoid of CO$_2$. Phase II represents a sharp increase of the CO$_2$ concentration. Phase III is the alveolar or expiratory plateau where the alveolar gas is sampled and measured.

Exhaled CO$_2$ enhances exhaled biomarker analysis by monitory ventilatory status (for volatile organic compound detection) tracing the breathing pattern and allowing normalization to CO$_2$ concentration. Commonly, the ventilatory status is monitored measuring the end-tidal CO$_2$ - concentration at the end of the exhalation cycle. In healthy subjects the end-tidal CO$_2$ levels are defined within the range of 4.5-5.1%. Lower levels (<4% CO$_2$) may indicate that the subject is unintentionally hyperventilating before the breath collection procedure. In particular, this is important when collecting VOCs [42, 43].

The exhaled CO$_2$ waveform is important as a marker of the breath cycle. A rapid rise in the inspiratory flow rate (phase III) and a sharp decrease in the end-tidal CO$_2$ concentration (the end of phase III) can be used to adjust and synchronize the target gas and CO$_2$ waveforms during the data recording or processing after the measurements [44]. For instance, this technique may be used in characterizing exhaled trace gases in critically ill patients (for instance, mechanically ventilated). Normalization of a target gas to CO$_2$ may decrease variability in breath measurements from an individual, improving the accuracy of the measurement and robustness [45].
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Figure 3.4: A typical capnogram representing the CO$_2$ waveform over time. Phase I represents the initial stage of expiration. Phase II represents the sharp upstroke of the waveform. Phase III - the alveolar (expiratory) plateau. The end-tidal CO$_2$ is the concentration at the end of the exhilation.

3.4 Experimental details

3.4.1 Sensor design

A schematic representation of the QCL-based sensor is shown in Fig. 3.5. A thermoelectrically cooled continuous wave QCL (Alpes Lasers), operating at 5.26 µm, generates an average output power of 3 mW. The laser is placed into a housing equipped with a 5 mm diameter collimating lens and a water cooled Peltier element. According to the specifications, the overall tuning range of the QCL is 8 cm$^{-1}$ (from 1897 cm$^{-1}$ at 0°C to 1905 cm$^{-1}$ at -30°C). To scan the transition at 5.26 µm (1900 cm$^{-1}$) the temperature of the laser is set to -19°C, with a current value of 500 mA. At this fixed temperature the frequency of the laser output is fine-tuned by modulating the QCL current with a 10 kHz triangular signal. The fine-tuning range is about 0.25 cm$^{-1}$. The beam is sent from the laser to a high finesse cavity (F $\approx$ 4500), formed by two 1” concave mirrors (1 m radius of curvature) with a reflectivity of R$\sim$99.93% at 5.2 µm (CRD Optics, Inc.).

The optical cavity consists of a 30 cm long aluminium tube and has a volume of 150 ml. The effective optical path length inside the cell is about 400 m. The cavity is aligned off-axis with respect to the incident laser beam by means of two translation stages. The
3.4. Experimental details

**Figure 3.5:** Schematic representation of the QCL-based sensor for NO and CO$_2$ detection at 1900 cm$^{-1}$. The laser beam is sent to a high finesse absorption cell with the effective pathlength of 400 m. The beam is focused on a 4 stage TEC infrared detector and the output signal is analyzed by the LabVIEW program.

Off-axis alignment provides better cavity mode suppression and effectively lowers the free spectral range (FSR) of the cavity [19, 21]. A pressure of 100 mbar is maintained inside the absorption cell by a vacuum pump with manually adjusted valves. To reduce the response time of the system, the flow rate through the gas cell is set to 50 l/h, producing a refresh time for the cell of approximately 1 sec. The output beam is focused by means of a 25 mm BaF$_2$ lens (5 cm focus distance) on a 4 stages thermoelectrically cooled (TEC) HgCdTe infrared detector with a built-in preamplifier (PV-5, D=1.5·10$^{10}$ cm-Hz$^{1/2}$/W, $\tau$=20 ns, Vigo System). The signal is amplified by 20 dB (Femto HVA-S, Germany) and acquired via a data acquisition card (GaGe Octopus-8325, USA) for computer analysis using LabVIEW software. The QCL-based sensor is calibrated with a reference mixture of 100±3 ppbv of NO in N$_2$ (VSL-National Dutch Metrology Institute). An N$_2$ gas cylinder is used to provide a gas reference free of NO.

In the spectral region of the QCL both CO$_2$ and NO absorption lines are present (1899.99 cm$^{-1}$ and 1900.07 cm$^{-1}$, respectively) and can be simultaneously measured. Fig. 3.6 represents simulated spectra of 1ppmv NO and 4% CO$_2$ (typical breath concentration) at working conditions (pressure 100 mbar, temperature 293 K) (from HITRAN 2008 data base [46]). Measurements of CO$_2$ dynamics in breath provide potential benefits, allowing for calculations to determine the physiological dead space volume [47–49].
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3.4.2 Breath sampling

In this study, the QCL-based sensor is applied for monitoring exhaled NO and CO$_2$ concentrations for both online and offline sampling of single exhalations. The study has been carried out in the Netherlands in accordance with the applicable rules concerning the review of research ethics committees and informed consent. The Institutional Review Board of UMC St Radboud (Nijmegen) has approved this study.

Online breath sampling

For online measurements a commercially available sampler (Loccioni, Italy) is used. This breath sampler monitors the mouth pressure together with the CO$_2$ concentration during the exhalation maneuver, fulfilling the American Thoracic Society (ATS) guidelines’ requirements for collecting breath [29]. With a back pressure of 10 mbar one can be sure that the soft palate is closed, so that the measured NO concentration is not affected by contributions from the nasal part. All the subjects were asked to maintain a constant exhalation flow rate. Several flow rates (15, 50, 100 and 300 ml/s) can be assessed by changing the resistance through the blowing tube. The CO$_2$ concentration and the airway pressure profiles are displayed in a graphical form on the screen of the

Figure 3.6: Simulated spectra of 1ppmv NO (solid line) and 4% CO$_2$ (dotted line) at 100 mbar pressure. Source: HITRAN 2008. The scanning range of the QCL allows a measurement of both lines simultaneously.
sampler, reducing possible errors while sampling. When the CO$_2$ concentration level reaches 2%, the subsequent part of the exhaled breath is sent to the QCL-based sensor. This value is chosen for several reasons. Firstly, it is easily reachable even for asthmatic children. Secondly, a CO$_2$ plateau begins for most of people at the concentration of 3%. Finally, the mouth pressure at this CO$_2$ value will be surely maintained constant by the subject. In order to prevent condensation, the breath sampling line is heated up to 38°C. The exhaled concentrations of NO and CO$_2$ are measured and displayed in real time.

**Offline breath sampling**

Offline sampling is most appealing when there is no possibility to measure the breath samples online. In order to do that, a custom-built hand held device is used (described in Section 3.2.2) to collect the breath samples from the patients in an NO-impermeable, aluminium-foil air bag of 500 ml capacity (Mylar balloon, ABC ballonnen, Zeist, The Netherlands) [50]. This apparatus is built in accordance with the ATS guidelines for the exhaled NO sampling and has been compared with a commercial sampler in a previous study [51]. The sampler consists of a mouth-piece connected to a discard bag (400 ml) and a Mylar bag. By changing the resistance of the sampling line, it is possible to adjust the exhalation flow to various values. The subjects maintain a constant flow rate thanks to a visual, optical LEDs feedback system. The device is simple, compact and can be used by medical staff.

**3.4.3 Collecting NO samples from skin**

Previous studies have reported endogenous NO in a range of biological pathways observed in several cell types that reside in the skin tissue [52–54]. A few attempts have been made to estimate the NO release from skin tissue in vivo, as well as from human sweat [55, 56]. In this study, a sampling method is developed to allow measurements of NO at trace concentrations from human skin using the QCL-based sensor. A glass cuvette (7 cm diameter, 35 ml volume) is placed on the skin at several positions on the body (arms, legs, chest and back) and fixed in a way to avoid leaks.

Compressed air from a cylinder is used as a carrier gas to transport trace gases released from the skin surface to the sensor. The flow through the first mass flow-controller (Brooks Instrument, USA) is 1.1 l/h. The flow through the cuvette and the second mass flow-controller is 1 l/h and maintained constant by a membrane pump. The sample is then transported to the QCL-based sensor. All the parts of the gas transport system (except mass flow-controller) are made of Teflon PFA or Teflon PTFE (PolyFluor Plastic, Hoevestein, The Netherlands). In order to avoid overpressure inside the Teflon sampling tube, a small portion of flow (0.1 l/h) is allowed to escape via an overpressure outlet. The outlet is placed before the glass cuvette to prevent dilution of the NO concentration.
produced by the skin tissue. The arrangement is schematically shown in Fig. 3.7. In addition, a simple 10 kOhm thermistor probe is placed near the cuvette to monitor the skin temperature.

3.5 Results

3.5.1 Performance of the QCL-based sensor

Before measuring breath samples, performance of the setup was estimated. The detection limit of the QCL-based sensor is 0.7 ppbv of NO for a 1 s average. The ultimate sensitivity of 100 pptv is obtained by using 128 s integration time. Fig. 3.8 represents the Allan standard deviation – a noise level of the signal as a function of the integration time of the measurement. Long term stability of the sensor was also tested.

Fig. 3.9 shows the noise spread in ppbv while measuring of NO free gas for the period of 52 h. Fig. 3.10 represents a dilution curve for the QCL-based sensor in the range 1-1000 ppbv.

Online exhalation profiles at a flow rate of 50 ml/s are shown in Fig. 3.11, where panel (a) represents airway pressure and exhaled CO$_2$ profiles measured by the commercial sampler and panel (b) represents NO and CO$_2$ profiles measured by the QCL-based sensor in real time. It can be seen that the CO$_2$ profiles measured by the sampler and the QCL-based sensor are in good agreement.
3.5. Results

Figure 3.8: Allan variance represents QCL-based detection limit as a function of the integration time. A detection limit of 0.7 ppbv for a 1 s average is achieved. The best detection limit of 100 pptv is reached for a 128 s acquisition time.

Figure 3.9: Long-term background stability of the QCL-based sensor over 52 h of continuous operation. Acquisition time – 1 s.

3.5.2 Comparison with a chemiluminescence analyzer

Since the chemiluminescence technique (CLD) is considered to be the ‘gold standard’ method, exhaled NO concentrations from 18 asthmatic children (5-15 years old) were
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Figure 3.10: Dilution curve for the QCL-based sensor in the range of 1-1000 ppbv.

Figure 3.11: Recorded airway pressure and exhaled CO₂ profiles measured by the sampler during single exhalation (a). Real time measurements of NO and CO₂ profiles with the QCL-based sensor during a single exhalation (b); acquisition time 1 s.

measured using both devices (QCL-based sensor and chemiluminescence analyzer NOA 280, Sievers). The breath samples were collected in Mylar bags at flow rates of 15, 50, 100 and 300 ml/s. At the 15 ml/s flow rate every subject experienced difficulties providing a sufficient volume of breath to be measured by both devices (data is not shown). Fig. 3.12 shows Bland-Altman plot [57] between NO values measured at the flow rate of 100 ml/s by the QCL-based sensor and the chemiluminescence device. The average
difference between NO values measured by the QCL-based sensor and the chemiluminescence devices is 0.2 ppbv and levels of agreement are 4.6 ppbv and -4.2 ppbv.

![Bland-Altman plot](image)

**Figure 3.12**: Bland-Altman plot between NO values measured by the QCL-based sensor and the CLD at the flow rate of 100 ml/s for 18 asthmatic children. Solid line is an average value (0.2 ppbv), dashed lines – levels of agreement (4.6 ppbv and -4.2 ppbv). SD – standard deviation; acquisition time 1 s.

For the flow rates 50 and 300 ml/s the average difference between the NO values are 0.6 and -0.1 ppbv, respectively, upper levels of agreement are 3.1 and 2.8 ppbv, respectively, and lower levels of agreement are -1.9 and -3.0 ppbv, respectively. Based on this data it can be concluded that the two methods of detection are in good agreement over a range of flow rates.

### 3.5.3 Comparison online/offline

With the present QCL-based sensor both online and offline sampling can be performed. Online sampling is advantageous if real time measurements are required. On the other hand, offline sampling might be a solution when clinical studies with a large number of samples are to be performed. In order to compare these two sampling methods, 15 healthy volunteers gave online single exhalations at a flow rate of 50 ml/s. Immediately following, a single breath sample was collected in a Mylar bag from each volunteer (offline sampling).

The bags were measured within 24 h after collection. Our previous measurement have proved that Mylar bags are suitable for offline NO breath sampling. Fig. 3.13 shows typical dynamics of NO concentration inside a Mylar bag which was filled at t=0 time point and measured for the next 35 h by CLD.
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Figure 3.13: Stability of breath NO concentration in a Mylar bag. The bag was filled in at t=0 time point and measured for the next 35 h by CLD.

Figure 3.14: Bland-Altman plot for offline and online values of NO (a) and CO$_2$ concentrations (b) measured by the QCL-based sensor (n=15). Solid line is an average value (-0.2 ppbv and -0.01%, respectively), dashed lines – levels of agreement (3.1 ppbv/-3.5 ppbv and 0.16%/-0.14%, respectively). SD – standard deviation; acquisition time 1 s.

Fig. 3.14(a) shows the Bland-Altman plot between offline and online NO values. The average difference between the online and offline values is -0.2 ppbv and levels of...
agreement are 3.1 ppbv and -3.5 ppbv. Fig. 3.14(b) shows Bland-Altman plot between offline and online CO$_2$ concentrations measured by the QCL-based sensor and Loccioni sampler. The average difference between the online and offline values is -0.01% and levels of agreement are 0.16% and -0.14%. Plots show the sampling methods to be in good agreement.

3.5.4 Skin measurements

With the simple sampling method described above, the release of NO from skin tissue in vivo was measured. Five healthy volunteers (24-27 years old) were selected. For each volunteer, the NO emission was monitored at 6 positions on the body, including left and right leg (m. gastrocnemius), left and right arm (m. biceps brachii), back (m. trapezius), and chest (m. pectoralis major). Before and after each skin measurement, the concentration of NO in the carrier gas was monitored. The temperature of the skin was monitored continuously by a thermistor probe placed near the sampling cuvette. A typical profile of NO release from skin tissue is depicted in Fig. 3.15, where raw data is shown in black and 200 points smoothed data using the Savitzky-Golay algorithm [58] is shown in solid red.

![Figure 3.15](image.png)

**Figure 3.15:** A profile of NO concentration during a measurement from skin tissue in vivo (arm) with a flow of 1 l/h through the cuvette; in black – raw data, in solid red – 200 points smoothed data; acquisition time 1 s.

The NO concentration as well as the skin temperature was different depending on the
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Figure 3.16: Panel (a) represents individual measurements of the NO production rate and the skin temperature for the following positions on a body: 1, 2 – left and right leg, respectively, 3 – back, 4 – chest, and 5, 6 – left and right arm, respectively. Typical standard deviation of the measurement is shown for subject 1 and remains the same for the rest of the group. Panel (b) shows a correlation between mean NO production rate and mean skin temperature. Each point is an average value for the group (n=5).

sampling position. However, the NO production values for left and right arms as well as for left and right legs were not significantly different. This tendency was valid for all the tested subjects. Fig. 3.16(a) represents individual measurements of the NO production rate and the skin temperature monitored at different sampling positions. Fig. 3.16(b) shows a correlation between mean NO production rate and mean skin temperature. Each point on the graph represents an average value for 5 test subjects for a particular sampling position.
3.6 Discussion

The development of a QCL-based sensor has been reported, employing integrated cavity output spectroscopy as a detection method. The sensor proved to be suitable for simultaneous monitoring of multiple trace gas concentrations in exhaled air (NO and CO$_2$) as well as for non-invasive detection of biogenic NO originating from the skin tissue in vivo. A detection limit of 0.7 ppbv of NO is achieved for an acquisition time of 1 s. This value is comparable to those reported in literature [16, 21, 51] and is sufficient to perform online breath sampling with acceptable time resolution. The small volume of the absorption cell provides for fast refresh times (approximately 1 sec) allowing real time measurements. It has been shown that values measured with the QCL-based sensor are in good agreement with those obtained with a commercially available NO analyzer (offering sensitivity <1 ppbv in 1 s acquisition time) for a wide range of exhalation flow rates, validating the QCL set-up for accurate measurements. Comparison of CO$_2$ concentrations is also performed with a commercial CO$_2$ sampler. The Bland-Altman plots show that the QCL-based sensor is reliable for medical applications. The QCL-based sensor was successfully tested on healthy subjects as well as on asthmatic children.

Extended NO analysis requires sampling at various exhalation flow rates [32, 59]. In this case, additional estimated parameters can provide valuable clinical information for further NO modeling. The QCL-based sensor, in combination with a breath sampler, allows exNO measurements at various flow rates without altering the sensor. Simple design allows coupling of the sensor to a custom-built hand held device and sampling NO at various exhalation flows for both online and offline sampling procedures. The importance of multiple flow rate sampling has been demonstrated not only for asthma, but also more recently for chronic obstructive pulmonary disease (COPD) [60]. Finally, the QCL-based sensor was successfully tested for online monitoring of NO release from the skin tissue in vivo. However, NO levels were found to differ depending on the position of sampling (except for left and right limbs, where no significant difference was observed). Up to now, these differences are not completely understood. Previous studies showed that endothelial NO has been found in dermal endothelial cells [61], in the cytosol of basal keratinocytes [62], and that calcium dependant NO was released from ultraviolet (UV) stimulated keratinocytes [63]. It has also been demonstrated that reduction of sweat nitrate generates NO on the skin surface [52]. In this case, production of NO from the skin surface was dependant on the nitrate concentration and was higher at low skin pH. Another study revealed that NO was involved in regulating the blood flow in the skin, which was lower after the intradermal injection of the inhibitor of nitric oxide synthase into forearm skin [64]. This might help explain the correlation between the NO production and the skin temperature observed in our study.

In conclusion, the results show that the QCL-based sensor employing integrated cavity output spectroscopy is a robust technique, suitable for fast and sensitive detection of
NO traces. This concept can be easily extended to the detection of other gases and can be applied for all sorts of investigations that involve biomedical background.

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References


References


Acetone measurements using a custom-made continuous wave EC-QCL in combination with a multipass cell

Abstract
We report on the development of a custom-made continuous wave external cavity quantum cascade laser (EC-QCL) in Littrow configuration suitable for the detection of broadband absorption features. The laser has an overall tuning range of 230 cm\(^{-1}\) and operates between 1150 and 1380 cm\(^{-1}\) (7.25-8.69 \(\mu\)m) producing \(\sim\)1 mW of output power at 243 K. In combination with a multipass cell offering 76 m path length, the EC-QCL allows fast and robust detection of volatile organic compounds such as acetone in breath. An absorption profile of acetone is recorded within 3 s acquisition time covering a wavelength region of \(\sim\)65 cm\(^{-1}\). Noise Equivalent Absorption Sensitivity (NEAS) for the EC-QCL-based spectrometer is estimated at \(8.6\cdot10^{-8}\) cm\(^{-1}\).Hz\(^{-1/2}\). Online measurements of acetone in exhaled breath are demonstrated.

This chapter is based on:
Chapter 4: Acetone measurements using a custom-made continuous wave EC-QCL in combination with a multipass cell

4.1 Introduction

Continuous wave Quantum Cascade Lasers (cw-QCLs) are infrared sources that became popular for a large number of applications in military and security purposes, medical research, environmental studies, industrial processes, analytical chemistry, and metrology [1–5]. Nowadays, operating at ambient temperatures with high output powers and excellent spectral quality, thermoelectrically cooled cw-QCLs have created a range of novel mid-IR gas sensors offering high sensitivity, selectivity and fast-response time measurements. Moreover, they triggered new applications for gas sensing due to their compact size, robust construction and low power requirements.

For spectroscopic applications single mode operation of the laser source is required. Generally, this is achieved by implementing a Distributed FeedBack (DFB) structure on the laser chip. However, DFB QCLs are typically designed for operation at a single target frequency and can be typically current tuned only over a narrow spectral range (0.1-2 cm$^{-1}$) that can be extended by temperature tuning of the laser up to 15-20 cm$^{-1}$ [6]. Sometimes, the tuning range at a specific temperature is only sufficient to resolve a targeted ro-vibrational absorption line of a single molecule. Therefore, for each specific wavelength another DFB-QCL has to be designed and manufactured. This restricts the range of applications and is not cost efficient. Besides, technical characteristics of the DFB QCLs make detection of the molecules with broadband absorption features rather challenging.

It is known that some volatile organic compounds (VOCs) in exhaled breath represent products of metabolism taking place in the human body. Some common VOCs in the exhaled breath of a healthy human are acetone, ammonia, acetaldehyde, isoprene, ethanol, methanol, and other alcohols [7–10]. Previously, it has been shown that elevated levels of some of these VOCs can be considered as indicators of a disease condition. For instance, elevated levels of breath isoprene or ammonia can indicate renal impairment [11–13], meanwhile acetone is related to diabetes or fat burning process [9, 14, 15] and ethanol to serum glucose levels [16]. In diabetic patients (type 1 diabetes) the level of acetone in exhaled breath can increase up to 12 ppmv [17]. Monitoring of exhaled breath acetone may be very useful in actuate diabetic patients as well as in early stage disease prevention, since with breath analysis technique this procedure is fast, reliable and non-invasive [18].

For the detection of molecules with broadband absorption spectra more advanced laser sources are required such as QC lasers operating in an external cavity. EC-QCLs have already proven to be suitable to these purposes [19–21] and have been proposed since many years to improve the scanning range of conventional infrared semiconductor lasers [19, 22–27]. The typical design consists of a laser offering a broadband emission gain with a grating to select one particular wavelength enabling single mode operational regime. The tuning is therefore achieved by rotating the grating. Two configurations are
the most common ways: the Littrow [28–30] and Littman-Metcalf configuration [31, 32]. Both cavity configurations have their own advantages and drawbacks, meanwhile Littrow cavities offer higher power and a simpler design [33]. In this paper, we report on the development of a custom-built, single-frequency, widely-tunable continuous wave external cavity QCL (EC-QCL) in a Littrow configuration. We demonstrate spectroscopic detection of acetone by combining EC-QCL with a multipass cell to increase the absorption path length. Several key issues have been implemented to improve the EC architecture and achieve a wide mode-hop-free tuning range (230 cm$^{-1}$ within the 7.25-8.69 $\mu$m spectral region). Possibilities for the online low-level breath acetone are also demonstrated.

4.2 Experimental setup

The external cavity arrangement employing a broadband thermoelectrically cooled QC gain chip in a Littrow configuration is shown in Fig. 4.1. It consists of: a QC gain chip (Alpes Lasers, Switzerland, AR coated at 8 $\mu$m on one facet and HR coated on the other facet), a collimating aspheric lens (AR coated for 7-14 $\mu$m with 4 mm focal length, Thorlabs, Germany), an aluminum diffraction grating (150 lines/mm, 93% reflectivity, blazed at 10.6 $\mu$m, Optometrics, model: ML-303, USA), and a gold mirror.

![Figure 4.1](image.png)

**Figure 4.1:** Panel (a) represents a schematic setup for the Littrow arrangement; panel (b) is the computer model of the EC-QCL housing. QC chip is placed in the copper block together with a collimating lens and cooled down to 243 K (-30°C). The mirror is mounted on the rotational platform together with the diffraction grating at the 90° angle to keep the direction of the laser beam. The external cavity length and the rotational angle of the grating are controlled by two step-motors, fine tuning is made by two piezo actuators. The housing is continuously flushed with a low flow of nitrogen to avoid water condensation.
The QCL chip has a gain profile centered at 7.9 µm (1,150-1,380 cm⁻¹). To operate at low temperatures (-30 °C), the gain chip is placed in a copper block on top of a Peltier element used to maintain constant low temperature of the copper. A collimating lens is placed in front of the laser head with a 3-axis manually adjusted stage. The 1ˢᵗ order diffraction of the collimated QCL beam is coupled back from the grating, providing optical feedback for lasing, while the 0ʰ order diffraction generates an optical output beam. The grating and the reflection mirror (placed at 90⁰ angle) are mounted on the same rotational platform. This arrangement allows keeping the optical output beam in fixed alignment (concerning the displacement variation of the angle from the mirror), while wavelength tuning is performed. The rotating platform (grating and mirror) is controlled by a linear translation stage (Physik Instrumente, model: M-014.00, Germany) for slow and wide wavelength tuning and a piezo actuator (Physik Instrumente: P-840.60, Germany) for fast scanning. In the same way, the cavity length can be adjusted with a piezo actuator and a linear translation stage. In this configuration, two independent parameters can be tuned: the external cavity length and the angle of the grating with respect to the incident beam from the EC-QCL. To ensure long-term temperature stability, the overall system is maintained at constant temperature by means of a second stage thermo-electrical cooling system consisting of 4 Peltier elements. All components are placed into a 30(L)x20(W)x15(H) cm housing box and continuously flushed with a low flow of nitrogen to avoid water condensation on the cooled parts including the laser chip. In this configuration, 1 mW maximum output power at -30°C is generated.

**Figure 4.2:** Schematic representation of the multipass-cell-based employing EC-QCL. The laser beam is sent to the astigmatic multipass cell with an effective absorption path length of 76 m and focused on a LN₂ HgCdTe infrared detector. The output signal is 40dB amplified and analysed on the PC with a LabVIEW program.

The light from the EC-QCL is sent to the astigmatic multi-pass cell (AMAC-76, Aerodyne, USA) with a path length of 76 m, 0.5 liter volume, filled with a gas mixture
at 1 bar pressure. To reduce the response time of the system, the flow rate through the
gas cell is set to 150 ml/s, producing a ventilation time of approximately 3 s for the cell.
Schematic representation of the setup is shown in Fig. 4.2. The output beam is focused
by means of a 25 mm BaF$_2$ lens (5 cm focus distance) on a LN$_2$-cooled HgCdTe infrared
detector ($D = 7 \times 10^{10}$ cm·Hz$^{1/2}$/W, bandwidth 30 MHz, Kolmar Technologies, USA). In
addition, the signal is amplified by 40 dB (Femto DLPVA-100-BLN-S, Germany) and
processed via a data acquisition card (GaGe Octopus-8325, 14 bit, USA) for further
computer analysis. The QCL-based system is calibrated with a reference mixture of
1 ppmv of acetone in nitrogen (VSL National Dutch Metrology Institute). A N$_2$ gas
cylinder is used to provide a gas reference free of acetone.

4.3 Results

Prior to the spectroscopic measurements the laser performance was determined. Fig. 4.4
shows the optical output power (depicted with black squares) of the laser chip at 800 mA
by angle-tuning of the grating over the full displacement range of the step-motor. The
single mode performance and wavelength is measured by a FTIR spectrometer (Bruker,
Germany). Our custom built EC-QCL has a maximum tuning range of 230 cm$^{-1}$ pro-
ducing the maximum output power of 1 mW at 243 K (-30$^\circ$C) with the lasing threshold
of 680 mA measured in the middle of the gain curve.

![Normalized FTIR spectrum of the QCL gain profile measured along the full displacement range of the external cavity step-motor and the corresponding optical output power of the EC-QCL (black squares) at 243 K and 800 mA current. The total scanning range is 230 cm$^{-1}$ (1150-1380 cm$^{-1}$), maximum power output – 1 mW.](image)

**Figure 4.3:** Normalized FTIR spectrum of the QCL gain profile measured along the full displacement range of the external cavity step-motor and the corresponding optical output power of the EC-QCL (black squares) at 243 K and 800 mA current. The total scanning range is 230 cm$^{-1}$ (1150-1380 cm$^{-1}$), maximum power output – 1 mW.
In the EC-configuration, the main challenge is to avoid frequency mode-hops during wavelength tuning. To achieve mode-hops free tuning, two parameters of the EC-QCL can be adjusted while tuning is performed: the laser current and the laser cavity length. As discussed before, the design of our systems provides independent control of the EC length, diffraction grating angle and laser current. At the same time that the grating angle is changed to tune the laser wavelength, the cavity length and current of the QCL are adjusted with computer control. Therefore, a mode tracking has been developed as proposed by Wysocki et al. [19] that allows mode-hop-free frequency tuning along the full tuning range of the EC-QCL. Here, such parameters of the EC-QCL as the laser current and the laser cavity length can be adjusted while the wavelength tuning is performed. The design of the EC-QCL-based spectrometer provides independent control of the EC length, diffraction grating angle and laser injection current. At the same time as the grating angle is changed to tune the laser wavelength, the cavity length and current of the QCL are adjusted with computer control to avoid hopping of the lasing laser mode.

In order to check the mode-hop-free capability of the system, the EC-QCL beam is sent though a germanium etalon (Free Spectral Range 0.049 cm$^{-1}$) as shown in Fig. 4.5. By just rotating the angle of the grating, the transmitted signal is completely disturbed (red line) because of the mode-hopping. By adjusting the cavity length and the laser current, the detected signal shows a smooth sine wave (black line), as expected whilst scanning the wavelength along the range of 1 cm$^{-1}$. This simple experiment with a short wavelength tuning allows finding the good parameters for the control system, providing mode-hops-free operation on the full tuning capability.
Figure 4.5: Mode-hop-free operation of the EC-QCL; in red – wavelength scan by only rotating the grating, in black – tuning of the wavelength by controlling the laser current, the cavity length at the same time while rotating the grating.

Figure 4.6: Calculated spectra for the compounds present in the scanning range of the EC-QCL (7.2-8.7 µm spectral region) under the following conditions: concentration – 1 ppmv, pressure – 1 bar, absorption path length – 1 m, and temperature – 298 K (25°C).
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Figure 4.7: Simulated absorption profiles of 1 ppmv acetone (in red) and 3% CO$_2$ (in grey) for the 76 m path length and 1 bar pressure and recorded over the width of $\sim$65 cm$^{-1}$ profile of 1 ppmv of acetone under the same conditions (in circles); resolution of the measurement – 1 cm$^{-1}$.

The EC-QCL operates in the middle of the so-called “fingerprint region” where a broad range of compounds can be identified. Figure 4.6 represents simulated spectra calculated from PNNL data base [34] for ethanol (red), isoprene (black), acetaldehyde (blue), ammonia (green), and methanol (magenta) for 1 ppmv mixture in nitrogen, using a path length 1 meter and a pressure of 1 bar.

For bio-medical applications acetone is important since it is seen in literature as a biomarker for diabetes and fat burning process. Monitoring breath acetone can be helpful to follow patients with a prescribed diet as well as to monitor diabetic patients [35]. Figure 6 shows simulated absorption profiles of 1 ppmv acetone (in red) and 3% CO$_2$ (in grey) for the 76 m path length and 1 bar pressure and recorded with EC-QCL-based spectrometer (in circles) over the width of $\sim$65 cm$^{-1}$ profile of 1 ppmv of acetone under the same conditions using a multipass cell. The acetone spectrum was acquired in 3 sec with a spectral resolution of 1 cm$^{-1}$ by rotating the diffraction grating by means of the step-motor at its maximum displacement velocity. The spectrum is obtained as a result of the subtraction of the acquired from the calibration mixture signal from pure nitrogen signal free of any absorbing compounds. The main factor limiting the recording time was the speed of the step-motor controlling the diffraction grating. The velocity of the displacement was limited to 0.75 mm/s. In order to estimate the noise level of the
4.3. Results

EC-QCL-based spectrometer, the total surface under the absorption curve of 1 ppmv of acetone was calculated per each scan. The calculated NEAS corresponds to $8.6 \times 10^{-8}$ cm$^{-1}$·Hz$^{-1/2}$.

![Figure 4.8: Calibration curve obtained for the EC-QCL-based spectrometer. Various prepared concentrations (0.1-1 ppmv) of acetone are applied and measured by the EC-QCL-based sensor in order to estimate how linear the response of the device is.](image)

Furthermore, the linearity of the EC-QCL-based system for different acetone concentrations was tested. Various concentrations (in the range 0.1 - 1 ppmv) of acetone were prepared by mixing the reference mixture of 1 ppmv with nitrogen by means of 2 mass-flow controllers. The laser wavelength was fixed at the position of the maximum intensity of the acetone absorption signal at $\sim 1218$ cm$^{-1}$, where the concentration of acetone was measured. The resulting graph is shown in Fig. 4.8. The applied and the measured concentrations are in a good agreement suggesting linear behavior of the device response. The mean value of the residuals is 4.3 ppbv with a standard deviation (SD) of 5.4 ppbv.

EC-QCL-based spectrometer allows for breath acetone measurements. For that, the grating of the laser was tuned over the wavelength region 1219-1224 cm$^{-1}$, which has low interference with water and CO$_2$ lines. Online exhalation patterns of acetone from human breath are presented in Fig. 4.9, where panel (a) shows two profiles of exhaled...
Figure 4.9: Panel (a) represents typical profiles of breath acetone measured by the EC-QCL-based spectrometer during single exhalation. The graphs represent the acetone concentrations of a volunteer measured after 12 hours fasting period (higher values) and those 1.5 h after meal (lower values); acquisition time 3 s. Panel (b) shows corresponding exhaled CO$_2$ profile (in red) and airway mouth pressure (in black).

acetone measured with an exhalation flow rate of 50 ml/s in a single exhalation. The graphs represent the acetone concentrations in a breath of a volunteer after a 12-hours fasting period (higher values) and 1.5 hours after a meal (lower values). The acquisition time per data point is 3 sec. Since there is no clear evidence in literature that breath acetone concentration is flow dependent [36, 37], a standard flow rate of 50 ml/s was used. Panel (b) depicts the corresponding exhaled CO$_2$ profile (in red) and airway mouth pressure (in black) measured by a commercially available breath sampler (Loccioni, Italy). The subject was asked to maintain a constant exhalation flow rate. The CO$_2$ concentration and the airway pressure profiles were displayed in a graphical form on the screen of the sampler reducing possible errors while the exhalation maneuver is
performed. Parallel measurements of CO\(_2\) dynamics in breath provide potential benefits, allowing to calculate the physiological dead space lung volume [38, 39].

### 4.4 Discussion

The performance of a custom-built EC-QC laser in combination with a multipass absorption cell is reported. The system allows for a broadband detection of absorption features in the mid-IR region (7.25-8.69 \(\mu\)m) and has proved to be suitable for the detection of molecules with broadband absorption features. Here, 1 ppmv acetone spectrum has been recorded in 3 sec over the range of 65 cm\(^{-1}\) with the resolution of 1 cm\(^{-1}\). Noise Equivalent Absorption Sensitivity was estimated at 8.6 \(\times\) 10\(^{-8}\) cm\(^{-1}\)·Hz\(^{-1/2}\). Linearity of the sensor has also been tested in the range of 0.1 – 1 ppmv. Furthermore, the possibility of online breath acetone measurements is demonstrated. For that, the laser was tuned over the wavelength 1219-1224 cm\(^{-1}\), representing a favorable region for breath acetone detection due to its low interference with water and CO\(_2\). It is well known that breath acetone concentrations may vary depending on the physiological condition of the body. That is why mean breath acetone concentration after fastening period of 12 h is significantly higher compared to the breath acetone concentration measured 1.5 h after a meal (430 and 140 ppbv, respectively). The current performance of the system has potential in clinical purposes, especially in breath analysis of diabetic patients where acetone levels can reach values up to 12 ppmv.

To perform the absorption measurements a pressure of 1 bar was used inside the cell. By reducing the pressure to lower values (100-200 mbar) the influence of the laser frequency mode-hopping can become significant. Further improvements to achieve fully automated mode-hop free running operational mode will be addressed in the next phase of the instrument development. In conclusion, the results clearly show that the EC-QCL-based system combined with a multipass cell is a robust detection scheme, suitable for fast and sensitive detection of broadband trace gas species, such as acetone in breath. The concept presented in this paper can be extended to the detection of other species of interest and can be applied for the trace gas analysis of any other medically relevant molecules.

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References

References

Chapter 5

Exploratory study on quantum cascade lasers operating in an external ring cavity resonator

Abstract
We describe a mid-infrared External Ring Cavity Quantum Cascade Laser (ERC-QCL). The Quantum Cascade gain chips are placed inside a compact X-shape resonator with an overall length of 45 cm. The material is designed to have a maximum gain at 7.72 µm (1295 cm⁻¹) at room temperature. Fabry-Perot QC (FP-QC) gain chips have end-facet angles of 0-, 3- and 7- degrees and they operate in a pulse mode. Characterization of the ERC-QCL is performed and includes the influence on the gain of the different active region designs processed either by deep dry or shallow wet etching and the effect of Anti-Reflection (AR) coated versus uncoated FP-QC gain chips on the gain within the ERC cavity are discussed. To make the ERC-QCL suitable for active mode-locking in the mid-infrared wavelength region, its potential to suppress Spatial Hole Burning (SHB) is investigated.
Chapter 5: Exploratory study on quantum cascade lasers operating in an external ring cavity resonator

5.1 Introduction

The mid-infrared wavelength range is of key technological importance for a wide range of spectroscopic applications. Many important molecular gases, such as hydrocarbons, nitrogen oxides, carbon dioxide, etc. have their strongest rotational-vibrational absorption features in the wavelength region between 3 and 25 $\mu$m. Due to this strong absorption, detection of molecular trace gases below part-per-billion and to low part-per-trillion can be achieved [1–4]. Such high sensitivities can lead to many potential applications in areas such as clinical diagnostics (breath analysis), process monitoring, atmospheric studies, plant physiology, metrology and analytical chemistry [5–9].

The first operation of the Quantum Cascade Laser (QCL) has been reported in 1994 [10] and rapidly the laser became one of the most commonly used coherent light sources in the mid-infrared region offering narrow line width, robustness, and high power at room temperatures [5, 11]. Thanks to its level of performance, the QC laser is widely used as laser source in studies for various gas sensing applications. However, to achieve single mode laser operation at a specific wavelength in order to target a molecular absorption transition of the gas under investigation is not straightforward. The use of Distributed FeedBack (DFB) gratings that are incorporated near top waveguide cladding of the QC ridge leads to a single frequency operation but as a result the wavelength tuning via the laser current is severely restricted. For state-of-art DFB-QCLs, typical current and temperature tuning coefficients are -0.01 cm$^{-1}$/mA and -0.16 cm$^{-1}$/K, respectively. This results in a total spectral range of 4 to 5 cm$^{-1}$ and 15 to 20 cm$^{-1}$ for maximum current and temperature tuning ranges, respectively [12]. QC lasers working in an external cavity configuration, with a grating serving as one of the resonator mirrors, have much wider wavelength tuning characteristics (over 200 cm$^{-1}$) but may suffer from mode hopping over the external cavity modes [13].

Inside a linear laser cavity a spatially inhomogeneous gain distribution can occur when a standing wave pattern is formed by two counter-propagating waves. This effect, known as Spatial Hole Burning (SHB), plays a central role in achieving single mode operation in lasers. SHB allows other laser cavity modes – with slightly different frequencies and therefore slightly different positions of nodes and antinodes in the standing wave pattern inside a laser cavity. These cavity modes fill in the regions of unsaturated population inversion and can, therefore, reach threshold for lasing. Under these conditions more than one laser cavity mode can oscillate, leading to multimode laser operation. A schematic representation of Spatial Hole Burning is depicted in Fig. 5.1.

In a travelling wave resonator such as ring cavity with two waves propagating in clockwise (CW) and counterclockwise (CCW) directions, the SHB effect can be suppressed in two situations [14]: only one mode (CW or CCW) is lasing; or both modes are lasing simultaneously, but there is no mechanism locking their relative phase. In practice, a unidirectional operational regime of the resonator is required to effectively
5.2 Experimental details

5.2.1 Quantum cascade laser processing

The QC devices studied in this work are grown by Metalorganic Vapor Phase Epitaxy (MOVPE, also called Metalorganic Chemical Vapor Deposition, MOCVD). They were manufactured at Sheffield University (UK) by Cockburn and co-workers. Details of the manufacturing process can be found elsewhere [15]. Here, a brief overview of the process will be given.
MOVPE technique is widely used in optoelectronics industry and has a number of important advantages over Molecular Beam Epitaxy (MBE). For example, the growth rate provided by MOVPE can be up to 10 times higher compared to MBE. Besides, MOVPE allows for multi wafer growth. Reactors employing MOVPE can typically reach higher wafer output at a lower cost per wafer.

Once epitaxial growth is complete, the QCL samples were processed into ridge waveguides using a dry-etched technique. Key fabrication steps are presented in Fig. 5.2.

**Figure 5.2:** Key steps of the QCL waveguide ridge fabrication: a) A hard mask is achieved by depositing of 800 nm of SiO$_2$ by Plasma Enhanced Chemical Vapor Deposition (PECVD). After this, the pattern was transferred into the SiO$_2$ layer using Reactive Ion Etching (RIE) with a SF$_6$/Ar plasma; b) The laser samples are dry-etched using an Inductively Coupled Plasma (ICP) RIE process with SiCl$_4$/Ar flow; c) The SiO$_2$ hard mask is removed using buffered HF solution; d) The waveguide ridges were covered with 300 nm SiO$_2$ by PECVD; e) The contact window was etched by RIE under a CHF$_3$/O$_2$ atmosphere using a pattern formed in photoresist using standard photolithography. f) A top contact consisting of Ti/Au (20/200 nm) was thermally evaporated and patterned using a photolithographic lift-off procedure. g) $\sim$1 $\mu$m of gold was electroplated on top of the contact to ensure electrical connection to the top of the ridge. h) The 350 $\mu$m thick substrate was thinned down to $\sim$200 $\mu$m by mechanical polishing to improve cleaving. i) A Ti/Au (20/200 nm) contact is again thermally evaporated on the bottom side.

Dry etching ensures reproducibility of the ridges and provides more accurate etch
5.2. Experimental details

depth processing as well as more vertical ridge profiles. However, the vertical wall of the ridge allows other lateral modes within the laser cavity may reach threshold for lasing leading to multimode operation regime of the FP-QCL. Taking this into account, it has been decided to process the ridges by wet chemical etching in later experiments to avoid the vertical side walls formation and suppressing higher order modes inside the resonator.

5.2.2 QC devices and experimental setup description

The QC devices described in this work use a gain medium centred at 7.72 µm (1295 cm⁻¹) at the room temperature. The laser ridges were processed by wet etching. The chips have 2 lasers each and are soldered with indium onto copper submount that has a size of 3x19x2 mm. Fig. 5.3(a) and Fig. 5.3(b) show SEM pictures of the two fabricated QC devices at Sheffield University with facets angles at 0- and 3- degrees, respectively.

Figure 5.3: SEM pictures of the fabricated QCL devices with 0- and 3-degrees facets and deeply etched ridges (panels (a) and (b), respectively).

During the process two angles, 3- and 7- degrees are used between the laser ridge direction and the intended as-cleaved facets. Initially, the lasers were used as Fabry-Perot laser devices and later both facets were anti-reflection (AR) coated (AR coating <2%) at Helia Photonics (UK). The chips were then placed inside of the ring cavity forming an External Ring Cavity Quantum Cascade Laser (Fig. 5.4) and driven by a commercially available pulse generator Avtech (AVOZ-DF1-B, Avtech, USA). Two types of lenses were used to collimate the radiation from both end facets. In the first experiments, plano-convex ZnSe lenses (diameter 0.5”) with numerical aperture (NA) of 0.5 and focal length (FL) of 8 mm were used. The AR coating of the lenses was optimized for 7.9 µm. In later experiments we used 0.22” Geltech aspheric lenses with NA – 0.85, FL – 1.873 mm that were AR-coated in the range of 8-12 µm.

The lenses were positioned in front of the laser facets by means of two translation stages allowing adjustments in x-y-z axes. The ERC-setup has X-shape configuration
Chapter 5: Exploratory study on quantum cascade lasers operating in an external ring cavity resonator

Figure 5.4: Schematic representation of the External Ring Cavity QCL (ERC-QCL) configuration. The radiation emitted from left (right) facet of the QC device is collimated by the aspheric lens $L_1$ ($L_2$), followed by 4 plane mirrors (M) and then eventually is re-injected in right (left) facet by the aspheric lens $L_2$ ($L_1$). The total length of the cavity is 45 cm. The out-coupling system is represented by CaF$_2$ beam splitter (BS).

provided by 4 flat mirrors (diameter 0.5") with a nominal reflectivity of R=99.9% at 8 $\mu$m. The total length of the ERC is 45 cm which corresponds to the Free Spectral Range (FRS) of 667 MHz and a roundtrip time of 1.5 ns. The 0.5 mm thick CaF$_2$ beam splitter with a nominal transmission of 0.95 at 8 $\mu$m was used as an output-coupler of the radiation out of the resonator. The light intensity was analyzed either by a standalone detector (Hamamatsu, Japan) or directed to an FTIR spectrometer.

5.3 Results and discussion

In the initial experiments lenses with a NA of 0.5 and a FL of 8 mm were used. In the case when the end facets of the gain chips were uncoated, the reduction in current threshold (indicator for the losses in the External Ring Cavity) was about 5% as compared to the same QC devices operating outside the ERC and lasing in its own FP cavity. Surprisingly, this reduction in current threshold remained almost the same for all the devices tested (with 0-, 3- and 7- degrees end facets angle). In Fig. 5.5(a) typical light-intensity curves are shown for 3- degrees angled facet laser. Beam shape measurements were performed by placing a blade on a XY-translation stage at 10 cm distance from the ERC. The corresponding graphs, shown in Fig. 5.5(b), revealed that the beam is not symmetrical being 2.5 mm in the horizontal direction and 4.6 mm in the vertical direction.

Such a low reduction in the current threshold may be explained by several factors:
5.3. Results and discussion

Figure 5.5: Panel (a): typical light-intensity curves for uncoated 3-degrees angled FP-QC device processed by deep dry etching (in black) and when inserted into an external ring cavity resonator (ERC, in red). Reduction in the current threshold observed was about 5%; panel (b): beam shape measurements.

on one hand the reflectivity of uncoated QCL facets is quite high (estimated value is RFP=30%), on the other hand – the coupling efficiency back into the QC device is low due to the low NA of the ZnSe lenses. This suggests that the waveguide coupling losses are much higher compared to the change introduced by the AR coating or tilting of the facets.

After the deposition of the AR coating on the facets, the FP-QC devices still remained lasing. However, the threshold was about 25% higher on average as compared to the uncoated chips (2.2 and 2.9 A, respectively). This might suggest that the deeply etched ridges with vertical side walls enable lasing at the higher order lateral modes in-
side the laser cavity since they might experience feedback from the walls of the ridge. Besides, the quality of the AR coating deposited on the QCL’s facets might also be compromised, particularly on the facet edges. Visual tests of the AR coating were performed in the first place by studying SEM images of the ridges (Fig. 5.6).

**Figure 5.6:** SEM images taken from left and right facets of a 0- and 3- degrees angled QC devices after deposition of the AR coating (panel (a) and (b), respectively).

Despite the fact that no obvious gross error was observed, further investigation on the quality of the AR coating was carried out. For that, the mid-infrared broadband light from a FTIR spectrometer was focused on the substrate below the laser ridge and away from the laser core region (to avoid the influence of the intersubband properties of the QCL). The measured reflection spectrum for the uncoated laser chip under the same geometrical conditions was used as a reference. The measurements were performed at the room temperature. The resulting reflectivity curve is depicted in Fig. 5.7 and clearly suggests that the reflectivity of the AR coating around 7.7-8 \( \mu \text{m} \) is good for our experimental purposes.

In the second experiment, QC designs were used with wet-shallow etched ridges, in order to suppress higher order modes inside the laser cavity and to ensure single mode operation regime. Moreover, to increase coupling efficiency into the resonator, aspheric lenses with a NA – 0.85 and FL – 1.873 mm were used. The positive effect of the new ridge design was directly observed in a strong current threshold decrease of 16% for the uncoated FP-QC devices. Deposition of the AR coating resulted in the absence of lasing (up to the maximum current of the power supply) for a FP-QC device while in ERC-QCL configuration laser action was observed.
5.3. Results and discussion

Figure 5.7: Reflectivity of an AR coated chip facet referenced to the reflectivity of an uncoated chip facet at T=300K.

Figure 5.8 shows typical examples of light-intensity curves for a shallow etched FP-QC device with 0-, 3- and 7-degrees end facets and a ERC-QCL (panel a). Panel b shows the effect of AR coating on the light-intensity curves of FP-QC and ERC-QC devices. All AR coated FP-QC devices exhibit similar behavior as the 3-degree device and do not lase till the current limit of the power supply (5 A). The current threshold reduction in the AR coated ERC-QCL as compared to the uncoated FP-QCL running outside the ring cavity was \( \sim 25\% \) for all the devices, which is in agreement with the results recently published by Malara et al. [14]. Malara et al. used QC gain medium centered at 7.9 \( \mu m \) (1250 cm\(^{-1}\)) [16] inserted in an ERC with a total length of 1.7 m and a roundtrip time of 5.7 ns yielding a FSR of 176 MHz.

In conclusion, we have demonstrated the mid-infrared External Ring Cavity Quantum Cascade Laser. Preliminary results revealed that our FP-QCL is capable of being exploited in the ERC, however, neither the tilted facets nor AR coating on the ridge facets did not provide good lasing suppression for the dry deeply etched ridges. The suitable design of the QCL device based on wet etching was developed and investigated. Further steps of the development will include integration of the coarse and fine wavelength tuning mechanisms as well as spectroscopic trials at later stage of the project.

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Figure 5.8: Panel (a): light-intensity curves for 0-, 3- and 7- degrees angled facets uncoated shallow etched devices running in FP and in ERC configurations; panel (b): the same dependencies after AR coating deposition.

References


References


Chapter 6

A compact laser-based spectrometer for detection of C$_2$H$_2$ in exhaled breath and HCN in vitro

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This chapter is based on:
Abstract
We report on the development of a compact prototype near-infrared DBR laser-based spectrometer employing Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS). The spectrometer is capable of simultaneous detection of acetylene (C$_2$H$_2$) and CO$_2$ at 1529.2 nm as well as hydrogen cyanide (HCN) at 1533.5 nm. The detection limits of 8 ppbv for C$_2$H$_2$ and 80 ppbv for HCN are achieved for the acquisition time of 1 sec. The setup has been tested for online measurements of C$_2$H$_2$ in exhaled breath of a smoking subject while HCN has been measured resulting from the metabolism of Pseudomonas aeruginosa (PsA) bacteria in vitro. Further improvements of the performance of the spectrometer are discussed.
6.1 Introduction

Nowadays, gas phase molecular spectroscopy is a well-established research field and is of great interest for applications in various areas including environmental studies [1–4], atmospheric chemistry [1] and laser physics [5–7]. Besides, there is an increasing need in sensitive, robust and fast detection systems to be exploited in biomedical applications [8, 9] and breath analysis in particular [6, 10–12]. Useful information can be extracted while studying gases in exhaled breath. They are often treated as reliable indicators of particular physiological processes or even certain metabolic disorders [13–16].

Acetylene (C$_2$H$_2$) is one of the most important hydrocarbons used in industrial technological processes and therefore it originated predominantly from anthropogenic activity including automotive industry and biomass burning [17]. It is usually used to estimate air quality [17, 18]. Several attempts have been made so far to perform sensitive detection of acetylene utilizing cavity-enhanced absorption techniques [19–21]. In some studies, pre-concentration of the sample was used, allowing low detection values down to 35 pptv which is sufficient for direct atmospheric detection at concentrations typical of both urban and rural environments [20]. However, this lead to an increase of the acquisition time up to 30 minutes. Recent study carried out by Schmidt et al. reports on the development of the diode laser-based continuous wave cavity ring-down spectroscopy (cw-CRDS) able to detect 0.34 ppbv for 70 s without sample pre-concentration [22].

Only since recently acetylene has been quantified in breath after test-persons had been exposed to tobacco smoke [23]. This study reports acetylene values up to 260 ppbv measured in breath directly after smoking with fast washout down to ambient levels within 3 h. Therefore, acetylene cannot be used as a biomarker for smoking status like 2,5-dimethylfuran, which is present in breath for more than 24 h after smoking [24].

Another interesting molecule that might serve as a potential indicator of physiological condition in humans is hydrogen cyanide (HCN). It results from the metabolism of Pseudomonas aeruginosa (PsA), one the most relevant pathogens for patients with cystic fibrosis (CF) [25]. Several studies report on the detection of HCN emitted by in vitro cultures of PsA using selected ion flow tube mass spectrometry (SIFT-MS) in exhaled breath [26–28]. However, only few publications report on detection of HCN by laser-based absorption techniques (i.e. Cavity Ring-Down and Photoacoustic Spectroscopy, respectively) [23, 29–31].

In this work, we report on the development of a compact prototype near-infrared laser-based spectrometer for fast and sensitive multi-compound detection of gases in exhaled breath and in vitro including acetylene, hydrogen cyanide and carbon dioxide. The spectrometer employs Integrated Cavity Output Spectroscopy [32, 33] in Off-Axis configuration (OA-ICOS) as the detection method. Possible improvements of the performance of the spectrometer are discussed.
Chapter 6: A compact laser-based spectrometer for detection of \( \text{C}_2\text{H}_2 \) in exhaled breath and HCN in vitro

6.2 Experimental details

6.2.1 Spectrometer design

A schematic picture, representing major parts of the laser-based sensor, is shown in Fig. 6.1 (a). A near infrared Distributed Bragg Reflector (DBR) laser was provided by VTEC Lasers & Sensors. The laser is fiber coupled and is based on the Oclaro LambdaFLEX\textsuperscript{TM} iTLA. The laser is a high performance Continuous Wave (CW) tunable laser source operating in the C-band window covering 1527-1564 nm (6394-6548 cm\(^{-1}\)) wavelength region split into 89 integrated channels. The laser is provided in a 26-pin butterfly package and connected to a Polarization Maintaining Fiber (PMF) for connection to the absorption cell. The temperature of the laser is set to 25\(^{0}\)C and controlled by an integrated Peltier module. In this configuration the laser generates an average output power of 20mW (13dBm). To scan the acetylene transition at 1529.2 nm (6539.46 cm\(^{-1}\)), the laser is set to channel 83 and for HCN transition at 1533.5 nm (6521.03 cm\(^{-1}\)) to channel 58. The frequency of the laser output is fine-tuned by modulating the laser current with a 10-kHz triangular signal. The fine-tuning range is about 0.19 cm\(^{-1}\). The laser beam is sent to a high finesse optical cavity (\(F = 1560\)), consisting of two 1 inch concave mirrors (1 m radius of curvature, R = 99.8% at 1550 nm, Layertec, Germany). A custom-made in-coupling system towards the optical cavity has been developed (Fig. 6.1 (b)). It is attached directly to the absorption cell and represents an aluminum holder for the optical fibre and a collimating lens aspheric lens (2.79 mm diameter, NA=0.18, 6 mm focal length, Lightpath, USA). It allows for a precise and flexible adjustment of a number of parameters: a focal length of the collimating lens (Z adjustment with a max. displacement of 10 mm), an injection angle of the laser beam with respect to the optical axis of the absorption cell (Angle adjustment with a max. angle of 60 off-axis) and position (X and Y adjustments with a max. displacement of 10 mm each) of the laser beam toward the absorption cell. After all the parameters have been determined, the in-coupling system can be mechanically fixed ensuring a robust optical alignment of the laser beam towards the absorption cell.

The optical absorption cell has a length of 25 cm and a volume of 310 ml. The cell has an effective optical path length of 150 m. The laser beam is injected off-axis with respect to the absorption cell. The laser beam is injected \(\sim 5^{0}\) off-axis with respect to the absorption cell. The off-axis alignment provides better intensity noise suppression, induced by the cavity modes [32, 34]. A pressure of 100 mbar is maintained inside the absorption cell by a vacuum pump and manually adjusted needle valves before and after the cell. To reduce the response time of the system, the flow rate through the gas cell is set to 50 l/h, producing a refresh time for the cell of approximately 2 s. The optical output beam is focused by means of a 25 mm BaF\(_2\) lens (5 cm focal length) on an InGaAs amplified infrared photo detector (PDA10CF, NEP = 1.2 \(\cdot\) \(10^{-11}\) W/Hz\(^{1/2}\), Thorlabs, Germany).
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Figure 6.1: Panel (a): schematic representation of the laser-based sensor for $\text{C}_2\text{H}_2$ and $\text{CO}_2$ detection at 1529.2 nm and HCN at 1533.5 nm. The laser beam is sent to a high finesse absorption cell with the effective pathlength of 150 m. The beam is focused on an InGaAs amplified detector and the output signal is analyzed by a LabVIEW program. Panel (b): custom-made in-coupling system allowing precise alignment ($X$, $Y$, $Z$ and angle) of the optical beam towards the absorption cell.

The electronic signal is amplified by 40 dB (Femto HVA-S, Germany) and acquired via a data acquisition card (GaGe Octopus-8325, 14 bit, USA) for computer analysis using LabVIEW software. The setup is mounted on a breadboard with the size of 40 cm L x 25 cm W. The overall weight of the setup together with the electronic equipment is about 25 kg. The laser-based sensor is calibrated with a reference mixture of 10 ppmv of acetylene and 5 ppmv hydrogen cyanide in nitrogen (VSL, National Dutch Metrology...
A nitrogen gas cylinder is used as a zero gas reference in breath acetylene measurements, free of any absorbing compounds.

### 6.2.2 Breath acetylene sampling

In this work, the laser-based spectrometer is applied for monitoring exhaled concentrations of acetylene and carbon dioxide concentrations for online sampling in a single exhalation. The study has been carried out in the Netherlands in accordance with the applicable rules concerning the review of research ethics committees and informed consent. A commercially available breath sampler device (Loccioni, Italy) is used for monitoring the exhaled CO\(_2\) concentration and the mouth pressure during the exhalation maneuver. Their profiles are displayed in a graphical form on the screen of the breath sampler device. To prevent condensation, the breath sampling line is heated up to 40°C. The exhaled concentrations of measured acetylene and CO\(_2\) traces are displayed in real time on a computer screen using LabVIEW software.

### 6.2.3 Measurements of HCN in vitro

We have developed a gas sampling system for measurements of HCN released by *Pseudomonas Aeruginosa* (PsA) in vitro. The bacteria strain (ATCC 27853) were stored in Brain Heart Infusion (BHI) broth at -80°C and were inoculated into 50 ml of liquid BHI medium with an initial concentration of approximately 5\(\cdot\)10\(^6\) [31] colony forming units (CFU)/ml. The PsA strain is placed in a 250 ml Erlenmeyer flask fixed on a rotating platform (rotating at 100 rpm, GFL 3005, Germany) to enable growing of the bacterium. Bacterial filters (FP 30/0.2 Ca/S, Whatman GmbH, Germany) are placed on the inlet and outlet of the flask to avoid bacterial contamination [35]. The flask and the rotating platform are placed in an environmental chamber (Sanyo MLR-350H, Japan) at 37°C. The headspace of the sample was constantly flushed with a mixture of 21% O\(_2\) in nitrogen, which is also used as a carrier gas to transport trace gases released from PsA bacteria to the spectrometer and the background signal reference. The flow through the mass flow controller MFC 1 (Brooks Instrument, USA) is set to 3.5 l/h. The flow through the cuvette and the mass flow controller MFC 2 (Brooks Instrument, USA) is 3 l/h. Before entering the spectrometer, in order to reduce interference with water, the air is dried by flushing through a cuvette with an absorber (CaCl\(_2\)). All the parts of the gas transport system (except mass flow controller) are made of Teflon PFA or Teflon PTFE (PolyFluor Plastic, Hoevestein, Netherlands). To prevent overpressure at the point of the biological sample, a small amount of flow (~0.5 l/h) is allowed to escape via an OverPressure outlet (OP). The outlet is placed before the Erlenmeyer flask to prevent dilution of the HCN concentration produced by the bacteria. The arrangement is schematically represented in
Fig. 6.2.

**Figure 6.2:** Schematic representation of the sampling system for HCN detection. The bacterium is placed in a 250 ml Erlenmeyer flask fixed on a rotating at 100 rpm orbital platform to enable growing of the bacteria. The flow rate of the carrier gas is maintained constant at 3.5 l/h by means of mass flow controller MFC 1. After the cuvette, HCN traces are further dried and carried to the spectrometer with a constant flow rate of 3 l/h controlled by the mass flow controller MFC 2. Excess air is led out via an OverPressure outlet (OP). The control interface (CI) monitors the flow rates of both flow controllers. The flask and the platform are placed in the oven at 37°C to simulate the temperature of a human body.

6.3 Results

In the spectral region of the laser-based spectrometer both acetylene (6539.46 cm\(^{-1}\)) and carbon dioxide absorption transitions are present (at 6539.51 and 6539.59 cm\(^{-1}\)). Parallel measurements of CO\(_2\) dynamics in breath provide potential benefits and allow calculation of the physiological dead space lung volume [36, 37]. Figure 6.3 represents simulated and experimentally measured spectra of 1 ppmv C\(_2\)H\(_2\) in the wavelength range 6539.35-6539.55 cm\(^{-1}\), and calculated spectra of 4% CO\(_2\) and 3% H\(_2\)O (typical found in exhaled breath) under the following conditions: pressure 100 mbar, temperature 293 K, path length 1 m (source: HITRAN database [38]).

Preceding the actual bio-medical measurements, the performance of the setup was evaluated. Figure 6.4 depicts the corresponding Allan variance curves for C\(_2\)H\(_2\) (in black) and HCN (in red) as a function of the integration time of the measurement. The measurements for both gases have been taken by tuning the laser wavelength over the absorption line of the gas of interest with a 10-kHz scanning rate and averaging the acquired signal 10000 times in 1 s by LabVIEW software.
Figure 6.3: Simulated and experimentally measured spectra of 1 ppmv \( \text{C}_2\text{H}_2 \) in the wavelength range 6539.35-6539.55 cm\(^{-1} \), and calculated spectra of 4\% \( \text{CO}_2 \) and 3\% \( \text{H}_2\text{O} \) (typical found in exhaled breath) under the following conditions: pressure 100 mbar, temperature 293 K, path length 1 m. The scanning range of the laser allows for a simultaneous measurement of \( \text{C}_2\text{H}_2 \) and \( \text{CO}_2 \) lines within a single scan.

Figure 6.4: Allan variance plot represents laser-based spectrometer detection limit as a function of the integration time. Detection limits of 8 ppbv for acetylene (black curve) and 80 ppbv for hydrogen cyanide (red curve) for a 1-s averaging time are achieved. The best detection limit of 1.5 ppbv for \( \text{C}_2\text{H}_2 \) and 12 ppbv for HCN is reached for a 128-s acquisition time.

Detection limits of 8 ppbv for acetylene and 80 ppbv for hydrogen cyanide are
achieved for 1 second averaging time. The best detection limits – 1.5 ppbv for acetylene and 12 ppbv for hydrogen cyanide – are reached for 128-s acquisition time. This is equivalent to Noise Equivalent Absorption Sensitivity (NEAS) of $2.1 \cdot 10^{-9} \text{ cm}^{-1} \cdot \text{Hz}^{-1/2}$.

Figure 6.5 shows calibration curve of the laser-based spectrometer. Various concentrations of acetylene were applied and measured by the laser-based spectrometer in the range from 3 ppbv up to 4 ppmv. The calculated and measured datasets are in a good agreement, implying a linear response of the system for a range of concentrations useful for breath analysis measurements.

**Figure 6.5:** Calibration curve obtained for the laser-based spectrometer. Various prepared concentrations (3-4000 ppbv) of acetylene were applied and measured by the laser-based system in order to estimate linearity of the spectrometer response.

### 6.3.1 Breath acetylene and hydrogen cyanide measurements *in vitro*

Typical online exhalation profiles of acetylene and carbon dioxide at a flow rate of 50 ml/s are shown in Fig. 6.6. Two breath samples were taken from the subject: one immediately after smoking a cigarette, another one – after 15 min. The acquisition time for online measurements was 2 s. The average concentration of 7 measurements of acetylene in breath of a test-person immediately after smoking a cigarette was $96.3 \pm 7.4$ ppbv. After 15 min the production was 6 times lower ($14.4 \pm 6.1$ ppbv) which is in a good agreement with the wash out kinetics model proposed by Metsala *et al.* [23].

The HCN production released in the headspace of the Erlenmeyer flask by PsA bacteria and the background reference signal (compressed air) were measured over 55 h in sequence of 30 min each. Resulting profile of HCN production is depicted in Fig. 6.7.
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Figure 6.6: Online measurements of C$_2$H$_2$ and CO$_2$ profiles measured with the laser-based spectrometer during single exhalation immediately after smoking (in blue) and 15 minutes afterwards (in black); acquisition time 2 s.

Figure 6.7: A profile of HCN production resulting from Pseudomonas Aeruginosa bacteria (strain ATCC 27853) in vitro continuously measured together with the background reference signal over 55 h. by the laser-based spectrometer with a flow of 3 l/h through the Erlenmeyer flask; acquisition time 30 s.

The acquisition time of the measurement was 30 s per data point resulting in the measurement noise of $\sim$20 ppbv. HCN production reaches its maximum in the first 35 h. and equals to $\sim$7 $\mu$l/h. After that the HCN production rapidly decreases most probably as a result of medium depletion [31].
6.4 Discussion

The development of a prototype laser-based spectrometer employing integrated cavity output spectroscopy has been reported. The spectrometer is used for simultaneous detection of acetylene and carbon dioxide during a single exhalation, as well as hydrogen cyanide resulting from *Pseudomonas Aeruginosa* bacteria *in vitro*. The detection limit obtained for acetylene is 8 ppbv for 1-s averaging time and 1.5 ppbv for 128-s averaging. The results prove that acetylene cannot be considered as a biomarker for an active smoking status due to fast elimination in exhaled breath.

The laser also allows the detection of HCN absorption transition in a different wavelength. It has been recently established that HCN can be considered as a biomarker for *Pseudomonas Aeruginosa* bacteria [26, 39]. Elevated HCN concentrations have also been detected in breath of patients infected with PsA [26, 40]. In this study, we have demonstrated potential possibilities for measurements of HCN production with our laser-based spectrometer. The setup provides sufficient detection capabilities (noise level of \(\sim 20\) ppbv) as well as long-term stability to perform continuous measurements over 55 h. We have detected levels of HCN production up to 7 \(\mu\)l/h (2.2 ppmv with 3 l/h flow rate through the PA sample) resulting from PsA strain ATCC 27853 *in vitro*. The measured HCN levels are somewhat lower compared to those previously assessed by our group with a continuous wave Optical Parametric Oscillator (OPO) based setup [31], however, the results were obtained from a different strain. Here, the authors observed maximum HCN concentration of 6.5 ppmv after 77 h. resulting from ATCC 10145 strain *in vitro* with a similar sampling arrangement. Compared to the OPO based setup employing pho-toacoustic spectroscopy, our near-infrared DBR laser-based spectrometer in combination with OA-ICOS offers more compact design suitable for the field measurements, however, at the cost of a lower detection sensitivity (0.4 ppbv of HCN in 10 s and 20 ppbv HCN in 30 s, respectively).

The radiation source used in this work is a prototype CW DBR laser tunable within the C-band (6394-6548 cm\(^{-1}\)) by switching to one of the 89 available channels. Current performance of the laser module allows only a few mode-hop-free spectral windows for the trace gas sensing. Within this paper, only 2 working channels made detection of already 3 different species possible. The on-going developments will offer continuous mode-hop-free tuning capabilities of the laser together with fast switching between the channels for the simultaneous multi-component gas detection. Furthermore, a faster laser modulation speed (up to 100 kHz) can potentially be realized in order to increase the laser performance.

The results obtained prove that the developed prototype of a compact laser-based spectrometer in combination with OA-ICOS represents a sensitive and robust technique, capable of rapid multi-component detection of acetylene, hydrogen cyanide and carbon dioxide traces with a sensitivity of \(2.1 \times 10^{-9}\) cm\(^{-1}\) Hz\(^{-1/2}\). The dedicated custom-made
in-coupling system allows fixed and robust alignment of the optical beam inside the absorption cell. This arrangement offers more flexibility and enables the spectrometer to be utilized in field campaigns as well as in hospital trials. In addition, small volume of the absorption cell provides fast refreshment time (less than 2 s) suitable for online measurements.

The sensitivity can be further improved by increasing the effective path length inside the absorption cell. This can be achieved by replacing the actual HR mirrors (99.8% at 1550 nm) with higher reflectivity ones. A reflectivity of 99.995% would result in an increase of the effective optical path length inside the absorption cell up to 5 km compared to current 150 m. Together with a more sensitive detector with NEP 75 fW Hz$^{-1/2}$ at 1550 nm this will grant higher sensitivity of the system up to 6.3·10$^{-11}$ cm$^{-1}$·Hz$^{-1/2}$. This will allow utilizing the laser-based spectrometer for the detection of HCN in vivo (in exhaled breath or emitted from skin), where the detection limit at ppbv or even sub-ppbv level is required [30].

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Summary

Trace gas detection spectroscopy is an attractive research area. It is of great importance for both fundamental research as well as for industrial applications. The advantages of the trace gas detection are evident: it is safe, non-invasive and reliable. Depending on the targeted application, various requirements should be satisfied while developing a trace gas sensor.

Nowadays, there is a range of well-established trace gas detection methods such as chemiluminescence, gas chromatography, electronic nose and mass-spectrometry. However, advances in the development of novel laser sources, especially in the mid-IR spectral region, with state-of-art performance (high power, narrow linewidth, widely tunable), have made laser-based absorption spectroscopy a powerful tool for the trace gas sensing. Laser-based spectrometers have significantly simplified the analysis of complex gas mixtures offering high sensitivity, selectivity and potential for further miniaturization.

Among the bio-medical applications targeted by the laser-based gas sensing, breath analysis represents one of the most important. Since Hippocrates people have been trying to investigate exhaled air of human. Back there, scientists established a close relationship between the smell of the exhaled air and the physiological condition of human. For example, fruity odor was attributed to uncontrolled diabetes while fishy exhaled air smell to the kidney failure. However, quantitative trace gas detection has become possible relatively recently – only about 200 years ago when Antoine Laurent Lavoisier and Pierre Simon Laplace analyzed oxygen uptake and carbon dioxide production in the exhaled breath of a guinea pig.

In the 20th century lots of Volatile Organic Compounds (VOCs) in exhaled air of a human were discovered by Linus Pauling using gas chromatography. It turned out that apart from main components (N₂, O₂, CO₂ and H₂O) there were dozens of other molecules in the exhaled breath, however, at significantly lower concentrations – at ppmv (1 : 10⁶, part-per-million by volume) or even ppbv (1 : 10⁹, part-per-billion by volume)
levels. Since that time breath analysis has a high impact on public health and quality of life in general. It has been shown that elevated levels of some of VOCs in breath can be considered as indicators of a disease condition. For instance, elevated levels of breath isoprene (C$_5$H$_8$) or ammonia (NH$_3$) can indicate renal impairment, ethanol (C$_2$H$_6$O) concentration is attributed to serum glucose levels, ethane (C$_2$H$_6$) – to lipid peroxidation and oxidative stress, methane (CH$_4$) – to colonic fermentation, etc. In the mid-infrared part of the spectrum most of these molecules exhibit their unique absorption properties. This region is known as ‘the fingerprint region’ and represents therefore a favorable area to access the absorption spectra of many gaseous compounds by laser-based absorption spectroscopy.

This thesis demonstrates possible experimental approaches for the sensitive trace gas detection (including breath analysis) based on laser absorption spectroscopy in the mid- and near-IR wavelength region. A brief introduction to the field of trace gas sensing and breath analysis is given in Chapter 1. Here, the importance of the monitoring and investigation of the exhaled breath compounds is briefly described as well as the requirements applicable for the development of trace gas sensors.

Chapter 2 introduces the concept of Quantum Cascade Lasers (QCLs), a recently developed laser source possessing such distinct features as high quantum efficiency, narrow linewidth, relatively high output power, availability in a broad wavelength region (3.4-24 µm, AlInAs/GaInAs and Terahertz – 60-250 µm, AlGaAs/GaAs) and room temperature continuous wave operation. These characteristics make the QCL a promising device for the various scientific applications. Furthermore, a description of the detection methods is given; these approaches include Wavelength Modulation Spectroscopy (WMS), Faraday Rotation Spectroscopy (FRS) and Integrated Cavity Output Spectroscopy (ICOS). The results for the detection of Nitric Oxide (NO) based on FRS technique are also discussed.

Chapter 3 provides a deeper investigation on exhaled and biogenic NO, describing its origin, flow dependency and sampling technique. For simultaneous detection of NO and CO$_2$ in exhaled human air a sensor based on a cw-QCL in combination with integrated cavity output spectroscopy has been developed, providing detection sensitivity of 0.7 parts-per-billion by volume of NO (acquisition time: 1 s). The sensor provides stable continuous operation over more than 2 days. Comparison is made with the standard chemiluminescence NO analyzer and a commercial CO$_2$ breath sampler. The QCL-based sensor is tested on healthy subjects at various exhalation flow rates (15, 50, 100 and 300 ml/s) for both online and offline sampling procedures as well as on asthmatic children (offline sampling). Possibilities for measurements of biogenic NO in vivo, i.e. NO originating from human skin tissue, are also demonstrated.

Chapter 4 describes a custom-made continuous wave external cavity quantum cascade laser (EC-QCL) in the Littrow configuration suitable for the detection of molecular gases with broadband absorption features. The laser has an overall tuning range of 230
cm$^{-1}$ and operates between 1150 and 1380 cm$^{-1}$ (7.25-8.69 µm) producing $\sim$1 mW of output power at 243 K. In combination with a multipass cell the developed EC-QCL allows fast and robust detection of volatile organic compounds in breath. An absorption profile of 1 ppmv acetone is recorded within 3 s acquisition time covering the wavelength region of $\sim$65 cm$^{-1}$. Noise Equivalent Absorption Sensitivity (NEAS) for the EC-QCL-based spectrometer is estimated at 8.6$\cdot$10$^{-8}$ cm$^{-1}$·Hz$^{-1/2}$. Possibilities for the online measurements of acetone in breath are demonstrated.

Chapter 5 represents an exploratory study on a prototype of the mid-infrared External Ring Cavity Quantum Cascade Laser (mid-IR ERC-QCL). The QC lasers with 0-, 3- and 7- degrees angled facets use a gain medium centered at 7.72 µm (1295 cm$^{-1}$) at room temperature and were placed inside a compact X-shape resonator. Preliminary characterization of the ERC-QCL is performed and some key features of the prototype are studied. Potential to suppress Spatial Hole Burning (SHB) effect is investigated, making ERC-QCL suitable for the active mode-locking in the infrared wavelength region.

Chapter 6 reports on the development of a compact near-infrared Distributed Bragg Reflector (DBR) laser-based spectrometer employing Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS). The spectrometer is capable of simultaneous detection of acetylene (C$_2$H$_2$) and CO$_2$ at 1529.2 nm as well as hydrogen cyanide (HCN) at 1533.5 nm. The detection limits of 8 ppbv for C$_2$H$_2$ and 80 ppbv for HCN are achieved for the acquisition time of 1 sec. The setup has been tested for online measurements of C$_2$H$_2$ in exhaled breath of a smoking subject while HCN has been measured resulting from the metabolism of Pseudomonas aeruginosa bacteria in vitro.

Despite recent advances in detection of exhaled breath gases, there is still lack of knowledge about the role of every particular compound and its impact on a physiological state. Accordingly, further investigation on breath biomarkers should be carried out and suitable experimental approaches and detection schemes are of great necessity. Besides, next generation of photonic devices integrated on a single chip will give a boost to further improvements in detection sensitivity.
Gassporen detectie spectroscopie is een aantrekkelijk onderzoeksgebied. Het is van groot belang voor zowel fundamenteel onderzoek als industriële toepassingen. De voordelen van gassporen detectie zijn evident: het is veilig, niet-invasief en betrouwbaar. Afhankelijk van de beoogde toepassing, moet er tijdens het ontwikkelen van een gassporen sensor aan verschillende eisen worden voldaan.

Tegenwoordig is er een scala aan gerenommeerde gassporen detectiemethoden zoals chemoluminescentie, gaschromatografie, elektronische neus en massa spectrometrie. Echter, de vooruitgang in de ontwikkeling van nieuwe laser-bronnen, voornamelijk in het midden-infrarode gebied van het spectrum, met state-of-art prestaties (hoog vermogen, kleine lijnbreedte, op grote schaal afstembaar), hebben gemaakt dat op laser gebaseerde absorptie spectroscopie een krachtig hulpmiddel is voor gassporen detectie. Op laser gebaseerde spectrometers hebben de analyse van complexe gasmengsels aanzienlijk vereenvoudigd door hoge gevoeligheid, selectiviteit en het potentieel voor verdere miniaturisatie.

Onder de biomedische toepassingen van op laser gebaseerde gas detectie, is ademanalyse een van de belangrijkste. Sinds Hippocrates heeft men geprobeerd om uitgeademde lucht van de mens te onderzoeken. Toen hebben wetenschappers een nauwe relatie vastgesteld tussen de geur van uitgeademde lucht en de fysiologische toestand van de mens. Zo werd een fruitige geur toegeschreven aan ongecontroleerde diabetes, terwijl een visachtige geur in uitgeademde lucht duidde op nierfalen. Echter, kwantitatieve gassporen detectie is slechts ongeveer 200 jaar geleden tot stand gekomen, toen Antoine Laurent Lavoisier en Pierre Simon Laplace zuurstofopname en de productie van koolstofdioxide in uitgeademde lucht van een cavia geanalyseerd hebben.

In de 20e eeuw werden veel Vluchtige Organische Componenten (VOCs) in de uitgeademde lucht van een mens ontdekt door Linus Pauling met behulp van gaschromatografie. Het bleek dat naast hoofdcomponenten $\text{N}_2$, $\text{O}_2$, $\text{CO}_2$ en $\text{H}_2\text{O}$ er tientallen
Samenvatting

Andere moleculen in de uitgeademde lucht aanwezig zijn. Echter, in aanzienlijk lagere concentraties - op ppmv (1 : 10^6, deel per miljoen per volume) of zelfs ppbv (1 : 10^9, deel per miljard in volume) niveaus. Sinds die tijd heeft ademanalyse een grote invloed op de volksgezondheid en de kwaliteit van leven in het algemeen. Het is aangetoond dat verhoogde hoeveelheden van sommige VOCs in de adem kunnen worden beschouwd als indicatoren van een ziektestoestand. Zo kan bijvoorbeeld een verhoogde isoproen (C_5H_8) of ammoniak (NH_3) concentratie in de adem nierfunctiestoornis aangeven en wordt de ethanol (C_2H_6O) concentratie toegeschreven aan serum glucose niveaus, ethaan (C_2H_6) — aan peroxidatie van lipiden en oxidatieve stress, methaan (CH_4) — aan darmfermentatie, etc. In het midden infrarode deel van het spectrum vertonen de meeste van deze moleculen unieke absorptie eigenschappen. Deze regio staat bekend als ‘the fingerprint region’ en dit is een gunstig gebied omdat vele gasvormige verbindingen in dit gebied te meten zijn door op laser gebaseerde absorptie spectroscopie.

Dit proefschrift toont de experimentele mogelijkheden voor gevoelige gassporen detectie (inclusief ademanalyse) gebaseerd op laser absorptie spectroscopie in het midden en nabij-infrarode golflengtegebied. Een korte inleiding op het gebied van het gassporen detectie en ademanalyse wordt gegeven in Hoofdstuk 1. Hier wordt het belang van de monitoring en het onderzoeken van verbindingen in uitgeademde lucht kort beschreven, evenals de eisen die van toepassing zijn op de ontwikkeling van gassporen sensoren.

Hoofdstuk 2 introduceert het concept van Quantum Cascade Lasers (QCLs), een recent ontwikkelde laserbron die in bezit is van zulke duidelijke kenmerken als hoge quantumsystatie, kleine lijnbreedte, relatief hoog uitgangsvermogen, beschikbaarheid in een breed golflengtegebied (3.4-24 μm, AllInAs / GaInAs en TeraHertz – 60-250 μm, AlGaAs / GaAs) en continue werking op kamertemperatuur. Deze eigenschappen maken de QCL een veelbelovend apparaat voor diverse wetenschappelijke toepassingen. Verder wordt een beschrijving van de detectiemethoden gegeven; waaronder Wavelength Modulation Spectroscopy (WMS), Faraday Rotation Spectroscopy (FRS) en Integrated Cavity Output Spectroscopy (ICOS). Daarnaast worden de resultaten voor de detectie van stikstofoxide (NO) op basis van FRS techniek besproken.

Hoofdstuk 3 geeft een diepgaander onderzoek van uitgeademde en biogene NO weer, het beschrijft de herkomst, de afhankelijkheid van uitademingssnelheid en de techniek voor het afnemen van monsters. Voor gelijktijdige detectie van NO en CO_2 in uitgeademde lucht is een sensor ontwikkeld op basis van een cw-QCL in combinatie met een Integrated Cavity Output Spectroscopy met de gevoeligheid van 0.7 parts per miljard in volume van NO (acquisitietijd: 1 s). De sensor maakt een stabiele continue werking mogelijk over een periode van meer dan 2 dagen. Een vergelijking is gemaakt met de standaard chemiluminescentie NO-analysator en een commerciële CO_2 adem sampler. De QCL-gebaseerde sensor wordt getest op gezonde proefpersonen op verschillende uitademingssnelheden (15, 50, 100 en 300 ml/s) voor zowel online als offline sampling procedures alsmede op astmatische kinderen (offline sampling). Mogelijkheden voor
het meten van biogene NO in vivo (afkomstig van menselijk huidweefsel) worden ook gedemonstreerd.

Hoofdstuk 4 beschrijft een op maat gemaakte continue external cavity quantum cascade laser (EC-QCL) in de Littrow configuratie, geschikt voor het detecteren van moleculaire gassen met breedband absorptie eigenschappen. De laser heeft een totaal afstembereik van 230 cm⁻¹ en werkt tussen 1150 en 1380 cm⁻¹ (7.25-8.69 µm), produceert ~1 mW uitgangsvermogen bij 243 K. In combinatie met een multipass cel maakt de ontwikkelde EC-QCL snelle en robuuste detectie van vluchtige organische stoffen in de adem mogelijk. Een absorptie profiel van 1 ppmv aceton wordt opgenomen binnen 3 s acquisitie tijd die het golflengtegebied van ~65 cm⁻¹ bedekt. Noise Equivalent Absorption Sensitivity (NEAS) voor het EC-QCL-gebaseerde spectrometer wordt geschat op 8.6×10⁻⁸ cm⁻¹·Hz⁻¹/². Mogelijkheden voor online metingen van aceton in de adem wordt gedemonstreerd.

Hoofdstuk 5 geeft een verkennende studie over een prototype van het midden-infra- rood Externe Ring Cavity Quantum Cascade Laser (mid-IR ERC-QCL). De QC-lasers met 0-, 3- en 7-graden hoekfacetten in een versterkingsmedium zijn gecentreerd op 7.72 µm (1295 cm⁻¹) bij kamertemperatuur en werden in een compacte X-vorm resonator geplaatst. Voorlopige karakterisering van de ERC-QCL wordt uitgevoerd en een aantal belangrijke eigenschappen van het prototype worden bestudeerd. Een potentieel mogelijkheid om Spatial Hole Burning (SHB) effect te onderdrukken wordt onderzocht, waardoor ERC-QCL geschikt is voor de actieve stand-vergrendeling in het infrarode golflengtegebied.

Hoofdstuk 6 beschrijft de ontwikkeling van een compacte infrarood Distributed Bragg reflector (DBR)-laser spectrometer in combinatie met Off-Axis Integrated Cavity Productie Spectroscopy (OA-ICOS). De spectrometer kan gelijktijdige detectie van acetylene (C₂H₂) en CO₂ op 1529.2 nm en waterstofcyanide (HCN) op 1533.5 nm uitvoeren. Het detectielimiet van 8 ppbv voor (C₂H₂) en 80 ppbv voor HCN wordt bereikt tijdens de opname tijd van 1 sec. De opstelling is getest voor online metingen van (C₂H₂) in de uitgeademde lucht van een rokende proefpersoon terwijl HCN is gemeten als gevolg van het metabolisme van Pseudomonas aeruginosa bacteriën in vitro.

Ondanks de recente vooruitgang in de opsporing van de uitgeademde lucht van gassen, is er nog steeds een gebrek aan kennis over de rol van elke bepaalde verbinding en de impact daarvan op de fysiologische toestand. Daarom moet verder onderzoek naar biomarkers in adem worden uitgevoerd en zijn geschikte experimentele benaderingen en detectieschema’s van groot noodzaak. Bovendien zal de volgende generatie van fotonische apparaten die geïntegreerd zijn op een enkele chip een impuls geven aan een verdere verbetering van de detectielimieten.
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