Miniaturized Gas Sensors based on Laser Absorption Spectroscopy
Application to exhaled breath gas diagnostics

DISSERTATION
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„Wer alleine arbeitet, addiert;

Wer zusammen arbeitet, multipliziert“.

(Arabische Weisheit. Autor unbekannt.)
Abstract

The research presented in this cumulative thesis focuses on the development of mid-infrared (MIR) gas sensors based on optically highly efficient miniaturized IR absorption cells and their application in various sensing scenarios. The dissertation is based on five peer-reviewed journal articles, which were the result of an interdisciplinary research involving analytical chemistry, laser and optical engineering, physics, and medicine being conducted at the Institute of Analytical and Bioanalytical Chemistry at Ulm University.

Within the framework of this thesis, absorption spectroscopy was combined with substrate-integrated hollow waveguides (iHWGs) for sensitive analysis of various molecules exhibiting distinctive infrared absorption features in the MIR spectral regime. There are two primary aims of the studies presented in this cumulative thesis: 1. Optimization of the performance of iHWGs as compact gas cells via testing of different substrate materials and coatings, fiber-coupling techniques and implementation of noise reduction techniques. 2. The development of compact sensor platforms that enable gas sensing in combination with newly developed infrared sources: A compact sensor combining iHWGs and surface-emitting ring quantum cascade lasers developed by the Vienna University of Technology with single-mode emission and symmetric low divergent beam profile was developed and tested. These QCLs offer highly collimated laser beams besides vertically emitting nature and are perfectly suitable for possible direct integration in iHWGs. A further contribution of this thesis is the development of gas sensors with novel MIR cascade lasers with very low power consumption. The so-called interband cascade lasers (ICL) enable continuous measurements of trace gases in the MIR range between 3 to 6 µm for various environmental, industrial, and biochemical sensing applications. An extensive study with main emphasis on the combination of ICLs and iHWGs
was performed for the detection and quantification of methane in the ppm range within a compact sensor design.

Moreover, within the scope of the EU-project Advanced Photonic SEnsor MAterials (APOSEMA), a new approach for laser based exhaled breath analysis utilizing dual-channel iHWGs and power-efficient ICLs was developed, prototyped, and tested for non-invasive continuous monitoring of CO$_2$ and the isotope ratio of $^{13}$CO$_2$/$^{12}$CO$_2$ in mouse breath. The developed sensor using laser based infrared spectroscopy allowed the integration of optochemical fluorescence sensors based on polymer nanofibers for ultra-fast oxygen sensing. The system was tested successfully for routine analysis in the mouse intensive care unit (MICU) at the Institute of Anesthesiologic Pathophysiology and Method Development. In summary, MIR sensing technologies developed within this dissertation indicate the high versatility of these iHWGs representing a key milestone in the development of compact sensor systems for fast and precise measurement of small volumes.
Zusammenfassung


Im Rahmen dieser Arbeit wurde die direkte Absorptionsspektroskopie mit Substrat-integrierten Hohlwellenleitern (iHWG) kombiniert, um empfindliche Analysen von verschiedensten Molekülen mit charakteristischen Absorptionseigenschaften im mittleren Infrarot-Spektralbereich durchzuführen. Es gibt zwei Hauptziele der Studien, die in dieser Dissertation präsentiert werden. 1. Die Leistungs-Optimierung von kompakten iHWGs durch das Testen von verschiedenen Materialien und Beschichtungen, Faserkopplungstechniken und die Implementierung von Techniken zur Rauschunterdrückung. 2. Die Entwicklung portabler Sensorsysteme basierend auf der Kombination innovativer Infrarotquellen (Quantenkaskadenlaser QCL und Interbandkaskadenlaser ICL) und optimierten iHWGs für die quantitative Analyse von relevanten Molekülen aus den Bereichen Umwelt- und Atemgasanalytik. Dazu wurde ein IR-Sensorsystem, das die entwickelten Gaszellen und einen von der Technischen Universität Wien entwickelten oberflächenemittierenden Ring-QCL mit Single-Mode-Emission und geringer Strahlbreite kombiniert, entwickelt und getestet. Ring-QCLs bieten neben der vertikalen Lichtemission hoch-kollimierte Laserstrahlen und eignen sich daher hervorragend für eine mögliche direkte Integration in die iHWGs. Ein weiterer...

Eine umfangreiche Studie mit Schwerpunkt auf der Kombination von ICLs und iHWGs wurde zur Detektion und Quantifizierung von Methan im ppm-Bereich innerhalb eines kompakten Sensordesigns durchgeführt. Darüber hinaus wurde im Rahmen des EU-Projektes Advanced Photonic SEnsor MAterials (APOSEMA) ein neuer Ansatz für die lasergestützte Atemanalyse mittels kompakter iHWGs und ICLs entwickelt, prototypisiert und für die nicht-invasive kontinuierliche Überwachung von CO₂ und des Isotopenverhältnisses von $^{13}$CO₂ zu $^{12}$CO₂ im Mausatem getestet. Der entwickelte Sensor ermöglichte neben der Infrarot-Analyse auch die Integration von optochemischen Fluoreszenzsensoren auf der Basis von Polymer-Nanofasern zur schnellen Sauerstoff-Sensorik. Das System wurde erfolgreich für die Routineanalytik in der Mausintensivstation des Instituts für Anästhesiologische Pathophysiologie und Verfahrensentwicklung getestet.

Zusammenfassend lässt sich festhalten, dass die entwickelten MIR-Sensortechnologien die hohe Vielseitigkeit dieser Substrat-integrierten Hohlwellenleiter zeigen und einen wichtigen Meilenstein in Richtung kompakter Sensorsysteme für die schnelle und präzise Messung von geringsten Gasproben darstellen.
# Table of Contents

Abstract ................................................................................................................................. I
Zusammenfassung .................................................................................................................... III
Peer-reviewed Publications .................................................................................................... 1
List of Abbreviations ............................................................................................................ 4
Aim and Overview of the Thesis ............................................................................................ 5

1. Introduction .......................................................................................................................... 7
   1.1 Motivation ....................................................................................................................... 7
   1.1.1 Laser Absorption Spectroscopy LAS ........................................................................ 11
   1.1.2 Exhaled Breath Analysis ............................................................................................ 12
   1.2 Theoretical Background ............................................................................................... 16
   1.2.1 Fundamentals of absorption-based spectroscopy ...................................................... 16
   1.2.2 Mid Infrared Laser sources ....................................................................................... 19
   1.2.2.1 Quantum cascade laser ......................................................................................... 19
   1.2.2.2 Interband cascade laser ........................................................................................ 21
   1.2.3 Hollow waveguide ..................................................................................................... 23

2. Results and Conclusions ..................................................................................................... 26
3. References ........................................................................................................................... 30
4. List of Figures ....................................................................................................................... 33
5. Journal Articles .................................................................................................................... 34
   5.1 Paper I. Instrumentation and applications of Cascade Laser Spectroscopy .................. 34
   5.2 Paper II. Advanced gas sensors based on substrate-integrated hollow waveguides and dual-color ring quantum cascade lasers .......................................................... 54
   5.3 Paper III. iHWG-ICL: Methane Sensing with Substrate-Integrated Hollow Waveguides Directly Coupled to Interband Cascade Lasers ...................................................... 62
   5.4 Paper IV. Fiber-Coupled Substrate-Integrated Hollow Waveguides: An Innovative Approach to Mid-Infrared Remote Gas Sensors......................................................... 68
   5.5 Paper V. Advanced Photonic Sensors based on Interband Cascade Lasers for Real-time Mouse Breath Analysis ......................................................................................... 76

Danksagung ................................................................................................................................. 84
Curriculum Vitae ....................................................................................................................... 86
APPENDIX ................................................................................................................................. 91
Declaration of Authorship ......................................................................................................... 98
Eidesstattliche Erklärung ........................................................................................................... 98
Peer-reviewed Publications

The presented thesis is based on the following publications, that are published in international peer-reviewed journals:

I. **Instrumentation and applications of Cascade Laser Spectroscopy**
   
   Erhan Tütüncü and Boris Mizaikoff.
   
   *Encyclopedia of Analytical Chemistry, R. A. Meyers (Ed.) (2018)*

II. **Advanced gas sensors based on substrate-integrated hollow waveguides and dual-color ring quantum cascade lasers**
   
   
   *Analyst, 141 (22), 6202-6207 (2016).*

III. **iHWG-ICL: Methane Sensing with Substrate-Integrated Hollow Waveguides Directly Coupled to Interband Cascade Lasers**
   
   Erhan Tütüncü, Markus Nägele, Peter Fuchs, Marc Fischer, and Boris Mizaikoff.
   
   *ACS Sensors, 1 (7), 847−851 (2016).*

IV. **Fiber-Coupled Substrate-Integrated Hollow Waveguides: An Innovative Approach to Mid-infrared Remote Gas Sensors**
   
   
   *ACS Sensors, 2 (9), 1287−1293 (2017).*
V. Advanced Photonic Sensors Based on Interband Cascade Lasers for Real-Time Mouse Breath Analysis


Furthermore, the presented PhD research herein has contributed to the following publications:

VI. Breath Analysis with Mid-Infrared Diagnostics

Vjekoslav Kokoric, Erhan Tütüncü, Felicia Seichter, Andreas Wilk, Paula R. Fortes, Ivo M. Raimundo Jr., and Boris Mizaikoff.


VII. Sensing hydrocarbons with interband cascade lasers and substrate-integrated hollow waveguides

Igor José Gomes da Silva, Erhan Tütüncü, Markus Nägele, Peter Fuchs, Marc Fischer, Ivo M. Raimundo, and Boris Mizaikoff.

Analyst, 141(14), 4432–4437 (2016).

VIII. Next-Generation Mid-Infrared Chemical and Biological Sensors: Combining Quantum Cascade Lasers with Thin-Film and Hollow Waveguides

Julian Haas, Erhan Tütüncü, Andreas Wilk, Vjekoslav Kokoric, and Boris Mizaikoff.

IX. polyHWG: 3D Printed Substrate-Integrated Hollow Waveguides for Mid-Infrared Gas Sensing

Robert Stach, Julian Haas, Erhan Tütüncü, Sven Daboss, Christine Kranz, and Boris Mizaikoff.

*ACS Sensors, 2(11), 1700-1705 (2017).*

X. Online monitoring of carbon dioxide and oxygen in exhaled mouse breath via substrate-integrated hollow waveguide - Fourier transform infrared - luminescence spectroscopy

Felicia Seichter, Erhan Tütüncü, Leila Tamina Hagemann, Josef Vogt, Ulrich Wachter, Michael Gröger, Sandra Kress, Peter Radermacher, and Boris Mizaikoff.


XI. Real-time and simultaneous monitoring of NO, NO₂ and N₂O using substrate-integrated hollow waveguides coupled to compact FT-IR spectrometer

João Flávio da Silveira Petruci, Erhan Tütüncü, Arnaldo Alves Cardoso, and Boris Mizaikoff.

*Applied Spectroscopy, First Published September 28, (2018).*
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>APOSEMA</td>
<td>Advanced Photonic Sensor Materials</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier Transform Infrared</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas Chromatography – Mass Spectrometry</td>
</tr>
<tr>
<td>HWG</td>
<td>Hollow waveguide</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>ICL</td>
<td>Interband Cascade Laser</td>
</tr>
<tr>
<td>iHWG</td>
<td>Substrate-Integrated Hollow waveguide</td>
</tr>
<tr>
<td>LLNL</td>
<td>Lawrence Livermore National Laboratory</td>
</tr>
<tr>
<td>MCT</td>
<td>Mercury Cadmium Telluride</td>
</tr>
<tr>
<td>MIR</td>
<td>Mid-Infrared</td>
</tr>
<tr>
<td>NDIR</td>
<td>Non-Dispersive Infrared</td>
</tr>
<tr>
<td>OAPM</td>
<td>Off- Axis Parabolic Mirror</td>
</tr>
<tr>
<td>POF</td>
<td>Polymer Optical Fiber</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>ppb</td>
<td>Parts per billion</td>
</tr>
<tr>
<td>QCL</td>
<td>Quantum Cascade Laser</td>
</tr>
<tr>
<td>riQCL</td>
<td>ring Quantum Cascade Laser</td>
</tr>
<tr>
<td>S/N</td>
<td>Signal to Noise Ratio</td>
</tr>
<tr>
<td>τ</td>
<td>Lifetime</td>
</tr>
<tr>
<td>TDLAS</td>
<td>Tunable Diode Laser Absorption Spectroscopy</td>
</tr>
<tr>
<td>TTR</td>
<td>Tracer to tracee ratio</td>
</tr>
<tr>
<td>VOC</td>
<td>Volatile Organic Compound</td>
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</table>
Aim and Overview of the Thesis

The present thesis focuses on the design, optimization, and testing of MIR gas sensors based on substrate-integrated hollow waveguides (iHWGs) serving as optically highly efficient miniaturized IR absorption cell and their potential application for environmental monitoring and biomedical diagnostics that require low volume samples and fast transition times. With increasing demand for fast, portable, user friendly sensors for the analysis of low gas volumes, MIR spectroscopy has attracted great interest since conventional applied analytical methods such as gas chromatography requires highly qualified personnel, expensive and bulky instrumentation. Thus, research presented in this cumulative thesis is focused on three main topics for the development of compact hollow waveguide–based gas sensors:

I. The first combination of a iHWG with ring-shaped vertically emitting quantum cascade laser (riQCL) providing two distinct emission wavelengths was demonstrated. A MIR spectroscopic sensor for multi-analyte gas sensing was developed and detailed results of this research are featured in the journal article ‘Advanced gas sensors based on substrate-integrated hollow waveguides and dual-color ring quantum cascade lasers’ presented in Chapter 5, II. Furthermore, a compact optical sensor device for rapid gas diagnostics of methane with MIR emitting type-II ICLs was developed. The analytical performance of the ICL-iHWG combination is presented in ‘iHWG-ICL: Methane Sensing with Substrate-Integrated Hollow Waveguides Directly Coupled to Interband Cascade Lasers’ in Chapter 5, III.

II. A detailed study on the remote operation of iHWGs via optical fiber coupling for broad- and narrow-band MIR (2.5–20 μm) sensing applications was conducted and presented in ‘Fiber-Coupled Substrate-Integrated Hollow Waveguides: An Innovative
**Approach to Mid-Infrared Remote Gas Sensors** in *Chapter 5, IV*. Optical fiber coupled iHWGs were fabricated and tested for highly sensitive sensing of various hydrocarbon molecules. For comparison studies, two cells with substrate dimensions of $50 \times 50 \times 12$ mm (L × W × H) and geometric channel lengths of 280.2 mm and 130.0 mm serving as miniature light guiding gas cell were used in combination with a Fourier-Transform infrared (FT-IR) spectrometer for broadband MIR sensing.

**III.** Within the framework of the project ‘Advanced Photonic Sensor Materials’ (APOSEMA), an ICL based breath sensor for real time monitoring of CO$_2$, $^{13}$CO$_2$ enrichment and oxygen sensing in mouse breath via optical oxygen sensors in mouse intensive care unit has been developed. The portable standalone breath sensor offering non-invasive and fast analysis of mouse breath has been tested successfully at the Institute of Anesthesiologic Pathophysiology and Method Development, Ulm University Medical Center. An extensive study on mouse breath samples was performed via the developed Tunable Diode Absorption Spectroscopy (TDLAS) based gas sensor combined with an innovative iHWG design comprising two channels is presented in *Chapter 5, V*, and published in ‘**Advanced Photonic Sensors Based on Interband Cascade Lasers for Real-Time Mouse Breath Analysis**‘.
1. Introduction

1.1 Motivation

The United Nation proclaimed the year 2015 ‘The International Year of Light and Light-Based Technologies’ (Houser, 2015) as a global initiative to draw attention to social, economic, and developmental role of optical technologies and their importance in solving world-wide challenges in education, energy, communications, and health. In particular, the analysis of trace gases based on optical technologies has become increasingly important for environmental sciences, industrial, and biomedical applications in the last three decades. These sensors play a major role from environmental monitoring, protection of workers from hazardous gases, reducing air pollution to the analysis of exhaled breath components for disease diagnostics.

The basis of the enormous demand for gas sensors in the coming years will be the increasing environmental awareness of the population and the associated stricter environmental laws in the industrialised countries. In order to detect and purify exhaust gases from households, industry and transport, it is necessary to know the composition of exhaust gases, for which appropriate chemical sensors are required. Furthermore, the fear of terrorist attacks with chemical warfare agents following the attacks of September 11, 2001 has increased interest in mobile gas analysis systems, particularly in the USA, and has led to an intensification of research work in this field. The global market for sensors has been estimated to grow at an average annual growth rate 7.9% between 2006 and 2011, and 9.0% between 2011 and 2016. (Schroeder, 2016) Conventional quantitative detection of trace gases is based on gas chromatography-mass spectrometry that has been widely accepted as the ‘gold standard’ for
chemical identification of volatile and semi-volatile organic compounds in gas mixtures. (Langford et al., 2014) Besides sophisticated and expensive instruments, substantial sample preparation has to be performed by highly skilled and well-trained operators and limits the use for real-time data. Thus, the demand for selective and sensitive solutions in the field of gas measurement technology providing simple, rapid and cost-efficient analysis is rising. There are nowadays a large number of different gas sensors in which different techniques are used for monitoring a multitude of gases. Electrochemical gas sensors offer sensitive analysis at ppm – ppb levels, but suffer from limited lifetimes and cross-sensitivity towards humidity and interfering gases. Other techniques offer small ultra-low-cost devices, such as pellistors and semiconductor devices with accurate sensitivity but however suffer from signal drifts and cross-sensitivity to varying humidity and other gases. In contrast, optical sensors offer minimal drift, fast responses and measurements can be realized in situ and in real-time, which is of great importance for a wide range of applications. The most important optical techniques are listed in Table I filling the gap between sophisticated laboratory equipment and lower cost sensors. Among these optical sensors, absorption spectroscopy is a powerful method for trace gas analysis, as the measurement is based on the molecules’s physical property (e.g., its absorption) at a specific wavelength. Transitions between energy states within the atom or molecule can be excited via photons which are absorbed thereby. The required energies of these transitions are characteristic for the respective molecule and thus allow precise identification. Vibrational transitions in the infrared spectral range play a special role. Many gases can be identified in this spectral range and quantified very selectively and sensitively. The most important optical techniques are Fourier-transform infrared spectroscopy (FT-IR), non-dispersive infrared spectroscopy (NDIR) and laser absorption spectroscopy (LAS).
Table 1: Optical gas sensing techniques reprinted with permission from ‘Detecting gases with light: a review of optical gas sensor technologies’ Robert Bogue Sensor Review 2015 35(2), 133-140. (Bogue, 2015)

<table>
<thead>
<tr>
<th>Luminescence/fluorescence</th>
<th>Chemiluminescence (CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV Fluorescence (UVF)</td>
</tr>
<tr>
<td>Absorption</td>
<td>UV Absorption</td>
</tr>
<tr>
<td></td>
<td>Non-Dispersive Infrared (ND-IR)</td>
</tr>
<tr>
<td></td>
<td>Fourier Transform Infrared (FT-IR) Spectroscopy</td>
</tr>
<tr>
<td></td>
<td>Differential Optical Absorption Spectroscopy (DOAS)</td>
</tr>
<tr>
<td></td>
<td>Tunable Diode Laser Absorption Spectroscopy (TDLAS)</td>
</tr>
<tr>
<td></td>
<td>Photoacoustic Spectroscopy (PAS)</td>
</tr>
<tr>
<td></td>
<td>Cavity Ring-Down Spectroscopy (CRDS)</td>
</tr>
<tr>
<td></td>
<td>Differential Absorption LIDAR (DIAL)</td>
</tr>
<tr>
<td>Ionization</td>
<td>Photoionization Detection (PID)</td>
</tr>
</tbody>
</table>

Although these optical techniques offer high selectivity and sensitivity, their usage is often limited due to required robustness and portability as well as the requirement of optical alignment as in the case for multi-pass gas cells that have a relatively large size and volume. Thus, the demand of low-cost, robust and field portable gas sensors is of high commercial interest. The development of optical gas sensors that have a potential to evolve from sophisticated laboratory technique towards widespread use and availability are bound to easy to use and align, compact and cost efficient optical components. Moreover, the required sample volume of optical gas cells with path lengths from tens of meters to km range from hundreds of mL to L. The bulkiness of these gas cell assemblies, their rather slow transient sample time
hinders their usage for studying of rapid concentration changes as in security/surveillance, process monitoring, or in exhaled breath diagnostics. Thus, the main aim of the present thesis is the development of cost-efficient optical gas cells (iHWGs) for analytical evaluation of relevant gas molecules and their integration into portable sensor systems for monitoring of small volumes of a variety of molecules. Figure 1 outlines the structure of the research towards compact MIR gas sensors in this thesis:

**Figure 1**: Research topics investigated in this thesis showing the combination of different techniques with substrate-integrated hollow waveguides (iHWGs) for the development of compact and portable systems.
1.1.1 Laser Absorption Spectroscopy LAS

In the last decades, infrared laser absorption spectroscopy (LAS) has made great progress due to recent developments and availability of novel compact MIR laser sources such as quantum cascade lasers QCL and interband cascade lasers ICL. These lasers offer optical power output in the range of 2 - 200 mW at low spectral width (< 30 MHz) with a tunability over a small range of wavelengths operated at room temperature. (Li et al., 2013) The wide range of available QCL/ICL light sources in the MIR spectral range enables various novel applications in gas phase analysis. Analysis with high sensitivity can be achieved by LAS due to fundamental vibration transitions in this area that are characterized by high absorption coefficients combined with a high spectral power density of the laser source. Thus, detection limits in the ppt range were achieved. (Ventrillard et al., 2017) In addition to sensitivity, the LAS offers a high temporal resolution (< 1 s), without the need of sample preparation enabling real-time in situ measurements. Compared to conventional used FT-IR spectroscopy, simultaneous multi-analyte determination is limited in LAS. Depending on the wavelength range of the tunable laser source, only one analyte can be detected at once. Certain laser sources, such as EC-QCL or ICLs (external cavity) have a larger tunable range and allow the detection of multi-analytes. In addition to the choice of the laser source, the detection scheme is also a central part of the system and decisive element of LAS. In the last decades, a considerable research effort was made to develop spectroscopic techniques to maximize the analytical signal and reduce noise sources. Various sensing systems have been developed and tested for a wide range of applications. Direct Absorption Spectroscopy (DAS)(Awtry et al., 2004), Tunable Diode Laser Absorption Spectroscopy (TDLAS), Wavelength modulated Spectroscopy (WMS)(Chao et al., 2012), Cavity-enhanced Absorption Spectroscopy
(CEAS)(Gorrotxategi-Carbajo et al., 2013) and Photoacoustic Spectroscopy (PAS) (Waclawek et al., 2016) are just a few examples that are currently established. Detailed principles of state-of-the-art LAS and its application to environmental monitoring, industrial applications, and exhaled breath analysis are described in Chapter 5, I within the invited review article ‘Instrumentation and applications of Cascade Laser Spectroscopy’.

1.1.2 Exhaled Breath Analysis

Exhaled breath analysis (EBA) is attracting widespread interest as it offers non-invasive, point-of-care (POC) disease diagnostics and metabolic status monitoring in real time. Exhaled breath mainly consists of nitrogen, oxygen, water vapour, argon, and carbon dioxide in relatively high concentrations, whereas volatile organic compounds (VOC), e.g., acetone, isoprene, propanole are present at parts per million (ppm) or sub-ppm levels. In 1971, Nobel Prize winner Linus Pauling and co-workers discovered approx. more than 250 VOCs in normal human breath with capillary gas chromatography. (Pauling et al., 1971) The study was based on the collection of breath from 10-15 exhalations in a cold tube and GC analysis of the released gases after heating of the tube. Further studies on EB carried out by the research group of Michael Philipps at the New York Medical College revealed that EB composition varies strongly and depends from different factors, such as life style, age, nutrition, activity and inhaled air composition. (Phillips et al., 1999) EBA can be divided into two categories: analysis of breath compounds that are formed endogenously in metabolic pathways due to a particular physiological status and breath metabolites after drug/substrate administration. A large number of these identified compounds have been established for the identification of particular medical conditions, e.g., diseases and metabolic disorders. In Tab. 2 some exhaled breath components
and their related physiological symptoms are summarized. Multidisciplinary research on the role of EB in diagnosis, therapy, and safety is an ongoing process, increasing the availability of breath sensors for use in health care settings and for individual use at home.

Breath acetone for example is produced endogenously and mainly from spontaneous decarboxylation of acetoacetate. Patients with newly diagnosed type 1 diabetes mellitus suffer from Diabetic ketoacidosis (DKA) due to the lack of insulin. Resulting fat metabolism process leads to ketone productions, such as acetone, β-hydroxybutyrate, and acetoacetic acid, when fatty acids are metabolized by the liver. The rise of blood glucagon concentration leads to an imbalance of sugar, protein, fat, water, electrolyte, and acid-base. As a novel diagnostic tool, monitoring of breath ketones, particularly acetone as a potentially useful biomarker of ketosis, would allow convenient and non-diagnosis and therapeutic monitoring possibilities. Conventional, daily blood glucose level monitoring by pricking a finger for blood drops could be avoided by EBA of breath acetone. (Jiang et al., 2016) Besides convenient monitoring of blood sugar levels via determination of acetone, EBA has a great potential as a diagnostic tool for metabolic status monitoring in intensive care units (ICUs) or therapy research in ICUs. Intensive care monitoring of exhaled breath with rapid and multi-factorial changes offers real-time monitoring capabilities compared to invasive blood sampling.
Table 2. Overview of various biomarkers occurring in the human breath and their typical physiological symptoms summarized from (Mürtz, 2005; Wang and Sahay, 2009).

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Metabolic Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>C₃H₆O, Lung cancer, diabetes, dietary fat losses, congestive heart failure, brain seizure</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>C₂H₄O, Alcoholism, liver related diseases, lung cancer</td>
</tr>
<tr>
<td>Ammonia</td>
<td>NH₃, Renal diseases, asthma</td>
</tr>
<tr>
<td>Butane</td>
<td>C₄H₁₀, Tumor marker in lung cancer</td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>CO, Oxidative stress, respiratory infection, anaemias</td>
</tr>
<tr>
<td>Carbon disulphide</td>
<td>CS₂, Schizophrenia, coronary, and artery diseases</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>CO₂, Oxidative stress</td>
</tr>
<tr>
<td>Carbonyl sulfide</td>
<td>OCS, Liver related diseases</td>
</tr>
<tr>
<td>Ethane</td>
<td>C₂H₆, Vitamin E deficiency in children, lipid peroxidation, oxidative stress</td>
</tr>
<tr>
<td>Ethylene</td>
<td>C₂H₄, Lipid peroxidation, ultra violet radiation damage of skin</td>
</tr>
<tr>
<td>Isoprene</td>
<td>C₅H₈, Blood cholesterol</td>
</tr>
<tr>
<td>Methane</td>
<td>CH₄, Intestinal problems, colonic fermentation</td>
</tr>
<tr>
<td>Methanol</td>
<td>CH₃OH, Nervous system disorder</td>
</tr>
<tr>
<td>Methyl nitrate</td>
<td>CH₃NO₃, Hyperglycemia in Type 1 diabetes</td>
</tr>
<tr>
<td>Nitrogen monoxide</td>
<td>NO, Asthma, bronchiectasis, hypertension, rhinitis, lung diseases</td>
</tr>
<tr>
<td>Oxygen</td>
<td>O₂, Respiration</td>
</tr>
<tr>
<td>Pentane</td>
<td>C₅H₁₂, Peroxidation of lipids, liver diseases, schizophrenia, breast cancer, rheumatoid arthritis</td>
</tr>
<tr>
<td>Pyridine</td>
<td>C₅H₅N, Periodontal disease</td>
</tr>
<tr>
<td>Sulfur compounds</td>
<td>Hepatic diseases and malordor, lung cancer</td>
</tr>
</tbody>
</table>
Exhaled breath sensing of respiratory CO₂ can help in the assessment of respiratory activity, disease severity, and treatment response in anaesthetized patients. Moreover, measuring stable carbon isotopes $^{12}$CO₂ and $^{13}$CO₂ in exhaled breath successfully provides information about metabolic processes within the body. After administration of $^{13}$C labelled sugar, metabolic state monitoring by detecting isotopic changes in the exhaled CO₂ in breath is possible with precise and selective sensor technology. Comparison of the sampled ratio ($^{13}$CO₂/$^{12}$CO₂) to a baseline ratio in the organism allows non-invasive determination of organ function and nutritional status. Furthermore, the $^{13}$C urea breath test (UBT) has established itself as one of the most important non-invasive methods for detecting *Helicobacter pylori* infection in the body. (Goddard and Logan, 1997) Although, breath analysis was established primarily as a diagnostics tool in human medicine, there has been a considerable interest for its use in animals (pigs, rats, mice, etc.) in veterinary diagnostics or surveillance and research in clinical animal models. (Amann and Smith, 2005)

Animal models offer the opportunity to investigate mechanisms of disease development and to test new forms of therapy and therapeutics. Even today, no causative drug treatment options are available for many chronic diseases. This is mainly due to the fact that molecular pathogenesis mechanisms of these diseases have not yet been fully clarified. Among the mammal species that are frequently used in preclinical research, the mouse model plays an important role as a laboratory animal. There are many reasons for their popularity as laboratory animals. One of the most important benefits is the availability of many different inbreeding strains, which ensures, for example, that the genetic variability in the experimental groups within a study is extremely low. In addition, the comparatively simple and inexpensive husbandry of the animals plays an important role. The use of mouse models to simulate clinical
intensive care conditions, allows repeated measurements and post mortem exploration of involved and affected organs. Conventional applied capnography sensors and blood gas analyzers as used in human medicine are limited for the small dimensions of mice and rats and their breath volume. These systems require large sample volumes and are only suitable for human breath analysis. IR spectroscopy techniques employing miniaturized gas cells has been proven to be a highly selective, sensitive, and accurate method. First applications of IR spectroscopy were based on hollow-core waveguides coupled to FT-IR spectroscopy (Wilk et al., 2011) (Fortes et al., 2013), EC-QCL (Wörle et al., 2013) and applying chemo-metric strategies (Seichter et al., 2013).

1.2 Theoretical Background

1.2.1 Fundamentals of absorption-based spectroscopy

Optical absorption spectroscopy for the analysis of molecules is based on the property of molecules to absorb electromagnetic radiation at a specific wavelength (discrete energy levels). A variety of chemical species exhibit strong absorption in the spectral region from 4000 cm\(^{-1}\) to 400 cm\(^{-1}\) (2.5 - 25 μm), commonly referred to as the mid-infrared MIR region. Due to the excitation of fundamental vibrational (including rotational and vibro-rotational) transitions, spectroscopy offers the identification of molecules by the analysis of their characteristic spectrum with appropriate infrared sensing technology. MIR spectroscopy for the determination of absorption lines or bands can be applied to most molecular species in solid, liquid/dissolved or gas state. Optical absorption gas sensors offer the measurement of the attenuation of laser light (e.g., globar, black body, infrared laser, etc.) which passes through a
gas sample in a transparent sample container with appropriate infrared detectors. The correlation between the attenuation of light and the particle density $N$ of the absorbing gas molecules can be described by the Lambert Beer law.

$$I = I_0 e^{-\sigma(\lambda)NL}$$  \hspace{1cm} (1)

$I$ is the intensity of the light after passing through the sample cell, where $I_0$ is the intensity of the incident light. The product of particle density $N$ and absorption cross section $\sigma(\lambda)$ can also be expressed as the linear absorption coefficient $\alpha(\lambda)$.

$$\alpha(\lambda) = \sigma(\lambda) \cdot N$$  \hspace{1cm} (2)

$$A = \alpha(\lambda) \cdot L$$  \hspace{1cm} (3)

The absorbance $A$ ($A = -\log I/I_0$) of the gas sample can thus be determined by the absorption coefficient $\alpha(\lambda)$ and the length $L$ of the absorbent medium. $A$ is thereby unit-less but often described in ‘absorbance units’ (AU).

An absorption spectrum displays the absorbance $A$ as a function of wavelength (e.g., in $\mu$m) or its reciprocal, wavenumber (in $\text{cm}^{-1}$). Typical absorption spectra are shown as inset in Fig. 2 for a single gas (methane) plotted at higher spectral resolution and for a series of gases in the MIR, measured with Fourier transform infrared (FT-IR) spectrometer using a broadband light source for multi-analyte analysis. FT-IR spectroscopy is based on the measurement of an interferogram through the use of a Michelson interferometer. To create spectroscopic data (frequency-domain), the Fourier transform technique is applied to the interferogram (time-domain). FT-IR spectrometers provide improved signal-to-noise ratios (S/N) compared to spectrometers using a moveable grating (dispersive infrared instruments). The so-called multiplex advantage is based on the measurement of the entire infrared spectrum with one scan, allowing signal averaging by combining individual scans. Moreover, FT-IR instruments
achieve a higher optical throughput (Jacquinot’s advantage) resulting in more energy reaching the sample and thus the detector. (Griffiths and De Haseth, 2007)

**Figure 2:** Schematic principle of a transmission absorption spectroscopy. Molecules absorbing infrared energy via vibrational transitions in a transparent cell generating distinctive infrared absorption signatures also termed ‘molecular fingerprint’. Top left: absorption spectrum for 10% methane at atmospheric pressure at 3028.7 cm$^{-1}$. Bottom right: MIR absorption spectra for exemplary six gases: CO, CO$_2$, CH$_4$, H$_2$O and N$_2$O (all at 100% vol). All spectra were calculated from HITRAN 2012. (Gordon et al., 2017)

Improved SNRs enhance the sensitivity of the instrument and allow the analysis of small absorptions. FT-IR spectroscopy has been used for applications ranging from molecular spectroscopy to industrial process for the analysis of a wide range of molecules. Portable systems are commercially available and allow in-field analysis. With increasing availability of QCLs and ICLs, the development of robust, highly selective and sensitive laser spectrometers
has gained high interest. In tunable diode laser absorption spectroscopy (TDLAS), the concentration of the target gas analyte can be measured by transmitting laser radiation through a sample cell containing the gas within a compact and robust design. The laser emission is thereby tuned over of a single absorption line and absorption is measured by appropriate detectors. Tuning of the emission can be performed by controlling of the drive current and laser temperature.

1.2.2 Mid Infrared Laser sources

1.2.2.1 Quantum cascade laser

Photon generation in conventional interband diode lasers is based on the recombination of electrons in the conduction band with holes in the valence band. The energy of the photons being generated in the conduction band is essentially determined by the energy gaps of the used semiconductor material. In 1971, R. F. Kazarinov and R. A. Suris proposed that photons are also produced and amplified by passing through transitions of electrons or holes between quantized sub-bands in the conduction band or in the valence band of quantum wells (QWs) in a semiconductor heterostructure. (R.F. Kazarinov and R.A. Suris, 1971) More than 20 years after the work of R. F. Kazarinov and R. A. Suris, the first experimental QCL was realized by J. Faist et al. at Bell Laboratories in 1994. (Faist et al., 1994) Instead of bulk semiconductor materials as in diode lasers, thin layers of varying materials are stacked to form a superlattice. Material systems based on InGaAs/InAlAs/InP cover the MIR spectral range from approximately 3 to 16 μm. (Gutowski et al., 2017) (Szerling et al., 2017) (Vitiello et al., 2006) (Vitiello et al., 2015) By changing the thickness of these quantum well layers, the series of
discrete energy levels can be directly controlled. Thus, emission wavelength can be tailored precisely over the entire MIR and into the far-infrared using the same material system.

Faist et al. were able to separate a period of superlattice into two regions – an active region where the actual laser transition takes place and the injection region that guides the electrons to the upper laser level in the active region. A schematic diagram of its basic principle is shown in Fig. 3:

![Schematic diagram of quantum cascade laser band structure with the active region and the relaxation/injection region.](image)

**Figure 3:** Schematic diagram of quantum cascade laser band structure with the active region and the relaxation/injection region.

In QCLs the gain medium consists of several repetitions of identical periods, where each electron undergoes multiple transitions, leading to multiphoton emission (cascade effect). Electrons are injected into the adjacent identical stage after photon emission by jumping from the upper level to the lower energy level. QCLs can be used in pulsed and continuous wave (cw) operation mode with combining a good beam quality with high output power besides easy handling. Wavelength selection can be obtained by various approaches. One approach is the
application of a layer with a modulated refractive index to the active regions. Due to this
distributed feedback (DFB) (Zeller et al., 2010) in the laser resonator, the wavelength
(corresponding to the period length of the modulation is preferred, while the other resonator
modes are suppressed. Thus, DFB-QCLs provide mode hop free tuning capability and single
mode behavior. Another approach to waveguide selection is the use of an external cavity (EC),
where a grating acts as wavelength filter element. (Luo et al., 2001) Different configurations
such as Littman-Metcalf, Littrow and Littrow back-extraction setup (Hawthorn et al., 2001)
have been realized.

1.2.2.2 Interband cascade laser

Interband cascade lasers (ICL) may be viewed as a hybrid of conventional diode lasers and
QCLs as they are based on electron-hole recombination and interband transitions with a
cascade scheme for multiphoton emission. Compared to QCLs, lower electrical input powers
are required due to the use of interband transitions instead of intersubband transitions. Due to
the fact that interband transitions are used, a more narrow range of wavelengths over which
lasing can be achieved at room temperature ($\approx$ 2.9 to 5.7 $\mu$m) is accessible. Thus, the gap (3-6
$\mu$m) in the MIR region between conventional room-temperature diodes and quantum-cascade
lasers could be filled by the development of ICLs which renders them as ideal light source for
the detection of strong C-H vibrational bands. The concept of ICLs was developed in 1995 by
R. Q. Yang (Yang, 1995), the first experimental realization was achieved by Lin et al. in 1997
with a 3.8 $\mu$m laser. (Lin et al., 1997)
**Figure 4:** One period of an interband cascade laser structure. Interband tunnelling to the next conduction band is shown (grey circle).

Material heterostructures (e.g., InAs/Ga (In)Sb) with a broken type II band structure are required for production. The possibility of a cascade structure is achieved by the possibility of electrons in the valence band being able to tunnel into the conduction band of the next period (see Fig. 4, grey circle).

This is also made possible by the use of Type II materials, which allow the production of extreme band structures in smallest dimensions. In ICL, single-mode operation can also be implemented by a DFB structure. (Yang et al., 2004) Since their first cw-operation at room temperature (RT) was first demonstrated in 2008 (Kim et al., 2008), subsequent advancements in tuning response and efficiency has led to a variety of spectroscopic applications for the analysis of hydrocarbons such as methane, ethane and aldehydes. (Ren et al., 2015; Song et al., 2017) Nowadays, commercially available DFB-ICLs with very low electrical drive power
pave the way for compact and portable chemical sensing applications by reducing overall sensor footprint and being able to be operated by battery or even solar power.

1.2.3 Hollow waveguide

Although hollow waveguides (HWGs) were initially designed for delivering high peak power laser light in industry and surgical applications, they have proven themselves as highly efficient miniaturized gas cells for low volume gas samples (few milliliters or less). HWGs are essentially hollow core light-pipes where interaction of radiation with the analyte takes place inside the hollow core. In the late 1970s, first HWGs were produced by Garmire from two strips of aluminum that are separated by dielectric spacers. (Garmire et al., 1976) Cylindrical HWGs were introduced by Miyagi by deposition of germanium into an aluminum pipe before plating nickel on top. (Miyagi, 1983) By dielectrically coating the inside of the hollow core wall with a metal (e.g., Ag), and a protective dielectric (e.g., AgI), these structural tubes (e.g., glass, metal, plastic) allow MIR radiation propagation via reflection along the inside walls of the HWG. A schematic illustration of the described waveguide concept is shown in Fig. 5. Via multiple reflections on the metallic inner wall light can propagate through the air core. Application of HWGs as gas transmission cells was shown by Harrington in 1991. (Saggese et al., 1991) A 150 mm long hollow sapphire fiber with a 1.06-mm inner diameter was used for optical spectroscopy of 20% CO₂. Although various research groups demonstrated that fiber-optic HWGs as optical gas cells provide short sample transition times due to small volume of probed gas, their integration in compact portable sensing systems is hindered by the mechanical flexibility and length.
**INTRODUCTION**

Figure 5: Cross-section of the hollow waveguide gas cell structure. For broadband MIR reflection, metallic Ag layer is coated by a thin dielectric film of AgI. The inner part consists of a metallic Ag layer coated by a single dielectric film of AgI.

Besides low insertion loss and nonlinearity, HWGs have a low beam divergence and losses are proportional to $a^{-3}$, where $a$ is defined by the bore radius. Moreover, HWGs suffer from high bending losses, which are proportional to $R^{-3}$, where $R$ is the bending radius. Signal attenuation losses and mechanical stresses due to coiling hamper their application as miniaturized IR absorption cells.

A new generation of HWG structures termed substrate-integrated hollow waveguides (iHWGs) has been developed by Mizaikoff and collaborators, thereby integrating the approach of an optical efficient waveguide embedded into a solid-state, planar substrate material. (Wilk et al., 2013) Commonly used multipass cells with path lengths of several meters are used for
trace analysis and quantitative precision measurements. In principle, the detection sensitivity is improved by increasing the total optic path length that travels through the analyte gas volume.

Nevertheless, conventionally used Herriott (Herriott and Schulte, 1965) and White cells (White, 1942) as bulky gas cells suffer from their requirement of huge gas volume and precise optical alignment. Compared to these cells, iHWGs achieve a higher volumetric optical efficiency due to optimal ratio of optical path length and cell volume. The feasibility of iHWG based gas sensors for detection of trace gases has been demonstrated for various sensing scenarios: environmental monitoring (Petruci et al., 2013; Petruci et al., 2015), process analytics (Rohwedder et al., 2014), catalysis research (Kokoric et al., 2016) and breath diagnostics (Perez-Guaita et al., 2014)(Kokoric et al., 2015).
2. Results and Conclusions

Innovative advances in MIR sensor technology for sensing of low gas volume are shown in the present thesis. It was successfully demonstrated, that gas sensing based on iHWGs is a highly versatile tool for various analytical applications requiring high robustness and sensitivity, molecular selectivity with a high potential for remote sensing applications. Smart combination of latest developments in advanced light source technology, efficient optical fiber transmission, and miniaturized gas cells offers great potential for compact sensing and diagnostic platforms. In the following, a summary of the most significant results achieved within this dissertation and corresponding published publications is provided.

The first journal article ‘Instrumentation and applications of Cascade Laser Spectroscopy’ is an invited review article, that summarizes recent technological developments of cascade laser spectroscopy. Since their introduction, QCLs and ICLs have made enormous strides and are now covering ranges in wavelength and output power larger than ever. With increasing efficiency, reliability and control of these lasers, various applications of laser based optical systems have been demonstrated for industrial process control, medical applications and standoff detection for security. A brief overview state-of-the-art spectroscopic techniques that have been utilized for these applications, including direct absorption spectroscopy (DAS), wavelength modulation spectroscopy (WMS), photoacoustic spectroscopy (PAS), and optical cavity enhanced spectroscopy (OCES) is provided.

The potential of hollow waveguide based MIR sensors for multi-analyte monitoring via vertically emitting ring-shaped two-color QCLs in gas/vapor phase sensing applications was demonstrated in ‘Advanced gas sensors based on substrate-integrated hollow waveguides and dual-color ring quantum cascade lasers’, which was resulting from a research
collaboration with TU Wien, Vienna, Austria. A miniaturized iHWG gas cell simultaneously serving as MIR waveguide with a sample volume of only 600 μL was directly coupled to a ring QCL with an embossed 2nd order DFB grating emitting at 1144 cm⁻¹ and 1170 cm⁻¹ at room temperature in pulsed mode. For various spectroscopic applications, compact coherent light sources are needed that allow the analysis of several molecules. With multi-wavelength single mode emission obtained from the same laser ridge, compact and cost-efficient optical setups can be realized as there is no need for separate light sources for each wavelength. Thus, spectroscopy of several molecules within the same optical path can be obtained by simultaneous emission or selection of single wavelength with the proper feedback. The developed optical system at Ulm University paired the dual-color ring QCL that offers vertically emitting and a collimated rotationally symmetric beam with a high volumetric optical efficient iHWG. This combination offers compact and simultaneous sensing of several molecules with fast transition times. In this study, light emission from the ring QCL was directly collimated by a ZnSe lens into the rectangular cross section of the optical channel for optimum coupling. As demonstrated, the system represents a proof of principle combination for analytical studies, such as quantitative vapor phase analysis of furan and 2-methoxyethanol. Due to the liquid nitrogen cooling requirement and collimation with an optical lens, the potential of entire miniaturization has not been fully exploited yet. Direct integration of the laser source and miniaturized detecting elements into the iHWG is the focus of an ongoing investigation. Avoiding the usage of any additional beam guiding optics and thermoelectrically cooled IR detectors will result in improved device robustness and compactness making it suitable for portable and in-field monitoring. Nevertheless, this study corroborates the potential
of miniaturized gas cells paired with novel laser sources for compact optical sensors clearing the way for completely new applications.

The high adaptability of iHWG technology as an optical sensor platform was illustrated in the article ‘iHWG-ICL: Methane Sensing with Substrate-Integrated Hollow Waveguides Directly Coupled to Interband Cascade Lasers’, where TDLAS with novel cascade lasers was presented. Combining compact gas cells with type II lasers with enhanced current efficiency at reasonable electrical power consumption ideally meets the demands for the development of compact MIR sensing systems. Using a room-temperature DFB-ICL emitting around 3.3666 μm, an optical gas sensors was built and used for comparison of various iHWG material and optical path length configurations. The comparison showed that gold deposition procedure that is applied to enhance reflectivity is crucial for analytical performance of the waveguides. When propagating along the HWG structure, surface scattering losses due to low surface quality of the fabrication occur and are highly affecting the attenuation. It could be shown that aluminum waveguides that were just polished to mirror like finish achieved superior sensitivity compared to gold coated brass waveguides. Based on these findings, iHWGs for the analyzer system presented in Paper V (Chapter 5, V) were fabricated as described in previous publication.

For application in hazardous environments with limited access opportunities, robust gas sensors with remote sensing capability are required for the fast analysis of toxic gases. In the article ‘Fiber-Coupled Substrate-Integrated Hollow Waveguides: An Innovative Approach to Mid-infrared Remote Gas Sensors’ the first prototype of a fiberoptically coupled iWHG was developed and characterized. The remote sensor concept was tested for
the analysis of isobutylene, cyclopropane and methane and demonstrated detection sensitivity in the low ppm range with rapid sample transition times.

Finally, the article ‘Advanced Photonic Sensors Based on Interband Cascade Lasers for Real-Time Mouse Breath Analysis’ presents the developed breath analyzer for the mouse intensive care unit. The sensor was developed for deployment as a real time monitor of exhaled CO$_2$ and the ratio of both stable isotopes $^{12}$CO$_2$ and $^{13}$CO$_2$. Moreover, with the addition of an optochemical sensor based on polymer nanofibers for ultra-fast oxygen monitoring, real time determination of the respiratory quotient was possible. Due to the unique possibilities of TDLAS as laser spectroscopy technique, a robust standalone system with no need of sample pretreatment was developed and installed. The analyzer system was tested during 14 experiments in the mouse intensive care unit of the Institute of Anesthesiologic Pathophysiology and Method Development, Ulm University Medical Center and successfully demonstrated highly accurate and reliable results. A CO$_2$ measurement precision of 1.6 ‰ at 480s integration time was verified via Allan variance analysis.
3. References


Kim, M., Canedy, C. L., Bewley, W. W., Kim, C. S., Lindle, J. R., Abell, J.,


4. List of Figures

Figure 1: Research topics investigated in this thesis showing the combination of different techniques with substrate integrated hollow waveguides (iHWGs) for the development of compact and portable systems. ................................................................. 10

Figure 2: Schematic principle of a transmission absorption spectroscopy. Molecules absorbing infrared energy via vibrational transitions in a transparent cell generating distinctive infrared absorption signatures also termed ‘molecular fingerprint’. Top left: absorption spectrum for 10% methane at atmospheric pressure at 3028.7 cm\(^{-1}\). Bottom right: MIR absorption spectra for exemplary six gases: CO, CO\(_2\), CH\(_4\), H\(_2\)O and N\(_2\)O (all at 100% vol). All spectra were calculated from HITRAN 2012. (Gordon et al., 2017) ........................................................................................................................................ 18

Figure 3: Schematic diagram of quantum cascade laser band structure with the active region and the relaxation/injection region. .................................................................................................. 20

Figure 4: One period of an Interband cascade laser structure. Interband tunnelling to the next conduction band is shown (grey circle). ................................................................. 22

Figure 5: Cross-section of the hollow waveguide gas cell structure. For broadband MIR reflection, metallic Ag layer is coated by a thin dielectric film of AgI. The inner part consists of a metallic Ag layer coated by a single dielectric film of AgI ................................................................. 24
5. Journal Articles

5.1 Paper I. Instrumentation and applications of Cascade Laser Spectroscopy

The scope of the paper was determined in close communication with B. Mizaikoff. E. Tütüncü wrote the manuscript. B. Mizaikoff edited the manuscript for the final version.


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Instrumentation and Applications of Cascade Laser Spectroscopy

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1 INTRODUCTION

Cascade laser spectroscopy is a relevant technique for analyzing trace gases in a diversity of applications ranging from fundamental molecular studies, industrial processes control, and atmospheric/environmental research to medical/clinical applications. Recent developments in the field of laser light sources and spectroscopic techniques pave the way toward advanced optical sensing systems. For the selective and sensitive detection of trace gases, spectral analysis in the MIR regime covering 400–4000 cm\(^{-1}\) (2.5–25 \(\mu\)m) is of particular importance, as many molecules have characteristic absorption features in this wavelength region sometimes also called the ‘fingerprint’ regime. In contrast to broadband light sources as used in conventional MIR absorption spectroscopy, laser technology offers light sources with narrow-line widths and resulting spectral power densities exceeding conventional broadband sources by orders of magnitude. Advances in semiconductor laser technology, and in particular the introduction of QCL utilizing intersubband transitions next to so-called ICL have led to a new generation of spectroscopic/sensing systems for ultra-sensitive and molecularly selective chemical sensing. Conventional laser sources such as lead salt diode lasers frequently require cooling besides providing rather limited output power (i.e. few mW), and gas lasers (e.g. CO and CO\(_2\)) with inherently limited tenability and large dimensions have somewhat limited their utility in spectroscopic sensing applications. Drawbacks of coherent light sources based upon difference frequency generation (DFG) and optical parametric oscillators (OPO) relate to their complexity and large dimension. In contrast, MIR cascade lasers are the latest addition to the family of coherent MIR laser sources and have attracted considerable attention since their introduction owing to a number of unique capabilities regarding output power, efficiency, and wavelength coverage. Hence, they nowadays populate a diversity of application scenarios reaching from laboratory settings toward industrial-scale sensing systems. Optimized band structure engineering and quantum well design nowadays allows tailoring QCL emissions from wavelengths as short as 2.6(\(\text{THz}\))–250 \(\mu\)m (i.e. far infrared or THz, respectively). While most THz lasers still require cryogenic cooling, most recently THz-QCLs operated at room temperature have been reported.
Infrared Spectroscopy

Infrared spectroscopy is a powerful tool for identifying molecules based on their vibrational and rotational transitions. It is widely used in various fields such as chemical analysis, pharmaceuticals, and environmental monitoring. The method relies on the absorption of infrared radiation, which results in the excitation of vibrational and rotational modes of molecules. The infrared spectrum provides unique fingerprints for different molecules, allowing for their identification and quantification.

The theoretical basis for infrared spectroscopy is the concept of quantum mechanics. The energy levels of molecules in the vibrational and rotational states are quantized, and transitions between these states occur when the energy difference matches the energy of an infrared photon. The spectrum is typically recorded as a function of wavelength or wavenumber, with the intensity of the absorption peak indicating the strength of the transition.

Infrared spectroscopy can be performed in various modes, including transmission, reflection, and attenuated total reflection (ATR). Each mode has its advantages depending on the sample nature and the specific application. Transmission mode is commonly used for transparent samples, while reflection and ATR modes are suitable for opaque or thick samples.

In recent years, infrared spectroscopy has been complemented by other spectroscopic techniques, such as Raman spectroscopy and nuclear magnetic resonance (NMR), to provide a more comprehensive understanding of molecular structure and dynamics. The combination of these methods is particularly useful in complex systems, such as in biological samples or inorganic materials, where multiple interactions contribute to the observed signal.

The development of advanced infrared detectors and data analysis techniques has further expanded the capabilities of infrared spectroscopy. This includes Fourier transform infrared (FTIR) spectroscopy, which enhances sensitivity and resolution compared to traditional infrared spectroscopy. Additionally, the use of infrared spectroscopy in combination with other techniques, such as mass spectrometry, enables the identification of trace compounds in complex mixtures.

In summary, infrared spectroscopy is a versatile and essential method for molecular analysis, providing valuable information about the structure, composition, and interaction of materials. Its applications continue to grow, driven by advances in technology and the expanding needs of various scientific and industrial sectors.
these semiconductor lasers,\(^{(4)}\) which utilizes bandstructure engineering of epitaxially grown semiconductor heterostructures composed of layers of indium arsenide (InAs), gallium antimonide (GaSb), and aluminum antimonide (AlSb). As schematically shown in Figure 1(b), light emission is based on transitions between the conduction and the valence bands of the heterostructure exploiting the type-II band alignment of material systems based on GaSb. When applying an external electric field to the gain material, electrons transit into the next quantum well, whereas holes move to the left. Compared to diode lasers and QCLs, electrons and holes needed for lasing activity are internally generated at semi-metallic interfaces of GaInSb/AlAs/InAs, which are located within each stage, and internally feed each cascade with carriers. Recent improvements in laser performance could be achieved with heavily n-doped electron injector regions for equalizing electron and hole populations within the active quantum wells (also known as carrier rebalancing). Moreover, ICLs offer substantial design flexibility regarding the emission wavelength within the MIR regime given by the tunability of the InAs layer thickness of typically applied W-shaped quantum wells (W-QWs). The optical interband recombination at these quantum wells requires low electrical input power, thus yielding radiation emission at substantially less energy compared to QCLs in the 3–6\( \mu \)m region. With the realization of ICLs, the way toward handheld or battery-operated laser spectroscopy and sensing applications, yet with sufficient optical output power appears to be paved. Since the first demonstration of CW ICLs emitting at 3.75\( \mu \)m at room temperature in 2008,\(^{(15)}\) significant advances toward compact narrowband ICLs including tenability and further improved efficiency have been reported. More detailed descriptions on their operation and application examples (e.g. detection of chemicals) taking advantage of distributed feedback ICLs are provided by Vurgaftman (see Sensitive Chemical Detection with Distributed Feedback Interband Cascade Lasers) in this book.

Depending on the resonator design, different configurations for wavelength tuning in QCLs and ICLs have been established. Laser resonators can be generally classified into three common types: (i) Fabry–Pérot (FP), (ii) distributed feedback (DFB), and (iii) external cavity (EC) devices. The most straightforward concept takes advantage of a Fabry–Pérot resonator, which creates multiple longitudinal modes resulting in multimode emission at several wavelengths. For most applications — for example spectroscopy and sensing in the gas phase — single mode emission is of paramount importance for achieving the frequently required high spectral resolution. Compared to FP-type lasers based on conventional laser chips with highly reflecting coatings at the end facets establishing the active cavity, DFB lasers typically have a Bragg grating embossed at the top ridge of the laser chip, thereby ensuring mode-hop-free single mode longitudinal emission. Spectral tuning of thus emitted single laser modes within a small range of wavelengths may be realized by changing the device operating temperature or the injection current. Broad spectral tuning is achieved by using external diffraction gratings, as realized in external cavity coupled laser systems. Thereby, broad spectral tuning across several hundreds of wavenumbers can nowadays be achieved via continuously changing/scanning the grating angle.

**Figure 1** Simplified scheme of the gain region under bias with associated electronic transitions. The diagrams indicate electron tunneling through a series of quantum wells, thereby giving rise to the emission of a cascade of photons for a quantum cascade laser (QCL) (a), and an interband cascade laser (ICL) (b).

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Beer–Lambert law writes as follows:

\[ I(\nu) = I_0(\nu) \cdot \exp(-\alpha(\nu) \cdot L) \]  

where transmitted and incident light intensity at a frequency of \( \nu \) is \( I(\nu) \) and \( I_0(\nu) \), respectively. The absorption coefficient \( \alpha(\nu) \) of the sample is proportional to the concentration of the absorbing material.

A convenient form of the Beer contribution when applied in high resolution laser spectroscopy for gas analysis is:

\[ \alpha(\nu, T) = S(T) \cdot \Phi(\nu, T, P, x_i) \cdot n(p, T) \]  

Here, the absorption coefficient is correlated with the line strength of a species \( S(T) \) in a transition \( \nu \), a line shape function \( \Phi(\nu, T, P, x_i) \), and the number density \( n(p, T) \).

High-resolution laser-based absorption spectroscopy with tunable diode lasers (TDLAS) can be performed with cascade lasers, which can be tuned across an absorption line of the analyte species. After propagating through the sample medium, the wavelength-dependent transmission is recorded at a suitable detector.

Tuning of the emission wavelength may be achieved by varying the operating current or the temperature of the laser. Current tuning may be accomplished at high modulation frequencies up to the MHz range, thus enabling high time resolution. A potential disadvantage of TDLAS is the low sensitivity (i.e. detection of absorbances of approx. \( 10^{-5} \)), as small signal changes frequently have to be determined on top of a sizeable background signal. In addition, low-frequency noise in the signal originating mainly from mechanical instabilities, laser intensity noise, and other external fluctuations may affect the signal. The signal-to-noise ratio (SNR) can be enhanced using modulation techniques such as wavelength modulation (also known as wavelength modulation spectroscopy, WMS) via, for example, a sinusoidal frequency modulation of the laser wavelength. The wavelength is tuned across an absorption line of the sample gas as in conventional DAS and additionally modulated at a high-frequency \( f \) (i.e. in the kilohertz regime). A schematic experimental setup of DAS and WMS is shown in Figure 2.

Infrared spectroscopy

3 INSTRUMENTATION

3.1 Tunable Diode Laser Absorption Spectroscopy

For the determination of quantitative absolute concentration, temperature, pressure, velocity, and mass flux of the analyte gas, DAS has been proven as an effective in situ technique. The basic principle relates to the Beer–Lambert law, which describes the exponential decay of light intensity \( I \) passing through a medium with an absorbing gas in dependence on the concentration of the absorbing species, and the optical path length \( L \). The Beer–Lambert law writes as follows:

\[ I(\nu) = I_0(\nu) \cdot \exp(-\alpha(\nu) \cdot L) \]  

If the wavelength-modulated light beam propagates through the sample cell, the change in intensity of the laser, and the absorption of the analyzed gas results in an amplitude modulation at the applied wavelength. Using a lock-in amplifier, the periodic detector signal is demodulated at a multiple integer \( n \) of the modulation frequency \( f \) (i.e. \( n=1, 2, 3, \text{etc.} \)). Using the second harmonic (also known as 2f-signal), background-free measurements can be performed. Compared to DAS, in WMS an assignment of the selected line(s) or determination of the integrated line strength is not required. Thus, the sensitivity can be significantly increased, and absorbances as low as \( 10^{-5} \) to \( 10^{-6} \) can be detected. Hence, WMS is a spectroscopic method of choice, if cost-effectiveness, facile implementation, and robustness is required.

For both spectroscopic techniques, an enhancement of detection sensitivity can be achieved by increasing the optical path length via, e.g. multipass absorption cells. Multipass cells (MPCs) such as white cells and Herriott cells(17) enable effective absorption path lengths of several tens of meters via folding the IR beam multiple times within a defined gas volume, thereby significantly increasing the absorption path length versus the actual dimension of the gas cell. A viable alternative for these rather complex and expensive gas cell types usually requiring several hundreds of milliliters of sample gas volume are recently introduced so-called substrate-integrated hollow waveguides (iHWG) pioneered by the Mizaikoff research group for analyzing minute gas volumes with high optical efficiency.(18) Early studies were conducted with photonic band gap hollow waveguides(19) requiring elongated hollow fibers in order to obtain suitable optical path lengths for gas analysis. In the iHWG concept, a hollow waveguide channel structure is embedded into a solid-state planar substrate material offering high mechanical robustness, extended absorption path length, flexibility in channel design, and low manufacturing costs (see Figure 3a). Trace gas analysis of \( \text{CO}_2 \), \( \text{CH}_4 \), \( \text{C}_2\text{H}_6 \), ozone, \( \text{H}_2\text{S} \), and \( \text{SO}_2 \) with iHWGs has been demonstrated for various sensing scenarios: environmental monitoring(20–24) catalysis research(25) breath diagnostics(26,27) and remote sensing.(28) For low volume analysis of furan and 2-methoxyethanol, a first combination of an iHWG with ring-shaped vertically emitting quantum cascade laser (riQCL) providing two distinct emission wavelengths (see Figure 3b) was demonstrated by Tüttüncü et al.(29) The ultra-compact dual color-iHWG gas sensing device enables the simultaneous detection of two vapor phase species due to distinctive absorption features at the emission wavelengths of the riQCL (i.e. \( 1144 \) and \( 1170 \text{cm}^{-1} \)) and small gas cell dimensions (required sample volume 600 \( \mu \text{L} \).
3.2 Photoacoustic Spectroscopy

PAS is based on the photoacoustic effect, which has been discovered by Bell in 1880.\(^{30}\) PAS initiates direct or indirect molecular oscillations via the absorption of electromagnetic waves, which result in inelastic interactions with the surrounding molecules, and thus local heating within the medium. If the light source is electronically or mechanically modulated, e.g. by using a chopper, the heat input into the system occurs periodically and can be detected as a pressure wave at acoustic frequencies. The frequency of the periodic pressure signal is thus determined by the modulation frequency. The created pressure wave (i.e. sound wave) can be detected using more or less complex microphones. By continuously tuning the wavelength of the laser light the resonances of the molecule of interest, a PA spectrum can be recorded. Recent developments taking advantage of exceptionally sensitive microphones, along with the evolvement of the laser light sources discussed herein renders PAS ideally suited for trace gas sensing applications, besides the analysis of liquid and solids. A conventional PAS experiment applied in gas sensing scenarios is shown in Figure 2b. A pulsed or a modulated CW laser is combined with a resonant cell using lock-in amplifiers at the modulation frequency for signal read-out. Alternatively, a quartz tuning fork (QTF) may used as a highly miniaturized acoustic transducer, as

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**Figure 2** Schematic principles of selected laser spectroscopic techniques. (a) Direct absorption spectroscopy (DAS), and wavelength modulation spectroscopy (WMS), (b) photoacoustic spectroscopy (PAS), and (c) optical cavity enhanced spectroscopy (OCES).
shown in so-called quartz-enhanced photothermal spectroscopy (QEPAS) for detection of a variety of trace gases.\(^{(31)}\) Due to the low losses, small dimensions, and low cost, quartz crystals – similar to the ones used in quartz watches – are ideally suited for PAS sensing applications. Besides their robustness toward environmental noise, QTFs offer high \(Q\)-factors at ambient conditions (i.e. \(10^{4}\) at 1 atm), and \(\geq 100,000\) if encapsulated and operated in vacuum. Typically, the quality factor \(Q\) of acoustic resonances in conventional PAS sensors ranges between 40 and 200. For a comprehensive review on recent developments and progress in PAS technology see chapter (see Photoacoustic Spectroscopy in Trace Gas Monitoring).

### 3.3 Cavity-enhanced Spectroscopy

Cavity-enhanced spectroscopy techniques have extensively been used for trace gas sensing applications due to the increased effective optical path length. Using these techniques, absorption path lengths up to several kilometers are achieved based on high-finesse optical cavities established between two highly reflective mirrors with reflectivity values \(R\) of 0.999. By analyzing the light intensity exiting the cavity instead of the strength of the absorption, noise is dramatically reduced. Compared to conventional absorption techniques, light source intensity fluctuations have no effect on the optical measurement, as the decay rate of an injected laser beam trapped within an optical cavity is observed rather than the change in light intensity. This direct approach using pulsed or CW laser sources takes advantage of several thousand reflections within the resonator, and thus, the optical path length may be increased by \(10^4\). Several different concepts including cavity ring-down spectroscopy (CRDS), intracavity-absorption spectroscopy, cavity-enhanced absorption spectroscopy (CEAS), and integrated cavity output spectroscopy (ICOS) have been reported.

Figure 2(c) illustrates the fundamental operating principle of CRDS, which has been pioneered by O’Keefe and Deacon in 1988.\(^{(32)}\) High finesse cavities using low-loss dielectric mirrors allow interaction of a gas phase sample with pulsed laser light within the cavity. For the determination of the characteristic decay time \(\tau\), pulsed laser light...
INSTRUMENTATION AND APPLICATIONS OF CASCADE LASER SPECTROSCOPY

is injected on-axis into the optical cavity. The decay time \( \tau \) is defined as a decrease by \( 1/e \) of the exponential intensity signal and is a function of the mirror reflectivity \( (R) \), the distance between the high reflecting mirrors \( (d) \), and the absorption coefficient \( (\alpha) \). The absorption coefficient can be determined by the measurement of the so-called ring-down times of a cavity filled with absorber \( \tau_{\alpha} \) versus an empty cavity \( \tau_{0} \). Mirror losses and scattering/refraction phenomena within the cavity predominantly contribute to the ring-down time for an empty cavity \( \tau_{0} \). In 1997, Romanini et al.\(^{(33)}\) realized a CW variation of the CRDS, i.e. so-called CWCRDS. Another approach for absorption spectroscopy utilizing high-finesse optical cavities is the integration of the entire signal to obtain equivalent absorption spectra. In 1998, O’Keefe et al.\(^{(34)}\) and Engeln et al.\(^{(35)}\) introduced yet another cavity-based technique, i.e. ICOS, and CEAS. In contrast to CRDS, these strategies are based on the off-axis injection of laser light, and sweeping across the desired gas absorption line rather than locking the laser line on each cavity mode. Thus, perfect mode matching of the laser frequency and the cavity mode to the absorption line, and highly accurate scanning over narrow bands as required in CRDS is not required. Progress in this area with a focus on theory, instrumentation, and applications has been reviewed by Young et al.\(^{(36)}\)

4 SELECTED APPLICATIONS

In the following, selected applications based on cascade lasers spectroscopy are highlighted ranging from the detection of byproducts during fuel burning to the analysis of relevant biomarkers in human breath. Given the wide range of application scenarios, the examples in this review focus on absorption-based spectroscopy and sensing applications taking particular advantage of the unique properties of QCLs and ICLs operated in CW or pulsed mode serving as advanced light source for analytical techniques including TDLAS, WMS, QEPAS, and CRDS.

4.1 Molecular Spectroscopy

High-resolution infrared spectroscopy is an important application area for cascade lasers with narrow linewidths, which renders them ideal candidates for the investigation of a variety of spectroscopic parameters such as spectral position, line strength, pressure broadening, and other – e.g. temperature-dependent – parameters. Precise measurements of these molecular and spectroscopic properties are essential in trace gas analysis and sensing applications. Several research groups are focusing on experimental, as well as computational molecular spectroscopy for the characterization of molecular line transitions including their temperature and pressure dependence.

Employing QCL-based spectroscopic techniques for the measurement of water vapor isotopologues \( H_{2}^{16}O, H_{2}^{17}O, \) and HDO were reported by the Groupe de Spectrométrie Moléculaire et Atmosphérique (GSMA) at Reims University in France. Joly and coworkers developed a CW DFB-QCL absorption-based sensor near 6.7 \( \mu \)m for the determination of water vapor isotopologues in the atmosphere. As water vapor is among the most relevant contributions to the greenhouse effect, studies are required that facilitate a more advanced understanding of the mechanisms involving the injection of tropical tropospheric \( H_{2}O \) into the stratosphere. Therefore, the spectral region ranging from 1483 to 1487 \( \text{cm}^{-1} \) was used to investigate eight accurate line strengths of water vapor isotopologues, which were then compared to the HITRAN database.\(^{(37)}\) Further research activities target the determination of line positions, line strengths, and self-broadening coefficients of sulfuric oxide \( (SO_{2}) \).\(^{(38)}\) A laboratory study of SO\(_{2}\) line intensities between 1088 and 1090 \( \text{cm}^{-1} \), and comparison with calculations was reported using a QCL. It was demonstrated that calculations of positions and strengths are in excellent agreement with experimental data. In 2012, Wang and Shariples observed the \( v_{6} \) vibrational band of formaldehyde \( (H_{2}CO) \) around 8 \( \mu \)m using a pulsed DFB-QCL, and determined pressure broadening and shift coefficients by introducing a variety of gases \( (\text{He, Ne, Kr, Ar, N}_{2}, O_{2}, \) and \( \text{CO}_{2}) \). Two transitions centered at 1252.11231 and 1253.14392 \( \text{cm}^{-1} \) have been assigned as \( (1,1,1) \leftarrow (2,0,2) \) and \( (10,1,9) \leftarrow (9,2,8) \) transitions.\(^{(39)}\) Recently, Sur et al. reported line intensity and temperature-dependent broadening coefficients of ammonia \( (NH_{3}) \) in the spectral region of 961.5–967.5 \( \text{cm}^{-1} \). This frequency range is perfectly suited for the development of MIR ammonia sensors due to strong absorption features of ammonia and minimal cross-sensitivity toward \( \text{CO}_{2} \) and \( \text{H}_{2}O \). Using a tunable DFB-QCL and a heated optical cell providing a path length of 101 cm, temperature broadening parameters were obtained between 300 and 600 K. Measurements of selected line intensities and self-broadening in the \( v_{2} \) region of ammonia were performed using a 3.36 \( \text{mm} \) cell with 99.99% pure \( \text{NH}_{3} \) at 296 K.\(^{(40)}\)

4.2 Combustion Diagnostics and Industrial Process Control

Recent advances in cascade laser sources particularly enable in situ measurements in harsh environments. QCL- and ICL-based spectroscopy has thus emerged among the most promising detection technologies in emission monitoring and process control applications.
In order to reduce pollutant emission or to improve the efficiency of combustion processes, environment protection agencies are continuously implementing increasingly stringent emission regulations. Hence, highly sensitive monitoring techniques for a wide variety of molecular species relevant in combustion scenarios (e.g. CO, CO₂, H₂O, NO, etc.) are in increasing demand. This challenging task for sensor systems frequently requires the application of highest fidelity light sources such as cascade laser for sensors operating at MIR wavelengths. For example, the combustion of fossil fuels in industry or combustion engine vehicles causes the emission of nitric oxides (NO) from the nitrogen-containing components in fuel. This severe air pollutant contributes to the formation of acid rain, smog, and tropospheric ozone. When operated in a time-division multiplexed mode. Rapid detection of NO₂ emissions after the exhaust gas treatment system of a heavy-duty diesel engine was also performed demonstrating the capability of the system for automotive emission studies. The obtained results were compared to measurements using a chemiluminescence analyzer, and excellent agreement between the sum of NO and NO₂ in a wide range of concentrations (i.e. 0.5–1000 ppm) was achieved.

Various QCL-based sensors for combustion diagnostics have been developed using WMS or QEPAS as well as CRDS. In 2014, an ICL-based sensor for NO monitoring has been realized requiring only 138 mW of power at room temperature and emitting at 5.2 μm. An in situ NO sensor based on a DFB-ICL was presented by Diemel et al. The sensor was tested for monitoring NO within the hot air co-flow of an auto-ignition test rig at temperatures between 800 and 1300 K. At 800 K, a noise-limited limit of detection (LOD) of 30 ppm within an observation period of only 10 ms was achieved using an optical path length of only 82 mm.

In general, cascade laser sources offer attractive options for in situ monitoring and control, e.g. for industrial plasma processes enabling improved understanding on the kinetics during chemically active discharge. Molecular plasma nowadays serves as an advanced matrix in etching and chemical vapor deposition procedures for

![Figure 4](image-url) Determination of NO emitted by local vehicle traffic versus time sampled from a rooftop performed comparing the performance of QCL- and TDL-based spectrometers. (left) Low concentrations. (right) High concentrations. The precision of the QCL system during 'zero' gas addition to the inlet is shown in the inset. (Reproduced with permission from Ref. 44. © Springer, 2002.)
removal or deposition of thin films yielding enhanced productivity, reproducibility, and reliability. Maximum performance is achieved, if transient or stable plasma reaction products such as CO are continuously monitored. The MIR spectral range offers highly sensitive detection schemes, as the pronounced fundamental absorption of CO near 4.7 μm enables sensing at the required sensitivity and molecular specificity. Researchers at the Leibniz Institute for Plasma Science and Technology (INP) have published seminal studies on related advances utilizing cascade laser spectroscopy for plasma process monitoring and control.\(^{(51-53)}\) For example, Lang et al. studied the correlation of etching rates of ultra-low-k SiCOH wafers vs. the concentration of the etching products, i.e. CO and SiF\(_4\).\(^{(54)}\) A fiber-based analysis system containing two pulsed QCLs was integrated with an inductively coupled plasma (ICP) reactor. An effective absorption length of 8 m was achieved implementing multipass optics with the detectors directly coupled via MIR fiber optics (i.e. PIR/CIR type fibers with a length of 2 m each) to avoid open optical pathways, as shown in Figure 5.

The sensor system comprised two pulsed QCLs emitting between 1030.90–1031.00 cm\(^{-1}\) and 2077.58–2077.72 cm\(^{-1}\) for SiF\(_4\) and CO analysis, respectively. Limits of detection for SiF\(_4\) and CO of 4.4 \times 10^{11} \text{ and } 5.5 \times 10^{11} \text{ molecules cm}^{-3} \text{ were obtained. It could be shown that the concentration of etching products nearly linearly depends on the etching rate, and thus, in situ monitoring can be used for direct online etching control. Further applications in industrial environments were demonstrated, e.g. for time resolved in situ monitoring of BCl\(_3\) to improve pulsed plasma-enhanced chemical vapor deposition (PECVD) processes.\(^{(55)}\)

4.3 Biomedical Applications

The MIR range is ideally suited for trace gas analysis of many (bio)medically and clinically relevant molecules with characteristic absorption features in this wavelength region. The application of cascade lasers – in contrast to other MIR light sources – allows for the design of particularly compact, sensitive, and user-friendly trace gas sensors suitable for medical diagnosis. Miniaturized and portable instrumentation was in part made possible by the development of particularly robust optical analyzer concepts benefiting from cascade laser technologies. Consequently, measurement techniques initially developed for industrial and environmental monitoring were adapted for biomedical applications including exhaled breath analysis and glucose monitoring. Selected examples highlight the utility of cascade laser sensing techniques for noninvasive (bio)medical analysis.

4.3.1 Exhaled Breath Analysis

The analysis of volatile organic compounds (VOCs) present in exhaled breath offers noninvasive, close to

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**Figure 5** Schematic of an experimental arrangement for QCL-based plasma monitoring. A fiber optic sensing system comprising two pulsed QCLs was integrated with an inductively coupled plasma (ICP) reactor. Etching products were detected via a multipass optical arrangement providing for an absorption path length of 8 m. (Reproduced with permission from Ref. 54. © Springer, 2015.)
real time, and point-of-care (POC) disease diagnostics and therapeutic management. In 1971, Linus Pauling and his team identified approximately 250 VOCs using gas chromatography coupled to mass spectrometry (GC-MS) within probed exhaled breath samples. These VOCs originate either from cellular metabolism and are produced endogenously, or from isotope-labeled species, which are released after administration of, e.g. $^{13}$C- or $^{15}$N-labeled substrates. Clinical breath analysis is based on the determination of these exhaled compounds after passing the blood/air interface within the lungs. Due to commonly encountered concentration levels in the low parts-per-billion (ppb) regime and below, and the rather large number of chemical species contained in exhaled breath, highly sensitive and selective analytical techniques are required for their qualitative and quantitative analysis as biomarkers. A variety of analytical techniques including (proton transfer reaction) mass spectrometry ((PTR)MS), nuclear magnetic resonance (NMR) spectroscopy, laser resonance spectroscopy, near-infrared (NIR) and MIR spectroscopic methods, isotope ratio mass spectrometry (IRMS), and nondispersive isotope-selective infrared spectroscopy (NDIRS) have been established for the detection of numerous breath biomarkers along with their routine quantification.

Cascade laser light sources promise significant advancements in exhaled breath analysis utilizing the mid-infrared spectral regime in such trace gas sensors. In contrast to many conventional analytical techniques, compact, easy-to-use, sensitive and accurate/reliable sensors providing results close to real time with minimal sample preparation are on the horizon enabling, e.g. continuous breath monitoring. The sensitivity of methods such as PAS, CRDS, and TDLAS utilizing MIR cascade lasers allows for the detection of breath biomarkers in the low ppb-to-ppt concentration range relevant for a wide variety of breath tracer gases such as NO, CO, CO$_2$, HCOH, C$_2$H$_6$, CH$_4$, C$_3$H$_2$, and others exhibiting characteristic spectral fingerprints in the 3–6 μm regime (see Fig. 6).

In the following, emerging MIR sensing techniques based on cascade lasers are highlighted with respect to their potential suitability in exhaled breath analysis.

As a nonspecific marker of inflammation in the lung, NO has been proven as a reliable VOC to diagnose respiratory infection and inflammation in the exhaled breath matrix. Moreover, high levels of NO can be found in atopic asthma, and in chronic obstructive pulmonary disease (COPD), whereas NO concentrations decrease in cystic fibrosis (CF). Next to chemiluminescence and electrochemical sensing devices, several publications have reported on the MIR-based laser spectroscopic detection of NO in breath evaluating the NO absorption feature at approx. 5 μm (i.e. 2000 cm$^{-1}$) using CRDS, multipass gas cells and cavity-enhanced techniques.

In 2011, the Laser Science Group at Rice University led by Titel reported a QEPAS sensor for the detection of NO using a broadly tunable EC-QCL emitting at 1900.08 cm$^{-1}$ (i.e. 5.26 μm). The QCL was operated at 16.5 °C and a maximum driving current of 450 mA was applied providing for continuous spectral tuning from 1763 to 1949 cm$^{-1}$. A time resolution of 13 s and a detection limit of 57 pptv were obtained. To improve NO vibrational-translational relaxation processes, water vapor was added at a concentration of 2.5%, and the experiments were performed at 210 Torr. High-resolution CRDS utilizing a CW EC-QCL operated mode-hop-free for the detection of NO at 5.2 μm was tested for breath analysis in 2016. In this example, an effective optical path of 1.7 km was provided by a high-finesse optical cavity with a dimension of 50 cm. A time resolution of 13 s and a detection limit of 57 pptv were obtained. In 2017, Ghorbani et al. reported the real-time detection of CO in exhaled breath using an ICL as the light source within a compact TDLAS sensor device. CO in exhaled breath was first identified as a potential biomarker in 1972 resulting from exogenous processes, hematological degradation, and nonhemic endogenous processes such as lipid peroxidation or bacteria. Other causes of elevated CO levels are asthma and chronic obstructive pulmonary disease (COPD).
An ICL operated at room temperature was coupled to a low-volume circular multipass cell using WMS detection schemes for enhancing the sensor performance (Figure 7). Compared to direct absorption spectroscopy, an improvement by a factor of 25 was achieved leading to a fringe-limited ($1\sigma$) sensitivity of $6.5 \times 10^{-8} \text{cm}^{-1} \text{Hz}^{-1/2}$, and a corresponding detection limit of $9 \pm 5 \text{ppb}$ at 0.07 s.

The CW DFB-ICL was operated at 4°C (i.e. TEC cooled) with a current scan interval of 38–80 mA at an average output power of 1.4 mW. The emission wavelength was locked to the absorption lines of the stable isotopes $^{12}\text{CO}$ and $^{13}\text{CO}$ in the range 2130.09–2131.87 cm$^{-1}$. The TDLAS sensor was used to perform online CO analysis of indoor air, and for real-time monitoring of the CO expirograms from healthy nonsmokers and a healthy smoker before and after smoking. Screening of exhaled CO has been widely used to access smoking status and behavior. In Figure 8, typical $^{12}\text{CO}$ and $^{13}\text{CO}$ exhalation profiles from a healthy occasional smoker before smoking (i.e. 19 h after the last cigarette) and 15 s after smoking are shown. It is immediately apparent that the eCO levels are increased by a factor of approx. 2.

A pulsed DFB-QCL with a balanced detection scheme in the spectral region around 2300 cm$^{-1}$ was used in a flow-through system for the analysis of the $^{12}\text{CO}/^{13}\text{CO}$ isotope ratio in human breath by Rubin et al.$^{(71)}$ Within a spectral window of 2 cm$^{-1}$, single rotational-vibrational lines for $^{12}\text{CO}$ and $^{13}\text{CO}$ were scanned via narrowly tuning cascade lasers, and enabled highly sensitive real-time measurements in the ppb concentration range. Carbon dioxide – and in particular the determination of its isotope ratio – is of high relevance in exhaled breath analysis for testing, e.g. *Helicobacter pylori* infections, liver malfunction, and drug administration using appropriately labeled substrates.$^{(72)}$ Although currently breath analysis is predominantly executed using mass spectrometric methods, several research groups have readily demonstrated the analysis of carbon isotopes via cascade laser spectroscopy.

In combination with miniaturized hollow waveguide gas cells (also known as iHWGs) offering the analysis of low-volume gas samples, the highly accurate determination of the $^{12}\text{CO}/^{13}\text{CO}$ isotope ratio in mouse breath samples was demonstrated by Wörle et al.$^{(73)}$ This first QCL-based sensor for applications in mouse intensive care scenarios employed a broadly tunable EC-QCL emitting from 2150 to 2450 cm$^{-1}$. Multivariate data evaluation and calibration techniques based on PLS regression were performed to

**Figure 7** Schematic of an ICL-based TDLAS setup with multipass cavity (MPC) for breath analysis. (Reproduced with permission from Ref. 67. © Optical Society of America, 2017.)

**Figure 8** Sequences of eCO expirograms. (a) $^{12}\text{CO}$, (b) $^{13}\text{CO}$ from a healthy occasional smoker before smoking (i.e. 19 h after the last cigarette), and 15 s after smoking. (Reproduced with permission from Ref. 67. © Optical Society of America, 2017.)
resolve and quantify overlapping spectral features with high fidelity.

Cascade laser-based breath analysis is attracting substantial interest in clinical diagnosis given ongoing advancements in laser technology and sensing techniques, which have already led to first commercially available laser-based exhaled breath analyzers with a small device footprint and robust operation facilitating real-time, point-of-care disease diagnosis and noninvasive monitoring of the metabolic status with minimal risk to the patient.

4.3.2 Noninvasive Glucose Sensing

Diabetes mellitus is a widespread metabolic disease in humans with around 400 million patients worldwide.\(^{74}\) Heart attack, stroke, blindness, and kidney failure are some of the common symptoms in the aftermath of diabetes. Since there is currently no treatment available, any therapy relies on regularly monitoring blood sugar concentrations and adjusting the level to approximately 60–120 mg dl\(^{-1}\). The requirement of drawing blood multiple times a day is severely affecting the quality of life of diabetic patients. Noninvasive glucose sensors promise significant quality of life improvements providing glucose readings, e.g. via the human skin.

While prior research toward noninvasive optical glucose sensing was predominantly based on near-infrared light between 1300 and 1900 cm\(^{-1}\), spectral interferences of other compounds such as proteins and acids in this wavelength range affect the quality of such readings.\(^{75}\) In contrast, the MIR region offers pronounced vibrational patterns characteristic for glucose in the 8–10 μm range promising and less interference with other (tissue) constituents.\(^{76}\) However, due to strong water absorptions, the penetration of MIR light into human skin is only approx. 100 μm.\(^{77}\) In recent years, various approaches have been proposed using cascade laser technology for noninvasive glucose sensing.

In 2014, Liakat et al.\(^{78}\) reported the noninvasive prediction of blood glucose levels for three healthy human subjects using a 500 μm hollow fiber coupled to s pulsed tunable QCL with a tuning range of 8–10 μm. Acceptable radiation intensity (i.e. on the order of magnitude of solar radiation) at the monitored skin areas was maintained at 55 kHz and 1% duty cycle with peak powers of 50–125 mW. Conventional chemometric techniques using partial least squares regression (PLSR) and derivative spectroscopy were used to analyze the radiation scattered from skin in the glucose-specific wavelength region (1075–1085 cm\(^{-1}\)). Clinically relevant predictions with an accuracy of 84% were obtained throughout a glucose concentration range of 80–160 mg dL\(^{-1}\). Kottmann et al.\(^{79}\) developed a dual-QCL-based photoacoustic sensor for glucose monitoring in human tissues. PAS was combined with two CW EC-QCLs operating at 8 mW and emitting at fixed wavelengths. One laser was used to address the glucose absorption around 1080 cm\(^{-1}\), while the second laser was emitting in a region of weak glucose absorption (i.e. approx. 1180 cm\(^{-1}\)). The maximum permissible exposure limit for skin was abided by reducing the incident power to 1 mW after modulation. The beams of the two QCLs were alternately directed to a power meter (PM), and to the PA cell by flipping mirrors (FM). A laboratory evaluation of the sensor system using the so-called ‘out-of-phase wavelength modulation method’ was successfully performed, and the compensation of long-term drifts due to variations of the sample or measurement conditions was achieved. The sensor was then used to perform feasibility tests for an oral glucose tolerance test at healthy individuals. With the dual-wavelength approach, an uncertainty of ±30 mg dL\(^{-1}\) of the blood glucose concentration level at a confidence level of 90% was achieved. Further progress in the field of MIR spectroscopy for biomedical applications has been recently reviewed in detail by Schwaighofer et al.\(^{80}\)

4.4 Defense and Security

During the last decades, detecting trace explosives and explosive compounds in public places including airports, railways or tourist sites is in the interest of security and public safety around the world. A number of factors such as the low vapor pressure of most explosive compounds, the emergence of novel explosive materials, and the need of nondestructive inspection processes severely obstruct their detection rendering such sensing scenarios a challenging task requiring rapid, selective, and sensitive instrumental techniques. A variety of cascade laser-based methods has been successfully demonstrated in either close contact or stand-off (i.e. tens of meters) detection situations. Stand-off scenarios inherently demand that the optical equipment and operator are located at a safe distance from the sample, thus unanimously requiring high-power light sources. As this technique allows analysis without any sample collection and pretreatment, it is perfectly suited for the detection, e.g. of low-vapor-pressure explosives.

Early reports on stand-off detection were presented by Brassington in 1982 using the so-called ‘photo-acoustic-detection-and-ranging’ (PADAR) technique. Using an optical parametric oscillator (OPO), a range resolution better than 10 mm was achieved with a probable maximum range of 100 m.\(^{81}\) A parabolic microphone located close to the laser was used to detect the acoustic wave generated by interaction of the laser pulse with the sample, and the concentration of analyte gases such as methane was determined.
Figure 9 Scheme of the experimental setup based on two pulsed QCLs. Reflect IR radiation of the target surface is focused onto the QEPAS device. (Reprinted with permission from Van Neste, C.W., Senesac, L.R., and Thundat, T. (2009) Standoff Spectroscopy of Surface Adsorbed Chemicals. Anal. Chem., 81 (5), 1952–1956. Copyright 2009 American Chemical Society.)

In 2007, stand-off explosives detection using QEPAS with a pulsed QCL was reported by researchers at the Oak Ridge National Laboratory (ORNL, USA) and collaborators. A pulsed QCL emitting between 1019.9 and 1080.2 cm\(^{-1}\) (9.2580–9.8041 \(\mu\)m) with a duty cycle of 5% and 100 mW output power was used. The beam spot size of the QCL was approx. 6 mm at the laser, and approx. 25 mm at a distance of 20 m. The sensitive and selective stand-off detection of tributyl phosphate (TBP) and three explosives (RDX, TNT, and PETN) at distances ranging from 0.5 to 20 m as surface-absorbed compounds was demonstrated with a detection limit on the order of 100 ng cm\(^{-2}\).\(^{82}\) As a further development of this approach, two tunable QCLs were simultaneously operated (Figure 9) and scanned across major identifying absorption peaks of the selected analytes.

The sample surface was illuminated and reflected/scattered radiation was focused using a mirror. Tuning forks with matching frequencies detected the pulses excited by the individual lasers. This dual laser approach allows scanning of a broader spectral window enabling molecular identification of explosive compounds.\(^{83}\) Most recently, Ostendorf et al.\(^{84}\) demonstrated imaging MIR laser backscattering spectroscopy for stand-off detection of residues of explosives or their precursor materials. For imaging backscattering spectroscopy, a system based on EC-QCL illumination combined with a large aperture optical telescope coupled to a high-performance cryogenically cooled MCT-based MIR camera for collection and detection of diffusively backscattered MIR radiation was developed. Using custom-made micro-opto-electro-mechanical systems (MOEMS) scanning gratings arranged in a Littrow configuration serving as wavelength selective elements, two EC-QCL units were combined into a single collinear output beam with a wavelength range from 7.36 to 10.14 \(\mu\)m (1358.7–986.2 cm\(^{-1}\)) resulting total tuning range of 370 cm\(^{-1}\). The twin EC-QCL module generated an average power >50 mW at a duty cycle of approx. 20% when operated in short-pulse-high-repetition-rate mode. In 2015, the system was tested in a scenario aiming at the identification of explosives derived from an improvised explosive device (IED) that was detonated near a car. Unexploded residues spread across the explosion site were determined from a vehicle over a distance of 17 m in this simulated real-world scenario. In Figure 10, the exploded vehicle, and the positive detection results for ammonium nitrate (AN) residues from various measurement areas are shown.

In summary, MIR spectroscopy based on advanced laser light sources provides a powerful tool for stand-off detection and identification of a variety of chemicals potentially used as explosives and their precursors. A commercial handheld stand-off detector using a broadly tunable QCL for the detection of substances at surfaces (LaserScan™ Analyzer) is available from Block Engineering operating at distances up to 12 in., and at longer range with appropriate collection optics. Stand-off detection at large distances remains challenging due to losses in light intensity, absorption losses, and scattering losses in the ambient air environment. Notwithstanding, the development of laser light sources enabling detection at extended distances is subject of ongoing research, and will further expand the utility of such cascade laser devices in military and homeland security applications.

5 SUMMARY

Taking advantage of the advancements and innovations in cascade laser light source technology, selective and sensitive spectroscopy and sensing systems for online analysis close to real time, and specifically for gas and vapor phase monitoring scenarios have been established. Advanced gas sensing systems utilizing various QCL- or ICL-based absorption spectroscopic techniques including DAS, WMS, PAS, and CEAS benefit from the increasing commercial availability of cascade lasers with extended wavelength coverage. Combined with more or less elaborate optical systems, QCLs and ICLs have found applications as advanced light sources in laboratories, clinics, and industrial scenarios due to the provided optical power in the mid-infrared spectral
Figure 10  QCL-based MIR backscattering spectroscopy for the detection of explosives residues at a car destroyed by a nearby exploded improvised explosive device (IED). Ion mobility spectrometry (IMS) was applied to cross check and confirm the obtained surface concentration of ammonium nitrate with an estimated average coverage of approx. 10 μg cm⁻². (Ostendorf, http://www.mdpi.com/2304-6732/3/2/28. Licensed under CC BY 4.0.)

regime, quality and robustness of the devices, and flexibility in tailoring their emission wavelength. In particular, their unprecedented tunability across almost the entire MIR spectral regime next to pulsed and CW operation renders QCLs and ICLs the most advanced light source technology for sensing principles based on coherent radiation. Although a variety of alternative MIR sources such as solid-state lasers, OPOs, VCSELs, and fiber lasers, as well as cost-effective thermopile sensors and LEDs are well established within the gas sensor market, MIR cascade laser technology has already proven to overcome several limitations of existing technologies due to their outstanding contributions to sensitivity and failsafe operation. In addition, the increasing demand for MIR applications benefits from cascade laser technology owing to their potential for miniaturization and on-chip integration. Cascade lasers have developed into a light source technology with a prosperous future, as they gap between conventional IR spectrometers covering the entire MIR band and laser-based technologies steadily decreases with increasingly broad tunability of QCLs, and lately, also ICLs. Future research targets are focused on faster tuning mechanisms and broader tuning ranges besides optimization in efficiency and power consumption. Next to external-cavity-coupled systems, optical frequency combs are providing multiple wavelength emission and are promising approaches toward expanded wavelength coverage. With increasing performance metrics and reduction of production cost, cascade laser technology is forecasted to not only meet future market demands but also to become the dominating light source in the 3–15 μm wavelength window and beyond.

Spectroscopy and sensing systems operating in gas phase detection scenarios utilizing cascade lasers particularly benefit from low-power consumption for potential battery operation, molecular selectivity inherent to operation in the MIR, and increased sensitivity/SNR while maintaining a small device footprint. While certainly not covering all technological and application aspects, this article aims at a contemporary summary of the benefits of cascade laser technology, i.e. QCLs and ICLs as currently the most mature mid-infrared light sources with particular emphasis on applications in molecular spectroscopy, combustion diagnostics, industrial process control,
### INSTRUMENTATION AND APPLICATIONS OF CASCADE LASER SPECTROSCOPY

(bio)medical/clinical applications, and safety/security scenarios.

### ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AN</td>
<td>Ammonium Nitrate</td>
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<tr>
<td>CEAS</td>
<td>Cavity-enhanced Absorption Spectroscopy</td>
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<tr>
<td>CF</td>
<td>Cystic Fibrosis</td>
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<td>COPD</td>
<td>Chronic Obstructive Pulmonary Disease</td>
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<tr>
<td>CRDS</td>
<td>Cavity Ring-down Spectroscopy</td>
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<tr>
<td>CW</td>
<td>Continuous Wave</td>
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<tr>
<td>DAS</td>
<td>Direct Absorption Spectroscopy</td>
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<tr>
<td>DFB</td>
<td>Distributed Feedback</td>
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<tr>
<td>DFG</td>
<td>Difference Frequency Generation</td>
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<tr>
<td>EC</td>
<td>External Cavity</td>
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<tr>
<td>FM</td>
<td>Flipping Mirrors</td>
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<tr>
<td>GC-MS</td>
<td>Gas Chromatography Coupled to Mass Spectrometry</td>
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<tr>
<td>GSMA</td>
<td>Groupe de Spectrométrie Moléculaire et Atmosphérique</td>
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<tr>
<td>ICL</td>
<td>Interband Cascade Lasers</td>
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<tr>
<td>ICOS</td>
<td>Integrated Cavity Output Spectroscopy</td>
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<tr>
<td>ICP</td>
<td>Inductively Coupled Plasma</td>
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<tr>
<td>IED</td>
<td>Improvised Explosive Device</td>
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<tr>
<td>iHWG</td>
<td>Integrated Hollow Waveguides</td>
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<tr>
<td>IRMS</td>
<td>Isotope Ratio Mass Spectroscopy</td>
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<tr>
<td>LOD</td>
<td>Limit of Detection</td>
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<tr>
<td>MBE</td>
<td>Molecular Beam Epitaxy</td>
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<tr>
<td>MIR</td>
<td>Mid-infrared</td>
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<tr>
<td>MOVPE</td>
<td>Metal-organic Vapor Phase Epitaxy</td>
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<tr>
<td>MOEMS</td>
<td>Micro-opto-electro-mechanical Systems</td>
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<tr>
<td>MPCs</td>
<td>Multipass Cells</td>
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<tr>
<td>NDIRS</td>
<td>Nondispersive Isotope-selective Infrared Spectroscopy</td>
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<tr>
<td>NIR</td>
<td>Near-infrared</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
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<tr>
<td>OCES</td>
<td>Optical Cavity Enhanced Spectroscopy</td>
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<tr>
<td>OPO</td>
<td>Optical Parametric Oscillators</td>
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<tr>
<td>ORNL</td>
<td>Oak Ridge National Laboratory</td>
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<tr>
<td>PADAR</td>
<td>Photo-acoustic-detection-and-ranging</td>
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<td>PAS</td>
<td>Photoacoustic Spectroscopy</td>
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<tr>
<td>PECVD</td>
<td>Plasma-enhanced Chemical Vapor Deposition</td>
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<tr>
<td>PLSR</td>
<td>Partial Least Squares Regression</td>
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<tr>
<td>PM</td>
<td>Power Meter</td>
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<tr>
<td>POC</td>
<td>Point-of-care</td>
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<tr>
<td>ppb</td>
<td>Parts-per-billion</td>
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<tr>
<td>(PTR)MS</td>
<td>(Proton Transfer Reaction) Mass Spectrometry</td>
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<tr>
<td>QCL</td>
<td>Quantum Cascade Lasers</td>
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<tr>
<td>QEPAS</td>
<td>Quartz-enhanced Photoacoustic Spectroscopy</td>
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<tr>
<td>QTF</td>
<td>Quartz Tuning Fork</td>
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<tr>
<td>rQCL</td>
<td>Ring-shaped Vertically Emitting Quantum Cascade Laser</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal-to-noise Ratio</td>
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<tr>
<td>TBP</td>
<td>Tributyl Phosphate</td>
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<tr>
<td>TDLAS</td>
<td>Tunable Diode Lasers</td>
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<tr>
<td>VOCs</td>
<td>Volatile Organic Compounds</td>
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<tr>
<td>W-QWs</td>
<td>W-shaped Quantum Wells</td>
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<tr>
<td>WMS</td>
<td>Wavelength Modulation Spectroscopy</td>
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INSTRUMENTATION AND APPLICATIONS OF CASCADE LASER SPECTROSCOPY


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INSTRUMENTATION AND APPLICATIONS OF CASCADE LASER SPECTROSCOPY

5.2 Paper II. Advanced gas sensors based on substrate-integrated hollow waveguides and dual-color ring quantum cascade lasers

E. Tütüncü performed all measurements, analyzed the data, and developed the measurement setup used in this study in close collaboration with V. Kokoric and R. Szedlak. D. MacFarland, T. Zederbauer, H. Detz, A. M. Andrews and W. Schrenk contributed in the development of the laser structure. G. Strasser and B. Mizaikoff contributed with revisions to the final manuscript. The scope of the paper was selected in agreement with all co-authors.


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The following image showing the combined ring QCL-iHWG sensor was selected for publication as Analyst cover image.
Boris Mizaiko et al.
Advanced gas sensors based on substrate-integrated hollow waveguides and dual-color ring quantum cascade lasers
Advanced gas sensors based on substrate-integrated hollow waveguides and dual-color ring quantum cascade lasers

Erhan Tütüncü, a Vjekoslav Kokoric, a Rolf Szedlak, b Donald MacFarland, b Tobias Zederbauer, c Hermann Detz, b Aaron Maxwell Andrews, b Werner Schrenk, c Gottfried Strasser b, c and Boris Mizaikoff a

This study shows the first combination of a ring-shaped vertically emitting quantum cascade laser (riQCL) providing two distinct emission wavelengths combined with a substrate-integrated hollow waveguide (iHWG). This ultra-compact riQCL-iHWG gas sensing device enables the simultaneous detection of two vapor phase species – here, furan and 2-methoxyethanol – providing distinctive absorption features at the emission wavelengths of the riQCL (i.e., 1144 and 1170 cm−1). Hence, multianalyte gas sensing via a unique mid-infrared (MIR) sensor concept is demonstrated.

Introduction

Infrared spectroscopy, and in particular the MIR spectral range (2–20 µm) provides a viable alternative to commonly used vapor phase analysis techniques (e.g., gas chromatography combined with various detection schemes, mass spectrometric techniques, etc.) for selectively, sensitively, and rapidly detecting or monitoring molecular constituents. Due to the substantial potential for miniaturization and given recent progress in IR waveguide, light source, and detector technology3–5 along with inherent molecular selectivity and minimal sample preparation, conventional infrared absorption spectroscopy has evolved into a versatile and modular sensing toolbox.6

The excitation of fundamental vibro-rotational transitions is usually initiated via either broadband light sources such as incandescent glow bars, or using narrowband emitters including initially lead salt laser diodes, and nowadays predominantly quantum cascade lasers (QCLs) and interband cascade lasers (ICLs). Clearly, MIR sensing applications benefit from a possibly broad wavelength coverage, if entire molecular spectra or the discrimination of a multitude of analytes with potentially overlapping spectral features is required. Alternatively, selected target analytes may be addressed via comparatively narrow spectral bands selecting a single yet analyte-specific vibro-rotational absorption line free from interferences with any other gas phase constituent present in the sampled scenario.

However, a variety of analytical applications demand for a compromise between these two scenarios (i.e., broadband vs. narrowband IR absorption spectroscopy) for addressing moderately complex samples containing a limited number of constituents, yet taking advantage of the spectral brightness and compactness of advanced laser light sources. This requirement may be met by either using tunable laser light sources, or lasers based on active gain media tailored to emit at two or more wavelengths. While the latter concept provides coverage of few selected wavelengths only, the advantages in terms of device complexity, laser control, compactness, robustness, and power consumption are immediately evident.

Quantum cascade lasers (QCL) were first reported in 1994,7 and offer nowadays access to almost any wavelength window in the MIR via engineering of the quantum heterostructure comprising the active gain medium. Efficient photon emission via intersubband transitions instead of electron–hole recombination prevalent in conventional heterostructure laser diodes has rendered QCLs the MIR light source of choice in a wide variety of applications, from state-of-the-art optical sensing and spectroscopy concepts. Thereby, one may directly capitalize on their compact dimensions, robustness, long life time, and superior emission characteristics.8–10

Edge-emitting Fabry–Pérot (FP) resonators are the most common optical configuration applied in diode laser light sources with the facets of the active laser chip acting as cavity mirrors. Recently, the research group of Strasser has reported a new generation of surface emitting QCLs based on ring-shaped active structures (riQCL; Fig. 1) utilizing a second order distributed feedback (DFB) grating fabricated into the ring waveguide for efficient vertical light outcoupling.9–11 Besides facilitating pronounced optical power12 and excellent
beam collimation, the integration of vertically emitting laser diodes facilitates their application within ultra-compact sensing concepts using a minimum of beam-guiding optical elements. From the perspective of optical efficiency, in addition a rotationally symmetric far field is achieved via dielectric meta-materials, thereby minimizing scattering losses.

Next to the light source, for the development of rapidly responding and portable gas analyzers miniaturized yet efficient gas cells are mandatory along with efficient coupling of the radiation emitted by the light source into the sample compartment. Conventional multipass gas cells are of limited utility in portable usage due to complex alignment, optical delicacy, and rather large required gas sample volumes. An innovative solution for highly miniaturized gas cells serving at the same time as efficient photon conduits has recently been presented by Mizaikoff and collaborators. These so-called substrate-integrated hollow waveguides (iHWGs) simultaneously serve as efficient optical waveguide and highly miniaturized gas cell. iHWGs enable addressing transient signals in minute gas sample volumes (i.e., few hundreds of microliters), and provide excellent signal-to-noise ratios (SNR) during absorption measurements. Hence, exquisite sensitivity along with ultra-fast sample transition times are achieved by the obtained so-called volumetric optical efficiency, which is superior to conventional long-path or multipass gas cells. Moreover, the design flexibility and mechanical robustness have proven iHWGs as a promising generic key component for a variety of infrared gas sensing scenarios including environmental analysis, process monitoring, and breath diagnostics.

The aim of the present study was demonstrating the first riQCL-iHWG prototype providing a compact yet high-performance gas/vapor mid-infrared sensor system comprising a low volume (i.e., 600 µL) iHWG gas cell combined with a multi-color riQCL enabling multi-component gas sensing.

**Experimental**

**Chemicals**

The vapor phase samples were prepared by collecting the head space gas above the corresponding liquid phase analytes. Furan (99% stab. with 250 ppm butylhydroxytoluen Alfa Aeser, Germany) and 2-methoxyethanol (Sigma Aldrich, Germany) were transferred into serum vials. After sealing the vial with a serum cap, the vial containing 2-methoxyethanol was transferred into a 40 °C water bath at a depth slightly above the liquid level inside the vial for enhancing the vapour pressure. In contrast, the furan-containing vial was placed in an icebox at 0 °C during the experiments in order to correspondingly decrease the vapor phase.

Head space samples were then collected by inserting the needle of 50 mL plastic syringe (B. Braun Melsungen AG, Melsungen, Germany) through the cap into the vial. Gas mixtures 0 to 100% of each analyte were prepared via a static mixing procedure using two 50 mL plastic syringes ensuring thorough mixing. Each sample was analyzed three times.

**Substrate integrated hollow waveguide**

Radiation from the QCL was collimated into the iHWG gas cell, and propagated along the waveguiding channel. The iHWG was fabricated from brass with dimensions of $150 \times 25 \times 20 \text{ mm}^3$ ($L \times W \times H$), and comprised a waveguiding channel with a cross-section of 2.0 mm$^2$ simultaneously serving as miniaturized gas cell. As optimum waveguiding is achieved by
Characterization of the riQCL

The experimental sensor setup is schematically illustrated in Fig. 2. MIR radiation emitted from the riQCL (A) was collimated via a 1.5" ZnSe lens (B), and then guided via a gold-coated iHWG with a waveguide channel/gas cell length of 15 cm. Radiation emanating at the distal end of the iHWG was directly illuminating a 4 mm² HgCdTe (MCT) detector element (FTIR-16-2.00 MSL-12, InfraRed Associates Inc., Stuart, FL, USA) located in a dewar cooled with liquid nitrogen (D). In a next step, it is anticipated that the riQCL and a thermoelectrically cooled MCT will be directly integrated into the iHWG structure, thus eliminating even the beam collimating ZnSe lens by coupling the emitted radiation instantaneously into the waveguide/gas cell channel.

Current pulses at the required repetition frequency were applied to the riQCL via a pulse generator (HP 8114A, Hewlett Packard, Houston USA). The riQCL was modulated at 10 kHz at a pulse duration of either 250 ns or 100 ns. The MCT detector output was coupled to a lock-in amplifier (Model 5209, Princeton Applied Research, NJ, USA) located in a dewar cooled with liquid nitrogen (D). Interferogram were recorded in a wavelength window of 400–4000 cm⁻¹ averaging 30 spectra at a spectral resolution of 2 cm⁻¹ using a Blackman–Harris 3-term apodization function.

Results and discussion

The characteristics of the riQCL in terms of optical emission power vs. applied voltage and current density are shown in Fig. 3. The threshold current density is 4 kA cm⁻² with a resulting optical output power of 5 mW (@ 5.5 kA cm⁻²), and 80 mW (@10 kA cm⁻²), respectively.

As the signal-to-noise ratio and background signal are dependent on the laser driving current, the SNR varied accord-
ingly from 344 for a driving current density of 5.5 kA cm$^{-2}$ to 1951 for 10 kA cm$^{-2}$.

In order to verify suitable spectral overlap between both riQCL emission lines (i.e., @ 1144 and 1170 cm$^{-1}$) and characteristic absorption bands of furan and 2-methoxyethanol, individual IR spectra of each gas phase constituent were recorded using an FT-IR spectrometer coupled to a suitable iHWG. The gas cell was filled with pure vapor of either furan or 2-methoxyethanol. Likewise, the FT-IR spectrum of the riQCL was recorded by coupling the emitted radiation in lieu of the broadband emitter of the spectrometer into the interferometer of the FT-IR spectrometer, thereby obtaining the emission characteristics of the riQCL. An emission wavelength of 1144 cm$^{-1}$ was obtained by applying 5.5 kA cm$^{-2}$ to the riQCL, whereas 10 kA cm$^{-2}$ led to the emission of both wavelengths (i.e., 1144 cm$^{-1}$ and 1170 cm$^{-1}$). The IR spectrum of laser emission characteristics (black) are overlaid with the spectral signatures of furan (red) and 2-methoxyethanol (blue) in Fig. 4.

Additional FT-IR spectroscopic studies of various furan and 2-methoxyethanol mixtures in a concentration range of 0–100% indicate distinctly changing spectral features, as shown in Fig. 5. After purging the iHWG with approx. 50 mL of gas mixture, the inlet/outlet gas ports of the iHWG were closed off via a T-valve, and 30 spectral scans were averaged.

The same experimental procedure was then applied for characterizing the performance and multicomponent detection capabilities of the riQCL-iHWG sensor system.

Fig. 6A shows the riQCL-iHWG sensor response as a function of increasing furan concentrations (2–20% in 2-methoxyethanol) at an applied laser voltage of 10 kA cm$^{-2}$, at which both wavelengths (i.e., 1144 cm$^{-1}$ and 1170 cm$^{-1}$) are emitted. As the absorption of furan overlaps with the laser emission at 1170 cm$^{-1}$, it is immediately evident that increasing concentrations of furan accordingly reduced the detected signal. The obtained response function is fitted with an exponential curve, thereby resulting in a calibration function with a coefficient of determination ($R^2$) >0.99.

Using the same procedure, the furan fractions were increased (20–80% in 2-methoxyethanol), and introduced into the iHWG. However, now the laser emission was tuned to 1144 cm$^{-1}$ at 5.5 kA cm$^{-2}$ of voltage applied to the riQCL, thus matching the absorption of 2-methoxyethanol (Fig. 6B).

Due to the decreasing concentrations of 2-methoxyethanol in the gas cell, a decreased in damping of the radiation emitted from the QCL readily verified that the narrow frequency band emitted at 1144 cm$^{-1}$ overlapped with the absorption feature characteristic. The positive slope of the exponential fit ($R^2 > 0.99$) reveals the expected behavior.
During these experiments, the riQCL-iHWG sensor system was operated at a repetition rate of 10 kHz with a pulse duration of 100 ns, whereby emission was achieved at room temperature for an applied voltage of 10 kA cm$^{-2}$. Similar characteristics were ensured at an applied voltage of 5.5 kA cm$^{-2}$ with a pulse duration of 250 ns at the same repetition rate.

The magnitude of the error bars shown for the experimental data points the calibration functions (Fig. 6) result predominantly from the manual gas mixing procedures, however suffice to demonstrate the analytical utility of the developed riQCL-iHWG sensor prototype in multicomponent vapor phase sensing scenarios.

Conclusions and outlook

In this study, we demonstrated the first combination of substrate-integrated hollow waveguides with vertically emitting ring-shaped two-color quantum cascade lasers for gas/vapor phase sensing applications. Specifically, the developed riQCL-iHWG sensor prototype comprises a riQCL with an embossed 2$^{nd}$ order DFB grating emitting at 1144 cm$^{-1}$ and 1170 cm$^{-1}$ at room temperature in pulsed mode, and a miniaturized iHWG gas cell simultaneously serving as MIR waveguide with a sample volume of only 600 µL.

By directly coupling the laser emission into the iHWG, two exemplary analytes – furan and 2-methoxyethanol – were simultaneously detected and quantified during proof-of-principle analytical studies. This innovative sensing concept not only promises exceptionally compact mid-infrared gas sensing platforms, but efficient quantitative vapor phase analysis in minute (i.e., few hundreds of microliters) sample volumes in sensing/monitoring scenarios requiring rapid measurements (i.e., few seconds) due to a transiently changing sample composition.

In a next step, a significant further reduction of the device footprint is anticipated by direct integration of the riQCL and a thermoelectrically cooled MCT detector or a pyroelectric detection device into the iHWG structure. Such hybrid integration not only ensures more efficient optical energy throughput, but also avoids usage of any additional beam guiding optics, while maintaining or even improving the device robustness (e.g., changes in optical alignment due to mechanical vibrations, etc.). Consequently, the application of such devices in sensing applications including environmental analysis, process monitoring, security and surveillance scenarios, and biomedical diagnostics appears a natural fit.

Acknowledgements

Partial support of this study by the project APOSEMA funded by the German BMBF within the M-Era.net program is greatly acknowledged. The Machine Shop at Ulm University is thanked for support during prototype development of the iHWG. This work was performed in part under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory (LLNL) under Contract DE-AC52-07NA27344. This project was funded in part under LLNL sub-contract No. B603018 and B607114. This work was also supported by the Austrian Science Fund (FWF) via project Next-Lite (F19-P09).

Notes and references

5.3 Paper III. iHWG-ICL: Methane Sensing with Substrate-Integrated Hollow Waveguides Directly Coupled to Interband Cascade Lasers

E. Tütüncü performed all measurements, conducted the data evaluation and established the analytical conclusions in agreement with all co-authors. B. Mizaikoff supervised the associated project APOSEMA. P. Fuchs and M. Fischer have developed the interband cascade laser and assisted during operation. M. Nägele and B. Mizaikoff contributed with revisions to the final manuscript.

This article is reprinted with permission from “E. Tütüncü, M. Nägele, P. Fuchs, M. Fischer and B. Mizaikoff, iHWG-ICL: Methane Sensing with Substrate-Integrated Hollow Waveguides Directly Coupled to Interband Cascade Lasers, ACS Sensors, 1, 2016, 847−851.” Copyright ©2016 American Chemical Society.
Sustainable analytical methods are ubiquitous needed for providing rapid and real-time monitoring of hydrocarbon gas concentrations. Within recent decades, advances in laser spectroscopic techniques including tunable laser absorption spectroscopy (TLAS) have been proven as a viable detection concept, in particular, if low sample volumes have to be addressed. Evolving from laboratory systems to in-field applications, optical sensing techniques have stimulated considerable interest in various application scenarios including the analysis of combustion processes, monitoring of environmental and industrial emissions, and biomedical diagnostics. In the present study we describe the development of the first MIR-TLAS sensor combining substrate-integrated hollow waveguide iHWG technology with a new generation of laser diodes, so-called interband cascade lasers (ICLs), a.k.a. iHWG-ICL sensor system. Thereby, we capitalize on the compactness, robustness, and simple operation of the involved components yet maintaining excellent detection sensitivity for gas analysis.

Methane (CH$_4$) is a relevant target analyte in various environmental and biomedical processes. Being continuously released resulting from anaerobic oxidation of organic materials or leaking from, e.g., methane hydrate deposits, methane is considered an important greenhouse gas besides CO$_2$, NO, halocarbons, and water. Other sources of methane include leakage from landfills, domestic animals, and the production of fossil fuels. Consequently, the quantitative determination of methane and related hydrocarbons is essential due to its climate impact, and potential safety risks occurring at instantaneously produced and localized high concentration levels. In addition, inhaling methane may be affecting human health causing suffocation. Other health effects range from dizziness or headaches to unconsciousness.

In applications requiring high accuracy, molecular resolution, and sensitivity, various embodiments of TLAS have been established as a versatile tool for determining trace gas concentrations. Using single mode semiconductor lasers emitting in the NIR or MIR spectral regime, isolated absorption lines may be addressed by tuning the emission of the diode laser across that wavelength. Hence, TLAS gas analysis provides fast response times and almost real-time sensing at sensitivities at parts-per-million (ppm) to parts-per-billion (ppb) concentration levels.

Until recently, for monitoring the isolated absorption line of methane in the v3 fundamental band (i.e., near 3 µm), laser sources emitting in continuous-wave (CW) mode and
operating at ambient temperatures have largely been absent. The wavelength gap in the 3–6 μm regime has therefore been referred to as “mid-infrared gap.” Recently, this spectral window has attracted substantial global interest for sensing hydrocarbons (e.g., methane, ethane, etc.) and other relevant analytes (e.g., CO, NO, H₂O, etc.), which have characteristic “fingerprint” spectra in this range. It was only in the past few years that interband cascade lasers (ICLs) have been made available as single mode light sources with reasonable optical output and low electrical power consumption as a viable alternative to quantum cascade lasers (QCLs) in this wavelength range. Using lasers emitting around 3 μm, ICLs have proven their utility for monitoring of acetylene impurities in ethylene, and for polyethylene manufacturing processes. These light sources are type II lasers based on antimonide materials, and may be characterized as a combination of conventional diode lasers and quantum cascade lasers, as they likewise facilitate voltage-efficient cascading schemes. Next to a particularly small device footprint, their enhanced current efficiency at reasonable electrical power consumption ideally meets the demands for the development of compact MIR sensing systems.

Besides the availability of miniature laser sources, conventional multipass cells commonly applied in spectroscopic gas analysis have frequently been the size-limiting factor for the development of portable optical sensors and systems. White and Herriott gas cells with internal volumes up to several liters restrict the development of compact and rapidly responding TLS sensing systems. Combining efficient optical wave-guiding and serving as a highly miniaturized gas cell, has established hollow waveguides (i.e., light-pipe structures) as a promising alternative in gas analysis. In 1992, Croitoru presented a first concept by coating silver/silver halide onto the interior of a hollow silica fiber. For more compact configurations, Mizaiko and collaborators have recently presented an entirely new generation of hollow waveguides, the so-called substrate-integrated hollow waveguide (iHWG). These iHWGs extend optical path lengths via meandered channels into small footprint devices, thus offering minute sample volume analysis at rapid sample transient and associated sensor response times. iHWGs provide a notably improved signal-to-noise ratio (SNR) despite requiring only small sample volumes (i.e., few hundreds of microliters), thus rendering them well suited as a key component for quantitative gas absorption spectroscopy. A variety of gas sensing applications combining, e.g., compact FT-IR spectrometers and iHWGs for sensing CO₂, CH₄, C₂H₆, ozone, H₂S, and SO₂, have been shown by Mizaiko and his team. Sonnenfroh et al. have reported an ICL-based sensor for ambient CH₄ analysis operating near 3.3 μm. CH₄ was determined using a multipass gas cell providing an optical path of 7 m, and an internal volume of 1 L. With an ICL operating in cw mode at cryogenic temperature, and a thermoelectrically cooled InAs detector a precision of 15 ppb for 60 s integration time was reported. Moskalenko et al. demonstrated laser spectroscopy for the analysis of methane in human breath evaluating the ν₁ fundamental band at 3.47 μm (2882 cm⁻¹) for the investigation of methane concentrations in the breath of smokers vs nonsmokers. Using an InSbAs diode laser source and a single-path cell providing 2 m of absorption path length, a detection limit of 0.5 ppm for methane was reported. As the average concentration of methane in exhaled breath is 3–8 ppm, the detection limit was sufficient for breath methane analysis.

In this Letter, we report the development of a compact iHWG-ICL gas sensor for the first time combining substrate-integrated hollow waveguides with interband cascade lasers, thereby paving the way toward ultracompact MIR gas analyzers.

**Experimental Setup**

The sensor architecture comprises three main components: an ICL, the iHWG, and an infrared detector. In Figure 1b, a schematic view of the setup with all components is depicted. The developed sensor was aligned via an optical rail system (X48–0.5, Newport, Irvine, CA, USA) mounted on a bread-board enabling rapidly exchanging iHWGs with different lengths via precisely realigning kinematic base plates (KB25/M, Thorlabs GmbH, Dachau/Munich, Germany). The setup comprises a thermoelectric cooled ICL emitting single mode radiation at 3.366 μm manufactured by nanoplus GmbH (S/N: 1541/4–21, mounted in TO66).

For convenient operation, the ICL is enclosed in a compact (5 × 5 × 5 cm³) heatsink assembly providing thermoelectric cooling. A laser diode controller (TLD001, Thorlabs GmbH, Dachau/Munich, Germany) with low current noise (<3 μA) was used along with a TEC Controller (TTCC01, Thorlabs GmbH, Dachau/Munich, Germany) for assuring stable temperature conditions.

The ICL output power is 4.8 mW at an operation temperature of 20 °C, and required a drive current of 35 mA. The current tuning rate of the ICL is 0.152 nm/mA. For determining the laser tuning characteristics, the beam was propagated through a 2-mm-long ZnSe etalon with a free spectral range (FSR) of 30 GHz for converting scan time into laser wavelength by recording the signal after the etalon during current tuning of the laser. Methane absorption spectra calculated from the HITRAN database were then compared with the experimentally obtained spectrum using the iHWG-ICL sensor system. Importantly, radiation from the ICL was directly coupled into the gas cell (i.e., without any additional optical element such as focusing lenses in between), and propagated along the iHWG gas cell. iHWGs were fabricated from either brass or aluminum. The assembled iHWGs had dimensions of 250 × 25 × 20 mm³ or 150 × 25 × 20 mm³ (L × W × T).
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with a waveguiding channel cross-section of 2.1 × 2.0 mm², which simultaneously serves as miniaturized gas cell. To ensure a high surface reflectivity for optimum waveguiding, the top- and base-substrates were polished to a mirror-like finish using commercially available diamond polishing suspensions. Then, gold was galvanically deposited only onto the brass substrate for improving the reflectivity yielding almost comparable results between neat Al and Au-coated brass iHWGs, yet with the latter requiring one more processing step. As an intermediate step, galvanic copper plating (copper film thickness approximately 1 μm) was used to protect brass from oxidation, and for enhancing the adhesion between the substrate and the gold layer. Detailed information on the surface quality and assembly of iHWGs has been published in detail elsewhere.7

Female V2A steel Luer lock connectors (EHS Medizintechnik GmbH, Leinfelden-Echterdingen, Germany) were attached to the iHWGs and sealed with PTFE o-rings providing top-surface access ports to the iHWG channel for the sampled gas. The channel itself was sealed via MIR-transparent BaF₂ windows (6.65 mm in diameter × 0.5 mm, OEC GmbH, Zusmarshausen, Germany).

For preparation of gas mixtures with different methane concentrations, a custom-made gas mixing system (GMS) based on mass flow controllers developed in collaboration with Lawrence Livermore National Laboratory (LLNL, Livermore/CA, USA) was used. A certified gas standard of methane (nominal concentration: 10 000 ppm in N₂, Western AG, Weilheim, Germany) was diluted by the GMS and fed into the iHWG.

Radiation emanating after the iHWG was directly transmitted onto a HgCdTe detector (FTIR-16–200 MSL-12, InfraRed Associates Inc., Stuart, FL, kept at 77 K via liquid nitrogen) with an active detector element area of 4 mm², which ideally matches the dimensions of the iHWG channel. Linear laser current modulation was applied by continuously and repetitively scan the iHWG emission wavelength across the selected methane absorption line. The function generator of a digital oscilloscope (Picoscope 5444B, PicoTechnology, Ltd., Cambridgeshire, UK) was used to generate a sawtooth wave with an offset of 2 V, and peak amplitude of 0.9 V. The loss in optical power after transmission through the iHWG was approximately 5–10%. The electrical signal is amplified using a custom-made amplifier, and digitized via the 16 bit digital oscilloscope at a sampling rate of 5 k Samples/s.

For the spectral data evaluation, the following procedure was applied: Nitrogen was used as an IR-inactive reference and background gas. The obtained methane absorption line was fitted with a third order (i.e., Savitzky–Golay filter) polynomial baseline, and a Voigt line-shape using a nonlinear Levenberg–Marquardt algorithm (ORIGIN software package; Microwal Software, Northampton/MA, USA). For quantitative data evaluation, a calibration function was established by evaluating the peak height after Voigt fitting, which was then plotted vs the methane concentration.

Two hundred successive measurements were averaged per sample, and each concentration was independently analyzed eight times. The limit of detection (LOD) and limit of quantification (LOQ) were derived from the slope of the calibration function using the 3σ and 10σ criterion, respectively, whereby σ is the standard deviation of the blank values.

RESULTS AND DISCUSSION

The ICL emission was scanned across the methane absorption line via current tuning within 5 ms at constant temperature averaging 200 successive spectra for a series of methane concentrations ranging from 50 to 400 ppm. Calibration functions were established for a series of iHWGs with a length of 15 and 25 cm, respectively, based on quantitative data analysis using a Voigt fit. The calculated LODs were determined as three times the standard deviation of the blank signal while purging the iHWG with pure nitrogen gas. All measurements were performed at atmospheric conditions.

data analysis was based on the evaluation of the maximum absorbance at 3.3888 μm via a Voigt profile fit to the shape of the absorption line. For each concentration, the mean value of eight independent replicate measurements was calculated. A linear response of the sensor systems within the selected concentration range of 50–400 ppm, CH₄ was obtained in compliance with the Lambert–Beer law. As anticipated, the slope of the regression function increased with increasing length of the waveguide due to the extended absorption path length, i.e., both waveguides with 25 cm nominal path length revealed an increase in sensitivity with LODs < 10 ppm, corresponding to a sensitivity improvement by a factor of 4–5.

In contrast to conventional FT-IR-based sensing techniques, TLAS provides reduced interference due to the narrow-band laser emissions, albeit at limited multicomponent analysis capabilities. In this study, even though the sensor setups were not enclosed in a housing, no interference from other atmospheric constituents in the spectral region of 3.367 to 3.370 μm (e.g., water, CO₂ etc.) were detected.

For completely regenerating the iHWG, a purging time of 60 s with pure nitrogen gas was applied after each sample. Within that period, the internal iHWG volume (approximately 100 μL) was exchanged multiple times, and a steady-state concentration of the next sample prepared by the GMS was ensured. The achieved LOD and LOQ values for each iHWG are summarized in Table 1. They were calculated according to

Table 1. Analytical Figures-of-Merit Obtained for the Investigated IHWG-ICL Sensing System Configurations

<table>
<thead>
<tr>
<th>LOD (ppm)</th>
<th>LOQ (ppm)</th>
<th>SNR</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold15</td>
<td>28</td>
<td>93</td>
<td>52</td>
</tr>
<tr>
<td>Au15</td>
<td>23</td>
<td>76</td>
<td>55</td>
</tr>
<tr>
<td>Gold25</td>
<td>67</td>
<td>20</td>
<td>209</td>
</tr>
<tr>
<td>Au25</td>
<td>7</td>
<td>23</td>
<td>164</td>
</tr>
</tbody>
</table>

Gold15: Gold-coated 15 cm iHWG; Au15: Au-coated 15 cm iHWG; Gold25: Gold-coated 25 cm iHWG; Au25: Au-coated 25 cm iHWG.

In Figure 2, calibration functions for the investigated sensor systems with corresponding errors bars are shown. Quantitative

Figure 2. Comparison between the calibration functions for the designed four waveguides. A higher sensitivity is observed for increasing optical path length (i.e., length of the HWG) of the sensor system, i.e., increased slope of the calibration function.

Figure 2
IUPAC using the 3σ/10σ criteria (i.e., three times/ten times the standard deviation of the blank value). All LODs were calculated between 6 and 28 ppm. If extended measurement times can be afforded, the achievable LOD may be further reduced by increasing the number of averaged scans (e.g., 2000 averaged scans decrease the LOD by a factor of $10^{23}$). An immediate improvement of the detection limit was achieved by increasing the absorption path length provided by the iHWG. Evidently, the polished aluminum waveguide with an integrated nominal channel length of 15 cm resulted in superior sensitivity compared to the same waveguide made from gold-coated brass, which is attributed to the high surface quality achieved for polished aluminum. Surface scattering losses are the dominant attenuation which is attributed to the high surface quality achieved for polished aluminum. Surface scattering losses are the dominant attenuation effect when propagating along hollow waveguide structures; thus, the surface roughness is the critical parameter from a fabrication point of view.

An exemplary experimentally obtained spectrum of the selected CH$_4$ line between 3.367 and 3.370 μm is shown in Figure 3. The solid line (black) represents the experimental signal, while the dotted line (blue) shows the correspondingly calculated HITRAN absorption spectrum at (1013 hPA and 296 K, absorption path length assumed as the physical length of the iHWG, i.e., 25 cm). The experimentally obtained line intensities are in excellent agreement with the anticipated calculated values (<10% deviation). Consequently, it is concluded that using the Voigt profile for data analyses adequately models the absorption line shape at the present experimental condition. The observed deviations may result from minute fluctuations from laser instabilities, the gas mixing procedure, and the actual absorption path length provided by the iHWG, which is exceeding the physical length of the iHWG channel.

After optimization and as previously reported, the applied polishing routines yielded a combined surface roughness/waviness of RMS = (44 ± 11) nm. According to the 2/10th criterion, the surface quality is more than sufficient for efficiently propagating MIR radiation at 3 μm. All sensor configurations showed a coefficient of determination ($R^2$) of >0.99, thereby confirming suitability of the established calibration model for quantitative analysis. The sensor system comprising the 15 cm Al iHWG revealed the highest precision ($R^2 = 0.9997$), thereby indicating an excellent linear fit of the regression. It should be noted though that neat Al and Au-coated brass iHWGs of the same length revealed comparable performance, yet with the latter requiring one more processing step during fabrication.

It is hypothesized that a significant component of the variance relates to the gas mixing procedure, as only one standard gas mixture (1%; 10 000 ppm, of methane in nitrogen) was used and correspondingly diluted (standard deviations in the range 1–10% are expected). In this study, direct absorption TLAS (dTLAS) was performed for the detection of CH$_4$. The detectability of the developed sensor is strongly affected by noise of the laser source and other utilized electronic devices. In order to remove stochastic noise from the ICL and detector and improve the signal-to-noise ratio successive ramps are signal averaged using the digital oscilloscope. With 200 averaged ramps, achieved SNR values from S2 to 209 are quite satisfactory. Finally, it is anticipated that further improvement of the sensitivity can be achieved using, besides more scans, frequency modulation or electronic common-mode noise-reduction techniques. For example, using wavelength modulation spectroscopy (WMS) strategies the SNR is improved by encoding and detection of the measured signal at high frequencies, as the system noise inherently decreases at increasing frequencies. Thereby, the analytical signal is detected at a harmonic of the modulation frequency.

On purpose, in the present study the ICL light source was directly coupled into the iHWG without collimating lenses, in order to demonstrate the principal feasibility of establishing a compact and robust sensor design minimizing alignment needs or potential misalignment during operation, yet potentially trading off sensitivity of the device. Hence, given the divergence of the beam multiple reflections along the propagation path inside the iHWG have to be considered resulting in corresponding reflection losses. Using a collimated beam, further improvement of the SNR is anticipated, in particular, if the collimating lens is directly integrated with the ICL source. In addition, upon availability of a wider selection of ICLs a stronger absorption line for the desired analyte may be selected, thus further improving the sensitivity.

Yet, the results obtained herein demonstrate the fundamental feasibility of iHWG-ICL sensor technology with significant potential for cost-effective in-field MIR gas phase sensing applications with small device footprint, low sample volumes, rapid response times, and future sensitivity improvements toward ultratrace gas diagnostics.

**CONCLUSIONS**

In this study, we described the first mid-infrared gas sensor combining substrate-integrated hollow waveguides with interband cascade lasers for rapidly sensing minute sample volumes. Due to the compact dimensions and adaptability of the iHWG, a modular and adaptable sensor system with a small footprint and low power consumption was established facilitating in-field usage. Monitoring of methane was performed by directly coupling MIR radiation from the ICL into the iHWG and current-tuning of the laser across a characteristic absorption line of methane. Comparison with calculated spectra from the HITRAN database revealed excellent agreement with the experimentally obtained results. Using the iHWG-ICL sensor system, LODs for methane as low as 6 ppm, were achieved.
Future work will incorporate DFB ICLs with integrated collimation lenses and optical higher output power along with advanced signal modulation techniques. Already now, the developed iHWG-ICL sensing platform enables the determination of ppm-level concentrations of relevant gas phase constituents in exceptionally low volume samples (few hundred microliters) associated with fast sample transient times when monitoring concentration fluctuations. Given the modularity and flexibility of the iHWG-ICL sensing technology, a robust and generic mid-infrared sensing platform is provided enabling gas sensing in a wide range of application scenarios ranging from environmental analysis to process monitoring and biomedical diagnostics such as exhaled breath analysis.

**REFERENCES**


All measurements and data analysis were performed by E. Tütüncü, the setup was organized in cooperation with V. Kokoric and A. Wilk, who designed the waveguides. F. Seichter and M. Schmid performed preliminary studies. W. E. Hunt, A.M. Manuel, P. Mirkarimi, J. B. Alameda from Lawrence Livermore National Laboratory contributed to the fabrication of the waveguides. Analytical and scientific conclusions after data analysis in close communication with J. C. Carter and B. Mizaikoff.

Fiber-Coupled Substrate-Integrated Hollow Waveguides: An Innovative Approach to Mid-infrared Remote Gas Sensors

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ABSTRACT: In this study, an innovative approach based on fiber-coupled substrate-integrated hollow waveguide (iHWG) gas cells for the analysis of low sample volumes suitable for remote broad- and narrow-band mid-infrared (MIR; 2.5–20 μm) sensing applications is reported. The feasibility of remotely addressing iHWG gas cells, configured in a double-pass geometry via a reflector, by direct coupling to a 7-around-1 mid-infrared fiber bundle is demonstrated, facilitating low-level hydrocarbon gas analysis. For comparison studies, two iHWGs with substrate dimensions of 50 × 50 × 12 mm (L × W × H) and geometric channel lengths of 138 and 58.5 mm, serving as miniature light-guiding gas cells, were fiber-coupled to a Fourier transform infrared spectrometer enabling broadband MIR sensing. In addition to the fundamental feasibility of this concept, the achievable sensitivity toward several gaseous hydrocarbons and the reproducibility of assembling the fiber-iHWG interface were investigated.

KEYWORDS: substrate-integrated hollow waveguide, iHWG, hollow waveguide (HWG), remote sensing, double-pass, optical fiber coupling, infrared spectroscopy, gas sensors

Since their introduction by Garmire et al. in the late 1970s, hollow waveguide (HWG) technologies for the transmission of infrared (IR) energy have continuously evolved and spread to new applications. A key development in 1983 was the demonstration that the addition of an optically transparent dielectric layer to a metallic waveguide surface significantly reduced IR transmission losses in straight and bent HWGs. Starting with a sacrificial aluminum tube, Miyagi and co-workers used a combination of sputtering, plating, and etching to fabricate and test nickel hollow waveguides with and without an inner germanium layer. This early work led to the study of other combinations of metallic and dielectric coatings including silver/silver iodide coatings on the inherently smooth inner surface of glass tubes, developed in 1994 by Abel and Harrington. Commercially available conventional HWGs for propagating IR radiation via reflections inside the hollow core are based on these early studies.

Although IR HWGs were originally developed for the delivery of high-energy coherent CO2 lasers, their use has grown to include IR radiometric and spectroscopic applications including gas sensing using broad- and narrow-band sources. For the latter application, HWGs simultaneously serve as waveguide and miniaturized gas cell, enclosing a minute gas volume on the order of a few milliliters. There are numerous papers on gas sensing that describe the merits of combining HWGs with Fourier transform infrared (FTIR) spectrometers.

Although the optical path length (OPL) achievable by HWGs is limited to a few meters at most (i.e., limiting also the achievable detection limits), conventional multipass gas cells (e.g., Herriott and White cells) suitable for ultratrace analysis have in turn limited sample transition times due to exceedingly large sample volumes. For the present study, we utilized a new class of HWG, the substrate-integrated hollow waveguide (iHWG), which was recently developed by our research team and described in detail by Wilk et al. In comparison to conventional, silica-based HWGs, the iHWG offers several key advantages: the optical channel is integrated into a mechanically robust substrate of compact size; the optical channel path length can be tailored along with the location of light in- and out-coupling ports via different geometries (e.g., straight, spiral, serpentine); and the entire optical channel is accessible, based

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on the iHWG two-piece design concept, enabling the addition of optical coatings and optics. Other advantages include improved mechanical ruggedness against drop, shock, and vibrations; ease of alignment; and compatibility with optical fibers. Hence, iHWGs are particularly suitable for field usage and applications in harsh environments, while at the same time being highly cost-effective. Since their introduction in 2012, the iHWG concept has proven useful in various challenging applications including compact NIR spectrometry, diagnostic conversion capabilities, and studies to show how the internal stresses of a coiled HWG from bending losses. Furthermore, there are no long-term demonstrated, the results show significant improvement in the overall lie of the sensor. These issues were the primary motivation for our developing the iHWG-based sensor concept.

The double-pass sensor configuration employed by Zhao and co-workers for remote detection of percent-level concentrations of methane in coal mines is similar in principle to our MIR iHWG-based sensing approach. In their 50 mm path length cell, a mirror is similarly used to effectively double the path length over which the gas is sampled. The gas cell is fiber-coupled back to a detector and NIR source operating at a wavelength that is appropriate for the gas being measured. This configuration of the “iHWG” makes it possible to observe the interference fringes, which are characteristic of the interference of the two light beams. This interference pattern can be used to monitor changes in the gas concentration. In addition, this configuration allows for easier alignment and reduces the risk of damage to the sensor. The iHWG is particularly suitable for applications in harsh environments, such as industrial environments, where traditional sensors may fail due to exposure to high temperatures, vibrations, and other demanding conditions.

The experimental setup is shown in Figure 1 and comprises a Bruker iCube FTIR spectrometer (1) (Bruker Optics GmbH, Ettlingen, Germany), a liquid nitrogen-cooled mercury–cadmium–telluride (MCT) detector (8) featuring a 2 × 2 mm active element (FTIR: 16–2000 cm⁻¹), a 0.05 cm focal length set, and a 7-around-1 silver halide fiber bundle. In the present study, we demonstrate double-pass iHWG-based sensors with a single integrated fiber bundle-coupling interface for detecting gas species, remotely and in situ. The integrated fiber minimizes MIR in- and outcoupling losses and enables embedding the iHWG within a device. Although the current iHWG-based sensor utilizes a 1 m long silver halide fiber bundle, this fiber can easily be disconnected from the iHWG and replaced with a different fiber bundle type and/or length, the installation of a new fiber requires minimal effort and does not affect the iHWG. In addition to describing the sensors, this paper discusses the figures-of-merit of FTIR-based iHWGs coupled to silver halide fiber bundles for advanced gas-sensing applications.

**Experimental Section**

The experimental setup is shown in Figure 1 and comprises a Bruker iCube FTIR spectrometer (1) (Bruker Optics GmbH, Ettlingen, Germany), a liquid nitrogen-cooled mercury–cadmium–telluride (MCT) detector (8) featuring a 2 × 2 mm active element (FTIR: 16–2000 cm⁻¹), a 0.05 cm focal length set, and a 7-around-1 silver halide fiber bundle. In the present study, we demonstrate double-pass iHWG-based sensors with a single integrated fiber bundle-coupling interface for detecting gas species, remotely and in situ. The integrated fiber minimizes MIR in- and outcoupling losses and enables embedding the iHWG within a device. Although the current iHWG-based sensor utilizes a 1 m long silver halide fiber bundle, this fiber can easily be disconnected from the iHWG and replaced with a different fiber bundle type and/or length, the installation of a new fiber requires minimal effort and does not affect the iHWG. In addition to describing the sensors, this paper discusses the figures-of-merit of FTIR-based iHWGs coupled to silver halide fiber bundles for advanced gas-sensing applications.
silver halide fiber with a core/cladding diameter of 630/700 μm (collection) and 900/1000 μm (delivery) has a length of ~1 m. The numerical aperture of the fibers are 0.28 ± 0.03, with a core refractive index of 2.15. A detailed cross section of the coupling between fiber bundle and iHWG is shown in the Supporting Information.

The iHWG (50 mm × 50 mm × 12 mm; L × W × H) was fabricated from an AlMG, alloy. The iHWG device comprised two layers, i.e., a base substrate with a milled (2 × 2.1 mm) optical channel, and a flat top substrate. Using a magnetron sputter deposition system, the channel and the top substrate of the iHWG were gold-coated. For achieving a double-pass configuration, a gold mirror was integrated at the end of the iHWG channel.

In Figure 2, three basic fiber bundle-to-iHWG coupling options are illustrated: noncontact mode (Figure 2a) and fiber insertion into the waveguide with (Figure 2b,d) and without window (Figure 2c). In noncontact mode (Figure 2a), fiber and iHWG are mounted separately and aligned in close proximity. Using this option, an air gap is always present between the fiber and window. The distance between the fiber and the sealing window can be adjusted for optimal coupling. Simple, noncontact coupling is of limited robustness and reproducibility during field usage, as several degrees of freedom are present. Because of the high refractive index of the window (e.g., BaF$_2$ or ZnSe), part of the radiation is always reflected directly back into the fiber and not coupled into the iHWG. Thus, stray light contributions are generated, which in turn limit the achievable linearity of the analytical signal, resulting in deviations from the Beer–Lambert law. Moreover, this assembly is sensitive to vibrations, and the alignment is difficult to sustain during field analysis. In the Figure 2b cross-section, a fiber coupling method with inserted fiber is shown; the Figure 2d model is an illustration of this type interface. Both fiber and window are placed into the iHWG. Using an o-ring or gasket with the window assures superior iHWG gas-tightness, because the fiber is coupled to the FTIR using free-space optics, resulting in the possible diffusion of ambient gas into the fiber bundle and along the fiber face; the addition of extra epoxy in each SMA connector helps to mitigate this. The major benefit of an integrated fiber coupling design is minimal coupling losses (unless the internal window is present) and ease of alignment, as the fiber is inserted in a designated connection port. The fiber is held in place tightly with a high level of shock resistance compared to the noncontact design. Furthermore, excellent repeatability between connections is provided, as positional variations arising from repeated insertions of the fiber are minimized. In the present study, full integration of the fiber bundle into the iHWG with no internal window (Figure 2c) is presented. Back-reflected radiation is eliminated with this windowless design. A miniaturized custom fiber guide was added to the iHWG, as shown in Figure 3a,b, to secure the fiber to the iHWG using two set screws. The only downside for this coupling is minimal centering errors caused by uneven tightening of the screws. An O-ring located inside the fiber guide provided a robust seal that sufficiently prevented ambient gas from diffusing into the iHWG during laboratory measurements.

Any possible ambient gas leakage along the face of the fiber bundle is tolerable, as measurements are performed while dynamically flowing calibrated gas mixtures through the iHWG device under controlled conditions to ensure known gas concentrations are measured and to speed up measurements for the laboratory sensor testing.

Each iHWG optical channel is fitted with a gold-coated retro-reflecting mirror located distal to the proximal fiber bundle/iHWG interface, effectively doubling the absorption path length over which the MIR radiation interacts with the gas molecules in the iHWG volume. Thereby, a nominal path length of 276 mm (138 mm × 2) had been achieved for the coiled waveguide geometry and 117 mm...
(58.5 mm × 2) for the straight channel sensor. In the Figure 3a,b, both iHWG sensors with illustrated optical/gas sensing channels (in red) are shown. The propagation of MIR radiation in the coiled iHWG channel as illustrated in Figure 3a demonstrates how the optical path length (OPL) can be tailored within the same overall footprint for a particular sensing application. It should be noted that the developed iHWG sensors not only propagate MIR radiation but also have proven applicable for near-infrared (NIR) gas-sensing scenarios.

### RESULTS AND DISCUSSION

**Sensor Setup.** A signal of 1.7 × 10^5 ADC (analog-to-digital converter) counts was obtained for the straight channel (i.e., 58.5 mm OPL) iHWG-based sensor (see Figure 1) equipped with a large cross-section (4 mm²) MCT element. MIR radiation traversing the straight channel structure is reversed via a reflecting gold mirror placed at one end of the channel, thereby doubling the absorption path length to 117 mm. The main loss mechanism in this configuration is associated with scattering and reflection losses inside the iHWG channel, despite having highly precision machined surfaces and gold coatings to mitigate losses to the extent possible. For the coiled iHWG channel structure, fewer detector counts were obtained as compared to the straight configuration (i.e., 4–4.4 × 10^5 ADC counts). Ray trace modeling confirmed that the coiled iHWG channel produces some reflections beyond the acceptance angle of the collection fibers, resulting in that fraction of light not making it to the MCT detector (Supporting Information). Transmission efficiencies were estimated to be 51.5% for coiled and 84.9% for straight iHWG designs. Another source of MIR radiation loss are the fibers, given their inherent transmission characteristics. In Figure S-1, a plot of a single-channel IR spectrum shows the transmission profile of the silver halide fiber and significant attenuation beyond 3000 cm⁻¹. In addition, absorption from ambient CO₂ (2300–2400 cm⁻¹) and H₂O (1700–3000 cm⁻¹) are also present in the spectrum. A cutoff below 700 cm⁻¹ confines the long wavelength transmission window of these fibers. Using a silver halide fiber-bundle therefore limits the useful spectral window to 3000–700 cm⁻¹ for analytical purposes, despite the fact that the iHWG structure would propagate radiation above and beyond these limits. In Figure S-2, the standard deviation of the noise is plotted across this spectral window. The mean spectrum of the noise was calculated by averaging 20 blank (N₂ gas only) spectra. Studying the mean noise absorption spectrum confirms the observations derived from the single-channel spectra (Figure S-1). Despite evident losses, it was confirmed that the extended absorption path length provided by the coiled iHWG outweighs the increase in reflection losses, thus providing enhanced analytical sensitivity versus the straight iHWG configuration.

**Analytical Performance.** Calibration samples were prepared via a custom dynamic flow gas mixing calibration system developed by IABC and LLNL based on mass flow controllers (Brooks SLA 7850 series, Brooks Instruments-Hatfield, USA ± 1% accuracy) and delivered into the iHWG sensor, as shown in Figure 3a,b. For testing purposes, cyclopropane, isobutylene, and methane (nominal concentrations, 1% balanced in N₂) were used. Sample gases were fed into the iHWG at a nominal flow rate of 200 sccm. Between consecutive measurements, the sensor was flushed with N₂ (purity >99.999%) for 90 s. Gas exchange times of both cells were estimated to be 170 ms (coiled iHWG, 579 μL inner volume) and 75 ms (straight iHWG, 245 μL inner volume) at a flow rate of 200 sccm. MIR spectra were recorded in the spectral range from 3000 to 700 cm⁻¹ at a spectral resolution of 2 cm⁻¹. Throughout the studies presented here, a Blackman–Harris three-term apodization was used, and 200 spectra were averaged for background and sample single-channel scans. The time for recording each spectrum is approximately 80 s. Data acquisition and peak area integration were performed via the OPUS 6.5 software package (Bruker Optics, Ettlingen, Germany). Further data acquisition parameters are listed in Table S-1. Figure 4 presents exemplary individual spectra of the investigated gases at 5000 ppm, measured with the developed fiber bundle-coupled iHWG MIR sensor system having a coiled (i.e., 276 mm OPL) channel.

![Figure 4](image)

**Table 1. Integration Boundaries for Peak Evaluation of Methane, Isobutylene, and Cyclopropane**

<table>
<thead>
<tr>
<th>analyte</th>
<th>integration area [cm⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>methane</td>
<td>1375–1225</td>
</tr>
<tr>
<td>isobutylene</td>
<td>925–850</td>
</tr>
<tr>
<td>cyclopropane</td>
<td>1100–800</td>
</tr>
</tbody>
</table>

The peak areas presented in Figure 3a,b are plotted as a function of both cells (i.e., 4 × 10^5 ADC counts). Ray trace modeling confirmed that the coiled iHWG channel produces some reflections beyond the acceptance angle of the collection fibers, thus providing enhanced analytical sensitivity versus the straight iHWG configuration. Because the coiled iHWG has an extended OPL of 276 mm (as compared to the straight channel iHWG with 117 mm) while maintaining the same form factor of 50 × 50 mm, absorbances of all analytes were increased as expected.

For evaluating and comparing the sensor performance of both iHWG designs (Figure 3a,b) configured as a fiber-coupled MIR double-pass sensor, characteristic absorption features of each analyte were selected for the estimation of univariate limits of detection. The peak areas presented in Table 1 were used for generating associated calibration functions. Each analyte comprises spectral bands located in spectral regions of highest transmission for the iHWG sensor and isolated from ambient H₂O and CO₂ absorption features.

Two exemplary calibration functions for isobutylene obtained for the iHWG sensing setups compared herein are shown in Figure 5 with corresponding error bars (displayed as ±5t). Each data point represents the mean of eight replicate measurements per concentration at five different concentra-
tions ranging from 500 to 7000 ppm. Figure S-3 shows the spectra of varying concentrations of isobutylene. It is evident that the slope of the regression line increases with increasing absorption path length, thus indicating an increased sensitivity. The strategy of a retro-reflectively doubled optical path length thus complies with the Beer–Lambert law, stating a linear relationship at low absorbance values.

The coefficient of determinations ($R^2$) for both systems revealed values of 0.9989 (coiled iHWG) and 0.9999 (straight iHWG). The error bars (±5σ) confirm precise and repeatable results. Figure S-4 shows an exemplary mean MIR absorption spectrum of eight consecutively recorded spectra, and the associated uncertainties reveal the obtained precision of 1.54 % expressed as the relative standard deviation of area.

Univariate limits of detection (LODs) were estimated for each analyte based on the linear calibration functions fitted in Figure S-5 using the method of Incze et al. The LOD was estimated by taking the ratio of the product of the confidence factor ($k = 3.29$) and the mean area of 20 blank (i.e., pure N2 gas) measurements integrated across the same spectral region as the analyte band by the slope of the regression function. The estimated LODs for all test analytes determined with both iHWG channel geometries are summarized in Figure 6. The tested iHWGs demonstrate LODs from 22 ppm, for isobutylene to 57 ppm, for cyclopropane.

When the estimated LODs for methane obtained with the coiled and straight channel iHWGs are assessed, it is apparent that the detection limits are quite similar. An improvement in sensitivity due to the coiled channel geometry is evident for the detection of cyclopropane, which yielded an improvement in LOD of 26%. Counterintuitively, for isobutylene the straight channel iHWG achieves a higher sensitivity. Figure S-5 shows isobutylene spectra at concentration levels of 10–50 ppm. As the method for the determination of the LOD considers both the standard deviation of blank samples (σblank) and the slope of the regression function, LOD values are also affected by the blank value variance. Low levels of noise (i.e., low blank variance) may be achieved by high-energy throughput translating into superior sensitivity and analytical performance. In Figure 7, the MIR absorption spectra between 930 and 840 cm$^{-1}$ recorded with both iHWG channel structures for 500 ppm, isobutylene (Figure 7a) and pure N2 (Figure 7b) are shown for elucidating the correlation between OPL and noise level. The coiled channel iHWG, providing an extended OPL, achieves a higher absorbance (gray line), as compared to the straight channel iHWG. However, this does not immediately translate into an improved analytical performance, as radiation losses tentatively attributed to increased reflection losses apparently result in an increased noise level, which is detrimental to the achievable LOD. In Figure 7b, exemplary MIR spectra of N2 are shown to illustrate the considerably smaller noise level for the straight channel iHWG (blue line). The standard deviation of the blank measurement is decreased by 75%. The reduced light throughput along with the increased baseline noise associated with the coiled iHWG structure apparently outweighs the gain in absorbance obtained via the increased OPL.

In the present study, it was demonstrated that a fiber bundle may be directly and efficiently interfaced with a hollow waveguide gas sensor without any optics in between, thereby...
providing a robust double-pass sensing geometry. Herein, the fiber bundle was directly inserted into the iHWG via a custom port ensuring gas-tight sealing. Furthermore, this assembly ensures permanent optical alignment along with readily replaceable interconnections, which is essential to field deployment. The results are summarized in Table 2.

### Table 2. Summary of the Obtained Results and Dimensions for the iHWGs Designs Studied Herein

<table>
<thead>
<tr>
<th></th>
<th>straight iHWG</th>
<th>coiled iHWG</th>
</tr>
</thead>
<tbody>
<tr>
<td>substrate dimensions (L × W × H)</td>
<td>50 × 50 × 12 mm</td>
<td>50 × 50 × 12 mm</td>
</tr>
<tr>
<td>optical path length (single pass)</td>
<td>58.5 mm</td>
<td>138 mm</td>
</tr>
<tr>
<td>retrieved mean area for isobutylene (7800 ppm) cm⁻¹</td>
<td>5.43</td>
<td>9.52</td>
</tr>
<tr>
<td>SNR</td>
<td>1049</td>
<td>499</td>
</tr>
<tr>
<td>limit of detection (ppm)</td>
<td>22</td>
<td>43</td>
</tr>
</tbody>
</table>

It should be noted that the comparability of the reported LOD values benefit from the realignment accuracy of this fiber-based coupling method avoiding any free-space optics and are all based on the same evaluation method (i.e., integration interval, spectral resolution, number of scans, etc.). However, the LOD for any specific analyte was not optimized during these proof of demonstration studies. Hence, LODs may be improved by several strategies including averaging more scans (e.g., by a factor of approximately 10 when averaging 2000 scans) or by using advanced light sources in lieu of the FTIR such as quantum cascade lasers (QCLs) and interband cascade lasers (ICLs) operating with significantly higher energy densities albeit within reduced spectral windows.

### CONCLUSIONS

In the present study, the first prototype of a fiber optically coupled remote mid-infrared gas sensor based on substrate-integrated hollow waveguides (iHWGs) were developed and characterized. A silver halide fiber bundle was integrated with the iHWG via a single access port for light delivery and collection avoiding any additional optical coupling elements. This innovative and robust optical sensor concept was tested for the analysis of isobutylene, cyclopropane, and methane facilitating detection limits at low ppm, concentration levels with rapid sample transition times while probing minute gas volumes. This device concept enables positioning a gas-sensing head remotely from the MIR light source (e.g., FTIR spectrometers), thereby enabling analytical applications in hazardous environments with limited access opportunities. It should be noted that although the experimental setup described herein utilized direct gas flow into the iHWG during measurements, this was done for convenience for testing purposes. We foresee these devices being used most beneficially as embedded sensors wherever the gas flow inlet and outlet ports allow gases to passively diffuse into and out of the iHWG. Due to an optical configuration that is entirely waveguide-based, a robust advanced gas-sensing tool with excellent analytical characteristics is provided. Given the modularity of the approach, the iHWG may be tailored and adapted to individual gas-sensing scenarios. The versatility of the developed sensing approach enables not only broadband IR sources but also advanced narrowband light sources including quantum cascade and interband cascade lasers for further improving the sensitivity in industrial, environmental, and biomedical application scenarios.

### ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssen-s.7b00253.

Further details on data acquisition, infrared spectra, and ray-tracing simulations (PDF)

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### REFERENCES

10. Seichter, F.; Wilk, A.; Wörl, K.; Kim, S.-S.; Vogt, J. A.; Wachtler, U.; Rademacher, P.; Mizaiko, B. Multivariate Determination of 13C/12C CO2 Ratios in Exhaled Mouse Breath with Mid-Infrared...
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5.5 Paper V. Advanced Photonic Sensors based on Interband Cascade Lasers for Real-time Mouse Breath Analysis

E. Tütüncü designed the dual-channel substrate integrated hollow waveguide, performed all measurements in the laboratory and in the intensive care unit, conducted the data evaluation, and wrote the manuscript. M. Nägele (OptoPrecision GmbH) developed the sensor setup and programmed the data control and evaluation system. M. Fischer and S. Becker (nanoplus GmbH) developed the laser source. C. Wolf, S. Köstler and V. Köstler (Joanneum Research mbH) were responsible for the oxygen sensor. A. Teuber assisted during the calibration and long term testing of the analyzer. M. Gröger, S. Kress, M. Wepler performed the mouse breath studies in the ICU, where Ulrich Wachter performed GC-MS reference measurements. J. Vogt assisted in data interpretation. P. Radermacher and B. Mizaikoff conceived and supervised the entire project. All authors contributed to preparation, writing, and editing of the manuscript.

Advanced Photonic Sensors Based on Interband Cascade Breath Lasers for Real-Time Mouse Breath Analysis

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ABSTRACT: A multiparameter gas sensor based on distributed feedback interband cascade lasers emitting at 4.35 µm and ultrafast electro-spun luminescence oxygen sensors has been developed for the quantification and continuous monitoring of 13CO2/12CO2 isotopic ratio changes and oxygen in exhaled mouse breath samples. Mid-infrared absorption spectra for quantitatively monitoring the enrichment of 13CO2 levels were recorded in a miniaturized dual-channel substrate-integrated hollow waveguide using balanced ratiometric detection, whereas luminescence quenching was used for synchronously detecting exhaled oxygen levels. Allan variance analysis verified a CO2 measurement precision of 1.6‰ during a 480 s integration time. Routine online monitoring of exhaled mouse breath was performed in 14 mechanically ventilated and instrumented mice and demonstrated the feasibility of online isotope-selective exhaled breath analysis within microliters of probed gas samples using the reported combined sensor platform.

KEYWORDS: substrate integrated hollow waveguides, breath analysis, interband cascade laser, electrospun polymer nanofiber, isotope enrichment

Exhaled breath analysis (EBA) has attracted a considerable amount of scientific and clinical interest, as noninvasive real-time identification and quantification of potential disease biomarkers offers substantial clinical potential. Due to the low concentrations of biomarkers, highly selective and sensitive analytical instruments and methods are required for the detection and quantification of relevant biomarkers for disease diagnostics and metabolic status monitoring. The latter is based on the analysis of endogenously produced breath compounds or the analysis of breath metabolites after substrate administration. Established biomarkers such as exhaled carbon dioxide (CO2) and the carbon isotopologues, 13CO2 and 12CO2, have been successfully demonstrated for the diagnosis of Helicobacter pylori infections, liver malfunction, bacterial overgrowth, and fat absorption. Changes in isotopic ratio of both isotopes is herein observed by administration of 13C-enriched sugars and may directly be assigned to alterations of metabolism processes. The increase of the isotope ratio in the expired breath (a.k.a., enrichment) allows one to noninvasively monitor glucose metabolism. Analytical techniques meeting the requirements of high precision and accuracy for breath gas analysis include gas chromatography (GC–MS),2,3 and isotope ratio mass spectrometry (IRMS).4 Although these techniques demonstrate excellent figures-of-merit for δ13C analysis, their routine establishment is limited due to large instrumental dimensions. Moreover, continuous online measurements next to mechanically ventilated animals are not feasible, as sampling is required. Online monitoring
Capabilities for exhaled breath analysis will only gain momentum if near-real-time optical/spectroscopic techniques are harnessed for the determination of relevant exhaled breath biomarkers in microliter sample volumes, as required in mouse breath analysis. Spectroscopic techniques for online monitoring and quantification of breath components have recently attracted attention due to continuous advancements in semiconductor lasers technology. High sensitivity and selectivity for trace concentration detection of breath biomarkers has taken advantage of tunable diode laser absorption spectroscopy (TDLAS), photoacoustic spectroscopy, optical methods based on infrared laser spectroscopy for CO and according isotope pairs exploit the pronounced fundamental vibrational band \( \nu_2 \) of CO in the mid-infrared (MIR) spectral range at 4.3 \( \mu \)m. With advancements in diode laser technology, and thus increasing availability of light sources emitting in the 3–6 \( \mu \)m regime at reasonable output power, interband cascade lasers (ICL) are of particular interest. ICLs offer emission in the wavelength regime of 3–6 \( \mu \)m at room temperature, provide the required wavelength tunability, and feature low power consumption, as needed for future portable devices. In addition to the determination of the isotope ratios in low-volume samples via the developed sensing system, fundamental insight into the metabolic status of the mechanically ventilated small animals is offered via calculation of the respiratory quotient (RQ). Significant advances in sensor response time of optochemical sensors have been achieved by processing the utilized polymer into nanofibers as introduced by Köstler and collaborators. Highly porous nanofiber structures were prepared by electrospinning the developed polymer formulation of PtTFPP (platinum(II)-5,10,15,20-tetrakis(2,3,4,5,6-pentafluorophenyl)-porphyrin) immobilized in polystyrene.

In this article, we describe the development of a compact multiparameter sensor system based on a combination of TDLAS and optical oxygen sensors for online and continuous multiparameter sensor system based on a combination of TDLAS and photoacoustic spectroscopy. The development of the developed breath sensor system was evaluated in terms of sensitivity, precision, and linearity. Figure 1a illustrates experimental direct absorption signal of both transitions, P(58) and R(14), respectively, for the \(^{13}\)C and the \(^{13}\)C isotopes around 2294 cm\(^{-1}\). The spectrum is obtained from a real mouse breath sample showing clearly enriched \(^{13}\)CO\(_2\) concentrations.

As shown in Figure 1a, the selected spectral window around 2294 cm\(^{-1}\) offers resolved absorption lines of \(^{12}\)CO\(_2\) P(58) and \(^{13}\)CO\(_2\) R(14) suitable for data analysis. The intensity ratio of both isotope lines has been calculated as 1.16/1.00 (based on line strengths derived from the HITRAN database\(^5\) for the ratio of natural abundance \((^{13}\text{CO}_2)^{12}\text{CO}_2 = 1.11/98.89\).

Further absorption lines (P 466 2294.34 cm\(^{-1}\), P47e 2293.38 cm\(^{-1}\)) are expected in this spectral region but, however, could not be resolved due to chirp-limited spectral resolution and rather broad absorption features at atmospheric pressure. As a consequence, a surface response fit was used for quantitative analysis of both isotopes in order to compensate for any occurring influence by underlying lines. Hence, despite the fact that underlying CO\(_2\) absorption lines are not resolved herein, their influence was compensated with sufficient accuracy.

The \( \delta \) value precision was evaluated via an Allan–Werle deviation analysis.\(^{10,11}\) The TDL was operated at a sample flow rate of 20 mL min\(^{-1}\) and a sampling interval of 2 measurements/min. As illustrated in the Allan deviation plot in Figure 1b, the developed isotopic ratio sensor achieved a precision of \( \pm 1.3\% \) when averaging 16 measurements (i.e., over a period of 8 min) for the TTR-%. Measurements of the CO\(_2\) mole fraction \((^{13}\text{CO}_2 + ^{12}\text{CO}_2)\) were made using certified gas standards of 3 vol % total CO\(_2\) in nitrogen for 5 h, indicating a TDLAS intra-assay precision of \( \pm 4.8\% \) for total CO\(_2\). As the TDLAS system is meant to be used during extended measurement periods (i.e., on average 8 h), the drift behavior was tested during a 16 h measurement of 3% CO\(_2\) (intra-assay precision of 8.85\%\)), and drift-free operation was obtained results were compared to GC–MS measurements for validation.

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**Figure 1.** (a) Experimentally determined transmission spectrum of a mouse breath sample at 303 K and 980 mbar. (b) Allan variance analysis revealing a precision of 1.3‰ when averaging 16 data points.
demonstrated. The obtained precision level is comparable to complementary approaches using laser spectroscopy for detecting isotope ratios, which operate at reduced sample gas pressures to achieve sufficient spectral resolution. The present approach offers real-time analysis of mouse breath at atmospheric pressure; therefore, vacuum equipment is not needed. Furthermore, the developed double-channel substrate integrated hollow waveguide (dciHWG) offers the ability to simultaneously record sample and reference signals in a balanced detection scheme to obtain the difference in $\delta$ that is, using the reference channel for the secondary isotope standard.

**Comparison TDLAS versus MS.** The performance of the sensor was tested for the determination of the TTR ratio (i.e., $^{13}$CO$_2$/C-$^{12}$CO$_2$) and for total CO$_2$ calculated via the $^{13}$CO$_2$ and $^{12}$CO$_2$ concentrations within real exhaled mouse breath samples and comparing the values validated via GC/MS studies performed using the same mouse breath samples. For glucose metabolism monitoring, $^{13}$C-enriched glucose was administered during the studies presented herein, resulting in exhalation of $^{13}$CO$_2$. The variation in exhaled tracer levels was monitored over an 8 h test duration, where breath samples were collected hourly for reference GC/MS measurements. Initial laboratory experiments indicated that the observed TDLAS versus MS offset was caused by pressure broadening due to N$_2$ serving as the background gas. This offset was avoided by calibrating the TDLAS system with CO$_2$ mixed with synthetic air (O$_2$ 20%, N$_2$ 80%). Finally, the developed sensor system was deployed for online breath analysis during 14 mouse breath studies.

The obtained results for CO$_2$ and TTR determination are shown in Figure 2a,b. The orthonormal regression analysis yields a slope of CO$_2$ GC-MS/TDLAS = 0.987 ± 0.008 ($R^2 = 0.995$) and TTR-% GC-MS/TDLAS = 0.994 ± 0.004 ($R^2 = 0.995$), corroborating excellent agreement between the developed TDLAS setup and GC/MS validation measurements. A mean deviation for the TTR of 12 and 14% for CO$_2$ determination was obtained. The concentration of exhaled CO$_2$ ranged from 2.36 to 3.97%, and the obtained TTR values ranged from 0.78 to 7.95% (112 measurements executed in total). Exemplary kinetic profiles obtained for four mice are shown in the Supplementary Figure S-9. All profiles show a fast increase in enrichment after administration of $^{13}$C-enriched sugar. Depending on the individual glucose metabolism of the surgically instrumented mice, the TTR values decrease over time.

The comparison shows that the developed sensor system provides high accuracy during continuous monitoring of $^{13}$CO$_2$ to $^{12}$CO$_2$ ratios and for total CO$_2$ in exhaled mouse breath without the need of sampling, sample storage, and time-delayed analysis as required by GC/MS. Moreover, the application of TDLAS enables obtaining real-time concentration values throughout the duration of the test, which could not be obtained by any other method. First approaches toward mouse breath analysis in the MICU were demonstrated by our research team based on Fourier transform infrared spectroscopy approaches using sophisticated chemometric strategies requiring advanced data treatment and complex calibration procedures.17–19 Due to accurate and robust optics and electronics in combination with integrative fitting algorithms, the TDLAS system uniquely facilitates online signal processing and evaluation.

**Oxygen Sensing.** In addition to the analysis of the isotope ratio, the developed sensor system is also capable of monitoring the respiratory quotient with additional oxygen sensing via the integrated optochemical polymer nanofiber sensor. As previously described, two absorption spectra and 12 oxygen measurement points were recorded every minute. The calculation of the respiratory quotient in exhaled mouse breath was based on measuring the ratio of the volume of exhaled carbon dioxide ($V_c$) to the volume of oxygen consumed ($V_o$). The precision and long-term stability of the oxygen sensor were analyzed. The oxygen sensor shows an average minimum 99% recovery after being purged with nitrogen, indicating high reversibility of the sensor. Relative standard deviations are in the range of 0.14% for oxygen-free medium to 0.19% for a 20% oxygen mixture, thus revealing the capability of high-precision oxygen measurements. Long-term stability of the utilized electrosprun oxygen sensor was tested by the continuous determination of the phase shift of a 20% oxygen mixture in nitrogen for 16 h. Every 5 s, a phase shift was determined and directly converted into an oxygen concentration. A relative standard deviation of 0.39% was found for a 16 h measurement period at a flow rate of 20 mL/min. No discernible drift of the signal was revealed, although permanent illumination is known to cause photoinduced dye
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oxidation and photobleaching. The limit of detection (LOD) of the oxygen sensor was calculated as LOD = (3.3 σ/k), where the standard deviation (σ) is the standard deviation of the y-intercepts of regression and k is the slope of the calibration graph. The LOD was determined at approximately 0.003% oxygen, indicating a sufficiently high sensitivity. The system response time was determined to be 10 s for a flow rate of 20 mL/min, resulting from gas exchange within the tubing volume. Increasing the flow rate of the pump to a flow rate of 200 mL/min led to a rise time of <2 s, indicating a higher gas exchange rate. It should be noted that the slow flow rate for system testing was selected to correlate with real mouse breathing periods (15–20 mL/min).

Monitoring of the Respiratory Quotient. The developed sensor system clearly provides sufficient sensitivity and time resolution for enabling real-time exhaled CO_2 and O_2 monitoring, as shown in Figure 3. Conventional capnography monitors and blood gas analyzers have only limited applicability in mechanically ventilated mice for characterization of the energy metabolism characterization, due to the low blood volume and small tidal volume.

To calculate the RQ in mouse breath, the difference in exhaled and inhaled oxygen (ΔO_2) is calculated by monitoring the oxygen content of both inhaled and exhaled air. Thus, long-term monitoring of the oxygen content in compressed air used for mechanical ventilation was performed, resulting in an average oxygen concentration of 20.77 ± 0.05%. Furthermore, precise synchronization of the two sensor signals—IR and luminescence—is essential. The implemented signal processing routine automatically determines the inhaled and exhaled oxygen concentration and the exhaled carbon dioxide concentration, which enables direct monitoring of the respiratory quotient in exhaled mouse breath almost in real time. In Figure 3b, an exemplary trajectory of the respiratory quotient response of a mouse experiment starting at 08:00 and ending at approximately 14:45 is shown.

A time delay of approximately 5 min between breath exhalation and signal generation was inevitable due to the required tubing for gas transportation, resulting from the rather slow respiratory flow from the mouse. All connection tubing was kept as short as possible to minimize the dead volume and reduce the delay in data acquisition. Prominent peaks and dips due to intermittent changes in ventilation settings and fluctuations were smoothed due to the gas reservoir between the expiratory branch of the respiratory unit and the breath analyzer. Using additional smoothing functions in the data evaluation procedure, that is, short moving average (3 data points) in combination with Savitzky–Golay filter, provided smoothed response data without losing any essential information. Data points in Figure 3 are shown every 60 s, resulting in sufficient temporal resolution for facilitating physiologically meaningful respiratory data interpretation.

Indicating a metabolic shift from carbohydrate metabolism to fat oxidation with increasing duration of the mouse experiment, the determination of RQ values of all 14 mouse experiments executed so far revealed a common trend with respect to the decrease in RQ value toward the end of the protocol to approximately 0.75. In addition to the significant drop of RQ values over time, sharp increases and declines are evident in intervals of 30 min. These peaks are caused by so-called recruitment maneuvers (RM). RMs are performed to reopen/re-expand collapsed lung tissue occurring by inflation of lung subunits. The usefulness of recurrent RMs in mechanically ventilated mice with the specific aim to maintain lung mechanics and other lung functions has been explored by Reiss et al. It could be shown that RMs induce the development of pulmonary inflammation and prevent atelectasis (i.e., incomplete expansion of the lung).

In summary, the successful combination of a low-volume dual-channel iHWG with ICL-based TDLAS and complementary yet synchronized luminescence oxygen sensors enabled the detection of various relevant biomarker molecules serving as an advanced diagnostic strategy in exhaled mouse breath analysis. To the best of our knowledge, this is the first report of a real-time multispectral gas sensor system enabling continuous metabolic status monitoring via in-line analysis of minute mouse breath samples during medical trials with a time resolution at the scale of seconds. The innovative diCIHWG gas cell offers rapid gas exchange while simultaneously serving as a photon conduit and is designed to support a balanced detection scheme for efficiently eliminating noise. In a next step, expanding the capabilities of this measurement technique via appropriate preconcentration schemes and extending the IR wavelength regime to address further trace biomarkers via their vibrational fingerprint is anticipated.
CONCLUSION

An innovative all-optical sensor system combining MIR interband cascade laser technology with miniaturized dual-channel substrate-integrated hollow waveguide gas cells and nanofiber-based luminescence schemes enables online monitoring of important metabolic parameters, including total CO$_2$, $^{13}$CO$_2$ isotope enrichment, and $O_2$ in exhaled breath of mechanically ventilated mice. An ICL tunable diode around 4.35 $\mu$m was used for time-resolved infrared absorption spectroscopy to determine $^{12}$CO$_2$ (PS8) and $^{13}$CO$_2$ (R14) during the respiration of mice in a mouse intensive care unit. The breath analyzer was integrated into the respiratory equipment of the so-called “mouse intensive care unit” (MICU) and enables real-time direct exhaled breath monitoring in microliter sample volumes. In combination with the integrated oxygen sensor, accurate determination of the basal metabolic rate based on online access to the respiratory quotient is facilitated. Excellent agreement of the obtained results was confirmed by validation via GC/MS. The developed breath analysis system is now in routine use in the MICU at the Institute of Anesthesiologic Pathophysiology and Process Engineering at the Ulm University Medical Center. The presented sensor is anticipated to facilitate online metabolic status monitoring in small animal models with the potential to be scaled for alternative sensing scenarios where addressing minute volumes of probed gas is challenging.

EXPERIMENTAL SECTION

Sensor Configuration. In Figure 4a, the developed portable sensing system comprising the TDLAS system for determination of $^{12}$CO$_2$/$^{13}$CO$_2$ ratio and the optical oxygen sensor is illustrated. The combined sensor architecture is packaged into a standard 19 in. housing with dimensions of 471 $\times$ 451 $\times$ 192 mm ($L \times W \times H$). In situ online monitoring of exhaled breath components was achieved by locating the sensor system in-line within the expiratory branch. A small animal respiratory system/ventilator was used for stable ventilation of treated mice. A 100 mL gas reservoir was placed between the expiratory branch and the sensor system to stabilize breath humidity and to reduce fluctuations.

For isotopic measurements, a thermoelectrically cooled distributed feedback (DFB) interband cascade laser (S/N: 2082/04–09, nanoplus GmbH), emitting single mode infrared radiation at 2294 cm$^{-1}$ (4.35 $\mu$m) was used. Further details on the ICL characteristics are provided in the Supporting Information. The laser diode was packaged in TO-66 housing and was equipped with a collimating lens. In the MIR spectral region, pronounced fundamental absorption features of CO$_2$ are located at 4.3 $\mu$m, which are 100 times stronger than the $\nu_2$ band centered at 1.5 $\mu$m and the combination band $\nu_3 + \nu_1$ centered at 2.7 $\mu$m. Several line pairs characteristic for $^{13}$CO$_2$/$^{12}$CO$_2$ can be addressed for direct absorption spectroscopy. High-precision isotopic ratio measurements for mouse breath analysis are accomplished by selecting absorption features with almost equal line strength. In order to prevent spectral interference of both isotopes, the respective absorption lines must be well-separated for accurate data analysis, yet need to be located within the tuning range of a single ICL.

Thus, absorption lines at 2293.81 and 2294.48 cm$^{-1}$ (4.3595 and 4.3582 $\mu$m) of $^{12}$CO$_2$ and $^{13}$CO$_2$, were selected, as these lines are sufficiently close in frequency, yet spectrally separated for line fitting. Furthermore, their absorption strength is almost equal ($^{12}$CO$_2$ = 3.055 $\times$ 10$^{-20}$, $^{13}$CO$_2$ = 3.547 $\times$ 10$^{-20}$) despite the disparity in relative concentrations of each isotope. It is clearly evident that the applied ICL readily covers the overlap of the P-branch of $^{12}$CO$_2$ and the R-branch of $^{13}$CO$_2$ of the (0,0,1)$-$(0,0,0) vibrational transition in this wavelength regime.

Laser diode modulation was performed via a custom current/temperature controller developed by OptoPrecision (OptoPrecision GmbH, Bremen, Germany), enabling highly reproducible scanning across absorption lines. Wavelength tuning of the DFB ICL was performed by temperature tuning from 25.6 to 31.5 $^\circ$C at a repetition frequency of $f$ = 4 MHz at a pulse width of 100 ns. A modulation frequency of 555 Hz was selected to ensure an optimized signal-to-noise ratio. Scanning via temperature was executed during the first 5 s of each measurement, and the data evaluation was carried out in the following 25 s.

Collimated radiation from the ICL was directly coupled onto a wedged ZnSe window, which was integrated into the dual-channel substrate-integrated hollow waveguide serving as 50/50 beam splitter and as window of the hollow waveguide gas cell. Thus, 50% of the emitted radiation was used as a reference beam (see Figure 4b). Both beams propagate through the dciHWG gas cell (94 mm $\times$ 50 mm $\times$ 81 mm).
isotopic ratio of absorption lines to Gaussian line profiles, both pyroelectric detectors were acquired and processed with a data reduction program. Volmer theory, converted into oxygen concentration units based on the Stern–Volmer theory. This phase shift may then be the excitation depending on the lifetime of the excited state of the fluorophore. Even more advanced, by excitation of the fluorophore, fluorescence is delayed versus fluorescence lifetime provides information on the temporal fluctuations of the fluorophore. For online mouse breath analysis via the developed TDLAS system, the analyzer was integrated into the respiratory equipment of the anesthetized mouse, enabling continuous online monitoring. As a measurement interval, two absorbance spectra and 12 oxygen data points per minute were collected, which provided the time resolution desired by physiologists. The mouse breathing with approximately 15 mL/min resulted in a gas exchange time of 1.2 s within the IHWG. Calibration. Calibration samples of $^{13}$CO$_2$, $^{15}$CO$_2$, and oxygen were established with a gas mixing pump (DIGAMIX 2 M 301, H. Woosthoff Messtechnik GmbH, Bochum, Germany) and, if needed, together with a lipoic acid tracer enrichment, the masses of m/z 44 and m/z 45 were analyzed and evaluated. CO$_2$ production rates were calculated as the product of tidal volume, respiratory rate, and CO$_2$ concentration. For online mouse breath analysis via the developed TDLAS system, the analyzer was integrated into the respiratory equipment of the anesthetized mouse, enabling continuous online monitoring. As a measurement interval, two absorbance spectra and 12 oxygen data points per minute were collected, which provided the time resolution desired by physiologists. The mouse breathing with approximately 15 mL/min resulted in a gas exchange time of 1.2 s within the IHWG.

SEM image of an electrospun layer of polymer nanofibers, output power characteristic at a heat sink temperature of 15 °C, wavelength tuning characteristic as a function of operating current and temperature, and response curve of the oxygen sensor based on electrospun nanofibers to four different oxygen concentrations (PDF)

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssens-8b00477.

SEM image of an electrospun layer of polymer nanofibers, output power characteristic at a heat sink temperature of 15 °C, wavelength tuning characteristic as a function of operating current and temperature, and response curve of the oxygen sensor based on electrospun nanofibers to four different oxygen concentrations (PDF)

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Author Contributions

E.T., M.N., and A.T. conceived the experiments. M.N. was responsible for the system configuration and design. The mid-infrared source was delivered by S.B., M.F., and J.K. The oxygen sensors were built by C.W., S.K., and V.R. Mouse breath experiments were performed by M.G., S.K., and M.W. Data interpretation was performed in cooperation with U.W., J.V., and P.R. The manuscript was written by E.T. and B.M. All
authors discussed the results and contributed to the preparation of the manuscript.

**Notes**
The authors declare no competing financial interest.

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**ABBREVIATIONS**

dcHWG, dual-channel substrate-integrated hollow waveguide; DFB, distributed feedback; EBA, exhaled breath analysis; GC, gas chromatography; HITRAN, HITRAN Molecular Spectroscopic Database; LOD, limit of detection; MS, mass spectrometry; RQ, respiratory quotient; TDLAS, tunable diode laser absorption spectroscopy.

**REFERENCES**

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**Konferenzbeiträge**


**Posterbeiträge**


Ulm, Oktober 2019
APPENDIX

Fig. A.1: Spectra and LI curves measured for the ring-QCL used in Chapter 5.2

Fig. A.2: Spectrum of the DFB-ICL used in Chapter 5.3
Fig. A.3: LI curve measured for the DFB-ICL used in Chapter 5.3

Fig. A.4: Tuning parameters measured for the DFB-ICL used in Chapter 5.3
Fig. A.5: Tuning parameters measured for the DFB-ICL used in Chapter 5.3

Fig. A.6: Spectrum of the DFB-ICL used in Chapter 5.5
Figure A.7: Output power characteristic at a heat sink temp. of 15 °C used in Chapter 5.5

Figure A.8: Wavelength tuning characteristic as a function of operating current and temperature.
Fig. A9 – A11: Technical drawings for the dual-iHWG used in Chapter 5.5
Declaration of Authorship

I hereby declare that this thesis is my own unaided work. All direct or indirect sources are acknowledged as references. The used literature is specified in the list of references.

Eidesstattliche Erklärung


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Ort, Datum                                Unterschrift