Real-time breath gas analysis of carbon monoxide

Laser-based detection and pulmonary gas exchange modeling

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Department of Applied Physics and Electronics
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To my family,

who valued education above all else
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Breath gas analysis is a promising approach for non-invasive medical diagnostics and physiological monitoring. Real-time, breath-cycle resolved biomarker detection facilitates data interpretation and has the potential to improve the diagnostic value of breath tests as exhalation profiles carry spatiotemporal information about biomarker origin and gas exchange in the respiratory tract. This thesis presents and scrutinizes a novel methodology for the analysis of real-time breath data, where single-exhalation profiles are simulated using a pulmonary gas exchange model and least-squares fitted to measured expirograms to extract airway and alveolar contributions and diffusing capacities. The methodology is demonstrated on exhaled breath carbon monoxide (eCO), a candidate biomarker for oxidative stress and respiratory diseases. The thesis mainly covers (1) the construction of a compact optical sensor based on tunable diode laser absorption spectroscopy (TDLAS) in the mid-infrared region (4.7 µm) for selective and precise real-time detection of CO in breath and ambient air (detection limit 9 ± 5 ppb at 0.1 s), (2) the design of an advanced online breath sampling system, (3) the implementation of a trumpet model with axial diffusion (TMAD) to simulate the CO gas exchange, and (4) the application of extended eCO analysis in clinical studies to establish the healthy non-smoker baseline of the eCO parameters and to study the response to CO and wood smoke exposure. It is shown that the TMAD adequately describes the gas exchange during systemic CO elimination for different breathing patterns, and that there is no difference between eCO parameters from mouth- and nose exhalations. Expirogram shape and eCO parameters exhibit a dependence on the exhalation flow rate, but for a given breathing maneuverer, the parameters lie in a narrow range. Airway CO is close to and correlates with ambient air CO, indicating negligible airway production in the healthy population. The alveolar diffusing capacity is independent of endogenous CO, even after exposure to elevated exogenous CO, and could be used to assess lung diffusion abnormalities. Compared to CO exposure, no clear additional effect of exposure to wood smoke particles on eCO is observed. The discrimination between endogenous and exogenous CO sources remains a challenge.
Sammanfattning

Detektion av spårgaser i utandningsluften har stor potential för icke-invasiv medicinsk diagnostik och fysiologisk övervakning. Realtid andningsgasanalys av enskilda andningscykler underlättar datatolkningen och kan förbättra det diagnostiska värden av andningstester, eftersom utandningsprofiler bär spatiotemporal information om biomarkörens ursprung och gasutbyte i andningssystemet. Denna avhandling presenterar och granskar en ny analysmetod, där utandningsprofiler simuleras med hjälp av en matematisk modell för gasutbytet, och anpassas till uppmätta expirogram för att bestämma luftvägs- och alveolära bidrag och diffusionsförmågor. Metoden demonstreras på utandad kolmonoxid (eCO), en potentiell biomarkör för oxidativ stress och respiratoriska sjukdomar. Avhandlingen omfattar huvudsakligen (1) konstruktionen av en kompakt optisk sensor baserat på mid-infraröd diodlaserabsorptionsspektroskopi (TDLAS) vid 4.7 µm för selektiv och precis realtidsmätning av CO i utandnings- och omgivningsluften (detektionsgräns 9 ± 5 ppb vid 0.1 s), (2) design av ett avancerat system för online provtagning, (3) adaption av en matematisk lungmodell med axiell diffusion (TMAD) för simulation av CO gasutbytet, och (4) tillämpningen av utökad eCO analys i kliniska studier för att fastställa baslinjen för eCO parametrarna i friska icke-rökare, och för att studera effekten av exponering för CO och trärök. Det visas att modellen väl beskriver gasutbytet under systemiskt CO utsläpp för olika andningsmönster, och att det inte finns någon skillnad mellan eCO parametrarna från utandning via mun och näsa. Utandningsprofilerna och eCO parametrarna ändras beroende på utandningsflödet, men för ett visst andningsmönstret ligger parametrarna i ett smalt område. Koncentrationen av CO i luftvägarna ligger nära och korrelerar med CO i omgivningsluften, vilket indikerar att CO produktionen i luftvägarna är försumbart hos den friska befolkningen. Den alveolär diffusionsförmågan är oberoende av endogen CO, även efter exponering för förhöjd exogen CO, och kan möjligtvis användas för att diagnosticera en nedsatt diffusionsförmåga. Jämfört med exponering för CO observeras ingen tydlig ytterligare effekt av exponering för trärökpartiklar. Att åtskilja endogena och exogena eCO källor förblir en utmaning.
The thesis is based on the following publications:

I  *Real-time breath gas analysis of CO and CO₂ using an EC-QCL*
   **R. Ghorbani** and F. M. Schmidt

II  *ICL-based TDLAS sensor for real-time breath gas analysis of carbon monoxide isotopes*
    **R. Ghorbani** and F. M. Schmidt

III  *Modeling pulmonary gas exchange and single-exhalation profiles of carbon monoxide*
    **R. Ghorbani**, A. Blomberg and F. M. Schmidt
    Front. Physiol. 9, 927 (2018)

IV  *Fitting of single-exhalation profiles using a pulmonary gas exchange model – application to carbon monoxide*
    **R. Ghorbani** and F. M. Schmidt
    Submitted to J. Breath Res.

V  *Extended breath CO analysis – baseline and diurnal variation of pulmonary gas exchange parameters*
    **R. Ghorbani**, A. Blomberg and F. M. Schmidt
    Submitted to J. Breath Res.
Publications not included in this thesis:

VI  *Calibration-free scanned wavelength modulation spectroscopy – application to H₂O and temperature sensing in flames*
Z. Qu, **R. Ghorbani**, D. Valiev and F. M. Schmidt

VII  *Tunable diode laser atomic absorption spectroscopy for detection of potassium under optically thick conditions*
Z. Qu, E. Steinvall, **R. Ghorbani** and F. M. Schmidt
### Notation

#### Acronyms

$A_{c,1}$ \hspace{1cm} cross-sectional area of the airways at generation 17 (cm$^2$)

$A_{c,A}$ \hspace{1cm} total cross-sectional area of alveolar compartment (cm$^2$)

$A_{c,aw}(z)$ \hspace{1cm} cross-sectional area of airway compartment at location $z$ (cm$^2$)

BAL \hspace{1cm} brochoalveolar lavage

BGA \hspace{1cm} breath gas analysis

BH \hspace{1cm} breath-holding

BMI \hspace{1cm} body mass index

$C_{ACO}$ \hspace{1cm} alveolar CO concentration at equilibrium with blood CO (ppb)

$C_{amb}$ \hspace{1cm} ambient air CO concentration (ppb)

$C_{CO}$ \hspace{1cm} concentration of gaseous CO in the respiratory tract (ppb)

$C_{CO,ET}$ \hspace{1cm} end-tidal CO concentration (ppb)

CEAS \hspace{1cm} cavity enhanced absorption spectroscopy

COHb \hspace{1cm} carboxyhemoglobin

$C_{tiss}$ \hspace{1cm} airway tissue CO concentration at equilibrium with blood CO (ppb)

$D'_{ACO}$ \hspace{1cm} diffusing capacity of CO in the alveoli per unit axial distance (pl.s$^{-1}$.ppb$^{-1}$.cm$^{-1}$)

$D_{ACO}$ \hspace{1cm} total diffusing capacity of CO in the alveolar region (pl.s$^{-1}$.ppb$^{-1}$)

DAS \hspace{1cm} direct absorption spectroscopy

$D'_{awCO}$ \hspace{1cm} diffusing capacity of CO in the airway per unit axial distance (pl.s$^{-1}$.ppb$^{-1}$.cm$^{-1}$)

$D_{awCO}$ \hspace{1cm} total diffusing capacity of CO in the airway (pl.s$^{-1}$.ppb$^{-1}$)

EC-QCL \hspace{1cm} external-cavity quantum cascade laser

EFR \hspace{1cm} exhalation flow rate

ICL \hspace{1cm} interband cascade laser

IFR \hspace{1cm} inhalation flow rate
<table>
<thead>
<tr>
<th>Notation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>$J'_{ACO}$</td>
<td>maximum volumetric flux of CO from the alveoli per unit axial distance (pl.s$^{-1}$.cm$^{-1}$)</td>
</tr>
<tr>
<td>$J_{ACO}$</td>
<td>total maximum volumetric flux of CO from the alveoli (pl/s)</td>
</tr>
<tr>
<td>$J'_{awCO}$</td>
<td>maximum volumetric flux of CO from the airways per unit axial distance (pl.s$^{-1}$.cm$^{-1}$)</td>
</tr>
<tr>
<td>$J_{awCO}$</td>
<td>total maximum volumetric flux of CO from the airways (pl/s)</td>
</tr>
<tr>
<td>$L$</td>
<td>total length of respiratory tract in trumpet model (27.20 cm)</td>
</tr>
<tr>
<td>LDH</td>
<td>lavage lactate dehydrogenase</td>
</tr>
<tr>
<td>MOL</td>
<td>method of lines</td>
</tr>
<tr>
<td>MPC</td>
<td>multipass cell</td>
</tr>
<tr>
<td>$N_{alv}(z)$</td>
<td>number of alveoli per unit axial distance</td>
</tr>
<tr>
<td>$N_{max}$</td>
<td>maximum number of alveoli at any axial position</td>
</tr>
<tr>
<td>$N_t$</td>
<td>total number of alveoli</td>
</tr>
<tr>
<td>$O_2$Hb</td>
<td>oxyhemoglobin</td>
</tr>
<tr>
<td>TDLAS</td>
<td>tunable diode laser absorption spectroscopy</td>
</tr>
<tr>
<td>TMAD</td>
<td>trumpet model with axial diffusion</td>
</tr>
<tr>
<td>$\dot{V}_E$</td>
<td>volumetric flow rate of air during exhalation (EFR; ml/s)</td>
</tr>
<tr>
<td>$\dot{V}_I$</td>
<td>volumetric flow rate of air during inhalation (IFR; ml/s)</td>
</tr>
<tr>
<td>$V$</td>
<td>average of inhaled/exhaled volume (ml)</td>
</tr>
<tr>
<td>WMS</td>
<td>wavelength modulation spectroscopy</td>
</tr>
<tr>
<td>$z$</td>
<td>axial position in the lung (cm)</td>
</tr>
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**Math Symbols**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\equiv$</td>
<td>defined to be identical</td>
</tr>
<tr>
<td>$:=$</td>
<td>equal by definition</td>
</tr>
<tr>
<td>$\sim$</td>
<td>asymptotically equal (in scaling sense)</td>
</tr>
<tr>
<td>$\approx$</td>
<td>approximately equal (in numerical value)</td>
</tr>
</tbody>
</table>
Physiological monitoring is the cornerstone of human physiology, a science concerned with how the human body works. Several simple and sophisticated monitoring systems have been developed to provide information on vital physiological parameters of an individual and their alterations. This information can be utilized in fundamental research or, at a later stage, in medical diagnostics and clinical applications. Early stage diagnosis, screening of health conditions and monitoring of disease progress have always been key factors in successful treatment of diseases. Particularly, in the case of deadly diseases, early diagnosis plays an important role and can significantly lower the rate of mortality and morbidity as well as the medical treatment expenses imposed on society. An investment in prevention and early treatment of the common chronic diseases can save many lives and decrease treatment costs and economic impact of diseases by hundreds of billion dollars per year [1]. A closer look at the in-vitro diagnostics market (IVD; Fig. 1.1) and the research in this field shows that there is a tendency towards personalized point-of-care (POC) diagnostics and monitoring tools that provide rapid results and ease-of-use operation [2]. This is going to shift the medicine from being a curative science to a predictive one.

Physiological monitoring systems can be classified with respect to different targets of interest. In terms of sampling, they can mainly be categorized as invasive and non-invasive. Methods such as blood sampling, bronchoscopy, lung biopsy and pulmonary artery catheterization are a few examples of invasive monitoring at different levels of invasiveness. Assessment of body temperature, electrocardiography, capnography, pulse oximetry, and arterial blood pressure measurement by an inflatable cuff can be named as examples for non-invasive physiological monitoring [3].

In terms of time resolution, it is important to know how often a system can provide information on the status of an individual: is it just once in a while or with a subsecond time difference between two successive data points? For some physiological techniques, the temporal resolution is fundamentally limited by the overall sample volume that can be extracted. For instance, blood sampling or lung biopsy cannot be performed many times per hour. The time required for data processing and sample analysis is another limiting factor of great importance.
In search for novel non-invasive diagnostic methods to assess the physiological and metabolic state of the body, the measurement of molecular biomarkers in exhaled breath has received wide attention [4, 5]. This is evident from the increasing number of papers published in the field (Figure 1.2). Breath gas analysis (BGA) has several advantages over the traditional monitoring methods. Apart from being a completely non-invasive approach, it offers unlimited sample volume and fast response time for tracking changes in response to interventions. Contrary to the main respiratory gases (oxygen, carbon dioxide and water) that are present in percent range, most of the other molecular biomarkers in breath appear in trace amounts at parts per trillion (ppt) to parts per million (ppm) levels, which require highly sensitive analytical methods.

During the past decades, several analytical techniques have been employed for detection of trace species, mainly volatile organic compounds (VOCs), in breath. BGA-based analytical tools can potentially cover different areas of applications including POC screening and treatment monitoring, early diagnosis of diseases, acute diagnosis, personalized medicine and medical research. The main techniques are mass spectrometry (MS)-based methods, optical absorption spectroscopy and solid-state gas sensors [4]. The efforts have been challenged not only by the low concentration of VOCs in breath and interference from water (humidity), but also by the lack of guidelines for standardized breath sampling for most of biomarkers. A suitable analytical technique should basically have high sensitivity, time-resolution,
Fig. 1.2 Publications in the exhaled breath area per year from 1995-01-01 to 2017-12-31, using the PubMed Advanced Search Builder, and the keywords breath OR exhaled air.

sufficient dynamic range and, ideally, be able to detect multiple compounds simultaneously (multispecies detection). Only few techniques satisfy these conditions.

The development of new physiological monitoring systems requires knowledge of multiple engineering disciplines as well as profound understanding of the underlying physiology and physiochemical properties of the biomarkers. Since for many potential biomarkers in breath the origin and the biochemical pathways are not well-understood, the detection systems should be reliable enough to enable physiological studies.

Despite the fact that modern BGA has been a subject of research for at least three decades, it seems still to be in its infancy. Only a few molecules have been approved as biomarker for certain diseases, but no volatile organic compound (VOC) is among them. For example, nitric oxide has already been established as biomarker for asthma and molecular hydrogen as a biomarker for lactose malabsorption and the $^{12}\text{CO}_2/^{13}\text{CO}_2$ isotope ratio for Helicobacter pylori bacterial infection. Stable, nonradioactive isotope tracers are used in in vivo human metabolic research. They can be labeled to track the biochemical reactions and metabolic pathways.

Mathematical models have been increasingly employed to better understand the gas exchange dynamics of species in the respiratory tract by comparison to experimental results. Mathematical formulations can provide insight, predictions, and possible guidelines for BGA, e.g. suggestions for optimizing the breath maneuvers and exhalation flow rates. For instance, molecules with higher water solubility or chemical reactivity can have an exchange source in the upper airways. Hence, the
airway tissue is considered in the model. It has recently been shown that highly

time-resolved and precise detection of single molecules combined with models can
provide information on physiological properties of the lung [6].

Respiratory diseases are among the leading causes of premature death worldwide.
It is generally accepted that air pollution triggers or significantly amplifies a variety
of respiratory diseases [7–10]. One of the promising biomarkers for non-invasive
assessment of systemic and local oxidative stress and respiratory diseases is carbon
monoxide (CO) [11, 12]. This molecule can conventionally be measured in mixed or
end-tidal breath samples. However, using the traditional approach, end-tidal eCO
congestion cannot distinguish between different factors that influence eCO level.
With the advent of sensitive and precise analytical techniques, online real-time
analysis of eCO can improve the diagnostic value of breath CO test.

![Fig. 1.3](image)

Typical breath cycles of two classes of molecular species with exchange locations in
the upper (dashed line) and lower (solid line) respiratory tract measured in real-time.
The three different phases of exhalation are indicated. Phase I and III represent the air
coming from conducting airways and alveolar region, respectively, and phase II is the
transition between airways and alveoli.

### 1.1 Real-time breath gas analysis

Real-time BGA refers to fast online breath sampling and subsequent breath-cycle
resolved detection of exhaled molecular compounds with high precision. It implies
fast sample exchange and acquisition times. The shape of the exhalation profile
carries valuable information about the gas exchange location of a gas species. For
instance, for a compound that is mainly produced in the airway wall tissue like
nitric oxide, a sharp peak may appear at the beginning of exhalation profile in
phase I (dashed line in Fig. 1.3). Whereas for a compound that originates from the lung like isoprene or carbon dioxide, the profile shows a steep rise in phase II and a high concentration in phase III (solid line in Fig. 1.3). Due to this, the profile shape can also depend on the exhalation flow rate. Not all the BGA techniques can offer real-time measurement of molecular biomarkers with high precision and time resolution. Even with the techniques capable of real-time measurement, in most studies still the end-tidal portion of breath is automatically separated and used for analysis. Optical sensors provide the possibility for real-time measurements of small molecular compounds with high precision and time resolution, and allow for exploiting the spatiotemporal information on the respiratory tract carried by expirograms, which facilitate data interpretation and improve the diagnostic value of breath tests.

1.2 Aim of the thesis

The main focus of this thesis is to provide a basis for the analysis of real-time breath data. As graphically shown in Fig. 1.4, this aim is achieved by coupling experimental breath cycles to simulated breath profiles using a model of gas exchange in the respiratory tract for CO. This methodology opens up for extended breath CO analysis and provides a procedure for extracting physiological parameters from a single-exhalation profile. The work presented in this thesis contributes to the improvement of mid-infrared tunable diode laser absorption spectroscopy (TDLAS) at around 4.7 μm spectral wavelength with a simple, compact setup, and to the construction of an advanced breath sampling system. It also presents for the first time (to the author’s knowledge) the use of trumpet model with axial diffusion (TMAD) to study the CO gas exchange dynamics. The baseline level of model parameters and their inter- and intra-individual variations were investigated for a group of healthy non-smokers. Moreover, the eCO and model parameter response to exposure to CO gas and to wood smoke from incomplete combustion are studied.

1.3 Thesis outline

Background knowledge on breath gas analysis and absorption spectroscopy are presented in chapters 2 and 4. Chapter 3 introduces the physiological model that is used for simulating the CO gas exchange dynamics in the respiratory tract (Paper III). The experimental setup and the measurement procedure are explained in chapter
Fig. 1.4  The combination of laser spectroscopy and gas exchange modeling provides a package that extends the real-time breath gas analysis of single-exhalation profiles to quantitative assessment of model parameters.

5 (Papers I and II). Chapter 6 describes the least-squares fitting formalism used for coupling of experimental results to gas exchange modeling (Paper IV). Chapter 7 summarizes the design of clinical studies, eCO baseline and diurnal variation studies and human exposure to exogenous CO and wood smoke from incomplete combustion (Papers V and VI). A compilation of results, conclusions and outlook are presented in chapters 8, 9 and 10, respectively. A short summary of the attached papers and the author’s contribution to each paper is presented in Chapter 11.
The diagnostic potential of breath testing has been recognized since the Age of Pericles\(^1\). The odor of a patients’ breath was one of the first indices based on which ancient physicians tried to diagnose some diseases. The association of a fruity smell with diabetes and an urine-like smell with kidney failure are only two examples of early use of BGA \([13–15]\). In the 10th century, Persian physician and polymath, Ibn Sina (aka, Avicenna) noted the role of breath in preserving a perfect equilibrium in the human body\(^2\) \([16, 17]\). In the 18th century, Antoine Lavoisier\(^3\) discovered the similarity between food metabolism and combustion by measuring carbon dioxide in breath\(^4\). He found that oxygen consumption and a release of water and carbon dioxide are common features of a flame and an animal body \([18]\). In the 19th century, exhaled ethanol was found to be a byproduct of alcohol metabolism and acetone was detected in the exhaled breath of diabetics \([14]\). But it was only less than five decades ago that the pioneering work of Linus Pauling\(^5\) opened a new window to the modern breath analysis era \([19]\). Using gas chromatography combined with mass spectrometry (GC-MS), he and his colleagues showed that breath gas contains more than 200 volatile compounds that might reflect the physiological and metabolic state of an individual. The interest in breath analysis has not been confined to the analysis of gaseous molecules, but it has also been developed towards non-volatile molecules within the exhaled breath condensate (EBC) \([20–23]\) and exhaled breath particles \([24–26]\).

\(^{1}\) Fifth-century Athens (480–404 BC).

\(^{2}\) “...it is the role of the vital force (breath) to maintain a perfect equilibrium within the elements of the body, and between the elements of the body and the environment” (The Canon of Medicine).

\(^{3}\) Antoine Laurent de Lavoisier (1743–1794).

\(^{4}\) “la respiration est donc une combustion”.

\(^{5}\) Linus Carl Pauling (1901–1994).
2.1 Breath gas composition

The breath of an individual human being is a mixture of more than 600 gases. Trace gases and volatile organic compounds (VOCs) constitute only less than 0.01% of the breath volume. Figure 2.2 illustrates the dry air and the human breath gas compositions. Many trace gases and VOCs are common among people and may have endogenous or exogenous origin. Molecular compounds are produced endogenously in the body due to biochemical processes during normal body metabolism. This results in a certain background level for each molecule in the breath. An observed value beyond the background level for a certain compound can be an indication of exposure to exogenous sources or a body disorder. In BGA, it is generally important to distinguish between different sources. A few established breath biomarkers and their associated diseases have been listed in Table 2.1.

![Breath gas composition chart]

---

**Fig. 2.1** Lavoisier human respiration experiment (ca. 1790).

**Fig. 2.2** (Left) Dry air, and (Right) human breath gas compositions.
Table 2.1  Breath biomarkers with more or less established connection to health conditions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>Alcohol exposure, liver diseases, lung cancer</td>
</tr>
<tr>
<td>Acetone</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Acetylene</td>
<td>VOC exposure, smoking</td>
</tr>
<tr>
<td>Ammonia</td>
<td>Liver and renal disease</td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>Oxidative stress, respiratory diseases</td>
</tr>
<tr>
<td>Carbonyl sulfide</td>
<td>Liver diseases</td>
</tr>
<tr>
<td>Ethane</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Gut bacteria</td>
</tr>
<tr>
<td>Ethylene</td>
<td>Oxidative stress, cancer</td>
</tr>
<tr>
<td>Hydrogen cyanide</td>
<td>Pseudom. aeruginosa, poisioning</td>
</tr>
<tr>
<td>8-Isoprostanine</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td>Isoprene</td>
<td>Cholesterol biosynthesis</td>
</tr>
<tr>
<td>Methane</td>
<td>Gut bacteria</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Asthma</td>
</tr>
<tr>
<td>Pentane</td>
<td>Lipid peroxidation, oxidative stress</td>
</tr>
</tbody>
</table>

2.2 Breath sampling

Controlled sampling is a crucial factor in breath analysis, as the reproducibility of the results greatly depends on the sampling methodology. There are many factors that may affect the results of measurements due to breath sampling condition [27?]. Body posture, hypo-/hyper-ventilation, exhalation flow rate and sampling via nose or mouth are just a few examples from a long list of influencing factors to be considered. Breath sampling is traditionally performed in an offline manner, where mixed or end-tidal breath is collected into sample containers, such as polymer sampling bags, for later analysis. This approach introduces several potential error sources. Considering the fact that molecular biomarkers in breath normally exist at trace levels, the measured concentrations are prone to significant alteration such as leakage, contamination or temperature dependence of the highly water-soluble
VOCs in humid breath. A storage temperature below the body temperature results in water condensation in the bag and consequently a decrease in the concentration of water-soluble compounds. The sampling bags may also be reactive and generate molecular compounds and cause an increase in measured concentrations.

In contrast to the traditional offline sampling, in online sampling approach the subject breathes directly into (an inline breath sampler connected to) the analytical instrument and either the entire breath cycle or the end-tidal part can be evaluated. In an end-tidal measurement, the phase I and II of the exhalations are overlooked. The fast changes in breath profile in response to interventions such as exposure or treatments are resolved in online analysis. Coupled to a pulmonary gas exchange model, this method offers the possibility to extract relevant physiological parameters.

### 2.3 Analytical techniques in breath gas analysis

A wide range of techniques with variety of capabilities are currently employed in breath analysis studies [28]. The main techniques are briefly described below. A comparison of techniques is given in Table 2.2.

**Gas chromatography combined with mass spectrometry**

Gas chromatography combined with mass spectrometry (GC-MS) utilizes the features of a gas-chromatograph and a mass spectrometer to identify substances in a sample. The sample is preconcentrated and its substances are separated by a chromatographic column at different retention times. Then, the compounds are ionized and sent to a mass spectrometer. The detection in this technique is done based on the mass to charge ratio of ionized atoms or molecules. GC-MS is considered as the gold standard for BGA. This technique has a high sensitivity (in the ppt range) and allows for identification of unknown VOCs and sample profiling. However, the system is not portable and requires sample preparation and known compounds for quantification. The required time for analysis process (tens of minutes) and offline breath sampling make it unsuitable for real-time BGA and clinical application. A thorough review of the technique can be found in Refs. [29, 30].
Soft-ionization MS methods

This class of analytical MS methods mainly includes proton-transfer-reaction mass spectrometry (PTR-MS) [31–33] and selected-ion flow tube mass spectrometry (SIFT-MS) [34, 35] which provide high sensitivity and real-time quantification of VOCs. These techniques are mainly based on soft chemical ionization of molecular compounds when they collide with a reactant gas particle (A+) of lower proton affinity and undergo the following reaction

\[ A^+ + R \rightarrow \text{Products} \]

For instance, if protonated water ion (H\(_3\)O\(^+\)) is used as a reagent, the products are RH\(^+\) and water. SIFT-MS and PTR-MS utilize NO\(^+\) and O\(_2^+\) as precursor (reagent) ions since these ions do not react with nitrogen, oxygen and carbon dioxide, the major constituents of ambient and exhaled air. Recently, five negative ions (OH\(^-\), O\(^-\), O\(_2^-\), NO\(_2^-\) and NO\(_3^-\)) have also been used. SIFT-MS allows for using multiple precursor ions and obtain multiple different spectra covering a wider variety of compounds. Both the PTR-MS and SIFT-MS techniques make use of mass spectrometric detection for real-time quantification of VOCs. However, in SIFT-MS, the reagent ions are rapidly mass selected and participate selectively in a well-controlled reaction with trace VOCs. Whereas, in PTR-MS, the reagent ions are directly injected without any mass filter into a drift tube where they react with VOCs in the gas sample and are drawn to the spectrometer by an electric field gradient. The detection is done either via a quadrupole mass analyzer or a time-of-flight mass spectrometer (TOF-MS). Recently, new types of PTR-MS instruments were introduced that can switch between H\(_3\)O\(^+\), O\(_2^+\), NO\(^+\), and even Kr\(^+\) and Xe\(^+\) as reagent ions as an option to lower the proton affinity of the instrument and increase the number of molecules that can be measured.

As mentioned above, the range of measurable molecular compounds is limited to the molecules with a proton affinity larger than that of the chemical ionization agents (reagent ions). Furthermore, the dynamic range of such techniques might be limited due to dilution of primary reagent ions, and the response of instrument might become nonlinear. Moreover, these analytical techniques are bulky and expensive.

Sensors

Novel sensor technology offers an alternative for simple, inexpensive and easy-to-use tools for breath gas analysis. Several gas sensors were developed during the last decade and been used actively in the field of BGA [4]. They mainly include nano-material-based sensors, sensor arrays, electronic nose and electrochemical sensors.
However, their efficient application in BGA is hampered by the lack of high sensitivity, selectivity and low time response. In addition, their performance might be influenced by humidity and temperature.

Optical techniques

Optical devices have been extensively used for research in BGA. These techniques are mainly laser-based techniques, chemiluminescence analyzers, nondispersive infrared sensors (NDIR) and semi-optical sensors. This class of BGA techniques are sensitive and selective and have fast response time, useful for real-time monitoring. However, they are usually suitable for detection of small molecules, and the number of species that can be detected simultaneously by them is limited. They might be relatively expensive and need for frequent calibration or need to be miniaturized.

In the current thesis, laser absorption spectroscopy is employed as one of the most sensitive and selective techniques for real-time detection of single exhalation profiles. A comprehensive survey of the technique is given in Chapter 4. A comparison between the more commonly used techniques in BGA is summarized in Table 2.2.

<table>
<thead>
<tr>
<th>Method</th>
<th>Real-time</th>
<th>Sensitivity</th>
<th>Selective</th>
<th>Compact/portable</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTR-MS</td>
<td>✓</td>
<td>H</td>
<td>I</td>
<td>×</td>
</tr>
<tr>
<td>PTR-TOF-MS</td>
<td>✓</td>
<td>H</td>
<td>I</td>
<td>×</td>
</tr>
<tr>
<td>SIFT-MS</td>
<td>×</td>
<td>H</td>
<td>I</td>
<td>×</td>
</tr>
<tr>
<td>GC-MS</td>
<td>✓</td>
<td>H</td>
<td>I</td>
<td>×</td>
</tr>
<tr>
<td>Ion mobility MS</td>
<td>✓</td>
<td>H</td>
<td>I</td>
<td>×</td>
</tr>
<tr>
<td>SESI-MS</td>
<td>✓</td>
<td>H</td>
<td>I</td>
<td>×</td>
</tr>
<tr>
<td>Sensor arrays</td>
<td>×</td>
<td>I</td>
<td>P</td>
<td>✓</td>
</tr>
<tr>
<td>Laser spectroscopy</td>
<td>✓</td>
<td>I/H</td>
<td>H</td>
<td>✓</td>
</tr>
</tbody>
</table>

Note: table has been extracted from [4].
H: high; I: intermediate; L: low; P: poor.

b can potentially be real-time.

c Laser spectroscopy can potentially be used in a calibration-free manner.

d For some techniques, can potentially be portable.
2.4 Current status and challenges

Apart from standardization and online/offline breath sampling, additional challenges include the fact that baseline level and variation of most of the biomarkers are not well-defined for healthy population. Also a biomarker might be relevant for several health conditions. There are usually many open questions concerning conditions under which the breath sample is taken, for instance if a certain number of breath samples should be taken, or if special breathing maneuvers like breath-holding should be performed before sampling or the effects of physiological parameters, such as respiratory rate or heart beat rate, on the amount of exhaled biomarker. In addition, biochemical pathways and sources and sinks are not well-known for many of the biomarkers. Moreover, the physical and chemical properties of the biomarkers also play role, e.g. inside the respiratory tract and for analytical instrument. For instance, if a highly reactive marker is stored in a bag for a long time. A set of comprehensive protocols of breath sampling and analysis might be needed to come close to answers for some of these questions. Such an effort should be made with special attention to the effects of pathological changes, physiological effects, confounding variables, and methodological effects. In this respect, physiological modeling can be a great tool to investigate and validate the dynamics of trace gases.

As a result, till now only a few breath molecules have been approved as biomarkers of certain health conditions. A list of breath tests approved by the U.S. Food and Drug Administration (FDA) is presented in Table 2.3.

2.5 Biomarkers of respiratory diseases

Several molecular compounds in the exhaled breath have been suggested as biomarkers of oxidative stress and respiratory diseases. These molecules are mainly NO, CO, Ethane (C$_2$H$_6$), hydrogen sulfide (H$_2$S) and pentane (C$_5$H$_{12}$) in breath gas [36, 37] and hydrogen peroxide (H$_2$O$_2$) and 8-isoprostane (C$_{20}$H$_{40}$) in EBC [21, 38]. Almost all of these molecules appear at low ppb level or even below in breath, except CO that is in the low ppm range in the mixed or end-tidal breath. CO is not reactive either. Hence, it might be a suitable biomarker. However, results from conventional mixed breath or end-tidal CO analysis with electrochemical sensors are ambiguous. More research is needed to decide if CO can be used as a biomarker [12]. In the current thesis, a single molecule, carbon monoxide is investigated as a potential biomarker for oxidative stress and respiratory diseases, as we believe that there are
still many open questions at the single biomarker detection level that need to be answered.

### 2.6 The biomarker carbon monoxide

Carbon monoxide is produced endogenously from catabolism of heme\(^{11}\) during erythrophagocytosis, i.e. senescent erythrocytes removal in the spleen [12, 39]. Figure 2.3 shows a schematic drawing of the heme removal pathway in which heme is first reduced to biliverdin by the heme oxygenase (HO-1) enzyme\(^{12}\) with CO released as the by-product. Then, biliverdin is reduced to bilirubin. Endogenous CO is transferred to blood plasma and is mostly taken up by the red blood cells (RBCs) and is exchanged in the respiratory tract.

Recent studies have shown that the background level of exhaled breath CO (eCO) falls in the range of 1-3 parts per million (ppm) for the healthy population [40], while this value can be significantly elevated for the diseased population. An increase in eCO level may be observed due to exposure to external sources (smoking, air pollution) [41] or increased HO activity due to oxidative stress-induced heme degradation [12, 42–45] or due to a change in lung diffusion properties [46]. The

\(^{11}\) an iron-containing compound that forms the protein-free part of haemoglobin and acts as the oxygen-carrier.

\(^{12}\) an enzyme catalysing the heme degradation.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Pathophysiological relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breath alkanes</td>
<td>Heart transplantation rejection</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>Capnography</td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>CO poisoning, neonatal jaundice</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Enforcing driving legislation</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>functional gastrointestinal disorders</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Asthma</td>
</tr>
<tr>
<td>Urea-(^{13})C-breath test (UBT)</td>
<td>Helicobacter pylori infection</td>
</tr>
</tbody>
</table>

\(^{a}\) Compiled from data in [4]
measured eCO level can also be influenced by breath sampling conditions [47, 48]. A traditionally-measured single eCO value is not able to distinguish between these influencing factors. Therefore, it is beneficial to have an analytical technique that can distinguish between systemic, induced and external contributions to the measured eCO.

Although much effort has been put towards improving the sensitivity of different techniques and obtaining a standard and clear picture of eCO as a diagnostic biomarker, its predictive value for the healthy and (different phases of illness in) the diseased population is still unclear.

The established BGA techniques capable of real-time measurements (PTR-MS and SIFT-MS) are unable to detect CO due to its low proton affinity. It has been shown that laser absorption spectroscopy (LAS) can nicely complement the established BGA techniques due to its high sensitivity, selectivity, and time-resolution in measurement of small molecules. It has recently been shown in an eCO study that LAS technique may offer the possibility to measure the local airway contribution to eCO [49].

**Carbon monoxide uptake and elimination**

In order to understand the influence of exposure on exhaled CO, it is important to understand the dynamics of respiratory uptake and elimination of carbon monoxide for example during and after exposure to external CO sources.
The relationship of inhaled CO and the carboxyhemoglobin (COHb) concentration in blood can be modelled using the differential Coburn-Forster-Kane (CFK) equation [50].

The Coburn-Forster-Kane model of COHb formation as a result of exposure to CO exists in two forms: (1) the linear CFK equation that assumes a constant level of oxyhemoglobin ($O_2Hb$), and (2) the nonlinear CFK equation that allows the $O_2Hb$ level to vary with the COHb level. As an approximation, the linear model which is less complicated may be used over a wide range of COHb levels. To improve the precision of linear CFK equation, Peterson et al. [51] introduced a “trial and error” procedure that incorporates a variable $O_2Hb$ into the linear equation. The CFK equation can be written as

$$[\text{COHb}]_t = \frac{1}{A} \left( [\text{COHb}]_0 \cdot A \cdot C + (1 - C) \cdot B \cdot \dot{V}_{CO} + (1 - C) \cdot P_{I,CO} \right),$$

(2.1)

with

$$C = \exp(-tA/V_bB),$$

(2.2a)

$$A = \frac{P_{O_2}}{M[O_2Hb]},$$

(2.2b)

$$B = \frac{1}{D} + \frac{P}{V_A},$$

(2.2c)

where $[\text{COHb}]_t$ is the COHb concentration at time $t$ (ml CO/ml blood), $[\text{COHb}]_0$ is the initial COHb concentration (ml/ml), $P_{O_2}$ represents the partial pressure of oxygen in lung capillaries (kPa), $\dot{V}_{CO}$ is the endogenous rate of CO production (ml/min), $D$ is the diffusivity of CO (ml.min$^{-1}$.kPa), $P$ is the barometric pressure minus saturated vapor pressure of water at 37 °C (kPa), $V_b$ is the blood volume (ml), $P_{I,CO}$ is the partial pressure of CO in inspired air (kPa), $V_A$ is the alveolar ventilation rate (ml/min), and $t$ represents the exposure time (min).

Several empirical equations have been proposed to express the relationship between COHb and alveolar CO concentration ($C_A$). One of the most commonly used one was proposed by [52] as follows:

$$\text{COHb} = 0.63 + 0.16 \cdot C_A.$$  

(2.3)
Exchange of respiratory ($O_2$ and $CO_2$) and inert gases in the lungs was among the first human body mechanisms that was modelled mathematically. Classically, the Farhi equation for pulmonary inert gas elimination is the simplest model proposed in 1967 to express the relation between blood concentration of a volatile compound and its corresponding concentration in the breath gas [53]. This equation is expressed as follows

$$C_A = \lambda_{\text{blood:air}} C_a = \frac{C_v}{\lambda_{\text{blood:air}} + \frac{V_A}{Q_c}},$$

(3.1)

where $C_A$, $C_a$, and $C_v$ are the alveolar, arterial blood and mixed venous blood concentrations of molecular compounds. $\lambda_{\text{blood:air}}$ is the blood:gas partition coefficient for the volatile compound of interest, $V_A$ is the alveolar ventilation, and $Q_c$ is the cardiac output. $C_A$ is assumed to be available from an end-tidal measurements. $V_A$ and $Q_c$ implicitly control the amount of compounds exchanged between the alveoli and the external environment and the amount of compounds carried to the lungs by the pulmonary blood flow, respectively.

In this classical theory, upper respiratory and lower airways are assumed to behave as inactive conducting airways that only provide the atmosphere-alveoli connection. However, by the advent of novel analytical instruments and more precise measurements of volatile compounds in the exhaled breath, the results of more experiments indicated that the measured end-tidal concentrations are not necessarily equal to the alveolar concentrations ($C_{\text{measured}} \neq C_A$). There are non-respiratory gases that exchange in the conducting airways due to their high water-solubility (e.g. acetone and ethanol) or high chemical reactivity (e.g. ozone). Also, they can be produced endogenously in the airway tissue (e.g. nitric oxide). These new findings motivated the development and application of more advanced models to investigate the exchange dynamics of volatile compounds in the respiratory tract in more detail.

In this chapter, a short overview of the main modeling approaches in breath gas analysis and a detailed description of a specific gas exchange approach adapted for carbon monoxide are given.
3.1 Pulmonary gas exchange models

The most commonly used models can be classified in two major classes of compartmental models [54–56] and morphological models [57, 58]. Based on the physical and biochemical characteristics of the target molecule, the tissue and blood layers can be added to each compartment when they take active parts in the gas exchange dynamics [54, 59, 60].

3.1.1 Compartmental models

Compartmental models are traditionally the most widely used approach to characterize the gas exchange dynamics in the respiratory tract [54, 55, 61]. A compartment consists of a group of tissues with similar physiological or anatomical properties. The number and configuration of the compartments can vary depending on the physicochemical characteristics of the molecular compound and the experimental observations. The respiratory tract is partitioned into multiple perfectly-mixed, lumped compartments that have no strict physiological or anatomic basis. In a two-compartment model, the bronchial compartment is considered rigid whereas the alveolar compartment is expansible [54]. Such models are simple and flexible, but compared to morphological models their performance can be limited.

3.1.2 Morphological models

Morphological models are more sophisticated since they consider the bifurcating structure of the lung and more complex mechanisms of gas mixing in the respiratory tract [58, 62, 63]. These models are usually described by a rigid trumpet-shape representation of the lung. The alveolar region is axially spread over a short distance. More details on the model is given in the next sections. This model has been used previously to study the pulmonary exchange characteristics of CO₂ [64] and NO [58].

Due to the more realistic structure and the comprehensive transportation mechanisms involved in this type of models, they have also been used to study the particle deposition as a function of particle size in exposure studies [65, 66].
3.2 A TMAD model for carbon monoxide

Considering the discussion above, morphological models are normally more suitable to simulate entire exhalation profiles. Since CO is mainly exchanged at the alveolar interface, a large gradient in CO concentration is expected between the airways and the alveoli. Hence, the mathematical model of carbon monoxide should incorporate axial diffusion and the assumption of an airway sink/source of carbon monoxide. Since CO exchange is diffusion-limited, bronchial and pulmonary blood circulations are not necessary in the model. Thus, a trumpet-shape model of the lung with axial diffusion (TMAD) (Fig. 3.1) is employed to simulate the CO distribution in the respiratory tract and the eCO expirograms (Papers III and IV).

In this model, \( N_{alv}(z) \) is the number of alveoli per unit axial distance in the alveolar region, \( N_t \) is the total number of alveoli, \( N_{\text{max}} \) is the maximum number of alveoli at any axial position, \( A_{c, A} \) the total cross-sectional area of the alveolar compartment, \( A_{c, aw} \) the cross-sectional area of the airway compartment, \( \dot{V} \) is the volumetric flow rate, and \( D_{CO, air} \) is the molecular diffusivity of CO in air. The gas exchange in the respiratory tract is characterized by four time-independent model parameters, the maximum volumetric fluxes of CO per unit axial distance in airways and alveoli, \( J'_{awCO} \) and \( J'_{ACO} \), respectively, and the corresponding diffusing capacities of CO per unit axial distance, \( D'_{awCO} \) and \( D'_{ACO} \). These parameters are and uniformly distributed per unit volume. The total maximum volumetric fluxes across the airway and the alveolar tissue membranes are by definition obtained when gas phase concentrations are zero in the airway and alveolar region, respectively. A constant plug flow is assumed along the trumpet shape. Airway tissue and alveolar concentrations at equilibrium conditions are determined from the ratios between the maximum fluxes and diffusing capacities [58].

3.2.1 Derivation of the governing equation

The unsteady-state governing equation for a TMAD model can be obtained by writing the mass balance equations over a differential control volume in the respiratory tract. The control volume is a disc of thickness \( \Delta z \) representing a differential volume in the airways and a washer of the same thickness representing a differential volume in the alveolar region [58].
Fig. 3.1  Schematic drawing of the trumpet shape representing the bifurcating structure of the lung [59, 67]. Airway and alveolar gas exchange parameters are indicated. AD – axial diffusion, $C_{\text{tiss}}$ – airway tissue CO concentration, $C_{\text{ACO}}$ – alveolar CO concentration. Not to scale.

The mass balance equation for CO can be written as follows [58]

$$
\frac{d}{dt} \left( C_{\text{CO}} \left\{ A_{c,\text{aw}}(z) + \left[ \frac{N_{\text{alv}}(z)}{N_t} \right] A_{c,A} \right\} \Delta z \right) =
\left[ \dot{V} C_{\text{CO}} \right]_{z + \Delta z}^{z} - D_{\text{CO,air}} \cdot \left[ A_{c,\text{aw}}(z) \frac{dC_{\text{CO}}}{dz} \right]_{z + \Delta z}^{z} + (J'_{\text{awCO}} - D'_{\text{awCO}} \cdot C_{\text{CO}}) \left[ 1 - \frac{N_{\text{alv}}(z)}{N_{\text{max}}} \right] \Delta z + (J'_{\text{ACO}} - D'_{\text{ACO}} \cdot C_{\text{CO}}) \left[ \frac{N_{\text{alv}}(z)}{N_t} \right] \Delta z.
$$

(3.2)
This equation states that the amount of CO in the control volume depends on the CO transferred to that volume by convection represented by the first term on the right hand side of the equation, by axial diffusion due to CO gradient along the $\Delta z$ represented by the second term, and the CO generated or absorbed in the airway and alveolar regions represented by the third and fourth terms, respectively.

The general mass balance equation is given by

$$\frac{d}{dt}\left(\rho \{ A_{c,aw}(z) + [\frac{N_{alv}(z)}{N_t}] A_{c,A} \} \Delta z \right) = \left[ \rho \dot{V} \right]_{z+\Delta z}, \quad (3.3)$$

Expanding Eq. 3.2 and then dividing both Eqs. (3.2) and (3.3) by $\Delta z$, the following equations will be obtained at the $\Delta z \rightarrow 0$ limit, respectively,

$$\left\{ A_{c,aw}(z) + [\frac{N_{alv}(z)}{N_t}] A_{c,A} \right\} \frac{dC_{CO}}{dt} + C_{CO} \frac{d}{dt} \left\{ A_{c,aw}(z) + [\frac{N_{alv}(z)}{N_t}] A_{c,A} \right\} = -\dot{V} \frac{dC_{CO}}{dt} + C_{CO} \frac{d}{dz} \left[ A_{c,aw}(z) \frac{dC_{CO}}{dz} \right] + (J'_{awCO} - D'_{awCO} \cdot C_{CO}) \left[ 1 - \frac{N_{alv}(z)}{N_{max}} \right] + (J'_{ACO} - D'_{ACO} \cdot C_{CO}) \left[ \frac{N_{alv}(z)}{N_t} \right], \quad (3.4)$$

and

$$\frac{d}{dt} \left\{ A_{c,aw}(z) + [\frac{N_{alv}(z)}{N_t}] A_{c,A} \right\} = -\frac{\dot{V}}{dz}. \quad (3.5)$$

Inserting Eq. (3.5) in Eq. (3.4) yields the final governing equation,

$$\left\{ A_{c,aw}(z) + [\frac{N_{alv}(z)}{N_t}] \right\} \frac{dC_{CO}}{dt} = -\dot{V} \frac{dC_{CO}}{dz} + D_{CO,air} \cdot \frac{d}{dz} \left[ A_{c,aw}(z) \frac{dC_{CO}}{dz} \right] + (J'_{awCO} - D'_{awCO} \cdot C_{CO}) \left[ 1 - \frac{N_{alv}(z)}{N_{max}} \right] + (J'_{ACO} - D'_{ACO} \cdot C_{CO}) \left[ \frac{N_{alv}(z)}{N_t} \right], \quad (3.6)$$

where

$$A_{c,aw}(z) = A_{c,1} \left( \frac{L - z}{z_1} \right)^{-2}. \quad (3.7)$$
with $z_1$, the length of alveolar region, equal to 0.6 cm.

### 3.2.2 Numerical solution

Writing the mass balance equations over a differential control volume of length $\Delta z$ yields a partial differential equation (3.6) for gas-phase CO concentration in the respiratory tract.

The governing equation (3.6) has the general form

$$u_t = -u_x + u_{xx} + f(u),$$

(3.8)

which represents a convection-diffusion-reaction equation that is geometrically classified as a hyperbolic-parabolic partial differential equation. The $u_x$ and $u_{xx}$ terms are the hyperbolic and parabolic terms representing the convection and diffusion, respectively. This equation is solved numerically using the method of lines (MOL) [68]. Temporally, a backward Euler method is utilized for discretization. The equation is discretized into $n$ sections and $n + 1$ grid points in spatial dimension. Figure 3.2 shows the grid points in such a discretization scheme. $NG$ is the total number of grid points. A stable solution is achieved using a one-sided, upwind approximation scheme to discretize the spatial dimension considering the direction of advection during inhalation and exhalation. Hence, the following relations are employed for spatiotemporal discretization,

$$C_t = \frac{C_j^{k+1} - C_j^k}{\Delta t} + \mathcal{O}(\Delta t),$$

(3.9)
A TMAD model for carbon monoxide

\[ C_x \approx \frac{C_j^{k+1} - C_{j-1}^{k+1}}{\Delta z} + O(\Delta z), \quad \text{(for inhalation, } \dot{V} > 0) \quad (3.10a) \]

\[ C_x \approx \frac{C_j^{k+1} - C_j^{k+1}}{\Delta z} + O(\Delta z), \quad \text{(for exhalation, } \dot{V} < 0) \quad (3.10b) \]

\[ C_{xx} \approx \frac{C_{j+1}^{k+1} - 2C_j^{k+1} + C_{j-1}^{k+1}}{\Delta z^2} + O(\Delta z^2), \quad (3.10c) \]

where \( C_j^k \) indicates the CO concentration at grid point \( j \) at time \( k \).

The discretized version of the gas exchange equation is obtained by inserting Eqs. (3.9), (3.10a), (3.10b), and (3.10c) in Eq. (3.6). Hence, the discretized equation for inhalation is given by

\[
\begin{align*}
\left\{ A_{c,aw}(z) + \left[ \frac{N_{alv}(z)}{N_t} \right] \right\} C_j^{k+1} - C_j^k \\
= -\dot{V} \frac{C_j^{k+1} - C_{j-1}^{k+1}}{\Delta z} + D_{CO,air} \cdot \frac{dA_{c,aw}(z)}{dz} \cdot \frac{C_j^{k+1} - C_{j-1}^{k+1}}{\Delta z} \\
+ D_{CO,air} \cdot A_{c,aw}(z) \cdot \frac{C_{j+1}^{k+1} - 2C_j^{k+1} + C_{j-1}^{k+1}}{\Delta z^2} \\
+ (J_{awCO}' - D_{awCO}' \cdot C_j^{k+1}) \left[ 1 - \frac{N_{alv}(z)}{N_{max}} \right] \\
+ (J_{ACO}' - D_{ACO}' \cdot C_j^{k+1}) \left[ \frac{N_{alv}(z)}{N_t} \right],
\end{align*}
\]

and for exhalation is expressed as

\[
\begin{align*}
\left\{ A_{c,aw}(z) + \left[ \frac{N_{alv}(z)}{N_t} \right] \right\} C_j^{k+1} - C_j^k \\
= -\dot{V} \frac{C_{j+1}^{k+1} - C_j^{k+1}}{\Delta z} + D_{CO,air} \cdot \frac{dA_{c,aw}(z)}{dz} \cdot \frac{C_{j+1}^{k+1} - C_j^{k+1}}{\Delta z} \\
+ D_{CO,air} \cdot A_{c,aw}(z) \cdot \frac{C_{j+1}^{k+1} - 2C_j^{k+1} + C_{j-1}^{k+1}}{\Delta z^2} \\
+ (J_{awCO}' - D_{awCO}' \cdot C_j^{k+1}) \left[ 1 - \frac{N_{alv}(z)}{N_{max}} \right] \\
+ (J_{ACO}' - D_{ACO}' \cdot C_j^{k+1}) \left[ \frac{N_{alv}(z)}{N_t} \right].
\end{align*}
\]

Rearranging the equations for \( C_j^k \) results in the following relations for inhalation and exhalation, respectively,
\( \text{inh} C^k_j = \left\{ -\frac{f_1(j)}{\Delta z} \dot{V} + \frac{f_1(j)f_2(j)}{\Delta z} - \frac{f_1(j)f_3(j)}{\Delta z^2} \right\} C^k_{j-1} + \left\{ 1 + \frac{f_1(j)}{\Delta z} \dot{V} - \frac{f_1(j)f_2(j)}{\Delta z} + \frac{2f_1(j)f_3(j)}{\Delta z^2} \right\} C^k_{j+1} + \left\{ -\frac{f_1(j)f_3(j)}{\Delta z^2} \right\} C^k_{j+1} + \left\{ -f_1(j) \left( J'_\text{awCO}f_4(j) + J'_\text{ACO}f_5(j) \right) \right\} \right\} \). \\

\( (3.13) \)

and

\( \text{exh} C^k_j = \left\{ -\frac{f_1(j)f_3(j)}{\Delta z^2} \right\} C^k_{j-1} + \left\{ 1 - \frac{f_1(j)}{\Delta z} \dot{V} + \frac{f_1(j)f_2(j)}{\Delta z} + \frac{2f_1(j)f_3(j)}{\Delta z^2} \right\} C^k_{j+1} + \left\{ \frac{f_1(j)}{\Delta z} \dot{V} - \frac{f_1(j)f_2(j)}{\Delta z} - \frac{f_1(j)f_3(j)}{\Delta z^2} \right\} C^k_{j+1} + \left\{ -f_1(j) \left( J'_\text{awCO}f_4(j) + J'_\text{ACO}f_5(j) \right) \right\} \right\} \). \\

\( (3.14) \)

with
The relations can be concisely expressed as a tridiagonal matrix as follows:

\[
C^k_j = M_j C^{k+1}_{j-1} + N_j C^k_{j+1} + P_j C^{k+1}_{j+1} + J_j ,
\]

(3.16)

where \(M_j, N_j,\) and \(P_j\) are the coefficients of \(C^{k+1}_{j-1}, C^k_{j+1}\) and \(C^{k+1}_{j+1},\) respectively, and \(J_j\) represents the terms in the last curly brackets. Using the boundary conditions, the matrix arrays can be expressed explicitly. The boundary conditions (BC) for solving the governing equation are given by Paper III.

BC for inhalation:

\[
z = 0 : C(0, t) = C^k_0 = C_{amb},
\]

(3.17a)

\[
z = L : C(L, t) = \frac{\partial C^k_{n+1}}{\partial z} = 0 \rightarrow C^k_{n+2} = C^k_n,
\]

(3.17b)

BC for exhalation:

\[
z = 0 : \frac{\partial C^k_0}{\partial z} = 0 \rightarrow C^k_1 = C^k_{-1},
\]

(3.18a)

\[
z = L : \frac{\partial C^k_{n+1}}{\partial z} = 0 \rightarrow C^k_{n+2} = C^k_n.
\]

(3.18b)

The initial condition (IC) for inhalation is defined as \(C(z, 0) = C^0_j = 0.\) For exhalation, the last axial distribution of CO concentration of the inhalation period is considered as IC. Here, \(n\) is the node before the last node and mouth is represented by the node at \(z = 0.\)

This matrix has a \((n+1)\times(n+1)\) dimension during inhalation and a \((n+2)\times(n+2)\) dimension during exhalation due to different boundary conditions during these phases of respiration. The main matrix arrays for inhalation or exhalation are calculated once and then is used iteratively to retrieve the concentrations at each node at each time step. The matrix relation for inhalation and exhalation are given below, respectively,
3.2.3 Model-simulated expirograms and limitations of the solution

Solving Eq. (3.6) raises a number of numerical challenges in terms of stability, speed and accuracy of the solution. It is important to consider the directions of
advection during inhalation and exhalation while discretizing the equation spatially. Otherwise, the solution will not be stable and huge oscillations will be seen in the distribution of CO along the respiratory tract.

Solving Eq. (3.6) for typical model parameters taken from literature for NO [58] and estimated for CO Papers III yields the exhalation profiles shown in Fig. 3.3 with three distinct exhalation phases that are indicated by Roman numerals. In this simulation an inhalation/exhalation flow rate of 200 ml/s and inhaled/exhaled volume of 1,400 ml were assumed. Inhaled CO and NO concentrations were 130 ppb and zero, respectively.

Moreover, the model provides the axial distribution of CO in the respiratory tract during different phases of breathing. Figure 3.4 shows the distribution of CO as a function of time and axial position during inhalation, breath-holding and exhalation. An initial zero concentration is assumed throughout the respiratory tract. During inhalation, the ambient air is taken in convectively and the CO from capillary blood and alveolar membrane tissue diffuses into the alveolar region. The last profile of inhalation represents the initial condition for exhalation. During exhalation, the alveolar gas is transferred towards the airways and mouth by convection while the systemic CO is still diffusing into the alveolar region. During breath-holding, the axial diffusion acts as the main gas transport mechanism due to a large gradient between airway and alveolar CO concentrations.

![Fig. 3.3](image)

Typical CO (solid line) and NO (dotted line) exhalation profiles simulated based on the estimated parameters given in Papers III and the NO parameters in [58], respectively. An eNO profile after 20 s breath-holding (dashed-dotted line) is also shown.

In the end, a summary of model applications for characterizing different exhaled gases and the corresponding extracted model parameters is given in Table 3.1.
Fig. 3.4  Typical spatiotemporal distribution of CO concentration along the respiratory tract, during inhalation (A) and subsequent exhalation (B) at 121 ml/s, and during a 20-s BH maneuver (C) followed by exhalation (D) at 151 ml/s. Inhaled CO and airway tissue concentrations are indicated in (A) and (C), respectively.
### Table 3.1 Comparison of extracted model parameters in different BGA studies.

<table>
<thead>
<tr>
<th>Species</th>
<th>Determined parameters</th>
<th>Model type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>$J_{awNO}$, $C_{ANO}$</td>
<td>C</td>
<td>[69, 70]</td>
</tr>
<tr>
<td>NO</td>
<td>$J_{awNO}$, $D_{awNO}$, $C_{awNO}$</td>
<td>C</td>
<td>[71]</td>
</tr>
<tr>
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<td>C</td>
<td>[55, 71–73]</td>
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<tr>
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<td>C</td>
<td>[74]</td>
</tr>
<tr>
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<td>M</td>
<td>[58]</td>
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<td>$J_{awNO}$, $C_{ANO}$</td>
<td>M</td>
<td>[75]</td>
</tr>
<tr>
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<td>C</td>
<td>[4]</td>
</tr>
<tr>
<td>Alcohol</td>
<td>$C_{ACO}$</td>
<td>C</td>
<td>[76]</td>
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<tr>
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<td>$C_{ACO}$</td>
<td>C</td>
<td>[77]</td>
</tr>
<tr>
<td>Isoprene</td>
<td>$C_{A}$</td>
<td>C</td>
<td>[4]</td>
</tr>
<tr>
<td>O$_2$, CO$_2$, N$_2$</td>
<td>$F_{VD}$, $F_{VD}$, $V_{A,tot}$, $V_{D,tot}$</td>
<td>C</td>
<td>[6]</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>$\dot{Q}_c$, $C_B$</td>
<td>M</td>
<td>[64]</td>
</tr>
<tr>
<td>CO</td>
<td>$J_{awCO}$ ($C_{tiss}$), $D_{awCO}$, $J_{ACO}$, $D_{ACO}$</td>
<td>M</td>
<td>P. III, IV</td>
</tr>
</tbody>
</table>

*a C: compartmental; M: morphological

$b J_{awNO}$: average volumetric conductive airway flux of NO

$c C_{ano}$: time-averaged alveolar NO concentration

$d \dot{Q}_c$: Cardiac output

$e C_B$: mixed venous partial pressure of CO$_2$ concentration
4 Laser absorption spectroscopy

Light interacts with matter in three ways: absorption, emission, and scattering. Absorption converts the radiant energy into internal energy and emission converts internal energy into radiant energy. In scattering, photons are first absorbed and then re-emitted. The principal characteristic of absorption is “energy selectivity”, which means that the energy is absorbed by matter at certain frequencies. Selective absorption is responsible for the color of most objects around us. The absorbed radiant energy in the near- and mid-infrared spectral ranges causes excitations of lattice vibrations and molecular rotational-vibrational states.

4.1 Overview of absorption spectroscopy

Absorption spectroscopy (AS) commenced by the pioneering work of Joseph von Fraunhofer (1787–1826) who studied the dark absorption lines in the optical spectrum of the sun. AS refers to methods that identify and quantify specific substances often in gaseous samples by making use of their wavelength-dependent absorption characteristics. The degree of absorption at a certain wavelength provides a measure of the concentration of a certain atom/molecule present in the sample. Using lasers as light sources, AS has been developed to a modern field of research, which has enabled measurements of trace substances at low parts per trillion (ppt) levels. Using the monochromatic laser light improved the spectral resolution significantly and reduced the spectrum acquisition time compared to traditional monochromators and broadband sources such as lamps and thermal sources. Detection of trace molecules in gaseous media using laser absorption spectroscopy (LAS) has found various applications in science and industry, spanning over atmospheric science to in-situ combustion diagnostics and medical diagnostics. LAS is one of the most suitable laser-based techniques that allows for real-time, non-intrusive and often calibration-free measurement of small-sized molecules in gas phase with high accuracy, precision, sensitivity and selectivity. If the energy of laser photons equals the energy difference between two specific atomic or molecular energy levels of a given
Overview of absorption spectroscopy

species, laser light will be partly absorbed by that species. All these features makes the LAS technique suitable for analysis of exhaled breath [78–81].

Using single-mode semiconductor lasers, tunable diode laser absorption spectroscopy (TDLAS) is one of the most common approaches of the LAS techniques. TDLAS is in fact a term that covers a wide range of techniques in which multi-pass cells and modulation techniques may be used to improve the sensitivity. In TDLAS, the laser is precisely tuned by careful control of the laser temperature and current to emit light at the specific resonant wavelength of certain species. This approach enables scanning over absorption features. As mentioned above, the strong fundamental rotational-vibrational absorption bands for many molecules are located in the near- and mid-infrared spectral region. Hence, detection of molecules in the mid-IR can further improve the sensitivity of the technique. For instance, Fig. 4.1 shows the fundamental ro-vibrational absorption band of CO in mid-IR range at \(\sim 4.7 \mu\text{m}\), and the 1st and the 2nd overtones in the near-IR spectral range with 2 and 4 orders of magnitude weaker linestrengths. Figure 4.3 shows a typical CO absorption line (red dash-dotted line) in the mid-IR alongside of background (blue solid line) and relative frequency scale (black dashed line) signals. Relative frequency scale signal will be explained later in this chapter.

![Carbon monoxide absorption bands with rotational-vibrational transitions in mid-IR with 1st and 2nd overtones in near-IR.](image)
4.2 Beer-Lambert’s law

In most situations, the attenuation of light due to absorption can be described by the Beer-Lambert law

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

where \( I_0 \) is the incident intensity, \( I(\nu) \) is the transmitted intensity and \( \alpha(\nu) \) is called the absorbance of the sample. The absorbance depends on the species concentration \( c \) (dimensionless), the pathlength through the analyte \( l \) (cm), the pressure of the gaseous sample \( p \) (atm), the line strength \( S \) (cm\(^{-2}\)/atm), as well as the frequency-dependent area-normalized absorption lineshape function \( \chi(\nu) \) (with \( \int_{0}^{\infty} \chi(\nu) \, d\nu = 1; \, 1/cm^{-1} \)), according to

\[ \alpha(\nu) = Scpl\chi(\nu). \]

![Fig. 4.2 Schematic drawing of the direct laser absorption spectrometry. The laser light is attenuated according to Beer-Lambert’s law.](image)

The line strength \((S')\) in HITRAN (high-resolution transmission) molecular absorption database is given in cm\(^{-1}\)/ (molecule.cm\(^{-2}\)) unit. This parameter can be expressed more appropriately in terms of a line strength \((S)\) in cm\(^{-2}\)/atm unit as follows

\[ S = \frac{n_i}{p_i} S', \]

where \( n_i \) (molecule.cm\(^{-3}\)) is the molecular density and \( p_i \) (atm) is the partial pres-
4.3 Lineshapes and broadening mechanisms

Spectral absorption and emission lines are not infinitely narrow, but are broadened by a number of processes depending on the behavior of molecules of a gas in thermal equilibrium. The line broadenings are generally divided in two major groups: homogeneous broadening, if the molecules behave in the same manner; and inhomogeneous if the molecules behave differently. Homogeneous broadening is represented a Lorentzian lineshape, whereas inhomogeneous broadening is represented by a Gaussian lineshape.

Fig. 4.3 Typical direct absorption background and transmitted signals alongside of frequency scale signal.

\[ S = 7.339 \times 10^{21} \frac{S'}{T}, \]  

(4.4)

where \( T \) (K) is the gas temperature, and the constant coefficient in the numerator in (molecule.cm\(^{-3}\).K) /atm unit is the product of Loschmidt number, which represents the density of molecules of an ideal gas at standard pressure and temperature, and the reference temperature \( (T_0) \) equal to 273.15 K.
4.3.1 Natural broadening

The natural broadening is categorized as a homogeneous broadening and is a direct consequence of the uncertainty principle. This occurs when an isolated molecule at rest is irradiated by an electroctromagnetic wave and the absorption is solely due to the natural life time of the excited quantum level. The Lorenzian profile is given by:

\[ \chi_N(\nu) = \frac{c}{\pi} \frac{\gamma_N/2}{(\nu_0 - \nu)^2 + (\gamma_N/2)^2}, \tag{4.5} \]

where \( \nu_0 \) is the on-resonance frequency, \( \nu \) is the frequency of the light, and the full-width-half-maximum (FWHM) of the profile and \( \gamma_N \) is the natural line width given by,

\[ \gamma_N = \frac{1}{2\pi \tau}, \tag{4.6} \]

where \( \tau \) is natural life time of upper level \[82\]

4.3.2 Pressure broadening

The pressure broadening is also a homogeneous broadening and is due to collisions between molecules that shortens the natural lifetime and broadens the line width. It depends on the number density and the temperature. Similar to natural broadening, the lineshape function is given by

\[ \chi_L(\nu) = \frac{c}{\pi} \frac{\gamma_L/2}{(\nu_0 - \nu)^2 + (\gamma_L/2)^2}, \tag{4.7} \]

where \( \nu_0 \) is the on-resonance frequency, \( \nu \) is the frequency of the light, and the full-width-half-maximum (FWHM) of the Lorentzian profile, \( \gamma_L \), is given by

\[ \gamma_L = \gamma_N + \gamma_{\text{col}}, \tag{4.8} \]

where \( \gamma_N \) is the natural linewidth given by Eq.(4.6) and \( \gamma_{\text{col}} \) is the Lorentzian FWHM of the profile that is given by

\[ \gamma_{\text{col}} = \sum_i \Gamma_i p_i + \gamma_{\text{col}}, \tag{4.9} \]

where \( p_i \) is the partial pressure of the species \( \Gamma_i \) and \( \Gamma_i \) is the corresponding pressure broadening.
4.3.3 Doppler broadening

The molecules of a gas in thermal equilibrium are free to move in all directions. This random thermal motion of atoms causes a Doppler shift in the transition frequency given by \( \nu = \nu_0(1 - \nu/c) \), where \( \nu \) is the velocity of the molecule in the direction of the incident electromagnetic wave. Hence, the electromagnetic wave can only interact with those molecules that have velocity \( \nu = c(\nu - \nu_0)/\nu_0 \). This process is an inhomogeneous effect subsequently culminates in the broadening of absorption line according to the Gaussian lineshape function [83],

\[
\chi_G(\nu) = \sqrt{\frac{4 \ln 2}{\pi \gamma_D}} \exp \left( -\frac{4 \ln 2 (\nu - \nu_0)^2}{\gamma_D^2} \right),
\]

where the Doppler width (FWHM) is given by

\[
\gamma_D = \frac{2 \nu_0}{c} \sqrt{\frac{2 \ln 2 k_B T}{m}},
\]

and \( k_B \) is the Boltzmann constant (J/K), \( T \) is the temperature (K) and \( m \) is the molecular mass (kg).

Figure 4.4 shows the difference between a Lorentzian and a Gaussian lineshape.

4.3.4 Voigt lineshape

If both Gaussian and Doppler broadenings are present (e.g. at atmospheric pressure), the lineshape is defined by a Voigt profile which is a convolution of Gaussian and Doppler lineshapes and is given by [83]

\[
\chi_V(\nu) = \frac{1}{\pi^{3/2} \gamma_L} \int_{-\infty}^{+\infty} \frac{\exp \left( -\frac{y^2}{2} \right)}{(y + a)^2 + b^2} \, dy,
\]

where the variable parameters \( a \) and \( b \) are given by

\[
a := \sqrt{4 \ln 2} \left( \frac{\nu_0 - \nu}{\gamma_D} \right),
\]

\[
b := \frac{\gamma_L}{\gamma_D}.
\]

There is no analytical solution for Voigt profile and it is usually calculated numerically from the error function. The FWHM of the Voigt lineshape, \( \gamma_V \), can be approximated with an accuracy of 0.02% as follows [84]

\[
\gamma_V(\gamma_L, \gamma_D) = \frac{1}{2} \left[ c_1 \gamma_L^2 + (c_2 \gamma_L^2 + 4 \gamma_D^2)^{1/2} \right],
\]

where \( c_1 = 1.0692 \) and \( c_2 = 0.86639 \).
The Voigt profile does not account for higher order effects like Dicke narrowing [85] and speed-dependent effects [86]. Therefore, deviations from the Voigt lineshape function are observed in precision spectroscopy. The higher order effects do not affect the measurements performed in this thesis are not within the scope of this thesis. Hence, the approximation form of area-normalized Voigt lineshape [84] has been employed in data analysis throughout the thesis, according to the following relation

$$\chi_{V}^{A,norm}(\nu) = \frac{\chi_{V}(\nu)}{\int_{-\infty}^{+\infty} \chi_{V}(\nu) \, d\nu},$$

(4.15)

where $\chi_{V}(\nu)$ is given by

$$\chi_{V}(\nu) = \frac{1}{\gamma_{D}} \left( \frac{\ln 2}{\pi} \right)^{1/2} K(x, y),$$

(4.16)

in which $K(x, y)$ is the real part of the complex error function [87] that is calculated approximately here as follows

$$K = ((1 - \kappa)G - \kappa L),$$

(4.17)

with

$$\kappa = \frac{y}{\gamma'},$$

(4.18a)

$$G = \exp \left( -\sqrt{\ln 2} \left( \frac{x}{\gamma'} \right)^2 \right),$$

(4.18b)

$$L = \frac{1}{1 + \left( \frac{x}{\gamma'} \right)^2},$$

(4.18c)

$$\gamma' = \frac{1}{2} \left( c_{1}y + \sqrt{c_{2}y^2 + 4 \ln 2} \right),$$

(4.18d)

$$x = \sqrt{\ln 2} \left( \frac{\nu - \nu_0}{\gamma_{D}} \right),$$

(4.18e)

$$y = \sqrt{\ln 2} \frac{\gamma_{L}}{\gamma_{D}}.$$  

(4.18f)

### 4.4 Tunable diode lasers in the mid-IR

Semiconductor lasers are lasers with a semiconductor as the gain medium. The working mechanism of semiconductor lasers is similar to that of a light-emitting
diode (LED) where the light emission occurs due to the electron-hole recombination at the p-n junction in forward bias operation mode. In most of semiconductor lasers, the population inversion is achieved electrically pumped. The two surfaces of semiconductor form the laser cavity due to different refractive indices of laser medium and air. The small size of gain medium (cavity) guarantees single-mode operation of the laser. Semiconductor lasers can be made over a wide spectral range by making use of bandgap engineering of materials. Distributed-feedback (DFB) lasers are the most common tunable diode lasers in the near- and mid-IR. DFB laser has a diffraction grating on its active medium that functions as a Bragg reflector, which provides the optical feedback for laser and enables single-mode operation.

### 4.4.1 Quantum cascade laser

Quantum cascade lasers (QCLs) are promising single-mode lasers for spectroscopy in 4-14 µm spectral range. Recently, a novel type of such semiconductor injection lasers based on quantum wells approach became commercially available that provides a high-power source of mid-infrared emission operating in continuous-wave (cw) mode at room temperature. The output frequency of QCLs can be tuned over a narrow range by tuning their input current and temperature, which makes them ideal portable sources for mid-IR spectroscopy, particularly for TDLAS.
4.4.2 Interband cascade laser

Interband cascade lasers (ICLs) are similar to QCLs, as the bandstructure engineering is also employed here to optimize the laser wavelength. Contrary to QCLs that benefit from the intersubband transitions (Fig. 4.5, left panel), photons are generated with interband transitions in ICLs as shown in Fig. 4.5, right panel. They provide laser light over a large part of the mid-infrared region of the electromagnetic spectrum (3-6 \( \mu \text{m} \)).

4.4.3 Diode lasers in external cavities

In LAS, in addition to sensitivity, spectral resolution, and acquisition time, spectral bandwidth is of great importance. Multispecies detection would be possible if the spectral bandwidth be wide-enough to cover the absorption features of multiple gas species. The narrow spectral bandwidth of free-running QCL and ICL limits the multispecies detection. Diode lasers can be put in an external cavity to widen the accessible spectral regions. The gain profile of a quantum cascade laser can be quite broad (> 500 cm\(^{-1}\)). Hence, installing a QCL in an external cavity (EC) containing a wavelength-selecting element enables it to emit at a single wavelength in a large part of its broad gain profile. The two most commonly used configurations of a diode laser in an external cavity, i.e. Littrow and Littman-Metcalf, are demonstrated in Fig. 4.6.
4.5 Noise sources and background signals

The absorption sensitivity is limited by different types of noise and structures in the measured signal. Depending on the employed spectroscopy technique, the optical and electronic devices used in the setup and the operating spectral range of the sensor, the significant types of noise in the system might be different. In general, several forms of noise have been identified in absorption spectroscopy, e.g. drift noise, etc. Here, only the relevant types are considered.

Types of noise

In the following, the main types of noise present in the TDLAS technique are briefly explained.

Shot noise is a direct consequence of quantum nature of light and puts a fundamental limit to the detection. The discrete nature of light photons impinging onto the detector results in a time-dependent fluctuations in electrical current. The minimum detectable absorbance that can be achieved for shot-noise-limited DAS is given by

$$ (\alpha_0)_{\text{min}}^{\text{DAS}} = \sqrt{\frac{2e\Delta f}{\eta P_0}}. $$

where $\Delta f$ is the detection bandwidth (Hz), $e$ is the electronic charge (C), $\eta$ is the detector current responsivity (A/W), and $P_0$ is the incident power on the detector (W). This limit is approximately on the order of $10^{-8}$ and is never reached conventionally due to the existence of other types of noise, specially flicker noise that is explained below.

Intensity noise of the laser beam translates directly into the noise in the spectrum.
This noise, referred to as the white noise or random noise, has a constant and frequency-independent spectral density. Thermal noise of the detector is one of the main sources of white noise in TDLAS. The random effect of white noise can be minimized by time-averaging the signal.

Flicker noise or $1/f$-noise has an inverse frequency-dependant spectral density. This type of noise in TDLAS originates mainly from the relative intensity noise (RIN) of the laser. Two minimize the effect of such a noise on the measured signal, modulation techniques (WMS and FMS) can be employed.

Technical noise may originate from electronic equipments in the setup and also mechanical vibration of optical components. Modulation techniques can often reduce this type of noise.

**Etalon signal**

A background noise or structure in the transmitted signal can also be originated from multiple reflections of laser beam between the optical components with relatively parallel surfaces. The wave behavior of light causes an interference between the multiple reflections and produces an interference pattern that appears as fringes on the signal, generally referred to as etalon fringes$^1$. The mechanism underlying generation of etalon fringes can be explained by the Fabry-Pérot Interferometer (FPI) that makes use of interference of multiple reflections. A FPI is made of two closely spaced partially transmissive surfaces. These surfaces can be the two surfaces of a single transparent slab or two mirrors that form a cavity. This type interferometer was used primarily as a narrowband filter in optical design. A schematic drawing of a typical Fabry-Pérot interferometer is shown in Fig. 4.7. The behavior of a FPI is mathematically described by the Airy function. An etalon can be used as a ruler to measure the relative frequency scale of a laser scan.

A monochromatic plane electric wave is assumed as the incident beam on the Fabry-Pérot interferometer with perfectly parallel surfaces shown in Fig. 4.7. The incident beam is partially reflected at both surfaces upon incidence. A series of partial reflections and transmissions at each surface forms a pattern shown in Fig. 4.7. The amplitude coefficients for reflection and transmission at each surface ($r_1$, $t_1$, $r_2$, $t_2$, $r'_1$, and $t'_1$) and the refractive indices of the interferometer and the surrounding media ($n_2$ and $n_1$, respectively) are indicated. The reflection and transmission coefficients can be complex numbers as the phase may change upon reflection. Equation 4.20 describes an electric plane wave with the amplitude $E_0$ and the frequency $\omega$.

---

$^1$ Etalon is a French word that means measuring gauge.
\[ E = E_0 e^{i(\mathbf{k}.r - \omega t)} , \]  

(4.20)

Assuming the absorption in the interferometer medium to be zero and \( r_1 = r_2 = r'_1 \) and \( t_1 = t_2 = t'_1 \), the total transmitted wave is given by the sum over partially transmitted waves

\[
E_t = E_0 t^2 + E_0 t^2 r^2 e^{i\delta} + E_0 t^2 r^4 e^{i2\delta} + \ldots
= E_0 t^2 \left(1 + r^2 e^{i\delta} + r^4 e^{i2\delta} + \ldots\right)
= \frac{E_0 t^2}{1 - r^2 e^{i\delta}} .
\]

(4.21)

where the Taylor expansion was utilized, and \( \delta \) is the phase difference between the two subsequent transmissions defined as

\[ \delta = 2knd \cos \theta . \]  

(4.22)

Considering the reflectance \((R = rr^* = |r|^2)\) and transmittance \((T = tt^* = |t|^2)\), the total transmitted intensity, \( I_t(= I_0 - I_r) \), is given by

\[ I_t = I_0 \frac{T^2}{|1 - Re^{i\Delta}|} , \]

(4.23)

where \( \Delta = \delta + \delta_r \) is the total phase difference between two successive waves, in which \( \delta_r \) is the phase change in one reflection. Equation 4.23 can be written in a more explicit form as follows
Laser absorption spectroscopy

\[ I_t = \frac{I_0 T^2}{(1-R)^2} \left( \frac{1}{1 + F \sin^2 \left( \frac{kd}{2} \right)} \right), \tag{4.24} \]

where the coefficient of finesse, \( F \), is defined as follows

\[ F = \frac{4R}{(1-R)^2}. \tag{4.25} \]

The term in the parantheses in Eq. 4.24 is known as the Airy function and is plotted for various \( F \) values in Fig. 4.24. The Airy function describes the power as a function of phase (path difference) and is characterized by free spectral range (FSR), resolution (FWHM of the peaks), and finesse. The FSR is given by the following equation

\[ \text{FSR} = \frac{1}{2n_2d}. \tag{4.26} \]

\[ \text{Fig. 4.8} \hspace{1cm} \text{The Airy function for different finesse values.} \]

**Allan-Werle plot**

As mentioned above, as long as the white noise is the dominant noise, the precision of a sensor can be improved by time-averaging the signal (slope \( \sim 1/\tau \)). However, at a certain integration the precision gets limited by thermal drifts and fluctuations in the background signal (slope \( \sim \tau^2 \)). Allan-Werle deviation plot demonstrates the precision and stability of a sensor as a function of integration time. Precisely speaking, it is defined as the deviation of the difference of two measured values.
Sensitivity improvement

$y(i + 1)$ and $y(i)$ at time $t_0 + i$ and $t_0 + (i + 1)$, respectively. The Allan-Werle deviation $\sigma_y(\tau)$ depends on the variable $\tau$ and is expressed as the mean square of all measured samples separated in time by $\tau$ over the entire measurement interval $T$, i.e. $[t_0 t_0 + T]$. The Allan-Werle deviation can be expressed mathematically as below

$$\sigma_y(\tau) = \left[ \frac{1}{2M} \sum_{i=0}^{M-1} (y(i + 1) - y(i))^2 \right]^{1/2}, \quad (4.27)$$

with

$$M = \left\lceil \frac{T}{\tau} \right\rceil - 1. \quad (4.28)$$

Figure 4.9 shows a typical Allan-Werle plot in which implies that the thermal drifts and fluctuations in the background signal become dominant above 10 s integration time, and a precision of 0.6 ppb is achieved at that time.

4.6 Sensitivity improvement

The main disadvantage of AS in general is that it relies on a measurement of a small change in light intensity on top of a large background intensity. Any noise introduced by the light source or the optical system destroys its limit of detection.
Although one can go to mid-IR, the sensitivity of direct absorption techniques is usually insufficient for many types of applications. Hence, AS is seldom used in its simplest mode of operation.

There are basically two ways to improve the sensitivity of a LAS-based sensor; one is to reduce the noise in the signal, the other is to increase the absorption signal. The former can be achieved by the use of modulation techniques such as wavelength modulation spectroscopy (WMS) or frequency modulation spectroscopy (FMS), whereas the latter can be achieved by placing the sample inside a multi-pass arrangement or optical resonator in which the light passes through the sample several times. If the technique is applied to trace species detection, it is also possible to enhance the signal by performing detection at wavelengths where the transitions have larger line strengths, e.g. using fundamental vibrational bands or electronic transitions.

4.6.1 Wavelength modulation spectroscopy

The sensitivity of a sensor operating in DAS scheme is limited by low frequency noise in the signal, which originates mainly from laser intensity noise, mechanical instabilities and other external fluctuations. This limitation can be efficiently overcome by modulation techniques that shift the measured signal to a higher detection frequency. Although the frequency of the laser is modulated in both cases, the modulation frequency is much lower in WMS approach (a few kHz to a couple of MHz, much lower than the absorption linewidth) than FMS one (several hundred of MHz).

In WMS approach, a sinusoidal modulation at a higher frequency (up to several hundreds of kilohertz) is used in addition to a low frequency (i.e. a few tens of hertz) scan across the absorption feature. The modulation signal is usually generated by a lock-in amplifier. Eventually, the measured signal is demodulated via the lock-in amplifier and different harmonics of the modulation frequency are extracted as the WMS signals.

The wavelength of a diode laser can be scanned by modulating its injection current \( i_{\text{inj}} \) with a sinusoidal modulation of frequency \( f_m \) [88, 89],

\[
i_{\text{inj}}(t) = i_c + i_a \cos(2\pi f_m t),
\]

where \( i_c \) is the center injection current, \( i_a \) is the modulation amplitude of injection current, and \( t \) is the time. The current modulation results in a modulation of both the wavelength and the intensity of the laser. The wavelength modulation is conventionally expressed in frequency units

\[
\nu(t) = \nu_c + \nu_a \cos(2\pi f_m t),
\]
where $\nu(t)$ is the instantaneous frequency, $\nu_c$ is the center frequency of the laser light and $\nu_a$ is the frequency modulation amplitude. Due to this modulation, a modulated detector signal, $S_D$, is obtained that can be expressed in terms of Fourier series as follows

$$S_D[\nu(t)] = \sum_{n=0}^{\infty} S_n(\nu) \cos(2\pi nf_m t),$$

(4.31)

where $S_n(\nu)$ are the $n^{th}$ Fourier coefficients of the detector signal, expressed as

$$S_n(\nu) = G\eta P_0 S' cpl \frac{2}{\tau} \int_0^\tau \chi(t) \cos(2\pi nf_m t) dt,$$

(4.32)

where $G$ is the detector gain (V/A), $\eta$ is the detector responsivity (A/W), $P_0$ is the laser beam power, $S'$ is the line strength (cm$^{-2}$/atm), $c$ is the concentration (dimensionless), $p$ is the total pressure of the gas (atm), $l$ is the path length (cm), and $\chi(t)$ is the area-normalized lineshape function (1/cm). The integral in Eq. (4.32) can be solved for Lorentzian and Gaussian lineshape functions. More detail on the solutions can be found in [89–92]. Figure 4.10 shows the first six WMS lineshapes of a typical Lorentzian profile.
The experimental setup comprised a laser absorption spectrometer for CO and an online breath sampling system Papers I-IV. This chapter gives an overview of the sub-systems used to provide the highly precise and time-resolved CO exhalation profiles.

5.1 TDLAS CO sensor

A laser-based absorption spectrometer was constructed for non-invasive, precise and real-time measurement of CO in ambient air and exhaled human breath. The spectrometer was based on a thermoelectrically cooled, DFB-ICL (Nanoplus GmbH) operating at 4.7 μm that offered a mode-hop-free tuning range of up to 50 GHz (Fig. 5.4) at nominal diode temperature and maximum injected current range with an average output power of 1.4 mW (Paper II). The spectrometer utilized second harmonic wavelength modulation spectroscopy (2f-WMS) to suppress the low-frequency noise, and a circular low-volume (38 ml) multipass cell (IR Sweep, IRcell-4M) for pathlength enhancement (4 m) (Fig. 5.5). The MPC accepts high optical throughput, and a fringe level of 0.39‰ rms of the dc intensity has been specified by the manufacturer. The laser beam was coupled to the MPC using a plano-convex lens with a focal length of 100 mm and the output beam from the MPC was focused on a mid-IR photodetector (VIGO System, PVI-2TE-5-1×1) using a parabolic mirror. The laser power was attenuated before coupling to the MPC to match the operating range of the detector (<1 mW). An analog lock-in amplifier (Stanford Research Systems, SR830 DSP) was used to provide the modulation signal and demodulate the detector signal of the WMS scheme to yield the 2f-WMS signal. A PC equipped with a 16-bit data acquisition card (Spectrum, M2i.4963-exp) was used to record the detector signal. An alternative beam path provided by the flip mirror allows for recording the relative frequency scale of the laser scan using an uncoated solid Germanium etalon (LightMachinery, OP-5483-50.8) with a free-spectral-range of 734.2 MHz. A typical etalon signal is shown in Fig. 4.3. The pressure in the MPC was monitored by a pressure transducer (Leybold, Ceravac
CTR100) and a shut-off valve (Swagelok, EL3233) at the MPC inlet allowed to achieve a 100-Torr sample pressure. A diaphragm vacuum pump (Leybold, Divac 1.4HV3C) was connected to the MPC outlet and provided a MPC gas exchange time of 0.1 s, fast enough for real-time CO detection. The sample flow rate from the buffer tube at atmospheric pressure to the cell was 50 ml/s.

With a slightly different configuration, the spectrometer could also be operated using a water-cooled, continuous-wave (cw) EC-QCL (Daylight solutions, TLS-41047-MHF) in Littrow configuration with a total tuning range of 205 cm$^{-1}$ and a mode-hop-free tuning range of $\sim$ 93 cm$^{-1}$ around 4.7 $\mu$m. The strong fundamental CO absorption bands were accessible in this spectral range (see Fig. 4.1). Three mechanisms for coarse and fine tuning offered the possibility for both broadband and narrowband spectroscopy. A laser temperature and current controller (Daylight solutions, 1001-TLC) was used to regulate the rotation of grating in step or sweep mode. The fine tuning of grating for narrowband scans was achieved via a piezo-electric element (PZT) attached to the grating and controlled by a high-voltage amplifier (Piezosystem Jena, 30V300 CLE). Injection current modulation provided a third possibility to scan over an even narrower bandwidth (Table 5.1). Table 5.1 shows characteristics of both lasers. More information on the laser specifications can be found in Paper I.

The schematic drawing of the spectrometer is shown in Fig. 5.1. Figure 5.1(a) illustrates the configuration of ICL-based sensor. A similar optical configuration is used in EC-QCL-based sensor, just the laser operation is slightly different that is shown in Fig. 5.1(b).

![Fig. 5.1](image)

(a) ICL-based, and (b) EC-QCL-based TDLAS CO sensor. The optical components in (b) are the same as in (a). ICL – interband cascade laser, MPC – multi-pass cell, PD – photodetector, PT – pressure transducer, LDC – laser diode controller, FGen – function generator, LiA – lock-in amplifier, LC – laser current and temperature controller, PZT – piezo amplifier, WCS – water cooling system.

Both narrowband and broadband detections offer the possibility to simultaneously
detect 1-3 species in exhaled breath samples in a single wavelength scan without interference from water molecules. Multiple species detection is more appropriate using the ECDL that enables broadband scan over a wide spectral range.

The spectrometer was operated in DAS and WMS modes. Figure 5.3 shows experimental DAS and $2f$-WMS raw signals (red markers) of the P(13e) and P(6) transitions at 5.9% $^{12}$CO$_2$ and 1.1 ppm $^{12}$CO, recorded with 0.14 s integration time (10 averages) by the EC-QCL. A least-squares fit (blue solid line) of a Voigt line shape to the raw data and the residual of the fit (lower panel) is shown for each spectrum. The Beer-Lambert law was employed to calculate the $^{12}$CO and $^{13}$CO$_2$ concentrations from the fit. The background signal was recorded using the nitrogen-filled MPC. A drift in background signal was accounted for by including a first-order polynomial function in the fit. The methodology proposed by Westberg et al. [89] was utilized to fit and extract the $2f$-WMS peak values. Finally, the $2f$-WMS peak values were calibrated with DAS to yield the $^{12}$CO and $^{13}$CO$_2$ concentrations. A detection sensitivity of $2 \times 10^{-7}$ cm$^{-1}$ and a 3 orders of magnitude dynamic range were achieved for the sensor using both lasers.

![Picture of the optical setup.](image)

### 5.2 $^{13}$CO isotope detection

Relatively strong $^{13}$CO absorption lines are available in the spectral ranges covered by both spectrometers. It is possible to find a spectral region in which the $^{13}$CO line is free of interferences. It is desirable if one can also find a $^{12}$CO absorption line nearby such that both $^{13}$CO and $^{12}$CO lines can be covered by a single scan of laser wavelength. The ability of the ICL to detect CO isotopes was presented in Papet II.
Table 5.1 Basic characteristics of the lasers

<table>
<thead>
<tr>
<th>Specification</th>
<th>EC-QCL</th>
<th>ICL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tuning range [cm(^{-1})]</td>
<td>2015 – 2220</td>
<td>2130.09 – 2131.87</td>
</tr>
<tr>
<td>Mode-hop-free range [cm(^{-1})]</td>
<td>2080 – 2173</td>
<td>2130.09 – 2131.87</td>
</tr>
<tr>
<td>PZT tuning range [cm(^{-1})]</td>
<td>1.9 (at 100 Hz)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>0.7 (at 500 Hz)</td>
<td>–</td>
</tr>
<tr>
<td>Current tuning range [cm(^{-1})]</td>
<td>0.18</td>
<td>–</td>
</tr>
<tr>
<td>Configuration</td>
<td>Littrow (EC)</td>
<td>DFB</td>
</tr>
<tr>
<td>Tuning mechanism</td>
<td>Grating</td>
<td>injec. current</td>
</tr>
<tr>
<td></td>
<td>PZT</td>
<td>temperature</td>
</tr>
<tr>
<td></td>
<td>injec. current</td>
<td></td>
</tr>
<tr>
<td>Average output power [mW]</td>
<td>180</td>
<td>1.4</td>
</tr>
<tr>
<td>Cooling</td>
<td>water</td>
<td>thermoelectric</td>
</tr>
</tbody>
</table>

Fig. 5.3 Typical (a) direct absorption, and (b) background-corrected 2f-WMS signals (markers) of \(^{12}\)CO and CO\(_2\) transitions recorded at 0.14 s integration time (10 averages), with least-squares Voigt fit (blue, solid line) and fit residuals. The sample was a 1.1 ppm and 5.9% CO and CO\(_2\) gas standard at 100 Torr, respectively. For clarity, only every 30th data point is shown in the DAS and WMS spectra.

5.3 Online breath sampling system

An advanced breath sampling system was constructed and used to record the respiratory data under controlled breathing conditions (Fig. 5.6). The main parts
Experimental setup

Fig. 5.4 Measured DAS spectra over the full ICL current tuning range from a breath sample collected during smoking (black line) and from a 28.24 ppm CO gas standard (inverted, red line).

Table 5.2 Transitions of CO and CO\(_2\) that have been used by the ECDL and ICL

<table>
<thead>
<tr>
<th>Species</th>
<th>Wavenumber cm(^{-1})</th>
<th>Line-strength cm(^{-1})/(molecule.cm(^{-2}))</th>
<th>Laser</th>
<th>Transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{12})CO</td>
<td>2119.6810</td>
<td>3.709E-19</td>
<td>ECDL</td>
<td>P 6</td>
</tr>
<tr>
<td></td>
<td>2131.6316</td>
<td>2.469E-19</td>
<td>ICL</td>
<td>P 3</td>
</tr>
<tr>
<td>(^{13})CO</td>
<td>2131.0045</td>
<td>4.338E-21</td>
<td>ICL</td>
<td>R 9</td>
</tr>
<tr>
<td>(^{12})CO(_2)</td>
<td>2119.596413</td>
<td>9.425E-24</td>
<td>ECDL</td>
<td>P 13e</td>
</tr>
</tbody>
</table>

Fig. 5.5 Circular multipass cell providing an effective path length of 4 m (51-reflections) in a compact design (38 ml) via a two-dimensional star-like beam-pattern (Copyright by IRSweep).
of this breath sampling system were an inline flow meter and a CO$_2$ capnograph mounted on a 15-cm long Teflon buffer tube. A computer interface was implemented to monitor the breathing pattern (IFR/EFR and breathing frequency). A mouth-piece and a nose-piece were interchangeably used at the inlet to make mouth- and nose-exhalations comfortable and easy to perform (Fig. 5.6). An antibacterial filter was mounted at the mouthpiece/nose-piece to prevent contamination. Both inhalation and exhalation were performed through the breath sampler. The separation of inhalation and exhalation at desired flow rates was achieved using a two-way non-rebreathing valve (Rudolph Inc.) connected to the outlet of the buffer tube and orifices with suitable diameters mounted at its inlet and outlet ports. A part of the sample (breath during exhalation, and ambient air during inhalation) was continuously drawn to the MPC at a flow rate of 50 ml/s for CO measurement.

**Fig. 5.6** Upper panel: (left) Picture of mouthpiece and nosepiece used on the breath sampler. (right) Schematic drawing of the breath sampler. Lower panel: Picture of the breath sampler equipped with the mouthpiece. TMP – Teflon mouthpiece; ABF – Antibacterial filter; CPN – Capnograph; FM – Flow meter; FLTK – FloTrak elite respiratory mechanics module; 2WV – 2-way valve;
5.3.1 Flow meter and CO$_2$ sensor

The inline flow meter was a fixed orifice differential pressure type that accurately measures proximal volume, flow rate, and pressure and potentially more than 60 other respiratory parameters on a breath by breath basis. This flow meter is a part of FloTrak Elite Respiratory Mechanics module designed by Phillips Respironics for low power applications, with a special attention to mechanically ventilated patients. It does not need calibration and has an upgrade path with CO$_2$ for volumetric capnography.

The mainstream CO$_2$ sensor is a non-dispersive infrared (NDIR) single beam optical device in a compact design that has light weight and no moving parts. It is fast with a 100 Hz sampling frequency and accurately measures CO$_2$ in a wide dynamic range (0-19.7%) and does not need calibration. Having all of the electronics located inside the Capnostat head has made it easy to integrate with the FloTrak Elite module. Only the communication and power are administered by the Elite module. All the communications with the computer interface were done using the RS232 protocol.

<table>
<thead>
<tr>
<th>Respiratory parameter</th>
<th>Resolution</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate [l/min]</td>
<td>0.01</td>
<td>-300.00 – 300.00</td>
</tr>
<tr>
<td>Airway pressure [cmH$_2$O]</td>
<td>0.05</td>
<td>-150 – 150</td>
</tr>
<tr>
<td>CO$_2$ [%]</td>
<td>0.1</td>
<td>0 – 19.7</td>
</tr>
<tr>
<td>Volume (Adult/Pediatric) [ml]</td>
<td>1</td>
<td>-1000 – 4000</td>
</tr>
</tbody>
</table>

5.3.2 Computer interface for sampler

A LabVIEW program was developed in our lab to control the laser and acquire the photodetector signal. This computer code also provided an interface with audiovisual indicators that helped the subjects to maintain the intended IFR/EFR and breathing frequency according to the protocol used for breath sampling, and enabled recording of respiratory data. Figure 5.8 shows the front panel of the LabVIEW code with monitoring panels for IFR/EFR, inhaled/exhaled volume, airway pressure and a panel to display the preliminary measured CO concentrations in
Fig. 5.7 On-line mouth-exhaled eCO breath sampling through the sampler.

Fig. 5.8 Front panel of LabVIEW interface.

real-time. A typical respiratory data set recorded for a breath cycle during normal breathing for a healthy non-smoker is shown in Figure 5.9.
Fig. 5.9 Typical respiratory data extracted from breath sampling system for one breath cycle (normal breathing). The mean IFR and EFR are indicated by dashed lines.
Extended breath gas analysis

Highly time-resolved and precisely measured single-exhalation profiles contain information about the gas exchange in the respiratory tract. Coupling the experimental expirograms to a model of pulmonary gas exchange can further improve the extent of information obtained from a breath test [61, 93] and provide a tool for non-invasive assessment of lung properties [6] Papers III. As mentioned above, most of the BGA studies were traditionally focused on measuring the end-tidal values. Consequently, the physiological models were also adapted to this type of measurements [56, 61, 93–95]. Only in a few cases, model-based simulations were compared or fitted to the entire single-exhalation profiles [72, 74, 76, 77, 96]. Recently, it was shown that least-squares fitting of simulated profiles to the real-time data of the respiratory gases O$_2$ and CO$_2$ (and the inert gas N$_2$) may enable the assessment of lung inhomogeneity [6].

The basic principles of laser-based real-time detection and pulmonary gas exchange modeling of eCO were summarized in Chapters 3-5. A least-squares fitting formalism can be used to couple these two features and yield an analytical tool for analysis of single-exhalation profiles of CO. The TMAD model described in Chapter 3 is used to implement a curve fitting routine for model parameter extraction from the experimental data. The extracted parameters are the maximum CO fluxes, equilibrium concentrations and diffusion capacities in airways and alveoli ($J_{awCO}$, $J_{ACO}$, $D_{awCO}$, $D_{ACO}$, $C_{ACO}$, $C_{tiss}$).

6.1 Least-squares fitting of expirograms

The model-simulated exhalation profiles are extracted from the solution of TMAD model at the first grid point representing the mouth. As seen above, the CO exhalation profile for a healthy subject exhibit a steep rise in the phase II. Linear least-squares fitting methods are sensitive to outliers in the data. The steep change in eCO profile mimics a similar situation. Because of that a linear fit fails to work properly in phases II and III (see Fig. 6.1(a)) and may cause more than 60% error in alveolar TMAD parameter values. The phase II would be even steeper if a
breath-holding maneuver is undertaken, and subsequently the fitting issue would become more severe. Since the sensitivity (parametric) analysis shows that variation in TMAD model parameters mostly affects phases I and III, but not phase II (Paper III), one solution to this problem is to have lower weight on the data points in phase II. Hence, a nonlinear least-squares fitting method that allows for weighting data points is employed. Practically, an array can be defined to put more weight on data points in phases I and III of exhalation and then a library function can perform nonlinear least-squares fitting. Figure 6.1 compares linear and nonlinear least-squares fits to the same real-time data. Using an array that puts 20 and 60 times more weight on the exhalation phases I and III, respectively, the nonlinear approach shows a much better match to the phase III of exhalation profile. The array defining the phases I and III of the breath cycle in fact includes the number of experimental data points in these regions. The number of points in each of these regions can be estimated based on the data acquisition rate, EFR, the exhaled volumes and considering the structure of the trumpet model. Based on these factors and the total number of data points recorded in a single-exhalation profile, rough estimates of 4% and 74% of the total data points in a breath cycle were considered for phases I and III, respectively, in our studies (Paper IV).

The main source of computational complexity of this algorithm stems from the fact that the $N_j$ and $J_j$ terms in Eq. 3.9 that include the TMAD fit parameters, and consequently the matrices in Eqs. 3.19 and 3.20, have to be recalculated in each iteration when the fitting routine varies the TMAD parameters. Nevertheless, the algorithm implemented in this work is computationally inexpensive Papers III-IV. It take around 45 s to fit a typical eCO profile for normal breathing at an exhalation flow rate of 250 ml/s and an exhalation volume of 1250 ml by a standard PC. The computational time is longer for eCO expirograms involving lower EFRs or breath-holding maneuver.

### Box 6.1 MATLAB code for fitting the breath cycle

F = @Physiol_Model_func;

diffF = @(Params,xdata) W.*(F(Params) - ydata);

[Coeff,Resnorm,Residual,Exitflag] = lsqnonlin(Fdiff,Params);

The basic MATLAB code for nonlinear least-squares fitting routine is given in Box 6.1. Here, F represents the theoretical (TMAD) model function outlined in Papers III, xdata and ydata are the experimental exhalation time and eCO data, respectively, W is the weight array, and diffF is a function that defines the weighted
The library function \texttt{lsqnonlin} solves the nonlinear least-squares equation for the gas exchange parameters, \texttt{Params}.

The precisely measured respiratory data during sampling, e.g. inhalation/exhalation flow rates and inhaled/exhaled volumes, as well as the ambient air CO are used as input parameters to the model. Initial estimates for the TMAD model parameters have been evaluated based on the healthy population physiological parameters found in the literature (\textbf{Paper III}). The model fitting parameters ($J_{\text{awCO}}$, $J_{\text{ACO}}$, $D_{\text{awCO}}$, $D_{\text{ACO}}$) are free to vary in a relatively wide range around the estimated values (\textbf{Paper IV}).

\subsection{6.1.1 Unique eCO parameters}

The nonlinear fitting routine explained above provides good fits to the experimental data recorded at different breathing conditions (normal breathing at different EFRs, breath-holding maneuver). The sensitivity analysis shows that the model is more sensitive to the individual TMAD parameters than to the $J_{\text{awCO}}/D_{\text{awCO}}$ and $J_{\text{ACO}}/D_{\text{ACO}}$ ratios. It also confirms that a specific eCO profile shape with a certain end-tidal value cannot be reproduced by different combinations of the TMAD parameters and the obtained parameters are unique (\textbf{Paper III}). The breath-holding maneuver increases the sensitivity to airway parameters due to the fact that airway tissue CO has more time to diffuse into the gas phase. Hence, the airway TMAD parameters are first determined from the BH profiles and then used as fixed parameters in the fits to the normal breathing expirograms.
The main parameters in constructing TMAD model are basically the anatomical data, input parameters (ambient air CO concentration and respiratory data, e.g. IFR, EFR, and the inhaled/exhaled volumes), zero-time point for exhalation, weight array, and the array defining the portions of a breath cycle corresponding to phases I and III. An up-to-date set of anatomical data based on Weibel’s lung structure has been employed to construct the TMAD model to minimize the errors due to anatomical information. The model is rather sensitive to the zero-time point. This point is determined based on the sampling (respiratory and capnography) data and is regulated slightly with respect to the time delay between CO measurement in the MPC and corresponding sampling data measured close to mouth. The repeatability of the results was tested over a sequence of multiple normal breathing cycles at 250 ml/s EFR for a healthy non-smoker. The standard deviation of the obtained alveolar TMAD parameters was around 5% intra-individually (Paper IV).

The alveolar diffusing capacity in the TMAD model ($D_{ACO}$) is vastly different from the standard diffusing capacity of the lung for CO ($D_{LCO}$) used in clinical setting, and the absolute values obtained for $D_{ACO}$ are considerably higher than $D_{LCO}$. The $D_{ACO}$ is measured during systemic elimination and tidal breathing, whereas the $D_{LCO}$ test involves vital capacity inhalation of CO gas at high concentrations (0.3%), breath-holding and fast exhalation. Moreover, morphological models often overestimate the diffusing capacity [97].

While using the weighted, nonlinear least-squares fit, a discrepancy between the fit and the data points would still be expected. This is mainly due to the fact that, although rather sophisticated, the 1D TMAD model does not fully account for the details of actual lungs anatomy, gas transport mechanisms and ventilation to volume heterogeneity. More discussions on the phase II discrepancy can be found in Papers III and IV.

All in all, the fit is, to a large extent, robust and provides reliable TMAD parameters under well-defined breath sampling conditions.
6.2 Workflow – from raw data to gas exchange parameters

Extracting TMAD model parameters from raw experimental data includes several experimental and analysis steps. The workflow procedure for measuring and analysing a breath cycle is briefly described in Table 6.1.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Practical steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>Recording DAS, WMS (for diluted CO gas standard) and frequency scale signals to obtain calibration curves,</td>
</tr>
<tr>
<td></td>
<td>Recording the $2f$-WMS signals and respiratory data for a subject while performing normal breathing at the desired EFR as well as for a 10-s BH maneuver,</td>
</tr>
<tr>
<td>Raw data analysis</td>
<td>Fitting the DAS and $2f$-WMS signals from diluted CO gas standard and obtain the calibration curves</td>
</tr>
<tr>
<td></td>
<td>Fitting the $2f$-WMS signals for the normal breathing and BH breath cycles ($\sim 200$ $2f$ signals per breath cycle)</td>
</tr>
<tr>
<td></td>
<td>Calibrating the peak value of the fits using the calibration curve to obtain the concentrations for breath cycles</td>
</tr>
<tr>
<td>Extended CO analysis</td>
<td>Using the respiratory data to correct the start time for breath cycles</td>
</tr>
<tr>
<td></td>
<td>Fitting the BH breath cycle and extracting the airway parameters</td>
</tr>
<tr>
<td></td>
<td>Fitting the normal breathing cycle using the airway parameters from BH as fixed parameters and extracting the eCO parameters</td>
</tr>
</tbody>
</table>
Design of clinical studies

In order to use extended eCO analysis in clinical practice, the first step is to investigate the baseline and inter-/intra-individual variations of the eCO parameters and their response to external exposure in the healthy non-smokers.

7.1 Baseline of eCO gas exchange parameters

The baseline for mixed breath or end-tidal CO concentration has mainly been investigated in medical trials to assess the smoking or poisoning status and in search for cut-off levels in diseased cohorts [98–105]. In addition to the influencing factors noted in chapter 2, the measured eCO levels in the healthy non-smoking population can also vary inter-individually as a function of fasting status, diet, age, gender, and body mass index (BMI). The background level and the effect of such factors on variations of the eCO parameters need to be investigated.

7.1.1 Baseline study population and protocol

The study population involved a cohort of 32 healthy non-smokers, 22 male and 10 female (mean age 37 years, range 26–69 years) with a BMI of 23.4 (range 18.4–28.1) kg/m², recruited from employees at Umeå University. The subjects were asked to refrain from food/drink intake and physical efforts (e.g. biking, heavy walking) for at least 1 hour prior to the measurement. The subjects were instructed to perform different breathing maneuvers via mouth and nose while sitting upright and relaxed. The protocol is summarized in Table 7.1. The respiratory data (inhalation/exhalation flow rate and volume) were continuously recorded by the online breath sampler. The ambient air CO was measured during inhalation. While providing the mouth-exhaled breath samples, the subjects were asked to use nose clips. Information on age, height, etc. were collected. The study was approved by the Regional Ethical Review Board at Umeå University (2017/306-31). Correlations between model parameters in different measurements ($J_{ACO}$, $D_{ACO}$, $C_{ACO}$, $C_{tiss}$ for mouth and nose)
Diurnal variation of eCO gas exchange parameters

Table 7.1 Timeline of the study protocol for real-time measurement of eCO profiles in the healthy population

<table>
<thead>
<tr>
<th>Timeline min</th>
<th>Event</th>
<th>Duration min</th>
</tr>
</thead>
<tbody>
<tr>
<td>-5</td>
<td>Arrival, rest, consent form and questionnaire information, getting instructed</td>
<td>5</td>
</tr>
<tr>
<td>0</td>
<td>Mouth-exhaled CO @ IFR/EFR of 250, 120 and 60 ml/sSequence of ca. 5 breath cycles</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Mouth-exhaled CO with breath-holding @ IFR/EFR of 120 ml/sBreath-holding time: 10 s (2 breath cycles)</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td><strong>Short break</strong></td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>Nose-exhaled CO @ IFR/EFR of 250, 120 and 60 ml/sSequence of ca. 5 breath cycles</td>
<td>5</td>
</tr>
<tr>
<td>17</td>
<td>Nose-exhaled CO with breath-holding @ IFR/EFR of 120 ml/sBreath-holding time: 10 s (2 single breath cycles)</td>
<td>5</td>
</tr>
</tbody>
</table>

were assessed by Spearman’s rank correlation with $p < 0.01$ and $p < 0.05$ denoting strong and moderate correlations, respectively.

### 7.2 Diurnal variation of eCO gas exchange parameters

Due to changes in metabolism in human body, the measured end-tidal CO value may vary during the day. To exclude part of clinical study that is biased by the time of measurement during the day, the diurnal variation of mouth- and nose-exhaled CO parameters were investigated.

Two healthy non-smoking male subjects (aged 42 and 37) from the cohort took a relaxed sitting position and provided mouth- and nose-exhaled breath samples at 250 ml/s normal breathing and at 120 ml/s after 10 s breath-holding every half an hour for a total period of 11 hours. Each time the breath sampling was first done for mouth and then for nose. The body mass index was 25 kg/m$^2$ for both subjects.
7.3 Acute exposure to CO and wood smoke

Exposure to exogenous sources of CO elevates the measured eCO concentration following an increase in blood COHb and changes the shape of CO exhalation profile. However, the lung diffusion properties are expected to stay unaffected. On the other hand, respiratory diseases can influence the lung diffusion properties.

As mentioned in Chapter 1, air pollution can significantly develop respiratory diseases and indirectly cause millions of premature deaths worldwide each year. The physiological mechanisms underlying the relationship between air pollution and respiratory diseases can be properly investigated if the short-term response of body due to acute exposure to air pollution can be measured, e.g., in human exposure studies [106–109]. In a recent human exposure study involving a 3-hours exposure to wood smoke from incomplete combustion at a concentration of 314 µg/m³ for particulate matter of 1 µm size (PM₁), an increase in the lavage lactate dehydrogenase (LDH) level as an indicator of tissue injury was observed in bronchoalveolar lavage (BAL) fluid that was sampled after 24 h [110]. Researchers could also observe signs of cell cytotoxicity and cell cycle arrest induced by the wood smoke particles in the respiratory tract with only minor inflammatory responses. Although CO is considered as an inflammatory biomarker, we may not expect to observe specific response since the inflammatory markers did not show up in standard tests either. However, correlation between the TMAD parameters and the medical test results can be of interest, e.g., a probable contribution to eCO rise due to wood smoke particles.

7.3.1 Exposure chamber

The effect of acute exposure to elevated CO levels on the physiological model parameters is investigated using a whole-body human exposure chamber with the size of 18 m³. Air pollution is introduced continuously into the chamber and a fan provided constant mixing. The details of human exposure chamber described by Unosson, et al. [106]. Figure 7.1 shows the chamber and the wood stove providing the controlled wood smoke including 10 ppm CO gas.

7.3.2 Exposure study population and protocol

The subjects were recruited mainly from students at Umeå University. A total of 14 healthy non-smoking subjects, 11 male and 3 female (mean age 26 years, range 19–35 years) with a mean BMI of 24.5 (range 20.7–29.0) kg/m², participated in
the study. They had normal lung function and no history of asthma, respiratory, or other illnesses or were taking any medication, and had no respiratory tract infections during the study period. The study was approved by the Regional Ethical Review Board at Umeå University (2018-35-32M) and subject gave their written informed consent.

In a double-blind trial (two visits) with at least 3 week interval, the subjects were randomly exposed either to filtered air (X) or wood smoke (Y) with an average PM concentration of 400 µg/m³ in a whole-body human exposure chamber. Controlled wood smoke was introduced continuously into the chamber and a fan provided constant mixing. During the exposure, the subjects performed 15 minutes cycling followed by 15 minutes rest. In each visit, eCO samples were taken about 10 min before and 7 min after completion of the 2 hours exposure. Bronchial wash (BW) and bronchoalveolar lavage (BAL) were collected by bronchoscopy around 6 h after exposure (Fig. 7.2). The eCO measurements before and after exposure were performed according to the protocol outlined in Table 7.2. The subjects were allowed to have a light breakfast. However, they were asked to refrain from food intake until the time after bronchoscopy procedure.

In order to roughly assess a potential contribution to eCO rise due to wood smoke particles, in another study, two healthy non-smoking male subjects were exposed to a mean CO concentration of 10 ppm and more than three weeks later to wood smoke for the same period of time (2 h). Similarly, the subjects performed 15 minutes cycling followed by 15 minutes rest during the exposure. The breath samples were measured before and 7 minutes after the end of the exposure.
The study design for each exposure session demonstrating sampling time points. Every subject undergoes two exposures.

### Table 7.2  Timeline of the study protocol for real-time eCO profiles measurements before and after exposure

<table>
<thead>
<tr>
<th>Timeline (min)</th>
<th>Event</th>
<th>Duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-5</td>
<td>Before exposure: arrival, rest, getting instructed</td>
<td>5</td>
</tr>
</tbody>
</table>
| 0             | Mouth-exhaled CO @ IFR/EFR of 250 and 120 ml/s  
|               | Sequence of ca. 5 breath cycles | 5 |
| 5             | Mouth-exhaled CO with breath-holding @ IFR/EFR of 120 ml/s  
|               | Breath-holding times: 10 s (2 breath cycles) | 5 |
| 10            | Short break | 1 |
| –             | After exposure: the same protocol as before exposure | 10 |
This chapter gives a brief summary of the results obtained in the thesis work.

8.1 TDLAS sensor performance

Using narrowband WMS detection, the mid-IR TDLAS sensor for carbon monoxide presented in Chapter 5 allows for highly precise and time-resolved, simultaneous detection of eCO and eCO$_2$ expirograms and ambient air CO based on the EC-QCL (Paper I). Using the ICL, the compact sensor offers the possibility for real-time detection of $^{12}$CO and $^{13}$CO exhalation profiles (Paper II). Basic parameters characterizing performance of the TDLAS sensor in the two configurations are given in Tables 8.1.

<table>
<thead>
<tr>
<th>Laser</th>
<th>Detected species</th>
<th>CO LOD ppb</th>
<th>CO$_2$ LOD ppm</th>
<th>Sensitivity cm$^{-1}$ Hz$^{-1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC-QCL</td>
<td>$^{12}$CO, $^{12}$CO$_2$</td>
<td>9 ± 2</td>
<td>650 ± 7</td>
<td>$8.5 \times 10^{-8}$</td>
</tr>
<tr>
<td>ICL</td>
<td>$^{12}$CO, $^{13}$CO</td>
<td>9 ± 5</td>
<td>–</td>
<td>$6.5 \times 10^{-8}$</td>
</tr>
</tbody>
</table>

A typical background-corrected 2f-WMS signal from 19 ppb $^{13}$CO in a diluted gas standard sample flowing through the MPC is shown in Figure 8.1 (left) together with a Voigt fit. Figure 8.1 (right) presents a 2f-WMS signal from 38 ppb e$^{13}$CO in alveolar breath of a healthy occasional smoker after smoking. The etalon fringes on the signal could be effectively filtered out by including an etalon term in the fitting procedure. Figure 8.2 shows the exhalation profiles for CO isotopes from an occasional smoker before (19 hours after the last cigarette smoke) and 15 s after smoking (Paper II). A more than two-fold increase in the eCO levels is observed.
after smoking, while the shapes of exhalation profiles for CO isotopes are similar. Comparison between the isotope ratios before and after smoking shows a depletion of $^{13}$CO in breath compared to natural abundance. Moreover, the absolute values obtained for isotope ratios before and after smoking have negative sign. This is in contrast to the results presented by Lee et al. [111], where they found positive isotope ratio values in the breath of healthy non-smokers.

**Fig. 8.1** $^{13}$CO lineshapes measured by TDLAS sensor using ICL for (left) 19 ppb $^{13}$CO concentration gas standard, and (right) 38 ppb $e^{13}$CO concentration in breath.

**Fig. 8.2** Multiple $^{12}$eCO (upper panel), and $^{13}$eCO (lower panel) breath cycles of a healthy occasional smoker before smoking (19 h after the last cigarette) and 15 s after smoking. $e^{13}$CO expirograms recorded 30 s after $e^{12}$CO. Gray – raw data, colored lines – smoothed raw data.
The EC-QCL-based spectrometer offers the possibility for broadband detection of species in DAS mode over a 93 cm\(^{-1}\) mode-hop-free range in the mid-IR (Paper I). Figure 8.3 (left) presents a broadband scan of mode-hop-free range of EC-QCL for an end-tidal breath sample of a healthy non-smoker. The absorption lines for CO, CO\(_2\) and water (H\(_2\)O) are observed in the spectra. The concentrations for CO, CO\(_2\) and H\(_2\)O are 1.2 ppm, 5% and 1.1%, respectively. A smaller section of the broadband scan is demonstrated in Fig. 8.3 (right), which is the spectral region found suitable for narrowband simultaneous detection of CO and CO\(_2\). A simulated spectrum based on the HITRAN 2012 database [112] is shown in the lower part of each graph (inverted for clarity). The experiment and the simulation are in good agreement. The poor repeatability of the sweep scan resulted in an error in the frequency scale and consequently in the spectrum. In addition, the background signal was measured separately and resulted in a noise on the baseline. The broadband performance of the spectrometer can be improved by simultaneous measurement of frequency scale, background and transmitted signals.

Figure 8.4 (left) shows the eCO and the two eCO\(_2\) expirograms for a typical breath cycles. The eCO and eCO\(_2\) expirograms have slightly different shapes, whereas eCO\(_2\) expirograms measured by capnography and TDLAS sensor have identical shape and concentrations, which confirms true real-time detection. Moreover, precise detection of eCO expirograms showed a first indication of dependence on exhalation flow rate and breath-holding time (Paper I). This is in contrast to results presented by Zetterquist et al. [113] and Pakmanesh et al. [114], but is in agreement with the findings of Fritch et al. [115].
8.2 TMAD model and extended breath CO analysis

The ability of the model to produce eNO and eCO expirograms based on the NO parameters found in the literature [58] and the estimated values for CO parameters (Paper III) was demonstrated in Chapter 3. It has further been discussed in Chapter 6 that the model can be used to least-squares fit the experimental eCO profiles and retrieve the optimum, unique TMAD parameters (Paper IV). Figures 8.5 shows the nonlinear least-squares fits (blue line) to the measured eCO profile (red markers) for a healthy non-smoker performing normal breathing at 250 ml/s of EFR. The breath sampling data (Fig. 5.9) and the ambient air CO concentration were continuously measured and used as input parameters. The exhalation starts at time zero. Roman numerals indicate the three exhalation phases. The airway TMAD parameters are fixed to those determined from the nonlinear fit to expirogram after 10 s breath-holding for the same individual shown in Chapter 6 (Fig. 6.1b). Because breath-holding increases the sensitivity to the airway TMAD parameters in exhalation phase I (Paper III).

The fitting routine provides a robust mean to extract the TMAD parameters from measured single-exhalation profiles at different conditions (breath-holding, exposure to CO gas, anticipated pulmonary diseases) during systemic CO elimination. This is demonstrated by the small intra-individual variations (standard deviations) of the TMAD parameters of successive breath cycles in Paper IV.

Figure 8.6 demonstrates the simulated end-tidal concentrations and elimination rates of CO and NO as a function of exhalation flow rate (Paper III). Model correctly simulates the previously observed dependence of end-tidal concentration and elimination rate of NO [4]. There is also a good agreement between the experimental data (star markers) and the simulations (dashed and dashed-dotted line) for CO,
Nonlinear least-squares fits (solid lines) to the measured eCO profiles (markers) for a healthy non-smokers at 245 ml/s of EFR (normal breathing). Lower panel shows the residuals of the fit. For clarity, only every 2nd data point is shown in the region of the fit. The three exhalation phases are shown in the figure by Roman numerals. $C_{amb}$ – ambient air CO.

and a dependence on the EFR is seen for both the end-tidal CO and CO elimination. At higher EFRs, the end-tidal CO decreases with increasing EFR. This is due to the diffusion-limited CO gas transfer from capillary blood to the gas phase in alveoli. At lower flow rates, end-tidal decreases abruptly with decreasing EFR, due to diffusion of CO into the airway tissue on its way out during exhalation. These indications show that airway may participate in the gas exchange. It is also shown that the end-tidal does not reach the model-predicted alveolar value.

As mentioned in Chapter 3, the respiratory diseases may affect the lung diffusing properties or influence the airway contribution to the eCO level. The ability of extended breath analysis methodology presented in the thesis to potentially assess such health conditions is demonstrated in Fig. 8.7 by fitting the relevant synthetic data with random noise. Figure 8.7(a) shows an eCO expirogram for a healthy subject (blue open markers) and a fit (solid black line) to synthetic eCO profile with an increased maximum airway flux (3 times more than in healthy subject, orange solid markers) after 10 s breath-holding. An increase in maximum airway flux that is expected from subjects with airway inflammation mainly affects the shape of profile in phase I. Figure 8.7(b) presents the eCO expirogram for healthy subject (blue open markers) alongside of two synthetic eCO profiles with a 60% reduction in alveolar diffusing capacity (as expected from subjects with severe chronic obstructive pulmonary disease(COPD)) with (orange circular markers) and without (green diamond markers) increase in COHb level performing normal breathin at 250 ml/s
Simulated end-tidal concentrations (a) and elimination rates (b) as a function of EFR for CO (dashed lines) and NO (solid lines). The average TMAD parameters for CO were 220 pl/s, 1.6 pl.s\(^{-1}\).ppb\(^{-1}\), 1.82E+7 pl/s, and 7767 pl.s\(^{-1}\).ppb\(^{-1}\) for \(J_{\text{awCO}}\), \(D_{\text{awCO}}\), \(J_{\text{ACO}}\), and \(D_{\text{ACO}}\), respectively, (Paper III) and for NO those from [58] were used. IFR was set to 127 ml/s. Dash line in (a) indicates the model-predicted alveolar CO concentrations (\(C_{\text{ACO}}\)). Experimental end-tidal values for the three EFRs (star markers) and breath-holding (diamond marker) are shown for comparison.

of EFR. The model fit to the synthetic simulated exhalation profiles representing different health conditions could retrieve correct gas exchange parameters used in simulations (Paper IV).

Nonlinear least-squares fit (solid black lines) to typical eCO profiles (markers) simulated for (a) a 3-fold increased maximum airway flux (including 10 s breath-holding), and (b) a 60% reduction in alveolar diffusing capacity with and without increased COHb. Blue hollow markers in both cases show the normal eCO profile.
8.3 Baseline of eCO gas exchange parameters

As outlined in Chapter 7, a clinical study was conducted to establish the healthy population baseline for the eCO TMAD parameters. A summary of the results is shown in Figs. 8.8, 8.9, and 8.10 for mouth- and nose-exhaled expirograms at 250, 120, and 60 ml/s EFRs and for 120 ml/s EFR after 10 s breath holding. A notched box-whisker plot representation is used to visualize the data. The box indicates the interquartile range. The median is shown by the box waist. The open marker shows the mean, and the whiskers show 1.5 times the interquartile range. Figures 8.8 presents the measured end-tidal CO and model-predicted alveolar CO concentration at equilibrium with blood for 32 subjects. Figure 8.9 shows corresponding data for the maximum alveolar CO flux and alveolar diffusing capacity for CO. Figure 8.10 shows the maximum airway CO flux determined from 10 s breath-holding maneuver for mouth and nose exhalation.

Fig. 8.8 Baseline levels of $E_{TCO}$ and $C_{ACO}$ in a group of 32 healthy non-smokers.

The eCO parameters spread over a limited range relevant for healthy population at each flow rate and follow the same pattern as presented in Paper III, e.g. the alveolar parameters are decreasing and approaching the equilibrium condition as the flow rate decreases. The alveolar CO is positively correlated with BMI and gender. Clear correlations of airway CO with ambient and alveolar CO indicate negligible CO contribution from airway tissue. No difference between the nose- and mouth-exhaled eCO is observed. Typical baseline range of TMAD parameters for mouth-exhaled expirograms and correlation coefficients between some of the parameters are summarized in Tables 8.2 and 8.3, respectively.

The end-tidal CO is in the expected range for healthy population (0.8-3 ppm).
The median end-tidal CO increases with decreasing EFR. In every case, it stays below the alveolar concentration. This could mean equilibrium with blood CO is never reached. The alveolar diffusing capacity is independent of blood CO as it does not correlate with the end-tidal and alveolar concentrations. The airway tissue concentrations are similar to or slightly higher than the ambient air CO and moderately correlate with end-tidal CO. Hence, airway tissue CO concentration is mainly influenced by the ambient CO and to some smaller extent by alveolar CO. The eCO parameters seem to be spread over a shorter range for 120 ml/s of EFR. This suggest that normal breathing at 120 ml/s is optimal.
8.4 Diurnal variation of eCO gas exchange parameters

Figure 8.11 shows the diurnal variation of the end-tidal CO concentration, alveolar CO concentration, and the alveolar and airway TMAD parameters for a healthy non-smoker (aged 42) over a period of 11 hours between 08:00 and 19:00 of a normal working day. The measurements were done at a 250 ml/s EFR. Although the eCO parameter may vary during the day, they still remain within the healthy population range. No difference between nose- and mouth-exhalation can be seen for each subject and no correlation with food intake was observed. Despite the change in the maximum alveolar flux, the alveolar diffusing capacity remains relatively unchanged (**Paper V**).
Fig. 8.11 Diurnal variation of TMAD parameters for mouth (square markers) and nose (circular markers) exhalations by a subject. Int 1 – coffee, Int 2 – lunch, Int 3 – coffee, Int 4 – fruit
8.5 Wood smoke exposure

Figure 8.12(left) presents the normalized end-tidal CO for a healthy non-smoker after 2 hours exposures to 10 ppm CO gas and after 2 hours exposures to wood smoke including 10 ppm CO in a separate session a few weeks later. The increase in blood CO level followed the CFK equation considering an approximate ventilation rate and normal blood properties. The decay rates of CO elimination curves are in line with the half-life of blood COHb (5-7 hours) after exposure to CO. The decays curves exhibit different time constants after 7 hours. This probably happens due to different control levels at the outset. The results do not indicate an additional effect on eCO due to wood smoke particles. This is in line with the previous studies, where no direct inflammatory marker was observed [116]. Figure 8.12(right) shows the corresponding model-derived alveolar diffusing capacities. Similar to baseline study, alveolar diffusing capacity is decoupled from the maximum alveolar flux.

Figures 8.13 and 8.14 summarize the variations of eCO parameters for a group of 14 healthy non-smokers before and after 2 hours exposure to wood smoke from incomplete combustion including 10 ppm CO and exposure to filtered air. As evident from Fig. 8.13, the maximum alveolar flux increases after exposure to wood smoke following the blood COHb, whereas the alveolar diffusing capacity remains within the same range as before exposure. The airway tissue concentration increases slightly after exposure to wood smoke, whereas a slight decrease is seen after exposure to filtered air. A correlation between maximum airway and maximum alveolar...
fluxes after exposure to wood smoke indicates a possible influence of alveolar concentration on the airway flux.

A paper on human exposure study is under preparation that is not included in the thesis.

**Fig. 8.13** TMAD parameters for CO gas exchange for a group of 14 healthy non-smokers before and after to wood smoke from incomplete combustion; (upper panel) $J_{ACO}$ and $D_{ACO}$, (lower panel) $J_{awCO}$. 
8.6 Clinical relevance

Carbon monoxide has been suggested frequently as a biomarker of oxidative stress and respiratory diseases [12]. There are several reports on the elevated eCO levels in chronic pulmonary diseases and lung inflammation or infections. However, its clinical usefulness has been under debate due to confounding factors such as exogenous sources. Although not as a biomarker of respiratory diseases, eCO has been clinically used as an index of smoking status and CO poisoning.

The clinical studies and sensitivity analysis of TMAD parameters in Papers III-V showed that a small change in model parameters can distinguishably affect the shape of eCO profile and be resolvd by the curve fitting methodology. The shape
of eCO₂ profile has been previously used to determine lung diffusion properties [117]. Since CO is diffusion-limited, the change in the profile shape might be more pronounced for eCO. The extended BGA methodology also provides more precise end-tidal and alveolar CO concentrations that is useful in those clinical applications where the end-tidal CO is used, e.g., to estimate COHb or red blood cell lifespan [118].

Moreover, the extended BGA of CO may enable to distinguish abnormalities in lung diffusion properties, and to identify CO sources and gas exchange location (Paper IV).
The aim of this thesis has been to introduce a novel method for the analysis of real-time breath data and to offer a tool to extract the information carried by single-exhalation profiles. To achieve this aim, three sub-projects were realized.

First, carbon monoxide as a biomarker of oxidative stress and respiratory diseases was chosen as the target molecule, and a sensitive mid-infrared TDLAS sensor was developed for real-time detection of CO in the breath and the ambient air. In parallel, an advanced breath sampler was constructed for online sampling. The experimental setup enabled the measurement of eCO expirograms with high precision and time-resolution. The measured eCO expirograms exhibited a clear dependence on exhalation flow rate and breath-holding time.

Second, a pulmonary gas exchange model was adapted to characterize the CO gas exchange dynamics in the respiratory tract during systemic CO elimination by four model parameters, maximum CO fluxes to the gas phase and diffusing capacities of the airways and the alveoli. The model was based on morphological approach that accounted for the bifurcating structure of the lung and could properly simulate eCO profiles.

Finally, a procedure was developed to least-squares fit the model-simulated single-exhalation profiles to real-time breath data and extract model parameters. Extended eCO analysis methodology was applied in two clinical studies to establish the baseline of eCO parameters for healthy non-smokers and to evaluate the response of model parameters to CO and wood smoke exposure.

Real-time eCO analysis coupled to mathematical gas exchange modeling provided means to retrieve accurate alveolar CO levels, the location of gas exchange and changes in lung diffusion properties. A small airway contribution might be resolved in the presence of high alveolar CO. Alveolar diffusing capacity was found to be decoupled from a variation in the maximum alveolar flux in both studies, which is a promising factor for potential clinical applications.

Conventional end-tidal CO measurement provides only one parameter, whereas the extended analysis provides at five independent parameters ($J_{ACO}$, $D_{ACO}$, $C_{tiss}$, $C_{ACO}$ and $C_{ETCO}$). In addition, not too specific and complicated maneuvers are
required (such as inhaling high concentration CO in $D_{LCO}$ clinical test). Extended eCO analysis based on real-time measurements and mathematical modeling has the potential to improve the diagnostic value and the understanding of CO physiology.

The extended BGA technique presented here can be refined by further improvement of the laser-based detection technique and the gas exchange model. Since both $1f$ and $2f$-WMS signals include the incident intensity (power) of the laser $I_0 (P_0)$, $2f$-WMS signal can be normalized by $1f$-WMS to discard the dependency on variations in laser intensity. This technique has been used before in harsh environments, specially in a calibration-free manner with a compact configuration [119–121]. Similar approach can be used in BGA to make the setup compact and obtain a more robust detection scheme that does not need calibration. The TMAD model can also be improved by considering more anatomical details of the respiratory tract and implementation of more comprehensive gas mixing mechanisms. Using the actual inhalation and exhalation flow rates measured by the breath sampler, instead of a fixed average value, can enhance the agreement between the model fit and the experimental data in phases I and II of the exhalation profiles. Mathematical formalisms for fitting the model-simulated profiles to the real-time data can always be improved by finding new approaches in defining the different phases of exhalation profiles based on deadspace volume and the total lung volume for each individual.

Moreover, more studies on diseased cohorts (COPD and asthma) can help to further understand the response of model parameters to actual disease conditions, and probably to define clear conditions under which model parameters can distinguish between healthy and diseased population and between different phases of a disease. In addition, future studies can be supported by additional medical tests such as blood hemoglobin monitoring and COHb measurements. This methodology can be applied to other biomarkers as well. Perhaps, simultaneous detection and extended analysis of other relevant biomarkers in breath would provide useful information.
Summary of the papers

Paper I

Real-time breath gas analysis of CO and CO₂ using an EC-QCL
R. Ghorbani, and F. M. Schmidt

In this work, an EC-QCL-based TDLAS sensor was developed for precise, breath-cycle resolved, simultaneous detection of exhaled CO (eCO) and carbon dioxide (eCO₂). A low-volume multi-pass cell and wavelength modulation spectroscopy were employed to achieve a noise-equivalent (1σ) sensitivity of $8.5 \times 10^{-8}$ cm$^{-1}$ H$^{-1/2}$ and (2σ) detection limits of 9 ± 2 ppb and 650 ± 7 ppm at 0.14 s spectrum acquisition time for CO and CO₂, respectively. The measured eCO expirograms by the sensor exhibited clear dependence on exhalation flow rate and breath-holding time.

I contributed to the construction and optimization of the experimental setup and implementation of data acquisition and breath sampling system. Analysed the data and prepared the first manuscript draft.

Paper II

ICL-based TDLAS sensor for real-time breath gas analysis of carbon monoxide isotopes
R. Ghorbani, and F. M. Schmidt

In this paper, a compact mid-infrared TDLAS sensor for carbon monoxide in air and breath was developed based on an interband cascade laser operating at 4.69 µm. Wavelength modulation spectroscopy and a low-volume multipass cell were employed to achieve a detection limit and precision in the low ppbv range. The
sensor enabled real-time detection of $^{12}$CO and $^{13}$CO exhalation profiles from non-smokers and an occasional smoker before and after smoking. Depletion of $^{13}$CO with respect to natural abundance was seen in breath samples.

I contributed to the construction and optimization of the experimental setup and implementation of data acquisition and breath sampling system. Analysed the data and prepared the first manuscript draft.

**Paper III**

**Modeling pulmonary gas exchange and single-exhalation profiles of carbon monoxide**

R. Ghorbani, A. Blomberg, and F. M. Schmidt


In this paper, a trumpet model with axial diffusion (TMAD) was used to simulate the CO gas exchange dynamics in the respiratory tract and corresponding eCO concentrations for the first time. The governing equation was numerically solved using the method of lines. The distribution of CO in the respiratory tract during inhalation, breath-holding, and exhalation was provided by the model at 1 mm spatial and 0.01 s temporal resolution. A very good agreement was obtained in exhalation phases I and III for different breathing maneuvers, yielding a unique set of TMAD parameters. The results confirm the recently observed EFR dependence of CO expirograms. An advanced breath sampling system for online sampling was constructed, which could provide the respiratory data in real-time.

I implemented the mathematical model, carried out the model simulations, performed the experiments and evaluated the raw data. I also contributed to results analysis and interpretation and to manuscript preparation.

**Paper IV**

**Fitting of single-exhalation profiles using a pulmonary gas exchange model – application to carbon monoxide**

R. Ghorbani, and F. M. Schmidt
Submitted to J. Breath Res.

In this paper, the TMAD model presented in the previous paper was used to implement a nonlinear least-squares fitting routine. Model-simulated eCO expirograms were least-squares fitted to real-time breath data from 2 healthy non-smokers before and after exposure to 10 ppm CO gas. A good agreement between was achieved and intra-individual variation in alveolar TMAD parameters was less than 6%. A simulation analysis showed the ability of the fit to resolve a change in alveolar diffusing capacity and a small contribution from airway.

I implemented the least-squares fitting routine, performed the experiments and evaluated the raw data. I also contributed to results analysis and interpretation and prepared the first manuscript draft.

Paper V

Extended breath CO analysis – baseline and diurnal variation of pulmonary gas exchange parameters
R. Ghorbani, A. Blomberg, and F. M. Schmidt
Submitted to J. Breath Res.

In this work, extended breath carbon monoxide (eCO) analysis methodology was employed to establish the healthy non-smoker baseline of the eCO parameters for mouth and nose exhalation. The measured end-tidal eCO was in the expected range of 1-3 ppm. There was no difference between moth and nose exhalation. The eCO parameters remained in a limited range at each exhalation flow rate.

In this work, I performed the experiments, analyzed the raw data and prepared the first draft of manuscript.
During my PhD studies, many people helped and supported me without whom this thesis would have not been possible.

First and foremost, I would like to express my sincere gratitude to my supervisor Florian Schmidt for introducing me to the interesting and exciting field of breath gas analysis, for his continuous support and patience. I would also like to warmly thank my assistant supervisors and reference person Anders Blomberg, Christoffer Boman, Staffan Schedin and Britt Andersson for their kind advices and critical comments.

I would also like to extend my sincere regards to all the members of staff at the Department of Applied Physics and Electronics and also Department of Physics for their timely support. In particular, I would like to thank TEC-Lab members and to express my gratefulness and reverence to my fellow student and specially my office-mates from past and present who have been great sources of encouragement and made my time in office enjoyable and memorable.

I also sincerely express my feelings of obligation to my friends in Umeå from past and present who have always been supportive. The list of your name would be too long to be put here, but you know who I mean.

Last but not least, my special thanks go to my family who always valued education above all else, for all their love, unconditional supports and continual efforts to make a calm and enjoyable space-time for me to work efficiently during my whole life.
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