Journal of Biomedical Optics

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Abstract. Fractional exhaled nitric oxide (F_ENO) is considered an indicator in the diagnostics and management of asthma. In this study we present a laser-based sensor for measuring F_ENO. It consists of a quantum cascade laser (QCL) combined with a multi-pass cell and wavelength modulation spectroscopy for the detection of NO at the sub-part-per-billion by volume (ppbv, $1:10^{-9}$) level. The characteristics and diagnostic performance of the sensor were assessed. A detection limit of 0.5 ppbv was demonstrated with a relatively simple design. The QCL-based sensor was compared with two market sensors, a chemiluminescent analyzer (NOA 280, Sievers) and a portable hand-held electrochemical analyzer (MINO®, Aerocrine AB, Sweden). F_ENO from 20 children diagnosed with asthma and treated with inhaled corticosteroids were measured. Data were found to be clinically acceptable within 1.1 ppbv between the QCL-based sensor and chemiluminescent sensor and within 1.7 ppbv when compared to the electrochemical sensor. The QCL-based sensor was tested on healthy subjects at various expiratory flow rates for both online and offline sampling procedures. The extended NO parameters, i.e. the alveolar region, airway wall, diffusing capacity, and flux were calculated and showed a good agreement with the previously reported values. [DOI: 10.1117/1.JBO.17.1.017003]

Keywords: fractional exhaled nitric oxide; breath analysis; quantum cascade laser; wavelength modulation spectroscopy; chemiluminescence; electrochemical; asthma.

Paper 11457 received Aug. 24, 2011; revised manuscript received Nov. 11, 2011; accepted for publication Nov. 14, 2011; published online Feb. 1, 2012.

1 Introduction

A method for non-invasive monitoring of inflammation from the respiratory system represents a challenge and so far, several methods have been used, i.e. exhaled breath condensate,[1] electronic nose,[2,3] and nitric oxide detection.[4,5] The discovery of nitric oxide (NO) in the exhaled breath[6] and its increased value in asthmatics[7] paved the way for technical developments. Measurement of F_ENO (Fractional Exhaled NO) is known to be flow dependent.[8,9] The American Thoracic Society/European Respiratory Society (ATS/ERS) statement recommends measuring F_ENO at a constant flow rate of 50 ml/s ($F_ENO_{0.05}$), reflecting a trade-off between sensitivity and patient comfort.[10] However, measurement of F_ENO at different flow rates, so-called extended NO analysis (or known as flow-independent parameters), can be used to calculate NO parameters providing NO production from different compartments of the lung.[11] The NO parameters are the NO from the alveolar region (C_ANO) and NO flux from the airways ($J'_{aw}NO$), derived from a linear model,[12] and the airway wall content of NO ($C_{aw}NO$) and diffusing capacity of NO over the airway wall ($D_{aw}NO$) that can be calculated from a non-linear model.[13] These NO parameters may give new insight into respiratory diseases.

For medical applications, there is a great requirement for a sensitive, accurate, compact, convenient and inexpensive sensor. Several instruments are now available making use of either chemiluminescence, electrochemical, or laser-based technologies.[14–18] Care has to be given to validate and calibrate each device to avoid conflicting reports between studies. Chemiluminescent detection of F_ENO is considered by many researchers in the field as the "gold standard." It provides accuracy and precision but is bulky and expensive, has high on-going running costs, and also requires technical expertise in calibration, limiting their use in routine patient care. Electrochemical sensors are convenient for development of portable hand-held analysers. However, the reproducibility of F_ENO measurements for absolute values is still subject to conflicting reports[19–21] and can have consequences of relying on a single estimate of the level of F_ENO in driving clinical management. The advances of quantum cascade laser (QCL) technologies have opened up new opportunities for novel mid-infrared (mid-IR) gas sensors. The QCL-based sensors are well suited for mid-infrared spectroscopic trace gas sensing due to their narrow linewidth, high power at room temperature, and continuous wave (CW) operation at mid-IR wavelengths (3 to 24 μm).[22,23] Several approaches for the optical sensing of NO have been reported. Sensors based on absorption spectroscopy using a multi-pass cell[24–26] or a high finesse cavity for cavity enhanced[15,27,28] or cavity ring-down,[29,30] photoacoustic spectroscopy,[31] and Faraday modulation spectroscopy[32,33] have been successfully

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implemented to reach a detection sensitivity in the order of single part per billion by volume (ppbv, $1:10^{-9}$) and below.

In this paper, we report on the development and performance evaluation of a laser-based NO sensor, utilizing a CW, thermoelectrically cooled QCL as a light source. The sensor was validated for F_ENO measurements; linearity, selectivity, precision, accuracy, and detection limit were determined. The QCL-based sensor was tested on healthy adults and asthmatic children. Performance comparisons were done with two other techniques, namely chemiluminescence and electrochemical sensor.

2 Experimental

2.1 *Gas Sensors*

The QCL-based NO sensor uses wavelength modulation spectroscopy technique (WMS) and consists of a continuous-wave distributed feedback quantum cascade laser (CW-DFB QCL) operating in the wavelength region of 5.2 μm (1891*to* 1908 cm^{-1}), a multi-pass cell, and a room temperature detector.[24] The laser beam is sent through an astigmatic Herriott absorption cell of 400 ml (AMAC-76, Aerodyne Research, USA), offering a total optical path length of 76 m and which consists of two concave mirrors where light is reflected multiple times, enhancing the effective path length through the breath sample. The absorbed amount of light at the output of the cell is proportional to the NO concentration present in the cell. The detection is performed by a photovoltaic detector (PV-6, Vigo Systems) working at room-temperature, thus eliminating the need for a highly sensitive liquid nitrogen-cooled IR detector, simplifying daily use of the system and allowing long-term automated operation. In order to improve sensitivity toward the NO target limit of ≤1 pbbv required for breath analysis, WMS is implemented by modulating the injection current of the laser at a frequency of 100 kHz. The absorption signal is transposed into a frequency domain where noise sources are weaker, and absorption spectra is obtained by demodulating the detected signal at the second harmonic (2f) using a lock-in amplifier (model SR844, Stanford Research Systems) with the time constant set to 100 μs. This approach allows a sensitivity of 0.5 ppbv within 1 s averaging. To reach a fast response of the system, a pressure of 70 cmH_2O is maintained in the multi-pass cell and a sample flow rate of 900 ml/ min is generated. Therefore, the cell is refreshed in less than 2 s, making the sensor suitable for online monitoring of exhaled breath. A schematic arrangement of the experimental setup is shown in Fig. 1. The QCL-based sensor was calibrated with a reference mixture of 100 ± 3 ppbv of NO in N_2 (prepared by VSL-National Dutch Metrology Institute) before analysis of a breath sample. A N_2 gas bottle was used as a NO-free gas reference.

In this study, the performances of the QCL-sensor were investigated. To do that, 11 gas mixtures were prepared from our reference gas mixture (100 ppbv NO in N_2) diluted in pure N_2 to cover the range 10 to 100 ppbv, and 14 gas mixtures were made from a 20 ppm ± 2% bottle of NO for concentrations up to 4 ppm. The different concentrations of NO were produced by using two mass flow controllers (Brooks Instrument, max flow: 25 l/h and 5 l/h with an accuracy of ±1%).

Contrary to the QCL sensor which directly measures the NO concentration, the chemiluminescence system is based on the reaction between NO and O_3, which generates NO_2 in the exited form.[34] When NO_2 returns to a stable state, light is emitted. The amount of light, which is proportional to the amount of NO, is measured by a photomultiplier. Calibration of the Sievers NOA 280 was performed each day prior to use with the same mixture of NO as with the QCL-based sensor, i.e. with N_2 gas bottle and 100 ± 3 ppbv of NO in N_2. The sample flow into the Sievers is 200 ml/ min. The chemiluminescence analyzer is connected to a computer with the NO analysis software, which provides a graphical display of F_ENO concentration during the analysis process.

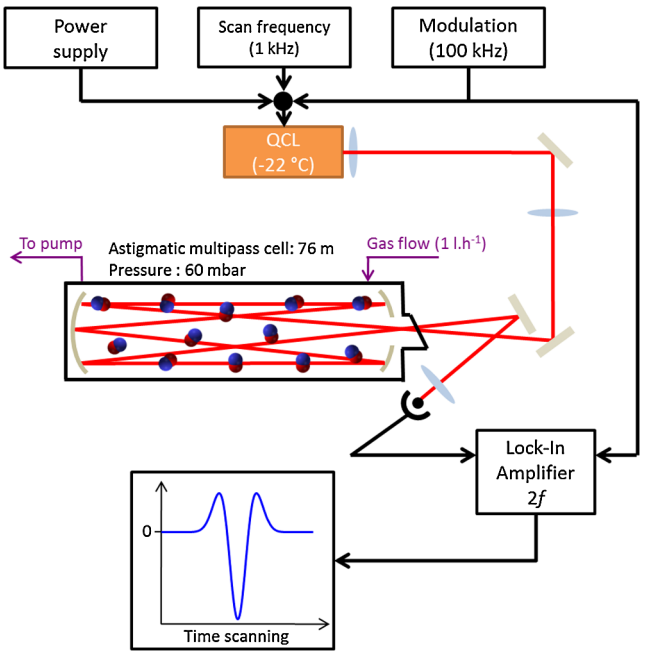

Fig. 1 Schematic of the QCL-sensor. The QCL beam at 5.2 μm is sent into a 30 -cm multi-pass cell, thus enhancing the absorption path length to 76 m. Wavelength modulation is performed by modulating the laser current at 100 kHz, and a 2f lock-in amplifier is used to demodulate the signal. (Color online only.)

Despite the chemiluminescence NO sensor being the gold standard reference for NO measurements in breath, its use for routine clinical practice is limited by its size and expense. Portable hand-held, relatively inexpensive NO analysers based on electrochemical analysis have been introduced on the market a few years ago. The NIOX MINO (Aerocrine AB, Sweden) Asthma Inflammation Monitor is one of the available sensors. This sensor is ideally suited for use in primary care, where the majority of asthma patients are managed. In this study, the accuracy of NIOX MINO measurements was assessed. The manufacturer stateed an accuracy of ±5 ppbv of measured value below 50 ppbv and ±10% at or above 50 ppbv.

2.2 *BreathCollection*

The QCL-based sensor was used for F_ENO monitoring with both online and offline breath-collection.

To perform online measurements, a commercially available breath sampler (Loccioni, Italy) was used to monitor the pressure of the exhaled breath within an acceptable range and to measure the breath CO_2 concentration level. The breath sampler meets the American Thoracic Guidelines (ATS) for collecting breath by providing a back pressure of 10 cmH_2O, to ensure soft palate closure and prevent nasal contamination, and allowing the patient to maintain constant exhalation flow.

The exhalation flow ranged from 15 to 250 ml/s. The target flow rate was maintained within 5%.

During the sampling, the collected breath entered the Loccioni breath sampler. The CO2 concentration profile and airway pressure were simultaneously displayed in graphical forms on the sampler display. When the CO_2 concentration reached 3%, a part of the breath was sent to the NO sensor. The 3% value has been chosen since it is the start of the CO_2 plateau for most of people. The F_ENO level in ppbv was acquired and plotted in real-time on a computer screen. In addition, the breath sampling pipe was heated to ≥38 °C to prevent water condensation. A typical recorded signal at flow 50 ml/s is shown in Fig. 2. F_ENO level was sampled after the estimated start of the CO_2 plateau region. The end-tidal of the CO_2 exhalation trend determines the point where the NO plateau is measured, i.e. 5 s before the end-tidal CO_2 point. One breath sample was collected at each flow rate (15, 50, 100 and 250 ml/s) from each patient.

Offline collections were also performed as samples can be collected at a distant collection site from the NO sensor. A custom-built breath-collection device was used to collect single breaths into bags with the subject exhaling at specific constant flow rates.[35] The breath-collection device was based on the guidelines of the ATS for the sampling of exhaled NO; it is very simple, inexpensive, and has been tested in previous studies. The exhaled breath line consists of a mouth-piece connected to a discard bag (400 ml) and an NO-impermeable aluminum-foil air bag of 500 ml capacity (Mylar balloon, ABC ballonnen, Zeist, The Netherlands).[36] To maintain a constant exhalation flow, the mouth pressure was monitored by the patient during the sampling process. Breath was collected at various constant flow rates from each subject by changing the resistance of the breath line to maintain a mouth pressure of 10 cmH_2O.

To assess the agreement of the two sampling procedures, offline F_ENO measurements in comparison to online measurements, 23 healthy individuals performed online and offline single breath maneuvers at a flow of 50 ml/s. To prevent systematic errors, 11 persons did first online followed by offline breath maneuvers, and 12 in the reverse order.

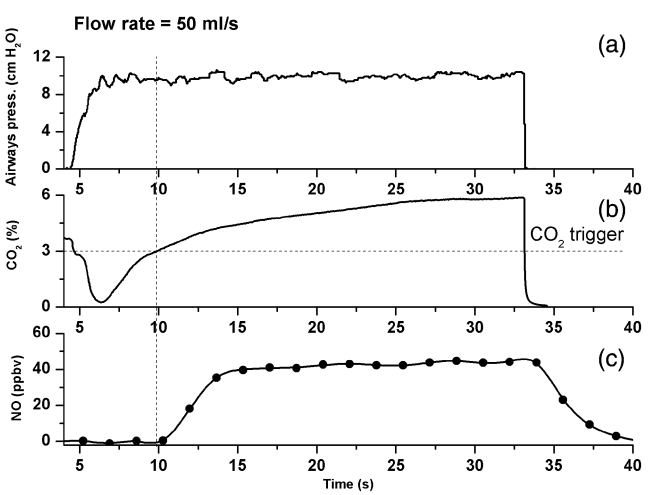

Fig. 2 Recorded data during online sampling of a single exhalation at 50 ml/s. During the exhalation process the mouth pressure is monitored to keep a constant flow rate (a). The exhaled breath is sent to the QCL-based sensor when the CO_2 concentration reaches 3% (b). The average of the last 5 seconds of the exhaled breath was used to calculate the F_ENO value (c).

2.3 *Extended NO Analysis*

Different exhalation flow rates can be performed with both online and offline exhaled breath measurements by changing the resistance of the sampling line and keeping a back pressure of 10 cmH_2O during the exhalation. Measuring the NO plateau for multiple flow rates allows an estimation of the NO extended parameters, which may provide additional useful clinical information. The simple two-compartment model[12] describes exhaled NO arising from two compartments: the airways and the alveolar region. It is based on three flow-independent exchange parameters, one describing the steady-state NO alveolar concentration (C_ANO, ppbv), and two describing the airway region (the airway NO diffusing capacity ($D_{aw}NO$, $pl \cdot s^{-1} \cdot ppbv^{-1}$) and the airway wall NO concentration ($C_{aw}NO$, ppbv).[11] The potential of these parameters lies in their ability to split exhaled NO into two important anatomic subdivisions of the lungs and also to provide both structural and metabolic information relevant to the NO pathways. To determine the airway and alveolar contribution to exhaled NO, multiple exhalation flow rates must be accomplished. Different approaches are described in the literature[11] and recently used in combination with a QCL-based sensor to determine extended NO parameters in chronic obstructive pulmonary disease.[37] We selected the method described by Högman et al.[13,38] as it requires only three exhalation flows. This method is based on a non-linear model with a quality control to notify erroneous NO values. NO values from different flow rates are included into a software program with a second order algorithm, which will render values of C_ANO, $C_{aw}NO$ and $D_{aw}NO$.

We measured F_ENO at different flow rates controlled by the Loccioni sampler: a low exhalation flow (15 ml/s), a medium flow (100 ml/s), and a high flow rate (250 ml/s). To validate the sampling system, the exhalation flow of 50 ml/s is also measured ($F_ENO_{0.05}$) and compared to calculated value by the model. A group of 20 subjects performed online single breath maneuvers at four expiratory flows as mentioned previously. After each maneuver, the subject was given a 1-minute pause before performing the next exhalation at a different flow.

3 Results

3.1 *QCL-Based Sensor Performance*

Prior to the study on patients, the performance of our QCL-sensor was evaluated. With the prepared NO concentrations, the QCL-sensor was tested in terms of linearity, precision, accuracy, sensitivity, and selectivity. To minimize random error, the

Table 1 Main characteristics of the QCL-sensor.

Sensitivity	<1 ppbv in 1 s
Repeatability (Precision)	±5%
Accuracy	99%–104%
Dynamic Range	1 ppbv–4 ppmv
Response Time	Lag Time: 2 s

concentrations were measured six times. One measurement consists of flushing the absorption cell for 5 min with the gas and by taking the last value displayed by our sensor. Table 1 summarizes the main characteristics of the sensor. The linearity response of the system, Fig. 3, is given by the coefficient of determination ($R^2 = 0.998$). Precision of the sensor was evaluated in conditions of their repeatability, and accuracy was determined by comparing the mixtures and the measured concentration. The calculated accuracy was between 99 and 104% and is limited by the accuracy of the mass flow controllers used for the dilution. The precision of the system expressed as RSD (relative standard deviation) was below 5%.

The best minimum detectable NO concentration achieved with QCL-based sensor with a 1 s averaging time is 0.5 ppbv. A better sensitivity can be reached by increasing the integration time. An Allan variance analysis of the data, which presents the variance of the data as a function of integration time of the sampling, is shown in Fig. 4. It displays the reduction in noise level as the integration time is increased.

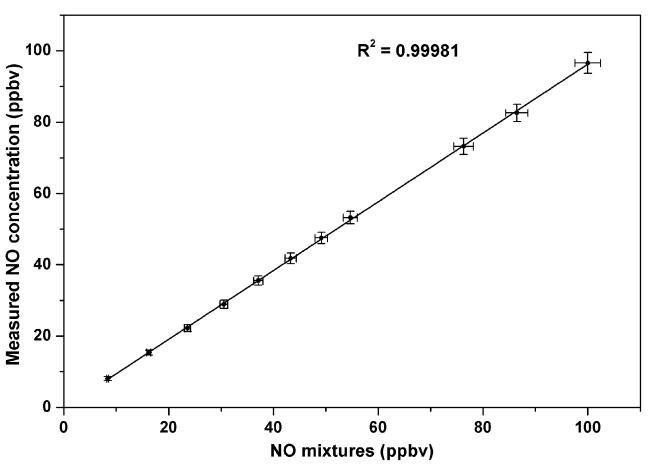

Fig. 3 Linearity of the QCL sensor. Measured NO concentrations versus prepared dilutions from a standard calibration mixture of 100 ± 3 ppbv of NO in N_2 are plotted ($R^2 = 0.9998$).

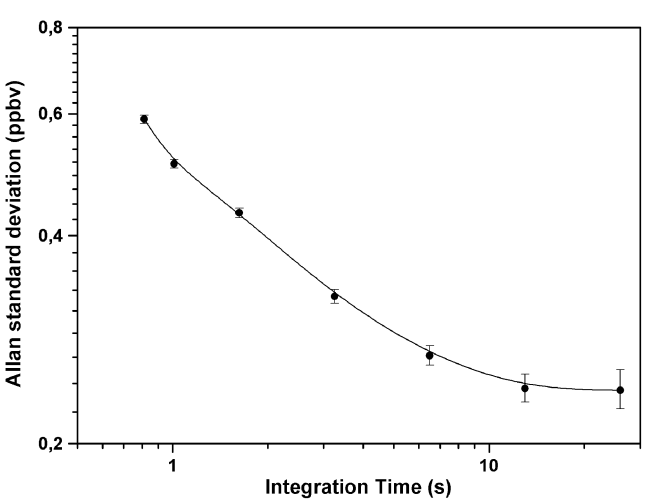

Fig. 4 Allan variance showing the QCL-based sensor detection limit. The sensitivity is 0.5 ppbv of NO with an acquisition time of 1 second. A minimum detection limit of 0.24 ppbv is reached with 26 s integration time.

As exhaled breath carries a number of compounds at high concentration such as water and CO_2, the selectivity of the sensor is important. The emission spectrum of the QCL allows accessing of the NO absorption line within the fundamental vibration band without interference of water and CO_2, as shown in the HITRAN simulation.[39] The response of the QCL sensor to humid air and 5% CO_2 has been experimentally investigated and has provided good agreement with the HITRAN calculations.

3.2 *Offline/Online Comparison*

The QCL-based sensor can perform F_ENO measurements online or offline. If the advantages of online analysis are obvious, the benefits of offline analysis are apparent when analyzing large sample numbers, for example during clinical studies. To examine the diagnostic capability of our sampling procedure, online and offline measurements in the same subjects are compared, using the Bland-Altman method.[40] The Bland-Altman plot, Fig. 5, consists of the difference between a pair of measurements (in this case offline–online) versus their average. On average, the difference in NO concentration between the two sampling methods (offline-online) was −0.1 ppbv and the limit of agreements was ±1.0 ppbv. By measuring the NO concentration in bags within 24 h of collection, no significant differences or combined effects of measurement technique or flow rate were noted. The sample is reportedly stable for up to 24 h, but it was observed that NO concentrations in bags decreased over time up to 7% after 48 h (data not shown).

3.3 *Validation of QCL-Based Sensor for Multiple Flows Analysis*

For each individual, the three independent parameters are calculated. Figure 6 shows the data as medians with lower and upper quartiles (25 to 75%). The level of agreement between the measured and calculated $F_ENO_{0.05}$ is determined by the coefficient of determination R^2 (average between calculated and measured: −0.4 ppbv, SD = 0.72, $R^2 = 0.9997$). Data

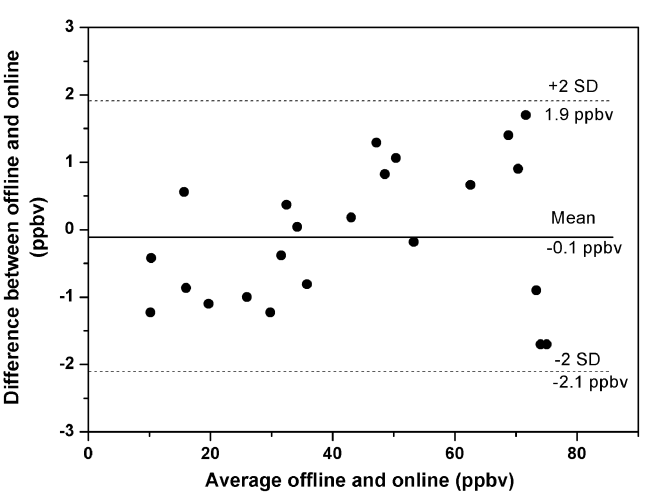

Fig. 5 Bland-Altman plot comparing 23 offline and online sampling procedures. Solid line represents the mean difference between values obtained using the two sampling methods (−0.1 ppbv). Dashed lines represent the limits of agreement −2.1 ppbv and 1.9 ppbv; SD is standard deviation. The plot indicates the sampling procedures are in good agreement.

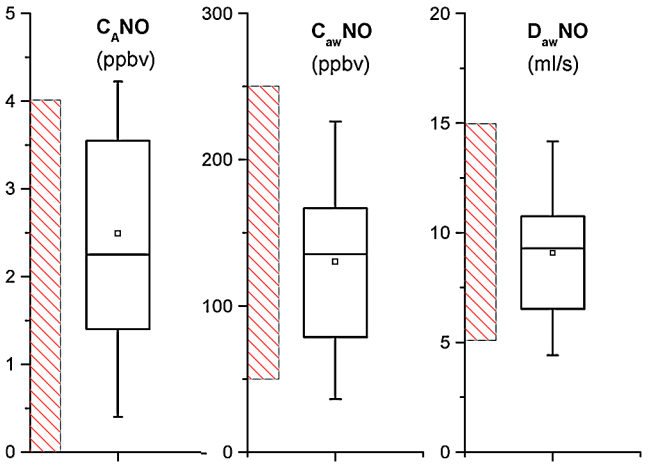

Fig. 6 Calculated NO extended parameters characterizing the alveolar region (C_ANO), airway wall ($C_{aw}NO$), diffusing capacity ($D_{aw}NO$), and flux ($J_{aw}NO$) NO exchanges using the model proposed by. Ref. 13 Minimum, median, maximum and, interquartile ranges (25%, 75%) are displayed. The data from the QCL-based sensor are included in the ranges suggested by Högmann and et al.[41] for healthy subjects (hatched boxes). (Color online only.)

are also compared to previous results, and these results are in agreement with values presented by Högman et al.[41] Regardless of the lack of agreement about what flow rates to use to calculate extended NO parameters, those results demonstrate the validity of our sampling procedure and confirm the efficiency of the theoretical model to give values which are consistent.

3.4 *Comparison of the QCL-Based Sensor with Chemiluminescence and Electrochemical Sensors*

F_ENO from 22 asthmatic children (age range: 6 to 16 years) was measured with three sensors. They provided offline samples in aluminum bags for the QCL-based sensor and the chemiluminescence, respectively, at flow 50 ml/s, followed by online F_ENO sample measured by the NIOX MINO two minutes later. Ambient NO varied between 1 and 4 ppbv over the study. The bags were measured simultaneously with the QCL-based and the chemiluminescent sensors on the same day as the sampling. Measurements were achieved with a 1 s integration time for both devices. The Bland-Altman plot, Fig. 7(a), shows a high degree of agreement between the QCL-based sensor and the chemiluminescence device with an average difference of −0.1 ppbv and a standard deviation of 1.1 ppbv.

The comparison between the QCL-based sensor and the NIOX MINO, Fig. 7(b), shows an average value of 1.6 ppbv with a SD of 1.7 ppbv.

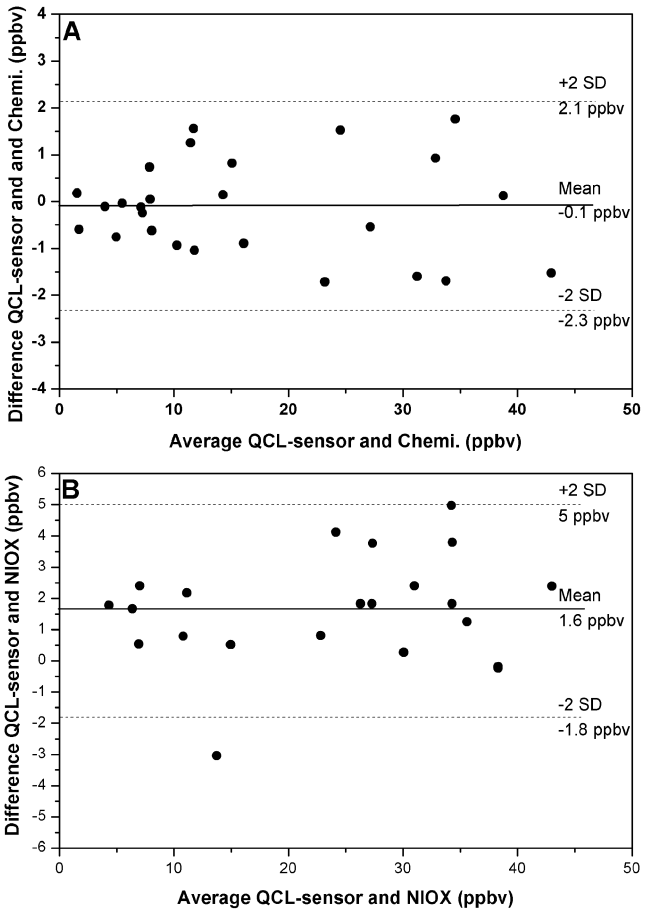

Fig. 7 Bland-Altman plot comparing the QCL-based sensor with chemiluminescence device (a) and with NIOX MINO (b), respectively. (a) Solid line represents the mean difference between values obtained using the QCL-based sensor and chemiluminescence (−0.1 ppbv). Dashed lines represent the limits of agreement, −2.3 ppbv and 2.1 ppbv. The plot suggests the two sensors are in good agreement. (b) Solid line represents the mean difference between values obtained using the QCL-based sensor and NIOX MINO (1.6 ppbv). Dashed lines represent the limits of agreement, −1.8 ppbv and 5 ppbv; SD is standard deviation. The plot suggests the QCL-sensor and NIOX MINO are quite similar and reveals one outlier.

4 Discussion

This study reports a QCL sensor, combining a multi-pass cell and wavelength modulation spectroscopy, suitable for F_ENO measurements. The achieved sensitivity of 0.5 ppbv within 1 s integration time, appropriate for F_ENO measurements, is comparable with other on-going optical sensor developments and does not represent the ultimate sensitivity. The fast response time achieved (<2 s), despite the use of a multi-pass cell, allows online analysis by providing enough time resolution to follow NO variations during a single breath, as well as offline measurements. Although presently used in a setup configuration, the QCL sensor has a real potential to be developed and integrated into a compact and convenient device configured for autonomous operation in clinical applications.

For the present work, the obtained results are directly comparable with those obtained by the chemiluminescent NIOX sensor, the device currently considered as the gold standard for measuring F_ENO. A Bland-Altman plot demonstrated good agreement between both devices for a wide range of NO concentrations, making the system trustable on any asthmatic patient regardless of the degree of airway inflammation. When coupled with a commercial or home-made sampler, single or multiple flow rates can be easily performed. The only requirement is to be able to monitor CO_2, the flow rate, and mouth pressure during the exhalation. The system offers a wide number of configurations fitting to each study, including extended NO analysis. The possibility of measuring multiple flow rates was demonstrated in healthy subjects by successfully comparing calculated extended NO parameters (from $F_ENO_{0.015}$, $F_ENO_{0.10}$, $F_ENO_{0.25}$) with previous papers.[41] In addition, the calculated $F_ENO_{0.05}$ from the model is in agreement with the measured $F_ENO_{0.05}$. By adapting the sampler device, any flow

rate can be used with the QCL sensor. A recent study also demonstrated the efficiency of a QCL-based sensor for multiple flow rates analysis in diseases other than asthma, as in chronic obstructive pulmonary disease.[37]

We also found a statistically significant difference (p-value = 0.0003) between F_ENO values measured by the QCL-sensor and the NIOX MINO. Ekroos et al.[42] reported on the exhaled NO from a selected group of healthy non-smoker adults and assessed their long-term variation. The variation of NO concentrations (95% confidence interval) was 1.1 ± 1 ppb within the interval of seven days. This is in agreement with the difference in the methods we report here. When patients start treatment this effect is much larger. Silkoff et al.[43] showed that the reduction in exhaled NO was around 40%. Therefore, we conclude that the difference between the two methods is not medically relevant for the measurement of $F_ENO_{0.05}$.

The validity of the NIOX MINO has been subject of conflicting papers. Some studies reported strong correlations and excellent reproducibility of the absolute value of F_ENO measurements obtained using the NIOX MINO.[44,45] They support the recommendation of performing only one measurement. Other studies did not support that conclusion.[20,46] Our data suggests that even by performing one F_ENO measurement, the level of agreement between the gold standard device, the QCL-sensor, and the NIOX MINO is certainly clinically acceptable. However, the 5 ppbv sensitivity prevents its use in determining the extended NO parameters.

In conclusion, this study shows that there is clinically acceptable agreement between the three main technologies used for the development of NO sensors. Whereas chemiluminescence analysers tend to be expensive, large and poorly portable, laser-based systems and electrochemical sensors demonstrate interesting opportunities to make F_ENO measurements in the primary care. Compared to the NIOX MINO and generally to electrochemical sensors, QCL-based systems, using highly selective optical spectroscopy principles, offer higher sensitivities and have already proven their advantages as a trustable and accurate technique over time. They are also suitable for multiple flow rates analysis as they can perform analysis at any flow without modification of the sensor. Compact devices based on laser spectroscopy are presently used in many fields of life sciences, and it is a matter of time before they will be used in any hospital for diagnosis, monitoring, and control of diseases such as asthma at single or multiple flow rates.

Acknowledgments

This work was supported by the GO-EFRO Ultragas gas analysis systems for quality control of agricultural products and medical diagnostics, project no. 2009-010034 of province of Gelderland (The Netherlands) and EU, EU-Marie Curie fellowships EU-people-2010-IEF, project no. 275584, QCLaser Nose and the EU-FP6-Infrastructures-5 program, project FP6-026183 'Life Science Trace Gas Facility.' The authors would like to thank the anonymous reviewers for their critical and constructive comments.

References

1. I. Horvath, J. Hunt, and P. J. Barnes and ATS-ERS-Task-Force-Exhaled-Breath, "Exhaled breath condensate: methodological recommendations and unresolved questions," *Eur. Respir. J.* **26**(3), 523–548 (2005).
2. A. P. F. Turner and N. Magan, "Electronic noses and disease diagnostics," *Nat. Rev. Microbiol.* **2**(2), 161–166 (2004).
3. S. H. Lee and T. H. Park, "Recent advances in the development of bioelectronic nose," *Biotechnol. Bioprocess Eng.* **15**(1), 22–29 (2010).
4. D. R. Taylor et al., "Exhaled nitric oxide measurements: Clinical application and interpretation", *Thorax* **61**(9), 817–827 (2006).
5. S. A. Kharitonov and P. J. Barnes, "Nitric oxide in exhaled air is a new marker of airway inflammation," *Monaldi Arch Chest Dis.* **51**(6), 533–537 (1996).
6. L. E. Gustafsson et al., "Endogenous nitric-oxide is present in the exhaled air of rabbits, guinea-pigs and humans," *Biochem. Biophys. Res. Commun.* **181**(2), 852–857 (1991).
7. K. Alving, E. Weitzberg, and J. M. Lundberg, "Increased amount of nitric-oxide in exhaled air of asthmatics," *Eur. Respir. J.* **6**(9), 1368–1370 (1993).
8. M. Hogman, S. Stromberg, U. Schedin, C. Frostell, G. Hedenstierna, and L. E. Gustafsson, "Nitric oxide from the human respiratory tract efficiently quantified by standardized single breath measurements," *Acta Physiol. Scand.* **159**(4), 345–346 (1997).
9. P. E. Silkoff et al., "Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide," *Am. J. Respir. Crit. Care. Med.* **155**(1), 260–267 (1997).
10. "ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005", *Am. J. Respir. Crit. Care. Med.* **171**(8), 912–930 (2005).
11. S. C. George et al., "Modeling pulmonary nitric oxide exchange," *J. Appl. Physiol.* **96**(3), 831–839 (2004).
12. N. M. Tsoukias and S. C. George, "A two-compartment model of pulmonary nitric oxide exchange dynamics," *J. Appl. Physiol.* **85**(2), 653–666 (1998).
13. M. Hogman et al., "Extended NO analysis applied to patients with COPD, allergic asthma and allergic rhinitis," *Respir. Med.* **96**(1), 24–30 (2002).
14. Z. Borrill et al., "A comparison of exhaled nitric oxide measurements performed using three different analysers," *Respir. Med.* **100**(8), 1392–1396 (2006).
15. M. R. McCurdy et al., "Performance of an exhaled nitric oxide and carbon dioxide sensor using quantum cascade laser-based integrated cavity output spectroscopy," *J. Biomed. Opt.* **12**(3), 034034 (2007).
16. K. Namjou et al., "Determination of exhaled nitric oxide distributions in a diverse sample population using tunable diode laser absorption spectroscopy," *Appl. Phys. B* **85**(2–3), 427–435 (2006).
17. J. H. Shorter et al., "Clinical study of multiple breath biomarkers of asthma and COPD (NO, CO(2), CO and N(2)O) by infrared laser spectroscopy," *J. Breath Res.* **5**(3), (2011).
18. T. H. Risby and F. K. Tittel, "Current status of midinfrared quantum and interband cascade lasers for clinical breath analysis," *Opt Eng* **49**(11), (2010).
19. K. Alving, C. Janson, and L. Nordvall, "Performance of a new handheld device for exhaled nitric oxide measurement in adults and children," *Respir. Res.* **7**(1), 67 (2006).
20. G. Roberts et al., "Are exhaled nitric oxide measurements using the portable NIOX MINO repeatable?," *Respir. Res.* **11**(1), 43 (2010).
21. C. R. Brooks, S. B. M. Brogan, van Dalen C. J., P. K. Lampshire, J. Crane, and J. Douwes, "Measurement of exhaled nitric oxide in a general population sample: A comparison of the medisoft HypAir FE(NO) and aerocrine NIOX analyzers," *J. Asthma* **48**(4), 324–328 (2011).
22. F. K. Tittel, D. Richter, and A. Fried, "Mid-infrared laser applications in spectroscopy," *Solid-State Mid-Infrared Laser Sources* **89**, 445–510 (2003).
23. R. F. Curl et al., "Quantum cascade lasers in chemical physics," *Chem. Phys. Lett.* **487**(1–3), 1–18 (2010).
24. S. M. Cristescu et al., "Laser-based systems for trace gas detection in life sciences," *Appl. Phys. B* **92**(3), 343–349 (2008).
25. L. Menzel et al., "Spectroscopic detection of biological NO with a quantum cascade laser," *Appl. Phys. B* **72**(7), 859–863 (2001).
26. J. B. McManus et al., "Pulsed quantum cascade laser instrument with compact design for rapid, high sensitivity measurements of trace gases in air," *Appl. Phys. B* **92**(3), 387–392 (2008).
27. D. S. Baer et al., "Sensitive absorption measurements in the near-infrared region using off-axis integrated-cavity-output spectroscopy," *Appl. Phys. B* **75**(2–3), 261–265 (2002).

28. M. L. Silva et al., "Integrated cavity output spectroscopy measurements of NO levels in breath with a pulsed room-temperature QCL," *Appl. Phys. B* **81**(5), 705–710 (2005).
29. B. A. Paldus et al., "Cavity ringdown spectroscopy using mid-infrared quantum-cascade lasers," *Opt. Lett.* **25**(9), 666–668 (2000).
30. A. A. Kosterev et al., "Cavity ringdown spectroscopic detection of nitric oxide with a continuous-wave quantum-cascade laser," *Appl. Opt.* **40**(30), 5522–5529 (2001).
31. V. Spagnolo et al., "NO trace gas sensor based on quartz-enhanced photoacoustic spectroscopy and external cavity quantum cascade laser," *Appl. Phys. B* **100**(1), 125–130 (2010).
32. H. Ganser, W. Urban, and A. M. Brown, "The sensitive detection of NO by Faraday modulation spectroscopy with a quantum cascade laser," *Mol. Phys.* **101**(4–5), 545–550 (2003).
33. R. Lewicki et al., "Ultrasensitive detection of nitric oxide at 5.33 microm by using external cavity quantum cascade laser-based Faraday rotation spectroscopy," *Proc. Natl. Acad. Sci. U. S. A.* **106**(31), 12587–12592 (2009).
34. A. Fontijn, A. J. Sabadell, and R. J. Ronco, "Homogeneous chemiluminescent measurement of nitric oxide with ozone—implications for continuous selective monitoring of gaseous air pollutants," *Anal. Chem.* **42**(6), 575–579 (1970).
35. M. M. L. Steeghs et al., "An off-line breath sampling and analysis method suitable for large screening studies," *Physiol. Meas.* **28**(5), 503–514 (2007).
36. P. H. Fischer et al., "Association between exhaled nitric oxide, ambient air pollution and respiratory health in school children," *Int. Arch. Occup. Environ. Health* **75**(5), 348–353 (2002).
37. M. R. McCurdy et al., "Exhaled nitric oxide parameters and functional capacity in chronic obstructive pulmonary disease," *J. Breath Res.* **5**(1), 016003 (2011).
38. M. Hogman and P. Merilainen, "Extended NO analysis in asthma," *J. Breath Res.* **1**(2), 024001 (2007).
39. L. S. Rothman et al., "The HITRAN 2008 molecular spectroscopic database," *J. Quant. Spectrosc. Radiat. Transfer* **110**(9–10), 533–572 (2009).
40. J. M. Bland and D. G. Altman, "Statistical-methods for assessing agreement between 2 methods of clinical measurement," *Lancet* **1**(8476), 307–310 (1986).
41. M. Hogman et al., "Extended No analysis in a healthy subgroup of a random sample from a Swedish population," *Clin. Physiol. Funct. Imaging* **29**(1), 18–23 (2009).
42. H. Ekroos, J. Tuominen, and A. R. A. Sovijarvi, "Exhaled nitric oxide and its long-term variation in healthy non-smoking subjects," *Clin. Physiol.* **20**(6), 434–439 (2000).
43. P. E. Silkoff et al., "Airway nitric oxide diffusion in asthma: role in pulmonary function and bronchial responsiveness," *Am. J. Respir. Crit. Care. Med.* **161**(4), 1218–1228 (2000).
44. B. Khalili, P. B. Boggs, and S. L. Bahna, "Reliability of a new hand-held device for the measurement of exhaled nitric oxide," *Allergy* **62**(10), 1171–1174 (2007).
45. B. J. Lipworth, D. Menzies, and A. Nair, "Portable exhaled nitric oxide measurement: comparison with the "gold standard" technique," *Chest* **131**(2), 410–414 (2007).
46. S. W. Turner, C. McGill, and G. Malik, "Validation of a hand-held exhaled nitric oxide analyzer for use in children," *Pediatr. Pulmonol.* **41**(11), 1053–1057 (2006).