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# A compact laser-based spectrometer for detection of $C_2H_2$ in exhaled breath and HCN in vitro

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**Abstract** We report on the development of a compact prototype near-infrared DBR laser-based spectrometer employing off-axis integrated cavity output spectroscopy. The spectrometer is capable of simultaneous detection of acetylene ($C_2H_2$) and $CO_2$ at 1,529.2 nm as well as hydrogen cyanide (HCN) at 1,533.5 nm. The detection limits of 8 ppbv for $C_2H_2$ and 80 ppbv for HCN are achieved for the acquisition time of 1 s. The setup has been tested for online measurements of $C_2H_2$ in exhaled breath of a smoking subject and HCN resulting from the metabolism of *Pseudomonas aeruginosa* bacteria in vitro. Further improvements of the performance of the spectrometer are discussed.

## 1 Introduction

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

Acetylene ($C_2H_2$) is one of the most important hydrocarbons used in industrial technological processes, and therefore, it originates predominantly from anthropogenic activity including automotive industry and biomass burning [17] and is usually used to estimate air quality [17, 18]. Several attempts have been made so far to perform sensitive detection of acetylene utilizing cavity-enhanced absorption techniques [19–21]. In some studies, pre-concentration of the sample was used granting low detection values down to 35 pptv which is sufficient for direct atmospheric detection at concentrations typical of both urban and rural environments [20]. However, this lead to an increase of the acquisition time up to 30 min. Recent study carried out by Schmidt et al. [22] reports on the development of the diode laser-based continuous-wave cavity ring-down spectroscopy (cw-CRDS) able to detect 0.34 ppbv in 70 s without sample pre-concentration.

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

Another interesting molecule that might serve as a potential indicator of physiological condition in humans is hydrogen cyanide (HCN). It results from the metabolism of *Pseudomonas aeruginosa* (PsA), one of the most common pathogens in cystic fibrosis (CF) patients [25]. Several

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studies report on the detection of HCN emitted by in vitro cultures of PsA using selected ion flow tube mass spectrometry (SIFT-MS) in exhaled breath [26–28]. However, only few publications report on detection of HCN by laser-based absorption techniques (i.e., cavity ring-down and photoacoustic spectroscopy) [23, 29–31].

In this work, we report on the development of a compact prototype near-infrared laser-based spectrometer for fast and sensitive multi-compound detection of gases in exhaled breath and in vitro including acetylene, hydrogen cyanide and carbon dioxide. The spectrometer employs integrated cavity output spectroscopy [32, 33] in off-axis configuration (OA-ICOS) as detection method. Possible further improvements of the current performance of the spectrometer are discussed.

## 2 Experimental details

### 2.1 Spectrometer design

A schematic picture, representing a laser-based sensor, is shown in Fig. 1a. A near-infrared distributed Bragg reflector (DBR) laser was provided by VTEC Lasers and Sensors Limited. The laser is fiber coupled and is based on the OclaroLambdaFLEX™ iTLA. The laser is a high-performance continuous-wave (CW) tunable laser source operating in the C-band window covering 6,394–6,548 $cm^{-1}$ (1,527–1,564 nm) wavelength region split into 89 integrated channels. The laser is provided in a 26-pin butterfly package and connected with a polarization maintaining fiber (PMF) to the absorption cell. The temperature of the laser is maintained at 25 °C and controlled by an integrated Peltier module. In this configuration, the laser generates an average output power of 20 mW (13 dBm). To scan the acetylene transition, the laser is set to 6,539.46 $cm^{-1}$ (1,529.2 nm), and for HCN transition—to 6,521.75 $cm^{-1}$ (1,533.3 nm). The frequency of the laser output is fine-tuned by modulating the laser current with a 10-kHz triangular signal. The fine-tuning range is about 0.19 $cm^{-1}$. The beam is sent from the laser to a high-finesse optical cavity ($F = 1{,}560$), consisting of two 1-inch concave mirrors (1 m radius of curvature, $R = 99.8$ % at 1,550 nm, Layertec, Germany).

A custom-made in-coupling system toward the optical cavity has been developed (Fig. 1b). It is attached directly to the absorption cell and consists of an aluminum holder for the optical fiber and a collimating lens aspheric lens (2.79 mm diameter, 0.18 numerical aperture, 6 mm focal length, Lightpath, USA). It allows for a precise and flexible adjustment of a number of parameters: a focal length of the collimating lens ($Z$ adjustment with a max. displacement of 10 mm), an injection angle of the laser beam with respect to the optical axis of the absorption cell (angle adjustment with a max. angle of 6° off-axis) and position ($X$ and $Y$ adjustments with a max. displacement of 10 mm each) of the laser beam toward the absorption cell. After all the parameters have been determined, the in-coupling system can be mechanically fixed ensuring a robust optical alignment of the laser beam toward the absorption cell. The optical absorption cell represents an aluminum tube with a length of 25 cm, volume of 310 ml and an effective optical path length of 150 m. The laser beam is injected ~5° off-axis with respect to the absorption cell. The off-axis alignment was chosen because this cavity-enhanced method demonstrates better intensity noise suppression, induced by the cavity modes [32, 34]. A pressure of 100 mbar is maintained inside the absorption cell by a vacuum pump and manually adjusted needle valves before and after the cell. To reduce the response time of the system, the flow rate through the gas cell is set to 50 l/h, allowing a refresh time for the cell of approximately 2 s. The optical output beams are focused by means of a 25-mm $BaF_2$ lens (5 cm focal length) on an InGaAs amplified infrared photodetector (PDA10CF, NEP = $1.2{\cdot}10^{-11}$ $W/Hz^{1/2}$, Thorlabs, Germany). The electronic signal is amplified by 40 dB (Femto

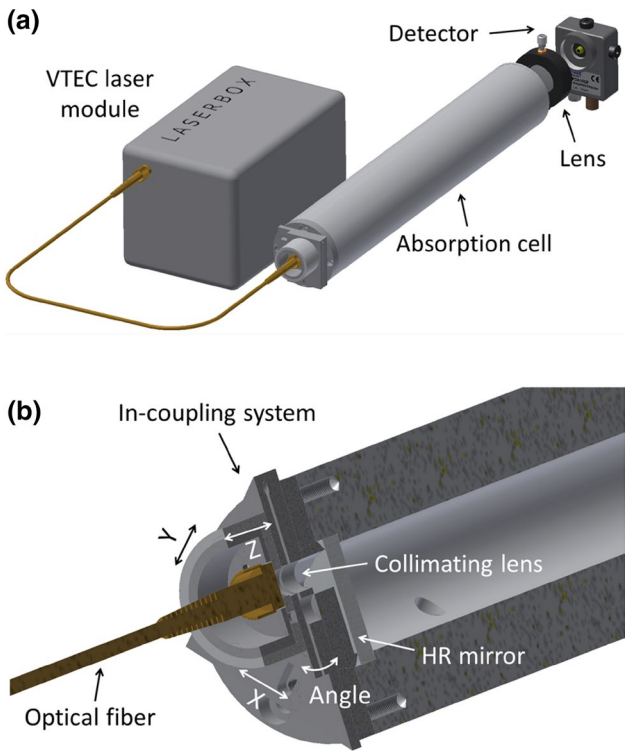


**Fig. 1** **a** Schematic representation of the laser-based sensor for $C_2H_2$ and $CO_2$ detection at 1,529.2 nm and HCN at 1,533.5 nm. The laser beam is sent to a high-finesse absorption cell with the effective path length of 150 m. The beam is focused on an InGaAs amplified detector, and the output signal is analyzed by a LabVIEW program. **b** Custom-made in-coupling system allowing precise alignment ($X$, $Y$, $Z$ and angle) of the optical beam toward the absorption cell

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

### 2.2 Breath acetylene sampling

In this work, the laser-based spectrometer is applied for monitoring exhaled concentrations of acetylene and carbon dioxide concentrations online in a single exhalation. The study has been carried out in the Netherlands in accordance with the applicable rules concerning the review of research ethics committees and informed consent.

A commercially available breath sampler device (Loccioni, Italy) is used for monitoring the exhaled $CO_2$ concentration and the mouth pressure during the exhalation maneuver. Their profiles are displayed in a graphical form on the screen of the breath sampler device. To prevent condensation, the breath sampling line is heated up to 40 °C. The exhaled concentrations of measured acetylene and $CO_2$ traces are displayed in nearly real time on a computer screen using LabVIEW software. In order to get a stable value of exhaled breath concentrations of acetylene and $CO_2$, a test-person was asked to perform artificially long exhalation.

### 2.3 Measurements of HCN in vitro

We have developed a gas sampling system for measurements of HCN released by PsA in vitro. The bacteria strain (ATCC 10145) were stored in brain heart infusion (BHI) broth at −80 °C and were inoculated into 50 ml brain heart infusion (BHI) broth (Mediaproduct BV, The Netherlands) in an initial concentration of approximately $5 \times 10^6$ colony forming units (CFU)/ml as previously reported [31]. The PsA strain is placed in a 250-ml Erlenmeyer flask fixed on a rotating platform (rotating at 100 rpm, GFL 3005, Germany) to enable uniform growing of the bacterium. Bacterial filters (FP 30/0.2 Ca/S, Whatman GmbH, Germany) are attached to the inlet and outlet of the flask to avoid bacterial contamination [35]. The flask and the rotating platform are placed inside an environmental chamber (Sanyo MLR-350H, Japan) at 37 °C. The headspace of the sample is constantly flushed with a compressed air mixture containing 21 % of oxygen in nitrogen, which is also used as a carrier gas to transport trace gases released from PsA bacteria to the spectrometer and the background signal reference. The flow through the mass flow controller MFC 1 (Brooks Instrument, USA) is set to 3.5 l/h. The flow through the cuvette and the mass flow controller MFC 2 (Brooks Instrument, USA) is 3 l/h. Before entering the spectrometer, in order to reduce interference with water, the air is dried by flushing through a trap filled with $CaCl_2$. All the parts of the gas transport system (except mass flow controller) are made of Teflon PFA or Teflon PTFE (PolyFluor Plastic, Hoevestein, Netherlands). To prevent overpressure at the point of the biological sample, a small amount of flow (~0.5 l/h) is allowed to escape via an overpressure outlet (OP). The outlet is placed before the Erlenmeyer flask to prevent dilution of the HCN released by the bacteria. The arrangement is schematically represented in Fig. 2.

## 3 Results

### 3.1 System performance

In the spectral region of the laser-based spectrometer, both acetylene (6,539.46 $cm^{-1}$) and carbon dioxide absorption transitions are present 6,539.51 and 6,539.59 $cm^{-1}$, respectively. These compounds can be covered within a single laser scan. Parallel measurements of $CO_2$ dynamics in breath provide potential benefits, allowing calculation of the physiological dead space lung volume [36, 37]. Figure 3 represents simulated and experimentally measured spectra of 1 ppmv $C_2H_2$ in the wavelength range 6,539.35–6,539.55 $cm^{-1}$ and calculated spectra of 4 % $CO_2$ and 3 % $H_2O$ (typically found in exhaled breath) under the following

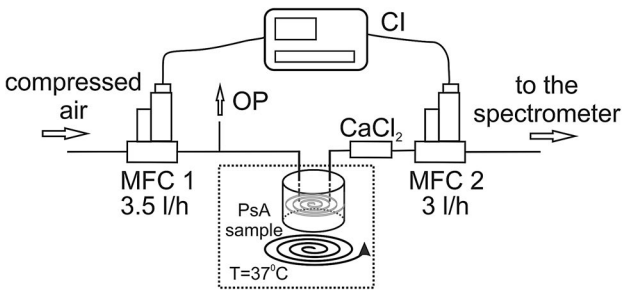


**Fig. 2** Schematic representation of the sampling system for HCN detection. The bacterium is placed in a 250-ml Erlenmeyer flask fixed on a rotating at 100 rpm orbital platform to enable uniform growing of the bacteria. The flow rate of the carrier gas is maintained constant at 3.5 l/h by means of mass flow controller MFC 1. The headspace is further dried and transported to the spectrometer with a constant flow rate of 3 l/h controlled by the mass flow controller MFC 2. Exceeding amount of air is led out via an overpressure outlet (OP). The control interface (CI) monitors the flow rates of both flow controllers. The flask and the platform are placed in the environmental chamber at 37 °C

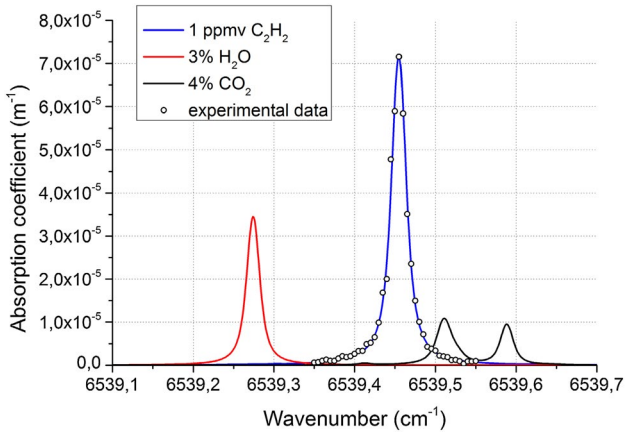


**Fig. 3** Simulated and experimentally measured spectra of 1 ppmv $C_2H_2$ in the wavelength range 6,539.35–6,539.55 $cm^{-1}$ and calculated spectra of 4 % $CO_2$ and 3 % $H_2O$ (typical found in exhaled breath) under the following conditions: pressure 100 mbar, temperature 293 K and path length 1 m. The scanning range of the laser allows for a simultaneous measurement of $C_2H_2$ and $CO_2$ lines within a single scan

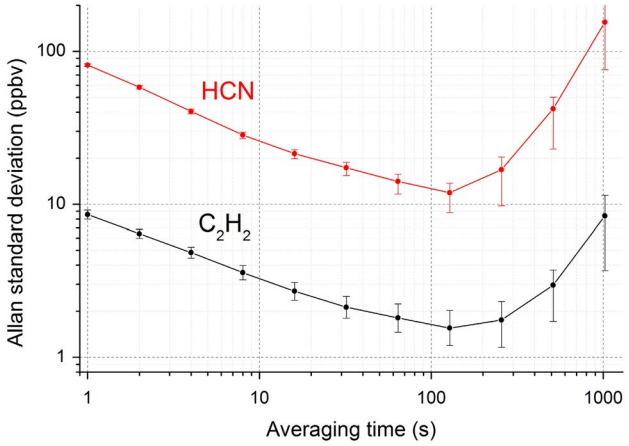


**Fig. 4** Allan variance plot represents laser-based spectrometer detection limit as a function of the integration time. Detection limits of 8 ppbv for acetylene (*black curve*) and 80 ppbv for hydrogen cyanide (*red curve*) for a 1-s averaging time are achieved. The best detection limit of 1.5 ppbv for $C_2H_2$ and 12 ppbv for HCN is reached for a 128-s acquisition time

conditions: pressure 100 mbar, temperature 293 K and path length 1 m (source: HITRAN database [38]).

Preceding the actual biomedical measurements, the performance of the setup has been evaluated. Figure 4 depicts the corresponding Allan variance curves for $C_2H_2$ (in black) and HCN (in red) as a function of the integration time of the measurement. The measurements for both gases have been taken by tuning the laser wavelength over the absorption line of the gas of interest with a 10-kHz scanning rate and averaging the acquired signal 10,000 times in 1 s by LabVIEW software.

Detection limits of 8 ppbv for acetylene and 80 ppbv for hydrogen cyanide are achieved for 1 s averaging time. The best detection limits—1.5 ppbv for acetylene and 12 ppbv for hydrogen cyanide—are reached for 128-s acquisition time. This is equivalent to noise-equivalent absorption sensitivity (NEAS) of $2.1 \times 10^{-9}$/cm $Hz^{1/2}$.

Figure 5 shows calibration curve of the laser-based spectrometer. Various concentrations of acetylene were measured by the laser-based spectrometer in the range from 3 ppbv up to 4 ppbv. The calculated and measured datasets agree within an error <1 % implying good linear response of the system for a range of concentrations useful for breath analysis measurements.

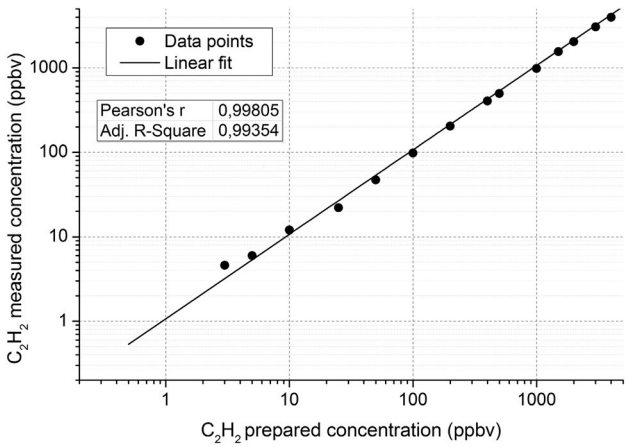


**Fig. 5** Calibration curve obtained for the laser-based spectrometer. Various prepared concentrations (3–4,000 ppbv) of acetylene were applied and measured by the laser-based system in order to determine the linearity of the spectrometer response

### 3.2 Breath acetylene and hydrogen cyanide measurements

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

The HCN production released in the headspace of the Erlenmeyer flask by PsA bacteria and the background reference signal (compressed air) were measured over 55 h in sequence of 30 min each. Resulting profile of HCN production is depicted in Fig. 7.

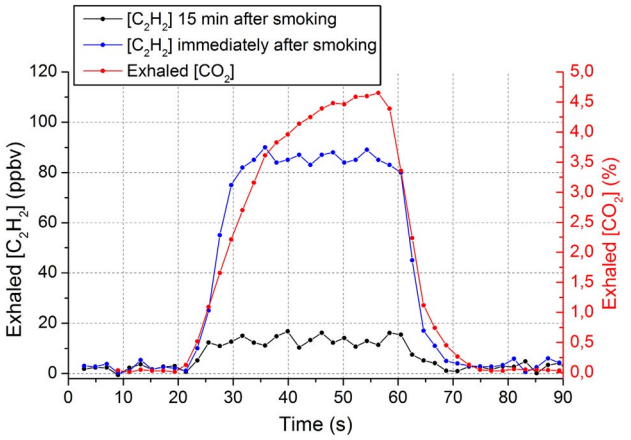


**Fig. 6** Online measurements of $C_2H_2$ and $CO_2$ profiles measured with the laser-based spectrometer during a single, artificially long exhalation immediately after smoking (in *blue*) and 15 min afterward (in *black*); acquisition time 2 s

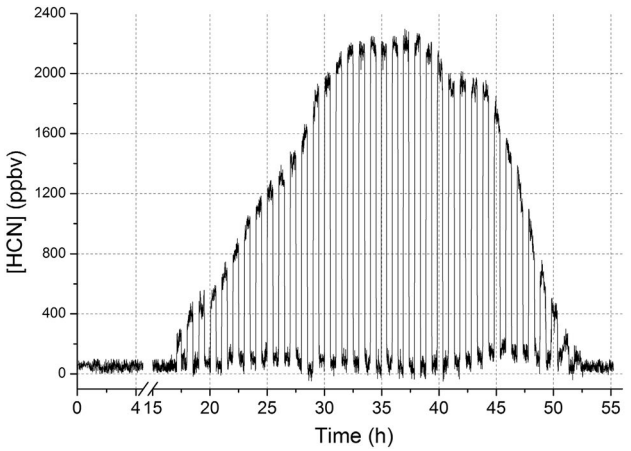


**Fig. 7** Dynamics of HCN production from *Pseudomonas aeruginosa* bacteria (strain ATCC 10145) in vitro culture. The headspace of the Erlenmeyer flask and the background reference signal (compressed air) were measured in sequence of 30 min each over 55 h by the laser-based spectrometer; acquisition time 30 s

The acquisition time of the measurement was 30 s per data point granting the detection limit of ~20 ppbv. The HCN production starts to rise after ~16 h when the bacteria enter the stationary growing phase and reach maximum in the first 35 h. After that, the HCN production decreases most probably as a result of medium depletion [31].

## 4 Discussion

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

The laser also allows the detection of HCN absorption transition in a different wavelength. It has been recently established that HCN can be considered as a biomarker for *Pseudomonas aeruginosa* bacteria [26, 39, 40]. In this study, we have demonstrated that our compact laser-based spectrometer employing off-axis integrated cavity output spectroscopy (OA-ICOS) is able to provide the required sensitivity (detection limit of ~20 ppbv for 30 s sampling time) and long-term stability for continuous measurements. We have detected levels of HCN production rate up to 7 μl/h (2.2 ppmv with 3 l/h flow rate through the PsA sample) resulting from PsA strain ATCC 27853 in vitro. The measured HCN levels are somewhat lower compared with those previously assessed by our group with a continuous-wave optical parametric oscillator (OPO)-based setup [31], however, from a different strain. Here, the authors observed maximum HCN concentration of 6.5 ppmv after 77 h from ATCC 10145 strain in vitro with a similar sampling arrangement. Compared with the OPO-based setup employing photoacoustic spectroscopy, our near-infrared DBR laser-based spectrometer in combination with OA-ICOS offers more compact design suitable for the field measurements, however, at the cost of lower detection sensitivity (0.4 ppbv of HCN in 10 s and 20 ppbv HCN in 30 s, respectively).

The radiation source used in this work is a prototype CW DBR laser tunable within the C-band (6,394–6,548 $cm^{-1}$) by switching to one of the 89 available channels. Current performance of the laser module allows for only a few mode-hop-free spectral windows for the trace gas sensing. Within this paper, the two available working channels made detection of three different species possible. The on-going developments will offer continuous mode-hop-free tuning capabilities of the laser together with fast switching between the channels for the simultaneous multi-component detection. Furthermore, a faster laser modulation speed (up to 100 kHz) can be realized in order to improve the laser performance.

The results obtained prove that the developed prototype of a compact laser-based spectrometer in combination with OA-ICOS represents a sensitive and robust technique, capable of rapid multi-component detection of acetylene, hydrogen cyanide and carbon dioxide traces with a sensitivity of $2.1 \times 10^{-9}$/cm $Hz^{1/2}$. The dedicated custom-made in-coupling system allows fixed and robust alignment of the optical beam inside the absorption cell. This arrangement offers more flexibility and enables the spectrometer to

be utilized in field campaigns as well as in hospital trials. In addition, small volume of the absorption cell provides fast refreshment time (<2 s) suitable for online measurements.

The sensitivity can be further improved by increasing the effective path length inside the absorption cell. This can be achieved by replacing the actual HR mirrors (99.8 % at 1,550 nm) with higher reflectivity ones. A reflectivity of 99.995 % would result in an increase of the effective optical path length inside the absorption cell up to 5 km compared with current 150 m. Together with a more sensitive detector with NEP 75 fW·Hz$^{-1/2}$at 1,550 nm, this will grant higher sensitivity of the system up to $6.3 \times 10^{-11}$/cm Hz$^{1/2}$. This will allow utilizing the laser-based spectrometer for the detection of HCN in vivo (in exhaled breath or emitted from skin), where the detection limit at ppbv or even sub-ppbv level is required [30].

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