Novel optical techniques for VOCs detection in breath

Arianna Elefante^{1,3}, Marilena Giglio¹, Angelo Sampaolo^{1,2}, Pietro Patimisco^{1,2}, Vincenzo Spagnolo^{1,2}

AFFILIATIONS

¹PolySense Lab, Dipartimento Interateneo di Fisica, University and Polytechnic of Bari, Via Amendola 173, Bari 70126, Italy ²PolySense Innovations srl, Via Amendola 173, Bari 70126, Italy ³National Research Council-Institute for Photonics and Nanotechnologies, Via Amendola 173, 70125 Bari, Italy

Abstract

Quartz-Enhanced PhotoAcoustic Spectroscopy and Light-Induced ThermoElastic Spectroscopy are optical techniques based on the use of a quartz tuning fork as detector. Their features are described to show their potentiality as real-time, sensitive, selective and compact sensors for breath sensing.

Keywords: QEPAS, LITES, breath analysis

1. Introduction

Breath analysis is a powerful non-invasive diagnostic tool based on the identification and quantification of several molecules present in the exhaled human breath which can be biomarkers of specific pathologies. The difficulty in this analysis relies on the nature and dynamics of human breath, which is a complex gaseous matrix composed of a large number of gas species, aerosol and particles present in concentrations varying in a wide range, from the percentage (nitrogen 74%, oxygen 13-16%, carbon dioxide 4-5%, water vapor 5-6%) down to the part-per-trillion (ppt), and with different origins. As a consequence, an ideal breath sensor should be able to discriminate the presence of a target molecule at the part per billion (ppb) or lower level, in presence of a matrix in the % level with high selectivity and sensitivity, and to precisely quantify its concentration. More capabilities are then required to employ breath analysis as mass screening test: on-line sampling, fast response time and compactness are capabilities required to allow real-time analysis on field clinical applications.

At the state-of-art, the gold standard is the analytical gaschromatography mass-spectrometry (GC-MS), which allows the identification of hundreds of volatile organic compounds (VOCs) in a single breath sample with high selectivity thanks to the temporal separation of different VOCs in the GC column, combined with the high sensitivity of the MS. GC-MS is certainly the best option for the initial research phase, when hundreds of VOCs and breath samples need to be investigated to identify the molecules biomarkers of specific pathologies. Nevertheless, some drawbacks limit the application of GC-MS in the clinical setting; it requires sample preconcentration and storage on a sorbing tube, increasing the possibility of contamination, as well as tens of minutes for the analysis of a single sample. It is not compact or portable and expensive, and requires well trained technicians for utilization and maintenance.

In this scenario optical techniques based on laser absorption spectroscopy (LAS) are emerging as a valid alternative to GC-MS for breath sensing. Infrared optical sensors offer highly sensitive and selective quantification of gas traces in the ppb range, with fast response time. The high selectivity is guaranteed by the strong molecular absorption features of the exhaled VOCs in the infrared region of the electromagnetic spectrum. The optical sensors' response time on the sub-second scale provides the possibility to record and resolve the exhalation profile of the single breathing cycle, which is rich of information on the processes in the respiratory tract and lung.

Compared to the GC-MS, LAS sensors do not require sample preconcentration and storage, allowing an on-line and real-time sampling and analysis of the breath. As additional advantage, the great progress achieved in the last decades in the miniaturization and integration of optical sensors for real-field applications (mainly environmental or industrial) can be directly inherited for the development of compact, light-weight and portable optical breath sensors.

Different potential breath sensing applications show the advantageous benefits deriving from optical sensors, compared to other sensing techniques. First, optical sensors are perfectly suitable for the detection of low molecular weight molecules, characterized by infrared spectra mainly composed of narrow separated absorption lines which can be selectively excited. On the other hand, GC-MS is more suitable for the detection of heavy (tens of atomic units) VOCs, whose broadband infrared spectra are more complex. Secondly, optical sensors can be used for the isotopic ratio measurement of the molecules, which is useful for several medical investigations. Finally, the single-breath resolving capability enables the study of the different phase of the respiratory's dynamics, supporting the research on physiological and gas exchange models.

2. Optical techniques for VOCs in breath detection

The principle of laser absorption spectroscopy is based on the transitions that an electromagnetic wave causes in a chemical species. If a molecule is irradiated by infrared light, it is excited to a rotational-vibrational energy level manifold which is specific for each chemical species. LAS sensors are based on application of the Beer-Lambert law:

$$I(\lambda) = I_0 e^{-\alpha(\lambda)l} \qquad \text{Eq. 1}$$

where I is the light transmitted through the cell containing the target gas, I_0 is the light incident on the gas cell, α is the absorption

coefficient at wavelength λ and l is the cell's optical pathlength. The absorption coefficient α is related to the number of molecules per unit volume n and the absorption cross section $\sigma(\lambda)$ through the relation $\alpha(\lambda) = \sigma(\lambda)n$. Thus, the simplest LAS sensor is composed by a laser source, a gas cell and an photodetector. Based on the Beer-Lambert law different approaches have been developed in gas spectroscopy through direct absorption techniques, including tunable diode laser absorption spectroscopy (TDLAS) and cavity-enhanced absorption spectroscopy (CEAS), and the indirect absorption photoacoustic spectroscopy (PAS).

2.1 Direct laser absorption techniques

TDLAS spectroscopy employes single mode emission semiconductor laser tuned across the absorption feature. In the basic single-pass configuration, it can be used to detect VOCs present in the breath sample at high concentration, such as carbon dioxide (CO₂) and water vapor (H₂O). To detect VOCs in the ppb range higher sensitivity are required, that can be reached combining modulation techniques to improve the signal-to-noise (SNR) ratio with the exploitation of multi-pass cell to increase the optical path-length, and consequently the absorption signal, according to Lambert-Beer law. The laser beam experiences multiple reflections at the surfaces of two large-diameter, focusing mirrors which compose the multipass cell, reaching optical pathlength as high as 100 m even if the gas cell length is in the order of tens of cm. However, long signal integration time (tens of second) are required, together with larger sample volume of multipass cell, limiting the capability of real time breath analysis. Then, the main application of real-time TDLAS for breath analysis is usually confined to species in the ppm range, such as CO, CH₄, NH₃, N₂O. Low volume circular multi-pass cell were implemented in (Ghorbani and Schmidt 2017a) to reconstruct the exhalation profile of CO and CO₂ using EC-QCL as excitation source, as well in (Ghorbani and Schmidt 2017b) for real-time breath analysis of CO isotopes.

As an alternative to multi-pass cell, the detection of breath biomarkers at the ppb level presenting weakly absorbing molecular transitions requires the implementation of cavity-enhanced absorption spectroscopy to achieve the required sensitivity. In CEAS, the breath sample cell is composed of a high finesse optical cavity comprised of two highly reflective mirrors (R > 99.99%), forming a Fabry-Perot etalon. An extensive review (Banik and Mizaikoff 2020) describes several implementations of CEAS for breath sensing, reporting the extreme sensitivity of the technique, reaching minimum detection limit (MDL) in the ppt-ppb range, and few implementations in medical settings (Ventrillard-Courtillot et al. 2009; Ciaffoni et al. 2016). Nevertheless, the cavity enhanced-based techniques are sensitive to optical misalignment, require laser beams with high spatial quality and narrow linewidth, and, being designed for a specific wavelength, are not suitable for multi-gas detection.

2.2 Indirect laser absorption techniques: Photoacoustic spectroscopy

Photoacoustic spectroscopy is also based on the absorption of electromagnetic radiation by the target molecules, but differs from the direct techniques on the physical phenomenon used for the detection of the absorption signal. After the absorption of the infrared radiation by the gas sample, the excited molecules relax to the ground state by means of collisional processes. These processes produce localized heating in the gas, which in turn results in an increase of the local pressure. Modulating the incident light intensity, a pressure wave is produced having the same frequency of the light modulation. A sensitive microphone is used as detector to convert the sound wave in an electrical signal, proportional to the amplitude of the detected sound wave. The resulting PAS signal linearly varies with the gas concentration allowing quantitative analysis of chemical species. PAS has demonstrated to be a highly sensitive trace-gas optical detection techniques, with a compact and relatively low-cost absorption detection module which is wavelength-independent, allowing its

implementation for breath analysis in several works, reported in (Dumitras et al. 2020).

3. Novel optical techniques

In this section, we focus on two optical techniques for VOCs detection whose features make them perfectly suitable for breath analysis: Quartz-Enhanced Photoacoustic Spectroscopy (QEPAS) and Light-Induced ThermoElastic Spectroscopy (LITES). Since both techniques rely on the use of a quartz tuning fork (QTF) as a detector, a preliminary description of the QTFs properties will be provided.

3.1 Custom Quartz Tuning Forks

Tuning forks have traditionally been used to adjust the pitch of musical instruments. Starting in the late 1960s, quartz tuning forks have been used as the timekeeping elements in modern clocks and smartphones. In timing applications, QTFs are made to vibrate by a small oscillating voltage applied to metal electrodes deposited on the quartz crystal's surface via an electronic oscillator circuit. QTFs are characterized by: (i) kHz–MHz resonance frequencies, (ii) a high frequency stability, and (iii) a high quality-factor (few tens of thousands in air) (Patimisco et al. 2017).

Standard 32.7 kHz QTFs, originally designed for timing applications, were first used in a gas sensing system in 2002 as transducers of an acoustic signal into an electrical signal through the piezoelectric properties of quartz. A tuning fork can be roughly approximated as two cantilever bars (prongs) connected at a common base. The inplane flexural vibration modes of QTFs can be categorized into two groups: symmetrical modes, where the prongs move in the same direction, and anti-symmetrical modes, where the prongs oscillate in opposite directions. QTF-based gas sensing techniques rely on vibrational anti-symmetrical modes induced by the pressure waves generated by a laser source absorption modulated at the QTF resonance frequency or at one of its subharmonics. The first

application of LITES for highly sensitive trace gas detection was reported in 2018 (Ma et al. 2018). In LITES, the laser beam directly hits the QTF prong, while in QEPAS, the beam is focused between the QTF prongs to generate acoustic waves. In the first case, the light absorbed by the QTF results in local heat accumulation, leading to an increase in the local temperature. In the second case, acoustic waves mechanically bend the QTF prongs. Both processes generate a stress field along the prong, which can be expressed by a longitudinal tensor $\sigma(x,y)$, producing a deformation within the material, as shown in Figure 1(a). This deformation generates in turn a strain field in the cantilever, which is related to the applied stress by the Hooke equation in the elastic regime:

$$\boldsymbol{\sigma}(x, y) = E \boldsymbol{\varepsilon}(x, y), \qquad \qquad \text{Eq. 2}$$

where E is the Young's modulus. Since quartz is a piezoelectric material, the stress field induces a local polarization $\mathbf{p}(x,y)$ of quartz, as shown in **Figure 1(b**), which depends on the stress field as:

$$\mathbf{p} = [\mathbf{d}] \boldsymbol{\sigma}$$
 Eq. 3

where [d] is the quartz piezoelectric tensor. Assuming that the QTF axes correspond to the quartz crystal axes and considering the effects of the polarization perpendicular to the tensile stress, the relation between **p** and σ reduces to the scalar expression:

$$p = -d_{11}\sigma = -d_{11}E \varepsilon \qquad \qquad \mathbf{Eq. 4}$$

where d_{11} is the longitudinal piezoelectric modulus.



Figure 1. a) Stress field generated along the QTF prong and b) polarization induced by the stress field.

The polarization field causes, in turn, a charge accumulation on the QTF prongs surface. This charge distribution generated by the inplane fundamental mode vibration appears on the prongs surface in a quadruple-pattern. The accumulated charges vary over time because of the prong's oscillation, thus generating an electric current, which is collected by gold electrical contacts appropriately deposited along the QTF prongs.

A quartz tuning fork can be modelled both electrically as an RLC resonant circuit (Patimisco et al. 2016), and mechanically using by the Euler-Bernoulli equation:

$$EI\frac{\partial^4 x}{\partial y^4} + \rho A\frac{\partial^2 x}{\partial t^2} = 0$$
 Eq. 5

where ρ is the density of the material, I is the moment of inertia and A is the cross-section area of the prong. Using clamped-free boundary conditions, the above equation can be solved to determine the resonance frequencies:

$$f_n = \frac{\pi T}{8\sqrt{12}L^2} \sqrt{\frac{E}{\rho}} v_n^2$$

where e L and T are the prong length and thickness, respectively, while the coefficients v_n are the mode constants.

Three main loss mechanisms contribute to the overall quality factor: (i) air damping, Q_{air}, related to the transfer of energy and momentum from the QTF prongs to the surrounding medium; (ii) support losses, Q_{sup}, related to the transfer of mechanical energy from the vibrating prong to the support; (iii) thermoelastic damping, Q_{TED} related to coupling between strain and temperature field inside the QTF (Giglio et al. 2019b). These dissipation mechanisms are assumed independent of each other and, therefore, the resonator Q-factor can be calculated as proportional to the inverse of the total energy dissipated. These contributions have been individually studied and they have been demonstrated to depend on the QTF geometry and on the gas pressure.

Since both the QTF resonance frequency and the related quality factor depend on the prong dimensions, the fork RLC values and the quartz crystal properties, starting from the standard 32.7 kHz QTF, several custom resonators have been designed and tested, to identify the QTF geometry optimizing its sensing performances when employed in a QEPAS or in a LITES setup. The guidelines followed for the optimization, based both on experimental results and stress-field simulations conducted using COMSOL MultiPhysics, will be discussed in the following sections for the two spectroscopic techniques.

3.2 Principles of Quartz-Enhanced Photoacoustic Spectroscopy

QEPAS is an indirect absorption spectroscopic technique based on the detection of the acoustic waves resulting from the absorption of light in a target gas using a quartz tuning fork as detector.

Eq. 6

In QEPAS, a modulated light source is focused between the QTF prongs and sound waves are generated via photoacoustic effect. The laser modulation frequency must match one of the QTF resonance frequencies, which are related to different prong motions. In particular, the piezoelectric active modes implemented in QEPAS are the first in-plane anti-symmetric flexural mode (fundamental vibrational mode) and the third in-plane anti-symmetric flexural mode (first overtone mode). The mechanical vibration of the QTF's prongs caused by the pressure wave generates a stress field along the prong. As explained in Section 3.1, the stress field induces a strain field and, for the piezoelectricity of the quartz, charges appear on the QTF surface that are collected by electrical contacts deposited along the QTF prong. The resulting QEPAS signal is proportional to:

$$S = \frac{Q P_L \alpha}{\sqrt{1 + (2\pi f_0 \tau_{V-T})^2}}$$
 Eq. 7

where τ_{V-T} is the V-T relaxation time and P_L is the excitation laser power, f_0 and Q are the QTF resonance frequency and quality factor.

QEPAS inherits the excellent detection sensitivity and selectivity and fast response time of conventional LAS techniques, as well as the small size, large dynamic range, long-term stability and wavelength-independence of PAS. The additional advantages of QEPAS sensor are connected to the innovative implementation of the QTF as detector. First, the replacement of the microphone in the acoustic gas cell with the QTF removes the restrictions imposed on the gas cell design by the acoustic resonance conditions. Moreover, the QTF is a sharply resonant acoustic transducer, characterized by high resonant frequency (kHz) and narrow bandwidth (a few hertz at atmospheric pressure), resulting in very high Q>10000. Being an acoustic quadrupole, the QTF is insensitive to environmental noise. The QTF is contained in a gas-tight cell called acoustic detection module (ADM) with volume as low as few cm³, enabling the trace gas detection of small samples, as the human breath.

Being the core element of the QEPAS sensor, a deep study of the QTF has been performed to optimize its performance for spectroscopy. The geometry of the OTF has been modified compared to the standard QTF driven by three fundamental guidelines. First, an increase of the prong spacing to facilitate the focalization of the laser beam through the QTF without touching the prongs and to avoid the generation of photothermal noise. Secondly, the QEPAS signal depends on the relaxation rate of the molecules (~µs). If the laser modulation frequency, which is determined by the QTF resonance frequency, is high compared to the relaxation time of the target gas, the molecules cannot efficiently release their vibrational energy during the modulation cycle, resulting in an inefficient generation of the acoustic wave. Consequently, a reduction of the fundamental resonance frequency of the QTF is advantageous, especially for the detection of slow relaxing gases, such as CO, CH₄, NO. Finally, the quality factor must be kept high, since the QEPAS signal is proportional to Q. Using the Euler-Bernoulli theory and studying the loss mechanism explained in section 3.1, custom QTF with different geometries have been developed and successfully tested in QEPAS sensor. Among the different geometries, the QTF showing the higher QEPAS SNR is the QTF with T-shaped cross section prongs, having a total prong length of 11.8 mm and thickness of 1.4 mm in the lower part of the prongs, which increases to 2.0 mm at the free end of the prongs. The resonance frequency and quality factor at atmospheric pressure are typically 12.5 KHz and 14000, respectively.



Figure 2 Acoustic Detection Module commercialized by Thorlabs (THORLABS).

In addition to the QTF geometry optimization, a significant enhancement of QEPAS signal is obtained acoustically coupling the QTF with one or two micro-resonator tubes to confine and amplify the sound waves. In the on-beam configuration, the tubes are aligned on both side of the QTF, close to the antinode point of the resonance mode; in off-beam configuration, the QTF is adjacent to the tubes. The system composed of the QTF and the tubes is called spectrophone and in contained in a gas-tight cell called acoustic detection module (ADM), shown in Figure 2.

3.2.1 QEPAS Setup

A schematic of a typical QEPAS setup is shown in Figure 3.



Figure 3. Left: Schematic of a QEPAS laboratory setup. TA – transimpedance amplifier, PM – Power Meter, DAQ – Data Acquisition Card, PC – Personal Computer; Right: picture of the QEPAS box with dimensions 48 cm x 40 cm x 15 cm commercialized by Thorlabs (THORLABS).

The excitation source for generating the laser signal is a laser source, typically a diode laser (DL), an inter-band cascade laser (ICL), or a quantum cascade laser (QCL). The ADM is mounted inside a vacuum-tight cell equipped with optical windows. The laser beam is focused by a lens through the tubes between the QTF prongs at the antinode point of the flexural resonance mode of the QTF.

The most common technique implemented for the generation of the signal in both QEPAS and LITES spectroscopy is the wavelength modulation (WM) approach, with the advantage that only the noise centered at the detection frequency and within the detection bandwidth affect trace-gas measurements. In WM a periodic sine function at the resonance frequency of the QTF, or its sub-harmonics is applied to the laser injection current, generating a modulation of both the laser power and wavelength. The modulated light interacting with the target molecules generates signals at the modulation frequency and its harmonics, that are then detected coherently using phase-sensitive detection electronics, such as a lock-in amplifier. In

the most common implemented detection scheme, the Lorentzian gas absorption line is recovered at twice the applied modulation frequency of the laser current (2f-detection). In the 2f-detection scheme, the acquired signal will exhibit a background-free second harmonic derivative line-shape with a distortion of the line-shape due to a residual amplitude modulation contribution. To implement the 2fdetection WM, a waveform generator is used to sinusoidally modulate the laser current at half of the QTF resonance frequency. A slow ramp (mHz) is added to the sinusoidal excitation to obtain QEPAS spectral scans by sweeping the laser current. The QTF signal is converted into a voltage signal using a trans-impedance preamplifier and then is demodulated at the QTF resonance frequencies using the lock-in amplifier, with typical integration time is 100 ms. The demodulated signals is recorded and analyzed on a personal computer by using a data acquisition card from National Instruments.

The gas line, consisting of a pressure controller, a flow meter, a pump and a needle valve, is used to set and control the pressure and the flow rate inside the ADM. Two tanks containing a certified concentration of the target gas and N_2 , used as dilution gas, are mixed using a gas mixer to calibrate the sensor, measuring the QEPAS signal corresponding to mixtures with different concentration of the molecule to be detected.

The QEPAS setup can be embedded into a compact case (48 cm x 40 cm x 15 cm), as the one shown in the right side of Figure 3 commercialized by Thorlabs for the detection of methane.

3.2.3 Potentiality of QEPAS for human breath analysis

Besides the vital research activities on QEPAS sensors in the last decades, most of the real-world applications have been focused on environmental or industrial issue. In Table 1 the demonstrated capabilities on the detection of molecules potentially interesting for breath analysis are reported. These results show the sensitive detection limit achieved, which are below the expected concentration

in human breath, and the fast response time of the sensor (\sim s). However, these sensors were not tested on human breath.

Table 1 Some representative molecules detected with QEPAS sensors.

| Biomarker | (hum) ک | Laser | (ddd) MDL | Time | Typical Level | Ref |
|---------------|---------|-------------------|--------------|------|------------------|----------------------------|
| Vitric oxide | 5.26 | EC-QCL | S | 1 s | 10 – 50 ppb | (Dong et al. 2011) |
| oon monoxide | 4.61 | DFB-QCL | 1.5 | 1 s | 0.01 – 10 ppm | (Ma et al. 2013) |
| Ethylene | 10.34 | DFB-QCL | 10 | 10 s | 0-10 ppb | (Giglio et al. 2019a) |
| rmaildehyde | 3.53 | DFB-ICL | 550 | 10 s | 0-10 ppb | (Horstjann et al. 2004) |
| Methanol | 76.31 | THz QCL | 160 | 30 s | 0 – 200 ppb | (Sampaolo et al. |
| bonyl sulfide | 1.53 | EDFA + DFB- DL | 29 | 1 s | 0-10 ppb | (He et al. 2018) |

An advantageous feature of QEPAS sensor, particularly suitable for the breath analysis, is the wavelength-independence of the QTF acoustic detector, which enables the multi-gas detection of molecules in different spectral ranges, without the need to change the detector or affecting its performance. Therefore, QEPAS sensor have been extensively implemented for multi-gas detection with different approaches, as reported in the review (Sampaolo et al. 2022). Particularly interesting for the breath analysis are two applications: the isotopic ratio measurement and the measurement of target gases in a fluctuating matrix.

QEPAS sensors for the isotopic ratio detection have been successfully demonstrated with different schemes. The simplest approach consists in using a single laser source whose emission range covers the absorption line of both the isotopes of the target gas. This configuration led to the detection of the ¹²CH₄ and ¹³CH₄ isotopes (Sampaolo et al. 2020) with detection sensitivities of 600 ppb and 110 ppb at 0.1 s of lock-in integration time, and of the ¹³CO₂ and ¹²CO₂ isotopic ratio with an average precision <1‰ (Wang et al. 2017) , which is perfectly suitable for most of the medical and biomarkerbased applications. A different approach was implemented in (Spagnolo et al. 2012) to measure the ¹⁸O/¹⁶O isotopic ratio in water vapor using two laser sources combined with the modulation cancellation method to reduce the background noise. The sensitivity achieved in determining the deviation from a standard sample δ^{18} O was 1.4‰, at 200 s of lock-in integration time.

When dealing with breath analysis, the target gas identified as biomarker is contained in a matrix with multi-components whose concentrations vary, like for H_2O or CO_2 generating a fluctuating background. These fluctuations must be considered in QEPAS spectroscopy since they affect the overall performance of the sensor in terms of (i) relaxation rate of the target molecule, (ii) resonance property of the QTF, due to the interaction with the surrounding matrix and (iii) enhancement factor of the mR tubes, due to the variation of sound speed. Different approaches have been developed to make QEPAS a robust and reliable techniques. One possibility is to combine the information of two ADMs, as developed in (Kosterev et al. 2010) for the detection of trace amount of H_2S in a fluctuating background of CO_2 and CH_4 in the percentage range. One ADM contained a QTF coupled with mR tubes to detect the H_2S with high sensitivity; the second ADM contained a standard bare QTF, which is unaffected by the non-linearity introduced by the tubes, and is used to monitor the gas matrix composition to correctly interpret or normalize the detected gas target concentration.

An alternative solution for dealing with the non-linear matrix effect consists in applying multivariate statistical tool to the experimental spectra to retrieve the correct concentrations of the target gas. In particular, the Partial-Least Square Regression analysis has proved its potentiality to accurately determine the analytes concentrations in mixtures presenting both highly overlapping QEPAS spectra (Zifarelli et al. 2020) or non-linear matrix effect (Menduni et al. 2022).

Another possible configuration to address for the variation of the QTF parameters in response to variations of the gas matrix (temperature, humidity, matrix composition), is the Beat Frequency QEPAS. BF-QEPAS is a variant of traditional QEPAS based on the detection of the beat frequency signal generated when the transient response signal of the QTF is demodulated at its non-resonance frequency (Wu et al. 2017). This approach allows to measure trace gas concentrations while continuously monitoring the electrical QTF parameters (frequency and quality factor). Tracking the QTF resonance frequency with a feedback-loop, consistent reduction of the signal drift has been demonstrated in (Rousseau et al. 2019).

Finally, the QEPAS setup described in (Elefante et al. 2019) has the capability to simultaneously detect two or more gas species. It is based on wavelength-modulation division multiplexing scheme: two laser sources, one for each species to be detected, are independently modulated to excite at the same time the fundamental and the first overtone mode of a custom QTF. The 2f-signals are then simultaneously demodulated by two different lock-in amplifiers. This apparatus, tested for the simultaneous detection of H₂O and CH₄ (or N₂O), can be exploit in breath analysis to measure a set of biomarkers at the same time, as the isotopes, or for an instantaneous self-calibration of the sensor.

3.2.4 QEPAS for breath

In the following section the few studies on QEPAS sensors tested on breath samples are reported and summarized in Table 2. A common issue is the influence of H₂O concentration on the gas target photoacoustic response, addressed inserting in line an humidifier to stabilize the humidity level inside the ADM. Most of the works have been demonstrated for the detection of exhaled NH₃, with different spectral range and sensor configurations. In the first demonstration (Lewicki et al. 2011) a compact MIR QEPAS sensor (12"x14"x10" housing) targeted the 10.34 µm absorption line of ammonia, leading to a MDL of 6 ppb at 1 s integration time. The sensor was connected to the Loccioni breath sampler to standardize the breath collection by maintaining a stabilized pressure of the exhaled breath and to measure the breath CO_2 concentration. All the system was heated to > 38°C to avoid NH₃ adsorption and water vapor condensation. The laser frequency was kept fixed at the NH₃ adsorption wavelength using a 3f wavelength locking scheme with a reference cell. With this configuration, it was possible to collect new breath samples every 3 mins, to allow the cleaning of the system between every collection.

In (Shang et al. 2022) the detection of exhaled NH_3 was demonstrated in the NIR at 1531.68 nm, reaching a sensitivity of 14 ppb at 1 s

integration time. To compensate for the lower line-strength in the NIR, a high power laser source was implemented, consisting in an erbium-doped fiber amplifier (EDFA) emitting up to 3 W of optical power. A custom QTF with 1 mm of prong spacing was selected to reduce the thermal noise generated by the 650 µm waist laser beam hitting the QTF. The sensor was tested for on-line detection of exhaled ammonia, introducing the breath into the ADM real-time. Figure 4 shows the real-time measurement of exhaled NH₃ by 3 healthy subjects, consisting in three breaths for each subject. As shown in the figure, levels of ammonia in the 170-230 ppb range were detected, while in the time interval between subsequent breath samples the QEPAS signal decreases rapidly to the noise level with a fall time of 15 s. The fast response of the sensor allows the detection of a small bump at the beginning of every breath, corresponding to a higher NH₃ concentration due to bacterial processes in the oral cavity.



Figure 4 Real-time QEPAS measurements of the exhaled ammonia concentration by three healthy subjects (Shang et al. 2022).

In a more recent work (Li et al. 2022), a NH_3 QEPAS sensor for breath analysis was developed with the alternative beat-frequency

configuration, providing faster response time and no need for calibration, achieving a MDL of 9.5 ppb at an integration time of 3 ms.

The first clinical implementation of a QEPAS sensor for breath analysis was reported in (Maurin et al. 2020) for the detection of endogenous exhaled CO using a QCL emitting at 4.7 μ m, reaching a MDL of 20 ppb at 1 s integration time. The sensor was clinically validated at the Montpellier Hospital, comparing its performance with the blood carboxy-hemoglobin (COHb%) reference model to demonstrating its reliability.

| Molecule | Laser | Spectrophone | MDL | T_{int} | Ref |
|-----------------|--------------------------------|------------------------------|-------|------------------|-----------------------------|
| | | | (ppb) | | |
| СО | QCL 4.7 μm | 32.7 kHz + mR off-beam | 20 | 1 s | (Maurin et al. 2020) |
| NH ₃ | DFB-QCL 10.34 μm | 32.7 kHz + mR | 6 | 1 s | (Lewicki et al. 2011) |
| NH3 | EDFA Diode Laser 1.53 μm | 9.4 kHz + mR On beam | 14 | 1 s | (Shang et al. 2022) |
| NH ₃ | DFB-QCL 10.36 μm | 32.7 kHz + mR On-beam | 9.5 | 3 ms | (Li et al. 2022) |

Table 2 QEPAS sensor for breath sensing.

3.3 Principles of Light-Induced ThermoElastic effect Spectroscopy (LITES)

LITES is a recent development of traditional TDLAS, with the QTF used as light detector, as explained in the previous section. In the typical configuration shown in Figure 5 the laser beam passes through an absorption cell containing the gas target and is then focused on the QTF surface. The QTF can be both inserted in the absorption cell itself, or outside.



Figure 5 Schematic of the sensing system of a LITES sensor.

To maximize the LITES signal, the laser beam is focused at the internal side junction of one of the prongs, since simulation based on the Finite Element Method on COMSOL Multiphysics revealed that in this area is generated the maximum strain field. The generation and detection of the signal, as well as the gas line system, are identical to that described for QEPAS sensors.

As anticipated in Section 3.1, an optimization of the QTF geometry was performed to optimize its performance as optical detector. In particular, the LITES signal is related to two parameters, which depend on the prong geometry: the strain field and the accumulation time. The accumulation time, defined as the ratio between the quality factor and the resonance frequency, influences the conversion efficiency of the photothermal energy deposited in the quartz crystal into mechanical oscillation of the prongs. Different QTF geometry were investigated (Russo et al. 2020), demonstrating a linear dependence of the measured LITES SNR as a function of the product $\tau^*\epsilon$. The results led to the identification of the most performing QTF as the T-shaped with 50 µm-deep rectangular grooves carved in both prong sides, with ~9.8 KHz of fundamental mode resonance frequency and quality factor at atmospheric pressure of 11500.

An extensive study of the performance of QTF compared to the commercially available PDs in terms of their figure of merits was investigated in (Wei et al. 2021), showing its capability to provide broad-band detection with fast response (tens of kHz), highresponsivity and narrow-bandwidth (1 Hz). The QTF employed was the T-shaped QTF with grooves shown in Figure 6 a). The spectral dependence of the QTF responsivity, defined as the output signal response to monochromatic radiation at an optical frequency incident on a photodetector, was measured using a TDLAS setup with five interchangeable single mode lasers covering the $1.65 - 10.34 \ \mu m$ spectral range. The experimental results are shown in Figure 6 b) and compared to the performance of commercial PDs: traditional PDs have to be selected according to the excitation laser source and exhibits a decreasing response when a broader spectral range has to be covered. Differently, the T-QTF exhibits a high (~2200 KV/W) and flat response for the whole infrared spectral range, without requiring any thermoelectrically cooling system. Therefore, the QTF is perfectly suitable as detector in multi-gas detection when laser sources emitting in several spectral range are combined.



Figure 6 a) T-shaped with grooves QTF; b) Responsivity of the QTF and commercially available detector as a function of the wavelength; c) FTIR spectrum of a 0.75 mm-thick Z-cut α -quartz slab.

The flat response of the QTF is explained analyzing its optical which determines the absorption properties, photo-induced thermoelastic conversion. The analysis of the transmittance spectrum of a 0.75 mm-thick Z-cut α -quartz (see Figure 6 c)) shows that for λ > 4.8 μ m almost all the electromagnetic energy is absorbed in the quartz sample, enabling an efficient thermoelastic conversion. In the $\lambda < 4.8 \ \mu m$ spectral range, where the light is transmitted through the quartz, the thermoelastic conversion is due to the 50 Å-thick chromium/ 250 Å-thick gold layers deposited on the back surface of the QTF used as adhesion promoter and electrodes for the charge collection, respectively. The combination of the MIR strong absorption of chromium and the highly reflectance of gold trap and deposit the electromagnetic energy at the quartz/chromium interface.

3.3.1 Potentiality of LITES for breath sensing

Different implementations of LITES sensors for the detection of molecules potentially interesting for breath analysis have been reported in the last few years. Some examples are summarized in Table 3. The main effort was focused on the optimization of the QTF surface to enhance the absorption of laser energy, and consequently the sensor sensitivity, using high thermal expansion and light-absorbing materials.

Ammonia was detected using a fiber-coupled NIR CW-DFB diode laser emitting at 1530.33 nm and a standard QTF with resonance frequency of 32.768 kHz, achieving a minimum detection limit of 5.85 ppm (Mi and Ma 2021). Ethane and methane were detected in a wide concentration range, from percentage to ppb (Zifarelli et al. 2023) using a single ICL emitting at 3.345 µm and a custom 9.8 kHz QTF as photodetector. Minimum detection limits of 650 ppb and 90 ppb with 10 s integration time were achieved for CH₄ and C₂H₆. CO was detected using a 1.57 µm DFB diode laser in combination with a fiber-coupled multipass cell (with optical path length of 40 m) to reduce optical interference and alignment's difficulty (Liu and Ma 2022), reaching a minimum detection limit of 9 ppm at an integration time of 200 s. The fluctuations of O₂ concentration were measured to monitor the storage and decay process of strawberries (Dai et al. 2023). A vertical-cavity surface emitting laser emitting a 760 nm with 1mW maximum power was employed to target the O₂ line at 13142.58 cm⁻¹. An optimization of the 32.7 kHz OTF was conducted to enhance the detection sensitivity: the QTF was coated with a film of CH₃NH₃PbI₃ perovskite designed to form a Schottky junction with the silver layer. The coupling of the photoelectric and thermoelastic effect led to an improvement in detection sensitivity of 2 orders of magnitude.

At the state of art, only one LITES sensor has been applied to the analysis of human breath, for targeting the exhaled CH₄ (Lou et al.

2023). A mid-infrared ICL emitting at 3.334 μ m to target the CH₄ line at 2999 cm⁻¹ and a Herriot gas cell with effective optical pathlength of 20 m were used. A MDL of 58 ppb was achieved, thanks to the use of a QTF optimized for LITES measurement. The 32.7 kHz QTF was coated with polydimethylsiloxane (PDMS) and reduced graphene oxide (rGO) to enhance the thermal-mechanical conversion efficiency: the PDMS shows a high thermal expansion coefficient, while the rGO layer reduces the reflection of the laser light on the silver layer on the surface of the QTF. This custom QTF showed an enhancement of the SNR of 3 times compared to the bare QTF. The sensor was employed to detect exhaled methane from volunteers, demonstrating its capability to distinguish between healthy/obese individuals, measuring an average CH₄ concentration of 2.8 ppm, 1.9 ppm and 3.6 ppm for healthy male, healthy female, and obese individuals, respectively.

| Ref | (Mi and Ma 2021) | (Zifarelli et al. 2023) | (Liu and Ma 2022) | (Dai et al. 2023) | (Lou et al. 2023) |
|----------|---------------------|-------------------------------|----------------------|--|--------------------------|
| F | 100 ms | 10 S | 100 s | 564 s | 145 s |
| MDL | 5.85 ppm | 90 ppb 650 ppb | mqq 9 | 83 ppm | 36 ppb |
| Cell | ~20 cm | ~few cms | Multipass (40 m) | Herriot (20 m) | Herriot (20 m) |
| QTF | 32.7 KHz | 9.8 kHz | 32.7 kHz | 32.7 kHz + CH ₃ NH ₃ PbI ₃ | 32.7 kHz + PDMS + rGO |
| Laser | DL 1.53 µm | ICL 3.345 µm | DL 1.57 µт | VCSEL 760 nm | ICL 3.334 µm |
| Molecule | Ammonia | Ethane Methane | Carbon monoxide | Oxygen | Exhaled Methane |

Table 3 LITES sensors for the detection of molecules potentially interesting for breath analysis.

Conclusion

The QEPAS and LITES optical sensors described in this chapter provide the potential tool to move from the state-of-art mass spectrometer to a real-time, in-line, compact point-care device for breath sensing, employable in mass screening. Both the sensors offer selectivity and sensitivity comparable with the MS, but with second or sub-second response time, enabling the reconstruction of the respiratory's dynamics of a single breath. The breath gas-cell has a volume of few cm³, allowing a fast gas exchange, and is inserted in a compact and portable sensor with total dimensions of a shoe box. Compared to the classical optical techniques, QEPAS and LITES are more suitable for multi-gas detection, thanks to the wavelengthindependence of the QTF when used as acoustic detector and to its flat spectral responsivity when employed as photodetector.

Nevertheless, the research is still focused on some issues that need to be addressed for the effective implementation of LITES and QEPAS for breath analysis. First, the capability of detection of multiple VOCs in the breath has to be improved, adapting the several approaches discussed in the chapter according to the specific implementation in medical research. Moreover, it is crucial to couple the optical sensors with real-time breath sampling system to guarantee a reliable and reproducible on-line analysis of the breath, monitoring several experimental parameters, as the exhalation rate and the exhaled CO₂ concentration.

References

Banik GD, Mizaikoff B (2020) Exhaled breath analysis using cavity-enhanced optical techniques: A review. J Breath Res 14

Ciaffoni L, O'Neill DP, Couper JH, et al (2016) In-airway molecular flow sensing: A new technology for continuous, noninvasive monitoring of oxygen consumption in critical care. Sci Adv 2:. https://doi.org/10.1126/sciadv.1600560

Dai J, Wang C, Liu P, et al (2023) Sensitive Light-Induced Thermoelastic Spectroscopy-Based Oxygen Sensor With a Perovskite-Modified Quartz Tuning Fork. IEEE Sens J 23:22380–22388. https://doi.org/10.1109/JSEN.2023.3304654

Dong L, Spagnolo V, Lewicki R, Tittel FK (2011) Ppb-level detection of nitric oxide using an external cavity quantum cascade laser based QEPAS sensor. Opt Express 19:24037. https://doi.org/10.1364/OE.19.024037

Dumitras DC, Petrus M, Bratu AM, Popa C (2020) Applications of near infrared photoacoustic spectroscopy for analysis of human respiration: A review. Molecules 25

Elefante A, Giglio M, Sampaolo A, et al (2019) Dual-Gas Quartz-Enhanced Photoacoustic Sensor for Simultaneous Detection of Methane/Nitrous Oxide and Water Vapor. Anal Chem 91:12866–12873. https://doi.org/10.1021/acs.analchem.9b02709

Ghorbani R, Schmidt FM (2017a) Real-time breath gas analysis of CO and CO2 using an EC-QCL. Appl Phys B 123:. https://doi.org/10.1007/s00340-017-6715-x

Ghorbani R, Schmidt FM (2017b) ICL-based TDLAS sensor for real-time breath gas analysis of carbon monoxide isotopes. Opt Express 25:. https://doi.org/10.1364/oe.25.012743

Giglio M, Elefante A, Patimisco P, et al (2019a) Quartz-enhanced photoacoustic sensor for ethylene detection implementing optimized custom tuning fork-based spectrophone. Opt Express 27:4271. https://doi.org/10.1364/OE.27.004271

Giglio M, Menduni G, Patimisco P, et al (2019b) Damping Mechanisms of Piezoelectric Quartz Tuning Forks Employed in Photoacoustic Spectroscopy for Trace Gas Sensing. physica status solidi (a) 216:. https://doi.org/10.1002/pssa.201800552 He Y, Ma Y, Tong Y, et al (2018) HCN ppt-level detection based on a QEPAS sensor with amplified laser and a miniaturized 3Dprinted photoacoustic detection channel. Opt Express 26:9666. https://doi.org/10.1364/OE.26.009666

Horstjann M, Bakhirkin YA, Kosterev AA, et al (2004) Formaldehyde sensor using interband cascade laser based quartz-enhanced photoacoustic spectroscopy. Applied Physics B 79:799–803. https://doi.org/10.1007/s00340-004-1659-3

Kosterev AA, Dong L, Thomazy D, et al (2010) QEPAS for chemical analysis of multi-component gas mixtures. Applied Physics B 101:649–659. https://doi.org/10.1007/s00340-010-4183-7

Lewicki R, Kosterev AA, Thomazy DM, et al (2011) Real time ammonia detection in exhaled human breath using a distributed feedback quantum cascade laser based sensor. In: Quantum Sensing and Nanophotonic Devices VIII

Li B, Feng C, Wu H, et al (2022) Calibration-free mid-infrared exhaled breath sensor based on BF-QEPAS for real-time ammonia measurements at ppb level. Sens Actuators B Chem 358:. https://doi.org/10.1016/j.snb.2022.131510

- Liu X, Ma Y (2022) Sensitive carbon monoxide detection based on light-induced thermoelastic spectroscopy with a fibercoupled multipass cell [Invited]. Chinese Optics Letters 20:031201. https://doi.org/10.3788/COL202220.031201
- Lou C, Wang Y, Huang L, et al (2023) Quartz tuning fork (QTF) coating enhanced Mid-Infrared laser Induced-Thermoacoustic spectroscopy (LITES) for human exhaled methane detection. Infrared Phys Technol 133:104824. https://doi.org/10.1016/j.infrared.2023.104824

Ma Y, He Y, Tong Y, et al (2018) Quartz-tuning-fork enhanced photothermal spectroscopy for ultra-high sensitive trace gas detection. Opt Express 26:32103. https://doi.org/10.1364/oe.26.032103

Ma Y, Lewicki R, Razeghi M, Tittel FK (2013) QEPAS based ppblevel detection of CO and N_2O using a high power CW DFB-QCL. Opt Express 21:1008. https://doi.org/10.1364/OE.21.001008

Maurin N, Rousseau R, Trzpil W, et al (2020) First clinical evaluation of a quartz enhanced photo-acoustic CO sensor for human breath analysis. Sens Actuators B Chem 319:. https://doi.org/10.1016/j.snb.2020.128247

- Menduni G, Zifarelli A, Sampaolo A, et al (2022) Highconcentration methane and ethane QEPAS detection employing partial least squares regression to filter out energy relaxation dependence on gas matrix composition. Photoacoustics 26:100349. https://doi.org/10.1016/j.pacs.2022.100349
- Mi Y, Ma Y (2021) Ultra-Highly Sensitive Ammonia Detection Based on Light-Induced Thermoelastic Spectroscopy. Sensors 21:4548. https://doi.org/10.3390/s21134548

Patimisco P, Sampaolo A, Dong L, et al (2016) Analysis of the electro-elastic properties of custom quartz tuning forks for optoacoustic gas sensing. Sens Actuators B Chem 227:539–546. https://doi.org/10.1016/j.snb.2015.12.096

Patimisco P, Sampaolo A, Zheng H, et al (2017) Quartz– enhanced photoacoustic spectrophones exploiting custom tuning forks: a review. Adv Phys X 2:169–187. https://doi.org/10.1080/23746149.2016.1271285

Rousseau R, Maurin N, Trzpil W, et al (2019) Quartz Tuning Fork Resonance Tracking and application in Quartz Enhanced Photoacoustics Spectroscopy. Sensors 19:5565. https://doi.org/10.3390/s19245565

- Russo S Dello, Zifarelli A, Patimisco P, et al (2020) Light-induced thermo-elastic effect in quartz tuning forks exploited as a photodetector in gas absorption spectroscopy. Opt Express 28:19074. https://doi.org/10.1364/OE.393292
- Sampaolo A, Menduni G, Patimisco P, et al (2020) Quartzenhanced photoacoustic spectroscopy for hydrocarbon trace gas detection and petroleum exploration. Fuel 277:118118. https://doi.org/10.1016/j.fuel.2020.118118

Sampaolo A, Patimisco P, Giglio M, et al (2016) Improved Tuning Fork for Terahertz Quartz-Enhanced Photoacoustic Spectroscopy. Sensors 16:439. https://doi.org/10.3390/s16040439

Sampaolo A, Patimisco P, Giglio M, et al (2022) Quartzenhanced photoacoustic spectroscopy for multi-gas detection: A review. Anal Chim Acta 1202:338894. https://doi.org/10.1016/j.aca.2021.338894

Shang Z, Li S, Li B, et al (2022) Quartz-enhanced photoacoustic NH3 sensor exploiting a large-prong-spacing quartz tuning fork and an optical fiber amplifier for biomedical applications. Photoacoustics 26:. https://doi.org/10.1016/j.pacs.2022.100363

Spagnolo V, Dong L, Kosterev AA, Tittel FK (2012) Modulation cancellation method for isotope ^18O/^16O ratio measurements in water. Opt Express 20:3401. https://doi.org/10.1364/OE.20.003401

THORLABS Acoustic Detection Module for QEPAS. https://www.thorlabs.com/newgrouppage9.cfm?objectgrou p_id=11241

THORLABS Quartz-Enhanced Photoacoustic Sensor for Methane. https://www.thorlabs.com/newgrouppage9.cfm?objectgrou p_id=16188

Ventrillard-Courtillot I, Gonthiez T, Clerici C, Romanini D (2009) Multispecies breath analysis faster than a single respiratory cycle by optical-feedback cavity-enhanced absorption spectroscopy. J Biomed Opt 14:. https://doi.org/10.1117/1.3269677

Wang Z, Wang Q, Ching JY-L, et al (2017) A portable low-power QEPAS-based CO2 isotope sensor using a fiber-coupled interband cascade laser. Sens Actuators B Chem 246:710– 715. https://doi.org/10.1016/j.snb.2017.02.133

Wei T, Zifarelli A, Dello Russo S, et al (2021) High and flat spectral responsivity of quartz tuning fork used as infrared

photodetector in tunable diode laser spectroscopy. Appl Phys Rev 8:. https://doi.org/10.1063/5.0062415

Wu H, Dong L, Zheng H, et al (2017) Beat frequency quartzenhanced photoacoustic spectroscopy for fast and calibration-free continuous trace-gas monitoring. Nat Commun 8:15331. https://doi.org/10.1038/ncomms15331

Zifarelli A, Giglio M, Menduni G, et al (2020) Partial Least-Squares Regression as a Tool to Retrieve Gas Concentrations in Mixtures Detected Using Quartz-Enhanced Photoacoustic Spectroscopy. Anal Chem 92:11035–11043. https://doi.org/10.1021/acs.analchem.0c00075

Zifarelli A, Sampaolo A, Patimisco P, et al (2023) Methane and ethane detection from natural gas level down to trace concentrations using a compact mid-IR LITES sensor based on univariate calibration. Photoacoustics 29:100448. https://doi.org/10.1016/j.pacs.2023.100448