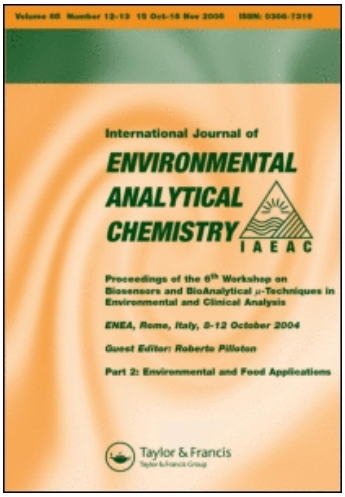


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# Laser sensors for trace gases in human breath

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Ethylene and ethane as biomarkers for lipid peroxidation in humans can be detected in the exhaled breath by means of very sensitive gas sensors based on high-resolution molecular spectroscopy. To this purpose, a laser photoacoustic spectroscopy sensor (PAS) for online ethylene monitoring and a tunable diode laser absorption spectroscopy sensor (TDLAS) for ethane detection were developed at ENEA Frascati Molecular Spectroscopy Laboratory. The sensors have been calibrated by certified mixtures. The main advantages of the breath analysis by spectroscopic technique are the high sensitivity and the absolutely non-invasive character. During an experimental study in cooperation with Umberto I Hospital (Radiology Institute) of Rome, we were able to detect very low concentrations (under 1 ppb) of trace ethylene content in the air exhaled by patients, following X-ray therapy; results are reported in this article.

*Keywords:* Medicine; Laser spectroscopy sensors; Breath analysis; Lipid peroxidation

## 1. Introduction

Many chemicals enter the body with food, through the air, through the skin or through drug administration. They take part in a complex chain of biochemical reactions and are converted into other molecular species. Some reaction products (mainly fast-diffusing small molecules) collect in the blood stream and are transported to the lung where they are dispersed on the exhaled breath. Thus, many different molecular species (both endogenous and exogenous) can be found in the human exhaled breath. In principle, the composition of exhaled air could give information about health condition, but in practice an extensive study on this topic is not yet ready. Anyway, some molecules detectable at trace level in a breath test have already been recognized as markers of pathology. The principle of the breath test is summarized in the following steps: (1) production of the marker molecule inside cells and organs, (2) diffusion through tissues, (3) input into haematic flow, (4) transport to the lungs, (5) release in the

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breathing air, (6) collection of a breath sample and (7) assessment of the marker in the breath sample.

Our aim was to develop high resolution sensors for fast and non-invasive monitoring of biomarkers in the exhaled breath. The systems described in this article were designed to detect ethylene and ethane produced as side products of lipid peroxidation (LP) in humans. The lipid peroxidation process consists of free radical induced oxidative degradation of polyunsaturated fatty acids constituting cell membranes [1]. Lipid peroxidation is an expression of the oxidative stress occurring inside the cells. The oxidative stress status is defined as an equilibrium between two concurrent processes: formation and removal of free radicals [2]. Under stress conditions (e.g. ionizing radiation, toxic chemical substances, chronic or acute diseases, etc.), the free radical production is significantly increased. In this case, the free radical scavenging capacity of the cell is overloaded and a complex chain of chemical reactions takes place. This leads to the cell membrane destruction, accompanied by the release of some volatile hydrocarbons, mainly ethylene, ethane and pentane. These markers can be found in the breath of healthy people at very low concentration (order of 1 ppb). The type of generated hydrocarbon depends on the polyunsaturated fatty acid (PUFA) involved in the lipid peroxidation process. If the PUFA involved in the LP process is the linolenic acid ($CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$), ethylene ($CH_2{=}CH_2$) and ethane ($CH_3{-}CH_3$) are produced, while the arachidonic acid generates pentane. Ethane and pentane are considered the most specific volatile markers for the investigation of lipid peroxidation [3]. The scheme of the ethylene generation is shown in figure 1.



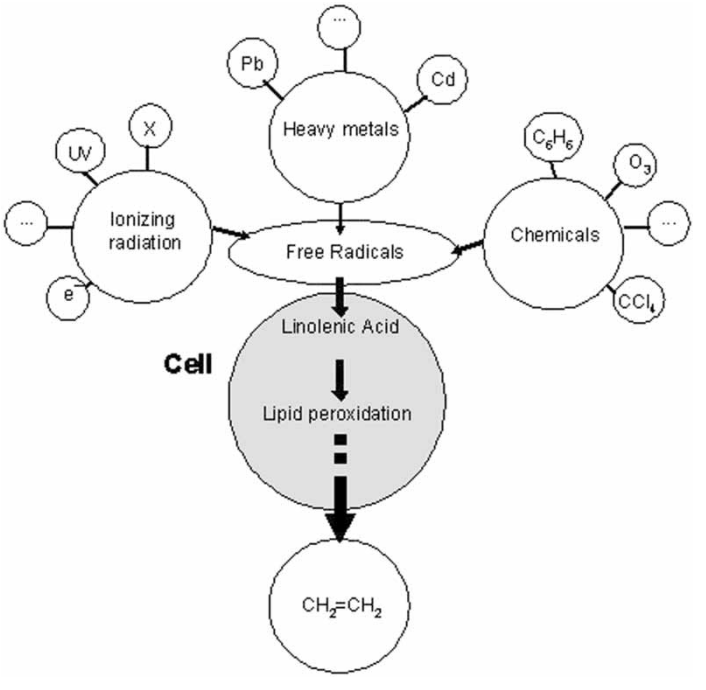


Figure 1. Ethylene production in a living cell under stress conditions.

The cell damage started by free radical action on biomolecules plays a very important role in the pathogenesis of some diseases, such as cancer, cardiopulmonary bypass [4], Alzheimer, atherosclerosis [5, 6], kidney or liver malfunction, asthma, neurological disorders, as well as the aging process.

The sensors used were a laser photoacoustic spectroscopy sensor (PAS) for ethylene detection and a tunable diode laser sensor (TDLAS) for ethane. The most common method for detecting hydrocarbons in the breath is gas chromatography/mass spectrometry (GC/MS). Due to insufficient sensitivity, the length of time required to perform measurements and the error propensity, laser spectroscopy sensors are thought to be more convenient than GC/MS in the medical praxis for breath testing. Laser spectroscopy techniques have already been proved to be a very selective and sensitive method for trace hydrocarbon detection in air at atmospheric pressure.

The optical systems we realized could be very useful to help medicine and science in understanding the life processes and build-up of non-invasive diagnostic procedures. The TDLAS and PAS sensors for ethane and ethylene trace detection can be used in medicine (breath test) as well as in biology (study of anaerobic processes in insects, soil and fruits), agriculture (plant physiology), environment study (*in situ* industrial, urban and rural atmospheric pollutant monitoring), spectroscopy (gases absorption spectra measurements). We have already performed experiments to determine the ethylene concentration emitted by seeds, plants and fruits in order to study the behaviour of different genotypes and their response to biotic and abiotic stresses [7].



In the following sections we will describe two kinds of optical apparatus and we will report some results on the related experimental study we are performing. A collection of breath samples was gathered in hermetic aluminized bags each saving half a litre of breath exhaled by a person. These were analysed in the spectroscopy laboratory. A secondary bag is connected to the mouth-piece, which discards the bronchial fraction of the breath and retains the alveolar fraction (which represents better the endogenous composition of the breath).

In this article, we report on the analysis of the exhaled ethylene in the case of patients affected by cancer and exposed to X-ray treatment. This technique could be useful for monitoring the radiation effect in the case of an accidental irradiation, for follow-up of radio-therapy, for monitoring personnel working in a radioactive environment (hospitals, nuclear power plants) or for studying some effects of ionizing radiation. Our main interest was focused on realizing a sensor sufficiently sensitive and selective to perform very accurate real-time high-resolution measurements with diagnostic value and to search the best parameters for a perfect condition of the experiment.

## 2. PAS spectroscopy

The principle of photoacoustic detection of ethylene can be summarized as follows: the laser radiation (modulated by a chopper at 550 Hz sound frequency) enters into the photoacoustic (PA) cell and is selectively absorbed by ethylene molecules that are able to pass on an excited level. This exciting energy is than transferred from the ethylene molecules to the surrounding nitrogen molecules (unable to use the laser energy to reach an excitation level) by intermolecular collision. The energy acquired by the nitrogen molecules serve to increase their kinetic energy; this means that the

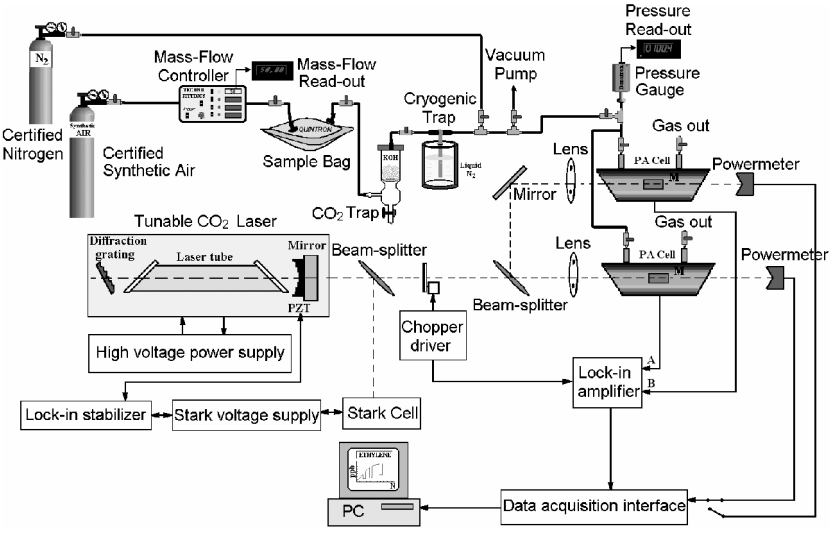


Figure 2. Block diagram of the experimental PAS sensor for differential measurement of ethylene traces.



temperature will be increased, and consequently, a pressure modulation is produced with the chopper frequency rate in the closed volume of the cell. This pressure modulation is detected by the sensitive microphones and represents the photoacoustic signal, its intensity being proportional with the ethylene content.

Unfortunately, the same effect is also shown by every gas species having strong absorption at the wavelength corresponding to the 10P(14) laser line, producing a strong measurement error. In human breath this undesired absorbing species are substantially carbon dioxide and water vapours. For this reason, it is necessary to eliminate them from the sample by a KOH scrubber (for $CO_2$) and a cryogenic trap or silica gel (for water vapours).

The experimental apparatus (see figure 2) is based on a water-cooled cw $CO_2$ laser (Ultra Lasertech Inc. Model 882X) emitting IR radiation between 9.2 and 10.9 μm. The main components are a Stark cell (Ultra Lasertech Inc. Model SC-700), a lock-in stabilizer (model 80.215 made by Lancing), two copper mirrors, a chopper, a Rk-570 powermeter by LaserProbe, a ZnSe lens, and a photoacoustic cell equipped with four high-sensitivity Knowles microphones (40 mV $Pa^{-1}$ total sensitivity). The signal produced by the microphones is transferred to a pre-amplifier followed by a pass-band filter with 3 kHz bandwidth amplitude and by a lock-in amplifier (model Stanford Research SR510). A PC Pentium III is used to control both the powermeter and lock-in, as well as to record the opto-acoustic signal coming out from the lock-in amplifier through a GPIB interface by using a program based on LabView software.

The wavelength used in the experiment (10P(14) laser line at 10.453 μm) can be selected by a diffraction grating. The laser is at a frequency stabilized by a feed-back loop based on a Stark cell filled by deuterated ammonia ($NH_2D$). Part of the emitted laser radiation (10%) is used to feed the Stark cell which is able to emit an electric signal directly proportional to the gap between the nominal wavelength of the setting and the instantaneous wavelength of the laser radiation. This signal is processed

by the lock-in stabilizer and used to adjust the cavity length through micrometric movements of the piezoelectric (PZT) device, following the well-known relation between laser wavelength $\lambda$ and optical cavity length $L$:

$$\lambda = \frac{2}{n} L.$$

Thus, the very stable frequency value of the laser radiation is maintained. The Stark cell stabilizes the laser emission on the 10P(14) laser line with 341 V voltage supply; 1 V variation in the Stark voltage supply corresponds to 7.74 MHz variation in the radiation frequency.

The system has been improved by inserting a second cell line. In this way, it is possible to allow either simultaneous or differential measurement of ethylene traces with two different gaseous samples. Using the differential PA instrument has some advantages: (a) the possibility to measure simultaneously the ethylene content in two different gaseous samples (the differential PA instrument allows the measurement of each sample in a different PA cell, avoiding the contamination of the cell with gases from the other sample); (b) the reduction of the measurement time in the case of a large number of samples. To obtain sensitivity at sub-ppb level, it is necessary to clean carefully the PA cell (this cleaning process could last a very long time, especially when different samples are measured, so one cell is used while the second cell undergoes the cleaning procedure).

The result of this conception are measurements of higher accuracy. The only drawbacks of the improved $CO_2$ laser-based differential photoacoustic sensor is that the laser power introduced in each PA cell (and consequently the duplication of the minimum detectable concentration) is reduced, the use of a supplementary ZnSe beam splitter and the complication of the laser beam alignments.

The photoacoustic sensor has been calibrated by using a certified bottle of reference mixture (1 ppm ethylene in nitrogen); 1 atm of the calibrating mixture was introduced in the PA cell and the PA signal was accurately recorded. The measure was repeated at different laser power values. Due to the linearity of the photoacoustic effect, the calibrating data have been expressed in $\mu$V/ppb, as summarized in figure 3.

## 3. Measurement of breath ethylene by PAS sensor in patients during radiotherapy

The effect of ionizing radiation on living cells is supposed to modify the oxidative stress status in the human body through an increase in the peroxidation processes started by the free water radicals generated by indirect radiation effect in the living tissue. A consistent part of the peroxidation events takes place in the cell membranes determining the release of small linear hydrocarbon molecules through the well-known lipid peroxidation pathways. A fraction of the hydrocarbon molecules generated in the tissue (between them is the ethylene) will be transported to the lungs by the blood and released in the exhaled breath. As the oxidative stress status affects the emission of ethylene in the exhaled breath, we analysed the ethylene in the breath exhaled from patients receiving a radiation treatment based on X-ray external beam.

In the reported experiment the patients received fractional doses as high as 1.6 to 1.8 Gy in a field as large as 222 to 1500 $cm^2$, depending on the pathology.

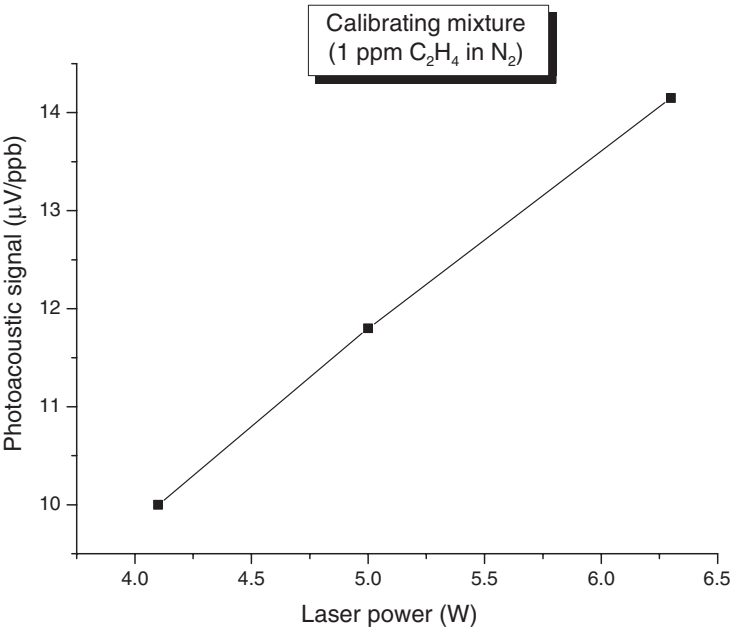


Figure 3. Calibration of the PA system by a standard mixture.



The radiation beam was provided by a linear electron accelerator, whose electron beam was converted into a photon beam. The analysis of the breath ethylene was performed by using the high resolution photoacoustic laser spectroscopy system developed in ENEA Laboratories. The ethylene was analysed before, as well as after, the radiation exposure, repeating the measurement at several time delays. The collection of data regarding this experiment has been summarized in figures 4–7. In figure 4, the photoacoustic signals recorded for each sample collected from one of the volunteer patients is reported. The PA signals were subsequently elaborated for detracting the background and the $CO_2$ contribution, in order to extract the value of the ethylene content in each sample. The resulting data are reported in the figures 5–7, representing the time evolution of the ethylene exhaled by each patient starting with the baseline value and continuing after the radio treatment (the time delays have been reported on the graphs).

The evolution of the breath ethylene concentration, showing an initial decrease which is slowly recovered with time, was shown to be similar in all patients. The small variations observed between different patients may depend on differences in absorbed dose, the extent of the pathology, the age and the individual response. The time evolution of the exhaled ethylene concentration was shown to be similar to the one found in a different group of patients treated by radio-iodine cancer therapy and previously reported [8]. To understand the behaviour of the results, we made the reasonable hypothesis that the fast development of oxidant free water radicals (locally generated by the radiation exposure of the tumour area) determines an increase in the total body anti-oxidant response, causing a temporary lowering in the oxidative stress status. Additional work is needed to confirm the reported results and to take some advantage from them, and in general from the possibility offered by the PAS facility.

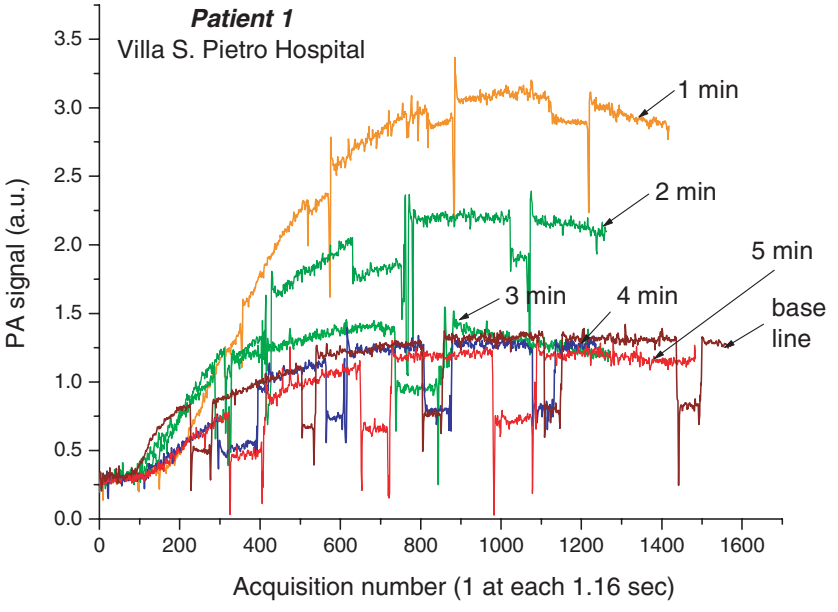


Figure 4. Photoacoustic signal records for patient 1 breath samples.

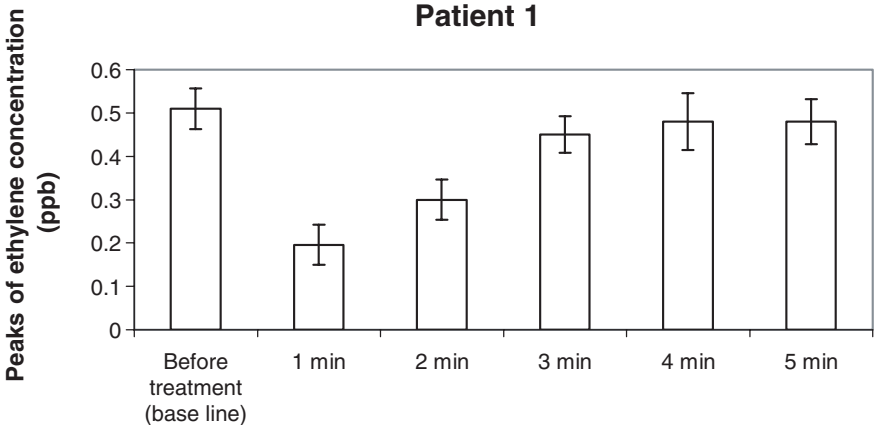


Figure 5. Time evolution of ethylene concentration in the breath of patient 1.

## 4. TDLAS spectroscopy

In the present section the IR absorption spectroscopy sensor based on a tunable diode laser source realized in the spectroscopy laboratory in Frascati for detecting ethane traces will be illustrated. The components of the system (see figure 8) are the laser source contained in the cryostat, the stabilized current supply feeding the laser, the monochromator, the reference cell, the multipass measurement cell equipped with ZnSe windows, the high-sensitivity MCT detector with its preamplifier, pressure and temperature sensors, germanium etalon, vacuum pump, optical components, and data acquisition system (the oscilloscope and PC).

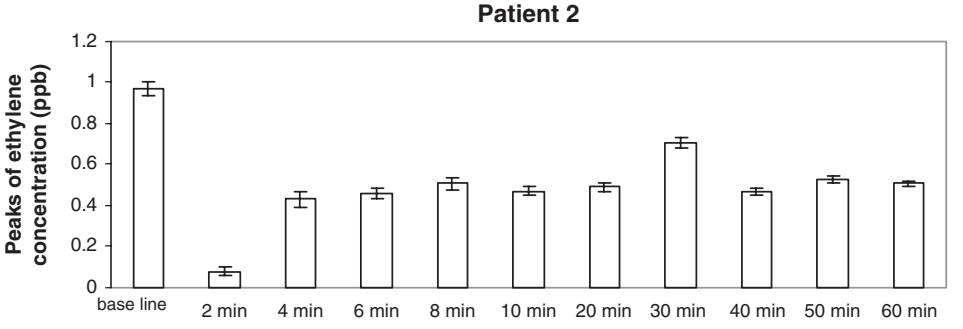


Figure 6. Time evolution of ethylene concentration in the breath of patient 2.

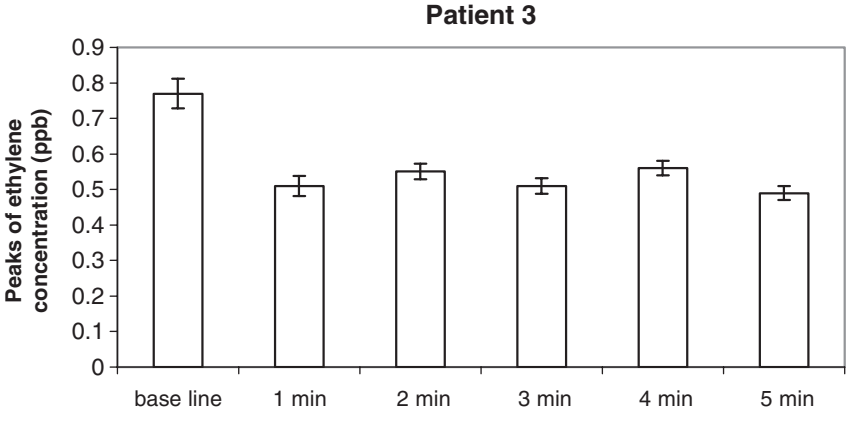


Figure 7. Time evolution of ethylene concentration in the breath of patient 3.



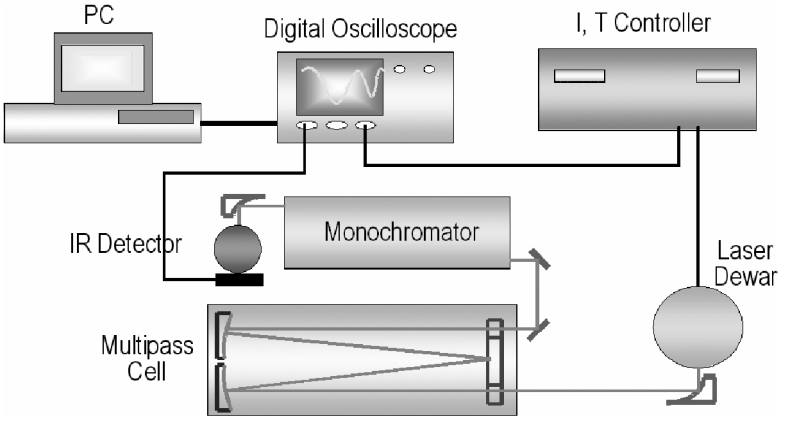


Figure 8. Tunable diode laser absorption spectroscopy sensor for ethane.

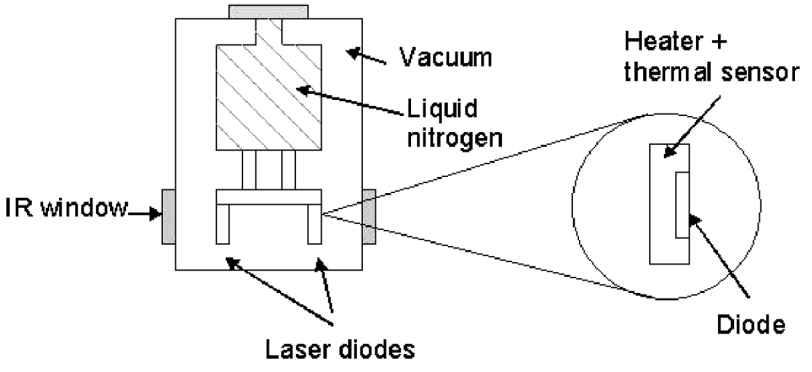


Figure 9. Scheme of the cryostat with the laser inside.



The multipass cell is equipped with a thermometric system based on a platinum element model Pt100 Sodern with a Memocal reader; the calibration was certified with 0.1 K sensitivity. The laser source used in this work is a lead salt semiconductor emitting 1 mW single mode IR radiation at 3.33 μm. It works at liquid nitrogen temperature and for this reason it needs to be kept inside the cryostat; a schematic view of the cryostat is given in figure 9.

The 1.5 L Dewar (L5736 model by Laser Photonics) has an operating temperature between 80 and 120 K and is able to run four lasers. The KBr windows allows the IR radiation to be extracted from the vacuum chamber. The laser is temperature controlled by a Si diode sensor and a heather with electronic regulation. The laser source is tunable, and the wavenumber $\nu_{\text{out}}$ of the emitted radiation can be modified by varying the injection current and the working temperature $\nu_{\text{out}} = \nu_{\text{out}}(i, T)$ with the following characteristic parameters:

$$\frac{\partial \nu_{\text{out}}}{\partial i} = 0.04 \frac{\text{cm}^{-1}}{\text{mA}} \quad \text{and} \quad \frac{\partial \nu_{\text{out}}}{\partial T} = 1 \frac{\text{cm}^{-1}}{\text{K}}.$$

The temperature is stabilized with a resolution as high as 0.0001 V (0.1 K) in the range between 80 and 120 K, while the injection current can be varied with 0.1 mA resolution. A Czerny-Turner 50 cm focal length monochromator (a 150 g mm$^{-1}$ grating) is used for accurate selection of the proper wavelength, and a germanium etalon (0.016 cm$^{-1}$ free spectral range) allows the measurement of the line width.

To collect the signal, the laser beam is focused on a MCT detector by a 50 mm focal length parabolic mirror; the HgCdTe semiconductor gap energy is 0.1 eV, convenient for detecting IR signals in the range 3–25 μm. The detector is cooled at liquid nitrogen temperature to keep the noise very low. At 77 K its response factor $F$ (defined as the voltage signal corresponding to 1 W power of absorbed radiation) is $10^3$ V W$^{-1}$, giving a $5.0 \times 10^{10}$ cm Hz$^{1/2}$ W$^{-1}$ detection factor $D$ defined as:

$$D = A^{1/2} \frac{F}{N},$$

where $A$ is the active surface area (in our case 1 mm$^2$) and $N$ is the noise signal. The electric signal produced by the detector is passed through a preamplifier

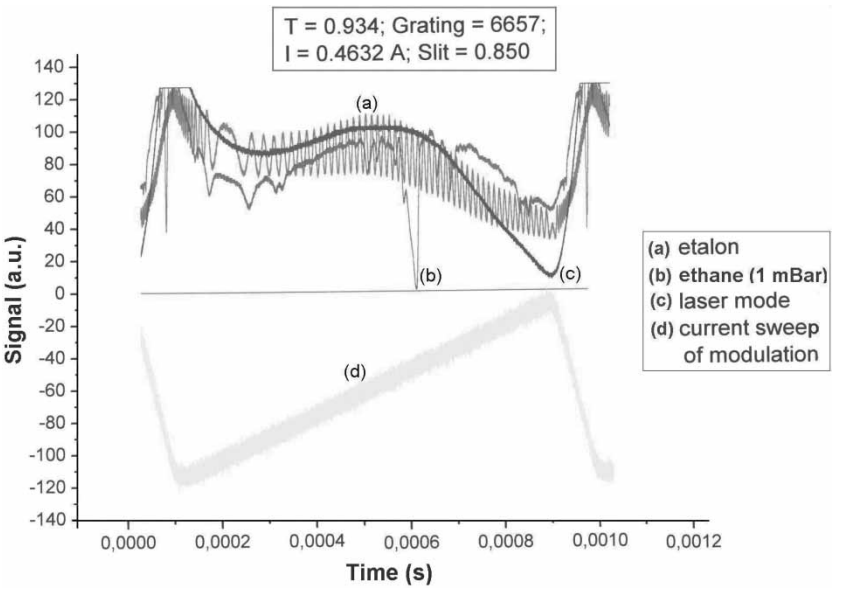


Figure 10. Experimental TDLAS acquisition of the following signals at 3.33 μm: (a) the etalon trace, (b) the absorbing lines of the calibrating ethane mixture modifying the laser mode, (c) the laser mode acquired without any sample inside the cell, (d) the current sweep modulating the laser wavelength.



(model LZ50 by Laser Photonics), then digitalized by a 500 MHz oscilloscope model LeCroy 9354C with 8 bit A/D converter and finally sent to a PC through a IEE 488 bus (GPIB interface) to be recorded. The data provided by the oscilloscope are acquired in real time by the Scope Explorer software running on the PC. Theoretical spectra are obtained by HITRAN simulation and compared with experimental data.

In figure 10, the ethane spectrum at 3.33 μm acquired by our TDLAS sensor is reported. In this figure you can see (a) the etalon trace, (b) the absorbing lines of the calibrating ethane mixture modifying the laser mode, (c) the laser mode acquired without any sample inside the cell, (d) the current sweep modulating the laser wavelength, all of them as shown on the oscilloscope screen. This experimental ethane spectrum has to be compared to the simulation of the ethane spectrum at the same wavelength given in figure 11. Moreover, the water and the methane IR spectra absorbing in the same region have been simulated; they were also reported in figure 10. In fact, $H_2O$ and $CH_4$ molecules represent the breath components showing the strongest absorbing lines in this IR region; the comparison is necessary to avoid any undesired absorption of the laser beam, causing an over-estimation of the ethane concentration. As can be seen, there is no contribution of $H_2O$ and $CH_4$ to the analytical $C_2H_6$ line. A detailed spectrum of water has been simulated (see figure 12) for calibrating the apparatus.

## 5. Conclusions

Laser spectroscopy has proved to be suitable in detecting trace gases in human breath for medical diagnosis [9]. Laser spectroscopy systems developed at ENEA Frascati

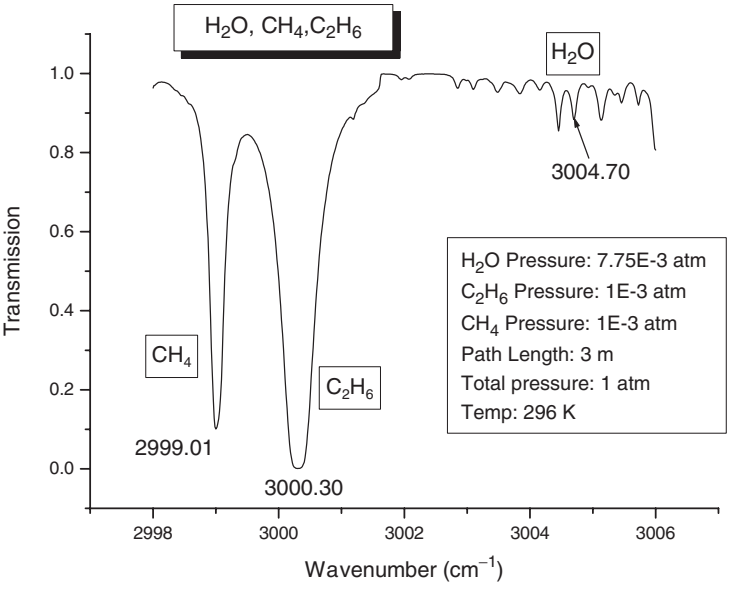


Figure 11. IR cumulative spectrum of $C_2H_6$, $CH_4$ and $H_2O$ simulated at 3.33 μm (HITRAN).

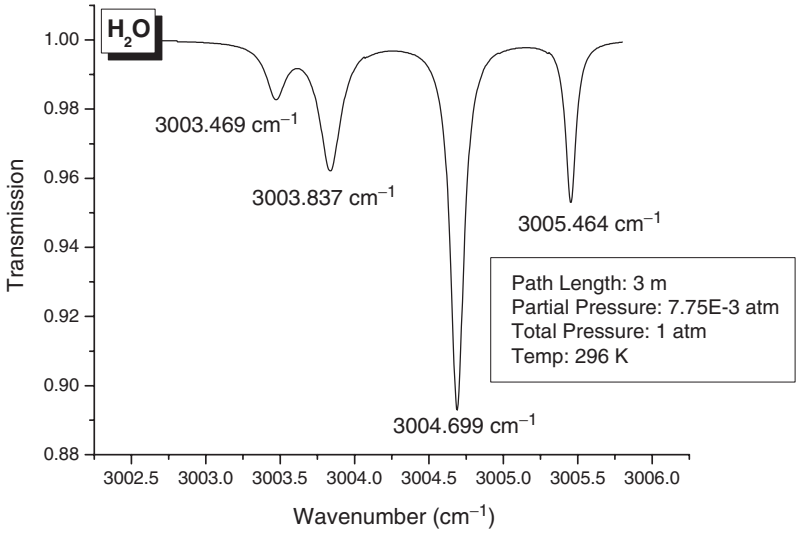


Figure 12. IR spectrum of $H_2O$ simulated by Hitran at 3.33 μm used for the system calibration.

Molecular Spectroscopy Laboratory we used because they revealed ethylene and ethane at very low concentration.

Advantages of the laser based sensor are high accuracy, high selectivity and sensitivity, real time data acquisition and simple and fast measurement; pre-treatment of the sample is not required. Moreover, by using the new diode laser generation operating at room temperature it will be possible to design more compact and mobile apparatus.

As a case study, ethylene concentration was determined in the breath exhaled from patients affected by cancer treated by external beam radiotherapy. The time evolution of the exhaled ethylene was shown to be similar to the one found in patients treated by radio-iodine therapy and previously reported.

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