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PO Box 117
221 00 Lund
+46 46-222 00 00

Simultaneous detection of molecular oxygen and water vapor in the tissue optical window using tunable diode laser spectroscopy

Linda Persson,^{1,*} Märta Lewander,¹ Mats Andersson,¹ Katarina Svanberg,²
and Sune Svanberg¹

¹Atomic Physics Division, Lund University, LTH, P.O. Box 118, SE-221 00 Lund, Sweden

²Department of Oncology, Lund University Hospital, SE-221 85 Lund, Sweden

*Corresponding author: Linda.Persson@Fysik.Lth.se

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We report on a dual-diode laser spectroscopic system for simultaneous detection of two gases. The technique is demonstrated by performing gas measurements on absorbing samples such as an air distance, and on absorbing and scattering porous samples such as human tissue. In the latter it is possible to derive the concentration of one gas by normalizing to a second gas of known concentration. This is possible if the scattering and absorption of the bulk material is equal or similar for the two wavelengths used, resulting in a common effective pathlength. Two pigtailed diode lasers are operated in a wavelength modulation scheme to detect molecular oxygen ~ 760 nm and water vapor ~ 935 nm within the tissue optical window (600 nm to $1.3 \mu\text{m}$). Different modulation frequencies are used to distinguish between the two wavelengths. No crosstalk can be observed between the gas contents measured in the two gas channels. The system is made compact by using a computer board and performing software-based lock-in detection. The noise floor obtained corresponds to an absorption fraction of approximately 6×10^{-5} for both oxygen and water vapor, yielding a minimum detection limit of ~ 2 mm for both gases in ambient air. The power of the technique is illustrated by the preliminary results of a clinical trial, noninvasively investigating gas in human sinuses. © 2008 Optical Society of America

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1. Introduction

Simultaneous detection of more than one gas has been a hot topic in the tunable semiconductor laser spectroscopy field during past years. The rapid progress in this field is mostly due to the invention of quantum cascade lasers (QCLs) operating in the mid-infrared region [1]. In this region many molecules such as CO, H₂O, NO, and CO₂ have strong absorption bands that might overlap spectrally. Because of the wavelength tunability of the QCLs, it is possible to scan over multiple absorption lines of different species and thus detect more than one gas simulta-

neously [2,3]. However, in tissue the absorption of constituents strongly limits the penetration depth in the midinfrared region ($1.5\text{--}4 \mu\text{m}$). In the tissue optics field it is common to refer to a tissue optical window ranging from 600 nm to $1.3 \mu\text{m}$, where tissue can be considered transparent due to the relatively weak absorption of major species making light penetration possible. The lower wavelength limit is due to absorption of hemoglobin and the upper limit is due to absorption of water [4,5]. In this optical window, where conventional diode lasers operate, the absorption lines of gases are weaker and fewer. This makes it difficult to achieve information about more than one gas by the use of a single laser source. A fruitful approach to improve on this is to use sum- and difference-frequency generation. An example of the for-

mer is given in [6], where water vapor, nitrogen dioxide, and sulfur dioxide can be measured using two diode lasers. The latter is illustrated in [7], where molecular oxygen, water vapor, and methane can be studied. However, the drawback of these techniques is the low light intensity of the generated frequency (normally a factor of ~ 1000 lower than the intensity of the light sources). Limited light intensity may be adequate in the application of free beam propagation in air, but is highly problematic, e.g., in strongly scattering media.

In this paper we report on a newly developed transportable system for dual-gas diode laser spectroscopy in biological and medical contexts. Since signals are highly geometry dependent, it is important to ensure measurement with identical conditions. By using two lasers operating spectrally close to each other, similar effective pathlengths can be achieved under the assumption that the absorption and scattering is the same for the bulk material at the two different wavelengths. Then, by normalizing the signal of a studied gas to the result obtained for a gas with known concentration, an absolute concentration value for the gas of interest can be determined. The present system uses two pigtailed diode lasers to simultaneously detect molecular oxygen at 760 nm and water vapor at 935 nm. The light beams from the two lasers are brought together with fibers before entering the sample. Different modulation frequencies of the lasers are used to distinguish between the two wavelengths. The system is not limited to these absorption lines. Any gas can be detected with the assumption that lasers operating at the corresponding wavelengths of the absorption lines of the gases are available. The system employs a computer board and software-based lock-in detection. The purpose of this paper is to describe the equipment and the multiplex advantages and to illustrate its applicability for clinical use. Examples of the detection of the two gases in the human sinuses are provided. The analyzed results of an ongoing clinical trial on human sinus cavities will be presented in future clinical publications focusing on the medical aspects.

2. Experimental Setup

A schematic drawing of the setup is shown in Fig. 1. Two pigtailed distributed feedback (DFB) lasers (Nanoplus, Germany) are scanned across the molecular oxygen absorption line R11Q12 at 760.445 nm (vacuum wavelength) and the water vapor absorption line at 935.685 nm (vacuum wavelength, vibration: $(000) \rightarrow (121)$, rotation: $J'' = 3 \rightarrow J' = 4$, $K_a'' = 0 \rightarrow K_a' = 0$, $K_c'' = 3 \rightarrow K_c' = 4$), respectively. A LabVIEW program controlling a computer board (NI-6120, National Instruments) creates the two input signals for the laser drivers. Each signal consists of a ramp with a superimposed sinus wave. The two ramps have the same frequencies (4 Hz), while the amplitude of the ramps differ due to the various responses of the wavelength of the lasers with regard to the driver current. The superimposed sinus waves on

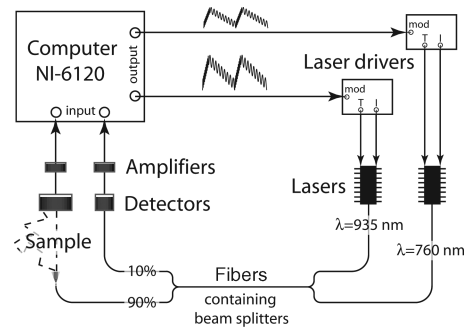


Fig. 1. Schematic of the experimental arrangement for simultaneous detection of molecular oxygen (760 nm) and water vapor (935 nm) using tunable diode laser gas absorption spectroscopy. The sample can either be an absorbing sample such as an air distance or an absorbing and scattering sample such as human tissue.

the ramps make lock-in detection possible. The laser used to detect oxygen is modulated by a frequency of 20.216 kHz and the laser used to detect water vapor is modulated by a frequency of 9.016 kHz. These frequencies are selected to avoid extensive noise at low frequencies and overlaps of the lower harmonics, and coherent sampling is enabled. The modulation depth is selected to optimize the signal amplitude without causing extensive line broadening [8].

The light beams from the lasers are each split into two arms, sample (90%) and reference (10%), by use of single-mode fiber-coupled beam splitters (Laser2000, Sweden). The light from the two reference arms and the two sample arms are fiber-optically brought together. The low-intensity light is directly guided to a photodiode (Hamamatsu S3590-01) providing the reference signal. The light from the sample arm is guided to the sample before being detected by a photodiode (Hamamatsu S3204-08). The sample in this study is either an air path or a scattering medium containing absorbing gas. The intensities reaching the sample are ~ 1 mW for both laser wavelengths. The signals from the detectors are amplified with two transimpedance amplifiers (Femto DHPCA-100 and DHPCA-200) before being synchronously recorded by two input channels on the computer board.

3. Data Analysis

The recorded signals are software-based lock-in detected using a LabVIEW program (Lock-in toolkit for LabVIEW). One advantage of this approach is the possibility to extract the different harmonics both in real time and after the data acquisition. All harmonics are available to process from the detected signals. A more detailed discussion of advantages using software-based lock-in detection is provided in [9]. The reference sinus waves needed to perform lock-in detection are internally created in LabVIEW. The $1f$ and $2f$ harmonics are computed for both the sample and the reference detector at the two frequencies corresponding to the two gases. The $2f$ signals of the two gases are normalized by dividing them with the offset of the corresponding $1f$ sig-

nal. This is done since the fractional absorption $\Delta I/I$ due to the gas has to be retrieved for concentration analysis with the Beer–Lambert law. The amplitude of the $2f$ signal is proportional to ΔI and the $1f$ signal offset is proportional to I . Before analyzing the normalized $2f$ sample signal, balanced detection is performed. This is done to suppress noise and perturbations like optical interference fringes. This procedure has been done in single-gas measurements and is described in detail elsewhere [10]. However, the same procedure can be performed with the simultaneous measurement since the two different wavelengths are modulation frequency tagged. A set of parameters is estimated by comparing the $2f$ reference signal and the $2f$ sample signal for each gas at frequency regions outside the expected gas imprint. A matched version of the reference signal is then subtracted from the sample signal over the whole frequency scan. An ideal signal obtained from measurements over a long air path resulting in a recorded signal with a gas imprint much larger than the noise is then fitted to the balanced-detection signal.

The gas content is obtained for each gas by evaluating the amplitude of the fitted curve of the normalized balanced-detection $2f$ signal. From this value a quantity called the equivalent mean path length, L_{eq} , is estimated by the use of the standard addition method [10]. The calibration curves were obtained in an atmosphere of $\sim 30\%$ relative humidity and a temperature of $\sim 27^\circ\text{C}$. The L_{eq} corresponds to how far the light has to travel in ambient air to obtain the same value.

It should be noted that when investigating scattering samples the estimated L_{eq} for each gas depends not only on the gas concentration but also on the optical properties of the sample such as scattering. A quantity independent of the latter and thereby directly proportional to the gas concentration can be estimated by forming the ratio between the two recorded L_{eq} for the different gases under the condition that similar optical properties for the two wavelengths can be assumed. In this way the concentration of one gas can be obtained if the concentration of the other gas is known.

4. Results and Discussion

A. Technique Performance Investigation

Typical recorded $1f$ and $2f$ signals from measurements over an air distance of ~ 20 mm are shown in Fig. 2. The signals have been averaged for ~ 25 s by repeatedly ramping the laser at 4 Hz and storing the signals. The balanced-detection signals are also included together with fitted ideal signals. The amplitude of the fitted signals correspond to an absorption fraction of approximately 5×10^{-4} and 9×10^{-4} for molecular oxygen and water vapor, respectively.

To investigate possible crosstalk between the two gas channels, an air distance of ~ 25 mm was studied. A fast Fourier transform was performed on the detected signals (see Fig. 3). Signals were recorded with

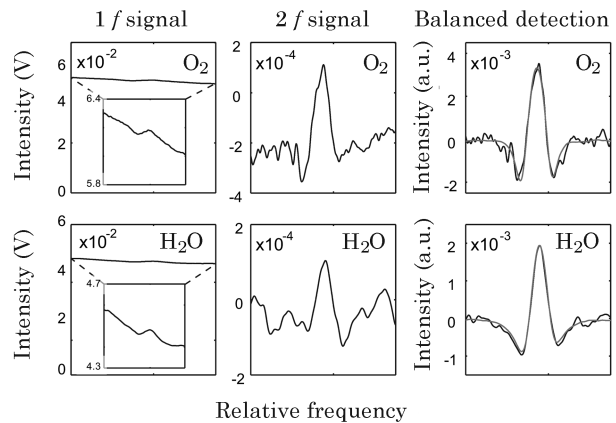


Fig. 2. Typical recorded oxygen and water vapor signals after background subtraction for measurements over an air distance of ~ 20 mm. Insets can be seen in the $1f$ signal plots enhancing the intensity region of interest for signals due to gas imprint. The balanced-detection $2f$ signal is shown (black) together with a fitted experimental ideal line shape (gray line). The relative frequency range for both gases corresponds to 35 GHz.

both lasers on, one laser on at a time, and no laser on. To the right in the figure, frequency regions of interest for crosstalk investigation are presented. As can be seen in the figure the modulation peaks occur only at multiples of the modulation frequencies of the individual lasers and no detectable crosstalk can be observed. To further study this issue ten measurements were recorded for each case. Signals were averaged for ~ 25 s. In Fig. 4 typical obtained balanced-detection signals can be seen, and the averages of the measured L_{eq} for each gas channel together with standard deviations are shown in Table 1. This test also verifies that no crosstalk occurs between the two gas channels.

By recording signals with no added air distance, the detection limit of the setup could be estimated. The detected noise floor corresponded to an absorption fraction of approximately 6×10^{-5} for both molecular oxygen and water vapor when the number of detected photons was not a limitation. This corresponds to an L_{eq} of ~ 2.5 mm for oxygen and ~ 1.5 mm for water vapor with calibration atmosphere. It was observed that the remaining pattern had a periodic structure for both cases. Hence the conclusion can be drawn that optical interference fringes [10] set the limitation of detectable signals.

B. Initial Clinical Test

In order to demonstrate the performance of the system developed, we present the results from test measurements on human frontal and maxillary sinuses performed in an initial clinical trial at the Lund University Hospital in collaboration with the Ear, Nose and Throat Clinic, the Radiology Clinic, and the Oncology Clinic. The cavities located within the human skull are surrounded by strongly scattering tissue, and gas signal recordings for such cavities have been previously demonstrated with laboratory equipment [11,12]. However, then separate setups were used for

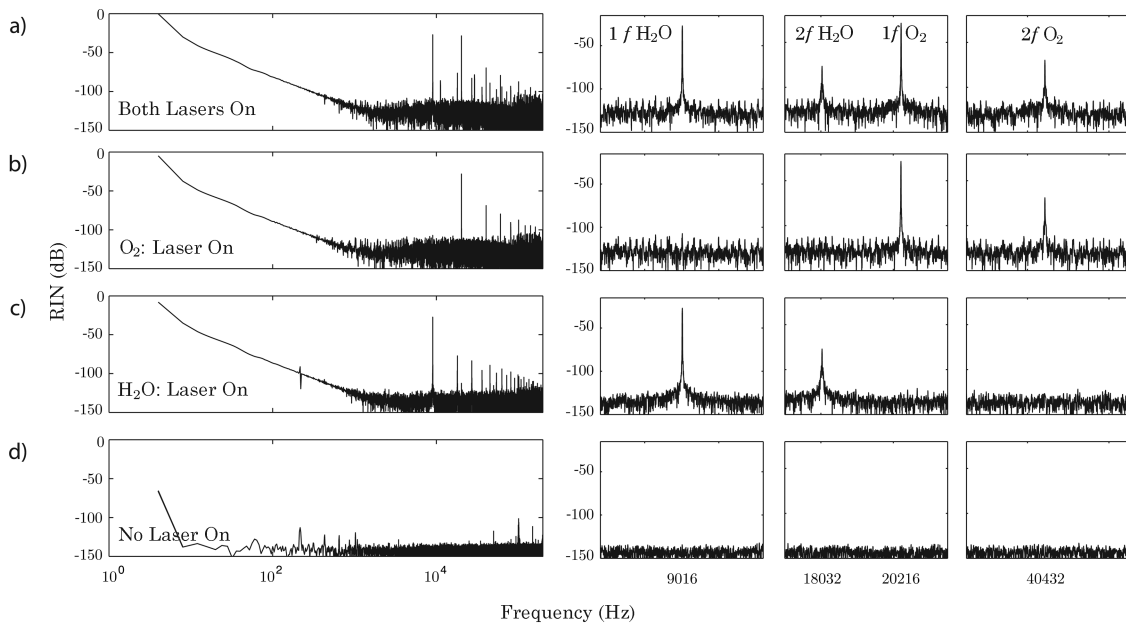


Fig. 3. Recorded laser relative intensity noise (RIN) expressed in relative dB, to investigate possible crosstalk between the two modulation frequencies used to detect two gases simultaneously. The fiber and the sample detector have been separated with an air distance of ~ 25 mm. To the right the frequency regions of interest are plotted. (a) Both lasers on. (b) Only laser for oxygen detection on. (c) Only laser for water vapor detection on. (d) No laser on.

molecular oxygen and water vapor measurements, and identical geometrical conditions for the measurements performed with separate probes could clearly not be ensured.

For a healthy patient the sinuses should be air filled, while in conditions like sinusitis (inflammation and infection of the sinuses), the mucosa is swollen and the sinuses may be filled with pus and liquid. The cause of sinusitis can in many cases be due to passage blockage between the nasal cavity and the sinuses preventing proper ventilation. The rationale behind the clinical trial is to investigate the pos-

sibility of using noninvasive diode laser-based absorption measurements of the sinuses for characterization regarding gas composition. The gas measurements are performed directly after the patients have undergone a computed tomography (CT) investigation of the skull, which is one of the most reliable diagnostic methods of sinusitis today. Unfortunately this method is expensive and uses ionising radiation. Therefore complementary and alternative techniques are desirable.

1. Frontal Sinus Investigation

To investigate the human frontal sinuses the optical fiber probe carrying the laser light to the sample is placed on the frontal bone below the eyebrow (the roof of the orbita) pointing up toward the frontal bone with the sinus cavity. The diffusely scattered light emerging out through the frontal bone with a fraction crossing the frontal sinus is detected by placing the detector on the frontal bone.

In Fig. 5(a) a CT image of the frontal sinuses can be seen of a patient participating in the clinical trial and in Fig. 5(b) balanced-detection signals for the left frontal sinus are shown. The signals correspond to an L_{eq} of 22 mm for oxygen and an L_{eq} of 123 mm for water vapor, which agree with the expected values reported in [12]. It was found that the ratio was ~ 0.18 . For air with 21% oxygen and water vapor at 27°C and 30% relative humidity, a ratio of 1.00 is expected with the definition chosen. However, the temperature within the body is $\sim 37^\circ\text{C}$ and the humidity is 100% due to water in liquid phase in the surrounding tissue. Both conditions (corresponding to enhancement factors of 1.8 and 3.3, respec-

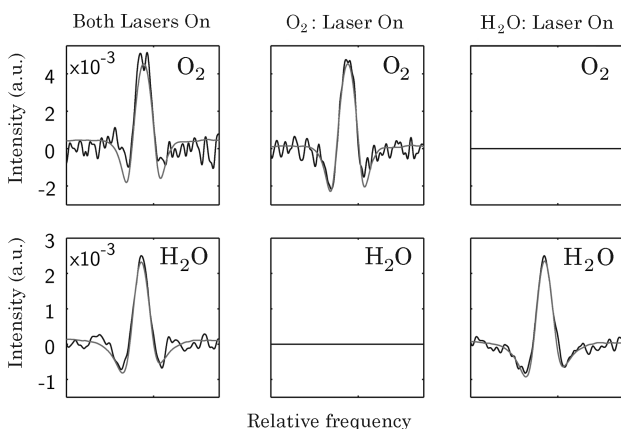


Fig. 4. Investigation of possible crosstalk between the two modulation frequencies used to detect two gases simultaneously. The balanced-detection signals are recorded from measurements over an air distance of ~ 25 mm. The balanced-detection $2f$ signals are shown (black line) together with a fitted experimental ideal line shape (gray line). The relative frequency range for both gases corresponds to 35 GHz.

Table 1. Average L_{eq} from Ten Recordings over an Air Distance of ~25 mm to Investigate Possible Crosstalk between the Two Modulation Frequencies Used to Study Two Gases Simultaneously^a

	L_{eq} (mm) O ₂	L_{eq} (mm) H ₂ O
Both Lasers On	23.5 ± 1.5	24 ± 1
O ₂ : Laser On	24 ± 1.5	—
H ₂ O: Laser On	—	26 ± 1

^aExamples of the detected signals used are provided in Fig. 4.

tively) will result in a reduction of the ratio by $1.00/(1.8 \times 3.3) = 0.17$. This is close to the value obtained for the sinus cavity indicating a close to ambient oxygen concentration (again, subject to the tissue property assumptions made).

At each occasion of measurements on patients during the clinical trial, a control recording is performed. The purpose of this control is to study any current offset of gas signals in the setup. During these measurements the fiber is placed on the inside of the cheek in the mouth and the light is detected on the outside of the cheek. This should result in non-detectable signals since no free oxygen or water vapor are expected in the tissue. In Fig. 5(c) recorded balanced-detection signals from the control performed just before the frontal sinus study are shown. As can be seen no offset of either gas could be detected.

2. Maxillary Sinus Investigation

For the maxillary sinus studies the optical fiber probe carrying the laser light to the sample is placed inside the mouth in contact with the palate in the area directly under the maxillary sinus investigated. The diffusely scattered light emerging out through the cheek bone with a fraction crossing the maxillary sinus is detected by placing the detector on the cheek bone [11,12].

In Fig. 6(a) the CT image of the maxillary sinuses can be seen for a different patient participating in the clinical trial, and in Fig. 6(b) the balanced-detection signals for the right maxillary sinus are shown. The signals correspond to an L_{eq} of 30 mm for oxygen and an L_{eq} of 185 mm for water vapor. The ratio of the gas signals are similar to the one recorded for the frontal sinus within the margin for error. The obtained balanced-detection control signals can be seen in Fig. 6(c). No offset of either gas was obtained.

Preliminary studies of the ventilation between the nasal cavity and the maxillary sinuses have also been performed. The nasal cavity on a healthy volunteer was flushed with pure nitrogen through the nostril for ~25 s while continuously measuring the gas composition within the sinuses. Signals were recorded before, during, and after termination of the flush. Each recorded signal was averaged for ~12 s. In Figs. 7(a) and 7(b) the measured L_{eq} for oxygen and water vapor, respectively, for one of the maxillary sinuses of the volunteer can be seen. In Fig. 7(c) the

ratio is shown and in Fig. 7(d) recorded oxygen and water vapor balanced-detection signals corresponding to indicated numbers in Figs. 7(a) and 7(b) are provided. A decrease of oxygen is recorded during the flush and the reinvasion of oxygen is observed after its termination. However, for the water vapor signal no noticeable change can be observed. This is not expected either since 100% humidity in the sinuses remains during the flush. For the recorded L_{eq} for oxygen during the time period no considerations have to be given with regards to the optical properties of the surrounding tissue since they remain unaffected. However, the water vapor signal is still advantageous to eliminate possible changes of recorded molecular oxygen signal due to movements of fiber and detector, and thereby different sampling volumes during the measurements. Thus the estimated ratio shown in Fig. 7(c) can be considered as a more reliable picture of how the oxygen concentration within the sinus changes than L_{eq} for

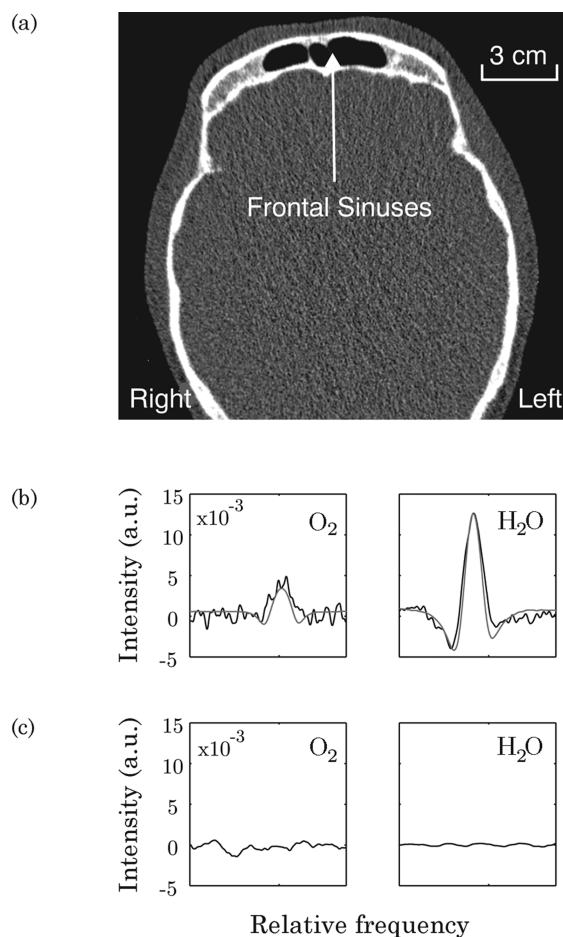


Fig. 5. (a) CT image of human frontal sinuses of a patient participating in the clinical trial; transversal section. (b) Corresponding recorded balanced-detection signals (black line) from the left frontal sinus of the patient with CT image shown in Fig. 5(a), together with fitted ideal signal (gray line). The oxygen signal corresponds to $L_{eq} = 22$ mm and the water vapor signal to $L_{eq} = 123$ mm. (c) Recorded balanced-detection control signal through the cheek. The relative frequency range for both gases corresponds to 35 GHz.

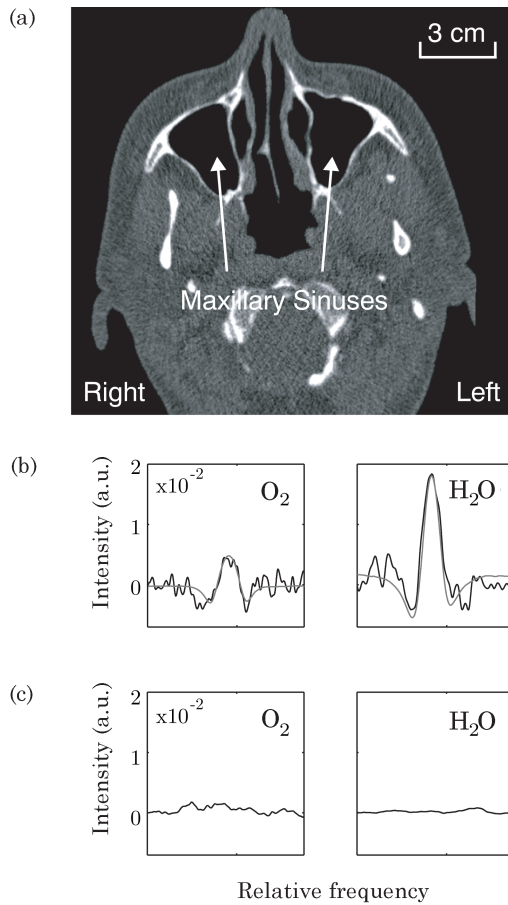


Fig. 6. (a) CT image of human maxillary sinuses of a patient participating in the clinical trial; transversal section. (b) Corresponding recorded balanced-detection signals (black line) from the right maxillary sinus of the patient with CT image shown in Fig. 6(a), together with fitted ideal signal (gray line). The oxygen signal corresponds to $L_{eq} = 30$ mm and the water vapor signal to $L_{eq} = 185$ mm. (c) Recorded balanced-detection control signal through the cheek. The relative frequency range for both gases corresponds to 35 GHz.

oxygen itself. The observations shown in Fig. 7 are typical for patients with good ventilation.

5. Conclusion

We show the feasibility to detect two gases simultaneously within the tissue optical window by applying wavelength modulation spectroscopy in a dual-diode laser setup. The technique is demonstrated on scattering and nonscattering absorbing samples. Two different modulation frequencies are used for two light sources to electronically frequency tag the two gases. The result shows no detectable crosstalk between the two gas channels interrogated simultaneously. Immunity to unwanted geometrical changes was also achieved. The detection limit was estimated by studying the noise floor of signals with no gas absorption. This resulted in a detection limit of approximately 6×10^{-5} for both molecular oxygen and water vapor. Lock-in detection is performed by using a lock-in toolkit provided by National Instruments. Fig-

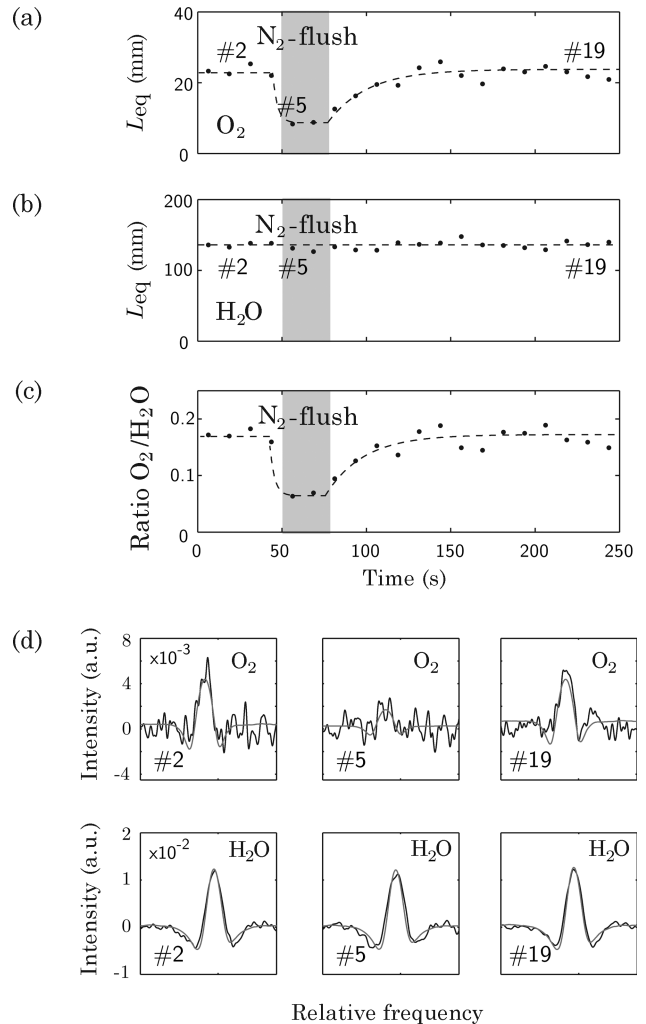


Fig. 7. Ventilation study between nasal cavity and maxillary sinus. (a) L_{eq} for oxygen (solid dots) measured as a function of time with N_2 flushing together with a curve drawn for guidance of the eye (dashed line). (b) L_{eq} for water vapor (solid dots) measured as a function of time with N_2 flushing. (c) Ratio between measured oxygen signal and water vapor signal (solid dots) as a function of time with N_2 flushing together with a curve drawn for guidance of the eye (dashed line). (d) Recorded balanced-detection $2f$ signal (black line) together with a fitted experimental ideal line shape (gray line). The numbers correspond to those indicated in (a) and (b). The relative frequency range for both gases corresponds to 35 GHz.

tailed lasers and fiber optics are used to guide the light from the lasers to the sample providing easy illumination of inaccessible locations. The system is made compact by utilizing a computer board to provide modulation signals imposed on the laser driver current and to collect the signals after interaction with the sample. The entire system is placed on a trolley (95 cm \times 45 cm \times 75 cm) making it possible to perform measurements outside the laboratory. Preliminary clinical results from gas measurements within the human frontal and maxillary sinuses recorded at the Lund University Hospital were obtained. In the initial clinical measurements substantial signal interference from different types of imaging equip-

ment typical for a radiological clinic was occasionally experienced. It should be noted that such an environment would not pertain to locations of intended future clinical use. A full analysis of the clinical data for all patients included in the study will be presented for the frontal and maxillary sinuses as well as for the mastoid bones (behind the ears) in future publications.

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