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Optical parametric oscillator-based photoacoustic detection of CO_2 at $4.23\ \mu m$ allows real-time monitoring of the respiration of small insects

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ABSTRACT A continuous wave, single frequency and continuously tunable optical parametric oscillator is used in combination with photoacoustic spectroscopy to detect trace emissions of CO_2 from insects under atmospheric conditions. The optical parametric oscillator (OPO) contains a periodically poled lithium niobate crystal and is tunable over the 3.9 to 4.8 μm infrared wavelength region. With the strong rotational-vibrational absorption band of CO_2 at 4.23 μm, it is possible to detect CO_2 down to 7 parts per billion volume using 20 mW of the OPO beam. This detection sensitivity was achieved by adding 4% of SF_6 gas to the atmospheric gas mixture to overcome the slow vibrational relaxation of the excited CO_2 levels. The usefulness of this system is demonstrated by real-time measuring of the fluctuations of the CO_2 concentration in the breath of a single ant (Lasius niger) and individual fruit flies (Drosophila melanogaster).

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1 Introduction

In the life sciences there is considerable interest in measuring minute quantities of trace gases released from biological samples. Trace gas detectors based on infrared laser spectroscopy are a good option, especially when fast on-line detection with a high time resolution is needed. Infrared laser-based trace gas detectors combine extremely high sensitivity and specificity with the possibility of rapid detection [1–3]. For example, using laser-based photoacoustic spectroscopy, concentrations can be measured for ethylene and acetaldehyde down to 10 and 100 parts per trillion volume (pptv), respectively [4, 5]. The performance of photoacoustic trace gas detection improves with higher laser power. That is why in the past high power gas lasers such as the CO_2, CO and CO overtone laser were used [1]. These lasers allow the detection of a large number of environmental, biological, and medical relevant gases, but their disadvantage lies in their limited (discrete line) tunability. A continuously tunable laser source would allow more gases to be measured with increased sensitivity combined with a better selectivity for gases that have an absorption line close to each other. Good examples of tunable, mid-infrared sources are quantum cascade distributed feedback lasers [6–8]. For photoacoustic spectroscopy, optical parametric oscillators (OPOs') are a better choice, because they combine a wide continuous tunability with a high output power [9].

Recently, we developed a continuous wave (cw), single mode, optical parametric oscillator able to generate high-power and widely tunable coherent radiation between 3.9 and 4.8 μm in the infrared wavelength region [10]. Amongst others, this wavelength region is very useful for the detection of CO_2 that has an absorption band near 4.234 μm [11]. Sensitive CO_2 detection allows on-line monitoring of small biological samples with a weight of only a few milligrams, providing cues for an understanding of the reactions of the metabolism in response to changing environmental conditions. Up to now the monitoring of small biological samples is limited by the sensitivity of commercial CO_2 detectors. Currently, the most sensitive on-line CO_2 detectors use direct absorption spectroscopy, employing a broadband infrared light source with an optical filter near 4.2 μm. This commercially available detector (Licor LI-7000) has a CO_2 detection limit of 90 parts per billion volume (ppbv) at a 2 seconds integration time. Here, by using a coherent light source instead of a broadband light source, we are able to improve this sensitivity by at least one order of magnitude.

Until recently, no suitable cw laser source with the required tunability and power has been available in this spectral range between 4 and 5 μm. Nonlinear frequency conversion based on optical parametric oscillation (OPO) is a promising method of infrared generation as wide tunability, high power, excellent stability and narrow linewidth are possible [12–17]. In this case, light from either a fixed frequency or tunable pump source is down converted in a nonlinear crystal into two tunable longer wavelengths, termed the signal and idler. The frequencies of the signal and idler sum to that of the pump and are typically tuned by varying properties of the nonlinear crystal such as temperature, angle, or in the case of quasi-

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phase matching (QPM) the crystal period is varied. For efficient continuous wave mid-infrared generation, quasi-phase matched periodically poled lithium niobate is an excellent choice. The high nonlinearity, broad transparency range and broad tunability make it ideal for many spectroscopic applications. High power (few watts) PPLN based sources have been demonstrated throughout the mid-infrared. However, operation is typically restricted to wavelengths shorter than 3.9 μm owing to intrinsic material absorption [17]. At longer wavelengths the transparency of lithium niobate decreases, which severely limits the generation of radiation [14, 18]. For pulsed operation, it has been shown that OPO operation is possible at idler wavelengths up to 7.3 μm despite the absorption loss in the PPLN crystal [19, 20]. However, for highly sensitive detection, pulsed lasers are not well suited due to their pulse-to pulse fluctuations, high intensities and saturation of the absorption. Furthermore, for high resolution spectroscopy cw excitation is usually preferred over pulsed excitation for its ability to use the narrow linewidth of cw lasers (~ 1 MHz). In continuous-wave operation, OPO generation of idler wavelengths above 3.9 μm has been developed only recently. Myers and Bosenberg [13] demonstrated that cw OPO operation is possible between 3.6 and 4.7 μm with very high pump powers, but their design was not suited for highly sensitive, spectroscopic applications due to multi-mode operation. For many spectroscopic applications, the OPO must have a narrow linewidth (< 10 MHz), good stability and broad tunability. Here we show that, using a suitable pump source, a successful attempt has been made to develop such an OPO operating at longer idler wavelengths.

2 Experimental setup

Our experimental setup is similar to the setup described earlier [16, 17]. However, for this setup the mirror and PPLN coatings have been adjusted to the new wavelengths (Fig. 1). In addition, a different PPLN crystal was used with poling periods ranging from 25.9 to 28.7 μm. As a pump source, we used an 11 W cw Nd:YAG source (Lightwave M6000) operating at 1064 nm. The generated signal and idler waves cover the wavelength ranges from 1370–1510 nm and 3620–4760 nm, respectively. Because the pump beam was single mode (single frequency) and the signal wave was constrained to a single frequency by an intracavity etalon, the idler frequency was, therefore, constrained to a single frequency which was determined by energy conservation between the pump and signal frequencies. For short frequency scans, the pump frequency was tuned over 24 GHz, which correspondingly tuned the idler wave over 24 GHz but in the opposite direction [16]. Pump tuning of the idler wave is an important advantage for mid infrared spectroscopic applications since the tuning is performed external to the OPO cavity and allows the use of sources for which tuning methods are well developed.

For wavelengths longer than 3.9 μm the absorption in the PPLN crystal becomes significant, causing a reduction in the idler output power. However, due to the high pump power the OPO can be operated from 3.62 μm (1.2 W output power) to 4.76 μm (120 mW) [10]. The decrease in output power with increasing idler wavelength is a direct reflection of the increased absorption in the PPLN crystal as a function of

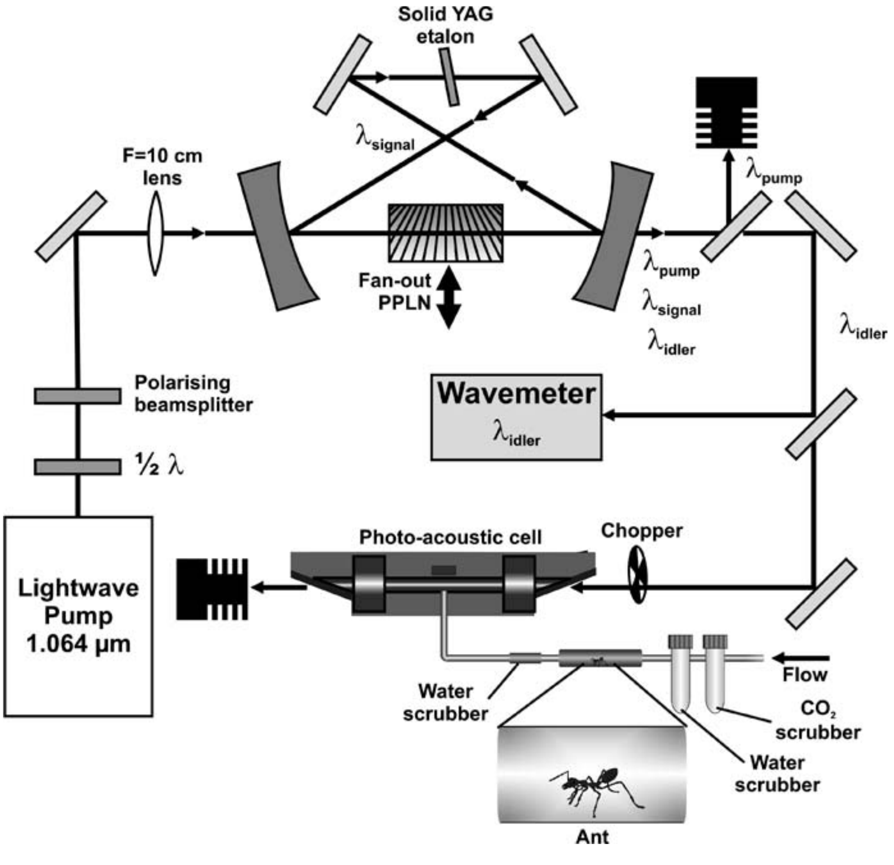

FIGURE 1 Experimental setup

wavelength. Despite the strong absorption and the subsequent thermal lens effect in the OPO crystal we observed a stable operation of the OPO, both in power and beam pointing stability. When we tuned the OPO with the intracavity etalon we observed strong output power fluctuations. We did not observe these effects when we tuned the idler frequency of the OPO by changing the pump laser frequency. In this scanning mode the OPO was stable, and we could observe a clear CO_2 absorption pattern [10].

The strongest CO_2 absorption is located at 4.235 μm (2361.47 cm^{-1}), which is well within the operating range of our OPO. At this wavelength, the OPO generates 110 mW of power. To ensure single-longitudinal-mode operation an intracavity etalon is placed into the OPO cavity, which reduced the output power further to 30 mW. In addition, the OPO setup was enclosed in a box and flushed with nitrogen gas to minimize additional losses due to the absorption by ambient CO_2 gas (ambient partial pressure 360 parts per million volume). In this way, the drop of power at the peak of the CO_2 absorption was limited to 30%, so 20 mW remains for the detection of CO_2.

3 Detection of CO_2

In order to determine the detection limit for CO_2 at these wavelengths, the absorption signal of a mixture of 7 parts per million volume of CO_2 in N_2 gas was measured using photoacoustic spectroscopy [1]. The photoacoustic effect is based upon conversion of electromagnetic into acoustical energy. Modulated infrared radiation is absorbed by a trace gas (here CO_2) in air or N_2. Through collisional relaxation, the periodically excited molecules transfer their vibrational energy to translational energy, which gives rise to a pressure modulation. The modulation frequency is adjusted to be equal to the lowest longitudinal resonance of the photoacoustic cell. The photoacoustic cell has a small inner radius (2 mm) and a length of 300 mm, which resulted in a resonance frequency of 560 Hz in air at STP conditions [1]. A small, highly sensitive electret microphone was used for detection of the acoustic signal. This, combined with resonant enhancement and ultranarrow bandwidth lock-in detection, enables ultra-sensitive detection. For the detection of CO_2 the OPO was scanned over the $R16$ and the $R50$ rotational lines of the ν_3 band at 2361.47 cm^{-1} and 2380.72 cm^{-1}, respectively. Their absorption strengths at the top of the absorption lines, according to the Hitran database are 365 $atm^{-1}\,cm^{-1}$ and 14.5 $atm^{-1}\,cm^{-1}$, respectively (Fig. 2) [11]. With a 0.3 s integration time the detection limit for CO_2 in N_2 was 88 ppbs (SNR $= 1$).

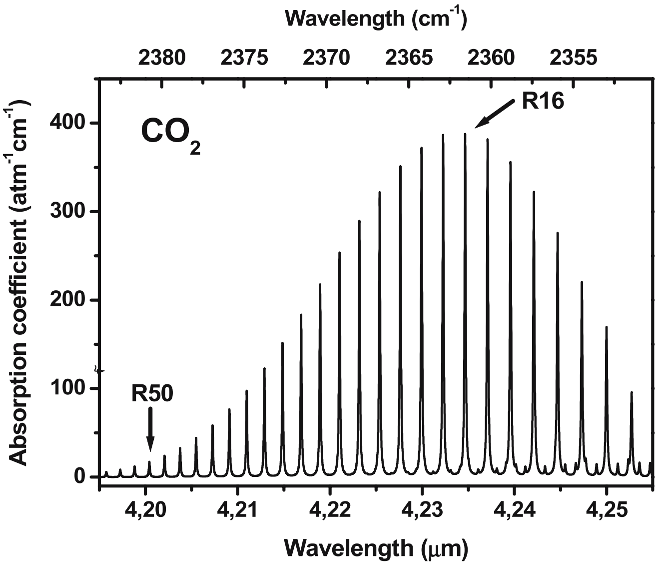

FIGURE 2 R-branch of the $\nu_3 = 1$ band of CO_2 at 4.2 μm. The $R16$ and the $R50$ rotational lines are indicated at 2361.47 and 2380.72 cm^{-1}, respectively. Their absorption strengths at the top of the absorption lines, are 365 $atm^{-1}\,cm^{-1}$ and 14.5 $atm^{-1}\,cm^{-1}$

Comparing these results with previous experiments detecting ethane [9], we expect at least an order of magnitude better absorption signal for CO_2. Furthermore, for CO_2 absorption a phase lag of the generated photoacoustic signal occurs, which indicates that kinetic energy is taken up by the molecules in the resonator at the modulation frequency, instead of modulated energy release. This effect is known as kinetic cooling, and is well documented [21]. In our case, the OPO excites CO_2 from the ground state to the $\nu_3 = 1$ state. Nitrogen has a near resonant vibrational level ($\nu = 1$ at a ΔE of $\sim 18\ cm^{-1}$) with the $\nu_3 = 1$ state of CO_2. Due to the overwhelming presence of N_2 in the air, all the energy will be transferred into the $\nu = 1$ state of N_2. For relaxation from this $\nu = 1$ state back to the ground state, a large quantity of energy must be transferred to translational energy by collisions. Due to this large energy gap, a low transfer probability in nitrogen exists in comparison with many other molecules. The nitrogen molecules at this $\nu = 1$ are metastable, and cannot readily lose energy radiatively, while their de-excitation lifetime via collisions is in the order of milliseconds [21]. The much larger number density of N_2 as compared to CO_2 creates a buffer of vibrational energy leading to an effective transient cooling of the translational degrees of freedom. Our chopping frequency of 560 Hz was too fast to observe the relaxation of N_2. Rather, we observed an inverse photoacoustic signal probably due to modulated cooling; this resulted in an amplitude decrease and a phase change relative to the signal caused by e.g., ethane in nitrogen gas. Water vapor is known to promote relaxation, and indeed by increasing the humidity in the CO_2-N_2 mixture the photoacoustic signal amplitude from CO_2 absorption was increased substantially and the phase lag was reduced. However, the use of water has disadvantages. Water vapor also absorbs in the OPO wavelength region, and its emission from biological samples is not always constant. For our application, in which we would like to measure the CO_2 emission from the respiration of small insect, the water emission and subsequently the concentration in the gas sample is changing periodically. This will have its effect on the photoacoustic detection of CO_2 from the same sample. To avoid this, we chose to add a small amount of SF_6 to the gas sample to ensure a stable photoacoustic detection of CO_2 independent of the water concentration in the sample. SF_6 does not have any absorption in this wavelength region, and due to its many vibrational modes it is known that it has a fast vibrational to translational relaxation [22]. In addition, SF_6 is chemically stable and not produced by any known biological system. Figure 3 shows the effect of the SF_6 concentration on the photoacoustic signal for the $R16$ and $R50$ CO_2 absorption lines. 4% of SF_6 added to the

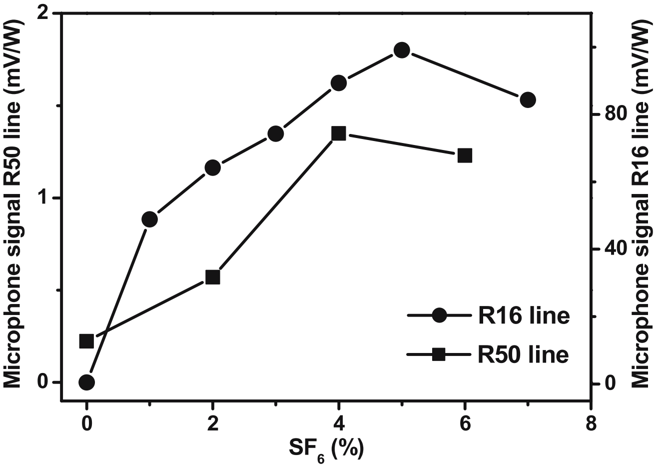

FIGURE 3 Increase of photoacoustic signal as function of the added SF_6 concentration. The effect is shown for the $R16$ and $R50$ CO_2 line. The size of the box is the error bar

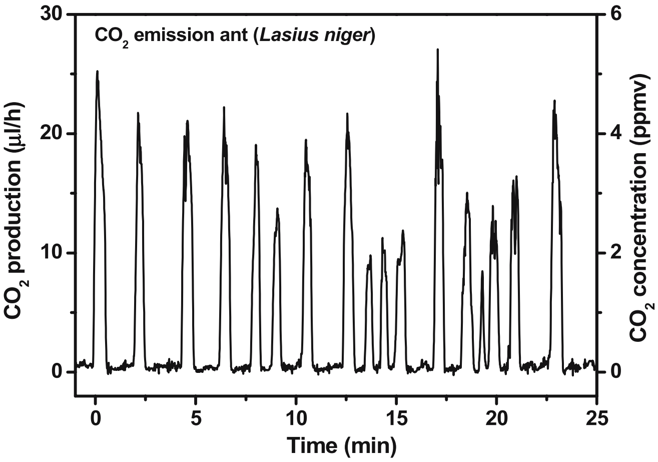

FIGURE 4 Real-time CO_2 emission of a small ant (Lasius niger, weight 3.7 mg). Analysis of the ants' respiration shows a periodic release of CO_2, on average every 90 s

gas mixtures was sufficient to recover the photoacoustic signal to the absorption values in the Hitran database, while the phase shift went back to zero. This lowered the CO_2 detection limit from 88 ppbv to 7 ppbv with a 0.3 s integration time.

4 Monitoring insect breath

To demonstrate the feasibility of an OPO based photoacoustic system for the real-time detection of trace amounts of CO_2, we examined the gas release during the respiration of small living insects. The insects were placed in a 2 cm long tube with 4.5 mm inner diameter (Fig. 4). Before air passed this sample volume, two scrubbers containing $CaCl_2$ and KOH, respectively removed water vapor and CO_2. After the sample volume a small scrubber removed water vapor introduced in the gas flow by the insect. For this experiment no SF_6 was added. The flow rate over the insect was 5 l/h, and since we introduced the trace gas flow at the centre of the resonator, the time response of the detector depended mainly on the volume of the resonator ($\sim$ 4 ml), which was determined to be 3 s. To determine the CO_2 concentration the OPO was set at the top of the ν_1 $R16$ CO_2 line (at 2361.47 cm^{-1}).

Insects take up O_2, and release CO_2 by breathing similar to vertebrates. However, insects do not possess lungs; they use a tracheal system for their gas exchange. This tracheal system consists of tubes connecting the various internal tissues via valves to the atmosphere. The valves are controlled by the ventral nervous system. Gas exchange between the tracheal system and the atmosphere could either take place by convection due to ventilation (like for mammals employing lungs) and/or by diffusion (no movement).

Figure 4 shows the periodic CO_2 release by a small ant (Lasius niger, weight 3.7 mg) over a time span of several minutes. Between the peaks, which reach maxima between 10 and 25 µl/h, a constant zero baseline could be measured. In the literature similar measurements have been shown with a decapitated and motionless Camponotus vicinis ant, which had a fresh weight of more than 100 mg [23]. These authors used a conventional Sable Systems TR-3 respirometry system (Sable Systems, Salt Lake City, USA) in order to study certain details of the so-called discontinuous gas-exchange cycle (DGC) [24, 25], which is characteristic for certain insects including ants. Such insects can control their gas exchange by opening and closing their spiracles, which control the connection of the insect's tracheal system with ambient air. During closing of the spiracles it has been assumed that the CO_2 release is zero. However, the determined detection limit in the above-mentioned publication did not allow detection of a CO_2 release at the closed-spiracle phase with sufficient resolution. In contrast to this commercial system, our setup did allow a quantitative determination of the CO_2 release during closed-spiracle phase, when using a free moving ant with a two orders of magnitude lower weight and with a time resolution of 3 s.

In a second experiment, we placed a single Drosophila melanogaster fruit fly (an Oregon R wild-type female with a weight of 1.4 mg) into the same setup (Fig. 5). CO_2 release from single Drosophila flies had been measured earlier [26] with the aid of a Licor LI-6251 infrared CO_2 analyzer. How-

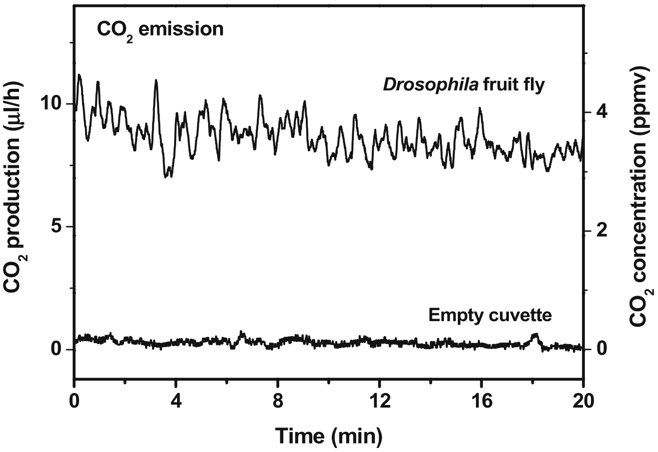

FIGURE 5 On-line detection of the CO_2 release of a fruit fly (Drosophila melanogaster, weight 1.4 mg). The comparison of the CO_2 trace from the fruit fly with the background (without fly) shows that the CO_2 release does not drop to zero. The accuracy of the CO_2 detection shows that the CO_2 peaks vary in height and frequency

ever, it remained unclear as to whether Drosophila flies exhibit a classical DGC, since the patterns of CO_2 release were rather irregular. Only Drosophila breeding lines, selected over more than a decade for desiccation resistance, showed a more regular pattern of CO_2 release. With our setup, we were able to obtain recordings of the CO_2 release similar to those published earlier [26], but with a better time resolution. In order to enhance the quality of the recordings 2% of SF_6 was added to the air passing by the Drosophila fly at a rate of 2.5 l/h. Our recordings show, that the pattern of CO_2 release by the fly is different from the classic DGC of the ant. Here, the CO_2 release does not drop to a close to zero base line. Rather, the Drosophila fly seems to enter a new CO_2 release cycle without closing its spiracles completely between the respiratory cycles.

There are various possibilities to improve the sensitivity of the system. For instance, by applying a different detection technique such as cavity ring-down spectroscopy [27]; or by using more powerful near infrared solid-state pump lasers. An example of a more powerful pump laser is the Versadisk (ELS GmbH, Germany), which has a slightly larger linewidth than the current pump laser, but can supply more than 20 W of radiation between 1020 nm and 1050 nm [28]. In a preliminary experiment, we have been able to generate 10 times more power at the maximum of the CO_2 absorption (200 mW including intracavity etalon). This increased the sensitivity by the same factor, pushing the CO_2 detection limit down to 0.7 ppb (with 4% SF_6 addition).

5 Conclusion

The high sensitivity and temporal response of photoacoustic spectroscopy, coupled with the ability to operate on fundamental molecular vibrational modes enabled by the use of this PPLN based OPO, makes this system ideal for life science applications. Other methods such as GC-MS (gas chromatography mass-spectrometry), while sensitive; do not have the required temporal resolution to observe such effects. If comparisons have to be made with established techniques such as direct absorption spectroscopy employing a broadband infrared light source, the OPO is much more complex. However, achievements have already been made to apply quasi-phase matched parametric light sources to practical infrared chemical sensing systems [29]. The use of vibrational relaxation by SF_6, coupled with operation on a fundamental vibrational mode, we derive a CO_2 detection limit of 7 ppbv, with a maximum OPO power of 20 mW. Preliminary measurements with a higher power system at this wavelength have shown that the detection limit can be reduced below the ppbv range. The ability to operate in the 3.9–4.7-μm region with continuous tuning and high power is unprecedented. This will allow access to a broad range of species, in their fundamental vibrational modes, thus expanding the range of applicability of the instrument. In addition, the newly developed laser system will open many new possibilities within molecular physics and physical chemistry such as molecular dynamics and chemical kinetics.

REFERENCES

1 F.J.M. Harren, G. Cotti, J. Oomens, S. te Lintel Hekkert, *Photoacoustic Spectroscopy in Trace Gas Monitoring*, In: *Encyclopedia of Analytical Chemistry*, ed. by R.A. Meyers (John Wiley, Chichester 2000) pp. 2203–2226
2 G. Berden, R. Peters, G. Meijer, Int. Rev. Phys. Chem. **19**, 565 (2000)
3 F.K. Tittel, D. Richter, A. Fried, Solid-state Mid-infrared Laser Sources, Topics in Applied Physics **89**, 445 (2003)
4 F.G.C. Bijnen, J. Reuss, F.J.M. Harren, Rev. Sci. Instrum. **67**, 2914 (1996)
5 F.G.C. Bijnen, H. Zuckermann, F.J.M. Harren, J. Reuss, Appl. Opt. **37**, 3345 (1998)
6 B.A. Paldus, T.G. Spence, R.N. Zare, J. Oomens, F.J.M. Harren, D.H. Parker, C. Gmachl, F. Capasso, D.L. Sivco, J.N. Baillargeon, A.L. Hutchinson, A.Y. Cho, Opt. Lett. **24**, 178 (1999)
7 D.D. Nelson, J.H. Shorter, J.B. McManus, M.S. Zahniser, Appl. Phys. B **75**, 343 (2002)
8 A.A. Kosterev, F.K. Tittel, IEEE J. Quantum Electron. **QE-38**, 582 (2002)
9 M. Van Herpen, S. Li, S.E. Bisson, F.J.M. Harren, Appl. Phys. Lett. **81**, 1157 (2002)
10 M. van Herpen, S.E. Bisson, F.J.M. Harren, Opt. Lett. **28**, 2497 (2003)
11 L.S. Rothman, A. Barbe, D.C. Benner, L.R. Brown, C. Camy-Peyret, M.R. Carleer, K. Chance, C. Clerbaux, V. Dana, V.M. Devi, A. Fayt, J.M. Flaud, R.R. Gamache, A. Goldman, D. Jacquemart, K.W. Jucks, W.J. Lafferty, J.Y. Mandin, S.T. Massie, V. Nemtchinov, D.A. Newnham, A. Perrin, C.P. Rinsland, J. Schroeder, K.M. Smith, M.A.H. Smith, K. Tang, R.A. Toth, J. van der Auwera, P. Varanasi, K. Yoshino, J. Quant. Spectrosc. Radiat. Transfer **82**, 5 (2003)
12 W.R. Bosenberg, A. Drobshoff, J.I. Alexander, Opt. Lett. **21**, 1336 (1996)
13 L.E. Myers, W.R. Bosenberg, IEEE J. Quantum Electron. **QE-33**, 1663 (1997)
14 P.E. Powers, T.J. Kulp, S.E. Bisson, Opt. Lett. **23**, 159 (1998)
15 M.E. Klein, C.K. Laue, D.-H. Lee, K.-J. Boller, R. Wallenstein, Opt. Lett. **25**, 490 (2000)
16 M.M.J.W. van Herpen, S. te Lintel Hekkert, S.E. Bisson, F.J.M. Harren, Opt. Lett. **27**, 640 (2002)
17 M.M.J.W. van Herpen, S. Li, S.E. Bisson, S. te Lintel Hekkert, F.J.M. Harren, Appl. Phys. B **75**, 329 (2002)
18 D.D. Lowenthal, IEEE J. Quantum Electron. **QE-34**, 1356 (1998)
19 P. Loza-Alvarez, C.T.A. Brown, D.T. Reid, W. Sibbett, M. Missey, Opt. Lett. **24**, 1523 (1999)
20 M.A. Watson, M.V. O'Connor, P.S. Lloyd, D.P. Shepherd, D.C. Hanna, C.B.E. Gawith, L. Ming, P.G.R. Smith, O. Balachninaite, Opt. Lett. **27**, 2106 (2002)
21 R.A. Rooth, A.J.L. Verhage, L.W. Wouters, Appl. Opt. **29**, 3643 (1990)
22 M.I. Buchwald, S.H. Bauer, J. Phys. Chem. **76**, 3108 (1972)
23 J.R.B. Lighton, D. Garrigan, J. Exp. Biol. **198**, 1613 (1995)
24 A. Kestler, *Respiration and respiratory water loss*, In: *Environmental Physiology and Biochemistry of Insects*, ed. by K.H. Hoffmann (Springer, Berlin 1985) pp. 137–183
25 J.R.B. Lighton, Physiol. Zool. 67 142 (1994)
26 A.E. Williams, M.R. Rose, T.J. Bradley, J. Exp. Biol. **200**, 615 (1997)
27 A. Popp, F. Muller, F. Kuhnemann, S. Schiller, G. von Basum, H. Dahnke, P. Hering, M.A. Murtz, Appl. Phys. B **75**, 751 (2002)
28 M.M.J.W. van Herpen, S.E. Bisson, A.K.Y. Ngai, F.J.M. Harren, Appl. Phys. B **78**, 281 (2004)
29 T.J. Kulp, S.E. Bisson, R.P. Bambha, T.A. Reichardt, U.B. Goers, K.W. Aniolek, D.A.V. Kliner, B.A. Richman, K.M. Armstrong, R. Sommers, R. Schmitt, P.E. Powers, O. Levi, T. Pinguet, M. Fejer, J.P. Koplow, L. Goldberg, T.G. Mcrae, Appl. Phys. B **75**, 317 (2002)