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Absolute, high resolution water transpiration rate measurements on single plant leaves via tunable diode laser absorption spectroscopy (TDLAS) at 1.37 μm

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Received: 21 January 2008/Revised version: 26 May 2008

Published online: 3 July 2008 • © Springer-Verlag 2008

ABSTRACT A new sampling-free and calibration-free multi-channel hygrometer using near infrared (NIR) tunable diode laser absorption spectroscopy (TDLAS) at 1.37 μm was developed and used to determine absolute transpiration rates of single plant leaves. Four $8 \times 6 \times 4 \text{ cm}^3$, fiber-coupled absorption cells are used to simultaneously measure absolute water vapor concentrations with an absolute accuracy of about 5% and a temporal resolution of about 2 s. Two chambers (BOTTOM, TOP) are directly attached to the leaf surface, while two chambers (IN, OUT) analyze the purge gas supplied to the plant leaf and the total outflow of the leaf chambers. The BOTTOM–TOP comparison provided a direct, leaf-side resolved ratio of stomatal conductance and—by taking into account the purge gas flow and the leaf area exposed—leaf side resolved water transpiration rates. The OUT–IN-difference yielded the total leaf transpiration rate with $2 \mu\text{mol}/\text{m}^2/\text{s}$ resolution. The new multi-point hygrometer was validated by monitoring of the transpiration dynamics of a plant of the species *Epipremnum pinnatum* (L.) Engl. during diurnal variation of the leaf irradiation. During these experiments the differential H_2O concentration resolution between two chambers was determined to be better than 3 ppm at $\Delta t = 2 \text{ s}$ (i.e. better than $711 \text{ ppb m Hz}^{1/2}$). This performance was verified by an Allan analysis over a 30 min time period using CH_4 as a surrogate absorber and yielded an average optimum optical resolution of 4.9×10^{-6} for 83 s measurement time, i.e. a CH_4 resolution of 892 ppb, which corresponds to the optical resolution needed for a water sensitivity of $454 \text{ ppb m Hz}^{1/2}$.

PACS 07.57.Ty; 42.62.Fi; 42.62.Be; 42.55.Px; 82.80.Gk

1 Introduction

The phytosphere as a main part of the biosphere plays a significant role for the atmospheric composition and thus for climatic processes. Even though plant transpiration, i.e. the emission of water from plant leaves, is one of the most important steps in the global water cycle, as 64% of the global rainfall returns into the atmosphere through evaporation and transpiration [1, 2], it is not fully understood. In

this context a more accurate understanding of plant transpiration, i.e. the coupling between atmosphere and phytosphere, is needed, to improve global water balance models, which, on the other hand, is very important for global hydrological cycle and climate modeling [3–5] as water is the most important natural green house gas. Thus the water transpiration of plants is of particular interest on all spatial scales, i.e. whole populations and complete plants as well as single leaves, and even down to the level of the stomata. Stomata are small ($15\text{--}30 \mu\text{m}$ lateral diameter) openings in the leaf surface through which water vapor, carbon dioxide, and oxygen is exchanged with the surrounding air [6–8]. Opening of the stomata is actively controlled by plant metabolism. Stomata conductance to water vapor greatly changes as response to environmental factors and the physiological status of the plant.

Gas exchange studies to determine plant transpiration parameters are realized via extractive measurements. Here a gas sample is taken and analyzed mostly by broadband infrared gas analyzers [9], which are the gold standard. On the single leaf scale, this is done with special, temperature and humidity controlled transpiration chambers, which enclose the leaf in order to enable an analysis of the inflow and outflow of the chamber, typically with a dual-channel infrared hygrometer connected to the chamber sampling lines. The surface averaged water transpiration rate (units of $\text{mmol of H}_2\text{O per m}^2$ and s) is determined by relating the total purge gas flow through the measurement chamber and the total leaf surface emitting into the chamber to the total water vapor concentration in the incoming and outgoing purge air supplied to the plant. Typically used sampling-based, extractive H_2O -vapor measurements are disadvantageous due to the strong adsorption of water: The transport of the sample gas to the measuring chamber within the hygrometer leads to problems with the absolute accuracy as well as the temporal response of such sensors. As a consequence they require a frequent and time consuming calibration, which is difficult as stable water vapor reference mixtures are not available but need to be generated near the instrument with additional equipment. Furthermore the common IR analyzers possess rather low spectral resolution, which leads to problems with spectral interference by atmospheric CO_2 . A time resolution, better than several tens of seconds, would be advantageous for many applications, e.g. the recording of fast and complex oscillatory transpiration

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patterns [10, 11] but is very hard to achieve with such extractive sensors.

Concerning the water vapor measurement on the single leaf scale, there are some more specific problems: The gas volume of interest surrounding the leaf is quite limited, so that absorption paths of only a few centimeters are possible. Simultaneously the expected transpiration rates are also quite small. Thus a high sensitivity is needed to isolate small variations on large absolute water background concentrations. The latter is needed to keep the plant healthy. Often the whole leaf is enclosed by the measurement chamber. A separation of the independent source terms from the top and bottom side of the leaf or localized measurement of isolated leaf areas is therefore not possible. Finally it is also desirable to measure close to the leaf surface to avoid boundary layer problems and to minimize the disturbance of the gas-column above the leaf.

These challenges have led to the development of our transpiration rate sensor. First of all the measuring technique should be able to detect the water vapor precisely and in situ, i.e. close to the leaf surface and without any gas sampling. The target value allows us to detect concentration differences with a resolution of only a few ppm on a background of 6000–30 000 ppm, which simultaneously requires a high dynamic range (ppm = parts per million = 10^{-6} mixture fraction). A temporal resolution of a few seconds is aspired. The measurement technique should be calibration-free, to avoid expensive and time-consuming sensor-calibration and to guarantee reproducible results with high accuracy. Taking into account, that the sensor should be adapted to single leaves, it also has to be lightweight, quite compact and should surround a small volume directly next to the emitting leaf surface. To separate it from the ambient atmosphere a closed system has to be realized. Small measurement chambers placed directly on the leaves avoid the gas exchange and permit independent measurement of both leaf sides, e.g. to study the side-resolved transpiration dynamics of hypostomatous, epistomatous or amphistomatous leaves, i.e. those which show stomata only on the lower or upper, or on both leaf sides. In the latter case it is quite interesting which fraction of the transpiration derives from the upper or lower leaf side.

Tunable diode laser absorption spectroscopy (TDLAS) in the near infrared (NIR) or mid-infrared (MIR) spectral region has been used to realize sensitivities close to the shot noise limit [12] and thus is a very promising technique to measure H₂O vapor. The application of TDLAS to water monitoring is very well documented (it is not possible to cover in this paper), but which indicates the high significance of TDLAS especially for water.

Taking advantage of the tremendous technical progress in near infrared (NIR) room-temperature diode lasers (DL) developed mainly for the telecommunication industry many of the TDLAS applications moved from the more traditional mid-infrared range into the NIR, to profit from the high spectral quality, simple operation and low costs associated with NIR-DLs, the excellent detector materials as well as the excellent optical transmission characteristics of glass fibers. While most NIR applications typically suffer to a certain extent from the relative weakness of the NIR molecular overtone and combination bands, the situation for the water molecule is different and by far not as severe. Here the H₂O overtone band

at 1.4 μm is only 2, respectively, 20 times weaker than the combination-band at 1.8 μm or the first fundamental oscillation band at 2.6 μm (see Fig. 3). Even the weaker band below 1 μm can provide sufficient sensitivity.

Hence it is not surprising that nearly all water absorption bands between 800 nm and 2.7 μm have been investigated for its suitability to humidity sensing. For in situ combustion monitoring in combustion processes with absorption paths of up to 20 m the weak 813 nm band was used for concentration measurements [13, 14] as well as for gas temperature monitoring by applying two line thermometry [15].

The stronger 940 nm transitions have, hence, been applied to a more sensitive detection of water [16], environmental monitoring with LIDAR systems [17] and in small laboratory burners [18]. The rather high 1.4 μm line strengths were soon exploited for real trace water detection mostly for environmental and atmospheric applications [19–22], but also for process monitoring, fire research [23], high temperature combustion environments [24, 25] or even for the isotopic analysis of water [26]. The 1.8 μm band offers rather powerful distributed feedback (DFB) lasers, widely tunable vertical cavity surface-emitting lasers, VCSELs, and relatively good extended InGaAs detectors, with recent applications in combustion diagnostics [27] and a planned laser hygrometer for a planetary mission on Mars [28]. But the lack of standard 1.8 μm fiber coupled components and the rather moderate increase in line strengths prevented up to now wide spread use of the band for laser hygrometry. The recent availability of 2–3 μm room-temperature diode lasers finally permits TDLAS-access to the strong fundamental vibrational H₂O transitions at 2.6 μm (see Fig. 1) which is quite promising for very compact or very sensitive diode laser hygrometers and would replace the rather problematic color center lasers, which have successfully demonstrated the high potential of that spectral range by permitting the first optical isotope ratio mass spectrometer [29]. First 2.6 μm TDLAS applications for spatially resolved water detection [30] or sensitive CO₂ detection [31] demonstrated the potential of the range, but significant problems with detector performance and efficient single mode fiber coupling still need to be solved. Hence a 1.4 μm TDLAS system was the optimum choice for our application so that we report here on a new, compact, fiber-coupled 1.4 μm TDLAS hygrometer that is able to determine absolute water vapor concentrations, non-invasively, calibration-free and at four spatially separated points simultaneously. The laser characterization, sensor design, its sensitivity and the performance of the different measuring channels will be presented and discussed. As an application the first double-sided, absolute in situ H₂O transpiration rate measurements on a single plant leaf will be described.

2 Experimental

For our concept and needs fiber-coupling is one of the most important demands and therefore we have chosen the 1.4 μm $\nu_1 + \nu_3$ -band for our application. Measuring at ambient pressures requires sufficient spectral line separation to minimize the disturbing influences of neighboring lines as far as possible. Using our new computerized line selection program [32] we isolated the (110 →

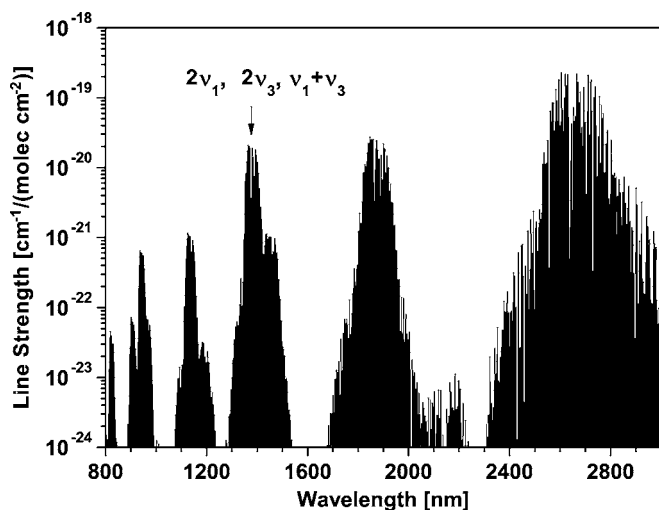


FIGURE 1 Line strengths of the water absorption bands between 800 and 3000 nm, according to HITRAN 2004. In this study we use the $2\nu_1$, $2\nu_3$, $\nu_1 + \nu_3$ overtone and combination band at 1.4 μm

211) transition at 7299.43 cm^{-1} (1369.97 nm) (line strength: $S = 1.005 \times 10^{-20} \text{ cm}^{-1}/(\text{molecule cm}^{-2})$, broadening coefficient $0.1032 \text{ cm}^{-1}/\text{atm}$ [33]) as one of the most suitable lines for this application.

This line is under the boundary conditions of the experiment free from possible spectral interference especially by CO_2 or other water lines. It has previously also been used for our open-path laser hygrometer to detect water vapor in cryogenic ice clouds with up to 15 ppb resolution [34–36] (ppb = parts per billion = 10^{-9} mixture fraction) or to quantify water vapor as well as liquid water mass fraction in dense water sprays during fire suppression experiments [23]. For the transpiration measurement we selected a typical telecom-packaged, single-mode fiber-coupled distributed-feedback (DFB) diode laser module (NEL) with up to 20 mW optical peak power (ex-fiber) and a typical laser line width of a few MHz, characterized its quasi-static thermal wavelength tuning behavior (Fig. 2) to $\partial\lambda/\partial T = 0.085 \text{ nm/K}$ and verified excellent coverage of the selected line as well as its nearest neighbors. Although the selected line does not represent the strongest line of the band we expect in the leaf scenario under ambient conditions at one volume percent water absorption of about 10% if we use an absorption length of 15 cm (see Fig. 2).

To evaluate the detector signal we use the principle of direct TDLAS which is based on the selective attenuation of laser light by the absorber molecule. In our case TDLAS can be described by an extended version of Lambert–Beer’s law:

$$I(\nu, t) = I_0(\nu)\text{Tr}(t) \exp(-S(T)\varphi(\nu - \nu_0)nL) + E(t).$$

With n being the number density of the molecular absorbers, $I_0(\nu)$ the initial laser intensity, and $I(\nu)$ the intensity detected after passing the absorbing medium of thickness L . The absorption line is characterized by the temperature dependent spectrally integrated line strength $S(T)$, and the normalized shape function $\varphi(\nu, \nu_0)$ of the absorption line, which is centered at the wavelength ν_0 [37].

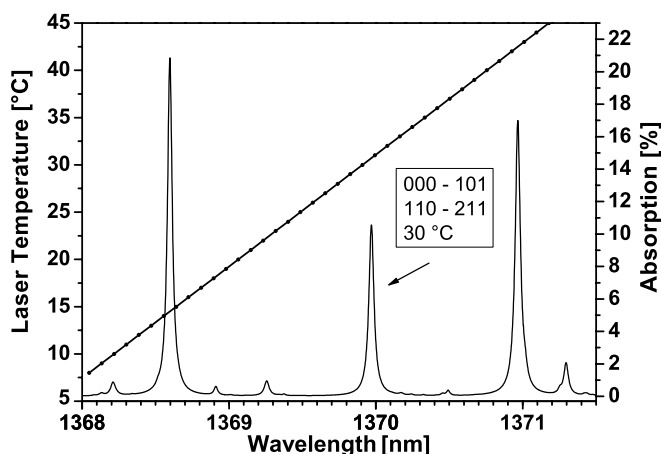


FIGURE 2 Comparison of the measured temperature tuning of the selected DFB-diode laser and the simulated H_2O water absorption spectrum around 1370 nm based on HITRAN2004 data. The ro-vibrational transition (110 \rightarrow 211) at 1369.97 nm generates for the typical plant leaf scenario (boundary conditions $p = 1 \text{ atm}$, $T = 296 \text{ K}$, $L = 15 \text{ cm}$ and $\text{c}_{\text{H}_2\text{O}} = 1 \text{ vol. \%}$) about 10% absorption

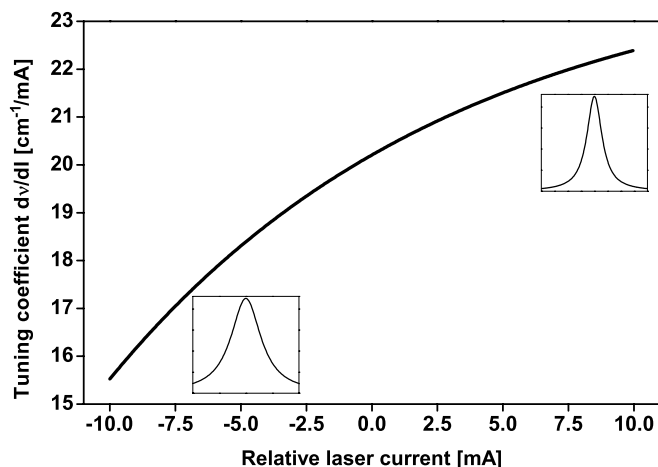


FIGURE 3 Experimental determination of the strongly nonlinear dynamic tuning coefficient $\partial\nu/\partial I$ of the 1370 nm diode laser of this study ($T_{\text{laser}} = 30.1 \text{ }^\circ\text{C}$, I_{laser} mean 44.77 mA, modulation amplitude/frequency of 1.7 V resp. 140 Hz). The line shape profiles in the insets depict the simulated effect of this tuning nonlinearity, i.e. a strong apparent change of the line width, hence, line area, which would lead to systematic errors of about 30% if not, it is not corrected for by the fitting routine

Using this principle we initially developed in situ laser absorption spectrometers for the harsh boundary conditions e.g. in combustion chambers of power plants [38–40] or in fire suppression experiments [23, 41]. Here, disturbances like thermal background radiation, $E(t)$, from particles or furnace walls can increase the detector photo current while dust, soot particles, or beam steering due to refractive index fluctuations lead to severe, time dependent variations of the background transmission, $\text{Tr}(t)$, of the measurement path. All have to be controlled and corrected by an adapted, highly robust, data evaluation software [38, 42].

For the leaf transpiration spectrometer the measuring environment is, on one hand, much less critical, and problems like misalignment or mirror degradation seem easy to handle. On the other hand it has to be kept in mind that the sensitivity requirements are one or two orders of magnitude higher

than in combustion applications. By adapting our data evaluation software to the transpiration measurements, we ensured excellent robustness and stability of the system. Resolving the extended Lambert–Beer’s Law to the absorber density n ,

$$n = -\frac{1}{S(T)L} \int \ln \left(\frac{I(v, t) - E(t)}{I_0(v)\text{Tr}(t)} \right) \frac{\partial v}{\partial t} dt$$

it becomes apparent that this method does not require calibration with a reference gas, as all quantities on the right-hand side of the equation can be determined either from the detector signal (i.e. $E(t)$, $\text{Tr}(t)$, $I(v)$, $I_0(v)$), from a spectroscopic data base like HITRAN [33] ($S(T)$) or from supporting measurements of the experimental boundary conditions (L , p , T , and the dynamic tuning coefficient $\partial v/\partial t$, which will be explained below).

This calibration-free property was recently successfully verified by a direct experimental comparison of an absolute TDLAS-based open path measurement of the atmospheric CH_4 background with a gas chromatograph, calibrated to NOAA (National Oceanic and Atmospheric Administration) precision reference gases [43, 44]. No calibration procedure was used for the TDLAS spectrometer. Relative systematic differences on the order of only 0.5% corresponding to an absolute bias of only 10 ppb CH_4 were found over several measurement periods of 24 h and more.

It is also obvious that the accuracy of the line strength S affects the quality of the measurement. Unfortunately, despite its usefulness, the HITRAN database may show relative errors of up to 20% for $S(T)$ [45]. Thus we also determined in a previous effort [46] the absolute line strengths for the 1370 nm line and confirmed the HITRAN values within an error margin of 3%.

Another important factor needed for an absolute concentration measurement is the dynamic tuning $\partial v/\partial t$ of the diode laser (Fig. 3), which is necessary to convert the detector signal measured in the time domain onto a linear spectroscopic wavelength axis, such as wave numbers or frequency.

Only then can one correlate the absorption line area, which is derived from the detector signal using a line fitting algorithm, with the tabulated integrated line strength S . This

nonlinear laser tuning behavior depends on the laser working point (i.e. laser current and temperature, modulation-amplitude and modulation-frequency) and needs to be determined with comparable precision like the line strength. By evaluating the measured frequency dependent transmission through an air-spaced Fabry–Pérot etalon [47, 48] we were able to determine the dynamic tuning coefficient $\partial v/\partial t$ with a resolution and with an error of 0.5%, which was verified from repeated measurements of the same laser. The importance of $\partial v/\partial t$ can be explained with the tuning curve in Fig. 3 by comparing the deformation of a molecular absorption line placed on the extreme ends of the laser scan. As indicated by the strong changes in line width and area (see insets in Fig. 3), the tuning non-linearity would cause signal deviations on the order of 30% if we would ignore the non-linearity and assume linear laser tuning, i.e. constant $\partial v/\partial t$.

The complete experimental setup of the leaf transpiration spectrometer is shown in Fig. 4.

The central part is the diode laser which was used to detect the $110 \rightarrow 211$ transition of the $\nu_1 + \nu_3$ -band at 7299.43 cm^{-1} . The laser contained in a telecom style laser module was temperature stabilized with a Peltier element and both powered via a combined diode laser/Peltier driver/temperature controller. A function generator supplied a triangular scanning modulation (140 Hz) to tune the laser across the absorption line. The laser light of the fiber-coupled laser module was guided via a 1×4 fiber optical splitter to the four independent measurement chambers (IN, TOP, BOTTOM, OUT) and here in each chamber directed onto the biased InGaAs detectors using a spherical 1" mirror. Low-noise current preamplifiers (500 kHz bandwidth) were used to convert the detector signals before they were fed in a AD-converter card (16 bit, 1.25 Megasamples/s) plugged into a personal computer.

Each measurement chamber consisted of a compact $8 \times 6 \times 4 \text{ cm}^3$ (length–width–height) sized aluminum housing containing a single mode fiber adapter, the folded absorption path ($L = 15 \text{ cm}$) the InGaAs detector and two gas supplies. To avoid etalons the number of light-transmissive optical surfaces was reduced to a minimum and only the spherical gold-coated 1" mirror was used to direct the light towards the detector. The gas temperature in each chamber is deter-

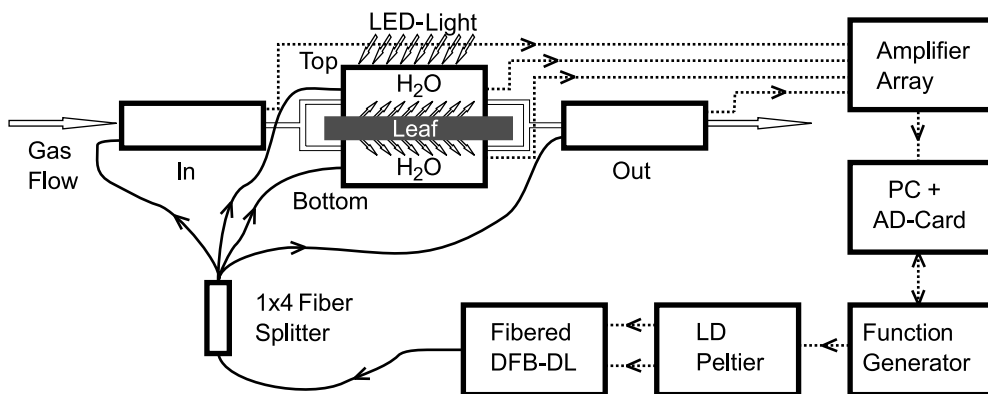


FIGURE 4 Setup of the 4-channel TDL hygrometer for absolute leaf transpiration measurements: The laser was powered and stabilized by a combined diode laser-Peltier driver/temperature controller and modulated by a function generator. The light was guided to the measurement chambers via a 1×4 fiber splitter module. The signals of four biased InGaAs photodiodes were amplified by current preamplifiers respectively, recorded and analyzed with an AD-Card assembled in a personal computer. The incoming humidified gas flow is analyzed, split and guided to the top and bottom leaf chambers, merged again behind the leaf chambers and passed through the output analyzer. Each sensor is determining absolute water vapor concentrations independently

mined by a fine-tipped thermocouple. The internal pressure is measured only in the first chamber as calculations have shown that the pressure drop inside the flow system is less than 1 mbar and therefore negligible. A calibrated membrane pump with an integrated moisturizer supplied the gas flow which was regulated with a 0.8–2 l/min flow controller. The incoming flow was analyzed for its humidity in the first measurement chamber (IN) and then evenly distributed on the two leaf chambers (TOP, BOTTOM) which were open on one side to be directly attached to the leaf surface. Additionally the top chamber was equipped with a large fused silica window to permit direct irradiation of the leaf surface to trigger the photosynthesis. The flows from both leaf chambers were merged again and analyzed in the fourth chamber (OUT) to determine the total amount of water emitted from the leaf.

A typical unprocessed detector signal from one of the measurement chambers is shown in Fig. 5.

Clearly visible is the dominating base-line slope due to the amplitude modulation of the laser caused by the current tuning. This base-line structure has to be removed precisely to extract the absorption line area. This is accomplished by a Levenberg–Marquardt-based fitting algorithm applying a signal model comprised of a 2nd order polynomial baseline and a Voigt line shape [49, 50]. Before the fit the raw signal has to be transferred from time to wavelength space using the measured dynamic tuning coefficients of the laser. The line area determined by the fitting procedure are converted into absolute water vapor concentrations applying the extended Lambert–Beer’s law, tabulated HITRAN 2004 line strengths and measured gas temperatures and pressures, assuming an ideal gas relationship. The extracted water absorption line (Fig. 6) shows excellent agreement with the fitted line model.

The residual between the Voigt fit and the measured profile is used to estimate the short term resolution of our spectrometer. During the plant transpiration measurements, we typically averaged 20 consecutive absorption scans (up-ramp only) which permitted a temporal resolution of about 1.1 s (0.9 Hz).

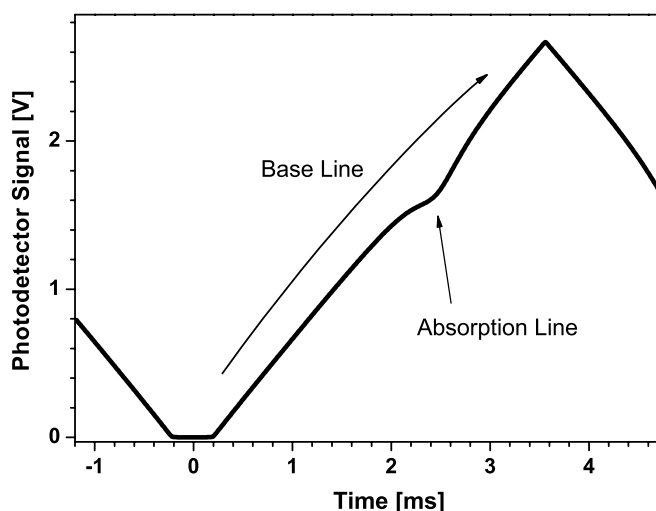


FIGURE 5 A typical unprocessed detector signal during a water vapor measurement at 1370 nm showing the strongly sloping background base line, caused by the laser current modulation with a triangular function, and the molecular absorption signal caused by the water molecule

This corresponds to a rather small duty cycle of only 12.8%, which was caused by the slow, multiplexed AD-card.

Under these conditions our residuals typically show an integrated standard deviation (1σ) of 1.6×10^{-4} OD (OD = $-\ln(I/I_0)$). With a peak absorption signal of 0.11 OD, we thus derive a 1σ signal to noise ratio (SNR) of 700 or a “scan-derived” H_2O resolution of 12.4 ppm, i.e. a normalized H_2O detectivity of 2 ppm $\text{m Hz}^{-1/2}$. As shown in the residual, there is no clear indication of spectral fringing. Furthermore the main residual structure proved to be quite stable in time, so that we attributed it to artifacts caused by the AD converter.

To determine a more realistic value for the resolution, investigate the long term spectrometer behavior and stability and determine the optimum averaging time and optimum spectrometer performance we applied the concept of the Allan-deviation [51]. This requires that a constant water vapor concentration is maintained in the measurement cells over a time span of about 30 min. As the gas conditioning and humidifying equipment used for the transpiration measurements turned out to be by far not stable enough for such measurements we decided to determine the *optical resolution* of our spectrometer by using a CH_4 calibrated gas mixture as a surrogate gas absorber and a 1.65 μm diode laser module and then calculate the concentration resolution from simulated H_2O and CH_4 spectra based on HITRAN data assuming that the concentration resolution will scale with the ratio of the $\text{CH}_4/\text{H}_2\text{O}$ absorption coefficients. To justify that approach, we only changed the laser modules, while the rest of the spectrometer as well as the boundary conditions – pressure and temperature – remained completely unchanged, i.e. no optics or detectors were changed or realigned. The lasers were both high quality InGaAsP telecom lasers from the same manufac-

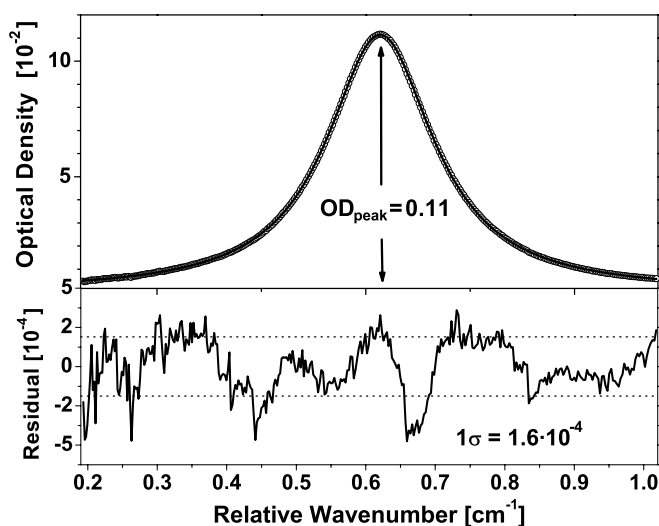


FIGURE 6 Top: Processed 1370 nm H_2O -absorption signal (average over 20 consecutive scans, i.e. 0.9 Hz concentration rate) from Fig. 5 after removal of amplitude modulation, corrections for transmission fluctuations and offsets and projection on a linear wavelength scale. Perfectly superimposed is the Voigt line shape model matched to the measured signal using a non-linear fitting algorithm and used to extract the absorption line area. Bottom: The effective optical resolution is estimated from the residual between model and measurement and quantified by the residual’s standard deviation (1σ) (here: 1.6×10^{-4} OD); from σ and the peak OD of 0.11 (corresponding to 8710 ppm) we deduce a “single fit” signal to noise ratio of 700 or a H_2O resolution of ± 12 ppm

Sensor number	1	2	3	4	Average
Optimum number of averages	110	140	95	120	116
Measurement time [s]	79	100	69	86	83
Optimum optical resolution [OD]	4.1×10^{-6}	4.4×10^{-6}	5.2×10^{-6}	5.9×10^{-6}	4.9×10^{-6}
Optimum CH ₄ resolution [ppb]	730	810	950	1080	892
Estim. opt. H ₂ O resolution [ppb]	272	301	353	401	332
Normalized H ₂ O resol. [ppb m Hz ^{1/2}]	362	452	439	556	454

TABLE 1 Performance intercomparison (at ambient conditions) of the measurement chambers using the Allan deviation approach from Fig. 7 with a certified CH₄ gas mixture as a surrogate gas and estimating the H₂O performance assuming an unchanged optical resolution

turer, with identical specifications (except laser wavelength), built up in the same butterfly module with the same fiber-optical connections. The intention was to minimize the contributions of the reference gas instabilities to the Allan deviation and only determined the spectrometer stability.

The Allan deviation plot shown in Fig. 7 is the result of such a measurement. CH₄ reference gas mixture (10 100 ppm \pm 2%) was supplied with a flow of about 0.2 l/min over a time period of 30 min to all four chambers while the absorption signals were recorded. With an identical laser scanning frequency of 140 Hz, we averaged 10 consecutive up-scan absorption profiles yielding a 1.8 Hz measurement frequency. During the 30 min measurement period gas temperatures/pressures were constant within 0.2 K and 0.5 mbar, respectively, but still taken into account in the data evaluation. The absolute, average CH₄ concentration extracted without any calibration was 9903 ppm and thus within the \pm 2% error margin, again validating our calibration-free approach. A minimum for the CH₄ concentration variance was found for averaging 140 to 500 absorption profiles. Averaging 140 absorption profiles, i.e. 1400 individual absorption scans taken in 100 s measurement time, an optimum CH₄ resolution of 810 ppb was determined. Using HITRAN data, one can convert this into an excellent optical resolution of 4.4×10^{-6} OD, which is comparable with standard $2f$ data evaluation schemes but offers the important advantage of an absolute, and calibration-free gas concentration measurement.

Assuming that the optical resolution of 4.4×10^{-6} OD will remain unchanged when switching the laser modules, we can convert the CH₄ into a H₂O resolution using simulated CH₄ and H₂O spectra for these boundary conditions. This procedure yields for this chamber an estimated optimum water vapor resolution of 301 ppb at 100 s measurement time (Table 1) or 452 ppb m Hz^{1/2}. It should be noted that the laser was not stabilized except the standard Peltier-based temperature controller; no line locking was used.

The significant improvement to the “single fit” optical resolution may be explained by the fact that the large baseline structures are temporarily very stable and thus don’t contribute to the noise of the absorption signal but only influence the absolute concentration accuracy at a level at least 10 times above the noise. Further experiments to verify this H₂O resolution using a certified, high quality permeation sources are in preparation.

We also compared the individual performance of the four separate measurement chambers (see Table 1) by operating them in parallel at identical conditions as mentioned above. Optimum averaging numbers ranged between 95 and

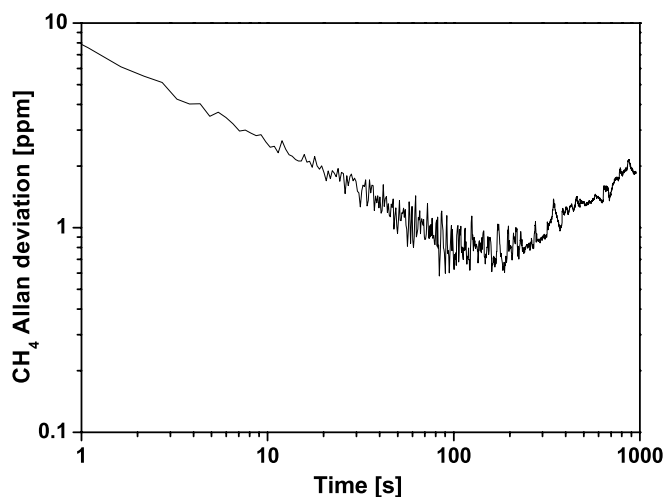


FIGURE 7 Allan deviation of the measured CH₄ signals determined for the BOTTOM chamber from a 30 min long data set measured with a reference gas (10 100 ppm). The optimal spectrometer performance is achieved by averaging 140 scans yielding an optical resolution of 4.4×10^{-6} OD corresponding to 810 ppb CH₄

140 and yielded comparable optical resolutions from 4.1 to 5.9×10^{-6} OD (average 4.9×10^{-6} OD) which were derived from the CH₄ concentration variances (730 to 1080 ppb – 892 ppb average). Using HITRAN simulations these were converted to expected optimum H₂O measurement resolutions of 272 to 401 ppb – average 332 ppb, and 362 to 556 ppb m Hz^{1/2} – average 454 ppb m Hz^{1/2}, respectively. It should be mentioned that due to nonlinearities of the data acquisition the measurement time is reduced to 64 s (four chambers average) if the averaging of the absorption scans is applied during the acquisition.

Finally we calculated the overall error budget of the sensor signals to give an indication on the absolute H₂O concentration accuracy achievable without a calibration procedure. Assuming absolute temperature and pressure uncertainties of 0.2 K and 1 mbar, we calculate the following contributions to the total relative uncertainty: Linestrength S 3%, temperature 0.1%, temperature dependence of S 0.3%, pressure 0.1%, absorption length 2%, tuning coefficient 0.5%. Hence the total absolute accuracy of our water vapor measurement is on the order of 5% and mainly dominated by temporarily invariant line strength and path length errors.

3 Transpiration measurements

As a first application of our fast, fiber-coupled, multi-point absolute water sensor we investigated the dynam-

ics of plant transpiration on the single leaf scale. For the initial experiments we selected a tropical plant of the species *Epipremnum pinnatum* (L.) Engl. and determined the absolute water emission dynamics during variable light stimulus to modulate photosynthesis (see Fig. 8). The experiments were performed in a laboratory environment. A single leaf (size $\sim 10 \times 8 \text{ cm}^2$), was clamped between top and bottom chamber. To prevent leakages at the leaf–chamber–interface a water-free sealing compound designed for medical applications was used. The leaf was not removed from the plant to minimize the physiological changes of the plant. A light stimulus was provided by a LED array which emitted sufficiently high light intensities especially at wavelengths of 430–460 and 640–670 nm to drive photosynthesis, while simultaneously avoiding excessive heating of the leaf or the measurement chambers.

As soon as photosynthesis is activated by light, the green photosynthetic tissue within plant leaves fixes gaseous CO_2 . As a consequence the leaf internal CO_2 concentration decreases and the stomata open to allow diffusion of CO_2 from the surrounding air into the leaf tissue and compensate the CO_2 consumption; as an unavoidable consequence the plant transpires, i.e. it loses water through the open stomata. To balance the necessary uptake of CO_2 and to prevent excess water loss, stomatal opening is precisely controlled by physiological reactions meant to ensure plant health, (see Fig. 8). The light induced transpiration modulation is then clearly detected using the laser absorption chambers on the top and bottom side of the leaf. No gas sampling is needed as the absorption path is right next to the leaf surface.

Similar to the natural scenario the leaf is irradiated from the top through a fused silica window integrated into the housing of the top chamber. The temporal history of the absolute water concentration in ppm in each measurement chamber is displayed in Fig. 8 over two night–day (light on/light off) periods.

During the dark periods when almost no water vapor is emitted by the leaf all measurement chamber show precisely congruent signals with offsets in the single digit ppm range.

No scaling or correction of the individual chamber signals was used, proving that all sensors were working well in the calibration-free mode, simultaneously showing the same concentrations, temporal resolution, and absolute accuracy. This feature was essential as it was the key for an excellent common mode suppression of the strongly drifting humidifier, which failed to provide a constant humidity in the feed air. Subtracting the water vapor concentration of the input chamber (IN) from the TOP, BOTTOM and OUT measurement chambers, respectively, (see Fig. 9 left scale), yielded the effective transpiration of each side of the leaf as well as the total transpiration. During the dark period the concentration noise (1σ) in these referenced signals was between 2 and 3 ppm, i.e. 474 to 711 $\text{ppb m Hz}^{1/2}$. Taking into account that this differential signal contains the noise of two sensor chambers, we should expect about a factor of $2^{1/2}$ better values for the unreferenced chambers, i.e. 360 to 560 $\text{ppb m Hz}^{1/2}$ which is in very close to the values determined from the CH_4 Allan experiment. Furthermore, due to the good time resolution, we could precisely determine the delay (5 min) between the light stimulus (LEDs on) and the stomata opening. Even small changes in the dynamics of the opening process would be detectable. In contrast to standard leaf chambers enclosing the full leaf, we could also demonstrate that the “double-sided” measurements with two independent chambers on both sides of the leaf not only show that the plant under investigation – as most plants – has hypostomatous leaves [7, 52] (i.e. that most stomata are located on the leaf bottom side). We could also quantify the 1 : 40 ratio of the top and bottom emission, which is a good indication for the ratio of the stomata spatial density on the leaf.

By taking into account the gas flow of 1 l/min and the effective leaf-area open to the sensor chambers of $4 \times 6 \text{ cm}^2$, we could precisely determine the total, leaf integrated, as well as the side resolved transpiration rate. As depicted in Fig. 8, transpiration was highest during late morning and midday when biochemistry and physiology of photosynthetic carbon uptake is fully activated and declined during the afternoon, indicating a present underlying endogenous diurnal control of

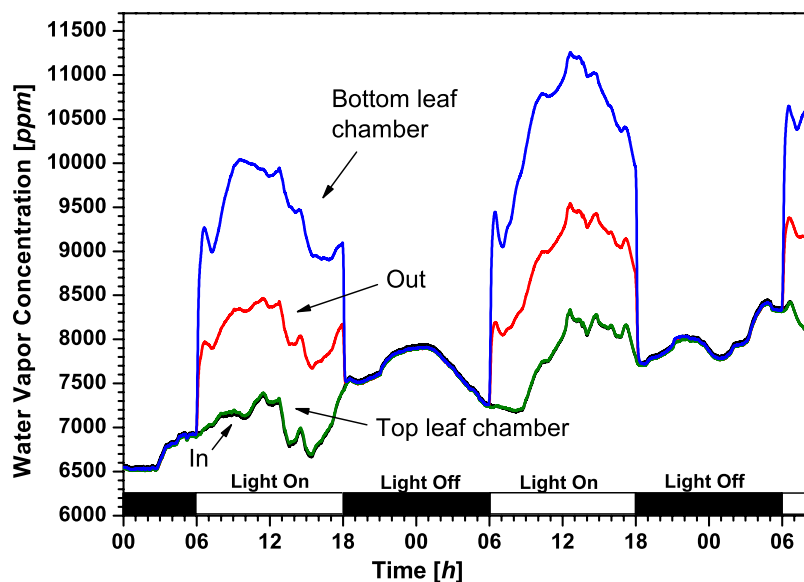


FIGURE 8 Typical H_2O vapor concentration time histories of a single plant leaf over a period of 2 days as measured with the four channel laser hygrometer during a physiological transpiration measurement with diurnal light variation. A strong drift of the purge gas humidifier is evident but very effectively suppressed due to the good absolute performance of the individual chambers. Note: No scaling of the signals is used to match them during the dark phase. The absolute water concentration in ppm is displayed for each channel with a resolution of 3 ppm respectively

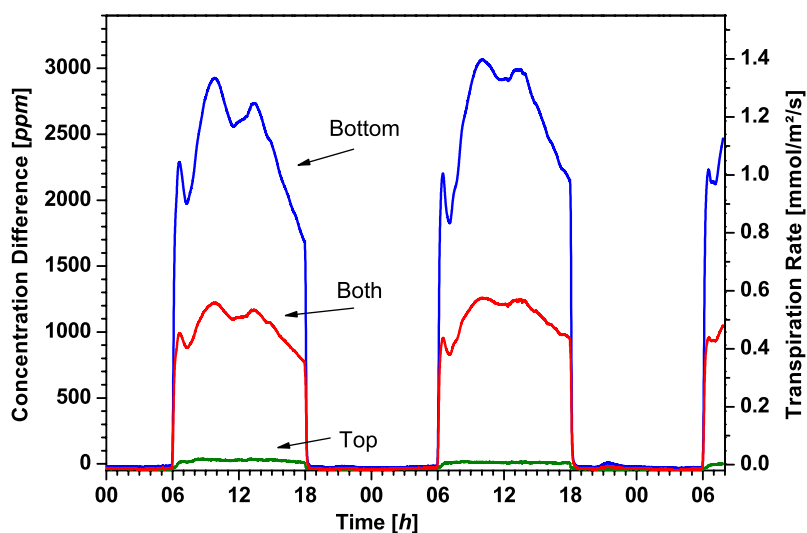


FIGURE 9 Concentrations differences to the incoming purge gas (*left scale*) and transpiration rates (*right scale*) in each measurement chamber, showing nicely that transpiration is triggered by the light stimulus on the leaf, as the plant has to open the stomata to get the CO_2 needed for photosynthesis. The transpiration ratio between top and bottom chamber reflects the different stomata densities on both sides of the leaf. The transpiration rates are measured with the laser hygrometer with a resolution of $2 \mu\text{mol}/\text{m}^2/\text{s}$ at a temporal resolution of 2.5 s

photosynthesis [7]. The comparably great afternoon decrease in transpiration is most likely a response of low light adapted plants that are exposed to high irradiance during measurements. Physiology of photosynthesis generally acclimates within a few days to a sudden transfer to high light intensities and can show signs of down-regulation because of high-light stress in late light period. This is supported by higher overall rates and a less pronounced afternoon down regulation at day 2. The respective transpiration dynamics showed the expected typical physiological response with a peak value of up to $1.4 \text{ mmol}/\text{m}^2/\text{s}$, which we determined with a transpiration rate resolution of $2 \mu\text{mol}/\text{m}^2/\text{s}$ ($\text{SNR}_{\text{max}} = 700$) and a 2.5 s temporal resolution.

4 Conclusion

In conclusion we developed a new type of a very compact, fiber-coupled, multi-point TDLAS based hygrometer which is able to determine absolute water vapor concentrations at four locations without the need for gas sampling or sensor calibration. Using a $1.37 \mu\text{m}$ NIR diode laser, we achieved with as little as 15 cm absorption path in a special dual path optical absorption cell and about 70 s measurement time an optimum optical resolution of 5×10^{-6} as determined by an Allan deviation measurement with CH_4 surrogate gas. This multi-point sensor was applied to enable the first, TDLAS-based, absolute and sampling-free, high-resolution measurements ($2 \mu\text{mol}/\text{m}^2/\text{s}$) of the plant transpiration dynamics on the single leaf scale, and the first side-resolved measurement of the leaf transpiration. The differential sensitivity of the spectrometer between two chambers corresponded to a concentration resolution of 2–3 ppm at 2 s temporal resolution. For a slower time response of about 70 s, we demonstrated a CH_4 concentration resolution of 900 ppb, which can be converted into a 350 ppb water resolution. Compared to previous transpiration based sensors the new approach provides higher temporal as well as concentration resolution, better absolute accuracy and is much easier to maintain. The unique compactness, the low weight and the high channel number possible will therefore open up new possibilities in the surveillance of the plant transpiration be-

havior, like the simultaneous comparison of multiple leaves or multiple plants. With the use of recently available $2.7 \mu\text{m}$ diode lasers it should even be possible to detect the CO_2 consumption of plants [53] on the single leaf scale by using miniaturized multi pass cells. Using such $2.6 \mu\text{m}$ lasers, we already realized first experiments towards a spatially resolved water detection on the single leaf scale to resolve spatial variations in the leaf transpiration also known as patchy stomatal conductance [54].

In the future the fiber coupled sensor architecture will be expanded to permit low-cost distributed sensor networks with absolute measuring capabilities and excellent sensitivity. Through the availability of high quality fiber couplers and the high laser power available channel numbers of up to 128 and more seem feasible. Recently we have already been successful in testing an eight-channel setup for the detection of CH_4 which permitted the same optical and temporal resolution than the sensor presented here.

ACKNOWLEDGEMENTS This work was financed by the Deutsche Forschungsgemeinschaft, DFG, in the framework of the DFG post graduate programme, GRK1114, and by the Forschungszentrum Jülich GmbH. We also would like to acknowledge support by Siegfried Jahnke and Roland Pieruschka.

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