

***In situ* measurement of dissolved methane and carbon dioxide in freshwater
ecosystems by off-axis integrated cavity output spectroscopy**

Rodrigo Gonzalez-Valencia,[†] Felipe Magana-Rodriguez,[†] Oscar Gerardo-Nieto,[†] Armando Sepulveda-Jauregui,[‡] Karla Martinez-Cruz,^{†,‡} Katey Walter Anthony,[‡] Doug Baer,[§] and Frederic Thalasso^{,†,‡}*

[†]Biotechnology and Bioengineering Department, Cinvestav, Mexico City, D.F., Av. IPN 2508, San Pedro Zacatenco, MX 07360

[‡]Water and Environmental Research Center, University of Alaska Fairbanks, AK 07360

[§]Los Gatos Research, Inc., Mountain View, CA 94041

Supporting information

17 pages (including references)

Extended description of materials and methods.

Figure S1. (A) Peak response of the UGGA to injections of known CH₄ concentrations;
(B) Integrated area of the peak response to increasing quantities.

Figure S2. Headspace concentrations in the sampling syringe for several shaking times.

Figure S3. Measured CH₄ concentrations by the M-ICOS after sudden changes in C_{CH₄}.

Figure S4. Example of the field response of the M-ICOS method to sudden change in water concentrations and calculated C_{CH₄}.

Table S1. Main characteristics of the selected lakes.

Table S2. M-ICOS prototype specifications.

Material and methods

UGGA details

Unlike conventional optical methods that rely on low-resolution spectroscopic techniques (e.g., non-dispersive infrared detectors), the UGGA uses a cavity enhanced laser absorption spectrometry technique that quantifies concentrations, based on measurements of high-resolution absorption lineshapes of the target molecules. The fully resolved lineshapes are recorded by tuning independent telecommunication narrow bandwidth (1 MHz) diode lasers, operating near 1600 nm (CO₂) and 1651 nm (CH₄, H₂O), over 1 cm⁻¹ wide spectral windows, respectively, which straddle the target molecular transitions. Real-time spectroscopic analyses of the measured spectra enables a direct continuous determination of the gas concentrations at rates up to 1 Hz, using Beer's Law, and assessments of gas temperature and pressure in the measurement cell. Because of the use of narrow linewidth tunable lasers to record high resolution, fully resolved lineshapes, no instrument deconvolution is required and cross interferences from other compounds are virtually eliminated.¹

Prototype details

The prototype included a continuous flow of CH₄- and CO₂-free analytical grade nitrogen (Infra, Mexico or Airgas, U.S.A.) and a continuous flow of water extracted at the desired depth from the freshwater ecosystem through a vacuum line (Figure 1). The gas and the liquid crossed at a gas exchange station. The water line (6 mm internal diameter

polyurethane tubing) included a suction probe to extract continuously the water sample. This probe consisted of a plastic tubing (25 mm diameter, 50 mm long), filled with a washable polyester wool filter to avoid line-clogging by sediment or suspended particulate matter. The probe was fixed on a thin pole for low depth environments or on a 200 g weight for deeper environments to ensure accurate location at the desired sampling depth. The water flow passed through the gas/liquid exchange station, which consisted of a silicone tubing array (Permselect, PDMSXA-1000, Medarray Inc., USA) and then ended in a vacuum graduated glass container with a vacuum gauge. The vacuum glass container was connected to a portable air sampling pump (PCXR4, SKC, USA) that created a fixed vacuum driving force. The water flow rate through the water sampling line was measured volumetrically several times prior to C_w profile determinations. The water flow rate was also controlled through the determination of t_d . As t_d is a direct function of the water flow rate, t_d was a clear indicator of constant water flow rate. It should be noticed that at each sampling location the M-ICOS method was calibrated against the H-ICOS method. As such, a constant water flow rate at approximately 600 mL min^{-1} was the main factor to ensure precise C_w measurement.

The gas line (6 mm internal diameter polyurethane tubing) included a 27" gas cylinder containing CH_4 - and CO_2 -free nitrogen (Infra, Mexico or Airgas, U.S.A.). The gas flow rate was regulated at 3 L min^{-1} with a mass flow controller (GFC17, Aalborg, USA). After passing through the gas/liquid exchange station, the air was filtered (AcroVent Filter $0.2 \mu\text{m}$, Pall, USA.) twice to avoid condensed water entering the UGGA detector. The complete set-up weighed approximately 30 kg, was powered by a 50 kWh boat battery, and

was easily operable by a single person from a small portable boat, although a two-person crew allowed an easier operation.

Details on H-ICOS method

The H-ICOS, described in the main body of the article, is an adaptation of the traditional gas/liquid equilibration technique, where the equilibrium between a water sample and a CH₄- and CO₂-free nitrogen headspace is obtained in a 60-mL sampling syringe. After measuring the syringe's headspace concentration by UGGA, the dissolved gas concentration in the water sample was determined according to Henry's law (eqs S1 and S2), where C_w is the dissolved gas concentration in the water sample (CH₄ or CO₂; mol L⁻¹), C_g^* the gas concentration measured in the headspace of the equilibration syringe (mol L⁻¹); V_l and V_g the water and gas volumes in the syringe, respectively (L); H' the CH₄ and CO₂ air/water partition coefficient (-), defined from eq S2; 1.013 is the conversion factor from atm to bars; R is the universal gas constant (0.082 L atm K⁻¹ mol⁻¹); T is the equilibration temperature (K) at the time of measurement; K_H is the Henry's law constant at 298.15 K (1.40x 10⁻³ and 34.0 x 10⁻³ mol L⁻¹ bar⁻¹, for CH₄ and CO₂, respectively);² and β is the temperature dependence coefficient of the Henry's law constant (1700 and 2400 K, for CH₄ and CO₂, respectively).²

$$C_w = \frac{(C_g^* \cdot V_g) + \left(\frac{C_g^*}{H'} \cdot V_l\right)}{V_l} \quad (S1)$$

$$H' = \frac{1}{1.013 \cdot R \cdot T \cdot K_H \cdot \exp\left[\beta \left(\frac{1}{T} - \frac{1}{298.15}\right)\right]} \quad (S2)$$

Details on the M-ICOS method

The M-ICOS method consisted of a counter flow of CH₄- and CO₂-free nitrogen and water, crossing in the silicone tubing array, as described in the main body of the article. The gas transfer can be described by a diffusion model, according to the Fick's second law (eq 1, repeated here for clarity);

$$\frac{dM}{dt} = 1000 \cdot K \cdot A_M \cdot \left(C_w - \frac{C_g}{H'} \right) \quad (1)$$

Since the transferred gas is directed to the gas phase and since the carrier gas contained no CH₄ and CO₂, eq 1 was modified to eq S3, which was obtained from a simple mass balance, where Q_g is the gas flow rate (L s⁻¹).

$$C_g \cdot Q_g = 1000 \cdot K \cdot A_M \cdot \left(C_w - \frac{C_g}{H'} \right) \quad (S3)$$

By rearranging eq S3, eq S4 is obtained, in which membrane, gas, and water transfer characteristics (Q_g , K , A_M and H') are combined into a single parameter α for easier calculation.

$$C_w = \frac{C_g \cdot Q_g}{1000 \cdot K \cdot A_M} + \frac{C_g}{H'} = C_g \cdot \left(\frac{Q_g}{1000 \cdot K \cdot A_M} + \frac{1}{H'} \right) = C_g \cdot \alpha \quad (S4)$$

Equation S4 shows the direct proportionality between C_w and C_g , which is directly detected by the UGGA, offering a convenient method for the measurement of the dissolved gas concentration. The parameter α can be determined by measuring first C_w by the H-ICOS method and then, with the same sample, by measuring C_g by the M-ICOS. This can be easily done in the field with actual water samples.

An important issue with the M-ICOS method is the delay time (t_d) and the response time (t_r) and of the system, as briefly described in the main body of the article. A continuous flow stirred tank reactor (CSTR) model of hydraulic residence time³ describes well the hydraulic behavior of the system (eq 3).

$$C_{wm} = C_w \cdot \left[1 - \exp\left(-\frac{t}{t_r}\right) \right] \quad (3)$$

By the derivation of eq 3, eq S5 is obtained;

$$C_w = \frac{dC_{wm}}{dt} \cdot t_r + C_{wm} \quad (S5)$$

Taking into account t_d , eq 4 was obtained.

$$C_{w,t} = \frac{dC_{wm,t+t_d}}{dt} \cdot t_r + C_{wm,t+t_d} \quad (4)$$

Laboratory testing

The precision and linearity of the UGGA were tested by injecting several CH₄ and CO₂ standards (from 2, 5, 20, 50, 200, and 500 ppm High Purity Standards, Infra) and by simultaneously measuring these standards with a gas chromatograph. We used a Clarus-500 (Perkin Elmer, USA) chromatograph equipped with a flame ionization detector (FID) detector and an Elite - Q Plot column for CH₄ and a thermal conductivity detector (TCD) and an Alltech Hayesep D 100/120 column for CO₂.

The first test of the H-ICOS method was to establish the peak response of the UGGA to several volumes and CH₄ and CO₂ concentrations injected in the gas line. With this purpose, a continuous CH₄- and CO₂-free nitrogen gas flow rate of 3 L min⁻¹ was established and controlled with a mass flow controller; then, 0.1 to 40 mL of 2 to 500 ppm CH₄ or 20 to 500 ppm CO₂ standards were injected. The peaks obtained were integrated (concentration over time using Wolfram Mathematica 8.0, USA). Then, synthetic water samples, containing a known C_w , were prepared in a 3 L lab-scale STR (ez-Control, Applikon, Netherlands) by injecting a continuous flow of 2 to 500 ppm standard gases in 2.5 L tap water with strong mixing (800 rpm), until saturation was obtained. To establish the time required to reach saturation in the STR, prior experiments were conducted by injecting air or nitrogen and measuring the dissolved oxygen concentration until 100% or 0% saturation was obtained (HI2400, Hanna Instruments, USA). Water containing a known dissolved gas concentration was prepared by mixing and gassing times at least two times greater. The dissolved gas concentration in water, with all standards, was established according to Henry's law (eqs S1 and S2). Samples of water were taken with a disposable

60 mL syringe, according to the H-ICOS method, and several shaking times were tested to establish the time required to reach equilibrium between the water sample and the headspace of the syringe. The headspace was injected in the UGGA and also measured by gas chromatography. Then, the M-ICOS method was tested, using the same water samples, in order to establish α (eq 2). Finally, t_d and t_r were determined with the M-ICOS method, by switching between water containing CH_4 and CO_2 to degased water using a 3-way valve. These experiments were also used to check the t_r model developed (eq 4).

Field-testing

The prototype and both methods were tested in four different lakes with contrasting climates, ecologies, and morphologies. The first lake was a eutrophic subtropical reservoir located in the Mexico metropolitan area (Lake Guadalupe, 19.6310 N, 99.2567 W). The second lake was a mesotrophic subtropical reservoir located in the same drainage basin as Lake Guadalupe (Lake Llano, 19.6577 N, 99.5069 W). Both subtropical lakes have been previously described.⁴ The third lake was a shallow yedoma-type, thermokarst lake (Lake Goldstream, 64.9156 N, 147.8486 W), and the fourth lake was a shallow non-thermokarst lake (Lake Otto, 63.8413 N, 149.0384 W). Lake Goldstream has been previously described.⁵⁻⁶ Lake Otto is a shallow tundra lake subject to high winds, which has been hitherto studied.⁷

Statistical and error analysis

The method detection limit (MDL)⁸ of both H-ICOS and M-ICOS were determined as the minimum concentration that can be distinguished from background noise with 99% confidence. The goodness of the correlation between the experimental data and the

response time model (eq 4) was quantified with the coefficient of determination (R^2). Measurement error was determined by the coefficient of variation, defined as the standard deviation divided by the arithmetic mean, and by the standard error of the mean, defined as the coefficient of variation divided by the square root of the replicate number. We also measured the signal to noise ratio of the UGGA, which is the arithmetic mean of the UGGA reading, divided by the standard deviation. Accuracy was calculated as the absolute difference between the measured and the expected concentration relative to expected concentration. Dynamic range was calculated as the logarithmic ratio between the maximum and the minimum concentrations measured. Maximum concentration was theoretically estimated from the UGGA specifications.

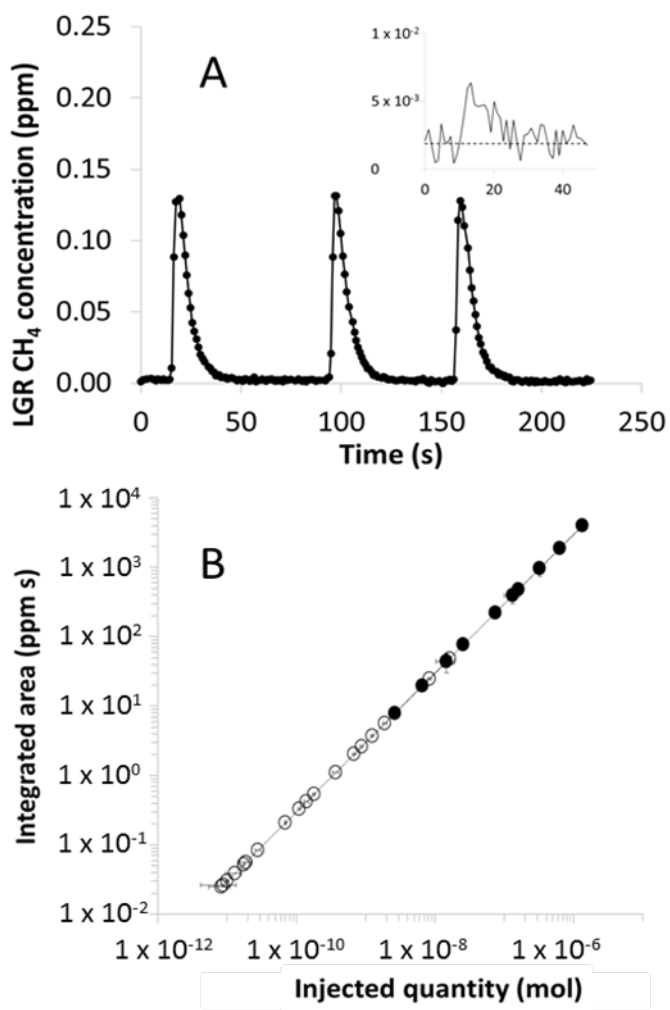


Figure S1. (A) Example of peak response of the UGGA to triplicate injection of 5 mL nitrogen containing 2 ppm of CH₄ and the minimum injected CH₄ quantity that was distinguishable from the background (inner Figure); (B) Integrated area of the peak response to increasing CH₄ (white dots) and CO₂ (black dots) quantities.

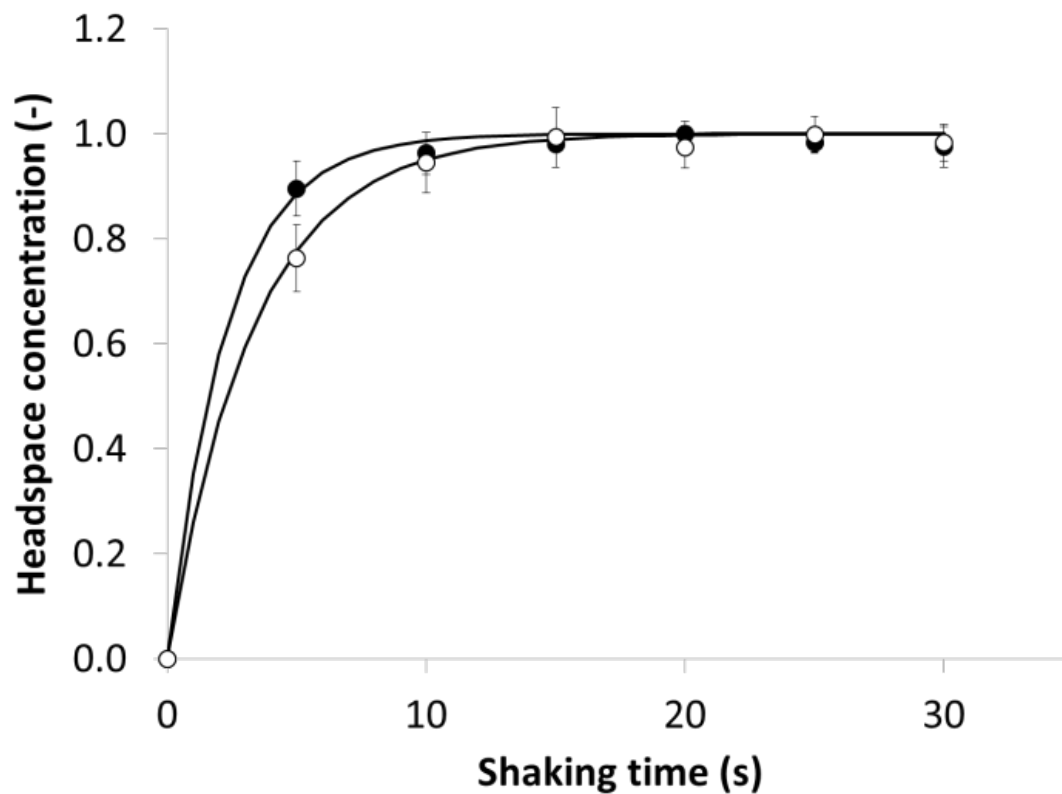


Figure S2. Normalized CH₄ (white dots) and) CO₂ (black dots) headspace concentrations in the sampling syringe for several shaking times. Error bars indicate the standard deviation of the triplicate measurements.

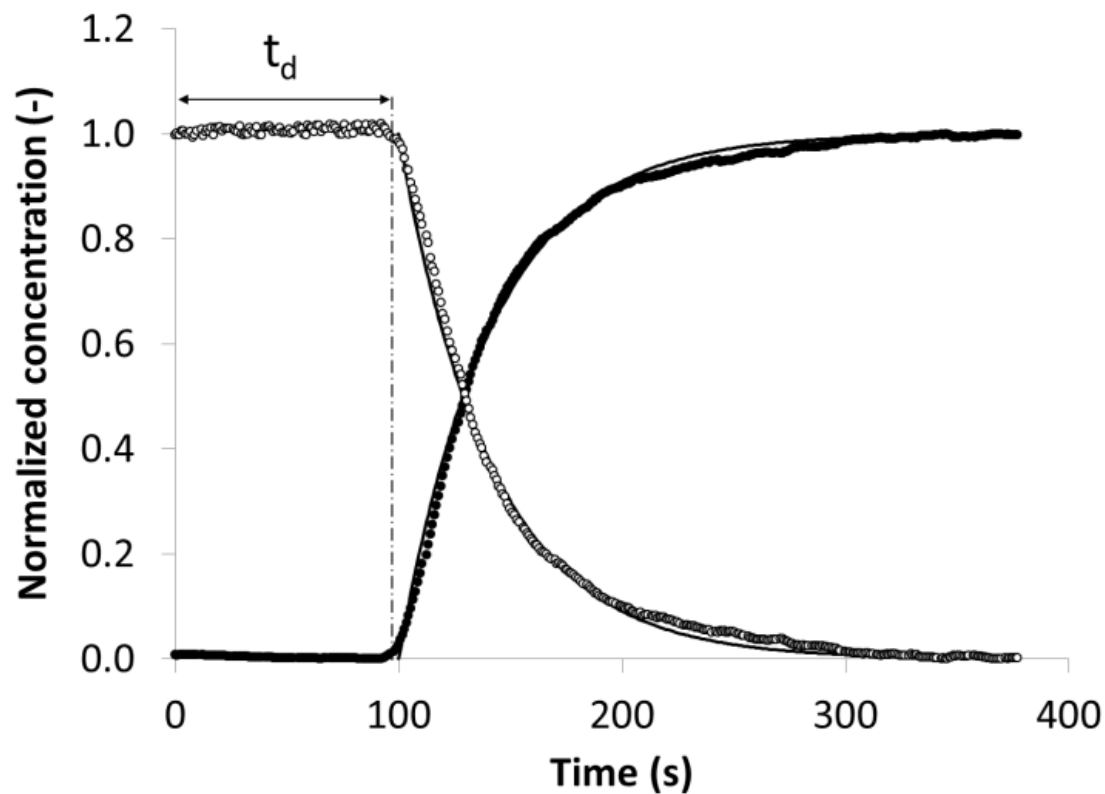


Figure S3. Example of measured CH_4 concentrations by the M-ICOS after sudden changes in C_{CH_4} ; decreasing concentration gradient (white dots) and increasing concentration gradient (black dots); normalized concentration, 1.0 being the initial or final steady state concentration.

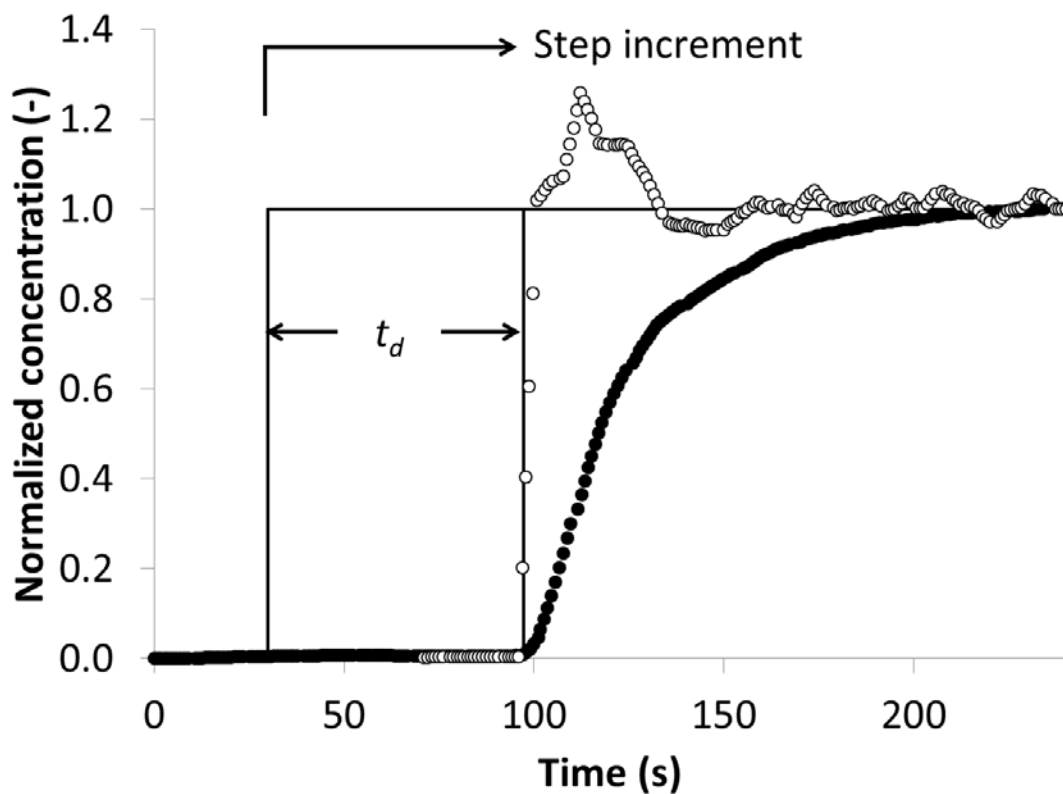


Figure S4. Example of the field response of the M-ICOS method to sudden change in water concentrations (black dots), as well as C_{CH_4} calculated from eq 4 (white dots); normalized concentration, 1.0 being the final steady state concentration.

Table S1. Main characteristics of the selected lakes.

Lake	Guadalupe	Llano	Goldstream	Otto
Area (km ²)	4.5	0.06	0.1	0.51
Mean depth (m)	13.3	9.49	3.3	2.5
TSI ^a (-)	Hypereutrophic	Mesotrophic	Distrophic	Oligotrophic
Secchi Depth (m)	0.55	2.13	1.0	1.6
pH	7.2	6.77	7.78	7.68
Surface DO (mg L ⁻¹)	2.89	6.48	8.50	7.94
Bottom DO (mg L ⁻¹)	0.00	4.02	0.01	7.93
Surface temperature (°C)	21.75	13.88	18.18	12.46
Bottom temperature (°C)	17.35	11.62	11.62	12.28
Mixed layer ^b (m)	0.50	0.50	1.10	2.5
Temperature gradient in the thermocline ^c (°C m ⁻¹)	2.80	2.44	3.01	No thermocline

^aTrophic State Index, measured as given by Carlson.⁹

^b Layer from the surface of the water to the depth at which temperature declined at a rate lower than 1 °C m⁻¹.

^c The thermocline was considered to be the layer between the depth at which temperature started to decline at a rate higher than 1 °C m⁻¹, to the depth at which it started to decline at a rate lower than 1 °C m⁻¹.¹⁰

Table S2. M-ICOS prototype specifications.

Weight (complete prototype)	30 kg
Shipping dimension (suitcase)	$0.47 \times 0.36 \times 0.18$ m (UGGA)
Power consumption (external battery)	12 VDC, 5.8 A (70 W)
Operating temperature	$0 + 40$ °C
Startup time	2 min
Response time UGGA	8 s
Response time M-ICOS	9.77 ± 1.01 s
Calibration time ^a	15 min
Data acquisition frequency	1 Hz
Signal to noise ratio CH ₄	1520 ± 415
Signal to noise ratio CO ₂	1803 ± 344
Standard error of the mean C_{CH_4} determination (n = 100) ^b	0.17 %
Standard error of the mean C_{CO_2} determination (n = 100) ^b	0.20 %
Method detection limit C_{CH_4}	2.76×10^{-10} mol L ⁻¹
Method detection limit C_{CO_2}	1.50×10^{-7} mol L ⁻¹
Accuracy for CH ₄	10.64%
Accuracy for CO ₂	12.22%
Dynamic range for CH ₄	5.00
Dynamic range for CO ₂	5.18

^a Triplicate calibration with H-ICOS method and determination of t_r and t_d .

^b n = number of replicates

REFERENCES

- (1) Baer, D. S.; Paul, J.B.; Gupta, J.B.; O'Keefe, A. Sensitive absorption measurements in the near-infrared region using off-axis integrated-cavity-output spectroscopy. *Appl. Phys. B-Lasers O.* **2002**, 75 (2-3), 261-65.
- (2) Wilhelm, E.; Batino, R.; Wilcock, R.J. Low-pressure solubility of gases in liquid water. *Chem. Rev.* **1977**, 77 (2), 219-262.
- (3) Fogler, H. S. *Elements of Chemical Reaction Engineering*; Prentice-Hall; Upper Saddle River, NJ, 1992.
- (4) Sepulveda-Jauregui, A.; Hoyos-Santillan, J.; Gutierrez-Mendieta, F. J.; Torres-Alvarado, R.; Dendooven, L.; Thalasso, F. The impact of anthropogenic pollution on limnological characteristics of a subtropical highland reservoir "Lago de Guadalupe", Mexico. *Knowl. Manag. Aquat. Ecosyst.* **2013**, (410); DOI 10.1051/kmae/2013059.
- (5) Walter, K. M.; Chanton, J. P.; Chapin, F. S.; Schuur, E. A. G.; Zimov, S. A. Methane production and bubble emissions from arctic lakes: Isotopic implications for source pathways and ages. *J. Geophys. Res Biogeosci.* **2008**, 113 (G2), G00A08.
- (6) Anthony, K. M. W.; Vas, D. A.; Brosius, L.; Chapin, F. S.; Zimov, S. A.; Zhuang, Q. L. Estimating methane emissions from northern lakes using ice-bubble surveys. *Limnol. Oceanogr. Meth.* **2010**, 8, 592-609.
- (7) Skaugstad, C.; Behr, A. *Evaluation of Stocked Waters in Interior Alaska 2007*; Fishery Data Series No. 10-90; Alaska Department of Fish and Game: AK, 2010.

- (8) Ripp, J. *Analytical Detection Limit Guidance & Laboratory Guide for Determining Method Detection Limits*, PUBL-TS-056-96; Wisconsin Department of Natural Resources: WI, 1996; <http://dnr.wi.gov/regulations/labcert/documents/guidance/-lodguide.pdf>.
- (9) Carlson, R.E. Trophic State Index For Lakes. *Limnol. Oceanogr.* **1977**, 22 (2) (361-369).
- (10) Gauthier, J.; Prairie, Y. T.; Beisner, B. E. Thermocline deepening and mixing alter zooplankton phenology, biomass and body size in a whole-lake experiment. *Freshwater Biol.* **2014**, 59 (5), 998-1011.