

Contents lists available at ScienceDirect

Sensors and Actuators: B. Chemical



journal homepage: www.elsevier.com/locate/snb

A novel, standalone and low-cost system for in-situ chemical imaging with planar optodes in soils

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ARTICLE INFO

Keywords: Chemical Imaging In-situ Planar Optode Soil Monitoring

ABSTRACT

Planar optodes are optical sensors that provide microscale 2D visualization of various chemical analytes in environmental and biological samples in real-time. The application of planar optodes to study the spatiotemporal dynamics in particular of oxygen, pH or ammonia is a valuable tool for investigating the drivers of many biogeochemical processes in soils. However, such applications are mainly limited to laboratory experiments due to practical limitations of the method that hinder accessibility for field measurements. This study describes a novel and low-cost multi analyte real time in-situ imaging system (MARTINIS) designed for in-situ or mesocosm applications. We show that imaging equipment used in traditional laboratory setups can be significantly scaled down to fit into a Ø250 mm cylinder that can be deployed in soils. To illustrate the functionality of MARTINIS, we demonstrate imaging of oxygen and temperature planar optodes to acquire "panoramic" images with high spatial ($\approx 10 \,\mu m$) and temporal (intervals ranging from minutes up to hours and days) resolution in a laboratory setup and in-situ. MARTINIS aims to increase accessibility for in-situ applications of planar optodes with a standalone solution for both hardware and software. In-situ applications are especially valuable in agricultural settings, where microscale soil dynamics leading to greenhouse gas emissions such as N₂O are affected by changing environmental conditions. Tools such as the system described in this study, which aims at long-term online monitoring of spatiotemporal in-situ soil conditions, could thus lead to new insights in the field of soil science.

1. Introduction

Soil is an essential resource for supporting life on land and its health is critical to maintaining ecosystem services that support food production, ensure water quality, regulate climate change, and protect biodiversity[1]. However, soil is often referred to as a "black box". This is largely due to the heterogeneous nature of the physical, chemical, and biological properties of soil and the processes within[2,3]. These properties and processes are dynamic and vary across spatial scales, ranging from microaggregates (μ m to cm) to changes in landscape topography (m to km). Furthermore, soil properties undergo changes over time, influenced by diurnal and seasonal cycles[4] or can rapidly shift within a few minutes, due to biogeochemical processes.

In addition to intrinsic soil heterogeneity, anthropogenic activities such as nutrient inputs from either synthetic or organic fertilizers affect soils[5,6] and also the exchange of greenhouse gases (CO₂, N₂O and CH₄) between agricultural systems and the atmosphere [7,8]. In particular N₂O emission from soils, fueled by fertilizer applications, is a major concern. N₂O is mainly produced by the aerobic microbial transformation of NH₄⁺ to NO₃ (Nitrification)[9] or the conversion of NO₃ to N₂ (Denitrification) under anaerobic conditions e.g. in soil aggregates[10,11]. Thus, the complex interplay between the heterogenous soil microenvironment with spatially distributed oxygen gradients and nitrogen availability acts as a driver for the formation of "hot spots" and

https://doi.org/10.1016/j.snb.2024.136894

Received 14 August 2024; Received in revised form 30 October 2024; Accepted 2 November 2024 Available online 8 November 2024

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"hot moments" for microbial N_2O production and determines which process is dominant[12,13].

However, the formation of dynamic microscale niches is not trivial to measure and detect with traditional electrochemical microsensors[14] or optical sensors that provide 1-dimensional information on soil chemistry[15]. While microsensors have been used to study microbial respiration and denitrification in small soil aggregates (4 mm radius) [16], extending this method to study soil heterogeneity at different scales and more dimensions is not feasible. Instead, the use of planar optodes, which allow the visualization of analyte concentrations as a 2-dimensional image[17,18], has gained popularity in soil research. Planar optodes can effectively resolve some of the chemical heterogeneities within the soil ranging from µm to cm, thus helping to illuminate chemical analytes within the "black box". Planar optodes have been used to visualize and quantify the spatiotemporal dynamics of oxygen and CO₂ around plant roots to study gas exchange in the rhizosphere [19] and pH around seagrass roots to study the solubilization of phosphorous and iron in sediments^[20]. Other studies have focused on the soil- amendment interface, using wheat straws and biochar to investigate the influence on soil micro heterogeneity of oxygen and pH and the correlation with N₂O[21] and CH₄[22] production. A recently developed planar optode was used to visualize NH₃ concentrations and dynamics after manure application[21] as well as synthetic fertilizer amendments^[23]. Due to the obtained real time spatial visualization of chemical microenvironments, optodes have also been used to perform informed destructive soil sampling for further soil analyses[24]. In addition to chemical parameters, optodes can also detect relevant soil environmental parameters such as temperature[25,26] or can even be designed to simultaneously measure multiple analytes at the exact same location[27-29], which is extremely valuable in complex samples.

Despite the increasing use of planar optodes in soil science, most studies to date have been limited to laboratory-based setups, which limits the transferability of trends and results from these experiments when upscaled to real-world scenarios in the field. One of the major uncertainties in this area is the influence of continuously changing environmental conditions, which can be hard to replicate in a laboratory setup. In addition, transporting soil samples from field sites to the laboratory can also introduce artifacts, as biochemical processes within the samples may change during transport. Both these factors indicate the importance of including in-situ measurements in soils. The current method of using planar optodes does not have the same small and compact form factor as commercially available 1D sensors for in-situ applications currently have. One reason for this is that the transition to in-situ applications for planar optodes is not straightforward. To enable chemical imaging with planar optodes not only the optode itself (sensor film) but also excitation lights, filters, cameras, and electronics have to be deployed directly in the soil. Any method applied for in-situ measurement will introduce a level of disturbance to a sample, however, while disturbance should be kept to a minimum it is an unavoidable consequence of getting planar optodes into the soil. The current state of the art procedure for in-situ application of optodes in soils requires the excavation of a relatively large trench (1 \times 1 \times 1.6 m), which facilitates the creation of a window into the soil profile[30]. The soil window and trench can then be used in combination with a traditional large form-factor imaging setup used for planar optodes in the laboratory. While this method is rather invasive, labor intensive and restricted to manual operation, it is currently the only method for measuring in-situ soil in field studies. An alternative method of applying optodes in-situ has been developed by Glud et al. and involves highly specialized equipment in the form of a periscope that can be deployed from a ship to measure oxygen or pH specifically in marine sediments[31]. Such a system effectively functions as a standalone planar optode sensor, providing the option to move the system around and measure at multiple in-situ locations. Unfortunately, this system is guite expensive and will be impractical for most applications in soils due to the periscope design and weight of the system.

There is an increasing demand for data to drive biogeochemical and soil emission models, which are essential for upscaling to relevant levels for legislators. To accommodate this need, future environmental studies require new methods that can deliver data with a high spatiotemporal resolution and less manual labor to operate. This study describes the development and application of a novel standalone imaging system for in-situ applications of planar optodes (called MARTINIS) using relatively low-cost components. Due to the small form factor and automated operation of MARTINIS we aim to overcome current barriers of in-situ applications and better understand in-situ spatiotemporal soil dynamics (e.g. O₂, pH, NH₃) and processes involved in production of climate relevant greenhouse gases. Moving planar optodes beyond laboratory-based soil studies is essential to support more realistic soil biogeochemistry and emission models in e.g. agriculture, and the development of MARTINIS seeks to aid in this transition.

2. Materials & methods

The following section describes the components of MARTINIS and applications with planar optodes in soils. For a detailed list of all parts, 3D files (.stl), electronics schematics and assembly information refer to repository: DOI: 10.5281/zenodo.13918877. For a guide and scripts (Python 3.10.4) on how to operate MARTINIS refer to repository: DOI: 10.5281/zenodo.13918973

2.1. System design

MARTINIS was designed as a novel tool to enable the potential insitu application of planar optodes in soils. The components in the setup were chosen to meet the requirements for the method of ratiometric oxygen imaging using planar optodes and RGB cameras[32,33]. Fig. 1 highlights individual electronic and mechanical components used in the construction of MARTINIS and the current prototype including a waterproof transparent, 70 cm long cylinder housing (PLEXIGLAS®XT, outer Ø 250 mm, inner Ø 240 mm) that allows it to be safely placed in a soil sample, mesocosm, or in-situ. Additionally, the housing provides a surface that interfaces with the soil where planar optodes can be attached.

A 12.3-megapixel RGB camera (Raspberry Pi HQ Camera, CSmount), with a resolution of 4056×3040 pixels is used for imaging. The CS-mount allows a wide range of commercially available camera lenses to be attached to the camera module. The lens used in this setup was a Raspberry Pi telephoto lens (16 mm telephoto lens - 10MP, Adafruit.com) with adjustable focus and aperture ranging from F1.4 - 16. Resulting in a field-of-view of 3.6 \times 2.7 cm. To illuminate the planar optodes a flexible arm was installed next to the camera to accommodate any 20 mm starboard mounted LEDs, as well as a bright 5 mm white LED that can function as navigational light when the system is deployed in the soil. In addition, camera lens filters are interchangeable via a 3D printed lens attachment. See supplementary material note 1 for evaluation of image distortion (Fig. S1) and emission filter efficiency (Table. S1). The system is designed to enable movement of the imaging unit (camera + LED) in 2-axis with free 360° rotational movement to cover as much of the soil sample as possible. Movement of the imaging unit is achieved by using two Nema 17 stepper motors (17HS08-1004S, Stepperonline) each connected to an A4988 stepper driver. A belt and pulley system controls the vertical movement while a gear system controls the rotational movement of the imaging unit. See supplementary material note 2 for calibrating movement (Eq. S1 and Eq. S2). A Raspberry Pi microprocessor (Raspberry Pi 4 model B 8 GB) is used as the main component for controlling the stepper motors, LED's, capturing images, and retrieving additional sensor data via the programmable GPIO pins and the dedicated camera connection. Other sensors, which are not essential for applications of using planar optodes, include a Time-Of-Flight sensor (VL53L0x, M5Stack) and a temperature/humidity sensor (DHT22, Adafruit.com) to retrieve absolute camera position and internal



Fig. 1. 3D model representation of the MARTINIS and photo of actual system inside a 70 cm long cylindrical plexiglass housing. 1) Raspberry Pi HQ camera with 16 mm telephoto lens for Raspberry Pi. 2) Emission filter, Blue-UV blocker. 3) Raspberry Pi 4 model B 4 GB. 4) 395 nm LED. 5) 5 mm white LED. 6) Stepper Motor (Rotational movement). 7) Stepper motor (Vertical movement). 8) Resistor for 395 nm LED. 9) 5 V relay. 10)10 mm Fan. 11) End-stop trigger. 12) Ball bearing 13) Gear ring for rotational movement. 14) Step-Down voltage regulator. 15) 2x A4988 Stepper drivers. 16) Gear for rotational movement. 17) Temperature/Humidity sensor. 18) Time-of-Flight distance sensor.

cylinder temperature and humidity, respectively. The system can be interfaced via remote desktop sharing (VNC) or directly via HDMI and is fully operated using Python programming language (Python 3.10.4) or through a custom graphical user interface (See repository: DOI: 10.5281/zenodo.13918973) to initialize automated image time-series, set motion patterns and control camera settings such as ISO and exposure time. The power consumption of the system is ≈ 2 W in standby and ≈ 9 W when imaging and can be powered from any 12 V 5 A DC power supply.

Overall, the MARTINIS is constructed using relatively low-cost components and has a total cost of approximately $650 \notin$ for a housing length of 70 cm excluding the cost of nuts and bolts and the production of planar optodes. 3D printed parts were printed in PETG (Spectrum filaments– 1.75 mm Deep Black).

2.2. Optode fabrication

The oxygen optode used in the setup was fabricated based on previous studies[34] using the following specifications: 11.2 mg indicator dye (Platinum(II)-5,10,15,20-tetrakis-(2,3,4,5,6-pentafluorphenyl)-por phyrin, Frontier Scientific), 11.2 mg reference dye (Macrolex fluorescence yellow 10GN, Lanxess), 448 mg diamond powder (Monocrystalline diamond powder, Microdiamant AG), and 10 g of 10 % Polystyrene (MW 192,000 g mol⁻¹, Sigma Aldrich) in chloroform (CHCl₃, Merck KGaA). The dye polymer mixture (cocktail) was applied on a transparent, 50 µm thick, polyethylene terephthalate (PET, Optimont®501, Bluher Folientechnik) sheet using a 4 milli inch gap film applicator (BYK-instruments) resulting in an optode layer thickness of ${\sim}10~\mu m$ after chloroform evaporation.

Temperature optodes were fabricated based on a previous study by Koren & Kühl[25]. In short regular A4 photocopy paper was soaked in 280 μ M Dichlorotris(1,10 phenanthroline)ruthenium(II) dissolved in milliQ water. The soaked paper was subsequently dried for 60 minutes at 60 °C, after which it was laminated using a lamination pouch and an office lamination machine.

2.3. Optode calibrations

As an excitation light a high-power UV 395 nm LED (Starboard, Luminus SST-10-UV-A130, 395 nm, NewEnergy™) was applied. A plastic UV and blue light blocker filter (821 Zircon UV Blue Blocker, LEE filters.com) with <1 % transmission below $\lambda{=}505$ nm was used as an emission filter for the camera. Calibration of the planar oxygen optode was done in a 1.5 L container, which was glued to the outside of the plexiglass housing (Fig. S2). PVC tubing for adding N₂ and atmospheric air were used to adjust the oxygen saturation in the calibration water. The oxygen saturation in the water was monitored by a reference oxygen optode (OXR430, PyroScience GmbH) and temperature sensor (TDIP15, PyroScience GmbH) connected to a FireSting GO2 pocket meter (PyroScience GmbH). The reference oxygen sensor was calibrated with a two-point calibration in an alkaline 14 % solution of sodium ascorbate (Sigma Aldrich) in water and 1 M NaOH, which served as the 0 % oxygen calibration point, and in air saturated distilled water as the 100 % oxygen calibration point. The calibration was performed at the

same temperature as the O₂ planar optode calibration. To adjust the temperature of the calibration water a thermostat (Alpha RA8, Lauda) was connected to a heat exchange coil placed in the container. Calibration images were captured in RAW 12-bit BGGR format (ISO: 100, exposure: 0.4 sec) from 0 % to 100 % air saturation in steps of 10 %. Technical replicates (n=3) were obtained for each step. The individual RGB color channels were then extracted to obtain the red and green luminescent signal for each pixel in the images and a Red/Green ratio for each pixel was calculated as in Eq. 1.

$$R = \frac{red}{\left(\frac{green_1 + green_2}{2}\right)} \tag{1}$$

Calibrations were performed at multiple temperatures (0.9, 10, 15, 20, and 25.3 °C) to compensate for the temperature dependency of the optode. To describe the response of the planar optode to oxygen a modified Stern-Volmer equation[35] was applied in Eq. 2.

$$\frac{R0}{R} = -\frac{1}{\frac{f}{1+K_{sy^*}[0_2]} + (1-f)}$$
(2)

Where K_{sv} describes the Stern-Volmer constant, f describes the quenchable fraction of the optode and *R*0 is the R value at 0 % air saturation at a given temperature. Calibrations at 20 °C resulted in a Ksv value of 0.0276 air saturation (%)⁻¹ and an f value of 0.623. See supplementary material note 3 for further calculations and temperature compensation procedures (Fig. S3 & Fig. S4). All image processing was performed using Python (Python 3.10.4)

Calibration of the temperature optode was performed in a similar setup to the oxygen optode. RAW 12-bit BGGR images were captured in 10-minute intervals while logging the reference temperature with an external temperature sensor (TDIP15, PyroScience GmbH) as the temperature of the calibration water increased from \approx 3 °C to \approx 20 °C. In contrast to the previous study, we did not use the red/blue signal ratio for calibration but only extracted the red channel intensity for each pixel in the images due to the choice of the blue light blocking filter on the camera. The temperature dependent signal of the red channel was then correlated with the measured reference temperature to obtain a linear calibration curve.

2.4. Soil imaging

Time-series images of microscale soil oxygen dynamics were obtained in the laboratory by placing the MARTINIS inside a large bucket filled with commercial potting soil (Grønne Fingrer Pottemuld, Denmark). After addition of \approx 500 mL water to the soil surface, the MARTINIS was programmed to image every 30 minutes for 17,5 hours at a fixed position covering the soil/air interphase. In-situ soil profile imaging was performed by deploying the MARTINIS in a field lysimeter in Garmisch-Partenkirchen, Germany. The system was deployed by digging a 50 cm deep hole with a diameter of 25 cm and further programmed to image a 40 cm long soil profile once every day for 3 1/2 months through late April until end of July 2024. See supplementary material note 3.1 for soil temperature profiles and compensation procedure. Any part of the cylinder housing above the soil surface was covered with black cloth to keep the camera unit under dark conditions. In both the laboratory and in-situ setups oxygen planar optodes were attached with black electrical tape to the outside of the plexiglass cylinder housing, creating an interface between the soil matrix and the planar optode when deployed in soil. The cylinder was carefully deployed to reduce any scratches on the optode caused by small stones. All images were taken with an ISO of 100 with the aperture set to 2.8 and the exposure time set to 0.4 seconds. For soil profiling, images were taken as multi-image captures with camera movement to cover a larger area of interest within the soil sample. The multi-image captures were then stitched together to create a single image representation, based on principles of panorama image processing[36], using the OpenCV[37]

library in Python. An individual laboratory experiment was performed to specifically highlight the stitching procedure, where anoxic water was added to the soil surface to create distinct layers in the soil profile. See supplementary material note 4 for a description of individual steps in the image stitching process (Fig. S6 & Fig. S7).

3. Results & discussion

The general purpose of this study was to develop a concept for a novel and low cost planar optode imaging system that would function as a stand-alone setup to measure in-situ soil environments in the field. Such a system is relevant for future research due to the limited options for applying planar optodes in-situ and the lack of dedicated stand-alone systems. The proof of concept presented here attempts to lay the groundwork for a system that functions in the same way as a laboratory setup using RGB cameras for the 2D visualization of oxygen concentrations using planar optodes and the ratiometric imaging approach. Moreover, this novel setup offers additional capabilities for automation and novel experimental approaches.

3.1. Temperature and illumination compensation

In addition to this new opportunity to enable 2D visualization of oxygen in-situ directly in the field, and thereby going beyond laboratory-based soil experiments, there is another immediate challenge that needs to be overcome when using planar optodes in-situ. This challenge is the inherent temperature dependence of luminescence intensity associated with all planar optodes, including oxygen, pH and NH3 optodes. However, it is possible to compensate for temperature changes during image processing by performing careful oxygen calibrations at multiple temperatures within the expected range of use. Fig. 2 shows Red/Green ratio calibration curves from multiple oxygen optode calibrations performed at five different temperatures relevant to soils using the here described in-situ system. While the calibrations clearly show temperature dependency of the Red/Green ratio we also observe a relatively large standard deviation of \pm 2.44 % at zero partial pressure of oxygen (hPa) compared to \pm 1,38 % at an oxygen partial pressure of 208.9 hPa (at 20 °C). However, this is caused by uneven illumination of the optode as the LED is placed to the left of the camera, which shows a more noticeable effect at low oxygen levels. Therefore, we apply an image "column by column" calibration process to compensate for the drop in luminescent intensity across the image from left to right (supplementary material note 5 Fig. S8 & Fig. S9). This process includes calculating individual calibration curves for each



Fig. 2. Image column averages of Red/Green luminescent intensity ratio over partial pressure of oxygen at various temperatures measured during optode calibrations. Error bars represent standard deviation of image column averages (n=2028). Exponential regression is fitted as $a * e^{-b*x} + c$.

column index in the calibration images. Thus, to achieve both illumination and temperature compensated measurements, we can apply Eq. 3.

$$[O_2] = \frac{f_{t,n}}{\left(\frac{R}{RO_{t,n}} - 1 + f_{t,n}\right) - 1} * \frac{1}{Ksv_{t,n}}$$
(3)

Where R0, f and K_{sv} are determined by temperature, t and column index, n.

To properly account for fluctuating temperature conditions in the field, all oxygen measurements must include simultaneous temperature measurements with high enough spatial resolution to achieve temperature compensation according to soil gradients. However, such measurements are not always trivial, for example, when measuring at nonresearch field sites where soil temperature data are not available. In such cases, we propose to incorporate simple laminated temperature optodes to obtain average soil temperature measurements and gradients that can be used to directly compensate for the temperature dependence of the oxygen optodes. Fig. 3 shows the average temperature measured with a simple laminated temperature optode, which is compared to reference temperature measurements. The addition of temperature optodes to the MARTINIS effectively allows for the implementation of temperature compensation. Although this type of sensor does not provide the same high microscale spatial resolution as the oxygen sensor, it is sufficient to estimate an average temperature for the area currently in view of the camera. While oxygen planar optodes can also be influenced by humidity (the change in water partial pressure) this effect is considered negligible compared to temperature and compensation is thus largely ignored in many applications.

The use of multi-parameter optodes, which allow simultaneous acquisition of temperature and oxygen signals or other parameters at the exact same position, would provide the most detailed and accurate data for complex soil samples. However, these types of planar optodes require different camera types, such as multi- or hyperspectral cameras or the ability to automatically switch between camera lens emission filters and/or switch between excitation light sources. The current MARTINIS does not have either of these capabilities, but they could potentially be introduced in future versions. Due to the inherently complex biogeochemistry of soils, multiparameter in-situ imaging would be a giant leap in further understanding the spatiotemporal variability and its interaction of soil biological and chemical processes. However, a first step towards such detailed multi-parameter insights into the soil microenvironment is to enable in-situ optode measurements in the field with new imaging systems such as the MARTINIS.

3.2. Proof of concept

A proof-of-concept setup was built and applied in soil to demonstrate the ability of the MARTINIS to capture a time-series of microbial respiration in the topsoil layer, as shown in Fig. 4C. While the optical components used in the system are relatively inexpensive compared to other commercially available camera setups used in other studies with planar optodes [38-40], we show that we can resolve the spatiotemporal dynamics of oxygen in a soil sample at a high spatial resolution of approx. 10 µm (Fig. 4A). The false color images show a clear distinction in O2 concentration between the soil and the air phase above the soil throughout the 17.5-hour time-series, as shown in the concentration profile of the region-of-interest (ROI) 5 compared to ROI 1-4 (Fig. 4B). A slight decrease in O_2 observed at ROI 5 from 20.9 % to around 20 %after 4 hours, which could be caused by limited air exchange within the bucket after covering it with a black cloth to ensure dark conditions during measurements. ROIs 1-4 show the heterogeneous respiration of oxygen in the soil top layer, where ROI 1 and 4, at a depth of 5–10 mm, reached anoxia after 5 hours (Fig. 4A and B). O₂ concentration at ROI 3, located at a depth < 5 mm, stabilized at around 7 % after 17.5 hours, while ROI 2 located just below the soil/air interphase decreased to \approx 12 % before slightly increasing after about 5 hours. This could be due to a relatively increased pore space in these two regions of the sample leading to more oxygen diffusion into the soil or due to water evaporation closer to the soil surface in areas with less compacted soil leading to decreased microbial activity. As the potting soil used in this experiment contains a high degree of nutrients, the observed differences in O₂ concentration between ROI 3 & ROI 2 compared to ROI 1 & ROI 4 could also be due to the random distribution of the compostable residues in the soil resulting in microbial activity hotspots and underlines the general heterogeneity of soil.

The MARTINIS was subsequently deployed in-situ in a field lysimeter over a 3.5-month period to monitor O_2 dynamics in a soil profile and to test the durability of the mechanical and electronic components. Fig. 5A shows how the MARTINIS was deployed inside a lysimeter to measure oxygen distribution along a section of the soil profile. Temperature sensors installed in the lysimeter at depths of 10, 20, 30, and 50 cm were used for temperature compensation of the oxygen optode (Fig.S5). To obtain reliable measurements it is important to assure sufficient contact between the soil and the planar optode during the deployment. However, the clayey and compact soil at the deployment location initially made full contact between soil and optode difficult to achieve. The soil/ optode contact, however, gradually improved over the following week after deployment as the soil settled with time. Other less compacted soil



Fig. 3. A) Average pixel values of the red channel signal intensity over temperature measured during calibrations with temperature optodes. A linear regression fit based on the mean temperature values is plotted in red. Error bars represent the standard deviation in red channel intensity. B) False color images showing the average temperature measured using the temperature optodes at 4 different temperatures. Reference temperatures measured with the FireSting GO2 temperature probe are shown for comparison.



Fig. 4. A) False color images of a soil respiration time-series (0–17 hours) showing the O_2 concentration in % after addition of water. The soil air interphase is marked as the black stapled line and region of interest (ROI) are marked as green boxes. B) Plot showing the average O_2 concentration within ROI 1–5 over time. Error bars display the standard deviation within the ROIs. C) Photo of the experimental setup where an early version of the MARTINIS is placed in a bucket filled with potting soil.

types such as loamy or sandy soils might naturally form better contact with the optode and cylinder over time than clayey soils.

Fig. 5B highlights a 5- day period of the soil profile measurements, where weather conditions changed the level of the water table within the lysimeter. Comparisons between the meteorological data in Fig. 5C and the O₂ profiles shows that the MARTINIS is able to clearly reflect changing soil O₂ conditions related to changes in water table created by heavy rain events. Thus, on May 27th prior to a rain event we observe O2 penetrate further into the soil when compared to May 28th where measurements were taken just after a rain event. Similarly, we observe that O₂ penetrates back into the soil when the rain stopped between May 28th and 29th before becoming completely anoxic when measuring during a rain event on the 31st. Furthermore, in all measurements except for the 31st, we observe microscale spatial heterogeneity in the O₂ distribution caused by soil aggregates where O2 consumption is relatively high and the formation of soil pore space where O₂ can more easily diffuse through. In addition, we can clearly observe root formation at the optode interface, which has a huge impact on soil oxygen dynamics at the microscale. The presence of plant roots can locally increase microbial respiration around the rhizosphere or leak oxygen through specialized aerenchyma tissue in the roots of some plant species. As demonstrated in Fig. 4 and Fig. 5, soil environmental conditions can change rapidly in both space and time introducing both microscale heterogeneity and larger scale gradients of oxygen in the soil. This type of 2-dimensional data is only achievable through the use of planar optodes and is very valuable data in many aspects of soil, plant and

microbial sciences.

The MARTINIS measured reliably throughout the entire 3.5-month period (Data not shown) without downtime due to electronic or mechanical malfunction, during both snow and heavy rain events. However, when working in water saturated soils the buoyancy of the cylinder can potentially force the cylinder out of the soil and securing the cylinder with additional straps can be necessary.

3.3. Image stitching

In addition to the ability to deploy a relatively small imaging system directly in the soil, we also show how we can implement an image stitching procedure to make "panoramic" images of a soil profile, to cover a larger area of the 2D oxygen distribution. This feature could potentially be expanded to a full 360-degree coverage of the deployed cylinder when fully equipped with optodes (Fig. 6). The ability to acquire a combined image of a large soil section enables a high degree of quantification for soil heterogeneity and improves the visual representation of soil profile data. However, when acquiring multiple images the increased demand for camera movement is accompanied by/results in a natural loss of temporal resolution. The high temporal resolution of a still image with no camera movement is between 0.4 and 1 second depending on the applied planar optode. The complete stitched image $(9.9 \times 12 \text{ cm})$ shown in Fig. 6, which consists of a total of 48 images with 50 % overlap, requires a total acquisition time of 4.5 minutes. By reducing the image overlap and/or adjusting the speed of the stepper



Fig. 5. A) Images showing how the MARTINIS is deployed in-situ in a 60 cm deep hole to measure soil oxygen profiles once per day over a 3.5-month period. B) Showing 2D oxygen profiles over a 5-day period from the 27th of May 2024 until 31st of May 2024. Each profile is 3,6 cm wide and 16 cm in height. The white dotted line indicates the soil/air interphase. Optode images are temperature compensated along the soil profile with a resolution of 0.1 cm. C) Meteorological data from the in-situ location covering the days from the 24th of May until the 2nd of June 2024. The black line represents hourly precipitation in mm and red vertical lines represents the timepoints where soil oxygen profiles were measured (only for dates included in Fig. 5B.) Blue bars shows the cumulative rainfall between each measuring timepoint.

motor movement this time can be modified to fit with the desired output. However, a minimum of 30 % image overlap is recommended to obtain good image stitching when applying the feature matching method (Fig. S6). By applying a feature matching approach, we can effectively correct for any small deviation in motor alignment between image sequences. To create the fully stitched images, image data is postprocessed from calibrated 8-bit false color images using a custom Python script. While this procedure functions well and allows for a much larger insight into the soil profile the image stitching approach can still be optimized further. This includes working with the original 12-bit RAW images and to better blend the images together in overlapping regions.

3.4. Further development and applications

The novel approach to in-situ applications of planar optodes presented in this study reduces some of the practical challenges associated

with using planar optodes in the field. This includes innovations to downscale the required hardware to produce a standalone system that operates automatically over long time periods using a dedicated user interface. Furthermore, having a system design relying on 3D printing, readily available and inexpensive components and a user interface for operations could potentially broaden the adoption of planar optode studies in environmental sciences. Although we have already displayed significant improvements in in-situ planar optode accessibility in comparison to other methods, further development of software for data processing will be a crucial next step. The 3D printed and modular design of the MARTINIS allows for new adjustments and versions to be quickly produced, and the Raspberry Pi ecosystem ensures that the system can stay up to date with new hardware installments as future development within this space will keep producing higher quality equipment at a fraction of the cost. Furthermore, the reduced cost of the system is important for large scale field studies that would need to



Fig. 6. Workflow for the image stitching process: Eight individual images (A-H) which have a 50 % overlap with the immediate neighboring image captured during a measurement sequence of a soil sample after addition of anoxic water to the soil surface. Images are progressively stitched together to produce a full vertical section of the total area of interest. The process is repeated for multiple vertical sections in the image capture sequence and are further stitched together horizontally to form the complete stitched image.

include multiple MARTINIS to obtain replicates and collect a representative dataset for landscape topography, field management or variation in vegetation. A large-scale field study may be carried out by installing plexiglass housings with planar optodes and manually moving the camera unit from housing to housing or by having individual camera units in each housing. In addition, imaging systems in cylindrical tubes as a novel concept for in-situ application can be adapted to fit a range of use-cases, where long tubes with a small diameter might be better for larger gradients (cm to meters), while shorter tubes with a wide diameter capture a larger degree of microscale local soil heterogeneity. However, there will always be a balance between the cost, size and quality of each camera used in each setup.

To date, deployment of the system can be accomplished by drilling or digging out soil cores to create a matching hole for the cylinder housing. However, the best practice for deployment has yet to be determined for different soil types, such as clayey, loamy, and sandy soils. The goal is to be minimally invasive and to ensure sufficient contact between the planar optode and soil. One approach for agricultural soils could be to deploy the system during tillage of the soil, as the soil disturbance at this time is already high. An unavoidable consequence of applying planar optodes in soils is the introduction of a "wall effect" at the measuring interface that can influence the distribution of O₂. Nevertheless, planar optodes remain the best practice for monitoring 2D microscale dynamics of O2 in soils with high temporal resolution. Furthermore, when deploying optodes in a matrix like soil, some scratches are to be expected. However, with careful deployment this can be greatly reduced and the optodes applied in this study were not damaged to an extent where obtaining data was obstructed. Furthermore, the photostable dyes used in production of the planar optodes provide a long lifetime of the optode when imaging with intervals of 12-24 hours. While we have demonstrated the application of oxygen planar optodes and temperature optodes in combination with the novel MARTINIS in this study, future work will be done to equip the system also with pH and ammonia planar optodes with only a few adjustments to the excitation light sources and

emission filters.

3.5. Conclusion

Planar optodes are an extremely valuable tool for laboratory experiments to study detailed microscale processes with high spatiotemporal resolution in complex soil samples. However, in-situ applications in the field are also highly valuable due to the rapidly changing environmental conditions, that cannot easily be mimicked in laboratory experiments. For planar optodes to have a part in the range of sensors that currently are feeding data to our prediction models of biogeochemical dynamics and greenhouse gas emissions we need to innovate on the accessibility to apply planar optodes in-situ. So far, such methods have not yet been feasible due to technological and practical limitations. With this study we aim to bridge the gap between laboratory and in-situ experiments using our novel imaging setup, MARTINIS. With MARTINIS we have significantly improved accessibility of applying planar optodes starting from the design perspective, using 3D printing and inexpensive components without compromising data quality. A smaller form factor than current methods simplify deployment, and automated operation reduces manual labor and increases temporal resolution. These new technical innovations allow online in-situ soil monitoring using planar optodes to obtain 2D data of important soil parameters such as oxygen. This new suite of soil data could potentially help to bridge the gap between soil biogeochemical processes and associated greenhouse gas emissions, of e. g. N₂O or CH₄, which can be measured with other types of sensors.

Funding Sources

This study was funded by the Pioneer Center for Research in Sustainable Agricultural Futures (Land-CRAFT), DNRF grant number P2.

CRediT authorship contribution statement

Theresa Merl: Writing – review & editing, Methodology, Investigation. Martin Reinhard Rasmussen: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. Simon Thomsen: Writing – review & editing, Validation, Conceptualization. Silvia Zieger: Writing – review & editing, Software, Formal analysis. Klaus Koren: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. Klaus Butterbach-Bahl: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. Ralf Kiese: Methodology, Resources.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Klaus Butterbach-Bahl reports financial support was provided by Danish National Research Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to extend a big thanks for the excellent technical support provided by Stefan Nahnsen, Ricky Haubro Nielsen, John Svane Jensen from the electrical and mechanical engineering workshop at Aarhus University, Lars Norge Andreassen from the pioneer center Land-CRAFT (Aarhus University), and Lars Borregaard Pedersen from the section of microbiology, Aarhus University. Fabian Steininger, Andrei Kalinichev and Pauline Sophie Rummel (Aarhus University) are thanked for their support throughout the project and many constructive discussions to help improve the MARTINIS and for inputs to data

processing.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2024.136894.

Data availability

Data will be made available on request.

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