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Toward scalable freshwater monitoring: an open-source sensor for chlorophyll-*a* fluorescence and turbidity

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Freshwater monitoring programs increasingly require affordable, field-deployable, and reliable sensors to expand the spatial and temporal coverage of key water quality indicators such as chlorophyll-*a*. However, data quality concerns currently limit the integration of community-based observations into operational monitoring frameworks, and the reliability of many low-cost technologies remains constrained by limited validation and inconsistent reporting practices. This work presents the design and validation of an open-source portable fluorescence sensor for the simultaneous quantification of chlorophyll-*a* and turbidity in freshwater systems. The proposed optical sensor integrates automated signal processing and IoT-enabled data transmission within a compact system costing less than US$100. Analytical performance was assessed under laboratory, mesocosm conditions. *In vitro* calibration demonstrated a linear response for chlorophyll-*a* over the 1–100 μg L^{-1} range (R^2 = 0.987–0.999), with limits of detection and quantification of 0.12 and 0.28 μg L^{-1}, respectively. Within the 6–100 μg L^{-1} working range, mean relative bias remained below 15%. Turbidity measurements showed a mean relative bias below 12% in the 10–150 NTU range, with a LOQ of 7.28 NTU. Instrument stability was maintained over a three-month evaluation period, with calibration slope variability below 9% RSD. *In vivo* testing on natural river samples showed strong agreement with a benchtop spectrofluorometer (R^2 = 0.95), and regression-based calibration across 59 samples reduced relative bias from 26.5% to 2.99% and RMSE from 12.7 to 5.3 μg L^{-1}, confirmed by 5-fold cross-validation. The proposed sensor offers a scalable and reproducible approach for integrating low-cost optical sensing into distributed freshwater monitoring frameworks.

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Environmental significance

Eutrophication and algal blooms are increasing worldwide due to nutrient enrichment and climate change. Effective management requires spatially and temporally resolved monitoring of key indicators such as chlorophyll-*a*. However, official programs systematically exclude small catchments and river tributaries that act as entry points for diffuse pollution into larger water bodies. Although community-based monitoring can expand coverage, low-cost sensors rarely provide the analytical robustness needed for environmentally meaningful observations. By combining rigorous analytical validation with open-source design and IoT connectivity, this study demonstrates that distributed fluorescence sensing can generate reliable measurements of chlorophyll-*a* and turbidity under field conditions, providing a scalable approach for expanding freshwater monitoring networks in underserved catchments.

1 Introduction

In vivo fluorescence signals associated with chlorophyll-*a* are widely used as a proxy for phytoplankton biomass and as an indicator of water quality and trophic state.[1] At the European level, chlorophyll-*a* is a key parameter for assessing the ecological status of surface waters. The Water Framework Directive (WFD) aims to prevent deterioration and improve water quality across different aquatic ecosystems. This directive mandates the assessment of the ecological status of water bodies and requires Member States to report key chemical and

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biological parameters, including chlorophyll-*a* as an indicator of phytoplankton biomass and eutrophication.[2] At the global scale it contributes to indicators such as Sustainable Development Goal 6, indicator 6.6.1, supporting the evaluation of water-related ecosystems and their health.[3] Despite the widespread implementation of freshwater monitoring programs, current *in situ* monitoring often does not provide sufficient spatial and temporal resolution.[4] Conventional *in situ* monitoring relies on specialized personnel, expensive fluorescence probes, or time-consuming laboratory analyses, limiting its scalability.[5] In this context, citizen science monitoring has emerged as a powerful tool to support monitoring frameworks, with the ability to expand monitoring coverage, particularly in rural areas and small river catchments.[6–9] Indeed, monitoring small catchments and river tributaries are of particular importance: these water bodies are frequently excluded from official monitoring programs, yet often represent key entry points for diffuse nutrient pollution from agricultural activities, with consequences for trophic conditions and algal bloom dynamics in downstream ecosystems.[8,10,11] The primary challenge in scaling freshwater monitoring is increasingly not data transmission or sensor availability, but the generation of sufficiently reliable observations under field conditions. However, the reliability of citizen science-generated data remain a critical concern.[12] This is particularly critical in citizen science contexts seeking to contribute data to be considered as complementary to official monitoring efforts, which imposes data quality requirements closer to scientific standards, while instruments must remain accessible to non-expert users. There is a need for low-cost sensing technologies capable of providing robust and reproducible measurements under real-world conditions. Growing interest in IoT have driven the development of low-cost optical sensors for water quality monitoring.[13]

Recent advances have substantially increased the availability of low-cost chlorophyll-*a* sensors.[14–16] However, there remains limited evidence that these technologies can generate reproducible and environmentally meaningful observations under real-world conditions. Most studies rely on a limited number of laboratory samples and emphasize correlation coefficients (R^2) and sensitivity alone, while neglecting analytical metrics such as accuracy, uncertainty, and long-term stability.[17] Consequently, many systems remain at the proof-of-concept stage, limiting their integration into community-based or operational freshwater monitoring frameworks.[18–21]

While low-cost technologies for water quality monitoring are abundant in the literature, individual studies typically claim their novelty along a single dimension: some report open-source CAD and firmware without a corresponding rigorous analytical validation, others report partial or exhaustive validation rather than CAD files, software, or data-sharing capabilities, and few combine both with accessible connectivity for non-expert users.[22] This lack of harmonisation represents a practical barrier, since analytical robustness, reproducibility and accessibility are all simultaneously required for a device to be deployable in citizen science and modern freshwater monitoring. Here, we address this gap by demonstrating that a platform integrating open-source hardware and firmware, a rigorous analytical validation protocol aligned with established guidelines, and simple data sharing can generate robust measurements of chlorophyll-*a* and turbidity across laboratory, mesocosm conditions. We present the design and performance assessment of a portable and low-cost optical sensor specifically developed for distributed freshwater monitoring. The proposed platform addresses the trade-off between affordability, analytical performance, and accessibility for non-expert users, providing a reproducible framework for expanding monitoring coverage in underserved catchments.

2 Experimental

2.1 Sensor design and optical configuration

The device presented here is an optical system, able to characterise a 10 mm sample. The optical subsystem is comprised of two light sources: an 820 nm LED (Chanzon) for turbidity measurements and a 430 nm SMD LED for chlorophyll-*a* excitation (HotRed) and a photodetector. Two 5 mm slits separate the light source compartments from the sample holder, which can host a 10 mm cuvette.

A long-pass optical filter (∅ 12.5 mm, cut-on wavelength 630 nm, thickness 1 mm, SCHOTT RG-630, Edmund Optics) separates the light source and detector compartments, preventing UV or visible excitation light from reaching the detector. The light detector is a Si PIN photodiode (S1223-01, Hamamatsu) positioned at 90° relative to the cuvette and light source. The photodiode exhibits high sensitivity in the visible-near infrared (NIR) range with a cutoff frequency of 20 MHz.

The voltage signal from the photodiode is acquired using a 4-channel, 16-bit analogue-to-digital converter (ADC) (ADS1115, Texas Instruments) and sent to a single-board computer (SBC) (Raspberry Pi Zero 2 W), which controls the system. Both LEDs and the photodiode are soldered onto three separate PCBs to ensure stability. Additional components include three LEDs (Elegoo) for status indication, a push-button to start measurements, and a 5000 mA h power bank (Cellularline) as a power supply. The fully assembled instrument (Fig. 1) is embedded in a 3D-printed box made of black polylactic acid (PLA) filament (∅ 1.75 mm) with dimensions 78 × 99 × 58 mm ($L \times W \times D$). The measurements require manual sampling and a four-window cuvette (PMMA, quartz, or borosilicate glass can be used). All design files, firmware, and calibration scripts are openly available at https://github.com/LowCostSensor/FROG-sensor.

2.2 Manufacturing and costs

The total production cost of the instrument is $99.05 (Table 1). All electronic components are RoHS-compliant, ensuring adherence to current restrictions on hazardous substances.[23]

2.3 Signal acquisition and processing

At system startup, a series of "zero" characterisation steps are performed. First, a "dark" measurement (light source off) is recorded as a baseline voltage measurement. Next, a "blank" measurement with deionized water is automatically registered, and used to verify system operation prior to sample

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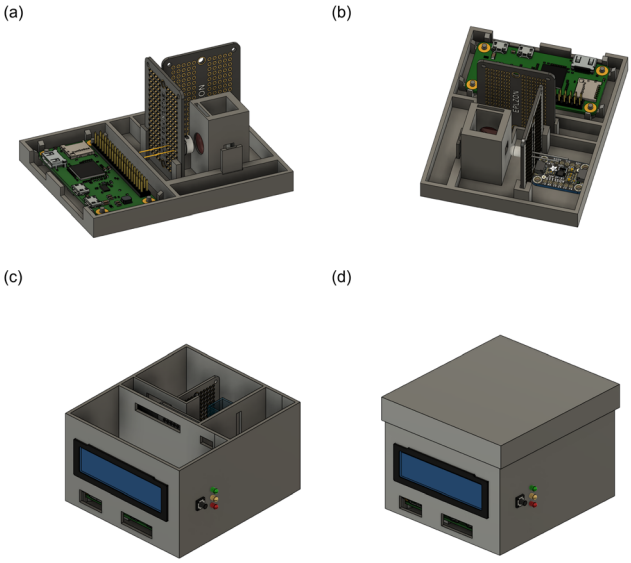


Fig. 1 (a) Side internal view of the microcomputer and detector section. The holder of the SMD LED is also shown. (b) Side internal view of the IR light source and analog-to-digital-converter section. (c) Top view of the assembled instrument. (d) Fully assembled view of the instrument.

measurements. The cuvette is manually filled with deionized water for this step.

Spectral data are obtained for each sample following 1500 data acquisitions, requiring 6 seconds. Collected data points are smoothed using a Savitzky–Golay filter (third-order polynomial; window length up to 51 points, adjusted to the largest odd value compatible with the signal length) to improve measurement robustness. Extreme outliers among the measurements are automatically identified using a z-score approach, and values exceeding ± 2.9 standard deviations from the mean are excluded. The remaining replicates are averaged to obtain the final reported signal. Averaging was applied to reduce the effect of random drift, although it was not expected during the acquisition time.

A built-in display allows real-time visualization of the measured averaged data during operation. All measurements are automatically stored locally on the Raspberry Pi SD-card to ensure data retention under offline conditions. In addition, the system enables data transmission to cloud-based platforms (such as ThingSpeak or ArcGIS Survey123), either in real time when a network connection is available or upon reconnection.

2.4 Laboratory assessment of analytical performance

Fifteen standard solutions covering 1–100 μg L^{-1} chlorophyll-*a* range were prepared using a stock solution obtained *via* solid–liquid extraction using ethanol (95%) following the EN 17899: 2024 standard method.[24] Chlorophyll-*a* was extracted from leaves of Medicago Sativa (Alfalfa). Before the extraction, the leaf samples were freeze-dried (48 h, 300 μbar, -45 ± 2 °C, 5 Pa), milled (Pulverisette 11, Fritsch), sieved with granulometry <500 μm and stored at -20 ± 1 °C until extraction.

Six calibration curves were performed, using independently prepared standard solutions rather than repeated measurements of the same samples. Each measurement was conducted with both the low-cost instrument and a benchtop spectrofluorometer (Cary Eclipse, Agilent) over a period of three months, to assess both short- and long-term repeatability. Analytical performance was evaluated to identify the device's range, limit of detection (LOD), limit of quantification (LOQ), standard deviation, bias, relative bias, relative standard error (RSE%) and relative percentage difference (RPD%) against the Cary Eclipse.[25] Fifteen replicates for each concentration were used.

For turbidity, a 4000 NTU stock solution of formazin (VWR International) was used to prepare seven standard solutions in the range 10–150 NTU. The same analytical parameters were calculated as in the case of chlorophyll-*a*.

The combined and expanded uncertainties were estimated using data from laboratory validation according to the Quantifying Uncertainty in Analytical Measurement (QUAM) Eurachem guidelines.[26] The combined uncertainty was calculated taking into account the intermediate precision from independent calibrations, bias, and the preparation of standard solutions. The expanded uncertainty (U) was calculated using a coverage factor $k = 2$, corresponding to a 95% confidence level, as suggested by the QUAM Eurachem guidelines.

2.5 Evaluation under dynamic phytoplankton conditions

Naturally occurring phytoplankton communities were monitored under contrasting nutrient conditions to evaluate the ability of the sensor to capture temporal variations in biomass

Table 1 Component costs for the production of the instrument

Component	Supplier	Qty	Cost ($)
Raspberry Pi zero 2 W	Melopero S.r.l., Italy	1	19.80
ADC (ADS1115)	Texas instruments, US	1	14.95
Photodiode (S1223-01)	Hamamatsu Photonics, Japan	1	15.24
Long-pass optical filter	Edmund optics BV, Netherlands	1	39.42
Mini PCB 2.0″ × 1.5″	EPLZON, China	2	1.64
Jumper wires	GTIWUNG, China	16	0.05
430 nm LED	HotRed, China	1	1.19
820 nm LED	Chanzon IT, China	1	0.15
Display	Freenove-EU, China	1	6.46
Button	Gikfun, China	1	0.15
Total			**99.05**

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under environmentally relevant conditions. Water samples for *in vivo* analysis were collected from locations in the Ombrone River basin (Tuscany, Italy; 43° 17′ 15″ N, 11° 22′ 36″ E, Tressa River, Fosso del Riluogo) in February and March 2026. The sampling sites are characterized by elevated macronutrient concentrations with spring blooms of green algae. Samples were transported to the laboratory, divided into four aliquots (2 L each) with different nutrient enrichments, and maintained in a growth chamber under constant aeration and a 16:8 h light–dark cycle. Nutrient concentrations were monitored daily and adjusted when necessary using a standard freshwater growth medium (BG-11).

Two independent cultures (BG-11 enriched and control) were monitored over a 30-day period to evaluate temporal variations in fluorescence associated with algal growth. Fluorescence measurements were performed daily using both the low-cost sensor and the Cary Eclipse.

Additionally, a total of 87 *in vivo* measurements were collected using both instruments to assess the correlation between the fluorescence signals obtained with the low-cost device and the Cary Eclipse.

2.6 Quantification of chlorophyll-*a* in real samples

A total of 59 river samples were analysed to compare the performance of the low-cost sensor and the reference instrument. *In vivo* fluorescence was measured on all untreated samples using both instruments. Chlorophyll-*a* extraction followed by spectrophotometric quantification was carried out on a limited number of samples, both to verify that concentrations remained within the instruments measurable ranges (up to 92.8 μg L^{-1}) and to establish a calibration curve. Specifically, five samples were used to derive the relationship between *in vivo* fluorescence and absolute chlorophyll-*a* concentration within the working range of the sensor.

For calibration purposes, *in vivo* chlorophyll-*a* estimates obtained with the Cary Eclipse fluorometer were treated as reference values (response variable), while the corresponding fluorescence signals measured by the low-cost sensor were used as predictor variables. A linear regression model was fitted to describe the relationship between the low-cost sensor signal and the Cary Eclipse measurements, thereby deriving the calibration function for the low-cost sensor within its operational range.

Model performance was evaluated using a 5-fold cross-validation to reduce overfitting. Performance metrics, including RMSE, absolute and relative bias, and RPD%, were computed for both training and validation datasets.

3 Results and discussion

3.1 Analytical performance and system stability

A prerequisite for the deployment of low-cost sensing technologies in distributed freshwater monitoring is the demonstration of analytical robustness under realistic operating conditions. Therefore, analytical performance was first evaluated under controlled laboratory conditions over a three-month

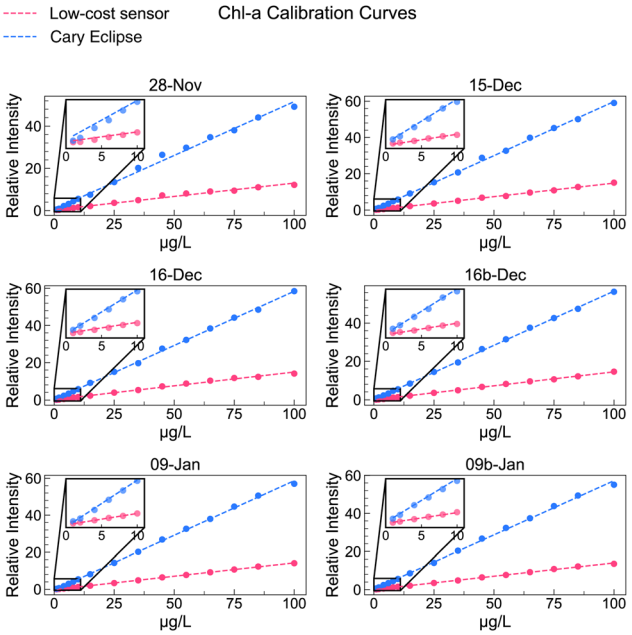


Fig. 2 Calibration curves made for chlorophyll-*a* using both the low-cost sensor (red) and the Cary Eclipse (blue).

period to assess sensitivity, precision, and system stability over 3 months. The proposed sensor exhibited a linear response for chlorophyll-*a* concentrations between 1 and 100 μg L^{-1} Fig. 2 and Table 3).

The limited variability observed in calibration slopes (RSD < 9%) over time suggests a stable instrumental response, with no evidence of significant systematic drift during the observation period. As expected, the proposed low-cost sensor exhibits lower sensitivity compared to the Cary Eclipse, as reflected by the lower calibration slopes (Table 3).

For visualization purposes only, the data shown in Fig. 2 were scaled by a factor $\alpha = 0.6$, as the unscaled calibration curve of the low-cost sensor would otherwise not be clearly distinguishable. The coefficient of determination for the low-cost sensor ranged between 0.987 and 0.999 (Table 2). Although high R^2 values are frequently reported in the literature, substantial fluctuations are often observed at low concentration levels, as the metric can be disproportionately influenced by high-concentration data points. Consequently, large relative errors at low concentrations yield small absolute residuals that contribute minimally to the residual sum of squares.[27,28] The calibration curves reported in this study maintain good linearity across the entire tested range, including low chlorophyll-*a* concentrations (Fig. 2).

The analytical performance of the proposed sensor shows good trueness and precision, as RSD% and RSE% are consistently below 2% and 0.5% (Table 3). LOD and LOQ are 0.12 and 0.28, respectively. However, the results indicate that measurement trueness becomes acceptable from 6 μg L^{-1} (Table 3). Within the 6–100 μg L^{-1} range, the mean relative bias was 5.67%, while the relative bias remained below 15%, falling within the range observed for other low-cost sensors designed

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Table 2 Determination coefficients and equations of chlorophyll-*a* calibration curves

Date	Equation	R^2
Low-cost sensor		
28-Nov	$y = 0.0049x + 0.0147$	0.987
15-Dec	$y = 0.0059x - 0.0033$	0.999
16-Dec	$y = 0.0060x + 0.0043$	0.995
16b-Dec	$y = 0.0061x - 0.0040$	0.999
9-Jan	$y = 0.0050x - 0.0061$	0.999
9b-Jan	$y = 0.0053x + 0.0019$	0.999
Cary-Eclipse		
28-Nov	$y = 0.5098x + 0.6310$	0.995
15-Dec	$y = 0.5959x + 0.1851$	0.999
16-Dec	$y = 0.5837x + 0.0474$	0.999
16b-Dec	$y = 0.5668x + 0.0239$	0.999
9-Jan	$y = 0.5909x - 0.4157$	0.999
9b-Jan	$y = 0.5703x + 0.1875$	0.999

Table 3 Analytical performance parameters of the FROG sensor for chlorophyll-*a*[a]

Std (μg L^{-1})	Rel. bias (%)	SD	RSD (%)	SE	RSE (%)	RPD (%)
2	−60.5	0.009	1.21	0.002	0.30	17.9
4	31.8	0.013	0.25	0.003	0.06	2.3
6	−6.0	0.009	0.16	0.002	0.04	7.2
8	−12.2	0.012	0.18	0.003	0.05	6.3
10	−14.1	0.008	0.09	0.002	0.02	10.5
15	−14.1	0.022	0.18	0.006	0.04	5.5
35	−4.5	0.042	0.12	0.011	0.03	9.3
45	−1.9	0.043	0.10	0.011	0.02	2.1
55	0.6	0.064	0.12	0.016	0.03	3.5
65	−4.9	0.055	0.09	0.014	0.02	2.5
75	−0.9	0.052	0.07	0.013	0.02	2.5
85	−2.0	0.090	0.11	0.023	0.03	2.6
100	1.2	0.041	0.04	0.011	0.01	1.6

[a] LOD: 0.12 μg L^{-1}, LOQ: 0.28 μg L^{-1}, *n* samples per standard: 15.

for *in situ* environmental monitoring.[15,29,30] These results, together with those obtained from the comparison with the Cary Eclipse (Table 3), indicate good performance of the proposed sensor for the quantification of chlorophyll-*a*.

Laboratory experiments for turbidity quantification demonstrated good precision and accuracy of the proposed sensor (Table 5). The results indicate a general underestimation of turbidity, with a relative bias ranging from 2.84% to 11.33%.

Table 4 Calibration equations and coefficients of determination for turbidity

Date	Equation	R^2
16-Dec	$y = 0.0078x + 0.0112$	0.9981
16-Dec b	$y = 0.0083x + 0.0111$	0.9997
18-Dec	$y = 0.0081x + 0.0154$	0.9993
09-Jan	$y = 0.0064x + 0.0002$	0.9936
09-Jan b	$y = 0.0071x + 0.0216$	0.9720
13-Jan	$y = 0.0074x + 0.0015$	0.9573

Table 5 Analytical performance parameters of the FROG sensor for turbidity[a]

Std (NTU)	Rel. bias (%)	SD	RSD (%)	SE	RSE (%)
10	−9.39	1.62	17.9	0.47	4.68
20	−11.33	0.52	2.9	0.15	0.75
40	−4.39	2.62	6.9	0.76	1.89
150	−2.84	2.15	1.5	0.62	0.41

[a] LOD: 2.18 NTU, LOQ: 7.28 NTU, *n* samples per standard: 15.

The LOD and LOQ were 2.18 NTU and 7.28 NTU, respectively. Within the working range 10–150 NTU, the RSD% varies between 6.9% and 1.5%, whereas below 10 NTU it increases to 17.9%, indicating reduced precision close to the LOQ. Results from intra-day and inter-day calibrations indicate good repeatability and a linear relationship between the signal and turbidity levels. With the exception of one calibration curve with $R^2 = 0.957$, all calibration curves showed R^2 from 0.972 to 0.999, with low variation in the slope of the regression equations (Fig. 3 and Table 4).

Lastly, the uncertainty was estimated for both chlorophyll-*a* and turbidity. As expected, the expanded uncertainty values calculated for chlorophyll-*a* (Table 6) are lower for the Cary Eclipse compared to the low-cost sensor. Nevertheless, both instruments the uncertainty varies proportionately to the concentration of chorophyll-*a*. For the turbidity measurements, it should be noted that the expanded uncertainty reported here was calculated exclusively from repeated measurements on formazin standards and therefore reflects uncertainty with respect to this reference material. In the absence of a direct comparison with a reference nephelometer, this estimate does not account for any systematic deviation from the NTU scale defined by a standard reference instrument, but still describes the variability of the low-cost sensor when measuring the same material (Table 7).

3.2 Tracking phytoplankton dynamics under environmental conditions

Natural samples cultured in different nutrient conditions over 30 days to cover a range of growth and senescence conditions, with concentrations ranging 1–100 μg L^{-1} of chlorophyll-*a*.

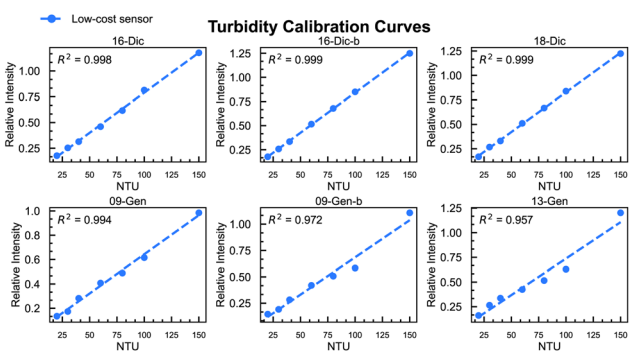


Fig. 3 Calibration curves obtained for turbidity using formazin standard solutions.

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Table 6 The expanded uncertainty values calculated for chlorophyll-*a* for both the low-cost sensor and Cary Eclipse

Std (μg L^{-1})	U low-cost sensor (μg L^{-1})	U Cary Eclipse (μg L^{-1})
6	1.734	0.875
8	2.099	1.058
10	2.207	0.795
15	2.668	2.581
35	5.639	2.226
45	8.590	4.315
55	11.501	5.661
65	13.479	7.941
75	16.292	11.640
85	14.803	10.863
100	22.847	15.655

Table 7 Expanded uncertainty values calculated for turbidity using formazin standards

NTU	U low-cost sensor (NTU)
10	14.645
20	6.989
40	21.946
150	36.119

Temporal variability enabled evaluation of instrument response across a range of phytoplankton biomass conditions. Regression analysis (Fig. 4) on the 87 paired measurements indicated strong agreement between the low-cost sensor and the Cary Eclipse (R^2 = 0.95). Time-series measurements showed that both instruments consistently tracked fluorescence dynamics in the two experimental cultures (Fig. 5). This was supported by Spearman's rank correlation coefficients, which indicated strong monotonic relationships for both conditions (ρ = 0.93 for BG11/40 and ρ = 0.98 for the control culture; $p < 0.001$). Although high correlation is expected due to the shared sensitivity of both instruments to chlorophyll-*a* fluorescence, these

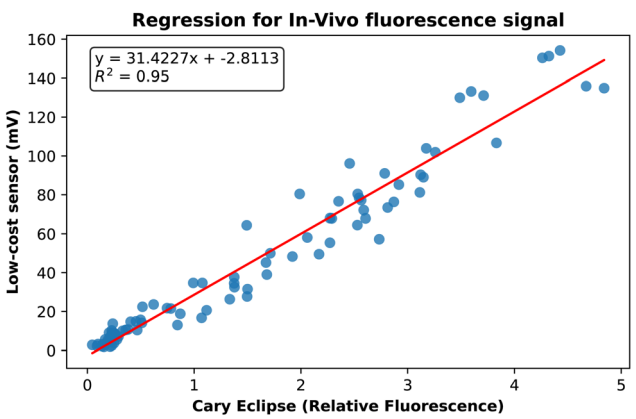


Fig. 4 Relationship between *in vivo* fluorescence measured by the low-cost sensor and the Cary Eclipse.

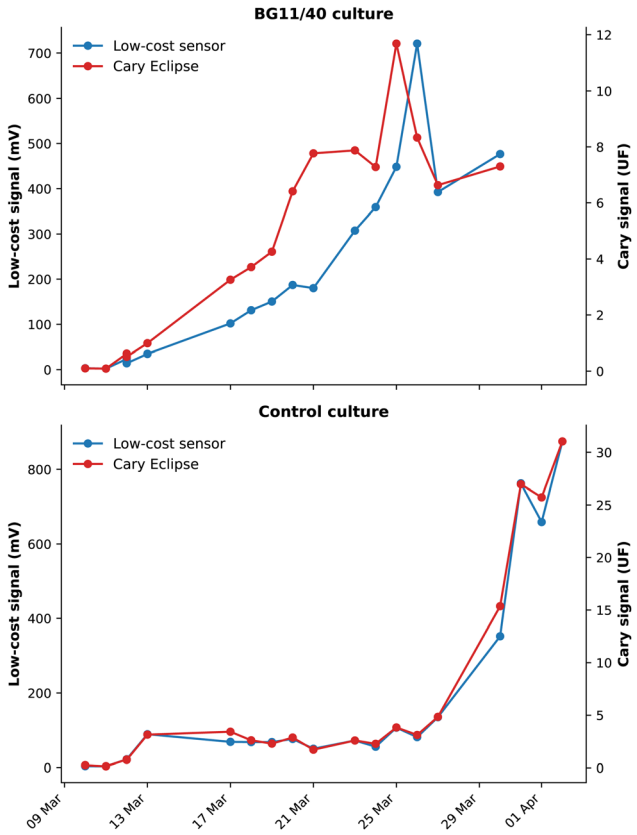


Fig. 5 Temporal monitoring of fluorescence dynamics in two different phytoplankton cultures. Each panel illustrates the comparison between the measurements acquired by the low-cost sensor (blue line) andthe Cary Eclipse (red line).

results demonstrate that the low-cost sensor reliably captures temporal variability in phytoplankton biomass. It should be noted that *in vivo* fluorescence reflects photosynthetic activity and pigment packaging effects, where intracellular pigment concentration affects fluorescence yield, not absolute chlorophyll-*a* concentration.[31] However, for tracking relative biomass dynamics, this limitation is acceptable.

3.3 Field deployment and implications for distributed freshwater monitoring

A site-specific calibration using the mesocosm samples was performed using both the low-cost sensor and the Cary Eclipse fluorometer. Similar to the *in vivo* experiments, calibrations conducted on natural samples exhibited high linearity and sensitivity. The coefficients of determination were R^2 = 0.994 and R^2 = 0.999 for the low-cost sensor and the Cary Eclipse, respectively, with corresponding slopes of 0.0013 and 0.061. The difference in slope reflects the higher optical collection efficiency and spectral discrimination of the Cary Eclipse fluorometer, leading to a greater fluorescence signal response per unit chlorophyll-*a* compared to the low-cost sensor.

Based on the *in vivo* calibration curves, chlorophyll-*a* concentrations ranged from 1.33 to 97 μg L^{-1}. Regression

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Table 8 Performance metrics of the low-cost sensor before and after linear calibration against the Cary Eclipse fluorometer

Dataset	RMSE (μg L^{-1})	Rel. bias (%)	R^2	RPD (%)
Raw data	12.71	26.48	0.94	24.43
Linear model (training)	5.30	2.99	0.943	15.92
Linear model (5-fold val.)	5.47	2.79	0.940	16.00

analysis between the two instruments showed a strong correlation ($R^2 = 0.94$; Fig. 8).

Using raw measurements from the low-cost sensor, the mean RPD% across the 59 samples was 24.4%, the mean relative bias was 26.5%, and the RMSE was 12.7 μg L^{-1}. After applying the linear regression model, there was a significant improvement in performance metrics (Table 8), with a RMSE of 5.3 μg L^{-1}, a mean relative bias of 2.99%, and a RPD% of 15.9%. Similarly, cross-validation results showed a RMSE of 5.5 μg L^{-1}, a relative bias of 2.8%, and a RPD% of 16%.

In order to further evaluate the agreement between the low-cost sensor and Cary Eclipse, and the potential of the linear calibration, the differences in concentration of chlorophyll-*a* between the two instruments were plotted using the Bland–Altman graphical method (Fig. 6 and 7). The mean bias is of −8 μg L^{-1} before the linear calibration, and a clear systematic underestimation trend of the chlorophyll-*a* concentration is shown, especially for high concentration values(>20 μg L^{-1}, Fig. 6). Nevertheless, the Bland–Altman plot after the linear calibration shows a mean bias of 0.4 μg L^{-1} and a narrowing of the limits of agreement, indicating a better agreement between the two instruments (Fig. 7). These results indicate that without the calibration, the quantification of cholorphyll-*a* using the low-cost sensor could produce false negative results in case of algal bloom. After the linear calibration, the results given by the low-cost sensor are still slightly underestimate when the real concentration is above 30 μg L^{-1}. Nonetheless, a water body is considered hypereutrophic when the concentration of chlorophyll-*a* is ≥ 40 μg L^{-1}. Given that the residual underestimation after calibration remains relatively small compared to this threshold, the calibrated low-cost sensor is expected to reliably detect hypereutrophic conditions and, more broadly, anomalous bloom events, despite a persistent tendency to slightly underestimate absolute chlorophyll-*a* concentrations at the higher end of the measured range. However, care should be taken when interpreting concentrations close to the hypereutrophic threshold itself, as the residual bias could in principle lead to borderline values being classified as eutrophic rather than hypereutrophic.

While the application of a linear calibration model substantially improved the accuracy of the low-cost sensor, it should be noted that the calibration remains dependent on specific environmental conditions. Its applicability outside the tested range and context should be carefully evaluated. Moreover, the fluorescence signal used for chlorophyll-*a* quantification is known to depend on multiple environmental and physiological factors, including temperature, phytoplankton

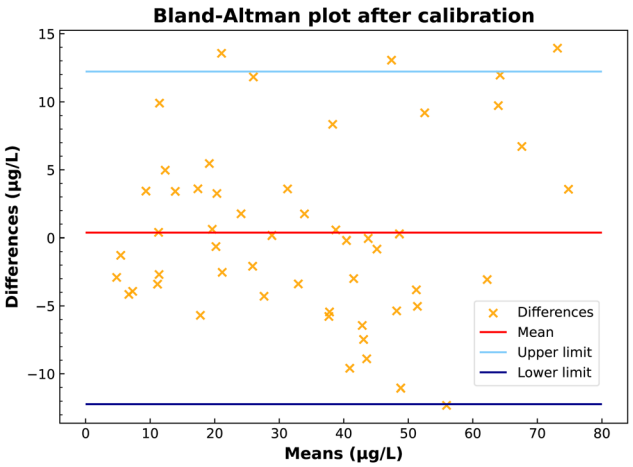


Fig. 7 Bland–Altman plot of differences between Cary Eclipse and the low-cost sensor after the linear calibration.

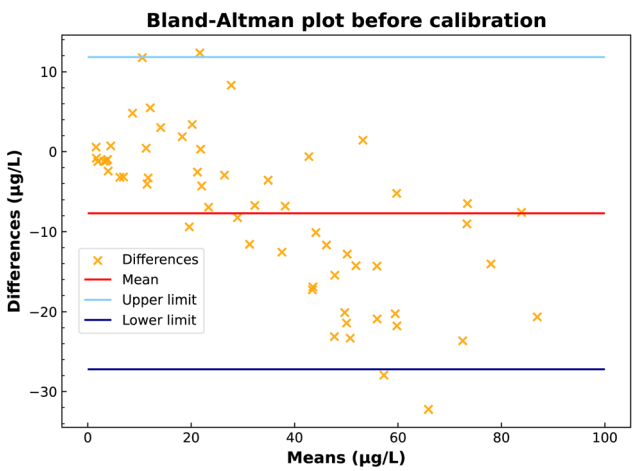


Fig. 6 Bland–Altman plot of differences between Cary Eclipse and the low-cost sensor before the linear calibration.

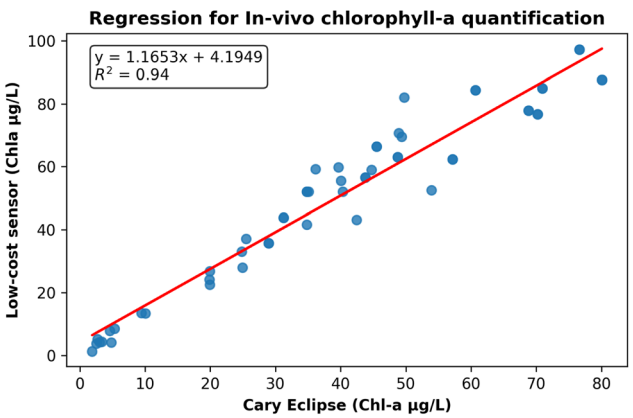


Fig. 8 Relationship between *in vivo* quantification of chlorophyll-*a* by the low-cost sensor and the reference spectrofluorometer (Cary Eclipse).

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community composition, and the physiological state of the photosynthetic system.[32,33] For instance, cyanobacteria contain phycobiliproteins that alter the relationship between 430 nm excitation and chlorophyll-*a* fluorescence, potentially requiring community-specific calibrations.[34] Consequently, while the proposed model improves the analytical performance of the low-cost sensor, its applicability remains site specific.

3.4 Scope and limitations of turbidity validation

The results of calibrations and the analytical validation for turbidity showed good performance of the low-cost sensor under laboratory conditions. However, the turbidity experiments were performed using formazin exclusively. Although formazin is a standard reference material recognized internationally for defining the NTU scale, no comparison against a reference nephelometer was conducted in this study, which represents a limitation for assessing turbidity trueness and precision on real samples.[35] Indeed, in environmental samples, suspended particles rarely share the size distribution and shape of formazin particles, and measurements may diverge from the formazin-based calibration, a well-known limitation of nephelometric turbidity measurement in general. A further potential limitation concerns light absorption by real samples. To minimise interference from dissolved coloured organic matter (CDOM), the proposed low-cost sensor uses a near-infrared LED, chosen specifically for consistency with ISO 7027-compliant nephelometers. However, this does not fully address potential absorption or scattering by particulate matter, which may behave differently from the optically near-transparent formazin particles used for calibration. Future work should prioritise cross-validation with a reference nephelometer on environmentally representative samples to assess trueness and precision also under operational conditions.

4. Conclusions

Reliable and affordable sensing technologies are essential for increasing the spatial and temporal coverage of freshwater monitoring as well as maintaining data quality standards required for environmentally meaningful observations. This study demonstrates that open-source fluorescence sensing, when combined with rigorous analytical validation, can provide robust measurements of chlorophyll-*a* and turbidity under field conditions.

Given this site specificity, the deployment of the sensor in a new context should follow a calibration procedure rather than relying on the coefficients reported here. This should include: (a) – collecting a set of local samples spanning the expected concentration range and the expected interference for that site (such as turbidity, dissolved organic matter, dominant phytoplankton taxonomic composition); (b) – using duplicate independent samples in order to separate sampling-related uncertainty from purely instrumental uncertainty, following the Eurachem "Uncertainty Arising from Sampling";[26] (c) – fitting a local regression model between the two instruments and validating it *via* cross-validation, as done in this study; (d) – periodically re-verifying the calibration, given the potential for temporal drift in community composition and environmental conditions. While a full site-specific calibration protocol is beyond the scope of this paper, we consider this a necessary step for operational deployment and an important direction for future work.

Unlike many low-cost systems that remain confined to proof-of-concept studies, the proposed platform underwent extensive analytical evaluation involving more than 200 measurements and direct comparison with a benchtop reference instrument. Although chlorophyll-*a* quantification remains inherently challenging due to environmental and physiological variability, the proposed sensor reliably captured temporal changes in fluorescence and associated variations in phytoplankton biomass. These findings suggest that low-cost distributed sensing can complement conventional monitoring approaches, provided that calibration and uncertainty are explicitly considered during deployment. With a production cost below US$100, FROG combines open-source design, automated data processing, and cloud connectivity within a reproducible and deployable framework for freshwater monitoring. Rather than replacing laboratory-based analyses, such systems may help address persistent monitoring gaps by increasing the spatial and temporal resolution of observations in small catchments and other underserved environments. By enabling distributed environmental observations while maintaining analytical robustness, this approach would support a more extensive integration of community-based monitoring into freshwater management strategies.

Author contributions

Riccardo Gaetano Cirrone: conceptualization, formal analysis, investigation, methodology, writing – original draft. Francesco Vesprini: investigation, formal analysis, writing – review. Óscar González: visualisation, writing – review & editing. Amedeo Boldrini: data curation, writing – review. Alessio Polvani: data curation, writing – review. Xinyu Liu: visualisation, writing – review. Anna Witter: writing – review & editing. Luisa Galgani: visualisation, writing – review & editing. Steven Arthur Loiselle: conceptualization, data curation, formal analysis, methodology, project administration, supervision, writing – original draft, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

All data, design files, firmware, and calibration scripts supporting the findings of this study are openly available at https://github.com/LowCostSensor/FROG-sensor.

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