Measuring fluorescence by means of smartphones with the new Citclops-Application

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SUMMARY

Why is it necessary to dedicate efforts in the development of a measurement system to determine fluorescence with smartphones? Because fluorescence is a proxy for algal biomass and dissolved material in water, and is therefore an indicator for different processes of societal concern. Fluorescence is conveniently used to measure such parameters within monitoring programs and scientific research. By utilizing smartphones citizens are enabled to actively participate in measurements of water quality to achieve a spacious data set of these parameters.

In the EU-project Citclops (Citizens' observatory for Coast and Ocean Optical Monitoring; *10*) two new devices were developed to measure algal fluorescence with smartphones. We present a) a method and sensor geometry for the use of internal smartphone elements for retrieval of Chlorophyll *a* (Chl *a*) fluorescence, and b) a multi-parameter fluorometer which can be directly accessed by multiple mobile end-devices.

Both approaches are easy to use, small and affordable for their respective field of use. Algorithms and applications to measure fluorescence of Chl *a* by means of smartphones were developed for citizen involvement for the two of them. The potential of the Citclops initiative is an almost synoptically and spacious data set of coastal seas based on participatory science.

INTRODUCTION

Coastal ecosystems are stressed by several anthropogenic factors e.g. tourism, fishery, and environmental pollution and their potential effects. In order to make a statement about the environmental state of the ecosystem, water quality is an important criterion.

As such, fluorescence is a standard procedure for the retrieval of water constituents, such as algal biomass, hydrocarbons, and colored dissolved organic material since many years.

There are several portable fluorometers *(8)* which are used from multiple platforms for retrieval of Chlorophyll *a* (Chl *a*). Some can be included into a FerryBox system for flow through measurements *(4, 7, and 1)*. Each of these has one excitation wavelength and one detection wavelength.

In order to measure different substances simultaneous more than one excitation wavelength is essential as given in the Algal Online Analyser® *(bbe-Moldaenke; 9, and 2)*. With multi-excitation but one emission wavelength the community composition of phytoplankton could be measured *(9)*. The measurement of different substances requires additional detection wavelengths as realized with the Advanced Laser fluorometer *(WET Labs; 3)*.

All these instruments are expensive and accurate, but restricted to use by professionals and deliver a restricted data set with limited spatio-temporal resolution. For observing and estimating the coastal environment, temporal-spatial data gaps could be filled by establishing a citizens' observatory.

The transfer of the common approach of measuring fluorescence to a mobile and low-cost device would allow citizens to actively participate in environmental monitoring. The wide availability of smartphones turns such devices into a perfect platform for low-cost sensory systems *(6, 5, and with photosynQ: 11)*.

Therefore, the objective of this study is to transfer the principles of fluorescence systems to smartphones. This is realized in two different ways a) a method and sensory system for the

smartphone and b) a multi-parameter fluorometer controlled by means of mobile end-devices. Professionals as well as citizens have the possibility to measure fluorescence by means of smartphones with the multi-parameter fluorometer and low-cost sensor, respectively.

METHODS

a) Method and sensory system for the smartphone

The transfer of the fluorescence measuring method is realized by means of 1) proof of principles, 2) prototype developing and 3) the retrieval of the Chl *a* concentration.

1) Proof of principle

Measuring fluorescence based on the knowledge that the fluorescence of each substance depends on the excitation wavelength and a certain emission wavelength which is detected by a detector, usually a photodiode. An external low-cost light emitting diode (LED) as excitation source, a low-cost filter, and a camera as detector is used to proof the principle of fluorescence measurements with smartphone components (Fig. 1a) partly based on the fluorometer by Leeuw et al. *(6)*. This measurement setup (Fig. 2b; camera not shown) is tested with a dilution series of Chl *a* standard from 1 to 10 µg/l and corrected with acetone.

2) Prototype of the sensory system

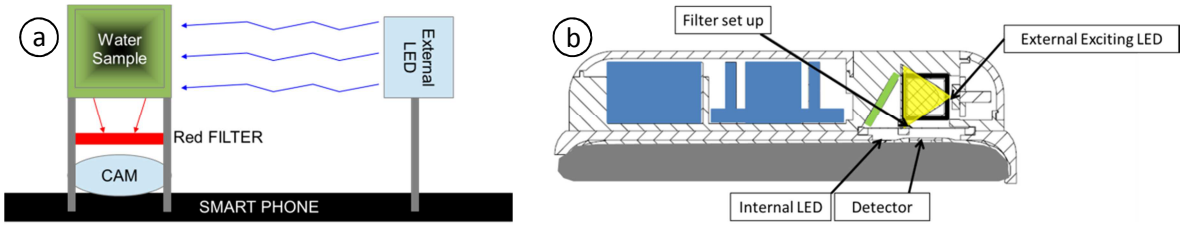

Figure 1: *Measure fluorescence with the smartphone. (a) A schematic diagram how the fluorescence signal is excited by an external LED in the water sample and how it is detected with the smartphone's camera (cam). (b) The top view shows how the schematic diagram given in (a) is realized on to the smartphone. Blue parts are electronic parts, the green square symbolized a mirror, and the black non-filled square indicates the position of the cuvette with the sample. The yellow triangle shows the light emitted by the external LED, the grey part is the smartphone and the shaded part is the holding system which is plugged at the smartphone (adapted after Nick Rüssmeier, pers.comm).*

For the application of the smartphone as fluorescence sensory system, the internal smartphone LED, as well as external LEDs were used as excitation light source while the smartphone's camera operated as detector. All these components and an additional filter setup are installed within an adapter attached to the smartphone (Fig. 1b). Within the adapter a cuvette with the sample is placed directly in front of the camera. The external LEDs and the control unit are connected to the smartphone via the earphone plug in as did similarly by Kuo et al. *(5)* and controlled by means of an application (APP).

3) Relation of the red, green and blue (RGB) images to Chl a concentration

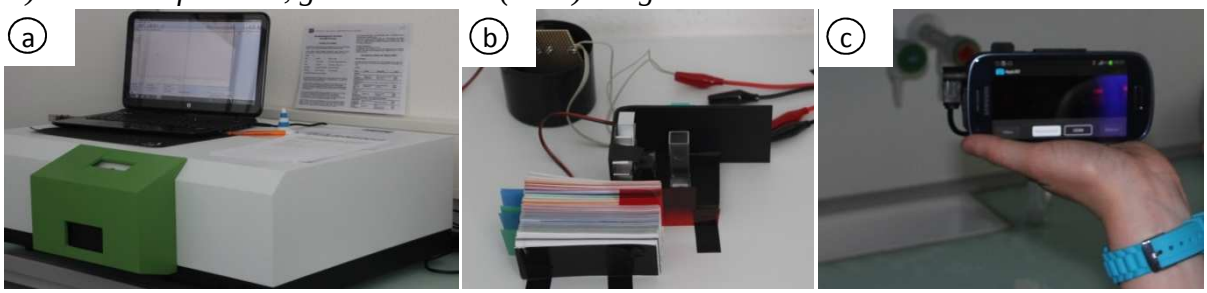

Figure 2: *The three different measurement systems are shown (a) the laboratory fluorometer, (b) the measurement set up with the external LED and the external camera (not shown) as detector, and (c) the prototype of the smartphone adapter.*

The measurement setup with a camera (Fig. 2b) and the prototype smartphone adapter (Fig. 2c) was tested with a fine dilution series of Chl *a* standard and corrected with acetone. Control measurements were conducted with a laboratory fluorometer (Fig. 2a; LS 55, PerkinElmer fluorescence spectrometer) and also corrected with acetone. The red to green (R2G) ratio of the RGB images taken by the camera are determined and related to the Chl *a* concentration.

b) Multi-parameter fluorometer using mobile end devices

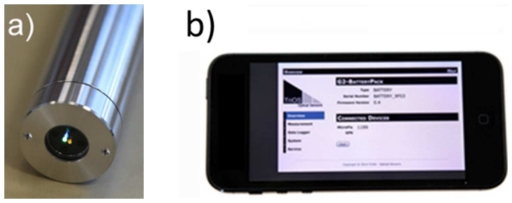

Figure 3: *The multi-parameter fluorometer (a) shows the housing with illuminated LEDs and (b) shows the web interface how the devices can be controlled with the mobile end-device. Instrument and web interface is manufactured by TriOS (Germany).*

The second fluorescence sensory system is a portable fluorescence sensor (Fig. 3a) which is usable with any mobile end-device. The sensor itself provides a wireless local area network (WLAN) signal (Fig. 3b) accessible for the mobile end-devices. Four different LEDs as excitation source, filters and detectors for different wavelengths were selected so that several parameters could be measured simultaneously.

RESULTS

The outcome of proofing the principles is transferred on to the mobile devices. A fine resolved dilution series between 1 µg/l and 10 µg/l was measured with a laboratory fluorometer (Fig. 2a), the measurement setup (Fig. 2b), and the prototype using the external LED (Fig. 2c).

1) Proof of principles

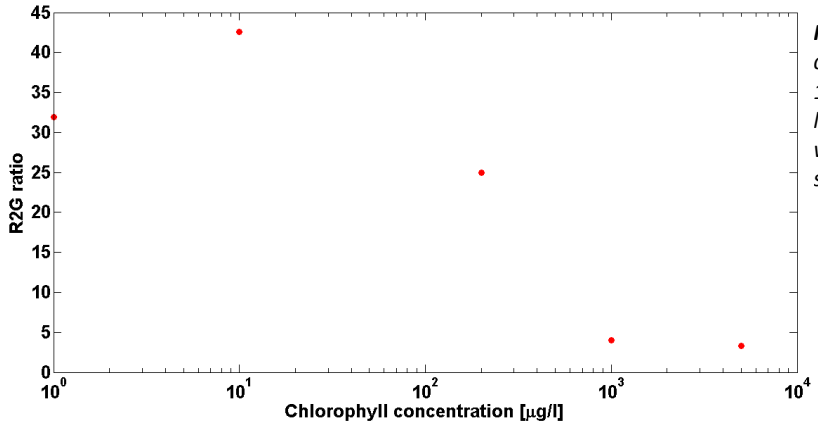

Figure 4: *The R2G ratio for Chl a concentration 1 µg/l, 10 µg/l, 200 µg/l, 1000 µg/l and 5000 µg/l are semi logarithmic presented. The data are derived with the measurement setup and a dilution series of Chl a standard.*

The principle of measuring fluorescence was proved with the measurement setup (Fig 2b). The samples of the coarse dilution series were excited by means of an external LED and the fluorescence is detected by an external camera. Scattered light and light of different wavelength are restricted with a red filter. The R2G ratio derived from the RGB images relates to a certain Chl *a* concentration (Fig. 4). The R2G ratio decreases from 10 µg/l Chl *a* concentration to higher Chl *a* concentration. An increasing occurs between the lowest Chl *a* concentration and 10 µg/l (Fig. 4).

2) Prototype of the sensory system

The fluorescence adapter and measurement components: LEDs, internal smartphone camera and a cuvette with the sample were successfully controlled with an APP and allowed fluorescence measurements. The APP activates the LED and takes the RGB images including metadata. The RGB images are taken for determination the R2G ratio and getting a relation of the RGB images to the Chl *a* concentration.

3) Relation of the RGB image to Chl a concentration

A finer dilution series was measured with the measurement setup (Fig. 2b), the prototype (Fig. 2c), and the laboratory fluorometer (Fig. 2a) to obtain in more details the relation of RGB images to Chl *a* concentration.

The control measurements show increasing fluorescence with increasing Chl *a* concentration. The maximum occurs around 670 nm (Fig. 5a).

The R2G ratio shows a general decreasing from lower Chl *a* concentration until higher Chl *a* concentration (Fig. 5b and c). Nevertheless, the R2G ratio is distributed widely and outliers exist. The R2G ratio value for 1 µg/l of the coarse resolved data set (Fig. 4) fits to the R2G ratio value for 1 µg/l of the finer resolved data set (Fig. 5b). The other values are too small for fitting into a declining regression line with the coarse data set.

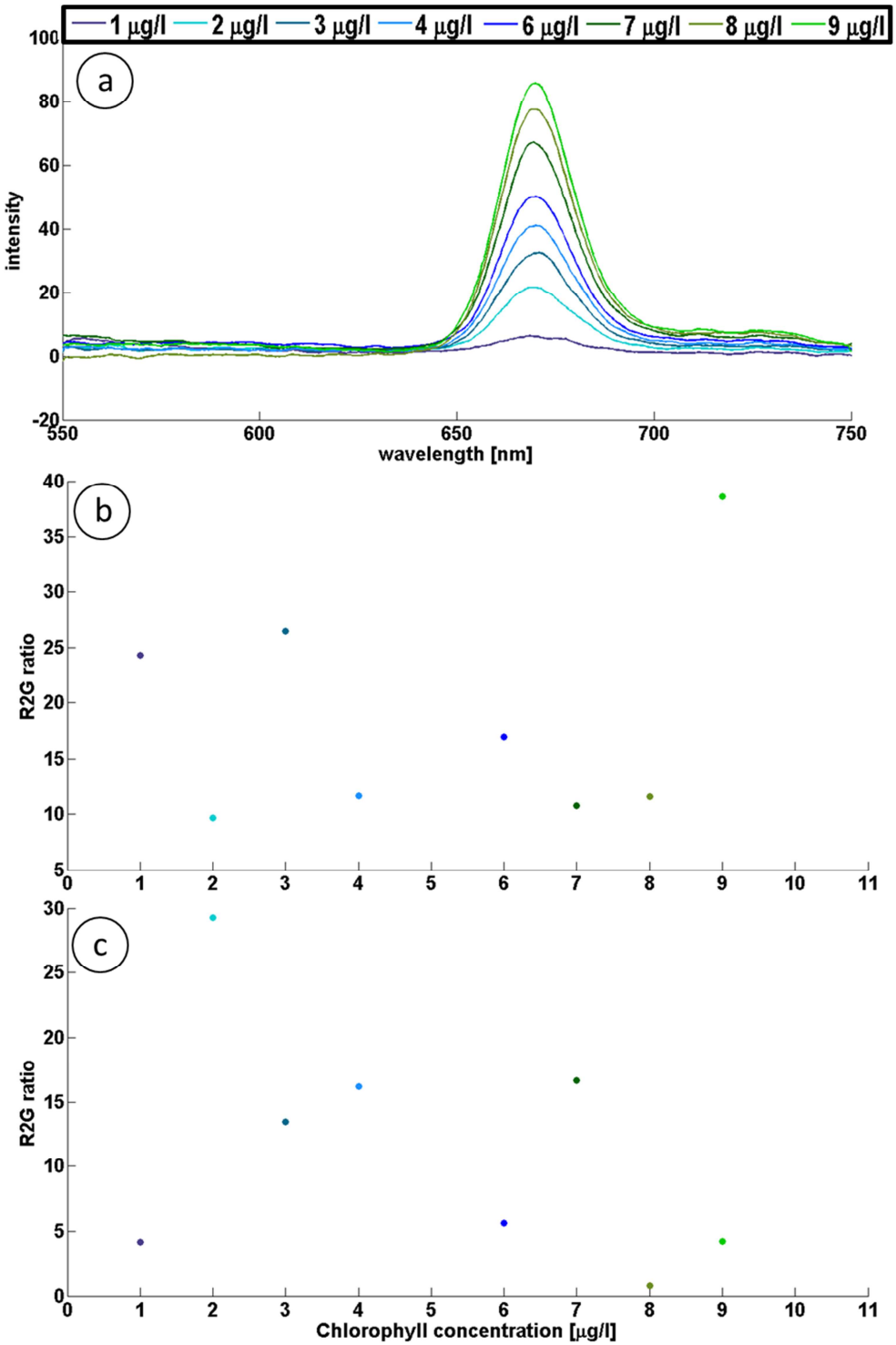

Figure 5: *The results of the different measurement methods. Results of (a) the laboratory fluorometer are given as intensity versus wavelength. For (b) the measurement setup and (c) the prototype the results are given in R2G ratio versus Chl* a *concentration. The color code for (b) and (c) is as in (a).*

The multi-parameter fluorometer is still under investigation so that no fluorescence data are available. Yet, the operation of portable fluorometers with smartphones by means of a WLAN connection was successful.

DICUSSION and OUTLOOK

Transferring the measuring principle of fluorescence on to the smartphones is accomplished with the adapter including the excitation source (LED), the detector (camera), the control unit, and a fixed place for the cuvette for the sample. The application of low-cost components in combination with smartphones is generally appropriate.

The relation between RGB images and Chl *a* concentration by determination the R2G ratio works well for the Chl *a* concentrations between 10 µg/l and 5000 µg/l. Therefore, the approach to use a camera as detector is promising. Yet, the determination of the R2G ratio for retrieval a relation between RGB images and Chl *a* concentration lower than 10 µg/l is not yet feasible and needs further investigation.

The assumption that the R2G ratio decreases with increasing Chl *a* concentration could neither be certified nor be confuted with the finer resolved dilution series. The results of the finer resolved dilutions series lack a clear decreasing or increasing of the R2G ratio with increasing Chl *a* concentration. A further investigation in this range is required as well as in higher Chl *a* concentration to get a fitting curve for the quality control. Also, the effect of scattering needs to be considered by replacing the Chl *a* standard by algal cultures.

Our study shows promising results towards the use of smartphones as fluorescence sensory systems. The smartphone works successfully as control system for external low-cost sensory systems for establishing a citizens' observatory. Additional the conversion of the smartphone into a sensory system by means of the shown prototype expands the number of monitoring tools within the citizens' observatory.

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