CyanoSensor
Low-Cost Early Warning System for Cyanobacteria

Graduation; Project: CyanoSensor; Final Report

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Advanced Sensor Applications
CyanoSensor
Low-Cost Early Warning System for Cyanobacteria

Submitted to
University of Applied Sciences Groningen

in partial fulfillment of the requirements
for the degree of
Fulltime Honours Advanced Sensor Applications

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<td>Version</td>
<td>2.15</td>
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<tr>
<td>Date</td>
<td>June 21, 2015</td>
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Abstract
During recreational activities, people can be exposed to toxins produced by cyanobacteria by ingesting contaminated water, inhaling toxic fumes or direct skin contact. Short term exposure may cause varying complaints like mild nausea and skin problems. Long term exposure may lead to sub-chronic conditions. Determining the presence of cyanobacteria is done by visual inspection and laboratory tests for over 650 recreational inland bathing waters in The Netherlands. A major obstacle is the time that is lost between sampling and taking actions. In this project was focused on the development of an optical sensor that can perform ad hoc measurements significantly faster. *In vivo* phycocyanin in cyanobacteria cells emits light at 610nm–630nm when excited at a wavelength of 645nm. The intensity of fluorescent light was measured with an optical sensor that was constructed for less than 70 Euro. The designed photospectrometer has measured *in vivo* phycocyanin in a *Microcystis* sample that contained between 534µg/L and 686µg/L phycocyanin.
Declaration

I hereby certify that this report constitutes my own product, that where the language of others is set forth, quotation marks so indicate, and that appropriate credit is given where I have used the language, ideas, expressions or writings of another.

I declare that the report describes original work that has not previously been presented for the award of any other degree of any institution.

Signed, Tim Stoppelenburg, Borger, 21 June 2015
Acknowledgements

In the second semester of the third year at Advanced Sensor Applications (2012), I started a project about blue-green algae with Anton Atanasov, Yaroslav Shuper and Ale-Watze Wiegersma. In that project, we designed a large buoy that could measure everything – except for blue-green algae. After the project ended, thoughts about how to measure blue-green algae followed me for a long time, until the point where I presented the subject as graduation project. Throughout the development process, my interest in the subject and excitement with the technology only kept growing, until the point where I almost forgot to graduate.

I would like to thank Imke Leenen for giving me the opportunity to continue with this project and for becoming my supervisor. Without her advice and feedback, it would not have been possible to complete even half of the research. I am also grateful to Wendy Beekman-Lukassen and Miquel Lurling, who made it took their time to help me to perform experiments with their equipment and to validate my prototype at Wageningen University & Research. Furthermore I would like to thank Corina Vogt for her constructive feedback and Johan Hekman for continuously making me question what I was doing.
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1. Introduction

This document is the final report for the graduation semester at Advanced Sensor Applications at the Hanze Institute of Technology in Assen (The Netherlands), part of the Hanze University Groningen.

1.1 Scope

The Netherlands has over 650 recreational inland bathing waters. A part of maintaining the water quality is the determining of the presence of cyanobacteria in the surface water[1]. Cyanobacteria that can produce toxins are a health risk to bathing guests, living organisms and animals that drink or play in the water. Several times per year, there are reports of inland beaches that are closed due to the health risks caused by cyanobacteria and yearly there are reports on dogs that have died after drinking water which contained cyanobacteria[2, 3].

Exposure to high concentrations of toxins produced by cyanobacteria is a serious health threat and can lead to a number of severe health complaints. Additionally, the water will attain a bad smell from the decay of algae scums. In compliance with the European Bathing Water Directive [1], the Nationaal Water Overleg established a protocol on cyanobacteria that describes the observation methods, hazard levels and intervals between measurements [4]. Determining the presence and amount of cyanobacteria is done by visual inspection and lab tests.

The 2012 blue-green algae protocol[4] describes how often and which types of measurements should be done and which management actions should be taken. A member of the responsible waterboard visits the location to see if there is a layer of algae floating on top of the water. The sample is analyzed in the laboratory and it takes a few days before the volume of phycocyanin or biovolume of cyanobacteria is determined.

1.2 Goal

The current measurement methods are time-consuming and expensive. In this project is focused on the development of a low-cost sensor that can indicate the presence of cyanobacteria according to the risk levels determined in the blue-green algae protocol[4]. The research question is defined as:

How can a low-cost photospectrometer be implemented as an early-warning system for hazardous cyanobacteria levels?

This leads to the following questions:

1. What are the properties of cyanobacteria?
2. Why are certain concentrations of cyanobacteria hazardous?
3. How are cyanobacteria currently measured?
4. Why is there a need for an early-warning system?


1.3 Structure

This project consists partly of research and partly of development. In this document, general research in cyanobacteria is described in chapter 2. Several experiments were performed to illustrate and understand the properties of cyanobacteria. These are described in chapter 5 and appendices A and B. In chapter 3 it is described which problems need to be solved in order to find a better system for measuring cyanobacteria. Chapter 4 describes the implementation of a proposed optical sensor system and in chapter 5 it is described with which accuracy the sensor can resolve measurements into cyanobacteria risk levels.

1.4 Partners

The project is done at the Center of Excellence for Intelligent Sensor Innovation at the Hanze Institute of Technology (HIT) under the supervision of external company supervisor Dr. Ir. Imke Leenen, director of H₂Oké Water & Gezondheid advies.

The HIT in Assen is part of the Hanze University Groningen. In September 2008, the HIT began as an European pioneer in the field of sensor technology education and works closely with partners such as Astron, the Nederlandse Aardolie Maatschappij (NAM), Shell and Sun Microsystems. The projects done at the HIT are research & development projects that require creativity, imagination and commitment to come to an innovative proof of concept. The education program is based on sensor appliances, which includes all aspects of the engineering disciplines.

H₂Oké is a consultancy that advises, executes studies or supports organizations in making choices in the field of water, health and spatial planning. In projects in which specifically water and people (or animals) are involved, H₂Oké supports with research and advice. Some examples are: recreational and bathing water, urban water (including management for climate-proof cities), water recycling and the development of new sanitation concepts.

Until 2014 Director Imke Leenen was a Senior Adviseur Water & Volksgezondheid at Grontmij Nederland, one of the largest water management consultants. After that she continued as director of H₂Oké. She is well acquainted with other experts in the field of water management and cyanobacteria at Wageningen University & Research, the University of Amsterdam, Deltares and the National Institute of Health & Environment.
2. Analysis

In this chapter it is explained what blue-green algae are, what their unique properties and hazards are. It is also described how these organisms are analyzed and which methods are used according to the Dutch protocol.

2.1 Cyanobacteria

Blue-green algae are among the oldest forms of life on earth and represent a stage in evolution of plant life. The name blue-green algae describes more than a thousand species of procaryotic organisms. Most organisms in this group are aerobic, photoautotrophic cells that are capable of photosynthesis[5]. Because these are procaryotic cells, the correct term for blue-green algae is blue-green bacteria or cyanobacteria[6]. With the presence of oxygen, water, carbon dioxide and light, they can survive and grow in a wide variety of habitats around the world[7]. They occur most often in freshwater ponds, but can also survive in brackish water and salt water[8, 9].

Cyanobacteria are capable of performing photosynthesis, which is achieved through the pigment phycocyanin[10]. The cells have the capability to produce massive populations which can cause blooms and scums[11]. With these properties, they pose a threat to ecosystems in different sorts of open water. In the Netherlands, cyanobacteria are a threat especially to fresh water bathing sites where there is a high probability of human and animal contact with the toxins that cyanobacteria produce[12].

Because of eutrophication, the occurrence of cyanobacteria blooms have increased. It is however important to note that cyanobacteria blooms are part of the ecosystems of lakes and ponds. It is not possible to consider that cyanobacteria blooms are maintained by the waterboards, nor is it desirable to remove the natural component of phytoplankton completely from these waters[8].

There are several factors that provide a suitable environment for algae growth. The conditions in which cyanobacteria growth is optimal are the following[13]:

- A water temperature between 20°C and 30°C;
- not too much/bright light;
- little to no water flow (stagnant water);
- water rich of nutrients like phosphate and nitrogen;
- calm weather conditions with limited wind and rain.

Many toxin-producing cyanobacteria are able to regulate their vertical position in the water. With intracellular gas vacuoles, they can level off to a depth where environmental factors such as light levels and oxygen levels are favourable. In calm weather conditions, cyanobacteria accumulate on the water surface in the form of scum formations. When cyanobacteria reach the end of their lifecycle and die, the cells rupture with the result that toxins are released and attain bad smell[8, 13].
2.2 Health risks

One of the major differences between cyanobacteria and regular algae is that many species are capable of producing a wide variety of cyanotoxins[14]. These toxins may contribute to human neurodegenerative diseases and serious health complaints. Exposure of cyanobacteria to humans may lead to several health conditions varying from mild nausea, skin problems and in rare cases, even death.

There have been cases where animals have died after exposure to cyanobacteria and where people have fallen ill after direct contact with cyanobacteria. Some years, dogs have been reported dead after drinking water which contained cyanobacteria blooms[2, 3, 15, 16]. In Wisconsin, USA, five teenagers went swimming in Anabaena-infested water in 2002. Two of them accidentally ingested the contaminated water which caused heavy nausea, vomiting and diarrhea resulting in the passing away of one of them[17]. In England, twenty soldiers were subjected to training in water that was infested with Microcystis. Half of the soldiers fell ill with symptoms such as vomiting, diarrhea, abdominal pain, sore throat and ulcerated lips. Two of them were hospitalized with a lung infection[18].

In 2006, the National Institute for Public Health and Environment (RIVM) in The Netherlands questioned 166 people who went swimming in water with more than 10 µg/L microcystin. 16% of the people who had direct contact with the water had light health complaints such as skin irritation, vomiting and diarrhea. No health complaints were reported by the people who did not get in contact with the water[8, 19].

Based on observations between 1998 and 2006, health complaints as a result of swimming in water which contained cyanobacteria were vomiting, diarrhea, abdominal pain, skin irritation, gastrointestinal symptoms, severe dizziness and lung infections. In several cases, the complaints were linked to a visit to the same recreational water. Leenen and De Roda Husman (2004) imply that mild complaints are not reported to the physician and that physicians generally do not relate the symptoms to swimming in cyanotoxins[8, 20].

People can come in contact with cyanobacteria in different ways: via drinking water, food (supplements) and via surface waters. Especially in recreational bathing waters, it is unclear what the relation is between coming in contact with cyanobacteria and the earlier mentioned health complaints. Most of the reported cases occurred in (semi-) natural waters. During recreational activities such as swimming, people can be exposed to cyanobacteria and their toxins by accidentally ingesting contaminated water, inhaling toxic fumes or direct skin contact. Short term exposure does not appear to lead to chronic conditions, but long term exposure may lead to sub-acute or sub-chronic conditions[13].

2.3 Cyanotoxins

Different species of cyanobacteria produce different toxins. Cytotoxins are found in the species Cylindrospermopsis, Umezakia, Aphanizomenon, Anabaena and Raphidiopsis[21]. These toxins slow down the synthesis of proteins and cause necrosis in the liver, kidneys, appendix and lungs[22]. Dermatotoxins cause irritation to the skin and eyes, but may also lead to fever and are often found in Lyngbya, Planktothrix and Schizothrix.

Hepatotoxins are the most occurring cyanotoxins and can consist of microcystins and nodularins. These toxins are often found in cyanobacteria blooms in freshwater and brackis water and are associated with bleeding and infection in the liver. The microcystins are produced by the species Anabaena, Microcystis, Planktothrix, Nostoc and Anabacenopsis[23]. Microcystins often cause the death of fish and birds in ecosystems where large cyanobacteria blooms are found. The toxins are present in the cells of cyanobacteria and are released when the blooms massively reach the end of their lives at the end of the cyanobacteria season[24].
Neurotoxins primarily poison organisms and animals in their direct environment. Exposure may cause the breathing of small animals to stop even within minutes. Neurotoxins such as anatoxine-a can be found in Anabaena, Planktothrix and Aphanizomenon[25]. Homoanatoxin is found in Planktothrix. Saxitoxins are often found in Aphanizomenon, Anabaena, Lyngbya and Cylindrospermopsis. In general, neurotoxins are found in almost twenty different species, not limited to Microcystis, Lyngbya, Nostoc, Planktothrix, Anabaena, Aphanizomenon and Cylindrospermopsis.

These species can be found in freshwater, brackish water, salt water, warmwater springs, hot water springs and even in terrestrial cyanobacteria that have symbiotic relationships with lichens. The amino acid β-Methylamino-L-alanine is associated with complaints such as limb muscle atrophy, degeneration and partial loss of pyramidal neurons of the motor cortex, behavioral dysfunction and Alzheimer[8, 26, 27].

2.4 Regulations

The World Health Organization has drafted guidelines for management actions for water with cyanobacteria in 2003. These guidelines are based on the toxicity levels reported in existing literature and are a subject of discussion as there is no certainty about the relation between cause, effect and prevention of cyanobacteria and health risks. Since 2006, there is a European directive for bathing water. Here, cyanobacteria are explicitly named as a potential risk for water quality, which requires special treatment/care[8].

There have been loads of publications about the negative effects of cyanobacteria which makes it reasonable to assume that there is need to be cautious when dealing with cyanobacteria and scum formations. Most of the health risks are expected when recreational waters show scum formations. Often when action is taken after a scum formation is observed, it would imply that earlier signs of growth had not been detected. By monitoring frequently at the start of spring, it should be possible to measure the formation of cyanobacteria blooms, so that in combination with weather conditions, scum formations can be detected earlier. This gives better opportunities to take management actions[13].

The Netherlands has a protocol for dealing with cyanobacteria in recreational inland bathing waters. This protocol was approved by the national board for water maintenance Landelijk Bestuurlijk Overleg Water in 2010. After an evaluation in 2011, the most recent protocol was established by the Nationaal Water Overleg in May 2012. This protocol is used throughout the project and focuses on natural inland bathing sites, swimming waters, surface waters and city waters/fountains.[4].

The protocol refers to the visual inspection of cyanobacteria by means of definitions of scum layers and fluorescence measurements to determine the concentration of cyanobacteria. There are three main categories in scum layers.

Figure 2.1: Category II scums on top of water[4]
In the first category, small wires (strings of cyanobacteria) or little green balls on the surface are observed. There are no compacted layers yet and also there is no typical smell. There is no mentioning of actual layers but more of clusters. These clusters can grow into layers under the right conditions.

Category II involves a lot of clusters that have stacked together to form a small layer, where one can still see through the medium now and then. There is still no typical smell, but these kinds of layers indicate that there has been accumulation due to the wind blowing them all in the same direction. They are commonly found at the shore lines.

Category III involves a lot of clusters that have stacked together to form a small layer, where one can still see through the medium now and then. There is still no typical smell, but these kinds of layers indicate that there has been accumulation due to the wind blowing them all in the same direction. They are commonly found at the shore lines.

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Figure 2.2: Category III scums on top of water

In category III, the layers have become so thick that not even the water can be seen. The layers have grown significantly and have volume and consistency. The colors of the layers have gone from the original green to white or light blue, indicating rot. One may assume that there are significant levels of dangerous toxins in the water.

2.5 Measurement Methods

The protocol describes that when scums and compacted layers are observed, a sample is to be taken to the laboratory for analysis. The most common methods in The Netherlands for determining the concentration of cyanobacteria are fluorescence measurements and counting. There are also several other methods that are capable of detecting the presence and/or quantity of cyanobacteria and toxins in water[7].

Polymerase chain reaction (PCR) is a biochemical technology to increase the volume of pieces of found DNA in a sample. DNA is extracted from cells in the water and duplicated with thermal cycling of heating and cooling the DNA, which allows enzymatic replication. With the correct primers, short DNA fragments, particular groups of DNA can be duplicated. By using the primers that are associated with particular cyanobacteria strains, the presence of a particular species can be confirmed when the amount of DNA is amplified above a detection point. This method is highly sensitive, widely available and uses only a small amount of sample, but is time-consuming, very delicate and requires very careful and time-consuming preparation[28, 29, 30].

High performance liquid chromatography is a separation method where eluent is pushed into densely packed columns under high pressure. A carefully determined amount of sample is injected,
after which carotenoids, chlorophyll-a and phycocyanin, the major photosynthetic pigments, can be separated by the chromatography process. From the presence of phycocyanin can be concluded that there are species of cyanobacteria present in the original sample. This method analyses a variety of substances, but also requires large and expensive equipment [31, 32, 33].

Gas chromatography is used to analyse compounds by vaporizing them without decomposition. The method separates different components in a sample and is able to display relative amounts of the individually present compounds. An nonreactive carrier gas moves the vaporized compounds into glass or metal columns. Gas chromatography is helpful in determining the presence of toxin-producing cyanobacteria by detecting the types and quantities of toxins [34]. The method is compact and very accurate, but is hard to automate due to the constant need to recharge the nonreactive carrier gas for the mobile phase.

Bioassay can also lead to the detection of toxicity due to cyanobacterial hepa-to-and neurotoxins [35, 36]. The biological assessment is a type of scientific experiment where the reaction of a living organism is evaluated when it is exposed to a compound, or in this case, a sample of cyanobacteria. The response of the organism tells whether there are cyanobacteria and/or cyanotoxins present and how they affect their direct environment. Bioassays are a diagnostic tool for research, but are not suitable for ad hoc measurements [37].

Cell counting is used in The Netherlands to determine the absolute number of cells per unit of volume of cyanobacteria [8, 13]. A sample of (suspected) contaminated water is brought to the laboratory and is cultivated up to several days. Specialists manually count (a part of) the amount of known cyanobacteria cells and calculate the approximate amount of cyanobacteria in the original sample. CASY® counting determines the cell viability based on the structural integrity of the plasma membrane. Living cells have intact plasma membranes that do not allow electric currents to pass through when they are exposed to a low voltage field. When the cells are aligned and pushed through an electric field, each intact cell can automatically be counted [9, 38].

In vivo fluorescence and reflectance measure the amount of phycocyanin inside cyanobacteria cells. When this pigment is excited with specific wavelength from the visible light spectrum, phycocyanin reflects light at a slightly different wavelength. The excitation and emission spectra of cyanobacteria are very different from those of eukaryotic algae, because they contain phycocyanin rather than chlorophyll-a. This is the fundamental difference between the fluorescence characteristics of green-algae and blue-green algae [39, 40, 41].

Mass spectrometry and Fourier Transform Infrared (FTIR) microspectroscopy are based on the same principle as fluorescence, but measure the entire wavelength range of visible or infrared light instead of a specific wavelength. The measured spectra are compared with reference spectra of pure cyanobacteria strains that have been recorded earlier. In this way, it is possible to screen the sample for microcystins and to discriminate cyanobacterial strains [42, 43].

Remote sensing or hyperspectral analysis uses data from ENVISAT and/or LANDSAT satellites. These satellites have (configurable) spectrometers on board which can provide large amounts of spectral data. Optically active pigments like chlorophyll-a and phycocyanin are detected and can be used to identify cyanobacterial blooms [10, 12, 44].

2.6 Management Actions

Cyanobacteria are an integral part of the ecosystem. It is neither desirable nor possible to remove all cyanobacteria from the water, but it is possible to take management actions to prevent excessive growth and blooms. When no actions are taken, it will take up to several decades before nature is able to restore balance in the ecosystem. Actions are necessary to make recreational waters safe for swimming and to prevent stench and toxic fumes in surface waters in city ponds.
The Dutch foundation for applied research in water management (Stichting Toegepast Onderzoek Waterbeheer, STOWA) collects and distributes knowledge to help water authorities monitor and maintain water quality. In 2010, STOWA presented an overview of management actions to combat cyanobacteria blooms. These measures focus on informing the public about swimming risks, slowing down algae growth and cleaning up (severely) contaminated waters (figure 2.3).

![Figure 2.3: Cleaning surface water by scooping out large clusters of cyanobacteria scums.](image)

There are several management actions to reduce cyanobacteria growth. One set of actions focuses on reducing the amounts of nutrients that end up in the water: fertiliser and manure from farmlands often end up in the trenches directly via the soil. The excess nutrients end up in the main flow (channel, river, nearby ponds and lakes) and contribute to cyanobacterial blooms. For farmers it is not an option to stop fertilising altogether, they do often opt for sealing the trenches that abduct the nutrient-rich water during the time the lands are being fertilised.

Dog toilets, street trash and active feeding of fauna in city parks, such as fish-feeding, are the main contributors of excess nutrients in urban areas. In both situations, preventive actions and active biological management (such as, but not limited to restoring the balance of water plants, fish/water fauna, making natural shores and river banks) is moderately effective, but costly[45].

When increased or excessive growth is observed in particular surface waters (category 2 risk level), there are methods available to disrupt further growth of cyanobacteria blooms. Methods such as a bubble screen or bubbling disrupt the cyanobacteria’s ability to use their intracellular gas vacuoles to level off to a depth where light intensities and oxygen concentrations are favourable. This disrupts the growth and reduces the rate in which cyanobacteria colonies grow.

Nature can not recover on its own from excessive amounts of cyanobacteria (category 3 risk level) within a reasonable timespan and requires external help to restore balance to the ecosystem. Algicides, UV-light and ultrasonic sound can break down cyanobacteria blooms, but not all applications are practical or cost-effective in surface waters. Other methods such as combinations of hydrogen-peroxide, aeration of hypolimnion, iron salts, aluminum salts and chalk help managing the amount of cyanobacteria, but may not immediately make the water safe for swimming[46, 45, 47].
3. Design

In this chapter is focused on the design of a sensor system that can help with estimating risk levels for cyanobacteria in surface waters.

3.1 Problem

When cyanobacteria start to bloom at the start of spring, shores of surface waters and bathing sites are visually inspected for scum layers. Water samples are collected when scum layers are observed or the presence of cyanobacteria is suspected. The samples are cultivated at a laboratory where specialists determine the amount of cyanobacteria. In combination with the weather conditions and the observation of scum formations, it is possible to anticipate on the formation of cyanobacteria blooms. This requires frequent monitoring and analyses at laboratories. If cyanobacteria blooms are detected early, there are opportunities to take management actions[13].

A major obstacle is the time that the procedure between sampling and taking actions consumes. Most time is consumed by the hours traveling to the water location, taking the sample and analysing the sample at the lab. The laboratory can perform the measurements fairly quickly, but it still requires a lot of time to report the result from all samples back to the different waterboards. That time is necessary for making a well-informed decision to act and implement management actions.

3.2 Proposed solution

The time can be reduced significantly when samples can be analyzed on the site. The costs can be reduced when these measurements are performed more often and without human intervention.

3.2.1 Desired situation

An autonomous sensor platform can automatically perform measurements on small intervals. Optical measurements can be performed much faster and much cheaper than a person who sequentially has to visit all different bathing sites. The optical measurements do not require refilling of chemical components and can be performed multiple times per day or on request. The measurement results are reported directly to the waterboards via any information infrastructure and can be reviewed from the desktop computer or mobile device.

A practical scenario could be the following: a number of portable buoys are placed and anchored on strategic places in any surface water at the start of spring. A solar panel provides the buoys with energy until the end of the season. The concentration of phycocyanin –as an indicator for the presence of cyanobacteria– is measured with the optical sensor system in the morning, afternoon and evening. Optionally, other information such as water turbidity, pH and temperature are measured to provide additional water quality parameters. The measurements are stored on a public or private server of the waterboards or maintained by a third party.

When a significant increase in concentration is measured, measurements are performed every few hours. The waterboard and Province decide that the increase in concentration is not significant enough to do harm or decides that the increase in concentration is severe enough to take action.
In compliance with the current protocol, a sample is taken at the bathing site and sent to the lab in order to determine the exact concentration and species. When these species are harmful to humans and/or animals, the waterboard acts according to the protocol and takes management actions, like closing the bathing site and informing the public that this water is not accessible. In the period that it is uncertain whether there is a threat, the waterboard takes water samples more often until it can be decided whether additional actions are necessary.

![Image of a map with markers indicating water quality](image)

Figure 3.1: Information about the quality of swimming water shown on the website zwemwater.nl

In the meanwhile, families who plan to visit a bathing water see on their tablet or smartphone that the bathing site has been closed due to cyanobacteria (figure 3.1) and decide to visit one of the other safe swimming sites instead. The sensor platform continues measuring regularly. When phycocyanin concentrations reduce, the waterboard performs measurements again to determine whether the toxins or cyanobacteria concentrations in the water have broken down sufficiently.

### 3.2.2 Requirements

To become a valuable asset in addition to the current measurement methods, the sensor platform must comply with a number of requirements.

1. **Accuracy**
   The Dutch protocol describes measurements that yields the amount of micrograms of phycocyanin or cubic millimeters of biovolume in a liter of sample water. The system must therefore be able to measure phycocyanin concentrations higher than $12.5 \mu g/L$ or cyanobacteria biovolumes higher than $2.5 mm^3/L$. In this project is aimed as a minimum requirement for accuracy to detect the highest risk level as it represents immediate hazardous levels. That means that the proof of concept should be able to measure phycocyanin concentrations higher than $75 \mu g/L$ or cyanobacteria biovolumes higher than $15 mm^3/L$.

2. **Autonomous**
   The platform must function at least one bathing season without human support. It does not require a refill of chemicals or replacement of batteries.
3. **Time-frame**
   Sensing of cyanobacteria must be faster than the current methods: once or twice per day.

4. **Low-cost**
   The system is only financially appealing when it saves significant amounts of costs. Current procedures are estimated at several thousands of Euros per year in The Netherlands [1]. In addition, early detection of cyanobacteria can also prevent the costs for reducing cyanobacteria blooms in an early stage. In this project, the costs for a development proof of concept are aimed at 250 Euro. The final product should cost a fraction of that price.

5. **Manageable**
   The autonomous platform should be easy to transport, portable and easy to deploy. The weight and size should be limited to a device of a maximum of 5 Kg and the size of a large toolbox.

6. **Information**
   The measurements must be made available to waterboards via an existing digital information infrastructure such as GPRS or GSM-R. The information should be comprehensible for people who have no knowledge of the technical process of measuring cyanobacteria. It is recommended to present the data as risk level, which is determined by the measured biovolume or phycocyanin concentration.

7. **Neutral**
   A small system is easily integrated in the environment and is far more manageable than a system that attracts the attention of bathing guests. A large buoy in the water may cause a risk to water skiers or surfers and is attractive for swimmers to dive from. Additionally, it could become very expensive to make such a device **watertight**. Liter-bottle sized buoys can be integrated in floater lines that are used to mark swimming areas. On-board materials can not be disposed into the water. No chemicals or toxins are allowed to be released into the water.

### 3.3 Measuring method

Not all measurement methods that have been described in chapter 2 are suitable or practicable in the field. Some methods require sample preparation, others require vast amounts of heat or electrical energy. The described methods are subjected to the following questions.

1. Is the measurement method direct or indirect?
2. Does the method require the use of perishable components, such as supplementary chemicals?
3. Can it be automated completely?
4. Does it require more energy than conventional solar panels can produce?
5. Does it require (excessive) heating or cooling?
6. Can the procedures be recreated using present laboratory equipment?

The only low-energy and low-maintenance methods after evaluating these characteristics are the optical methods fluorescence, absorbance and reflectance. These methods do not require any external sample preparation and can be recreated with available laboratory equipment, specifically the OceanOptics USB4000-UV-VIS Miniature Fiber Optic Spectrometer, which is available at HIT. The optical methods do not require moving parts and do not require perishable components. Furthermore, the methods have low power consumption and do not depend on cooling or heating. All three methods are also applicable with relatively few electrical components.
3.3.1 Fluorescence

Fluorescence is a natural phenomenon where a molecule is excited with a particular wavelength of light and emits this light at a different wavelength. When a molecule is irradiated with light at a certain wavelength (band), its electrons are instantaneously promoted to a higher energy state. The neighbouring electrons in the molecule re-equilibrate quickly. If the energy threshold is equal to the energy of a photon of certain wavelength, the molecule can fall back to its original ground state and emit a photon at a different wavelength.

To an observer, this happens almost instantaneously[48]. A schematic overview of this process is drawn in figure 3.2: a source of light emits light at one wavelength, represented by a green arrow onto a molecule. The molecule, here represented as a sample cuvette, excites the light at a different wavelength in all directions, as represented with the red arrows.

The major difference between fluorescence, absorbance and reflectance is the angle between the excitation source and the placement of the sensor. Absorbance measures the fluorescent light in the same direction with the light source and the sample, where reflectance measures the light reflected back at (or right next to) the source. Fluorescence is generally measured under a 90° angle.

![Figure 3.2: Schematic overview of the concept of fluorescence](image)

Fluorometry is chosen for its extraordinary sensitivity, broad measurement range, high specificity, simplicity, and low cost as compared to other analytical techniques. Fluorometry is ordinarily more sensitive than absorbance measurements. It is a widely accepted and powerful technique that is used for a variety of environmental, industrial, and biotechnology applications. It is a valuable analytical tool for both quantitative and qualitative analysis[49].

3.3.2 Pigment Excitation & Spectra

Fluorescence has a large role in the photosynthesis process. Broad-spectrum light is absorbed by pigments and stored as chemical energy in the form of carbohydrates. The responsible pigments are chlorophyll-a, chlorophyll-b and carotenoids in plants and phycocyanin in cyanobacteria[50].
The absorption mechanism for chlorophyll-a is used as a template for the optical measurement system that measures phyocyanin of in vivo cyanobacteria cells. The fluorescence spectrum shown in figure 3.3 is the result of excitation of chlorophyll-a with blue light. When excited with orange light, phyocyanin shows the spectrum in figure 3.4.

Though the spectra for chlorophyll-a and phyocyanin have significant differences, the concept of fluorescence remains the same. Chlorophyll-a is present in all plants and algae that depend on photosynthesis and therefore provided a good base to develop an optical sensor. Experimentation (see also chapter 5) and literature established that chlorophyll-a is excited with a light source at approximately 450nm (blue) or 700nm (red) and reflects light at 670nm. Phycocyanin is excited with light at 645nm (orange) and reflects light at 620nm[49, 51, 52, 53, 54, 55].

### 3.4 Sensor Design

This project focuses on the development of a low-cost optical sensor that can measure concentrations of phyocyanin. This optical sensor is a customized photospectrometer that utilizes the fluorescent properties of phyocyanin.
3.4.1 Concept

The design for the optical sensor is based on the following principles:

1. Cyanobacteria contain phycocyanin.
2. Phycocyanin is visible in fluorescence measurements around 620nm.
3. Other wavelengths can be rejected from the measured spectrum with an optical bandpass filter.
4. A photodiode can measure the intensity of light that passed through the filter and returns this as voltage.

The voltage corresponds to the concentration of phycocyanin in a sample. The concentration phycocyanin corresponds to the biovolume of cyanobacteria[56]. The concentration of phycocyanin and the biovolume of cyanobacteria correspond to risk levels in the protocol.

There are several restraints to this method.

1. Not all cyanobacteria contain equal amounts of phycocyanin. The amount of phycocyanin varies between species of cyanobacteria, but also within different strains of the same species[55].
2. The wavelength on which phycocyanin reflects light varies between 610 and 630. Optical filters for this specific wavelength are expensive.
3. Low concentrations of phycocyanin reflect very little amounts of light. The low light intensity may be below the threshold of low-cost photosensitive sensors.

Fluorescence measurements are not a new method to determine cyanobacteria concentrations. Existing methods for measuring cyanobacteria in the field are however expensive and for beyond what is affordable for waterboards. Commercial buoys or fluoroprobes cost several thousands of Euros per device[57] and have the ability to measure fractions of micrograms of phycocyanin per liter sample.

The difference with the proposed sensor platform and commercial devices is in the lower accuracy and price of the proposed sensor. The sensor platform only requires to detect cyanobacteria concentrations that correspond with risk levels, rather than highly precise concentrations such as other commercial devices do.

3.4.2 Focus & Constraints

The proposed solution described a low-cost autonomous sensor platform that can measure hazardous cyanobacteria concentrations. In this project is focused on developing a sensor that can approximate risk levels. The following parts were part of the project scope:

- Construct a proof of concept for a sensor that can determine the presence of cyanobacteria;
- making the sensor sufficiently accurate to determine different risk levels[4];
- adapting an optical sensing methods so it can operate in an autonomous environment;
- developing an optical sensor that can measure the wavelength that corresponds with phycocyanin present in cyanobacteria.

Other parts that are considered outside the scope of the project are:

- building a mini-buoy or sensor platform, its wireless communication or power generation;
- hardware optimization for commercial purposes and large quantities (lean production);
- extreme accuracy and sensitivity, higher than determining different risk levels;
- commercial aspects (market research, mass production, marketing, sales);
- quantification of what is “cost-effective” for waterboards and detailed estimations of the expenses on monitoring cyanobacteria;
- user aspects (how should the device be operated and what will the user want to see on his/her screen).
4. Implementation

In this chapter it is described how the proof of concept for the optical sensor is developed.

4.1 Proof of Concept

Fluorescence measurements are performed by exciting a sample with light of a defined wavelength. If \textit{in vivo} pigments such as chlorophyll-a or phyocyanin are present in cells in the sample, they emit light at a slightly shifted wavelength in all directions. The intensity of the fluorescent light is measured with an optical sensor such as a light dependent resistor, photodiode or phototransistor. When the intensity of the excitation source remains constant while the concentration of \textit{in vivo} pigments in the sample vary, the measured intensity of the fluorescent light varies too.

![Figure 4.1: Schematic overview of fluorescence under a 90° angle.](image)

The schematic overview in figure 4.1 displays the fluorescence process for phyocyanin. When this pigment is excited with light at 645nm, the sample emits fluorescent light at 620nm. The intensity of this light is measured under a 90° angle. Various light sources (light bulbs, LEDs) emit light at a rather broad spectrum. It is therefore not possible to excite the sample directly with a narrow-band light source at 645nm. To narrow the band, it is necessary to place an optical band-pass filter with a peak of 645nm between the excitation source and the sample. General optical sensors can measure wavelengths between 200nm and 1100nm with peaks between 500nm and 800nm, depending on the specific sensor and purpose for which it was designed. Phyocyanin concentrations are measured at a wavelength of 620nm. To achieve this, an optical band-pass filter with a peak of 620nm is placed between the sample and the optical sensor to reject all other wavelengths from the sensor.

4.2 Optical Sensors

There are different types of optical sensors. Fluorescence measurements are often performed with a pulsating light source, so there is a clear distinction between measurements where the sample is excited. In order to measure this light at the moment of excitation, fluorescence measurements require a fast and accurate optical sensor.
4.2.1 Sensor Types

There are three general types of sensors that are considered for fluorescence.

- Light Dependant Resistors (LDRs) vary in resistance when exposed to light. LDRs are slow and inaccurate and are commonly used in environments where light varies slowly. Applications include day/night detection or determining whether lights are turned on/off. In series with a fixed-value resistor and power source, the configuration acts as voltage divider where the LDR is a variable resistor.

- Photodiodes and Light Emitting Diodes (LEDs) have similar properties. Both LEDs and photodiodes have a semiconducting p-n junction\[58\] where an electrical current is converted to light and vice versa. Photodiode are optimized for collecting light: a relatively large intrinsic area between the p-n junction collects photons, whose impact cause a small electrical current to flow.

- In phototransistors, the transistor base is replaced by the same photosensitive p-n junction as a photodiode. The sensitivity of the photosensitive layer is similar to the sensitivity and accuracy of a photodiode, whereas phototransistors have a typical internal gain between 500 and 1500\[59\].

Both photodiodes and phototransistors are suitable optical sensors for fluorescence measurements. In this project a photodiode is used since it has the same form and properties as a phototransistor, but allows for better tinkering with the reverse bias, offset and amplifier circuitry.

4.2.2 Photodiode Selection

There are many types of photodiodes on the market for a wide variety of purposes. In order to perform a fluorescence measurement, the photodiode must be able to measure at the wavelengths of \textit{in vivo} chlorophyll-a and phycocyanin. Furthermore, the photodiode must be sufficiently fast to measure fluorescent light during the excitation period (in the order of milliseconds). A photodiode is selected based on the following properties.

- Wavelength sensitivity at least between 600nm and 800nm;
- relatively large photosensitive area (>5mm$^2$);
- fast response/rise time (<10ms);
- operational in outside conditions in The Netherlands (temperatures between -10°C and 45°C);
- can operate in an environment with relatively low energy (<15V);
- analog current output;
- low-cost (<10 Euro).

In table 4.1 a number of photodiodes are compared. Many other photodiodes have been reviewed in addition to this table, but were discarded in earlier selection due to incorrect wavelengths, area and/or application.

<table>
<thead>
<tr>
<th>Type</th>
<th>EPD-365-0/1.4</th>
<th>QP5.8-6-TO5</th>
<th>QP50-6-18U-TO8</th>
<th>BPW20RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>EPIGAP Optoelektronik</td>
<td>Mouser Electronics</td>
<td>Mouser Electronics</td>
<td>Vishay Semiconductor</td>
</tr>
<tr>
<td>Spectral range (nm)</td>
<td>245–400</td>
<td>400–1100</td>
<td>400–1100</td>
<td>400–1100</td>
</tr>
<tr>
<td>Peak wavelength (nm)</td>
<td>365</td>
<td>633</td>
<td>633</td>
<td>920</td>
</tr>
<tr>
<td>Active area (mm$^2$)</td>
<td>1.2</td>
<td>5.76</td>
<td>49.2</td>
<td>7.5</td>
</tr>
<tr>
<td>Rise time (ns)</td>
<td>140</td>
<td>20</td>
<td>40</td>
<td>3400</td>
</tr>
<tr>
<td>Operating temp (°C)</td>
<td>40 to +125</td>
<td>40 to +100</td>
<td>40 to +100</td>
<td>-55 to +100</td>
</tr>
<tr>
<td>Storage temp (°C)</td>
<td>40 to +125</td>
<td>-55 to +125</td>
<td>-55 to +125</td>
<td>-55 to +100</td>
</tr>
<tr>
<td>Dark current (nA)</td>
<td>20</td>
<td>0.4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Reverse voltage (V)</td>
<td>10</td>
<td>50</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Peak current (mA)</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Responsivity (A/W)</td>
<td>0.07</td>
<td>0.4</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Case style</td>
<td>TO-46</td>
<td>TO-5</td>
<td>TO-8</td>
<td>TO-5</td>
</tr>
<tr>
<td>Cost</td>
<td>$77.00</td>
<td>$44.54</td>
<td>$138.95</td>
<td>E5.27</td>
</tr>
</tbody>
</table>

Table 4.1: Comparison of different photodiode specifications.
The development process is started with the **BPW20RF** from Vishay Semiconductor as it has a reasonable sensitivity-to-price relation. In the development process should become clear whether this photodiode is sufficiently accurate to measure low concentrations of phyocyanin. Other factors are not directly important for the development process. If future prototypes of the proof of concept yield sufficiently accurate results and are considered suitable for mass-production, more optimized sensors and electrical components will be chosen at a later stage.

### 4.2.3 Optical Filters

In chlorophyll-α fluorescence, there is a clear difference between the excited light at 450nm and the reflected light at 670nm. The difference in wavelengths when measuring fluorescence of phyocyanin is significantly smaller (respectively 645nm and 620nm). It is possible to filter out excess light with optical filters. These filters consist of coated glass and has properties that allow certain wavelengths to pass, while rejecting other wavelengths. In figure 3.2 it is shown how an orange-colored filter allows fluorescent light from the sample to pass, while blocking the different color of the excited light before it reaches the optical sensor.

![Transmittance spectra for a band-pass filter (phyocyanin) and a long-pass filter (chlorophyll-a).](image)

Optical filters exist in the following forms.

- long-pass filters (higher wavelengths pass while shorter wavelengths are rejected);
- short-pass filters (shorter wavelengths to pass while higher wavelengths are rejected);
- band-pass filters (a small band of wavelengths pass while all other wavelengths are rejected);
- band-reject filters (all other wavelengths pass while a small band of wavelengths is rejected).

![Absorbance spectra of phycocyanin in *Spirulina*, *Phormidium* and *Lyngbya*[60](image)

On the websites of three optics vendors (**EdmundOptics**, **Newport** and **Omega Optical**) it was shown that a 620nm bandpass filter is not a standard filter and costs between 200-600 Euro. An alternative filter with a 610nm band is available for 35 Euro. In literature, the fluorescent light of phyocyanin has a peak wavelength of 620nm. Closer inspection of the fluorescent fingerprint of...
phycocyanin reveals that the highest intensity of fluorescent light occurs between 600nm and 630nm as seen in figure 4.3[60]. The 610nm band-pass is therefore considered the best choice. Figure 4.2 shows the measured transmittance spectrum of a 610nm 10nm band-pass filter for phycocyanin and the transmittance spectrum of a 655nm long-pass filter chlorophyll-a as described in appendix B.

4.3 Electronics

A photodiode is only a small part of the optical sensor. To get a clean and useful signal, the photodiode output must be amplified and filtered.

4.3.1 Amplifier

When light falls on the photosensitive area of a photodiode, it creates a small current. Even with excessive amounts of light, the current is so small that the impedance of a bipolar operational amplifier is not high enough to prevent that the current of the photodiode is drained. Field-effect amplifiers or unipolar amplifiers use an electric field to control the output of the amplifier and do not have an input impedance. The photodiode signal is amplified as shown in figure 4.4.

![Amplifier schema for a photodiode](Figure 4.4: Amplifier schema for a photodiode)

Photons (light) impact the intrinsic layer of a photodiode and generate a small current in the direction of resistor $R_1$, which causes a potential difference between the positive and negative inputs of the amplifier. The output of the amplifier will rise and cause a current through $R_1$ in the direction of $R_3$. The output of the amplifier rises until the potential between the positive and negative inputs are 0V, following Kirchhoff’s Law[58]. The netto potential over the photodiode is 0 Volt, while the output of the amplifier is proportional to the amount of light on the photodiode.

For the first proof of concept, a general purpose CA3140 amplifier is used, manufactured by Intersil. Circuits later in the development process use LM6211MF low-noise amplifiers, manufactured by Texas Instruments.

4.3.2 Excitation Source

Standard LEDs require little power (5–25mA) and can produce enough light to saturate the chosen BPW20RF photodiode. LEDs are available in various colors. A broad-spectrum white LED combined with an optical 645nm band-pass filter is capable of producing sufficient amounts of light to excite the sample. Figure 4.5 shows the spectrum after it has been filtered with a 640nm 10nm band-pass filter.
To reduce the risk of errors, fluorescence measurements can be performed at short intervals by exciting the sample at a chosen frequency. When the sample is excited at a frequency of 100Hz, other frequencies can be filtered out. The frequency of 100Hz is chosen because it allows excitation faster than the ever-present electrical noise of 50Hz (Europe) or 60Hz (United States of America) and slower than the maximum sample rate of general purpose analog-to-digital converters.

### 4.3.3 Filtering

When the sample is excited at a frequency of 100Hz, an electrical band-pass filter of 100Hz can reject all other unwanted frequencies. An electrical band-pass filter is realized with a series RC network and a series CR network (figure 4.6).

![Figure 4.6: A low-pass filter realised with a series RC network (left) and a high-pass filter realised with a series CR network (right).](image)

The values for both filters are calculated with equation 4.1. To prevent that the two filter networks affect each other, the second network must be chosen with values that are at least an order of 100 smaller. After trial and error, the cutoff frequencies $f_{lpf} = 500Hz$ and $f_{hpf} = 90Hz$ were chosen. This allows variability between 100Hz–500Hz in the frequency with which the sample is excited.

$$f_{lpf,hpf} = \frac{1}{2\pi RC}$$

### 4.3.4 Analog-to-Digital Converters

The measurements are automated with an Arduino Uno. This development kit consists of various I/O pins, a microcontroller (Atmel Atmega328) and a USB interface to connect to a host computer. The Atmega328 has a 10-bit Analog-to-Digital Converter (ADC) over a range of 5Volt, which allows a maximum resolution of 4.88mV.

To gain a higher resolution, an alternative ADC was chosen. The ADS1115T DGST, manufactured by Texas Instruments, has a resolution of 7.63µV over a range of 5V and interfaces with the Arduino over the I²C protocol. The ADS1115 has a number of settings such as the address, the amount of samples per second and the ability to amplify the gain of the analog signal. In the development process should become clear whether this increased accuracy is necessary, or whether a 10-bit ADC suffices.
4.3.5 System

When all components are combined, the outlines of the optical sensor become visible. The photodiode signal is amplified as shown in figure 4.7 (left). Immediately after amplification, a high-pass filter is added to filter out bias from the amplifier circuit and other frequencies below 100Hz. The signal is then further amplified and supplemented with a 2.5V bias. A low-pass filter rejects all high-frequent noise such as radiosignals. The ADC measures the signal, which is now properly centered around 2.5V, the area where the ADS1115 is most precise. The ADC is connected to the I²C bus of the Arduino (right).

![Figure 4.7: The optical sensor schematic with a photodiode (left), filters, offset and ADC (right).](image)

4.4 Software

The test chamber where fluorescence measurements take place, is controlled by an Arduino Uno. The software for this development kit consists of three parts: excitation of the sample at various frequencies, reading the values of the optical sensor and reporting the measurements to a host computer.

![Figure 4.8: Microcontroller program flow for measuring and sending data](image)
4.4.1 Excitation Interval

The logic behind excitation of the sample at 100Hz is implemented with interrupts. Before interrupts can be configured, interrupts are disabled to prevent interruption of the interrupt configuration. Default interrupt settings are overwritten to create a recurring timer. The variable for the interrupt compare match is calculated by dividing the clock speed by the preferred frequency and prescaler (equation 4.2). A timer prescaler of 1024 is necessary for the Atmega328 to scale the clock speed to a frequency of 200Hz. After the calculation, one is subtracted as digital technology counts down to 0 rather than 1.

\[
\text{OCR0A} = \frac{\text{clock speed}}{2 \times \text{frequency} \times \text{prescaler}} - 1 = \frac{16,000,000}{2 \times 100 \times 1024} - 1 = 79 \quad \text{(4.2)}
\]

To create one 100Hz period, two interrupts are necessary: one to put the output high, one to put the output low. Therefore, the timer interrupt frequency is twice as large as the wanted frequency. After setting the correct timer variable and prescaler, interrupts are enabled. While the program enters the main loop, the timer counts from 0 until 78. The compare match then triggers the interrupt service routine, which switches the LED on or off. The timer is reset automatically and continues counting until the next compare match as shown in listing 4.1.

```c
#define LED 9  // Define the LED on pin 9
boolean LED_STATE = HIGH;

void setup(){
  // set LED pin as output
  pinMode(LED, OUTPUT);
  cli(); // first disable all current interrupts
  // we don't want to interrupt the interrupt configuration.

  // set timer0 interrupt at 100Hz
  TCCR0A = 0; // set TCCR2A register to 0
  TCCR0B = 0; // set TCCR2B register to 0
  TCNT0 = 0; // init counter at 0

  // set compare match register for 100Hz
  // NB: to oscillate at 100Hz, states must change twice per Hz
  OCR0A = 78; // = \frac{(16 \times 10^6)}{(200 \times 1024)} - 1

  // turn on CTC mode
  TCCR0A |= (1 << WGM01);

  // Set CS12 and CS10 bits for 1024 prescaler
  TCCR1B |= (1 << CS12) | (1 << CS10);

  // enable timer compare interrupt
  TIMSK0 |= (1 << OCIE0A);

  sei(); // turn interrupts back on
}

ISR(TIMER0_COMPA_vect){
  // reverse the LED state
  LED_STATE = !LED_STATE;

  // make the LED pin high/low
  digitalWrite(LED, LED_STATE);
}
```

Listing 4.1: Source code for constructing a 100Hz timer interrupt.

4.4.2 Reading

The ADS1115 Analog-to-Digital converter is accessed over the I^2C protocol. Arduino has a library for I^2C and allows direct-to-device writing. The settings in table 4.2 are used to measure a maximum of 250 samples per second at 1× gain.
### Byte data Function

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0b01001000</td>
<td>Device address 0x48, ground</td>
</tr>
<tr>
<td>2</td>
<td>0b00000001</td>
<td>Points to config register</td>
</tr>
<tr>
<td>3</td>
<td>0b10000000</td>
<td>Bits 11:9 = FS +/-4.096V (=gain 1x), OS1: Begin a single conversion when in power down mode</td>
</tr>
<tr>
<td>4</td>
<td>0b10100011</td>
<td>bit 7:5 = 101 for 250 samples per second</td>
</tr>
</tbody>
</table>

**Table 4.2: Configuration options for the ADS1115TDGST**

After configuration of the settings from table 4.2, an interrupt routine or threaded loop calls the function `readADC()` to obtain a reading. The analog signal is converted by the ADC and sent in two parts: the most significant bits (MSB), followed by the least significant bits (LSB) which correspond to the first two bytes and last two bytes of an unsigned integer. This function is shown in listing 4.2.

```c
#include <Wire.h>
#define I2Caddress 0x48 // device address

// init function
void setup () {
  Wire.begin();

  // configuration
  Wire.beginTransmission(I2Caddress);
  Wire.write(0b00000001); // point to the config register
  Wire.write(0b10000000); // +/-4.096V (=gain 1x), begin a single conversion pdm
  Wire.write(0b10100011); // 250spsc
  Wire.endTransmission(); // end initialisation

  // open a new connection
  Wire.beginTransmission(I2Caddress);
  Wire.write(0b00000000); // point to conversion reg
  Wire.endTransmission();
}

// get ADC value as unsigned integer
unsigned int readADC (void) {
  Wire.requestFrom(I2Caddress,2); // request a reading
  // receive the measurement in two parts (MSB and LSB)
  while (Wire.available() == 0) {};
  byte MSB = Wire.read();

  // wait until I2C becomes available and receive the LSB
  while (Wire.available() == 0) {};
  byte LSB = Wire.read();

  // return the combined MSB|LSB as unsigned integer
  return (MSB * 256)+LSB;
}
```

Listing 4.2: Source code for reading the ADS1115TDGST Analog-to-Digital Converter

### 4.4.3 Sending

When data is recorded by the Arduino, it is sent over USB to a host computer. This is done via Arduino’s `Serial` library, which utilizes the RS232 protocol. This is shown in listing 4.3.

```c
void setup () {
  // set baudrate to 9600Baud/s
  Serial.begin(9600);
}
```
The host computer can either read the data over Hyperterminal (Windows XP/7/8) or with the `screen` command (Unix) as shown in listing 4.4.

### Measurement Chamber

Both a dark measurement chamber and mathematical compensation for stray light help improve the accuracy of a fluorescence measurement. Natural light (sunlight) and industrial light (light bulbs, street lights) have a wide spectrum that includes earlier discussed wavelengths. It is therefore necessary that fluorescence measurements take place in a dark measurement chamber. A measurement of background light before and after the fluorescence measurement can help compensate for this mathematically.

As earlier discussed, the proof of concept focuses on an optical sensor to measure the concentration of phycocyanin. The development setup consists of a dark measurement chamber of black polymethylmethacrylaat (PMMA) with a thickness of 8mm. The dark test chamber is 6cm high, 3cm wide (excluding the 7x7cm base plate) and can hold one generic 5mL plastic, square sample cuvette. PMMA is available for 0.011 Eurocent per square centimeter and can be carved out in any preferred shape with a 70W lasercutter. Both large sheets of PMMA and the lasercutter are available at HIT\(^1\).

---

\(^1\)All layout files and software are attached to this document separately.
5. Evaluation

In this chapter it is discussed whether the designed sensor can indicate hazardous cyanobacteria concentrations. Experiments were performed to prove the detection of phycocyanin in cyanobacteria and to measure the response of the designed sensor.

5.1 Phycocyanin in Cyanobacteria

Experiments were performed at Wageningen University and Research on 9 January, 2015 and at the Hanze Institute of Technology on 13 January, 2015. The purpose of those experiments was to determine the fluorescence wavelength of phycocyanin present in cyanobacteria and to determine whether phycocyanin is exclusively present in cyanobacteria.

5.1.1 Experiments

The experiment used established equipment to determine the cell count in a sample and the amount of phycocyanin in the same sample. A more detailed description of the experimental procedure is given in appendices A and B. The experiments are performed with Microcystis (cyanobacteria) and Monoraphidium (green-algae), which are both common species in bathing waters in The Netherlands[8]. The tests are performed with these two types of bacteria/algae to determine whether the sensor discriminates between cyanobacteria and common, non-harmful algae.

Samples of Microcystis and Monoraphidium were diluted in steps of 0.50, 0.25, 0.10 and 0.01 percent. Of every sample, the amount of µg/L phycocyanin/chlorophyll-a was determined with a Phyto-PAM. The cell count in every sample was determined with a CASY® counter. After that, the full optical absorbance spectra and fluorescence spectra were determined with a Beckman Coulter DU 730 and Ocean Optics QEB1800 photospectrometer.

5.1.2 Phycocyanin and Chlorophyll-a

Both series of Microcystis and Monoraphidium samples were measured with the Phyto-PAM to determine the quantity of phycocyanin and chlorophyll-a. In Microcystis only phycocyanin was detected, but no chlorophyll-a. In Monoraphidium, only chlorophyll-a was present, but no phycocyanin (figure 5.1).
5.1.3 Cell counting

The same Microcystis and Monoraphidium samples were used for the CASY®-counting measurements. The results were recorded as following: CML – number of particles per mL (mL⁻¹), VML – total volume of particles per ml (µm³/mL) and MVL – mean volume per particle (µm³).

<table>
<thead>
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<th>Mvl</th>
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<tr>
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<td>2.42E+008</td>
<td>1.78E+002</td>
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<td>0.01</td>
<td>2.38E+005</td>
<td>6.36E+008</td>
<td>2.67E+003</td>
</tr>
</tbody>
</table>

Table 5.1: Particles in Monoraphidium samples.

5.1.4 Spectra

Spectrometry measurements were performed to identify the typical fluorescence spectra of phycocyanin and chlorophyll-a. The following absorbance spectra were observed with a Beckman Coulter DU 730 at 100%, 50%, 25%, 12.5% and 6.25% sample dilutions.
The measurements were performed with clear glass cuvettes. At the left side of the spectrum can be seen that the glass cuvette partially blocks ultraviolet light between 200nm and 300nm. Infrared light above 950nm is mostly absorbed by the glass. A number of distinct peaks are seen in the spectra of both samples at approximately 450nm and 670nm. These peaks are associated with the photosynthesis process[49], as discussed in chapter 2.

The following fluorescence spectra were observed with an Ocean Optics QEB1800 photospectrometer for 100%, 10% and 1% sample dilutions, measured under an angle of 90°.

In the Monoraphidium, a distinct peak at 670nm is observed. This emission spectrum is associated with chlorophyll-a[49, 54], as discussed in chapter 2. The expected peak for phycocyanin[51, 54] as seen in figure 3.4 can not be distinguished in the emitted spectrum in figure 5.4a.

5.1.5 Discussion
The species Microcystis and Monoraphidium were chosen to determine whether the sensor discriminates between cyanobacteria and common, non-harmful algae. Monoraphidium has chlorophyll-a to perform photosynthesis, while Microcystis has phycocyanin for this purpose[54]. Therefore it is expected that chlorophyll-a is not present in cyanobacteria and that phycocyanin is not present in green-algae. The measurements with the Phyto-PAM have shown that this is true for Microcystis and Monoraphidium.

The deviation between cell counting measurements is sizable. The amount of counted particles between the two samples of the same species and original sample has almost doubled. Cell counting, as described in chapter 2, counts every particle as a cell. Bubbles and dust particles in the water also are counted as intact cells. This may explain the large deviation between two recorded samples. The measurements are too widespread to draw a valid conclusion about the relation between cell count and the concentration of phycocyanin/chlorophyll-a per cell. Another possible explanation can be due to small errors in the measurements performed. While Phyto-PAM measurements were completed, the diluted samples settled and particles sank to the bottom of the cuvettes. When CASY®-counting measurements were made, samples were taken...
from the bottom of the vial. Different samples had different amounts of time to where particles were allowed to sink to the bottom of the vial, which may explain the large difference between samples of the same source.

In both the absorbance spectra and the fluorescence spectra of *Microcystis* and *Monoraphidium* it can be seen that peaks that are associated with the photosynthesis process can be clearly recognized. The fluorescence spectrum of chlorophyll-a is clearly visible, while phycocyanin can not clearly be recognized. In both absorbance and fluorescence measurements, the sample is excited with the full visible light spectrum (200nm–1000nm). Because there is a clear difference between the excitation wavelength at 450nm and the reflected light at 670nm in chlorophyll-a fluorescence, chlorophyll-a can absorb a lot more light around the 450nm band. In order to measure fluorescence of phycocyanin, the excitation wavelength band is significantly smaller, with the result that less fluorescent light is emitted.

To obtain a better fluorescence spectrum of *in-vivo* phycocyanin, it is recommended to repeat the experiments with an excitation wavelength centered closer around 645nm.

### 5.2 System Performance

Before fluorescence measurements were performed with the prototype, the response of the electrical systems were tested.

#### 5.2.1 Simulated Input

The performance of the electrical system was tested with a function generator. The photodiode in figure 4.7 was replaced with the output of a function generator with an impedance of 50Ω. The output signal was measured at the input of the ADC and compared with the input signal over X1.

As input signal, a sine wave was generated with an offset between 0V–2V and a frequency between 10Hz–500Hz.

![Figure 5.5: The output signal (left) and the frequency spectrum (right) when the input over X1 is a 500Hz sine wave.](image)

In figure 5.5 is seen how the system responds to a 500Hz input signal with an amplitude of 1.5V and an offset of +0.5V. The output is a clean 500Hz sine wave with an amplitude of 1.5V and an offset of 2.5V, as intended. The spectrum (right) shows a clear peak at 0Hz (offset) and 500Hz (sine wave) and peaks as a result of aliasing caused by the HandyScope S3 software at 1000Hz and 1500Hz.
5.2.2 Decoupling

During the development process, all resistors were decoupled with 10pF ceramic decoupling capacitors. While simulating with above-mentioned input signals, the output of the photodiode and amplifier (built according to figure 4.4) revealed that these decoupling capacitors had an adverse effect. In figure 5.6 is shown how the output signal decreases when exciting light directly onto the photodiode with different intervals.

![Figure 5.6: The effects of exciting light directly onto the photodiode with different intervals when decoupling capacitors are used.](image)

The circuit was tested with a function generator that produced an adjustable voltage with an output impedance of 50Ω. A photodiode creates a small current through resistors $R_1$ and $R_3$, which cause a potential difference over these resistors. Decoupling capacitors effectively act as a low-pass filter, in this configuration with a cut-off frequency of approximately 25Hz. The first high-pass filter after amplification has a cut-off frequency of 90Hz, which caused the signal to be rejected completely. When the decoupling capacitors were removed, the signal was restored.

5.3 Fluorescence tests

Before water samples can be taken from surface waters and analyzed for cyanobacteria with the designed prototype, the device must be validated. During the development process, cyanobacteria were not widely available. Cyanobacteria are hazardous and can only be kept and cultivated by universities and institutions that have the proper equipment and knowledge to prevent hazardous situations.

5.3.1 Chlorophyll-a

The prototype is designed to perform fluorescence measurements. As said, cyanobacteria were not available until a later stadium in the development process. Before measurements at Wageningen University & Research were performed, regular tropical aquarium algae, which contain an unknown
amount of chlorophyll-a, were used. These algae were cultivated with 1 part Pokon per 100 parts tap water and supplemented with oxygen by an aquarium pump.

To distinguish between the spectra of the blue-LED excitation source from the fluorescence spectra of chlorophyll-a, a 655nm long-pass filter was used. Two dilution samples of 100% and 50% were made. A tap water sample was used as reference. The Textronics TBS1052B-EDU oscilloscope is connected directly after the first photodiode amplifier, to allow inspection of the unfiltered signal.

![Image](a) An unknown algae, 100% sample  
(b) An unknown algae, 50% sample

Figure 5.7: Measuring chlorophyll-a with the prototype

The prototype showed a signal with significant difference between 100% (figure 5.7a) and 50% (figure 5.7b) sample. The tap water sample yielded no signal other than the 50Hz noise that is observed in both the 100% and 50% samples. The signal has a signal-to-noise ratio of respectively $\text{SNR}=\frac{6}{2}$ for 100% sample and $\text{SNR}=\frac{4}{2}$ for 50% sample.

5.3.2 Phycocyanin

Various samples of cyanobacteria are cultivated at Wageningen University & Research. With the Phyto-PAM, the amount of phycocyanin is measured in various *Microcystis* sample dilutions. With the prototype, these measurements are repeated. Samples are excited with a blue LED to measure chlorophyll-a and with an orange LED to measure phycocyanin. The data is automatically recorded after the photodiode amplifier with a Textronics TBS1052B-EDU with a horizontal timescale of 2.5s and a horizontal scale of 20mV at an offset of $-0.386\text{mV}$.

In figure 5.8 a sample of 100% *Monoraphidium* is measured and recorded by the oscilloscope with a horizontal timescale of 2.5s and a horizontal scale of 20mV at an offset of $-0.386\text{mV}$. As expected, the sensor is able to indicate the presence of phycocyanin. The sensor is also able to indicate a considerable amount of chlorophyll-a.

![Image](a) With 610nm 10nm band-pass filter  
(b) With 655nm long-pass filter

Figure 5.8: Measuring phycocyanin (left) and chlorophyll-a (right) in a 100% *Monoraphidium* sample.
In figure 5.9 a sample of 100% *Microcystis* is measured and recorded by the oscilloscope with a horizontal timescale of 2.5s and a horizontal scale of 50mV at an offset of −0.200mV. Note that in figure 5.9a, the oscilloscope is configured with a horizontal timescale of 2.5s and a horizontal scale of 20mV at an offset of −0.200mV. It was expected that the sensor is able to indicate phycocyanin. The sensor is also able to indicate that there is no chlorophyll-a present.

(a) With 610nm 10nm band-pass filter  
(b) With 655nm long-pass filter

Figure 5.9: Measuring phycocyanin (left) and chlorophyll-a (right) in a 100% *Microcystis* sample.

### 5.4 Low-cost

The purpose of this project is to develop a low-cost sensor. In chapter 3 it was defined that the system is only financially appealing when it saves significant amounts of money. The costs for a prototype are aimed at 250 Euro. The final product should cost a fraction of that price. To evaluate the low-cost aspect of the project, the costs of development materials have been reviewed. There are three classes of costs that are considered. Of the first class, costs have been recorded in table 5.2.

1. **Component costs**  
The costs of special components that have been selected on criteria described earlier in this report. These costs are aimed to be below as 250 Euro.

2. **Standard material costs**  
The costs of standard components such as resistors, capacitors, circuit board manufacturing, casing, shipping and soldering materials (assuming large orders). After the prototype has been optimised for mass production, these components are expected make up for less than 25% of final product cost. These costs are therefore no part of the low-cost scope.

3. **Development costs**  
These costs are made during the development process. This includes the costs of sample cuvettes, equipment and development kits such as the Arduino Uno. These costs are not part of the low-cost scope.

<table>
<thead>
<tr>
<th>Description</th>
<th>amount</th>
<th>costs (Euro)</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>Filter 640mm 12.70mm Mtd</td>
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<td>28.50</td>
</tr>
<tr>
<td>Photodiode BPW20RF</td>
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<td>5.27</td>
</tr>
<tr>
<td>ADC ADS1115</td>
<td>1</td>
<td>5.63</td>
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<tr>
<td>Low-noise OpAmp LM6211MF</td>
<td>2</td>
<td>3.26</td>
</tr>
</tbody>
</table>

Table 5.2: Special component costs
5.5 Discussion

In this project it is described how a low-cost photospectrometer can be implemented as an early-warning system for hazardous cyanobacteria concentrations. In this chapter, a proof of concept for the proposed sensor has been evaluated with the aspects of functionality, cost and time-frame as described in chapter 3.

1. Accuracy
   From figure 5.9a is concluded that phycocyanin is measured in a 100% Microcystis sample. In graph 5.1a is seen that the sample contained between 534µg/L and 686µg/L phycocyanin. The protocol specifies that concentrations higher than 75µg/L are considered hazardous[4]. That demonstrates that the prototype is capable of detecting hazardous concentrations of cyanobacteria. There is however a large gap between 686µg/L and 75µg/L phycocyanin. A more thorough validation process is required in order to determine how accurate the prototype really is.

2. Time-frame
   The fluorescence measurements are performed within a fraction of a second. In the development process, up to several tens of measurements have been performed to evaluate the prototype. This is more often than the requested interval of one or two measurements per day.

3. Low-cost
   From table 5.2 is seen that the costs of the components on which the prototype is based do not exceed 250 Euro. It can be argued that the rest of the components that make up an autonomous sensor platform are not included in the table of costs. This is an early prototype that is used to prove whether it is possible to measure phycocyanin with a low-cost sensor.

4. Manageable
   The autonomous platform should be easy to transport, portable and easy to deploy. The prototype is 6cm high, 3cm wide (excluding the 7x7cm base plate) and does not include the Arduino Uno board that was used to read out data. This is not a complete system, therefore no valid conclusions can be drawn on the aspect of portability.

5. Information
   The information is currently read as analog voltage at the input of an ADC. This information is not comprehensible. In order to understand the information, a empirical conversion table or calibration curve must be made in order to determine the relation between output voltage of the sensor and concentration of µg/L phycocyanin.

The validation process should answer the following: can the prototype cyanosensor indicate hazardous concentrations? This was tested by measuring various samples of cyanobacteria and algae. Phycocyanin has only been measured once in the described validation process. The concentration measured in figure 5.9a could have been a successful measurement, but could also have been a false positive; overlap or stray excitation light that found its way around the optical filter. In order to be sure that phycocyanin is correctly measured, an extended validation procedure should be followed. A draft for this procedure is written in appendix C.
6. Conclusions

In this project is focused on the development of a low-cost sensor that can indicate the presence of cyanobacteria according to the risk levels determined in the blue-green algae protocol.

What are the properties of cyanobacteria algae?  
Cyanobacteria are procaryotic cells that are capable of photosynthesis which is achieved through the pigment phycocyanin. With the presence of oxygen, water, carbon dioxide and light, they can survive and grow in freshwater ponds, brackish water and salt water. Cyanobacteria blooms are part of the ecosystems of lakes and ponds, but also pose a health risk to bathing guests, living organisms and animals that drink or play in the water.

Why are certain concentrations of cyanobacteria levels hazardous?  
One of the major differences between cyanobacteria and regular algae is that many species are capable of producing cyanotoxins. During recreational activities, people can be exposed to these toxins by accidentally ingesting contaminated water, inhaling toxic fumes or direct skin contact. Short term exposure may cause varying complaints from mild nausea, skin problems and even death. Long term exposure may lead to sub-chronic conditions.

How are cyanobacteria algae currently measured?  
When cyanobacteria start to bloom at the start of spring, shores of surface waters are visually inspected for scum layers. Collected samples are cultivated at a laboratory where specialists determine the amount of cyanobacteria. The most common measuring methods in The Netherlands are fluorescence measurements and cell counting.

Why is there need for an early-warning system?  
It is possible to take management actions to prevent excessive growth and blooms. When no actions are taken, it will take up to several decades before nature is able to restore balance in the ecosystem. Most time is consumed by the hours traveling to the water location, taking the sample and analysing the sample at the lab. A major obstacle is the time that is lost between sampling and taking actions.

How can a low-cost photospectrometer be implemented as an early-warning system for hazardous cyanobacteria levels?  
An optical sensor that can perform ad hoc measurements significantly faster was developed in this project. In vivo phycocyanin in cyanobacteria cells emits light at 610nm–630nm when excited at a wavelength of 645nm. The intensity of this fluorescent light was measured with an optical sensor that was constructed for less than 70 Euro.

The designed photospectrometer has measured in vivo phycocyanin in a Microcystis sample that contained between 534µg/L and 686µg/L phycocyanin with a signal-to-noise ratio of SNR=$\frac{1}{2}$ on one single occurrence. The measured concentration of phycocyanin could have been a successful measurement, but could also have been a false positive. In order to relate measurements with hazardous cyanobacteria concentrations, extended validation should be considered.
7. Recommendations

The prototype has measured phycocyanin in a Microcystis sample with a signal-to-noise ratio of SNR=$\frac{1}{2}$ on one single occurrence. This does neither prove nor disprove that the prototype is capable of measuring hazardous cyanobacteria concentrations. There are a several steps necessary to relate measurements from the prototype with hazardous cyanobacteria concentrations.

1. Extended validation
   To relate measurements from the prototype with hazardous cyanobacteria concentrations, multiple cyanobacteria species should be measured. Various concentrations should be measured to establish an empirical conversion table or calibration curve. A draft for this procedure is described in appendix C.

2. Mixed samples
   Only concentrated samples of one species have been measured at the time. Different species should be mixed to determine whether this affects the output of the prototype. This include mixing with green-algae and inorganic compounds (trash).

3. Pure phycocyanin
   When the prototype measures *in vivo* phycocyanin in cyanobacteria and mixed samples correctly, pure phycocyanin should be used to create an absolute calibration curve.

4. Compact design
   The current design uses an Arduino Uno, which is connected to the sensor with unshielded wires. A more compact design reduces the electrical noise at the output.

5. Lower-cost filters
   The optical filters are the most expensive parts of the system. The filters are large and fragile. Perhaps alternative ways of optical filtering, such as colored plastic, could present a solution.

When these steps have successfully been followed, the optical sensor can be developed further into a low-cost autonomous early-warning system for hazardous cyanobacteria levels.
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<td>Spectra of dilutions of Microcystis and Monoraphidium samples together in a single plot.</td>
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Appendices
A. Wageningen Experiments

This appendix describes the experimental plan to learn more about the optical properties of cyanobacteria. The experiments are performed at Wageningen University and Research.

A.1 Synopsis

Find the optical absorbance spectra for cyanobacteria to translate wavelength amplitude into quantities of phycocyanin and/or cyanobacteria biovolume. Determine the required sensitivity and accuracy with which the designed proof of concept “cyanosensor” can approximate the absolute concentration of cyanobacteria in a controlled environment.

A.1.1 Safety

Various cyanobacteria can produce toxins that may cause symptoms such as irritation of mucus membranes, skin irritation, nausea, diarrhea and fever. Longer term exposure may lead to damage to the nervous system, skin and liver. Experiments require a lab coat, glasses and nitril/latex gloves. The experiments are performed with cuvettes up to 5mL per sample.

If a cyanobacteria sample makes contact with the skin, wash the skin with water and soap. Alert the lab supervisor and determine the threat that the particular cyanobacteria sample poses. Follow further instructions from the lab supervisor.

After the experiments, the cyanobacteria are returned to a bottle labeled “waste” and killed with bleach at the end of the experiments. The waste bottle is left exposed to sunlight to allow the toxins that were released to break down over a timespan of at least three weeks.

A.1.2 Rationale

The objective of the experiments below is to determine the absorbance wavelength of phycocyanin present in cyanobacteria. This experiment uses established equipment to determine the cell count in a sample, the amount of phycocyanin in the same sample and the optical properties of diluted cyanobacteria concentrations. Properties of different species are compared in order to find a trend in amount of phycocyanin in cyanobacteria and how diluting the concentrations affects optical properties.

A.2 Tasks

The following steps and experiments will be conducted:

- Make a dilution series for available species as shown in table A.1;
- determine the amount of microgram phycocyanin/chlorophyll-a in every sample;
- determine the cell count of the sample;
- observe the spectrogram of the sample;
- approximate the difference in intensities with the proof of concept “cyanosensor”.
A.2.1 Species

The following available species will be analyzed:

- Microcystis
- Monoraphidium

A.2.2 Dilution Series

To map the amount of phycocyanin, cell count and spectra of different strains, not all cyanobacteria strains have to be diluted in the same series or concentrations. The following dilutions should provide all relevant information.

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<td>PS</td>
<td>-</td>
<td>o</td>
<td>-</td>
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Table A.1: Dilution series and the experiments in which this dilution will be used.

Different dilutions are intended for different and combined experiments. In the table, these experiments are indicated with the following letters:

- P - Phyto-PAM, measures chlorophyll-a + phycocyanin;
- C - CASY® counter, cell counting;
- S - spectrometry with Beckman Coulter DU 730 (WUR Wageningen);
- X - cyanosensor, own device measures light intensity;
- o - optional measurement to establish whether established equipment can measure very low concentrations.

It is not certain until what accuracy and absolute amount all equipment can measure. The 100% sample may saturate the equipment, whereas the 0.001% sample might be below the threshold that equipment can register. Microcystis serves as an indicator to determine the concentrations with which to perform the other measurements.

Another dilution series must be made to be able to reference to the 2012 blue-green algae protocol[4]. For this series, Microcystis is diluted in concentrations to match the following phycocyanin concentrations or biovolumes in table A.2.

<table>
<thead>
<tr>
<th>Risk level</th>
<th>level 0</th>
<th>level 1</th>
<th>level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>phycocyanin</td>
<td>&lt;12.5µg/L</td>
<td>12.5 – 75µg/L</td>
<td>&gt;75µg/L</td>
</tr>
<tr>
<td>biovolume</td>
<td>&lt;2.5mm³/L</td>
<td>2.5 – 15mm³/L</td>
<td>&gt;15mm³/L</td>
</tr>
</tbody>
</table>

Table A.2: Reference dilutions for Microcystis matched with the 2012 blue-green algae protocol[4].

A.2.3 Procedure

Dilution

Place the amount of 100% in a 5 mL cuvette with label and fill up the cuvette up to 4.0mL with tap water. See the table below for the ratios cyanobacteria sample and tap water. Repeat for all species, following table A.1.
Phyto-PAM

Pipet the diluted sample in the sample cuvette, place it in the measurement chamber and close it. Start the measurement at the PC. Use the ‘gain’ button to scale automatically. Record the output. Repeat for all samples following table A.1. If no saturation occurs at the 100% sample for Microcystis, measurements with higher concentrations can be considered.

### Table A.3: Ratio 100% sample and tap water.

<table>
<thead>
<tr>
<th>cuvette label</th>
<th>amount 100% sample</th>
<th>amount tap water</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>4 mL</td>
<td>0 mL</td>
</tr>
<tr>
<td>50</td>
<td>2 mL</td>
<td>2 mL</td>
</tr>
<tr>
<td>25</td>
<td>1 mL</td>
<td>3 mL</td>
</tr>
<tr>
<td>10</td>
<td>400 µL</td>
<td>3.6 mL</td>
</tr>
<tr>
<td>1</td>
<td>40 µL</td>
<td>3.96 mL</td>
</tr>
<tr>
<td>0.1</td>
<td>4 µL</td>
<td>3.996 mL</td>
</tr>
<tr>
<td>0.01</td>
<td>0.4 µL</td>
<td>3.9996 mL</td>
</tr>
<tr>
<td>0.001</td>
<td>0.04 µL</td>
<td>3.99996 mL</td>
</tr>
</tbody>
</table>

CASY® counter

The CASY® has been subjected to the cleaning procedure in advance of this experiment. Pipet 0.10 mL in the sample vial and add 9.90 mL CASY®TON, an electrolyte that causes the cells to flow through the counter at a constant speed. Roll the closed sample vial a few times slowly over the table to remove air bubbles from the inside walls of the vial. Place the vial under the input tube. Start the measurement with the ‘Start’ button on the display. Record the following outputs: CML – number of particles per mL (mL⁻¹), VML – total volume of particles per mL (µm³/mL) and MVL – mean volume per particle (µm³). Repeat for all samples following table A.1.

Figure A.1: The Phyto-ED measurement chamber for chlorophyll-a/phycocyanin measurements at Wageningen University and Research.

Figure A.2: The CASY® Counter setup with display and sample at Wageningen University and Research.
Spectrometer

Place the sample cuvette in the holder and start the measurement at the display. Store the output on a USB device. Repeat for all samples following table A.1.

![Figure A.3: The Beckman Coulter spectrometer at Wageningen University and Research.](image)

Cyanosensor

Place the sample cuvette in the holder and start the light excitation program. Record the output with an oscilloscope. Make a photo or screenshot to record the output. Repeat for all samples following table A.1.

![Figure A.4: The proof of concept for the cyanosensor between measurement sessions.](image)

A.3 Results

The experiments were performed at Wageningen University and Research on 9 January 2015. The experiments were performed by Tim Stoppelenburg and Svitlana Shtyforuk under the supervision of Wendy Beckman-Lukassen and Imke Leenen.

A.3.1 Phycocyanin and Chlorophyll-a

Both the *Microcystis* and *Monoraphidium* samples were measured with the Phyto-PAM to determine the amount of phycocyanin and chlorophyll-a. The following quantities were recorded.
Table A.4: $\mu g$ phycocyanin per % *Microcystis* sample.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>576.76</td>
<td>534.87</td>
<td>686.78</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>294.04</td>
<td>274.54</td>
<td>352.51</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>117.01</td>
<td>126.61</td>
<td>162.57</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>54.58</td>
<td>44.69</td>
<td>57.39</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>9.45</td>
<td>6.17</td>
<td>7.19</td>
<td></td>
</tr>
</tbody>
</table>

Figure A.5: Plot of table A.4 of $\mu g$ phycocyanin per % *Microcystis* sample.

Table A.5: $\mu g$ chlorophyll-a per % *Monoraphidium* sample.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>2977.55</td>
<td>3131.94</td>
<td>2297.02</td>
<td>2749.49</td>
</tr>
<tr>
<td>0.50</td>
<td>1474.39</td>
<td>1606.99</td>
<td>1582.01</td>
<td>1215.39</td>
</tr>
<tr>
<td>0.25</td>
<td>637.24</td>
<td>671.58</td>
<td>670.28</td>
<td>647.05</td>
</tr>
<tr>
<td>0.10</td>
<td>187.92</td>
<td>194.71</td>
<td>214.56</td>
<td>233.89</td>
</tr>
<tr>
<td>0.01</td>
<td>16.92</td>
<td>18.63</td>
<td>35.08</td>
<td>38.01</td>
</tr>
</tbody>
</table>
The dilutions series were continued until a sample-water ratio was found where the concentration of phycocyanin was lower than the concentration described in the protocol. At 1.0% sample, the concentration of phycocyanin was lower than $12.5 \mu g$ phycocyanin per liter.

### A.3.2 Counting

The same *Microcystis* and *Monoraphidium* samples that were used at the Phyto-PAM measurements were used for the CASY®-counting measurement. The results were recorded as following: CML – number of particles per mL (mL$^{-1}$), VML – total volume of particles per ml ($\mu m^3$/mL) and MVL – mean volume per particle ($\mu m^3$).
Number of particles in mL$^{-1}$ (figure A.7), total volume of particles in $\mu$m$^3$/mL (figure A.8) and mean volume per particle in $\mu$m$^3$ (figure A.9) of *Microcystis*. When a sample contains more than 2.00E + 006 particles (Cml), the measurements become unreliable. The concentration of *Monoraphidium* was too high to yield valid results. Below a dilution of 10% sample, the CASY®-counter returned the results shown in table A.6.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Cml</th>
<th>Vml</th>
<th>Mvl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>1.36E+006</td>
<td>2.42E+008</td>
<td>1.78E+002</td>
</tr>
<tr>
<td>0.10</td>
<td>1.49E+006</td>
<td>7.32E+007</td>
<td>4.90E+001</td>
</tr>
<tr>
<td>0.10</td>
<td>1.47E+006</td>
<td>8.01E+007</td>
<td>5.43E+001</td>
</tr>
<tr>
<td>0.01</td>
<td>2.38E+005</td>
<td>6.36E+008</td>
<td>2.67E+003</td>
</tr>
</tbody>
</table>

Table A.6: Particles in *Monoraphidium* samples.

### A.3.3 Photospectrometer

New sample dilutions of *Microcystis* and *Monoraphidium* were used for the photospectrometry measurement. The following spectra were observed.
Figure A.10: Spectra of dilutions of *Microcystis* samples.

Figure A.11: Spectra of dilutions of *Monoraphidium* samples.

A sample of both *Microcystis* and *Monoraphidium* are shown in the same plot in figure A.12 to determine the characteristic differences between general green-algae and bluegreen-algae.
A.3.4 Cyanosensor

With the proof of concept for the cyanosensor, three samples are measured to establish the proper functionality of the sensor. The recording device is a Textronics TBS1052B-EDU oscilloscope which is configured with a horizontal timescale of 2.5s and a horizontal scale of 20mV at an offset of $−0.386mV$. The data is automatically recoded to a USB drive when the ‘record’ button is pressed. The first sample is demiwater.

![Image](image.png)

(a) With 610nm 10nm band-pass filter  (b) With 655nm long-pass filter

Figure A.13: Testing the proof of concept with different filters on a demiwater sample.

The second sample is a 100% *Monoraphidium* sample. The oscilloscope is configured with a horizontal timescale of 2.5s and a horizontal scale of 20mV at an offset of $−0.386mV$. 

![Image](image.png)
The third sample is a 100% *Microcystis* sample. The oscilloscope is configured with a horizontal timescale of 2.5s and a horizontal scale of 50mV at an offset of −0.200mV. Note that in figure A.15a, the oscilloscope is configured with a horizontal timescale of 2.5s and a horizontal scale of 20mV at an offset of −0.200mV.
B. Spectrometry Experiments

This appendix describes the experimental plan to observe the fluorescence spectra of cyanobacteria. The experiments are performed at the Hanze Institute of Technology in Assen.

B.1 Synopsis

Find the optical fluorescence spectra for cyanobacteria to translate wavelength amplitude into quantities of phycocyanin and/or cyanobacteria biovolume. Determine the optical properties of used filters.

B.1.1 Safety

Various cyanobacteria can produce toxins that may cause symptoms such as irritation of mucus membranes, skin irritation, nausea, diarrhea and fever. Longer term exposure may lead to damage to the nervous system, skin and liver. Experiments require a lab coat, glasses and nitril/latex gloves. The experiments are performed with cuvettes up to 5mL per sample. If a cyanobacteria sample makes contact with the skin, wash the skin with water and soap. Alert the lab supervisor and determine the threat that the particular cyanobacteria sample poses. Follow further instructions from the lab supervisor. After the experiments, the cyanobacteria are returned to a bottle labeled “waste” and killed with bleach at the end of the experiments. The waste bottle is left exposed to sunlight to allow the toxins that were released to break down over a timespan of at least three weeks.

B.1.2 Rationale

The objective of the experiments below is to determine the fluorescence wavelength of phycocyanin present in cyanobacteria. This experiment uses established equipment to determine the fluorescence spectra of dilutions of Microcystis and Monoraphidium. Additionally, the transmittance of two optical filters is measured to display the spectrum of transmitted light.

B.2 Tasks

The following steps and experiments will be conducted:

- Make a dilution series for available species for 100%, 10% and 1% sample;
- observe the spectrogram of the sample;
- observe the spectrogram of the two filters;

B.2.1 Species

The experiment will be performed with the following species, provided by Wageningen University and Research.

- Microcystis
- Monoraphidium
B.2.2 Procedure

Dilution

Place the amount of 100% in a beaker and pipet 3 mL in a cuvette with label ‘100%’. Pipet 1mL of this sample into a beaker with label ‘10%’ and add 9mL water. Pipet 2mL of this sample in a cuvette with label ‘10%’. Pipet 1mL of this sample into a beaker with label ‘1%’ and add 9mL water. Pipet 2mL of this sample in a cuvette with label ‘1%’. Fill a cuvette labeled ‘blank’ with approximately 2mL demiwater. Repeat this dilution series so that there are two series of each species.

Spectrometer

Place the sample cuvette in the holder and start the measurement at the computer. Store the output on a USB device. Repeat for all samples. Place the filter in the appropriate filter holder and perform an absorbance measurement for each filter, without sample.

B.3 Results

The experiments were performed at the Hanze Institute of Technology in Assen on 13 January 2015. The experiments were performed by Tim Stoppelenburg under the supervision of Johan Hekman.

B.3.1 Photospectrometer

The following spectra were observed for Microcystis and Monoraphidium. The measurements were made with an Ocean Optics QEB1800 photospectrometer and a host computer with SpectraSuite.

![Figure B.1: Spectra of dilutions of Microcystis samples.](image)
The filters were placed in the holder designed for this purpose. The following spectra are the absorbance spectra for a 610nm 10nm band-pass filter (orange) and a 655nm long-pass filter (dark red).

Figure B.2: Spectra of dilutions of Monoraphidium samples.

Figure B.3: Absorbance spectra for two different filters.
C. Validation Experiments

This appendix describes the experimental plan to validate the designed proof of concept. The experiments are to be performed at Wageningen University & Research.

C.1 Synopsis

Determine the accuracy of the designed proof of concept.

C.1.1 Safety

Various cyanobacteria can produce toxins that may cause symptoms such as irritation of mucus membranes, skin irritation, nausea, diarrhea and fever. Longer term exposure may lead to damage to the nervous system, skin and liver. Experiments require a lab coat, glasses and nitril/latex gloves. The experiments are performed with cuvettes up to 5mL per sample.

If a cyanobacteria sample makes contact with the skin, wash the skin with water and soap. Alert the lab supervisor and determine the threat that the particular cyanobacteria sample poses. Follow further instructions from the lab supervisor.

After the experiments, the cyanobacteria are returned to a bottle labeled “waste” and killed with bleach at the end of the experiments. The waste bottle is left exposed to sunlight to allow the toxins that were released to break down over a timespan of at least three weeks.

C.1.2 Rationale

The objective of the experiments below is to determine the response of the designed proof of concept and to determine the accuracy of the current design. This experiment uses established equipment to determine the cell count in a sample, the amount of phycocyanin in the same sample and the optical properties of diluted cyanobacteria concentrations. Properties of different species are compared in order to find a trend in amount of phycocyanin in cyanobacteria and how diluting the concentrations affects optical properties.

C.2 Tasks

The following steps and experiments will be conducted:

- Make a dilution series for available species as shown in table C.1;
- determine the amount of microgram phycocyanin/chlorophyll-a in every sample;
- determine the cell count of the sample;
- approximate the difference in intensities with the proof of concept “cyanosensor”.

C.2.1 Species

If available, the following species will be analyzed:

- Microcystis
- Anabaena
- Planktothrix
- Aphanizomenon
- Woronichinia
- Cryptomonas
- Cylindrospermopsis
- Monoraphidium
- Phormidium (optional)

### C.2.2 Dilution Series

To map the amount of phycocyanin, cell count and spectra of different strains, not all cyanobacteria strains have to be diluted in the same series or concentrations. The following dilutions should provide all relevant information.

<table>
<thead>
<tr>
<th></th>
<th>100%</th>
<th>50%</th>
<th>25%</th>
<th>10%</th>
<th>1%</th>
<th>0.1%</th>
<th>0.01%</th>
<th>0.001%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (ref)</td>
<td>PC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Microcystis</td>
<td>PC</td>
<td>-</td>
<td>P</td>
<td>PC</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Anabaena</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>PC</td>
<td>P</td>
<td>P</td>
<td>-</td>
<td>o</td>
</tr>
<tr>
<td>Planktothrix</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>PC</td>
<td>P</td>
<td>P</td>
<td>-</td>
<td>o</td>
</tr>
<tr>
<td>Aphaniizomenon</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>PC</td>
<td>P</td>
<td>P</td>
<td>-</td>
<td>o</td>
</tr>
<tr>
<td>Woronichinia</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>PC</td>
<td>P</td>
<td>P</td>
<td>-</td>
<td>o</td>
</tr>
<tr>
<td>Cryptomonas</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>PC</td>
<td>P</td>
<td>P</td>
<td>-</td>
<td>o</td>
</tr>
<tr>
<td>Cylindrospermopsis</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>PC</td>
<td>P</td>
<td>P</td>
<td>-</td>
<td>o</td>
</tr>
<tr>
<td>Monoraphidium</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>PC</td>
<td>P</td>
<td>P</td>
<td>-</td>
<td>o</td>
</tr>
<tr>
<td>Phormidium</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>PC</td>
<td>P</td>
<td>P</td>
<td>-</td>
<td>o</td>
</tr>
</tbody>
</table>

Table C.1: Dilution series and the experiments in which this dilution will be used.

Different dilutions are intended for different and combined experiments. In the table, these experiments are indicated with the following letters:

- **P** - Phyto-PAM, measures chlorophyll-a + phycocyanin;
- **C** - CASY counter, cell counting;
- **X** - cyanosensor, prototype low-cost early warning system;
- **o** - optional measurement to establish whether established equipment can measure very low concentrations.

*All samples are measured with the prototype cyanosensor.* It is not certain until what accuracy and absolute amount all equipment can measure. The 100% sample may saturate the equipment, whereas the 0.001% sample might be below the threshold that equipment can register. Microcystis serves as an indicator to determine the concentrations with which to perform the other measurements.

Another dilution series must be made to be able to reference to the 2012 blue-green algae protocol[4]. For this series, Microcystis is diluted in concentrations to match the following phycocyanin concentrations or biovolumes in table C.2.

<table>
<thead>
<tr>
<th></th>
<th>level 0</th>
<th>level 1</th>
<th>level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>phycocyanin</td>
<td>&lt;12.5µg/L</td>
<td>12.5 – 75µg/L</td>
<td>&gt;75µg/L</td>
</tr>
<tr>
<td>biovolume</td>
<td>&lt;2.5mm³/L</td>
<td>2.5 – 15mm³/L</td>
<td>&gt;15mm³/L</td>
</tr>
</tbody>
</table>

Table C.2: Reference dilutions for Microcystis matched with the 2012 blue-green algae protocol[4].

### C.2.3 Procedure

**Dilution**

Place the amount of 100% in a 5 mL cuvette with label and fill up the cuvette up to 4.0mL with tap water. See the table below for the ratios cyanobacteria sample and tap water. Repeat for all species, following table A.1.
Final Report

Appendix C. Validation Experiments

<table>
<thead>
<tr>
<th>cuvette label</th>
<th>amount 100% sample</th>
<th>amount tap water</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>4 mL</td>
<td>0 mL</td>
</tr>
<tr>
<td>50</td>
<td>2 mL</td>
<td>2 mL</td>
</tr>
<tr>
<td>25</td>
<td>1 mL</td>
<td>3 mL</td>
</tr>
<tr>
<td>10</td>
<td>400 µL</td>
<td>3.6 mL</td>
</tr>
<tr>
<td>1</td>
<td>40 µL</td>
<td>3.96 mL</td>
</tr>
<tr>
<td>0.1</td>
<td>4 µL</td>
<td>3.996 mL</td>
</tr>
<tr>
<td>0.01</td>
<td>0.4 µL</td>
<td>3.9996 mL</td>
</tr>
<tr>
<td>0.001</td>
<td>0.04 µL</td>
<td>3.99996 mL</td>
</tr>
</tbody>
</table>

Table C.3: Ratio 100% sample and tap water.

Phyto-PAM

Pipet the diluted sample in the sample cuvette, place it in the measurement chamber and close it. Start the measurement at the PC. Use the ‘gain’ button to scale automatically. Record the output. Repeat for all samples following table C.1. If no saturation occurs at the 100% sample for Microcystis, measurements with higher concentrations can be considered.

CASY® counter

The CASY® has been subjected to the cleaning procedure in advance of this experiment. Pipet 0.10 mL in the sample vial and add 9.90 mL CASY®TON, an electrolyte that causes the cells to flow through the counter at a constant speed. Roll the closed sample vial a few times slowly over the table to remove air bubbles from the inside walls of the vial. Place the vial under the input tube. Start the measurement with the ‘Start’ button on the display. Record the following outputs: CML – number of particles per mL (mL⁻¹), VML – total volume of particles per ml (µm³/mL) and MVL – mean volume per particle (µm³). Repeat for all samples following table C.1.

Cyanosensor

Place the sample cuvette in the holder and start the light excitation program. Record the output with the Arduino. Save the output in a spreadsheet. Repeat for all samples following table C.1.

C.3 Results

These experiments have not taken place yet.