Automated quantification of the phototaxis of microalgae

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Entry to the Stockholm Junior Water Prize 2019

Germany

Summary

We have developed an experimental setup that can automatically and autonomously investigate the phototaxis of unicellular green algae. The fields to be tested are phototaxis in terms of wavelength and intensity of the triggering light. Other parameters to be considered are contrast, time of day, cell concentration and the speed of the source of light.

With our apparatus we investigate the phototaxis of the microalga Chlamydomonas reinhardtii.

Acknowledgements

We would like to express our gratitude to our teacher Wolfgang Bindel for constantly being open for discussions on questions from biology and on the presentation of our work, as well as to Dr. Volker Wagner from the Faculty of Biological Sciences of the Friedrich Schiller University of Jena, Germany, for scientific advice and for the supply of algae.

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1 Introduction

What can the phototaxis reaction of microalgae do?

When we first heard about plants actively moving (towards light), we were excited and decided to investigate this problem.

In general, we consider algae to be very important and undervalued.

Microalgae have recently received more and more attention in science, as they probably represent a great potential for research and development and are still not sufficiently investigated.

There are very impressive examples of climate neutral oil produced from algae with technical applications from road surfacing to fuel. There are now plants in which algae are fed with CO_2 and then processed into diesel-like fuel. Like other fuels based on vegetable oil, this fuel is almost CO_2 neutral, because the algae absorb as much CO_2 during growth as is released later during combustion. However, the biomass obtained from algae is ten times higher than that of rapeseed, for example.

The phototaxis of algae has not yet been used to generate energy. It is likely that what used to work with the donkey driving the mill wheel will not work so effectively with millions of algae. But what if it does? In our opinion the phototaxis of microalgae deserves investigation in any case.

It is difficult to observe phototaxis in microalgae without technical tools, because it takes place in a world of microscopic organisms. Many individuals are needed to recognize anything at all. And then the movement is relatively slow for us. Furthermore, there is the danger that the algae colonies will be destroyed by the introduction of harmful germs. During the observation itself, changes of environmental influences must be avoided as far as possible or has to be measurable and documentable. Our goal is therefore to build a closed system in which the parameter "light" can be controlled. The system should be as automated as possible and must be able to trigger and observe phototaxis simultaneously.

To our eye only large numbers of algae are visible, then appearing as green water, possibly in motion. But as that is not fascinating enough, the algae are interesting even in low numbers. Single algae - like active nano particles - could show prescribed behaviour, well controlled by light.

Therefore we want to study phototaxis with regard to wavelength and intensity of the triggering light. Other parameters considered are contrast, time of day, cell concentration and the given speed of movement of the light source.

We are fascinated by the small plants, and we hope that our work will contribute to the systematic study of microalgae.

2 Our technical approach

In the following, we would like to show how we have tried to achieve our goals and explain how our results and insights have been obtained.

Already during the initial basic research our next steps were clear:

1. to investigate the theoretical background
2. to develop a suitable experimental setup
3. to provide algae and to secure suitable living conditions for them
4. to carry out experiments

2.1 Theoretical basics

First of all, we would like to clarify a few terms.

2.1.1 Thallophytes (lower plants)

are unicellular or multicellular organisms which are not subdivided into root, stem and leaves. They also do not form flowers or seeds. Thallophytes include fungi, algae and lichens.

2.1.2 Algae

are plants of simple structure that are adapted to life in water. Some algae can move actively.

2.1.3 Green algae

are a group of plants, they belong to the systematic organizational level of thallophytes, and are adapted to life in water.

Green algae live mainly in fresh water, some species also thrive in coastal waters of the seas or on wet soils, some algae can move.

Green algae occur as unicellular organisms, colonies or multicellular organisms. Their cells have a nucleus, chloroplasts and are surrounded by a cell wall.

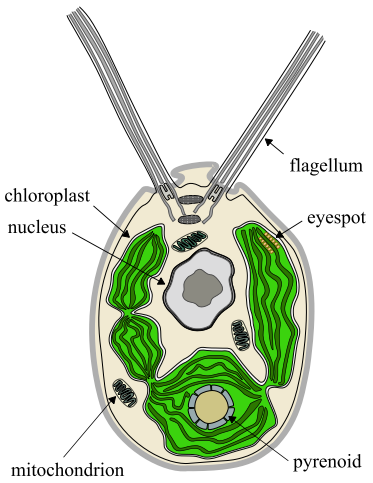
2.1.4 Microalgae (microphytes)

are a group of microscopically small and often unicellular algae.

2.1.5 Chlamydomonas reinhardtii

is a microalgae of the genus Chlamydomonas. Important characteristics of this unicellular microalga are two equally long anterior flagellae, which emerge closely to each other from the cell body. With these flagellae the alga moves actively. In addition, the cell has an eye spot for detecting light stimuli. Chlamydomonas reinhardtii has all the cell components of a plant cell.

Chlamydomonas reinhardtii are also called Chlamys in the following.



2.1.6 Taxis

is a locomotion, as a reaction to a certain stimulus effect (e.g. aerotaxis - orientation to oxygen, thermotaxis - motion due to temperature, and phototaxis – triggered by light).

2.1.7 Phototaxis

is a locomotion which is influenced by differences in light irradiation, light intensity and light colour. Positive phototaxis is the orientation towards higher illuminance. Conversely, if one moves in the direction of the low-light areas, one speaks of negative phototaxis. If the organism moves at a certain angle to the source of light, the behavior is referred to as transverse phototaxis.

2.2 Experimental requirements

The following questions are to be answered by our experimental setup.

-Do Chlamys (to a noticeable extent) make phototaxis at all?

-How fast do they move?

-How uniformly do they move?

-To which spectra of colour do they react (how much)?

-How much light do algae prefer?

In order to answer these questions we need the position data of the algae at different times and with different illumination.

2.2.1 Developed experiments

- Experiment 1: Test for the reaction of algae in the experimental setup.

- Experiment 2: Testing of different algae concentrations for phototaxis optimum.

  This means that we test if the density of organisms influences phototaxis. If so, what effects appear? Our assumption was that we cannot recognize a few cells with our experimental setup or the naked eye, and that many cells interfere with each other in phototaxis.

- Experiment 3: Determination of the light intensity preferred by the Chlamys.

- Experiment 4: Determination of the colour preference of Chlamys.

  The algae get the chance to choose between two different coloured objects.

- Experiment 5: Study of the effect of wavelength on the phototaxis of Chlamys.

- Experiment 6: Determination of the speed of the Chlamys, with different influences.

2.3 Experimental set-up for the inspection of the phototaxis of microalgae

The experimental setup described in the following is intended to make it possible to carry out the planned experiments and thus to analyze the phototaxis reaction of microalgae according to our requirements.

In order to achieve a phototactic reaction that is as controlled as possible, it is necessary (or at least very useful) to let only light impinge on the algae, which can be controlled in wavelength, intensity and location. This is important, because otherwise decisive quantities of input are not known and the evaluation becomes much more difficult than it already is.

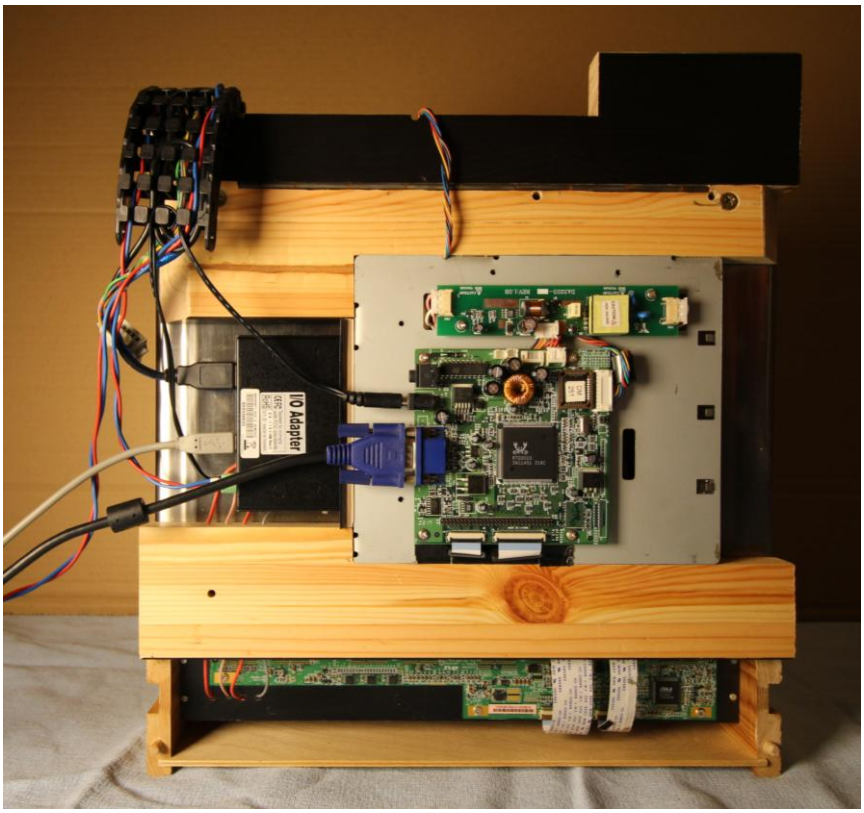
On the other hand, the phototactic reaction should also be detectable. Since it is useful for our project to generate many accurate and fine-grained measured values over a longer period of time, this should be done as automatically as possible.

The setup described here is the current version at the time of writing. This version has already undergone some changes and will be further optimized, adapted and extended.

The previous optimizations are mostly based on results, which were not actually searched for, e.g. influence of temperature or time of day (see results). The remaining changes and enhancements are described in the final discussion.

2.3.1 Hardware for triggering phototaxis

In order to be able to vary the intensity and colour of the light impinging on the algae pixel by pixel, there is an LCD (Liquid Crystal Display) in front of the light source. The algae are then supposed to swim above the light source. The used TFT-LCD has 1200 x 800 pixels which can display the colors red, green and blue in different intensities, the refresh rate is 30 fps.



The LCD can be controlled via a VGA interface.

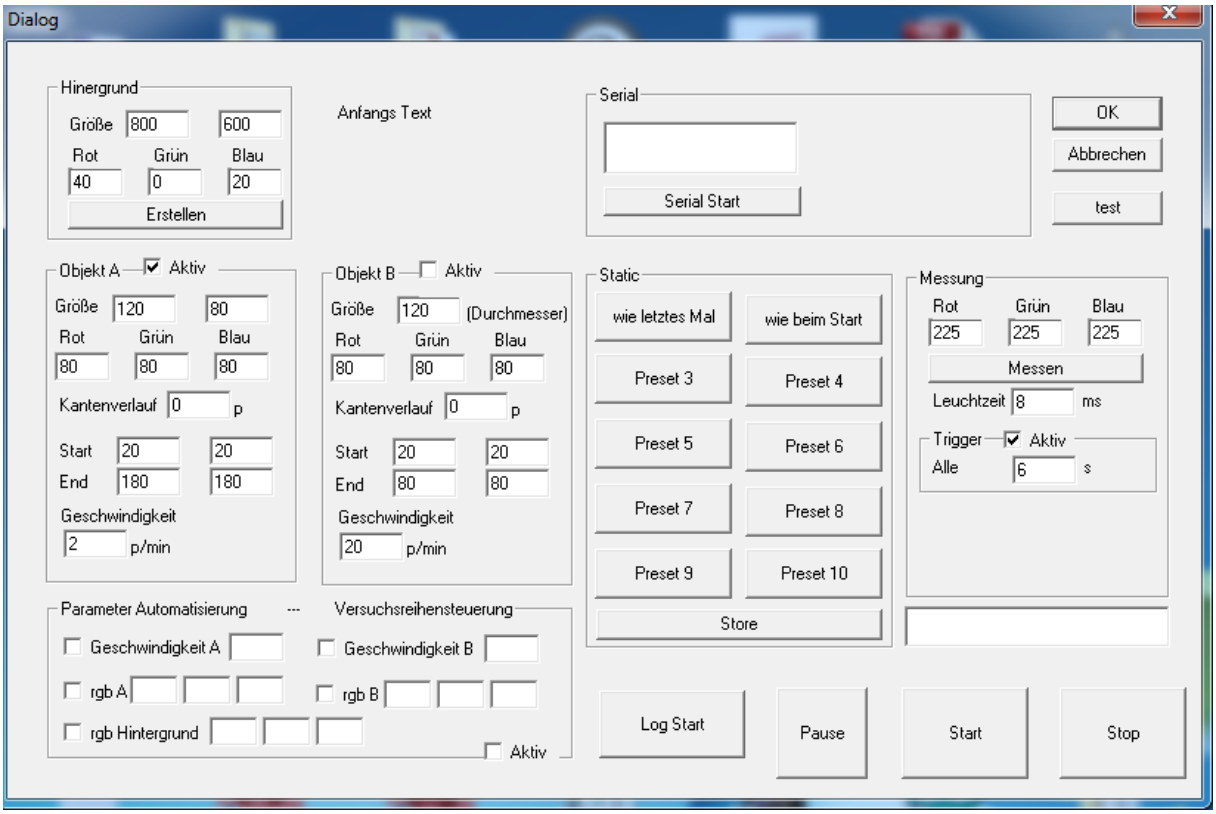
Under the LCD screen there is a diffuser whose task is to distribute the light coming from the light source as uniformly as possible on the LCD.

A mixture of warm white LEDs and cold cathode tubes is used as the light source in order to ensure a uniform output colour spectrum. Some incandescent lamps would also be predestined for this, but their waste heat is simply too large to be adequately dissipated or cooled down. It is important to keep the interior at a constant temperature despite all the surrounding electronics, as the temperature may influence the activity or even the well-being of the algae.

2.3.2 Control software

In order to control the display appropriate to the species, software is required that runs on commercially available PCs as far as possible and drives the mounted LCD via a VGA interface. The software generates an animation on the LCD to which the algae can react.

In order to keep the effort within limits, a common Windows operating system is used as a basis.

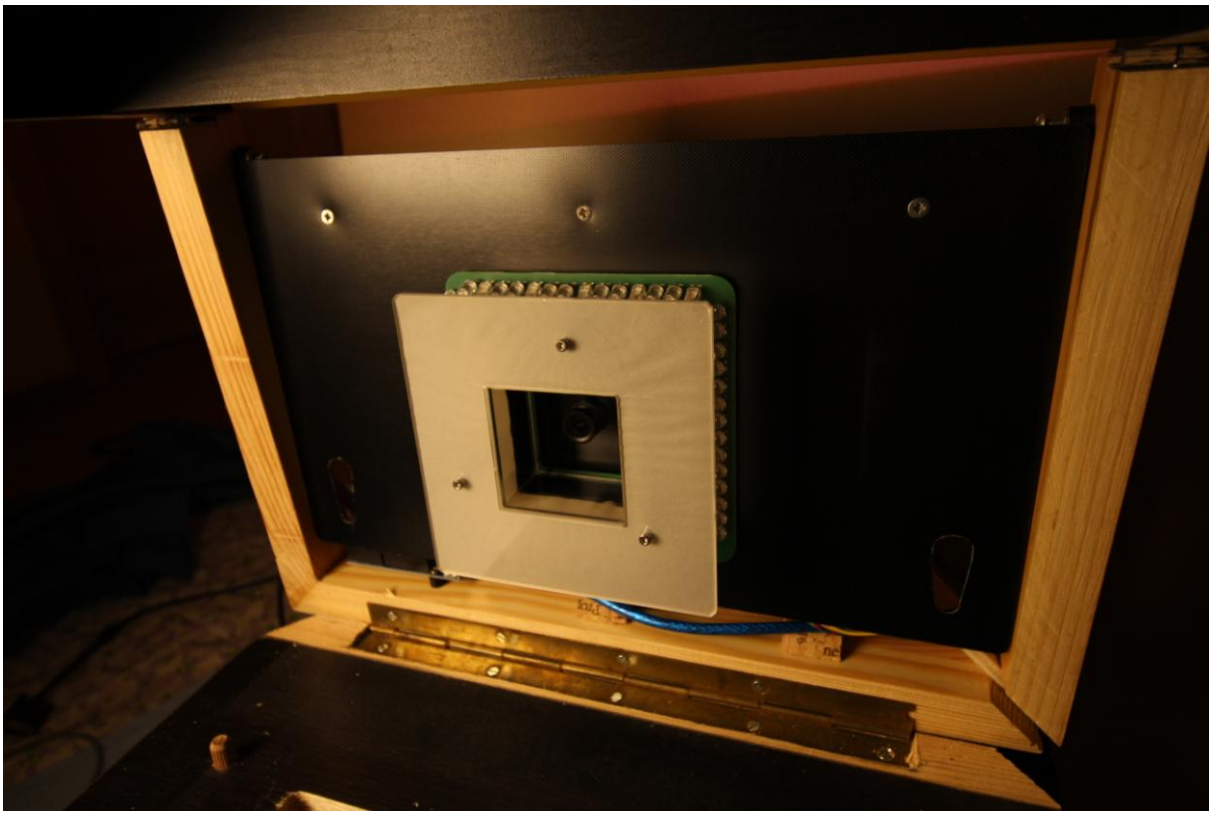


This software generates a background of any color and brightness over which a rectangle or a circle, both in any color, brightness and size, moves.

Because the Chlamys do not only behave positively but also negatively phototactically, which means they also swim off the light into the dark, if it is too bright for them (see Phototaxis), there is an additional function, which makes it possible to represent the forms with brightness gradients. So the Chlamys can choose their preferred light intensity. This function can and should also be used for calibration in order to find the light intensity preferred by the majority of algae.

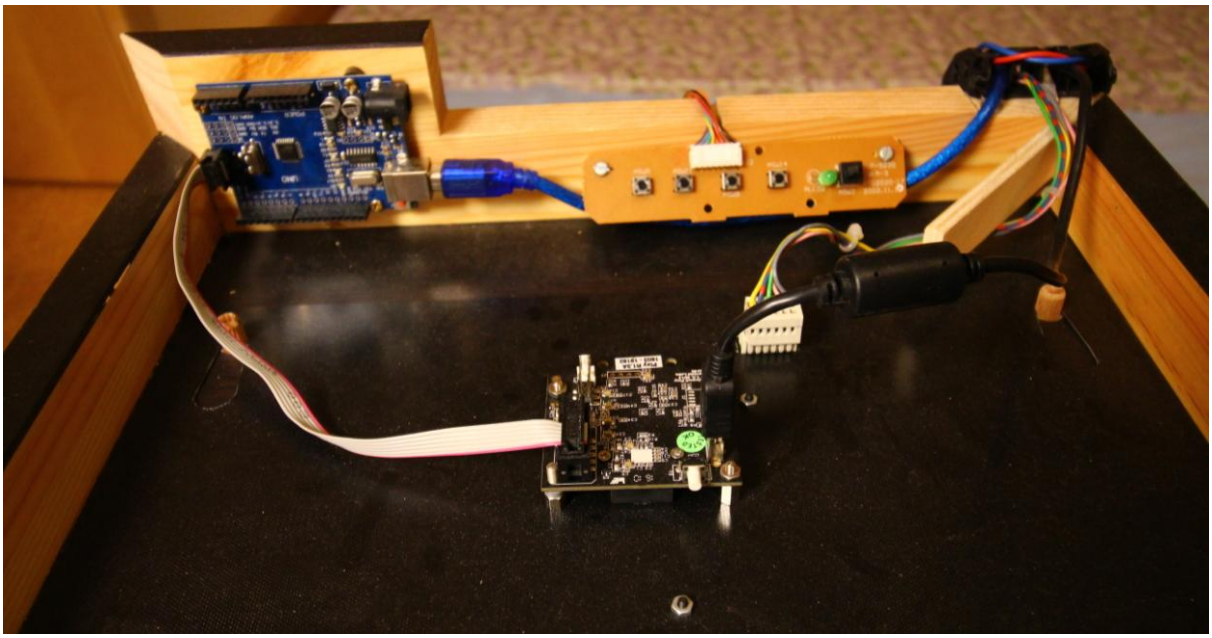
2.3.3 Hardware for detecting phototaxis

To detect phototaxis, more hardware is needed. Since our camera cannot detect the approximately 10 µm large algae individually, only the intensity of green colour of water indicates where a large amount of the algae are. Recognizing different concentrations of greens in front of the unevenly illuminated LCD screen during an animation is practically impossible. Therefore, in order to measure the position of the algae, a different, uniform background is briefly shown and a photo of the algae is taken during this time. This photo is then analysed by image evaluation software.



The light arranged around the camera allows the regeneration of the algae. This light ensures optimal conditions for photosynthesis between experiments and helps to prevent the Chlamys from being exhausted and becoming sluggish.

A RGB-UV-LED matrix is used as light source. The Pixy cam with a resolution of 1280x800 detects the algae.



The camera has its own microprocessor (NXP LPC4330) for image processing. This communicates via SPI with an Arduino (AT MEGA 328P). The Arduino then passes the data filtered and sorted per serial interface (via USB) to the connected PC.

2.3.4 Process of construction

The housing consists mainly of wood and metal. The electronic components were mainly recycled from damaged (household) appliances and modified if necessary. The camera and the Arduino were still in stock (from previous projects).

The software is written in C++ with Visual Studio Professional 2010. It consists of the PC program and the script for the Arduino. The PC program provides the user interface and the stimulating light for the algae, and also manages the final processing and storage of data. The Arduino acts as a bridge between PC and hardware.

2.4 Carrying out the experiments

Once the first version of the experimental setup had been developed, we still needed suitable test candidates. During our research, we learned that the Faculty of Biological Sciences of the Friedrich Schiller University of Jena also deals with phototactic algae, especially with the circadian cycles of the green alga Chlamydomonas reinhardtii. At our request, Dr. Volker Wagener gave us great support in the form of knowledge, literature, references and Chlamy

cultures. The Chlamydomonas reinhardtii have proven to be suitable for our purposes. Therefore all our experiments so far are based on that species.

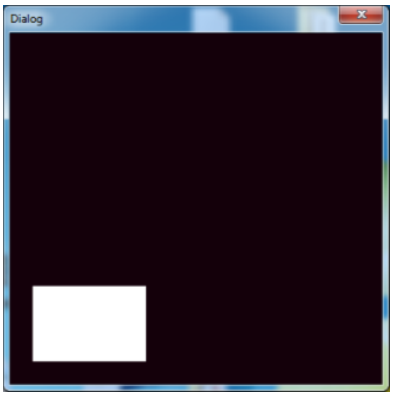
2.4.1 Experimenting

Parts of our experiments have been carried out or at least prepared at the Faculty of Biological Sciences, because the Chlamy cultures are susceptible to germs and sterile working is possible there. Particularly in the growth phase of the culture, sterility is necessary (otherwise the germs grow with it). Later, when the Chlamys live on a nutrient-poor medium and feed upon photosynthesis, this is less critical.

In the following, a basic experiment is presented as an example.

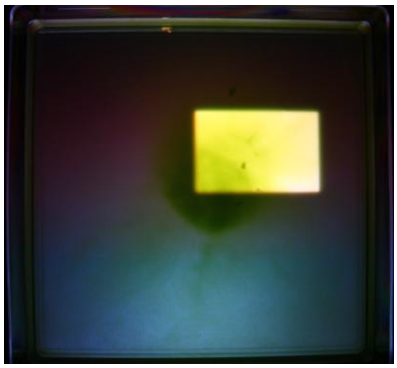
We first transfered a Chlamy culture of approx. 14,00,000 cells per millilitre to a specimen holder of size 10cm x 10cm. The holder was filled approx. 2mm high with algae and nutrient medium. We then inserted it into our experimental set-up. The experiment started at 4:20 p.m. at a temperature of approx. 20C°.

The LCD displayed a dark background with a small portion of red and blued. On that background the algae were offered a bright white rectangle, which moved across the screen at one pixel per second, to swim after.

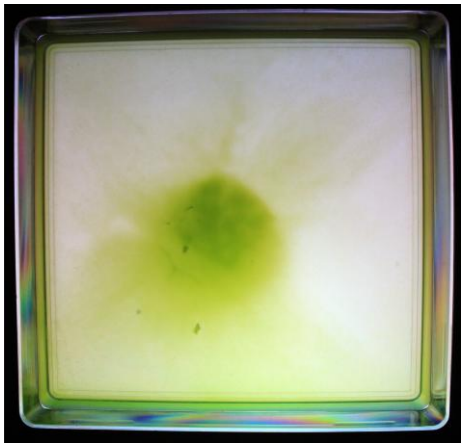


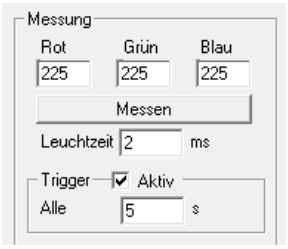
Before the rectangle moved out of the algae's field of vision, it was stopped.

One could see from above, looking through the colony of algae, how the rectangle moved. Already at that stage one could guess that the algae followed the source of light, but this was not clearly visible with this illumination.



Therefore, a white monochrome background is displayed for the duration of every recording.

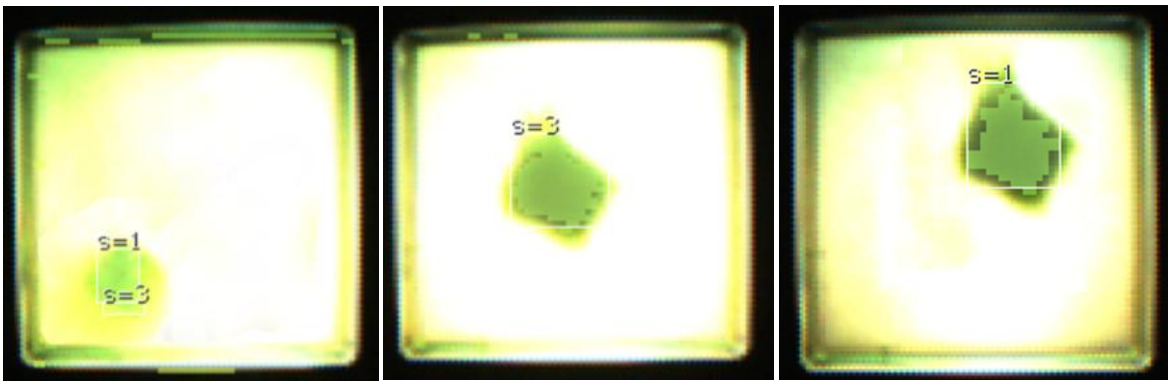




Measurements are processed every 5 seconds. For that a uniform bright white light is displayed for 2 milliseconds.

This way the monitoring of the algae is essentially easier.

From different pictures (which were created at different times) one can now deduce the movement.



The illustration shows that the video processing has identified the colonies of algae. Different signatures indicate the detected relative density of organisms.

The first experiments were actually successful. As suspected, the Chlamys reacted to what they were shown! But the effects were very different. We conducted experiments similar to those described above and were able to observe phototaxis well (early afternoon). After 6 p.m. there was only little movement to observe.

While testing different parameters we increased the brightness of the background and found an interesting phenomenon. The Chlamys grouped into small hotspots randomly distributed in the culture medium.

We also tried to measure the maximum speeds of different algae cultures between 10 a.m. and noon (actually the best time for observing phototaxis). Among others, we investigated another strain of algae. However, this culture was completely unimpressed by our experiments and did not even begin to move (visibly). It was not even possible to affect the algae to accumulate on the illuminated side.

3 Results

Our goal was to find a suitable way to study the phototaxis of microalgae and to use it for investigations with Chlamydomonas reinhardtii. As results of these investigations we decided to find out how the phototaxis behaves with regard to wavelength, contrast, cell concentration and the given speed of motion of the light source.

3.1 Technical design

We have invented an experimental setup that is able to trigger the phototaxis of microalgae in a contolled setting. This setup is able to output pixel by pixel light variable in color and intensity.

Our software makes it possible to calculate and display different animations. It is possible to create two objects in different shapes, colors and brightness. These can also be moved differently. It is also possible to create color and intensity gradients. Our optical, automated evaluation by image recognition makes it possible to carry out a large number of experiments with relatively little effort.

All components are built up in a system with housing, with outsourced computer technology, and with optical and thermal insulation to keep the test conditions as constant and stable as possible.

3.2 First biological results

In the case of Chlamydomonas reinhardtii, the intensity of phototaxis depends on the time of day. Before realizing that, we did many of our experiments in the evening. At that time we could find out only little about phototaxis. Especially on days when we started experimenting

earlier, we noticed that the later it got, the worse our experiments worked and the more unspectacular our results became. This is the typical performance of the Chlamys from the Faculty of Biological Sciences. There, the mechanism of biological circadian clocks is being investigated for Chlamys. This means that their behaviour (=circa) depends on the course of the day (= diem), or more precisely on the status of their internal circadian clock, which runs through a 24-hour cycle. This leads to the conclusion that comparable experiments should be carried out at the same time of day.

During one of our first attempts, a strange lump or hotspot formation of the algae occurred. We had placed a culture of about 1.000.000 algae in the just finished experimental setup and started to search and test suitable parameters for the software. However, the Chlamys did not swim after the object as expected, but grouped themselves into randomly arranged green spots on the entire surface, regardless of the lighter rectangle or the dark background. One hypothesis is that the algae, which are not yet used to this kind of light, would like to hide from it and do it behind their fellow species, because there is nothing else there. Thus small accumulations appear, which should turn upside down constantly, because the lower ones swim into the shadows of the others.

A further insight is that phototaxis does not only depend on the external circumstances, but also on the Chlamys themselves. Some strains of Chlamys show more phototaxis than others. Also how the algae have been shaken or bubbled previously seems to have an effect. Probably the behaviour depends even on the habits (for example to a certain light source) of the cultures.

If it is no longer necessary for the algae themselves to move over a longer period of time, for example because the culture is constantly stirred, the algae of this culture lose their ability to move actively after some time. Chlamys can adapt quite quickly. Thus different Chlamydomonas reinhardtii cultures, which are separated over a longer period of time (a few months are enough), also have different properties. For example, an American strain of Chlamys behaves substantially differently than, for example, Chlamys from the University of Göttingen. According to eyewitnesses, the American Chlamys are said to show much stronger positive phototaxis than the European Chlamys. However, we have not yet been able to prove this. Our American Chlamys do not yet show any phototaxis.

4 Discussion of results

The stimulation of the algae is done by an LCD-TFT display, as it is also used in entertainment electronics (for humans). Due to the way the LCDs work, this display is unable to show light of arbitrarily prescribed wavelength. Rather, each pixel is represented by three partial pixels, one with a red, one with a green and one with a blue color filter (filters that only allow a certain range of wavelengths to pass through). This means that it is not possible to let a desired wavelength pass through a pixel. It is only possible to control the intensity of each of the three wavelength ranges. This is a pity, because it restricts the possibilities of experiments. Nevertheless, with the three wavelength ranges red, green and blue it is possible to test the reaction of the algae to certain sufficiently different colours.

Moreover, it is also possible to mix these colours, but we do not know how this is noticed by the algae. In fact, the Chlamys with their size of 10 micrometers are so small compared to the 300 micrometer pixels that they are usually only above a partial pixel of the monitor and therefore only see one color. We suspect that it is thanks to the scattering of the light by the nutrient medium and the farsightedness of the Chlamys that it still works.

Furthermore, it should be noted that the LCD, even if it should not let pass any light, is not completely black. At least a some light remain visible.

Since our system is based on a camera, it can be used very flexibly. Compared to solutions based on LDRs (individual photo resistors), our setup has to process much larger data sets, but we can enjoy the advantage of extreme versatility. We can therefore not only calculate where the algae are by analyzing the distribution of brightness, but also recognise different colours and objects. This is especially useful when the structure is extended. For example, loads moved by the algae could be detected. It would also be possible to add small particles to the water whose colour differs from that of the algae. The recoil of the algae would then have to interact with these particles. Such particles could be monitored at the same time as the algae.

We are satisfied with our experimental setup for the time being. We have developed something that, to our knowledge, has never existed before and that can investigate the phototaxis of microalgae. We made sure that the device is easily expandable and partly modular. For example, the lower light source, the interior for inserting the algae or the camera unit can be exchanged and adapted to a given situation. Our device is therefore very flexible and allows versatile examinations in a comfortable way.

5 Conclusion

With our experimental setup we have created a (presumably) solid basis that allows to carry out experiments. Due to the flexibility of our device, it is easy to extend it for further experiments and to adapt it to further situations. So we intend to continue our research in this area. Our fascination for these small, actually mobile plants, has not been decreased, on the contrary.

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