Master Thesis

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15/01/2021

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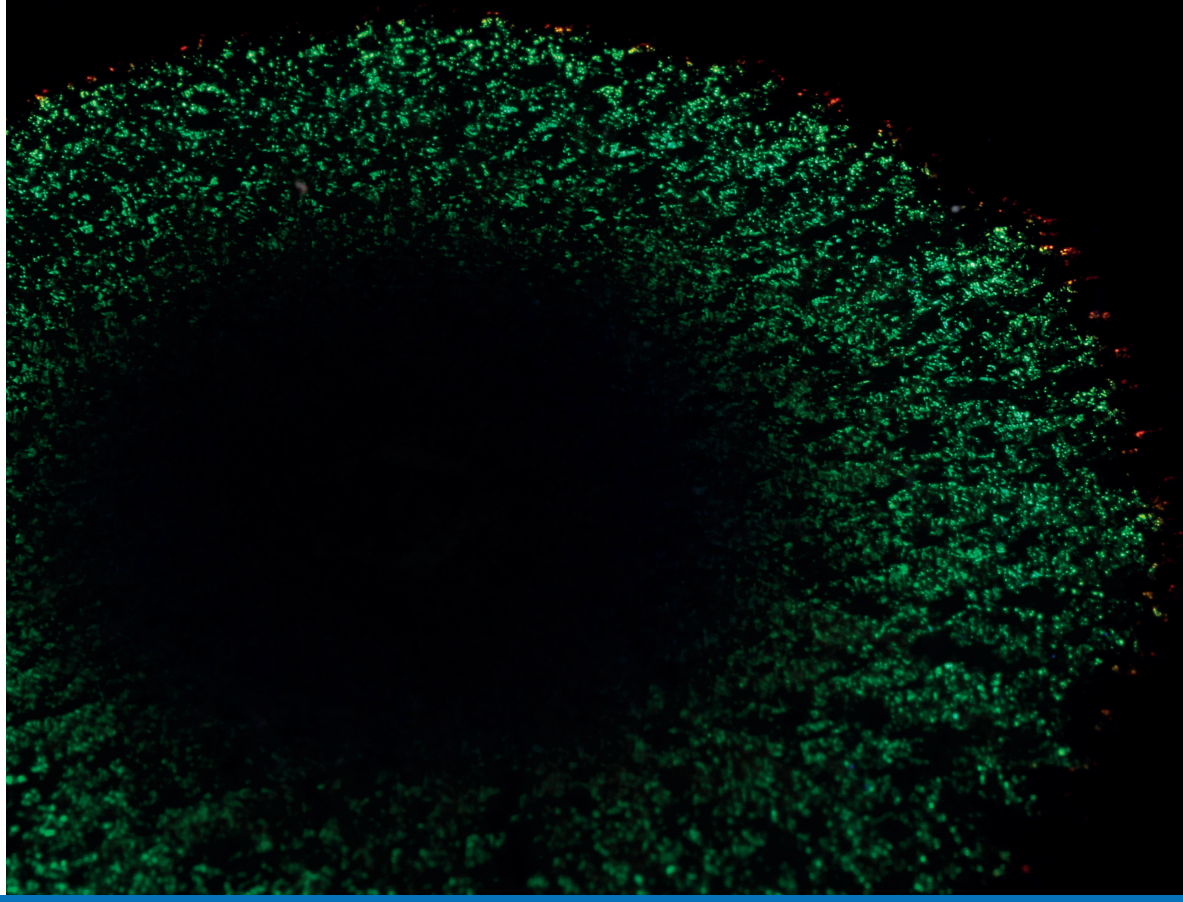

FLAVOBACTERIA'S STRUCTURAL COLOUR

Characterizing, Capturing and Communicating the Temporal and Iridescent Appearance of Flavobacteria

TU Delft

MSc. Integrated Product Design
Master Thesis of Clarice Risseeuw

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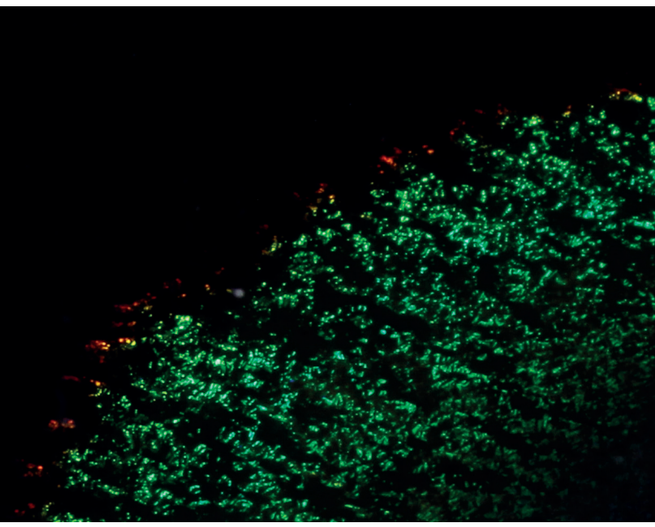
January 2021



PREFACE

Whenever I find myself wandering through nature, I often get frustrated by all the litter lying around. People consume more and more and the consumables often end up in the wrong places as trash. During my studies at the faculty Industrial Design Engineering, I sometimes felt a bit depressed by the idea of contributing to this problem by creating more and more. However, the more I learned about sustainable design strategies, the more I realised that you can decide as a designer not to contribute to this.

My interest and knowledge about sustainable design strategies increased during my studies and it felt like, this way, I could combine my passions for nature and design. Therefore, I was thrilled that I could start a bio design project as graduation project. Within this project, I learned to be more flexible as a project with a living microorganisms can be hard to steer. Besides, I truly enjoyed conducting research and experimenting with Flavobacteria. Even after 20 weeks, the dazzling appearance remains mind blowing. The fact that this project contributes to a more sustainable world makes it even better.

In particular I want to thank my supervisory team, Elvin Karana, Willemijn Elkhuizen and Hazal Erktürkan for keeping me challenged due to the refreshing feedback. Secondly, I want to thank Colin Ingham, Radi Hamidjaja and Ward Groutars for introducing me to Flavobacteria and supporting me with healthy bacteria. My appreciation also goes out to all the people who have supported me during this project by letting me use facilities and equipment and/or giving me advice. A final thanks to my family, friends and especially Stan, for all the love and support. Particularly during corona times, I could not have done this without you.

I hope you enjoy reading my master thesis!

ABSTRACT

Flavobacteria could become a sustainable alternative for the colouring industry by using them to grow colours on artefacts, as they are capable of creating dazzling structural colour. To contribute to this development, the temporal and iridescent appearance is characterized, captured and communicated within this graduation project.

The appearance of naturally grown Flavobacteria on marine growth medium is characterized regarding its size, iridescence and dominant colour. This is done by capturing colonies of Flavobacteria every 8 hours of their lives from different angles. It turns out the surface area grows quadratically; the colony appears the brightest when looking at the retroreflection from a height of 45˚ degrees; the colony reaches its green colour with red edges within 24 hours.

A setup is created to capture samples of Flavobacteria consistently and densely. To properly capture the temporal and iridescent appearance, the setup is automated and able to tilt and rotate the sample, as well as change the azimuth angle (i.e., the angle between the light and the camera).

To create straightforward visualizations that communicate Flavobacteria's unique appearance, an interactive webpage is created. This communication tool shows the captured sample and allows users to control the variables of the data (i.e., the tilt and rotation of the sample, the azimuth angle and the time). Besides communicating Flavobacteria's appearance to designers and artists, the communication tool can be used during experiments regarding influences on the appearance. These experiments need to be conducted in the future for designers to be able to steer the appearance to grow colours on artefacts to our own liking. To easily draw conclusions from these experiments, a second version of the communication tool is created, showing two samples side by side.

At the end of the project, design guidelines are formulated based on the characterization and experience of working with Flavobacteria, for designers that will integrate Flavobacteria's structural colour. Finally, further research is recommended regarding the definition of properties, the communication and perception of Flavobacteria and the search for potential application areas.

GLOSSARY

Agar	A jelly-like substance which is extracted from seaweed and used to create solid growth medium
Altitude angle	Angle in the vertical plane between the direction of the camera/light and the horizontal plane
Azimuth angle	Angle in the horizontal plane between the directions of the camera and light
Characterization	A description of the distinctive features of something
Genera	Category of biological classification that ranks between the family and the species
Illumination angle	Angle in the vertical plane between the direction of the light and the surface of the sample
Inoculation	The process of introducing microorganisms into a new substance
Iridescence	Phenomenon of a surface that gradually changes colour as the positions of the viewer or light changes
Isotropic materials	Materials of which the properties remain the same value when measuring in different directions
Structural colour	Colour as a result of light interacting with the micro- or nanostructure of a material
Symbiosis	Two different organisms living in close physical alliance while interacting to the advantage of both
Temporal	Time-related
Viewing angle	Angle in the vertical plane between the direction of the camera and the surface of the sample

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Project Overview

Within this first chapter, an overview of the project is given, starting with an introduction. The identified knowledge gaps are elaborated on, as well as the contribution of the project to these gaps. Throughout the project, the contribution to the knowledge gaps was slightly adapted, relative to the contribution defined in the project brief (appendix A), as this definition turned out to be too ambitious for the time available. Finally, the structure of the project is visualized.

1.1 | INTRODUCTION

Nature's light manipulation strategies—in particular those at the origin of bright iridescent colours —have fascinated humans for centuries. In recent decades, insights into the fundamental concepts and physics underlying biological light-matter interactions have enabled a cascade of attempts to copy nature's optical strategies in structurally coloured materials (McDougal, Miller, Singh, & Kolle, 2019). Structural colour is a result of the micro- or nanostructure, which interacts with light. Existing attempts to copy nature's optical strategies often result in materials that contribute to resource scarcity, have a large amount of invested energy and/or are not ideal for recyclability.

In the field of bio design, designers, artists and engineers are collaborating with living organisms to produce new materials with ecological benefits. Recently, we have seen a growing interest to explore alternative, more sustainable ways of living. The Flavobacteria turns out to be capable of creating structural colour and could serve as a sustainable alternative to petroleum-based pigments and dyes in the future.

To be able to design with new materials, it is important that the properties of these materials are well communicated between the designers, artists and engineers. Designing with living organisms additionally requires knowledge on their needs and temporal qualities (i.e., how they change over time). Because of the complex nature of the structural colour created by the Flavobacteria, its temporal properties are to be defined further as well as communicated better.

The aim of this project is to give people an initial idea on how the bacteria behave by characterizing the temporal and iridescent appearance.
This will lead to the first design guidelines of Flavobacteria's structural colour effects. Secondly, the project aims to provide a setup and tool which can be used to capture and communicate the appearance consistently. Last but not least, the project aims to spark interest of other designers by creating beautiful captured data of the temporal and iridescent appearance. This will stimulate further research and promote the idea of growing colour on artefacts.

1.2 | IDENTIFICATION OF KNOWLEDGE GAPS

To be able to contribute to the development of Flavobacteria as an alternative, sustainable way of colouring artefacts, first, the knowledge gaps were identified.

1

Properties

Although microbiologists have been experimenting with the Flavobacteria's structural colour, a lot remains unknown on the behaviour and resulting properties of the microorganism. In order to integrate Flavobacteria's colour in applications, more research is needed on:

- Influences on the material appearance
- Temporal qualities (i.e., how the appearance changes over time)
- Potential efficient fixation of the colour
- Possible substrates for the bacteria to grow on (e.g. textile)
- Mechanical properties
- Thermal properties

2

Communication

Currently, the angle-dependent colours created by the Flavobacteria are communicated through graphs and pictures. However, this medium lacks the communication of the temporal properties and might not be adequate for designers and artists as it is not comprehensible at first sight. Research is therefore needed in order to provide an efficient way of communicating the material appearance and hereby improve the communication between the designers, artists and engineers.

3

Perception

To articulate the material's unique role (in contrast to alternative materials) both the technical as the experiential characterization of a material is needed (Karana, Barati, Rognoli, & Zeeuw van der Laan, 2015). Researchers and scientists describe the colours of the Flavobacteria as vivid and glittering. Besides this, no research was found on how the material is perceived by people.

4

Application areas

The structural colour of the Flavobacteria is currently not yet integrated into applications. Since the material is novel, a search is needed for applications through which unique user experiences and meanings will be elicited.

1.3 | SCOPE/CONTRIBUTION

To contribute to the knowledge gap on the properties, a technical characterization study is conducted as described in the first step of the Material Driven Design (MDD) method by Karana et al. (2015). The appearance of Flavobacteria is characterized using the size, the iridescent properties and the colour of the colony as pillars.

To contribute to the knowledge gap on the communication, a setup and communication tool is designed to capture and communicate the temporal and iridescent appearance of the Flavobacteria. While conducting literature research on setups to specifically capture Flavobacteria, little was found. Most researchers used a setup with fixed light altitude of 60˚ or microscopy to capture the appearance. A setup which is able to capture more of Flavobacteria's temporal and iridescent appearance, is therefore expected to be a valuable contribution.

The communication tool can in the future also be used to conduct an experiential characterization study as described in the first step of the MDD method, which will contribute to the knowledge gap on perception.

A benchmarking analysis pointed out the unique aspects of Flavobacteria and the technical characterization lead to design guidelines for integrating Flavobacteria's colour. These could lead to insight on potential application areas in further research.

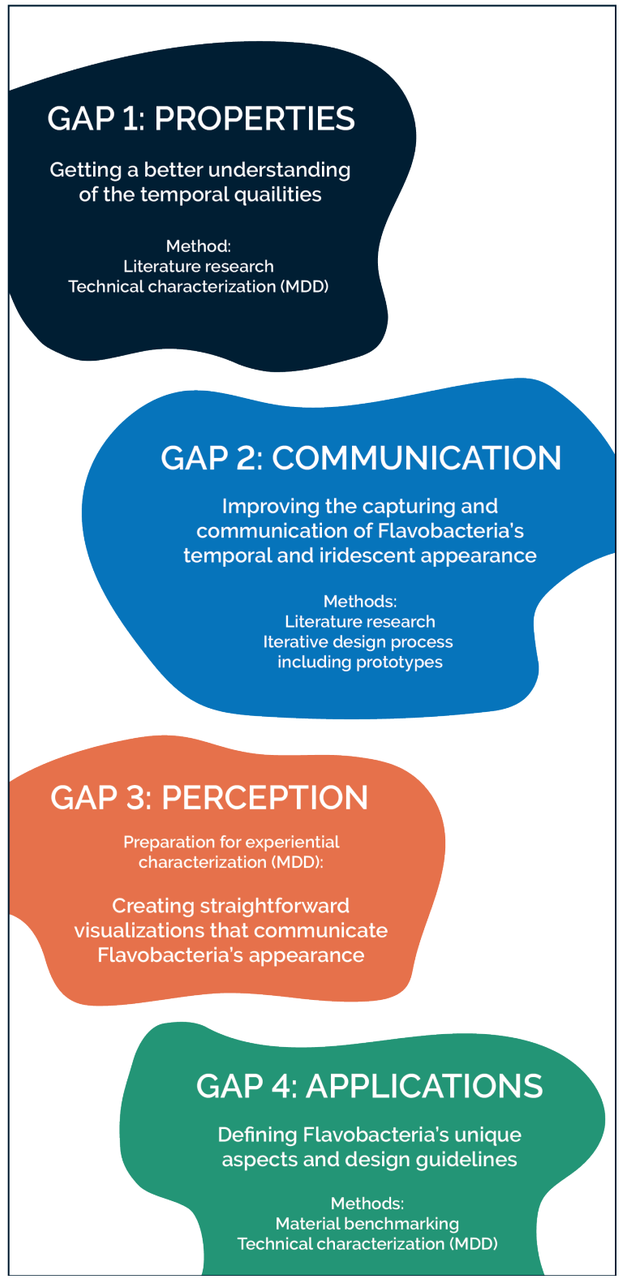
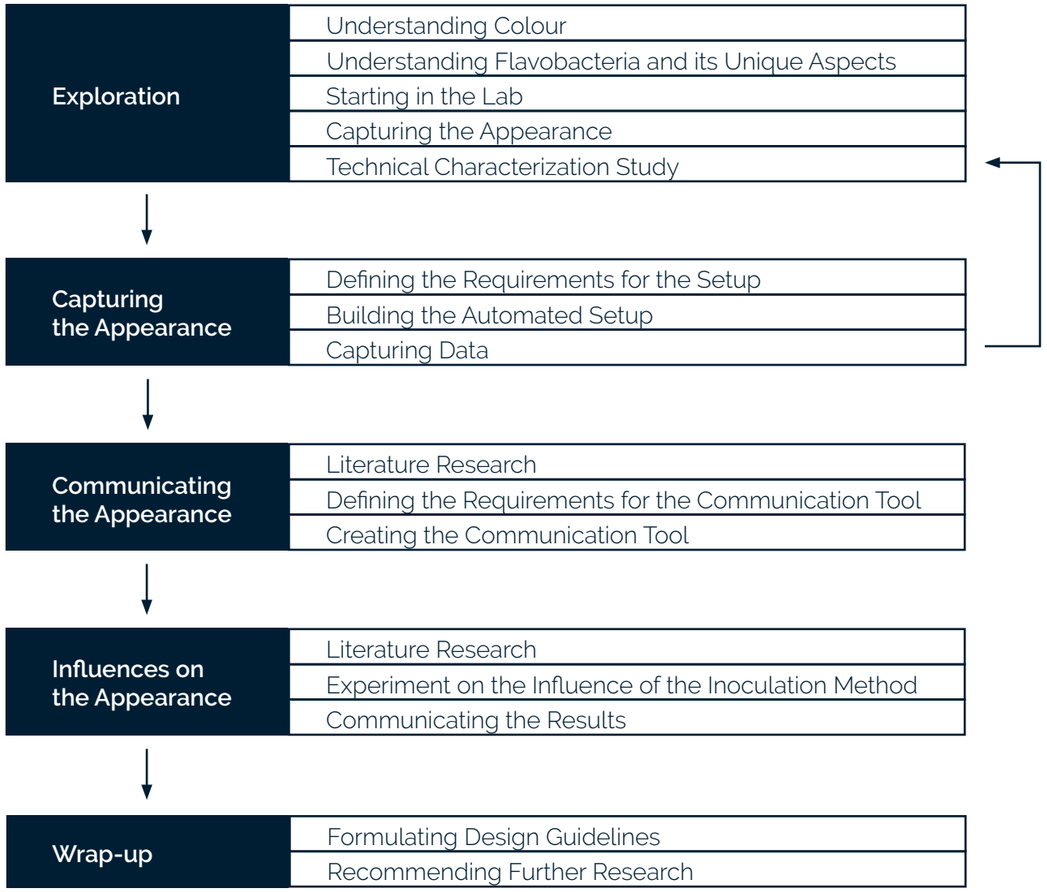


Fig. 1.1: The contribution of the project to the knowledge gaps

1.4 | STRUCTURE



2

Background Information

This chapter provides background information for the project. It includes information on colour, bio design related to colour and Flavobacteria, which is needed to understand the microorganism and its structural colour effects. The information was gathered by visiting Hoekmine B.V., a biotechnology company specialized in Flavobacteria, and conducting literature research and lab experiments.

2.1 | COLOUR

The perception of a colour depends on three factors: the object, the light source and the viewer. The appearance of colour is a result of light interacting with the object, which is perceived by the viewer. The colours that appear in nature are created using three different principles: colourants, bioluminescence and structural colour. Colourants include pigments and dyes, which are respectively insoluble and soluble in water, oil or other common solvents. Pigments particles are larger than molecules of dyes, making them insoluble but more resistant to UV light. Both pigments and dyes create the appearance of colour because they absorb certain wavelengths of visible light whilst others are reflected. Bioluminescence is light produced by a chemical reaction within a living organism. Unlike colourants and bioluminescence, structural colour is not based on biochemistry but created by the micro- or nanostructure of a material. The interaction between light and the structure causes optic effects on different scales, which create the unique, dazzling colouration effects. Another important advantage of structural colours is its resistance to fading, unlike colourants in which the absorbed photons transfer their energy to the electrons causing damage and fading over time (McGuire, Stephenson, & Xiao, 2014).

Structural colour found in nature is a result of thin- or multifilm interference, coherent or incoherent scattering, diffraction grating or 1D, 2D or 3D photonic crystals (Sun, Bhushan, & Tong, 2013). These mechanisms can be seen in figure 2.1. Some examples of the resulting effects can be seen in figure 2.2 on the next page.

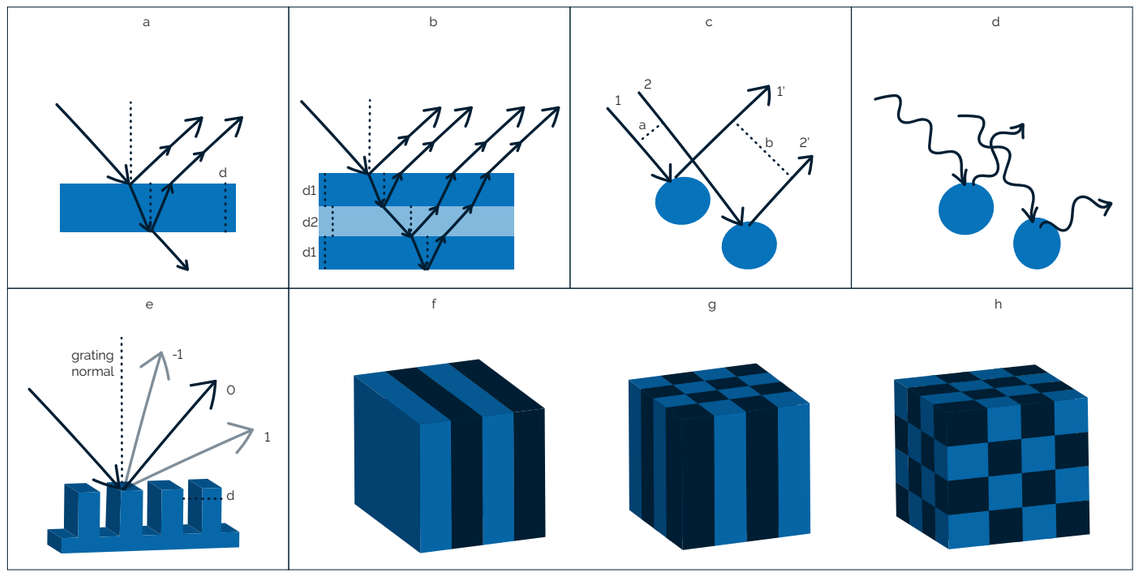


Fig. 2.1: Structural colour arise via (a) thin-film interference, (b) multifilm interference, (c) coherent scattering, (d) incoherent scattering, (e) diffraction grating and (f-h) 1D, 2D and 3D photonic crystals.

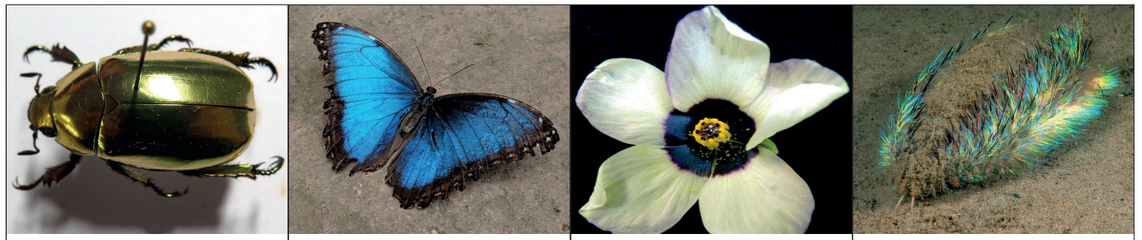


Fig. 2.2: Examples of structural colour in nature. From left to right: the beetle *Chrysina Resplendens*: multifilm interference; the wings of the *Morpho Peleides*: coherent scattering; the *Hibiscus Trionum*: diffraction grating; the sea mouse *Aphrodita Aculeata*: 2D and 3D photonic crystals.

Structural colours can be angle dependent and independent, caused by respectively the well-ordered or disordered nanostructure (McGuire et al., 2014). Angle dependent structural colours are iridescent. This means that the angle of incidence of both the eye and the light source changes the perceived colour. Nevertheless, it is sometimes difficult to distinguish between iridescent and non-iridescent colours, since structural colours often involve multiple scales of organization (Sun et al., 2013). The spines of the sea mouse are an example of high angle dependent structural colour (fig. 2.2). The feathers of the bird Cotinga Maynana (fig. 2.3) reveal a low angle dependent blue structural colour, although they are described as non-iridencent (Takeoka, 2012).

Some living organisms use their structural colour functionally by adjusting their micro- or nanostructure, and thus their colour, for instance for camouflage or reproductive behaviour (Sun et al., 2013). The chameleon is a well-known example that can change their colour for camouflage due to structural adjustments (fig 2.4).

Fig. 2.3 [top]: The low angle dependent structural colour of the Cotinga Maynana (Uribe, 2012)
Fig. 2.4 [left]: The adjustable structural colour of a chameleon

For many millennia, humans have been implementing colours in their lives. Pigments are for instance used to make paint by granulating pigments to powder and thoroughly mixing them with a liquid, called dispersing agent. Dyes are used for colouring substances such as textile and paper. Until the nineteenth century, all colourants were of natural origin. The world's first synthetic colourant, serendipitously discovered by William Henry Perkin in 1856, was called aniline purple (Hicks, 2017). Today, chemists arrange and manipulate complex organic compounds to make all kinds of synthetical colourants. Both the natural as the synthetic colourants industry can be harmful for the environment. These harmful effects include material derivation from scarce resources, polluting emissions during manufacturing, high energy consumption, creation of waste and enormous water consumption and pollution (Lellis, Fávaro-Polonio, Pamphile, & Polonio, 2019; Porwal, 2015). In fact, the wastewater from textile plants is known to be the most polluting of all the industrial sectors, considering the volume as well as its composition (Drumond Chequer et al., 2013).

In the recent decades humans have made many efforts to copy nature's non-fading synthetic structurally coloured materials. However, we tend to create these materials via methods that have little in common with the processes used by biology (McDougal, Miller, Singh, & Kolle, 2019). To reproduce the Morpho-butterfly quasi-structure, Watanabe, Hoshino, Kanda, Haruyama and Matsui (2005) for instance used ion beam chemical vapor deposition (FIB-CVD), a tool in fabricating 3D micro- and nanostructure (fig. 2.5). McDougal et al. (2019) stated that although human-made materials often exceed the biological structurally coloured materials with regards to application-specific performance characteristics, biological materials possess some significant advantages. Especially regarding sustainability the biological optical materials sweep the board. They can be formed close to room temperature and rely exclusively on biocompatible materials and sustainable chemical processes, unlike many synthetically created replica's. Therefore, biological structural colour has great potential as a high-performance, sustainable colour in the future.

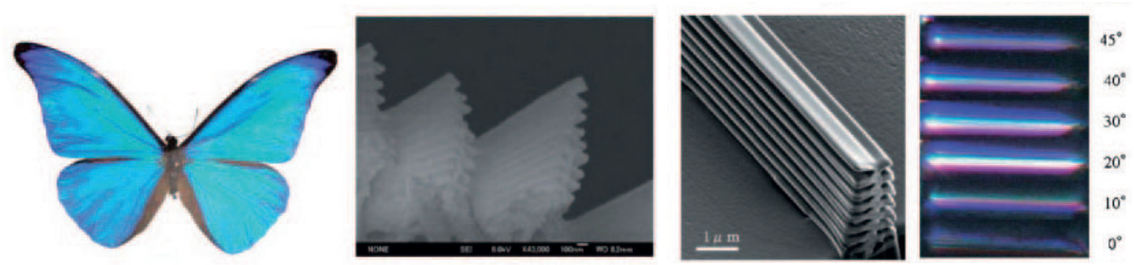


Fig. 2.5: Morpho-butterfly quasi-structure and replica (Watanabe et al., 2005). From left to right: microscope image of Morpho-butterfly; Morpho-butterfly structure; Morpho-butterfly structure fabricated by FIB-CVD; microscope image of replica observed with a 0 to 45° incidence angle of white light.

2.2 | BIO DESIGN RELATED TO COLOUR

Bio design is an emerging and radical approach that can lead to new possibilities for design, art and architecture (Myers, 2012). This approach builds upon bio fabrication, in which complex biological products are produced from living cells or biomaterials. Within the field of bio design, scientists, artists, and designers integrate biological processes and materials into the creation of buildings, everyday artefacts, fashion, etcetera. By incorporating living organisms, such as algae, fungi and bacteria, into the design process, bio design goes further than bio-inspired approaches in which nature's designs are simply imitated.

Fig. 2.6 [top]: The microbial billboard at day 2 (Lowe Roche, 2012)
Fig. 2.7 [bottom]: The microbial billboard at day 4 (Lowe Roche, 2012)

An example of a bio design is the microbial billboard designed by CURB media, ad agency Lowe Roche, microbiologists and immunologists to publicize Steven Soderburgh's 2011 film Contagion (Meyers, 2012). The team inoculated two giant petri dishes with a mix of multi-coloured bacterial and fungal strains to create a dynamic billboard revealing the logo of the film (fig. 2.6-2.7). In this project the microorganisms were kept alive to create a new design expression in an unpredictable way.

Bio design leads to novel aesthetic expressions, biophilic designs (i.e., designs that (re)connect people to nature) and new materiality for art and design. Technological and economic opportunities alongside its ecological benefits, point to bio design as a new industrial paradigm for the production of artefacts in 21st century (Karana, 2020).

In the recent decade, more creations of colour within the field of bio design and fabrication like the microbial billboard, have been developed. To create an overview of these developments and get a better understanding of the design domain, a benchmarking analysis was performed. The projects selected for this benchmarking analysis (described in appendix B), involve a novel and unique way of creating colour by collaborating with bacteria, algae and/or fungi.

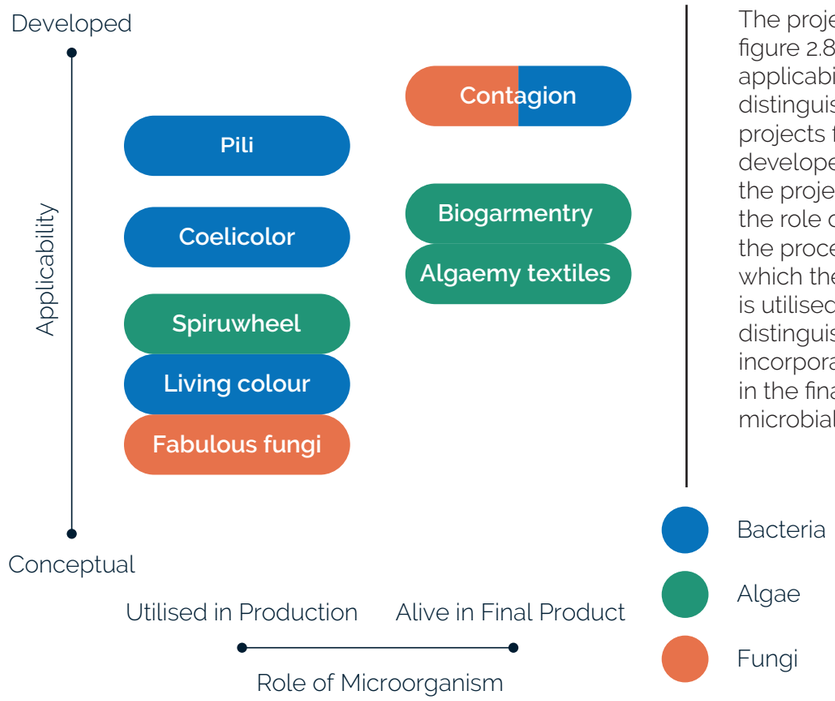


Fig. 2.8: The benchmarking analysis

The projects, as visualized in figure 2.8, are arranged by their applicability along the vertical axis, distinguishing the more conceptual projects from those that are further developed. On the horizontal axis, the projects are divided based on the role of the microorganism in the process. Here, the projects in which the microorganism's growth is utilised in the production are distinguished from those that incorporate living microorganisms in the final product, such as the microbial billboard.

Fig. 2.9: Coelicolor (*Project Coelicolor*, 2018)

The project Coelicolor (fig. 2.9) of the Faber Futures LAB in collaboration with the University College London's Department of Biochemical Engineering, is an example that utilises the microorganism's growth in the production of textile by collaborating with a bacteria that produces a colourant.

The project Biogarmentry (fig. 2.10) of Roya Aghighi is an example that really incorporates the biophilic attempt to connect to nature by creating textiles with living algae that require care and attention of the owner.

The unique aspect of Flavobacteria compared to these bio design projects is that it creates angle-dependent structural colour, known for its dazzling, non-fading colouration effects. Therefore Flavobacteria has great potential for bio design projects in the future. But first, more knowledge is needed to understand the behaviour and the structural colour effects of this bacteria. Besides, before living Flavobacteria could be incorporated in products, design guidelines should be carefully formulated that focus on the needs and care of the organism. Or, to use the created colour in the production process, fixation methods that maintain the structure created by the bacteria should be developed.

Fig. 2.10: The biogarmentry project which uses living algae that purify the air (Aghighi, 2019)

2.3 | FLAVOBACTERIA

Structural Colour Effects

The family Flavobacteriaceae contains at least 90 genera and hundreds of species (McBride, 2014). Members of the family are yellow and orange pigmented and often create iridescent structural colour. In this report, "Flavobacteria" will refer to the iridescent structurally coloured family members. The Flavobacteria are abundant in the natural environment and can for example be found in the harbour of Rotterdam (Johansen et al., 2018). Flavobacteria are gliding, rod-shaped marine bacteria of which the colony biofilms display angle dependent structural colour (fig. 2.11). The green iridescence appearance is dominant but yellow, orange, red, and violet "pointillistic" iridescences are easily observed at the colony edges (Kientz et al., 2016).

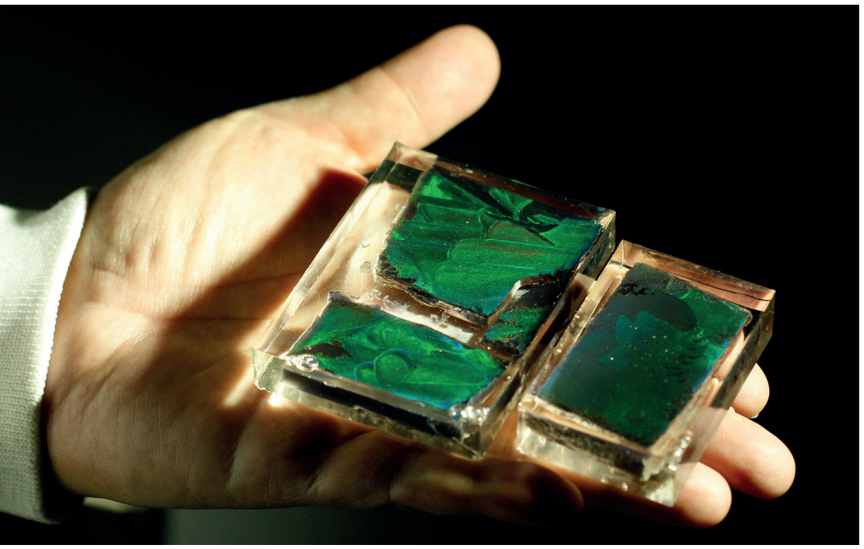


Fig. 2.11: Spread structural colour of Flavobacteria in epoxy resin (Hoekmine B.V., 2020a)

Fig. 2.12: Electron microscopy showing the highly ordered cells packed in a photonic crystal (Hoekmine B.V., 2020b)

At Hoekmine B.V., a biotechnology company located at the iLAB within the Hogeschool Utrecht, microbiologists have been experimenting with the Flavobacteria's structural colour for over 10 years. The colonies of the Flavobacteria reflect light as a 2D photonic crystals. This means the bacteria cells are forming 2D ordered, sub-wavelength lattices (fig. 2.12) that effect the propagation of light in a similar fashion as atomic crystals control electrons (Sun et al., 2013). The interference of reflections and diffraction of photons create the optical phenomenon. Accordingly, the structural colour and glitter-like iridescence is a result of the highly ordered spatial organizations formed by the bacteria cells, which interact with light.

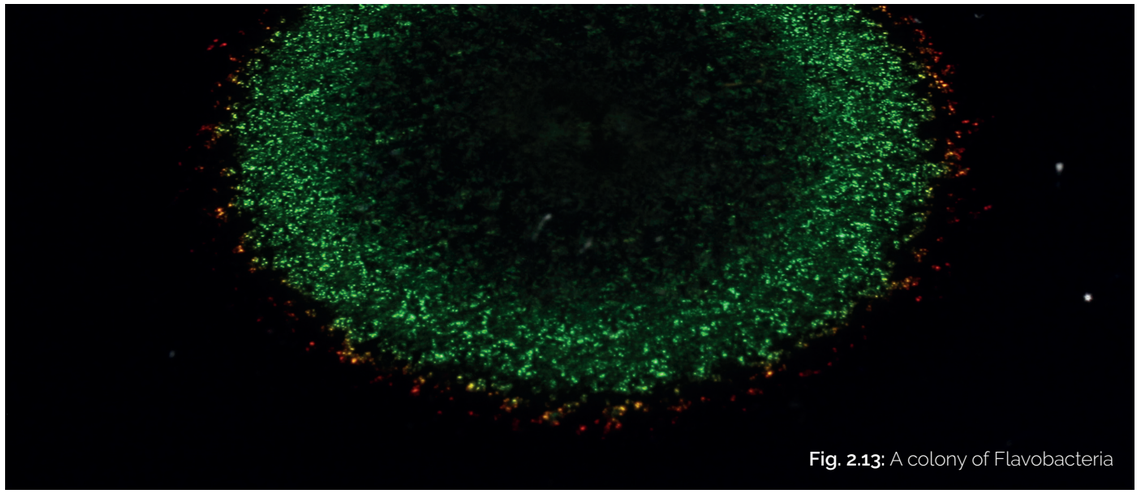


Fig. 2.13: A colony of Flavobacteria

Johansen et al. (2018) found that genes control the organization of bacteria cells and thus, the appearance. This means that genetic modifications can alter the material appearance of the bacteria. The microbiologists of Hoekmine B.V. also experiment with this and have some mutants and genetically engineered strains in their extensive collection of structural coloured microorganisms. This project will however focus on the non-modified Flavobacteria strains, or more accurately on the brightly iridescent Cellulophaga Lytica (PlyA2) strain (fig. 2.13). This strain was isolated from marine algae off the coast of Plymouth by Colin Ingham in 2018.

Scientists have speculated on various functions of the structural colour, including mechanisms to protect the microorganism from extreme levels of irradiance. Very few species were found to be structurally coloured in deep (>20 m) water, suggesting that structural colour may function to provide protection against high levels of solar radiation in shallow subtidal areas or intertidal rock pools (Chandler, Wilts, Brodie, & Vignolini, 2017). Kientz, Ducret, et al. (2012) linked the presence of bacterial iridescence to the gliding mobility, which is the form of movement used by bacteria. Hamidjaja, Capoulade, Catón and Ingham (2020) have recently found that the Flavobacteria strain IR1 is a predator of an unrelated bacterium, Enterobacter Cloacae B12. The observations in this study suggest that the organization required to create structural colour has a biological function: facilitating predation. This work is the first experimental evidence supporting a role for this widespread type of cell organization in the Flavobacteria.

When cells of Flavobacteria die, the created structural colour disappears. Therefore Hoekmine B.V. is also experimenting with fixation methods that will kill the bacteria while leaving the created structure undamaged. However, up until now, no sustainable fixation method is found.

Flavobacteria can potentially be used as future biomaterial since it only requires living cells and a suitable surface and has rapidly self-organizing capabilities (Johansen et al., 2018). The colour is biocompatible and biodegradable and would be a disruptive, sustainable innovation for the colouring industry. On top of using the Flavobacteria to grow low cost, biodegradable materials, Johansen et al. (2018) envisions viable pathways for engineering bacteria toward living sensors with intrinsic self-healing capabilities. The Flavobacteria could be fine-tuned for changing colouration under external stimuli with genetic modification and for instance detect specific chemicals.

Temporal Qualities

Flavobacteria are living micro-organisms, which rapidly expand at the edge of the colony (fig. 2.14) by creating new bacteria cells. To give an initial idea, Flavobacteria can approximately last ten days in a petri dish of 94mm at room temperature. The appearance of the bacteria cells changes over time. Temporal qualities refers to this changing appearance. Kientz, Marié and Rosenfeld (2012) briefly characterized the temporal qualities of the Cellulophaga lytica, in a research letter.

> *"Violet, red and yellow first observed. The dominant green iridescence with red edges appeared after 12h of growth. At later stages of growth the inner parts of the colonies contained few blue glitters and were losing the bright effect."*

In this research letter, the changes in the colony's colour over time were linked to cell density.

The temporal qualities are influenced by many factors, such as the amount of nutrients, the moisture level of the habitat and the storage temperature (as discussed in chapter 7).

Because of these temporal qualities, time is an important variable for capturing and communicating the appearance of Flavobacteria.

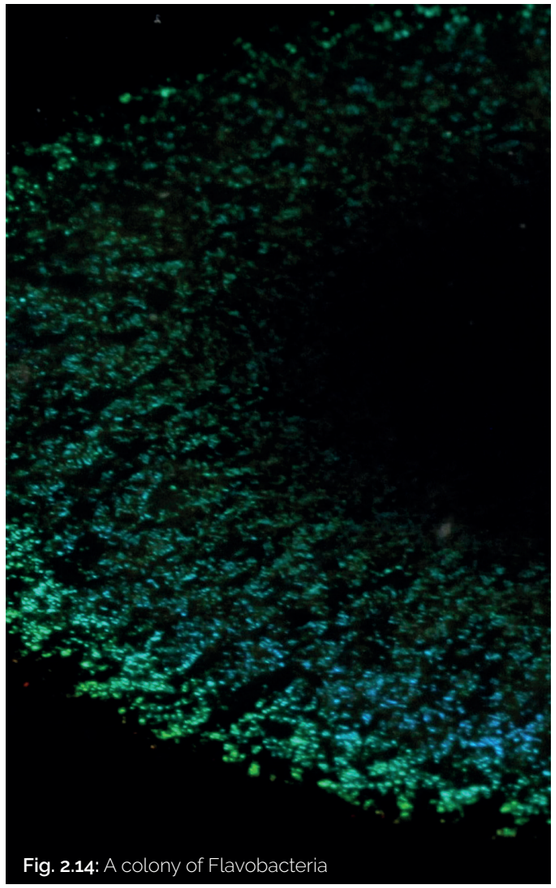
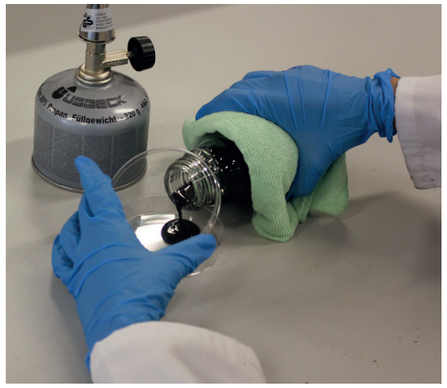


Fig. 2.14: A colony of Flavobacteria

Growing Flavobacteria

A protocol was written in order for designers and artists to understand how to work with Flavobacteria in the lab (fig. 2.15-2.23). The entire protocol and a list of the equipment can be found respectively in appendix C and D.

Bacteria are provided with nutrients and a place to grow by growth medium, a solid or liquid substance. Growth medium can be made solid by adding agar, which is extracted from seaweed. Agar allows the growth medium to be poured into petri dishes and hold it shape once it is set (polymerized). This is needed in this case, to analyse and capture the structural colour. Also nigrosine, a pigment, is added to make the growth medium black in order to see and analyse the colour better. For the Flavobacteria two different types of marine growth media are used: called MAR and MAR+. Both contain salts to provide the desired salinity, peptone and yeast as nutrients, and water. The MAR growth medium is used for the stock at room temperature. The MAR+ contains 10 times more yeast to enable faster growth and is used for analyses of the appearance.



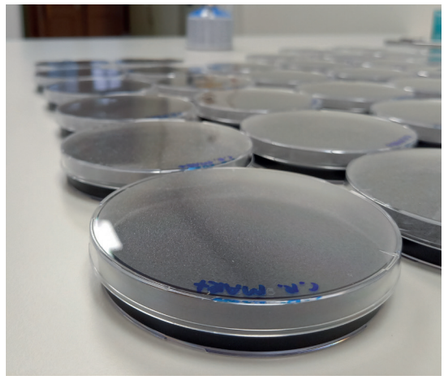
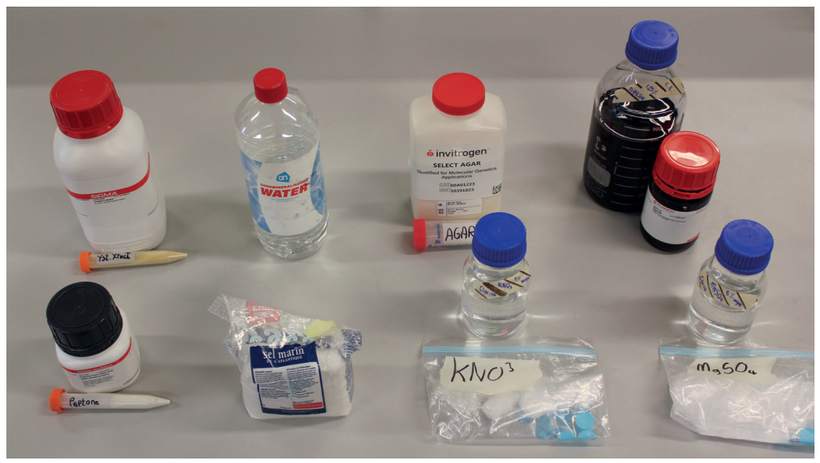
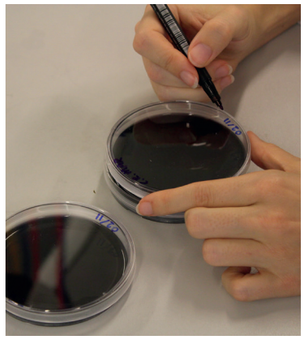


Fig. 2.15 [left]: Lab outfit
Fig. 2.16 [top]: Pouring the growth medium
Fig. 2.17 [mid]: Polymerization of growth medium
Fig. 2.18 [bottom]: Ingredients for growth medium





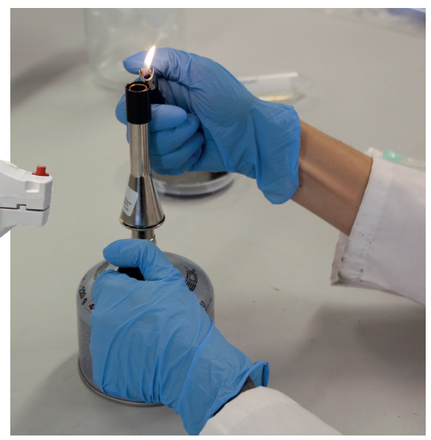


Fig. 2.19 [top]:
Cultivating bacteria
Fig. 2.20 [mid left]:
Marking petri dishes
Fig. 2.21 [mid right]:
Freezer stock
Fig. 2.22 [bottom left]:
Autoclave
Fig. 2.23 [bottom right]:
Bunsen burner

Flavobacteria can be cultivated on this solid growth medium by applying bacteria from the stock to the petri dishes and letting them grow at room temperature. The creation of structural colour is not influenced by light exposure and plate orientation during the growth (Kientz, Marié, & Rosenfeld, 2012). Optionally, Flavobacteria can be spread into patterns after 24 hours of growth.

Flavobacteria can be stored in the freezer and will stay healthy for many years in -80°C and for at least 2 months in -20°C. To make a freezer stock, the bacteria are dissolved in a solution of liquid MAR+ growth medium and glycerol. Glycerol is added to avoid water crystals, which are too sharp and can damage the bacteria cells.

A sterile environment is important while working with microorganisms. Such can be established by using a laminar airflow cabinet or working close to a Bunsen burner with a blue flame, which creates an updraft that pushes dust and microbes away. The future habitat of the Flavobacteria, the petri dish containing growth medium, should also be sterilized. This is realised by autoclaving the growth medium before pouring it into the petri dishes.

3

First Explorations

This chapter includes the first explorations in the lab and within the field of capturing material appearances.

Flavobacteria was new in the materials lab of the faculty Industrial Design Engineering of the Technical University in Delft. Therefore, some preparation experiments were conducted regarding the work environment and storage methods. Besides these experiments, learnings from the initial failures in the lab are discussed in this chapter.

The field of capturing materials appearances was explored by conducting literature research on lights and existing setups and by running tests regarding polarizers and optical mixing with an initial setup.

3.1 | STARTING IN THE LAB

Preparation of the Lab

During the first weeks in the lab, a feel was created for working sterile and for the behaviour and lifetimes of the bacteria. This was done by cultivating and observing Flavobacteria.

Flavobacteria can be stored for many years in a -80˚C freezer. However, the materials lab of the faculty Industrial Design Engineering is not equipped with this freezer. By conducting an experiment (appendix E), the -20˚C freezer proved to be capable of storing healthy Flavobacteria for at least two months.

Since a laminar airflow cabinet was not available in the materials lab of the faculty Industrial Design Engineering, a Bunsen burner was used to create a sterile work environment.

A pressure cooker was used to sterilize the growth medium in the materials lab of the faculty Industrial Design Engineering. Because only the small bottles of 200 ml fit, larger amounts of growth medium were autoclaved and poured into petri dishes at the lab in Science Centre.

Learnings from the Initial Failures

As working in the materials lab was a new experience, failures were inevitable. The failures included:

- Contamination (fig. 3.1)

  Possibly due to hanging over petri dish while pouring the growth medium or opening the petri dish too far from the Bunsen burner

- Too much condensation in the petri dishes which ruined the colonies (fig. 3.2 on next page)

  Possibly due to not turning the petri dishes upside down when polymerized or due to immediately closing the lid after pouring very hot growth medium

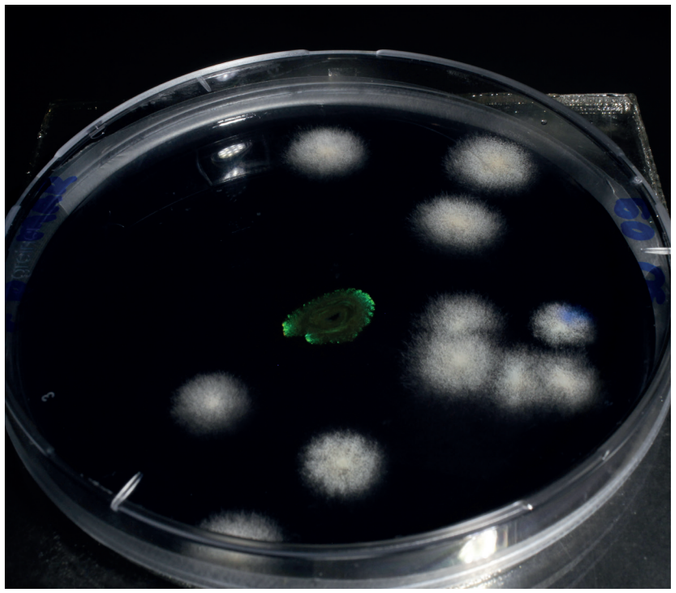


Fig. 3.1: Contamination

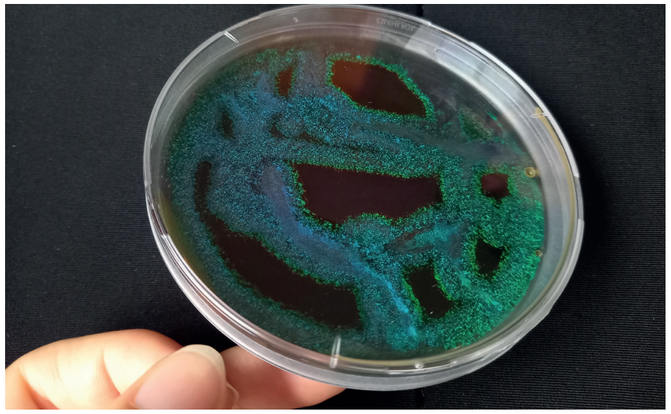


Fig. 3.2: Ruined colony by too much condensation

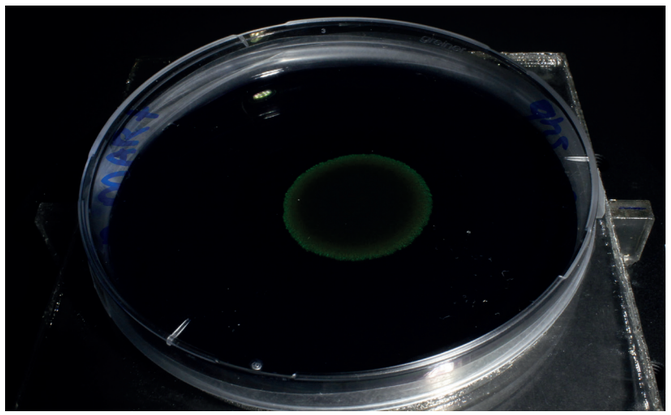


Fig. 3.3: Dried colony due to ripped parafilm

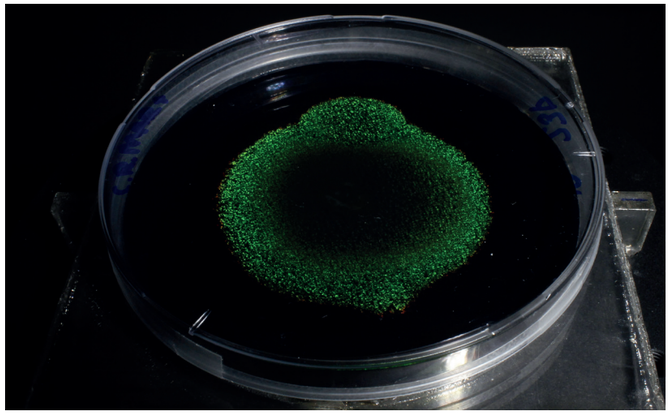


Fig. 3.4: Colony that does not grow in a circle

- Dried, dying colonies due to ripped parafilm by the sharp sample holder of the initial capture setup (fig. 3.3)

- Colonies that do not grow in a circle (fig. 3.4)

  Possibly due to not have properly shaken the growth medium before and after autoclaving it

These initial failures resulted in the following learnings:

- The petri dish should be opened with extreme care; close to a Bunsen burner and without hanging over it.
- The parafilm should be handled with care to avoid ripping.
- The protocol (appendix C) should be followed accurately. This will make sure that the growth medium is properly mixed and that the petri dishes do not contain too much condensation.

In general, one should work with extreme care and focus in order to avoid failures in the lab.

3.2 | CAPTURING

Lights

There is only a small part of the electromagnetic radiation spectrum that is visible to the human eye (fig. 3.5), which we call the visible light. In wavelength it ranges from approximately 400 nm (violet) to 700 nm (red.) To make sure all the structural colour effects of the Flavobacteria that are visible to the human eye are revealed and captured, it is important to use a light that emits all wavelengths of the visible spectrum. Therefore the spectra of different lights were measured using a spectroradiometer. During the first explorations, the spectra of two halogen lights differing in colour temperature and a 6500K LED were measured. All of the lights turned out to emit all wavelengths of the visible spectrum. However, the halogen lights also emit a lot of infrared which might affect the bacteria during long exposures. Therefore, a LED is preferred to illuminate Flavobacteria.

The perceived appearance of the Flavobacteria strongly depends on the position of the light. Therefore, a directional light is preferred to capture the Flavobacteria's unique, iridescent properties.

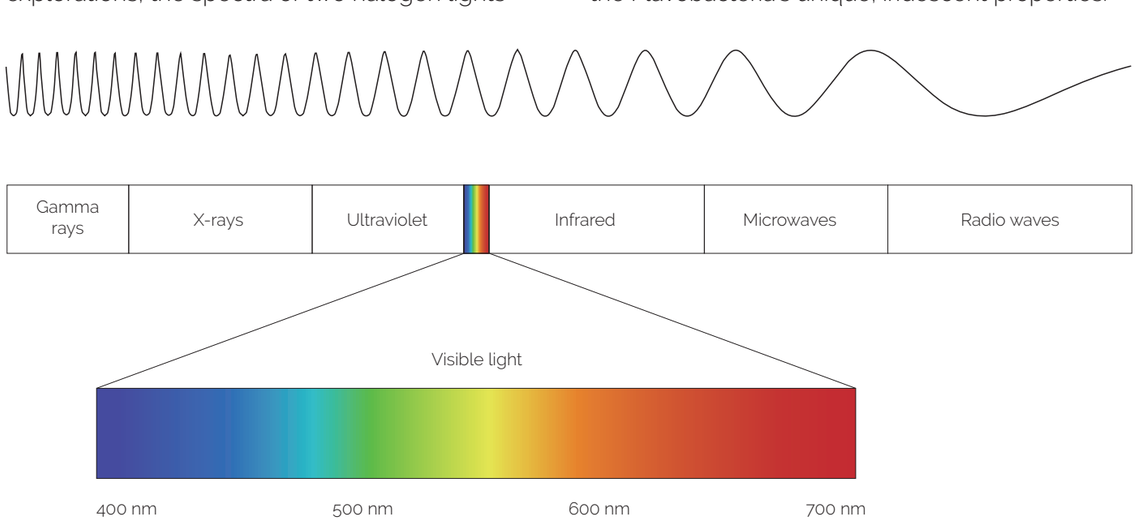


Fig. 3.5: The visible spectrum

Existing Setups

In the recent decades, many methods for capturing and rendering material appearances were developed for different types of materials. The first step of these methods involves acquisition of data: capturing the material appearance.

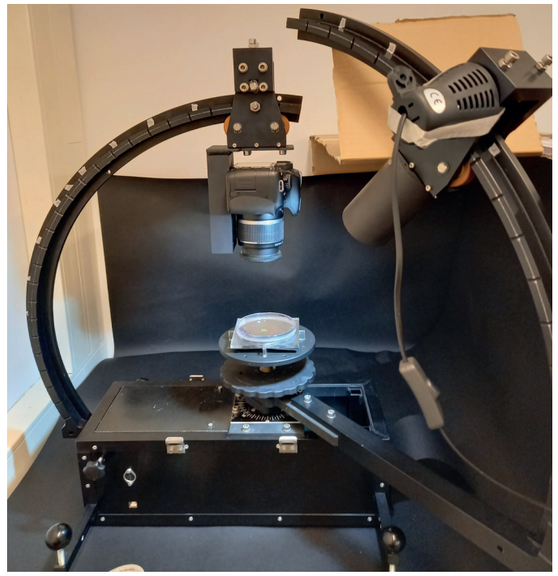


Fig. 3.6: The camera and LED on a gonioreflectormeter

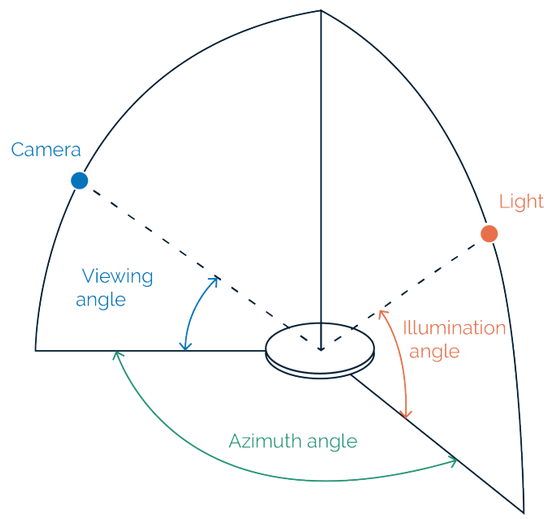


Fig. 3.7: The adjustable angles of the setup

The most densely approach to capture the material appearance is by using gonioreflectormeters or domes of lights and cameras. A gonioreflectormeter is a device for measuring the reflectance of materials and consists out of a light source and a sensor that captures light reflected from the material. Obviously, the variation in angles during the capture process can be varied endlessly, but make the acquisition more time-consuming. Recently, many approaches were developed for a more efficient acquisition using algorithms which estimates either the geometry, the reflectance or the illumination during the rendering (Dana, 2016). However, in order to develop an appropriate, more efficient capture method, the micro-structure and light interaction of the material should be thoroughly understood (Guarnera, Guarnera, Ghosh, Denk, & Glencross, 2016). Therefore, a setup is needed which can densely capture Flavobacteria's appearance to contribute to the understanding of the microorganism's light interaction.

The Initial Setup

An initial setup was created to explore how to capture the temporal and iridescent appearance of Flavobacteria the best way. This setup was created by mounting a Canon camera and LED to a gonioreflectormeter (fig. 3.6 and appendix F). The altitude angle of the camera and light are adjustable, as well as the azimuth angle between the camera and light. As the sample remains in a flat position within this initial setup, the altitude angles of the camera and light (i.e., the angles relative to the surface) are respectively identical with the viewing and illumination angle (i.e., the angles relative to the sample). Fig. 3.7 shows the viewing, illumination and azimuth angle and will be used in the following chapters to visualize the settings of the setup.

Polarizers

Polarizers were added to the gonioreflectormeter setup to try to get rid of the reflection in the petri dish. The polarizers turned out to get rid of the structural colour effects as well (fig. 3.8). Thus it can be concluded that they cannot be used to eliminate the petri dish reflection.

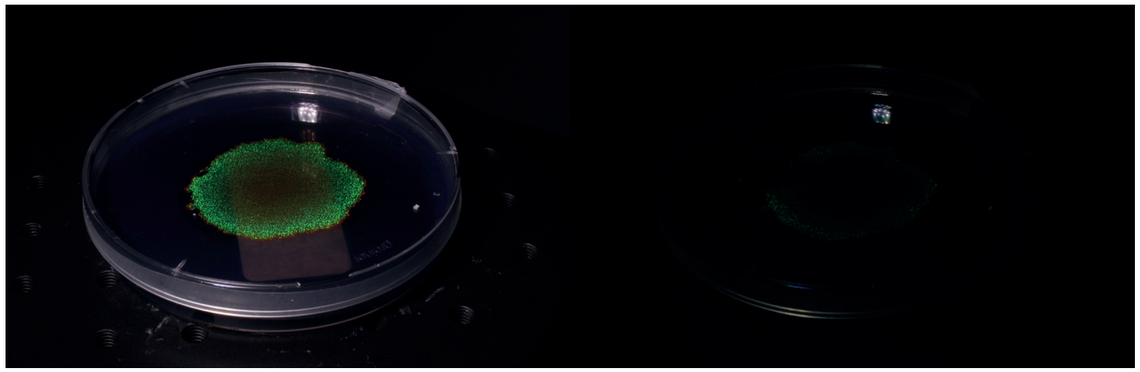


Fig. 3.8: The colourful sample without (left) and with (right) polarizers

Optical Mixing

Optical mixing is used to efficiently capture textures illuminated from different angles. This convincing technique uses only eight pictures with different azimuth angles and blends the pictures to visualize the in-between results. An experiment was conducted to find out whether optical mixing also works for the structural colour of Flavobacteria.

Eight pictures, thus skipping 45˚ of the azimuth angle, turned out to be insufficient for the optical mixing of the Flavobacteria (appendix G). However, optical mixing with a total of 12 pictures, thus skipping only 30˚ of the azimuth angle, did have a convincing result. This was concluded from picture B and a blended image of picture A and C (fig, 3.9), which looked almost exactly identical. The pictures A, B and C and the blended image can be seen in fig. 3.10-3.12 on the next page.

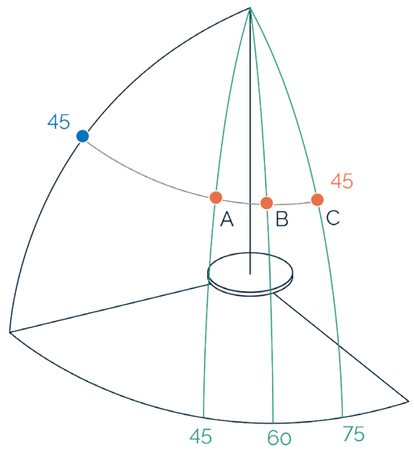


Fig. 3.9: The settings of the setup

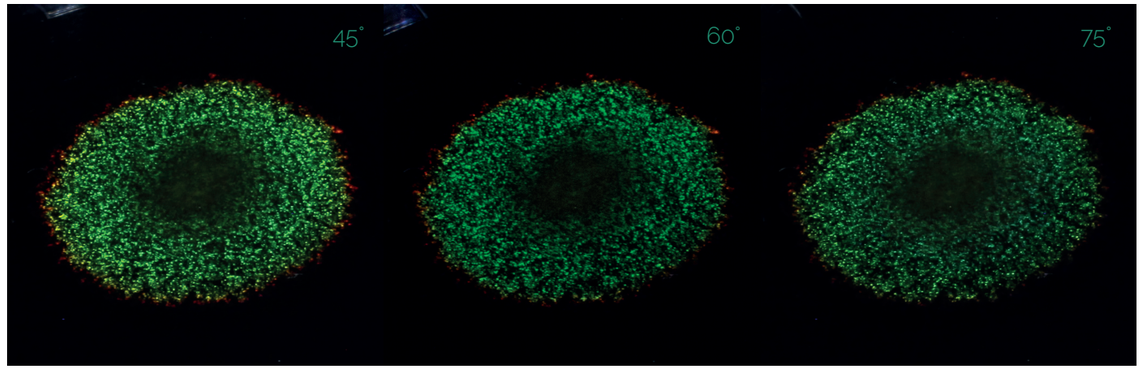


Fig. 3.10: The pictures A, B and C with an azimuth angle of respectively 45˚, 60˚ and 75˚.

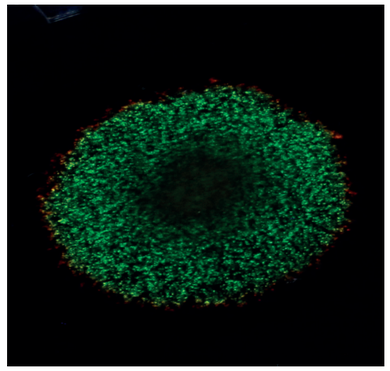


Fig. 3.11: The blended image of A and C

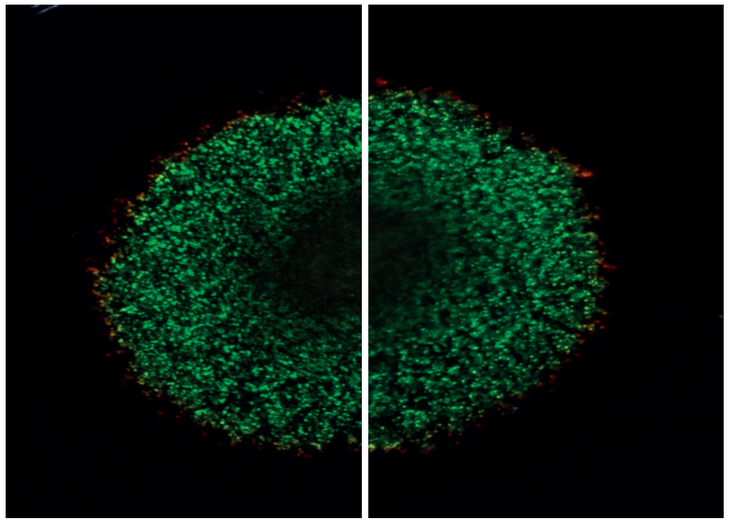


Fig. 3.12: The optical mixing result while skipping 30 degrees: picture B (left) and the blended A and C (right)

This exploration suggests that optical mixing can be used to visualize the effect of the azimuth angle on the colony's colours. To properly create the in-between results, Flavobacteria has to be captured with a maximum step size of 30˚ in azimuth angle.

4

Technical Characterization

In this chapter the technical characterization study is discussed. This study aims to characterize the appearance of Flavobacteria's standard growth by exploring the change over time regarding the size, influence of the positions of the camera and light (i.e., the iridescence) and colour. Standard growth is defined as the growth of the bacterial colonies which are cultivated from a room temperature stock on MAR+ petri dishes and not spread, following the protocol as described in appendix C. During the study, several samples of Flavobacteria were captured every 8 hours of their lives with different view and light directions and analysed. To avoid working during the nights and weekends, four batches of samples were created.

The characterization can be used as control study when exploring potential influences on the bacteria's appearance. Besides, the understanding of the bacteria is needed for designers and artists to articulate its unique role when applied in products.

4.1 | FLAVOBACTERIA'S SIZE OVER TIME

To characterize the size of the bacteria's standard growth, the diameters of the colonies were measured and plotted against the age of the colonies. By measuring the diameters of the colony, the overall shape and thickness of the colony is not taken into account.

Protocol

Several samples of Flavobacteria were captured every 8 hours of their lives using a setup with a camera and light mounted to a gonioreflectormeter (fig. 3.6 on page 26). The Canon eos 550d camera was set to an aperture of f22, a shutter speed of ¼ seconds and iso-400. In order to ease the distinguishing of the structural colour, the pictures were taken from a viewing angle of 60˚ and straighten in Photoshop based on the square of the sample holder (fig. 4.1). Afterwards, the diameter of the colony was determined using a size template (fig. 4.2). Since not all the colonies were perfect circles or in the middle of the petri dish, the diameter was defined by taking the average of four sides.

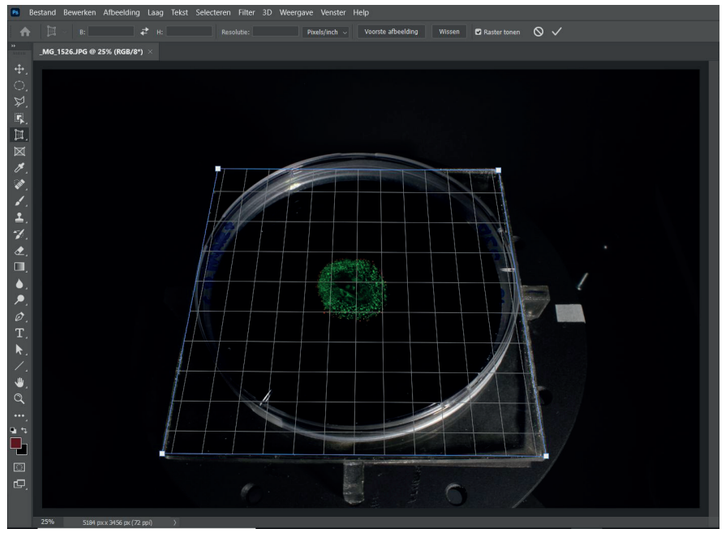


Fig. 4.1: The straightening of the pictures

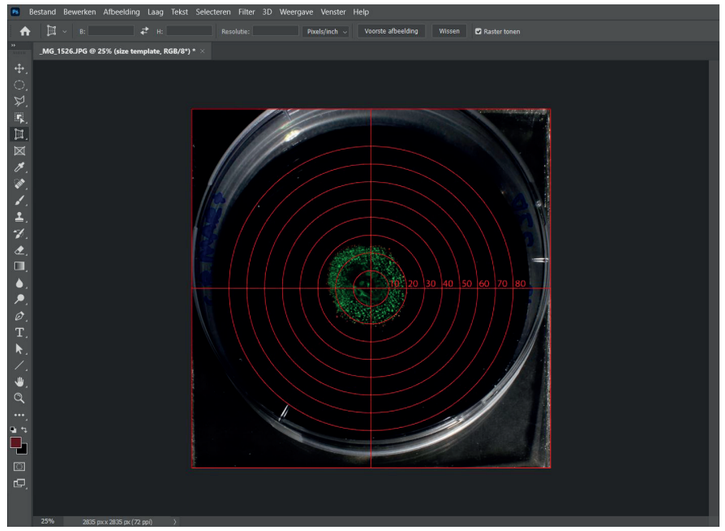
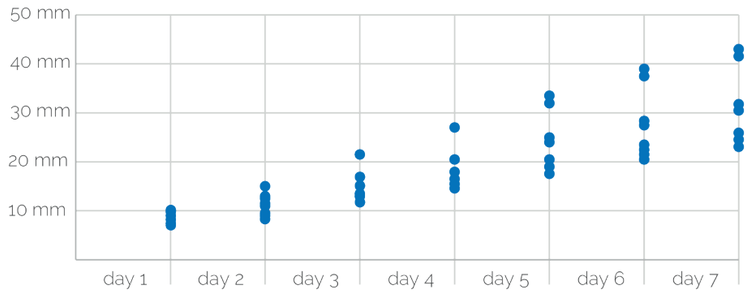


Fig. 4.2: The size template

Results



The colonies showed more variation in diameter as they grew older, as can be concluded from fig. 4.3 in which the diameters of all samples were plotted for every 24 hours of the colony's first week. This means the expansion speed differed per sample.

Fig. 4.3: The variation in the colonies' diameters during the first seven days

However, this expansion speed seems to be constant for each sample, as the diameter of the colonies showed a linear growth. This can be concluded from fig. 4.4, in which the diameters of four samples (one from each batch) are plotted against their age. A linear growing diameter of the colony means that the surface area of the bacteria grows quadratically.

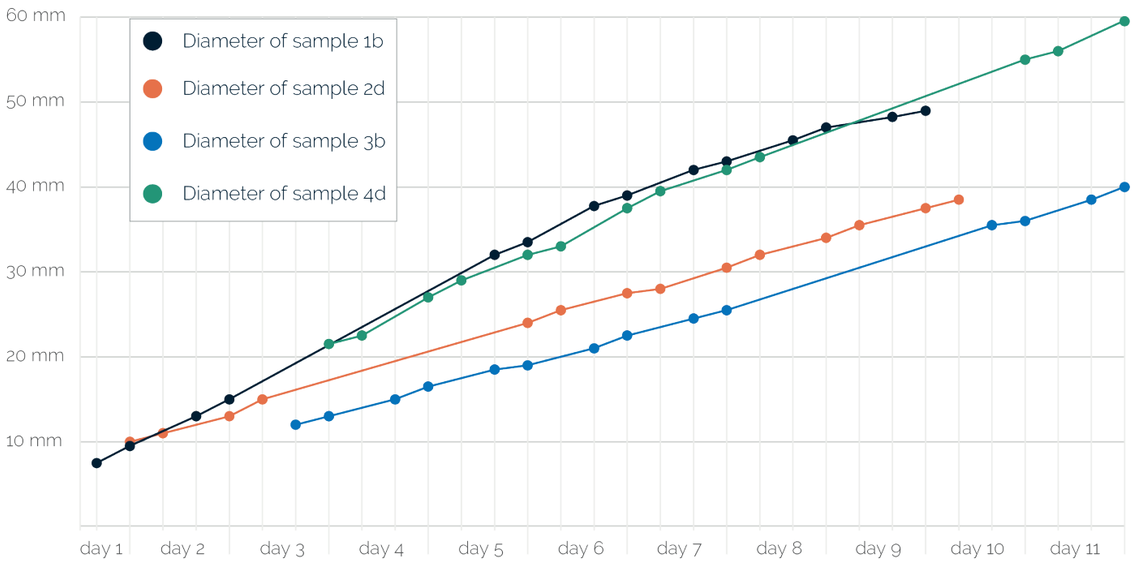


Fig. 4.4: The linear growth of the diameter of four samples (one from each batch)

Note: The slowing down of sample 1b on day 9 can be explained by ripped parafilm (as discussed in chapter 3.1).

4.2 | FLAVOBACTERIA'S IRIDESCENCE OVER TIME

The incident angles of the viewer and the light influences the appearance of Flavobacteria as it gradually changes colour while varying these angles. This is called iridescence. As the iridescence affects the appearance, it is used to characterize the appearance of Flavobacteria's standard growth. The iridescence is characterized by defining the angles that result in structural colour and the occurring colour shifts while varying the angles.

Protocol

To characterize the iridescence of the bacteria's standard growth, several picture series and movies were made while varying the rotation of the sample and the viewing, illumination and azimuth angles. These picture series and movies lead to insights about Flavobacteria's iridescence, regarding:

- The direction-dependency of the colony's appearance
- The retroreflection
- The specular reflection
- The azimuth angle
- The time-dependency

Several samples of Flavobacteria were captured in two movies every 24 hours of their lives using a setup with a camera and light mounted to a gonioreflectormeter (fig. 3.6 on page 26). The Canon EOS 550d camera was set to an aperture of f22 and iso-400. In one movie, the azimuth angle was varied from 45˚ to 135˚. In the other movie, the sample was tilted resulting in a view angle of 30˚ to 90˚. These movies lead to the insights on the iridescence's time-dependence.

The picture series that lead to insights regarding the azimuth angle were also made using the gonioreflectormeter setup. The Canon EOS 550d camera was set to an aperture of f22, a shutter speed of ¼ seconds and iso-400.

The movie and picture series that lead to insights regarding the direction-dependency of the colony's appearance, the retroreflection and the specular reflection were made using a 48 megapixel smartphone camera (fig. 4.5). This way, the positions of the camera and light could be almost identical, which was impossible with the big Canon camera on the gonioreflectormeter setup. The smartphone camera was set to iso400 and manually focused on the sample.

Fig. 4.5: The smartphone camera in front of the LED

The Direction-dependency of the Colony's Appearance

The appearance of the bacteria's standard growth is isotropic. This was concluded from a movie in which the sample was rotated. The smartphone camera and light were positioned at a fixed altitude angle of 45˚ with an azimuth angle of approximately 0˚ (fig. 4.6). As can be seen in the snapshots (fig. 4.7) and the complied image of the snapshots (fig. 4.8), the rotation of the sample does not affect the appearance.

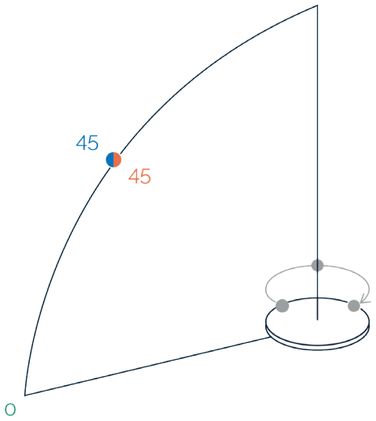


Fig. 4.6: The settings of the setup

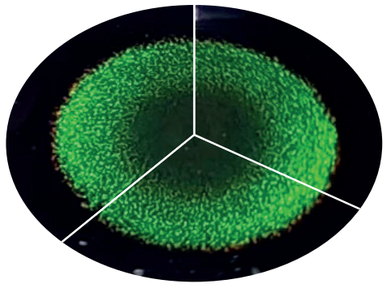


Fig. 4.8: A complied image of the colony created with snapshots of the movie at a rotation of 0˚, 120˚ and 240˚

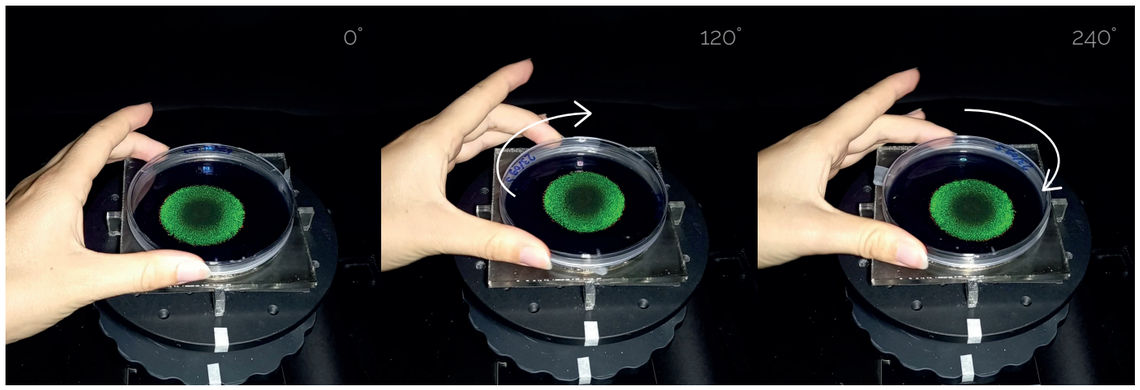


Fig. 4.7: Snapshots of the movie at 0˚ (left), 120˚ (middle) and 240˚ (right) rotation

The Retroreflection

The retroreflection, which can be captured close to the light source (fig. 4.9), results in the brightest structural colour, meaning that most of the light is being backscattered. This was concluded from two series of pictures in which either the azimuth angle was varied (fig. 4.10-4.11) or the viewing angle (fig. 4.12-4.13 on the next page).

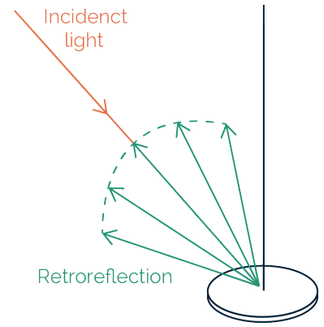


Fig. 4.9: Retroreflection

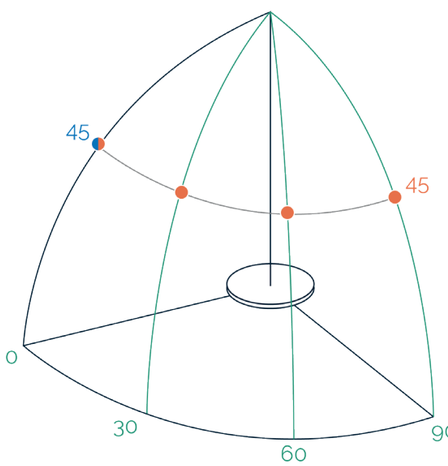
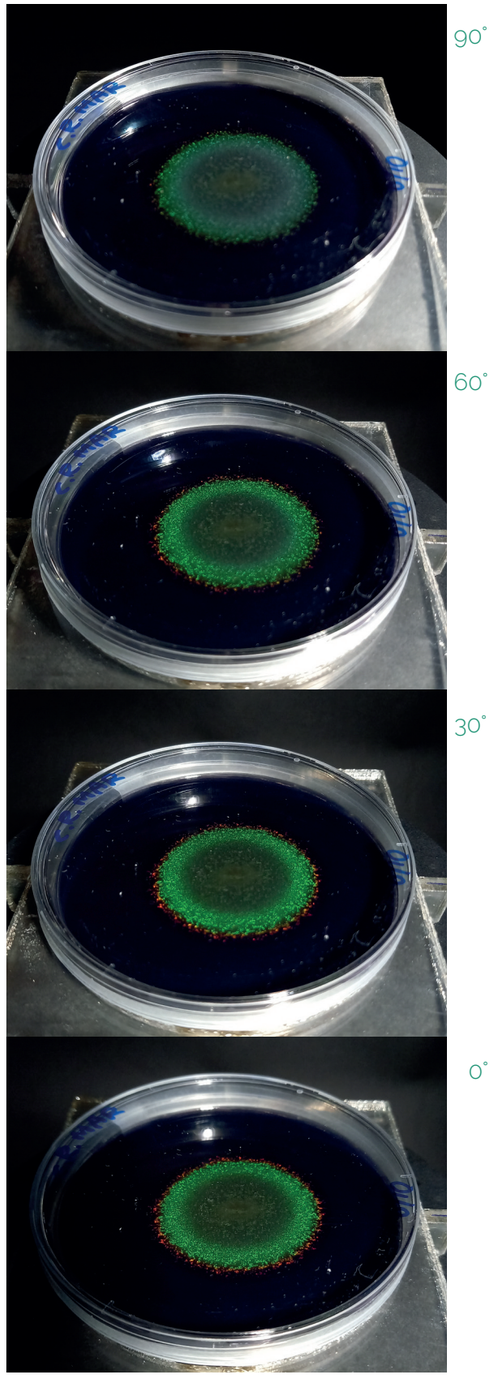
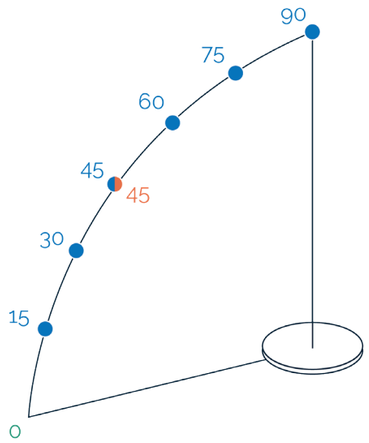


Fig. 4.10 [top]: The settings of the setup
Fig. 4.11 [right]: The colony with an azimuth angle of 90˚, 60˚, 30˚ and 0˚ (top to bottom)





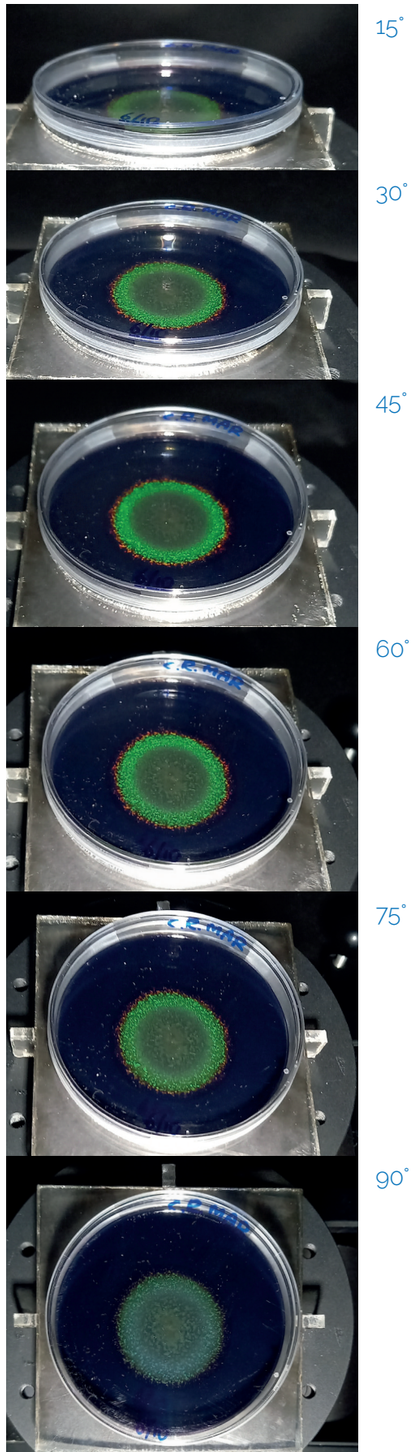
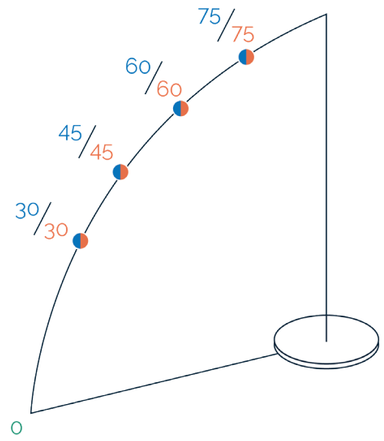


Fig. 4.12 [top]: The settings of the setup
Fig. 4.13 [right]: The colony from a viewing angle of 15˚, 30˚, 45˚, 60˚, 75˚ and 90˚ (top to bottom)

The brightness of the retroreflected structural colour strongly depends on the viewing and illumination angle. The brightest retroreflection appears at a viewing and illumination angle around 45˚. This was concluded from a picture series in which the viewing and illumination was simultaneously varied from 30˚ to 75˚ (fig. 4.14-4.15).



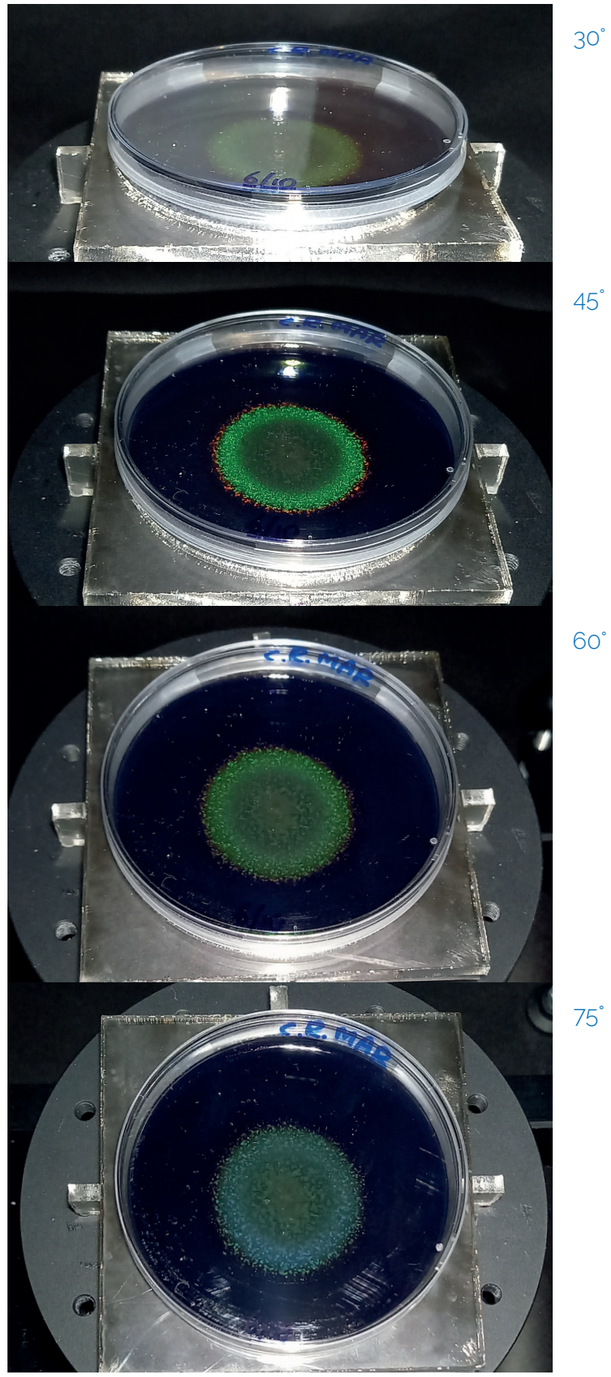


Fig. 4.14 [top]: The settings of the setup
Fig. 4.15 [right]: The retroreflection of the colony from a viewing angle of 30˚, 45˚, 60˚ and 75˚ (top to bottom)

Because of the big step size between the pictures, the brightest appearance might be skipped. Therefore, the automated setup (as discussed in chapter 5) was used to take pictures with a smaller step size. Pictures were taken for every 1˚ variation in the viewing and illumination angle. From these pictures is concluded that the brightest retroreflection appears at 45˚/46˚. As the variation in brightness is hard to detect when seeing the pictures next to each other, a complied image is created (fig. 4.16) showing the colonies ranging in viewing and illumination angle from 35˚ to 55˚.

This brightest structural colour will from now on be called the dominant colour of the colony.

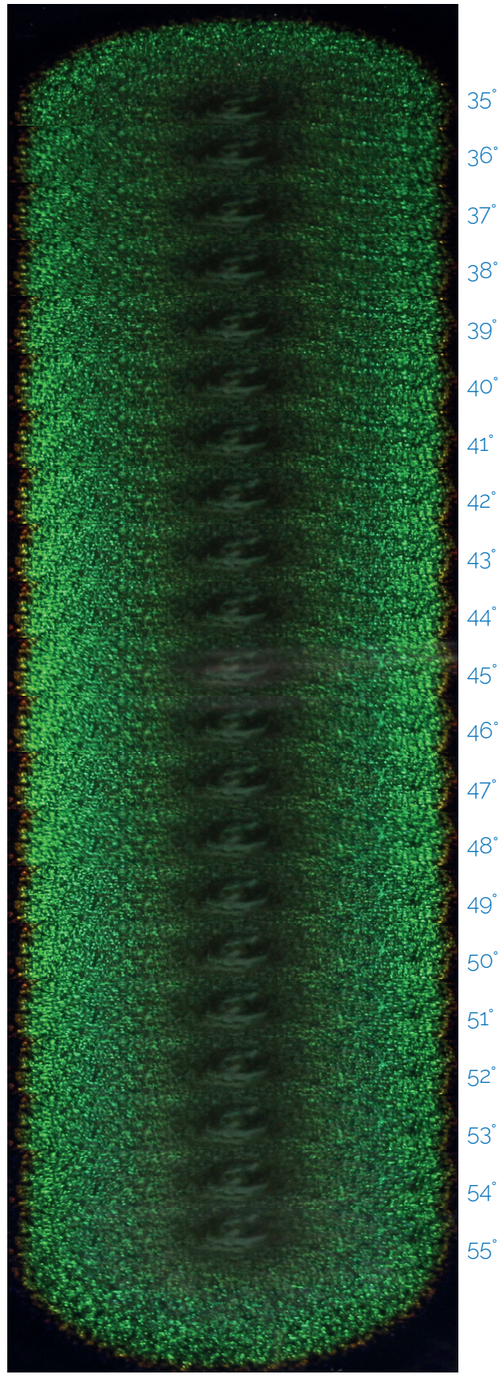


Fig. 4.16: Pictures taken with the automated setup of the retroreflection ranging in viewing and illumination angle from 35˚ to 55˚ (top to bottom)

The Specular Reflection

The specular reflection of the light results in a blue/purple structural colour (fig. 4.17). This colour is most present from a viewing angle of 90˚ and fades around a viewing angle of 60˚. This was concluded from a picture series in which the specular reflection was captured from several viewing angles (fig. 4.18-4.19). As can be seen in the reflection of the sample, a LED ring was used to capture this picture series.

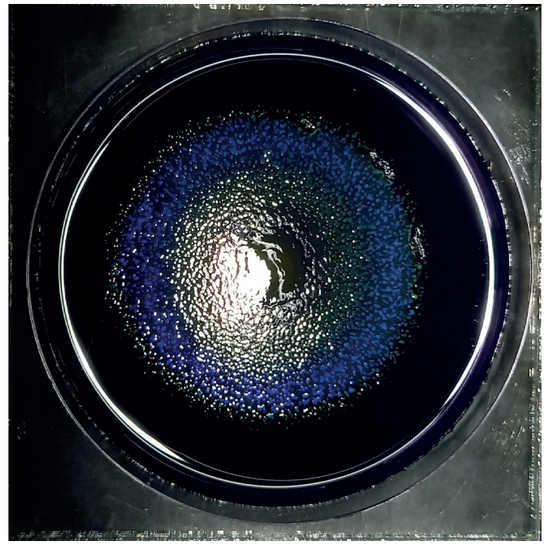


Fig. 4.17: The specular reflection captured without petri dish lid from a viewing angle of 90˚

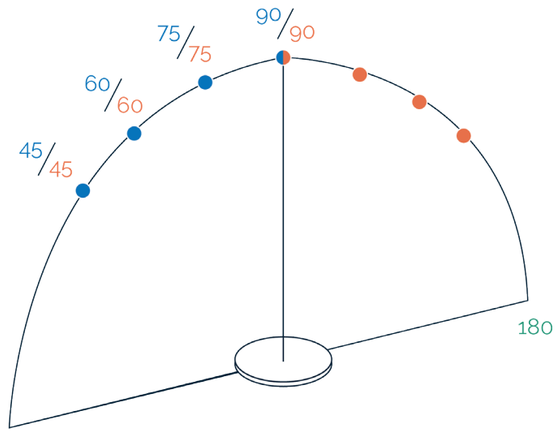
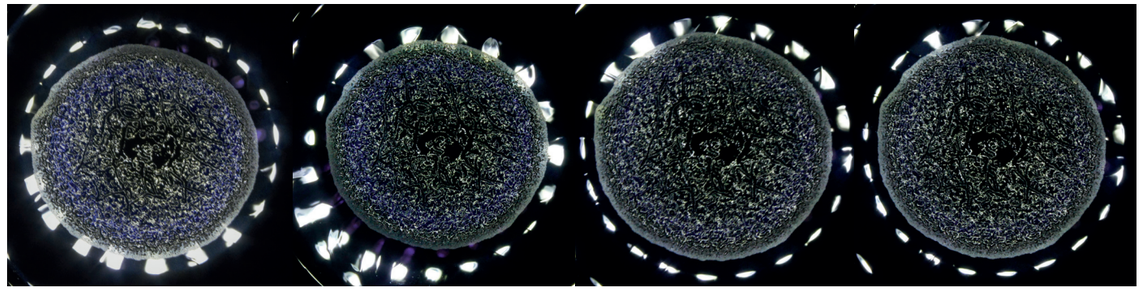
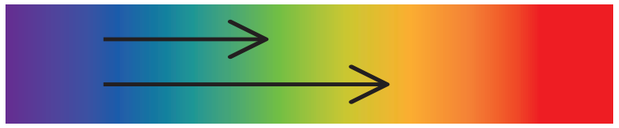


Fig. 4.18 (left): The settings of the setup
Fig. 4.19 (bottom): The specular reflection of the colony from a viewing angle of 90˚, 75˚, 60˚ and 45˚ (left to right)



The Azimuth Angle

Although the brightest colours are a result of retroreflection, the colony does display an interesting colour change while varying the azimuth angle (fig. 4.20-4.21). This shift in colour goes from blue/purple (large azimuth) to the dominant colour (small azimuth). Young colonies show a more interesting shift in colour since the dominant colour shifts from red to green in time (as discussed on page 43-44) and the colour change, as a result of the varying azimuth angle, shifts corresponding to the wavelength order.



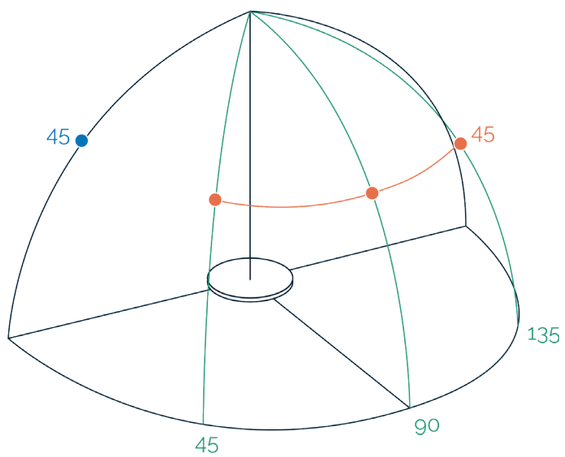
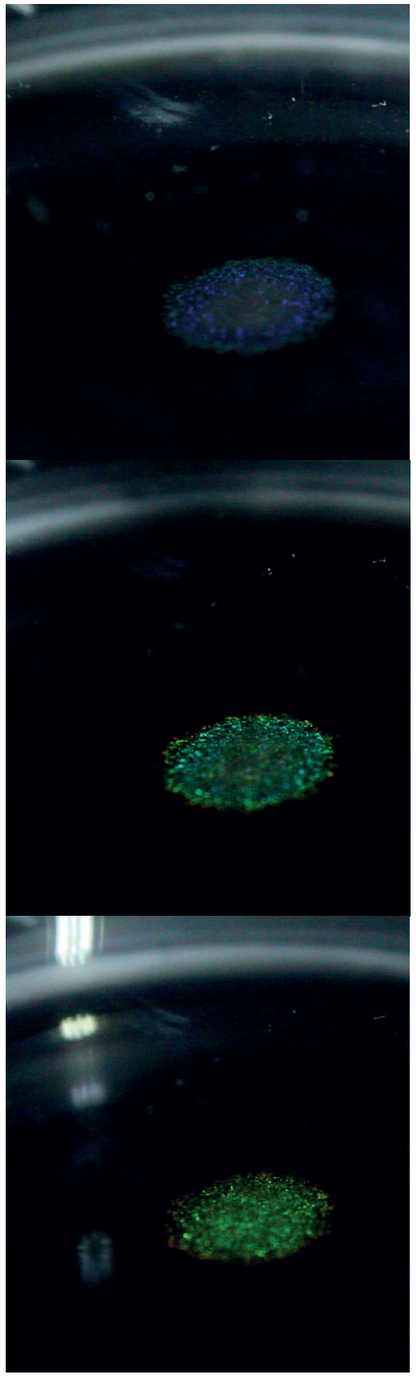


Fig. 4.20 [top]: The settings of the setup
Fig. 4.21 [right]: The colony with an azimuth angle of 135˚, 90˚ and 45˚ (top to bottom)



Varying the azimuth angle appears to especially be interesting from low viewing angles, such as 30˚ (fig. 4.22-4.25).

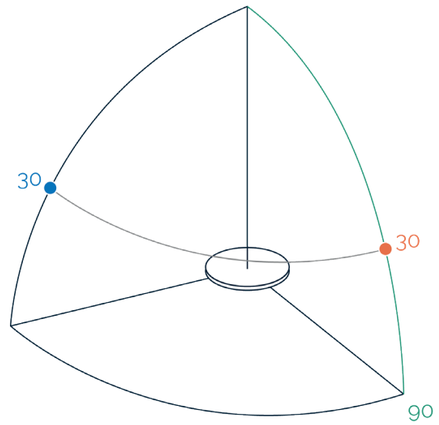
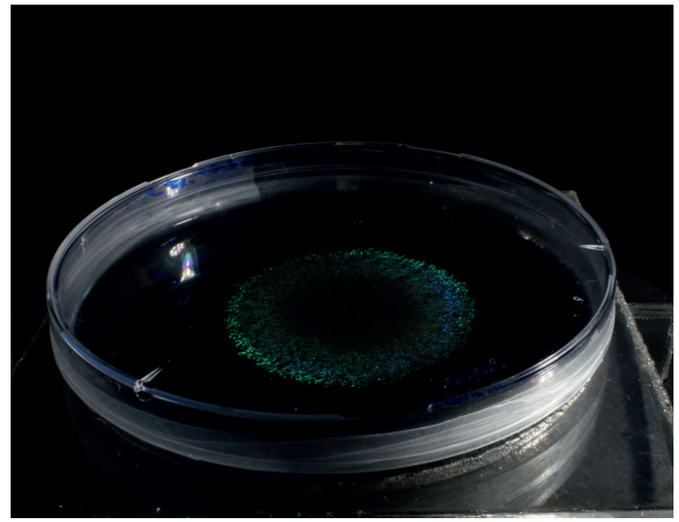


Fig. 4.22 [top]: The settings of the setup
Fig. 4.23 [right]: The colony from a viewing angle of 30˚



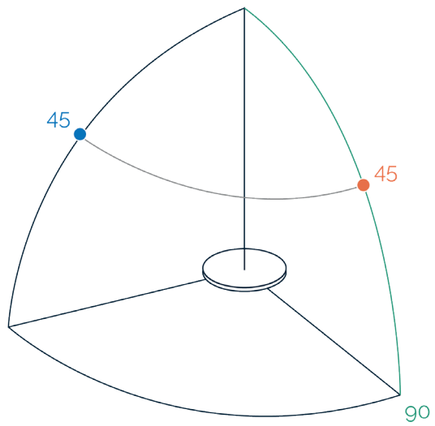
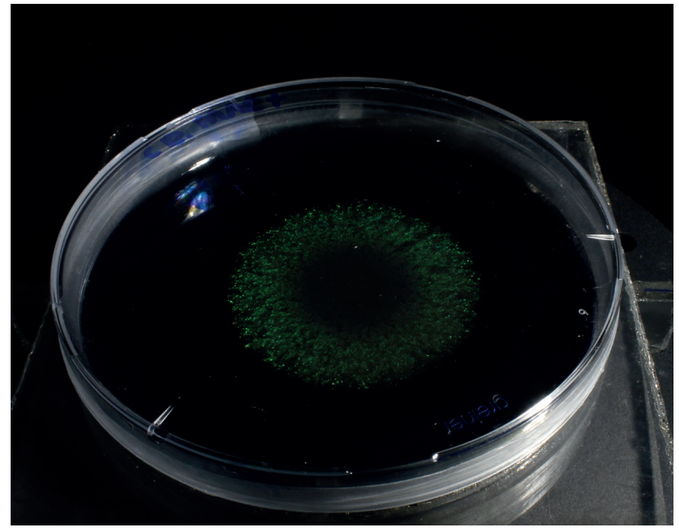


Fig. 4.24 [top]: The settings of the setup
Fig. 4.25 [right]: The colony from a viewing angle of 45˚



The Time-dependency

The range of angles that result in structural colour is identical for most of the colony, consisting out of different-aged bacteria cells. The only part of the colony that does not share this identical range is the faded middle. However, as this part run out of nutrients and presumably therefore lost its colour, this part does not contain healthy bacteria cells. In that case, it can be stated that the range of angles that result in structural colour is not dependent on the age of bacteria cells as long as they are healthy. Accordingly, an expanding colony that grows in a habitat from which the nutrients are not replenished, is dependent on time. This conclusion has to be verified by proving that the bacteria would retain their colour if the nutrients would be replenished.

The shifts in colour are dependent on time as younger colonies show the most interesting colour shift (as discussed on page 39). However, the colour appears to always shifts from blue/ purple to the dominant colour.

These statements are based on two series of movies that were made every 24 hours of the colony's lifetimes in which either the sample was tilted, or the azimuth angle was varied. Snapshots of these series of movies can respectively be found in fig. 4.26 and fig. 4.27.

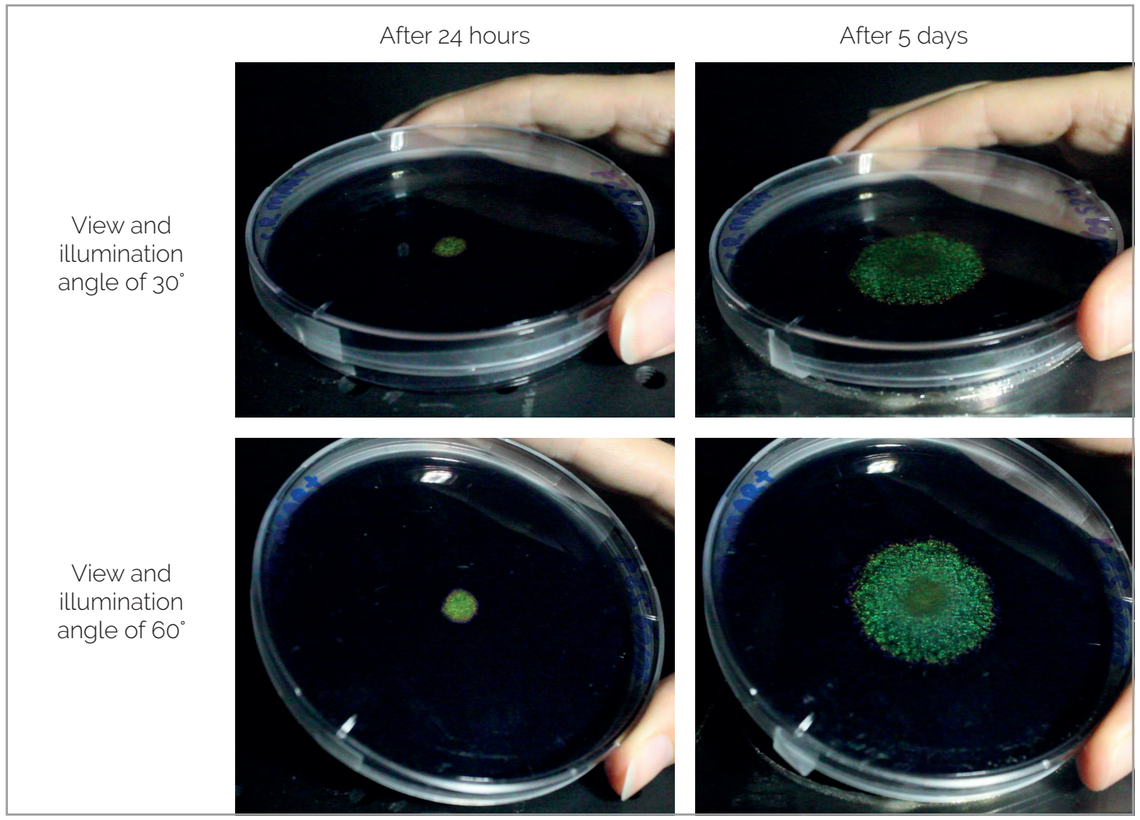


Fig. 4.26: Snapshot of the series of movies in which the sample was tilted

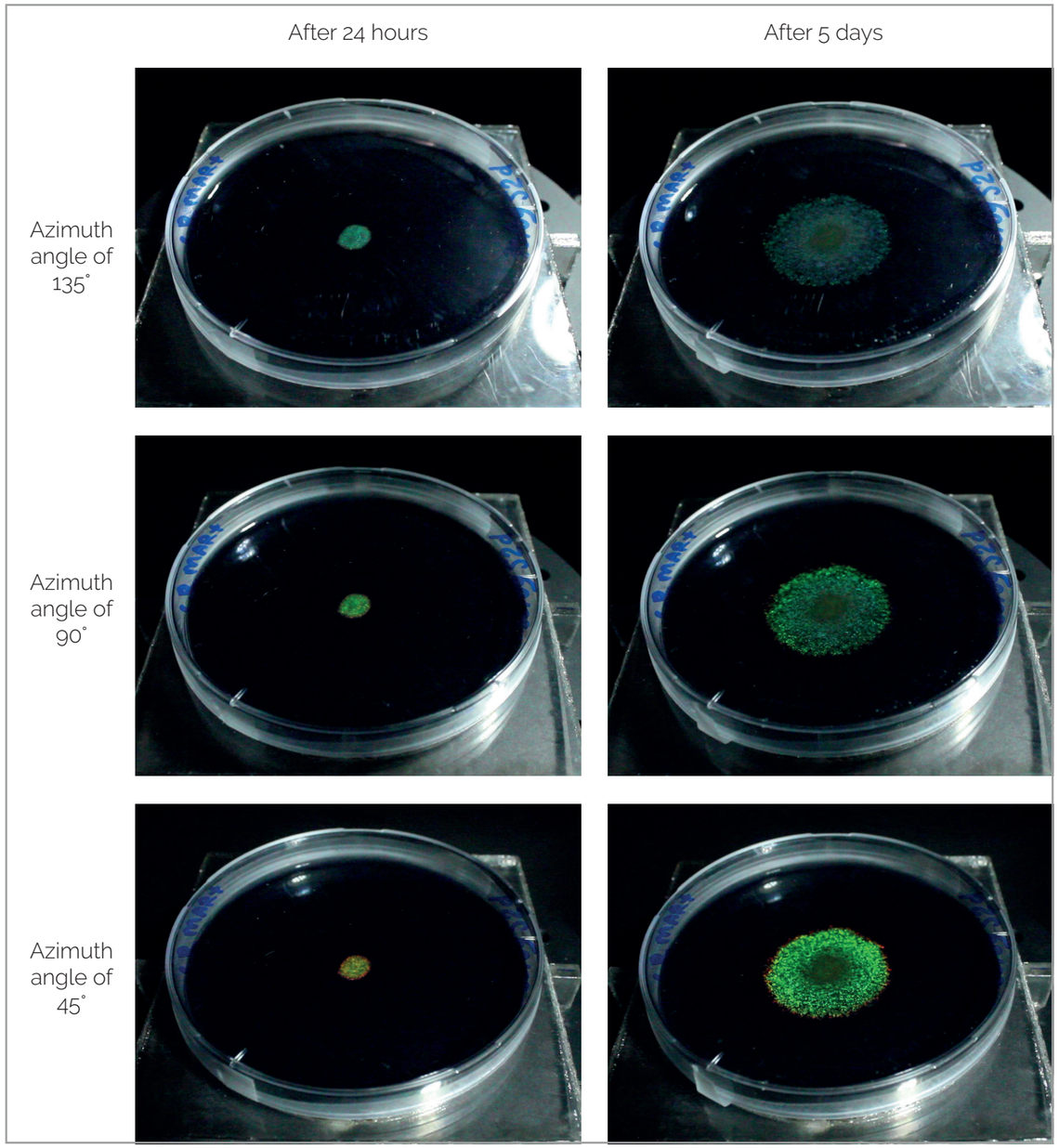


Fig. 4.27: Snapshot of the series of movies in which the azimuth angle was varied

4.3 | FLAVOBACTERIA'S COLOUR OVER TIME

To characterize the colour of the bacteria's standard growth, the colours of the colonies were described and analysed. This characterization focuses on the dominant colour of the colonies. The dominant colour refers to the brightest colour which can be seen close to the retroreflection from a viewing/illumination angle of 45˚ (page 37).

Protocol

Several samples of Flavobacteria were captured every 8 hours of their lives using a setup with a camera and light mounted to a gonioreflectormeter (fig. 3.6 on page 26). The Canon EOS 550d camera was set to an aperture of f22, a shutter speed of ¼ seconds and iso-400. The colonies were photographed and illuminated from an angle of 45˚ (fig. 4.28). The colours in the pictures were analysed using an online colour summarizer, developed by M. Krzywinski (2020). This tool reports a summary of the colours in a picture by using k-means clustering to group similar colours together and derive a set of colours that are representative for the picture. This set of colours shows the average colour of each group and takes the amount of pixels per group into account. The colours in the pictures were clustered into 6 groups (k-means). Since the tool summarizes the colours into groups, small details like the red-orange edge do not show in the set of colours. However, the analysis is useful to show the most present colours of the colonies. To focus only on the Flavobacteria in the colour analysis, the black of the background was removed from the set of colours. All the sets of colours are shown above the pictures on the next page.

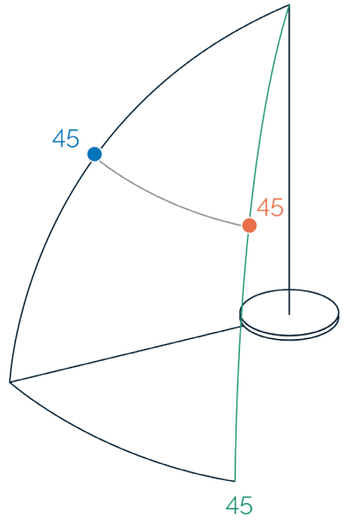


Fig. 4.28: The settings of the setup

Results

In the first 24 hours, the colour exhibits the most extensive change (fig. 4.29-4.34). It shifts from violet-red, via red, orange and yellow, to green. While the inner part of the colony turns yellow and eventually green, the edge of the colony retains its red-orange colour. This green dominant colour with its red-orange edge will remain for the next days. In these days, however, the inner parts of the colonies turn more turquoise and start to show blue glitters (fig. 4.35-4.36). On the fourth day, the inner parts of the colonies (i.e., the oldest bacteria cells) start to lose their bright colour. This faded inner part of the colony will become bigger in time, as more bacteria cells lose their colour, while the red-orange edge moves further away from the middle (fig. 4.37). When the colony stops expanding, the red-orange edge will turn to green and fade as well.

8 hours

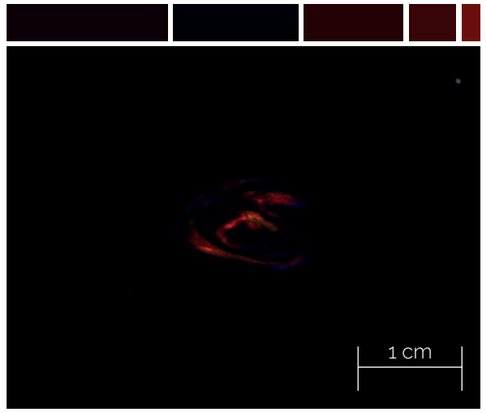


Fig. 4.29: Flavobacteria, eight hours after cultivation

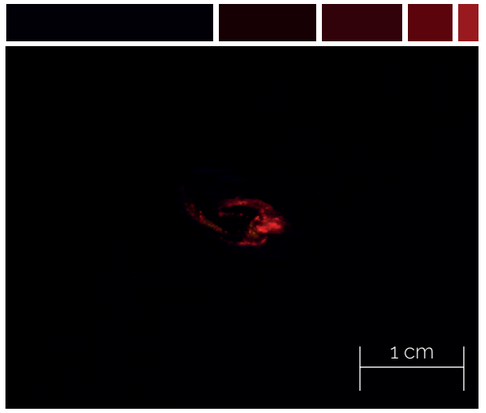


Fig. 4.30: Flavobacteria, eight hours after cultivation

16 hours

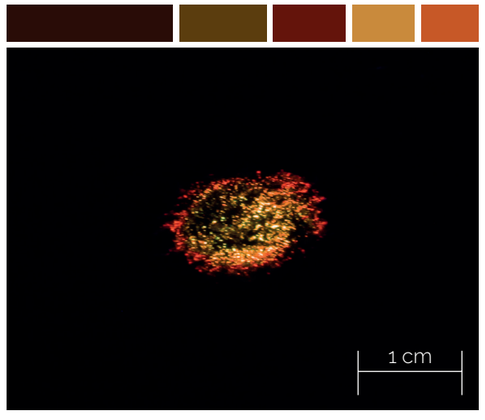


Fig. 4.31: Flavobacteria, 16 hours after cultivation

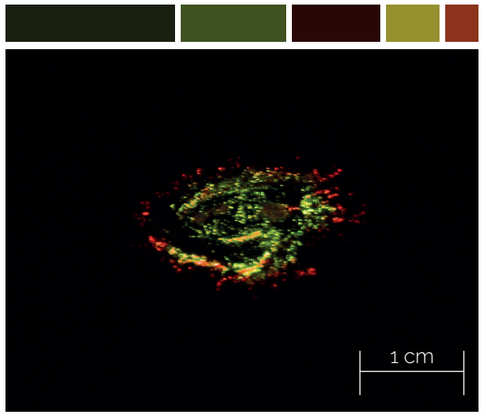


Fig. 4.32: Flavobacteria, 16 hours after cultivation

24 hours

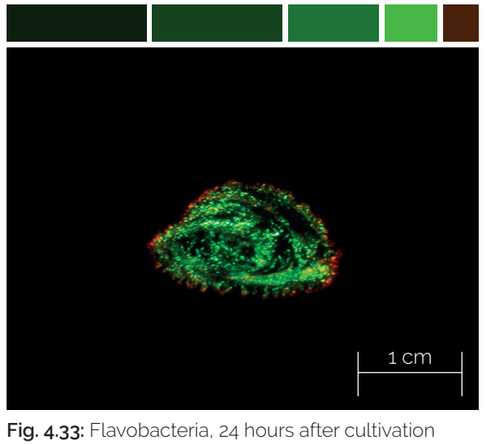


Fig. 4.33: Flavobacteria, 24 hours after cultivation

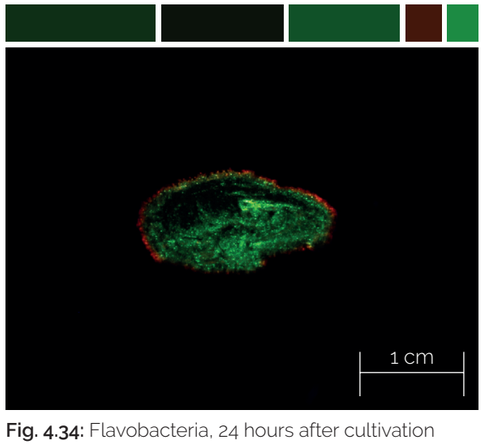
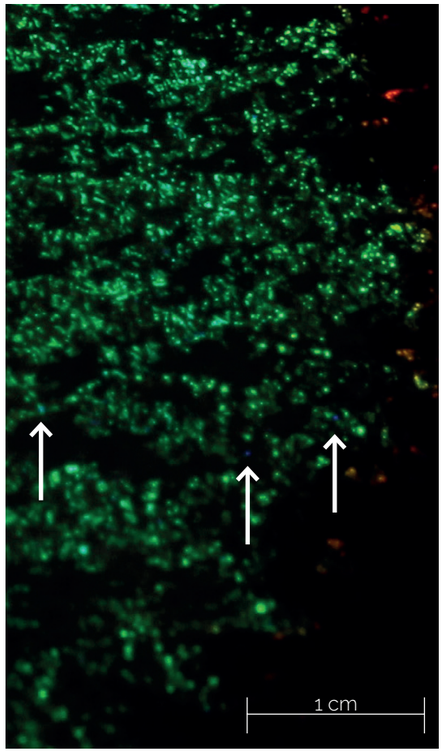
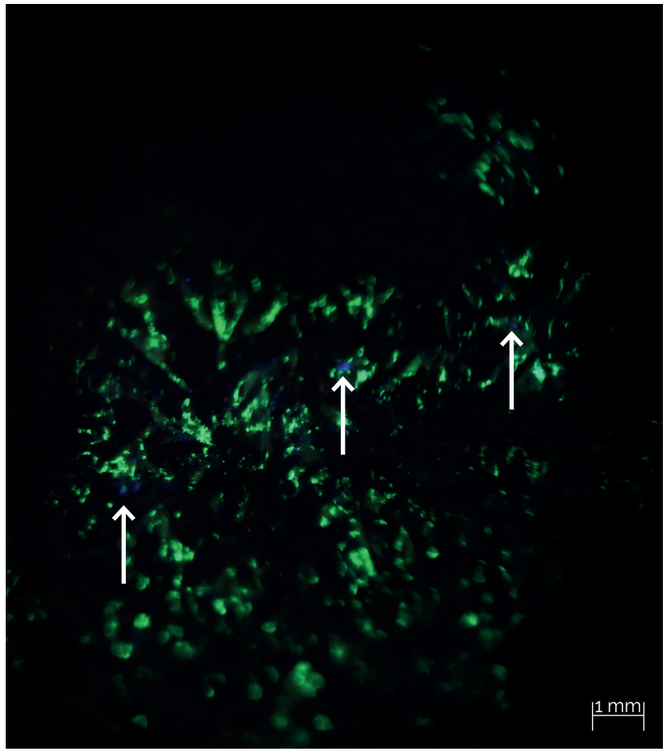


Fig. 4.34: Flavobacteria, 24 hours after cultivation





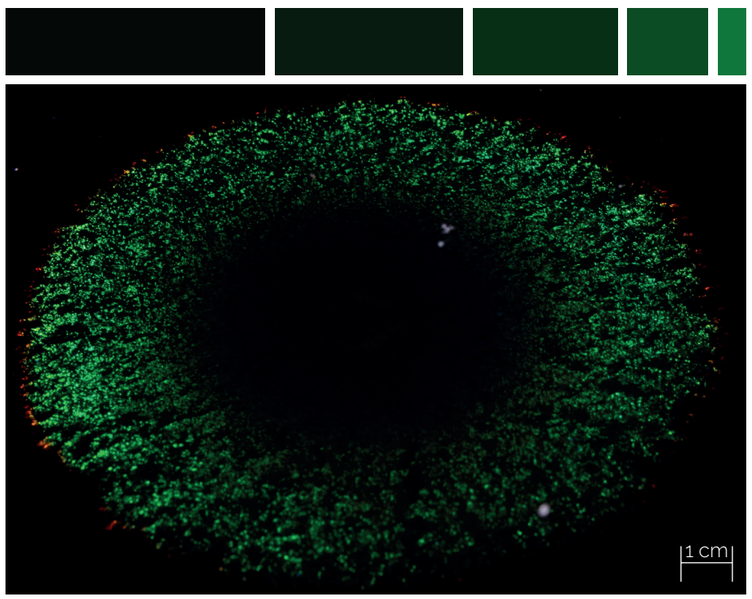


Fig. 4.35 [top left]: Close-up of colony with blue glitters
Fig. 4.36 [top right]: Blue glitters under microscope
Fig. 4.37 [left]: Colony with expanding red-edge and faded inner part

The glitter-like appearance is visualized by fig 4.38, which shows how the pixels of a colony, including the black background, were partitioned into the different clusters during the analysis.

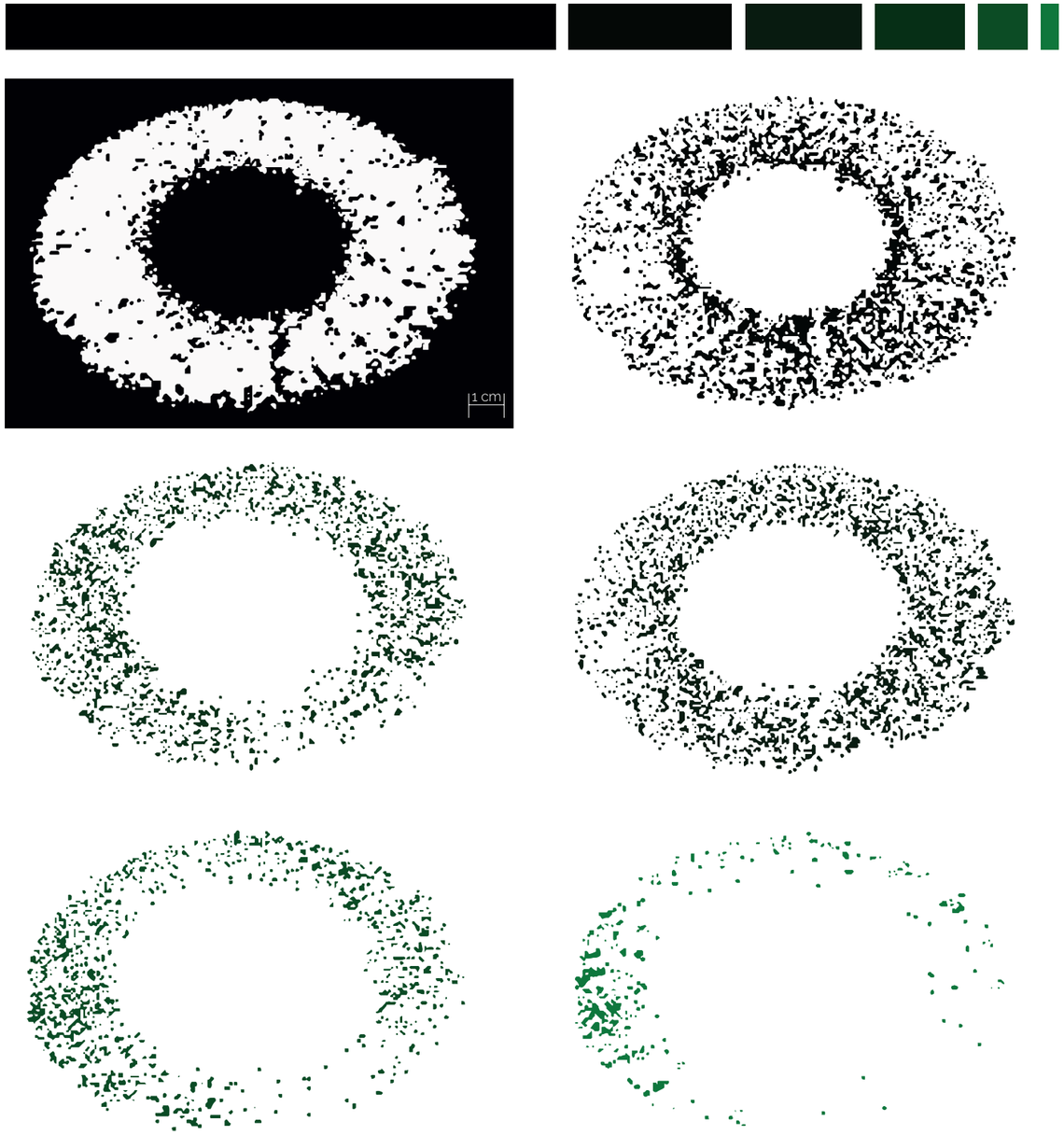


Fig. 4.38: Partitioned pixels into the different clusters during the analysis

5 Capturing the Appearance

This chapter discusses the design process of the setup that was created to capture Flavobacteria's unique, temporal and iridescent appearance. While using the gonioreflectormeter setup to capture flavobacteria for the characterization, it became clear that an automated setup was more practical and thus preferred. Besides, it was imagined that moving the sample instead of the camera's position would create more intuitive data as one would also move the sample around in real life. Based on these insights and the first explorations within the field of capturing material appearances (chapter 3.2), requirements were formulated and a setup was built. While using the setup to capture data, a small iteration on the setup was made.

5.1 | THE REQUIREMENTS

Reasoning from the final goal (last alinea of chapter 1.1), the setup should create data for:
- detailing the characterization of the appearance;
- a movie to spark interest of other designers;
- straightforward visualizations to communicate Flavobacteria's appearance;
- computer graphic models and rending.

Therefore:

the setup should be able to capture the iridescent appearance of the Flavobacteria;
- The sample should be able to consistently move by tilting and rotating around its centre in order to create intuitive data (i.e., data that is easy to understand and looks natural, which is in this case realised by simulating a hand movement).
- The middle of the sample should be consistently aligned with the centre of the camera and the light.
- The setup should be able to capture the dominant colour (i.e., the brightest colour which is a result of the retroreflection from a viewing/illumination angle of 45˚).
- The setup should be able to capture the blue/purple specular reflection (i.e., the specular reflection around a viewing angle of 90˚).
- The setup should be able to capture the colour shift as a result of a variation in azimuth angle (at least 0˚-135˚).
- The altitude angles of the camera and light and the azimuth angle of the setup should be adjustable to finetune the settings later on if needed.

the setup should be able to capture the temporal appearance of the Flavobacteria;
- The setup should be safe in order to capture the material appearance during the night and weekends.
- The setup should be able to automatically enable the light from the preferred direction.
- The setup should be able to automatically take pictures.
- The setup should be able to automatically tilt or rotate the sample.

and in general:
- the light should emit all the wavelengths of the visible spectrum;
- the light should uniformly illuminate the sample;
- the sample holder should fit a standard Greiner petri dish of 94mm wide and 16mm high;
- the camera should be able to focus on the sample;
- interreflections from the surrounding should be avoided;
- the setup should be customizable regarding the data it will capture.

5.2 | AUTOMATED SETUP

Based on the requirements, ideas were generated and selected for realising the setup (appendix H). The selected ideas were combined in a design, which was built in the workshop as discussed in appendix I.

Mechanics

A sample holder, made of laser cut MDF which was painted black, was created for the petri dish (fig. 5.1). A servo motor was included that could tilt the sample 180˚. A stepper motor was added that could rotate the entire platform 360˚. Small foam parts were added to hold the petri dish in the middle, without ripping the parafilm (fig. 5.2).

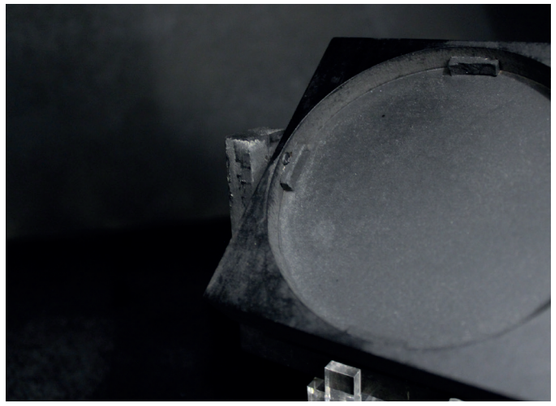
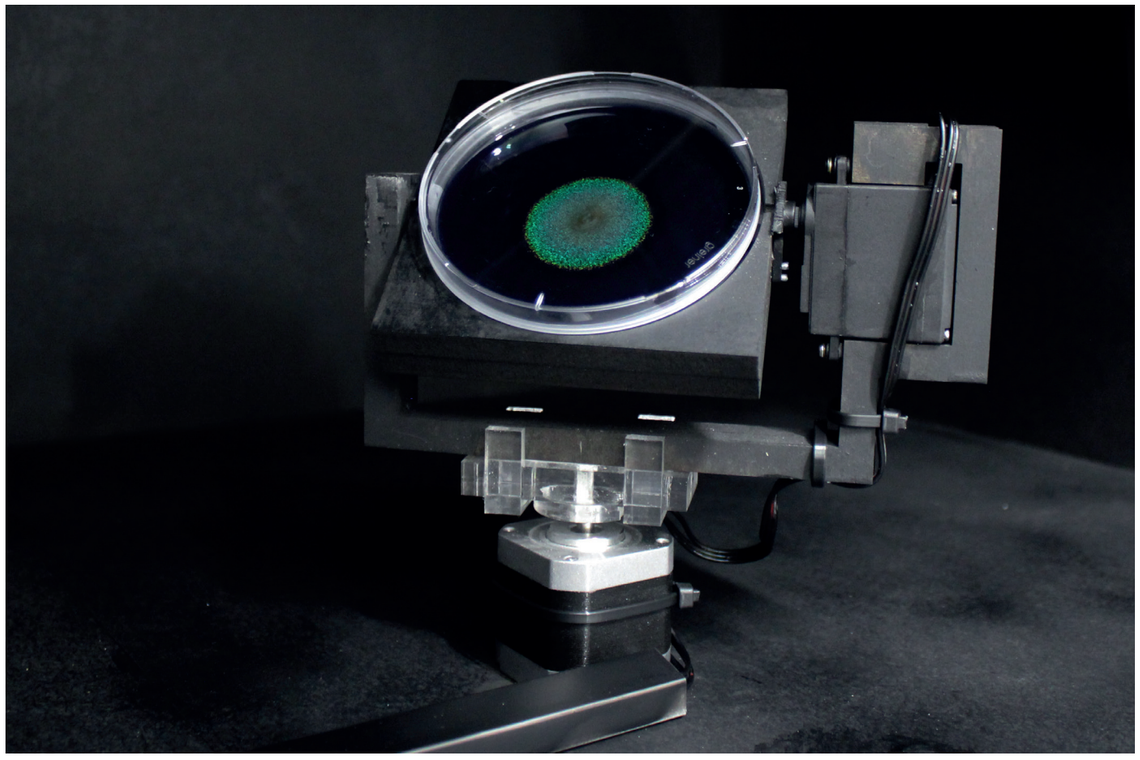
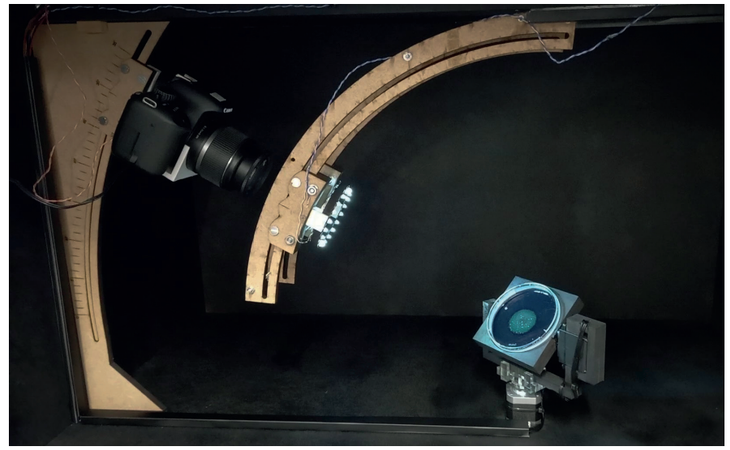


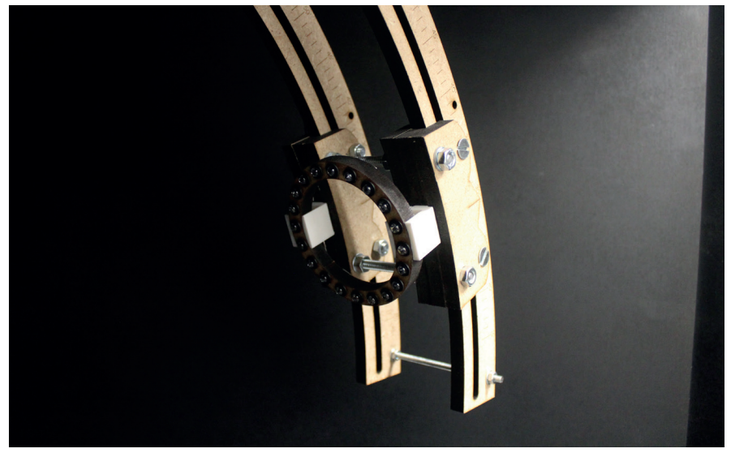
Fig. 5.1 [bottom]: The tilting and rotating sample holder
Fig. 5.2 [right]: Small foam edges to hold the sample in place



In order to capture the retroreflection, a LED ring was created through which the camera could capture the sample (fig. 5.3). The LED ring consists out of 20 white LEDs which emit 280 lumen and all the wavelengths of the visible spectrum (fig. 5.4 on the next page). However, some wavelengths are emitted stronger than others. Therefore, a colour calibration is recommended for further analyses.

The LED ring was connected to a moving arm, that can be rotated with a stepper motor for at least 180˚. To uniformly illuminate the sample, the arm made sure the LED ring was 280mm away from the sample. The LED ring is clamped on the moving arm, making it possible to adjust the altitude angle from 15˚ to 90˚ (fig. 5.5-5.6). To reduce the momentum as a result of the arm on the motor's axis, a counterweigh was included (fig. 5.7-5.8 on the next page). This counterweight can be adjusted by shifting the weight in the aluminium profiles as the altitude angle of the LED ring is adjusted.





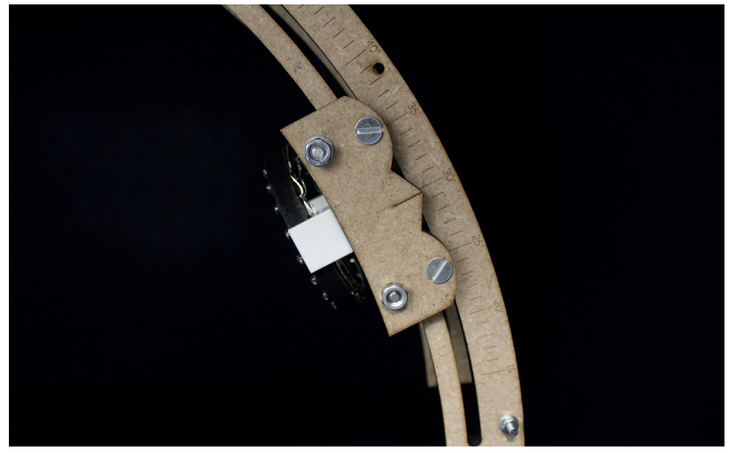


Fig. 5.3 [top]: The LED ring illuminating the petri dish
Fig. 5.5 [middle]: The LED ring connected to the moving LED arm
Fig. 5.6 [bottom]: The adjustable altitude angle of the LED ring

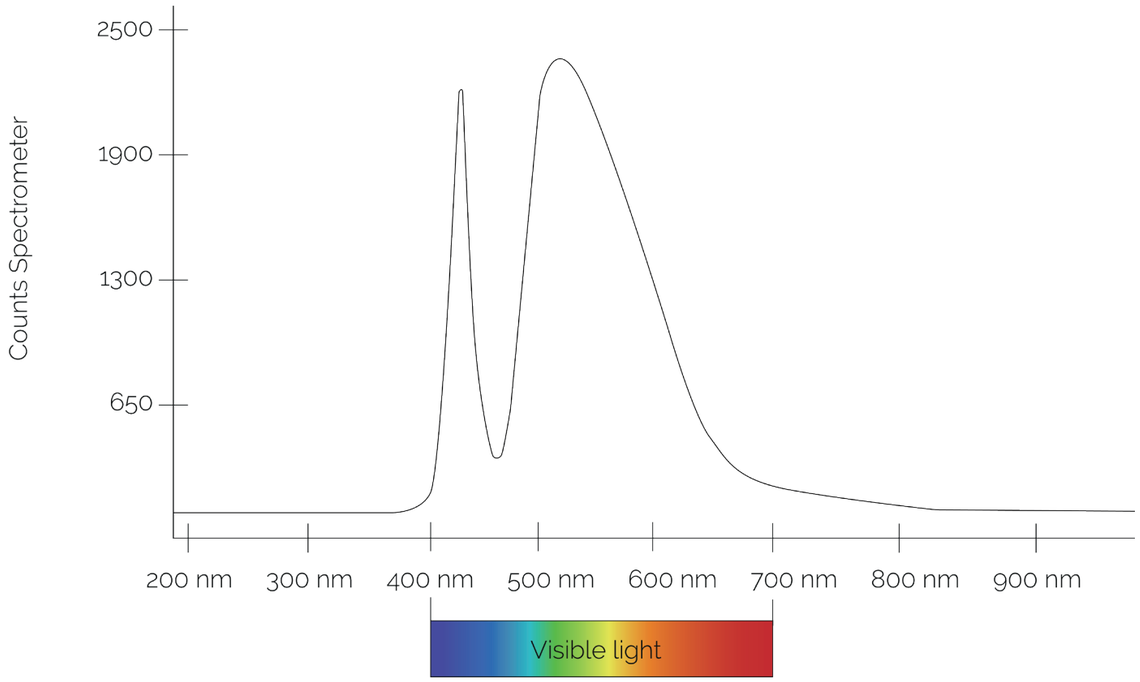


Fig. 5.4: The emitted wavelengths by the LED ring

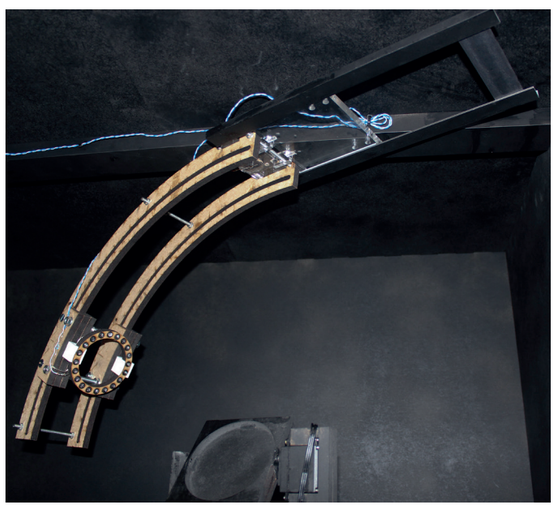


Fig. 5.7: The counterweight connected to the moving LED arm

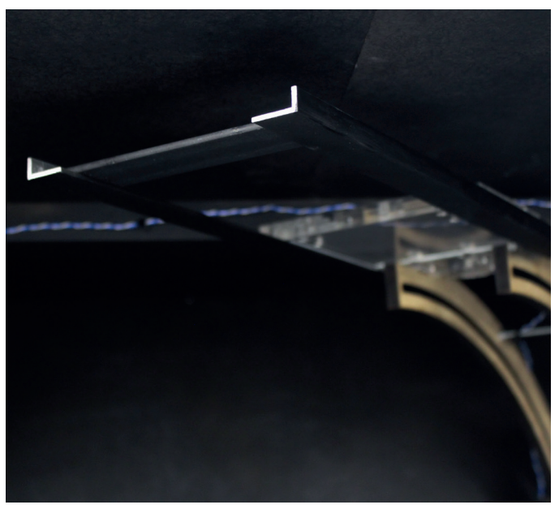


Fig. 5.8: The adjustable counterweight in the aluminium profiles

A camera holder was 3d-printed for a Canon EOS 550d to fixate the camera's position (fig. 5.9-5.10). The camera holder was clamped on an arm, making it possible to adjust the altitude angle from 0° to 35°.

Fig. 5.9: The 3d-printed camera holder clamped on an arm

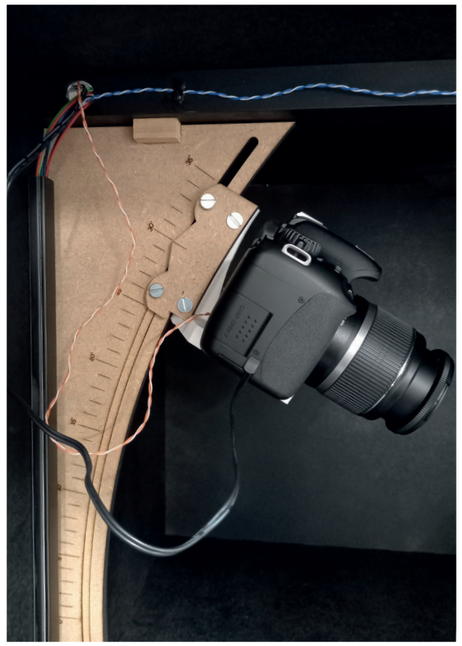


Fig. 5.10: The camera in position

A wooden box (fig. 5.11-5.12) was created to create a dark capture environment. The box also made sure all the components are in the correct position in order to keep the sample consistently in the centre of the light and camera (fig. 5.13). The parts of the box were made by laser cutting MDF and were painted black on the inside. The stepper motor of the moving LED arm was connected to an aluminium profile, which was enclosed by the box (fig. 5.14).

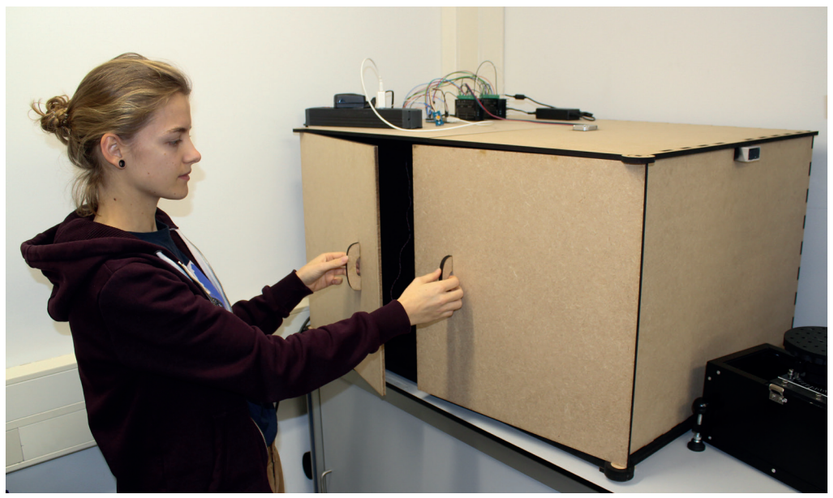


Fig. 5.11: The wooden box of the setup with doors

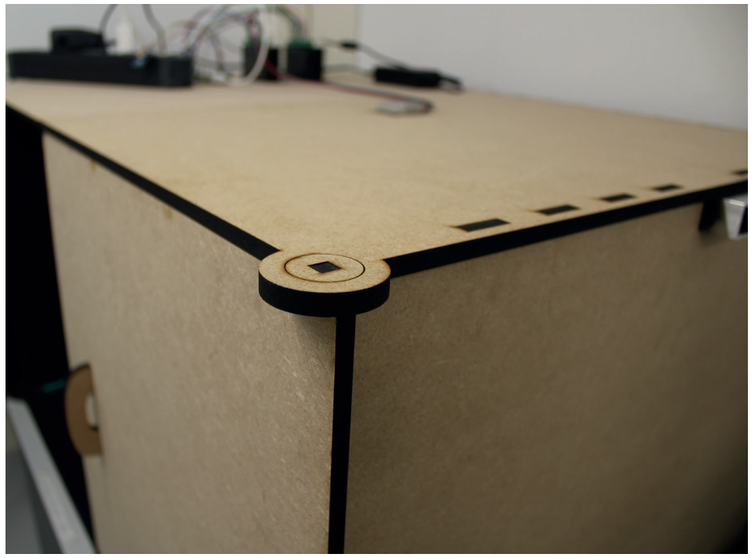


Fig. 5.12: Details of the wooden box

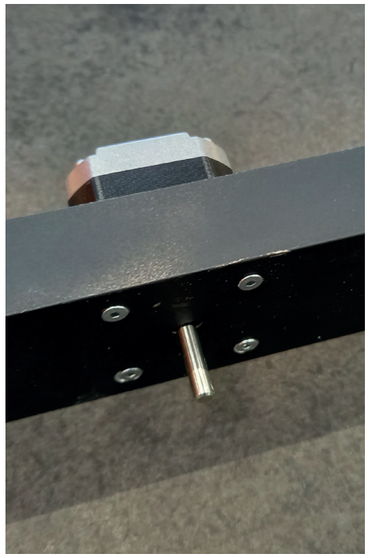


Fig. 5.14: The stepper motor of the moving LED arm in the aluminium profile

Fig. 5.13: The box with all the components in the correct position

Electronics

An Arduino Uno is used as microcontroller to control the S3003 servo motor, the LED ring, the two nema17 stepper motors and the Canon camera (fig. 5.15). To be able to control the stepper motors with the Arduino, two TB6600 micro stepping driver are added. By adjusting the dip switches on these micro stepping drivers, the nema17 stepper motors are able to make smaller steps resulting in a smooth movement. The Canon is controlled by an optocoupler and jack plug connected to the connection of the shutter release control.

A 12V 3A adapter is used to power the nema17 stepper motors and their micro stepping drivers. To make sure the camera does not run out of battery, an adapter is also used for the Canon. The LED ring and S3003 servo motor get their power supply through the Arduino, which is powered through a 5V 1A adapter.

Unlike the servo motor, the stepper motors cannot recall their current position. Therefore the moving LED arm and the sample holder should be calibrated before starting the setup. This is done by rotating the parts to the defined angles of 0°, which are implemented in the code. To make sure the setup does not start over after a power failure without being calibrated, a safety button is added to the setup. This button should be pressed in order for the setup to start.

The entire circuit is visualized in appendix J.

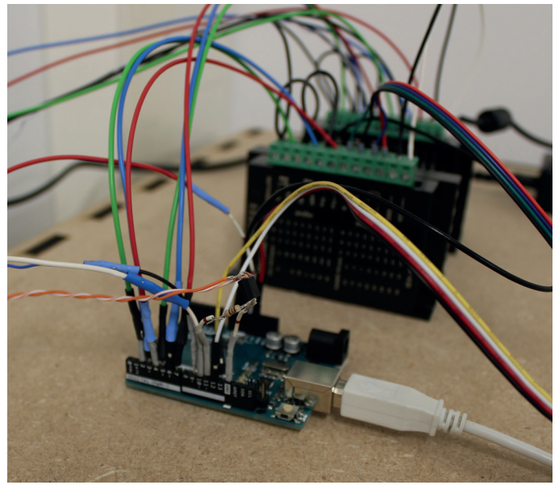


Fig. 5.15: The Arduino connected to all the components

Software

A code was written to control the Arduino and thus, the setup. The code was segmenting into functions, one for each component, making it easier to change the settings of the setup, such as the movement of the LED ring or, how often a picture should be taken. This makes the setup easy to customize regarding the data it will capture. The different functions are called upon in a looping function at the end of the code (called void loop). To change the settings of the setup, only this looping function has to be adjusted.
In practice the looping function does not loop continuously, as the safety button activates the looping function. Therefore, the button also prevents the setup from restarting the captures session once it is finished. The entire code can be found in appendix K.

Instructions on how to use the automated setup can be found in appendix L.

5.3 | ITERATION ON SETUP

The first data captured with the automated setup was satisfying but did not have a stunning quality. Therefore, a Canon EOS 5DS with a TS-E 90mm lens was installed in the setup. The camera needed a new connection with the Arduino, as the camera had a three-pin connection instead of a 2.5 jack plug for the shutter control. Because the new camera was too heavy for the plastic camera holder, a tripod was installed inside the box (fig. 5.16). Due to the tripod, the new camera is no longer aligned by the camera holder and arm, which makes it harder to align the new camera in the correct position. However, as can be seen in fig. 5.17, the captured data definitely improved.

Fig. 5.16: The Canon EOS DS camera on a tripod in the setup

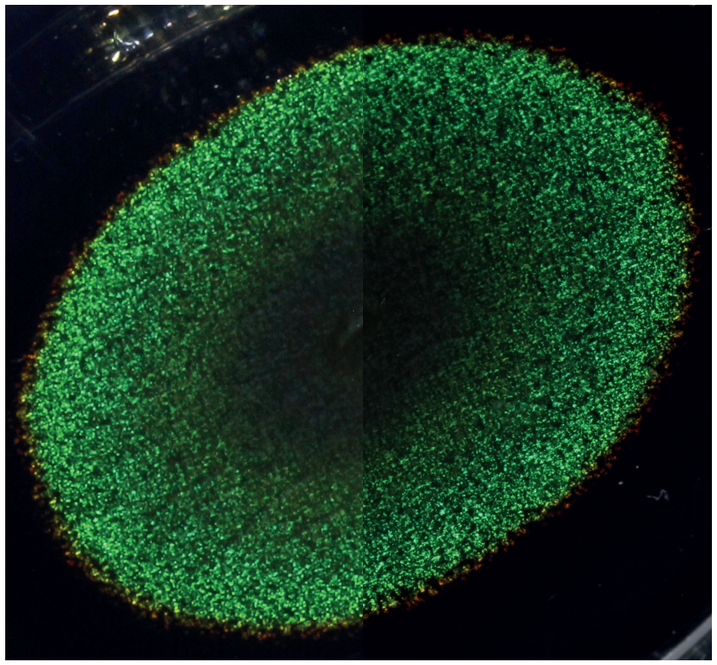


Fig. 5.17: The captured data of the old camera (left) and the new camera (right)

5.4 | CAPTURING DATA

Using the automated setup, data was captured for detailing the characterization of the appearance, sparking interest of other designers and for straightforward visualizations to communicate Flavobacteria's appearance. The settings of the setup were adjusted for each capture session. Within this subchapter, the different capture sessions are discussed.

The data for detailing the characterization of the appearance was captured with a Canon EOS 550d which was set to a shutter speed of 1.6 seconds, an aperture of f25 and iso800. As discussed on page 37, the automated setup was used to capture Flavobacteria for every 1˚ variation in the viewing and illumination angle. This data was used to determine the angle of the brightest retroreflection. Besides, the automated setup was used to capture the specular reflection of Flavobacteria. To reduce the reflections, these pictures were taken of a sample without the petri dish lid.

To spark interest of other designers, a movie was created that simulated a hand movement. The movie was not filmed by the camera but created with pictures and the stop motion technique. This was done because the movies filmed by the camera turned out too dark due to the shutter speed and because the stepper and servo motor moved step by step resulting in a bumpy movement. The pictures for the stop motion were captured with a Canon EOS 5DS, which was set to a shutter speed of 1.3 seconds, an aperture of f25 and iso1250.

To create data for the straightforward visualizations that communicate the appearance, a sample was captured for an entire week with the Canon EOS 550d. The camera was set to a shutter speed of 1.6 seconds, an aperture of f25 and iso800. It took a picture with an interval of 2 hours during the first 24 hours and an interval of 4 hours during the following 6 days. At every time interval the sample was captured 60 times while varying the tilt, the rotation and the azimuth angle. While translating this large set of data into straightforward visualizations (chapter 6), the capture session turned out to have some flaws. Therefore, an iteration was made for this acquisition of data. This second set of data was captured with the Canon EOS 5DS, which was set to a shutter speed of 1.6 seconds, an aperture of f25 and iso1250. This time, the camera captured the sample with an equal time interval of 3 hours to ease the visualization of the variable in the communication tool (chapter 6.3). Besides, the sample was also rotated in a flat position and captured. This data was needed to communicate the fact that the growth of natural spread flavobacteria is isotropic. The last acquisition of data was repeated with the exact same settings for a sample that was spread at the inoculation. This data was used to communicate the influences on the appearance and is elaborated on in chapter 7.

These large sets of data can also be used for the first computer graphic models and rending in the future.

6

Communicating the Appearance

This chapter discusses the design process of the communication tool, which creates straightforward visualizations to communicate Flavobacteria's appearance using the data of the automated setup. In order to find a proper way to communicate the temporal and iridescent appearance of Flavobacteria, existing methods on communicating time and/or angle-dependent data were analysed. After defining the requirements for the communication tool, an interface was designed using the existing methods as inspiration. Finally, a mock-up was of the communication tool created.

6.1 | EXISTING METHODS TO COMMUNICATE TIME- AND/OR ANGLE-DEPENDENT DATA

Several existing methods to communicate time- and/or angle-dependent data were analysed. The data that was communicated by these methods, varied from 3d models to pictures, movies and 2d graphic computer models. The methods gave insights on how to communicate data based on the variables.

More and more web shops include a 3d model of products which allow users to spin the object around by using their cursor. This method communicate data that is dependent on the view angle.

The biologist Johann Mourier and underwater photographer Laurent Ballesta collaborated to create a new documentary on the hunting behaviour of sharks (Cailloce, 2018). For the acquisition of data, 32 cameras were fixed around an arch of 4 meters in diameter, filming the scene from several angles simultaneously (fig. 6.1). This made it possible to observe predation scenes in stop motion from all angles. The data is visualized by showing the first part of the movie from one angle, then, shifting to another angle while pausing the movie and showing the final part. This method communicates data which is both time-dependent as dependent on the view angle.

Fig. 6.1: Acquisition of data using an arch of cameras (Ballesta, 2018)

The non-profit organization Cultural Heritage Imaging developed a technique called reflectance transformation imaging (RTI). This technique uses cameras and light to capture subtle surface details and generates detailed surface models of objects with interactive light settings ("Cultural Heritage Imaging | Reflectance Transformation Imaging (RTI)", 2020). To visualize these results, the picture is shown next to a control panel in which you can use your cursor to adjust the position of the light (fig. 6.2). This method communicates data that is dependent on the incident angle of the light.

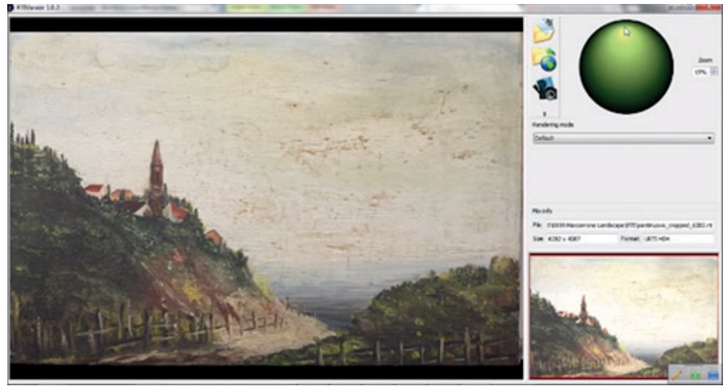


Fig. 6.2: The control panel for the position of the light next to the image (Cultural Heritage Science Open Source, 2014)

Tyler Sloan developed a computer model using Processing which simulates the growth of 19 species of algae under different circumstances (fig. 6.3). These circumstances, the temperature and the luminance, can be adjusted with sliders. The growth simulation can be restarted by clicking on the screen. This method communicates data that is dependent on time, illuminance and temperature.

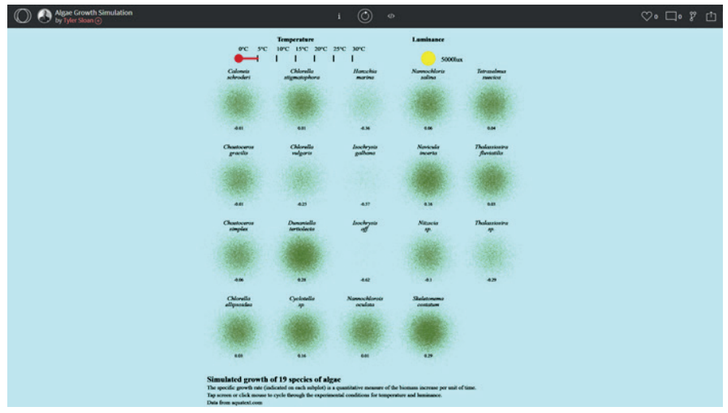


Fig. 6.3: The algae growth simulation (Sloan, 2018)

Dirk Brockmann developed a similar computer model for pattern formation in bacterial colonies (fig. 6.4). By adjusting the settings on the right, such as the initial amount of nutrients, the pattern can be influenced. This method communicates data that is dependent on time and other factors that affect the appearance such as the amount of nutrients.

Certain aspects of these relevant examples are implemented in the interface design of the communication tool (chapter 6.3).

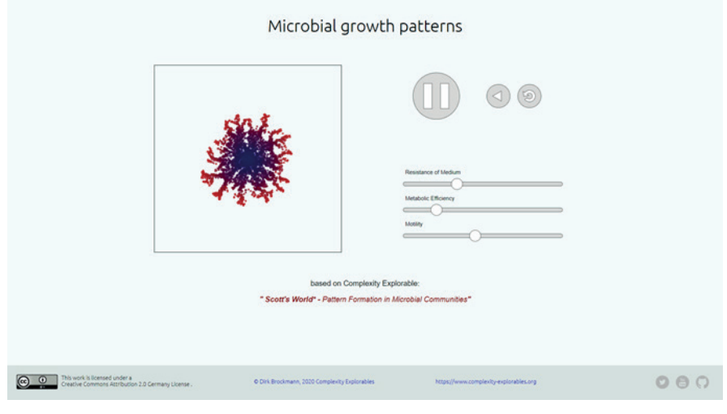


Fig. 6.4: The model for pattern formation in bacterial colonies (Brockmann, 2018)

6.2 | REQUIREMENTS

The main requirement of the communication tool is that it should create straightforward visualizations that communicate Flavobacteria's temporal and iridescent appearance by giving insights on the influences of the positions of the viewer and light and time.

To be more specific, the communication tool should:

- use the captured data from the automated setup as an input.
- visualize the data intuitively in order to be straightforward.
- visualize or communicate the variables of the setup (i.e., the tilt and rotation of the sample, the azimuth angle and the time) in order for the user to understand what changes when they manipulate the variables. This is needed for the user to be able to link the appearance to the variables.
- be interactive by allowing the user to control the variables, in order for the user to get a clear understanding of their influences in their own pace.
- be easy to access in order to reach as many designers and artists.
- be adjustable regarding the step sizes and limits of the variables to be able to also communicate data which is captured with different settings as discussed in chapter 5.4.

6.3 | INTERFACE OF THE COMMUNICATION TOOL

The communication tool will be an interactive webpage, showing the captured data and its variables. This way, it will be easily accessible for many designers and artists.

To visualize the variables of the data, the visual used throughout this report to show the settings of the gonioreflectormeter setup (fig. 3.7 on page 26), turned out to be not very useful. This is due to the fact that the sample is no longer in a fixed, flat position. Specifically, the sample is tilted and rotated in the automated setup while the altitude angles of the camera and the light are fixed. Therefore, the angles had to be visualized another way.

The designed interface can be seen in fig. 6.5 on the next page, which shows the interface for two different pictures as input.

Unlike the azimuth angle, the tilt and rotation of the sample can be seen in the captured pictures due to the shape of the petri dish. Therefore, these variables do not require a visualization next to the picture. Looking at the picture, it is not clear where the light comes from, therefore this should be visualized next to the picture in the interface, just like the light control of the RTI results (fig. 6.2 on page 59). A visual of the top view is added in order for the user to clearly see the azimuth angle. In this visual, an eye is added to indicate the position of the viewer relative to the light.

All variables are controlled by sliders, like the variables of the pattern formation model (fig. 6.4 on page 59). Sliders were assumed to result in an interactive tool and quick insights, as the user can easily play around with the variables. Besides, sliders make it easier to adjust the step size and limits of the variables compared to the clickable variables of the algae simulation model (fig. 6.3 on page 59). The slider that controls the azimuth angle is implemented in the top view visual. A visualized lamp can be used as the handle of this slider, making it even clearer that you are adjusting the azimuth angle.

Optical mixing (page 27-28) can be activated for the azimuth slider by checking the checkbox beneath the slider. This function will create the in-between results by blending the adjacent pictures and thus, make the slider continuous. Information about this function is provided when hovering over the information button with the cursor.

The slider for the variable time is placed on the bottom of the picture, as is usually done in videos. Besides, the variable time has the smallest step size, making it require a long slider. The variable time can also be auto-played, creating a stop motion of the growing Flavobacteria. Just like is done with the example of the shark hunting documentary, the angle of the data can be adjusted during the stop motion.

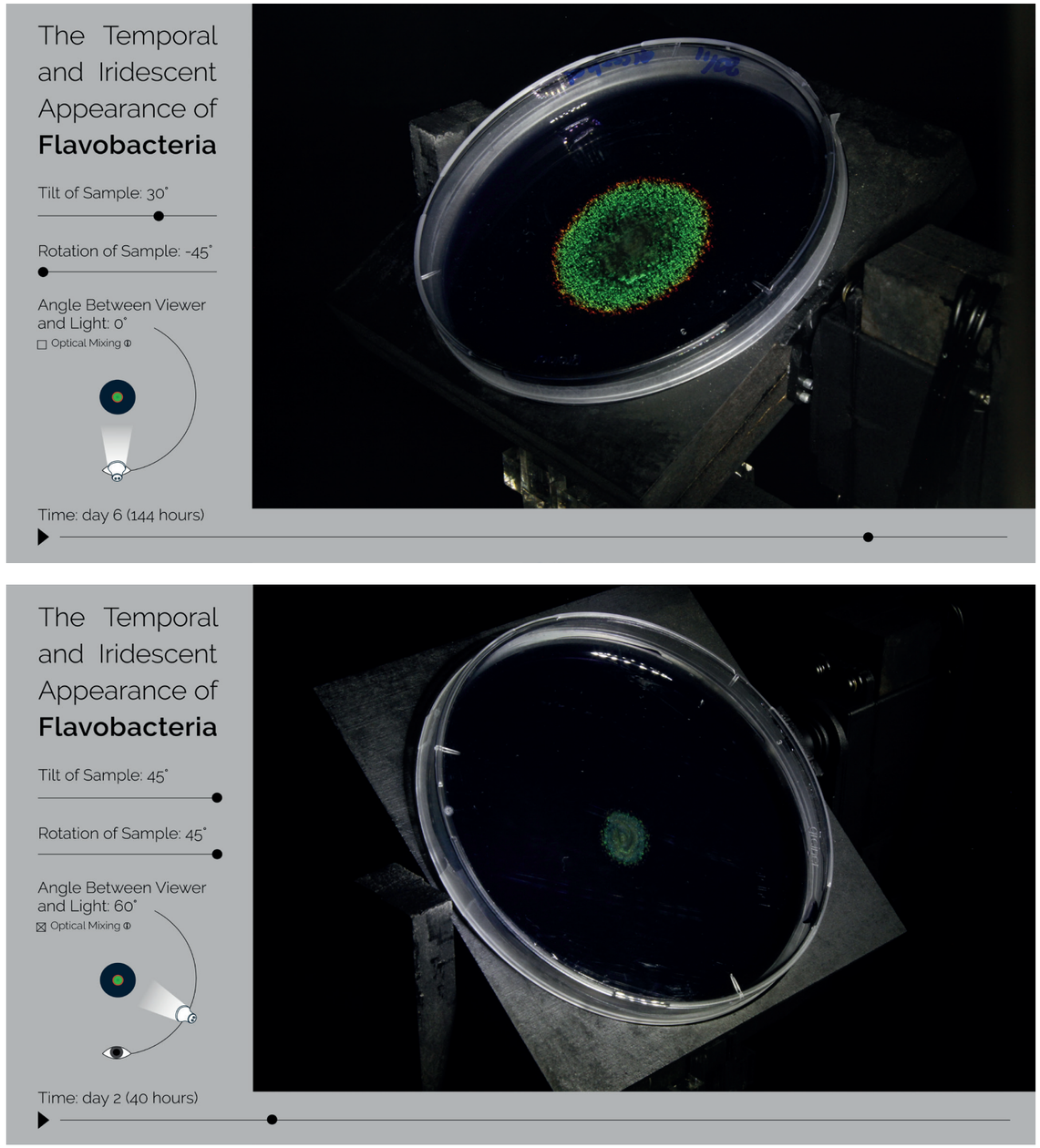


Fig. 6.5: The interface for the communication tool for two different pictures

6.4 | MOCK-UP OF THE COMMUNICATION TOOL

A mock-up was created of the communication tool using a combination of HTML, JavaScript and CSS programming. Within the HTML file, the basic structure of the webpage is determined. The layout of this structure is controlled by the CSS file. Through the JavaScript file, the interactive behaviour of the structure's different elements is controlled. The entire code is written within the software Atom and can be found in appendix M.

The mock-up (fig. 6.6) includes the stop motion which displays a time lapse of the Flavobacteria, the option to activate optical mixing and the sliders for the tilt and rotation of the sample, the azimuth angle and the time. However, the slider for the azimuth angle is simplified. This is the only element in the mock-up that differs from the designed interface in chapter 6.3.

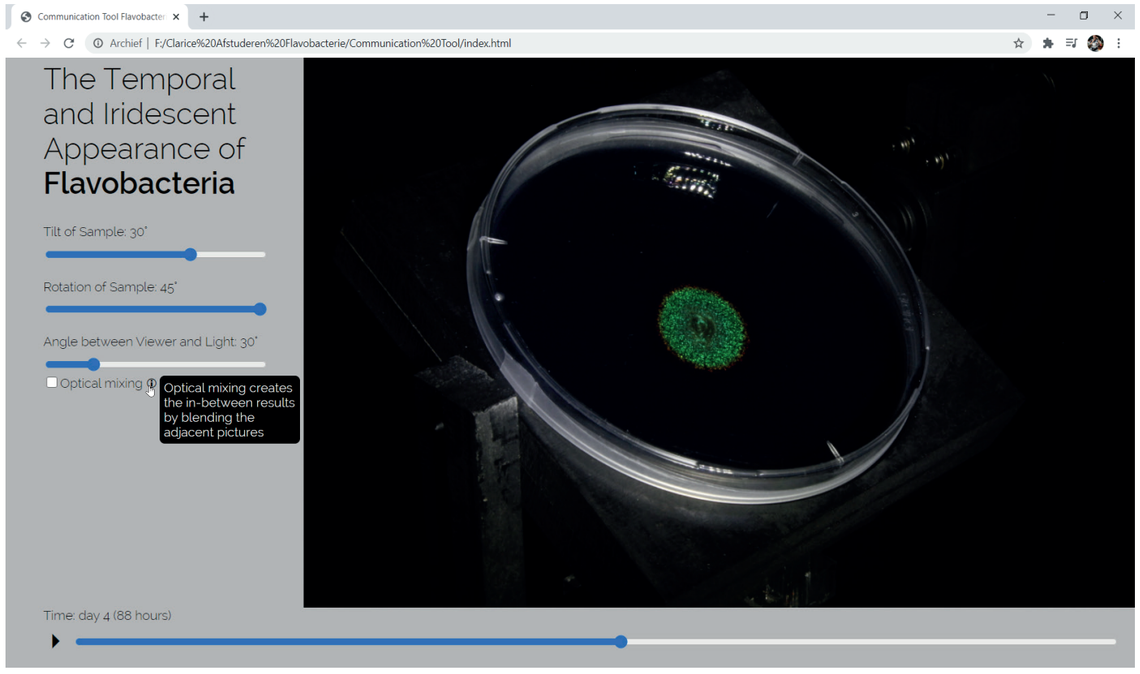


Fig. 6.6: A screenshot of the mock-up

The communication tool can be easily adjusted to visualize a different set of data by changing the values of the variables on top of the JavaScript file (fig. 6.7). These variables define the step sizes and limits of the variables as well as the location of the data and its lowest photo number. The lowest photo number enables the communication tool to calculate which photo it has to display.

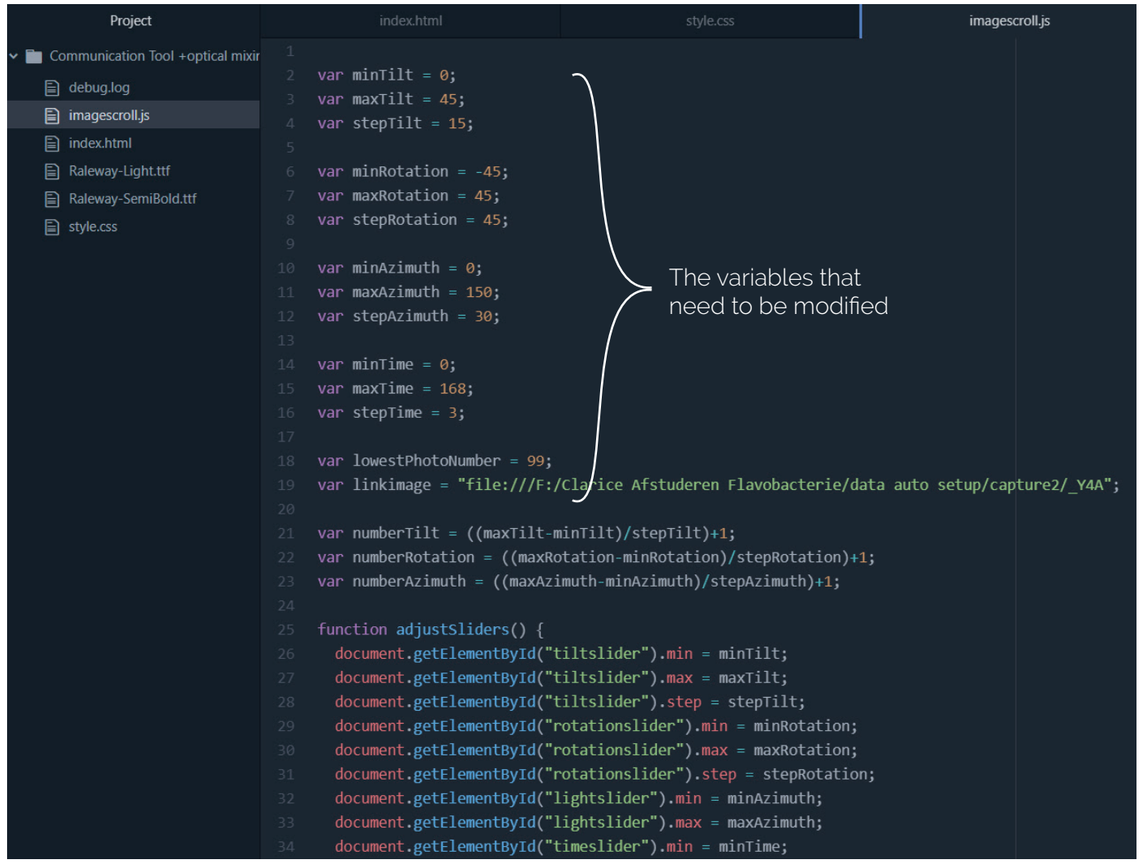


Fig. 6.7: The variables within the JavaScript file for changing the data set

7

Influences on the Appearance

The appearance of Flavobacteria is influenced by many different factors, such as for example the moisture level of the habitat. Ideally, the designers that will integrate Flavobacteria's structural colour effects in the future can steer the appearance to grow colours on artefacts to our own liking. This requires knowledge on how the appearance can be steered through adjusting factors that have a significant influence.

Literature research was conducted regarding influences on the appearance, as many research papers have already gained knowledge on this topic. However, more experiments are needed in the future to solve the still existing knowledge gap. Therefore, it is demonstrated how the automated setup and communication tool can contribute to these experiments by properly capturing and communicating the results.

7.1 | LITERATURE REGARDING INFLUENCES ON THE APPEARANCE

An expanding and iridescent Flavobacteria colony requires healthy bacteria cells. Therefore, not only the well-being but also the appearance of Flavobacteria depends on the habitat conditions. The amount of nutrients in the growth medium and the moisture level in the habitat are for example highly relevant for the appearance, as this influences the well-being of the bacteria cells.

The salinity and the agar concentration of the growth medium also affect the appearance of marine microbes, as stated in the research journal of Livingston (2019). Both a high salinity as a high agar concentration appeared to inhibit the growth of the colonies, while a low salinity or a low agar concentration inhibited the iridescence.

Kientz, Marié and Rosenfeld (2012) have researched the effect of light, gravity and storage temperature on the temporal qualities of Cellulophaga Lytica. They found that exposure to light and the plate orientation had no effect. However, the storage temperature did have a significant effect. After growing Flavobacteria for 24 hours at 25˚C, it was conserved several days at 4˚C. Afterwards, the colonies showed larger red edges, which were intensely iridescent.

Hamidjaja et al. (2020) have found that the cell organization causing the structural colour effects is related to the predation of Flavobacteria. The presence of other bacteria can therefore influence the appearance. As explained in the research paper, Flavobacteria invade colonies of other bacteria by infiltration, undercutting and hollowing it out. The Flavobacteria displayed an intense green colour within the attacked bacteria, Enterobacter Cloacae B12, suggesting a high degree of local organization due to this interaction. Flavobacteria also showed an increase of 60% in spreading rate from the moment it infiltrated the other colony.

The presence of other bacteria can besides predation lead to symbiosis, which also influences the appearance of Flavobacteria. The microbiologists of Hoekmine B.V. discovered that Flavobacteria can live with Sulfitobacter in symbiosis. The effect of this interaction on the appearance can be seen in fig. 7.1.

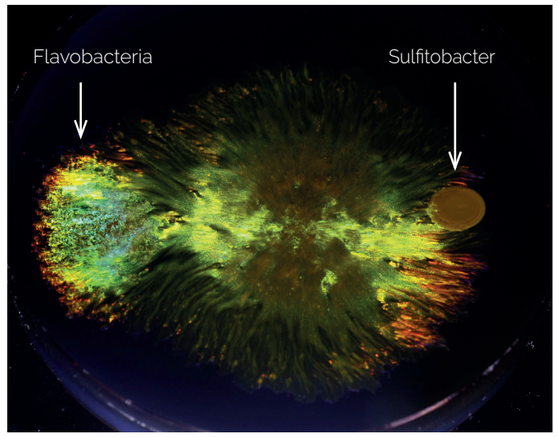


Fig. 7.1: A symbiosis between Flavobacteria isolated by Colin Ingham (left) and Sulfitobacter isolated by Sanne Schouten (right) (reproduced with permission from Hoekmine B.V.)

To summarize, the appearance of Flavobacteria is influenced by habitat conditions such as the ingredients of the growth medium, the temperature and the presence of other bacteria. However, not enough is known to be able to completely steer the appearance. For example, we know that changes in colour over time are linked to cell density (page 19), but we do not know yet how to control the cell density and thus, Flavobacteria's colour and appearance. Therefore, more experiments regarding influences on the appearance are needed in the future.

7.2 | CAPTURING AND COMMUNICATING EXPERIMENTS

To easily draw conclusions during experiments regarding influences on Flavobacteria's appearance, the results have to be captured and communicated in a consistent manner. This can be done using the automated setup and an adjusted version of the communication tool. In this version, the communication tool shows two samples next to each other to ease the comparison between the appearances. The sliders of the variables control both samples simultaneously. This version of the communication tool enables people to conduct experiments as the results can be easily visualized.

To demonstrate how the setup and adjusted communication tool can be used during experiments, such an experiment was conducted. The influence of spreading the bacteria on the appearance was chosen to dive into.

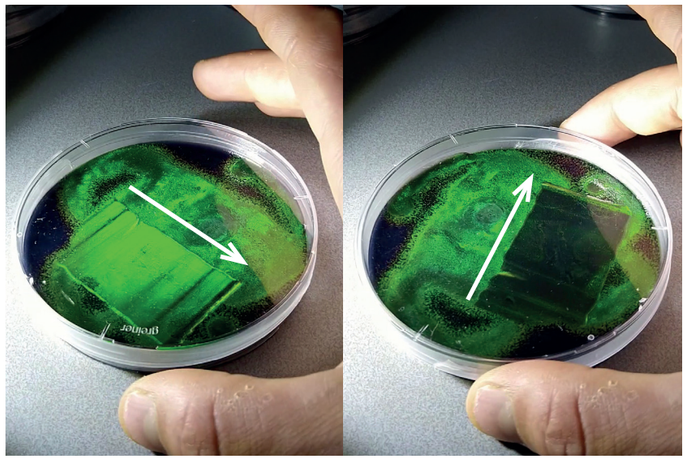


Fig. 7.2: The anisotropic appearance of spread Flavobacteria (reproduced with permission from Ward Groutars) with the spreading direction indicated in white

As mentioned in chapter 2.3, Flavobacteria can be spread to create a pattern (fig. 2.11 on page 17). Spreading the bacteria results in brighter colours from certain angles. More specifically, the structural colour effects appear brighter when light is reflected transverse to the spreading direction. While at the same time, little light is reflected along the spreading direction, with almost completely faded structural colour effects as result. Accordingly, the appearance of spread Flavobacteria is, unlike the natural grown Flavobacteria, not isotropic (fig. 7.2).

During the experiment to convey the influence of spreading on the appearance with the automated setup and adjusted communication tool, two samples of Flavobacteria were captured with the automated setup. One sample was naturally spread by the gliding motility and captured for a week. The other one was spread after 24 hours following the protocol (appendix C) and afterwards, placed in the automated setup for 6 days. The samples were captured in multiple rotated positions to be able to demonstrate the anisotropic appearance of the spread Flavobacteria.

Since one sample was spread 24 hours after the cultivation and only since then captured by the automated setup, the minimum time variable of the communication tool was set to 24 hours. To make this understandable, the communication tool was adjusted to show both the time after the cultivation as well as the time after the spreading. Besides, the optical mixing is left out, since this technique was only verified for the standard growth (page 27-28). Fig. 7.3 shows how the adjusted communication tool communicates the results of the experiment.

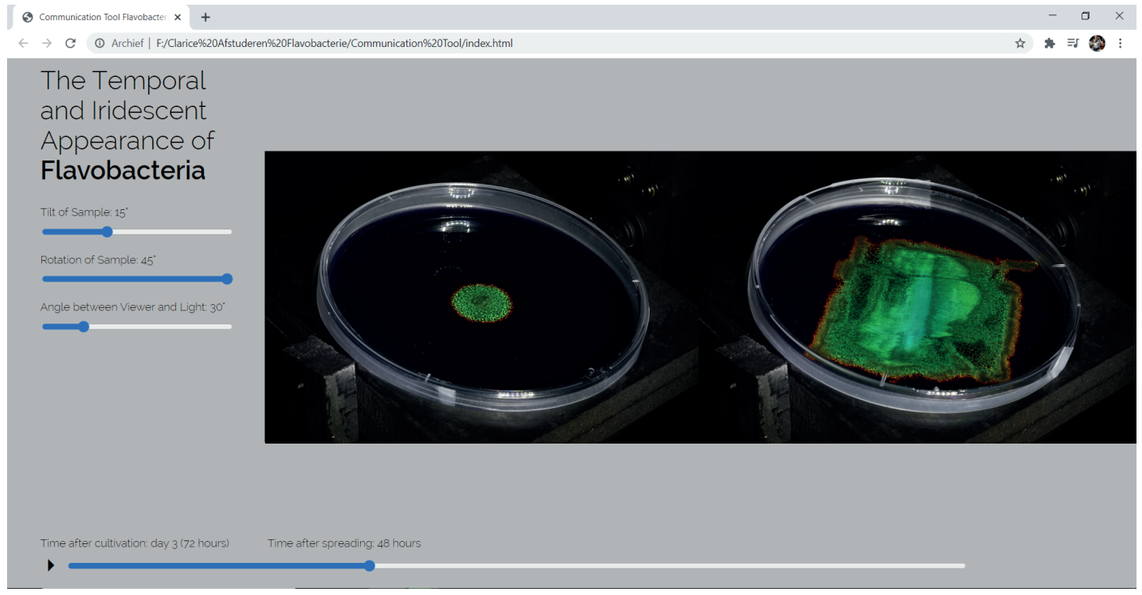


Fig. 7.3: The adjusted communication tool to convey the influence of spreading on the appearance

8 Design Guidelines

While working with Flavobacteria in the lab, characterizing the appearance of the standard growth and building a proper setup to capture its appearance, insights were gained that could be translated into design guidelines. As designing with living organisms requires knowledge on their needs and temporal qualities, these design guidelines are highly relevant and valuable for designers and artists that want to integrate Flavobacteria's structural colour into applications.

The design guidelines are divided into two sections. The first section considers the structural colour effects of Flavobacteria and suggests how the application can maximise the dazzling appearance. The second section of design guidelines focuses on the microorganism's needs as working with living organisms requires extra care and attention.

Design guidelines regarding Flavobacteria's structural colour effects:

- The application should be used close to a bright light, or implement one, as this is required for the structural colour to appear.
- The light that is close to the application or implemented in it, should emit white light that covers all of the visible wavelengths. This is important as it results in the most arising colours.
- The light that is close to the application or implemented in it, should ideally illuminate the Flavobacteria from (approximately) the same direction as the user. For example, by illuminating the Flavobacteria over the shoulder of the user. This is because the retroreflection results in the brightest colour.
- The user should ideally view the Flavobacteria from a viewing/illumination angle of approximately 45˚, as this results in the brightest colour.
- Ideally, specular reflections on the Flavobacteria's habitat should be avoided.

While searching for applications through which unique user experiences and meanings will be elicited, it is important to remember that:

- The Flavobacteria show a glittering iridescent appearance when the positions of the viewer or light is varied.
- Young bacteria cells, which are present in the edge of a colony or throughout young colonies, show the most interesting colour changes while varying the positions of the viewer or light.

Design guidelines regarding Flavobacteria's needs:

- The application should offer Flavobacteria a sterile habitat as Flavobacteria are sensitive to several other microorganisms.
- The application should offer Flavobacteria consistent and ideal habitat conditions (e.g., the level of moisture, temperature, etcetera) as Flavobacteria are sensitive to their habitat conditions.
- The application should offer Flavobacteria nutrients at all times, as Flavobacteria die when they run out of nutrients. Living, healthy Flavobacteria are needed for the structural colour effects.

9

Recommendations

Days where we grow colour on our artefacts, are not there yet. But with all the relevant research being conducted, the integration of Flavobacteria's colour into applications is getting closer. Throughout the project, recommendations regarding further research on Flavobacteria were gathered, that will contribute to the knowledge gaps and thus, to the development of Flavobacteria as an alternative, sustainable way of colouring artefacts.

The recommendations regarding further research on Flavobacteria are linked to the knowledge gap(s) they will contribute to, as discussed on page 8.

Communication

- Calibrate the colours in the captured data by photographing a colour checker and editing the data digitally. Subsequently, extensive colour analyses can be performed to create a graphic representation of how the Flavobacteria's dominant colour changes over time.
- Capture Flavobacteria from a great deal of different angles and use this data to create a graphic model of the iridescent appearance. A graphic model can, for example, be realised by an interactive tool that shows the appearing colours of the Flavobacteria from different angles on a sphere, in which the lights are controlled by the cursor. This graphic model can be verified by growing Flavobacteria on a curved surface.
- Improve the communicating tool by changing the default slider of the azimuth angle into the proposed visualization (chapter 6.3).

Perception

- Conduct an experiential characterization study as described in the MDD method, while evaluating the communication tool. This can be done by analysing people's perception towards either a real sample of Flavobacteria or the communication tool and comparing the results. This study will determine how realistic the representation is and point out Flavobacteria's experiential qualities, which are useful for finding proper application areas.
- Conduct research on how to create realistic computer graphic models and renders for iridescence materials.

- Use the communication tool to find out what people find the most interesting of the appearance by tracking what sliders are used most.

Application areas

- Conduct the 2nd, 3rd and 4th steps of the MDD method (creation of experience vision, experience patterns and potential application areas through which unique user experiences and meanings will be elicited).

Properties

- Tinker with Flavobacteria in the lab to determine the influences on the appearance and therefore, how to steer the appearance and to find possible substrates. Findings might lead to wider applications areas.
- Conduct research to find a sustainable, efficient way to fixate the colour. This would lead to different application areas in which living cells are not required.
- Determine the mechanical and thermal properties of Flavobacteria on different substrates. Findings might lead to restricted application areas.

- Experiment with the effect of diffused light on Flavobacteria's appearance. This will presumably lead to less contrast in the colony, but more brightness as more sparkles might lit up.

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Personal Project Brief - IDE Master Graduation

TU Delft

Growing, capturing and communicating colours created by Flavobacte project title

Please state the title of your graduation project (above) and the start date and end date (below). Keep the title compact and simple. Do not use abbreviations. The remainder of this document allows you to define and clarify your graduation project.

start date 17 - 08 - 2020 22 - 01 - 2021 end date

INTRODUCTION **

Please describe, the context of your project, and address the main stakeholders (interests) within this context in a concise yet complete manner. Who are involved, what do they value and how do they currently operate within the given context? What are the main opportunities and limitations you are currently aware of (cultural- and social norms, resources (time, money,...), technology, ...).

Nature's light manipulation strategies—in particular those at the origin of bright iridescent colours —have fascinated humans for centuries. In recent decades, insights into the fundamental concepts and physics underlying biological light-matter interactions have enabled a cascade of attempts to copy nature's optical strategies in synthetic structurally coloured materials. However, we tend to create these materials via methods that have little in common with the processes used by biology (McDougal et al., 2019).

These attempts to copy nature's optical strategies often result in materials that contribute to resource scarity, have a large amount of invested energy and/or are not ideal for recyclability. In the field of biodesign designers, artists and engineers are collaborating with living organisms to produce new materials with ecological benefits. The Flavobacteria turns out to be useful for creating structural colour and could serve as a sustainable alternative to petroleum-based or mined pigments and dyes. The bacteria shows a formidable capacity and flexibility to organize as a colony, drastically modifying its optical appearance in terms of spectral and angular response under different growth conditions and with genetic modification (Johansen et al, 2018). Structural colour created by the Flavobacteria can be seen in figure 1.

In this project, we consider 'Flavobacteria' as a novel living material for design, which is aimed to be incorporated in the design process. But how would designers design with such living materials, which can sense, grow, adopt and eventually die? How would they understand its unique temporal qualities? Because of the complex nature of the structural colour created by the Flavobacteria its properties are to be further defined and better communicated between the designers, artists and engineers. This project will involve creating a better understanding of the material, including more knowledge on the influences and time-changing abilities of the colour. This knowledge will be gained by tinkering with the material following the first part of the material driven design (MDD) method as developed by E. Karana in 2015 (figure 2) as guidance and by literature research. Knowledge gained by the microbiologists of Hoekmine B.V., who have been working with the bacteria for 10 years, will serve as a starting point. Secondly, the project will involve creating a setup to capture and visualize the angle-dependent vibrant colours in order to communicate the properties and potential of this new material, using the expertise and research of Willemijn Elkhuizen on capturing material appearances as guidance. Furthermore, since the material is novel, it is in need of the designer to generate potential application areas.

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Personal Project Brief - IDE Master Graduation

TU Delft

introduction (continued): space for images

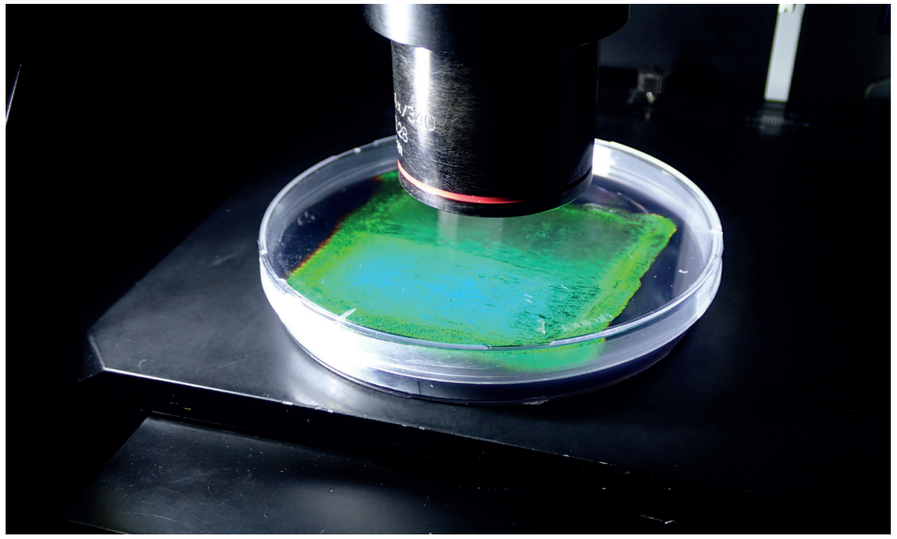

image / figure 1: Structural colour created by the Flavobacteria

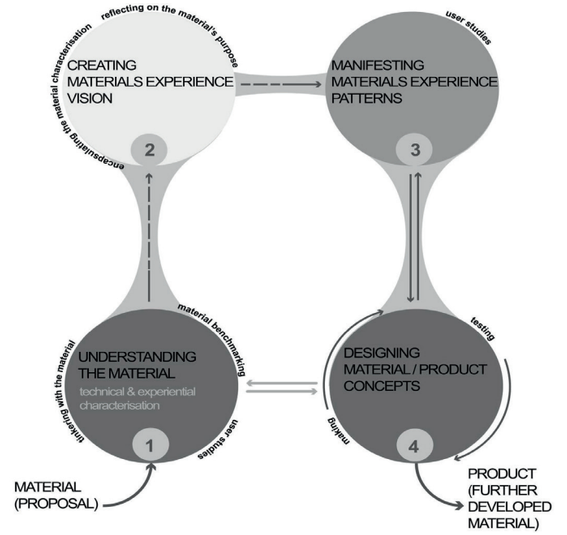

image / figure 2: The material driven design (MDD) method (Karana et al, 2015)

Personal Project Brief - IDE Master Graduation

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PROBLEM DEFINITION **

Limit and define the scope and solution space of your project to one that is manageable within one Master Graduation Project of 30 EC (= 20 full time weeks or 100 working days) and clearly indicate what issue(s) should be addressed in this project.

The aim of the project is to prepare the structurally coloured material for future use by:

- conducting literature research and lab experiments to understand variables that influence appearance
- creating a setup to capture and communicate the grown appearances
- generating potential application areas, which would exemplify the unique appearance properties of the material

Literature research and experiments with the Flavobacteria will lead to a better understanding of the material. Technical characterization studies will help us understand the temporal qualities of the Flavobacteria (i.e., how the appearance changes over time) and its influences. Some experiential studies will also be run to investigate how the material is preceived (i.e., what it makes people think, feel and do). This knowlegde on the propeties and experience of the material will help to solve the current research gap and further develop the material, which is essential for future use.

Currently, the angle-dependent colours created by the Flavobacteria are communicated using graphs and pictures. The setup to capture and visualize the vibrant colours will improve this communication and help designers to get a better understanding of the Flavobacteria's possibilities. The setup will contribute to the future use of the material by communicating the possibilities of the new material in a catchy way and thereby improving the communication between the designers, artists and engineers.

ASSIGNMENT **

State in 2 or 3 sentences what you are going to research, design, create and / or generate, that will solve (part of) the issue(s) pointed out in "problem definition". Then illustrate this assignment by indicating what kind of solution you expect and / or aim to deliver, for instance: a product, a product-service combination, a strategy illustrated through product or product-service combination ideas, ... In case of a Specialisation and/or Annotation, make sure the assignment reflects this/these.

Growing, researching and tinkering with the Flavobacteria, which creates vibrant colour appearances, in order to further define the material's properties. While at the same time, designing, prototyping and evaluating a setup to capture and visualize these grown appearances in order to communicate the potential of this new material.

The expected results of this assignment are:

- The findings on the literature research and technical and experiential characterization that contribute to the better understanding of the material and help solve the current research gap.
- A prototyped and evaluated setup to capture and visualize the vibrant colours. The setup will be evaluated by comparing the response of 2 groups of people who received either graphs and photos or outputs of the setup.
- A communication of grown appearances (e.g. in form of an interactive video) created by the setup.
- Insight on potential application areas which could be used as a starting point for design projects in the future. To keep the target group of designers broad, several different application areas will be expected. This is to ensure that the material can grow to its full potential in as many design fields as possible.
- Design guidelines for designing with Flavobacteria that will instruct the designers who will incorporate this new material in the design process.

Initials & Name C.C. Risseeuw Student number 4438426

Title of Project Growing, capturing and communicating colours created by Flavobacteria

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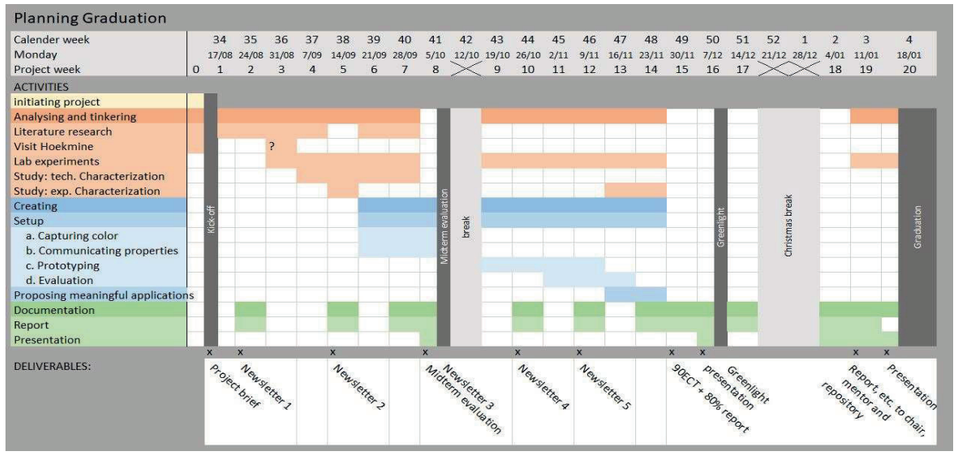
PLANNING AND APPROACH **

Include a Gantt Chart (replace the example below - more examples can be found in Manual 2) that shows the different phases of your project, deliverables you have in mind, meetings, and how you plan to spend your time. Please note that all activities should fit within the given net time of 30 EC = 20 full time weeks or 100 working days, and your planning should include a kick-off meeting, mid-term meeting, green light meeting and graduation ceremony. Illustrate your Gantt Chart by, for instance, explaining your approach, and please indicate periods of part-time activities and/or periods of not spending time on your graduation project, if any, for instance because of holidays or parallel activities.

start date 17 - 8 - 2020 22 - 1 - 2021 end date



The project will start after the kick-off meeting in mid august with conducting literature research and possibly visiting Hoekmine B.V. for a second time. Once the lab in Delft is ready to grow structural colours, the lab experiments can start.

The technical characterization studies will be run before the midterm. The experiential studies will be run twice, once before creating the setup, and once during or after the evaluation of the setup. The lab experiments will continue since living, colorful bacteria are preferred for the capture-setup.

After the midterm evaluation a short break is scheduled to relax a bit and be able to keep on the hard work.

The greenlight meeting will be just before the christmas break, which will give enough time to make the report and presentation, and perhaps gather some presentation material from the lab, before the graduation ceremony in the end of January.

Initials & Name C.C. Risseeuw Student number 4438426

Title of Project Growing, capturing and communicating colours created by Flavobacteria

Appendix A | Project Brief

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Personal Project Brief - IDE Master Graduation

MOTIVATION AND PERSONAL AMBITIONS
Explain why you set up this project, what competences you want to prove and learn. For example: acquired competences from your MSc programme, the elective semester, extra-curricular activities (etc.) and point out the competences you have yet developed. Optionally, describe which personal learning ambitions you explicitly want to address in this project, on top of the learning objectives of the Graduation Project, such as: in depth knowledge a on specific subject, broadening your competences or experimenting with a specific tool and/or methodology, ... Stick to no more than five ambitions.

In addition to design, I have a passion for nature. In my spare time, you will either find me outside or taking care of the plants in my room (approximately 80). As a result of these interests in design and nature, I wanted to do a graduation project related to bio-based design. Besides, as a true industrial designer student, I really like colours. I've been painting and colouring since I was a little kid and last year, I really enjoyed the course Basic Colour Skills during the master electives. Because this project is about creating colour with living materials, the assignment fits my wishes very well.

I hope to learn more about the iridescent colour effects created by the Flavobacteria and the material driven design method. I expect that this project will take my knowledge about the composition of colour and the creation of it to a higher, professional level.

By working with the material driven design method, I expect to learn how to see the material and its properties as the starting point of a design project. This is especially a valuable method for new sustainable materials in the circular economy of the future. Since I want to work within this field, getting acquainted with this method during my graduation project is very beneficial.

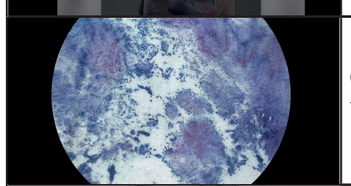
At last, I feel excited to prove the negative perception of bacteria, that most people have, wrong by showing that bacteria can also be practical and create beautiful things.

FINAL COMMENTS
In case your project brief needs final comments, please add any information you think is relevant.

Initials & Name C.C. Risseeuw Student number 4438426

Title of Project Growing, capturing and communicating colours created by Flavobacteria

Appendix B | Projects of Benchmarking Analysis

Image	Desciption	Source
	Spiruwheel by atelier SUMIOSIS is a dyeing process that uses colourants made by algae.	https://atelier-sumbiosis.com/Spiruwheel
	Algaemy textiles by Rosa Weber are pieces of textiles that changes over time when exposed to sunlight by incorporating algae.	https://www.rasaweber.com/algaemy-textiles
	Biogarmentry by Roya Aghighi is a piece of clothing that uses living photosynthetic microalgae cells to create patterns and purify the air.	http://www.royaaghighi.com/biogarmentry.html
	Fabulous Fungi by Ilse Kremer is a fungal dye created by extracting colourants made by fungi.	https://www.ilsekremer.nl/fabulous-fungi.html
	Contagion by CURB media, ad agency Lowe Roche, microbiologists and immunologists is a dynamic billboard to publicize Steven Soderburgh's 2011 film Contagion	Myers, W. (2012). Bio design. London: Thames & Hudson.
	Coelicolor by Faber Futures LAB is a bacteria dye created by extracting colourants made by bacteria.	https://faberfutures.com/projects/project-coelicolor/
	Phili is a biotech company in France that uses colourants made by bacteria to create colours for the textile industry on a large scale.	https://www.pili.bio/
	Living Colour by Laura Luchtman & Ilfa Siebenhaar is a bio design research project exploring the possibilities of natural textile dyeing with bacteria that produce pigment	https://livingcolour.eu/

A protocol was written in order for designers and artists to understand how to grow flavobacteria.

Sterile work environment

Always make sure you are wearing a lab coat and nitrile gloves (fig. C.1) and clean your workspace with propanol before and after each experiment (fig. C.2). A sterile work environment is being created by a laminar airflow cabinet or a Bunsen burner (fig. C.3). After each experiment, throw away all your waste and wash all used equipment (fig. C.4). Biowaste should be thrown in the bin for biomaterial waste after making it sterile with the autoclave or pressure cooker (fig. C.5).

Fig. C.1: Lab coat and nitrile gloves

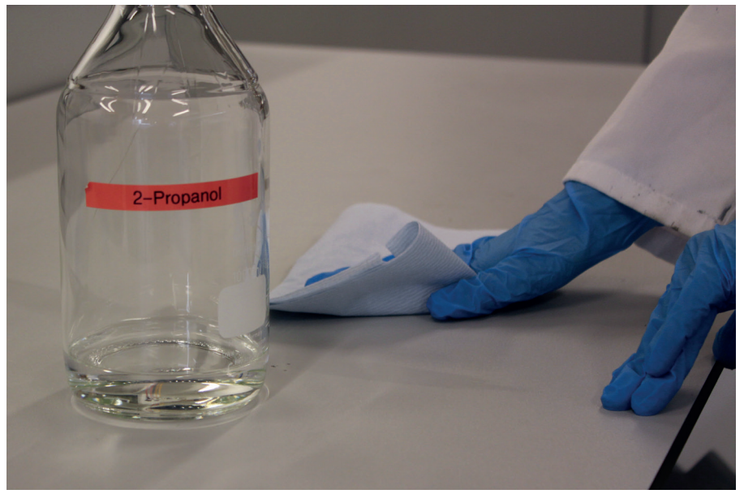


Fig. C.2: Cleaning with propanol

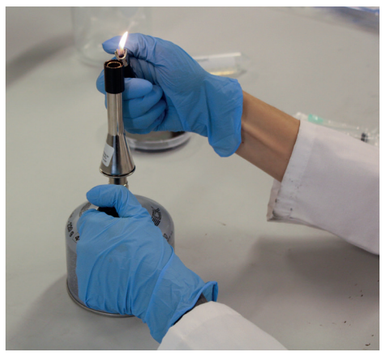


Fig. C.3: Bunsen burner

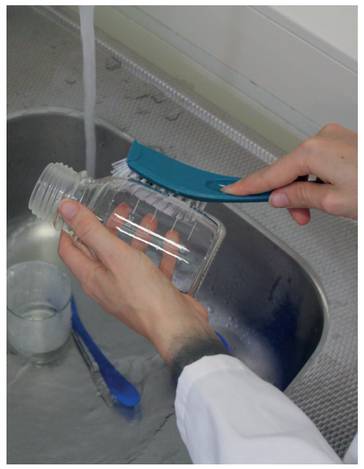


Fig. C.4: Washing equipment

Fig. C.5: Disposing biowaste

Preparation of the growth medium

Growth medium is a solid or liquid substance which provides the bacteria with nutrients and a place to grow. Culture media is one of the five basic types of growth media and is used for the Flavobacteria.

For the Flavobacteria two different types of culture growth media are used:
- MAR medium to keep the Flavobacteria alive as long as possible. This is used for the bacteria stock at room temperature.
- MAR+ medium to provide the Flavobacteria with a lot of nutrients to grow fast and create lots of colours. This is used for the tests and analyses of the material appearance.

The difference between the growth media is the amount of yeast (respectively, 100 mg versus 1 g).

The growth media can be made solid by adding agar, which is extracted from seaweed. Agar allows the growth medium to be poured into petri dishes and hold it shape once it is set (polymerized). This is needed to analyse and capture the structural colour. Also a pigment can be added, nigrosine, to make the medium black so that the bacteria and its colour can be seen and analysed better. Petri dishes containing growth medium are always stored upside down to avoid condensation on the lid.

The ingredients (fig. C.6):
- 1 g peptone (nutrient)
- 1 g/100 mg yeast extract (nutrient)
- 13 g sea salt (salt)
- 50 mg $MgSO_4$ (salt)
- 10 mg KNO_3 (salt)
- 6 g agar
- 100 mg nigrosine
- 400 ml demineralized or distilled water

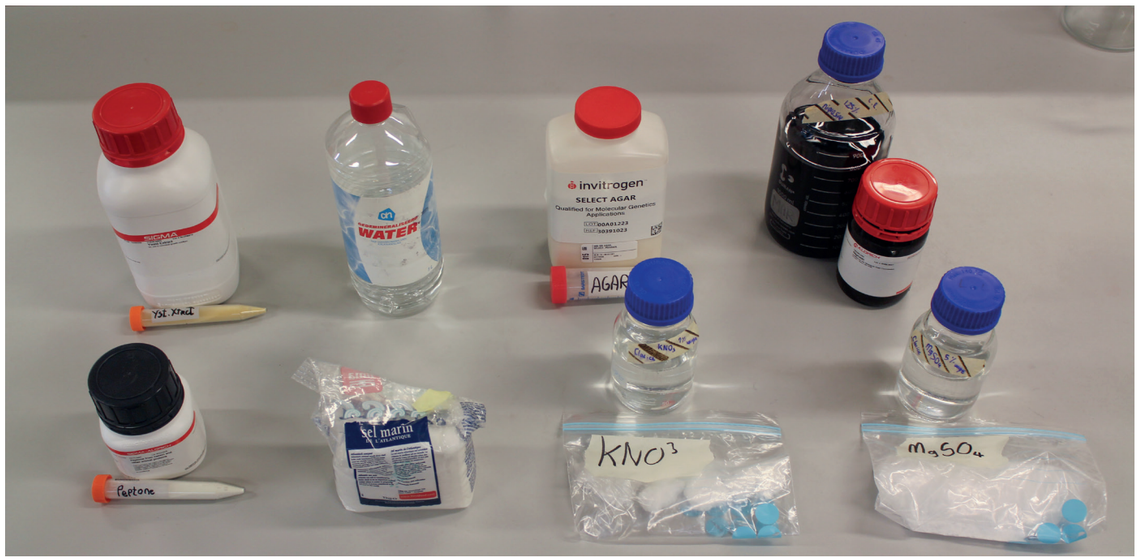


Fig. C.6: The ingredients for the growth media of Flavobacteria

The steps (fig. C.7-C.11):

1. Clean some spoons, a few beakers, a funnel and a 500 ml bottle for the medium with hot water. The bottle should also be rinsed with demineralized water. Dry the equipment with clean paper.
2. Weigh all the ingredients and deposit them in the glass bottle using the funnel. Use the precise scale for the small amounts (e.g. the MgSO4) and a clean spoon for every ingredient. Tare the scale with the bottle after each ingredient to remain accuracy. Use the demineralized water as final ingredient to rinse all the leftover ingredients from the funnel into the bottle.
3. Shake the bottle well and add some sterilization tape. Write the type of medium, date and your name on it.
4. Sterilize the growth medium by using an autoclave or a pressure cooker. Always make sure the cap of the bottle is loosely on the bottle so that the air can escape.
5. Mark the lid of the petri dishes with the type of medium and your name.
6. Create a sterile work environment. Shake the bottle thoroughly, (in case of a Bunsen burner, sterilize the opening with the flame) and pour it into the petri dishes.
7. Spread the petri dishes out on a table to fasten the polymerization. Once they are set, turn the petri dishes upside down.

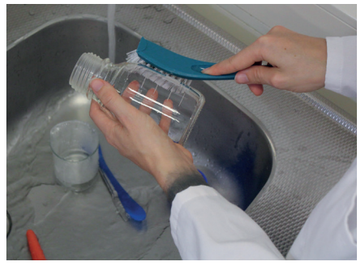


Fig. C.7: Step 1: cleaning

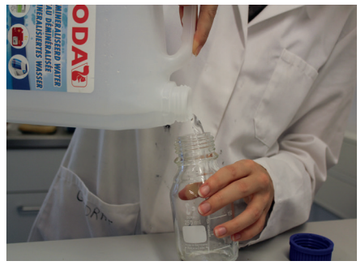


Fig. C.8: Step 1: rinsing with demineralized water

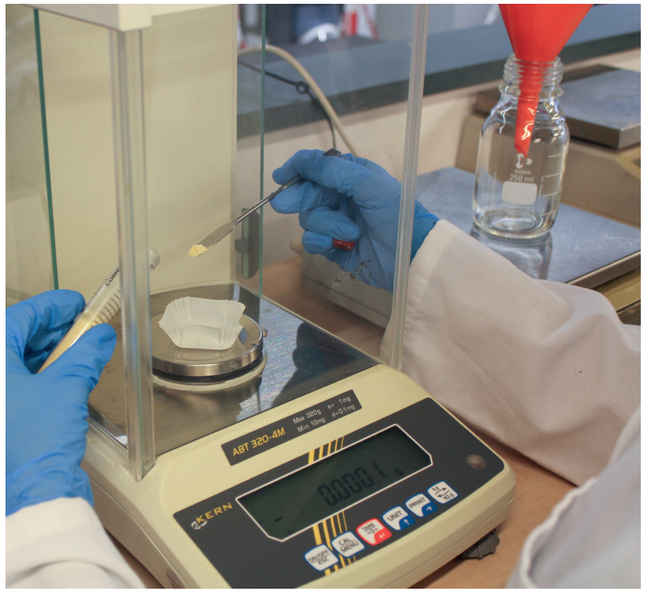


Fig. C.9: Step 2: weighing a small amount on the precise scale

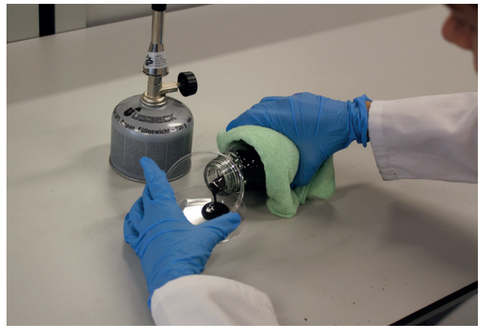


Fig. C.10: Step 6: pouring into the petri dishes

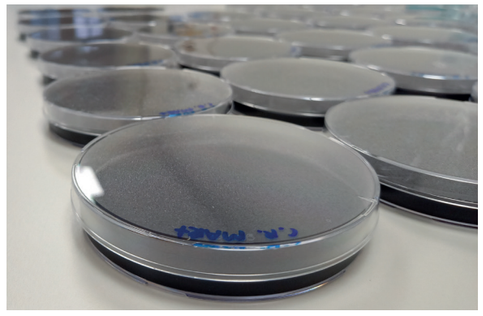


Fig. C.11: Step 7: spread petri dishes

Cutlivating the bacteria

The stock bacteria are growing on a MAR petri dish or frozen (-80°C or -20°C) in a MAR + glycerol solution.

The steps (fig. C.12-C.15):

1. A) Stock on MAR petri dish
   Create a sterile workspace and use a inoculation loop to derive bacteria from the MAR petri dish making a stroke of approximately 2 mm (depending on the thickness of the bacteria) on the colony's edge.
   B) Frozen stock
   Create a sterile workspace and take the bacteria out of freezer. In case of a metal inoculation loop, briefly heat it in the flame and use it to derive some bacteria of the frozen stock. In case of a plastic inoculation loop, melt the stock of bacteria and use the loop to derive the bacteria from the melted stock.
2. Make a tiny circle on growth medium in the new petri dish with the inoculation loop. Close the petri dish as quickly as possible.
3. Close the stock as quickly as possible, mark that it has been opened and put it back where it came from.
4. Close the new petri dishes with parafilm and mark the lid of them with the date and possibly with an experiment number.

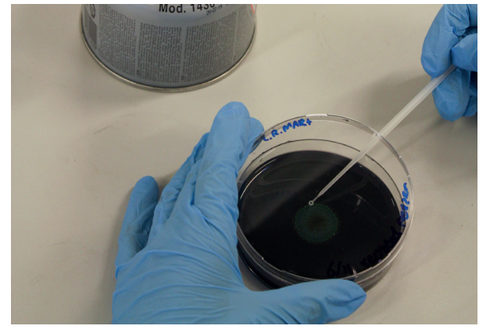


Fig. C.12: Step 1A: deriving bacteria from petri dish

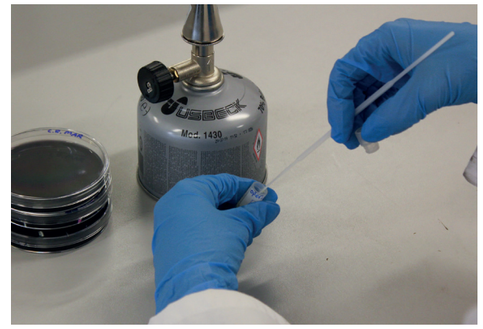


Fig. C.13: Step 1B: deriving bacteria from frozen stock

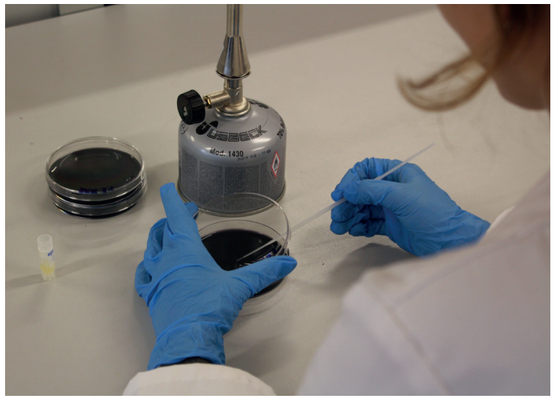


Fig. C.14: Step 2: making a tiny circle on growth medium in the new petri dish with the inoculation loop

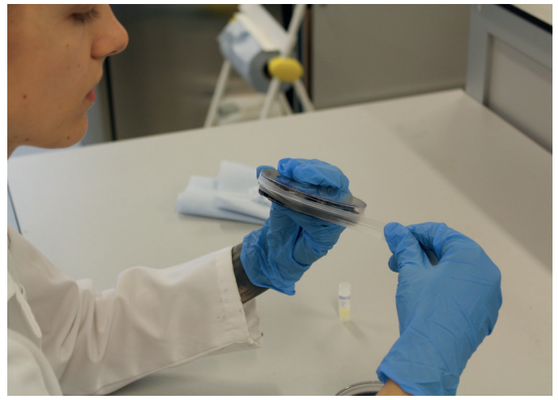


Fig. C.15: Step 4: closing the petri dish with parafilm

Growing

Let the bacteria grow while storing the petri dish upside down at a temperature between 10 and 30°C, preferably at room temperature.

Appendix C | Protocol Flavobacteria

Spreading (optional)

The bacteria can be spread to create a pattern after one day of growth. This can for instance be done with a brush, inoculation spreader or with small drops of liquid medium.

The steps:
1. Create a sterile workspace and sterilize the spreading tool with propanol in case you are using a non-sterile tool, such as a brush. Give the spreading tool one minute to make sure all the leftover propanol is evaporated.
2. Spread the bacteria in the petri dish with the spreading tool. Make sure not to put too much pressure as the agar will be damaged.
3. Close the petri dish again with parafilm.

Making a freezer stock

A stock of flavobacteria in the freezer requires less care and attention and will stay healthy for at least 2 months in -20˚C and for many years in -80˚C. To make a freezer stock, bacteria are dissolved in a solution of liquid MAR+ growth medium and glycerol. Glycerol is added to avoid water crystals, which are too sharp and can damage the bacteria cells. The steps (fig. C.16-C.19):
1. Create a sterile work environment and add 4ml of the MAR+ glycerol solution to a colourful petri dish. Let this rest for a minute.
2. Use an inoculation spreader to carefully mix the bacteria with the solution.
3. Derive the solution mixed with the bacteria with a sterile pipette and put them in a tube container.
4. Mark the tube container with a letter for example and put them in a glass marked with “Flavobacteria [your name] [the date]”.

Fig. C.16: Step 1: adding 4ml of the solution to a colourful petri dish

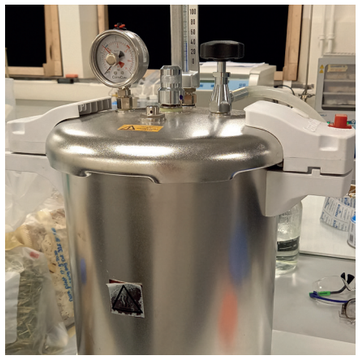
Fig. C.17: Step 2: mixing the bacteria with using an inoculation spreader

Fig. C.18: Step 3: deriving the bacteria with a sterile pipette

Fig. C.19: Step 3: putting the bacteria in a tube container

Appendix D | List of Equipment

Autoclave



A machine that uses high temperature (121˚C), delivered by pressurized steam, to kill microorganisms and thereby provide sterilization

Bunsen burner

A burner that can provide a blue flame to create a sterile field by creating an updraft which pushes dust and microbes away

Inoculation loop

A simple tool used to pick up and transfer microorganisms

Inoculation spreader

A simple tool used to spread microorganisms

Lab coat

Coat which has to be worn in the lab

Nitrile gloves

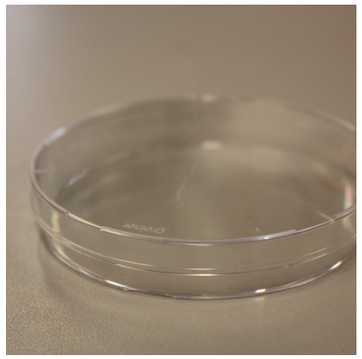
Glove used to protect both the designer as the microorganisms

Appendix D | List of Equipment

Parafilm

Tape used to close the petri dish, which allows only air to pass through, keeping the habitat sterile

Petri dish

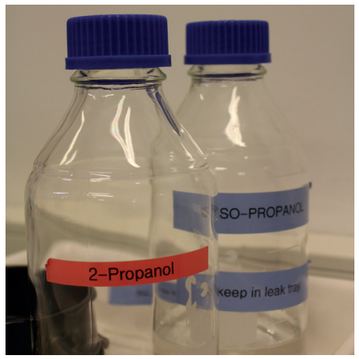


A transparent lidded dish used to hold growth medium,v in which the bacteria cells can be cultivated

Pressure cooker

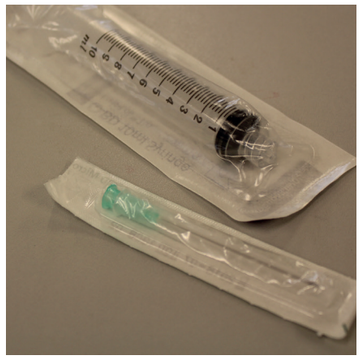
A machine that can be used as an autoclave by using the steam function for 30 minutes

Propanol



Liquid alcohol used to sterilize the work area

Sterile pipette



A simple tool used to derive liquid solutions

Sterilization bags

Bags used to sterilize biowaste that resist temperatures used in the sterilization process

Appendix D | List of Equipment

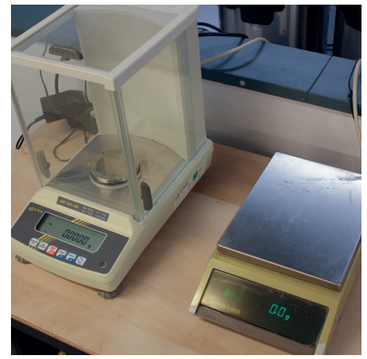
Sterilization tape

Tape which changes colour after being exposed to temperatures used in the sterilization process and is used to validate the sterilization

Tube container

A small plastic container used to store liquids in the freezer

Weighing scales



Scales used in the lab to measure the weight of small amount of ingredients

Appendix E | Preparation experiment: -20 ˚C stock

Goal: Finding out whether the bacteria stored in the -20˚C freezer for two months are still healthy

How: Comparing the growth of the bacteria on MAR+ petri dishes of the -20˚C stock with the growth of new bacteria from Hoekmine B.V.

Step by step:
1. Inoculate 3 MAR+ petri dishes with bacteria from the -20˚C stock
2. Inoculate 3 MAR+ petri dishes with healthy Hoekmine B.V. bacteria
3. Observe and compare the growth of bacteria of both groups

Results: 2 out of 3 petri dishes that were inoculated with the -20˚C stock were spread by condensation (fig. E.1). These petri dishes showed a colourful colony after two days, with its peak on day 3. The 3rd petri dish inoculated with the -20˚C stock took some more time to develop a colourful colony and was comparable with the petri dishes inoculated with the Hoekmine B.V. bacteria regarding size, colour and iridescence. Both groups showed colourful colonies and glitter-like iridescence (fig. E.2), proving that the -20˚C stock still contained healthy bacteria after 2 months.

Discussion:
In 2/3 petri dishes from freezer stock the bacteria were spread by condensation. This lead to faster creation of colour, making it difficult to compare the growth of the two groups.
The amount of inoculated bacteria was hard to define, since the freezer stock was liquid and the Hoekmine B.V. bacteria were derived from a petri dish. Less cultivated bacteria might require more time to multiply into a colourful colony but on the other hand there are more nutrients available. This might have influenced the growth of the bacteria.

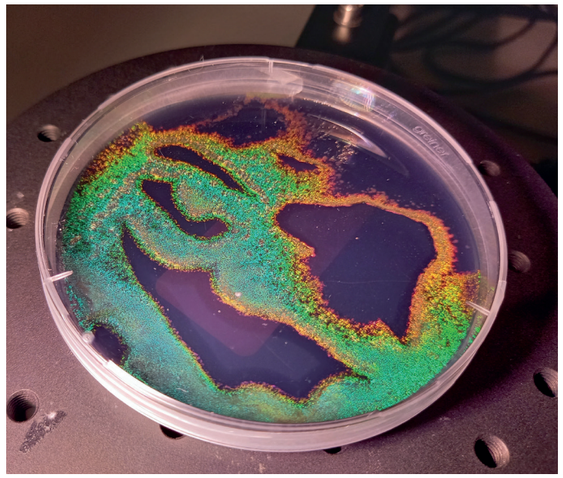


Fig. E.1: The cultivated -20˚C stock spread by condensation (day 3)

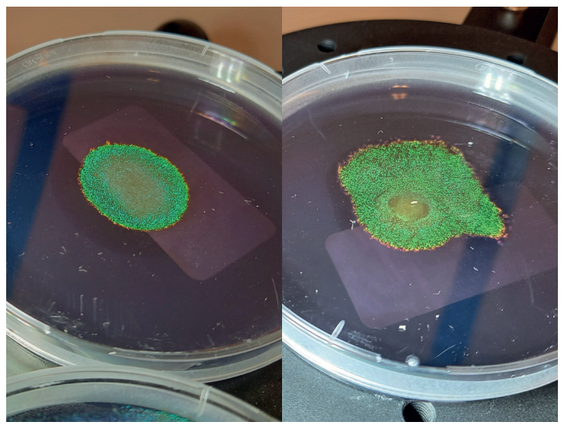


Fig. E.2: The cultivated -20˚C stock (left) and the cultivated Hoekmine B.V. bacteria (right) on day 6

Appendix F | The Gonioreflectormeter Setup

A Canon eos 550d camera was connected with a 3d printed holder to the gonioreflectormeter to avoid movements (fig. F.1-F.2).

The camera's shutter speed, aperture and iso were consistently set to 1/4, f22 and iso400 respectively, during the characterization.

A 5W, 300 lumen, 6500K LED was connected to the moving arm of the gonioreflectormeter, enabling the variation of the azimuth angle (fig. F.2).

The altitude angles of the camera and light could be varied in steps of 5 degrees.

A sample holder was made of plexiglass using a laser cutting machine to position the sample consistently in the centre point of the camera and light (fig. F.4-F.5 on the next page).

Black paper was added to the background.

During the photographing the background light was switched off.

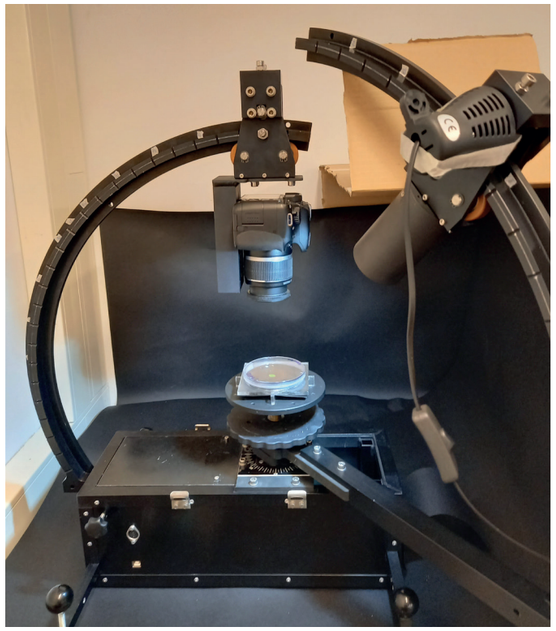


Fig. F.1 [bottom]: The 3D printed camera holder
Fig. F.2 [right top]: The gonioreflectormeter setup
Fig. F.3 [right]: The LED mounted to the setup

Appendix F | The Gonioreflectormeter Setup

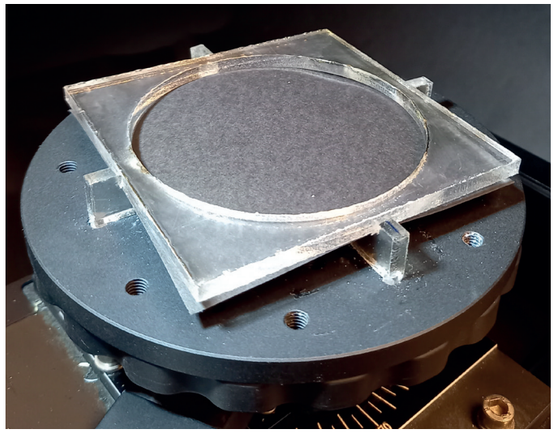
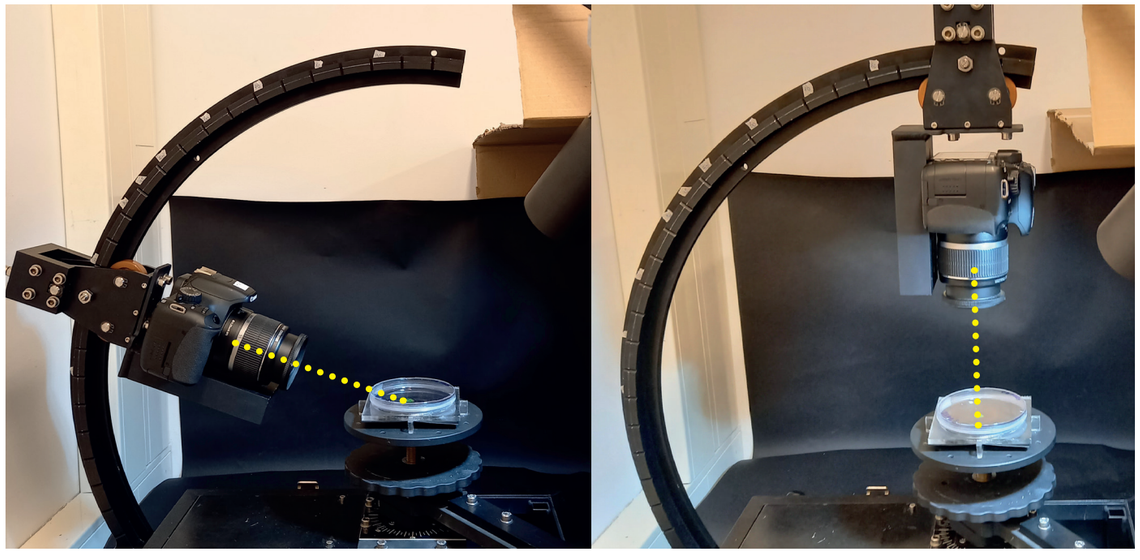


Fig. F.4 [left]: The sample holder
Fig. F.5 [bottom]: The centre point of the setup



The pictures series for the iridescence characterization that included capturing retroreflection and specular reflection were made with a 48 megapixel smartphone camera and the 5W LED. This way, the positions of the camera and the LED were almost identical, which was impossible with the big Canon camera. The smartphone camera was set to iso400 and manually focused on the sample.

The data for the colour characterization was, however, created with the Canon camera. This was possible since the pictures were taken with an azimutha angle of 45˚, since the perceived colour of the sample with an azimuth angle of 0˚ and an azimuth angle of 45˚ was identical (fig. 4.11 on page 32).

Appendix G | Optical Mixing with Eight Steps

Three pictures were made with the gonioreflectormeter setup using the settings of fig. G.1. Next, picture A and C were blended and compared to the picture B (fig. G.2-G.3). The images looked alike but also showed small differences. Therefore, optical mixing with a total of eight pictures turned out to be insufficient for the structural colour of Flavobacteria.

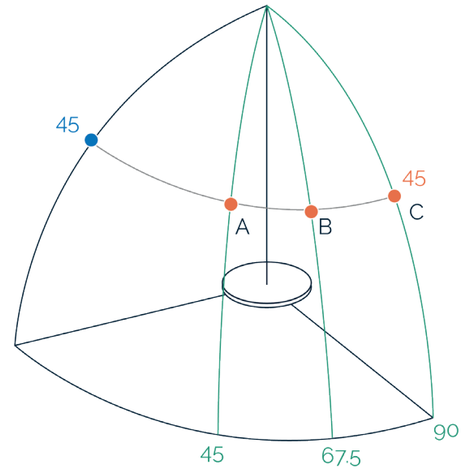
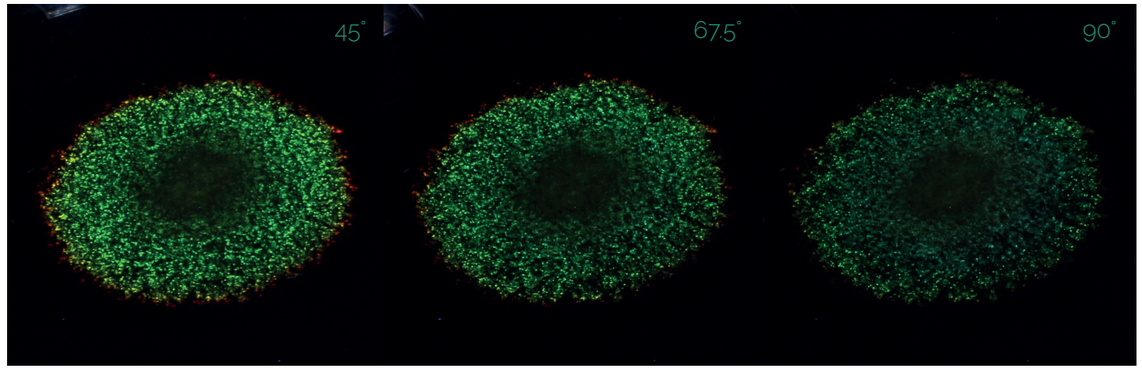
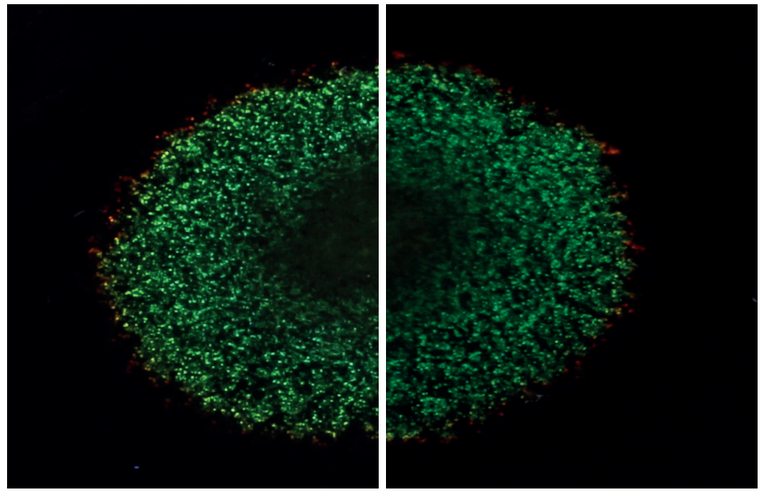


Fig. G.1 [right]: The settings of the setup
Fig. G.2 [middle]: The pictures A, B and C with an azimuth angle of respectively 45˚, 67.5˚ and 90˚.
Fig. G.3 [bottom]: The optical mixing result while skipping 45 degrees: picture B (left) and the blended A and C (right)





The moving sample holder

1 Four pins that move up and down and make the petri dish tilt in every possible direction. Since natural growth is isotropic, rotation is not needed to capture all different light interactions.

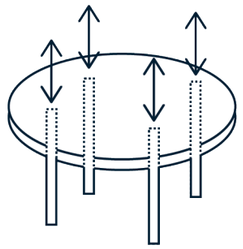


Fig. H.1: Idea 1 to move the sample

2 One motor that tilts the sample and one motor that rotates the entire platform. Since the natural growth is isotropic, all different light interactions can be captured this way.

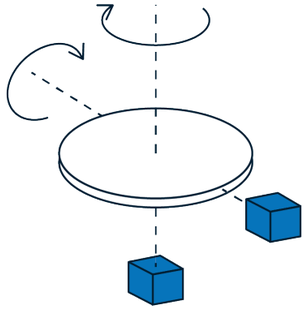


Fig. H.2: Idea 2 to move the sample

Idea 2 is preferred because it can also rotate the petri dish to capture proof that the natural growth is isotropic and that spread colonies are not.

Important to consider: Rotary axes should cross the middle of the sample

Since the sample does not weigh a lot, the tilting can be done with a servomotor which can turn 180˚. A servo motor was tested to make sure it does not vibrate when standing still.

Since a light-weighted servo motor will be used, the tilting part of the sample holder can lean on the rotating motor without a bearing (fig. H.3-H.4). For rotating the sample, a stepper motor will be used. These can rotate at least 360˚ and are stiff enough to keep the platform horizontal.

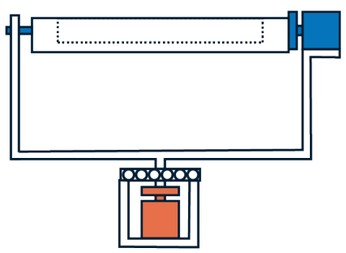


Fig. H.3: Side view of the bearing that holds the weight of the tilting part

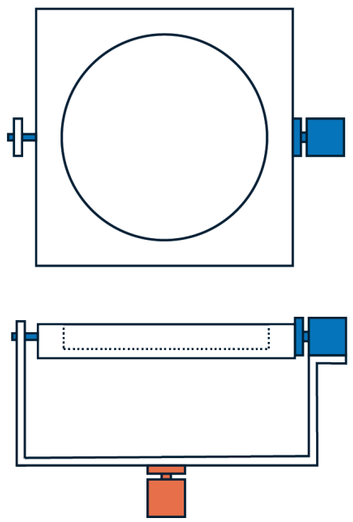


Fig. H.4: Top and side view of the tilting part leaning on the stepper motor (orange)

The light

1 A semi-transparent mirror could be used to capture the retroreflection.

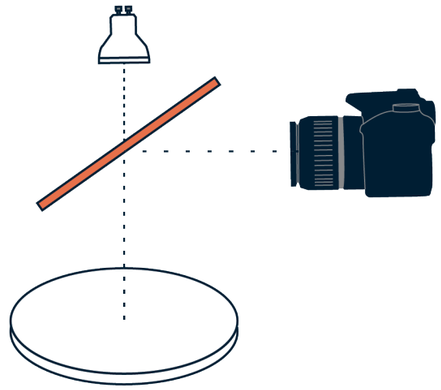


Fig. H.5: Idea 1 to illuminate the sample

2 A dome with six LED rings could be created. With optical mixing, the appearance of the sample for all azimuth angles could be created.

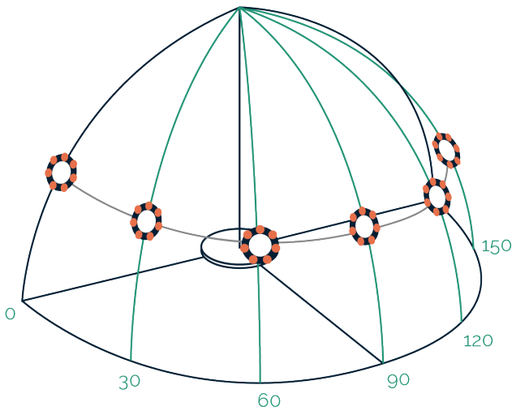


Fig. H.6: Idea 2 to illuminate the sample

3 A LED ring could be placed on a moving arm.

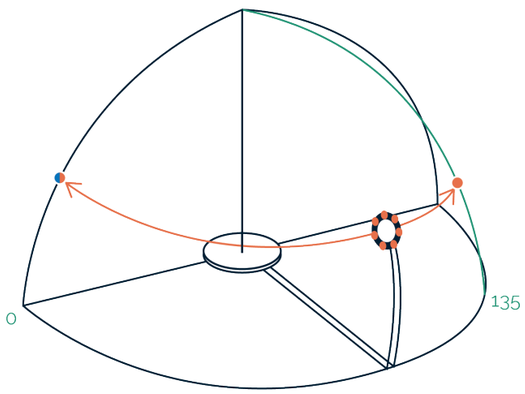


Fig. H.7: Idea 3 to illuminate the sample

The advantage of a light with a semi-transparent mirror is that the direction of the light is exactly the same as the one of the camera. Besides, there is more freedom in choice of light source. However, with this solution it would be complex to capture the retroreflection and the variation in azimuth angle, without having differences in light intensity due to the mirror. Besides, the light reaching the camera will be reduced in intensity and result in a higher shutter speed, which is not possible for movies.

Idea 3 is preferred to keep the azimuth angle fully adjustable.

Appendix H | Setup ideation

Since the sample should be the centre point, the rotatory axis of the sample holder should be in line with the axis of the moving arm. Two different constructions are designed for this.

3A The rotatory axis of the moving arm could be placed beneath the entire sample holder platform. An overhanging platform would avoid restricting the rotation of the moving arm.

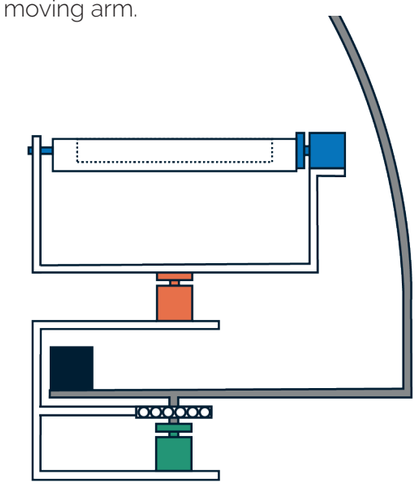


Fig. H.8: Side view of the overhanging platform of the moving LED arm (grey)

3B The rotatory axis of the moving arm could be placed above the sample, connected to the ceiling of a black box.

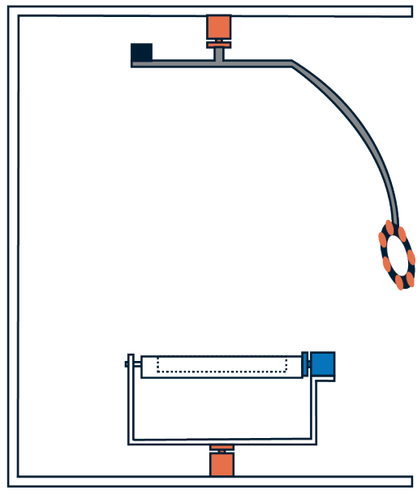


Fig. H.9: Side view of the moving LED arm (grey) above the sample holder

Idea 3B is preferred as a black box is needed anyway and this construction restricts the LED arm less than the overhanging platform.

A counterweight is used to reduce the moment of force on the motor axis due to the weight of the LED arm. Because of the stiff and strong stepper motor, a bearing was not needed to hold the weight of the LED arm. To make sure the rotary axes of LED arm and the sample holder are aligned, the sample platform will be connected to the box.

The LED ring will be clamped onto the moving arm to be able to adjust the altitude angle in the future.

To make sure the camera can capture the sample through the LED ring, the moving arm consists out of two parallel arms. The parallel arms are made stiff with screw thread and nuts connections, which can be moved in case they are in the way of the camera's view in the preferred altitude angle.

The camera
The camera will be clamped on an arm, just behind the rotating LED ring arm, to be able to adjust the altitude angle in the future.

Appendix I | Building the Automated Setup

First, the parts for the sample holder and moving LED arm were laser cut (fig. I.1). The LEDs were placed in the ring with plastic LED holders and soldered (fig. I.2).

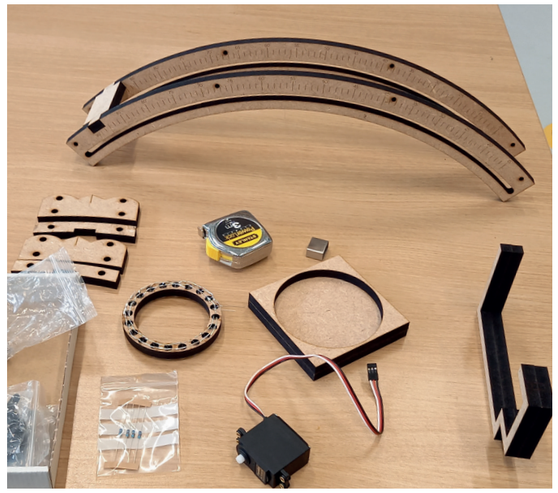


Fig. I.1: Laser cut parts for the sample holder and moving LED arm

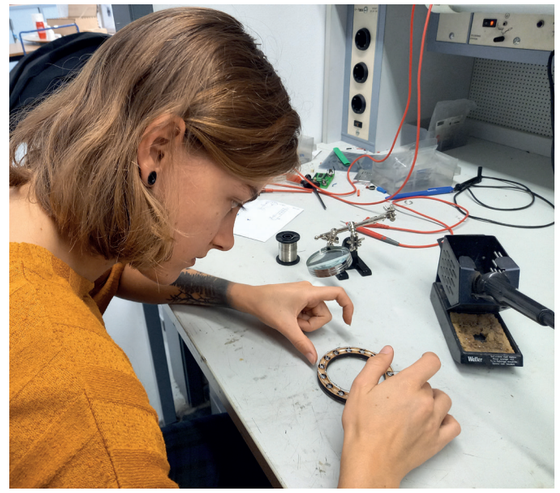


Fig. I.2: Soldering the LED ring

The two parts that hold the sample in place, were glued together and the servo motor was connected to the third part with screws. Since the servo motor can only turn 180°, the range of the movement was first defined by connecting it to an Arduino. Afterwards, the two parts that hold the sample in place were connected to the servo with a plastic part, that comes with the motor, and screws. A piece of plexiglass was laser cut to mount the sample holder on the stepper motor. All wooden parts and the electric wires of the servo motor were painted black (fig. I.3).

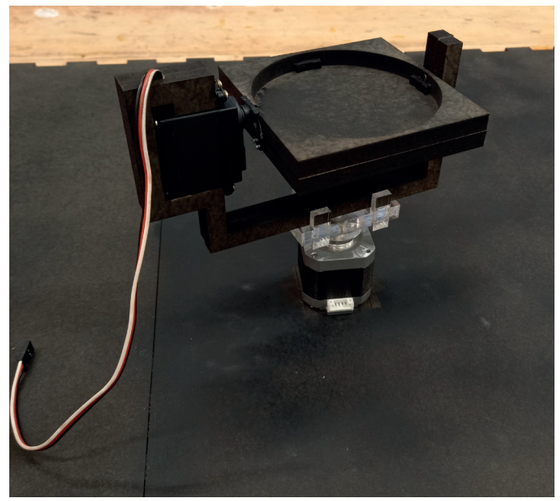


Fig. I.3: The sample holder mounted on the stepper motor

The two parallel arms were connected with screw thread and nuts connections. The LED ring was connected with a small 3d print to two clamps, made with four pieces of MDF, screw thread and nuts (fig. I.4). By pushing bolts through the slots of the arms and the clamps, the LED ring was connected to the moving LED arm. A piece of plexiglass was laser cut to mount the moving LED arm on the stepper motor and to connect a counterweight. To make sure the moving LED arm could not slide of the motor axis, a pin was placed through the motor axis and a small circle of plexiglass (fig. I.5). This small circle of plexiglass was connected to the bigger piece of plexiglass with epoxy glue. Two aluminium L-profiles were connected to the bigger piece of plexiglass as well, using double sided tape (fig. I.6). In these profiles, a piece of steel could be shifted in the preferred position as a counterweight. The stepper motor of the moving LED arm was placed in a rectangle aluminium profile (fig. I.7). All the aluminium profiles were painted black.

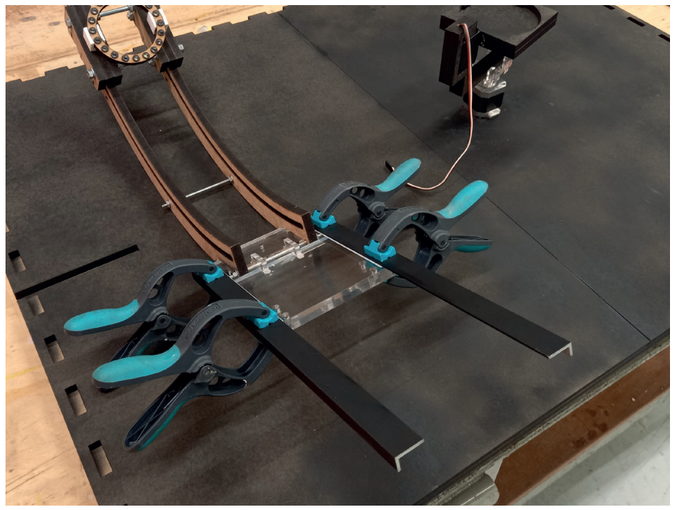


Fig. I.6: The aluminium profiles for the counterweight mounted to the plexiglass

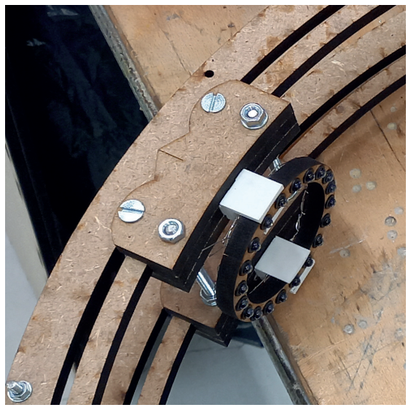


Fig. I.4: The LED ring on the moving arm

Fig. I.5: The pin through the small plexiglass circle and the motor axis

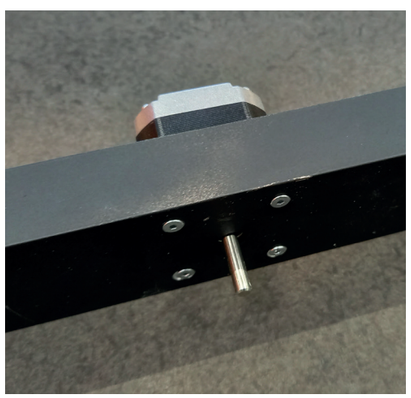


Fig. I.7: The stepper motor in the aluminium profile

After mounting the moving LED arm and the sample holder on the stepper motors, the size of the box could be determined. The size of the box depend on these parts, since the moving arm should be able to rotate around the sample, the camera should fit behind the LED ring and the sample should align with the centre of LED ring and the camera. The MDF parts were laser cut (fig. I.8) and the sides that would face the inside of the box were painted black. As MDF turned out to absorb paint a lot, the pieces were painted twice (fig. I.9-I.10).

A camera holder was 3d-printed for the Canon EOS 550d. The camera holder is connected to the slot of the camera arm with a clamp, made with two pieces of MDF, bolts and nuts (fig. I.11).

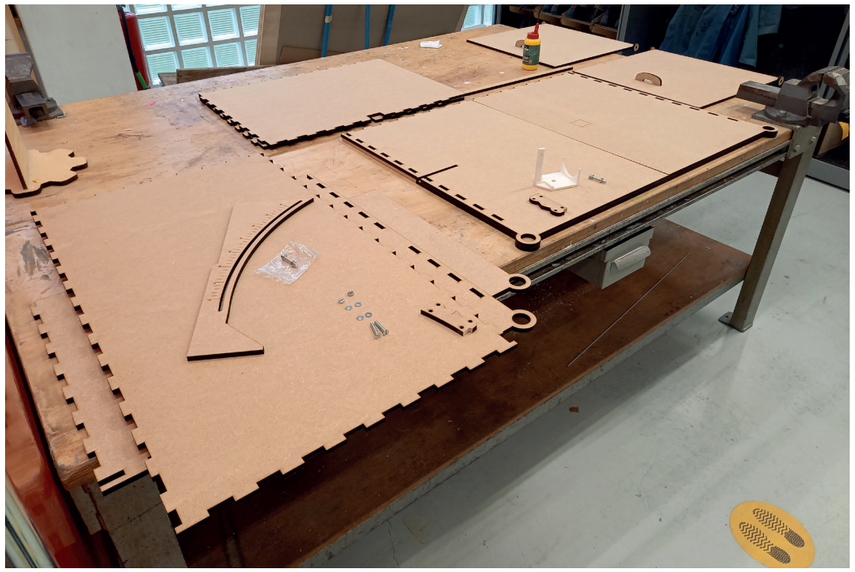


Fig. I.8: The laser cut parts for the box

Fig. I.9: In the paint room

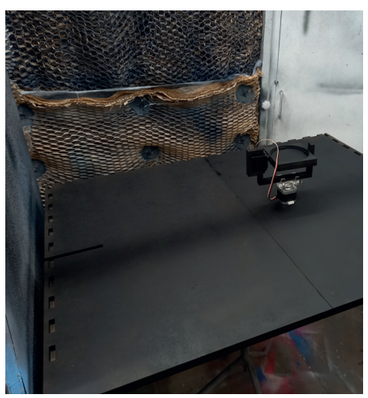


Fig. I.10: Painting a second layer

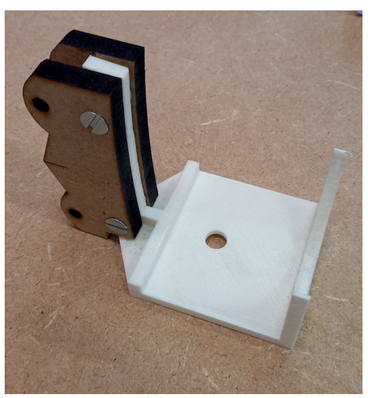


Fig. I.11: The camera holder with the clamp

Long electric wires were soldered to the servo and stepper motor of the sample holder and concealed with a electric wiring channel that was painted black (fig. I.12). In order to withhold the electric wires of the servo motor from wrapping around the axis of the stepper motor, they were connected with a tie rap, allowing the sample holder to rotate at least 180˚ (fig. I.13).

Long wires were also soldered to the LED ring, together with a resistor. The electric wires of the LED ring were connected to the aluminium profile, allowing the moving LED arm to rotate at least 270˚. In the rectangle aluminium profile, a hole was made to transport the electric wires to the outside of the box (fig. I.14).

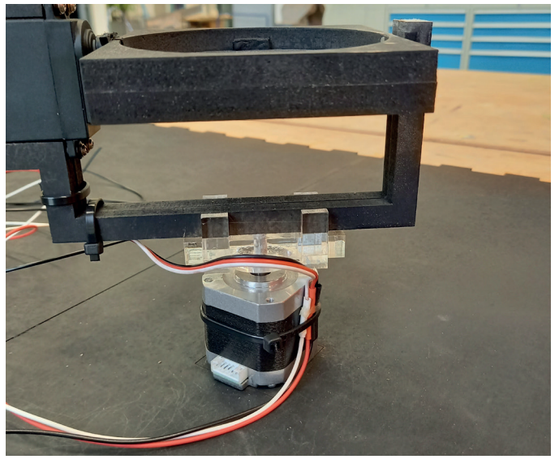


Fig. I.13: Electric wires of servo connected with tie rap

Fig. I.12: Electric wiring channel

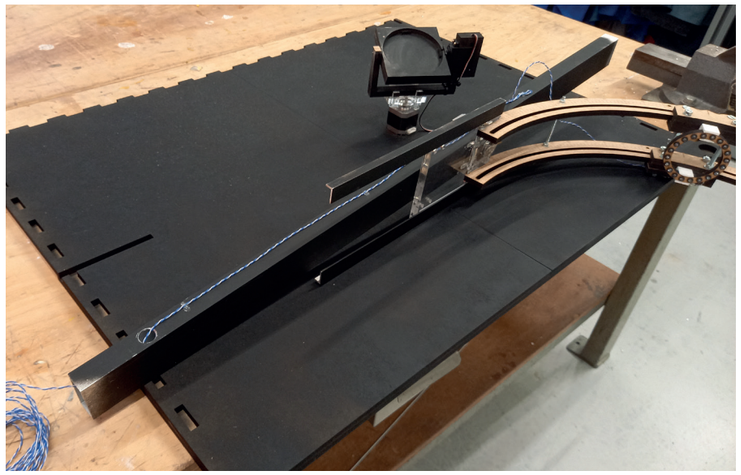


Fig. I.14: Electric wires of the LED ring connected to and going through the aluminium profile

During the final stage of the building process, all the parts were placed into each other (fig. I.15). As the parts of the box were designed as puzzle pieces, no glue or screws were needed.

The electronic parts were placed on the top of the box (fig. I.16). After soldering pin headers to the wires, all the components were connected (fig. I.17).

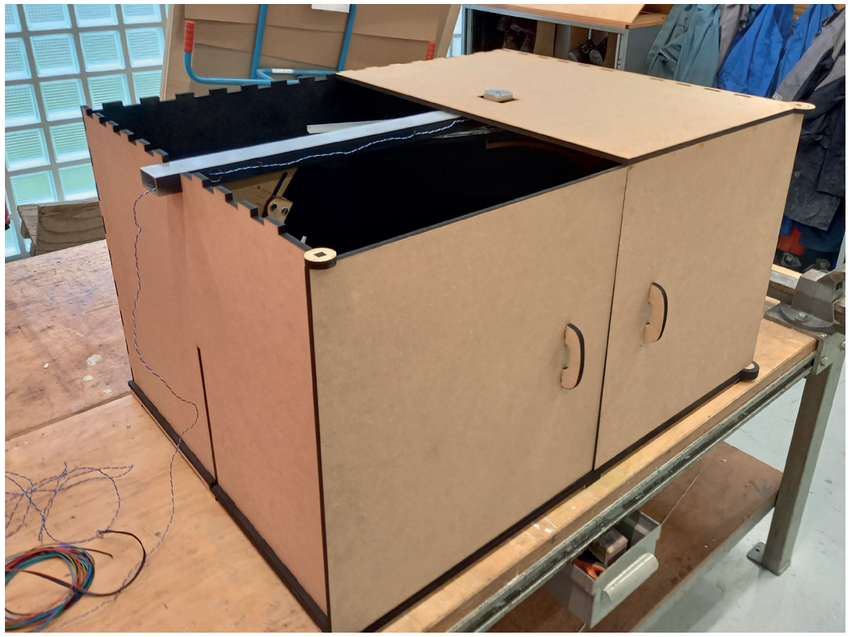


Fig. I.15: Placing the parts into each other as a puzzle

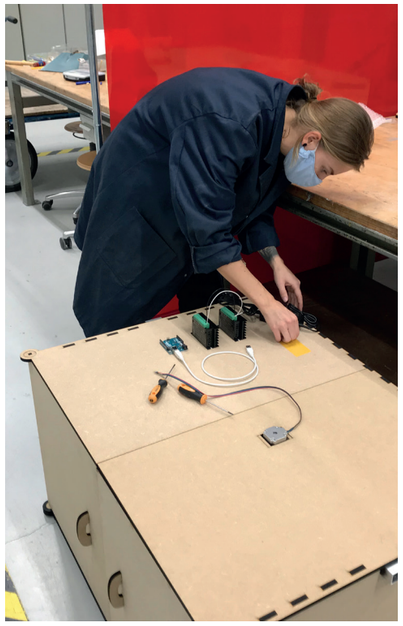


Fig. I.16: Placing the electronics on top

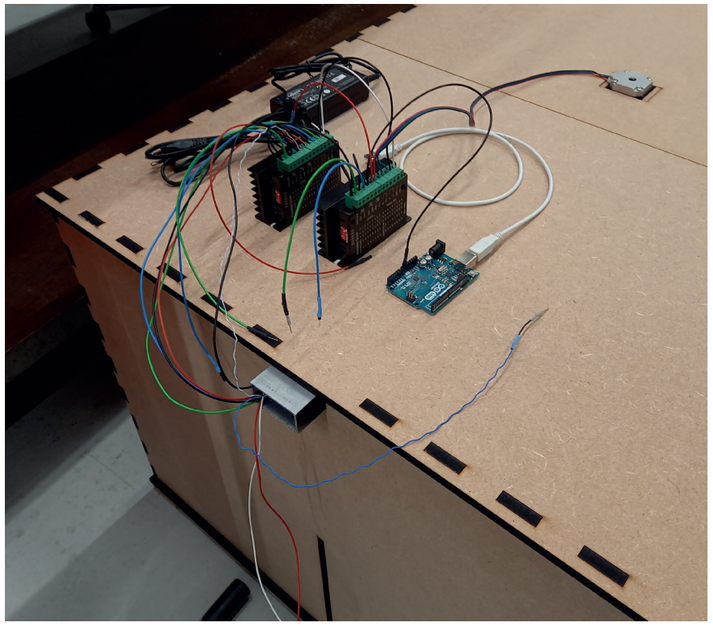
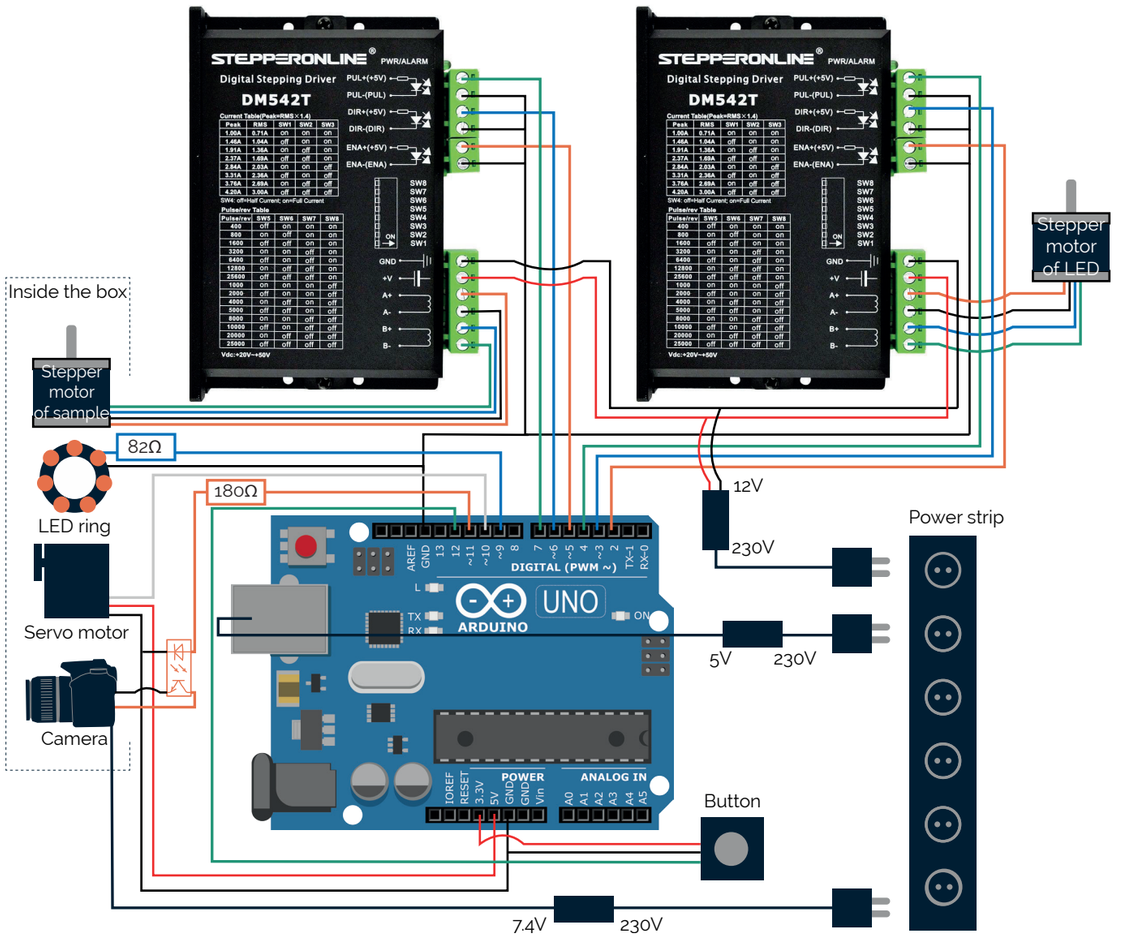


Fig. I.17: Connecting all the electronic components

Appendix J | The Circuit of the Automated Setup



Appendix K | The Arduino Software Code

Capture1 | Arduino 1.8.13

Bestand Bewerken Schets Hulpmiddelen Help

Capture1

```
#include <Servo.h>
Servo tiltingservo;
int servoposition;

const int enaLED = 2; //enable
const int dirLED = 3; //direction
const int pulLED = 4; //step
float nemaLEDposition = 0;

const int enaSample = 5; //enable
const int dirSample = 6; //direction
const int pulSample = 7; //step
float nemaSampleposition = 0;

const int camerapin = 11;

const int LEDpin = 9;

const int Button = 12;


//functions
int NemaLEDTurnTo(int degree, float variablespeed){
   //degree = position to which motor turns. Between 0 and 270
   //variable speed = speed of rotation. Between 0.1 and 1
   float stepsize = (1.8/4);

   if(degree > 270){
    degree = 270;
   }
   if(degree < 0){
    degree = 0;
   }
   if(variablespeed > 1){
    variablespeed = 1;
   }
   if(variablespeed < 0.1){
    variablespeed = 0.1;
   }

   digitalWrite(enaLED, LOW); //enable motor

    if(degree > nemaLEDposition){
    digitalWrite(dirLED, HIGH); //set direction CW
    for(float i = nemaLEDposition; i < degree; i=i+stepsize){
      digitalWrite(pulLED, HIGH); //step
      delayMicroseconds(8000/variablespeed);
      digitalWrite(pulLED, LOW); //not a step
      delayMicroseconds(8000/variablespeed);
      nemaLEDposition = nemaLEDposition + stepsize;
    }
    //step back when overshooting more than 0.9 degrees
    if(nemaLEDposition > (degree + (stepsize*0.5) )){
      digitalWrite(dirLED, LOW); //set direction CCW
      digitalWrite(pulLED, HIGH); //step
      delayMicroseconds(8000/variablespeed);
      digitalWrite(pulLED, LOW); //not a step
```

```
    delayMicroseconds(8000/variablespeed);
    nemaLEDposition = nemaLEDposition - stepsize;
  }
}
    else if(degree < nemaLEDposition){
 digitalWrite(dirLED, LOW); //set direction CCW
 for(float i = nemaLEDposition; i > degree; i=i-stepsize){
   digitalWrite(pulLED, HIGH); //step
   delayMicroseconds(8000/variablespeed);
   digitalWrite(pulLED, LOW); //not a step
   delayMicroseconds(8000/variablespeed);
   nemaLEDposition = nemaLEDposition - stepsize;
 }
 //step back when overshooting more than 0.9 degrees
 if(nemaLEDposition < (degree - (stepsize*0.5) )){
   digitalWrite(dirLED, HIGH); //set direction CW
   digitalWrite(pulLED, HIGH); //step
   delayMicroseconds(8000/variablespeed);
   digitalWrite(pulLED, LOW); //not a step
   delayMicroseconds(8000/variablespeed);
   nemaLEDposition = nemaLEDposition + stepsize;
 }
}
  delay(5000);

}

int LEDaan(){
  digitalWrite(LEDpin, HIGH);
}

int LEDuit(){
  digitalWrite(LEDpin, LOW);
}

int NemaSampleTurnTo(float degree, float variablespeed){
   //degree = position to which motor turns. Between 0 and 360
   //variable speed = speed of rotation. Between 0.1 and 1
   float stepsize = (1.8/32);

   if(degree > 360){
    degree = 360;
   }
   if(degree < 0){
    degree = 0;
   }
   if(variablespeed > 1){
    variablespeed = 1;
   }
   if(variablespeed < 0.1){
    variablespeed = 0.1;
   }

   digitalWrite(enaSample, LOW); //enable motor

   if(degree > nemaSampleposition){
```



```
   digitalWrite(dirSample, LOW); //set direction CW
   for(float i = nemaSampleposition; i < degree; i=i+stepsize){
     digitalWrite(pulSample, HIGH); //step
     delayMicroseconds(800/variablespeed);
     digitalWrite(pulSample, LOW); //not a step
     delayMicroseconds(800/variablespeed);
     nemaSampleposition = nemaSampleposition + stepsize;
   }
   //step back when overshooting more than 0.9 degrees
   if(nemaSampleposition > (degree + (stepsize*0.5) )){
     digitalWrite(dirSample, HIGH); //set direction CCW
     digitalWrite(pulSample, HIGH); //step
     delayMicroseconds(800/variablespeed);
     digitalWrite(pulSample, LOW); //not a step
     delayMicroseconds(800/variablespeed);
     nemaSampleposition = nemaSampleposition - stepsize;
   }
}
else if(degree < nemaSampleposition){
 digitalWrite(dirSample, HIGH); //set direction CCW
 for(float i = nemaSampleposition; i > degree; i=i-stepsize){
   digitalWrite(pulSample, HIGH); //step
   delayMicroseconds(800/variablespeed);
   digitalWrite(pulSample, LOW); //not a step
   delayMicroseconds(800/variablespeed);
   nemaSampleposition = nemaSampleposition - stepsize;
 }
 //step back when overshooting more than 0.9 degrees
 if(nemaSampleposition < (degree - (stepsize*0.5) )){
   digitalWrite(dirSample, LOW); //set direction CW
   digitalWrite(pulSample, HIGH); //step
   delayMicroseconds(800/variablespeed);
   digitalWrite(pulSample, LOW); //not a step
   delayMicroseconds(800/variablespeed);
   nemaSampleposition = nemaSampleposition + stepsize;
 }
}
   digitalWrite(enaSample, HIGH); //disable motor
}

int ServoTurnTo(int degree){
   if(degree > 160){
    degree = 160;
   }
   if(degree < 20){
    degree = 20;
   }
   degree = degree - 4; //adjust to correct slight tilt of servo

   if(degree > servoposition){
    for(int s = servoposition; s < degree; s++){
      tiltingservo.write(s);
      delay(50);
    }
   }
   else if(servoposition > degree){
    for(int s = servoposition; s > degree; s--){
```

Appendix K | The Arduino Software Code

```
      tiltingservo.write(s);
      delay(50);
    }
   }
   //tiltingservo.write(degree);

   servoposition = degree;
}

int CameraClick(){
  delay(1000);
  digitalWrite(camerapin, HIGH);
  delay(600);
  digitalWrite(camerapin, LOW);
  delay(5000);
}

////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////

void setup()
{
pinMode(enaLED, OUTPUT);
pinMode(dirLED, OUTPUT);
pinMode(pulLED, OUTPUT);

pinMode(enaSample, OUTPUT);
pinMode(dirSample, OUTPUT);
pinMode(pulSample, OUTPUT);
pinMode(camerapin, OUTPUT);
pinMode(LEDpin, OUTPUT);
pinMode(Button, INPUT);

tiltingservo.attach(10);

tiltingservo.write(86);
servoposition = 86;
}

////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////

void loop()
{
  while (digitalRead(Button) != LOW) {
    //do not start program until button is pressed.
    //also ensures that program does not run twice.
    //calibrate nema motors to 0.
  }

  //NemaLEDTurnTo(20, 0.1);
  //LEDaan();
  //LEDuit();
  //NemaSampleTurnTo(10, 0.5);
  //ServoTurnTo(90);
  //CameraClick();
```

These functions can be used to write a code which determines the settings of the capturing

Appendix L | Instructions for Using the Automated Setup and Communication Tool

- Always calibrate the stepper motors to their starting point (fig. L.1) before starting the setup, to make sure the motors do not tear apart the electric wires
- Always test a code with the doors open to be able to tell when it goes wrong and switch the setup off.
- Do not take extra photos while shooting for communication tool as the communication tool uses the numbers of the pictures to calculate which photo it has to display.
- Use stop motion to create movies with the setup as filming with the camera makes the result too dark due to the fast shutter speed.
- Use so-called for loops in the void loop to define the settings of the capture session in order to reduce the length of the code and increase the overview.
- Use the Canon EOS 550d camera on the camera holder when comparing samples for an experiment and use the camera of the iteration (i.e., the Canon EOS 5DS) or another high-quality camera on the tripod for shooting high-quality images. This is recommended as the camera holder positions the camera very consistently.
- Use an equal step size in time when shooting for the communication tool in order to easily adjust the ranges of the sliders. This was not done for example during the first capture session of a week. Since the step size of this session was smaller during the first 24 hours, the code of the communication tool had to be modified significantly.
- Use a lens calibration tool (fig. L.2) to precisely focus the camera on the middle of the sample holder.
- Create a real life connection with the camera and a laptop. This way the data can be checked during the capture session for failures without disrupting the capture session.

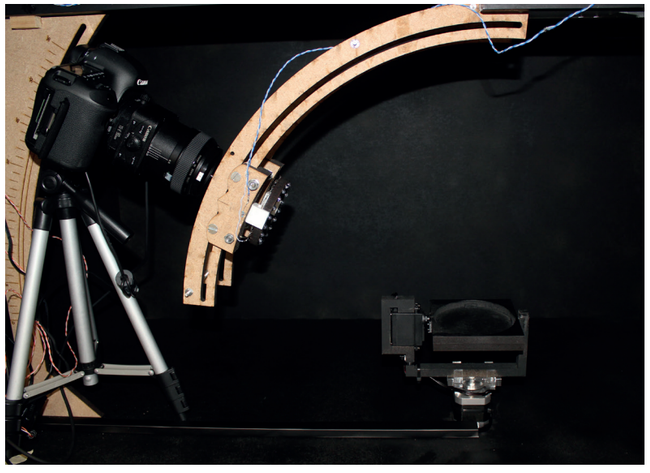


Fig. L.1: The starting points of the stepper motors

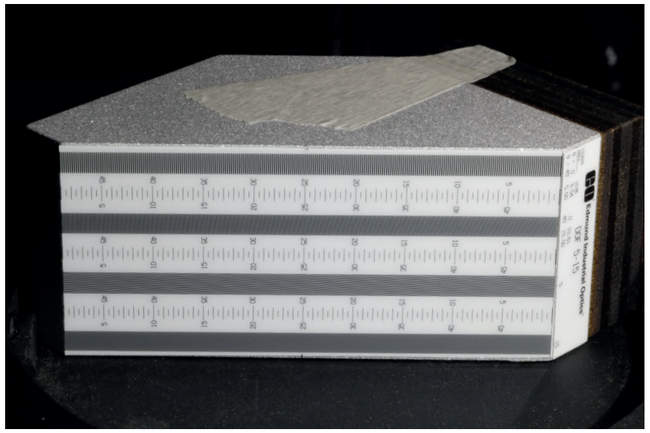


Fig. L.2: Lens calibration tool

Appendix M | The Code of the Communication Tool

index.html

```
<!DOCTYPE html>
<html>
  <head>
    <meta charset="utf-8">
    <title>Communication Tool Flavobacteria</title>
    <script type="text/javascript" src="./imagescroll.js"> </script>
    <link rel="stylesheet" href="style.css">
  </head>

  <body>
    <div class="controlpanel">
      <div class="controltitle">
        The Temporal and Iridescent Appearance of <div style="font-family: Raleway-SemiBold;">Flavobacteria</div>
      </div>
      <br>
      <div class="tiltcontainer">
        Tilt of Sample: <div style="display:inline" id="currenttiltvalue">0</div>°
        <input type="range" min="0" max="45" value="0" step="15" class="smallslider" id="tiltslider" oninput="changePicture()">
      </div>
      <br>
      <div class="rotationcontainer">
        Rotation of Sample: <div style="display:inline" id="currentrotationvalue">0</div>°
        <input type="range" min="-45" max="45" value="0" step="45" class="smallslider" id="rotationslider" oninput="changePicture()">
      </div>
      <br>
      <div class="lightcontainer">
        Angle between Viewer and Light: <div style="display:inline" id="currentlightvalue">0</div>°
        <input type="range" min="0" max="150" value="0" step="30" class="smallslider" id="lightslider" oninput="changePicture()">
        <br>
        <input type="checkbox" id="opticalmixing" oninput="activateOpticalmixing(); adjustSliders();">Optical mixing
        <div class="tooltip">&#x1F6C8;
          <span class="tooltiptext">Optical mixing creates the in-between results by blending the adjacent pictures</span>
        </div>
      </div>
    </div>

    <div class="data">
      <img id="image" src="" class="image">
    </div>
    <div class="data">
      <img id="upperimage" src="" class="image" style="opacity:50%; display:none">
    </div>

    <div class="timepanel">
      <div class="timecontainer">
              Time: day <div style="display:inline" id="currentdayvalue">0</div> (<div style="display:inline" id="currenttimevalue">0</div> hours)
              <br>
              <div class="playbutton" id="playbutton" onclick="togglePlay()">
```


Appendix M | The Code of the Communication Tool

style.css

```
@font-face {
  font-family: Raleway-Light;
  src: url(Raleway-Light.ttf);
}
@font-face {
  font-family: Raleway-SemiBold;
  src: url(Raleway-SemiBold.ttf);
}

body {
  background-color: #BDBDBE;
  color: black;
  font-family: Raleway-Light;
  font-size: 15px;
}

.controlpanel {
  width: 350px;
  height: 90%;
  position: absolute;
  top: 0px;
  left: 0px;
  word-wrap: break-word;
}

.data {
  width: calc(100% - 350px);
  height: 90%;
  position: absolute;
  top: 0px;
  right: 0px;
}

.timepanel {
  width: 100%;
  height: 10%;
  position: absolute;
  bottom: 0px;
  left: 0px;
}

.controltitle {
  position: relative;
  margin: 10px 45px;
  font-size: 35px;
}

.tiltcontainer {
  margin-left: 45px;
}

.rotationcontainer {
  margin-left: 45px;
}

.lightcontainer {
  margin-left: 45px;
}

.timecontainer {
  margin-left: 45px;
}

.smallslider {
  margin-top: 10px;
  width: 260px;
}

.bigslider {
  margin-top: 10px;
  width: 82%;
  display: inline;
}

.playbutton{
  display: inline;
  font-size: 28px;
  cursor: pointer;
}

.image {
  height: 100%;
}

.upperimage {
  height: 280px;
}

.tooltip {
  position: relative;
  display: inline-block;
}

.tooltip .tooltiptext {
  visibility: hidden;
  width: 155px;
  background-color: black;
  color: #fff;
  text-align: left;
  border-radius: 6px;
  padding: 5px 5px;
  position: absolute;
  z-index: 1;
}

.tooltip:hover .tooltiptext {
  visibility: visible;
}
```

index.html | style.css | imagescroll.js

```
var minTilt = 0;
var maxTilt = 45;
var stepTilt = 15;

var minRotation = -45;
var maxRotation = 45;
var stepRotation = 45;

var minAzimuth = 0;
var maxAzimuth = 150;
var stepAzimuth = 30;

var minTime = 0;
var maxTime = 168;
var stepTime = 3;

var lowestPhotoNumber = 99;
var linkimage = "file:///F:/Clarice Afstuderen Flavobacterie/data auto setup/capture2/_Y4A";

var numberTilt = ((maxTilt-minTilt)/stepTilt)+1;
var numberRotation = ((maxRotation-minRotation)/stepRotation)+1;
var numberAzimuth = ((maxAzimuth-minAzimuth)/stepAzimuth)+1;

function adjustSliders() {
  document.getElementById("tiltslider").min = minTilt;
  document.getElementById("tiltslider").max = maxTilt;
  document.getElementById("tiltslider").step = stepTilt;
  document.getElementById("rotationslider").min = minRotation;
  document.getElementById("rotationslider").max = maxRotation;
  document.getElementById("rotationslider").step = stepRotation;
  document.getElementById("lightslider").min = minAzimuth;
  document.getElementById("lightslider").max = maxAzimuth;
  document.getElementById("timeslider").min = minTime;
  document.getElementById("timeslider").max = maxTime;
  document.getElementById("timeslider").step = stepTime;
  if(document.getElementById("opticalmixing").checked == true){
    document.getElementById("lightslider").step = 1;
  }
  else{
    document.getElementById("lightslider").step = stepAzimuth;
  }
}

function activateOpticalmixing(){
  var azimuthStepDecimals = (document.getElementById("lightslider").value - minAzimuth) / stepAzimuth;
  var fade = 100*(azimuthStepDecimals - Math.floor(azimuthStepDecimals));
  if(document.getElementById("opticalmixing").checked == true){
    document.getElementById("upperimage").style = "opacity:" + fade + "%; display:inline;";
  }
  else{
    document.getElementById("upperimage").style = "opacity:" + fade + "%; display:none;";
    changePicture();
  }
}

function changePicture() {

```

The variables that need to be modified


```
  var tiltvalue = (document.getElementById("tiltslider").value - minTilt) / stepTilt;
  var rotationvalue = (document.getElementById("rotationslider").value - minRotation) / stepRotation;
  var lightvalue = Math.floor((document.getElementById("lightslider").value - minAzimuth) / stepAzimuth);
  //lightvalue is rounded down because of opticalmixing's continious slider
  var timevalue = (document.getElementById("timeslider").value - minTime) / stepTime;

  var imagenumber = lowestPhotoNumber + (tiltvalue*1)+(rotationvalue*numberTilt)+(lightvalue*numberRotation*numberTilt)+(time
  var imagenumberstr = "" + imagenumber;
  var pad = "0000";
  var imagenumber4digitals = pad.substring(0, pad.length - imagenumberstr.length) + imagenumberstr;
  var imagesrc = linkimage + imagenumber4digitals + ".JPG";

  console.log(imagenumber);
  document.getElementById("image").src = imagesrc;

  document.getElementById("currenttiltvalue").innerHTML = document.getElementById("tiltslider").value;
  document.getElementById("currentrotationvalue").innerHTML = document.getElementById("rotationslider").value;
  document.getElementById("currentlightvalue").innerHTML = document.getElementById("lightslider").value;
  document.getElementById("currenttimevalue").innerHTML = document.getElementById("timeslider").value;
  document.getElementById("currentdayvalue").innerHTML = (Math.ceil(document.getElementById("timeslider").value/24));
  if(Math.ceil(document.getElementById("timeslider").value/24) == 0){
    document.getElementById("currentdayvalue").innerHTML = 1;
  }

  if(document.getElementById("opticalmixing").checked == true){
    var upperimagenumber = imagenumber + (numberRotation*numberTilt);
    var upperimagenumberstr = "" + upperimagenumber;
    var upperimagenumber4digitals = pad.substring(0, pad.length - upperimagenumberstr.length) + upperimagenumberstr;
    var upperimagesrc = linkimage + upperimagenumber4digitals + ".JPG";

    document.getElementById("upperimage").src = upperimagesrc;

    activateOpticalmixing();
  }
}

var playing = false;
var autoPlay;

function togglePlay(){
  if(playing == true){
    playing = false;
    document.getElementById("playbutton").innerHTML = "▶"
    clearInterval(autoPlay);
  }
  else {
    playing = true;
    document.getElementById("playbutton").innerHTML = "⏸"
    autoPlay = setInterval(function(){
        if(document.getElementById("timeslider").value == maxTime){
          clearInterval(autoplay);
          return;
        }
        document.getElementById("timeslider").value = parseInt(document.getElementById("timeslider").value) + stepTime;
        console.log(parseInt(document.getElementById("timeslider").value) + stepTime);
        changePicture();
      }, 200
      );
  }
}
```

FLAVOBACTERIA'S STRUCTURAL COLOUR

Characterizing, Capturing and Communicating
the Temporal and Iridescent Appearance of Flavobacteria

Master Thesis | Clarice Risseeuw