GROWING LIGHT

MASTER THESIS
M.S.C. DESIGN FOR INTERACTION
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EXECUTIVE SUMMARY

Within this graduation project, the qualities and potential of bioluminescent micro-algae will be explored with the use of the Material Driven Design Method, as described by Karana et al.(2015). This project aims to provide designers first insights in the behaviour of the bioluminescent algae by looking at the relation between movement and light.

The first step will be to understand the different factors that influence the growth of the organisms, in order to be able to create a sustainable living environment that allows us to effectively maintain and grow the organisms. Afterwards, reflecting upon the growing process and creating general guidelines for effectively growing material.

Next, the response of the material in regards to different types of stimuli was explored and three types of agitation forms [rotation, pulse and vibration] were evaluated; providing first insights in how the type of stimuli affects the characteristics of the light, with a focus on changes in total light output over time, differences in experiential qualities and boundaries of the material.

Based upon the questions that resulted from the exploration phase, a research structure was defined. Within the research, key insight in the flash characteristics and overall behaviour of the light, with regards the temporal form, textural qualities and spatial distribution of the light, will be analysed; taking the first steps in describing the complex relation between movement and experiential qualities of the light. In the end, providing an overview of factors that can be adjusted to alter the flash characteristics and general behaviour of the light.

The project is concluded with the design of a research object, in the form of a small scale interactive exhibition; displaying the findings of the research phase and sparking the interest of other designers; stimulating further research and promoting the use of bioluminescent materials.

FLASH CHARACTERISTICS	**General behaviour and properties of the light that is produced by bioluminescent dinoflagellates; described from both an experiential and technical point of view.**
AGITATION	**Generating shear force through movement; stimulating the cells in order to trigger the bioluminescent process.**
BRIGHTNESS	**Perceived luminance of a light source; generally used to (subjectively) describe changes in light intensity over time.**
LUMINANCE	**A photometric measurement of the luminous intensity per unit area of light travelling in a given direction; describing the amount of light that passes through, is emitted from or is reflected from a particular area**
TMSL	**Total mechanically stimulated light; measured in the amount of photons emmited per cel (Widder and Case, 1981).**
RESPONSE CURVE	**A curve which graphically illustrates the magnitude of the a response to a varying stimulus. In this case: a graphical depiction of the total (relative) light output over time as the result of a (mechanical) stimulation.**
TEXTURAL QUALITIES	**Term used to describe the perceived texture of a light soure; looking at both the density and the distribution of individual visible luminous clusters.**
DIFFUSED LIGHT	**Evenly spread and uniform light radiating homogeneously in all directions.**
GLOWING STATE	**Little to no fluctuations in brightness for an extended period of time, resulting in a steady light.**
FLASHING STATE	**Spiky variations in brightness over time, resembling a light that 'flickers'.**
DYNAMIC LIGHT PATTERN	**Variation of light over time and space; describes how the light varies through the liquid while agitated, looking at both the kinetics and brightness.**
CLUMPING	**Cells sticking together, forming larger 'blobs' of cell matter.**

PROJECT OVERVIEW

General overview of the project; defining the scope, goals and methodologies used while providing an overall structure.

C1

CONTENTS

AIM

Providing an overview of the project by defining the scope and goals, as wel as describing the general structure of the project and report.

- Describing the motivation and relevance of the project in regards to the practice of Growing Design
- Defining the general scope and direction of the project, as wel as the overall aim of the project
- Understanding and describing the used methodology, in order to get a better idea of the different elements of the project and to be able to define the main points of interest
- Determining the overall structure of the project; defining the different phases and key elements of the process

METHODS

- Literature research

1.1

PROJECT BRIEF

Recently, we have seen a growing interest among designers and design researchers in investigating living organisms and their application in everyday products. A key incentive is to explore their potential for alternative and more sustainable ways of living with less impact on the environment. Within the practice of Growing Design, designers make use of living organisms, such as fungi, bacteria or algae, to grow and shape new materials for product design purposes.

RELATION WITH GROWING DESIGN

In Growing Design, designers work with living organisms in a collaborative manner; guiding their growth by adjusting the conditions in which the organism is growing (Karana et al., 2018). In most cases, the focus is on the creation of the material itself; exploring the properties by adjusting certain living conditions. However, in certain cases designers try to grow and shape the organism directly into the desired product or research object.

While the process of working with algae is slightly different from that of the traditional Growing Design materials, as designers do not directly engage in the growing process of the algae, it still remains to be seen as an important part of the Growing Design practice. As most species of algae have a great rate of growth, they are a promising and almost beyond renewable resource that can be used and processed for many potential applications (Wijffels et al., 2013).

GENERAL OVERVIEW OF THE PROJECT

Within this graduation project, the qualities and potential of bioluminescent micro-algae for product design purposes will be explored with the use of the Material Driven Design Method, as described by Karana et al.(2015). The first step will be to create a sustainable living environment or 'bio-reactor' system to grow and maintain the algae. Only once a proper system that effectively maintains the algae has been designed and sufficient algae matter has been grown, it will be possible to start with the following phase: understanding and exploring the behaviour of the light.

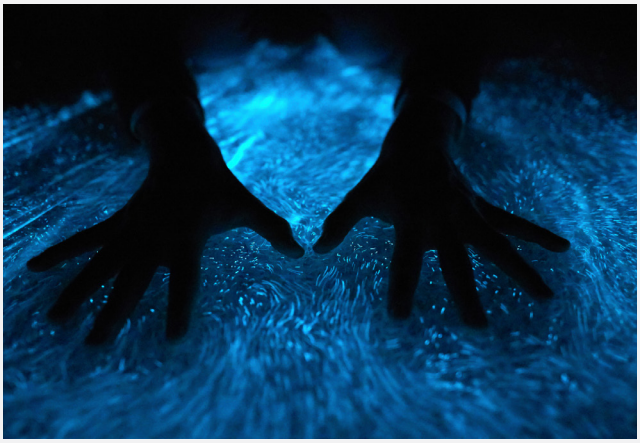
Within this second phase of the project, the focus will be on the exploration of the material design space in order to discover the unique performative and experiential qualities of the bioluminescent algae (Karana et al., 2015). The main goal of this phase is to discover and document the unique flash characteristics of the bioluminescent algae for certain types of mechanical stimulation; building a foundation and providing first insight for future uses in product design solutions.

The end result of the project will be a research object that shows the potential and unique experiential characteristics of the bioluminescent algae by encouraging people to actively explore the material affordances through movement.

MAIN GOAL OF THE PROJECT

This project aims to provide designers the first insights in the behaviour of the bioluminescent algae by looking at the relation between movement and light. The end goal will be to create an object that displays these findings and sparks the interest of other designers; stimulating further research and promoting the use of bioluminescent materials.

IMAGES [1-2]
TOP - BIOLUMINESCENT ALGAE IN NATURE (DAVID RODGERS, 2018)
BOTTOM - GLOWING NATURE: ART INSTALLATION (STUDIO ROOSEGAARDE, 2018)



SCOPE

Within the project the following topics and points of interest will be discussed:

LIGHTING DESIGN
The main focus of the project will be within the field of lighting design. At the core, defining the unique characteristics of the light on a technical level; looking at properties such as the luminance, amount of diffusion and emission spectra.

- Defining the technical characteristics of the light
- Enhancing or optimizing the light characteristics
- Benchmarking or comparison to other light sources

GROWING DESIGN
In most cases, the core of a Growing Design project is to design with a living material by guiding or influencing its growing process. However, as it is assumed that it will not be possible to intervene the growing process of the algae (with the goal to adjust its properties), the main focus will be on investigating the overall behaviour and possible implementations of the material for product design purposes.

- Designing with a living organism
- Embodiment of algae matter into a material
- Determining potential applications for the algae material
- Implementation of algae material into an object

MATERIAL EXPERIENCE
Materials are known to be a important factor in shaping and affecting the overall user experience of a product, especially within material driven design projects (Karana et al., 2008). In this case, the main focus will be on understanding the material with the goal to discover the qualities and limitations.

- Discovering experiential and performative qualities
- Tinkering with the algae material (ways of embodiment)
- Finding the limitations or boundaries of the material

BIOFYSICA
An important aspect of the project will be understanding the biological processes that happen within the organism, not only looking at the production of light, but also at general behaviour and growth.

- Getting a basic understanding of the behaviour
- Understanding the core processes of the light production
- Knowing the different factors that influence the growth
- Creating a sustainable living environment for the algae

HCI
One of the key factors, in regards to the field of HCI, will be evaluating the response of the light in regards to stimulation; defining the relation between the input, seen as a mechanical stimulus and the output, the light produced by the organisms.

- Understanding the response of light to stimulation
- Describing the input - output relation

FIGURE [3]
SCOPE - FOCUS ON TOPICS

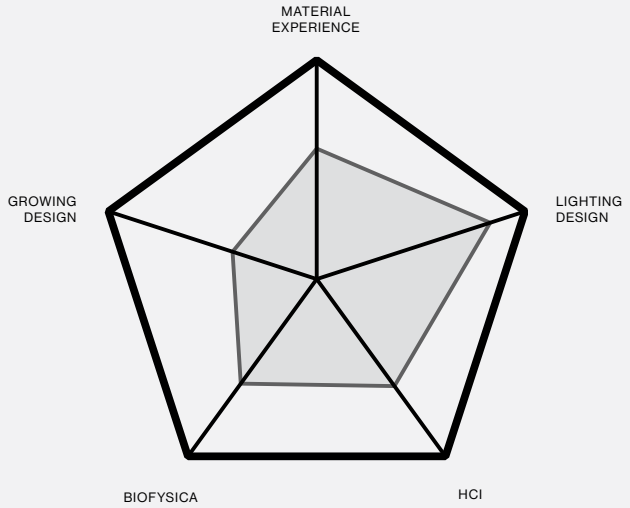
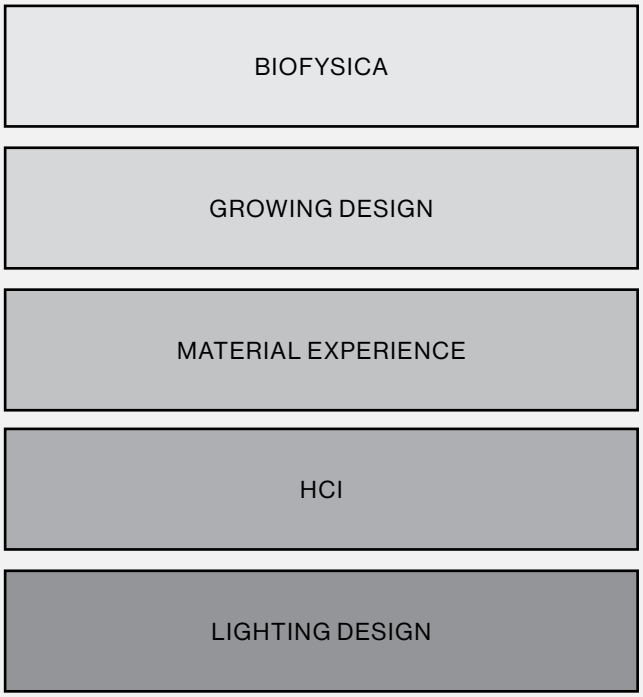


FIGURE [4]
SCOPE - HIERARCHY OF TOPICS



1.3

METHOD

The core of the project will be based on the Material Driven Design method (see figure 5).

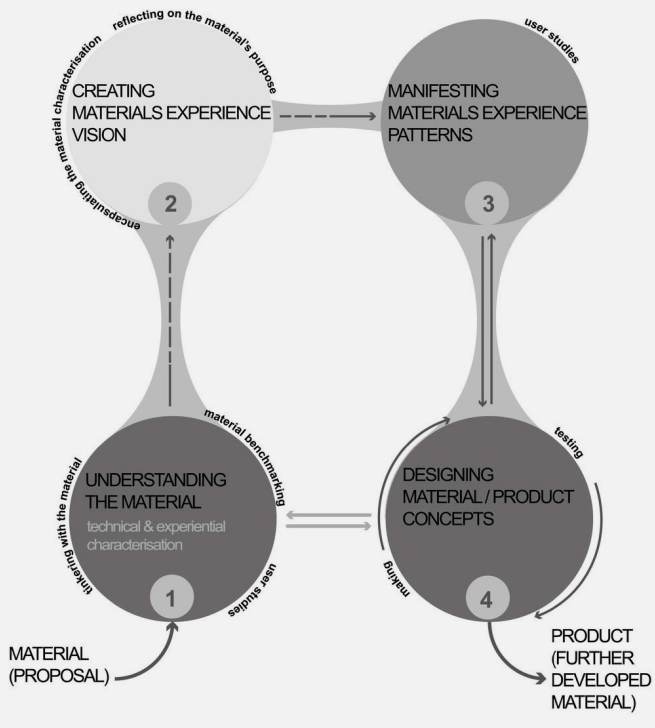
One of the first steps of the Material Driven Design method, consists of understanding the material at hand, in order to be able to characterize it both on a technical and experiential level (Karana et al., 2015). This step requires the designer to start tinkering with the material to be able to get new insights on the affordances of the material, its technical or mechanical properties and ways or shapes in which it could be embodied. At this point, the designer should start with benchmarking the material; determining its strenghts and weaknesses compared to similar materials. By performing user test, the designer will be able to determine how the material at hand is perceived; defining the experiential qualities of the material.

The following step in the MDD model would be to create a Materials Experience Vision, which shows how the designer envisions its unique experiential qualities and functionality within a product, as wel as the purpose of the material within the envisioned context.

After the Materials Experience Vision has been created, the designer is expected to manifest the Materials Experience patterns. During this step, the designer will begin to learn and understand how and when people will experience or interact with the envisioned material.

The final step will be to combine all of the important findings of the previous steps into the product design phase. Note that in some cases, the designer might already have an idea for a type of application or even a product concept. This allows the designer to combine material considerations with the creation of a product design solution; shaping both the material and the concept at the same time.

FIGURE [5]
METHOD - OVERVIEW OF MDD STEPS
(KARANA ET AL., 2015)



FOCUS WITHIN THE MDD MODEL

Within this project, the focus will be on the first step of the MDD model; understanding the material. As working with bioluminescent materials, is still relatively new, especially within the field of product design, a lot is yet to be discovered about the behaviour of the material. Therefore, technical and experiential characterisation of the material will be the most valuable at the current moment.

In order to fully explore the relations between movement and form, a Research through Design approach will also be used while defining experiential characteristics of the material, as well as during the creation of the bio-reactor system.

LITERATURE STUDY	**UNDERSTANDING THE ORGANISM**
	DETERMINING CURRENT KNOWLEDGE GAPS
	DEFINING THE SCOPE OF PROJECT
RESEARCH SETUP	CREATING A BIO-REACTOR SYSTEM
	GROWING THE ALGAE CULTURES
	DESIGNING A SHAKING DEVICE
EXPERIMENTATION	EXPLORING THE BEHAVIOUR OF LIGHT
	DOCUMENTING FIRST INSIGHTS
	DEFINING P.O.I FOR RESEARCH
	PERFORMING TEST SEQUENCES
ANALYSIS	REVIEWING GROWING RESULTS
	CREATING GROWING RECOMMENDATIONS
	DOCUMENTING TEST SEQUENCES
	ANALYSING TEST RESULTS
	DETERMINING KEY FINDINGS
	DISCUSSING TEST RESULTS
DEMONSTRATION	CREATING A RESEARCH OBJECT
	DISPLAYING KEY FINDINGS OF RESEARCH
	DISCUSSING POTENTIAL OF ALGAE MATERIAL
	DEFINING P.O.I. FOR FURTHER RESEARCH

UNDERSTANDING BIOLUMINSCENCE

Understanding the basic principles of bioluminescence and getting a key understanding of how the organisms function on a basic biological level.

C2

CONTENTS

AIM

Gathering in depth information about the organisms and the the light produced; where do they live, how do they produce light, what are the differences between species, etc.

- Understanding the basic principles of the phenomenon of bioluminescence [focus on marine bioluminescence]
- Analysing the current uses of bioluminscence within the field of product design; getting first insights on possible implementations of bioluminescent organisms and the possible relations to HCI
- Gathering information about the type of bioluminescence displayed by dinoflagellates, with the goal to be able to compare the two main dinoflagellates used within the project [focus on species of the Pyrocystis family]
- Understanding the process behind the production of light, in order to get initial insights on the characteristics and limitations of the organisms and the light they produce
- Collecting and combining available knowledge on the flash characteristics of the Pyrocystis Fusiformis and Lunula; getting insights on the behaviour of the light
- Defining current knowledge gaps in order to determine inital points of interest for further research

METHOD

- Literature research
- Information from dinoflagellate suppliers
- Information from experts [Richard Groen]

2.1

BIOLUMINESCENCE

Bioluminescence, the production of light by a living organism, is a commonly seen phenomenon in nature. Bioluminescent organisms are mostly found in the marine environment, where it is estimated that more than 75% of the organisms have the ability to produce light (Martini, 2017). While bioluminescence is more common among deepwater and planktonic organisms, it is found in wide variety of species, ranging from shrimp and algae to octopuses and fishes. In most cases, the light is produced by the organisms themselves, known as autogenic bioluminescence, while in other cases the light is produced by a bacterial symbiont, commonly referred to as bacteriogenic bioluminescence (Haddock, 2009).

Even though bioluminescence is rather common in the marine environments, it is almost absent in freshwater environments and also rarely found on land (Haddock, 2009). While one of the best known examples of terrestrial bioluminescence is the firefly, bioluminescence can also be found in some species of fungi, worms and beetles.

BASIC PRINCIPLES BEHIND BIOLUMINESCENCE

All variants of bioluminescence are the result of chemical reactions happening within the cell of the organism and are therefore classified as a type of chemoluminance. In most cases, bioluminescence is the result of a reaction between a light-emitting molecule, known as luciferin, and a catalysing enzyme, referred to as luciferase. As the luciferase enzym catalyses the oxidation of the luciferin, energy is released in the form of cold light (Pieribone & Gruber, 2005).

The light that is emitted from marine organisms is mainly expressed in the blue-green part of the visible light spectrum between 400 to 500nm, as these shorter wavelengths tend to travel further through water, thus making them more easily visible (Valiadi, 2013). Besides, as longer wavelengths, such as yellow, orange, and red, are almost completely absorbed within a few meters of water, most deep sea organisms have become sensitive to only blue-green light (Haddock, 2009).

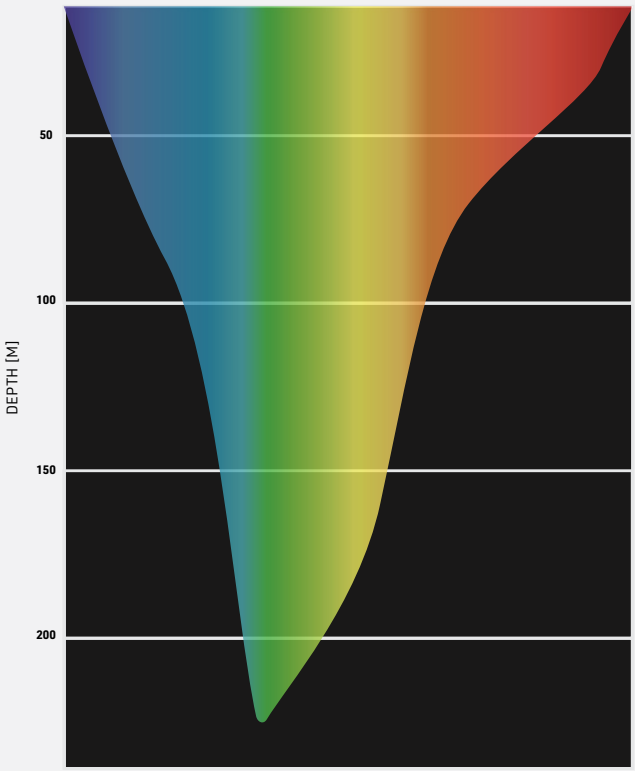
Organisms that live at greater depths tend to show a shift in expressed bioluminescence towards the shorter wavelenghts; resulting in a more blue or even slightly purple light (Haddock & Case 1999; Douglas et al. 1995).

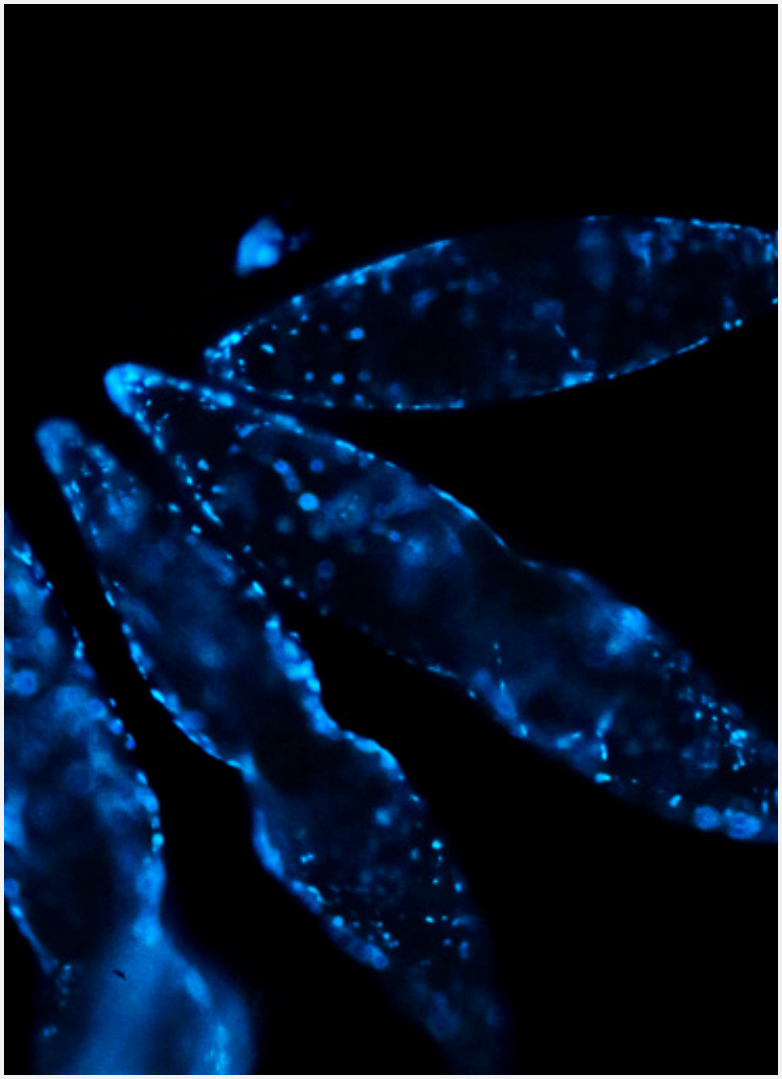
PURPOSE OF BIOLUMINESCENCE

Bioluminescence is known to serve several different purposes depending on the type of organism and for some organisms, bioluminescence may even have multiple functions. In general, bioluminescent glow is seen as a form of attraction, while more rapid flashing is assume to be a form of repellent. Although it is difficult to determine the exact function of bioluminescence for a certain organism, it is generally assumed to be either a form of defence, offence or communication (Haddock, 2009).

It appears that bioluminescence is most commonly used as a defence mechanism, often in an attempt to either startle a predator, act as a form of camouflage or even in order to attract higher order predators (Haddock, 2009). In cases where the light is used as an offensive mechanism, it generally acts as a lure; attracting and illuminating prey. Marine bioluminscence used as a form of communication appears to be mostly used for reproductive purposes; acting as a form of mating display.

FIGURE [6]
INFLUENCE OF DEPTH ON LIGHT SPECTRUM
(NOAA OCEAN EXPLORER, 2010)





TOP [7]
BIOLUMINESCENT SHRIMP
(TDUB PHOTO)

LEFT [8]
BIOLUMINESCENT DINOFLAGELLATES
(MR LOWNDESS)

RIGHT [9]
BIOLUMINESCENT JELLYFISH
(DIOGO BRANDAO)

2.2

DINOFLAGELLATES

One of the most frequently encountered forms of marine bioluminescent is that of the dinoflagellate. Dinoflagellates, also commonly referred to as micro-algae or phytoplankton, are single celled organisms that share characteristics of both plants and animals (Encyclopædia Britannica, 2017). At times when their populations are dense, disturbances in the water will result in displays sparkling bright blue light (see image 10). Currently, there are at least eighteen different genera of dinoflagelLates known to exhibit bioluminescence. The most well-known species of bioluminscent dinoflagellates are those of the Pyrocystis genus, which are often referred to as the fireflies of the sea.

FIGURE [11]
OVERVIEW OF CELL STRUCTURE AND SIZE COMPARISION
(SAL ET AL., 2013

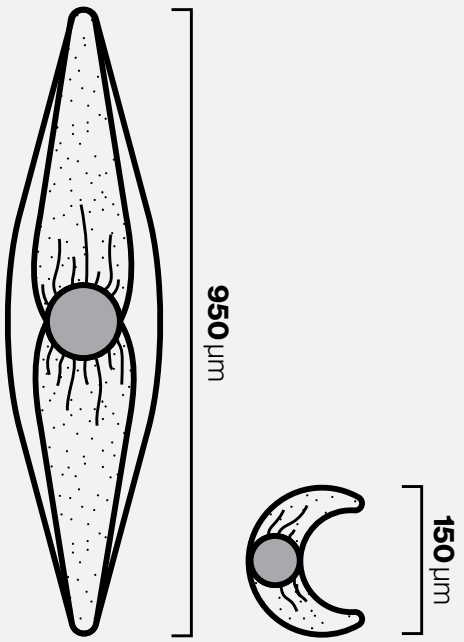


IMAGE [10]
BIOLUMINESCENT ALGAE IN NATURE
(DAVID RODGERS, 2018)

TYPES OF BIOLUMINESCENT DINOFLAGELLATES

Even though all dinoflagellates share the same fundamental characteristics, each species tends to have its own unique and defining features. Dinoflagellates from the Pyrocystis genus are especially interesting as they are known for their relatively high intensity light emissions (Seliger et al., 1969). Species of the Pyrocystis genus also appear to be the most abundant dinoflagellates in tropical and sub-tropical areas of the ocean (Swift and Durbin 1971; Swift et al. 1973). As the species of the Pyrocystis genus are a major part of the marine phytoplankton community, they also are one of the biggest contributors to the earth's oxygen supply. Through the process of photosynthesis they are able to consumes large amounts of carbon dioxide by converting it into oxygen (Swift and Durbin 1972; Miler 2010). Within this project, the focus will be on the two species of the Pyrocystis genus: Pyrocystis Fusiformis and Pyrocystis Lunula.

COMPAIRING THE TWO MAIN SPECIES OF DINOFLAGELLATES

While both organisms appear to behave in a similar manner, the main differences are seen in the shapes and size of cells. Both species are non-motile, meaning that they are unable to 'swim' and can only move in a vertical orientation through adjusting their buoyancy. This characteristic is shared by all dinoflagellate species of the Pyrocystaceae family, which lose their flagellum, a tail-like appendage that allows the organism to move through the water, by the time they reach adulthood (Classification - Pyrocystis Fusiformis, 2015).

Pyrocystis Fusiformis a relatively large and abundant species of bioluminescent dinoflagellates. On average, the cells of the Fusiformis are about 950µm long and 150µm wide; having an average cell volume of about 94 x 10^3 µm^3 (Sal et al., 2013). In comparison, the average cell of Pyrocystis Lunula will be about 150 µm long and about 50 µm wide with a total volume of roughly 17 x 10^3 µm^3 (Sal et al., 2013). Pyrocystis Fusiformis is named after its characteristic spindle-shaped cell, while Pyrocystis Lunula is easily recognized by its unique cresent moon-shaped cell (see figure 11).

Both species of dinoflagellates appear to be most frequently found at a depths of around 50-100 meters, however are also able to live at depths of up to 200 meters (Swift et al., 1976). Pyrocystis Fusiformis is most common in coastal marine waters and tropical or sub-tropical bays, but is also known to survive in oligotrophic waters, where nutrients are sparse. It has been found in many different continents, ranging from North, Central and South America to Asia, Australia and even Europe. While Pyrocystis Lunula can be found in many of the same regions as the Fusiformis, it also has been found in less tropical and colder waters, such as the North Sea or Atlantic (International Hydrographic Association, 1953).

FLASH FORMS

FIGURE [11]
COMMONLY FOUND LOCATIONS OF PYROCYSTIS
(INTERNATIONAL HYDROGRAPHIC ASSOCIATION, 1953)

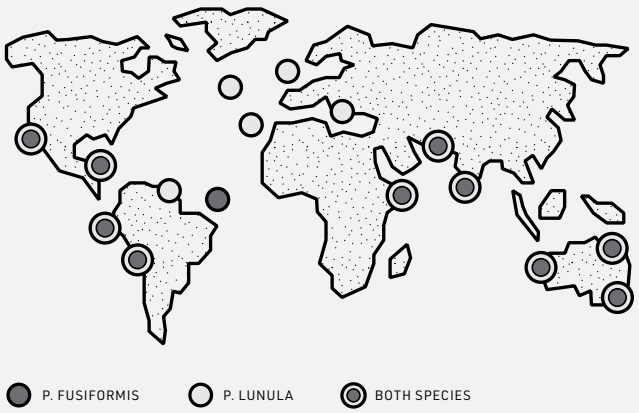


TABLE [12]
OVERVIEW OF CHARACTERISTICS
PYROCYSTIS FUSIFORMIS AND LUNULA

NAME	Pyrocystis Lunula
CELL SIZE	150 µm [A]
CELL VOLUME	17 x 10^3 µm^3 [A]
TSML PER CELL	20 - 50 x 10^8 photons [B]

NAME	Pyrocystis Fusiformis
CELL SIZE	950 µm [A]
CELL VOLUME	94 x 10^3 µm^3 [A]
TMSL PER CELL	230 - 630 x 10^8 photons [C]

[A] SAL ET AL. 2013 [B] BIGGLEY 1969 [C] SWIFT ET AL. 1973

Dinoflagellates are known to invest heavily in their ability to produce light, allocating their energy to bioluminescence even before using it to grow. However, the ability to produce light does seem to come second to the ability to be able to 'swim' and move through the water column (Latz & Jeong, 1996).

DINOFLAGELLATE FLASH FORMS

In general, bioluminescent dinoflagellates are known to have two distinct ways of emitting light: flashing and glowing. The most commonly seen and described form of bioluminescence among dinoflagellates is flashing; the production of short discontinuous bursts of light. Flashing primarily occurs when shear stress is induced on the cell through hydrodynamic movements, for example through larger animals swimming by or waves breaking on the surface (Rohr et al., 1998). Due to the microscopic scale of the organisms, movements within the water are experienced as shear force. A widely accepted theory regarding the biological advantage of bioluminescence for dinoflagellates is a phenomenon called: the burglar alarm (Abrahams & Townsend, 1993). Within this theory, the main function of the bioluminescence is to act as a form of defence, as when a dinoflagellate comes into contact with a predator, the flash of light will bring attention to the predator in turn making it vulnerable to attacks of higher order predators. (Mensinger and Case, 1992; Fleisher and Case, 1995)

Natural movements of the sea, such as the changes in the current or tides, do not appear to trigger the bioluminescence of the dinoflagellates. Dinoflagellates appear to have become less sensitive to these smaller shear forces and changes in flow stress, as it would be highly inefficient for the organism to constantly light up and exhaust its bioluminescent potential (Latz et al., 2004).The threshold shear stress level tend to differ between each species of dinoflagellates and is mainly influenced by the thickness of the cell wall and the internal cell structure (Namdev & Dunlop, 1995). In general, the process of bioluminescence is triggered with fluid shear stresses of around 0.02 to 0.3 Nm^2 (Latz et al., 2004). Not only the amount of force, but also the rate of changes in the flow stress appears to affect the bioluminescent response, as gentle changes in flow stress will result in a decrease in the total stimulated light intensity (von Dassow et al., 2005).

Some species of bioluminescent dinoflagellates are also able to glow, but this continuous emission of light is at a much lower intensity, often not invisible to the naked eye (Krasnow et al., 1980). The amount of glow in relation to the amount of flashing strongly varies among the different species; with the intensity of the glow being rather small for dinoflagellates that produce high intensity flashes, such as species of the Pyrocystis genus (Valiadi, 2013).The intensity of glow fluctuates cyclically, following the circadian rhythm of the organism; being at its maximum during the night and minimum during the day.

Another factor that is known to influence the total light output of dinoflagellates is the cell size (Swift & Meunier, 1976). Research has shown that for Pyrocystis Fusiformis a twofold increase in cell size, will result in a twofold increase in the total amount of light produced as a result of mechanical stimulation (Widder & Case, 1981). This also explains why larger species of dinoflagellates tend to produce more light per cell. Where Pyrocystis Fusiformis has a total mechanically stimulated light output, or in short TMSL, of about 230 to 630 x 10^8 photons per single cell, Pyrocystis Lunula only has a TMSL of roughly only 20 to 50 x 10^8 photons for each single cell (see table 12).

2.3

FLASH BEHAVIOUR

The behaviour of the light or changes in flash characteristics over time are still relatively unknown at the moment. One of the most noteable research articles is that of Widder and Case (1981); describing a number of key flash characteristics, such as the two distinct flash forms, the recovery of these first flash form and the effects of stimulus frequency.

FIRST FLASH CHARACTERISTICS

Pyrocystis fusiformis appears to have two different types of flashing: the initial flash, referred to the as the first flash, and the subsequent flashes. The first flash of the Fusiformis is relatively bright, has a quick rise time of about 10ms and a short biphasic decay; being 90% complete after 200ms from the flash onset. Each of the following flashes is much more dim, but longer lasting; having a rise time of approximately 150ms and a longer monotonic decay that is 90% complete after roughly 500ms from flash onset (Widder & Case, 1981). Similar flash behaviour has also been found in specimens of Pyrocystis Lunula (Tesson &Latz, 2015).

The characteristics of the first flash appear to recover over time (see figures 13-14). After a paused interval of 1 minute the total light emission of the second flash will be about 10% the intensity of the first. From an interval of 3 minutes and upwards, the recovery time appears to steadily decrease as the durations of the intervals increase. After 12 minutes the total stimulated light intensity of the second flash even gets up to 120% of the value of the first flash. However, the rise time of the second flash at this point is only at 70% of its initial time. The increase in total stimulated light is the result of a longer overall flash duration, as the decay of the flash is extended for the second flash after a 12 minute pause. After 30 minutes, both the rise and fall times of the second flash appear to be back to their original and the first flash characteristics have appeared to be fully recovered (Widder & Case, 1981).

However, if one stimulates a cell till exhaustion the first flash characteristics appear to recovery much more slowly. An exhausted cell will produce only 60% of the total light output of after a period of 6 hours. Even after 24 hours, the first flash characteristics have yet to be fully recovered as the total light output sits at around 90% of its initial value. When compared to the recovery times of a cells that have been agitated only once, these times are exceptionally long (Widder & Case, 1981).

STIMULUS FREQUENCY AND LIGHT OUTPUT OVER TIME

Another interesting finding noted by Widder & Case (1981), is the influence of stimulus frequency on the number of flashes a single cell is able to produce until it is exhausted. It appears that the number of flashes per cell is highly dependent on the frequency of the pulse stimulus. Cells that were stimulated with 0.083 pulses per second, which equates to about 5 pulses per minute, produced on average about 77 flashes. While cells stimulated at a 1.33 pulses per seconde or about 80 pulses per minute only produced 20 flashes on average.

FIGURE [13]
RECOVER OF FIRST FLASH KINETICS
(WIDDER & CASE, 1981)

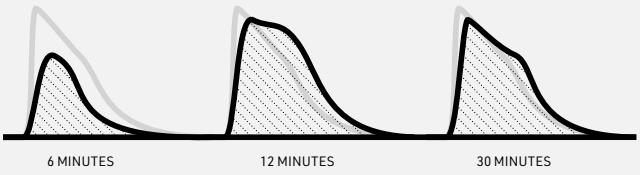
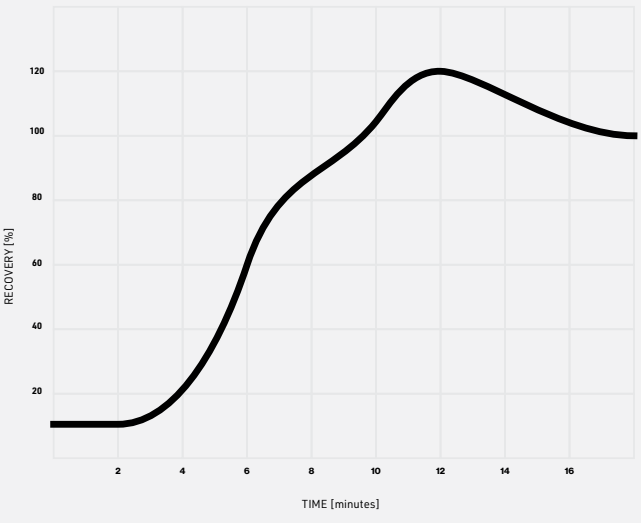


FIGURE [14]
RECOVERY OF FIRST FLASH INTENSITY
(WIDDER & CASE, 1981)



INTERCELLULAR PROCESSES

Bioluminescent dinoflagellates show a distinct rhythm in the intensity of their flashing, being much brighter during during the night than during the day. As the bioluminescent processes are regulated by an endogenous circadian clock. This clock regulates the internal process with a day and night cycle.

FIGURE [15]
DEPICTION OF INTERCELLULAR PROCESS
(VALIADI, 2013)

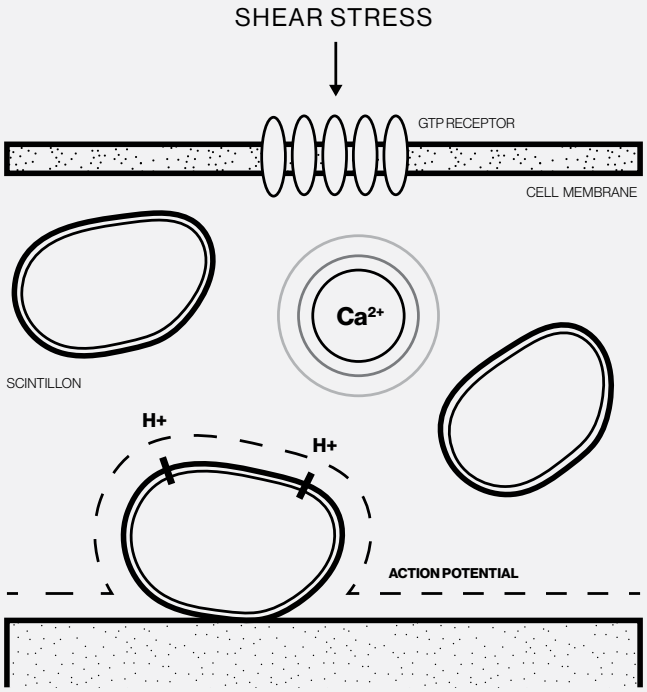
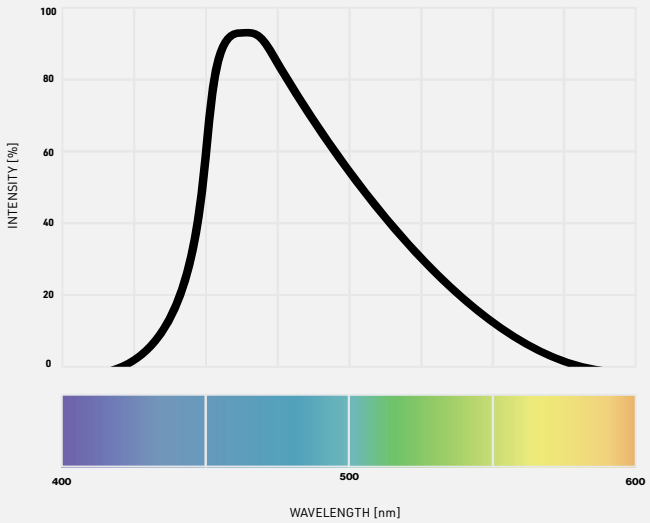


FIGURE [16]
EMISSION SPECTRUM OF PYROCYSTIS LUNULA
(SELIGER ET AL., 1969)



INTERCELLULAR PROCESS

Within the cells of the dinoflagellates, light is produced in the scintillions (Desa & Hastings, 1968). Scintillions are small vesicles with a diameter of approximately 0,5 to 0,9 µm that lie near the edge of the cell. These small organelles contain the luciferin substrate and the catalysing enzyme luciferase, which are the main components responsible for the production of light (Nicolas et al., 1987).

Before light is produced, a sequence of cellular and molecular processes need to happen within the cell. Once shear stress is exerted on the outside of the cell membrane, a GTP protein coupled receptor is activated, which transducts the force from the outside to the inside of the cell (Chen et al., 2007). Next, this mechanical signal is translated to an electrical signal, as there is an increase in Ca^{2+} within the cytoplasma. Most of the Ca^{2+} is sourced from within the cell, but it can also be derived from the medium surrounding the organism (Von Dassow and Latz 2002). As the increase in Ca^{2+} depolarizes the vacuole membrane, an action potential is created. This action potential will travel across the membrane of the scintillions touching the vacuole membrane, activating the voltage gated proton channels on the membrane (Smith et al., 2011). As the protons from the acidic vacuole enter the scintillion, the pH within the organelle quickly drops, in turn activating the bioluminescent process within the scintillion.

When the scintillions are acidified, the luciferase within the organelles become active and opens the luciferin binding sites. Next, the lucifein is oxidized by the luciferase, resulting in the emission of photons (Schultz et al., 2011). These photon emissions can be seen as short flashes of bright blue light with wavelengths peaking at 475nm (Seliger et al., 1969).

REGULATION OF BIOLUMINESCENT PROCESSES

The regulation of the bioluminescence and circadian rhythms tends to vary significantly between species (Vialdi, 2013). Some species of dinoflagellates regulate the processes by adjusting the amount of luciferase and luciferin within the cell; breaking down the scintillons themselves at dawn in order to resynthesize them at dusk. Pyrocystis Lunula, however, is known to regulate its bioluminescence by relocating, instead of destroying, its scintillions. During the night, the scintillions are located near the edge of the cell, while during the day they are moved towards the centre of the cell; preventing their stimulation (Knaust et al., 1998).

Another important factor that plays a role in the regulation of the bioluminescent processes within dinoflagellates is the act of photoinhibition, a mechanism shared by all photosynthethic organisms. Photoinhibition is described as a reduction in the efficiency in the use of light in the process of photosynthesis (Kok, 1956). Neither the mechanism of photoinhibition, nor the identity of the receptor that inhibits the light, have been found. Regardless, photoinhibiton is still seen as one of the fundamental mechanisms that regulates the bioluminescent behaviour of the dinoflagellates (Vialdi, 2013).

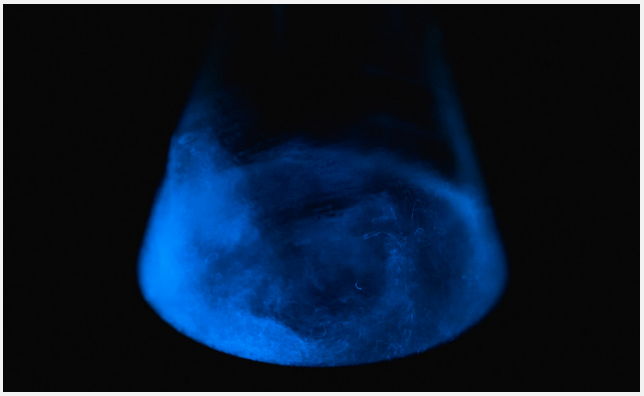
2.5

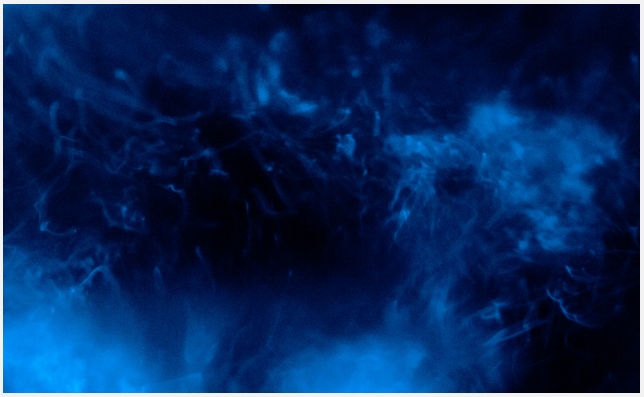
BENCHMARKING

In order to further define the scope of the project, it will be important to define the current knowledge gaps. In this case, by reviewing available research on the flash behaviour and the growth of bioluminescent dinoflaggelates, as wel as looking into the current uses of bioluminescence within the fields of product design and arts.

FLASH CHARACTERISTICS

IMAGES [17 - 18]
TOP - BEHAVIOUR OF A COLLECTIVE OF CELLS
BOTTOM - BEHAVIOUR OF SINGLE CELLS





RESEARCH ON THE BEHAVIOUR OF THE LIGHT

Currently, most of the available research is either focussed around the biological or mechanical characterisation of the light produced by dinoflagellates. Commonly described topics include: the absolute photon emissions of a single cell, the amount of force required to trigger bioluminescence and the influences of the time of day on the light output (Seliger et al., 1969; Tesson & Latz, 2015; Biggley, 1969).

While this type of technical characterisation provides valuable insights in the fundamental properties of the light produced by certain species of dinoflagellates, it does not provide much information on the actual behaviour of the light within real life scenario's. Especially, as almost all of the current research is focussed on the response of a single cell, rather than the combined response of a group of cells. In order to effectively implement any form bioluminescence within a product design solution or exhibitional setup, it will be much more valuable to have an idea of how the light will behave over time. For example, by looking at changes in intensity for different types of agitations, differences in sensitivity to different kinds of stimuluses and overall behaviour as a group.

One of the articles that stands out the most is the paper from Widder & Case (1981), which describes the two 'flash forms' of Pyrocystis Fusiformis. This paper can be seen as one of the first steps into the characterization of the flash behaviour of bioluminescent dinoflagellates; providing valuable insights in the behaviour of the light over time. While the core of this research is still highly technical and focusses on the responses of a single cell, it is the first to describe the changes in flash characteristics over time; giving first insights in the behaviour, as wel as the limitations of the light produced by the algae

No research appears to be performed yet with regards to the experiential characterisation of bioluminescent materials. Nevertheless, the unique experiential qualities of the light are still assumed to be one of the most promising features of bioluminescence. However, as bioluminescent materials are living organisms that need to be contained or embodied within a material, the experiential qualities will not only be based on the behaviour of the light itself, but also highly depend on way in which it is embodied. In a way, making it even harder to determine the experiential qualities of an organisms as it will depend on many different factors.

While there is still remains a lot to discover about the flash characteristics of bioluminescent dinoflagellates, the most important knowledge gaps appear to be within the following topics:

- **Changes in the behaviour of the light over time**
- **Response to stimuli of the organisms as a collective**
- **Overall response to different types of stimuli**
- **Experiential qualities of the light**

2.5

LIVING CONDITIONS

In order to successfully make use any kind of bioluminescent materials within a product design solution, the most important step will be to keep the organisms alive. As if designers are not able to grow and maintain the bioluminescent material, they will not be possible to create sustainable product. Therefore, the first step in designing with living materials should always be to understand the material at hand, not only from a technical perspective, but also on a biological level.

RESEARCH ON THE GROWTH OF THE ORGANISM

While the effects of changes in environmental conditions, such as temperature, light and nutrients, have been thoroughly researched and documented for many different species of phytoplankton, it remains challenging to determine the optimal living conditions of a certain species. As you start to narrow down and search for more species-specific information, going from phytoplankton to dinoflagellates or even to a specific species of dinoflagellate, information begins to become scarce (see figure 19). As each species of dinoflagellates appears to respond differently to environmental changes, it is not possible to precisely define the optimal living conditions by just looking at response of dinoflagellates in general (Boyd et al., 2013). At the moment there is no source that fully describes the effects of changes in certain environmental conditions for the different kinds of bioluminescent dinoflagellates.

INFORMATION OF SUPPLIERS

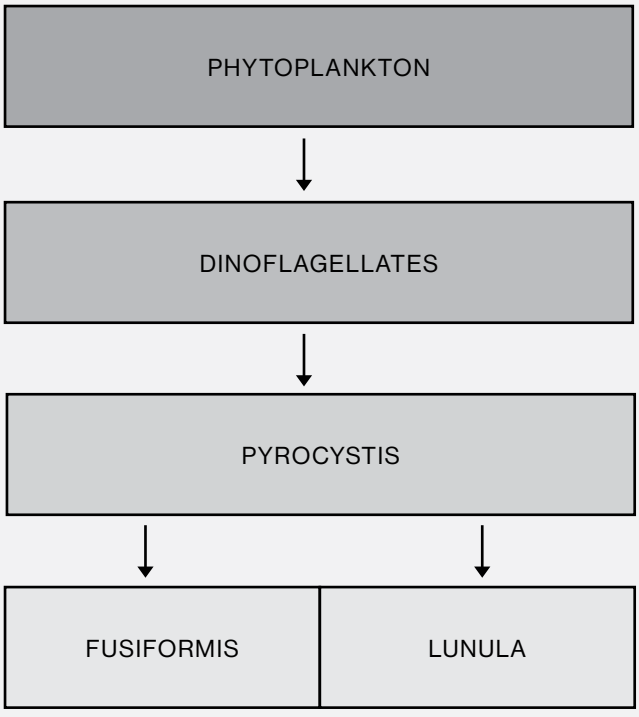
Most suppliers of the bioluminescent dinoflagellates also do not appear to provide sufficient information to allow designers to create a sustainable living environment. Growing manuals or guides are often simplified and seem to mostly focus on extending the death of the organisms for as long as possible, instead of providing detailed instructions on how to properly grow and maintain them. While other general tips and tricks can be found online from various sources, there is no real concicive guide on how to effectively grow bioluminescent dinoflagellates at home or in a lab environment.

Also, there currently is not much information available on how the organism should be handled. While it is generally known that these organisms are highly prone to contamination and therefore hard to culture without the right equipment, there is not much information available showing how to properly handle and store the organisms.

In order to be able to stimulate further research and the use of bioluminescent dinoflagellates within the field of product design, it will be important to create a concise overview of the optimal conditions for each of the key environmental factors that have shown to influence the growth; providing sufficient information for others to grow and maintain the organisms.

- **Lots of information, but not specific for species**
- **Lack of (shared) knowledge from suppliers**
- **No source of combined information available**
- **Lack of information regarding handling**

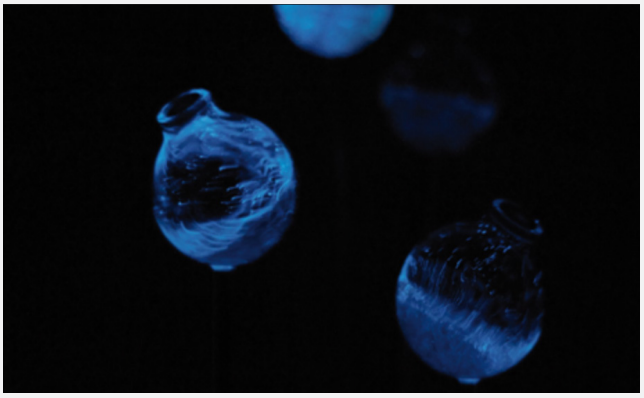
FIGURE [19]
HIERARCHY OF SEARCH TERMS

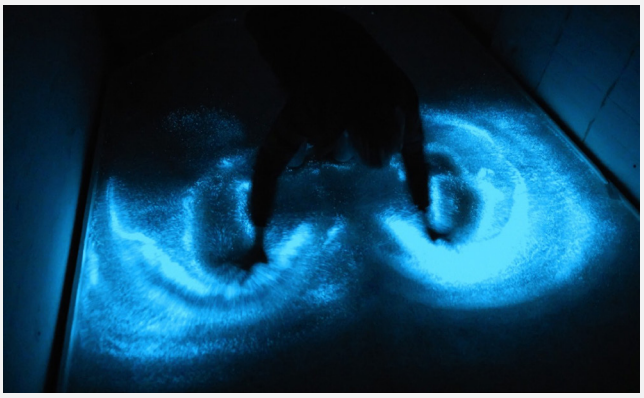


2.5

BIOLUMINESCENCE & DESIGN

IMAGES [20 - 21]
TOP - BIOLUMINESCENT FIELD (NICOLA BURGGRAF)
MID - GLOWING NATURE (STUDIO ROOSEGAARDE)
BOTTOM - THE BLUE ISLAND (ISLAND CHEN)





While the use of bioluminescence within product design solutions and art installations appears to have gotten more attention over the last few year, it still remains a relatively new and unknown material for most designers.

BIOLUMINESCENCE AND EXHIBITONAL DESIGN

At the moment, bioluminescence is mainly used in exhibitional design projects, such as Growing Light by Studio Roosegaarde and the Bioluminescent Field by Nicola Burggraf. A common theme of these interactive exhibition setups is to allow visitors to explore the behaviour of the bioluminescent dinoflagellates through movement; in a way by replicating the interactions that would happend within natural habitat of the organisms.

Glowing Nature uses transparent polymer bags embedded into the floor, which are filled with large amount of dinoflagellates. As visitors walk over the tiles, the algae within the tiles will be triggered by the force that is generated, in a way mimicking the experience of walking through a bunch of bioluminescent dinoflagellates as they are washing up onto the shore.

The Bioluminescent Field consists of a dark room filled with a large amount of small flasks containing the bioluminescent dinoflagellates. As the containers are attached to moving rods, the vials will start to move once visitors walk through the installation, which in turn triggers the bioluminescence of the algae. Within this installation the algae act as a bioluminescent sensor that responds to the movement of the visitors.

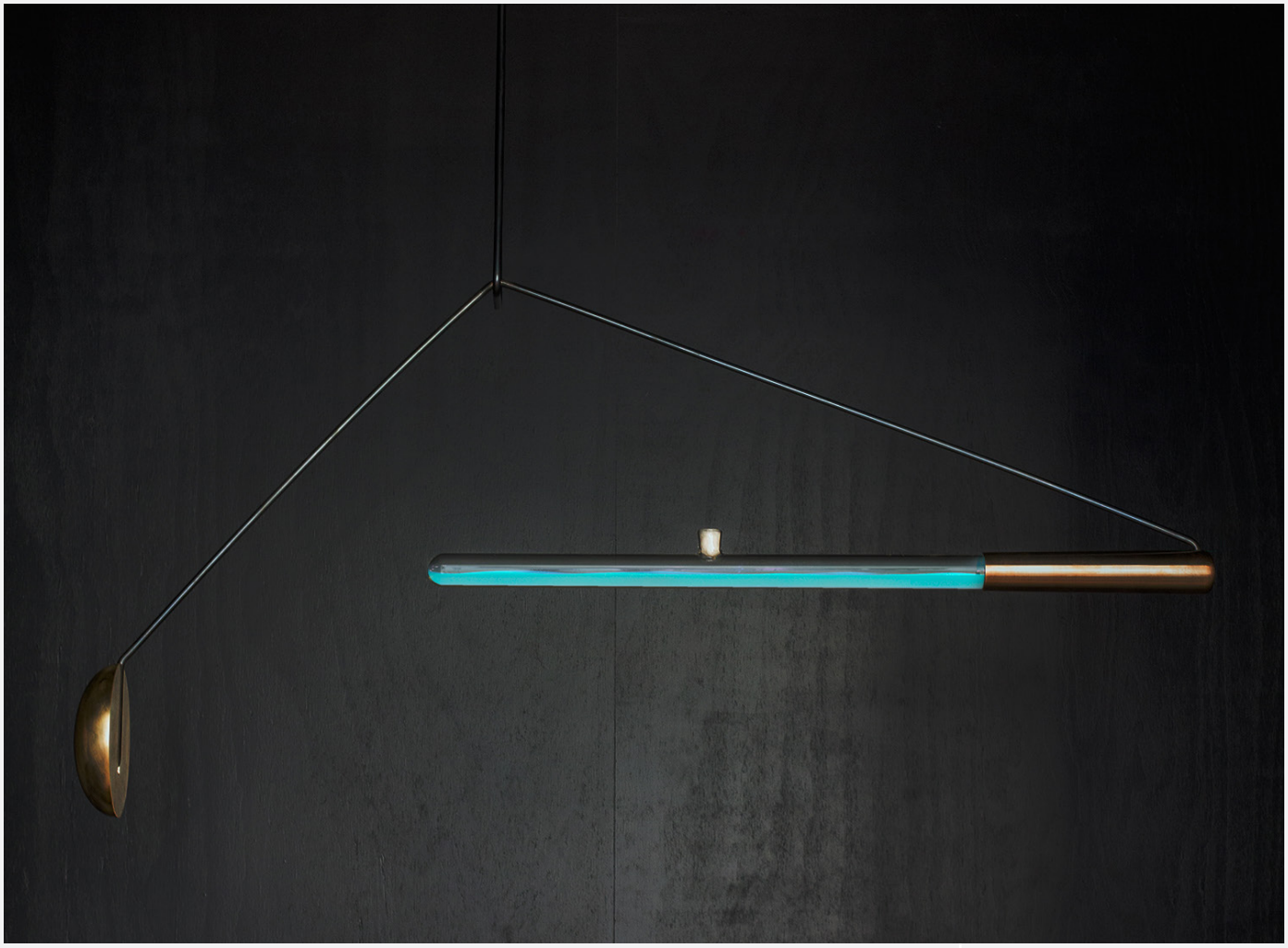
While the interactive elements of both exhibitions are clearly different, both exhibitions try to replicate the behaviour of the organisms in their natural habitat in some form. These type of interactive exhibitions are a great way to show the potential of bioluminescent materials, as they require the vistor to actively engage in exploring the material affordances.

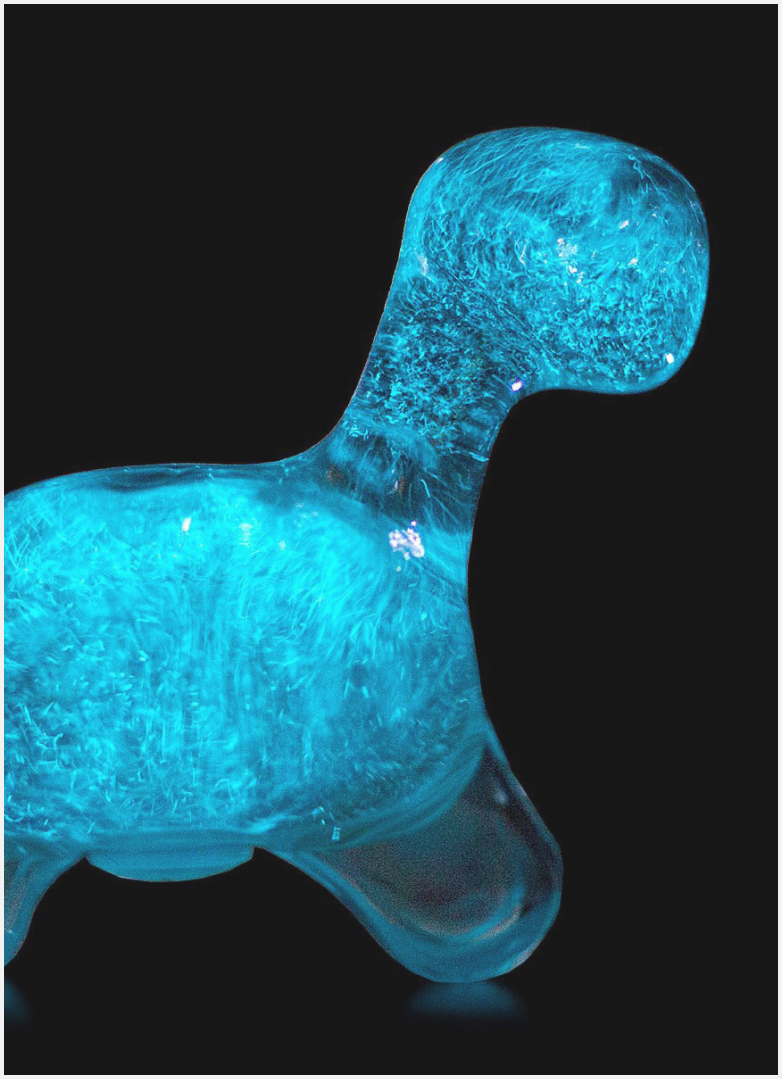
BIOLUMINESCENCE AND PRODUCT DESIGN

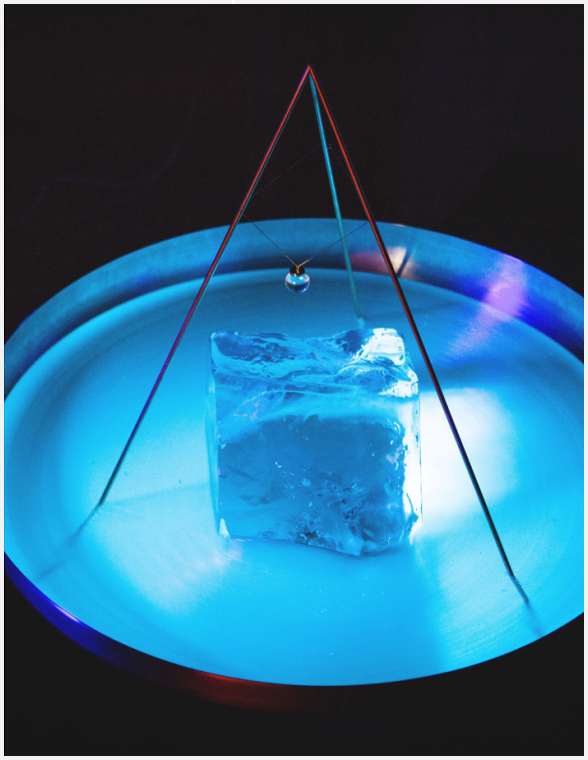
Other designers, such as Teresa van Dongen, make use of bioluminescent bacteria to create not only art installations, but also try to move towards product design solutions for everyday use scenario's. With AMBIO, a lamp powered by bioluminescent bacteria, she is one of the first designers that attempts to make use of bioluminescent materials beyond exhibitional design.

While there currently are some others products available that make use of bioluminescent algae, these objects often tend to have no clear purpose. Commercial products, such as the PyroOrb or the Mermaid's Lunchbox pendant, are merely decorative containers for the organisms and have no function aside from aesthetics. These products clearly show the current lack of knowledge surrounding the behaviour of the material and make poor use of the unique experiential and performative qualities of the bioluminescent organisms. Another issue with these type of products is the fact that the organisms are often treated as a disposable element of the product. In most cases, no real care appears to be taken in creating a sustainable living environment for the organism within the product.

In order to further stimulate the use of bioluminescence within the field of product design, it will become necessary to create sustainable products that move beyond using the material purely for decorative purposes. Instead, stimulating people to actively discover the unique experiential and performative qualities of the bioluminescent material through interaction.







TOP [22]
AMBIO - BACTERIAL LAMP
(TERESA VAN DONGEN)

LEFT [23]
DINO PET - LIVING BIOLUMINESCENT PET
(BIOPOP)

RIGHT [24]
MERMAID'S LUNCHBOX PENDANT
(BOMPAS & PARR)

GROWING & MAINTAINING

Determining the optimal living conditions of the organisms, in order to create a sustainable living environment allowing us to effectively maintain and grow the organisms.

C3

CONTENTS

AIM

Understanding the different factors that influence the growth of the organisms, in order to be able to create a sustainable living environment that allows us to effectively maintain and grow the organisms.

- Understanding the conditions that the organisms require in order to survive and grown; looking at their natural living environment
- Defining the requirements needed to create a sustainable living environment that allows us to maintain and grow the organisms
- Creating an environment or bio-reactor that can be used to grow the algae; with the goal to grow algae for further testing, as wel as determining what conditions actually work for the growing process
- Describing and analysing the growing 'results' to get a view on the different factors and characteristics of the growing setup that affect the growth;
- Reviewing the growth process, with the goal to create [basic] guidelines for growing te algae.

METHOD

- Literature research
- Information from dinoflagellate suppliers
- Information from experts [Richard Groen]

3.1

LIVING REQUIREMENTS

Bioluminescent dinoflagellates are known to be challenging to culture, especially without proper lab equipment. Therefore, an essential part of the project will be creating a sustainable living environment for the organisms; allowing us to grow and maintain the algae for further research.

Dinoflagellate growth responds in a complex manner to the combined effects of temperature, light, salinity and nutrition. (Jitts et al., 1964).While it is possible to increase the growth rate of the organisms by optimising the growing conditions, there is a certain limit to which the growth rate can be pushed. In optimal conditions, Pyrocysistis Fusiformis, tends to divide every 4 to 5 days. However, as conditions vary the division times generally lies between 5 to 14 days (Swift et al., 1976).

The following chapters analyses the optimal conditions for each of the key environmental factors that have shown to influence the growth of Pyrocystis Fusiformis and Pyrocystis Lunula. Note that the optimal growing conditions will vary between species.

TEMPERATURE

In general, the lower and upper temperatures limits for growth of phytoplanktonic organisms, such as dinoflagellates, lies between 5 and 30 degrees C, with an optima usually between the 15 and 27 degrees. The precise temperature values for optimum growth differ per species and conditions, such as the light intensity and the presence of other organisms or bacterial contaminants (Boyd et al., 2013).

The optimal temperature range for most marine phytoplankton appears to be correlated with the temperatures found in their natural habitat (Thomas, 1975). For Pyrocystis Fusiformis, which is usually found in more tropical marine environments, the water temperature fluctuates from around 16 to 24 degrees (Global Sea Temperatures). Pyrocystis Lunula, which can be found in many of the same regions as the Fusiformis, also can be found in less tropical climates (such as in the North Sea and Atlantic Ocean) and therefore is expected to withstand even lower temperatures.

Generally, suppliers of the algae cultures suggest keeping them at room temperature (most commonly referred to as around 18 to 22 degrees Celsius). Pyrofarms, the supplier of the Fusiformis, mentions that dinoflagellates can withstand a wider temperature range if the changes are gradual and light conditions are complimentary. In comparison, the growing setups used in most of the research papers generally try to maintain a control temperature of approximately 20 degrees Celsius (Widder & Case, 1981; Tesson & Latz, 2015; Sweeny 1982; Craig et al., 2013).

Higher temperatures, ranging from 24 to sometimes even 30 degrees Celsius, are known to promote the division rate of some dinoflagellates species (Thomas, 1975). However, as most phytoplankton also show a steep decay in growth rate once they pass a certain high temperature threshold, using growing temperatures above 24 should generally be avoided. It is important to note that higher temperatures also makes it easier for other unwanted organisms to start growing inside the container, increasing the chance of contamination. Lower temperatures (ranging from 15 to 18 degrees) appear to result in a slight decrease in the division rate. However, slight fluctuations in temperature within this range will have less of a noticeable effect on the growth rate (Thomas, 1975).

FIGURE [25]
EFFECTS OF TEMPERATURE ON THE DIVISION RATE
OF A TROPICAL DINOFLAGGELATE CULTURE [GYMNODINIUM]

SOURCE: (THOMAS, 1975)

LIGHTING CONDITIONS

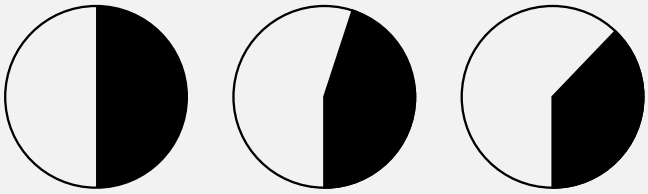
As dinoflagellates are autotrophic organisms, meaning they derive their energy through photosynthesis, they require light in order to charge the bioluminescent processes. The amount of energy the algae can generate during the light cycle depends on the several aspects of the light source, such as the intensity, spectrum and duration.

DURATION OF LIGHT

As the bioluminescence of the dinoflagellates is controlled by an endogenous circadian clock, a constant lighting schedule is required. It is important to provide cultures with approximately 12 hours of light, followed by 12 hours of darkness. Most of the research setups tend to use either a 12 or 14 hour light cycle (Widder & Case, 1981; Tesson & Latz, 2015; Sweeny 1982; Craig et al., 2013). It is assumed that by increasing the duration of the light cycle from 12 to 14 hours, the algae will be able to generate more energy, in turn resulting in increase in TMSL. However, it is important to note that at the moment there is no research available on the effects of changes in the lighting schedules and its relation with the flash characteristics.

While it is possible to shift the timing of the circadian rhythm, it should generally be avoided as it tends to put a lot of stress on the organisms, in some cases even resulting in a decrease in bioluminescence. For research purposes, it is recommended to provide the algae with light during the night time (from 18:00 till 8:00), while keeping them in a dark area during the day time (from 8:00 till 18:00); making it possible to perform test during the day time. However, it is important that during the dark cycle the algae are kept in a fully darkened environment. Note that is possible to bring cultures that currently are in their dark cycle into the light for a short period of time (Richard Groen, 2020).

FIGURE [26]
COMPARISON OF DIFFERENT LIGHTING SCHEDULES
RATIO [LIGHT : DARK]



INTENSITY OF LIGHT

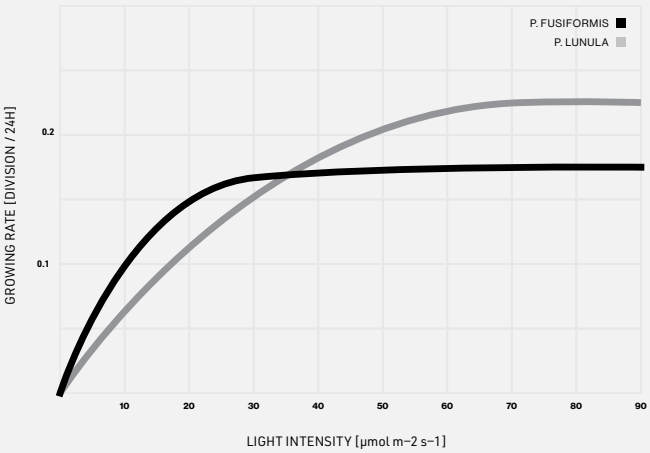
Research from Swift and Meunier (1976) has shown that the cell numbers of Pyrocystis Fusiformis and Lunula do not further increase from light intensities higher than 5 to 10 µmol m^{-2} s^{-1}. It also shows that the division rate (amount of doubling per 24H) was saturated at 30 µmol m^{-2} s^{-1}, while the capacity of stimulable bioluminescence was already saturated at 0.15 µmol m^{-2} s^{-1}. While both Pyrocystis Fusiformis and Lunula will continue to divide even at light intensities below the saturation rates, over time the lack of light will result in a decrease in stimulable bioluminescence and a reduction in cell size.

Suppliers of the bioluminescent algae generally recommend a 400 lumen LED light source to be more than sufficient. Using this type of light will result in a light intensity of approximately 10 µmol m^{-2} s^{-1} (depending on the spectrum characteristics of the light and distance of the light to the cultures). However, as the research of Swift and Meunier (1976) shows that higher light intensities (up to 140 µmol m^{-2} s^{-1}) do not have negative effects on the growth, a more powerful light source of about 800 to 1600 lumen is recommended if one wants to enhance the growth.

Note that while it is difficult to give the algae to much light, high intensity light sources often tend to produce a lot of heat. In certain setups, this heat can be transferred to the medium; resulting in an undesirable fluctuation in temperature or even overheating of the culture.

FIGURE [27]
EFFECTS OF LIGHT INTENSITY ON THE DIVISION RATE
COMPARISON BETWEEN P. FUSIFORMIS AND P. LUNULA

SOURCE: (SWIFT & MEUNIER, 1976)

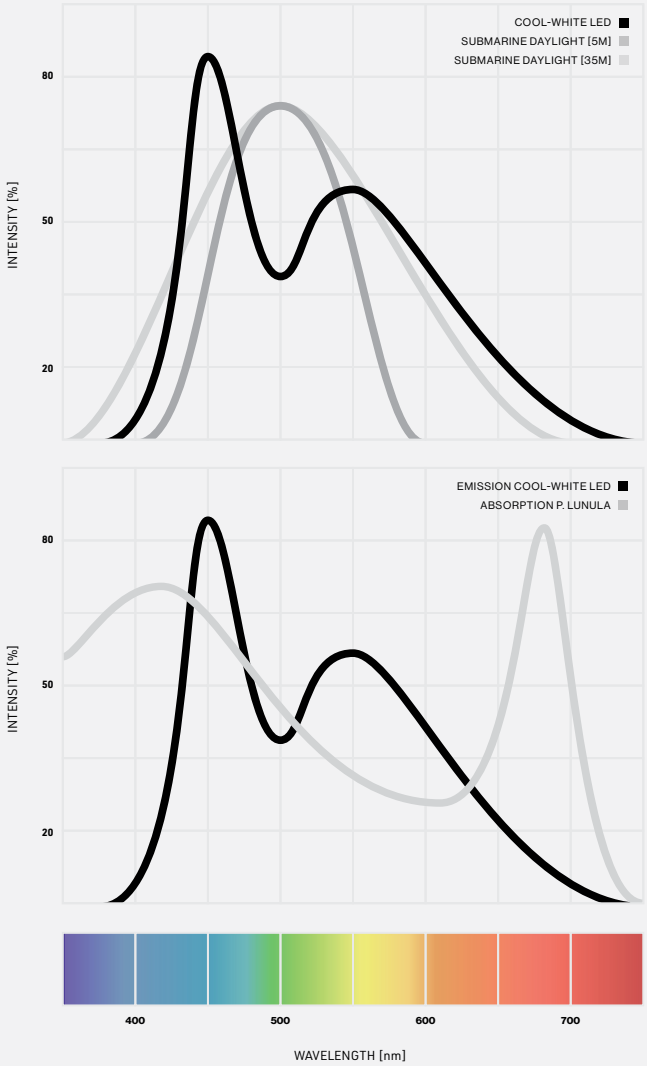


3.1

FIGURE [28 - 29]
EMISSION & ABSORPTION SPECTRUM COMPARISIONS

TOP - SUBMARINE DAYLIGHT / COOL-WHITE LED
BOTTOM - ABSORPTION PYROCYSTIS LUNULA / EMISSION COOL-WHITE LED

SOURCE: (JITTS ET AL., 1964)



SPECTRAL DISTRIBUTION
Not only the intensity, but also the spectral distribution of the lighting is important. Especially, as autotrophic organisms can only absorb certain wavelengths of light depending on the type of pigments they exhibit. Since the amount of light that is being absorbed depends on the composition and concentration of the pigments in the chloroplasts, some type of algae absorb more light at a given wavelength; being able to convert more light energy of that specific wavelength to chemical energy (Andersen & Lewin, 2019).

Pigment compositions tend to vary between different types of algae and even between different species of dinoflagellates (Johnsen et al., 1994). While some research has been done to determine the absorption spectra of certain species of bioluminescent dinoflagellates, it remains difficult to precisely describe these spectra, as they tend to vary throughout the day. These variances appear to be caused by changes in the internal cell structure (Stephens, 1995).

Also, while the effects of different wavelengths on the process of photosynthesis are well known, the relation between the different type of wavelengths and the cell division kinetics of dinoflagellates is less evident (Jitts et al., 1964).

RECREATING NATURAL LIGHT
Another problem occurs when one tries to recreate similar lighting conditions with regards to the spectral distribution, as in the natural environment of the algae. As the shape of the curve will depend on the depth, the clarity of the water and the conditions of the sky (Jitts et al., 1964). Within a few metres of water most of the red wavelengths are absorbed. If the water contains organic material, such as other algae, plants or coral, most of the blue wavelengths will also be absorbed. Therefore most deep water environments will only receive light with primarily green wavelengths (see figure 28).

No form of daylight, even when observed only a few meters below the water surface, appears to spectrally resemble most of the commonly used light sources to grow dinoflagellates, such as LEDs or fluorescent bulbs.

RECOMMENDED LIGHT SOURCE
At the moment, the best type of lighting source used to grow dinoflagellates appears to be LED. The main benefit of using a LED light source, in comparison fluorescent or incandescent lighting, is its energy efficiency and durability. LEDs also do not produce a lot of excess heat, which is important if the light source is not properly isolated or close to the algae culture.

While there are numerous types of LEDs, it is generally recommended to use a cool-white LED (5000-6500K). These types of LEDs show a significant increase in emission near the blue wavelengths (between 400-500 nm), while warmer LED variants (2000-4000K) show less of a pronounced peak and a slight increase in the yellow and orange wavelengths (around 600 nm). Cool-white LED light strips also appear to have a tendency to produce slightly more lumen, when compared to their daylight or warm color variants.

It is important to note that the lighting characteristics of LEDs can differ quite a bit between manufacturers. Therefore, not all LED's marked as cool-white will have a similar spectral distribution as the one shown in figure 29.

MEDIUM

Dinoflagellates require nutrients to be able to grow and divide. In order for the algae to be able to effectively grow, they are best kept in a liquid medium. The medium is used to replenish nutrients, as wel as dilute cultures that have overgrown.

Generally, the liquid medium for dinoflagellates consists of a nutrient enriched seawater. The most commonly used recipe is the F/2 medium as described by Guillard and Ryther (1962). The original F/2 medium consists of filtered ocean seawater with additional vitamins and trace metals. Throughout the years, several derivatives of the F/2 medium have been made. One of the most commonly used modifications of the F/2 medium is the L1 medium (see appendix A1 for full recipe and instructions on how to make it).

The L1 medium is a general-purpose marine medium used to grow coastal algae. While it shares all of the key ingredients with the F/2 medium, it has some additional trace metals. A commonly used derivative is the L1-Si medium. In this case, one prepares the L1 medium, but omits the silica source. This version of the medium is preferred for growing dinoflagellates, as less precipitation tends to form (NCMA Bigelow).

While filtered open ocean seawater is commonly used to create F/2 or L1 medium, it is generally recommended to use artificial seawater. As one does not know the exact composition of the elements in natural seawater, it is seen as less reliable. By creating artificial seawater one will be certain all necessary elements are available in the medium in sufficient quantities. Also, it will be easier to recreate the exact same medium as the ingredients do not change over time.

Apart from the required nutrients and trace metals, the salinity and pH of the medium are also important. The pH of the final solution should be between 8.0 to 8.2, while the salinity should be around 3.5% (NCMA Bigelow).

TABLE [30]
OVERVIEW OF MAIN COMPONENTS MEDIUM
(SEE APPENDIX A1 FOR FULL RECIPE)

COMPONENT	CONCENTRATION	UNIT
Artificial seawater	1000	mL
N/P source*	2.0	mL
Trace metals*	1.0	mL
Vitamins*	0.5	mL

* pre-mixed stock solutions

IMAGES [31-32]
COMPARISON OF DIFFERENT CULTURE DENSITIES

TOP - LOW DENSITY P. FUSIFORMIS CULTURE
BOTTOM - HIGH DENSITY P. FUSIFORMIS CULTURE

RATIO OF MEDIUM TO ALGAE

Generally, suppliers recommend not adding more than 20 to 30% of medium to a culture every 3 to 4 weeks. Adding too much medium to a culture can result in a noticeable decrease in bioluminescence or even death of a culture. The optimum percentage of new medium will depend on the condition and density of the culture. Dense and undiluted cultures will most likely be able to cope with a slightly higher percentage of new medium. For now, we assume the maximum amount of medium added will be around 50% of the current volume if one has a dense and previously undiluted culture.

The density of a culture can be easily determined by looking at the transparency and color of the liquid. A low density culture generally results in a clear and almost fully transparant liquid. As the culture grows over time the liquid will start to become more yellow or orange (depending on the species of algae) and turn more opaque. While there are several methods to precisely measure the optical density of a culture, such as with a spectrophotometer, in most cases a visual inspection will provide enough insights on the density of a culture and the amount of medium that should be added. Note that in most cases, it is best to stick to adding 20 to 30% of medium, as this limits the risk of overdiluting the culture.

3.1

STORAGE

One of the most challenging aspects of growing dinoflagellate cultures in a lab environment, is the risk of contamination. As the living conditions of the algae are also appropriate for many other types of microorganisms, such as bacteria or other phytoplankton, it is difficult to keep a dinoflagellate culture isolated and free of contamination. While in some cases the contamination will be only visible after a couple of weeks, in other cases the contamination can outgrow the dinoflagellate culture within a few days; resulting in a quick death.

TYPE OF CONTAINER

In order to reduce the chance of contamination, it is important to store the cultures in sterile containers. Small volumes of algae cultures are best kept in glass Erlenmeyers, as these can be easily sterilised with the use of an autoclave. Erlenmeyer flasks are also beneficial for the process of photosynthesis, as the tapered bottom of the flask results in a larger surface area; improving the gas exchange between the liquid and the air in the flask. With most flasks the surface area of will be optimal when it has been filled to about 1/4 of its maximum capacity. It is generally recommended to not fill a flask more than 1/2 of its prescribed maximum volume (see image 33).

In order to further support the process of photosynthesis, a constant sterile air exchange will be necessary. Therefore, containers are best sealed with non-absorbant cotton wool or a 0.2 µm filter cap. Both plugs allow gasses to enter and leave the container, while blocking other organisms and particles. In both cases it is important that the caps or plugs have been sterilised before getting into contact with the flask. Also, when using cotton wool plugs it will be important to ensure that the container is fully sealed. If there are any gaps between the cotton wool and the flask, the chances of contamination are greatly increased and almost certain.

HANDLING THE CULTURES

Once the culture has been transferred to a sterile container, which has been properly sealed, the risk of contamination has been greatly decreased. However, it is important to note that once the plug is removed the culture once again becomes prone to contamination. Therefore, it is recommended to only open the container if necessary. If one needs to open the container, it will be important to work as sterile as possible in order to limit the chances of contamination.

It is recommended to work underneath an extractor hood while using the proper lab equipment, such as a lab coat and gloves. Make sure the inside of the plug remains sterile and does not touch anything outside of the container once it has been removed. Also, the container should remain open for as little time as possible in order to limit the risk of contamination. Be aware of particles that could fall into the container once it is opened. Do not sit above an opened container and try to keep your hands away from of the opening and neck of the glass.

IMAGE [33]
EXAMPLE OF PROPER STORAGE
100ML OF CULTURE IN 300ML ERLENMEYERS

GROWING **REQUIREMENTS**

A conclusive guide for effectively and safely growing and maintaining the algae, based upon the previous literature research and information from experts [Richard Groen].

R1 TEMPERATURE

A relative safe and efficient temperature range to maintain the algae will be between 18 and 20 degrees.

Algae are best placed in a temperature controlled environment to avoid major fluctuations in temperature. If one would like to enhance the growth rate of the algae it is possible to raise the temperature to 22 - 24 degrees. However, it is important to note that at temperatures above 24 degrees the growth rate will rapidly decline and at even higher temperatures (26 degrees and above) the algae will most likely die.

Maintaining the algae at lower temperatures (15-18 degrees C), although assumed to slightly limiting the growth rate, appears to be a viable and safe alternative.

R2 LIGHTING

Algae cultures will require between 12 to 14H of light (with a minimal intensity of 10 PAR) per day in order to grow and have sufficient energy for the production of light.

It is recommended to use cool-white LED's, as these appear to be the best match to the absorption spectrum of the algae, are energy efficient and do not produce a lot of heat. A LED bulb or strip with an output of at least 400 lumen is recommended.

To verify if the light intensity of the LED's is sufficient, one could use a PAR meter to measure the light intensity. Aim for a PAR reading of at least 10 µE m−2 s−1.

R3 MEDIUM

Bioluminescent dinoflagellates are best kept in an L1-Si liquid medium with a salinity of approximately 3.5 percent and a pH between 8.0 and 8.2.

Add 20 percent of new medium every 3 to 4 weeks for a culture with an average density concentration and 30 to 50 percent for high density cultures. Be carefully not to add too much medium, as this can result in a decrease in bioluminescence or even death of the culture.

Make sure no contamination is present in the medium before adding it to a culture. Also, work as sterile as possible during the process of adding the new medium, as the cultures can be easily contaminated once the cotton wool plug has been removed. (see appendix X for proper handling procedures)

R4 STORAGE

Small volumes of algae culture are best kept in sterilised erlenmeyers, which are sealed with non-absorbant cotton wool (in order to block contamination, but still allowing gasses to exchange]

Containers are best sterilised with the use of an autoclave. While it is possible to sterilise the container with the use of ethanol, this is not recommended as there will be a bigger risk of contamination.

Make sure to only fill the glass to 1/4 or 1/2 of its maximum capacity to maintain proper gas exchange (large surface area). Gently shake the erlenmeyers once every few days to avoid clumping of the cells.

3.2

GROWING SETUP

In order to effectively grow and maintain the algae cultures, a growing setup had to be built. The following chapter provides a detailed description of setup and points of improvement for further iterations.

DESCRIPTION

During the project, the algae cultures were kept within an adapted Severin KS 9889 wine cooler standing in at the lab of the TU Delft Science Centre. The cooler allows for a constant temperature, ranging from 14 to 16 degrees Celsius depending on the temperature of the lab environment. The cooler also isolates the cultures; providing an extra barrier, making them less prone to contamination. An analog thermometer has been placed inside the cooler to monitor the temperature. Within the current setup there is room for a maximum of four to five 300ml Erlenmeyers or about three 500ml Erlenmeyers.

A light panel, consisting of 120 cool-white (3528 SMD, 5500K) LEDs, which are linked and housed within aluminium profiles has been attached to the front door of the cooler. As this model of cooler features a transparant front, it was possible to mount the LEDs on the outside of the cooler; further reducing the heat transfer of the light to the cultures.

The light from this panel will provide the algae with sufficient light (about 800 lumen), while also blocking the light from outside of the cooler. The LEDs are connected to a 12V adapter plugged into an analog power outlet timer, which switches on the lights from 8:00 till 18:00; providing the cultures with 14 hours of light per day.

Reflective panels, made from aluminium tape and cardboard, have been place inside of the cooler in order to optimize the distribution of the light. As the panels reflect the light, it is possible to ensure the cultures get a more evenly distributed light. Also, as the black coating on inside of the cooler absorbs most of the light, the reflective panels will help increase the overall light intensity that reaches the algae matter.

FOR FURTHER IMPROVEMENT

Discussion of the current growing setup and possible points of improvement for next iteration (in relation to the growing requirements from the previous chapter).

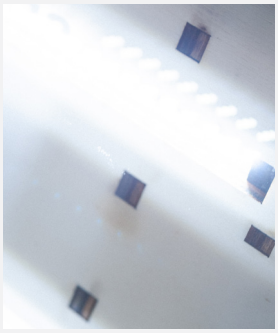
TEMPERATURE CONTROL

Temperatures within the cooler appear to be below the optimal growing temperature of 18 to 20 degrees. While the manual of the cooler mentions it should run at 18 degrees at its lowest setting, actual temperatures appear to be between 14 to 16 degrees Celsius. Further iterations should have accommodate a wider range of temperatures (15 to 20 degrees Celsius) in order to be able to enhance the growth rates.

INCREASE IN AVAILABLE SPACE

The space inside the cooler is fairly limited and only sufficient for small batches of algae cultures, especially as even small volumes need large containers which can not be placed onto the rack. Due to the current space limitations the Erlenmeyers will also have to stand very close to the light source. By being able to place the containers a bit more further to the back it would be possible to ensure all the light from the panel reaches the cultures, as at the moment the algae mostly receive the light from the bottom two LED strips.

IMAGES [35-36]
INSIDE OF GROWING SETUP
COOL-WHITE LED LIGHT PANEL



IMAGES [37]
OUTSIDE GROWING SETUP
LIGHT PANEL WITH ALUMINIUM HOUSING

3.3

GROWING PROCESS

The following chapter will describe the different procedures used to transfer the algae cultures between containers, as wel as a checklist for weekly visual inspections to determine the condition and growth of the different algae cultures.

OBSERVATION

In order to monitor the condition and growth of the culture, weekly visual inspections have been performed. During the visual inspection of the cultures, each of the following aspects has been taken into consideration:

RESPONSE TO AGITATION

The condition of a culture can be best monitored by looking at its response to agitation. By gentle swirling around the liquid inside the container, the algae within the culture should be stimulated enough to start emitting light. Note that this can only be done when the culture is currently in its night cycle, as there will be no bioluminescent response during the day cycle.

A decrease in bioluminescence often is a good indicator that a culture is starting to decline. However, determining the exact cause of this decline will be more difficult. Nutrients inside the medium might be depleted, cultures might not get enough light or the temperature of the enviroment is either too high or too low. Note that if one moves a culture to a different container or shifts the day and night cycle, there will most likely also be a decrease in observable bioluminescence. It usually takes a few days for the culture to adapt to changes in the environment.

APPEARANCE OF THE MEDIUM

By looking at the color and consistency of the medium and the culture, it is possible to monitor the condition as wel as the growth of a culture.

Over time, a healthy culture will become more dense; getting less transparant and have a slight yellow or orange tint. Severe contamination within a culture is usually easy to detect, as it influences the color and transparancy of the liquid. After a while, contaminaton might even result in other bits (strings or flakes) of organism visually floating through the liquid.

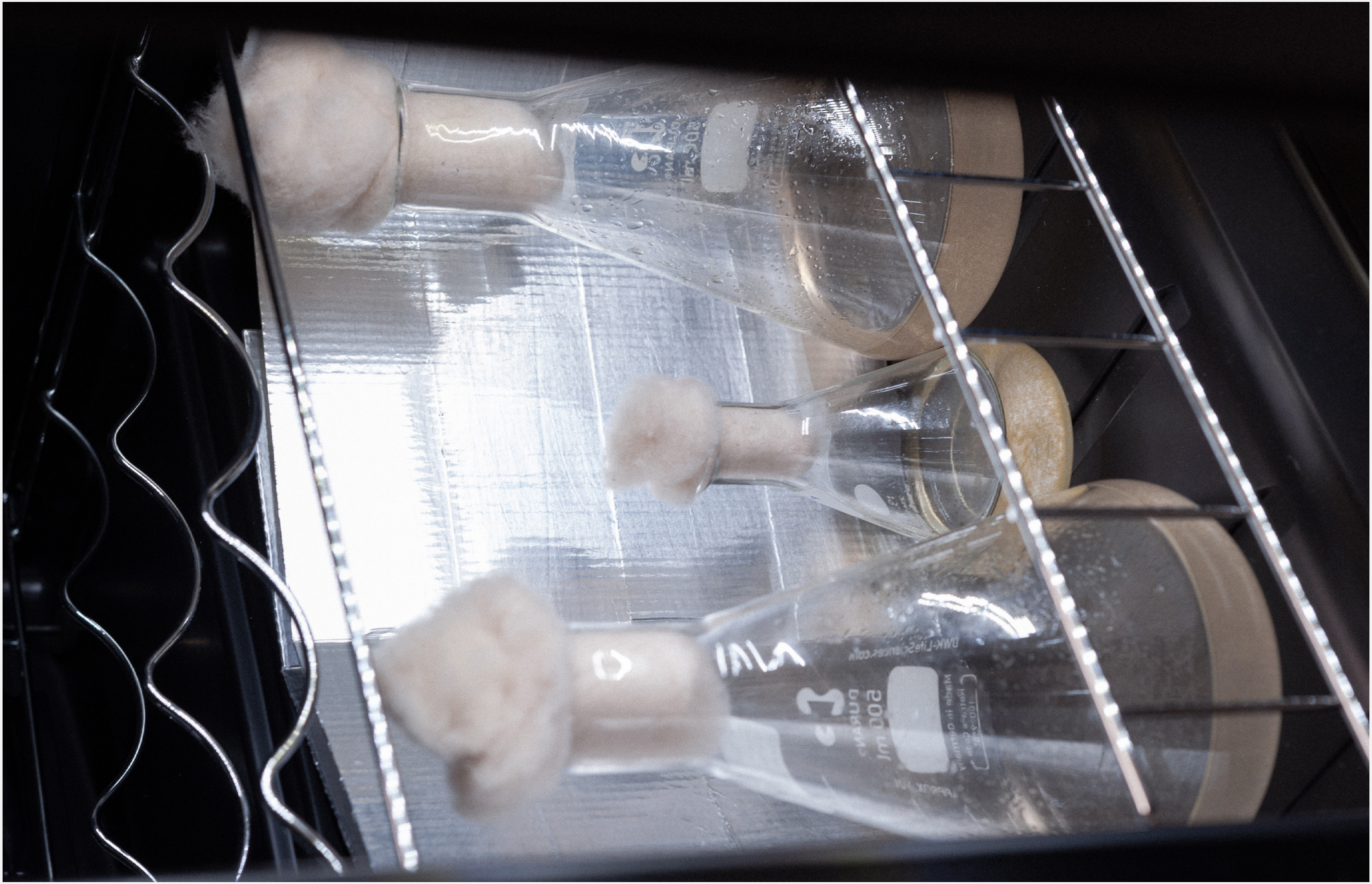
CHANGES IN TEMPERATURE

While the temperature itself does not provide much valuable information about the condition of a culture itself, it remains important to monitor the temperature inside the growing setup; making sure the temperature is consistent and remains within the desired range.

OVERVIEW

The following pages show a selection of observations for the different batches of algae cultures used within the project. Providing a description of the steps taken during the transfer of the algae from the shipping bag to their new containers, as wel as the notes from the visual inspections.

- BATCH 01: Pyrocystis Lunula (2x 50ml)
- BATCH 02: Pyrocystis Fusiformis (2x 200ml)
- BATCH 03: Pyrocystis Lunula (2x 50ml)



B1

SPECIES	Pyrocystis Lunula
SUPPLIER	Bio-Glow
LOCATION	Utrecht, The Netherlands

PROCEDURE

Detailed description of steps taken when transferring the algae from the plastic tubes to new glassware

1. 500ml clear glass laboratory bottles were cleaned and sterilised with ethanol (97% alcohol). First, pouring about 100ml of ethanol inside the container, carefully swirling it around. Next, pouring out all of the liquid and letting the traces of liquid evaporate underneath the extractor hood.

2. 150 ml of Bio-Glow growth medium was poured into each sterilised bottle, followed by the 50ml of algae culture: the liquids were mixed by gently shaking the bottles.

3. Non-absorbant cotton wool was twisted into the top of the bottle; making sure the container was fully sealed.

4. Both containers were placed on the bottom row of the cooler at approximately 20cm from the light source.

OBSERVATION

WEEK 01 – 17.10.2019
Both the culture concentrate and the medium look clear and show no signs of contamination. Individual algae cells are clearly visible throughout the whole culture. Most of the algae appear to be clustered together and float on top of the liquid.

- **No signs of contamination in both liquids**
- **Transparent liquid with visible bits of algae matter**
- **Clusters of algae floating at the top of the liquid**

WEEK 02 – 24.10.2019
Liquid in both bottles has turned cloudy and the concentration of algae sits at the bottom of the flask. No light is emitted once the culture is shaken (at 4H into dark cycle).

The supplier (Bio-Glow) mentioned this could be caused by the high percentage of medium to algae ratio or the 'colder' temperature inside the cooler, causing certain elements in the medium to react and turn the liquid opaque. However, as the container with only the remaining medium was left in the cooler as well and did not show any signs of cloudiness, this is most likely not the case.

Richard assumes the culture has been contaminated during the transfer process. In this case, the ethanol most likely did not fully sterilise the container, resulting in the contamination and eventually other organisms growing inside the container.

- **Liquid has turned 'cloudy'**
- **Culture appears to have been contaminated**
- **No signs of bioluminescence once shaken**

WEEK 03 – 1.11.2019
The liquid has become even more opaque and small strings of matter float through the liquid; a clear sign of contamination. No light is emitted once shaken [at 4H into dark cycle]. At this point, almost all of the algae have most likely died and the culture can be noted as lost.

- **Liquid has become almost milky-white and opaque**
- **Clear signs of contamination: strings of unknown matter**
- **Culture has most likely died**

IMAGES [38-39]
STERALISATION OF THE CONTAINERS
WITH THE USE OF ETHANOL

IMAGE [40]
CONTAMINATION OF P.LUNULA
'MILKY' COLORED LIQUIDS

B2

SPECIES	Pyrocystis Fusiformis
SUPPLIER	Pyro Farms
LOCATION	Carlsbad California, USA

PROCEDURE

Detailed description of steps taken when transferring the algae from the plastic tubes to new glassware

1. 500ml clear glass Erlenmeyers were sterilised with the use of the autoclave: sealing the erlenmeyers with non-absorbant cotton wool covered by aluminium foil and placing them in the autoclave (20 min. at120 degrees)

2. After the glassware was back to room temperature, 200ml of the algae concentrate was carefully poured into each sterilised erlenmeyer underneath the extractor hood

3. Non-absorbant cotton wool was twisted into the top of the bottle; making sure the container was fully sealed

4. Both containers were placed on the bottom row of the cooler, approx. 20cm from the light source

OBSERVATION

WEEK 01 – 1.11.2019
Both the culture concentrate look clear and show no signs of contamination. Lots of individual algae cells are visible throughout the whole culture and the concentration appears to be very promising.

- **Healthy concentration of algae within liquid**
- **No signs of contamination in both liquids**
- **Transparent liquid with visible bits of algae matter**

WEEK 02 – 13.11.2019
The liquid of both cultures is clear and there are no signs of contamination. Cultures appear to have survived the shipping and are adapted to the new lighting schedule , as they clearly emit light once shaken.

- **Light emission from both cultures**
- **No signs of contamination in both liquids**

WEEK 03 – 20.11.2019
Both cultures are clear and show no signs of contamination. At 8:00 (2H into dark cycle)in the morning the cultures are sensitive and produce quite a bit of light even when slightly disturbed. Perceived light intensity appears to be increased. The light output remains fairly consistent even after agitating multiple times. Culture appears to be ready for testing, as the light produced can be clearly captured by a camera in the dark.

- **Bright light emissions from both cultures**
- **No visual difference between both liquids**
- **Transparent liquid with easily visible bits of algae matter**

NOTE

There were no notable visible changes in either the color and consistency of the medium, as wel as bioluminescent response in the following weeks. Therefore, the culture could be noted as stable.

IMAGE [41]
STERALISATION PROCESS
PREPARINGS ERLENMEYER FOR AUTOCLAVE

IMAGES [42-43]
TRANSFER PROCESS
POURING CULTURE INTO STERILISED ERLENMEYER

3.4

GROWING CONCLUSIONS

Within this chapter, we will reflect upon the growing results: discussing various aspects that might have influenced the growing behaviour of the cultures.

IMAGE [44]
CLUMPING OF CELLS
BATCH OF PYROCYSTIS LUNULA

POSSIBILITY OF MAINTAINING AT LOWER TEMPERATURE

Even though the suppliers usually suggest to keep the algae around 18 to 24 degrees Celsius, they appear to be able to continue to survive and grow effectively at lower temperatures down to 15 degrees without any negative effects. Especially, P. Fusiformis appears to grow at a steady pace even at a lower than optimal temperature and showed a noticeable increase in light output over time.

However, for P. Lunula these lower temperatures might not be optimal, as after a few weeks the light produced seems to stay the same. While it is difficult to say if the minimal growth and light production is caused by the lower than recommended temperature, as there are many other factors that could play a role in this, such as the composition of the medium or the initial condition of the algae.

It is important to note that we have no clear result on the true influence of the temperature of the growth rate. While higher temperatures are likely to increase the growth rate to a certain extent, it can also be more risky. The most important aspect will be maintaining a consistent temperature, in order to avoid temperature shocks. In this case, maintaining the algae at a lower temperature has proven to be effective and provides us with consistent results in both growth and light output.

IMPORTANCE OF PROPER STERILISATION

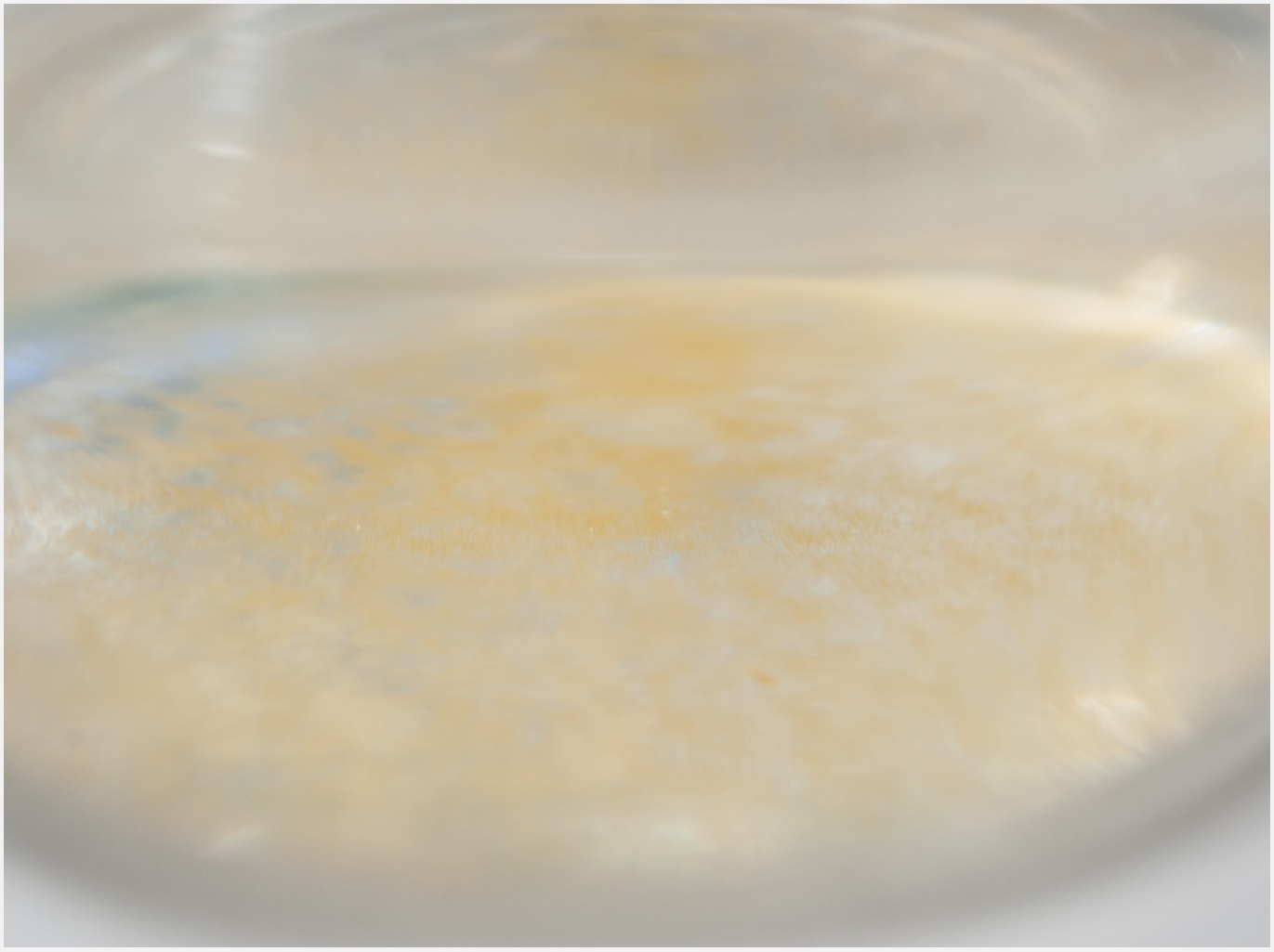
The rapid contamination of the first batch of P. Lunula made clear how important it is to ensure all materials that come in contact with the organisms should be sterile.

The best way to achieve this is with the use of the autoclave. While it is possible to clean or kill most of the organisms with ethanol, this is seen as a more risky approach. Contaminations can spread even quicker than thought and have show to be able to outgrow a culture within a few days. However, once the algae are in a closed environment, which allows the exchange of gasses, the risk of contamination is minimal. Therefore, it is suggested to keep the amount of transferring procedures to new containers minimal, as each time the container gets opened there will be a risk of contamination.

FREQUENT AGITATION TO PREVENT CLUMPING

As the algae cultures sit still over a few days, the cells start to clump together and form compact blobs within the liquid. Especially, Pyrocystis Lunula appeared to be sensitive to this 'clumping' effect. In order to avoid this, it is best to shake or swirl the cultures every couple of days.

As cells clump together they might not be as efficient in taking up the light as the light is blocked by the surrounding cells. While this should not be a problem with small volumes, it could make a significant difference for larger containers. Especially, when doing tests it is important to make sure the algae are well spread in the liquid, as clumps of cells are more difficult to trigger, thus will most likely influence the test results.



TOP [45]
PYROCYSTIS LUNULA
WEEK 6

LEFT [46]
PYROCYSTIS FUSIFORMIS
WEEK 6

RIGHT [47]
PYROCYSTIS FUSIFORMIS
WEEK 1

3.4

IMAGES [48 - 49]
OVERVIEW OF LAB ENVIRONMENT

REVIEW OF "SELF-MADE' MEDIUM

As it took a while to gather all the ingredients to create the Li-Si medium, all cultures remained undiluted for up to 10 weeks. Even though this resulted in highly concentrated algae cultures, the lack of fresh medium might not have been optimal for the growing rate and overall condition of the cultures. As generally, it is recommended to add fresh medium every 3 to 4 weeks. If there are no nutrients left in the medium a culture will slowly decline, in turn resulting in a decrease in light output over time. However, as there was no visible decrease in light output visible, there was no rush to add new medium.

In order to determine the effects of the new medium, without taking the risk to contaminate or perhaps even destroy all of the cultures, a small percentage was firstly added to a test culture. It is always important to test a new batch of medium, as it is hard to determine how a culture will react to the addition of new medium. Certain elements of the old medium will react with some elements of the new medium and even though the medium may look like it is free of contamination, this might not always be the case.

While in most scenario's, it is recommended to add only up to 20 to 30% of fresh medium to a culture, for the test batch we decided to add up to 50% of new medium. As the cultures had been undiluted for such a long period, most of nutrients were most likely gone. In addition, as the cultures had become so dense, the risk of overdiluting seemed a lot less significant. As the test culture appeared to respond well to the addition of the new medium and showed no decrease in light output, the medium was also added to the other cultures. Over time, the cultures began to show a visible increase in bioluminescence. No signs of contamination in both the medium itself and the cultures were visible.

Note that as the creation of the Li-Si medium is a fairly difficult and risky process, in particular if one is not used to working in a lab environment. Therefore, it is generally recommended to purchase pre-made medium. Especially, as the chemicals required to make the trace metal stock solutions are hazardous and require delicate handling.

DIFFERENCES BETWEEN CULTURES

While all batches of Pyrocystis Fusiformis appeared to grow and properly responds to agitations, both Pyrocystis Lunula cultures were less responsive. Although both species had been kept in the same conditions, Lunula showed almost little to no response to agitation. While throughout the weeks, the Pyrocystis Lunula cultures did consistently show slight signs of bioluminescence, it was never as bright and sensitive to agitation as the Fusiformis cultures.

Also, where the light intensity of the Fusiformis cultures showed a noticeable increased over time, the light output of the Lunula cultures remained fairly similar. Even after the addition of new medium, there was no noticeable difference in response and light output. The reason behind these strong differences in behaviour and growth are not clear ye. It might be possible that Pyrocystis Lunula has slightly different growing requirements; demanding more light or higher temperatures in order to properly grow. However, It could also be that the condition of the culture was not optimal to begin with.


300
250
150
100

INITIAL EXPLORATION

First insights on the behaviour and unique characteristics of the light produced by the bioluminescent algae.

C4

CONTENTS

AIM

Getting first insights on the behaviour and characteristics of the light produced by the bioluminescent algae; with the goal to define the points of interests and boundaries for further research.

- Building a orbital shaking device that allows us to create a wide variety of consistent agitation patterns.
- Getting an overview of the different types of agitation patterns and their effect on the light characteristics.
- Getting first insights in the possible boundaries of the light, such as the intensity, duration and recovery.
- Determining possible points of interest and research questions [with hypothesis] for experimentation phase; using the explorations as preparation for full scale testing

METHOD

- Iterative process of experimentation and observation
- Use of video editing software to analyse brightness

4.1

ORBITAL SHAKER

In order to be able to consistently perform different types of 'shaking' patterns, an orbital shaker was built. The following chapter provides a detailed description of the shaker and notes points of improvement for further iterations.

CONSTRUCTION

The orbital shaker consists of three main components: the base housing the electronics, the rotating arms translating the rotation of the motor and the top plate holding the containers. At the core of the shaker is the 6 volt DC motor, which can be controlled with the use of an Arduino Uno. In addition, two potentiometers, one for controlling the speed of the motor and one for switching between the agitation patterns, have been added. Different types of holders have been made in order to securely hold various sizes of Erlenmeyers. The rotating arms make use of ball bearings to reduce friction; resulting in a smooth and consistent rotation.

SHAKING POSSIBILITIES

Two types of attachments were made in order to agitate the algae in various ways: an orbital shaking top and a vibration plate. Both fit on the same base and are controlled with the same Arduino Uno; allowing for easy use.

ROTATIONAL VARIABLES

The orbital shaker is a fairly simple device with only a few parameters that can be adjusted, including the power to the motor, the direction of rotation and the duty cycle. This allows for a large variety of shaking or agitation patterns.

- POWER OUTPUT: 3 to 5 volt
- DIRECTION: forward - reverse
- DUTY CYLE: on - off and duration

VIBRATION VARIABLES

In order to further increase the amount of possible agitations, a vibration plate was added. This wooden plate consists of 7 small vibration coin motors, which are able vibrate at speeds of around 3000 to 10.000 rpm depending on the voltage supplied.

- POWER OUTPUT: 3 to 5 volt
- DUTY CYLE: on - off and duration

FOR FURTHER IMPROVEMENT

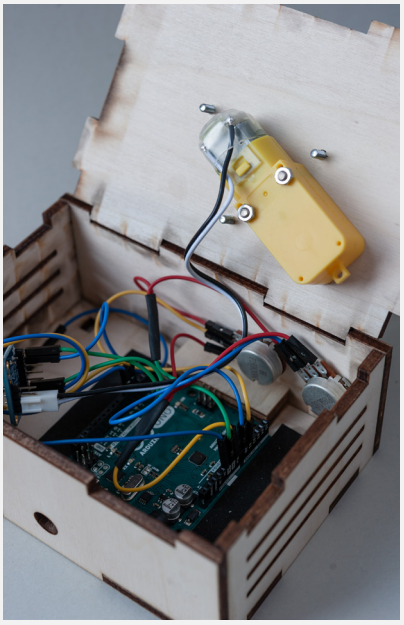
LIMITATIONS OF DC MOTOR

As the motor only runs when it is supplied at least 3 volt, it is not possible to gradually ramp the speed of the shaker. Also, there currently is not much room for differences in rotational speeds as the voltage supplied to the motor has to be between 3 and 5 volt. Therefore, it is recommended to use a stronger DC motor (minimum of 9V) for the next iteration.

STABILITY OF BASE

While the small and light housing makes the shaker easy to carry the shaker, it can become a bit unstable at times. Especially when a more aggressive shaking pattern is being performed. Adding weights or simply building the base out of a heavier and more durable material would increase the stability and usability of the shaker.

IMAGES [50-51]
INSIDE OF SHAKER HOUSING
CONSTRUCTION OF ROTATION ARMS



IMAGES [52]
OVERVIEW OF ORBITAL SHAKER
SILICON MAT TOP

4.2

FLASH EXPLORATION

In order to get first insights on the general behaviour and characteristics of the light produced by the bioluminescent algae, a large number of 'agitational explorations' have been performed. The goal to of these exploration was to define points of interests and boundaries for further research.

AGITATION FORMS

During the explorations, three different types of stimuli have been used: rotation, pulse and vibration.

Note that as the current orbital shaker is limited to rotational movement, pulse stimuli are created with very short duration alternating rotations: rotating one-way for approximately 50ms, before coming to an abrupt stop as the motor is forced to rotate the other way. As the motor is only powered for a short period of time, the container will move in an almost linear manner, in a way mimicking a shaking motion.

The exploration phase was based around an iterative process of trial and error. For each of the three types of stimuli a base form had been defined. All of the following rotational form were created by slightly adjustment the properties of the base form, for example by adjusting the rotational speed or through the addition a paused interval between each stimuli. This allowed for a wide variety of different agitation patterns and provided first insights in the effects of certain parameters on the behaviour of the light.

OBSERVATION

A combined overview of all the explored agitation patterns (in chronological order, as each exploration gave new insights and ideas for the next) is provided on the following pages. Each exploration has been captured with the same camera settings, makinge it possible to compare the differences in luminance. To create a consistent way of analysing, the following aspects were described for each of the base agitational patterns:

DESCRIPTION
A general overview of visible changes in light intensity over time based on observation and further defined with the help of the luma scopes within Adobe Premiere, which provides a graphical overview of changes in luminance over time.

- **Describing how the intensity changes over time**
- **Noting peaks and drops in intensity**

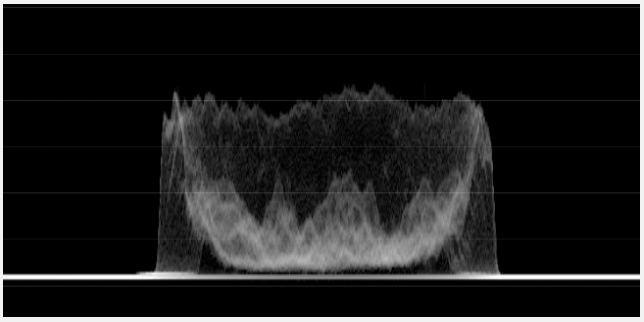
QUALITY OF LIGHT
A detailed description of the visual characteristics of the light, looking the movement, patterns and textural qualities. Extra care is taken into thoroughly describing the motion of the light in regards to the motion of the stimuli or input.

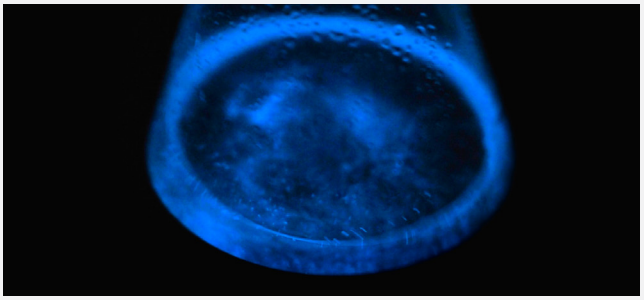
- **Describing the general visual characteristics of the light**
- **Looking at texture, glow, movement and patterns**

BOUNDARIES
Noting any deviations and mentioning first insights on possible boundaries of the light, looking at the rate of energy depletion or a loss of sensitivity

- **Noting deviations (e.g. sudden spikes in intensity)**
- **Looking at energy depletion or visible loss of sensitivity**

IMAGES [53]
OVERVIEW OF LUMA SCOPES
MAPPING LUMINANCE VALUES

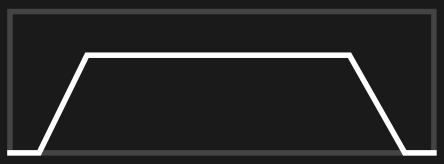




DUTY CYCLES
FLASH EXPLORATION PATTERNS

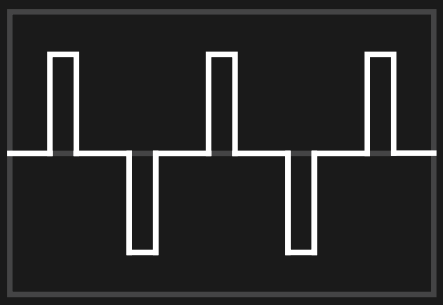
T1 ROTATION

R1 CONTINUOUS ONE-WAY ROTATION AT 90 RPM

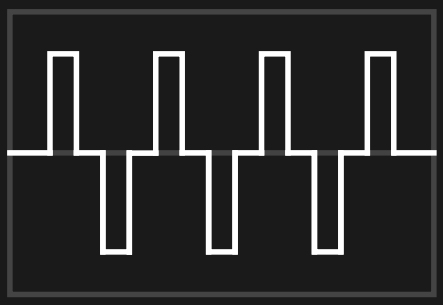


R2 CONTINUOUS ONE-WAY ROTATION AT 120 RPM

R3 SINGLE ROTATION AT 120 RPM



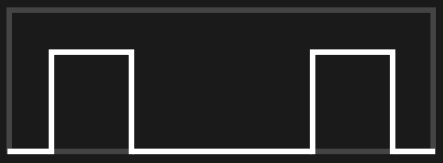
R4 ALTERNATING ROTATION AT 120 RPM [1:2 INTERVAL]



R5 ALTERNATING ROTATION AT 120 RPM [1:1 INTERVAL]

R6 RAMPED ALTERNATING ROTATION AT 120 RPM

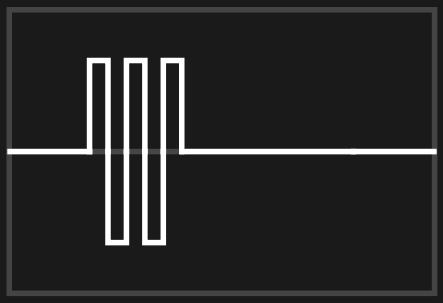
R7 SINGLE ROTATION AT 120 RPM WITH 5 MIN. INTERVAL



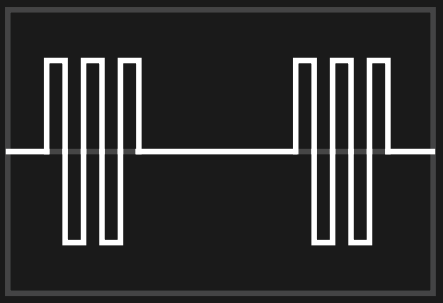
R8 SINGLE ROTATION AT 120 RPM WITH 10 MIN. INTERVAL

R9 RAMPED CONTINUOUS ROTATION

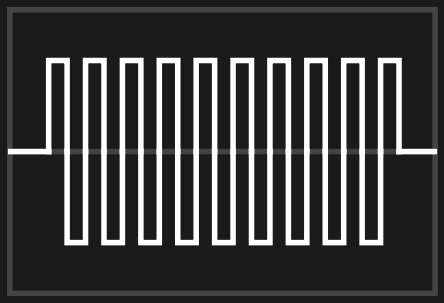
T2 PULSE



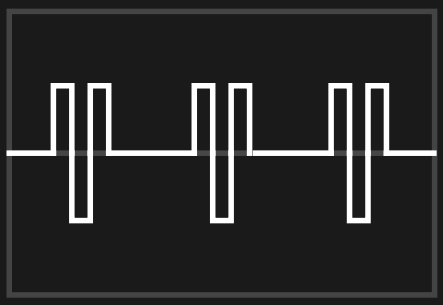
P1 1 X 20MS PULSES WITH 5 MIN. INTERVAL



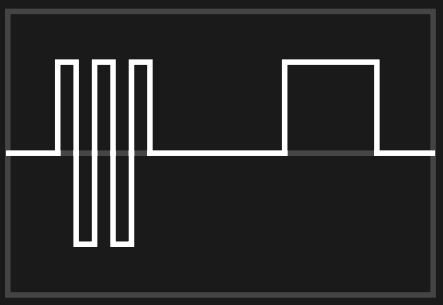
P2 5 X 50MS PULSES WITH 10 MIN. INTERVAL



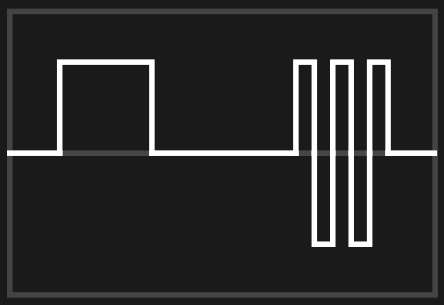
P3 CONTINUOUS 50MS PULSES



P4 3X 20MS PULSES WITH 1 SEC. INTERVAL

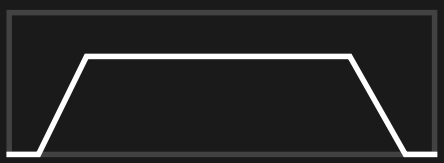


P5 5 X 50MS PULSES - 10 MIN. INTERVAL - SINGLE ROTATION



P6 SINGLE ROTATION - 10 MIN. INTERVAL - 5 X 50MS PULSES

T3 VIBRATION



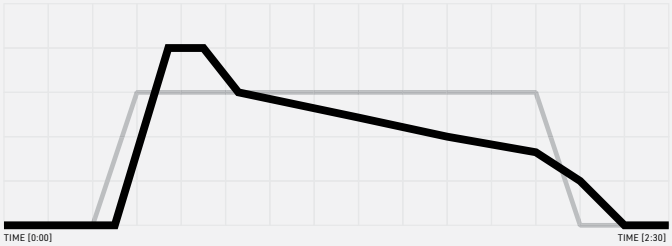
V1 CONTINUOUS VIBRATION AT 5 V

V2 100MS PULSES AT 5V WITH 1 SEC. INTERVAL

V3 200MS PULSES AT 5V WITH 1 SEC. INTERVAL

R1

FLASH EXPLORATION
ROTATIONAL AGITATION
VIDEO ID [EX-R1]



MOTION	Rotation
DIRECTION	One-way
POWER	3 volt, 90 rpm
INTERVAL	No pause, continuous
DURATION	2:30 minutes

DESCRIPTION

As the liquid inside the erlenmeyer starts to move once the container gets rotated, a strong inital glow occurs. This initial burst of light quickly fades to a lower intensity [0.4 to 0.2]. This second 'flashing state' is maintained for over 2 minutes, as it slowly fades over time. After nearly 2 minutes the flashing of the algae has almost completely faded and is barely detectable by the camera anymore. However, it is still slightly visible to the human eye at this point.

- **Strong initial glow – quickly fades**
- **Slow fade of light intensity over time**
- **Barely visible after 2 minutes of agitation**

QUALITY

The rotational motion creates a swirling movement within the liquid, resulting in an interesting lighting pattern. First, a cluster of 'individual algae' passes the glass. This is followed by a bright blue glow when the liquid bounces into the glass as it is being rotated. For as long as the speed of the rotation is not altered, this cycle continues.

Algae in the centre of the glass appear to be triggered first, but are only visible for only a few milliseconds before they are swirled to the edge of the glass. As the rotational force continues to push the algae to the side of the glass, no light is emitted from the centre of the glass.

While some individual 'dots of light' are still visible in the first seconds of the motion, they start to blend together once the swirling motion is in full effect.

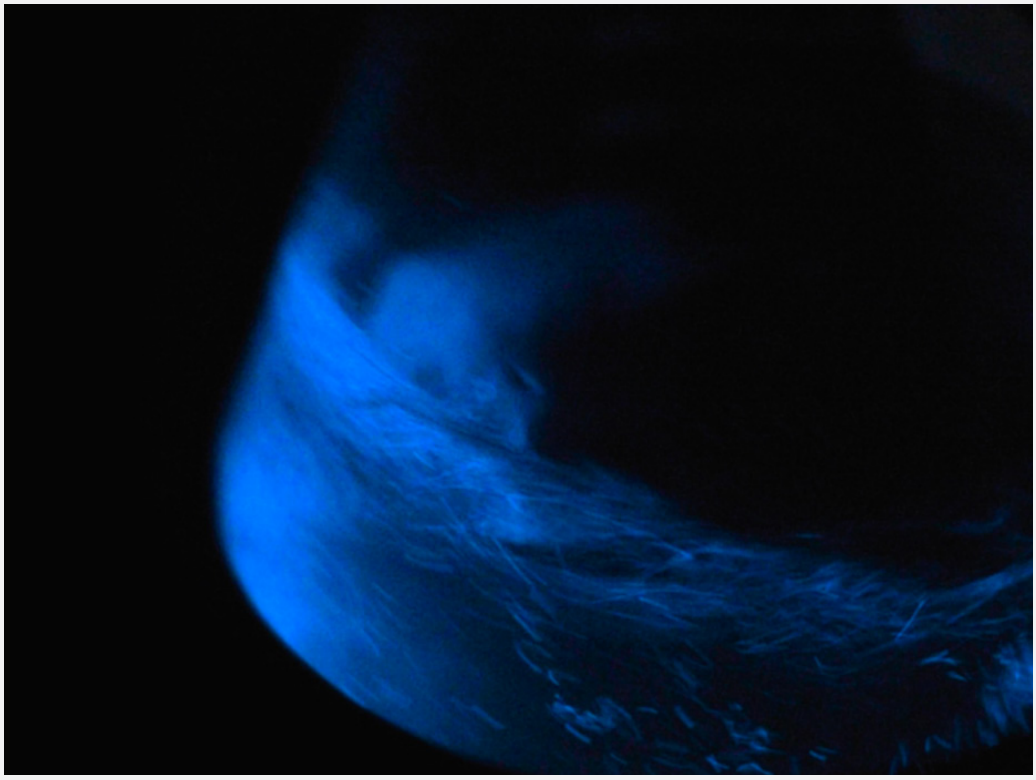
- **Swirling motion of light – direct relation to movement**
- **Pulsating effect as algae bounce against glass**
- **Slight blending and blurring of individual light dots**

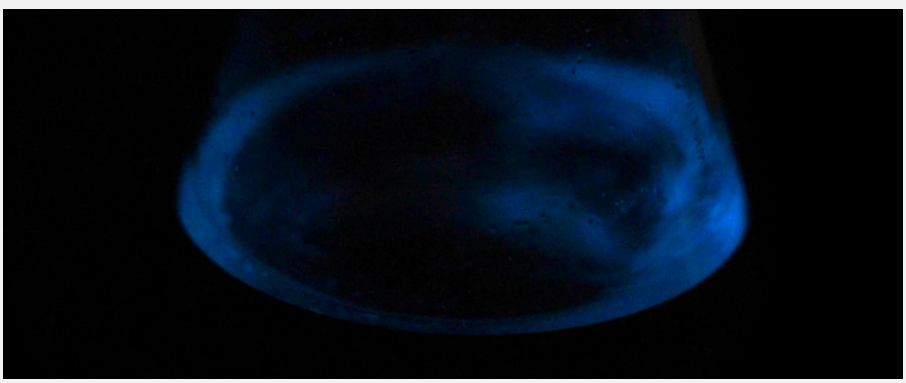
BOUNDARY

Something interesting happens at the end of the test when the speed of the shaker is slowly decreased. As the shaker slows down, the algae start to bouncing against the glass with more force, resulting in bright sparks of light (even although the culture appeared to be depleted of energy). This gives the impression that the decrease in light intensity during the agitation is most likely not only caused by the limit in available energy for light production, but also due to a loss in sensitivity by mechanical stimulation.

- **Energy depletion v.s. loss of sensitivity**
- **Bright spark of light even after 2 minutes of agitation**

IMAGE [54]
FIRST ROTATION OF SEQUENCE





TOP [55]
TIME STAMP - 0:01
LUMA VALUE - 0.4

MIDDLE [56]
TIME STAMP - 1:15
LUMA VALUE - 0.1

BOTTOM [57]
TIME STAMP - 2:25
LUMA VALUE - 0.3

R1

VARIANTS OF R1

Descriptions of rotation variations similar to sequence R1. Within each description, the differences in the motion are mentioned and displayed in a graph. The most interesting observations are noted; providing more insights into the behaviour of the light in regards to small changes in input.

R2 INCREASE OF SPEED

DIFFERENCES WITH R1
- Rotational speed increased to 120 RPM
- Performed after test P2: previously agitated culture

OBSERVATIONS
An increase in rotation speed appears to result in overall higher luminance values. Within the first few seconds of the agitation there is a bright spike in luminance, which fairly quickly fades resulting into a more consistent and glow-like light. It appears that this decrease in light output from the initial spike to the glowing state happens slightly faster when compared to that of R1. At this point, the luminance values start to slowly decrease (in a similar manner as with variant R1). Once the movement stops, the light quickly fades.

Another noticeable difference is seen in the textural qualities of the light. The increase in rotational speed causes individual dots of light to blend together, resulting in a more uniform and diffused light.

ADDITIONAL NOTE
It is important to note that this sequence has been performed after sequence P2. As it has been previously agitated, it might behave in a different manner. However, it also shows that sequence P2 has barely depleted the energy of the culture as there was no noticeable decrease in luminance.

FIGURE [58]
INPUT MOTOR [R2]
CHANGES WITHIN DUTY CYCLE

R3 SINGLE ISOLATED ROTATION

DIFFERENCES WITH R1
- Rotational speed increased to 120 RPM
- Duration limited to 1000ms
- Filmed at 50fps, instead of 24fps (slowmotion)

OBSERVATIONS
By isolating the rotation (limiting the duration to 1000ms) and increasing the number of frames per second, it is possible to get a better view of how the light behaves and moves through the container as it is swirled around.

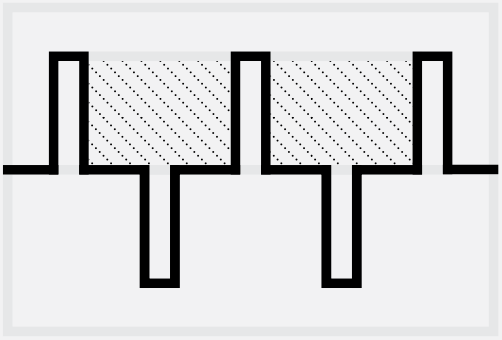
Powering the motor for 1000ms appears to result in a rotation of about 1080 degrees (2.5 full rotations). Once during each rotation a cluster of algae appears to 'crash' into the side of the glass, resulting in a bright spike of light. These spikes create a certain flashing behaviour, similar to a flickering light. As the container rotates about two times, there are also two distinct spikes in light visible. The first spike appears to have a slightly higher peak luminance value than the second one.

Once the shaker stops rotating, the light slowly starts to fade (similarly as with R1). However, now it is clearly visible how the liquid inside the container continues to move and swirl around inside the glass even after the rotation has stopped. It seems that this continuation of the motion is the reason why the light appears to slowly fade at the end of each rotation sequence.

FIGURE [59]
INPUT MOTOR [R3]
CHANGES WITHIN DUTY CYCLE

R4-5 ADDITION OF PAUSED INTERVAL

FIGURE [60]
INPUT MOTOR [R4-5]
CHANGES WITHIN DUTY CYCLE



DIFFERENCES WITH R1
- Rotational speed increased to 120 RPM
- Addition of paused interval of 2 or 1 sec.
- Alternating rotations between each interval

OBSERVATIONS
The first rotation results in a high luminance burst of light, which is noticeable brighter than that of R1. As soon as the container stops rotating the light slowly fades, until it is barely visible anymore. At this moment, the container starts with the next rotation in the opposite direction. This movement creates a similar burst of light, but with a slightly lower intensity. The following rotations progressively produce slightly less light. After approximately 35 rotations, the light of the algae is barely visible anymore.

The increase in luminance appears to be the result of the alternating rotational motion. As the liquid continues to swirl around even after the motion has stopped, the counter rotation will most likely generates more shear force; resulting in an increase in light output.

Another interesting observation is the fact that light during the first rotation appears almost completely smooth. While there still are some individual dots of light visible in the centre of the glass, almost all of the dots near the edge appear to blend together, resulting in an almost uniform glow.

ADDITIONAL NOTE
Decreasing the paused interval time to 1 second showed no change in the overall behaviour of the light. However, where a 2 second pause provides the culture with just enough time to gradually fade and stop emitting light, the 1 second pause agitates the culture again before the light has fully faded.

R6 RAMPED ALTERNATING ROTATION

FIGURE [61]
INPUT MOTOR [R6]
CHANGES WITHIN DUTY CYCLE

DIFFERENCES WITH R1
- Rotational speed increased to 120 RPM
- Linear ramped acceleration
- Alternating rotation without a pause

OBSERVATIONS
The shaking motion causes the liquid to move from side to side within the container, pushing the algae into to edge of the glass causing them to emit light. Every once in a while, the 'waves' within the liquid synchronise, creating a stream of algae that gets pushed up the glass and falls back down into the culture, emitting light both as they move upwards and fall down.

The most interesting part of the sequence is the randomness of the behaviour of the light, as the time between different spikes of light tends to vary. The shaking motion itself does appear to create enough force to trigger the bioluminescence and the algae only appear to produce light when the ripples inside the liquid clash into each other or against the edge of the glass

Another interesting aspect is the visibility of the individual dots of light. It is easy to spot and identify single cells, resulting in a light that appears to be highly texturised. At times, small groups or even single cells of algae stick to the side of the glass after they have been launched upwards. This results in an interesting afterglow or ghost image, which shows the delayed movement of the algae throughout the liquid.

R1

R7-8 SINGLE ROTATION WITH INTERVAL

DIFFERENCES WITH R1
- Rotational speed increased to 120 RPM
- Duration limited to 1000ms
- Addition of 5 or 10 minute interval between rotations

OBSERVATIONS
Both of the testing sequences consist of a 3 single rotations with a paused interval of 5 minutes in between each rotation. The main goal of these sequences is to get first insight in the recovery of the flash characteristics over time.

At a first glance, no significant differences in luminance or overall behaviour can be seen between each of the rotations. However, when comparing the luma scopes of the agitations it becomes clear that there is a very slight decrease in luminance over time, especially between the first and third rotation.

Rise and fall times of the light output also appear to be almost identical and differences in kinetics are even harder to define. Therefore, it seems reasonable to conclude that a 5 minute interval will be sufficient for the algae to recover most of their first flash characteristics.

Further increasing the time between agitations to 10 minutes appears to have no significant effect. Differences between in peak luminance values have become even more difficult to spot and all three of the rotations appear to be almost identical.

ADDITIONAL NOTE
For further research it would be interesting to see how the flash characteristics will change over time as the amount of rotations within a sequence is increased. Especially, as it is expected that over time the differences between the 5 and 10 minute intervals will become more apparent.

FIGURE [62]
INPUT MOTOR [R7-8]
CHANGES WITHIN DUTY CYCLE

R9 GRADUAL RAMPED ACCELERATION

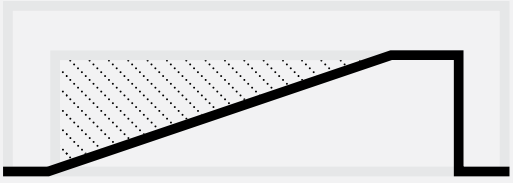
DIFFERENCES WITH R1
- Rotational speed increased to 120 RPM
- Duration limited to 1000ms
- Addition of 5 or 10 minute interval between rotations

OBSERVATIONS
Slowly increasing the rotational speed has shown to result in a more gentle increase rise in light intensity. However, it also appears to result in a decrease in peak luminance values. As the container slowly starts to accelerate, more and more algae get triggered.

Once the movement has reached its maximum speed the behaviour and intensity of the light is almost identical to that of R1. The main difference between both sequences appears to be within the first few seconds.

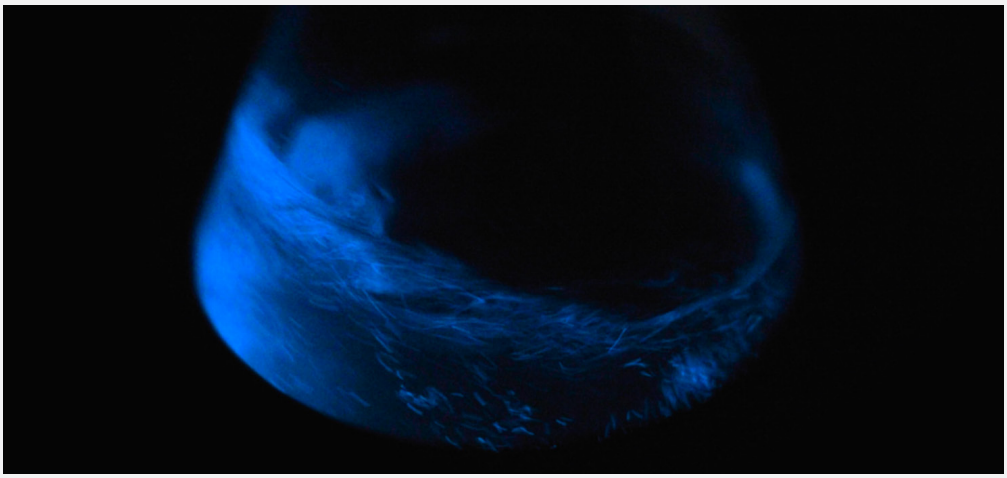
ADDITIONAL NOTE
It was not possible to fully ramp the acceleration of the motor from 1 volt to 5 volt, as the with the current orbital shaker setup the motor only runs when it is supplied at least 3 volt. This lead to a fairly quick ramped acceleration and a slight abrupt start of the rotational motion.

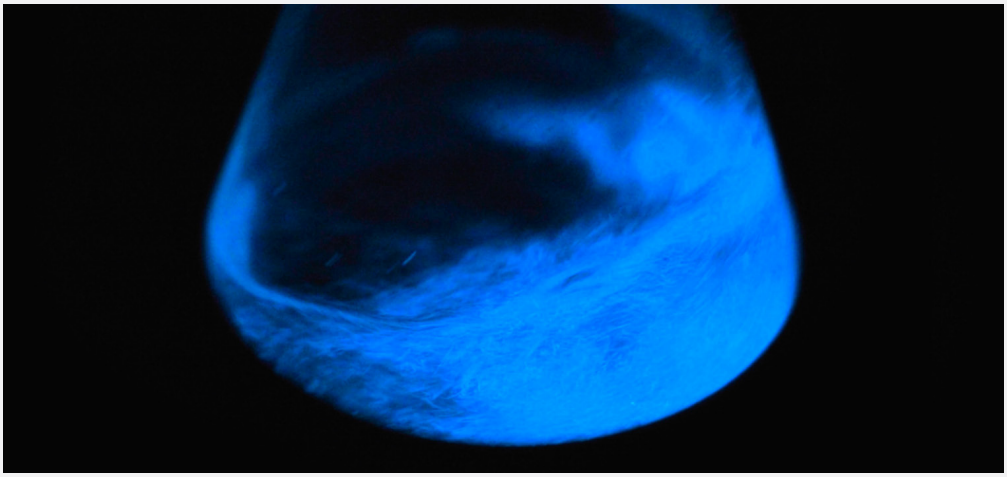
FIGURE [63]
INPUT MOTOR [R3]
CHANGES WITHIN DUTY CYCLE



TOP [64]
90 RPM ROTATION
SEQUENCE [R1]

BOTTOM [65]
120 RPM ROTATION
SEQUENCE [R4]





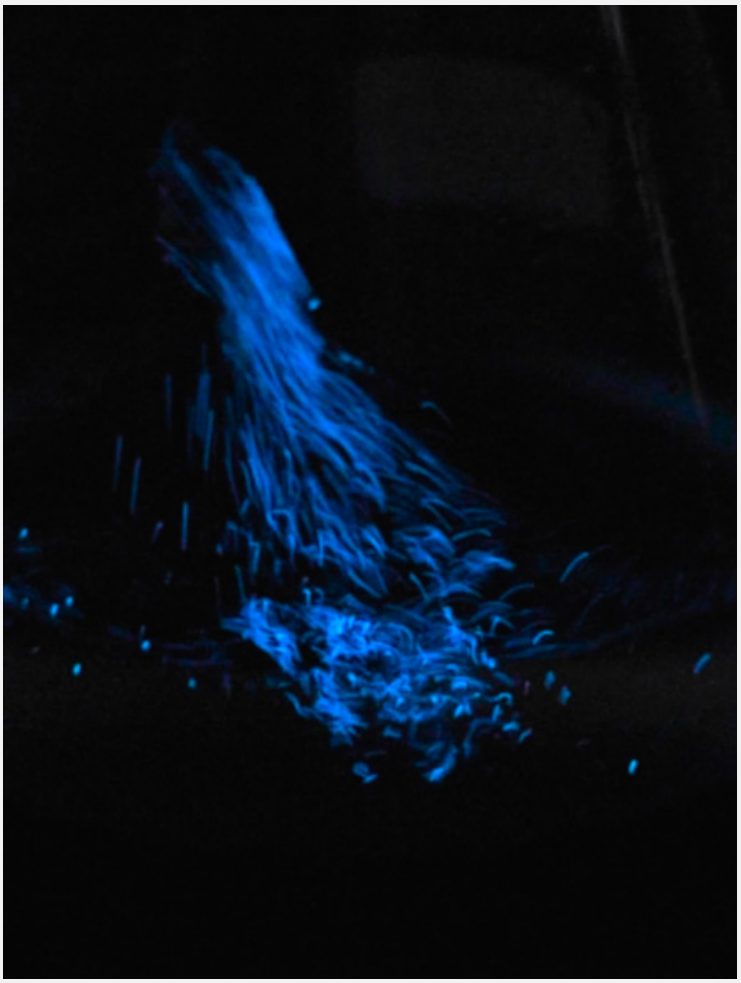
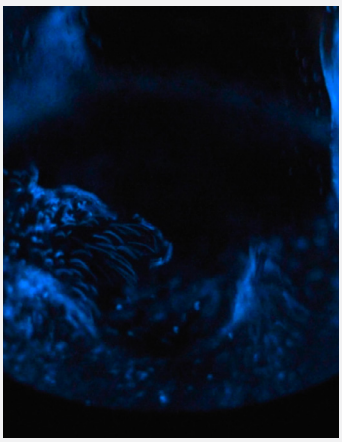
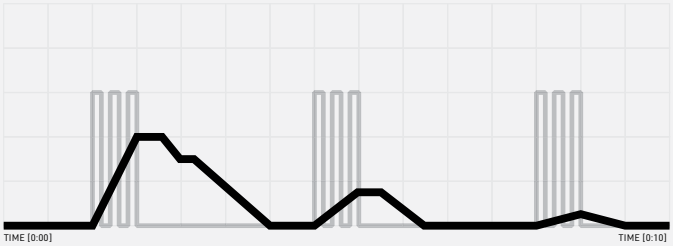


IMAGE [66]
RAMPED ALTERNATING ROTATION
SPIKES OF LIGHT



P1

FLASH EXPLORATION
PULSE AGITATION
VIDEO ID [EX-P1]



MOTION	Pulse
DEGREE	20ms, 5 degrees
POWER	5 volt, 120 rpm
INTERVAL	3 pulse - 2 second. pause
DURATION	30 seconds

DESCRIPTION

As soon as the first pulse is initiated the edges of the liquid start to light up [max. intensity of 0.2]. This initial burst of light quickly fades [within 50ms] and is followed by a small, yet concentrated and bright cluster of light emerging from the centre of the liquid [max. intensity of 0.4], which also fades in a similar manner.

The second pulse results in some scattered dots of light near the edge of the glass [max. intensity of 0.05], but does not appear to trigger algae in the centre of the culture.

The following pulses have a similar effect, but only manage to trigger a few individual cells near the edge of the glass per agitation. After three to four pulses the light from the culture is barely visible anymore.

- **Bright initial burst of light – quickly fades**
- **Two distinct peaks in light intensity**
- **Relatively low maximum luminance values**

QUALITY

While the intensity of the light is much lower compared to that of the rotational agitation, the light pattern created by the three pulses is unique and more difficult to correlate with the movement of the glas, resulting in a certain element of surprise as the light moves in unexpected ways.

First, the algae near the edge of the glass light up. At the point when the light on the edge starts to fade, a cluster of algae in the middle light up, creating a bright blue blob of light. This is most likely caused by the 'waves' within the liquid colliding in the middle of the glass.

- **Light pattern consisting of two distinct phases**
- **Element of 'surprise' – unexpected movement of light**
- **Highly texturised light**

BOUNDARY

Pulses do not appear to fully stimulate all the algae within the culture, as the light emission is not nearly as bright when compared to the rotational agitations. This might be caused by a decrease in shear force or the shorten agitation duration.

The rapid decrease in luminance over time appears to be the result of a loss of sensitivity to mechanical stimulation. This becomes even more apparent as the culture still produced a lot of light once it was swirled around after the test sequence.

- **Not all cells appear to be triggered - lack of shear force**
- **Rapid decrease in luminance - loss of sensitivity**

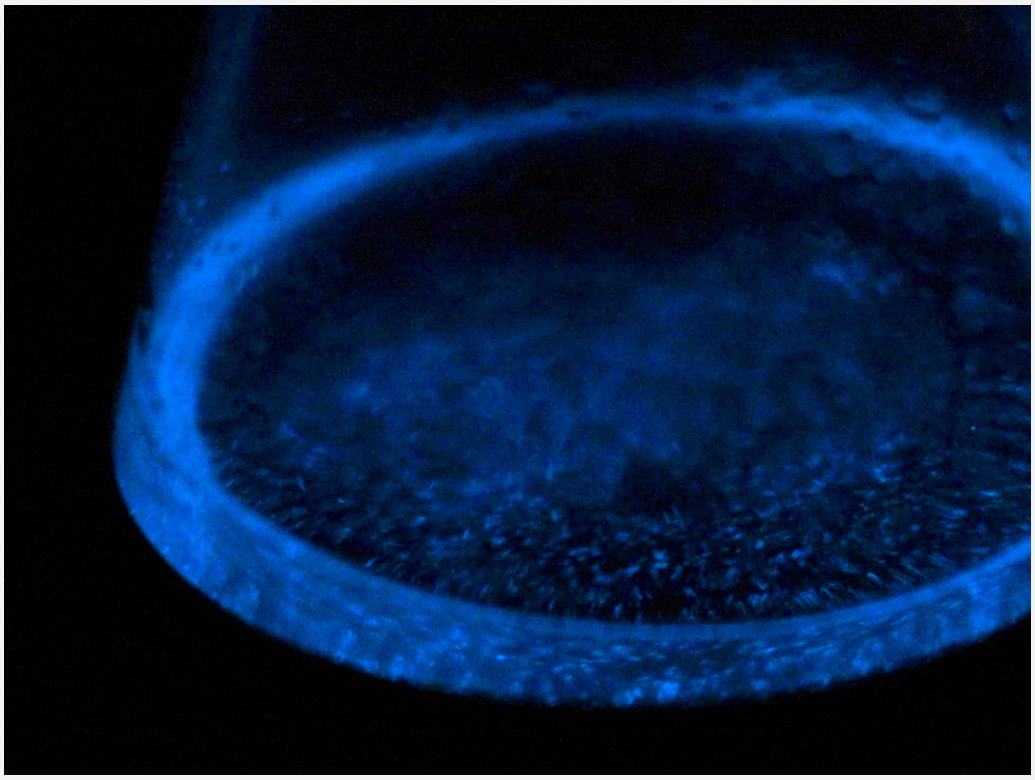
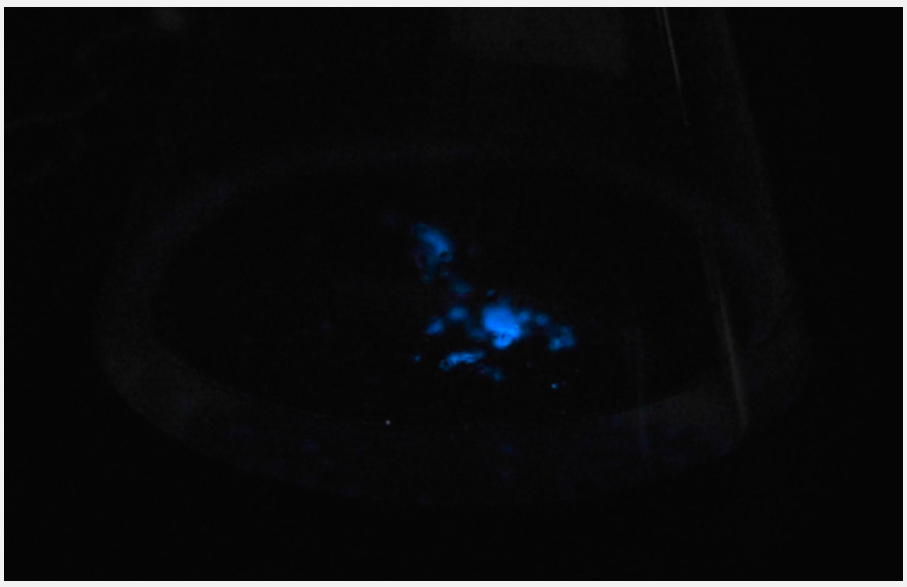


IMAGE [67]
VISIBILITY OF INDIVIDUAL ALGAE



TOP [68]
LIGHTING UP OF EDGES
PHASE [1]

BOTTOM [69]
DOT OF LIGHT WITHIN CENTRE
PHASE [2]

P1

VARIANTS OF P1

Descriptions of rotation variations similar to sequence P1. Within each description, the differences in the motion are mentioned and displayed in a graph. The most interesting observations are noted; providing more insights into the behaviour of the light in regards to small changes in input.

P2 INCREASED PULSE INTENSITY

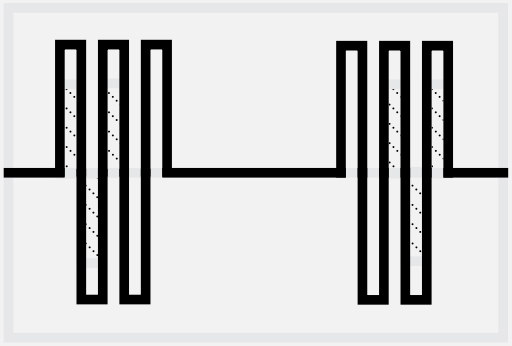
DIFFERENCES WITH P1
- Duration of pulse increased to from 20ms to 50ms
- Amount of pulses increased from 3 to 5

OBSERVATIONS
With the slight increase in the duration of the pulse the shaker is able to accelerate just a bit more, in turn resulting in a more powerful and abrupt stop. The combination of the longer duration and the increase in the amount of pulses appears to greatly enhance the total light output.

While the light still shows the two distinct phases as seen in variant P1, noticeably more algae are triggered; resulting in an overall increase in luminance over time. The second phase, in which a blob of light forms in the centre of the glass, appears to be extended. The blob of light also appears to have become slightly bigger, resulting in an overall increase in luminance. The increase in duration and light output of the second phase is most likely caused by the increase in the amount of pulses. For future research, it would be interesting to see till what extend this second phase can be elongated.

Similar to P1, each of the following pulses results in slightly lower total light output. However, the differences between the peak luminance values of each pulse are not as extreme as with pulse sequence P1.

FIGURE [70]
INPUT MOTOR [P2]
CHANGES WITHIN DUTY CYCLE



R3 CONTINUOUS PULSES

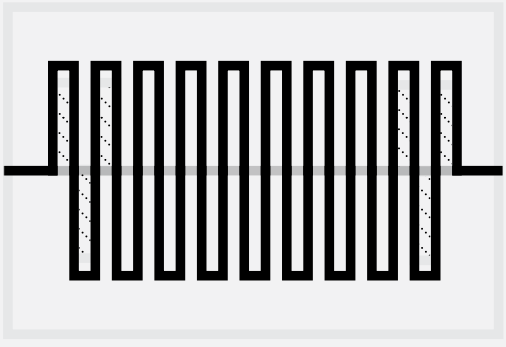
DIFFERENCES WITH P1
- Duration of pulse increased to from 20ms to 50ms
- Continuous: no pauses between the pulses

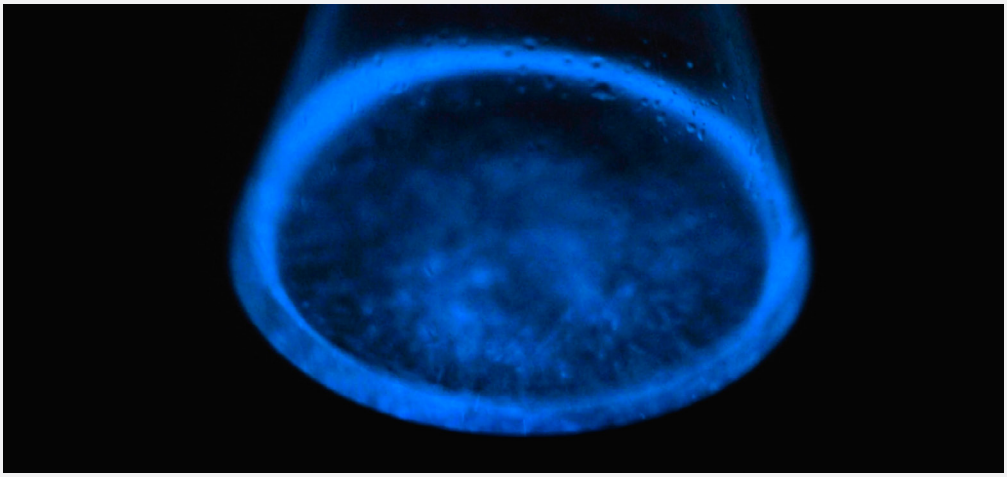
OBSERVATIONS
Similar as with the ramped alternating rotation sequence (R6), this pulsating motion causes the liquid to bounce around within the glass, pushing the algae up and into to edge of the glass; resulting in peaks of light that move up the container. However, as the shaking motion is much more intense and fast paced, the behaviour of the light is more chaotic when compared to that both sequence P1 and R6.

Within the first few seconds of the sequence, the movement of the light is almost identical to that of P1; showing the same two phases. However, after a few seconds the movement of the liquid appears to fall into sync with the motion of the shaker, resulting in multiple spikes of light moving upwards from the edge of the glass. In comparison to rotation sequence R6, the frequency and number of spikes is much higher. While the movement of the light appears fairly random at a first glance, the distinct peaks of light can be regularly seen at the same place. Almost no light appears to originate from the centre of the glass.

Over time there is no significant visible decrease in luminance, however, big spikes of light do appear to become more scarce.

FIGURE [71]
INPUT MOTOR [P3]
CHANGES WITHIN DUTY CYCLE

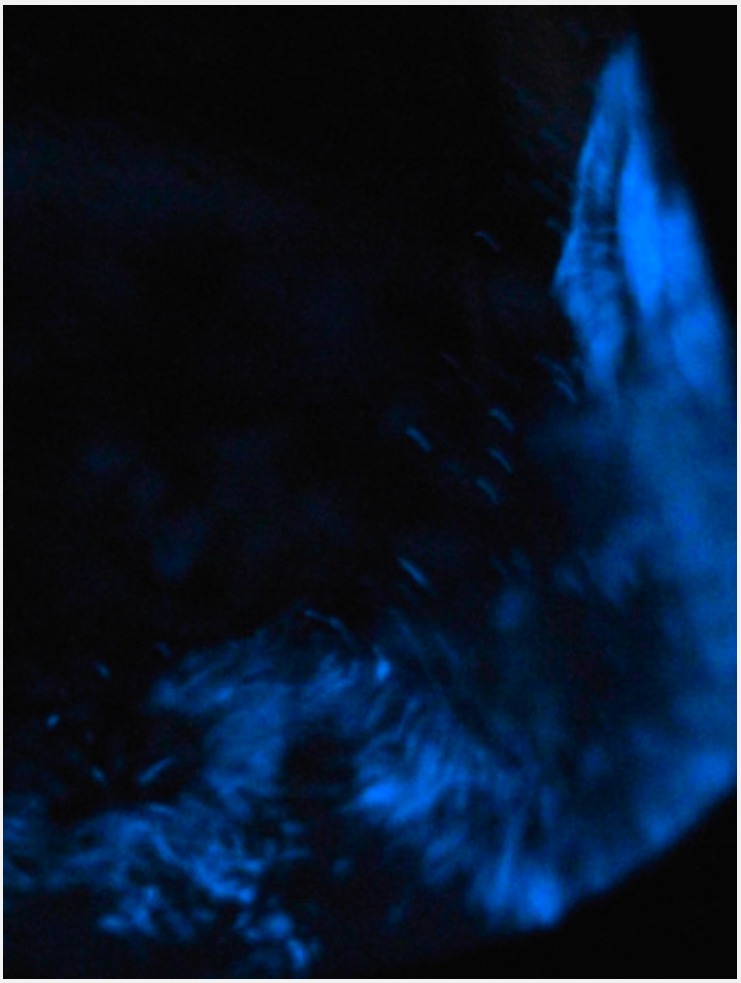




TOP [72]
20MS PULSE AGITATION
SEQUENCE [P1]

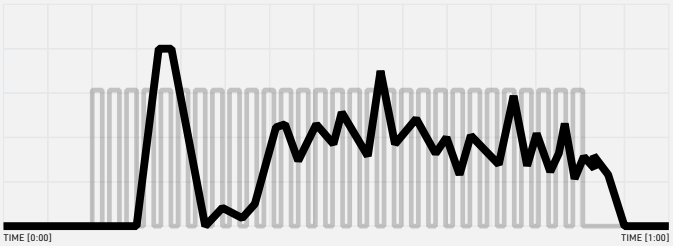
BOTTOM [73]
50MS PULSE AGITATION
SEQUENCE [P2]

IMAGE [74]
CONTINUOS PULSE AGITATION
SPIKES OF LIGHT



V1

FLASH EXPLORATION
VIBRATION AGITATION
VIDEO ID [EX-V1]



MOTION	Vibration
POWER	5 volt
INTERVAL	No pause, continuous
DURATION	1:30 minutes

DESCRIPTION

As soon as the vibration motors are initiated the edges of the liquid slowly start to light up. However, as only individual algae are triggered the overal light intensity remains very low [max. 0.2] Over time more clusters of algae start to light up; creating a glittering effect throughout the liquid. Each algae appears to get only triggered once, giving light for about 50 to 100ms.

After approximately 10 seconds there is a visible decrease in luminance as less of the algae appear to get triggered. From this point on, every once in a while a cluster of algae quickly lights up. Over time, these peaks in luminance become less frequent and after approximately 1 minute there is almost no response in bioluminescence.

- **Bright dots of light, but low overall light output**
- **Fairly quick decrease in light intensity**

QUALITY

While the overal light intensity and peak luminance values are much lower when comparison to those of the other agitation types, the stimuli from the vibration plate resulted in a unique lighting pattern.

Most of the algae near the edges appear to be agitated first, creating a ring of light around the edge of the glass. Some of the dots of light near the edge are slightly extended or even deformed as they pass through the curvature of the glass; creating streaks of light within the liquid.

As each individual cell only lights up for a short period of time and each cell appears to be only triggered once, the light starts to flicker. While the algae quickly light up, they do appear to fade slightly slower; resulting in a slight ghosting effect. The most unique aspect of this type of agitation is the fact that even though the liquid does not move the algae still get triggered.

- **Unique flickering effect - resembling glitter**
- **Lack of movement, but not static**
- **Clear view of individual cells lighting up**

BOUNDARY

As it was not possible to increase the intensity of the vibration plate, further explorations of this type of agitation were not succesful. A decrease in the voltage supplied to the motors or the addition of a paused interval both resulted in barely any responses in bioluminescence.

The culture appears to quickly lose its sensitivity to the pulse agitation. If the culture has been agitated before, even in the slightests manner, there will be almost no response.

- **Highly affected by loss of sensitivity**
- **Barely visible response to agitation**

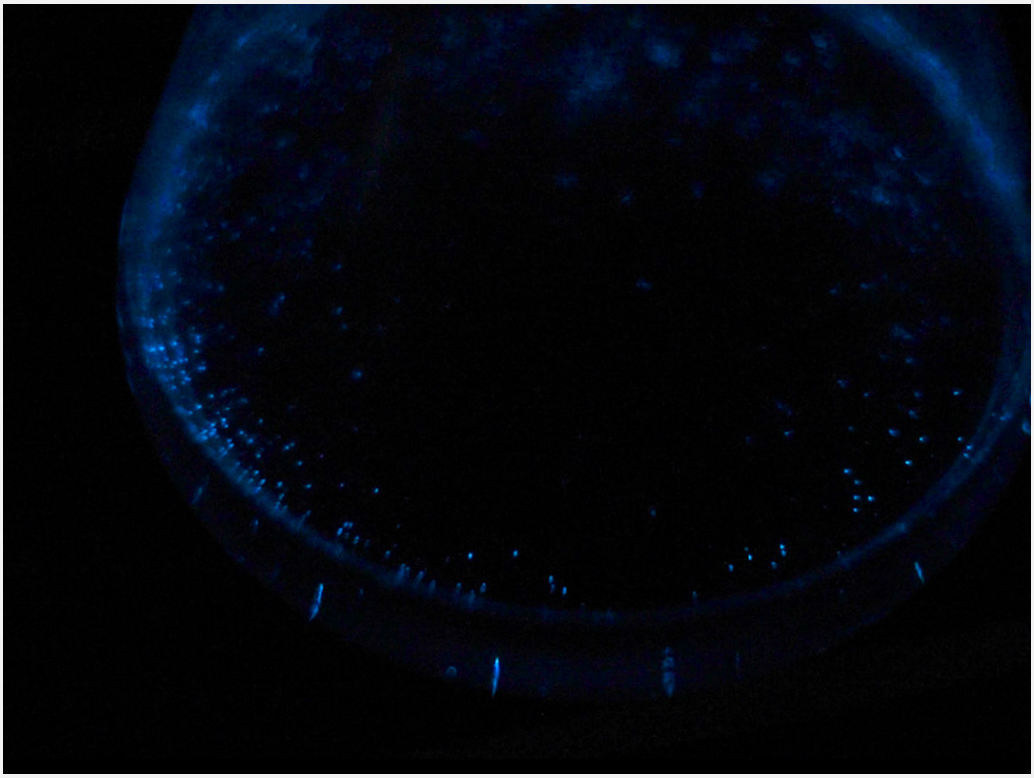


IMAGE [75]
CONTINUOUS VIBRATION
DOTS OF LIGHT NEAR THE EDGE

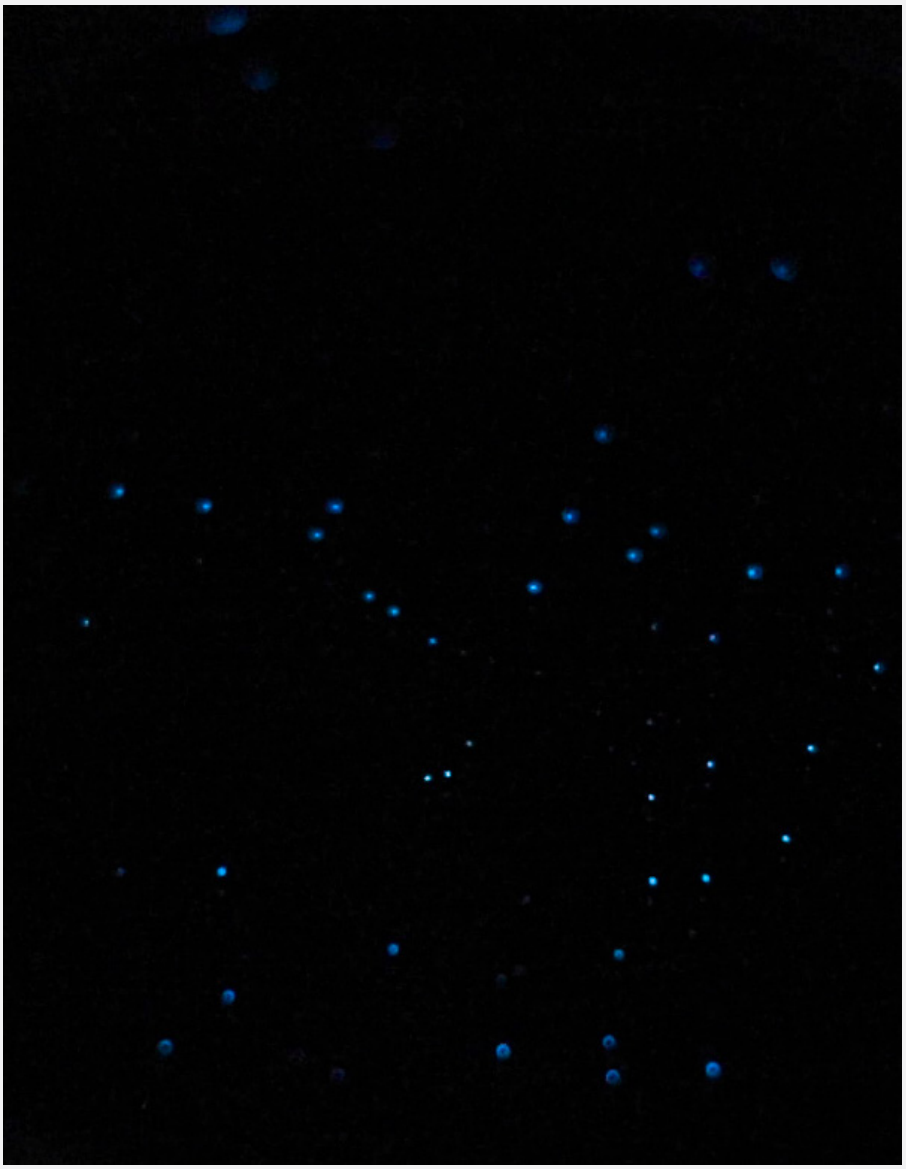


IMAGE [76]
CONTINUOUS VIBRATION
INDIVIDUAL DOTS OF LIGHT

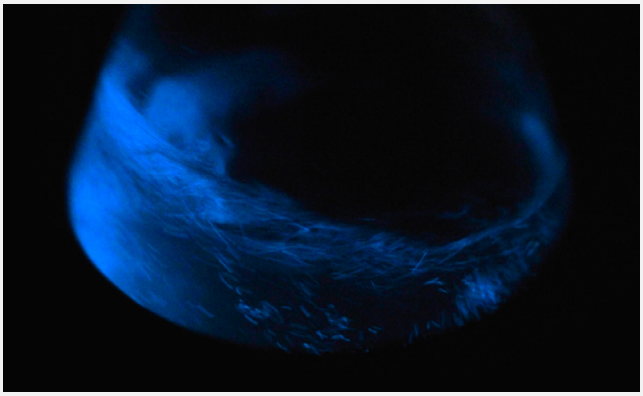
4.3

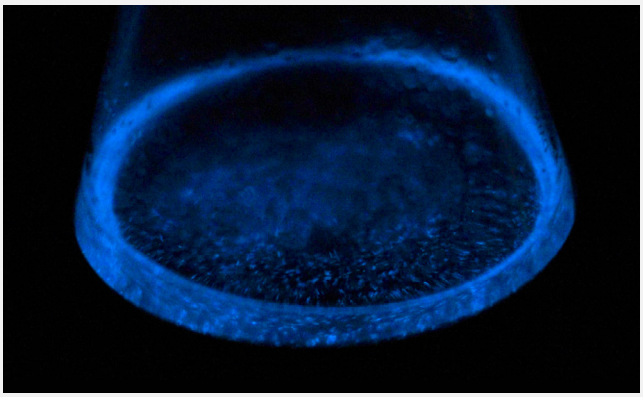
CONCLUSION

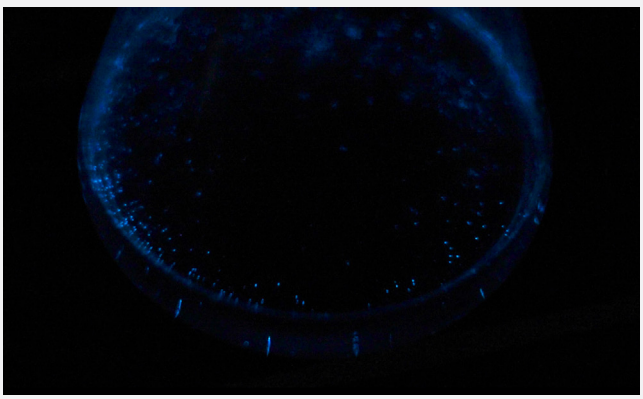
Within this chaper an overview of characteristics for each of the three different types of agitations will be provided; describing key elements looking at the boundary conditions and unique qualities of the light.

OVERVIEW OF QUALITIES

IMAGES [77 - 79]
TOP - ROTATION [R2]
MID - PULSE [P2]
BOTTOM - VIBRATION [V1]







ROTATION
Most forms of rotational agitations tend to result in a high total light output over time, especially within the first few seconds of the sequence. Rotation will also result in a fairly consistent and uniform light source. However, as rotational speeds increase some of the textural qualities of the light tend to get lost. While rotational agitations produce a lot of light, they also appear to deplete the energy of a culture rather quickly.

+ **High intensity light (mostly during first seconds)**
+ **Consistent responses in light output over time**
+ **Less affected by losses of sensitivity**

- **More uniform glow: loss of texture**
- **Quickly depletes energy of algae**

PULSE
In most cases, forms of pulse agitations will result in a highly texturised and unique lighting pattern. However, the overal intensity of the light will be slightly lower when compared to most rotational agitations.The algae cultures also appear to lose their sensitivity to pulse agitations rather quickly, further decreasing their total light output over time.

+ **Unique two phased lighting pattern**
+ **Many individual dots of light: lots of texture**
+ **Less energy intensive**

- **Overal lower intensity light**
- **Noticeably affected by loss of sensitivity**
- **Short duration of visible light**

VIBRATION
While forms of vibrational agitation create exceptional and almost incomparable lighting patterns, the overall intensity of the light is much lower when compared to the other types of agitation. Especially, as in most cases the response of the algae to the vibrations is barely visible. However, if one is able to increase the force of the vibrations, it might become a viable way to trigger the bioluminescence of the algae.

+ **Exceptional and intriguing lighting pattern**
+ **Clearly separated dots of light: highly texturised**
+ **Appears to deplete almost no energy**

- **Very low light intensities: barely visible**
- **Highly affected by loss of sensitivity**
- **Lack of movement within light**

BOUNDARY CONDITIONS

Determining the general characteristics of the light by looking at the intensity over time, total duration and recovery times of the cultures.

B1 INTENSITY OVER DURATION

An increase in the rotational speed appears to result in a higher [initial] light intensity, however it also appears to deplete the energy of the algae noticeably quicker in comparison to the more controlled and slower rotations.

What is the relation between the 'shaking' intensity [defined as a combination of the rotational speed, duty cycle and acceleration] and the duration of light?

B2 RECOVERY OF FIRST FLASH

Cultures showed a recovery in flash characteristics similar to the two flash forms as described by Widder [even after being continuesly agitated for longer periods of time]. However, it is not clear yet how different types of agitation will affect the characteristics of this first flash recovery.

How does the type of agitation [rotation, pulse or vibration] affect the first flash recovery characteristics?

B3 SENSITIVITY TO STIMULATION

There appears to be a noticeadecrease in sensitivity to mechanical stimulation over time, as an increase in force is required to achieve similair outputs in light intensity once a culture has been previously agitated.

To what extend is there a loss of sensitivity over time and how does this affect the flash characteristics?

QUALITY OF LIGHT

Determining the unique visual qualities of the light by looking at its 'liveliness' , the textural qualities and the movement or trace of light.

Q1 TEMPORALITY OF LIGHT

Different types of agitation appear to have different effects on the 'response curve' of the light in relation to the mechanical stimulation [looking at the delay, rise and decay of the light].

What is the response curve of the light to certain types of agitations? [determining the relation between stimulation and light output over time – liveliness of the material]

Q2 TEXTURAL QUALITIES

Fast rotations appear to result in a more uniform glow, while pulse agitations and vibration tend to create a more textured light [where individual dots of light are still visible].

How do the different types of agitations affect the 'textural qualities' of the light?

Q3 TRACE OF LIGHT

The movement of the light inside the container [pattern of light] differs depending on the type of agitation. However, it is not clear yet how certain agitations affect the pattern of light.

How do the different types of agitations affect the pattern and movement of light within a container? [determine how the light moves over time – position and intensity]

CHAPTER 5

FLASH CHARACTERISATION

Determining the general characteristics of the light by looking at the temporal form, textural qualities and spatial distribution

C5

CONTENTS

AIM

Discovering the unique qualities of the light produced by the bioluminescent algae; looking at the temporal form, textural qualities and spatial distribution

- Defining points of interests and research questions, based upon the results of the exploration phase
- Describing the test setup, as wel as the procedures and methods used to analyse the results
- Providing a structured overview of the test results both in text and graphically
- Discussing test results, looking at points of improvement for further testing

METHOD

- Use of general research structure
- Comparing results to known literature
- Information from experts

5.1

RESEARCH STRUCTURE

Based upon the questions that resulted from the exploration phase, the focus of the research will be on the following topics: the temporal form, textural qualities and spatial distribution of the light produced by the bioluminescent dinoflagellates.

Each of the subjects has a different effect on the way the light is experienced. While in reality, there are many different other factors that will influence how one perceives the light (such as the embodiment, overall environment, etc). The focus will be on determining the relation between movement directly related to the experiential qualities; providing an overview of factors that can be adjusted to alter the flash characteristics.

TEMPORAL FORM

An overview of the factors that influence the behaviour of the light over time; looking at changes in luminance, the overall response curve and the loss of sensitivity.

INTENSITY OVER TIME
Determining how the light intensity changes over time, by looking at the relation between the 'shaking' intensity (defined as a combination of the type of rotation, the rotational speed and duty cycle) and the light output.

INPUT - OUTPUT RESPONSE
Analysing the response curve of the light with regards to different types of mechanical stimulation. Looking at the delay between input and output, as wel as other aspects that define the behaviour of the light.

LOSS OF SENSITIVITY
Evaluating to which extend the assumed loss of sensitivity interacts with the behaviour of the light over time, as wel as analysing which factors influence the decrease in sensitivity.

TEXTURAL QUALITIES

An overview of the factors that have an influence on the textural qualities of the light with a focus on the scattering properties.

COUNTING INDIVIDUAL DOTS OF LIGHT
Quantitating the level of texture by counting the amount of individual dots in order to a better insight on which factors influence the textural qualities of the light.

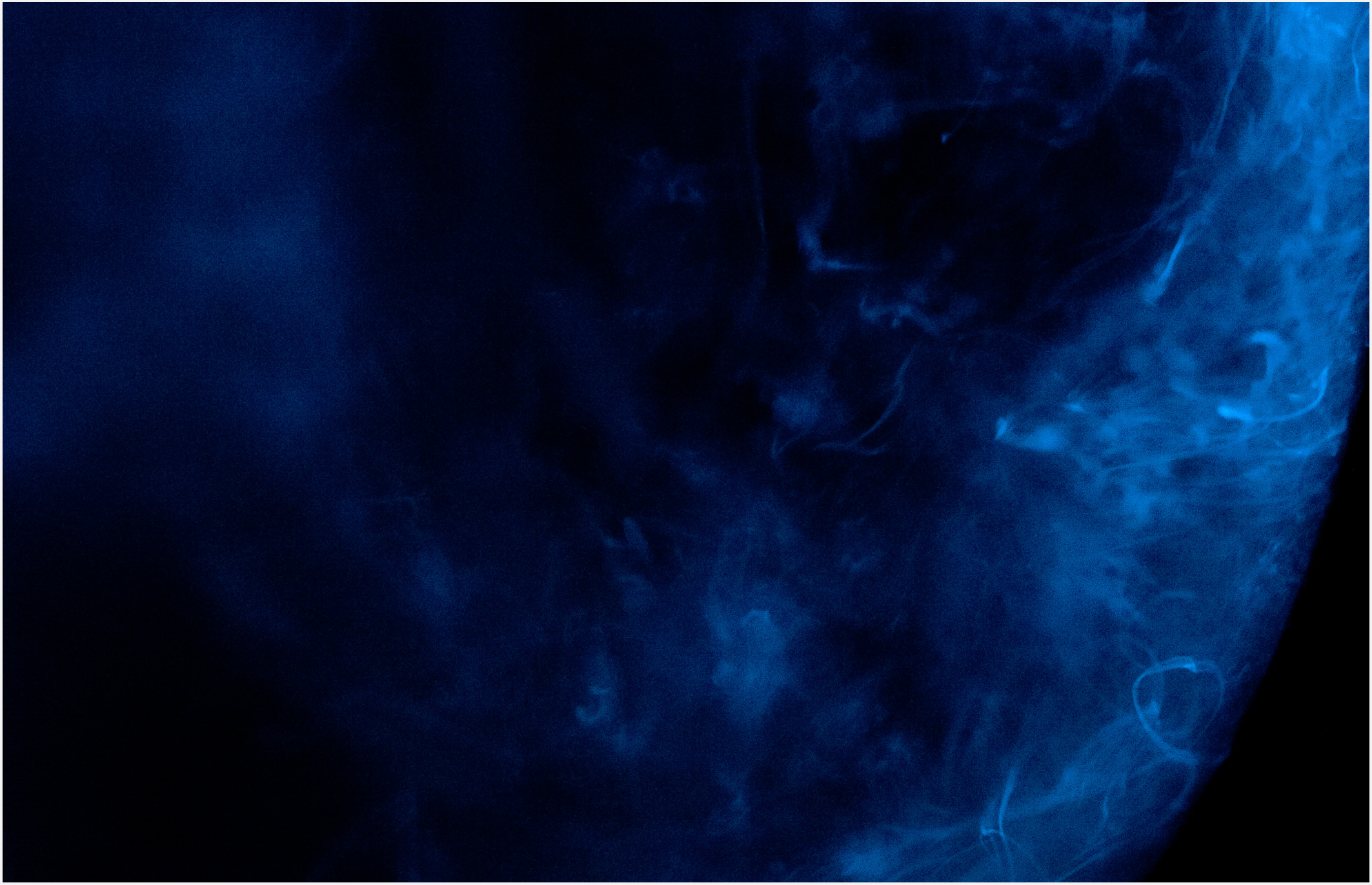
EXAMPLES FOR ROTATION TYPES
Describing the differences in textural qualities for certain types of agitation sequences.

SPATIAL DISTRIBUTION

An overview of the factors that influence the movement of the light; providing first insight in the spatial distribution of the light over time for different types of agitations.

FACTORS INFLUENCING MOTION
Determining the factors that have an influence on the spatial distribution and movement of the light over time.

EXAMPLES FOR ROTATION TYPES
Describing the movement of the light over time for certain types of agitation sequences.



5.2

TEST SETUP

The following chapter provides a detailed desciption of the test setup with an overview of the equipment, the camera capture settings and overall condition of the algae cultures.

LOCATION & TIME

All tests were performed at lab at the TU Delft Science Centre between 8:00 and 9:00 in the morning. At this point the algae cultures were approximately 2 hours into their dark cycle.

EQUIPMENT

In order to ensure all tests were performed in total darkness, a light-tight box was built. This setup consisted of a holder for the shaker, covered with a black lightproof cloth. To make sure no stray light would reflect onto the glass, an extra lightproof cloth was added in order to fully enclosing the setup.

During all test sequences, the cultures were agitated with the use of the orbital shaker, as described in Chapter 4.

CAPTURE SETTINGS

Videos were captured with a Nikon D5500 and a Nikkor 50mm f/1.4 G lens. In order to ensure the settings remained the same for all tests, the camera was put into manual mode. Each video was capture with the following settings:

- RESOLUTION: 1920x1080 at 50fps
- APERTURE: f/2
- SHUTTER SPEED: 1/50
- ISO: 800

The camera was placed on a tripod and the angle and distance of the lens to the glass were carefully measured and the same for each test (see image 90).

IMAGE [80]
TEST SETUP
[DAYLIGHT & WITHOUT ADDITIONAL CLOTH]

CONDITION OF ALGAE CULTURES

Two cultures of Pyrocystis Fusiformis (from the same batch) were used for the research. Both cultures were bought from PyroFarms (arrived on 1.11.2019) and had been grown in the growing setup, as described in Chapter 3, for approximately 6 weeks. During this period, no medium had been added, which resulted in highly concentrated cultures.

The two batches of algae concentrate (180ml and 200ml) were split up into three batches of 100ml. The remaining 80ml was used as a test batch for the new medium. The algae cultures were divided and transferred to sterilise 300ml Erlenmeyer one week before testing. During the transferring process of the culture extra care was taken to ensure each container had the roughly the same amount of algae matter in it. This was done by continuously swirling the liquid in opposite directions trying to evenly spread the algae within the medium and create a more homogenous liquid. Once the algae matter appeared to be wel mixed with the medium, the culture was poured into the Erlenmeyer as quickly as possible, trying to minimize the amount of algae sinking to the bottom.

It is important to note that even though this way of splitting a culture often results in evenly distributed algae matter, it does not ensure that all batches give the same amount of light. Especially, as not all cell matter within the liquid might still be alive. Therefore, it will be necessary to do benchmark tests in order to accurately define the differences between each batch.

IMAGES [81-82]
CONDITION OF ALGAE CULTURES
CLOSE-UP

IMAGES [83]
OVERVIEW OF ALGAE CULTURES
BATCHES [A, B, C]

5.2

DATA ANALYSIS

All video files were adjusted (color-corrected to match real life colors and brightness), to be trimmed and exported as H.264 files with the use of Adobe Premiere. A Matlab script, based of the Video Processing Tutorial of Image Analyst (2020), was used as a base for the video analysis. As this script is able to read video files frame by frame, it is possible to extract various types of information, such as the mean or max gray values, for each individual frame.

CALCULATING INTENSITY OVER TIME

In order to determine the differences in luminance over time, each frame was first converted to a grayscale image with the use of the rgb2gray command. This command converts the RGB values to grayscale values by forming a weighted sum of the R, G, and B components:

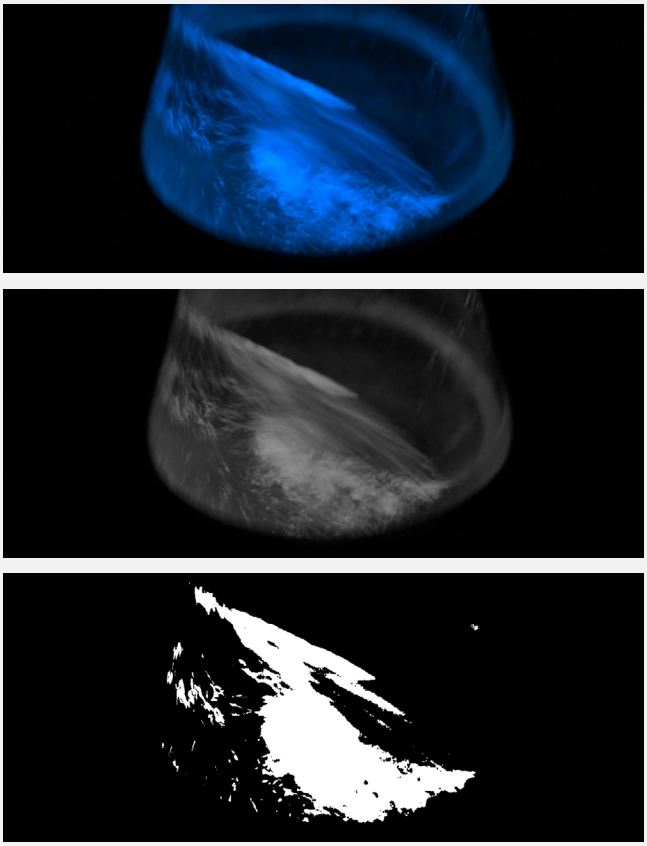
[0.2989 * R + 0.5870 * G + 0.1140 * B]

With the use of this weighted sum it is possible to ensure that the grayscale values will have the same perceived luminance as the original color image (in relation to its original color space).

Next, in order to determine the change in light intensity over time, either the mean or max luminance value of all pixels in the frame was calculated and plotted for each individual frame. While the mean gray value will look at the average luminance value of all pixels in the frame, the max gray value only looks at the brightest single pixel in each frame.

Once all frames have been processed, the area underneath the plotted graph is calculated, representing the total mean or max luminance over time.

IMAGE [84]
IMAGE CONVERSION STEPS
TOP TO BOTTOM: (COLOR - GRAYSCALE - BINARY)



CALCULATING THE AMOUNT OF 'BLOBS'

In order to get insights on the textural qualities of the light, the grayscale image was first converted to a binary image; replacing all pixels with a luminance above the threshold level with the value 1, while replacing all other pixels with the value 0. Next, all pixels with the value 1 become white, all the pixels with the value 0 are turned into black.

In order to get the most detail within the binary image, it is important to use the lowest possible threshold level. However, if the threshold level is too low background noise will become visible.Trial and error showed that he ideal threshold level is between a value 0.02 and 0.05.

Once the binary image has been created, it will be possible to quantise the amount of texture for each frame. With the use of the bwlabel command, the pixels that lie next to each other and have similar luminance values are connected and formed into so-called 'blobs'. If the light becomes more scattered, there will be an increase in the amount of blobs, while if the light is starts to blend together there will be a decrease in the amount of blobs (as the light forms one big blob).

To get a better view on the textural qualities light in relation to the light intensity, a relative threshold that is linked to the mean gray value has also been used. In this case, a base threshold level of 0.01 was used. The relative threshold was calculated based upon the following components:

THRESHOLD = 0.01 + (MEAN GRAY VALUE / 255)

DATA VISUALISATION

All graphs shown within the next chapters will be based around the following structure:

DESCRIPTION OF GRAPHS
The x-axis of the graph shows the duration, where each step represents a single frame. As all videos are shot at 50 frames per second, each frame will have an actual duration of 20ms.

The y-axis of the graph shows the measured value, which can either be the mean gray value, the max gray value or the amount of blobs. Both the mean and max gray value can have values ranging from 0 to 255. However, as each video frame consists of mostly black pixels, the mean gray value will be relatively low throughout all tests.

GLOSSARY

Overview of general terms used to describe changes or states within the graphs.

AMPLITUDE
Height of the curve [max. value]

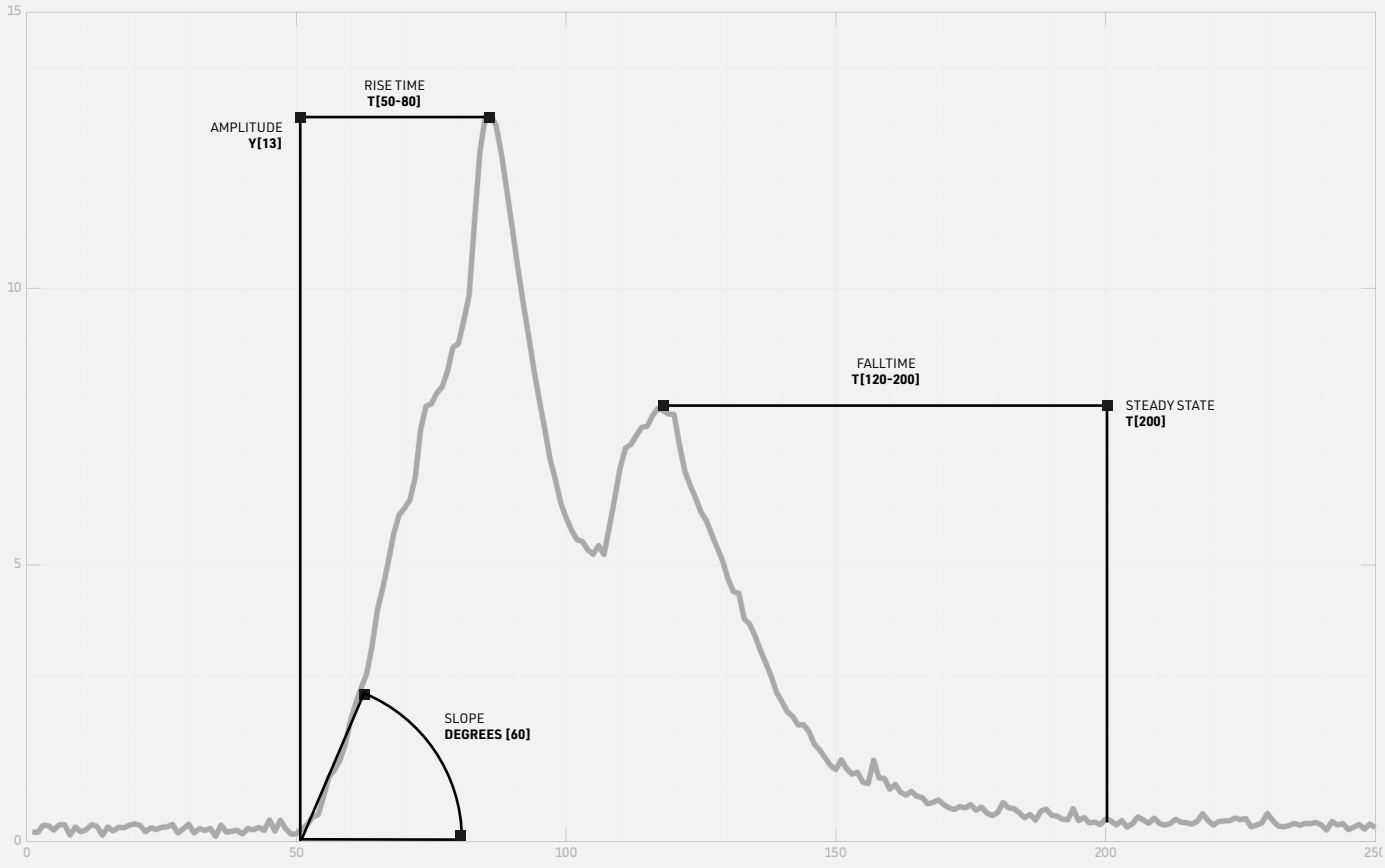
RISE TIME
Time from start of response till initial peak [increase]

FALL TIME
Time from peak till baseline state [decrease]

SLOPE
Angle of the response curve [degrees]

STEADY STATE
Point at which the amplitude remains constant [min. value]

FIGURE [85]
EXAMPLE GRAPH
COMMONLY USED TERMS



5.2

PROCEDURE

IMAGE [86]
TEST PROCEDURE
NEUTRAL POSITION OF SHAKER

HANDLING THE CULTURES
Before the beginning each test, one of the Erlenmeyers containing the algae was removed from the growing setup and placed inside the the holder of the orbital shaker. During this process it was key to disturb the algae as little as possible, as even the slightest movement would trigger some of the algae. In order to be able to monitor wether the cultures were agitated before the tests, all of the following procedures were performed in complete darkness.

Note that even although the algae were moved with extreme care, slight disturbances were unavoidable. As the cultures are super sensitive to any minuscule motion, even picking the glass containing the culture out of the fridge already resulted in some visible light emissions.

While in an ideal scenario the glassware containing the algae would be placed in the shaker without any disturbance, this was not fully possible. As the growing setup was placed on the other side of the lab (near the windows), it was not possible to do tests next to the growing setup.

POSITION OF ORBITAL SHAKER
Before starting the agitation sequence, the orbital shaker is put in its neutral position (pointing forwards and to the middle). This ensures each agitation starts from the same position, creating an equal starting point for each test. As the size of the glass and therefore also the 'size' of the light source within the video frame slightly changes as the glass starts rotating: becoming smaller when it moves to the back and larger when it moves to the front.

BASELINE AGITATION
Most of the tests started out with a baseline agitation, which was added in order to check the condition of the culture as wel as remove some of the 'clumping' of the cells. As the cells of the algae have a tendency to stick together over time, there will be clumps of algae matter suspended within the liquid. Tests in the exploration phase have shown that these clumps will influence the behaviour of the light, causing significant variances in luminance and creating undesirably and random behaviour of the light. By rotating the algae for just a second, almost all of the clumping is removed.

In order to ensure that the cultures could recover and regain some of their first flash characteristics, following the research of Widder & Case (1981), a 5 minute pause was added after each baseline agitation. Note that in an ideal scenario, the pause between the baseline agitation and the test sequence would be even longer (optimally between 10 to 15 minutes). However, as our timeframe did not allow for such long time intervals between test sequences, a 5 minute pause was used.

LIST OF TEST SEQUENCES

	DESCRIPTION	ID
T1 BASELINE AGITATION	**BEFORE TEST SEQUENCE**	
	MOTOR [255] - 120 RPM	R[1-6]-B
	AFTER TEST SEQUENCE	
	MOTOR [255] - 120 RPM	R[1-6]-A
T2 ROTATIONAL AGITATION	**CONSTANT ROTATION**	
	MOTOR [130] - 90 RPM	R1-T
	MOTOR [255] -120 RPM	R2-T
	PAUSED ONE-WAY ROTATION	
	MOTOR [130] - 90 RPM	R3-T
	MOTOR [255] -120 RPM	R4-T
	PAUSED ALTERNATING ROTATION	
	MOTOR [130] - 90 RPM	R5-T
	MOTOR [255] -120 RPM	R6-T
T3 PULSE INTERVAL AGITATION	**WITHOUT BASELINE AGITATION**	
	1 MIN. INTERVAL - 5X	P1-T
	3 MIN. INTERVAL - 5X	P3-T
	5 MIN. INTERVAL - 5X	P5-T
	WITH BASELINE AGITATION	
	1 MIN. INTERVAL - 5X - IN DARK	P1-T-BD
	1 MIN. INTERVAL - 5X - IN LIGHT	P1-T-BL
T4 SINGLE ROTATION	**DIFFERENCE IN SPEED**	
	MOTOR [130] - 90 RPM	S1-T
	MOTOR [255] -120 RPM	S2-T
	DIFFERENCE IN VOLUME	
	WITHOUT MEDIUM - 120 RPM	S3-T
	WITH MEDIUM - 120 RPM	S4-T
T5 VARIATION IN AGITATION SEQUENCES	**ORDER OF AGITATION**	
	PULSE - INTERVAL - ROTATION	O1-T
	ROTATION - INTERVAL - PULSE	O2-T

T1

BASELINE AGITATION

AFTER TEST SEQUENCE

A clockwise rotational motion with a speed of 120 rpm for 1000ms, performed before each rotational test sequence.

OVERVIEW OF SHAPE: MEAN GRAY VALUE CURVES

When looking at the changes in mean gray value over time, all of the baseline agitations appear to have similarly shaped curves. Each curve starts with a steep linear rise at T[50]; only milliseconds after the rotation of the shaker is initiated. After approximately 30 to 35 frames from the start of the rotation, the mean gray values reach their peak values, which range between 13.2 and 14.9 (see figure 89).

In all cases, this spike in intensity is quickly followed by a steep linear fall (with an almost identical slope as the initial rise) to values ranging from 5.0 to 5.5 at T[100]. At this point, the mean gray values start to rise again, resulting in a second peak at T[120]. For the majority of the baseline agitations, this second peak in light intensity has less of a pronounced spike at the top and reaches only 50 to 60% of the intensity of the first peak. It is important to note that there are some variances in the maximum values and overal shapes of the second peaks. For example, as the second peak of R5-B has a distinct spike: with a similar shape as its first peak and a maximum value of 11.5, R3-B has less pronounced and almost flat second peak that only reaches a maximum value of 6.0.

After the second peak, the mean gray values of all the baseline agitations start to fall, once again with a similar kind of slope as the inital rise. At approximately T[150], the linear curve starts to fade into a more gradual downward concaved curve. This gradual fade continuous till the intensity has reached a value of 0.5 at T[200]. At this point the curve appears to have reached its steady state and there is no light visible anymore.

OVERVIEW OF SHAPE: MAX GRAY VALUE CURVES

The graphs of the agitations displaying the maximum gray values over time are similar to those of the mean gray values; showing two distinct peaks at T[80] and T[120]. However, in most cases the max. gray value curve has more spikes in intensity, resulting in a more 'jagged' appearance. The biggest difference is seen in the decay after the second peak. When comparing both curves, the max value curve shows a more gradual decay with lots of spikes in intensity.

COMPARING PEAK AND TOTAL VALUES

All of the maximum gray values are seen in the first peaks of the agitation. Most of the agitations fall within a range of 0.1 to 0.5 of the average maximum gray value of 14.4. When looking at the maximum values,one of the main outlier appears to be R1-B, which has a peak value of 13.2 (approximately 9% lower than the average maximum value).

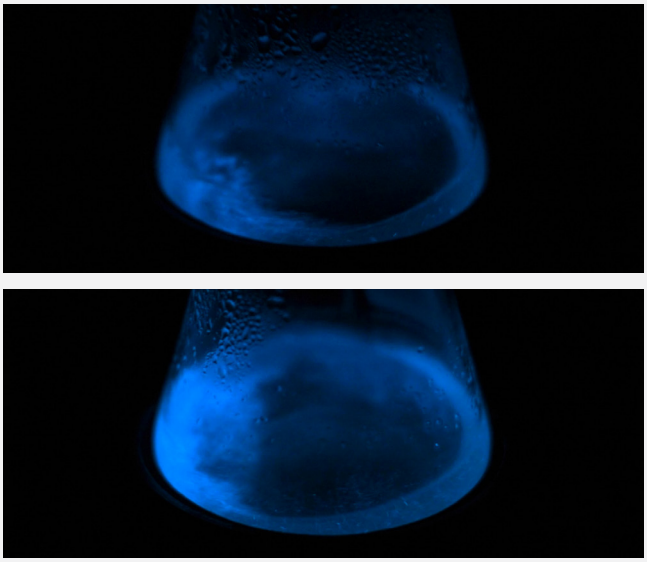
However, when looking at the total mean gray value over time of R1-B, it appears to be almost identical to the average. There seems to be more variance between the total values, where the lowest total value (R3-B) is approximately 15% lower than the average total value.The differences in total values appear to be mostly caused by differences in shape and maximum value of the second peaks.

N5-B has both the highest maximum and total values, making it one of the most notable outliers (see table 87).

TABLE [87]
MEAN GRAY VALUES OVER TIME
BASELINE AGITATIONS

TEST [NO.]	MAXIMUM	TOTAL
R1-B	13.2	1.59×10^5
R2-B	14.3	1.71×10^5
R3-B	14.6	1.37×10^5
R4-B	14.8	1.54×10^5
R5-B	14.9	1.85×10^5
R6-B	14.5	1.72×10^5
AVERAGE	**14.4**	**1.63×10^5**

IMAGE [88]
TOP [R3-B] - BOTTOM [R5-B]
FRAME NUMBER [120]



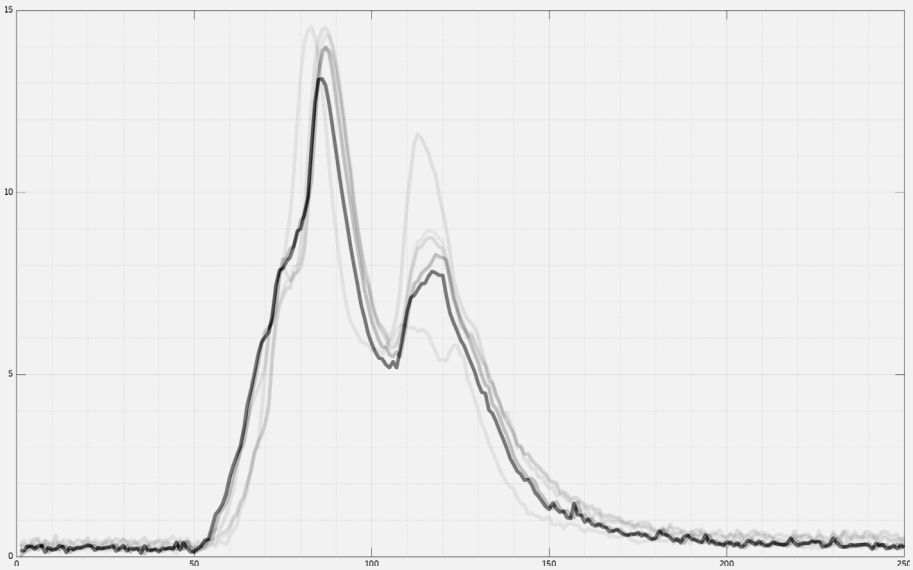


FIGURE [89]

MEAN GRAY VALUE OVER TIME
COMPARISON OF AGITATIONS
DURATION [0-250]

■ 120 RPM - BASELINE AGITATION

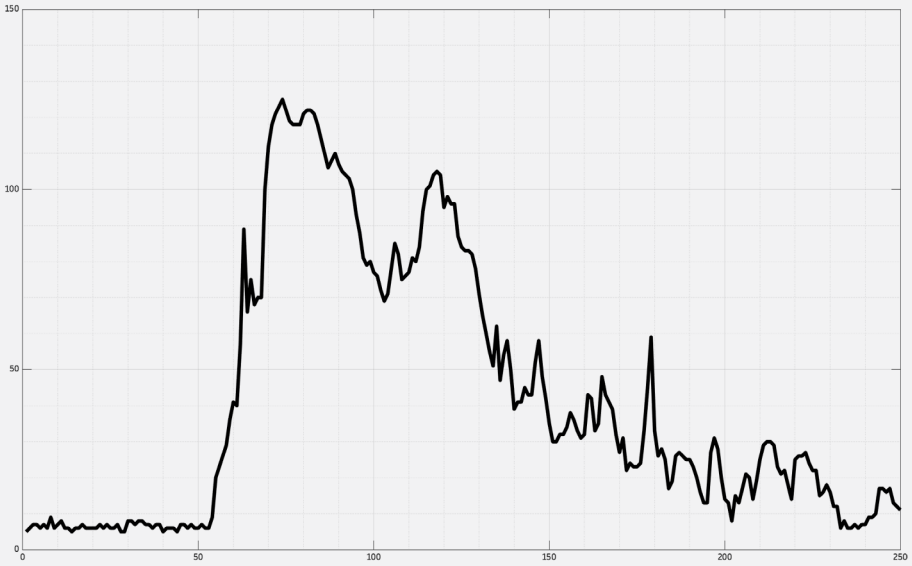


FIGURE [90]

MAX GRAY VALUE OVER TIME
TEST NO. [R1-B]
DURATION [0-250]

■ 120 RPM - BASELINE AGITATION

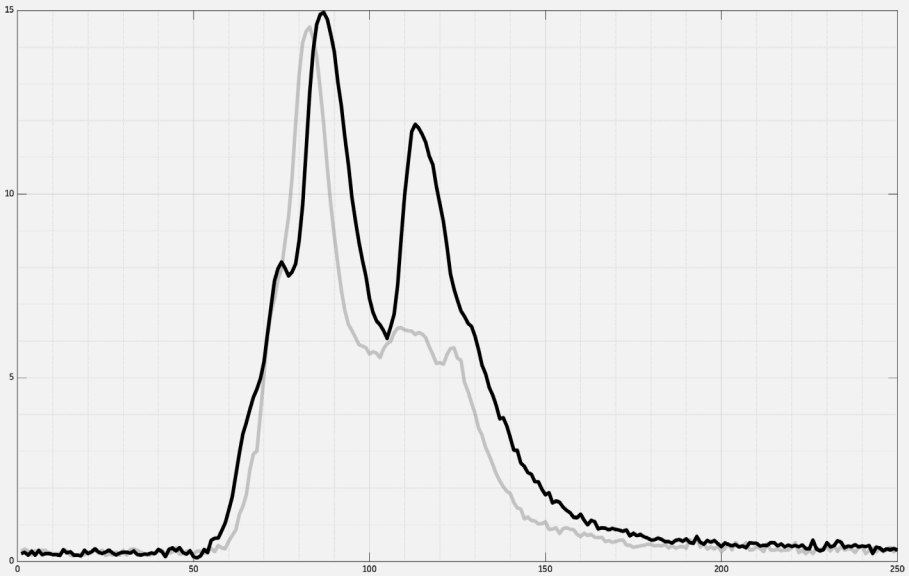


FIGURE [91]

MEAN GRAY VALUE OVER TIME
TEST OUTLIERS [R5-B / R3-B]
DURATION [0-1500]

■ N5-B
■ N3-B

T1

BASELINE AGITATION

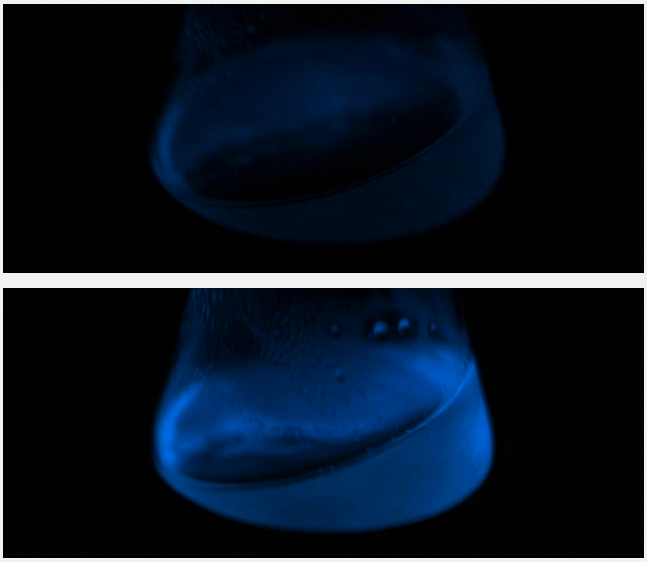
AFTER TEST SEQUENCE

A clockwise rotational motion with a speed of 120 RPM for 1000ms, performed after each rotational test sequence.

TABLE [92]
MEAN GRAY VALUES OVER TIME
BASELINE AGITATIONS

TEST [NO.]	MAXIMUM	TOTAL
R1-A	3.2	3.05×10^4
R2-A	9.5	7.99×10^4
R3-A	1.9	2.51×10^4
R4-A	3.2	3.29×10^4
R5-A	0.4	1.72×10^4
R6-A	8.9	7.92×10^4
AVERAGE	**4.5**	**4.41×10^4**

IMAGE [93]
TOP [R1-A] - BOTTOM [R2-A]
FRAME NUMBER [80]



OVERVIEW OF SHAPE: MEAN GRAY VALUE CURVES

There appear to be some big differences between the mean gray value curves for each of the baseline agitations done after the different rotational test sequences. When comparing the shapes of the curves, there appear to be two distinct groups: rotational sequences at 90 RPM (R2, R4 and R6) and at 120 RPM (R1, R3 and R5).

90 RPM VARIANTS

Performing a baseline shake after agitation after a rotational sequences with a speed of 90 RPM tends to result in a curve that is similarly shaped as its initial baseline agitation. N2 and N6 for example, both have a curve that starts with a steep linear rise at T[50], reaching a peak value of 9.5 at T[80]. At this point, the graph falls down to a value of approximately 3.0 at T[100]. Next, there is a small rise in intensity, resulting in another peak around T[110]. Note that this second peak only reaches values of 3.3 to 3.5, which is at 35% of intensity of the first peak. At T[125], the light intensity begins to rapidly fade until it reaches its steady state at approximately T[160].

While the curves of R2-A and R6-A have almost identical maximum and total values, the curve of R4-A (which is also a 90RPM variant) shows significantly lower values. However, the overal shape of the curve of R4-A still appears to be quite similar.

120 RPM VARIANTS

The baseline agitations performed after a rotational sequence with a speed of 120 RPM, results in almost no response in light output. The curves of R1-A and R3-A show a small spike in intensity that starts to rise in a linear manner at T[60]; reaching a peak value of 3.2 at T[85]. From this point, the mean gray values starts to fall until it reaches a value of 0.9 at T[110], where it stays for another 20 frames before continuing to gradually fall. At T[150], the curve has appeared to have reached its steady state and no light is visible.

Where the curves of R1-A and R3-A still show response, the curve of R5-A shows no significant changes in luminance. Only when looking closely there is a slight bump visible at T[85], which reaches a peak value of 0.4. However, as the steady state of the curve sits around a value of 0.2, this small change in intensity is hardly visible.

COMPARING PEAK AND TOTAL VALUES

The absolute differences between the curves of the baseline rotations done after either 90 or 120 RPM rotation sequences become even more clear when looking at the maximum and total values (see figure 92). As R2-A reaches a peak value of 9.5, R1-A (the 120 RPM variant of the same type of rotation sequence), has maximum value of just 3.2. This difference is also clearly visible in the amount of total gray value over time for both agitations, where the total value of R1-A is at about 40% of the total value of R2-A.

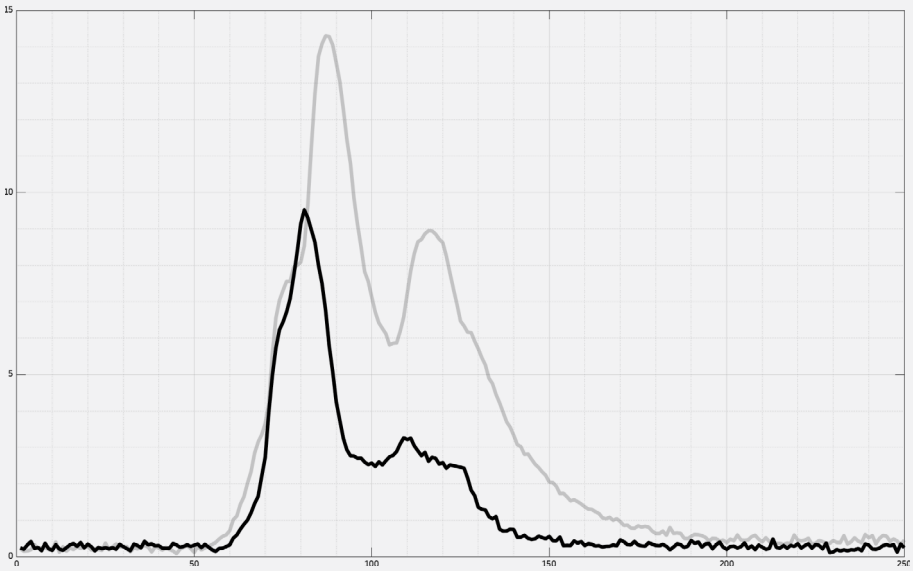


FIGURE [94]

MEAN GRAY VALUE OVER TIME
COMPARISION R2-A / R2-B
DURATION [0-250]

■ 120 RPM - BASELINE AGITATION

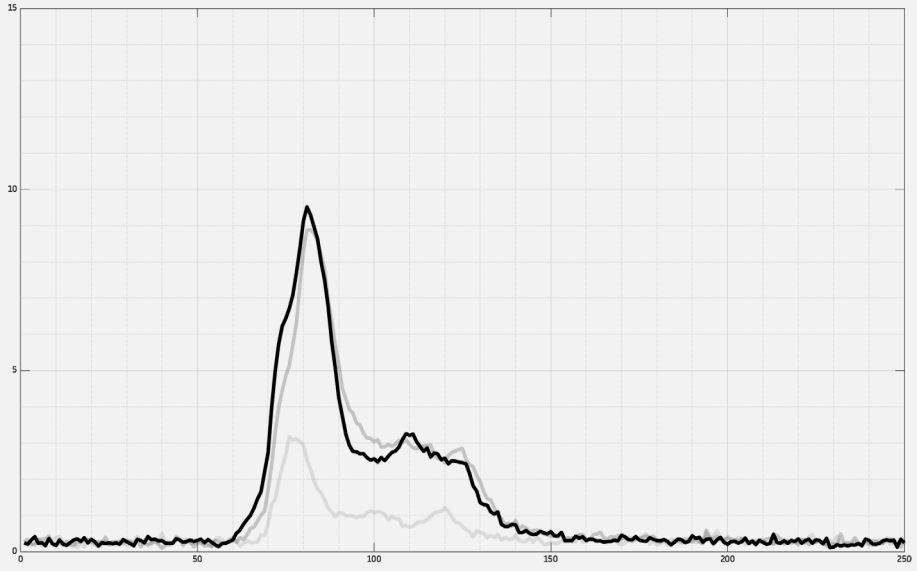


FIGURE [95]

MEAN GRAY VALUE OVER TIME
90 RPM VARIANTS GROUP
DURATION [0-250]

■ 120 RPM - BASELINE AGITATION

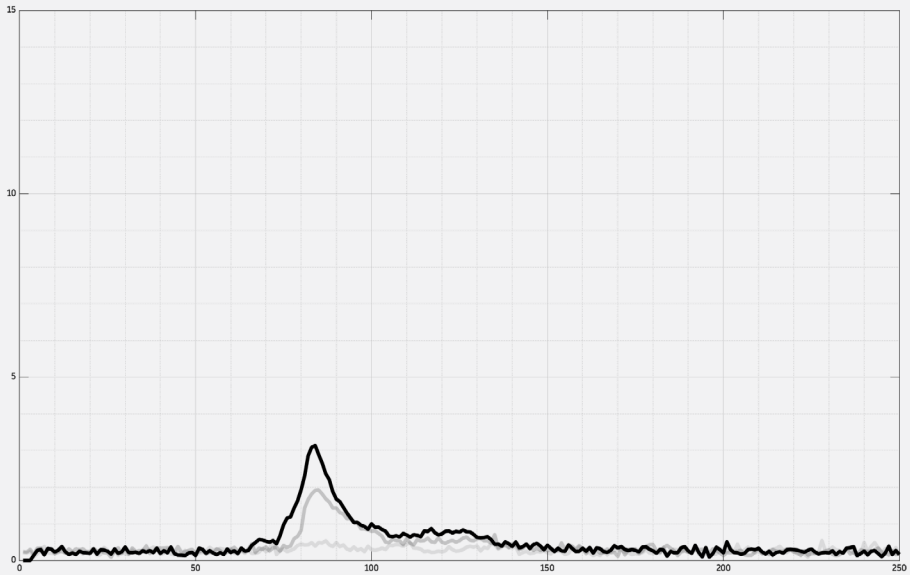


FIGURE [96]

MEAN GRAY VALUE OVER TIME
120 RPM VARIANTS GROUP
DURATION [0-1500]

■ N5-B
■ N3-B

T2

FLASH CHARACTERISTICS
ROTATIONAL AGITATION
RESULTS

CONSTANT ROTATION

A clockwise rotational motion at a speed of either 90 or 120 RPM, performed 5 minutes after a baseline agitation.

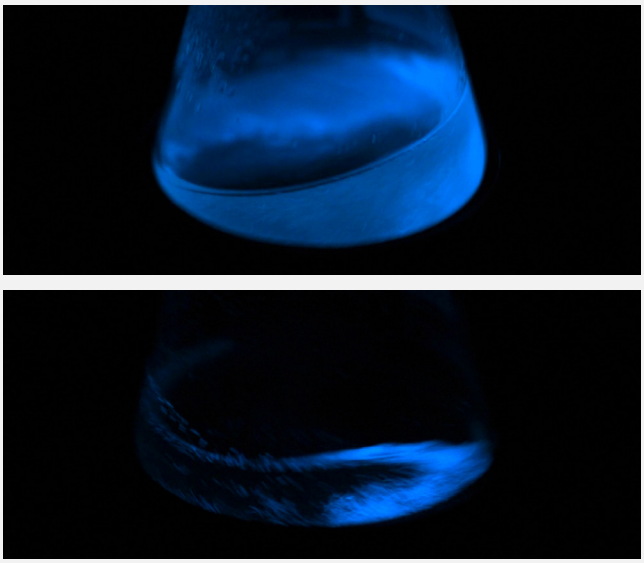
OVERVIEW OF SHAPE: R1-T [120 RPM]
When looking at the beginning of the curve of R1-T (from T[0] to T[130] it appears to be similar to that of its baseline agitation; starting with a a steep linear rise at T[50] until it reaches its peak value of 13.5 at T[85]. At this point the light intensity drops with a similarly linear steepness, until it rises again at T[105] This second peak in light intensity is at only 50% of the intensity of the first peak. As the rotational motion continues, the peak intensity of each of the following peaks gradually drops; with the curve following a downwards concave. Over time (after peak no. 3-4) the spikes in intensity become less frequent, resulting in a more consistent light output over time. At T[550], there are almost no peaks in intensity anymore and the culture seems to have reached its steady state. From this point on, the light is barely visible anymore while it slowly continues to fade, going from 1.0 at T[550] to 0.5 at T[6000].

OVERVIEW OF SHAPE: R2-T [90 RPM]
In general, the mean gray value over time curve of R2-T is comparable to that of R1-T. One of the biggest differences is the shape and size of its initial peak; being at a value of only 3.5 at T[85], roughly 25% the intensity of R1-T's first peak. The slope of the curve leading into the first peak has become more gentle and now follows a slight upwards concave. As the curve starts to fall at T[90], the intensity after the first peak has a similar shape as the inital rise. This slower fall results in a delayed second peak, which now occurs at T[130] instead of T[110].The second peak has a similar slope as the first and 50% of the intensity of the initial peak (similar behaviour as with N1-T). At this point, there is another peak every 40 frames. Each of the following spikes has a slightly lower peak value; decreasing with steps of 0.3 to 0.5. This flashing behaviour is continued till approximately T[900], as at this point the curve has appeared to be in its steady state.

TABLE [97]
MEAN GRAY VALUES OVER TIME
CONSTANT ROTATION

FRAME [NO.]	R1-T [120]	R2-T [90]
T [250]	1.82×10^5	5.48×10^4
T [1500]	3.50×10^6	1.31×10^6
T [6000]	3.24×10^7	1.40×10^7

IMAGE [98]
TOP [R1-T] - BOTTOM [R2-T]
FRAME NUMBER [85]



MAX INTENSITY COMPARISION
When comparing the maximum gray value curves of both agitation sequences, there does not appear to be much of a difference. While R1-T does have slightly higher peak values in the first few seconds the agitation, the curves start to level out at around T[500]. From this point on, the path of both curves can be seen as identical. Both showing a gradual fade in maximum intensity with big spikes every few rotations. Over time, these big spikes tend to become less apparent.

COMPARING PEAK AND TOTAL VALUES
The difference in total mean gray value over time is the most visible within the first 250 frames of the agitation sequence (see table 97). As at T[250], R1-T has produced 3 times the amount of light as R2-T. Over time, the differences begin to decrease and at T[6000] the factor has decreased to 2.3.

It is important to notice that there also is a difference in light output in the steady state (at T[1000]). While R1-T has a mean gray value of approximately 1.0 in its steady state steady state, R2-T's steady state lies around a value of 0.5.

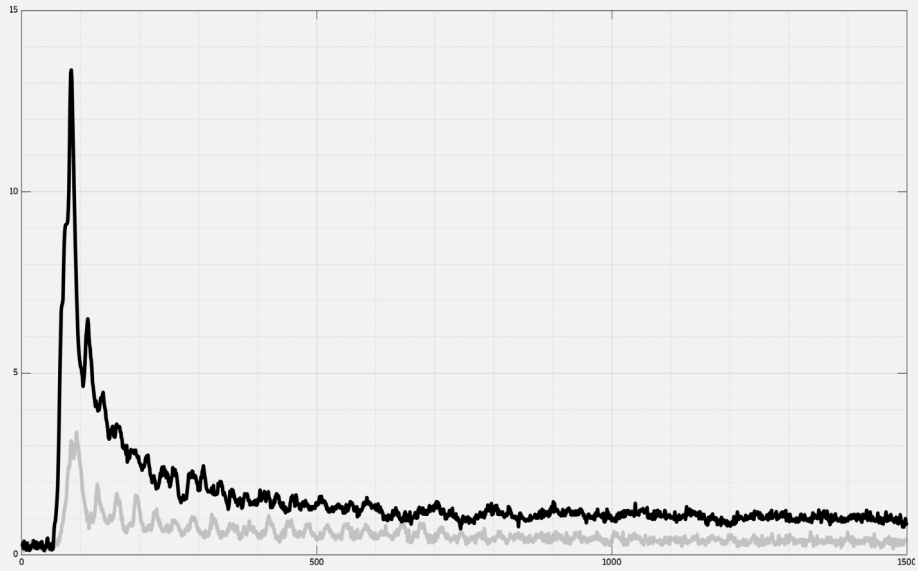


FIGURE [99]

MEAN GRAY VALUE OVER TIME
DURATION [0-1500]

■ 120 RPM - CONSTANT ROTATION
■ 90 RPM - CONSTANT ROTATION

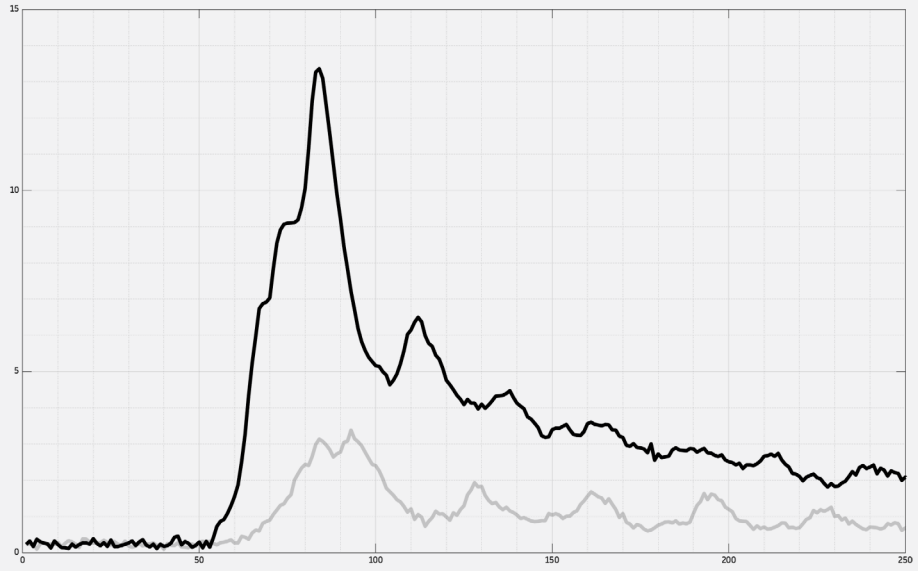


FIGURE [100]

MEAN GRAY VALUE OVER TIME
DURATION [0-250]

■ 120 RPM - CONSTANT ROTATION
■ 90 RPM - CONSTANT ROTATION

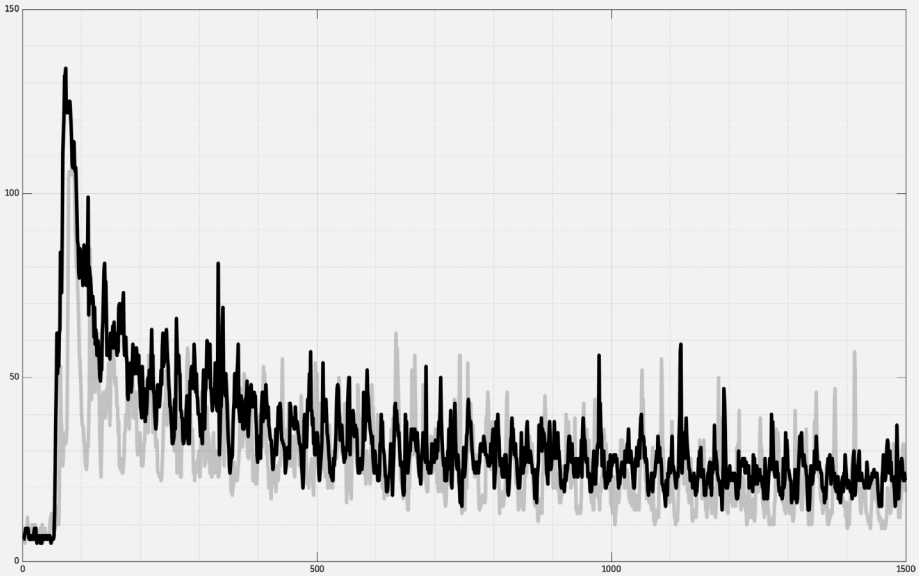


FIGURE [101]

MAX GRAY VALUE OVER TIME
DURATION [0-1500]

■ 120 RPM - CONSTANT ROTATION
■ 90 RPM - CONSTANT ROTATION

T2

PAUSED ROTATION

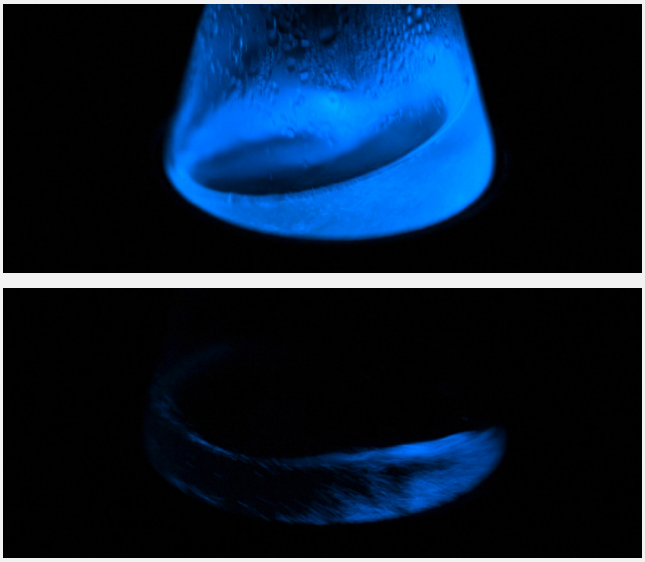
ONE-WAY ROTATION

A clockwise rotational motion at a speed of either 90 or 120 RPM for 1000ms, followed by a pause of 2000ms. Sequence repeated until light was barely visible anymore; performed 5 minutes after a baseline agitation.

TABLE [102]
MEAN GRAY VALUES OVER TIME
CONSTANT ROTATION

FRAME [NO.]	R3-T [120]	R4-T [90]
T [250]	1.72×10^5	2.47×10^4
T [1500]	3.62×10^6	8.70×10^5
T [6000]	3.77×10^7	1.18×10^7

IMAGE [103]
TOP [R3-T] - BOTTOM [R4-T]
FRAME NUMBER [85]



OVERVIEW OF SHAPE: R3-T [120 RPM]
When looking at the first rotation of test R3-T (from T[0] to T[200], the shape of the curve shows strong similarities with that of its baseline agitation (R3-B). At T[50] the mean gray value start to rise with a steep linear curve; reaching its peak value of 19.2 at T[85]. This spike is quickly followed with a steep linear fall (with a similar slope) to a value of 8.0 at T[90], where it stays constant for another 20 frames before falling to a value of 5.0 at T[110]. Note that this second fall has a slightly more gentle slope compared to its inital fall. At this point the mean gray value starts to rise again, creating a second peak in intensity of 7.8 at T[120]. This second peak is at 40% of the intensity of the first peak and quickly fades after T[120]; starting out with a steep linear decline that turns into a more gradual downward curve from T[130] onwards.

Each of the following rotations, creates a peak with similar characteristics (comparable rise and fall times, slopes and overall shapes). However, the differences in maximum values between the first and second peak gradually decreases (see table 102). Also, the maximum value of the first peak tends to fluctuate quite a bit for each rotation. As for the second agitation the second peak has an intensity of about 70% of that of the first, the third agitation shows a bigger difference, where the second peak is at 40% of the intensity. Over time, the curve gradually 'flattens' out, however every once in a few shakes there still is a bright peak of light in the first peak of each agitation.

OVERVIEW OF SHAPE: R4-T [90 RPM]
Similarly as with the comparison between R1-T and R2-T, the 90 rpm paused rotation (R4-T) has a much lower first peak; being at a value of 3.1 at T[85], which is about 15% of intensity of the first peak from R3-T. While the curve stills shows two distinct peaks per rotation, the time between the first and second peaks of each rotation, which have become shorter. Another noticeable difference is the rise and fall have become more gradual and concave, similar as with test R2-T . From the second rotation at T[200] onwards, each of the following rotations results in a similar sized curve. Over time, the peak values start to slowly decrease, going from 1.0 at T[1000] to 0.5 at T[6000].

COMPARING PEAK AND TOTAL VALUES
When comparing the total mean gray values over time of both sequences, the differences in light intensity become even more apparent. Differences in total gray value are the most significant within the first few second of the sequence, as at T[250] agitation R3-T has most almost 7 times the total value of R4-T. Over time the difference between the total mean gray values gradually decreases and at T[6000] agitation R3-T has only 3 times the total value of R4-T.

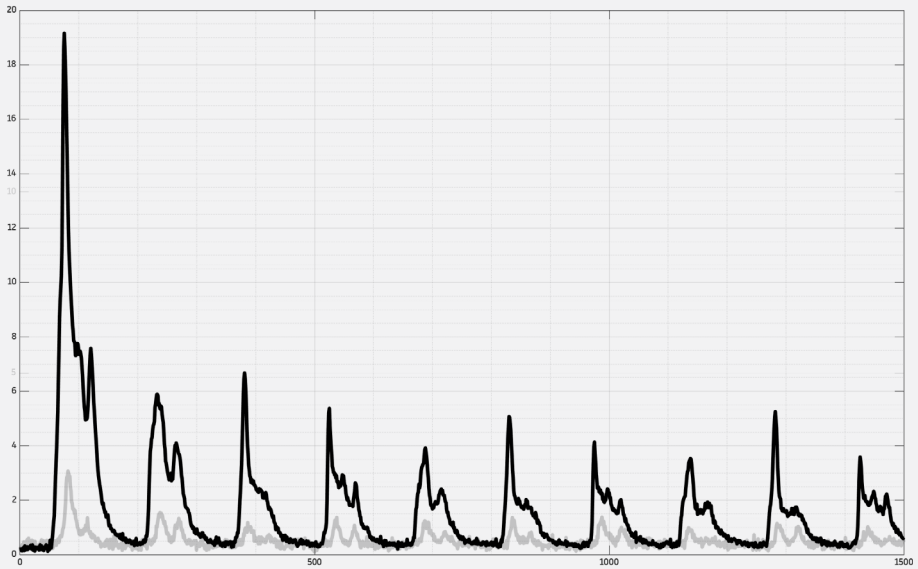


FIGURE [104]

MEAN GRAY VALUE OVER TIME
DURATION [0-1500]

120 RPM - PAUSED ROTATION
90 RPM - PAUSED ROTATION

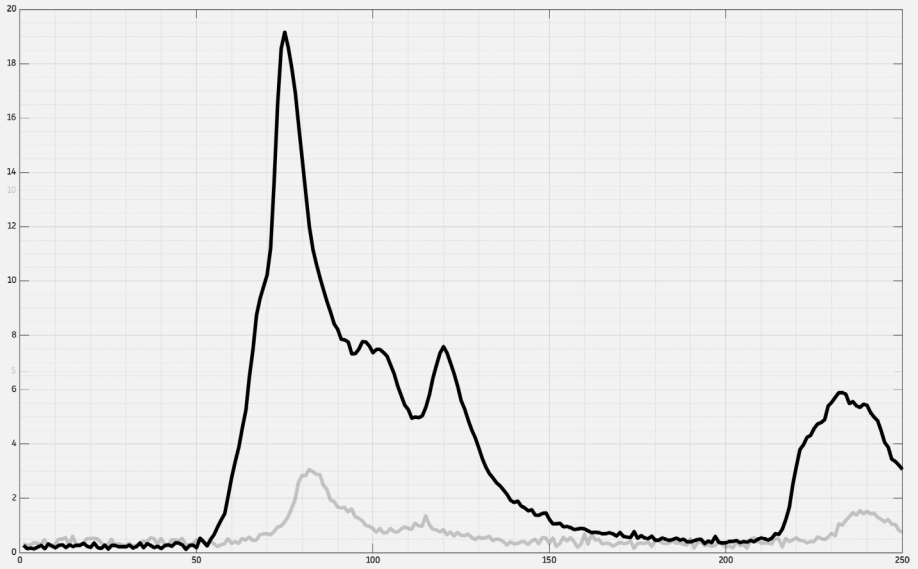


FIGURE [105]

MEAN GRAY VALUE OVER TIME
DURATION [0-250]

120 RPM - PAUSED ROTATION
90 RPM - PAUSED ROTATION

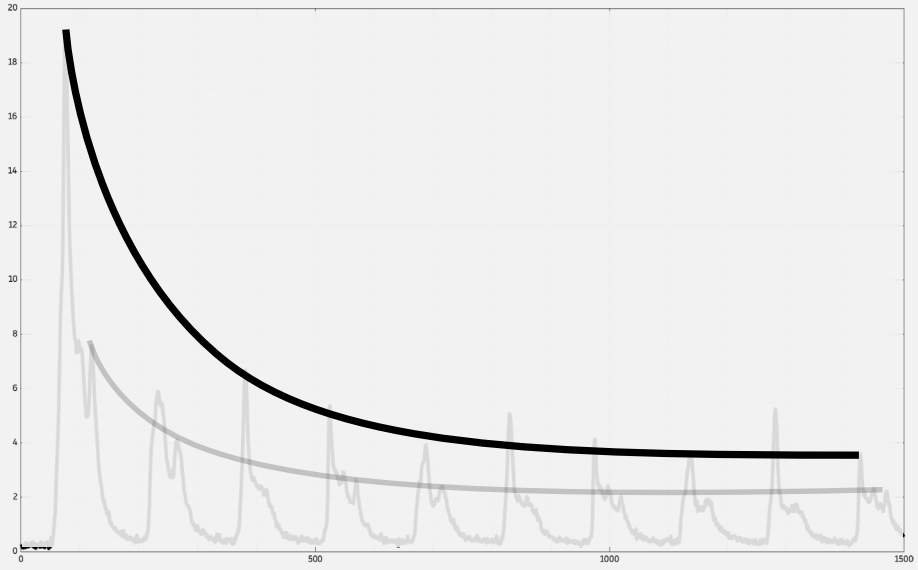


FIGURE [106]

MEAN GRAY VALUE OVER TIME
SLOPE OF THE CURVES
DURATION [0-1500]

120 RPM - PAUSED ROTATION
90 RPM - PAUSED ROTATION

T2

PAUSED ROTATION

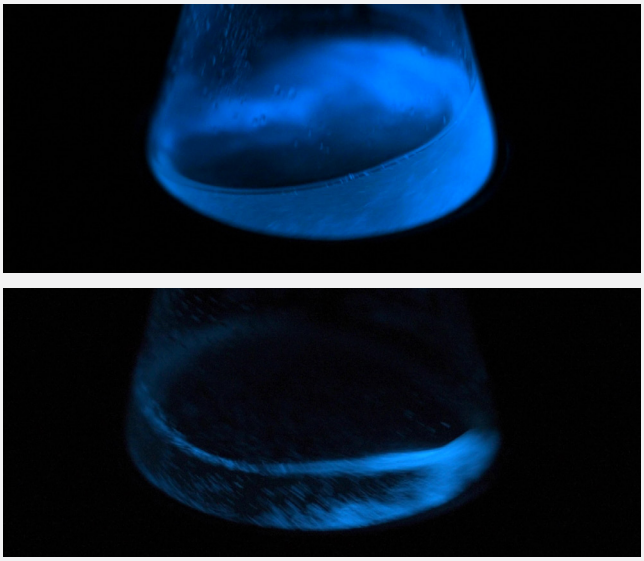
ALTERNATING

A clockwise rotational motion at a speed of either 90 or 120 RPM for 1000ms, a pause of 2000m follows. Direction of rotation alternates for each sequence; performed 5 minutes after a baseline agitation.

TABLE [107]
MEAN GRAY VALUES OVER TIME
CONSTANT ROTATION

FRAME [NO.]	R5-T [120]	R6-T [90]
T [250]	2.28×10^5	8.46×10^4
T [1500]	8.58×10^6	3.22×10^6
T [6000]	7.79×10^7	4.38×10^7

IMAGE [108]
TOP [R5-T] - BOTTOM [R6-T]
FRAME NUMBER [85]



OVERVIEW OF SHAPE: PAUSED ROTATION 90 RPM
The shape of the first rotation of R5-T appears to be almost identical to that of R3-T and its baseline agitation; having a steep linear rise from T[50] till it reaches its peak value of 17.9 at T[85]. This peak is followed by a rapid linear fall to a value of 8.0 at T[90], before rising once again to create a second peak with an intensity of 10.5 at T[115]. This second peak is at 60% of the intensity of the first peak and has a less of a distinct spike at the top and shows small fluctuations in light intensity. At T[130] the mean gray value starts to decrease again. First following a steep linear curve, before gradually transitioning into a more gentle downwards concave. From T[200] the curve has appeared to have reached its steady state and as at this moment the motor is powered on once again, the mean gray value starts to rise again. Note the slight delay between the start of the agitation at T[200] and the start of the rise at T[210]. Also, the slope of the initial rise of the second agitation appears to be slightly steeper.

Each of the following rotations creates a peak with similar characteristics as seen in the second rotation (comparable rise and fall times, slopes and overall shapes). However, the differences in maximum values between the first and second peak gradually decreases (see table 107).This is mostly caused by the decrease in intensity for the first peak of each agitation, as the second peak does not decrease as much over time. As for the second agitation the second peak has an intensity of about 70% of that of the first, the third agitation shows a bigger difference, where the second peak is at 40% of the intensity. Over time, the curve that follows the first peaks of each rotation gradually flattens out from values of 5.0 at T[3000] to values of 2.0 at T[6000].

OVERVIEW OF SHAPE: PAUSED ROTATION 90 RPM
Similarly as with the comparison between R1-T and R2-T, the 90 RPM variant (N4-T) has a much lower first peak; being at a value of 3.1 at T[85], which is about 15% of intensity of the first peak from N3-T. While the curve stills shows two distinct peaks per rotation, the 'distance' in intensity between the first and second peaks of each rotation, which have become very low. Another noticeable difference is the rise and fall have become more gradual and concave, similar as with test N2-T . From the second rotation at T[200] onwards, each of the following rotations results in a similar sized curve with a peak value of approximately 4. Over time, the peak values start to slowly decrease, going from 4.0 at T[1000] to 0.5 at T[6000].

COMPARING PEAK AND TOTAL VALUES
The most remarkable aspect, is the high total mean gray value of R5-T over time. While during the first few seconds of the agitation, the total value appears to be relatively normal (in comparison to the other 120 RPM variants), the total value at T[6000] is roughly 2 times as R1-T and R3-T. There also appears to be less of a significant difference in total mean gray values between both R5-T and R6-T. Once again, the biggest difference in intensity is seen within the first few seconds of the agitation sequence.

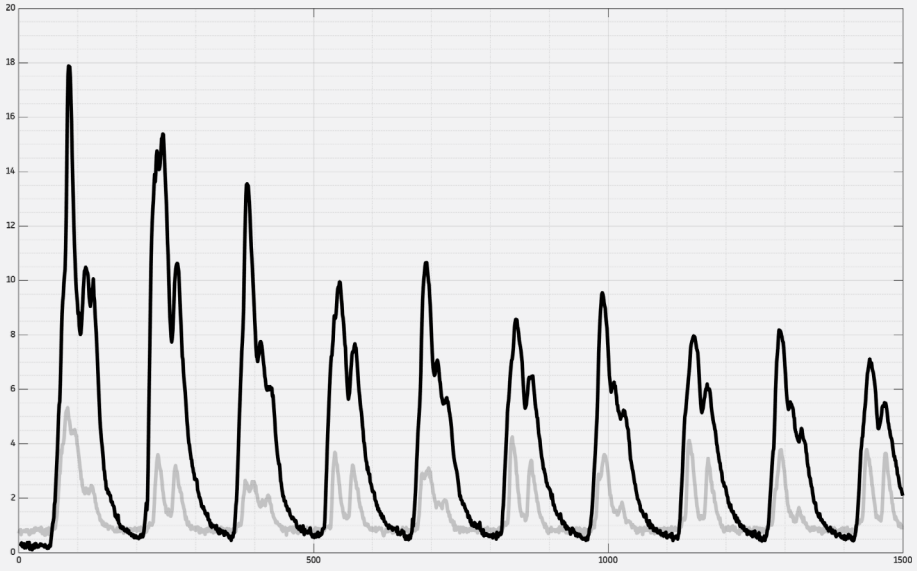


FIGURE [109]

MEAN GRAY VALUE OVER TIME
DURATION [0-1500]

120 RPM - PAUSED ALTERNATING ROTATION
90 RPM - PAUSED ALTERNATING ROTATION

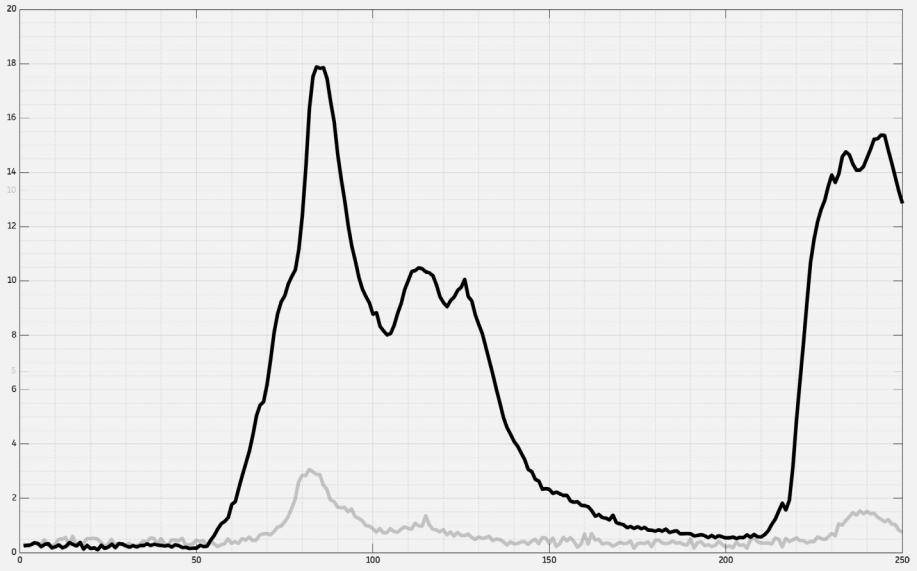


FIGURE [110]

MEAN GRAY VALUE OVER TIME
DURATION [0-250]

120 RPM - PAUSED ALTERNATING ROTATION
90 RPM - PAUSED ALTERNATING ROTATION

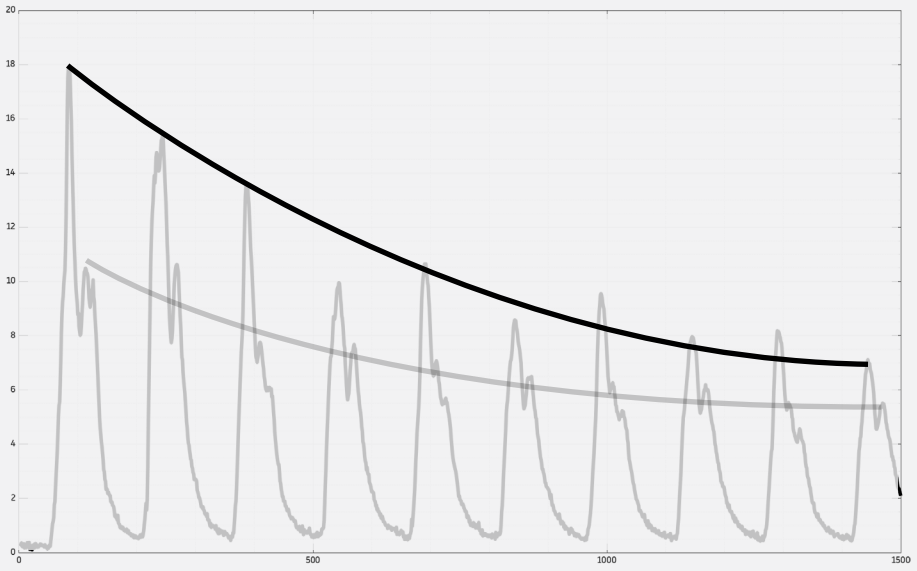


FIGURE [111]

MEAN GRAY VALUE OVER TIME
SLOPE OF THE CURVES
DURATION [0-1500]

120 RPM - PAUSED ALTERNATING ROTATION
90 RPM - PAUSED ALTERNATING ROTATION

T3

PULSE INTERVAL

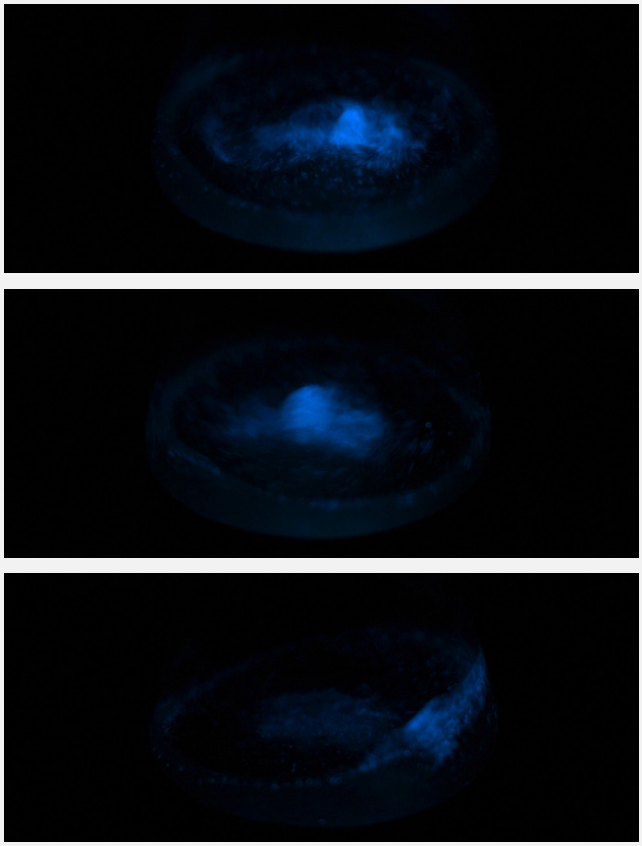
WITHOUT BASELINE AGITATION

A pulse agitation constructed of 50ms clockwise rotation, directly followed with a 50ms counter clockwise rotation. Both rotations are done at 120 RPM and repeated 5 times for each pulse. Times between each pulse are either 1, 3 or 5 minutes depending on the type of sequence.

TABLE [112]
MEAN GRAY VALUES OVER TIME
INDIVIDUAL PULSES

TEST [NO.]	MAXIMUM	TOTAL
P1-T-2	8.9	9.93×10^4
P3-T-2	8.0	9.56×10^4
P5-T-3	12.3	1.25×10^5

IMAGE [113]
TOP: T[70] - MID: T[90] - BOTTOM : T[100]
PULSE NO.3 [P1-T]



OVERVIEW OF SHAPE: GENERAL CHARACTERISTICS OF PULSE
Although there are some variances between the peak values of the different pulse interval sequences, the curves of the individual pulses appear to have comparable shapes. The general shapes of the curves, when looking at the rise and fall times, the amount of peaks and total area, appear to be fairly consistent throughout all test sequences (see table 112).

In general, the curve start to rise 5 to 10 frames after the initiation of the motor at T[50]. At this point there is a steep linear rise in the mean gray values, in most cases resulting into a sharp peak with values ranging from 5.2 to 12.3. Some of the lower intensity agitations more of a concave rise, where the slope gradually decreases over time. These agitations also tend to have less of a pronounced spike at the first peak.

For most of the pulse agitations, the mean gray value has reached its maximum at approximately T[70]. From this point on, the light intensity drops with a similarly linear steepness, until it rises again at T[90] or T[100]. In some cases there are three distinct peaks, the inital peak at T[70], a second peak at T[90] and a third peak at T[100]. While the rise and fall times of the last two peaks are similar, the third peak tends to have a slightly lower peak value (ranging from 60 to 80% the intensity of the previous peak). After the last peak (either at T[90] or T[100]), the light intensity starts to gradually fade until the curve has reached its steady state at T[180].

COMPARING THE CURVES: PATTERNS WITHIN SEQUENCES
When looking at the pulse interval sequences as a whole, the peak values appear to follow a certain pattern (see figure 106). For both the 1 and 3 minute intervals (P1-T and P3-T), the second pulses had the highest peak values (as wel as highest total mean gray values). From the second pulse onwards, each of the following pulses showed a decrease in peak values. The 5 minute interval sequence (P5-T) shows a similar pattern, but reaches its peak value at the third pulse, showing a slight delay in response.

Another clear difference between P5-T and the other interval sequences are the relatively high peak values. While P1-T and P3-T both have peak values at around 8.5, P5-T has peak values at around 12.5; an increase of about 45%.

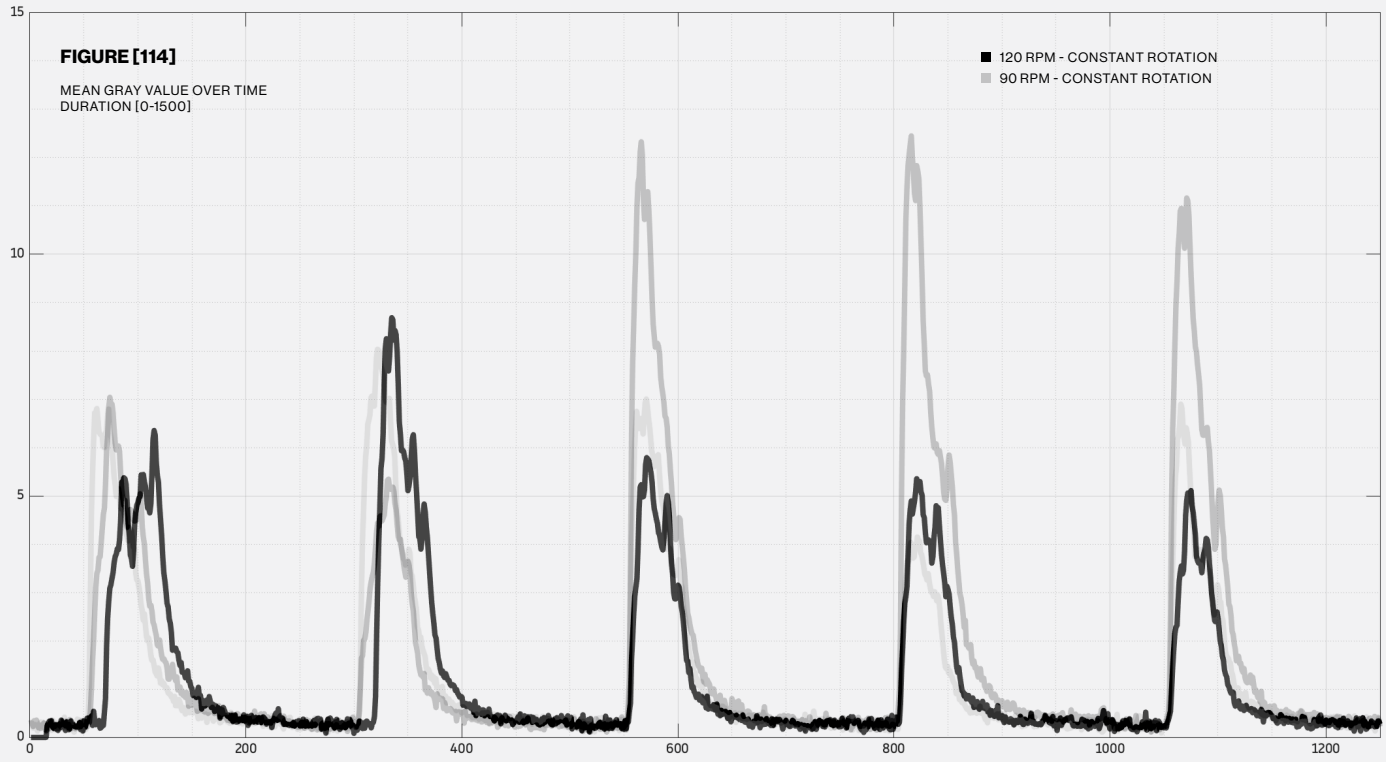


FIGURE [114]

MEAN GRAY VALUE OVER TIME
DURATION [0-1500]

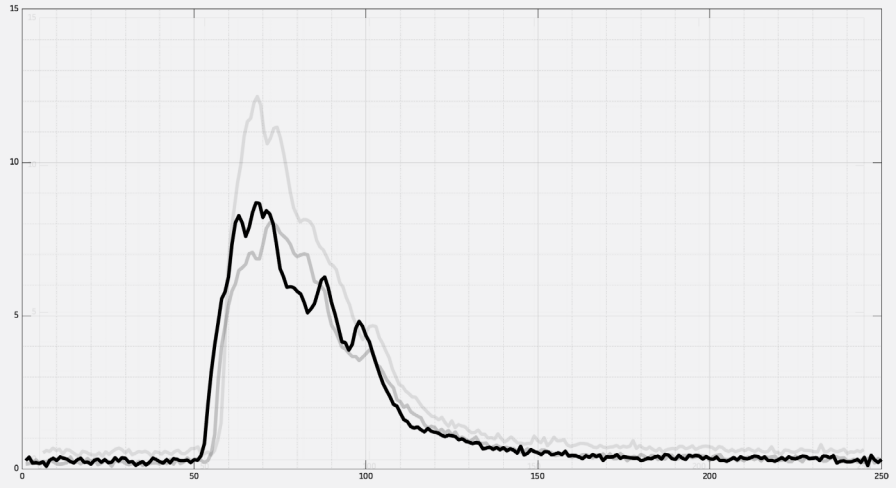


FIGURE [115]

MEAN GRAY VALUE OVER TIME
HIGHEST PEAK OF EACH SEQUENCE
DURATION [0-250]

120 RPM - CONSTANT ROTATION
90 RPM - CONSTANT ROTATION

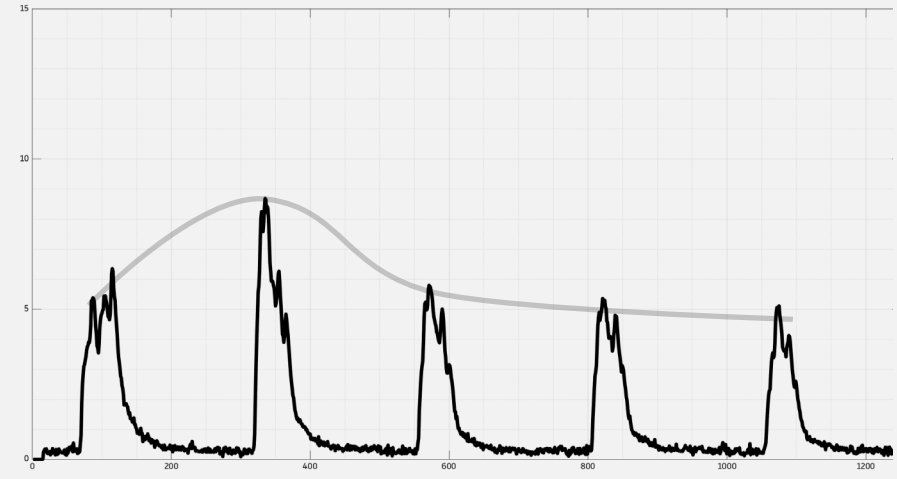


FIGURE [116]

MEAN GRAY VALUE OVER TIME
PATTERN FOLLOWING PEAKS
DURATION [0-1500]

120 RPM - CONSTANT ROTATION
90 RPM - CONSTANT ROTATION

T3

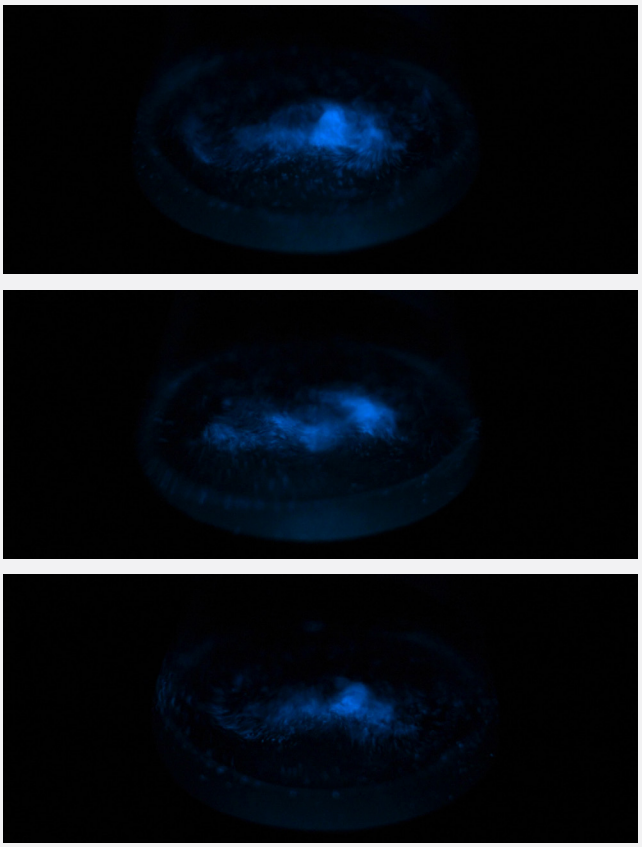
PULSE INTERVAL

WITH BASELINE AGITATION

TABLE [117]
MEAN GRAY VALUES OVER TIME
INDIVIDUAL PULSES

TEST [NO.]	MAXIMUM	TOTAL
P1-T-2	8.9	9.93×10^4
P1-BL-2	3.2	4.56×10^4
P1-BD-2	2.0	3.53×10^4

IMAGE [118]
TOP: P1-T, MID: P1-BL, BOTTOM : P1-BD
FRAME [70], PULSE [2]



ADDITION OF BASELINE AGITATION

In order to determine wether the clumping of the cells had influence on the irregular results of the pulse interval tests, the 1 min interval sequence was repeated with a baseline shake before. As the clumping of the cells is assumed to be the most obvious reason of the irregularities in the results, additional tests have been done.

For the first test, a baseline agitation has been done before the pulse agitation sequence was initiated. This sequence is referred to P1-BD. After the baseline agitation there was a 5 minute pause, just as with the rotational test sequences. As the research suggests handling the algae in the light will not disturb or agitate them, as of their photoinhibition mechanism (see Chapter 3), there was also one version where the baseline shake was performed in the light [called P1-BL]. The previous 1 minute pulse agitation sequence (P1-T) is used as a reference, as this showed the most consistent results, represented the agitation the best, this was chosen as the interval to do the baseline tests with.

COMPARING THE DIFFERENT PULSES

For both pulse sequences with the baseline shakes (P1-BL and P1-BD), the mean gray values remain similar throughout the whole test. The only notable differences are seen in the first pulse, where P1-BL has a slightly higher peak intensity compared to that of P1-BD. The following pulses all appear to result in similarly shaped curves: starting with linear rise at T[50] that leads into a peak at approximately T[70]. Even though all sequences have their first peak in intensity at T[70], there do appear to be some differences between the rise times of each curve. While P1-T has a noticeably quick rise with a slope of 85 degrees, P1-BL shows a slightly more gentle rise with a slope of 65 degrees. The rise of P1-BD has become even more gentle, resulting in a slope of approximately 45 degrees.

From T[70] onward, the curves of each pulse agitation sequence begin to differ even more. Where P1-T shows three distinct spikes in intensity, both P1-BL and P1-BD have only one apparent peak. After the initial peak, the curve of P1-BL stars to slowly decrease in a linear manner, going from a value of 3.2 at T[70] to a value of 0.5 at T[115]. The curve of P1-BD, however, continues to stay at its peak value of 2.0 until T[100]. At this point, the mean gray value starts to decrease in a similar manner as with P1-BL. In the end, all pulses appear to reach their steady state at the same time (at approximately T[180]).

COMPARING PEAK AND TOTAL VALUES

The most noticeable difference are the substantial decreases in the total and maximum mean gray values, especially for the pulse sequence with the baseline agitation performed in the dark (see table 117). Where P1-T had a peak value of 8.9, P1-BD only reaches a peak value of 3.2; a decrease in intensity of roughly 80% When comparing the total mean gray values over time, there appears to be a similar trend. The sequence with the baseline agitation performed in the light shows slightly higher peak and total values, when compared to P1-BD. However, its peak value is still at only 65% of that of P1-T.

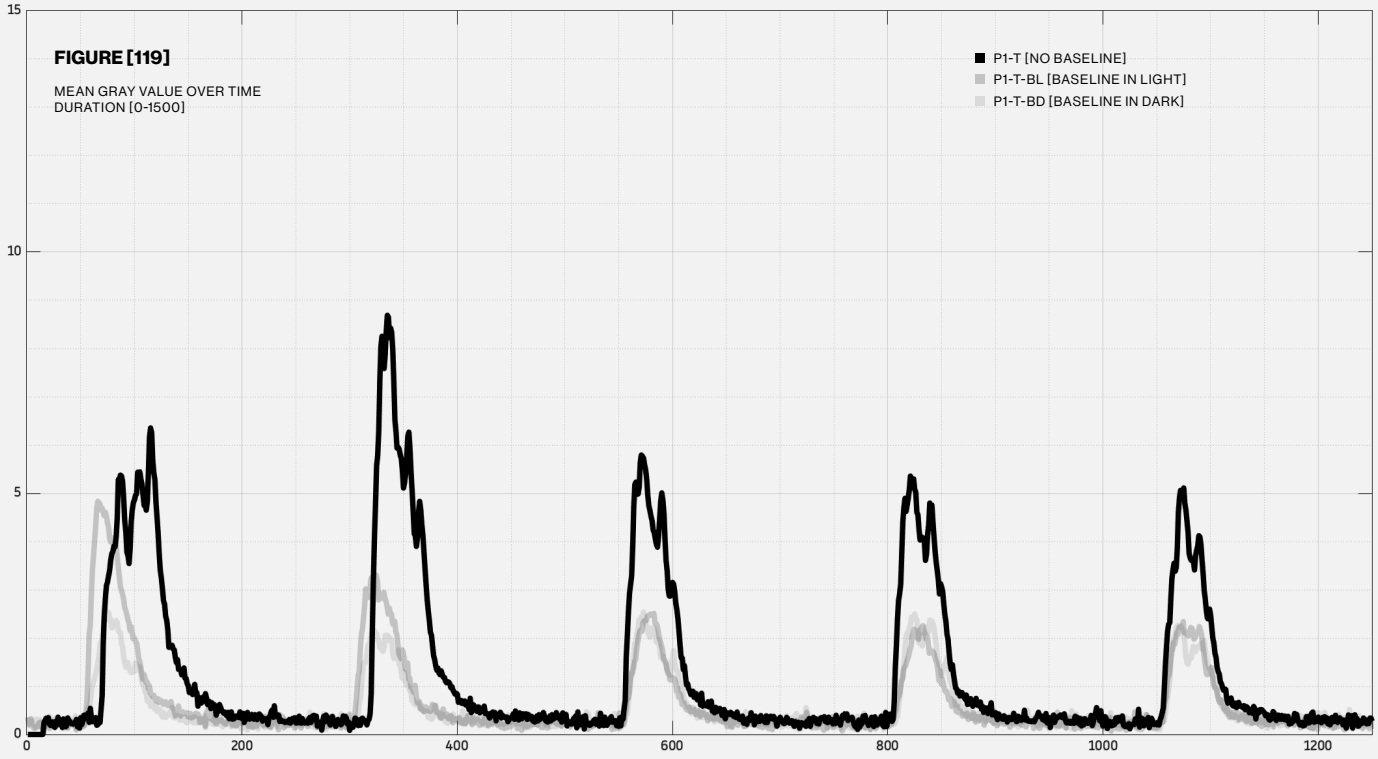


FIGURE [119]

MEAN GRAY VALUE OVER TIME
DURATION [0-1500]

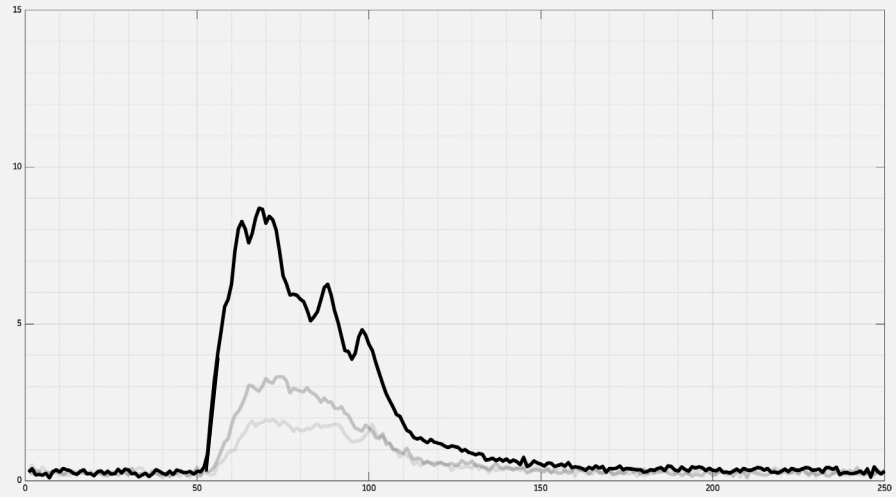


FIGURE [120]

MEAN GRAY VALUE OVER TIME
COMPARISION OF 2ND PEAKS
DURATION [0-250]

P1-T [NO BASELINE]
P1-T-BL [BASELINE IN LIGHT]
P1-T-BD [BASELINE IN DARK]

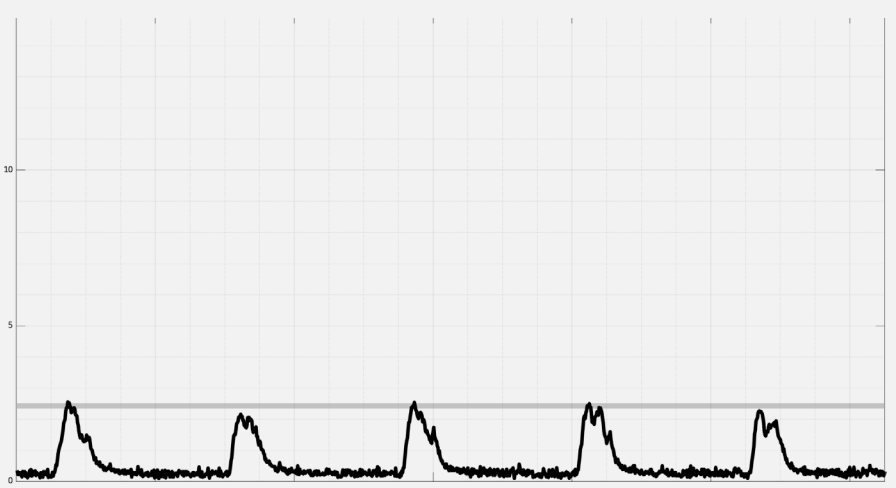


FIGURE [121]

MEAN GRAY VALUE OVER TIME
PATTERN FOLLOWING PEAKS
DURATION [0-1500]

P1-T-BD [BASELINE IN DARK]
PEAK REGRESSION

T4

SINGLE ROTATION

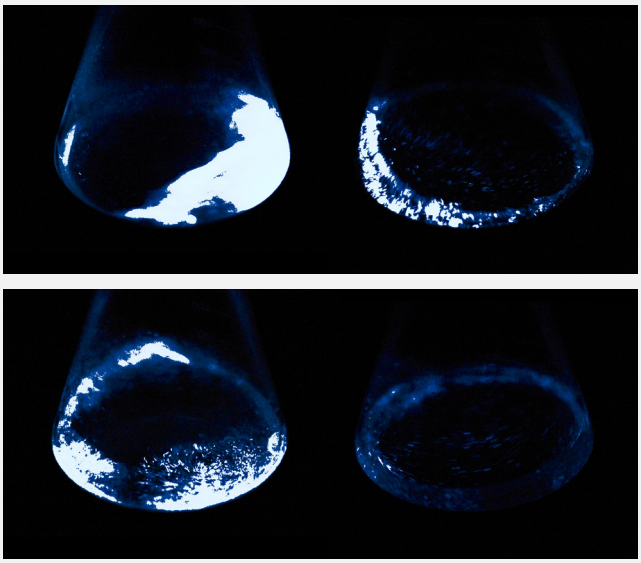
DIFFERENCE IN SPEED

A clockwise rotational motion for 1000ms, performed at either 90 or 120 RPM.

TABLE [122]
MEAN GRAY VALUES OVER TIME
INDIVIDUAL PULSES

TEST [NO.]	PEAK	TOTAL
S1-T-A	520	5.9×10^5
S2-T-A	200	3.9×10^5
S1-T-R	480	10.9×10^5
S2-T-R	760	15.5×10^5

IMAGE [123]
COMPARISION OF THRESHOLDS
TOP: FRAME [45], BOTTOM: FRAME [60]



OVERVIEW OF SHAPE: 'ABSOLUTE' NUMBER OF BLOBS

The most noticeable similarity between both the graphs of S1-T and S2-T is the jagged appearance and the large number of spikes. The single rotation at 120 RPM (referred to as S1-T) shows a gradual increase in the amount of blobs starting from T[25], until it reaches its maximum amount of 520 at T[35]. At this point, most of the dots of light start to merg together, resulting in a decrease in the amount of blobs. Around T[45], only a few milliseconds after the glass has stopped rotating, the amount of blobs start to increase once again; reaching a second peak of 340 blobs at T[60]. From this point, the amount of blobs starts to gradually decrease with a similar slope until the curve has reached its steady state at T[70]. Note that there still are some small spikes in the curve from this point on.

The curve of the single rotation at 90 RPM (referred to as S2-T) also starts rising at T[25], but has a much more gentle slope with an increased amount of spikes. At T[50] the curve of S2-T has reached its peak value of 200. From this point on, the curve starts to gradually decrease until it reaches its steady state at T[65]. The fall of S2-T from its peak appears to have slightly steeper slope than its rise. Once S2-T has reached its steady state there still are some noticeable peaks in the amount of blobs every 10 frames.

OVERVIEW OF SHAPE: 'RELATIVE' NUMBER OF BLOBS

In order to get a better idea of the relation between the rotation speed and the amount of blobs over time, another analysis was performed. As it is difficult to find a threshold value that is able to recognize lower intensity blobs, but still is able to separate higher intensity blobs, the threshold value was linked to the mean gray value. In this case, an increase in the mean gray value would lead to an increase in the threshold value. In order to increase the sensitivity of the threshold, a lower baseline threshold value was used (0.01 instead of 0.05).

When comparing the curves that depict the amount of blobs over time, relative to the mean gray value (S1-T-R and S2-T-R) with the initial curves showing the 'absolute' values, there are some noticeable differences. First, the increase in sensitivity during the lower light intensities (beginning and end of sequence) results in a higher blob count during these phases. While the curves of S1-T-R and S1-T-A are almost identical and both show the characteristic dip in the amount of blobs at T[45], the curve of S2-T-R has changed more noticeably. When comparing S2-T-R to S2-T-A , we see a great increase in the amount of blobs over time. The most significant difference appears to be the large initial spike of S2-T-R at T[35].

COMPARING PEAK AND TOTAL VALUES

The difference between the different threshold levels become even more apparent when looking at the peak and total values. While the peak values of S1-T remain similar, there is a big difference between the peak values of S2-T-A and S2-T-R. Where S2-T-A has a peak value of only 200, S2-T-R has a peak value of 760; an increase of almost 400%.

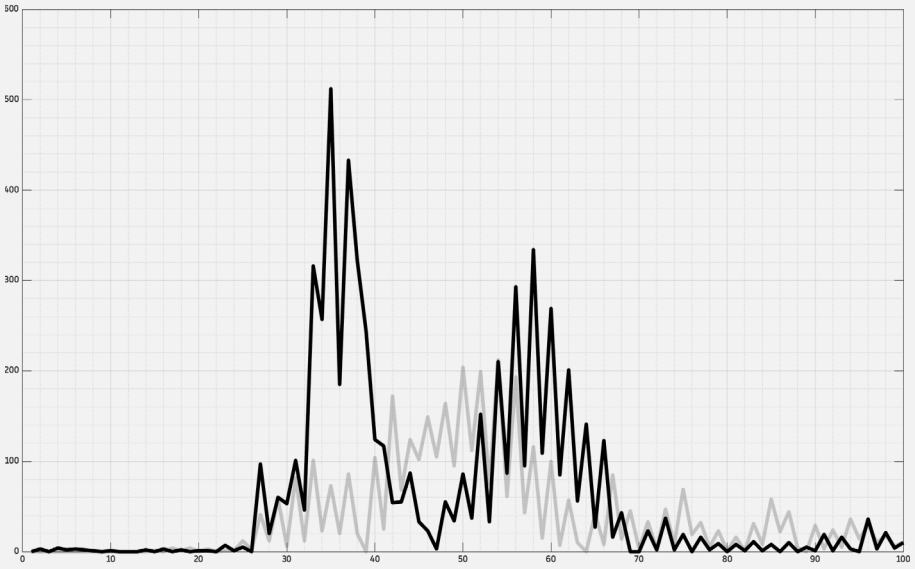


FIGURE [124]

AMOUNT OF BLOBS OVER TIME
DURATION [0-100]

■ 120 RPM - SINGLE ROTATION
■ 90 RPM - SINGLE ROTATION

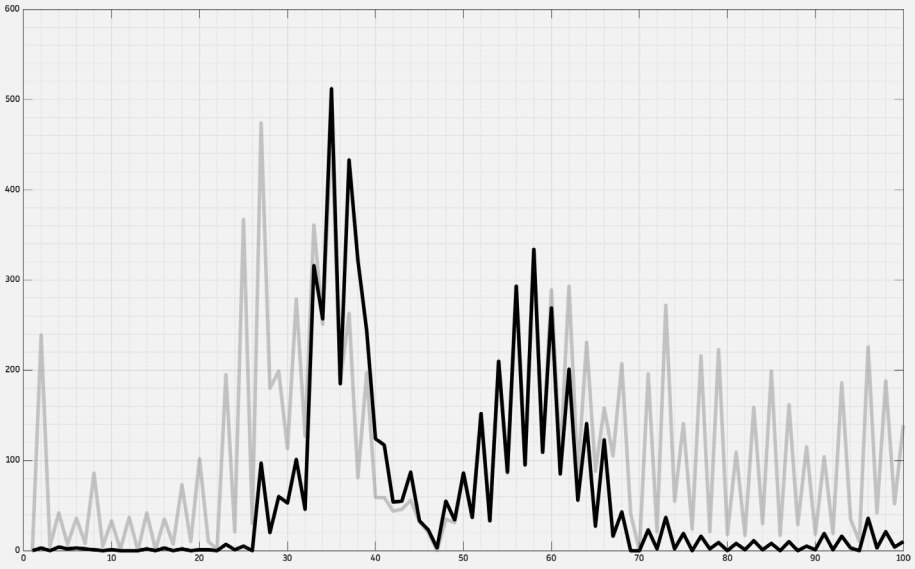


FIGURE [125]

AMOUNT OF BLOBS OVER TIME
ABSOLUTE / RELATIVE
DURATION [0-100]

■ 120 RPM - ABSOLUTE
■ 120 RPM - RELATIVE

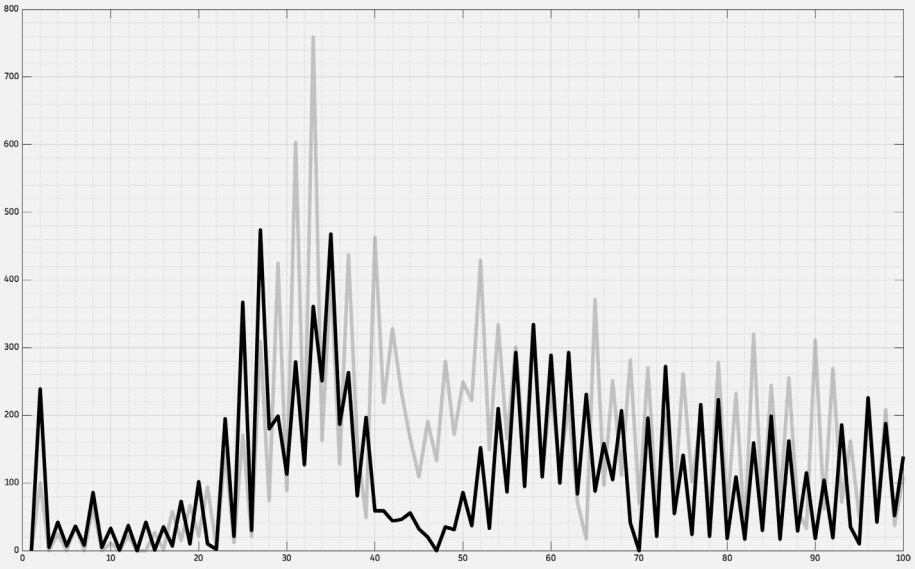


FIGURE [126]

AMOUNT OF BLOBS OVER TIME
RELATIVE TO MEAN GRAY VALUE
DURATION [0-100]

■ 120 RPM - SINGLE ROTATION
■ 90 RPM - SINGLE ROTATION

T4

SINGLE ROTATION

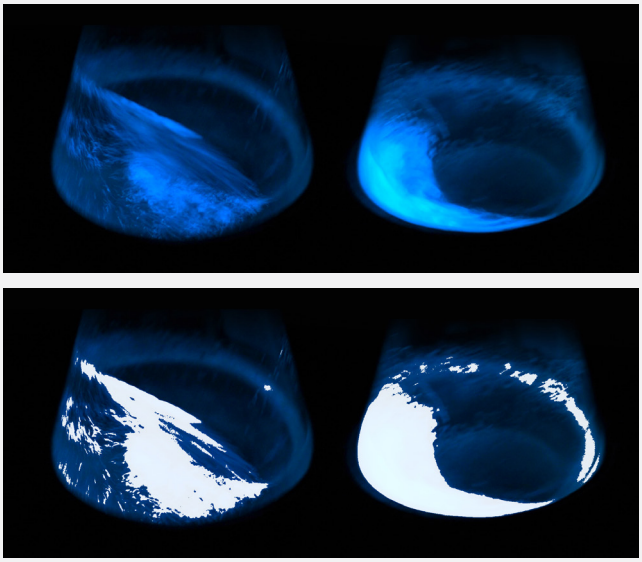
DIFFERENCE IN VOLUME

A clockwise rotational motion for 1000ms, performed at a speed of 120 RPM with two different batches of algae.

TABLE [127]
MEAN GRAY VALUES OVER TIME
INDIVIDUAL PULSES

TEST [NO.]	PEAK	TOTAL
S3-T-A	240	3.4×10^5
S4-T-A	180	3.9×10^5
S3-T-R	480	4.5×10^5
S4-T-R	700	9.8×10^5

IMAGE [128]
COMPARISION OF THRESHOLDS
TOP: FRAME [45], BOTTOM: FRAME [60]



OVERVIEW OF SHAPE: 'ABSOLUTE' NUMBER OF BLOBS
Similar to the graphs of S1-T and S2-T, the graphs of both S3-T and S4-T have a the large number of spikes in the amount of blobs. The single rotation of the batch without any added medium (referred to as S3-T or the 100ml variant) shows a linear increase in the amount of blobs starting from T[15], until it reaches its peak amount of 240 at T[30]. Note that there is a slight drop at T[20]. From T[30] onwards, many of the blobs of light appear to blend together, resulting in a strong decrease in the amount of blobs. Around T[35], the curve has stopped declining and stays around a value of 30 for a number of frames. At T[45] the curve starts to rise again, leading into a second peak of 150 blobs at T[50]. From this point, the amount of blobs starts decrease with a similar slope until the curve has reached a value of 30 at T[60]. From T[60] till T[90] there still appear to be some small spikes, but the average value continues to stay around 30.

The curve of the single rotation with the added medium (referred to as S4-T or the 150ml variant) also starts rising at T[15], but already reaches its first peak value of 180 at T[20]. At this point the curve drops to a value of 50 at T[25], before rising once again to a value of 150. From this point on, the curve starts to fall again until it reaches a value of 30 at T[35]. From T[35] till T[90], the curve stays around a value of 30. Note that during this period there still are some noticeable spikes in the amount of blobs. The most significant spikes are visible at T[45], T[55] and T[80]. These spikes all have peak values ranging between 90 and 110.

OVERVIEW OF SHAPE: 'RELATIVE' NUMBER OF BLOBS
When comparing the curves of S3-T-R and S4-T-R, where the amount of blobs over time is relative to the mean gray value, there do not appear to be that many differences. The most noticeable difference is seen in the shapes and sizes of the first peaks. Where S3-T-R has a high initial peak with an extremely steep rise that reaches values of almost 700 at T[20], S4-T-R reaches its peak value of 480 at T[30]. Note that S4-T-R also does have a small peak at T[20]; reaching a value of 260. At T[35] both curves have fallen down to their baseline values. From this point on, both curves continue to show some spikes from time to time. Note that during this phase the peak values of S4-T-R slowly start to increase over time, usually being about twice the size of the peak values of S3-T-R.

COMPARING PEAK AND TOTAL VALUES
While the peak and total values of both S3-T and S4-T are similar and show no significant differences, the values of S3-T-R and S4-T-R show some clear differences. Although the shapes of both curves appeared to be fairly similar, there is a big difference in the total amount of blobs over time, as S4-T-R has more than twice the total amount.

The difference in peak values appear to be less extreme.Where S4-T-R has a peak value of 700, S3-T-R has a peak value of 480; a decrease of just over 30%.

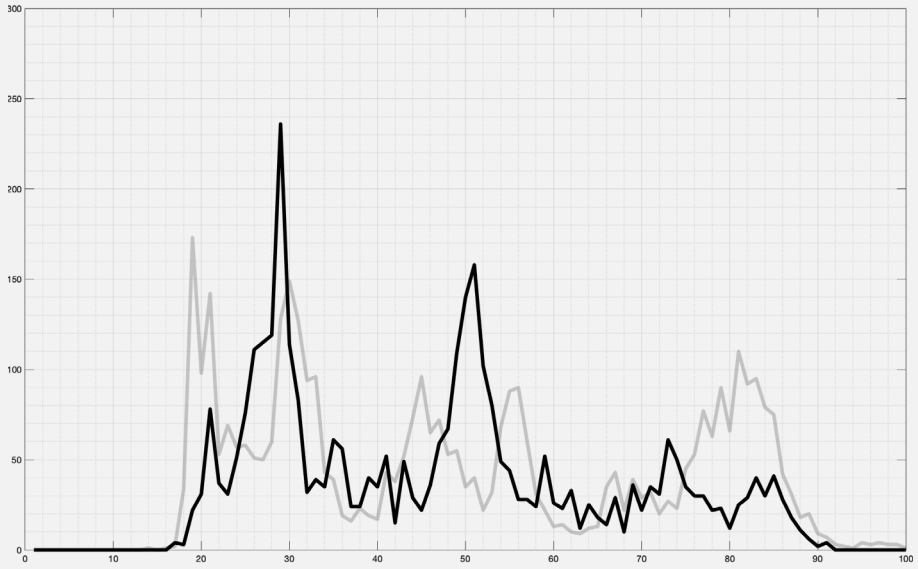


FIGURE [129]

AMOUNT OF BLOBS OVER TIME
DURATION [0-100]

■ 100 ML - SINGLE ROTATION
■ 150 ML - SINGLE ROTATION

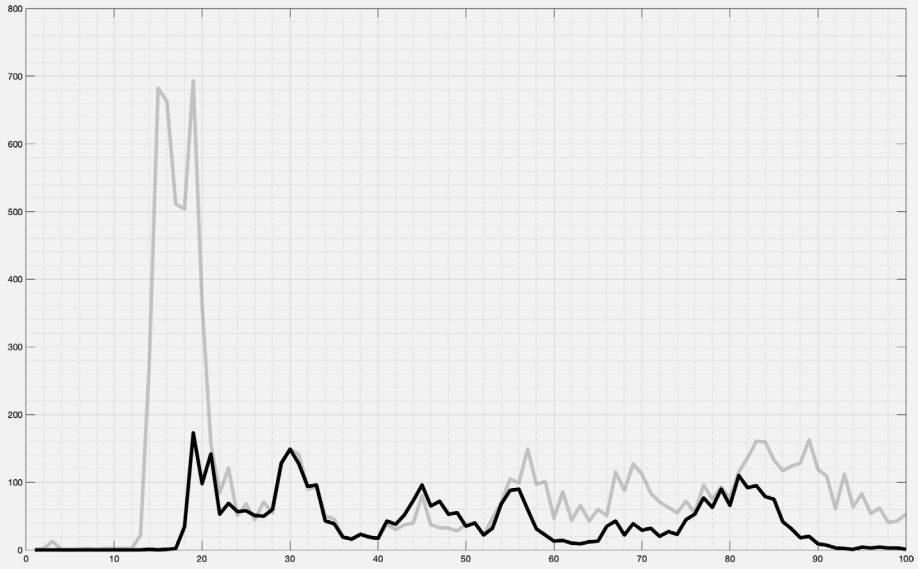


FIGURE [130]

AMOUNT OF BLOBS OVER TIME
ABSOLUTE / RELATIVE
DURATION [0-100]

■ 150 ML - ABSOLUTE
■ 150 ML - RELATIVE

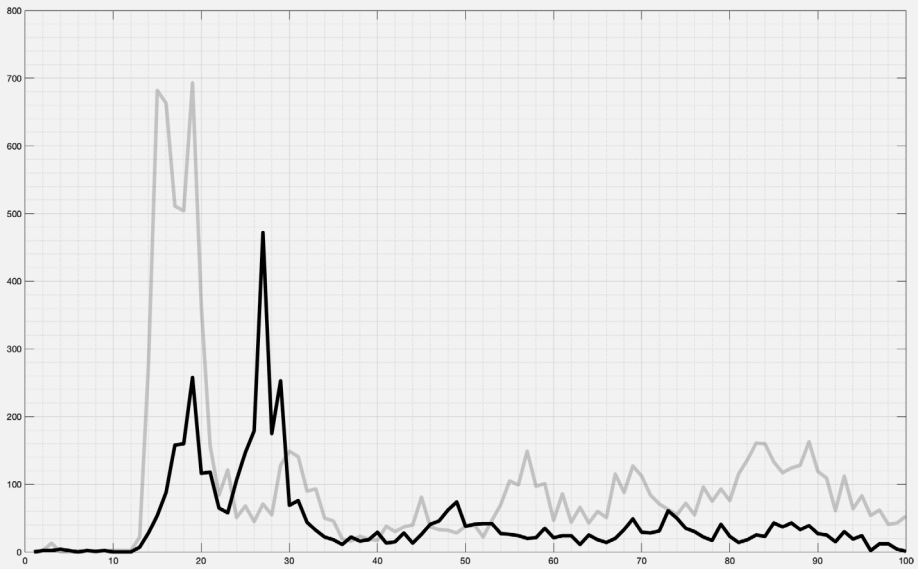


FIGURE [131]

AMOUNT OF BLOBS OVER TIME
RELATIVE TO MEAN GRAY VALUE
DURATION [0-100]

■ 100 ML - SINGLE ROTATION
■ 150 ML - SINGLE ROTATION

T5

VARIATION IN ORDER

WITHOUT BASELINE AGITATION

A clockwise rotational motion for 1000ms with a speed of either 90 or 120 RPM, a 2000ms pause, followed by a pulse agitation (similar to the pulse interval sequences).

OVERVIEW OF SHAPE: V1-T [120 RPM]
At a first glance, there does not appear to be a distinct pattern in both the curves of V1-T and V2-T, especially when looking at the peaks created by the rotational movement. However, there does appear to be a more clear pattern in the peaks created by the pulse agitation.

The first pulse of the sequence of V1-T (starting at T[50]) reaches a peak value of 6.5 at T[60]. This pulse is followed with a rotational agitation at T[200], which reaches a peak value of 9.8. The following pulse agitation at T[300] has a significantly lower peak value, only reaching a maximum value of 2.5. Each of the following pulse agitations shows a slight decrease in peak intensity, going from a value of 2.3 during the third pulse at T[600]to a value of 1.5 during the seventh pulse at T[1500]. From this point on, each of the following pulse agitations does not result in any visible changes in light intensity. However, at this point each of the rotational agitations still result in a clearly visible response and high peak values; reaching a value of 6.8 at T[1600].

The peak values of the rotational agitation of V1-T appear to behave in a similar manner as the pulse interval sequence P1-T; having the maximum peak value during the second agitation. From the second rotational agitation onwards, the peak values appear to gradually decrease for each of the following rotations. However, every once in a while there are some outliers with significantly lower peak values (e.g. the rotation at T[900], which only reaches a peak value of 2.6)

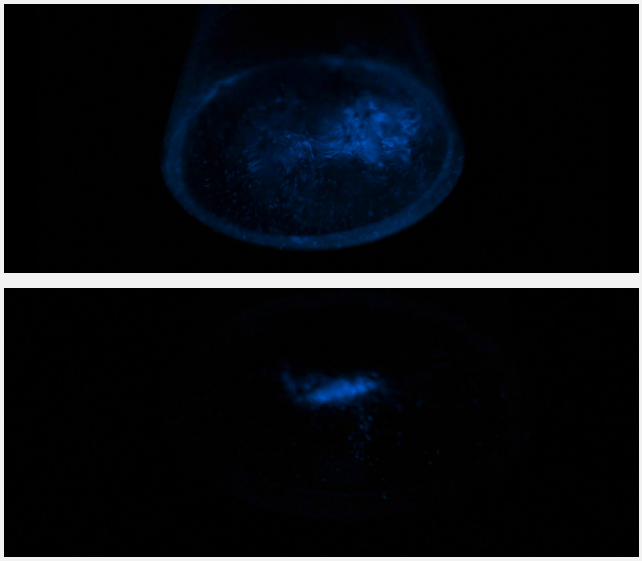
OVERVIEW OF SHAPE: V2-T [90 RPM]
While there appears to be no distinct patter in the peak values of the rotational agitations of V2-T, as the values tend to fluctuate quite a bit between rotations. Where the rotational agitation at T[3100] only results in a peak value of 3.2, the following rotation results in a peak value of 7.8; the highest peak value of the whole sequence. Another clear difference between V2-T and V1-T, is the lack of response to the pulse agitation. While the first pulse does manage to result in a slight increase in mean gray value (reaching a maximum of 1.2 at T[70]), each of the following pulses does not results in a change in light intensity.

NOTE: as the pulse agitation of V2-T was also performed at 90 RPM this is most likely the cause of the low values.

TABLE [132]
MEAN GRAY VALUES OVER TIME
CONSTANT ROTATION

FRAME [NO.]	V1-T [120]	V2-T [90]
T [300]	1.76×10^5	7.88×10^4
T [1500]	3.35×10^6	1.98×10^6
T [6000]	3.99×10^7	3.12×10^7

IMAGE [133]
TOP [V1-T] - BOTTOM [V2-T]
FRAME NUMBER [65]



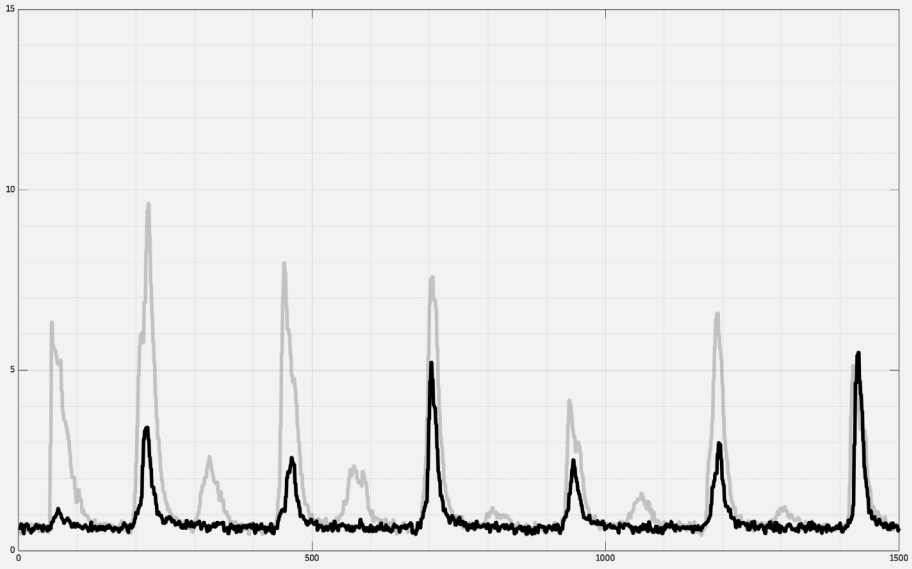


FIGURE [134]

MEAN GRAY VALUE OVER TIME
COMPARISION V1-T AND V2-T
DURATION [0-1500]

V2-T
V1-T

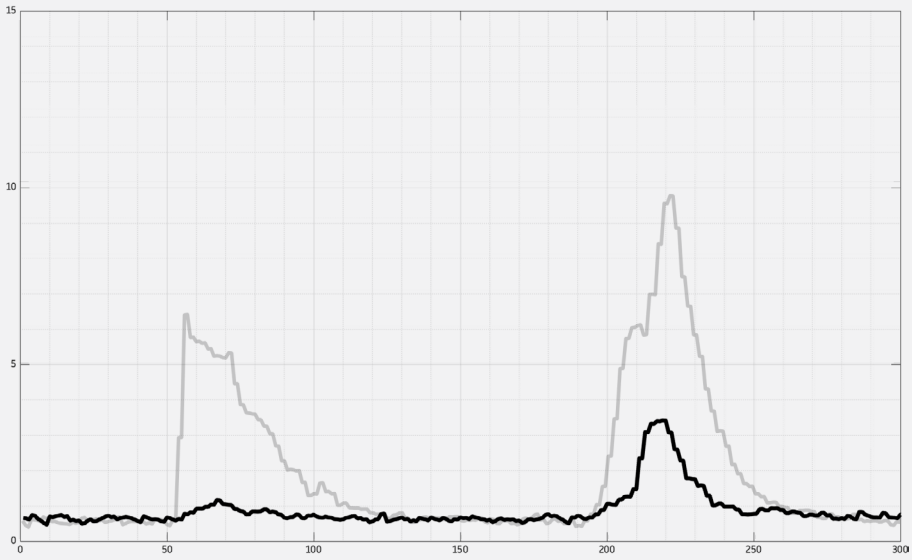


FIGURE [135]

MEAN GRAY VALUE OVER TIME
COMPARISION V1-T AND V2-T
DURATION [0-300]

V2-T
V1-T

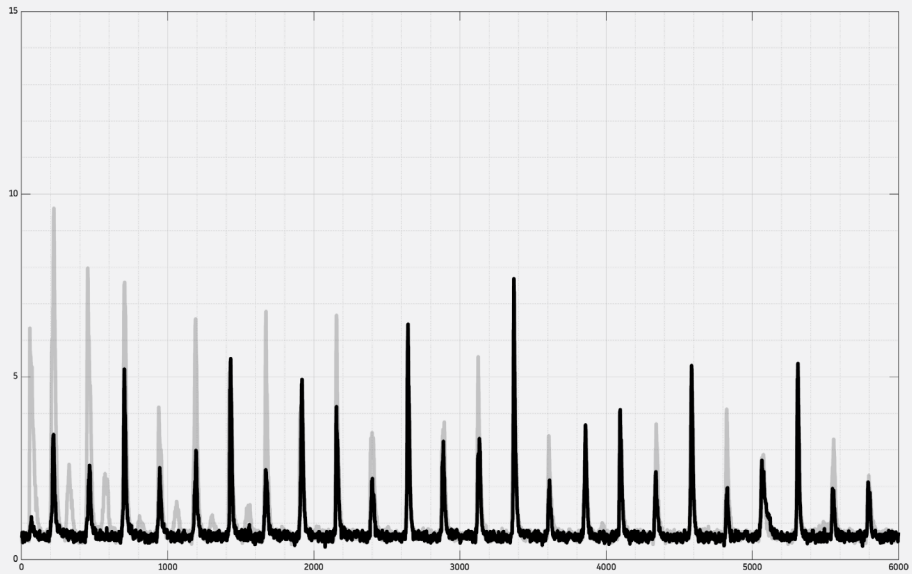


FIGURE [136]

MEAN GRAY VALUE OVER TIME
COMPARISION V1-T AND V2-T
DURATION [0-6000]

V2-T
V1-T

5.4

TEMPORAL FORM

Within this analysis chapter we will look into the behaviour of the light over time; referred to as the temporal form of the light. The main focus will be on the changes in luminance over time, the relation between input and ouput, as wel as the sensitivity to mechanical stimulation.

INTENSITY OVER TIME

When looking at the differences in luminance over time for rotational agitation sequences, the most important factors appear to be the rotational speed, the duty cycle of agitation and the direction of the rotation.

Each factor and its effects will be evaluated with the goal to create general guidelines; providing useful insights in the behaviour of the light with regards to the described factor.

FACTOR: ROTATIONAL SPEED
A rotational agitation at 90 RPM appears to agitate less of the algae within a culture, in comparison to its 120 RPM variant; resulting in significantly lower peak intensities and an overal decrease in total light output over time. Peak values of the 90 RPM rotational sequences generally tend to be 60 to 80% lower than those of the rotational sequences performed at 120 RPM. Differences in the total light output over time for both variants appear to be slightly less significant, as the values of the 90 RPM sequence are generally about 40 to 50% lower.

While rotational sequences performed at 120 RPM tend to have a steep decay (with the exception of sequence N6: the paused alternating rotation), rotational agitations at 90 RPM appear to have a much more gradual decay; resulting in a more (visually) consistent light output over time.

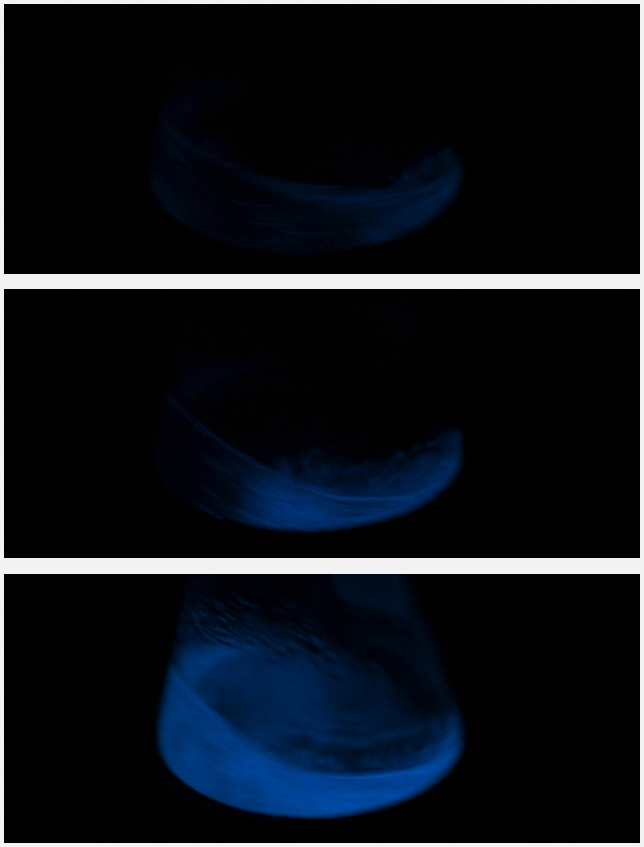
By increasing the rotational speed, one can increase the peak and total amount of light produced over time. However, a faster rotational speed will also result in a more rapid and noticeably drop in intensity over time.

FACTOR: DUTY CYCLE
The addition of a paused interval to a rotational sequence of 120 RPM has shown to slightly increase the total light output over time with approximately 20%. For the rotational sequence at 90 RPM, however, the addition of a paused interval appeared to have no significant effect on the total light output over time.

The most noticeable difference of the addition of a paused interval is the greatly extended decay in light output over time. The addition of a 2 second interval after each rotation makes it possible to extend the period of 'useable' light (in this case a response with a mean gray value of 2.0) for up to 10 times. However, it is important to note that while the light output over time will be stretched, the overall light intensity will fluctuate more noticeably, especially as there are periods of darkness between each agitation.

By adding a paused interval to a rotational sequence, one can extend, as wel as slightly increase the total light output over time. However, the pause in rotational agitation does lead to a less visually consistent light output over time.

IMAGE [137]
TOP: R1-T, MID: R3-T, BOTTOM: R5-T
FRAME [1000]



FACTOR: DIRECTION

One of the most important factors that affect the total light output over time appears to be the direction of the agitation. By alternating the direction of rotation during a paused interval rotation sequence, it is possible to greatly increase the light output over time (more than 200% of a constant rotation and one-way paused rotation at the same speed].

This significant boost in light intensity appears to be caused by an increase in shear force resulting from the sudden change of direction. As the liquid with the algae continues to swirl around for a few seconds after to movement of the shaker has stopped, the counter rotation goes against the motion of the liquid; resulting in an increased shear force acting on the algae, triggering a larger portion of the culture, which in turn results in a bright spike of light.

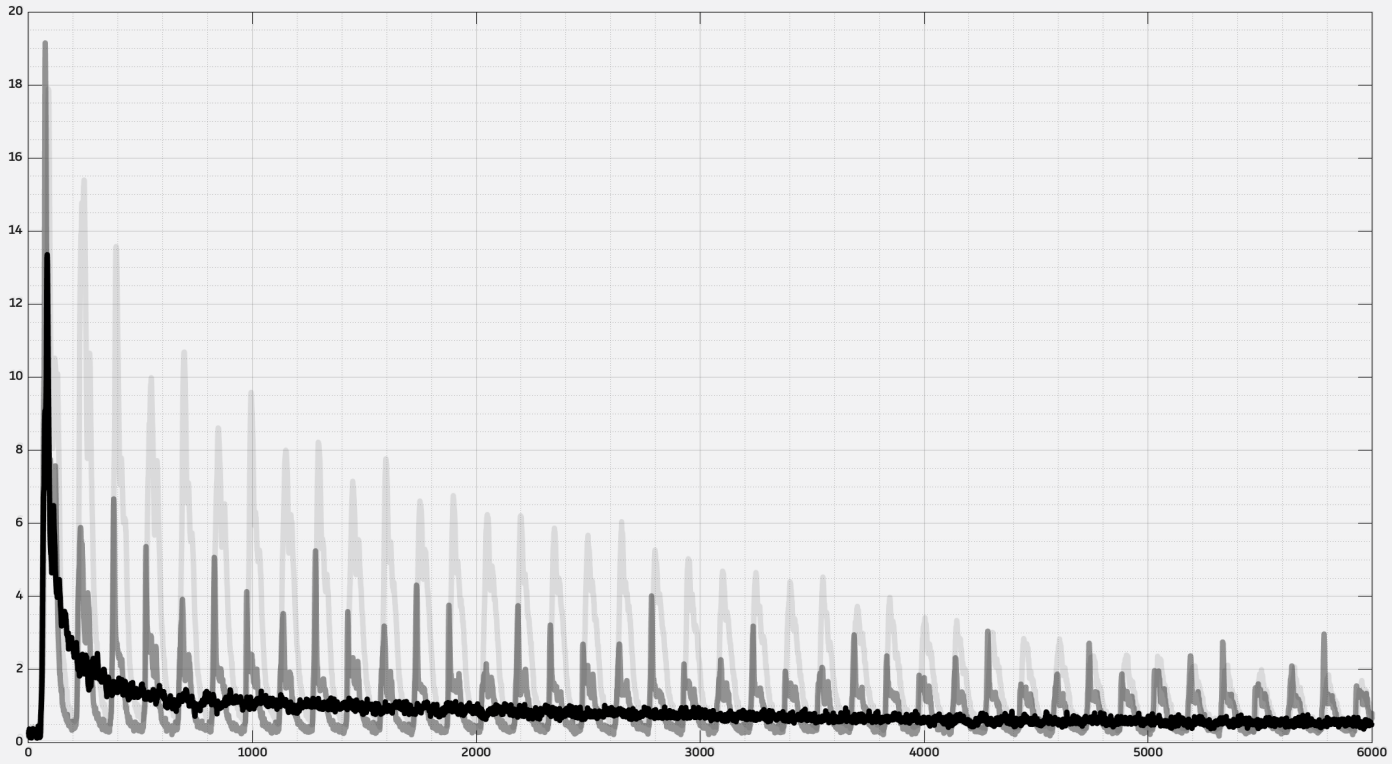
Alternating the direction of rotation also leads to a much more gradual decay of light intensity and tends to result in less variances between the peak values of each agitation. Similarly as with the one-way paused agitations, a paused alternating rotation will extend the duration of light (till the point no light is visible anymore) about 10 fold compared to the duration of a regular constant rotation at the same speed. However, it is important to note that during this period (from T[500] to T[5000])the peak intensities of the alternating rotation sequence are generally about 50% higher than those of the regular one-way paused rotation sequence.

By changing the direction during a paused interval rotation, one can significantly increase the total light output over time, as wel as create a more consistent and gradually fading light.

TABLE [138]
ROTATIONAL SEQUENCE COMPARISON
PEAK AND TOTAL MEAN GRAY VALUE OVER TIME

TEST [NO.]	MAX. VALUE	TOTAL VALUE
N1-T-6000	13.5	3.24×10^7
N2-T-6000	3.5	1.40×10^7
N3-T-6000	19.2	3.77×10^7
N4-T-6000	3.1	1.18×10^7
N5-T-6000	17.9	7.79×10^7
N6-T-6000	5.4	4.38×10^7

FIGURE [139]
ROTATIONAL SEQUENCE COMPARISON
DURATION [0-6000]



5.4

INPUT - OUTPUT RELATION

FIGURE [140]
INPUT VS MECHANICAL DELAY

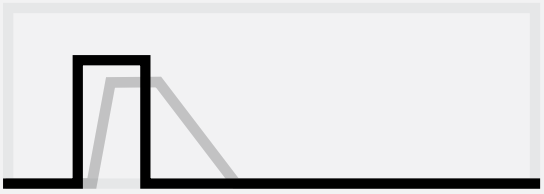
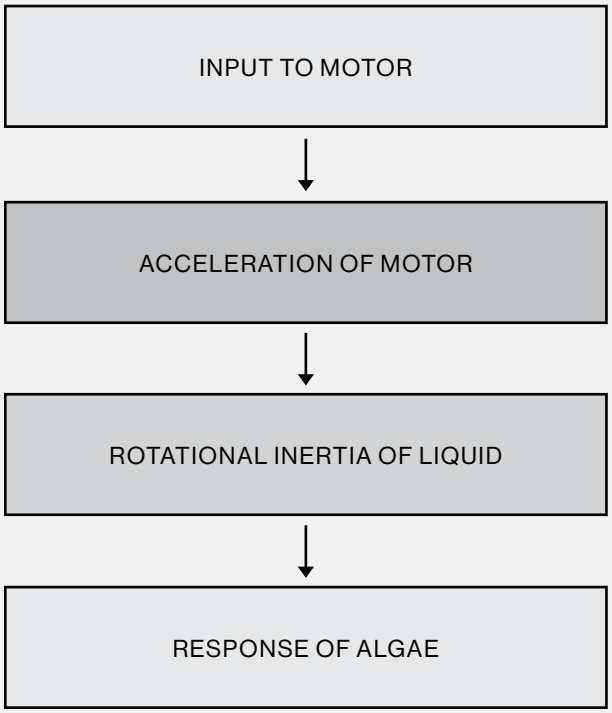


FIGURE [141]
FACTORS INFLUENCING RESPONSE TIME



While it is one could see the relation between input and output as the overall behaviour of the light in regards to stimulation, this chapter will mainly focus on determining the factors that influence the shapes of the response curves; showing the changes in intensity over time. Therefore, the focus will be on the changes in the descriptive properties of the curves, such as difference in rise and fall times or slope angles.

FACTOR : AGITATIONAL MOVEMENT AND SPEED
One of the most interesting aspects of the behaviour of the light produced by the bioluminescent algae, is its tendency to behave in a random manner. Even for sequences with the exact same agitation settings, the response in light output can differ quite noticeably. These variances in both luminance and general behaviour or kinetics of the light appear to be the more common for rotational sequences performed at 90RPM. While for the majority of the 120 RPM rotational agitations distinctive trends can be seen across the peak intensities, the response curves of the 90 RPM variants generally shown less distinct patterns and tend to have lots of irregular spikes in luminance.

Variances in the peak values appear to be the result of minor irregularities within each the motion. As the liquid continuous to swirl around after each rotation, tiny offsets (differences in milliseconds) in the timing of the following rotation can result in noticeable differences in peak intensities. In some cases, the rotation will end a bit earlier, counteracting the motion of the liquid, leading to an increase in shear force and a spike in light intensity. In other cases, the rotation ends just a bit later and falls in sync with the movement of the liquid, resulting in a smoother stop and thus a less pronounced spike in luminance.

FACTOR: ACCELERATION SPEED OF MOTION / ROTATIONAL INERTIA
The initial delay in response to mechanical stimulation appears to be influenced by a number of factors (see figure 141).

First, the micro-controller (in this case an Arduino UNO) will send an input to the DC Motor of the shaker. Next, the motor will start to accelerate until it reaches the desired speed. At a certain point, the rotational speed of the motor will result a rotational force larger that exceeds the moment inertia of the liquid; causing the liquid to start moving, in turn creating a shear force that triggers the bioluminescent process within the cell of the algae.

As research of Latz et al.(2008) has shown that the biological process of light production within the cells of P. Fusiformis (from detecting the stimulus to the emission of light) only takes 20 milliseconds and the research of Widder et al.(2018), determined that the rise time of the light itself will vary between 10 to 150ms (depending on the amount of previous stimulation), the combined response time of the algae will be ranging from 30 to 170ms (approximately 1 to 3 frames at 50fps). Therefore, it is safe to say that the response time is mainly influenced by the acceleration of the motion and the moment of inertia, which is directly related to the total volume) of the liquid. Where an increase in acceleration will lead to a shorter response time (as seen with test results [T2]), an increase in volume will result in a delay in response (as seen with test results [T4]).

By either increasing the acceleration of the movement or decreasing the volume of the liquid, one can decrease the delay in response time (up to a certain extent).

FACTOR: STATE OF ALGAE CULTURE

In order to get a better view on the changes in response over time, three single rotations from N5-T at T[0], T[1500] and T[3000] have been isolated and compared. (see figure 143).

While there are visible delays in response times for the later rotations of up to 20 frames, the moment at which the light reaches its first peak appears to only slightly shift over time; having a delay of about 5 to 10 frames (see table 142).

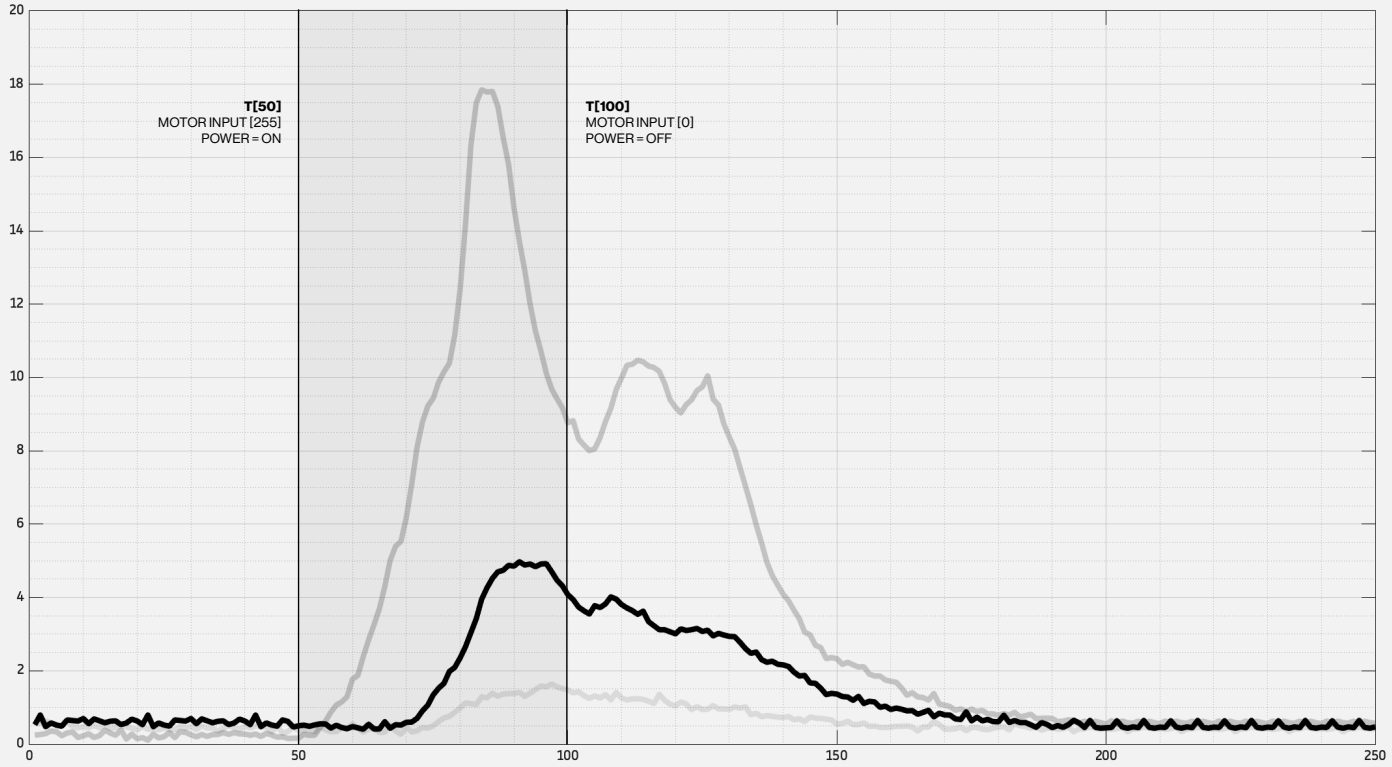
The delay in response and the more gentle slopes, appear to be a loss of sensitivity to mechanical stimulation. As the algae have been agitated over a while, they lose their sensitivity to stimulation, thus requiring more shear force in order to trigger their bioluminescence. Therefore, in the first few frames of the motion, as the rotational force starts to build up, the shear force will not strong enough to trigger the algae. This increase in the force that is required to stimulate the algae results in the slight delay in response time, as it takes longer to generate enough force to pass the threshold value. However, as the general motion of the agitation remains the same over time, the moments at which the algae crash into the glass will still be at the same points in time. Therefore, the timing of the peak values will remain within a similar timeframe.

By using a 'fresh' culture, which has not been agitated before, the response to mechanical stimulation be almost instantly. However, if one uses a culture that has been agitated before there will be a slight delay in response, which depends on the intensity and duration of the previous stimulations.

TABLE [142]
RESPONSE CURVE COMPARISION [N5-T]
SLOPE DEGREE, START OF RISE AND PEAK TIMES

TEST [NO.]	SLOPE	RISE AT	PEAK AT
N5-T-0	75 °	T[50]	T[85]
N5-T-1500	60 °	T[70]	T[90]
N5-T-3000	20 °	T[70]	T[95]

FIGURE 143
RESPONSE CURVE COMPARISION [N5-T]
DELAY BETWEEN INPUT-OUPUT



5.4

LOSS OF SENSITIVITY

Evaluating to which extend the assumed loss of sensitivity interacts with the behaviour of the light over time, as wel as analysing which factors influence the decrease in sensitivity.

FACTOR: DIFFERENCES IN AGITATION 'INTENSITY'
While at first the decrease in available energy or fatigue of the organisms was seen as the most important factor in the decrease in light output over time, it appears that in most cases the decrease in bioluminescence is mostly caused by a loss of sensitivity to mechanical stimulation.

Algae appear to be able to quickly adapt to a certain stimulus; becoming less sensitive to it over time. Once a culture has been agitated with by a large shear force, for example a high speed rotational agitation, it appears to lose some of its sensitivity and show almost no bioluminescent response to following lower intensity agitation sequences. This phenomenon can be clearly seen in both the baseline agitations after the 90 RPM rotation sequences and the variations in order tests.

Where baseline agitations performed after a 90 RPM rotation sequences, generally resulted in an almost identical response in bioluminescence when compared to their initial baseline agitations (see figure 147), baseline agitations performed after a rotational sequences with speeds of 120 RPM, resulted in almost no bioluminescent response.

This loss of sensitivity over time is regarded to be the reason behind the relative short durationa of the constant rotational sequences, especially when compared to the paused interval agitation sequences. As once the liquid is in motion and moves in sync with the movement of the orbital shaker, the shear force will remain fairly similar over time. The lack of variance in shear force, in combination with the loss of sensitivity over time, results in a rapidly decrease in bioluminescent response.

Research by von Dassow et al. (2005) also describes this effect and notes that not only the amount of force, but also the rate of changes in the flow stress affect the bioluminescent response. There appears to be a slow inactivation process, referred to as the loss of sensitivity, which results in a decrease in total light output if there is a lower rate of increase in flow stress.

For more 'intense' agitation sequences, such as the paused alternating rotation, energy depletion appears to be a more prominent factor in the decrease of light output. However, as it was not possible to further increase the rotational speed in these agitation sequences it is not possible to accurately conclude this.

In order to be able to fully utilize most of the bioluminescent potential and enhance the total light output of a culture, one should pay attention to the order of agitations. Starting with lower intensity agitations in order to maintain some of the sensitivity to mechanical stimulation. Once a culture has been exposed to large shear forces, it will show little to almost no response to lower intensity agitations.

TABLE [144]
MEAN GRAY VALUES OVER TIME
BASELINE AGITATIONS

TEST [NO.]	MAXIMUM	TOTAL
R2-B	14.3	1.71×10^5
R2-A	9.5	7.99×10^4

IMAGE [145]
BASELINE BEFORE AT AFTER (R2-T)
FRAME [85]

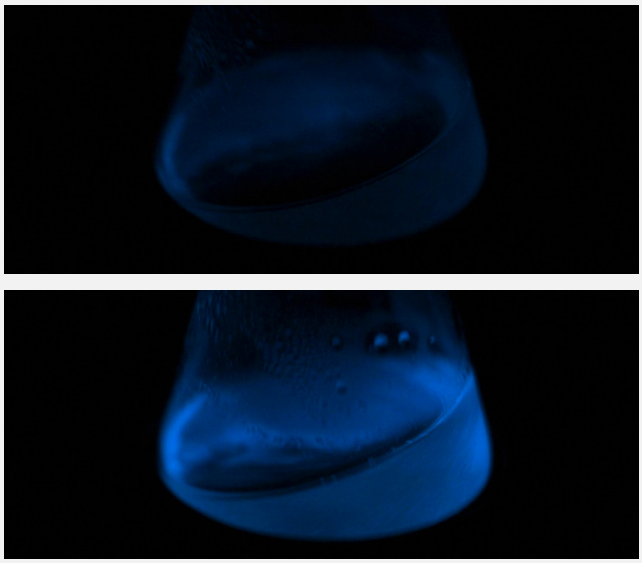
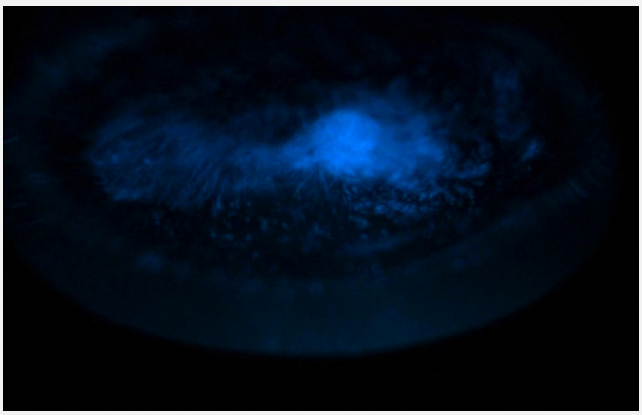


IMAGE [146]
EFFECTS OF CLUMOING ON RESPONSE
PULSE AGITATION SEQUENCE

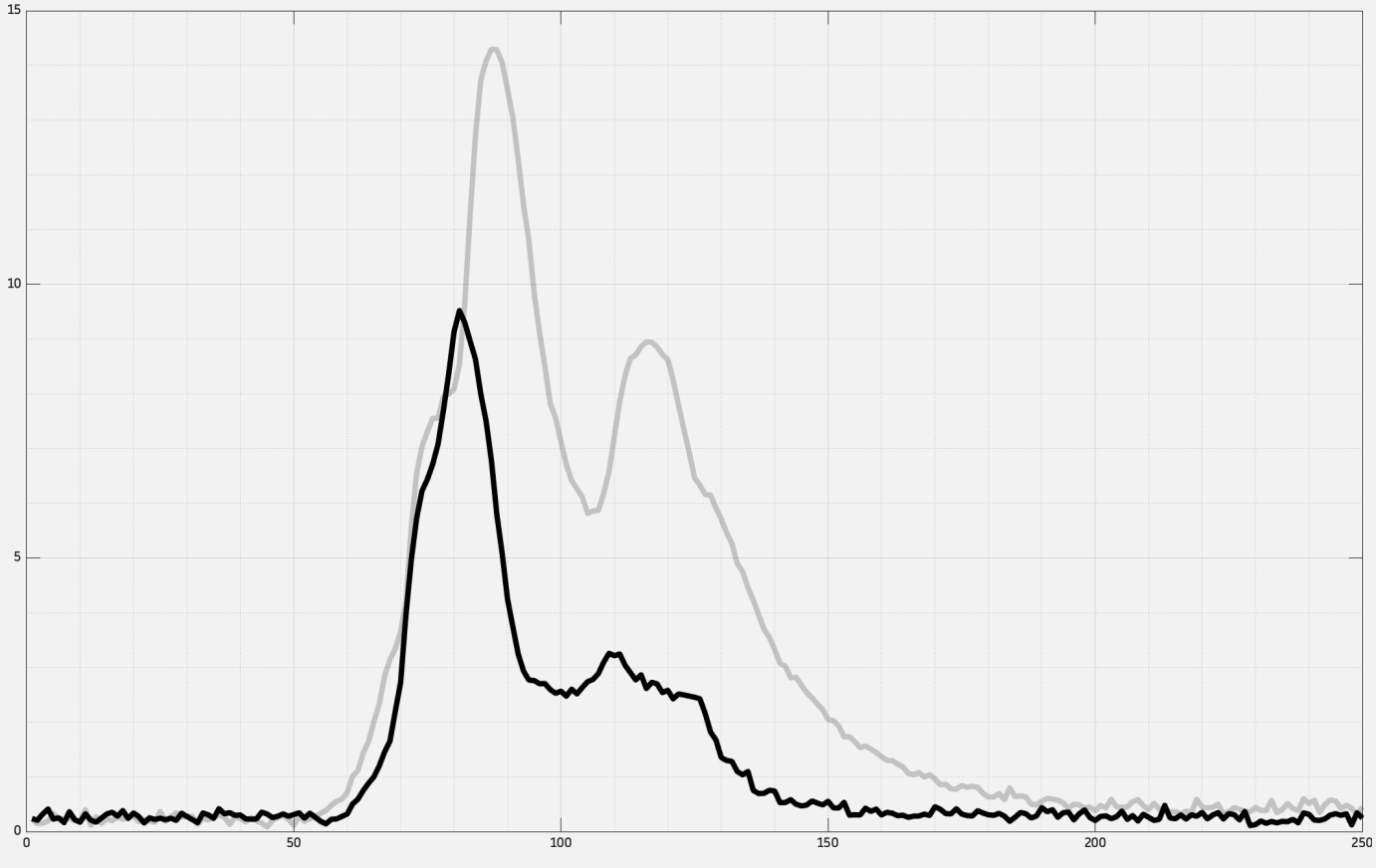


FACTOR: CONDITION OF ALGAE CULTURE
The condition of a culture, especially when looking at the consistency of the liquid and culture, also appears to have a significant effect on the sensitivity of a culture. As cells stick together over time, they appear to become less sensitive to shear force. This is most noticeable within the pulse interval agitations (T3), where one could clearly see a big difference in peak luminance values between the first and second pulse. While rotational agitations do not appear to be affected by the clumping of the cells as much, less intense types of agitations show significant variances in response in cases where the cells appear to stick together.

Not only does the clumping causes cells to stick together, thus increasing their resistance to force, these larger blobs of cells also tend to move less throughout the liquid; resulting in a more static lighting pattern, but also a decrease in the amount of shear force exerted on the cells. However, it is difficult to determine which of the above mentioned phenomenons (the resistance to movement of a larger cluster of cell matter or the connectedness of the cell membranes) has a bigger influence on the loss of sensitivity.

Be aware that as cells clump together over time, the culture becomes less sensitive to lower intensity stimulates, such as pulses or vibrations. Therefore, it is recommended to agitate the cultures once every few days in order to minimize the clumping effect.

FIGURE [147]
RESPONSE CURVE COMPARISION [N5-T]
DELAY BETWEEN INPUT-OUPUT



5.4

TEXTURAL QUALITIES

Evaluating the factors that influence the textural qualities of the light through quantitating the amount of texture by counting the amount of individual dots over time

FACTOR: TYPE OF ROTATION
The textural qualities of the light appears to strongly differ between each type of agitation.

In general, forms of rotational agitations will result in more of a uniform glow with barely any visible texture. As the rotational force pushes the all algae into a tight cluster near the edge of the glass, the individual dots of light start to blend together. This in combination with the slight motion blur that is created by the high rotation speeds, results in an almost textureless orb of light that moves throughout the liquid. While most cases this results in a fairly even glow throughout the liquid, in other cases there are some remaining algae floating in front and behind of the main cluster, creating some some slight form of texture within the light.

Pulse agitations tend to create more evenly spread and highly texturised lighting patterns, as cells throughout the whole liquid are triggered, but not pushed together as much. As the algae tend to travel at a much lower speeds in comparison to most rotational agitations, there is less of a noticeable motion blur. This results in clearly separated and almost circular dots of light, instead of the almost linear stripes of light that are seen with the rotational variants (see images 148). These dots of light are mainly seen within the first few seconds of the agitation (first phase of the lighting pattern).

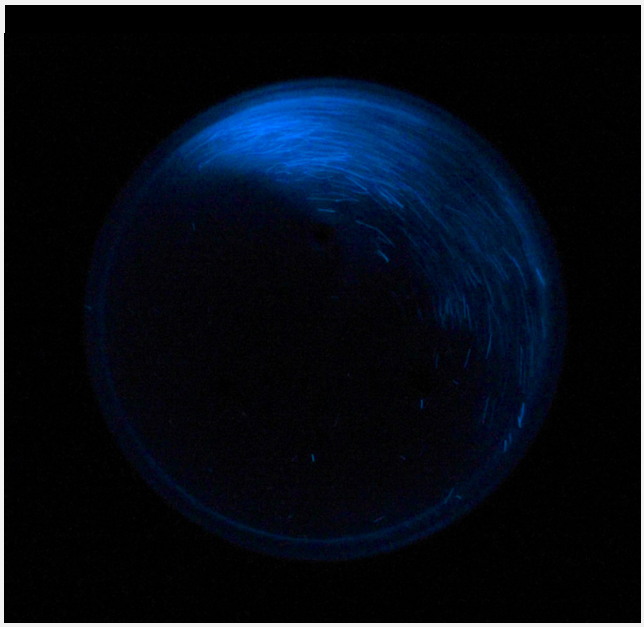
Vibration agitations appear to result in an even more texturised light, where the light of each individual algae can be clearly seen. As there is no movement within the liquid, individual sources of light do not blend together; resulting in a unique and highly scattered light.

FACTOR: ROTATIONAL SPEED
Differences in textural qualities are mostly visible within changes of the rotational speed. While rotational sequences performed at 120 RPM will generally result in a more glow-like appearance where the light almost no individual dots of light are visible anymore, slower rotational speeds usually result in a more glittery-like appearance where individual sources of light are easily distinguished from each other.

Note that the relation between the type of agitation and the textural qualities of the light is also influenced by the shape of the container. Therefore, it is best to look at the movement of the liquid, rather than the movement of the shaker.

While the textural qualities are mainly influenced by the type of agitation form, it is possible to increase or decrease the amount of texture by adjusting the speed or intensity of the agitation. In general, a higher rotation speed will lead to a decrease in visible texture as individual dots of light start to blend together.

FIGURE [148]
DIFFERENCES IN 'SCATTERING'
TOP: PULSE AGITATION, BOTTOM: ROTATIONAL AGITATION



FACTOR: CONDITION OF CULTURE

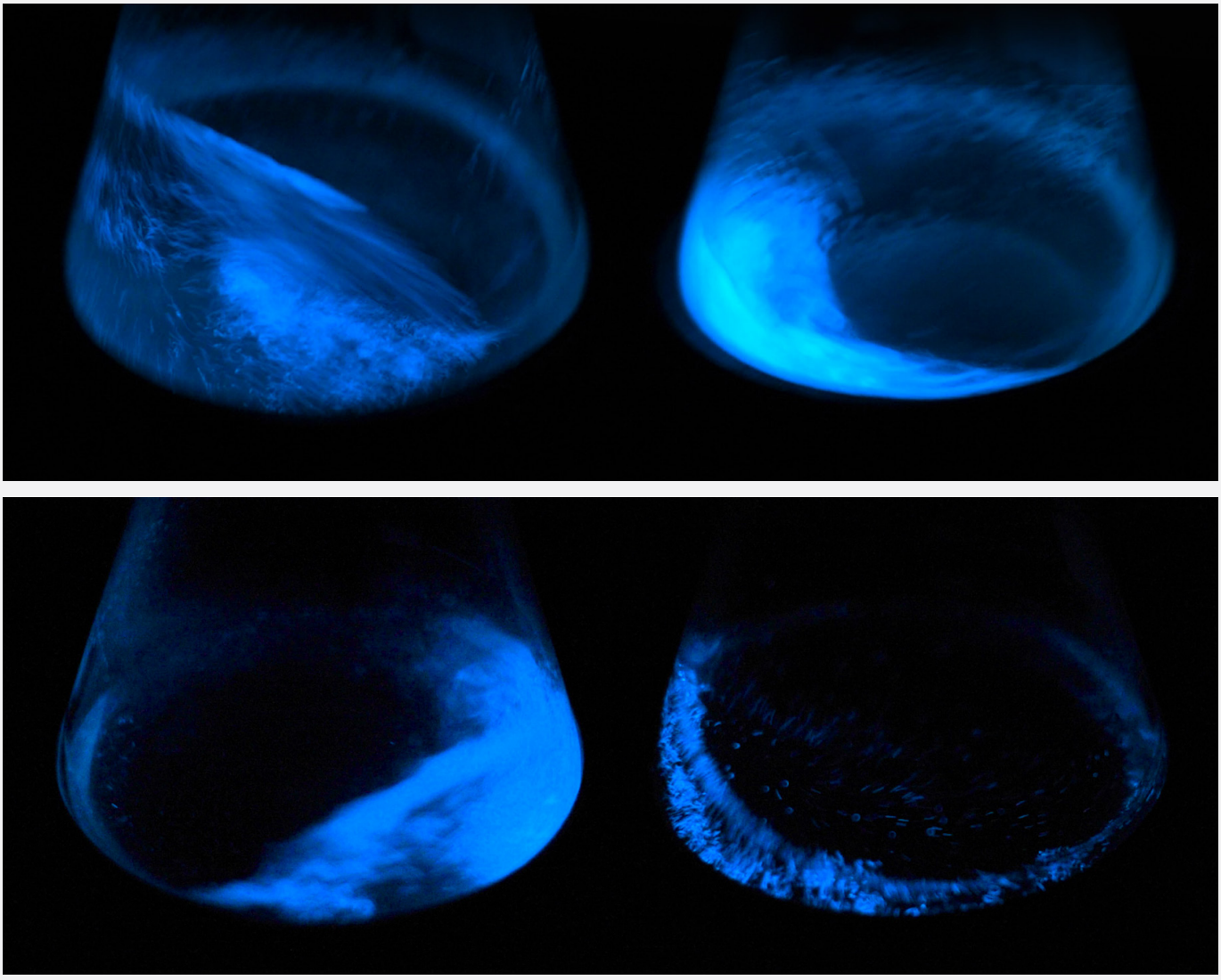
Another important factor that appears to effect the textural properties of the light, is the condition of the culture. A dense culture, with a high ratio of algae matter to liquid volume, has a tendency to create a more uniform glow as it is agitated.

As the density increases, individual dots will sit closer to each other and appear to blend together. This appear to not only be caused be the blending of the dots. The refractive properties of the cells themselves also are assumed to have an effect on the textural qualities. As within a more dense culture, the light emitted by the algae to pass through more cell matter, which results in a more diffused light.

Over time, there will be an increase in dead cell matter within the liquid, further diffusing the light. Increasing the volume, by either adding new medium or increasing the surface area, results in a decrease in density; in turn resulting in a more scattered and texturised light.

By increasing the density of a culture, one can create a more uniform glowing light. Decreasing the density, for example through the addition of fresh medium, will generally result in an increase in texture, individual dots of light are more spread and thus easier to distinguish from each other.

IMAGE [149]
TOP: DIFFERENCES IN VOLUME
BOTTOM: DIFFERENCES IN ROTATIONAL SPEED



5.4

SPATIAL DISTRIBUTION

Within this chapter, we will determine which factors have an influence on the spatial distribution of light and movement of the light, as wel as give first insights in how these factors can be adjusted to adjust the behaviour of the light.

As the spatial distribution of the light is directly linked to the movement of the liquid and the shear forces that are generated through this movement, it will be difficult to predict the precise movement of the light without using advanced computational models. In order to accurately determine all the factors that influence the spatial distribution of the light over time, one will have to make use of fluid dynamic analysis techniques; simulating the movement of particles and the forces acting upon them.

However, with the current data it is possible to make general assumptions on the movement of the light and the factors influence the spatial distribution. The main factors that appear to influence the 'movement' of the light are the following:

FACTOR: AGITATION TYPE
The main factor that influences the spatial distribution of the light will always be the type or rather the motion of the stimulus. As the movement of the shaker is directly related to the movement of the liquid, it will also be the main element influencing the spacial distribution of the light.

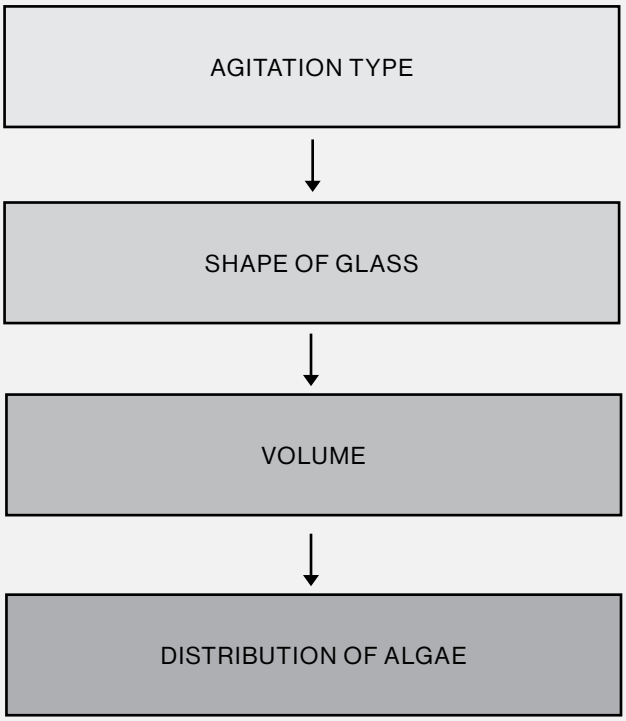
Certain type of agitations, such as rotations, have a tendency to result in recognizable and predictable spatial distributions of the light. While other types of agitations, such as pulses or vibrations, provide a certain element of surprise with regards to the behaviour of the light as the movement of the shaker does not appear to have a clear correlation with the spatial distribution and movement of the light.

FACTOR: SHAPE OF CONTAINER
Another important factor that influences the behaviour and movement of the light is the shape of the container. Round containers, such as Erlenmeyers or even petridishes, allow the liquid to smoothly move along the edge of the container. On the contrary, a square container will most likely result in less of an irregular and chaotic movement, as the algae will crash against the corners of the glass.

A smooth container allows the movement of the shaker to be directly translated to the movement of the liquid, as there are no obstacles. Containers with irregular surfaces and sharp transitions tend to have more of an influence the movement light as the liquid bounces against multiple different surfaces.

Note that it is hard to predict how a certain shape of container will influence the spatial distribution of the light. However, it is possible to assume that round containers (with smooth transitions between the different surfaces) will result in a more predictable movement of the light. Containers with sharp surface transitions or irregularities will most likely result in a more random distribution of light, as the liquid is going to 'bounce' around in the container.

FIGURE [150]
LAYERS OF FACTORS INFLUENCE SPATIAL CHARACTERISTICS
FIRST INSIGHS IN MOVEMENT OF LIGHT



FACTOR: VOLUME
The total volume of a culture also appears to have a visible effect on the movement of the light. As a larger volume results in a bigger moment of inertia, it will take longer for the liquid to start moving; in turn resulting to an overal slower response time of the algae in regards to the stimulation (as seen with test sequence T4). However, once a liquid with a larger volume has started moving, it will also continue to rotate for a longer period of time; once again showing a slight delay in response.

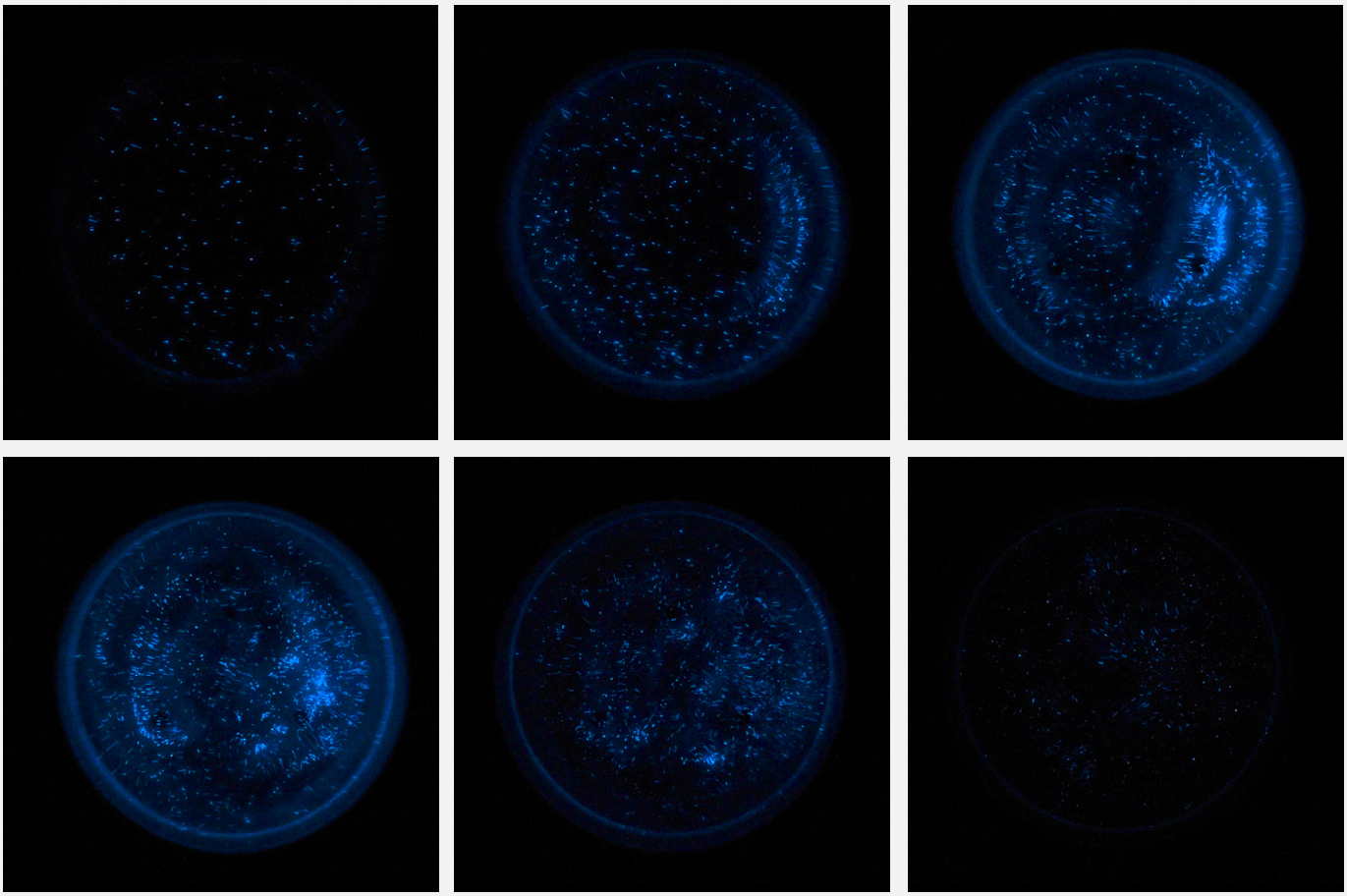
Another important factor, related to the volume of a culture, is the layer height of the liquid. As within a tall container the algae matter will be more evenly spread (in a vertical manner), while within a low-profile container, such as a petridish, there will be almost no vertical distribution of the light.

By increasing the volume of a culture, for example through the addition of extra medium, it is possible to slow down the acceleration of the liquid; resulting in a slight delay in response time of the algae.

FACTOR: DISTRIBUTION OF ALGAE MATTER
For agitations types that do not generate any noticeable movement of the liquid, such as small pulses or vibrations, the distribution of the algae matter throughout the liquid will also become a factor that influences the spatial distribution.

However, as in most cases the algae matter will be naturally be fairly evenly spread within the liquid, this factor will not be crucial in predicting the behaviour of the light.

IMAGE [151]
PULSE AGITATION SEQUENCE
TOP LEFT TO BOTTOM RIGHT



5.5

DISCUSSION

Evaluating the results of the research: discussing factors that might have had an influence on the results, defining outliers, and looking at the overall relevance of the results.

REFLECTION ON METHOD

While the current method of analysing and comparing the bioluminescent responses of the algae through video analysis, showed to be effective and provided reliable results, there were a few notable issues.

CAMERA SETTINGS

The main issues with the current method are mostly related to the camera settings that were used to capture videos. As the light produced by the algae is dim and thus difficult to capture with a camera, a fairly high ISO value in combination with a slow shutter speed and a large aperture are required in order to be able to capture the bioluminescent response of the algae.

While the noise in the videos generated by the relatively high ISO value of 800 resulted in small fluctuations in luminance, which are clearly visible in the first 50 frames before the start of the agitation sequence, it does not appear to have had a significant influence on the overal test results.

The relatively slow shutter speed of 1/50, however, appears to have a slightly more noticeable effect on the results. As the use of slower shutter speeds generally results in a slight motion blur, especially if the subject quickly moves across the frame. While this should not have a noticeable influence on the luminance values, it might influence the overall appearance of the light. In some cases, exaggerating the 'glowing effect' as dots of light appear to be slightly more blurred and create trail of light as they move across the frame. These trails of light can be seen in many the 120RPM rotational variants and are a clear sign of a low shutter speed.

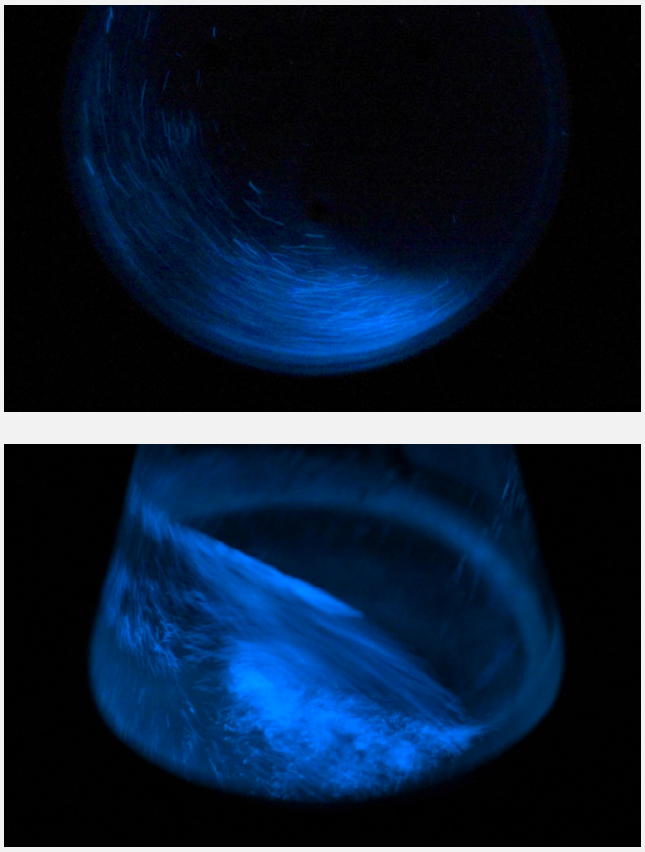
At the moment, the biggest issue appears to be the relatively large aperture of f/2, which results in a very shallow depth of field. With the current depth of field and camera angle it was not possible to keep the whole glass in focus, even when it was standing still. Once the glass start rotating and begins to move backwards, both the glass and liquid will be slightly blurred. While this does not appear to be an issue if one purely looks at the changes in luminance over time, it does become more problematic if one tries to accurately define the textural qualities of the light.

In order to create a wider depth of field, the aperture value will need to be increase, preferably to f/8 or even f/16 if possible, as this makes it possible to have almost all of the glass in focus even as it rotates.

POINT OF VIEW

In an ideal scenario, the camera would be placed above the glassware, as this provides a more even and undisturbed view of culture (see image 153). Most importantly, it does not lead to an changes in the 'size' of the light, as the container it moves towards and away from the lens during the rotation. This will result in an overal more consistent and accurate reading of the light output over time. However, as in this case the luminance readings were purely used for comparison purposes and the camera angle as wel as the distance to the glass were the same for each test sequence, the current point of view should not have resulted in any significant deviations.

IMAGE [153]
DIFFERENCES IN 'SCATTERING'
TOP: PULSE AGITATION, BOTTOM: ROTATIONAL AGITATION



VARIANCES IN RESULTS

While there are various factors that might have lead to some of the variance seen within the test results, the most important appear issues appear to be: variances in algae concentrations between batches, changes in the conditions of the organisms themselves and irregularities within the shaking motion.

DIFFERENCES IN CONCENTRATION
Even though extra care was taken in order to ensure the initial algae culture was evenly distributed among the three batches and each container had the same amount of liquid, there still could be slight differences concentrations of algae matter between each batch. Variances in algae density could easily result in significant differences in luminance values. However, as the starting culture was very dense, slight discrepancies in density will have less of a noticeable effect.

DIFFERENCES IN CONCENTRATION
Even when assumed that two cultures have the exact same concentration of algae matter, there still could be noticeable differences in bioluminescent response. As the total light output and behaviour of a culture will not only depend on its density, but also on its overal condition; looking at factors such as its age, general living conditions and previous stimulation (Latz et al., 2004).

Slight differences in environmental factors, such as the amount of previous agitation, the distance to light source or the amount of available nutrients, over time will result in more noticeable differences in the behaviour and total light output between the batches. Furthermore, as each individual cell within a culture behaves and grows in a slightly different manner, the combined response of a culture will also naturally vary over time.

IRREGULARITIES WITHIN MOTION
As it was not possible to precisely control the movement of motor with the current shaking setup, there were some minor irregularities within the movements of each agitation sequence. While the variances between the motions were miniscule and often not visible, even the slightest offsets in the timing of the rotations can result in noticeable differences in the total light output (see chapter T1).

RELATION BETWEEN FACTORS

Within in the current research, the intensity over time, textural qualities and the spatial distribution are treated as isolated properties of the light that each have a different effect on the way the light is experienced. While in reality, all properties are closlyconnected and affected by each other.

For example, where an increase in rotational speed does not only result in a change in the total light output over time, it also affects the textural qualities of the light and the way in which the light moves throughout the container.

The behaviour of the light has shown to be influenced by a large number of factors, which all appear to have some sort of relation with one another.

CHAPTER 6

DEMONSTRATION

Communicating the key findings of the research on the flash characteristics and general behaviour of the light; with the use of a small scale interactive installation.

C6

CONTENTS

AIM

Concluding the research by showing the potential and unique characteristics of the light produced by the bioluminescent algae; demonstrating the effects of certain parameters on the behaviour of the light.

- Building a research objects that shows the potential and unique characteristics of the light that is produced by the bioluminescent algae
- Providing an overview of the construction of the object, focussing on certain design choices
- Evaluating the key findings of the research by reflecting upon the potential of the bioluminescent algae
- Determining possible points of interest and research questions for further research

METHOD

- Iterative process of prototyping
- Research through design approach

6.1

DEMONSTRATION

In order to communicate the key findings of the research on the flash characteristics and general behaviour of the light, as wel as to spark the interest of other designers, a small scale interactive installation has been constructed.

GOAL AND PURPOSE

The main goal of the installation is to show the potential and unique characteristics of the light that is produced by the bioluminescent algae; demonstrating the effects of certain parameters, such as the type of agitation and rotation speed, on the behaviour of the light.

This current version of the installation functions as a prototype and is seen as a proof of concept for the full scale installation to be displayed at STILL ALIVE: an exhibition and symposion within Het Nieuwe Instituut, which focusses on biodesign research and interactive living artefacts.

DESCRIPTION

The installation will consist of a number of cubes, each with its own orbital shaking device and a small amount of algae culture. The movement of the containers within the cubes is regulated with the use of a motion sensor; being triggered by the movement of the visitors

ENVISIONED INTERACTION

As soon as a visitor enters the installation space, the nearest cube will initiate its movement; starting with a gentle pulse agitation to only trigger a slight portion of the algae. Once the visitors comes closer towards the cube, the agitation will start to become more intense, either through an increase in the rotational speed or by varying the amount of pulses.

After having performed a certain number of agitation patterns, the movement of the shaker will stop as the algae cultures will need to rest in order to recover some of their bioluminescent capabilities. This pause in interaction is seen as an important part of the exhibition as it clearly shows the limitations of the material, but also enforces the notion that the material is alive.

Not only will the cubes respond to the visitors, but they will also respond to each other; creating a dynamic relation and lighting pattern.The type of agitation will vary over time, demonstrating the differences in the behaviour of the light for both pulse and rotational agitations.

It is important to note that as at this moment it is unclear for how long the algae are able to respond to high intensity stimuli, it might not be possible make use of movements that generate a lot of shear force (e.g. alternating rotations at high speed or continuous rotation).

- **Response to movement - based upon distance to object**
- **Displaying various types of agitation sequences**
- **Interlinked cubes: intricate response patterns**

IMAGE [154]
TOP AND SIDE VIEW OF INSTALLATION
EXAMPLE OF SETUP AND PROPORTIONS

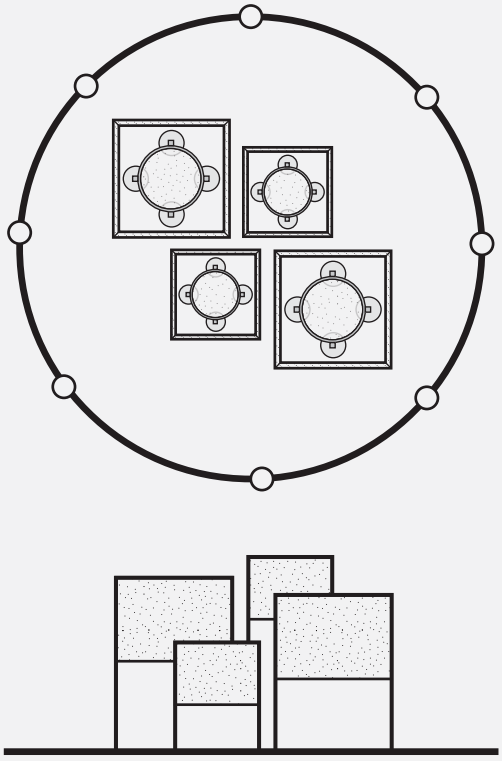


IMAGE [155]
OVERVIEW OF CUBES

IMAGES [156]
CLOSE-UP OF LIGHTING
PULSE AGITATION

6.1

CONSTRUCTION

At the core, the construction of the cubes are based around of that of the orbital shaker as described in Chapter 4; consisting of a base, rotating arms and a holder for the glass containing the bioluminescent algae.

BASE FRAME
Each cube is built around a steel frame, constructed out of 15mm square tubing. This steel frame functions as a stable base structure to which the acrylic plates and orbital shaking mechanisme will be attached.

ROTATING ARMS
The orbital shaking mechanism is powered with the use of a 9V DC motor, which is attached to the bottom plate and directly connected to one of the rotating arms. As the motor is able to reach speeds of up to 200RPM, various types of agitational patterns can be performed.

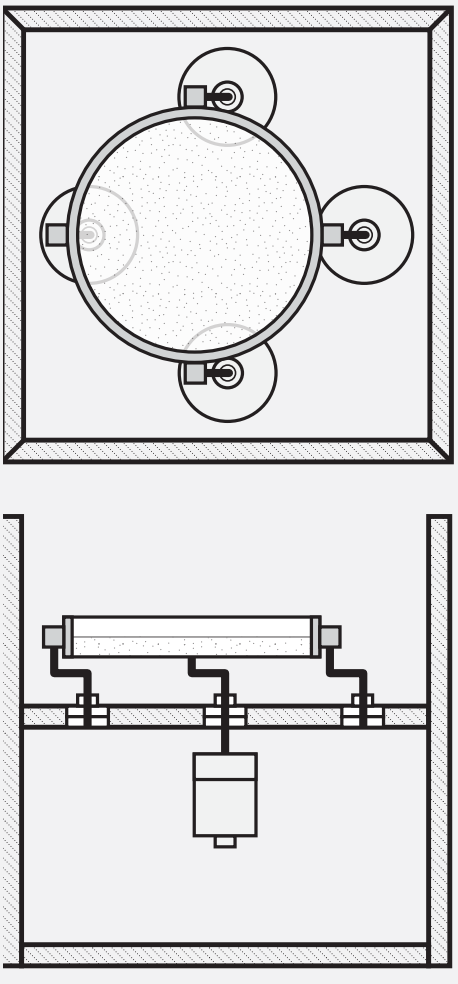
There are a total of four rotating arms which translate the spinning motion of the motor to a larger rotational motion of the container. Each arm is kept into place with the use of sliding bearings, which are housed in 3D printed holders that are attached below the bottom plate. Each holder is attached to the the bottom plates with screws, resulting in a stable base.

CONTAINER HOLDER
The glass container, in which 30ml of algae culture is kept, consists of two sterilised glass petridishes placed on top of each other and sealed with the use of parafilm; creating an almost watertight seal that still allows some gas exchange. A 3D printed clamp is used to securely hold the two petridishes together. The clamps are squeezed together and secured with the use of bolts, ensuring a tight fit. An additional piece of rubber tape is added in between the clamps and the petridish construction in order to further reduce the chance of leaking. The steel rods of the rotating arms snugly fit into the sliding bearings embedded within the clamps, resulting in a smooth rotation of the container.

ELECTRONICS
Both DC Motors are connected to a DRV8833 motor driver, which makes it possible to control the speed and direction of the both motors through an Arduino Uno. An external 9V 1A power source has been attached to the motor driver in order to be able to use the full potential of the motors.

Within the current prototype, no motion sensors have been implemented yet. However, for the final exhibition the motion of the shakers will linked to IR distance sensors housed in the base of the shaker.

IMAGE [157]
TOP AND SIDE VIEW OF INSTALLATION
EXAMPLE OF SETUP AND PROPORTIONS



IMAGES [158]
DETAILS OF CUBES
CLOSE-UP

IMAGES [159]
OVERVIEW OF CUBES

6.2

CONCLUSION

Evaluating the key findings of the research and reflecting upon the potential of the bioluminescent algae for product design purposes.

KEY FINDINGS

Within this research, key insight in the flash characteristics and the behaviour of the light, with regards the temporal form, textural qualities and spatial distribution of the light, were discussed.Resulting in first insights in the complex relation between movement and the qualities of the light. In the end, providing an overview of factors that can be adjusted to alter the flash characteristics and behaviour of the light.

In addition, three types of agitation forms [rotation, pulse and vibration] were evaluated; providing first insights in how the type of stimuli affects general characteristics of the light, focussing on changes in intensity over time, differences in experiential qualities and boundaries of the material.

POTENTIAL OF MATERIAL

While the unique experiential qualities of the light are still assumed to be one of the most promising features of the light that is produced by bioluminescent algae, there are many other properties that show the potential of the algae as a material.

LIVELINESS OF THE LIGHT

One of the most intriguing aspects of the light produced by the algae, is its randomness and fairly unpredictable behaviour in regards to stimuli. As the algae never appear to respond in the exact same way, the light that is generated is truly unique for each agitation. This sporadic behaviour further enforces the notion that the material is alive. As the liveliness of the material is one of its core strengths, products making use of bioluminescent algae should always try to emphasise the fact that the material is alive.

CONNECTION WITH THE MATERIAL

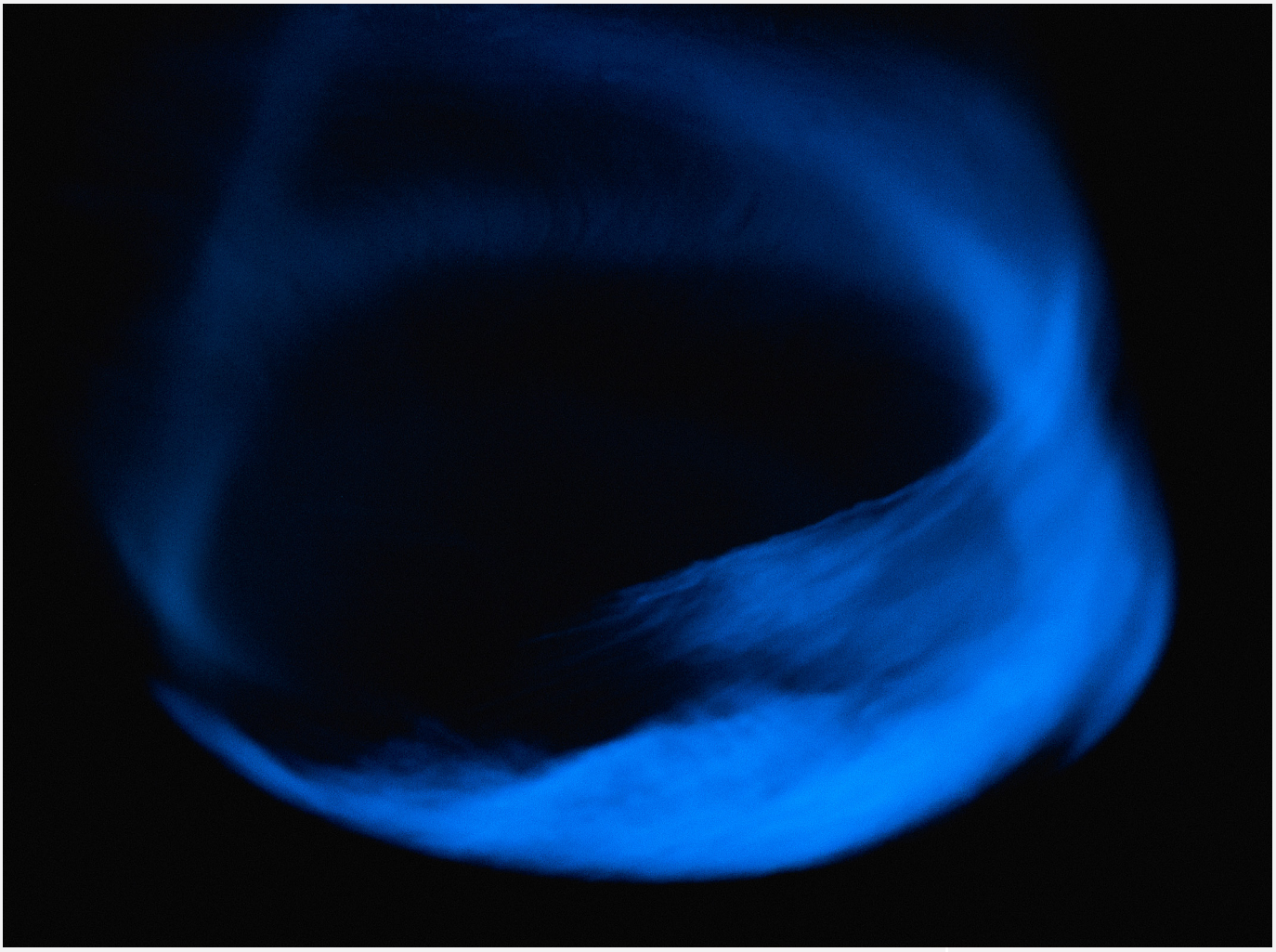
Since bioluminescent dinoflagellates are difficult to culture; having strict requirements in terms of living conditions and handling procedures, it remains difficult to create a sustainable living environment within a product. Especially, if the product will need to be maintained by user in their home environment. However, the sense of fragility of the organism and its need to be nurtured, does appear to result in an enhanced connection between the user and the material.

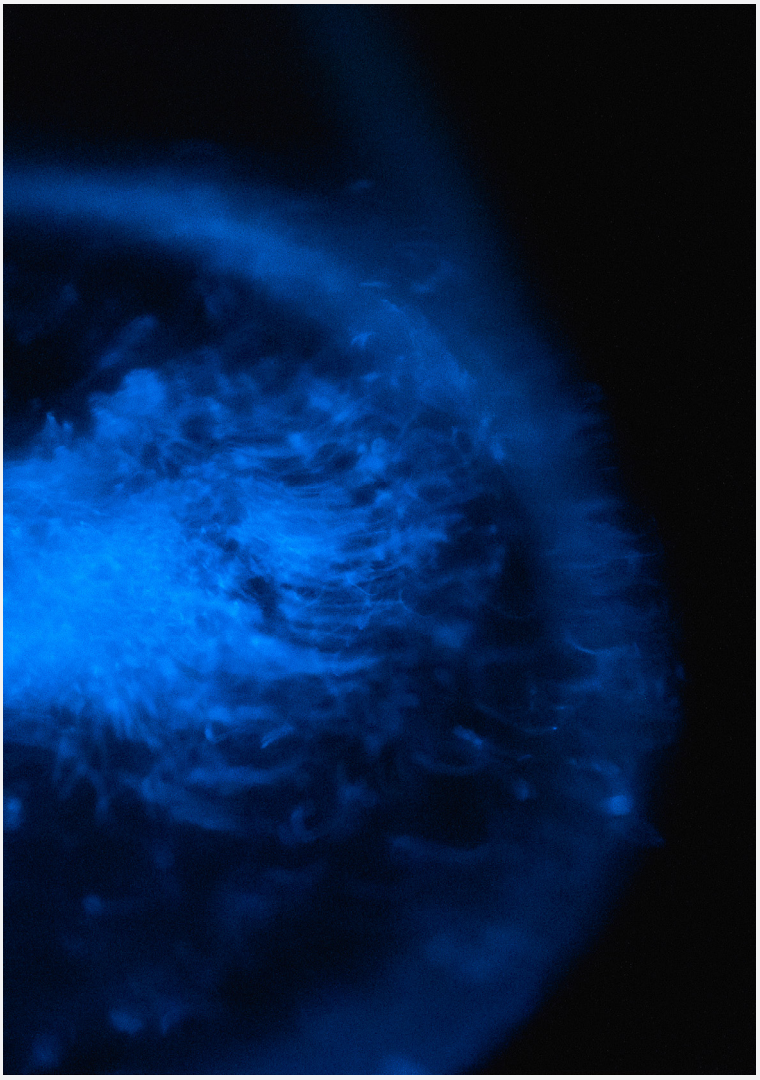
Working with the algae is best described as taking care of a new plant; first carefully transferring it to its new container and ensuring the conditions are right, before trying your best to enhance its growth by monitoring its behaviour and giving it extra nutrients every once in a while.

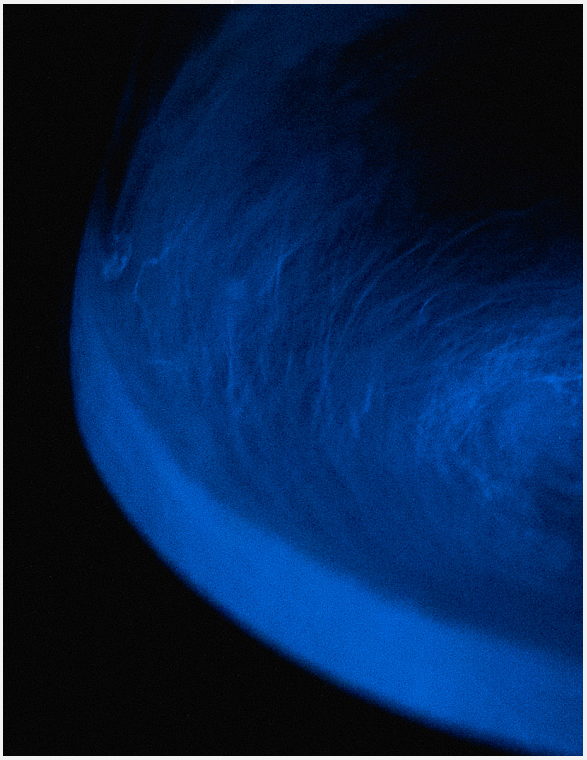
LIMITATIONS IN LIGHT OUPUT

One of the main limitations of using the algae as a source of light, is their extremely low light output compared to more traditional light sources.

As the light produced by the algae is dim and in some cases even barely visible, one should not try to use the material for purely functional purposes. Instead, one should rather focus on emphasizing the unique characteristics of the light, such as its response to movement or the textural qualities.







TOP [160]
FAST ROTATIONAL AGITATION

LEFT [161]
CLOSE-UP OF PULSE

RIGHT [162]
SLOW ROTATIONAL AGITATION

6.2

FOR FURTHER RESEARCH

An overview of points of interest for further research; focussing on topics relevant to the field of product design

FLASH CHARACTERISTICS

As this project acts as a starting point for future research, there still remains a lot to discover about the behaviour and characteristics of the light produced by bioluminescent algae.

EXPERIENTIAL QUALITIES

No research appears to be performed yet with regards to the experiential characterisation of bioluminescent materials. Nevertheless, the unique experiential qualities of the light are still assumed to be one of the most promising features of bioluminescence.

INCREASING DURATION - TOWARDS REALLIFE USE

For the implementation of the algae material in everyday use objects, it will be important to know more about the changes in flash characteristics over a longer period of time. By either increasing the overall duration of the test sequences or the number of agitations within a sequence, it will be possible to get a better view on the recovery of the flash characteristics over a longer period of time.

IMPLEMENTATION OF FLUID DYNAMICS PRINCIPLES

In order to get to be able to more accurately predict and control the behaviour of the light, it would be interesting to make use of fluid dynamics principles and modeling. First, comparing the computational models with the actual responses of the algea in order to determine the accurate of the model.

DIFFERENCES BETWEEN SPECIES

While the inital plan of the project was to compare the flash characteristics of two species of dinoflaggelates (Fusiformis and Lunula) this was not possible in the end, as the Lunula culture never produced enough ligt. However, defining the differences in flash characteristics and behaviour of various species of bioluminescent algae still remains a valuable topic.

EMBODIMENT

In order to further simulate the use of bioluminescent algae within the field of product design, it will be important to have more knowledge on how to properly implement the material and create a sustainable living environment for the organisms.

STERALISATION OF DIFFERENT MATERIALS

At the moment, using the autoclave appears to be the only real viable option to ensure that the material which comes into contact with the algae is sterile. However, as the number of materials that can be placed inside the autoclave is limited, there currently are not that many options with regards to the type of embodiment.

MAINTAINING A STERILE ENVIRONMENT

In order to further stimulate the use of bioluminescent algae within everyday use products, it will be important to find ways on how to maintain a sterile environment for the culture as it is being kept within a home environment.

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A1

Full description of the procedure for creating the artificial seawater medium with additional nutrients and trace metal used for growing bioluminescent dinoflagellates

RECIPE

COMPONENT	CONCENTRATION	UNIT
Artificial seawater	1000	mL
$NaNO_3$*	1.0	mL
NaH_2PO_4*	1.0	mL
Trace metals*	1.0	mL
Vitamins*	0.5	mL

* pre-mixed stock solutions

C1 ARTIFICIAL SEAWATER

COMPONENT	CONCENTRATION	UNIT
NaCl	31.10	g/L
KCl	0.77	g/L
$CaCl * 2H_2O$	1.60	g/L
$MgCl_2 * 6H_2O$	4.80	g/L
$MgSO_4 * 7H_2O$	3.50	g/L
$NaHCO_3$	0.11	g/L

C2 N/P SOURCE

COMPONENT	CONCENTRATION	UNIT
$NaNO_3$	8.82×10^{-4}	M
NaH_2PO_4	4.17×10^{-5}	M

C3 TRACE METALS

COMPONENT	CONCENTRATION	UNIT
$Na_2EDTA * 2H_2O$	1.17×10^{-5}	M
$FeCl_3 * 6H_2O$	1.17×10^{-5}	M
$MnCl2 * 4H_2O$	9.09×10^{-7}	M
$ZnSO_4 * 7H_2O$	8.00×10^{-8}	M
$CoCl_2 * 6H_2O$	5.00×10^{-8}	M
$CuSO_4 * 5H_2O$	1.00×10^{-8}	M
$Na_2MoO_4 * 2H_2O$	8.22×10^{-8}	M
$Na_2O_4Se * 10H_2O$	1.00×10^{-8}	M
$NiSO_4 * 6H_2O$	1.00×10^{-8}	M
Na_3VO_4	1.00×10^{-8}	M
K_2CrO_4	1.00×10^{-8}	M

C4 VITAMINS

COMPONENT	CONCENTRATION	UNIT
Thiamine * HCl	2.96×10^{-7}	M
Biotine	2.00×10^{-9}	M
Cyanocobalamine	2.00×10^{-9}	M

EQUIPMENT

- Clear glass laboratory bottle: 1000ml [note: bottle does not have to be sterilised, however no particles and obvious signs of contamination should be visible]
- Beaker glass (approx. 300ml)
- High resolution scale (min. resolution of 0.01 grams)
- Plastic weigh boats, small spoon and two funnels
- Demineralised water and spray bottle (approx. 1.5L)
- Magnetic stirrer and stir bar
- Laboratory autoclave
- Precision pipet (0.5 to 1.0 ml resolution)
- Safety glasses

A1

PROCEDURE*

*using stock solutions of the N/P source, trace metals and vitamins

STEP 01 – ORGANISING WORKPLACE

1. Arrange bottles containing the chemicals to order following the recipe list, going from left (first chemical) to right (last chemical).

2. Carefully drop stir bar into 1000ml bottle, holding the bottle sideways to avoid bar hitting the bottom.

3. Place the big funnel into the bottle [note: this funnel is only used for pouring liquids]

STEP 02 – FILLING WITH WATER

4. Place the 1L bottle (with the funnel and stir bar) onto the scale and calibrate the scale [make sure the scale is properly leveled].

5. Fill the bottle with demi water until the scale has reached a value of 1000g. The last couple of grams are best added with the use of a spray bottle, as this makes it easier to add small amounts of liquid.

6. Mark the height of the liquid on the bottle (waterline) with a permanent marker [aim for the 'belly' of the liquid]

7. Remove the funnel and pour approximately 300ml of the water into the beaker glass [safe the water for later use]

8. Place the bottle onto the magnetic stirrer and turn it on. [note that the stirring bar should stay in the centre of the glass to create a consistent vortex within the liquid]

STEP 03 – ADDITION OF CHEMICALS

9. Add the other (smaller) funnel into the bottle

10. Place the weigh boat onto the scale and recalibrate it

11. Open the container and use the spoon the 'grab' some of he chemicals [be carefull not to spill anything]

12. Carefully add the chemicals onto the weigh boat until scale shows the required amount [note: be as precise as possible and follow the recipe]

13. Lift the weight boat from the scale and carefully pour the chemicals into the bottle. Use the spray bottle with demi water to spray the weight boat and the funnel to ensure all of the chemicals are poured into the liquid.

14. Repeat steps for all of the required chemicals [note: use safety glasses when handling $CaCl * 2H_2O$]

15. Once a chemical has been added be sure to remove the container from the order, maintaining a clear overview of which chemicals have been added so far.

STEP 04 – 'REFILLING' TO 1L

- Replace the small funnel with the larger funnel that was previously used to pour the demi water
- Carefully pour the demi water from the beaker into the bottle until it reaches the marked line. Once again, use the spray bottle for the last few milliliters.
- Remove the stirring rod from the bottle with the use of a metal stick [note: ensure the bottle is not standing on the magnetic stirrer before trying to remove the stirring rod]

STEP 5 – STERILISATION

- Twist the bottle cap onto the bottle. Be sure to not fully tighten the cap, as it should sit loosely onto the glass to ensure pressure can escape.
- Remove the lid of the autoclave and place the bottle containing the artificial seawater mixture, in the centre of the rack [note: the bottle shoul not touch the sides of the autoclave]
- If not sterilised before, add the stock solutions of the remaining chemicals (vitamin mix, trace metals and n/p source) to the autoclave as wel.[note: it is important to autoclave all 'components' individually before mixing them together to avoid precipation].
- Close the lid and turn on autoclave at 121 degrees celsius; following intructions provided by the manual included with the equipment

STEP 6 – ADDITION OF STOCK SOLUTIONS

- Once all the autoclave solutions have cooled to room temperature and are cool to the touch, it is possible to add them to the artificial seawater solution [made in steps 1-4].
- With the use of the pipet, gather the required amount of the stock solution.
- Carefully deposite the liquid from the pipet into the bottle containing the 1L of seawater medium. It is important not to touch the edges of the glass to avoid contamination
- Repeat the steps for all the stock solutions. In order to avoid contamination use new 'pipet tips' for each solution
- Once all the solutions have been added, the bottle will need to be sealed with the cap, before being gentle stirred and shaken to ensure solutions are wel mixed.
- Mark the bottle containing the solution (name / date) before putting it in the fridge.[note: medium is best stored at a temperature of around 7-15 degrees]

GROWING LIGHT
DESIGNING WITH BIOLUMINESCENT ALGAE

MASTER THESIS
TIM VAN DORTMONT

28.02.2020
DELFT