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Luminacell-Biostim

Diploma Thesis

Autonomous School Specialization
Bionik

implemented in school year 2025/2026 by:

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Abstract[VV], [GW]

Retinal prostheses represent a promising approach for the treatment of degenerative retinal diseases. This diploma thesis investigates the suitability of organic semiconductor materials (D18 and Y6), for use in such systems. The aim of this work is to experimentally evaluate whether the cultivation of retinal pigment epithelial cells (RPE cells) is feasible under school laboratory conditions and how these cells respond to contact with the mentioned materials.

For this purpose, RPE cells are cultured in a school laboratory and subsequently brought into direct contact with D18- and Y6-based layers. The influence of these materials on cell growth and cellular activity is then analyzed using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid (MTT) and immunofluorescence (IF) assays. A major challenge in this context is the long cultivation period required for the cells, which significantly slows down experimental progress.

To address this limitation, a device was developed as part of this work that enables targeted light stimulation of the cells. This system is intended to influence cellular processes and facilitate faster acquisition of experimental results.

Kurzfassung[VV, GW]

Retinale Prothesen stellen einen vielversprechenden Ansatz zur Behandlung degenerativer Netzhauterkrankungen dar. Im Rahmen dieser Diplomarbeit wird untersucht, inwiefern organische Halbleitermaterialien (D18 und Y6), für den Einsatz in solchen Systemen geeignet sind. Ziel der Arbeit ist es, experimentell zu überprüfen, ob die Kultivierung von retinalen Pigmentepithelzellen (RPE-Zellen) unter schulischen Laborbedingungen möglich ist und wie diese Zellen auf den Kontakt mit den genannten Materialien reagieren.

Dazu werden RPE-Zellen im Schullabor gezüchtet und anschließend in direkten Kontakt mit D18- und Y6-basierten Schichten gebracht. In weiterer Folge wird der Einfluss dieser Materialien auf das Zellwachstum und die Zellaktivität mittels 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid] (MTT) und Immunofluoreszenz (IF) tests analysiert. Ein zentrales Problem stellt dabei die lange Kultivierungsdauer der Zellen dar, die den experimentellen Fortschritt erheblich verlangsamt.

Um diesem Hindernis entgegenzuwirken, wurde im Zuge der Arbeit ein Gerät entwickelt, das eine gezielte Lichtstimulation der Zellen ermöglicht. Dieses System soll dazu beitragen, zelluläre Prozesse zu beeinflussen und experimentelle Ergebnisse schneller zugänglich zu machen.

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1 Introduction

1.1 Retinal Prostheses - Problem Setting and Research Qestion[VV]

Several degenerative diseases of the retina, including Retinitis Pigmentosa and Age-related Macular Degeneration, result in serious progressive degeneration of photoreceptors. [48, 45] Patients affected by these diseases experience a gradual worsening of vision, which can eventually lead to complete blindness in advanced stages. [34, 15] Currently, there is no complete cure for degenerative retinal diseases. Available treatments focus primarily on slowing disease progression. However, the regeneration of lost photoreceptors is currently not possible. [48, 53, 45] Even though there have been different approaches to restoring vision, most current technologies face significant challenges regarding long-term stability and chronic immune responses.

To overcome these limitations, a promising approach by **Ahmad Salti** (2022) and his research team utilizes organic semiconductors. [17] While the toxicological effects of these materials, specifically D18 and Y6, on the viability and structure of RPE cells have been successfully tested in specialized university facilities, such environments often exceed standard laboratory capabilities. Consequently, this thesis investigates whether these results can be replicated and the cell quality assessed within a setting of more limited technical resources, such as a school laboratory.

1.2 Retinal Prostheses - Solution[VV]

There are currently no medical treatments that restore the photoreceptors lost due to diseases like AMD and RP. Current research aims to replace the dead photoreceptors with electronic retinal prosthesis using organic photovoltaic polymers such as D18 (see Section 2.6.1) and Y6 (see Section 2.6.2). These materials are placed directly under the retina (subretinal), between the remaining retinal neurons and the RPE. [26] Because they are in direct contact with RPE cells, it is essential to evaluate their biocompatibility. This is tested by cytotoxicity assays. [17] In this diploma thesis, this will be assessed using cell viability assays and immunofluorescence tests, specifically MTT and ICC tests.

1.3 Cell Growth - Problem Setting and Research Question GW

Cell research is a major part in biomedical research. These processes and experiments often require a defined developed stage or a specific density before they can be used in experiments. The cell growth is a time-consuming and very sensitive process, often extending over days or even weeks. RPE cells take round about three weeks to reach a confluency where they can be passaged or experiments can be peformed with them. [8] While this time the cell-cultures require optimal conditions regard, sterility, temperature, humidity or gas composition. As a result, important time and resources in medical research are wasted and may delay workflows and solutions. If these growth periods could be shortened, there would be more time for effective research. This could lead to faster discoveries and faster treatments.

This raises the question, how can cell cultures be encouraged to grow faster, to minimize risk of suffered or damaged cells and to fasten research? There are already research results that show that light influences the growth of cells and increases the ATP production in the cells. [43] This effect is used in high-performance sports, after intensive training sessions, athletes go to the infrared cabin to prevent muscle soreness and regenerate more quickly. In addition, the heat penetrates so deeply, blood circulation increases, and the cells are supplied with oxygen and nutrients more quickly. [65]

This thesis aims to find out whether light at different wavelengths actually influences growth. To investigate this, a device that shines light at different intensities and wavelengths onto the cells for a certain amount of time each day, over several days is build. If it is not possible to cultivate RPE cells in a school environment, the device will be tested with lactic acid bacteria, as these grow more slowly than *E.coli*.

1.4 Cell Growth - Solution GW

The experimental setup consists of a programmable lighting unit designed to deliver specific light treatments to cell cultures. Adjustable parameters include wavelength, intensity, start time, and duration per day, enabling automated long-term studies over multiple days. It should be a robust housing that is easy to use and functions independently of power connections. It is also very important that the housing can be sterilized. If this is not possible, at least the surface should be easy to disinfect, so without many ridges and recesses. The user interface should be accessible by a simple display, and individual settings should be able to be made via buttons. The device is operated using a program specifically configured.

1.5 Structure of this Thesis[VV,GW]

First, the biological foundations are introduced, beginning with the anatomy of the human eye and a detailed description of the retina. This is followed by an overview of retinal dystrophies, including major diseases that lead to retinal degeneration.

Subsequently, the focus shifts to stem cells, their generation, and their differentiation into RPE cells. In addition, the principles and development of retinal prostheses are presented.

The theoretical part is complemented by methodological foundations, including cell culture techniques, MTT assays, and immunofluorescence analysis.

The practical section describes the experimental work, including the cultivation of RPE cells, their interaction with D18 and Y6 materials, and the performed analyses. Furthermore, the design and implementation of a light stimulation device are explained.

Finally, the results are presented and discussed.

This thesis includes a resume of the team members, an appendix detailing project management and acknowledgments. References, a list of figures, a list of tables and listings are included at the end.

2 Theoretical Background

2.1 Explantation of Terms[VV],[GW]

"π–π stacking" - describes the position of the molecules in the material. In this case, they are stacked like playing cards. This ensures that the electrons can move easily between [29]

"RP" - Retinitis Pigmentosa - eye disease

"(A(R)MD)" - age-related macular degeneration - eye disease also known as atrophic AMD

"w-AMD" - wet Age-Related Macular Degeneration - subcategory of AMD

"d-AMD" - dry Age-Related Macular Degeneration - subcategory of AMD

"photoreceptors" - rods and cones

"rods" - for sight in the dark

"cones" - for color vision and sharpness

"RPE" - retinal pigment epithelium

"VEGF" - a protein causing more blood vessels to grow

"D18 and Y6" - organic photovoltaic polymers

"Pluripotent stem cells" - cells that can differentiate into almost any cell type in the human body

"RO" - retinal organoid - organoid similar to the retina

"PWM" - pulse width modulation - variable on and off cycles of light to adjust brightness

"IF" - Immunofluorescence

"ICC" - Immunocytochemistry

"MTT" - 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid

"APT" - Adenosintriphosphat - main energy source of living cells

"MSCs" - mesenchymal stem cells

"DSMO" - Dimethylsulfoxid

"$\Delta\Psi_m$" - mitochondrial membrane potential

"ROS" - are oxygen-containing molecules with a very high chemical reactivity

"G-CFS - Granulocyte-Colony Stimulating Factor - is a hormone that stimulates the production of white blood cells in the bone marrow

"NTP" - Network Time Protocol

"PFA" - Paraformaldehyde

"PBS" - Phosphate Buffered Saline

"FBS" - Fetal Bovine Serum

"TBST" - Tris-buffered saline + 0.1% Tween-20

"DAPI" – 4′ 6-diamidino-2-phenylindole

"AB" – Antibody

2.2 The Human Eye[GW]

The human eye consists of many different parts. The cornea focuses the light and allows it to enter. The iris regulates the amount of light that enters the eye. The lens bundles the light so that a sharp image can be formed on the retina. The exact structure can be seen in Figure 2.1.

Task of the retina is converting light into nerve impulses that can be transmitted to the brain. This happens due to the approximately 127 million receptors covering the retina. Cones are for seeing with colour and rods are for distinguishing between light and dark. The ciliary muscle ensures that the eye can focus at different distances. The macula is in the centre of the retina and contains more light receptors than anywhere else. Therefore, the vision is particularly sharp in this area. This point aligns itself automatically while looking around. [63]

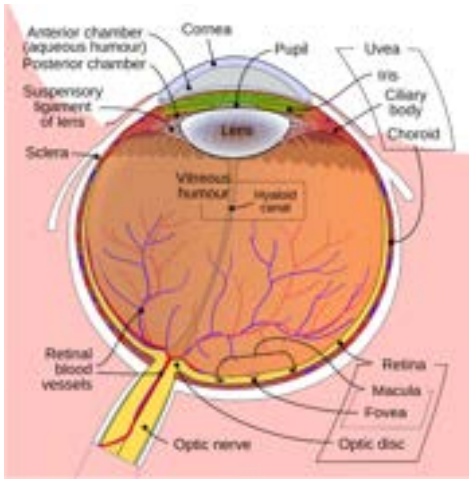


Figure 2.1: Structure of the Human Eye [58]

2.2.1 The Retina

The retina is divisible into four different cell-layers.

• pigment epithelium: responsible for supplying oxygen and nutrients to the retina and absorbing excess light.

• receptor layer: This is where the cones and rods sit. These transmit the light stimulations as a chemical signal.

• ganglion cell layer: Converts light stimulations into electrical signals that are transmitted to the brain. [61]

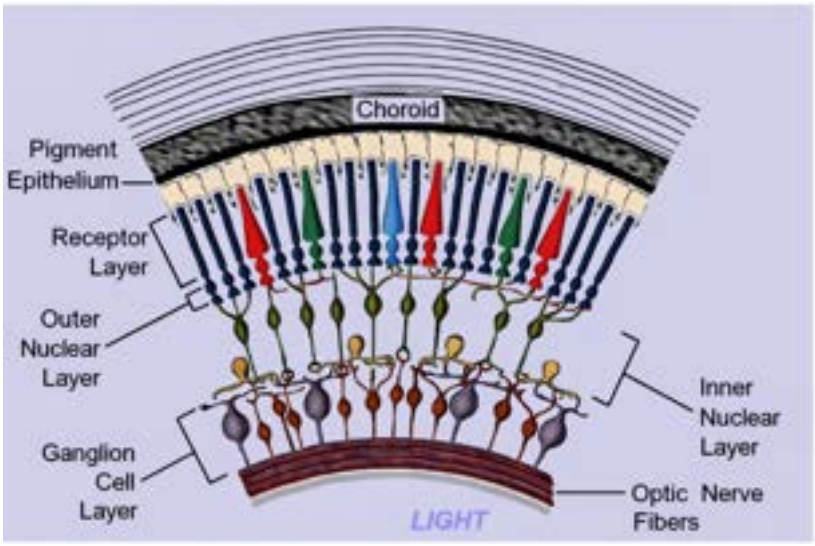


Figure 2.2: Retinal Structure [7]

2.3 Retinal Dystrophies[VV]

2.3.1 Age-related Macular Degeneration A(R)MD

AMD is considered as a leading cause of irreversible and currently uncurable vision loss, most common with people from the age of over 50 to 60. [15] Figure 2.3 shows a simulated view of a patient in advanced stages. It can appear in two different types: dry and wet. While d-AMD is the most frequent type, affecting about 80 – 90 percent of patients, w-AMD is much less common with about 10 – 20 percent affectivity. [34, 22] It is currently not known what exactly causes the disease, however some factors like smoking and genetics may have a negative influence engaging the risk of suffering from the disease. [48]



Figure 2.3: Simulated View of a Patient with Advanced AMD (right) Compared to Healthy Vision (left). [4]

Dry AMD

Dry AMD, also known as atrophic AMD, progresses slowly and is usually painless, as there are no pain receptors in the retina. [37, 21] In contrast to the wet form, vision loss occurs gradually rather than rapidly. Patients with d-AMD experience blurred vision or reduced central vision due to degeneration of the macula, the part of the retina responsible for sharp, detailed and color central vision. [34, 60, 41] The cause of the retinal degeneration is metabolic products, called drusen, accumulating as shown in Figure 2.4.

This damages the retinal pigment epithelium, a layer of cells that supports and nourishes the photoreceptors. In result, the receptors will be damaged or even die. Therefore, the vision will worsen. [34, 56, 41, 52] Since the disease often affects only one eye in early stages, the patient might not even notice any difference in their vision, because the other eye can

compensate the loss. For this reason, d-AMD often goes unnoticed at the beginning. It is currently researched about possible treatments for decelerating the progression of the disease, however, there hasn't been found a method to restore or repair the photoreceptors. Also, prevention does not guarantee absolute protection. [34, 41]

Wet AMD

Wet AMD can emerge out of d-AMD, but this happens in only 10 – 20 percent of affected individuals. [41, 37] However, it is the more acute version of AMD, which leads rapidly to complete vision loss if not immediately treated. It occurs when the body reacts to the undersupply of the RPE with a protein (VEGF) causing more blood vessels to grow. When those new vessels get too many, the new ones will grow abnormally beneath the macula, the choroid as shown in Figure 2.4. [48, 60] When those instable vessels start to leak blood or fluid, the macula will be severely damaged and the patient will lose vision. [34] Current treatments for the disease consist of periodic injections containing inhibitors of the protein that initiates the growth of vessels. [5, 40] This method, however, cannot restore dead photoreceptors but decelerate the progression of w-AMD. [48]

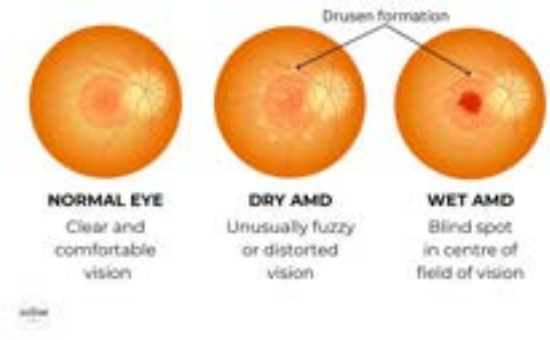


Figure 2.4: Left: Healthy Eye. Middle: Accumulation of Metabolic Waste Products (drusen) Beneath the Retina. Right: Abnormal Blood Vessels Growing Beneath the Macula and Leaking Fluid. [4]

2.3.2 Retinitis Pigmentosa (RP)

RP refers to a group of retinal diseases which can be inherited. Worldwide, it affects about 1 in 4,000 to 1 in 5,000 people, corresponding to around 1.5 to 2.5 million people. RP is caused by mutations in many different genes that are involved in the structure and function of photoreceptors. As a result of these mutations, photoreceptors become dysfunctional and gradually degenerate. Typically, rods are affected first, followed by cones. Due to this degeneration, patients experience vison loss, which is expressed as a gradual narrowing of the visual field resulting in tunnel vision and night blindness in early stages. Currently, gene therapy is available for the RPE65 gene mutation, which however is only one of many different genes which cause RP when mutated. [53, 36, 18, 23]

2.4 Stem Cells[GW]

2.4.1 What is a Stem Cell

Stem cells are cells, whose function has not yet been determined. When they divide, they can either remain stem cells or develop into other cell types with specific functions. Every stem cell has three specific characteristics. They are undifferentiated, which means they do not have specific functions, such as nerve cells that transmit impulses. Stem cells can self-renew and divide continuously. A single stem cell can give rise to millions of cells through repeated division. Another characteristic is that stem cells can develop into specialized cells. This process is called differentiation. To enable the cell to adapt to the required function, various genes are activated within the cells. These are called pluripotent. [44]

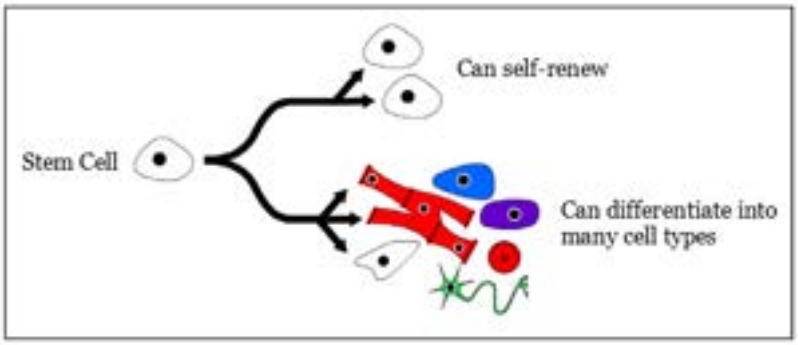


Figure 2.5: Stem Cell Development [44]

2.4.2 How to Generate Stem Cells

When stem cells are harvested from blood rather than bone marrow, the procedure is called peripheral blood stem cell transplant. Since these are present in the blood only in small amounts, the patient is given a medication, for example G-CFS, a few days before the collection to stimulate the stem cells to enter the bloodstream from the bone marrow. Since these are present in the blood only in small quantities, the patient is given a medication a few days before the collection to stimulate the stem cells to enter the bloodstream from the bone marrow. [28]

2.4.3 Reprogramming Stem Cells

In order for blood stem cells, they must then be reprogrammed. To do this, they are cultured in Petri dishes and treated with molecules, such as proteins or chemical substances. That send signals to activate gene expression and instruct the cells to divide and differentiate into various cell types. The whole system is modeled after the natural developing organism. After a few weeks, the cultured stem cells begin to resemble the cells they are meant to become. Once they are fully developed, these reprogrammed cells function just like “real” retinal cells and are ready for research. [32] This process is used to cultivate the RPE cells needed for research on retinal prostheses.

2.5 Retinal Prostheses - History[VV]

Retinal prostheses have been investigated since the 1960s. [6] There have been different approaches to restore vision irreversibly destroyed by retinal diseases since then. A primary distinction is made between classic electronic implants and organic retinal prostheses. The majority of those treatment approaches are not yet approved. However, gene therapy for the specific gene RPE65 causing retinal degeneration when mutated, is currently the only therapy approved and available. [66] Electronic implants, such as Argus II were approved, but are not available anymore due to suspending the manufacturing. [54, 51, 30]

Classic electronic implants often rely on external devices such as a camera to capture the surroundings and transmit the information into a small microchip implanted into the eye to pass on the information with electrical stimuli, simulating the photoreceptors. This method works because mostly only the photoreceptors are damaged and the remaining nervous system stays functional. However, inorganic materials such as microchips may increase the risk of inflammation. One of the reasons for this is the stiffness of the circuit boards with the microchips on them. [51, 35] Therefore, research has also moved towards stem-cell-based approaches which offer better biocompatibility. Pluripotent stem cells, which are either derived from embryonic stem cells or human cells that have been reprogrammed back into a stem-cell state (induced pluripotent stem cells), can be differentiated into retinal organoids *in vitro*. Those ROs contain different retinal cell types including photoreceptors. ROs can be used in two main ways. Either the whole organoid is transplanted to replace damaged retinal tissue, or photoreceptor cells are isolated from the organoid and transplanted to replace lost photoreceptors. Also, those organoids are used for modelling diseases for investigation, and many other tests can be performed with them *in vitro*. [10, 46]

Due to problems such as immune rejection and poor survival of transplanted retinal organoids, a new approach involves implanting organic semiconductor polymers that are light-sensitive, mechanically flexible, and biocompatible. These materials convert light into electrical signals that can stimulate the remaining retinal neurons and transmit visual information to the brain. In particular, the polymers D18 and Y6 are currently being investigated for this purpose. [25]

2.6 MTT-Test[GW]

The MTT-assay [(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid] is a widely used method in research to determine cell viability and proliferation. An enzymatic reaction of the cells is used. Only living cells can convert the yellow tetrazolium salt-MTT into violet coloured formazan crystals. This reduction is mainly carried out by succinate dehydrogenase. Succinate dehydrogenase is an enzyme in the mitochondria. If it is very active, this indicates that the cell has a very active metabolism, produces a lot of energy, and is healthy. Therefore, the intensity of the formazan formed can be used to determine the number of living cells. The intensity is determined spectrometrically at a wavelength of approximately 570-590 nm. For correct measurement results, it is very important that the formazan dissolves completely before measurement. This test is very sensitive to cell density and incubation time. These must be adjusted for each cell type. [1]

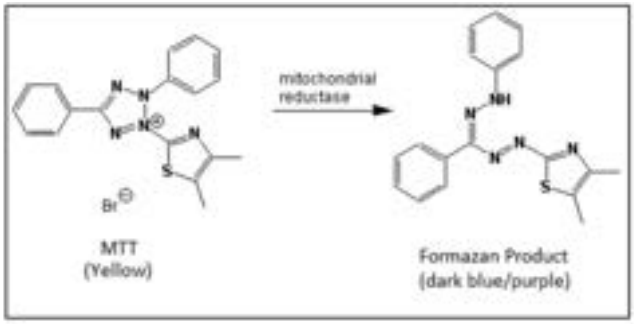


Figure 2.6: MTT Reaction [11]

It is often used to obtain information on toxicity as a summary parameter. Since the intensity of the staining correlates with the number of living cells you can tell by the intensity of the solution if the material killed many of the cells or if it is harmless. This is particularly useful if many toxins or unknown substances are expected in the sample. [33]

For the investigation of organic photovoltaic semiconductor materials for retinal prostheses, these were also subjected to a cytotoxicity test. The human RPE cells were grown directly on the materials D18 and Y6, then the cytotoxicity of these materials was tested using the MTT assay. For the retinal prosthesis a mix between D18 and Y6 is used, because these materials cover pretty much the entire transmission range of the human retina, shown in Figure 2.7. [20]

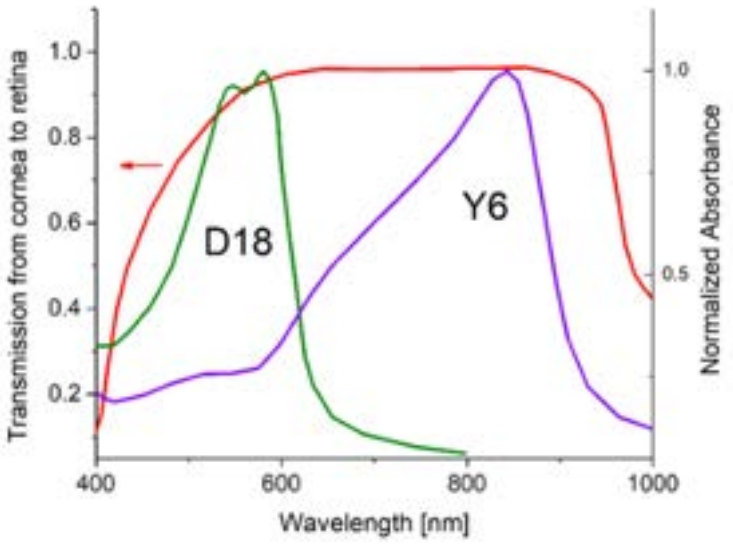


Figure 2.7: Comparison Transmission from the Cornea to the Retina of a Human Eye and Absorbance of D18 and Y6 [20]

While working with photovoltaic materials it is important to keep in mind that the materials can release coloured substances or absorb light. This could affect the intensity or colour of the MTT-solution before testing and so as to distort the results of the MTT-test.

2.6.1 Organic Photovoltaic Material D18

The material D18 is based on a fused-ring thiadiazole unit. It has a large, rigid, and planar molecular structure and contains alkyl side chains for good solubility and film processing. [42] This is particularly important because the material is used for *in vitro* experiments and must be vapor-deposited onto glass coverslips. The material has locomotion ($1.6 \times 10^{-3}\,\mathrm{cm^2\,V^{-1}\,s^{-1}}$), meaning how fast positive invoices move in the material. The higher the value, the better the current flows. In addition, strong lamellar and π–π stacking efficiently transport charges within the material. [27]

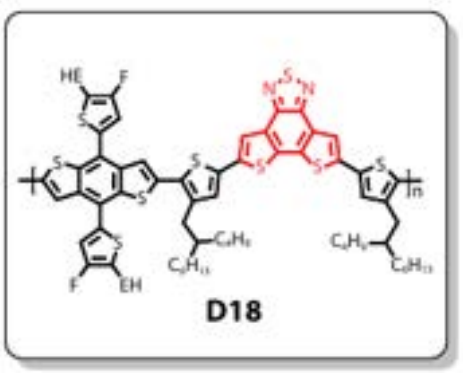


Figure 2.8: Structure D18 [42]

2.6.2 Organic Photovoltaic Material Y6

The material Y6 belongs to the class of non-fullerene acceptors. This means that they have acceptor molecules and are therefore very effective and have good absorption. Also, Y6 has low energy loss and efficient electron transport because of the 3D networked electronic coupling and the π–π stacking. Like D18, it is also highly soluble and forms a good film due to its branched alkyl chains. [38, 68]

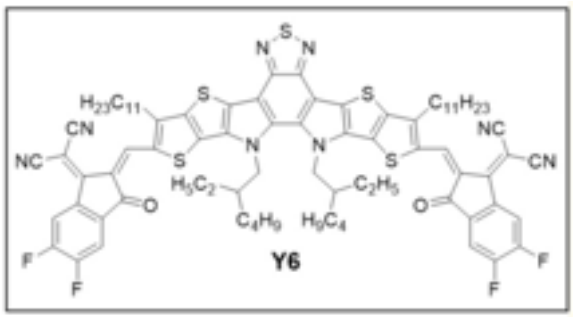


Figure 2.9: Structure Y6 [68]

2.7 Immunofluorescence[VV]

Immunocytochemistry (ICC) refers to a group of techniques that use antibodies to detect specific proteins within individual cells. Immunofluorescence (IF) is a specific type of ICC in which antibodies are conjugated to fluorescent dyes, known as fluorophores, allowing the visualization of target proteins in cells or tissue samples using a fluorescence microscope. This enables the determination of the presence and localization of specific proteins within the sample. [47, 19]

2.7.1 Immunofluorescence Staining Methods

In IF staining, the primary antibody binds specifically to the target protein, the antigen. Two main approaches can be used: direct and indirect immunofluorescence as shown in Figure 2.10. In direct IF, the primary antibody is directly conjugated to a fluorophore. In indirect IF, an unlabelled primary antibody first binds to the target antigen. After washing away unbound antibodies, a fluorophore-labeled secondary antibody is added, which binds to the primary antibody.

The fluorescent signal can then be detected using a fluorescence microscope, allowing the analysis of protein localization and cellular morphology. [19, 39, 16] Counterstaining is an additional fluorescent staining step used after immunofluorescence labelling to visualize other cellular structures such as nuclei or cytoskeletal components. Common counterstains include DAPI and Hoechst which bind DNA and stain nuclei blue. When designing an experiment, it is essential to choose fluorophores with minimal spectral overlap to ensure that counterstains and target signals can be clearly distinguished. [13, 24]

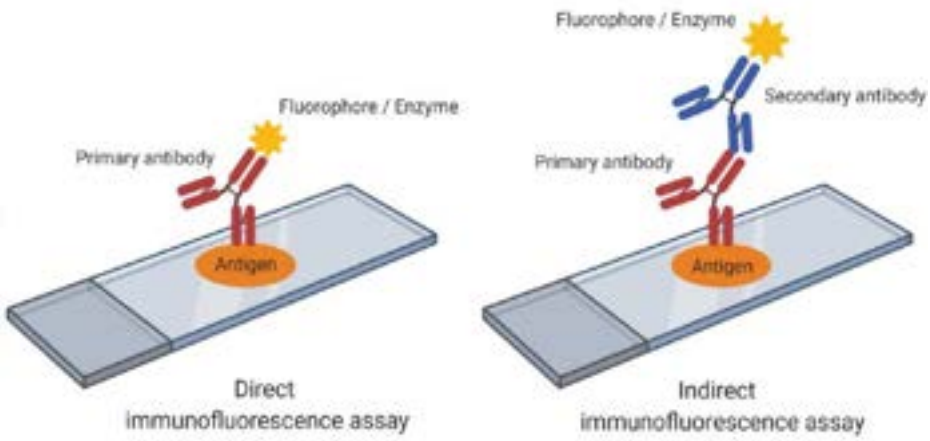


Figure 2.10: Difference between Direct and Indirect IF Assay. [12]

2.7.2 Process

Figure 2.11 shows the workflow of IF. It is an essential first preparation step to **fix** the sample cells before performing IF to immobilize the target proteins and preserve the cell morphology. Without fixation, cells can undergo autolysis, which means self-degeneration after death and therefore losing their structure, meaning that immunofluorescence analysis would become unreliable. [39, 59, 62, 59]

As a second step, **permeabilization** is performed to open the cell membrane by partially disrupting its lipid layer, allowing the large antibodies to access intracellular proteins.

As the next step, **blocking** is performed to minimize non-specific binding of antibodies and reduce background signals. This involves treating the sample with a protein solution, normal serum, or a commercial blocking buffer, which covers non-target sites but leaves the target proteins available for the antibodies to bind. The choice of blocking reagent depends on the antibody type and sample. [39, 59, 62]

Following sample preparation, the **staining** steps are carried out. First, the **primary** antibody is applied, which binds specifically to the target protein. After sufficient incubation, the sample is washed several times with buffer to remove any unbound primary antibody. In the case of indirect immunofluorescence, a fluorophore-labeled **secondary antibody** is then applied to bind the primary antibody, followed by additional washing steps to remove excess secondary antibody. For **direct immunofluorescence**, this secondary antibody step is omitted since the primary antibody is already labelled. After antibody staining, **counterstaining** can be performed to visualize additional cellular structures. [19, 39, 16, 13, 24]

Lastly, the sample is **mounted** to keep it hydrated and to prepare it for microscopy. Mounted slides can be stored in the dark at $-20,^{\circ}\,\mathrm{C}$ for several months. However, the fluorescence intensity gradually decreases over time, even when anti-fading reagents are present in the mounting medium. [14, 2, 3]

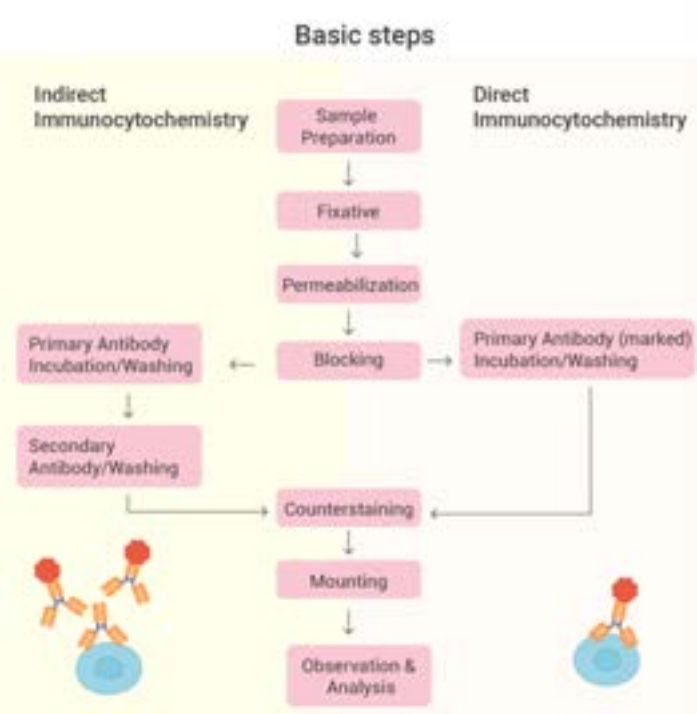


Figure 2.11: Process of ICC. [49]

2.7.3 Antibody Selection and ICC Reagents

The reagents and antibodies used in this study were provided by Assoc. Prof. Ahmad Salti and were selected to analyze RPE cell identity, proliferation, and structure.

Samples

The cell samples on coverslips were received already **fixed** and stored in 12 well plates as shown in Figure 2.12. Originally, 4 coverslips were stored in the 12 well plate for each sample type (D18/Y6, Terpanol).

Fixation had been performed using 4% paraformaldehyde (PFA) in 1× (already diluted accordingly) Phosphate Buffered Saline (PBS) for 10–15 minutes at room temperature. PBS buffers the pH to help preserve cellular structures. PFA is specifically suitable for cytoskeletal and intracellular proteins. After fixation, the coverslips were washed carefully three times for 5 minutes each with PBS (500μl per wash). The fixed samples were stored in PBS at 4°C sealed with Parafilm.

Two sets of cells were prepared this way: one grown on D18/Y6 and one on Terpanol before fixation. [19]

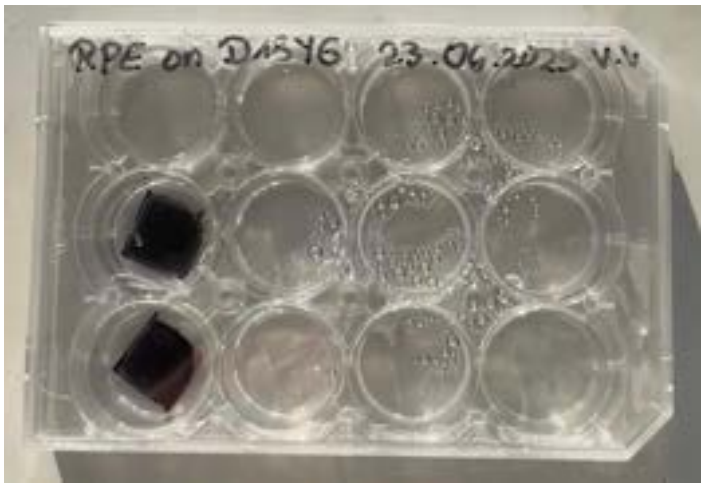


Figure 2.12:
D18Y6 with Fixed RPE Cells in 12 Well Plate (Sample)

Preparation

For **permeabilization**, 0.3% Triton X-100 in TBST was used, as it is specifically suitable for cytoskeletal and intracellular proteins.

For **blocking**, 10% FBS in TBST + 0.025% Triton-X-100 was used. FBS contains proteins that bind to nonspecific sites, which helps to reduce background staining. [19]

Antibodies

Primary AB/ Fluorescent Dye	Secondary AB	Target	Fluorophore
Phalloidin*	—	cell structure	red
rabbit Ki67	anti r488*	cell proliferation	yellow
mrpe65	anti m664*	identify RPE cells	green
DAPI	—	cell nucleus	blue

Table 2.1: Antibodies and Markers used for ICC

*Phalloidin conjugated 555
*Alexa 488 anti-rabbit
*Alexa 647 anti-mouse

Phalloidin marks the cytoskeleton. It is a network of protein filaments that determines cell shape and enables cell movement. Phalloidin binds directly to actin filaments and produces a fluorescent signal through its conjugated fluorophore.

Ki67 is a nuclear protein that is only found in dividing cells and is therefore used as a marker for cell proliferation.

mrpe65 specifically marks RPE cells and is used to identify, count, and determine their localization within the sample.

DAPI is not an antibody but a fluorescent dye used for counterstaining as it marks all cell nuclei (not only RPE cells). [19, 3]

This specific selection of antibodies and fluorescent dyes was used to analyze the effects of D18/Y6 and Terpanol on RPE cells. Using these markers, it is possible to determine whether cells are proliferating, to evaluate changes in cell structure, to identify RPE cells, and to visualize all cells within the sample.

2.8 Light Stimulation for Organisms - History[GW]

Light is an important component in the lives of many organisms. Especially for plants, which use light for photosynthesis; without it, they would not be viable. Human cells are particularly affected by infrared light, which penetrates deep into the tissue, right into the mitochondria. Typical effects include increased ATP production, promotion of cell growth and regeneration. [31]

There are studies that prove that low-power laser irradiation, at a wavelength of 532 nm and a power of 30 mW, promotes the proliferation of mesenchymal stem cells (MSCs) and cardiac stem cells, among other cells. Low-intensity red and infrared light acts on the cells via a primary photoacceptor. The absorption of light by cytochrome c oxidase increases $\Delta\Psi_m$, ATP and ROS, which leads to greater energy availability and signal transduction. These cellular changes can lead to increased cell growth. [67]

This type of light irradiation has also been tested on fungi. These were exposed to red, green, and blue light at various intensities for 14 days. They showed changes in growth, spore formation, pigmentation, and fungal morphology. They built a customized light box to provide optimal lighting for the mushrooms in Petri dishes. This could be adjusted using specific software. This light box was used as inspiration for the Luminacell-Biostim device, with some improvements and adjustments to meet the specific needs of the university.

As can be seen in Figure 2.13, there is no way to directly adjust settings such as exposure time, wavelength, intensity, and the like on the device. With the Luminacell-Biostim, this is possible via touch buttons and a built-in display. In addition, the Petri dishes in the device shown are illuminated from above, which can cause light scattering. To prevent this, the light in the Luminacell-Biostim comes from below, ensuring that the maximum possible beam power reaches the cells.

The Luminacell-Biostim is extra user friendly, due to its built-in positioning aid and battery operation. This means that the device can be used in any laboratory, regardless of power outlets. Because of the open design of the Luminacell-Biostim a ventilator with a kind of climate chamber, seen in Figure 2.13, is unnecessary. [55]

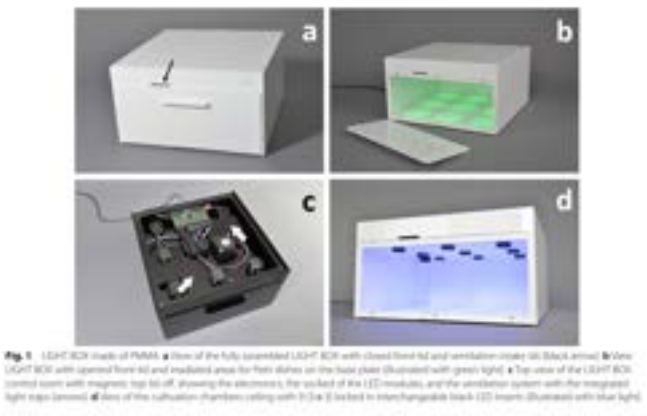


Figure 2.13: Light Box that was used as Inspiration for Luminacell-Biostim [55]

2.9 Device Requirements[VV]

The device must be able to operate reliably in the high-humidity environment of a cell incubator, with a fully sealed enclosure that prevents any moisture from reaching the electronics. The housing should be easy to disinfect from the outside, as autoclaving is not possible due to the sensitive electronics. It should consist of materials that are resistant to disinfection agents. The system should be completely wireless to fit inside the incubator and designed for user-friendly operation, allowing non-technical personnel to use it without difficulty. It must provide adjustable and precise wavelengths suitable for specific cellular applications, with accurate, programmable illumination durations and automatic shutoff once the settings are applied to save battery over time. Light intensity should be calibrated and reported in W/cm^2. PWM control should be carefully considered, as certain cell types may respond to light flickers. The device should ensure homogeneous illumination across all wells to avoid variability in experimental conditions. Heat generation should be minimalized to maintain stable culture environments. The overall design must prioritize user safety and robustness, preventing any electrical hazards in a humid environment.

2.10 Sterile Working[VV],[GW]

The cells worked with are very sensitive; if they get contaminated they are dead and useless for experiments. Therefore, it is very important to work in a sterile environment, including a laminar flow hood. The cells should be stored in a sterile environment, in the best case an own incubator only for the RPE cells is used. For every work step which involves direct or indirect contact with the cells, it is important to wear a clean lab coat, gloves, and, if necessary, shoe covers and to regularly disinfect your hands.

When working in the laminar flow hood, first, everything must be cleaned with 70% ethanol. Then everything that goes under the hood needs to be disinfected again. Proper work planning and having all necessary utensils ready reduces the risk of contamination under the hood. When unscrewing bottles or containers, the lid should always be held in the hand with the opening facing downwards. It is best not to put it down during the work process. After use, the bottles should be sealed with parafilm.

For the pipette tips, centrifuge tubes, culture bottles, well plates, and similar products used in contact with the cells it is important that they are sterile, tested disposable materials. If this is not possible, autoclaving is also an option.

3 Practical Work

The following methods were carried out according to instructions from Assoc. Prof. Ahmad Salti, PhD. All following steps were performed under sterile condition as in Section 2.10.

3.1 Growing Cells[GW]

3.1.1 Materials

- RPE cells [Retinal Pigment Epithelia cells]
- Dmem media [Dulbecco's Modifies Eagle's Medium]
- RPE media [Retinal Pigment Epithelium Medium]
- Centrifuge [Hettich Universal 320]
- Eppendorf pipettes + tips
- Cell counting chamber (optional)
- Cultivating flask

3.1.2 Culturing Cells

In order to be able to grow the cells under sterile conditions, it is important to clean the working environment and prepare everything in the first step. Then fill a flacon tube with 9 ml of DMEM medium. This should be at room temperature. The cells can now be removed from the -80°C freezer and thawed by rolling them in the palms of your hands. It is important not to thaw the cells completely, a small block of ice should remain in the tube.

Then the 1ml of cells can be pipetted into the tube to the medium. Normally, the cells should be counted using a cell counting chamber, because the quantity should be 500.000 cells/well. To be on the safe side, we took the entire cell mass. Since the cells should not heat up during centrifugation, we repeatedly cooled the centrifuge with ice cubes before using it. This mixture is now centrifuged at 200g for 3 minutes, in our case more times because no pellet formed. The used centrifuge only works with rpm, so it was converted using the radius [r=9cm].

$$\text{rpm} = \sqrt{\frac{g}{r \times 1.118 \times 10^{-5}}} \tag{3.1}$$

Table 3.1: cell seeding rpm/minute

rpm	Min
1330	3
1330	3
1500	3
2000	3



Figure 3.1: Cell Pellet

Once a pellet formed, as in Figure 3.1, the supernat can be aspirated carefully. The remaining pellet is now dissolved in 10 ml of RPE medium. The RPE medium is DMEM media with a small amount of fetal bovine serum and the antibiotic penicillin-streptomycin. It is there to provide the cells with additional nutrients during growth. To produce 50ml of RPE media 44,5 mL DMEM media, 5 mL FBS 10% and 500 µL Pen/Strep are mixed. The resuspended pellet can then be pipetted into the culture bottle, seen in Figure 3.2. The cells are now ready to grow.

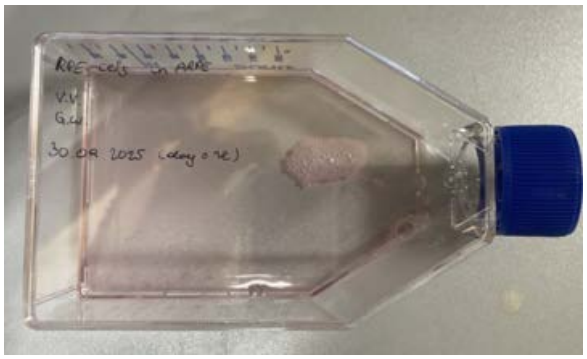


Figure 3.2: RPE Cells in Cultivating Flask

After thawing, the media needs to be changed the next day to get rid of Dimethylsulfoxid (DSMO) found in freezing media because this gets harmful for the cells during prolonged contact in thawed state. It is used in combination with media, when freezing cells to prevent the formation of crystals, which could destroy the structure of the cells. [57] The media needs to be changed every second day for 10 days.

After 10 days the cells are ready for the MTT-Test [3.6] or for fixing if you want to perform an ICC-test. The confluency of the cells can be checked under the microscope. Once it reaches 70-80%, the cells can be passaged. In addition, a microscope can be used to check whether the cells have their typical shape. This can provide information about whether they are healthy or not. Figure 3.3, taken with the university's microscope, shows how the RPE shape typically look when they are healthy.

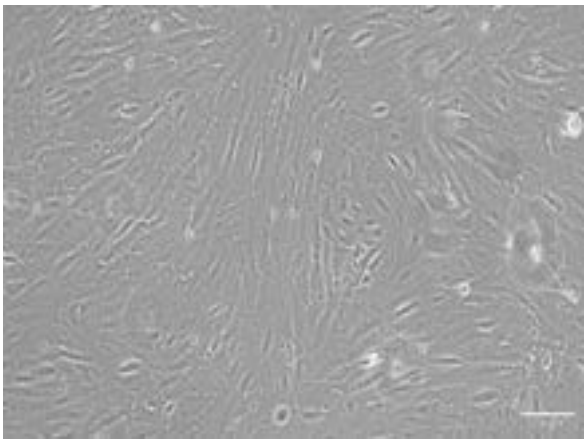


Figure 3.3: RPE Cells - 10 Days Grown

Unfortunately, the magnification of the HTL microscope is too low to be able to examine the cells in enough detail. Therefore, we can only estimate the confluence.

3.1.3 Seeding Cells in Direct Contact with Materials

If cells need to grow in direct contact with certain materials, for example, to test their cytotoxicity, a few procedural steps will be modified. The steps remain the same until resuspension. However, it is then important to determine the exact cell count to ensure that there are enough cells in each well. To do this, mix 10 µl of the resuspended pellet with 10 µl of trypan blue and place it in a counting chamber. This can be used to calculate how many ml/µl of the cell mixture should be added to each well if a specific population density is to be achieved there. The materials to be tested must first be added to the well plate into which the cells will later be placed. In our case, these were vapor-deposited onto glass coverslips. Place those into the wells with the material facing upwards. The exact amount can then be filled into the wells with the corresponding amount of RPE medium, seen in Figure 3.4. In our case, 500µl and 500µl.



Figure 3.4: Seeding Cells on Material

3.1.4 Media Change

Materials

- DMEM media
- Aspirator/Eppendorf pipette

Execution

Before changing the medium, it is important to sterilize everything as usual and prepare the media at room temperature. When performing the change, the cultivating flask must be held at an angle to be able to aspirate all the old media without absorbing the cells. Also, it is important to not touch the ground of the flask to prevent the pipette tip from dislodging or damaging cells from the bottom. And make sure to never touch the bottle with the pipette tip when filling the new media, because this could affect sterility. The flask is held at an angle, and the old media can be carefully removed, like shown in Figure 3.5. If you can't get everything out, it's better to leave a small amount behind than to damage the cells by scraping the bottom. Once the old media is aspirated, the same amount of new media (10ml) can be filled.



Figure 3.5: Media Change of RPE Cells

3.1.5 Passaging

Material

- DMEM media
- Trypsin
- PBS [phosphate buffered saline]
- Aspirator/Eppendorf pipette
- Centrifuge
- cultivating flask / well plates

Execution

In order to passage the cells, they must first be detached from the bottom. This is done with the help of trypsin. First, the old medium must be aspirated from the cultivation bottle. Then 1ml of phosphate buffered saline is added to clean the cells from the media. Then the trypsin is added (the amount is any desire). The plate or flask is then incubated for 5 minutes, after this time it should be examined under a microscope to see whether the cells have detached.

This can be seen when you shake the bottle and the cells inside move along with it. If they haven't detached, then you can incubate a little longer. When detached, DMEM medium twice the amount of trypsin is added to deactivate the trypsin. (500µl trypsin – 1ml media) This mixture can then be poured into a centrifuge tube and centrifuged for 5 minutes at 300g with 25°C. The supernat can be removed and the pellet resuspended in 1ml of media. The cell count should be determined using a cell counting chamber and then diluted accordingly.

Counted number: 25×10^4 cells/mL $= 3 \times 10^5$ cells/mL

Need: 1×10^4 cells/100 μL $\Rightarrow$ Dilution 1:3

Calculation: 3×10^5 cells/mL $\Rightarrow$ 1 mL $= 1 \times 10^4$ cells in 100 μL

The appropriate number of cells can now be added to the new cell bottle or well plate with medium. The cells can then continue to grow normally. The medium is then changed again every 2 days.

3.2 MTT-Test[GW]

3.2.1 Materials

- Trypsin
- DMEM media
- MTT solution
- 0.05% TritonX in MEM
- Isopropanol >95%
- 70% Ethanol for disinfection
- Eppendorf pipettes
- Centrifuge
- Incubator
- Microplate reader

3.2.2 Execution

In order to perform the MTT-Test, we allowed the cells to grow for 14 days. The time may vary, the main thing is that the cells are confluent. Since the MTT solution is light-sensitive, it is important to work in a dark room and also to switch off the light in the hood. The medium should be brought to room temperature beforehand and the trypsin (stored at -20°C) thawed.

Since the cells have grown in a culture bottle, they must first be dissolved. To do this, remove 10 ml of medium and add 5 ml of trypsin. This must be swirled well to dissolve all cells from the bottom. This liquid can then be placed in a centrifuge tube with 10 ml of DMEM. The double amount of medium deactivates the trypsin.

This cell suspension then is centrifuged for 3 minutes at 200g. Since our centrifuge can only be set in rpm, we used this formula:

$$\text{rpm} = \sqrt{\frac{g}{r \times 1.118 \times 10^{-5}}} \tag{3.2}$$

to convert g into rpm. r is the radius of the centrifuge, in our case r was 9 centimetres. Since the cells should not become too warm during centrifugation, we cooled the centrifuge with ice cubes. Unfortunately, no pellet had formed after 1x centrifugation, so we tried 2 more times, with increased rpm.

Table 3.2: MTT-Test rpm/minute

rpm	**Min**
1330	3
2200	3
2500	3

Once a pellet has formed, Figure 3.6, we removed the supernatant, and resuspended the pellet in 10 ml DMEM. The optimal cell number for seeding would be 50.000 cells/well, since we don't have a counting chamber, we took 1/3 of the total pellet which will then be divided into 9 wells. 1/3 equals 3.33ml of the 10ml, these were centrifuged again for 3 minutes at 2350rpm.



Figure 3.6: Cell Pellet MTT-Test

The supernatant is discarded, and the pellet is resuspended in 3.33 ml DMEM. The solution is now divided among the 9 wells = 360µl/well. Then 500µl of MTT-solution are added into every well.

For the positive control wells 500µl of 0.05% TritonX in MEM are added into two empty wells. The well plate is now incubated at 37°C in the dark for 1.5 hours. During this time, formazan crystals form.

After these 1.5 hours, the MTT solution can be carefully removed from the wells. This must be done with extreme caution, as otherwise the crystals that have formed could be sucked out as well. The formazan crystals formed are insoluble in water, so they are dissolved with 1 ml of isopropanol/well.



Figure 3.7: Dissolved Formazan Crystals

Now the absorption can be measured in the microplate reader, Figure 3.8. Our microplate

reader can only read 96 well plates, so we split each well into 6 wells of the 96 well plate (150µl). We read the absorbance of our test at 562nm.



Figure 3.8: Microplate Reader EZ Read 800 Plus

3.3 ICC-test[VV]

3.3.1 Materials

Samples
For sample preparation see Section 2.7.3

- RPE cells grown on D18/Y6 (fixed)
- RPE cells grown on Terpanol (fixed)

Blocking and Permeabilization

- blocking solution: 10% FBS in TBST + 0.025% Triton-X-100
- permeabilization solution: TBST + 0.3% Triton-X-100

Antibodies
For further information about antibody selection see Section 2.7.3

- primary: Ki67 - secondary: anti r488
- primary: mrpe65 - secondary: anti m664
- Phalloidin
- DAPI (300nM in TBS)
- dilute ABs in incubation solution: 5% FBS in TBST + 0.025% Triton-X-100
- washing solution: TBST + 0.025% Triton-X-100

Sterile Working Conditions

- 70% Ethanol

Additional Devices

- tweezers
- Eppendorf pipettes
- water (deionized water through Filter 0,2µm)
- PBS
- glass coverslips
- glycerin
- polyethylene glycol 400

3.3.2 Execution

All following steps were performed under sterile condition as in Section 2.10. Once secondary antibodies and fluorescent dyes were applied, the samples were protected from light by closing the blinds and turning off the lights to prevent photobleaching and the resulting loss of fluorescence signal. All steps were performed for both 12 well plates with each containing four samples separated in four wells.

Permeabilization and Blocking
As the cell samples were received already fixed and stored at 4°C in the fridge, the procedure started with sample preparation. First, the PBS in which the cells were stored was carefully removed using Eppendorf pipettes to avoid damaging the cells. For further preparation, the blocking and permeabilization solutions were thawed from -20°C.

For the permeabilization step, 190μl (to cover the samples completely) of permeabilization solution was added to each well with a cell sample. The solution was carefully added at the edge of the well to avoid direct contact with the cells, as this could cause them to detach. The samples were then incubated for 15 minutes at room temperature.

After incubation, the incubation solution was removed using Eppendorf pipettes again. For the blocking step, 190μl of blocking solution was carefully added to each well with a cell sample. The samples were incubated for two hours at room temperature.

Antibodies
First, a dark environment was established. The incubation solution, primary antibodies and Phalloidin were removed from the fridge and thawed from -20°C. The aluminium foil used to protect the Phalloidin from light was then removed.

The antibodies were diluted in incubation solution as follows: mrpe65 (1:1000), Ki67 (1:1000), and Phalloidin (1:2000). Accordingly, 1.6μl of mrpe65, 1.6μl of Ki67, and 0.8μl of Phalloidin were added to 1600μl of incubation solution. This antibody cocktail was prepared for eight wells, with 200μl per well.

First, the blocking solution was removed with Eppendorf pipettes. Then, 200μl of the antibody cocktail was carefully added to each well containing a sample. The samples were

incubated overnight at 4°C. To protect them from light, the well plates were wrapped in aluminium foil.

On the next day, the antibody cocktail was removed using Eppendorf pipettes. To remove any remaining primary antibodies, 500μl of washing solution was added to each well containing a sample. The well plates were carefully moved in a circular motion to wash away any residues without damaging the cells. The samples were then incubated for 5 minutes at room temperature. After incubation, the solution was removed again. This washing step was repeated three times.

For the secondary antibody cocktail, the antibodies were diluted in incubation solution as follows: anti r488 (1:1000) and anti m664 (1:1000). Accordingly, 1.6μl of each antibody was added to 1600μl of incubation solution, resulting in a total volume sufficient for eight wells with 200μl per well. The secondary antibodies were then carefully added to the samples and incubated for one hour at room temperature in the dark.

After incubation, the secondary antibody solution was removed using Eppendorf pipettes. To remove any unbound secondary antibodies, 500μl of washing solution was added to each well. The well plates were carefully moved in a circular motion to wash away residues without damaging the cells. The samples were then incubated for 5 minutes at room temperature and after that, the solution was removed again using Eppendorf pipettes. This washing step was repeated three times.

Lastly, the samples were incubated for 5 minutes at room temperature in the dark with 200μl DAPI solution per well.

Finally, the samples were washed three times with 500μl of washing solution. Next, the samples were intended to be washed with 500μl ddH_2O. However, deionized water filtered through a GD/X Sterile 0.2μm CA Filter Media was used instead, as ddH_2O was not available.

Mounting
For mounting, a solution of 9:1 glycerin and PBS was used, as no commercial mounting medium was available. Alternatively, a 9:1 mixture of polyethylene glycol 400 and PBS was used. This should prevent the samples from drying out and to reduce fluorescence fading. PBS served as a buffering agent to maintain stable conditions.

For mounting, the samples on glass coverslips were removed from the well plates using tweezers. For each sample, two coverslips were mounted using the glycerin solution and two using the polyethylene glycol solution. For mounting, one drop of the mounting media was placed onto a glass slide, and the coverslip was positioned with the cells facing downward into the mounting medium as shown in Figure 3.9.

The mounted samples were stored flat at 4°C in a slide box wrapped in aluminium foil to prevent fluorescent fading.



Figure 3.9: RPE Cells on D18/Y6 Stained and Mounted on Coverslip with Polyethylene Glycol Solution

Observation The samples were analyzed after being stored for two weeks. Fluorescence microscopy was performed at JKU using a DMi8 microscope (Leica Microsystems). For imaging, immersion oil type G (Leica Microsystems) was used to improve image quality. It was started with low-energy filters to avoid bleaching.

3.4 Housing[GW]

3.4.1 Housing Construction

For the housing it was important to choose a material that can be disinfected easily and many times without dissolving. Since it is kept in an incubator with high humidity for long periods of time, it is important that the material is durable against water damp. PETG brings all the characteristics that are needed. 3D printing allows for great flexibility in terms of structure.

The housing has space inside for the capacitive buttons. These can be glued in from the inside, just like the display. A glass panel glued to the outside of the display ensures a seamless transition. The glass plate, on which the well plate can later be placed, is attached to the offset using two-component adhesive.



Figure 3.10: Front View Housing, Created with Fusion 360



Figure 3.11: Bottom View Housing, Created with Fusion 360



Figure 3.12: Bottom View Lid, Created with Fusion 360

A centering plate is installed, to ensure perfect alignment of the LED rings with the 6 well plate. The LED rings are attached to this plate.



Figure 3.13: Side View Centering Disc, Created with Fusion 360

The bottom can be unscrewed, as the box has to be opened repeatedly to load the battery. A seal inserted within the framework, ensures that it is watertight.



Figure 3.14: Top View Basement, Created with Fusion 360

3.4.2 Spectral Analyse

A simple glass plate serves as a layer between the electronics and the well plate. Spectral analysis was used to measure that the absorption of the glass plate in combination with the well plate does not affect the incoming wavelength and does not affect the irradiation of the cells, these can be seen in Figure 3.15.

The head of the spectroscope is attached directly behind the glass and the well plate, the light ring was installed behind the glass plate.

Table 3.3: Wavelength Ranges [50] and Measured Values

Color	**Wavelength (nm)**	**Measured wavelength (nm)**
blue	420–500	450–480
red	600–780	620–640
green	500–570	510–540
white	410–780	410–640

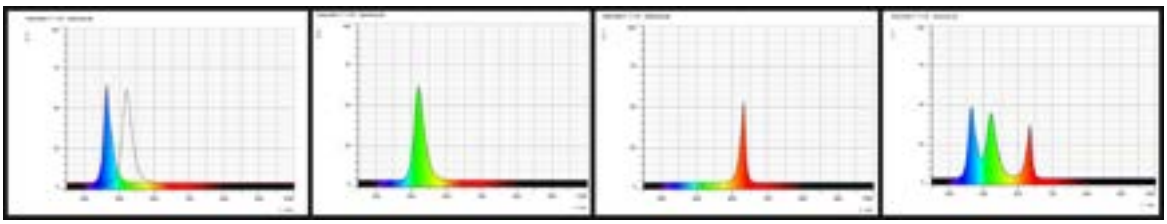


Figure 3.15: Spectral Analyses

From this results, can be concluded that the glass and the well plate absorb so little light that it is negligible.

3.4.3 Capacitive Buttons

Since the housing should be as sterile and as little contamination as possible should remain on the surface for long periods of time. It is important to incorporate as few recesses and edges as necessary. That's why capacitive buttons are best, as they respond to touch and can be installed under the housing.

The ESP32 has integrated capacitive touch sensors. These sensors are capable of waking the ESP32 from deep sleep. [9]

Table 3.4: Capacitive Touch Sensors of the ESP32, Seen in Figure 3.16

GPIO	Capacitive Touch Pin
GPIO 0	Yes (T1)
GPIO 2	Yes (T2)
GPIO 4	Yes (T0)
GPIO 12	Yes (T5)
GPIO 13	Yes (T4)
GPIO 13	Yes (T4)
GPIO 14	Yes (T6)
GPIO 15	Yes (T3)
GPIO 27	Yes (T7)
GPIO 32	Yes (T9)
GPIO 33	Yes (T8)



Figure 3.16: Pinout ESP32 [64]

We use copper plates as buttons. The limits of capacitive buttons can be tested using a simple test program.

```
const int touchPin = T0;    // alternativ z.B. T3, T5, T7 ...
const int uG = 980;
const int OG = 1050;

void setup() {
  Serial.begin(115200);
  delay(1000);
  Serial.println("ESP32 Touch Sensor Test");
}

void loop() {
  int touchValue = touchRead(touchPin);

  Serial.print("Touch Wert: ");
  Serial.println(touchValue);
  delay(1000);    // 5 Messungen pro Sekunde

  if(touchValue >= OG)
  {
    Serial.println("Knopf nicht gedrückt");
  } else if(touchValue <= uG)
  {
    Serial.println("Knopf gedrückt");
  } else {
    Serial.println("Keine Auswertung möglich");
  }

  delay(1000);
}
```

Listing 3.1: ESP32 limits capacative buttons - test programm

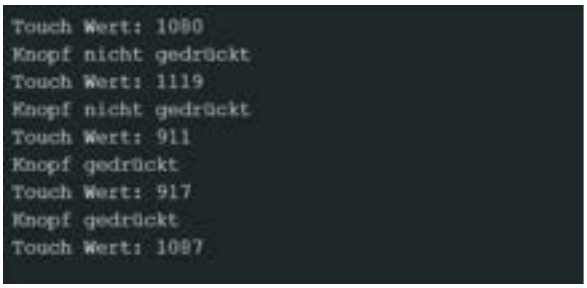


Figure 3.17: Limits Capacative Buttons

Because of this results a button counts as pressed when the value is <= 980, untouched counts from >=1050.

3.5 Electronic Structure[GW]

3.5.1 Components and their Function

- LED-rings
- 3,7V Lithium-ionen-battery
- ESP32
- dc-dc step up module
- oled-display (I2C)

The led-rings are connected to a one-wire data bus. This allows us to connect the rings in series. Because these rings need a voltage of 5V and the lithium-ionen-battery delivers only 3.7V, a dc-dc step up module is used. The module converts the voltage to the required level. The ESP32 is also supplied with 5V, it supplies the data for the LEDs and controls our program. An OLED display with I2C bus is used as the output device.

Since the circuit is located in an incubator, it is permanently exposed to increased humidity, we attempted to seal the housing with gaskets.

The battery can be easily charged via a USB-A port using a charging board. To prevent incorrect connection, polarity-protected plugs were retrofitted to both the battery and the charging plug.

3.5.2 Schematic

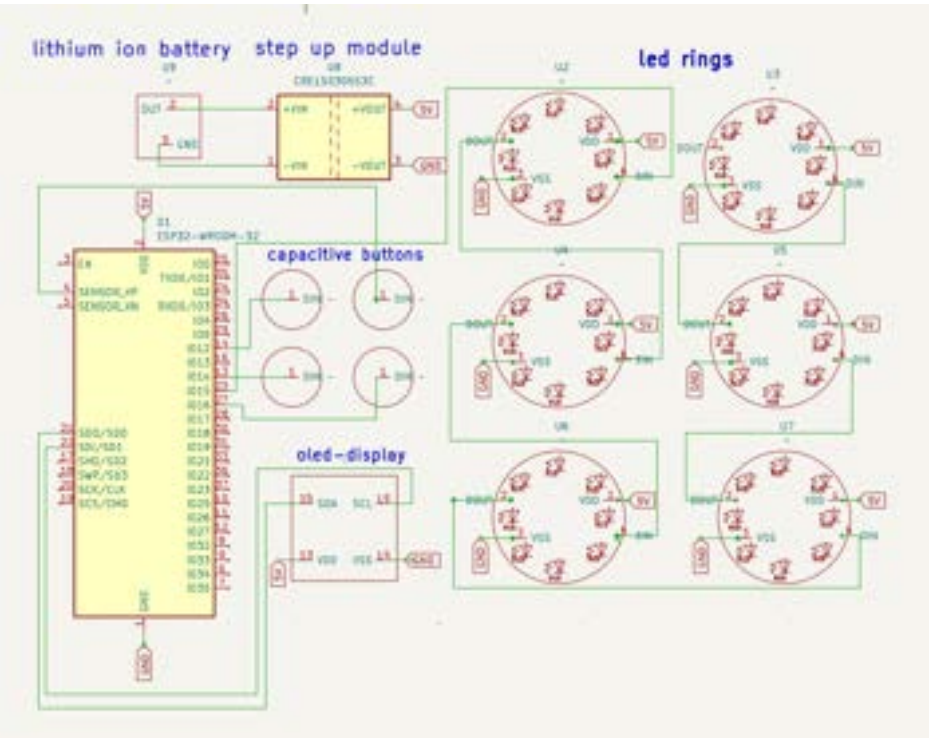


Figure 3.18: Circuit Diagram of Luminacell-Biostim

3.6 Software[VV]

3.6.1 System Architecture

Libraries

The program utilizes several libraries for hardware interaction and extended functionality:

- **Wire.h** – facilitates communication over the I^2C bus.
- **Adafruit_GFX.h** – provides basic graphics functions for display rendering.
- **Adafruit_SSD1306.h** – allows control of OLED displays based on the SSD1306 driver.
- **Adafruit_NeoPixel.h** – enables the control of individually addressable RGB LED rings/strips.
- **esp_sleep.h** – provides access to the ESP32 microcontroller's power-saving and sleep modes.
- **Preferences.h** – allows storing and retrieving configuration data in non-volatile memory.
- **time.h** – provides functions for time synchronization and management.
- **WiFi.h** – enables connection to Wi-Fi networks.

Hardware Architecture

Figure 3.19 illustrates the overall system architecture. The ESP32 microcontroller acts as the central control unit and manages all components. User input is received through capacitive touch buttons, while system information and menus are displayed on an OLED screen. The light output is generated by individually addressable NeoPixel LED rings. Additionally, the ESP32 connects to a WiFi network to synchronize the internal clock using the Network Time Protocol (NTP). Configuration parameters are stored in non-volatile flash memory, and the device uses the ESP32 deep sleep mode to reduce power consumption between lighting cycles.

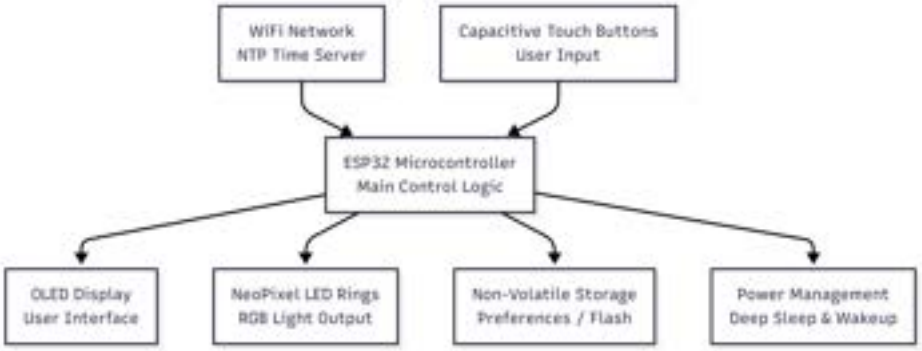


Figure 3.19: Overall System Architecture

Flowchart

The flowchart was generated using ChatGPT (GPT-5 Mini, OpenAI) and refined with Mermaid.ai.

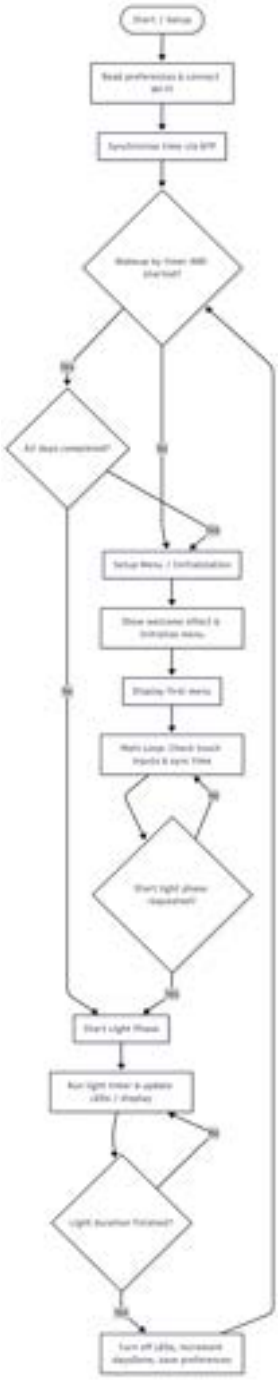


Figure 3.20: Flowchart of the ESP32-Based Light Control Program Architecture

3.6.2 User Interface

Touch Button Configuration

The user input of the device is implemented using the capacitive touch sensors integrated in the ESP32 microcontroller. Each touch button is connected to a dedicated touch-capable GPIO pin and is evaluated using a predefined threshold value.

```
#define TOUCH_POWER 4
#define THRESH_POWER 60

#define TOUCH_BACK 13
#define THRESH_BACK 62

#define TOUCH_OK 14
#define THRESH_OK 67

#define TOUCH_NEXT 27
#define THRESH_NEXT 68
```

Listing 3.2: ESP32 Touch Pin Definitionen

Each button is defined by two parameters: the GPIO pin used for the touch sensor and a corresponding threshold value. The threshold determines the sensitivity level at which the system interprets a touch event. The threshold values differ for each button because the electrical characteristics of the individual connections vary slightly. Factors such as trace length, parasitic capacitance, and environmental influences affect the measured touch values. Additionally, the touch sensors are located behind a 1 mm thick 3D-printed enclosure layer. The system must therefore reliably detect touch input through this material.

To ensure proper operation, the threshold values were adjusted experimentally until stable detection was achieved without false triggers.

Menu Navigation System

Stack

To manage the navigation between different menu screens, a stack-based menu system is implemented. The available menu states are defined using an enumeration:

```
enum MenuState {
    MENU_COLOR,
    MENU_TIME,
    MENU_DAYS,
    MENU_STARTTIME,
    MENU_BRIGHTNESS,
    MENU_OVERVIEW
}
```

Listing 3.3: MenuState Enumeration

Each value represents a specific menu screen of the user interface. The navigation itself is implemented using a stack data structure:

```
#define MENU_STACK_SIZE 7

MenuState menuStack[MENU_STACK_SIZE];
int menuLevel = 0;
```

Listing 3.4: Menu Stack Definition

The stack stores the currently active menu path. When the user moves forward to a new menu, the new menu state is pushed onto the stack. When the user navigates back, the last menu state is popped from the stack, returning to the previous screen. The variable `menuLevel` represents the current position within the stack and therefore indicates which menu is currently active. This mechanism allows hierarchical navigation through the menu structure while preserving the previously visited menu states.

Menus User Interface

The possible menu states are:

- Colour selection
- Light duration configuration
- Number of treatment days
- Start time configuration
- Brightness adjustment
- System overview
- Light phase



Figure 3.21: Menu User Interface - Screen Example

The transitions between these states are triggered by user input. Pressing the `OK` button moves the system forward to the next configuration step, while the `POWER` button returns to the previous menu. Parameter values can be adjusted using the `NEXT` button to increase and the `BACK` button to decrease the selected value.

To ensure reliable touch input, a simple software debounce is implemented. After a touch is detected on any button, the program waits for a short delay before reading inputs again, preventing false or multiple triggers:

```
if (touchRead(TOUCH_NEXT) < THRESH_NEXT) {
    delay(250); // debounce to prevent multiple triggers
    // ...
}
```

Listing 3.5: Touch Button Debounce Example

Once all parameters have been configured, the user reaches the overview screen where all parameter values are displayed. Also, a WiFi symbol, as shown in Figure 3.22, is displayed when successfully connected or a warning symbol (!) if not. Confirming this screen stores the configuration parameters. At this point, the configuration is saved in non-volatile memory using the ESP32 `Preferences` library. The system enters deep sleep mode, where it waits until the scheduled start time.

During the lighting phase, the `POWER` touch button allows the user to interrupt the session and start from the menu stack again.



Figure 3.22: WiFi Symbol

3.6.3 Time Management

The device requires an accurate time reference to start the lighting cycle at the configured start time. For this purpose, the ESP32 connects to a WiFi network and synchronizes its internal clock using the Network Time Protocol (NTP).

The time synchronization is performed during system startup using the `configTime()` function. Once the connection to the NTP servers is established, the ESP32 retrieves the current time and applies the configured time zone settings. The system uses Central European Time (CET) with automatic daylight saving time adjustment.

In case of a failed time synchronization at startup, the internal clock cannot be used to calculate the next wakeup time for the lighting cycle. Consequently, the device will not enter the scheduled lighting phase until a valid time has been obtained from the NTP server. This ensures that the therapy sessions always start at the correct, user-configured time.

```
1  WiFi.mode(WIFI_STA);
2  WiFi.begin(WIFI_SSID, WIFI_PASS);
3
4  while (WiFi.status() != WL_CONNECTED) {
5    delay(500);
6  }
7
8  configTime(0, 0, "pool.ntp.org", "time.google.com");
9
10 setenv("TZ", "CET-1CEST,M3.5.0,M10.5.0/3", 1);
11 tzset();
```

Listing 3.6: WiFi Connection and NTP Synchronization

The synchronized time is then used to calculate the delay until the next scheduled lighting cycle. This calculation is performed by the `calculateWakeupTime()` function, which determines the remaining time until the next configured start time.

Because the internal clock may drift slightly during deep sleep, the exact lighting duration is calculated again after the device wakes up. When the lighting phase begins, the system determines the end time by adding the configured lighting duration to the current time. This ensures that the LEDs remain active for exactly the configured number of minutes, independent of small timing deviations that may occur during the sleep phase.

To maintain time accuracy during longer light periods, the system periodically refreshes the NTP synchronization (after every 5 minutes). If the refresh fails, it is continued with the internal clock.

```
uint64_t calculateWakeupTime() {
  struct tm ti;
  if (!getLocalTime(&ti)) return 60ULL * 1000000ULL;

  int targetHour = startHour % 12;
  if (isPM) targetHour += 12;

  time_t now = time(nullptr);
  struct tm nextTime = ti;

  nextTime.tm_hour = targetHour;
  nextTime.tm_min = startMinute;
  nextTime.tm_sec = 0;

  time_t nextWake = mktime(&nextTime);
  if (nextWake <= now) nextWake += 24 * 3600;

  return (uint64_t)(nextWake - now) * 1000000ULL;
}
```

Listing 3.7: Wake-up Time Calculation

```
time_t endTime = time(nullptr) + (lightTimeMin * 60);

while (time(nullptr) < endTime) {
  time_t now = time(nullptr);

  if (touchRead(TOUCH_POWER) < THRESH_POWER) {
    if (handleQuitLightMode()) {
      showCurrentMenu();
      return;
    } else {
      display.clearDisplay();
    }
  }
}
```

Listing 3.8: Lighting Duration Control

3.6.4 Power Management

The device is designed to operate efficiently and minimize energy consumption, which is particularly important to save battery power and ensure long operation times. To achieve this, the system uses the ESP32 deep sleep mode, a low-power state where most components of the microcontroller are powered down.

During normal operation, the ESP32 is only active when:

- the user interacts with the device via touch buttons, or
- a lighting cycle is running.

After the user completes the configuration process, the device enters deep sleep. In this state:

- the LEDs and the display are turned off,
- the main processor is suspended, and
- power consumption is reduced to a minimum.

The system can wake up in two ways:

Timer Wakeup: The device automatically wakes up at the next scheduled start time for the lighting cycle, calculated using the configured start time and current system time.

```
1 esp_sleep_enable_timer_wakeup(calculateWakeupTime());
2 esp_deep_sleep_start();
```

Listing 3.9: Timer-Based Wakeup using Deep Sleep

Touch Wakeup: Only available while the light mode has not started yet, for example when the device was manually turned off using the power button. If the user touches the power button, the ESP32 wakes up and the menu is displayed.

```
1 touchSleepWakeUpEnable(TOUCH_POWER, THRESH_POWER);
2 esp_sleep_enable_touchpad_wakeup();
3 esp_deep_sleep_start();
```

Listing 3.10: Touch-Based Wakeup using Deep Sleep

3.6.5 LED Rings

The lighting output of the device is generated using individually addressable RGB LEDs (NeoPixel/WS2812). These LEDs are arranged in six rings with eight LEDs each, resulting in a total of 48 LEDs.

The LEDs are controlled via a single-wire communication protocol, implemented using the Adafruit NeoPixel library. Although only one data line is required, each LED contains an integrated controller that receives the signal, updates its color and brightness using PWM (Pulse Width Modulation), and forwards the remaining data to the next LED in the chain. This allows the ESP32 to control all LEDs individually while using only one GPIO pin. The LEDs serve several purposes:

Feedback during configuration: When a menu selection is confirmed, a short fade animation in the previously selected color is displayed to indicate that the input has been accepted (`flashFade()` function).

```
void flashFade(uint32_t color) {
  for (int b = 0; b <= 150; b += 10) {
    strip.fill(strip.Color(((color >> 16) & 0xFF) * b / 150,
                           ((color >> 8) & 0xFF) * b / 150,
                           (color & 0xFF) * b / 150));
    strip.show();
    delay(20);
  }
  for (int b = 150; b >= 0; b -= 10) {
    strip.fill(strip.Color(((color >> 16) & 0xFF) * b / 150,
                           ((color >> 8) & 0xFF) * b / 150,
                           (color & 0xFF) * b / 150));
    strip.show();
    delay(20);
  }
  strip.clear();
  strip.show();
}
```

Listing 3.11: Fade Animation for User Feedback

Startup indication: During the startup sequence, a "Knight Rider" animation runs across the LED rings to visually indicate that the system is powered on and ready

(`knightRiderEffectBlue_welcome()` function).

```
void knightRiderEffectBlue_welcome(int rounds, int delayMs) {
  for (int r = 0; r < rounds; r++) {
    for (int i = 0; i < NUM_LEDS; i++) {
      strip.clear();
      strip.setPixelColor(i, strip.Color(0, 0, 150));
      strip.show();
      delay(delayMs);
    }
    for (int i = NUM_LEDS - 2; i > 0; i--) {
      strip.clear();
      strip.setPixelColor(i, strip.Color(0, 0, 150));
      strip.show();
      delay(delayMs);
    }
  }
  strip.clear();
  strip.show();
}
```

Listing 3.12: Startup Animation (Knight Rider Effect)

Lighting therapy phase: During the active phase, the LEDs illuminate with the user-selected color and brightness for the configured duration. The brightness is calculated based on the selected percentage and applied uniformly to all LEDs.

3.7 Testing Luminacell-Biostim with Lactic Acid Bacteria[GW]

To test whether light really causes visible differences in growth with bacteria, lactic acid bacteria growing on yeast agar media were seeded.

3.7.1 Materials

- yeast extract
- agar-agar
- deionised water
- plain yogurt
- petri dishes
- trigalski spatula
- eppendorf pipettes
- autoclave

3.7.2 Testing Luminacell-Biostim with Lactic Acid Bacteria

To prepare 250 ml of culture medium, weigh 3.75 g of agar-agar and 3 g of yeast extract into a 500 ml laboratory bottle. Then add 250 ml of deionized water and stir the mixture until it is a uniform liquid. Then the laboratory bottle can be half-closed and placed in the autoclave for one hour.

The culture media must be poured while the solution is still warm, otherwise it will solidify in the laboratory bottle. In order to work as quickly and as sterile as possible, it is important to label the Petri dishes in advance and to use a Bunsen burner to protect the bottle opening from flying bacteria. The laboratory bottle is swirled once over the Bunsen burner, then the Petri dish can be poured with culture medium and sealed and set aside to solidify.

In order to be able to add the lactic acid bacteria, a dilution series is made 1:10, 1:100, and 1:1000. Always 1ml of sterilised water is added into a epi, and 0,1ml, 0,01ml and 0,001 ml of plain yogurt are added and mixed.

100 µl of each dilution series can be taken and spread onto the culture medium using the Trigalski spatula. This step should be carried out under a hood, and the Trigalski spatula should always be passed through a Bunsen burner before use, to kill any foreign bacteria so that the culture is as pure as possible.

The bacteria can then grow in the incubator. To have a good basis for comparison as to whether the light actually influences bacterial growth, we placed the 1:100 dilution in our device once and exposed it to red light at full intensity for 10 minutes a day for 7 days. At the same time, a 1:100 dilution grows in the incubator without irradiation, so that differences can be analysed. This can be seen in Figure 3.23



Figure 3.23: Lactic Acid Bacteria in Incubator

4 Results

4.1 MTT-Test[GW]

Since working with cells that grew without contact with materials in the test, formazan crystals should have formed everywhere, as the cells should have been alive.

Unfortunately, nothing was stained, and the result barely showed any differences between the positive controls, blank controls and our cells.

We suspect that the cells were already dead or damaged before the experiment. Based on these results, we determined that the cells in our school laboratory were not growing and discontinued the experiment. Therefore, we were unable to use the MTT-Test to investigate how materials D18 and Y6 affect the cells.

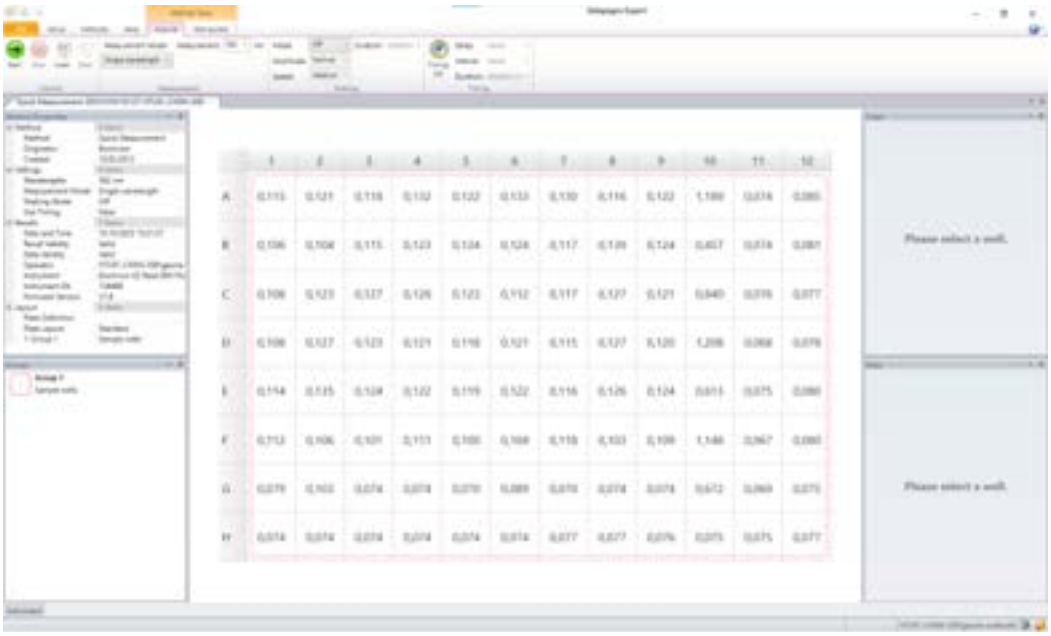


Figure 4.1: Results MTT-Test

row 1-9: cells

row 10&11: positive control

row 12: DMEM media – blank control

4.2 ICC-Test[VV]

Since the slides were stored for two weeks and not mounted professionally, the Ki67 staining faded too much to observe any data that could be analyzed. Also, the stainings on D18/Y6 faded too much to observe properly. However, the DAPI, the Phalloidin and mrpe65 on Terpanol showed promising results.

DAPI was used to label cell nuclei, Ki67 as a proliferation marker, Phalloidin for actin filaments and therefore cell structure, and mrpe65 to identify RPE cells.

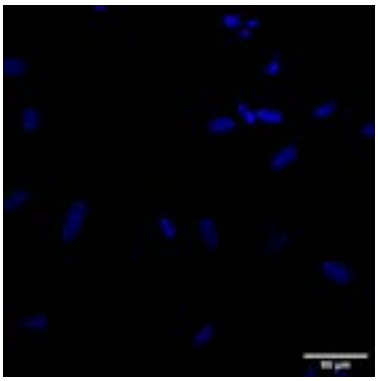


Figure 4.2: DAPI Staining on Terpanol with 60x magnification

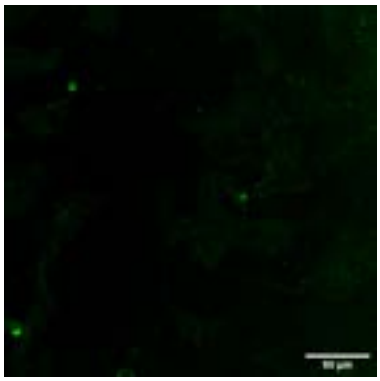


Figure 4.3: mrpe65 Staining on Terpanol with 60x magnification

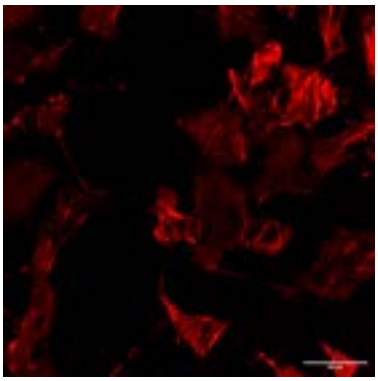


Figure 4.4: Phalloidin Staining on Terpanol with 60x magnification

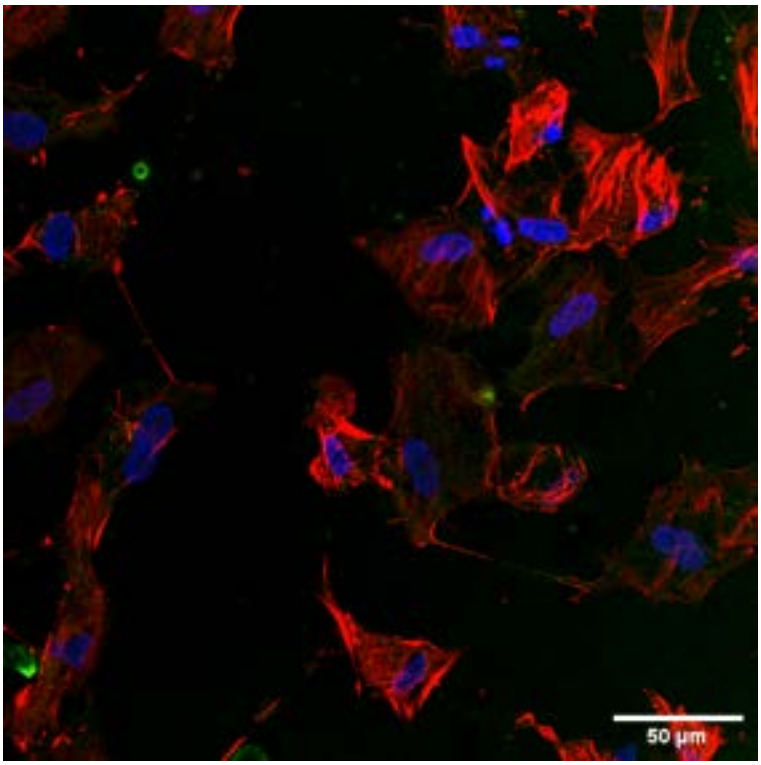


Figure 4.5: Merged Images of All Stainings

As a result, DAPI staining revealed a large number of cells, indicating that the nuclei remained intact and the cells survived the treatment. However, only three RPE cells were detected with mrpe65 staining. Ideally, each green mark for an RPE cell should be surrounded by the blue DAPI signal, as DAPI stains all nuclei. Similarly, the red Phalloidin staining should outline the nuclei, reflecting the cytoskeletal structure. As the Ki67 staining was unsuccessful, no conclusions regarding cell proliferation could be drawn.

These results suggest that the low detection of RPE cells is likely due to technical limitations, such as fading of the fluorescent signal, prolonged storage, or suboptimal antibody staining, rather than negative effects of D18/Y6 or Terpanol.

Studies have shown that Terpanol and D18/Y6 do not negatively affect cells, as demonstrated by ICC and MTT assays, and are therefore considered biocompatible materials suitable for retinal and other prostheses. [19] Figure 4.6 illustrates an example of a successful ICC staining. In this reference image, Hoechst was used to stain cell nuclei (blue), Phalloidin to visualize the cytoskeleton (red), and m65 to specifically mark RPE cells (green).

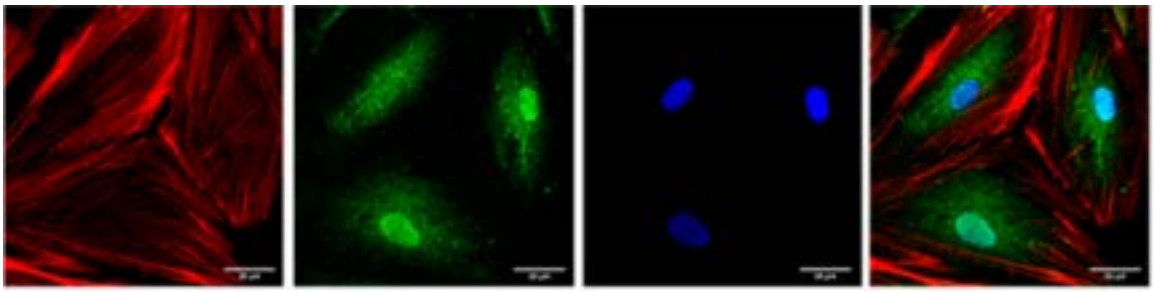


Figure 4.6: Reference for Successful Stainings of D18/Y6 [19]

4.3 Luminacell-Biostim[VV,GW]



Figure 4.7: Luminacell-Biostim Front View



Figure 4.8: Luminacell-Biostim Top View



Figure 4.9: Luminacell-Biostim in Use



Figure 4.10: Luminacell-Biostim Lights On

4.4 Power Density Luminacell-Biostim^{GW}

In order to make precise statements in scientific studies about the extent to which light exposure influences cell growth, it is important to know the exact power density at which the cells are illuminated. An ammeter was used to measure the current flowing through the rings at various intensities. Given the known voltage, always 5V, the power of the LEDs could then be calculated. This value was then extrapolated to the entire surface area. This allows us to determine very precisely the power at which the cells are illuminated.

Table 4.1: Power per cm² Luminacell-Biostim

Intensity (%)	**Current (A)**	**Power (W)**	**W/cm^2**	**mW/cm^2**
100%	0.585	2.925	0.026571584	26.57
90%	0.260	1.300	0.011809593	11.81
80%	0.238	1.190	0.010810320	10.81
70%	0.141	0.705	0.006404433	6.40
60%	0.117	0.585	0.005314317	5.31
50%	0.143	0.715	0.006495276	6.50
40%	0.129	0.645	0.005859375	5.86
30%	0.088	0.440	0.003997093	4.00
20%	0.055	0.275	0.002498183	2.50
10%	0.022	0.110	0.000999273	1.00
Sleep Mode	0.014	0.070	0.000635901	0.64

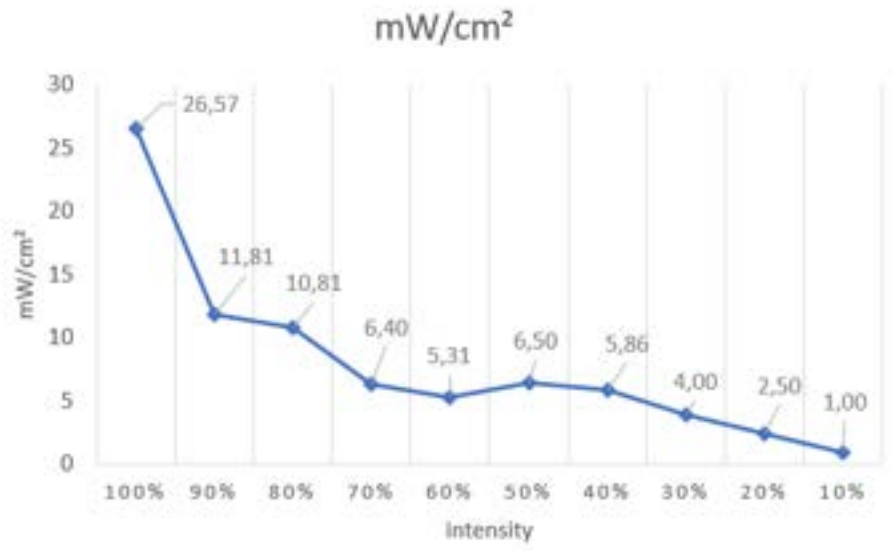


Figure 4.11: measured power density

4.5 Growing Lactic Acid Bacteria in Luminacell-Biostim^{GW}

After 7 days, we counted the bacteria that had grown. On the left side of Figure 4.12, you can see the bacteria that grew with light, and on the right side, those that grew without light. We counted them and found that there were 270 individual bacteria exposed to light and 250 unexposed. This suggests that light has an influence on growth, which would probably be even more pronounced in other bacteria or fungi.

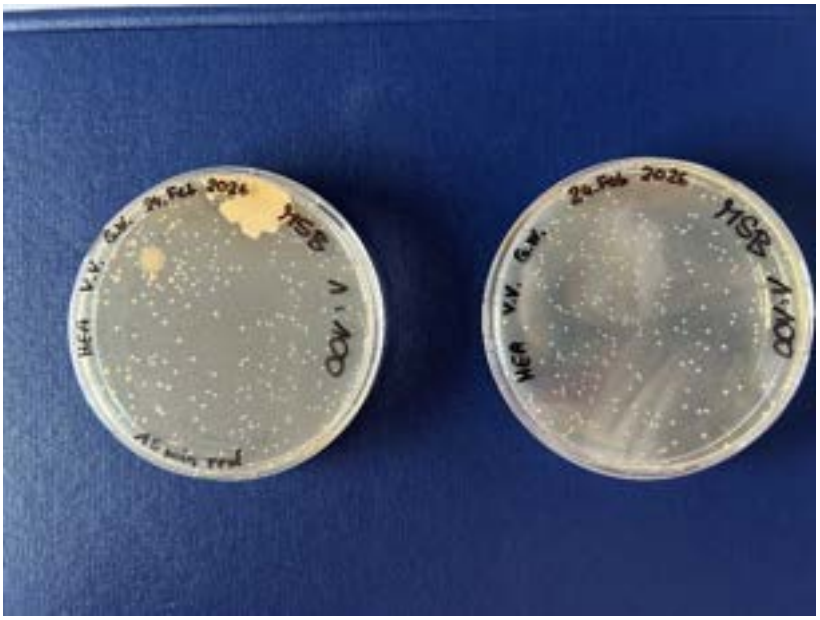


Figure 4.12: Lactic Acid Bacteria after Incubating

5 Project Management

5.1 Planning

For effective planning and deadline tracking, we used the Teams Planner Board. To monitor free time, upcoming goals, and completed objectives, we used various Excel tables.

5.2 Freetime Hours

5.2.1 Viola Vorhauer

Table 5.1: Freetime Hours - Viola

Date	**Hours**	**Activity**
06.06.2025	02:30	Research/Videos on analysis method
13.06.2025	03:00	Reading documents from Mister Salti
23.06-25.06.2025	05:00	Travel time Apartment - JKU
23.06-25.06.2025	05:00	Travel time Linz and home
23.06.2025	04:30	JKU Lab guided tour and observing, onboarding Sara
23.06.2025	03:30	seeding cells with Sara and Alessandro
23.06.2025	01:30	perparing questions for Mister Salti and Mister Schmeitzl
24.06.2025	04:00	Media change and theory overview with Alessandro - reviewing papers
24.06.2025	01:30	brainstorming for additional project
25.05.2025	04:00	Observing refreshing media, inspecting devices + explanation and testing
25.06.2025	02:00	Creating a list of required materials
13.07.2025	01:00	Topics and scheduling: diploma thesis theory
16.07.2025	01:00	Breakdown of topics
18.07.2025	01:00	Merging ideas and creating red thread
07.08.2025	01:00	Detailed research on immunofluorescence, specifying assays for process
09.08.2025	01:00	Reading, translating, and summarizing immunofluorescence paper
13.09.2025	01:00	Meeting for organizing transport and materials from JKU
14.09.2025	02:00	Email to Alessandro concerning missing items for IF and ICC, reading papers for cell cultivation, preparing questions for Tuesday
17.09.2025	02:00	Lab exercise protocol, register ABA Portal, checking missing items, administrative tasks

Date	Hours	Activity
22.09.2025	01:30	Entering new dates in schedule, organizing dry ice transport, reading ABA Portal, revising time log, recipes and further ideas for thesis day
23.09.2025	02:00	preparing PBS in lab
29.09.2025	05:00	Trip to Linz to pick up cells
29.09.2025	00:30	Entering new dates, sorting tasks, reading Gesine's plan
01.10.2025	00:30	Media change
03.10.2025	00:30	Media change
06.10.2025	00:30	Media change
06.10.2025	01:00	Updating schedule, follow-up on last week, tasks, setting up programming environment, updating Fuchs project management, searching for programs, reviewing
10.10.2025	05:00	MTT-Test
20.10.2025	01:00	Preparing ICC
22.10.2025	03:30	ICC Test - Secondary antibodies and finalizing the test
24.10.2025	02:30	Light project questions, registering for competitions, creating photo collection, make a PowerPoint for Mister Salti, read research report, contacting Sara for visit/ICC update, informing supervisor about project
29.10.2025	00:30	Teams meeting regarding additional project
30.10.2025	02:00	Reading papers from Mister Salti and Mister Schmeitzl
31.10.2025	02:30	Reading papers from Salti and Schmeitzl, taking notes for Mister Salti, working on PowerPoint
02.11.2025	01:30	Arranging meeting with Salti, finalizing PowerPoint
04.11.2025	03:00	Trip to Linz, picking up new materials and inspecting ICC test under microscope
10.11.2025	02:00	Brainstorming for Tuesday, work plan for cell seeding and planning MTT
12.11.2025	00:30	Media change
18.11.2025	02:00	Measuring LED heat output, rewriting program with CSV file
28.11.2025	02:00	Completing 3D construction
30.11.2025	01:30	Newspaper article
05.12.2025	06:00	Open House day
01.02.2026	02:00	3D drawing: buttons
02.02.2026	03:30	Improving 3D drawing (display), removing borders, heat-set insert holes, selecting material for 3D print (durability)
06.02.2026	08:00	Procuring new materials, gluing glass, final soldering of cables, revising program
13.02.2026	05:00	Building device, writing diploma thesis, heat-set inserts, gluing lid
19.02.2026	01:30	Time log, informing about Zotero, reading Gesine's diploma section, reading Mister Salti paper
20.02.2026	03:30	Repairing detached parts, programming, gluing glass, sanding housing
20.02.2026	01:30	Meeting concerning diploma thesis

Date	Hours	Activity
22.02.2026	03:00	Researching current limiting for circuit, compiling Uni documents, programming, listing component names
23.02.2026	03:30	Programming
25.02.2025	02:30	Programming, competition entry, prepare Uni documents/user guide
28.01.1900	03:00	Repairing battery connector, assembling housing, programming (%)
01.03.2026	03:00	Testing program and fixing time errors
02.03.2026	02:30	Fixing WiFi time problem and testing program, writing diploma thesis
05.03.2026	05:00	Writing diploma thesis
09.03.2026	02:30	Writing diploma thesis (improvements)
11.03.2026	02:00	Writing diploma thesis
13.03.2025	01:00	Writing diploma thesis
14.03.2025	02:00	Soldering switch for battery operation, improved time drift, attempted OTA, testing
15.03.2026	04:00	Writing diploma thesis
16.03.2026	04:30	Ordering LED rings, improving flowchart, writing diploma thesis, LaTeX sources/Zotero
17.03.2026	02:00	Writing diploma thesis, writing offline code
18.03.2026	00:40	Writing diploma thesis
18.03.2026	07:00	handing over Luminacell-Biostim in Linz
19.03.2026	06:30	Writing and fixing diploma thesis, "red thread"
23.03.2026	00:30	3D printing
24.03.2026	01:30	Improving diploma thesis, CV
25.03.2026	01:00	Improving diploma thesis
26.03.2026	01:00	Writing diploma thesis
27.03.2026	03:00	Writing diploma thesis
29.03.2026	01:30	Writing diploma thesis
30.03.2026	03:00	Writing diploma thesis
31.03.2026	04:30	Finalizing diploma thesis
Total	**200:10**	

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5.2.2 Gesine Wollboldt

Table 5.3: Freetime Hours- Gesine

Date	Hours	Activity
06.06.2025	02:30	Research/Videos on analysis method
13.06.2025	03:00	Reading documents from Mister Salti
23.06-25.06.2025	05:00	Travel time Apartment - JKU
23.06-25.06.2025	05:00	Travel time Linz and home
23.06.2025	04:30	JKU Lab Guided tour and observing tasks, onboarding Sara
23.06.2025	03:30	seeding cells with Sara and Alessandro
23.06.2025	01:30	questions for Mister Salti and Mister Schmeitzl
24.06.2025	04:00	Media change and theory overview with Alessandro - reviewing papers
24.06.2025	01:30	brainstorming for additional project
25.05.2025	04:00	Observing refreshing media, inspecting devices + explanation and testing
25.06.2025	02:00	Creating a list of required materials
13.07.2025	01:00	Topics and scheduling: diploma thesis theory
14.07.2025	01:00	Breakdown of topics and creating a red thread
16.07.2025	01:00	Reading papers
13.09.2025	01:00	write down what materials are needed for the MTT test
13.09.2025	01:00	Meeting for organizing transport and materials from UNI
14.09.2025	01:30	Translating and reading papers
14.09.2025	01:30	Translating and reading papers
17.09.2025	02:00	Lab exercise protocol, registering ABA Portal, checking missing on items for experiments, administrative tasks
21.09.2025	02:00	filling ABA Portal, schedule work instructions for microbiology exercise
27.09.2025	01:00	Updating annual plan, cell growth instructions with media change plan, brainstorming incubator monitoring
29.09.2025	05:00	Trip to Linz to pick up cells
01.10.2025	00:30	Media change
03.10.2025	00:30	Media change
05.10.2025	01:00	Searching for circuits for light measurement and temperature
06.10.2025	00:30	Media change
10.10.2025	05:00	MTT-Test
22.10.2025	03:30	ICC Test - Secondary antibodies and finalizing the test
22.10.2025	00:30	Recording ICC and schedule
24.10.2025	02:30	Research log, checking competitions, updating photo collection, informing supervisor about additional project, reading Salti paper, research on IR lamps
29.10.2025	00:30	Teams meeting regarding additional project
30.10.2025	01:30	Reading papers from Mister Salti and Mister Schmeitzl, taking notes for Mister Salti, create PowerPoint
02.11.2025	01:30	Arranging meeting with Mister Salti, finalizing PowerPoint, organizing lab day in Linz

Date	Hours	Activity
02.11.2025	00:30	Reading summary of papers
04.11.2025	03:00	Trip to Linz, collecting materials and inspecting ICC test under microscope
05.11.2025	01:00	Testing materials for light
09.11.2025	03:00	Updating schedule overview, starting poster for open day, writing Jugend Innovativ entry, preparing material tests
12.11.2025	00:30	Media change
22.11.2025	02:00	Resubmitting Jugend Innovativ entry
27.11.2025	01:30	Newspaper article, project bonus
05.12.2025	06:00	Open House day
08.12.2025	00:30	Updating project planning
23.12.2025	03:00	Fusion sketch: final product
01.02.2026	01:00	Breaking down diploma thesis content
10.02.2026	01:00	Writing diploma thesis
11.02.2026	02:00	Writing diploma thesis
13.02.2026	05:00	Building device, writing diploma thesis, heat-set inserts, gluing lid
16.02.2026	04:30	Writing diploma thesis
17.02.2026	08:00	Writing diploma thesis
18.02.2026	03:30	Writing diploma thesis
19.02.2026	01:20	Writing diploma thesis
20.02.2026	02:30	Writing diploma thesis, updating time log, inform about current limiting circuit
20.02.2026	01:30	Meeting for diploma thesis
21.02.2026	02:30	Writing diploma thesis
22.02.2026	03:00	Writing diploma thesis
25.02.2026	00:30	Competition entry: FH Kärnten
05.03.2026	08:00	Assisting with "Jugend Forscht" competition
07.03.2026	02:00	Improving diploma thesis
08.03.2026	04:00	Improving diploma thesis
11.03.2026	01:30	Writing diploma thesis
16.03.2026	01:00	Writing diploma thesis
16.03.2026	02:00	Writing diploma thesis, Excel intensity calculation
17.03.2026	02:00	transfer Diploma thesis in LaTeX
18.03.2026	07:00	handing over Luminacell-Biostim in Linz
18.03.2026	04:30	transfering Diploma thesis in LaTeX
19.03.2026	03:30	writing diploma thesis, updating project planning, optimizing red thread
20.03.2026	01:00	CV LaTeX template
23.03.2026	00:30	3D printing
24.03.2026	03:00	Correcting diploma thesis, creating list of abbreviations, separating time log
25.03.2026	01:00	Writing acknowledgments
27.03.2026	00:30	Inserting product photos
29.03.2026	07:00	creating Scientific poster, formating diploma thesis
31.03.2026	02:00	Writing conclusion, inserting project planning

Date	Hours	Activity
01.04.2026	03:00	Competition entry: Bosch, FH Salzburg
Total	**193:50**	

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6 Future Work

Ahmad Salti's team is continuing its research on retinal prostheses. Luminacell-Biostim represents one avenue of this research.

For Luminacell-Biostim a key focus is expanding the control and monitoring functions. A web server would allow the device to be monitored and controlled remotely. Information such as the current battery level, operating status, and remaining runtime could then be viewed in real time via a smartphone or computer. It would also be beneficial to include a function that allows for the flexible adjustment of lighting cycles or time intervals remotely. Such a solution would significantly improve ease of use, especially during long experiments.

Furthermore, the system could be enhanced by integrating additional sensors. For example, temperature and light sensors could be used to continuously record and log the actual environmental conditions. Combined with data storage, these parameters could be analyzed over the long term and used to optimize experimental conditions.

Additionally, automatic notifications for low battery levels would be conceivable, for example, via the aforementioned web interface.

The device's housing could be improved by using industrial manufacturing processes such as injection molding. This would result in greater stability, better reproducibility, and a more professional finish.

7 Conclusion

This diploma thesis investigated the biocompatibility of organic semiconductor materials, D18 and Y6, for retinal prostheses, tested possibilities of growing RPE cells in a school lab and developed a device, the Luminacell-Biostim, which should improve cell growth. While the primary goal of culturing Retinal Pigment Epithelial cells in a school laboratory proved challenging due to viability issues and technical limitations in staining, specifically fading Ki67 signals, the experimental design yielded critical insights. The MTT assay indicated that cells were compromised prior to testing, and the ICC results, though limited by storage conditions, confirmed that the materials themselves did not exhibit toxicity, aligning with existing literature on their biocompatibility.

A significant contribution of this work is the development and characterization of the "Luminacell-Biostim", a device enabling targeted light stimulation. Preliminary trials with lactic acid bacteria suggested that light exposure can influence microbial growth, hinting at the device's potential to modulate cellular processes. Although the direct assessment of RPE cell proliferation was hindered by technical constraints of the school setting, the successful development of the stimulation device and the confirmation of material safety provide a solid foundation for future research. The Luminacell-Biostim is now tested in experiments with RPE cells by Mister Saltis research team.

Acknowledgements

We would like to take this opportunity to express our sincere gratitude to all the teachers who contributed to this project through their inspiration, problem-solving skills, and expertise.

In particular, we would like to thank our advisor, Bernhard Schmeitzl, for introducing us to the idea of collaborating with Mr. Salti and for his support, valuable input, and trust throughout the entire project.

We would also like to thank Mr.Salti and his research team for giving us the opportunity to gain practical experience during our internship at the university. Thanks for the willingness to share your knowledge, answer our questions, and provide the necessary materials for our cell research.

Furthermore, we would like to thank our classmates for their support and for creating a motivating working environment.

Finally, we would like to express our deepest gratitude to our families for their constant support, patience, and encouragement throughout the entire process.

Artificial Intelligence Usage

AI tools were used as supportive instruments in the preparation of this thesis, primarily for improving grammar and spelling, as well as assisting with LaTeX formatting and document structure. Additionally, flowcharts were generated with the assistance of AI tools. However, all research, content development, and source selection were carried out independently by the authors without the use of AI.

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A Appendix

A.1 Complete Code

```
#include <Wire.h>
#include <Adafruit_GFX.h>
#include <Adafruit_SSD1306.h>
#include <Adafruit_NeoPixel.h>
#include "esp_sleep.h"
#include <Preferences.h>
#include "time.h"
#include <WiFi.h>

// ---------------- OLED ----------------
#define SCREEN_WIDTH 128
#define SCREEN_HEIGHT 64
#define OLED_RESET -1
Adafruit_SSD1306 display(SCREEN_WIDTH, SCREEN_HEIGHT, &Wire, OLED_RESET)
    ;

// ---------------- WiFi ----------------
#define WIFI_SSID ""
#define WIFI_PASS ""

// ---------------- LED RINGS ----------------
#define LED_PIN 23
#define LEDS_PER_RING 8
#define RINGS 6
#define NUM_LEDS (LEDS_PER_RING * RINGS)
Adafruit_NeoPixel strip(NUM_LEDS, LED_PIN, NEO_GRB + NEO_KHZ800);

// ---------------- TOUCH ----------------
#define TOUCH_POWER 4
#define THRESH_POWER 60

#define TOUCH_BACK 13
#define THRESH_BACK 62

#define TOUCH_OK 14
#define THRESH_OK 67

#define TOUCH_NEXT 27
#define THRESH_NEXT 68

// ---------------- MENU STACK ----------------
enum MenuState { MENU_COLOR,
                 MENU_TIME,
                 MENU_DAYS,
```

```
                  MENU_STARTTIME,
                  MENU_BRIGHTNESS,
                  MENU_OVERVIEW };
#define MENU_STACK_SIZE 7
MenuState menuStack[MENU_STACK_SIZE];
int menuLevel = 0;

// ---------------- STATE ----------------
bool quitMode = false;
bool timeSynced = false;
int colorIndex = 0;
int selectedColor = 0;
int lightTimeMin = 5;
int daysInRow = 5;
int startHour = 9;
int startMinute = 0;
bool isPM = false;
int brightnessPercent = 50;
unsigned long lastSync = 0;
const unsigned long syncInterval = 300000;

// ---------------- COLORS ----------------
const char* colors[] = { "red 620-650nm", "blue 440-480nm", "green
    500-560nm" };
const int COLOR_COUNT = 3;
uint32_t colorValues[] = {
  Adafruit_NeoPixel::Color(150, 0, 0),
  Adafruit_NeoPixel::Color(0, 0, 150),
  Adafruit_NeoPixel::Color(0, 150, 0)
};

// ---------------- HELPERS ----------------
MenuState currentMenu() {
  return menuStack[menuLevel];
}
void pushMenu(MenuState m) {
  if (menuLevel < MENU_STACK_SIZE - 1) menuStack[++menuLevel] = m;
}
void popMenu() {
  if (menuLevel > 0) menuLevel--;
}

void drawWifiIcon(bool synced) {
  if (synced) {
    display.drawPixel(120, 5, SSD1306_WHITE);
    display.drawCircle(120, 5, 2, SSD1306_WHITE);
    display.drawCircle(120, 5, 4, SSD1306_WHITE);
  } else {
    display.setTextSize(1);
    display.setCursor(110, 0);
    display.print("!")
  }
}

```

```
97  // ---------------- LED EFFECTS ----------------
98  void flashFade(uint32_t color) {
99    for (int b = 0; b <= 150; b += 10) {
100     strip.fill(strip.Color(((color >> 16) & 0xFF) * b / 150, ((color >>
            8) & 0xFF) * b / 150, (color & 0xFF) * b / 150));
101     strip.show();
102     delay(20);
103   }
104   for (int b = 150; b >= 0; b -= 10) {
105     strip.fill(strip.Color(((color >> 16) & 0xFF) * b / 150, ((color >>
            8) & 0xFF) * b / 150, (color & 0xFF) * b / 150));
106     strip.show();
107     delay(20);
108   }
109   strip.clear();
110   strip.show();
111 }
112
113 void knightRiderEffectBlue_welcome(int rounds, int delayMs) {
114   for (int r = 0; r < rounds; r++) {
115     for (int i = 0; i < NUM_LEDS; i++) {
116       strip.clear();
117       strip.setPixelColor(i, strip.Color(0, 0, 150));
118       strip.show();
119       delay(delayMs);
120     }
121     for (int i = NUM_LEDS - 2; i > 0; i--) {
122       strip.clear();
123       strip.setPixelColor(i, strip.Color(0, 0, 150));
124       strip.show();
125       delay(delayMs);
126     }
127   }
128   strip.clear();
129   strip.show();
130 }
131
132 // ---------------- DISPLAY MENUS ----------------
133 void showHelloJKU() {
134   display.clearDisplay();
135   display.setTextColor(SSD1306_WHITE);
136   display.setTextSize(2);
137   display.setCursor(0, 25);
138   display.println("Hello JKU!");
139   display.display();
140 }
141
142 void showColorMenu() {
143   display.clearDisplay();
144   display.setTextSize(1);
145   display.setCursor(40, 0);
146   display.println("color:");
147   display.setCursor(0, 50);
148   display.print("<");
```

```
149   display.setCursor(120, 50);
150   display.print(">");
151   int w = strlen(colors[colorIndex]) * 6;
152   display.setCursor((128 - w) / 2, 30);
153   display.println(colors[colorIndex]);
154   display.display();
155 }
156
157 void showTimeMenu() {
158   display.clearDisplay();
159   display.setTextSize(1);
160   display.setCursor(0, 0);
161   display.println("min of light per day:");
162   display.setCursor(0, 50);
163   display.print("<");
164   display.setCursor(120, 50);
165   display.print(">");
166   display.setTextSize(2);
167   char buf[10];
168   sprintf(buf, "%d min", lightTimeMin);
169   int w = strlen(buf) * 12;
170   display.setCursor((128 - w) / 2, 28);
171   display.println(buf);
172   display.display();
173 }
174
175 void showDaysMenu() {
176   display.clearDisplay();
177   display.setTextSize(1);
178   display.setCursor(0, 0);
179   display.println("days in a row:");
180   display.setCursor(0, 50);
181   display.print("<");
182   display.setCursor(120, 50);
183   display.print(">");
184   display.setTextSize(2);
185   char buf[12];
186   sprintf(buf, "%d days", daysInRow);
187   int w = strlen(buf) * 12;
188   display.setCursor((128 - w) / 2, 28);
189   display.println(buf);
190   display.display();
191 }
192
193 void showStartTimeMenu() {
194   display.clearDisplay();
195   display.setTextSize(1);
196   display.setCursor(0, 0);
197   display.println("start time:");
198   display.setCursor(0, 50);
199   display.print("<");
200   display.setCursor(120, 50);
201   display.print(">");
202   display.setTextSize(2);
```

```
203   char buf[12];
204   sprintf(buf, "%02d:%02d %s", startHour, startMinute, isPM ? "pm" : "am
          ");
205   int w = strlen(buf) * 12;
206   display.setCursor((128 - w) / 2, 28);
207   display.println(buf);
208   display.display();
209 }
210
211 void showBrightnessMenu() {
212   display.clearDisplay();
213   display.setTextSize(1);
214   display.setCursor(30, 0);
215   display.println("brightness:");
216   display.setCursor(0, 50);
217   display.print("<");
218   display.setCursor(120, 50);
219   display.print(">");
220   display.setTextSize(2);
221   char buf[10];
222   sprintf(buf, "%d %%", brightnessPercent);
223   int w = strlen(buf) * 12;
224   display.setCursor((128 - w) / 2, 28);
225   display.println(buf);
226   display.display();
227 }
228
229 void showOverviewMenu() {
230   display.clearDisplay();
231   display.setTextSize(1);
232   display.setCursor(20, 0);
233   display.println("--- SUMMARY ---");
234   display.setCursor(0, 11);
235   display.printf("Color:%s", colors[selectedColor]);
236   display.setCursor(0, 20);
237   display.printf("Time: %d min", lightTimeMin);
238   display.setCursor(0, 29);
239   display.printf("Days: %d days", daysInRow);
240   display.setCursor(0, 38);
241   display.printf("Start:%02d:%02d %s", startHour, startMinute, isPM ? "
          pm" : "am");
242   display.setCursor(0, 47);
243   display.printf("Brightness:%d %%", brightnessPercent);
244   struct tm ti;
245   if (getLocalTime(&ti)) {
246     int h12 = ti.tm_hour % 12;
247     if (h12 == 0) h12 = 12;
248     display.setCursor(0, 56);
249     display.printf("Now:%02d:%02d %s", h12, ti.tm_min, (ti.tm_hour >=
            12) ? "pm" : "am");
250   }
251   drawWifiIcon(timeSynced);
252   display.display();
253 }
```

```
254
255 void showQuitPrompt() {
256   display.clearDisplay();
257   display.setTextSize(2);
258   display.setCursor(30, 0);
259   display.println("Quit?");
260   display.setTextSize(1);
261   display.setCursor(0, 45);
262   display.println("OFF again: YES");
263   display.setCursor(0, 55);
264   display.println("Any other: NO");
265   display.display();
266 }
267
268 void showCurrentMenu() {
269   if (currentMenu() == MENU_COLOR) showColorMenu();
270   else if (currentMenu() == MENU_TIME) showTimeMenu();
271   else if (currentMenu() == MENU_DAYS) showDaysMenu();
272   else if (currentMenu() == MENU_STARTTIME) showStartTimeMenu();
273   else if (currentMenu() == MENU_BRIGHTNESS) showBrightnessMenu();
274   else if (currentMenu() == MENU_OVERVIEW) showOverviewMenu();
275 }
276
277 // ---------------- LOGIC ----------------
278 uint64_t calculateWakeupTime() {
279   struct tm ti;
280   if (!getLocalTime(&ti)) return 60ULL * 1000000ULL;
281   int targetHour = startHour % 12;
282   if (isPM) targetHour += 12;
283   time_t now = time(nullptr);
284   struct tm nextTime = ti;
285   nextTime.tm_hour = targetHour;
286   nextTime.tm_min = startMinute;
287   nextTime.tm_sec = 0;
288   time_t nextWake = mktime(&nextTime);
289   if (nextWake <= now) nextWake += 24 * 3600;
290   return (uint64_t)(nextWake - now) * 1000000ULL;
291 }
292
293 Preferences prefs;
294 bool handleQuitLightMode();
295
296 void startLightPhase() {
297   int realBrightness = (brightnessPercent * 255) / 100;
298   strip.setBrightness(realBrightness);
299   strip.fill(colorValues[selectedColor]);
300   strip.show();
301
302   time_t endTime = time(nullptr) + (lightTimeMin * 60);
303
304   while (time(nullptr) < endTime) {
305     time_t now = time(nullptr);
306     if (touchRead(TOUCH_POWER) < THRESH_POWER) {
307       if (handleQuitLightMode()) {
```

```
308         showCurrentMenu();
309         return;
310       } else {
311         display.clearDisplay();
312       }
313     }
314
315     int diff = (int)(endTime - now);
316     int remM = diff / 60;
317     int remS = diff % 60;
318     struct tm ti;
319     localtime_r(&now, &ti);
320     int h12 = ti.tm_hour % 12;
321     if (h12 == 0) h12 = 12;
322
323     display.clearDisplay();
324     drawWifiIcon(timeSynced);
325     display.setTextSize(1);
326     display.setTextColor(SSD1306_WHITE);
327     display.setCursor(0, 10);
328     display.printf("Rem: %02d:%02d", remM, remS);
329     display.setCursor(0, 30);
330     display.printf("Time: %02d:%02d:%02d %s", h12, ti.tm_min, ti.tm_sec,
          (ti.tm_hour >= 12) ? "pm" : "am");
331     display.display();
332     delay(500);
333   }
334
335   strip.clear();
336   strip.show();
337   prefs.begin("lightConfig", false);
338   prefs.putUInt("daysDone", prefs.getUInt("daysDone", 0) + 1);
339   prefs.end();
340 }
341
342 // ---------------- TOUCH & QUIT ----------------
343 bool handleQuitLightMode() {
344   showQuitPrompt();
345   delay(500);
346   while (true) {
347     if (touchRead(TOUCH_POWER) < THRESH_POWER) {
348       delay(300);
349       prefs.begin("lightConfig", false);
350       prefs.putBool("started", false);
351       prefs.putUInt("daysDone", 0);
352       prefs.end();
353       strip.clear();
354       strip.show();
355       display.clearDisplay();
356       display.setCursor(10, 25);
357       display.println("Restarting...");
358       display.display();
359       delay(1000);
360       ESP.restart();
```

```
361       return true;
362     }
363     if (touchRead(TOUCH_NEXT) < THRESH_NEXT || touchRead(TOUCH_BACK) <
            THRESH_BACK || touchRead(TOUCH_OK) < THRESH_OK) {
364       delay(300);
365       return false;
366     }
367     delay(50);
368   }
369 }
370
371 void handleQuit() {
372   quitMode = true;
373   showQuitPrompt();
374   while (quitMode) {
375     if (touchRead(TOUCH_POWER) < THRESH_POWER) {
376       delay(300);
377       strip.clear();
378       strip.show();
379       display.clearDisplay();
380       display.display();
381       touchSleepWakeUpEnable(TOUCH_POWER, THRESH_POWER);
382       esp_sleep_enable_touchpad_wakeup();
383       esp_deep_sleep_start();
384     }
385     if (touchRead(TOUCH_NEXT) < THRESH_NEXT || touchRead(TOUCH_BACK) <
            THRESH_BACK || touchRead(TOUCH_OK) < THRESH_OK) {
386       quitMode = false;
387       showCurrentMenu();
388       delay(300);
389       return;
390     }
391     delay(50);
392   }
393 }
394
395 void checkTouch() {
396   if (touchRead(TOUCH_POWER) < THRESH_POWER) {
397     delay(300);
398     if (menuLevel == 0) handleQuit();
399     else {
400       popMenu();
401       showCurrentMenu();
402     }
403     return;
404   }
405   if (touchRead(TOUCH_NEXT) < THRESH_NEXT) {
406     delay(250);
407     if (currentMenu() == MENU_COLOR) colorIndex = (colorIndex + 1) %
            COLOR_COUNT;
408     else if (currentMenu() == MENU_TIME && lightTimeMin < 60)
            lightTimeMin++;
409     else if (currentMenu() == MENU_DAYS && daysInRow < 30) daysInRow++;
410     else if (currentMenu() == MENU_STARTTIME) {
```

```
411       startMinute += 15;
412       if (startMinute >= 60) {
413         startMinute = 0;
414         startHour++;
415         if (startHour > 12) {
416           startHour = 1;
417           isPM = !isPM;
418         }
419       }
420     } else if (currentMenu() == MENU_BRIGHTNESS && brightnessPercent <
          100) brightnessPercent += 5;
421     showCurrentMenu();
422   }
423   if (touchRead(TOUCH_BACK) < THRESH_BACK) {
424     delay(250);
425     if (currentMenu() == MENU_COLOR) colorIndex = (colorIndex - 1 +
          COLOR_COUNT) % COLOR_COUNT;
426     else if (currentMenu() == MENU_TIME && lightTimeMin > 1)
          lightTimeMin--;
427     else if (currentMenu() == MENU_DAYS && daysInRow > 1) daysInRow--;
428     else if (currentMenu() == MENU_STARTTIME) {
429       startMinute -= 15;
430       if (startMinute < 0) {
431         startMinute = 45;
432         startHour--;
433         if (startHour < 1) {
434           startHour = 12;
435           isPM = !isPM;
436         }
437       }
438     } else if (currentMenu() == MENU_BRIGHTNESS && brightnessPercent >
          5) brightnessPercent -= 5;
439     showCurrentMenu();
440   }
441   if (touchRead(TOUCH_OK) < THRESH_OK) {
442     delay(250);
443     MenuState state = currentMenu();
444     if (state == MENU_COLOR) {
445       selectedColor = colorIndex;
446       flashFade(colorValues[selectedColor]);
447       pushMenu(MENU_TIME);
448     } else if (state == MENU_TIME) {
449       flashFade(colorValues[selectedColor]);
450       pushMenu(MENU_DAYS);
451     } else if (state == MENU_DAYS) {
452       flashFade(colorValues[selectedColor]);
453       pushMenu(MENU_STARTTIME);
454     } else if (state == MENU_STARTTIME) {
455       flashFade(colorValues[selectedColor]);
456       pushMenu(MENU_BRIGHTNESS);
457     } else if (state == MENU_BRIGHTNESS) {
458       flashFade(colorValues[selectedColor]);
459       pushMenu(MENU_OVERVIEW);
460     } else if (state == MENU_OVERVIEW) {
```

```
461       prefs.begin("lightConfig", false);
462       prefs.putUInt("color", selectedColor);
463       prefs.putUInt("minutes", lightTimeMin);
464       prefs.putUInt("days", daysInRow);
465       prefs.putUInt("startHour", startHour);
466       prefs.putUInt("startMin", startMinute);
467       prefs.putBool("isPM", isPM);
468       prefs.putUInt("bright", brightnessPercent);
469       prefs.putBool("started", true);
470       prefs.putUInt("daysDone", 0);
471       prefs.end();
472       flashFade(colorValues[selectedColor]);
473       display.clearDisplay();
474       display.display();
475       esp_sleep_enable_timer_wakeup(calculateWakeupTime());
476       esp_deep_sleep_start();
477     }
478     if (state != MENU_OVERVIEW) showCurrentMenu();
479   }
480 }
481
482 // ---------------- SETUP ----------------
483 void setup() {
484
485   prefs.begin("lightConfig", true);
486   bool started = prefs.getBool("started", false);
487   selectedColor = prefs.getUInt("color", 0);
488   lightTimeMin = prefs.getUInt("minutes", 5);
489   daysInRow = prefs.getUInt("days", 5);
490   startHour = prefs.getUInt("startHour", 9);
491   isPM = prefs.getBool("isPM", false);
492   brightnessPercent = prefs.getUInt("bright", 50);
493   startMinute = prefs.getUInt("startMin", 0);
494   prefs.end();
495
496   esp_sleep_wakeup_cause_t wakeupReason = esp_sleep_get_wakeup_cause();
497
498   WiFi.mode(WIFI_STA);
499   WiFi.begin(WIFI_SSID, WIFI_PASS);
500
501   while (WiFi.status() != WL_CONNECTED) {
502     delay(500);
503   }
504
505   configTime(0, 0, "pool.ntp.org", "time.google.com");
506
507   setenv("TZ", "CET-1CEST,M3.5.0,M10.5.0/3", 1);
508   tzset();
509
510   int retry = 0;
511   while (time(nullptr) < 1000000000L && retry < 10) {
512     delay(500);
513     retry++;
514   }
```

```
515
516   if (time(nullptr) > 1000000000L) {
517     timeSynced = true;
518   }
519
520   if (wakeupReason == ESP_SLEEP_WAKEUP_TIMER && started) {
521
522     prefs.begin("lightConfig", true);
523     unsigned int done = prefs.getUInt("daysDone", 0);
524     unsigned int total = prefs.getUInt("days", 5);
525     prefs.end();
526
527     if (done >= total) {
528
529       prefs.begin("lightConfig", false);
530       prefs.putBool("started", false);
531       prefs.putUInt("daysDone", 0);
532       prefs.end();
533
534     } else {
535
536       strip.begin();
537       Wire.begin(21, 22);
538       display.begin(SSD1306_SWITCHCAPVCC, 0x3C);
539
540       startLightPhase();
541
542       strip.clear();
543       strip.show();
544       display.clearDisplay();
545       display.display();
546
547       delay(200);
548
549       esp_sleep_enable_timer_wakeup(calculateWakeupTime());
550       esp_deep_sleep_start();
551     }
552   }
553
554   Wire.begin(21, 22);
555   display.begin(SSD1306_SWITCHCAPVCC, 0x3C);
556   strip.begin();
557   strip.show();
558
559   knightRiderEffectBlue_welcome(1, 20);
560   showHelloJKU();
561
562   delay(1000);
563
564   menuStack[0] = MENU_COLOR;
565   showCurrentMenu();
566 }
567
568 void loop() {
```

```
569
570   if (millis() - lastSync > syncInterval) {
571     configTime(0, 0, "pool.ntp.org", "time.google.com");
572     lastSync = millis();
573   }
574
575   checkTouch();
576 }
```

Listing A.1: Complete Code

A.2 Instruction for Luminacell-Biostim



Explanation Dimming

We are controlling the brightness of the LEDs using software dimming instead of a traditional constant-current driver since the LED rings can't be controlled that way. Here's what that means:

How it works:

1. Each LED has an internal circuit that pulses the full light intensity on and off very quickly.
2. When we reduce brightness in software, the LED is on less of the time.
3. The result is that the average light intensity decreases, even though the LED is still switching on and off very fast.
4. At 70% intensity, the light is on 70% of the time and off 30% of the time – for example 7ms on and 3ms off.

Difference from constant-current regulation:

1. Constant-current regulation provides a smooth, unchanging light intensity.
2. Software dimming only controls the average intensity, not the exact instantaneous current.
3. This can make a small difference for experiments sensitive to light pulses.

Practical effect on experiments:

1. The samples see approximately the intended light intensity over time.
2. Very rapid pulses may slightly affect sensitive reactions.
3. This method also makes it easy to adjust brightness safely without changing the hardware.

What happens at 100% intensity? At 100% intensity, the light is on 100% of the time and off 0% of the time. This results in a steady current flow, which means the LED does not flicker at this setting.

Time Synchronization & Time Zone Handling

The device synchronizes its time via NTP using public servers (e.g. pool.ntp.org). NTP provides universal UTC time and works worldwide as long as an internet connection is available.

The local time, including automatic daylight saving time adjustment, is defined by a fixed time zone setting in the firmware (our program).

This configuration corresponds to Central European Time (Austria/Germany). If the device is operated in another country (e.g. UK or USA), the time zone string must be adjusted accordingly.

WiFi

The device requires a WiFi SSID (WiFi name) and password in the code because it needs to connect to a wireless network to synchronize time.

A small WiFi symbol is displayed on the top right of the screen to indicate whether the device was able to synchronize the current time via the internet. It appears in the overview menu and during light phases. If the symbol is visible, the time synchronization was successful, and the system is operating normally. If a warning symbol (!) appears instead, the device was not able to connect to the network or retrieve the current time.



In this case, the scheduled light cycle may not start at the correct time. To resolve this issue, restart the device and allow it a few moments to reconnect to the WiFi network. If the problem persists, make sure that the device is within range of the WiFi network. Also, checkt if the WiFi password or name has changed (make those changes in the software and upload new software).

User guide

The device is an ESP32-based light therapy system with six RGB LED rings, a 128×64 OLED display, capacitive touch controls, and WiFi connectivity. It is designed to run automated daily light sessions based on user-defined parameters while minimizing power consumption through deep sleep functionality.

When powered on, the system performs a short LED welcome animation and initializes the display. It then connects to the configured WiFi network and synchronizes the current time using public NTP servers. The device operates with a fixed Central European time zone configuration (CET/CEST), including automatic daylight saving time adjustment. An active internet connection is required for correct time synchronization.

After initialization, the user is guided through a structured menu. Using four capacitive touch buttons (Power, <, OK, >) the following parameters can be configured sequentially: light color (red 620–650 nm, blue 440–480 nm, or green 500–560 nm), daily light duration (1–60 minutes), number of consecutive days (1–30 days), and daily start time (12-hour format with AM/PM). Each selection is confirmed with the OK button, and a summary screen displays all chosen settings together with the current synchronized time before activation.

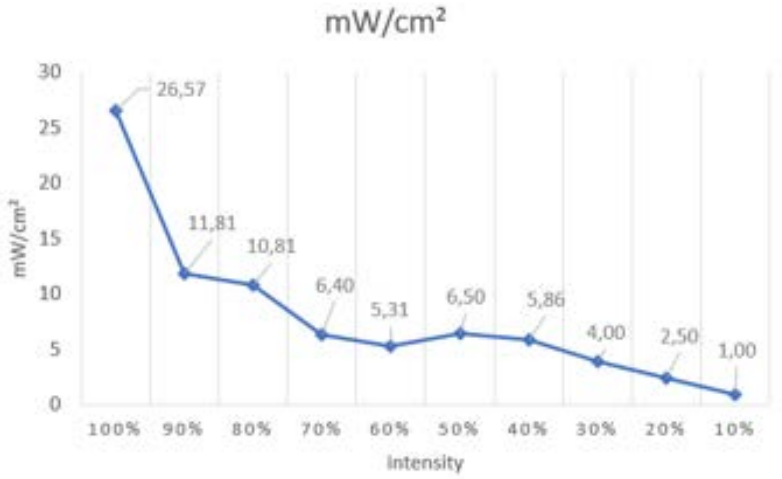
Once confirmed, the configuration is stored in non-volatile memory (ESP32 Preferences), ensuring that settings persist across power cycles. The device then calculates the next scheduled start time and enters deep sleep mode to conserve energy.

At the programmed time, the ESP32 wakes automatically via timer interrupt and starts the light phase. During the session, the selected LEDs illuminate continuously in the chosen color for the defined duration. The display shows the remaining time and the current clock time. The user can interrupt the session manually via the Power touch button on the side of the device. After completion, the system increments the internal day counter, recalculates the next activation time, and returns to deep sleep.

Once the predefined number of consecutive days has been completed, the device remains in deep sleep. The user may then reactivate the system manually to configure and start a new cycle.

Energy input through light

We estimated the power density (W/cm²) rather than measuring it directly. Since we couldn't measure the exact optical output of the LEDs on the surface, we used the electrical input (current and voltage) along with typical efficiency values to approximate the result. This gives a reasonable order-of-magnitude estimate, but not a precise measurement. For calculations see Excel file.



Battery

The device uses a rechargeable 5000 mAh lithium-ion battery with 3,7V output voltage. It is equipped with a reverse-polarity-protected connector to prevent incorrect insertion. The connector indicator lights up red when plugged in, as it would during charging, but it remains red continuously even when finished charging. Recharge the battery every 3-4 Days when you use the device. After recharging the settings have to be set again

Instruction manual



Make sure that the battery inside is connected and the device is closed.

1. If display is off – press Power once. Now an animation starts.
2. After animation - choose colour by pressing < or >. Press OK to select.
3. Navigate through all other menus with < and > and always confirm selection with OK button.
4. Check settings in –Summary– menu
   a) Check for WiFi symbol on top right – when shown then you are ready to confirm again with OK. When (!) is shown: see WiFi section in user manual.
   b) For correction press Power button to move back to previous menu.
   c) Confirm settings with OK button. Device will shut down and wake up at time you set. During this sleep time all buttons and display are turned off. No interaction with device is needed and possible.



5. When device wakes up and starts light phase you can press Power to reset and start over again. Confirming with Power button will bring you back to first menu. For common usage no interaction is needed. Device will shut down and wake up again automatically.

When device is not in use turn it off by pressing Power button until quit dialogue shows and confirm with pressing Power button again. When device is not in use for longer periods, simply disconnect battery – no shut down with Power button necessary.

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