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SANTA CRUZ

BRIDGING IMAGING AND TRAINING: OPEN SOURCE DEVICE FOR BIOLOGICAL IMAGING AND AN INTERACTIVE VIRTUAL LAB TRAINING

A dissertation submitted in partial satisfaction of the
requirements for the degree of

Doctor of Philosopy

in

COMPUTER ENGINEERING

by

Victoria Ly

March 2024

The Dissertation of Victoria Ly
is approved:

Dr. Mircea Teodorescu, Chair

Dr. David Haussler

Dr. Sri Kurniawan

Peter Biehl
Vice Provost and Dean of Graduate Studies

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2024

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Abstract

Bridging Imaging and Training: Open Source Device for Biological Imaging and an Interactive Virtual Lab Training

Victoria Ly

This dissertation explores the utilization of internet-connected devices to address the accessibility challenges associated with microscopy in the field of biology. Traditional microscopy devices are often costly, limiting their widespread adoption in laboratories. To overcome this barrier, we developed a cost-effective multi-well imaging device integrated with an open-source pump and stimulation system. This device facilitates longitudinal cell studies and reduces the time required for capturing cell images, thereby enhancing efficiency for biologists.

Moreover, our internet-connected imaging system serves as an innovative educational tool for aspiring scientists. Through remote project-based learning activities and engaging serious games, complex biological concepts become more accessible, fostering enthusiasm and understanding among high school and undergraduate students. The dissertation discusses the application of this system in an educational setting and the implementation of an algorithm for determining organoid size through computer vision. Additionally, it introduces an interactive virtual lab, designed to provide a low-stakes environment for students to learn scientific and laboratory safety protocols.

Overall, this work highlights the potential of internet-connected devices in revolutionizing both biological research and education. By making microscopy more accessible and engaging, it empowers scientists and students alike to explore the intricacies of biology with enthusiasm and curiosity.

The work in this dissertation is dedicated to my friends and family that have supported me on my PhD journey.

Acknowledgments

"Here's a toast to my friends [lab mates and family]" Taylor Swift

I wish I could say thank you to each of my friends and lab mates that have supported me along the way, but then this section would probably be longer than my actual dissertation.

First and foremost, I extend my sincerest appreciation to Dr. Mircea Teodorescu, who has not only served as an exceptional mentor and advisor but has also become a cherished lifelong friend. Alongside Dr. Sri Kurniawan, they have extended their care and support to their students as if they were family. Their home has been a sanctuary of celebration for every milestone, where warmth, food, and hospitality were generously shared. Their selflessness knows no bounds, and their kindness has profoundly impacted my life.

To my family, I am immensely grateful for your unwavering support. To my parents and my brother Dylan, thank you for your endless love and encouragement.

I would be remiss not to include a nod to Taylor Swift, whose music has provided the soundtrack to many late-night study sessions and moments of inspiration throughout this journey.

To my husband, Dr. Erik Jung, our shared experience of embarking on our PhD adventures in the same lab has been something I cherish. Your unwavering support and belief in me have been my anchor through the highs and lows of this endeavor. I am immensely proud of you, and I eagerly anticipate the adventures that lie ahead for us.

Finally, I extend my heartfelt gratitude to my esteemed committee members: Dr. Mircea Teodorescu, Dr. Sri Kurniawan, and Dr. David Haussler. Your invaluable guidance, insights, and unwavering support have been instrumental in shaping this significant milestone in my academic journey.

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Chapter 1

Introduction

1.1 Introduction

Biologists engage in a plethora of hands-on tasks essential for pioneering research. Each researcher employs distinct protocols tailored to their experiments, often derived from prior studies or developed through trial and error. Despite the expectation for detailed and replicable protocols, studies reveal that approximately 70% of researchers struggle to reproduce experiments published in papers [Baker, 2016]. Manual execution of tasks introduces variability in experiment procedures and sample handling, potentially leading to protocol inconsistencies. Standardized protocols are paramount, and automated systems ensure uniformity in experiment execution, minimizing errors during training sessions for handling specific biological samples.

In addition to detailed protocols, the monitoring and manipulation of live cell cultures are fundamental in biological and biomedical research. Microscopy advancements have transformed the observation and analysis of cellular processes across various biological samples, from frog embryos and zebrafish to both 2D and 3D mammalian models. [Specht et al., 2017]. The field has witnessed significant

advancements, enabling scientists to utilize diverse types of microscopes. These range from super-resolution microscopes facilitating the imaging of individual biomolecules [Godin et al., 2014, Miller et al., 2010]to conventional benchtop microscopes widely used in academic research laboratories [Selinummi et al., 2009,] [Miller et al., 2010,] industrial and corporate settings [Yao et al., 2020, Zamxaka et al., 2004] and teaching laboratories and classrooms [Ferreira et al., 2019].

Despite the availability of diverse microscopy devices, their substantial costs often limit laboratories, forcing them to rely on a centralized unit shared among multiple researchers. Which often can lead to the need for advanced planning and potential scheduling conflicts. This financial constraint hinders the widespread adoption of multiple microscopy units across laboratories, impeding research accessibility and collaboration.

With the uproar of open source maker devices and 3d printers there are ways to help reduce the costs of laboratory equipment [Baden et al., 2015]. 3-D printing has empowered the scientific and engineering communities to fabricate essential components swiftly, facilitating quicker and smoother establishment of laboratories than previously possible [Baden et al., 2015]. Moreover, consumer-friendly laser-cutting technology simplifies the process of swiftly shaping raw materials like plywood, acrylic, or aluminum into intricate forms within seconds. The potential for innovation expands significantly when these mechanical components are integrated with readily available electronics, budget-friendly microcontrollers like Arduino boards [Uno, 2019], and single-board computers such as Raspberry Pi [Pi, 2015]. Following an initial investment typically under a thousand dollars (e.g., for establishing a 3D printer), the additional materials required to construct a wide array of items are minimal, including a few hundred grams of plastic (approximately US$30/kg), cables, and basic electronic components[Baden et al., 2015]

with these parts, we can build devices that can become internet-connected allowing for more technology to be accessible in the laboratory without paying for proprietary or commercial devices or software. By embracing an open-source sharing culture, researchers and makers can openly adapt device designs or construction plans to tailor creations to their specific requirements without needing to begin from scratch. With affordable maker options available, we can also extend our reach to communities worldwide, fostering greater interconnectedness among diverse groups.

The integration of internet-connected devices introduces a transformative avenue for resource sharing. This connectivity not only addresses the challenge of limited access to microscopy resources but also opens up opportunities for remote learning experiments through project based learning (PBL) and gamified educational supplemental tools.

Typically, educational authorities such as the National Association of Biology Teachers strongly advocate for the inclusion of hands-on activities in biology classes, such as those conducted in field or laboratory environments [Holstermann et al., 2010]. However not all schools are able to provide high quality hand's on experiments for students due to budget constraints. Which can lead to students not having an interest in biology. Historically, three fields—medicine, civil engineering, and law—have been regarded as the most lucrative options for steady income, leading to limited enrollment in natural science subjects, and even fewer individuals pursuing active research roles [Baden et al., 2015]. This dissertation explores the potential of leveraging internet-connected devices to facilitate remote learning experiences and gamified educational approaches. Such initiatives aim to inspire and engage high school and undergraduate students with an interest in biology, fostering a new era of accessibility and enthusiasm in the field

of biology.

1.1.1 Motivation

In our endeavor to enhance the efficiency of cell imaging processes for biologists and lab technicians, we're committed to developing a cost-effective multi-well imaging device that seamlessly integrates with an open-source pump and stimulation system. Our aim is to construct a system using easily accessible off-the-shelf components, ultimately facilitating a significant increase in the number of experiments conducted in biological labs. By enabling longitudinal cell studies and reducing the time required for capturing cell images, our proposed solution has the potential to greatly improve efficiency for biologists, who often engage in manual tasks. Manual labor can introduce variability in how scientists conduct their experiments or handle biological samples, leading to potential discrepancies in protocols and care husbandry. Automated machinery offers a solution by simplifying tasks such as imaging, feeding, and waste removal for biological samples.

Beyond the immediate benefits for researchers, our internet-connected imaging system is poised to support both current and future biological researchers. By incorporating remote project-based learning activities and interactive serious games, this system can not only aid existing researchers but also serve as a valuable educational tool for aspiring scientists. This innovative device, when coupled with educational programs, has the potential to demystify complex topics, facilitating a smoother transition for budding scientists into the intricacies of biological research.

1.2 Contributions to the Field

The work in this dissertation describes the design, development, experiments, and analysis to create a an open source longitudinal imaging system with image analysis and an interactive approach to enhancing undergraduate understanding of 3D cell culture concepts as well as laboratory safety. The contributions are listed below.

- A methodology and protocol for building an open source longitudinal parallel imaging system
- An application of using an open source imaging system in an educational setting
- An algorithm for determining the size of an organoid through computer vision
- A low stakes environment for students to learn about a scientific protocol as well as laboratory safety and conduct.

The dissertation concludes with a summary of potential applications for future research.

Chapter 2

Background

2.1 Introduction

The literature review, encompassing Chapter 2 and sections 2.1 - 2.5, delves into fundamental techniques for cultivating organoids, devices utilized in longitudinal cell studies, low-cost imaging systems, remote education and serious games in biology.

2.2 Overview

From research, we know the human brain continues to develop as the human develops from birth. According to Johnson et al., there are multiple theories of how the brain develops functionally [Johnson, 2001].

- "The maturational perspective states that cognitive abilities develop as the cortical areas mediating them mature."
- "The interactive specialization approach suggests that cognitive abilities develop as the networks of cortical areas that mediate them develop appropri-

ate interactions.

- "The skill-learning hypothesis proposes that certain regions will be active during the development of skills in infants, but the other regions will be active once the skill has been learned (as in adult motor learning)"

To confirm or deny these theories, many have studied how the brain develops. Some methods to study the human brain include: 2D and 3D cell culture studies, post mortem analyses via positron emission tomography (PET), or magnetic resonance imaging (MRI) [Johnson, 2001].

Typically, biologists will use 2D cell cultures as their baseline models to determine how effective a protocol is; however, growing cells in 3D cell cultures is more similar to studying cells *in vivo* [Breslin and O'Driscoll, 2013]. In 2013, Lancaster et al. demonstrated that cerebral organoids could model human brain development, shown in figure 2.1. The results of this paper have influenced the Braingeneers to use cerebral organoids as well as Lancaster et al. method [Lancaster et al., 2013], to model the human brain as well.

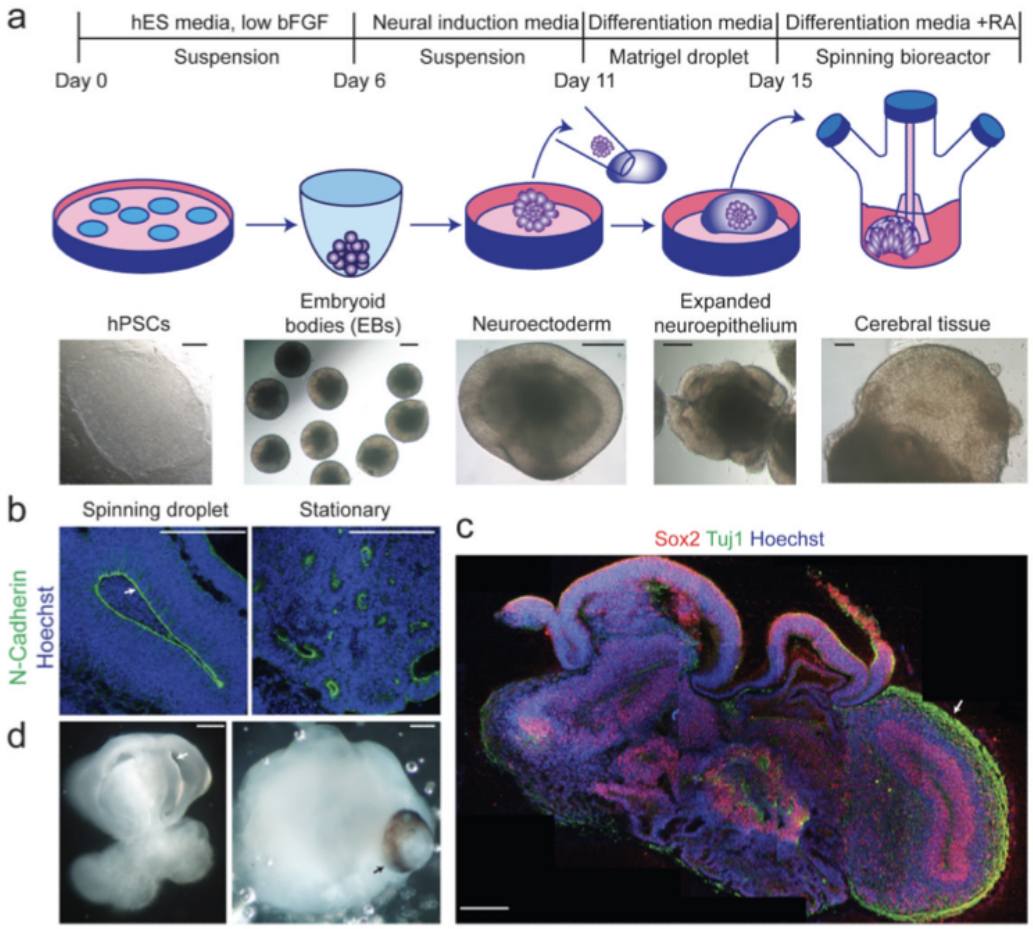

Figure 2.1: Detailed explanation of cerebral organoids cultures. (a) The methods used to culture the organoids. (b) The growth of neuroepithelial tissues. (c) It shows a cross-section of the tissues from figure b, showing the complicity of the morphology that has begun to grow in the organoid. (d) Exhibits low light brightfield microscopy. [Lancaster et al., 2013]

Lullo et. al also used brain organoids to understand how neurons and diseases develop [Di Lullo and Kriegstein, 2017], figure 2.2.

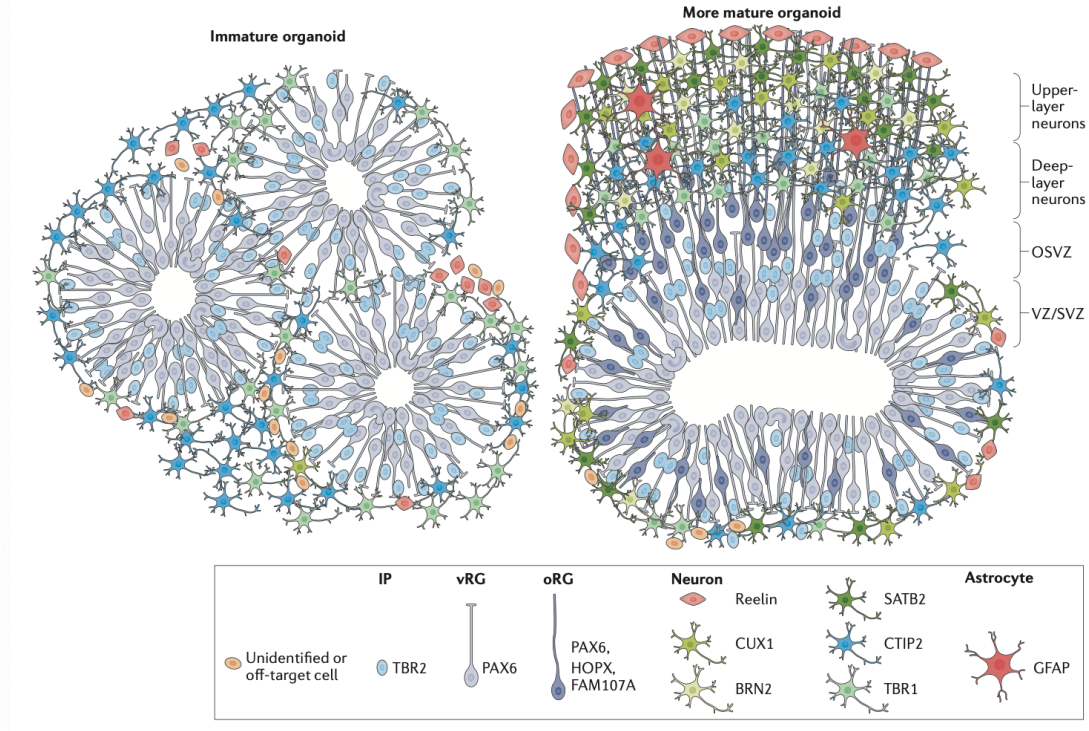

Figure 2.2: Using a particular protocol, Lullo et. al compares an immature organoid versus a more mature organoid[Di Lullo and Kriegstein, 2017].

The primary objective is to use cerebral organoids as a model for the human brain. This particular mammalian model will require imaging from within a controlled environment for warmth, humidity, and proper gas exchanges, all available through the use of an incubator connected to a gas tank.

The purpose of this literature review is to highlight what has already been achieved in the following fields:

- Microscopes Used for Cell Biology
- Longitudinal Live-Cell Studies
- Microscope with Incubators
- 3D Printing Lab Equipment

- Low-Cost Maker Scopes
- Remote Education
- Educational Biology Games
- Virtual Reality Technology in Education

2.3 Microscopes Used for Cell Biology

Microscopes allow the user to closely examine specimens with magnification and high resolution. Several different types of microscopes are used in cell biology: the inverted microscope, confocal microscope, and fluorescence microscope, just to name a few. The first microscope was built in the 1590s by, dutch eyeglass manufacturers, Zaccharias and Hans Janssen, who first observed magnification by putting various lenses in a tube [Masters, 2001]. A single-lens microscope was used by Antonie Van Leeuwenhoek in the 1670s to observe cells and microorganisms [Masters, 2001]. The next major advance in microscope technology was when Joseph Jackson Lister invented microscopes with objectives in the 1800s. [Masters, 2001].

One of the most notable names in the microscope field is Carl Zeiss, and his microscope company invented the prototype microscope capable of fluorescent microscopy in the 1900s [Masters, 2001]. There are different versions of microscopes that have been invented throughout the years. Starting in 1957, the confocal microscope was developed by Marvin Minsky [Masters, 2001]. Minsky designed his microscope with a pinhole aperture and could do point-by-point illumination, which helps reject the light that is out of focus; these features are still used in modern confocal microscopes today [Semwogerere and Weeks, 2005][Gu, 1996].

The confocal microscope allows researchers to see cellular structure and function due to the optical sectional ability that the confocal microscope provides. This particular microscope is preferred by biologists over widefield microscopy because raw images can be viewed without deconvolution being applied, the signal to noise ratio of images produced from a confocal microscope is much lower, and the theoretical resolution is better when compared to a widefield microscope. [Francis et al., 2019]. The confocal microscope is best explained by thinking about a system with two lenses. These lenses assist by focusing the light at one focal point to the other lens.

Some microscopes are able to do fluorescent microscopy (figure 2.3B) by exciting the fluorophores with a proper excitation light (figure 2.3C). Fluorescent microscopy is useful when trying to highlight certain features in cell biology and tracking while performing longitudinal imaging.

Another important factor to consider when preforming live cell imaging is photobleaching. This occurs quite frequently when over exciting fluorophors during fluorescent microscopy. It is believed that photobleaching can occur when the fluorophor molecules react with oxygen and the reaction can cause the cells to no longer fluoresce [Semwogerere and Weeks, 2005].

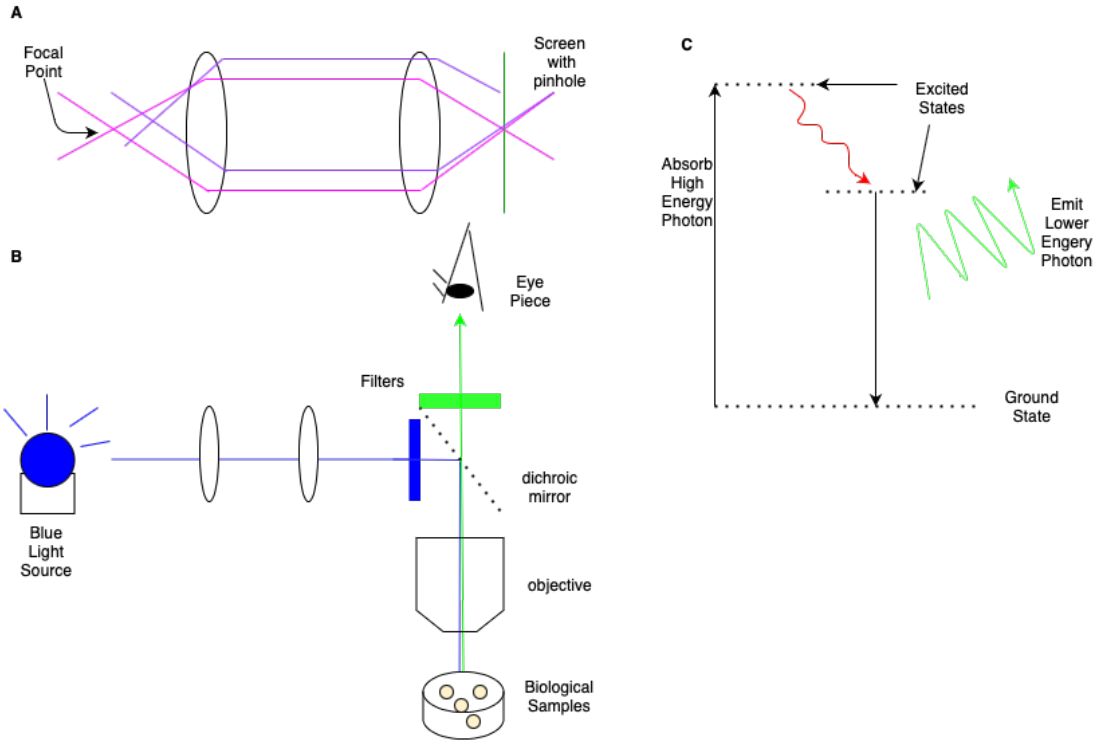

Figure 2.3: A. If the light is not focused on the focal plane, it gets rejected by the confocal microscope. For instance, two different light rays, dark blue and light blue, and if you look at the screen with the pinhole, only the dark blue light is allowed to reach the screen to view the focused plane while other unfocused light, light blue, is rejected. B.Typical setup diagram of a fluorescence microscope. The light bounces off a dichroic mirror and directs towards the specimen you want to observe under the microscope. C. Fluorescent dyes are excited by a certain wavelength of light and, when excited, emit a different wavelength of light. These images were inspired by [Semwogerere and Weeks, 2005]

Minimizing the chances of photobleaching is very critical for a successful fluorescent microscopy experiment [Swedlow and Platani, 2002]. The easiest solution is to reduce the intensity of the excitation light or minimize the cell's exposure time to the excitation light [Swedlow and Platani, 2002].

2.4 Longitudinal Live Cell Studies

The conventional practice among biologists involves capturing images of their experiments during feeding sessions, necessitating the removal of cells from the incubator. However, this approach introduces temporal gaps between successive images and poses challenges in maintaining an optimal cellular environment crucial for cell viability. Temperature control and regulation are some of the most essential parameters for maintaining healthy cells [Jensen, 2013]. Jeffrey Stern, the husband of Sally Temple, co-founder of the Neural Stem Cell Institute, said, "If the cells are living well in the incubator, you have to put the microscope in the incubator" [Baker, 2010]. To accomplish this, many biologists like Temple have retrofitted microscopes by attaching a camera and drilling a hole to allow the camera to view the cells in the incubator [Baker, 2010]. This design allowed Temple to capture images as often as she wanted; however, there was one caveat, Temple could only see the cells that were in the microscope's field of view since she could not move the microscope slide without disturbing the experiment[Baker, 2010]. Longitudinal live cell studies have shown promising results [Baker, 2010]; however, it is a very tedious task especially depending on the frequency of the images and if biologists are attempting to do this without a device that automatically captures images and provides cells with their proper living conditions [Jensen, 2013].

Figure 2.4: Sally Temple's automated imaging and incubation device [Baker, 2010]

Many major microscope companies like Zeiss and Thermofisher have developed their own imaging devices specifically for time-lapse images. Thermofisher's device, the EVOS M7000 imaging system provides automated imaging with an onboard incubator that allows the user to do time-lapse imaging while the cells are in an incubator [ThermoFisher, 2020]. The base price of these devices is around $ 14,190.00 [ThermoFisher, 2020]. Many labs can only afford one of these devices at this price point on top of the price of multiple incubators, microscopes, and other necessary lab equipment.

2.4.1 Fast Manufacturing of Lab Equipment

In the early 1900s, fabrication in industry relied on subtractive fabrication, and it was not until the 1980s that additive manufacturing became prominent in industrial fabrication [Savini and Savini, 2015]. Subtractive manufacturing stems from removing unnecessary material from a block to create the object. This method typically refers to CNCing, sculpting, or carving. The opposite is additive

manufacturing is when you add material to nothing and create the object. A 3D printer is usually commonly associated with additive manufacturing. This particular method has become quite popular due to the low cost of desktop 3D printers. With this device, you can quickly test various designs and rapidly iterate through different designs before you send out the design to be manufactured by a company, which could be costly depending on the material and how many times you would have to iterate the design to get it right.

The inventor of stereolithography (STL) is Charles Hull. In the 1980s, Hull described a method in which he was able to deposit and solidify layers of a liquid polymer that hardens with UV light [Savini and Savini, 2015]. Hull inspired others to contribute to additive manufacturing; for example, the University of Texas created Selective Laser Sintering (SLS). However, it wasn't until the early 1990s, when 3D printing became an accurate coined phrase by a Massachusetts Institute of Technology group. They took inspiration from the Canon inkjet printer [Savini and Savini, 2015].

C.S. Crumps is credited with the development of Fused Deposition Modeling (FDM) technology which is capable of depositing material layer by layer via a robot with 3-axis [Savini and Savini, 2015]. Crumps founded Stratysis Inc in 1992, and the company is significant in manufacturing 3D printers. It is important to note that at this time, 3D printers were costly and not commonly found in a household but rather part of industrial manufacturing. It was not until 2005 when companies began committing to making 3D printers more affordable [Savini and Savini, 2015]. University of Bath by A. Bowyer was able to create a 3D printer that could manufacture its own parts, which was part of a project called Rep Rap (Replicating Rapid Prototyping)[Savini and Savini, 2015]. With open-source hardware and software, it encouraged makers to create and modify

their own printers. This leads us to the creation of MakerBot industries in 2006. MakerBot created at-home kits for enthusiasts to get their hands on their own FDM 3D printer [Savini and Savini, 2015]. With the increased numbers of at home kits and 3D printers being able to produce parts to create another printer, the price of 3D printers has gone down and is easily accessible for makers at home. Another benefit of additive manufacturing and 3D printing is the large array of materials you can print with. For example, Acrylonitrile Butadiene Styrene (ABS), Polyactic Acid (PLA), Poly Cyclohexylenedimethylene Terephthalate glycol-modified (PCTG), Polypropylene, ceramic, some metals, and Nylon are all examples of materials that can be printed with a 3D printer. With access to various types of materials, 3D printing has changed manufacturing processes by allowing customization of material choice as well as the ability to make stuff at home [van Wijk and van Wijk, 2015].

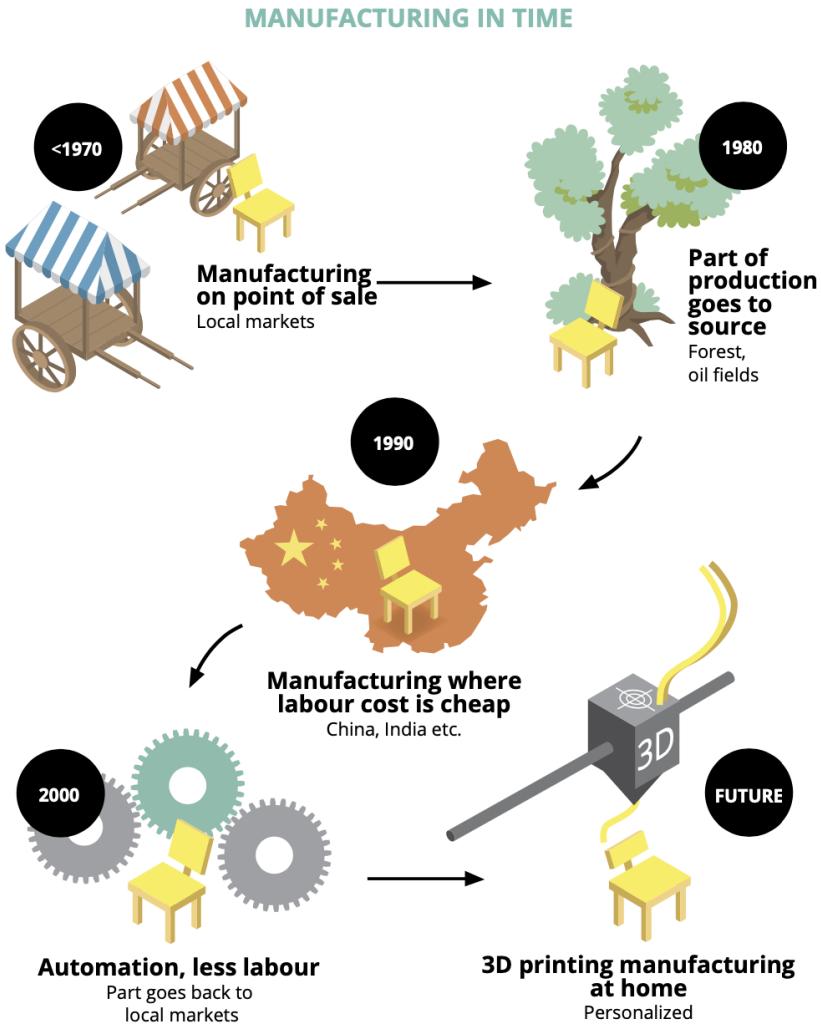

Figure 2.5: A timeline of how manufacturing has changed and how it could change due to the accessibility of 3D printers [van Wijk and van Wijk, 2015]

There exists bio-based plastics which can be found in the common LEGO brick. These plastics are made out of crops, which is converted into biomaterials and then into biobased plastics such as PLA [van Wijk and van Wijk, 2015]. PLA is a very affordable biobased plastic, and it is excellent for rapid prototyping since most 3D printers can easily print with PLA. This is a common plastic many 3D printer

hobbiest use in order to rapidly prototype. However PLA isn't able to withstand high temperatures. PETG and PCTG are both another alternative due to PCTG belongs to the glycol-modified copolyester family of terephthalate polyesters, sharing molecular similarities with PETG. PCTG offers distinct advantages such as heightened chemical resistance, expanded printing capabilities at elevated temperatures, and enhanced strength compared to other polymers. These remarkable attributes render PCTG conducive to manufacturing across diverse temperature ranges and printing orientations, complemented by its safety in handling and recyclability, further bolstering its appeal in various applications[Bhosale, 2023].

Typically 3D manufacturing has two main approaches: additive and subtractive, where both require dedicated equipment. In the past, some of these devices were limited to specialized manufacturing facilities. Over the past couple of decades, 3D manufacturing has gone through a revolution. Equipment, including 3D printers and computer numerical control (CNC) machinery, have become affordable and ubiquitous in most university engineering laboratories. Research in the areas of labs-on-chip, optofluidics, microscopy, in combination with developments in consumer-oriented tools for "makers," has the potential to democratize access to cell biology based research [Zhang et al., 2013, Baden et al., 2015, Alessandri et al., 2017]. Laboratories are now able to more easily develop custom devices that can be shared with the greater research community as open-source projects[Baden et al., 2015]. Laboratories can develop custom devices that are often shared with the greater research community as open-source projects [Baden et al., 2015].

In 2014, Gross et al. assessed the potential impact that a 3D printer could have in a lab setting. Rapid prototyping is a term typically referred to when a 3D printer is involved due to standardization of .STL file format from computer-aided

design (CAD) programs like Solidworks or AutoDesk Fusion360 [Gross et al., 2014].

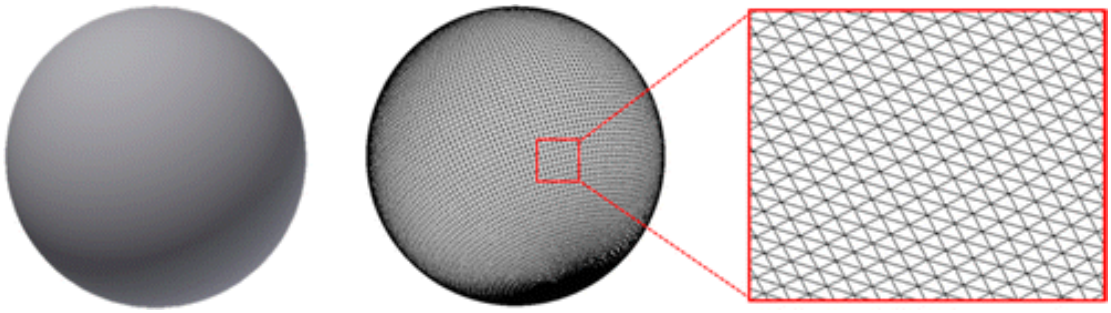

Figure 2.6: The first sphere is what the design looks like in CAD, and the second sphere shows what the CAD design looks like once it is saved as an .STL file. The object saved as an .STL file has triangular shapes on this surface which dictate coordinates for the vertices of the triangles to be stored in the .STL file. With this information different 3D printers can print out the file even if the design was made on different CAD software and printed pieces should look identical [Gross et al., 2014]

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2.4.2 Microscopes with Incubators

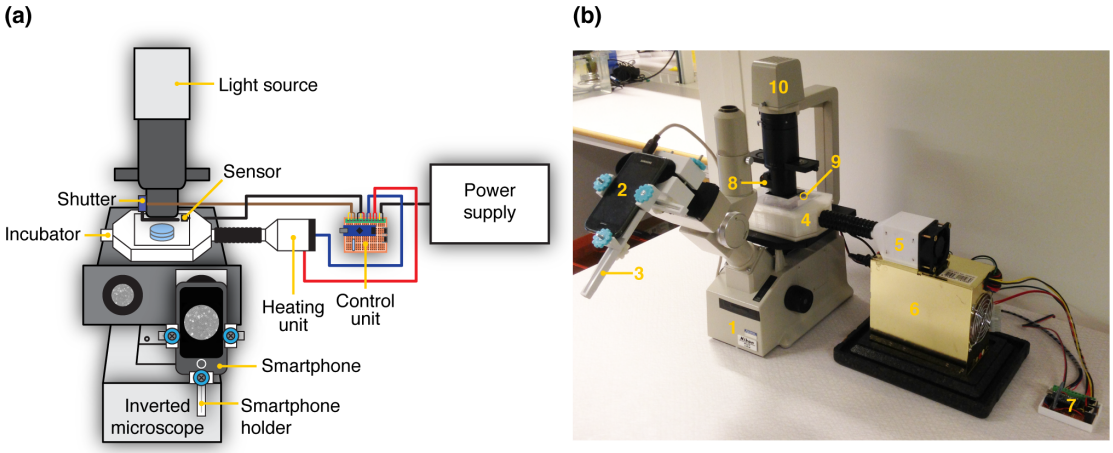


Figure 2.8: a. Schematic diagram of the device b. the actual microscope equipted with a smart phone and in house made incubator [Vera et al., 2016]

Hernandez Vera et al. created a low cost time-lapse imaging device using easily accessible items, a 3D printer, an inverted microscope and a smart phone

[Hernández Vera et al., 2016] This particular imaging device attaches to a typical microscope that can be found in research labs to assist in longitudinal cell studies that can be performed in the onboard incubator on the microscope. Merces et al. developed an open-sourced low-cost microscope with a custom GUI that can image from within an incubator [Merces et al., 2020].

2.4.3 Maker Scopes: 3D Printed/low Cost/Off the Shelf Components

Single Well Microscopes

The Flypi Scope is a 3D printed open-source microscope that is capable of fluorescence and white light imaging [Chagas et al., 2017]. This particular system uses a raspberry pi computer with one raspberry pi camera and the user can view either petri dishes or microscope slides in brightlight or fluorescent microscopy.

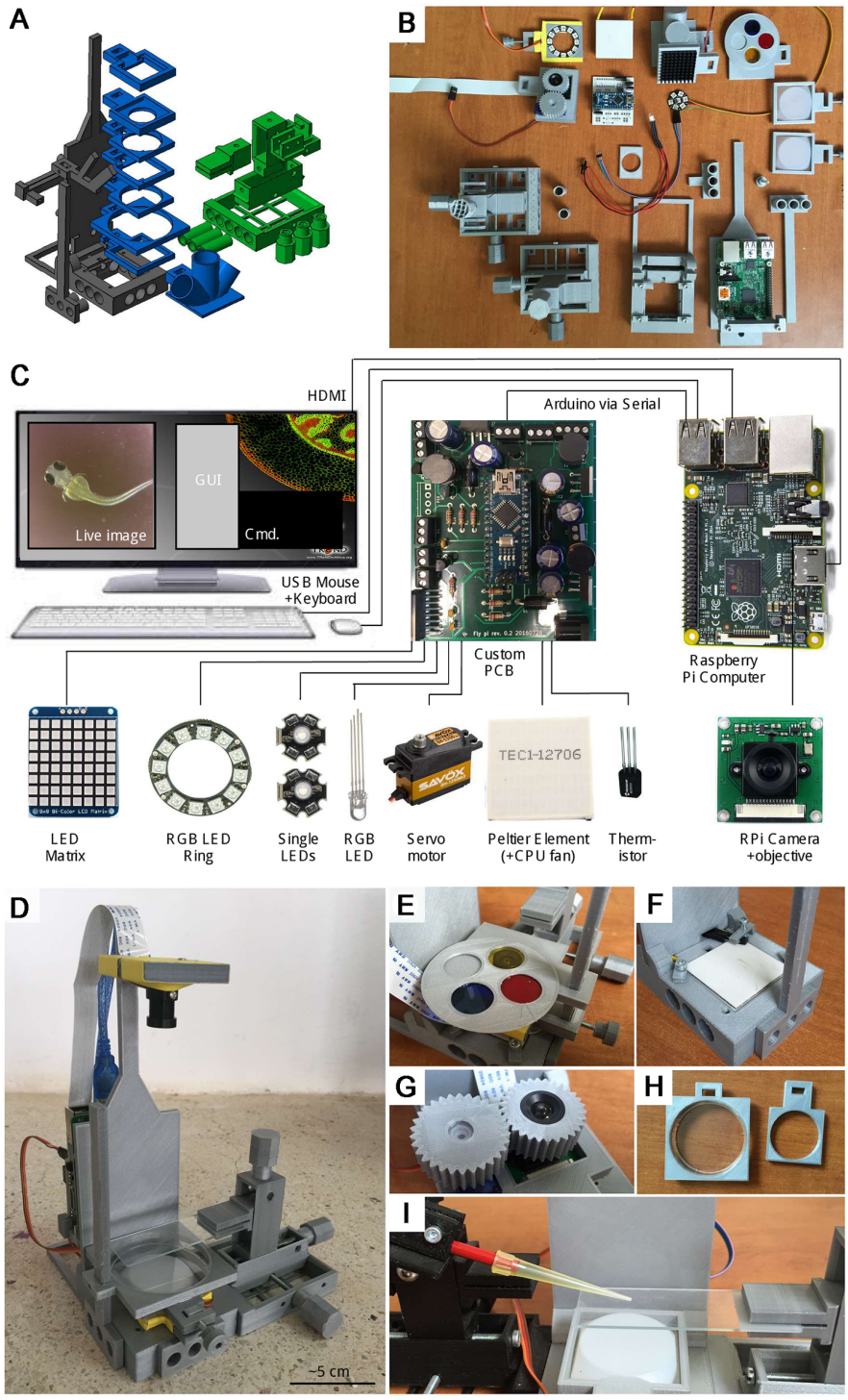


Figure 2.9: FlyPi Scope diagram [Chagas et al., 2017]

Brown et al. 3D printed a confocal microscope, called AttoBright, which has

the ability to do single-molecule detection [Brown et al., 2019].

A miniature 3D printed open source wireless microscope capable of fluorescence microscopy[Liberti III et al., 2017]. This device was originally constructed to be placed monitor activity in the brain of a songbird. This particular microscope can also do fluorescence microscopy.

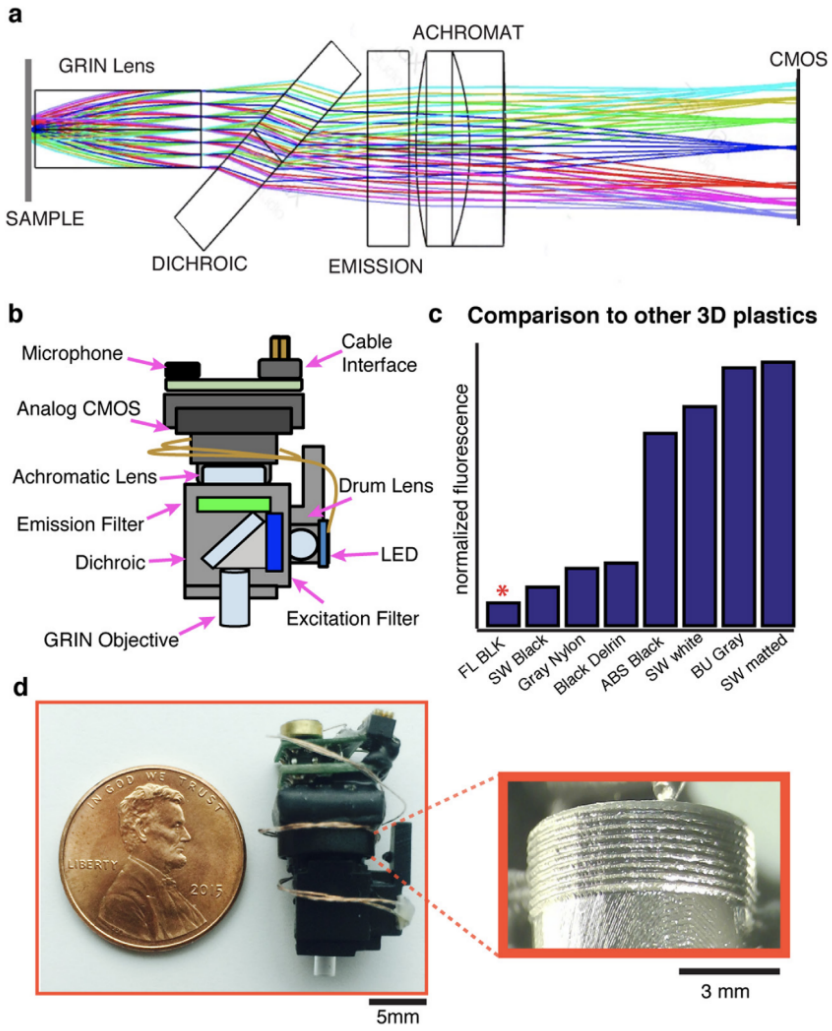


Figure 2.10: a. shows how various wavelengths pass through. b. is a diagram of the device. c.is a comparison between different black plastics d. shows the size of the device [Liberti III et al., 2017].

The foldscope is a low cost paper microscope that is based on origami

[Cybulski et al., 2014]. This particular device has demonstrated that it can preform brightfield, darkfield and fluorescence microscopy. The foldscope comes down to approximately a dollar per device.

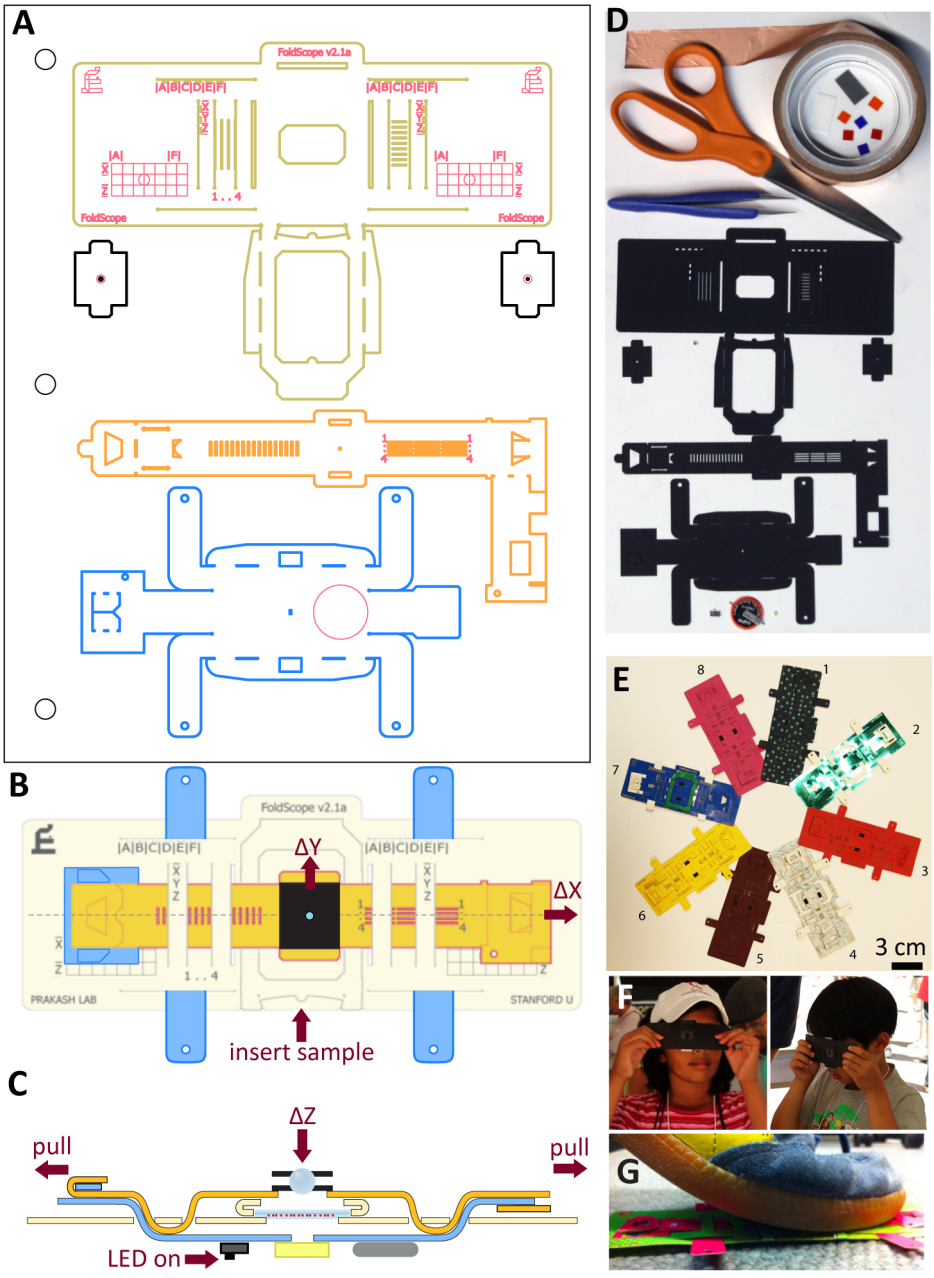


Figure 2.11: Foldscope Schematics [Cybulski et al., 2014]

Aidukas et al. built a low-cost microscope with sub-micron resolution using

a Raspberry Pi in conjunction with a color camera with Fourier ptychography [Aidukas et al., 2019]. This particular device also has a wide field of view (FOV) and provides images with sub-micron resolution.

Kim et. al created a low cost microscope with a webcam, that they retrofitted by reversing the lens on the webcam, to image cells through Matlab from within the incubator[Kim et al., 2012]. Diederich et al. created UC2 (You. See. Too), which is a reconfigurable open-source inexpensive microscope made from off the shelf components [Diederich et al., 2020]. This particular device is capable of brightfield and light sheet microscopy (depending on the configuration).

Walzik et al. created a portable device, low cost, and capable of long-term live-cell imaging. This device can be quickly re-built since it is comprised of 3D printed parts, off-the-shelf components, and utilizes the open-source microcontroller the Arduino [Walzik et al., 2015].

Nuñez et. al. created an open-source and low-cost multi fluorescence imaging system[Nuñez et al., 2017]. The single-camera imaging system can even do time-lapses with acrylic filters that are inexpensive and off-the-shelf electronics.

Another fluorescence microscope created by Tristan-Landin et. al with 6 3D printed components. It also is comprised of off-the-shelf electronic components [Tristan-Landin et al., 2019].

Multi-Well Microscopes

Bohm created a gantry system based automated imaging system [Bohm, 2018]. The system is comprised of mostly off the shelf components and can image samples in 12, 24, and 96 well plates. This particular imaging device can image multiple wells with one camera that travels between the wells. With the ability to monitor more than one well at a time, researchers can do parallel longitudinal imaging

across many wells.

Gürkan et al. created incu-Stream, which is an automated imaging device that images from within an incubator [Gürkan and Gürkan, 2019]. This particular device has one imaging component that moves from well to well through motors, similarly to a gantry system. THe incu-stream is different from most other maker microscopes because it has the ability to image multiple wells however the images will not all be taken at the same time. There will be a slight delay on when the pictures are taken.

Merces et al. created a 3D printed microscope that can image from within an incubator [Merces et al., 2020]. The incubot is created from easily accessible hardware components and can image multiple wells by moving the single camera unit.

These devices were created with off-the shelf components, easy to reproduce through fast manufacturing techniques, and assisted with live-cell imaging at a lower cost.

Table 2.1: Comparison between 3D printed microscopes

Low Cost Maker Microscope	Single/ Multi-Camera	Price/ Camera	Resolution	FOV
Foldscope [Cybulski et al., 2014]	Single	$1	submicron	N/A
LudusScope [Kim et al., 2016a]	Single	$30-100	4.4 μm	18 mm
FlyPi [Chagas et al., 2017]	Single	$120.93	10 μm	N/A
A versatile and customizable low-cost 3D-printed [Diederich et al., 2020]	Single	$120.92 - $483.69	2 μm	N/A
Incu-Stream 1.0 [Gürkan and Gürkan, 2019]	Single	$184	1.8 μm	6.5mm
Portable, Battery-Operated, Low-Cost, Bright Field [Miller et al., 2010]	Single	$240	0.8 μm	N/A
Microscopi [Wincott et al., 2020]	Single	$419.06	N/A	N/A
The Incubot [Merces et al., 2021]	Single	$1,208.95	4 μm	1300 μm x 925 μm
An inexpensive system for imaging the contents of multi-well plates[Bohm, 2018]	Single	$3400	N/A	N/A
A mini-microscope for in situ monitoring of cells[Kim et al., 2012]	Single	$N/A	1.4 μm	1.52 $\times$ 1.13 mm*
11 Low-cost, sub-micron resolution, wide-field computational microscopy [Aidukas et al., 2019]	Single	$N/A	submicron	4-mm2
EmSight [Kim et al., 2016b]	Single	$N/A	4.5 μm	5.7 mm $\times$ 4.3 mm
The Picroscope	Multi-Camera	$83 ($2000 for 24 cameras)	7 μm	5mm

Table 2.2: Brightfield and Florescence capability comparison between 3D printed microscopes

Low Cost Maker Microscope	Brightfield	Fluorescence
Foldscope[Cybulski et al., 2014]	x	x
LudusScope[Kim et al., 2016a]	x	N/A
FlyPi[Chagas et al., 2017]	x	x
A versatile and customizable low-cost 3D-printed open standard for microscopic imaging[Diederich et al., 2020]	x	N/A
Incu-Stream 1.0[Gürkan and Gürkan, 2019]	x	N/A
Portable, Battery-Operated, Low-Cost, Bright Field and Fluorescence Microscope [Miller et al., 2010]	x	x
Microscopi[Wincott et al., 2020]	N/A	x
The Incubot[Merces et al., 2021]	x	x
An inexpensive system for imaging the contents of multi-well plates[Bohm, 2018]	x	N/A
A mini-microscope for in situ monitoring of cells[Kim et al., 2012]	x	N/A
Low-cost, sub-micron resolution, wide-field computational microscopy using opensource hardware[Aidukas et al., 2019]	x	N/A
EmSight[Kim et al., 2016b]	x	x
The Picroscope	x	N/A

2.5 Remote Education

Traditional in person classes require students to be in the laboratory to learn, however not all educational intuitions can provide students with a state of the art lab. [Grout, 2017] During the COVID-19 pandemic, many schools and students had to adjust to remote education. In some classes, remote experiments were the

only way to continue the curriculum.

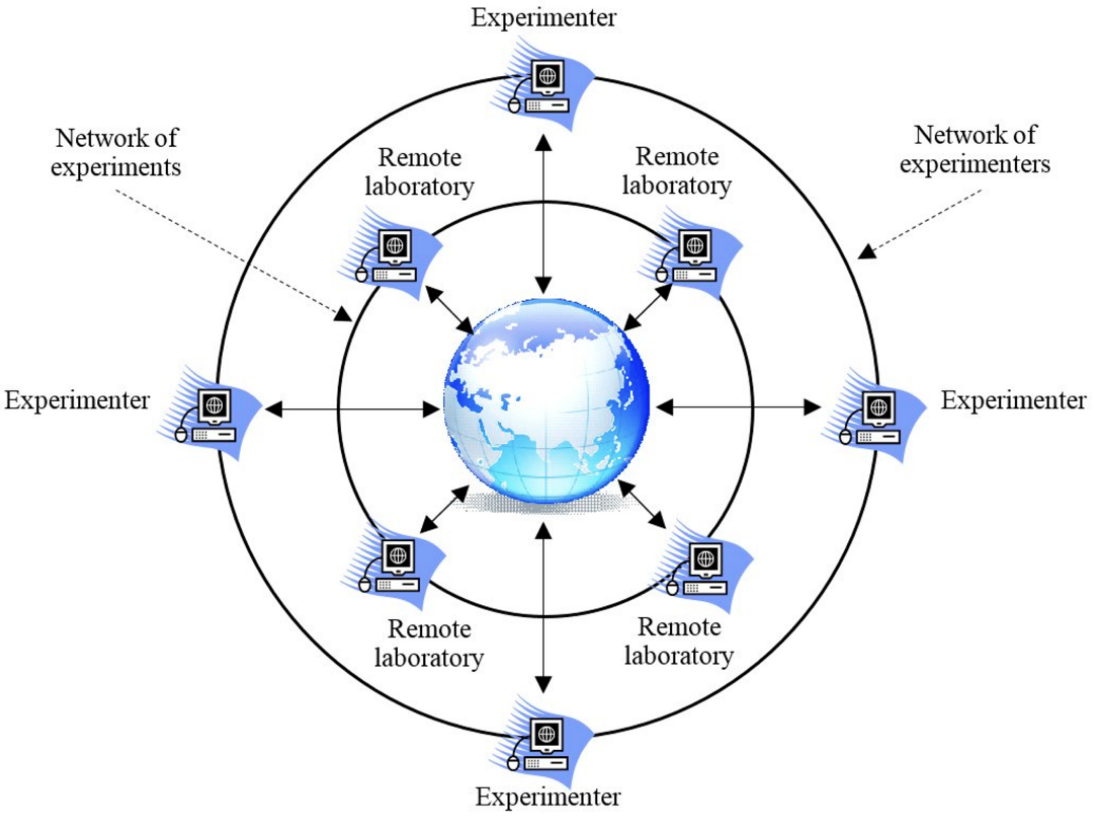


Figure 2.12: A global online and remote laboratory community [Grout, 2017]

Remote experiments is a way in which students who do not have access to a fully equip laboratory have the ability to conduct and control real world experiments [Scanlon et al., 2004, Grout, 2017] instead of fully virtual and fully simulated experiments with a prescribed outcome. Remote education is can be paired with virtual reality learning, which allows for more ease of access to underfunded communities.

2.5.1 Laboratory Safety/Education and Cell Culture Care

Undergraduate research experiences (UREs) are widely recognized as crucial for nurturing future generations of scientists, prompting substantial investments

from governmental and philanthropic agencies annually to support undergraduate research internships [Rodenbusch et al., 2016]. The traditional apprenticeship model of UREs, wherein undergraduates collaborate closely with seasoned researchers such as faculty members, postdoctoral scientists, or graduate students, inherently limits the number of undergraduates who can engage in research. This limitation, combined with the growing interest in expanding research opportunities and addressing the high costs associated with apprenticeships, has led to the development of courses aimed at actively involving students in research endeavors [Rodenbusch et al., 2016].

Course-based undergraduate research experiences (CUREs), which are accessible at the introductory level, offer significant potential to shape students' educational and career trajectories compared to research internships typically available in later undergraduate years. Research by Rodenbusch et al. highlights that students dedicating more time to CURE projects demonstrate improved learning outcomes and a heightened interest in STEM subjects and fields [Rodenbusch et al., 2016]. This underscores the notion that early engagement in course-based research, starting as early as the first year of undergraduate studies or even earlier in educational pathways like high school, contributes to increased graduation rates and enhanced completion rates of degrees in science, engineering, and mathematics [Rodenbusch et al., 2016].

Before stepping foot into any laboratory, there are comprehensive safety trainings to be completed and academic literature to be read in order to even be present in the laboratory, let alone work there. Precautions and trainings are set into place due to multiple factors. Many laboratories are shared spaces, and researchers must be respectful of other ongoing experiments. Various chemicals have specific containment and waste protocols that must be followed to ensure

safety practices are followed within the laboratory. Equipment in the laboratory is expensive and can be damaged easily if proper care is not taken. Proper authorities must be notified to maintain the machines as well as fix devices if necessary. Not properly understanding safety protocols or correct use of equipment can be incredibly dangerous and has caused injury in the past [Ménard and Trant, 2020].

Laboratory safety training is typically taught through slides or exams. Some laboratories have alternatively used games[Gublo, 2003]) or digital laboratory safety platforms [Viitaharju et al., 2021, aal,]. Learning safety protocols at the undergraduate level or even earlier will help enforce good safety practices in both teaching and research laboratories as well as classrooms[Ménard and Trant, 2020, Fivizzani, 2016, Alaimo et al., 2010, Annetta et al., 2014].

Cell culture is hands-on, and requires attentiveness to detail and procedure following to ensure the highest viability and repeatability for the cells being grown, and the lowest chance of contamination by foreign microorganisms. Contamination is a serious risk for cell cultures and can lead to disastrous problems for the laboratory. [Langdon, 2004]. Additionally, specific and differing procedures needing to be followed for different experiments. Due to the nature of the biology being used, additional hazards relating to bloodborne pathogens are present and require additional knowledge to operate safely.

2.5.2 Serious Games in Biology

A Serious Game is crafted with a primary objective beyond mere entertainment. Its purpose varies based on industry and application, ranging from training, learning, and simulations to diagnostics and testing. Over the past decade, there has been a surge in the release of Serious Games spanning various fields such as healthcare, defense, education, communication, and politics [Mittal, 2018]. There

have been many instances of games being used in the sciences, Khatib et. al created a game called Foldit [Khatib et al., 2011], which was successful of crowd-sourcing protein folding algorithms through ordinary people in the community to play a puzzle game [Good and Su, 2011]. This game has been analyzed and adapted for other topics,like biochemistry [Kleffner et al., 2017]. Which led on to create an education mode of foldit [Miller et al., 2020].

This game enabled players of all backgrounds to participate in groundbreaking science[Khatib et al., 2011, Good and Su, 2011]. Serious games have been used in conjunction with lessons learned in the classroom. Studies have shown the effectiveness and issues of playing games in the classroom[Lee et al., 2004, Randel et al., 1992, Ishak et al., 2021, Kinchin, 2018].

From: Introducing Foldit Education Mode

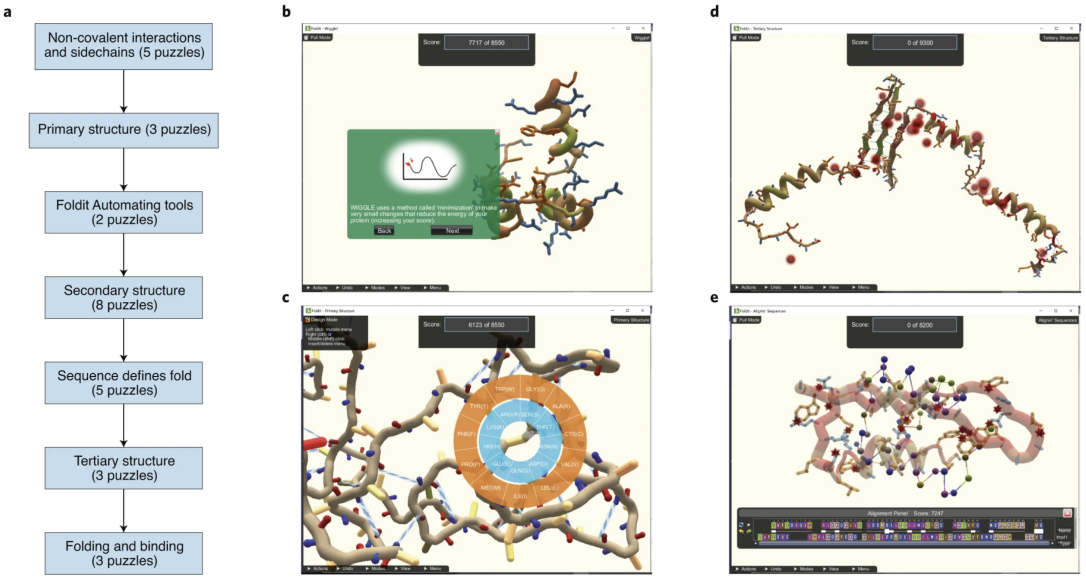


Figure 2.13: a, A flowchart depicting the current topics and number of puzzles within Foldit Education Mode. b, Wiggle puzzle, showing a tutorial bubble example. Students click back or next to proceed through the tutorials within each puzzle. c, An example of a protein design puzzle, "Primary Structure," in which students are asked to choose amino acids that optimize the protein's stability. d, An example of a protein folding puzzle, "Tertiary Structure," in which students are given a protein with preformed secondary structure and are asked to fold it into a stable tertiary structure. e, An example of a sequence alignment puzzle. In this puzzle, "Alignin' Sequences," students are asked to align the sequence of a protein to that of a protein of known structure to enable structural threading.[Miller et al., 2020]

In addition, they consider the importance of the teacher's role in designing and in playing a game; the need to consider the issue of feedback to students and how this will be achieved in a game environment, and how student motivation can be harnessed to maximize engagement. Finally, we need to see this as a potential activity that can be developed in partnership with students and invite them to provide feedback to us."

Particularly in math and the sciences, serious educational games have been shown to increase motivation in students. Serious games have also improved

retention of knowledge about the material presented [Chorianopoulos et al., 2014, Cheng et al., 2015, Mayfield et al., 2019, Hellings et al., 2019].

In biology in particular, games like "Discovering the cell" [Spiegel et al., 2008] and Gutierrez et al.'s card game that teaches biology vocabulary [Gutierrez, 2014] stand as examples of serious games that helped improve the knowledge of disciplinary material. Sadler et al. showed the success of games like these to have a positive effect on science education for students [Sadler et al., 2015].

To further supplement traditional classroom materials, some schools have turned to virtual reality (VR) companies like Labster[Stauffer et al., 2018] to teach students basic biological laboratory experiments.

2.5.3 Virtual Reality Technology in Education

Virtual Reality technology constructs immersive, interactive, and multi-sensory three-dimensional environments. Virtual Reality has garnered increasing attention in educational contexts, offering immersive and interactive learning experiences across various disciplines [Winn, 1993]. Its application in education traces back to the early 1990s, where researchers began exploring its potential to enhance learning [Held and Durlach, 1992]. Initial experiments primarily focused on simulating real-world environments for training purposes, such as flight simulation for pilots and medical procedures for healthcare professionals.

As technology progressed, so did the applications of VR in education. The late 2000s and early 2010s witnessed the development of affordable VR headsets as wkek as VR software platforms, opening new possibilities for educational use [Dalgarno and Lee, 2010]. Today, educators are harnessing VR to create virtual classrooms, interactive simulations, and immersive field trips, enabling students to explore subjects like history, science, and art in engaging ways [Wu et al., 2013].

One of the primary advantages of VR in education is its ability to accommodate diverse learning styles and preferences. Visual learners, for instance, can benefit from immersive 3D visualizations, while kinesthetic learners can engage in hands-on simulations [Akçayır and Akçayır, 2017]. Moreover, VR provides access to experiences that would otherwise be impractical or impossible to replicate in a traditional classroom setting, such as exploring the surface of space or depths of the ocean.

Despite its potential, the widespread adoption of VR in education is still in its nascent stages, with challenges such as cost, accessibility, and technical limitations needing to be addressed [Dede et al., 2009]. However, as technology advances and becomes more affordable, VR holds the promise to revolutionize the teaching and learning process, making education more engaging, interactive, and accessible to students globally.

Virtual reality training eliminates the cost of maintaining an active laboratory and mitigates safety risks. Jones' work on virtual laboratories highlights this, describing a safer laboratory environment that can also benefit those who cannot afford to attend a real laboratory [Jones, 2018].

Virtual reality,in education, is a solution from schools that cannot provide students with all the necessary equipment, for example in physics classes [Bogusevschi et al., 2020], chemistry courses [Georgiou et al., 2007], or language sciences [Peeters, 2019]. So far, the case study results from the students are all positive, and the students are demonstrating that their understanding is still high considering they are learning in a virtual environment [de Vries and May, 2019].Most recently, Arizona State University (ASU) has converted to have fully virtual online biology courses using labster [Jones, 2018, Stauffer et al., 2018]. With the adapatation of labster in the classroom, universities are able to provide a safer

learning platform at a cheaper option due to the cost of VR headsets as technology improves. While virtual reality is an effective learning tool for undergraduate students, a basic cell culture VR class was developed by Labster [cel,]. There are some potential downsides to adopting VR in educational settings including internet addiction, inflexibility with the curriculum, functionality problems with the software and interpersonal interactions due to the lack of face-to-face interaction [Ahlawat et al., 2024].

2.6 Conclusion

In conclusion, the literature review has provided valuable insights into microscopes with incubators, 3d printed lab equipment, low-cost maker scopes, educational biology games and virtual reality technology used in education. The review revealed several key themes, including how 3D printing is paving the way for cheaper manufacturing and can be used to create laboratory equipment including open source microscopes and how serious games can help students learn and providing a softer landing pad to help students understand difficult topics. Overall, the literature review has laid the groundwork for advancing our understanding of how to create a an open source longitudinal imaging system with image analysis and a gamified approach to enhancing undergraduate understanding of 3D cell culture concepts as well as laboratory safety.

Chapter 3

Prototyping

3.1 Introduction

The literature review, encompassing Chapter 2 and sections 2.1 - 2.5, delves into fundamental techniques for cultivating organoids, devices utilized in longitudinal cell studies, low-cost imaging systems and serious games in biology. While these methodologies and tools are prevalent in contemporary biology labs, their widespread adoption is impeded by certain limitations. Off-the-shelf imaging systems, in particular, tend to be costly, leading many labs to acquire only one unit, which must be shared among multiple research groups. The labor-intensive process of imaging cells compounds this challenge, as biologists grapple with the intricacies of managing numerous experiments while also attending to the feeding, waste removal and imaging of cells.

These challenges could be addressed by an open-source system designed to automate feeding, image capture, and stimulus detection in cortical organoids. Such a system aims to provide valuable insights into the developmental processes of cortical organoids, offering a model for understanding the temporal evolution of the human brain. Utilizing cortical organoids as a representative model allows

for a comprehensive exploration of how the human brain develops over time.

Chapter 3 chronicles the progress made over the past four years, with a primary focus on the design, construction, and enhancement of the advocated open-source, low-cost multi-well imaging platform. Section 3.1 provides an account of the outcomes obtained through the replication of other low-cost microscopes identified in the literature review from Chapter 2. Section 3.2 delves into the various design iterations undertaken, while section 3.3 explains the computer vision algorithms applied to analyze the captured images. This comprehensive overview showcases the evolution and advancements in our pursuit of an innovative imaging platform, contributing to the broader understanding of organoid development and cellular dynamics.

3.2 Generations of the Picroscope

3.2.1 Single Unit Microscope

In the realm of microscope optics, there exists a delicate balance between magnification and field of view. Opting for a broader field of view necessitates a compromise in magnification, while seeking higher magnification inherently reduces the observable field. This inherent trade-off is fundamental to microscope design, where adjustments in magnification directly impact the extent of the observable sample area. Consequently, researchers must carefully assess their experimental needs to determine the optimal magnification levels, as they dictate the level of detail captured in the sample versus the viewing frame. Such considerations play a vital role in refining microscopy protocols and ensuring the acquisition of precise and comprehensive data across various scientific inquiries.

$$f = \frac{1}{\frac{1}{o} + \frac{1}{i}} \tag{3.1}$$

f = focal length o = object distance i = image distance

$$M = \frac{i}{o} \tag{3.2}$$

M = linear magnification

In designing the device, our aim was to achieve a balance between maximizing the field of view within the well and attaining a suitable magnification level for observing cellular structures. This approach facilitates clear visualization of the growth and progression of biological samples over time.

To achieve this goal, we experimented with adjusting the distance between the lens and the camera sensor. Utilizing 3D printed components, specifically designed camera barrels as depicted in figure 3.1, allowed us to fine-tune this distance.

Our experimental setup, illustrated in figure 3.2, involved testing various configurations to determine the optimal distance between the lens and camera sensor for capturing the desired images.

Following rigorous trial and error, we ultimately opted for an off-the-shelf lens manufactured by Arducam, which effectively met our requirements for imaging the biological samples with the desired field of view and magnification. The cameras are attached to the base by pieces printed in a dental model on the Formlabs form2 printer, figure 3.1.

In order to increase the magnification, to allow us to see cells, we chose the Arducam 1/2" M12 Mount 16mm Focal Length Camera Lens. This lens has a wide field of view that allows us to see the entire well (approx 5 mm diameter well) of a 24 well plate (figure 3.3).

Observing figures 3.4a and 3.4b, it becomes apparent that the squares appear

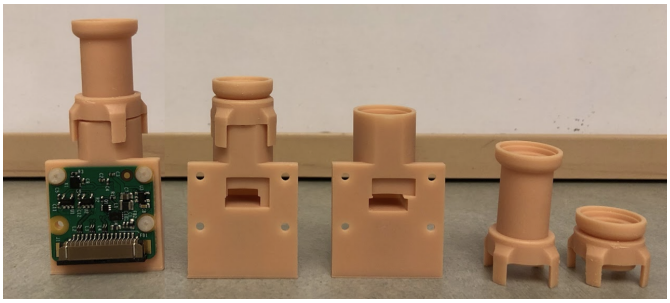


Figure 3.1: 3D printed Camera Barrel extensions to just the zoom of our imaging unit

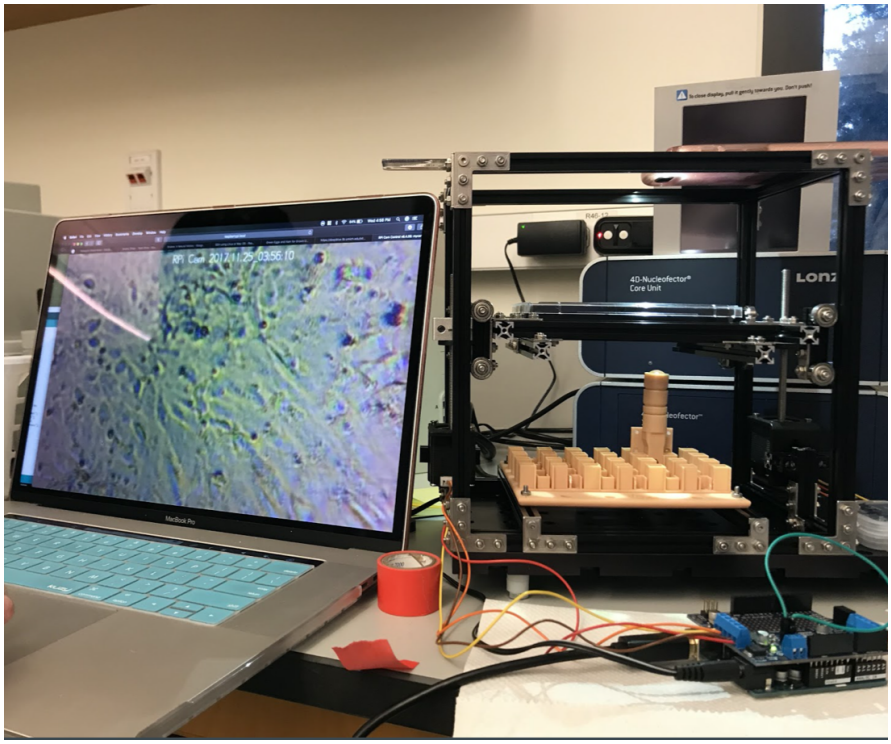


Figure 3.2: Adjustable magnification through 3D printed pieces

distorted visually, despite the expectation that they should all be of equal size.

Upon visual inspection, we observed that the center appears to be the most focused, while the surrounding outer areas appear stretched.

The Picroscope is equipped with a fixed lens system, providing a field of view of approximately 5 mm. It can resolve Group 7, Element 1 targets (1951 USAF Targets), corresponding to a resolution of approximately 7 μm (Figure 3.6). If

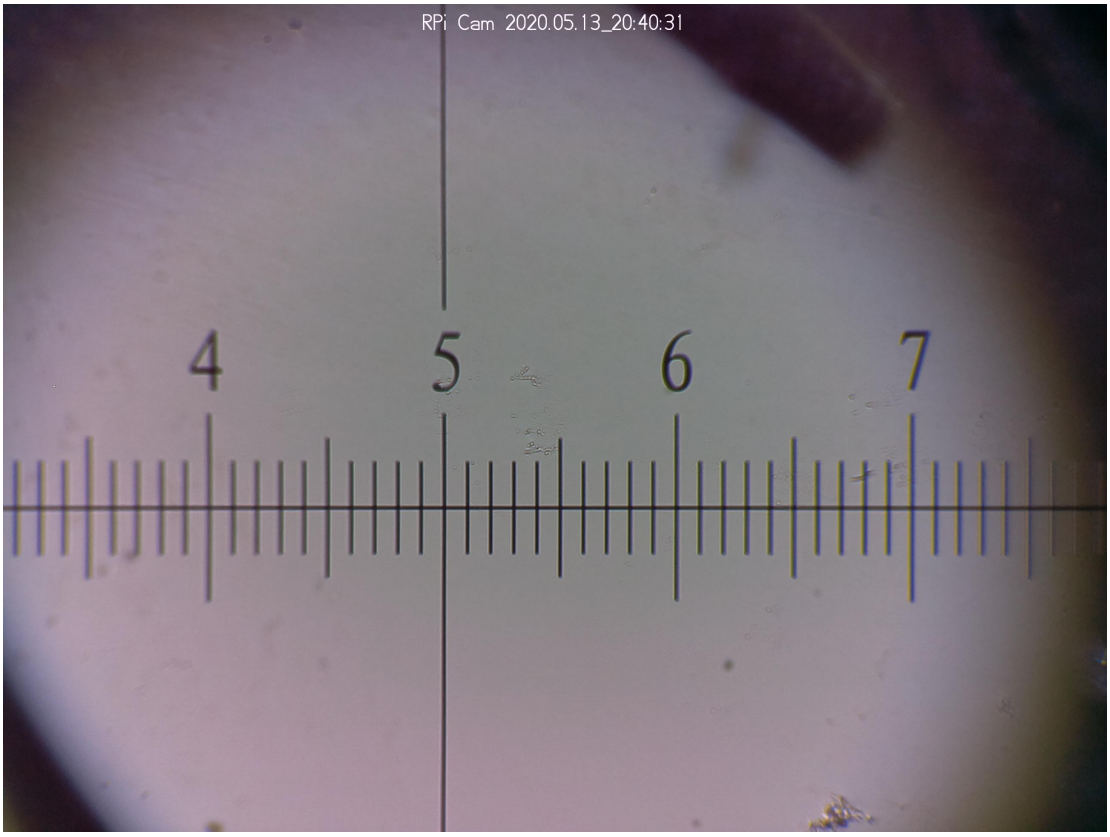
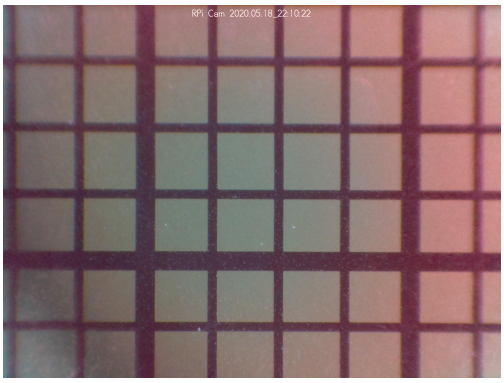
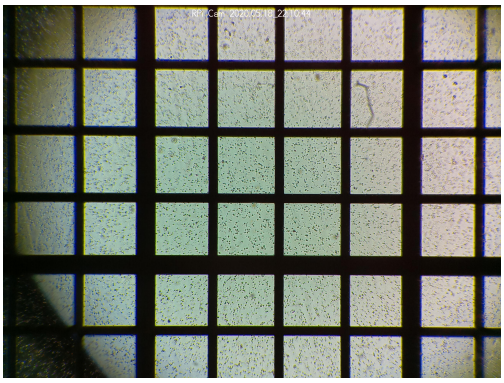


Figure 3.3: Current FOV image

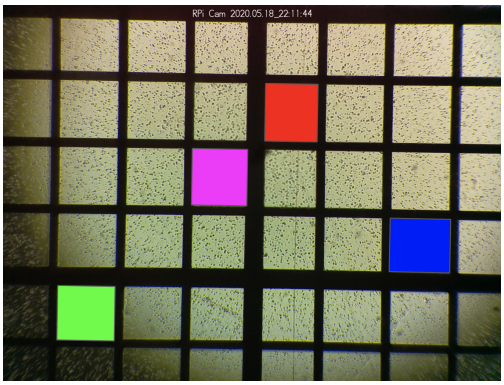


(a) Microscope grid calibration slide with only lights from below

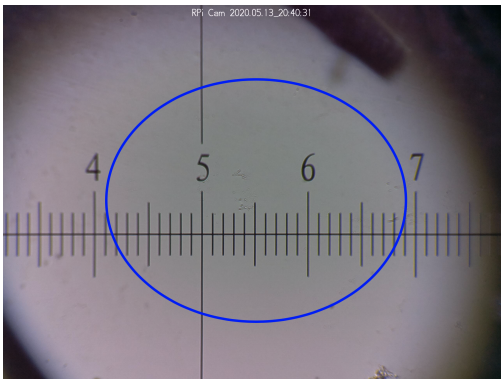


(b) Microscope grid calibration slide with only lights from above

higher resolution is required, the lens can be replaced with a higher magnification option (Table 2.1). The current lens selection was based on our focus on imaging whole organisms.



(a) By comparing the various colored squares that are laid on top of the grid image, we can see that the squares are different sizes, and this is due to distortions from the lens. The blue square is further away from the center and seems to be stretched, whereas the pink and red squares are more similar in width and height, and they are located closer to the center, where we believe the images are more in focus.



(b) The blue oval represents the most in focus portion of the images

3.2.2 Microscope Array: First Generation Design

While the design proved to be functional, it possessed certain drawbacks that rendered it less optimal. Firstly, manual adjustment of the four butterfly nut screws was necessary, leading to challenges in achieving perfect leveling, as depicted in Figure 3.8A. Additionally, cabling emerged as an issue due to limited space, accommodating only six Raspberry Pis instead of the intended 24 along with their corresponding cameras.

Furthermore, the substantial size of the base necessitated the use of a 3D printer with a large build area. For imaging, we utilized the V2 Raspberry Pi 8-megapixel camera, although the original lens was replaced with a commercially available lens sourced from Edmund Optics. In order to connect the new lense, we

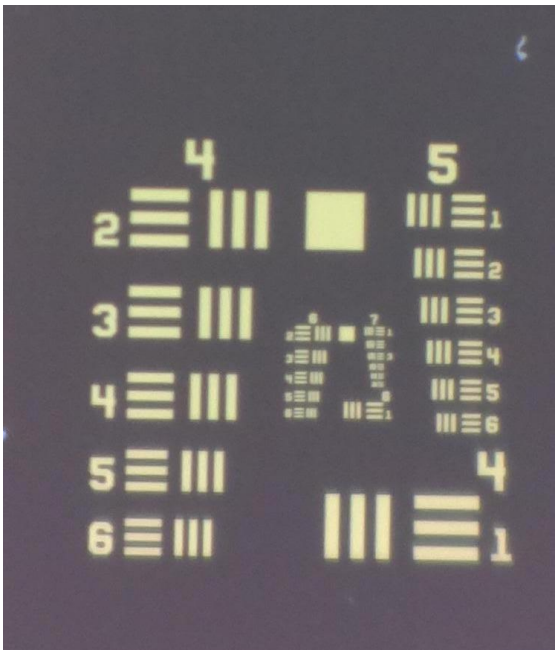


Figure 3.6: Using a 1951 USAF Target slide we are able to find the resolution of the Picroscope

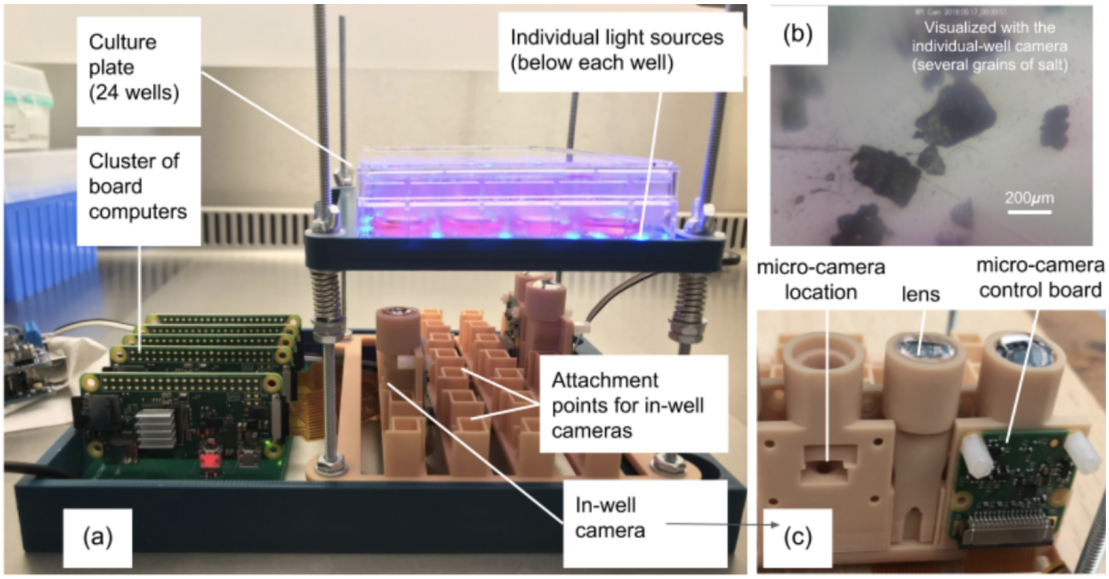


Figure 3.7: First working version of the Picroscope

have 3D printed camera barrel. The camera barrel is printed out of black PCTG and has printed threads that match the M12 threads of the lens from edmund optics.

3.2.3 Second Generation Design

Subsequently, our focus shifted towards enhancing the design by incorporating a motorized mechanism to eliminate the need for manual adjustment, as illustrated in Figure 3.8B. While this addition proved beneficial, we encountered challenges with plate leveling, attributed to lifting the plate from only one side. Moreover, the realization that moving the cell plate in the z-direction could potentially disrupt the cells under observation prompted further reconsideration of our approach. Additionally, limitations in accommodating Raspberry Pis on the base and the ensuing complexities in cable management exacerbated the design's shortcomings.

3.2.4 Third Generation Design

In this iteration, we constructed a robust frame capable of adjusting the plate's focus uniformly along the Z-axis using two motors, as depicted in Figure 3.8C. However, upon further consideration, we concluded that moving the plate might cause excessive vibration, which could potentially disrupt the cells. To address this, we devised extension barrels to modify the distance between the sensor and the lens, allowing for zoom adjustments (Figure 3.1 and Figure 3.2).

This particular design utilizes Makerbeam components, readily available for purchase. However, its assembly complexity posed a challenge, requiring multiple screws and careful alignment to ensure all beams were precisely flush at 90° angles. For imaging, we employed the V2 Raspberry Pi 8-megapixel camera coupled with the Arducam 1/2.5" M12 Mount 16mm Focal Length Camera Lens M2016ZH01, selected based on our optical calculations.

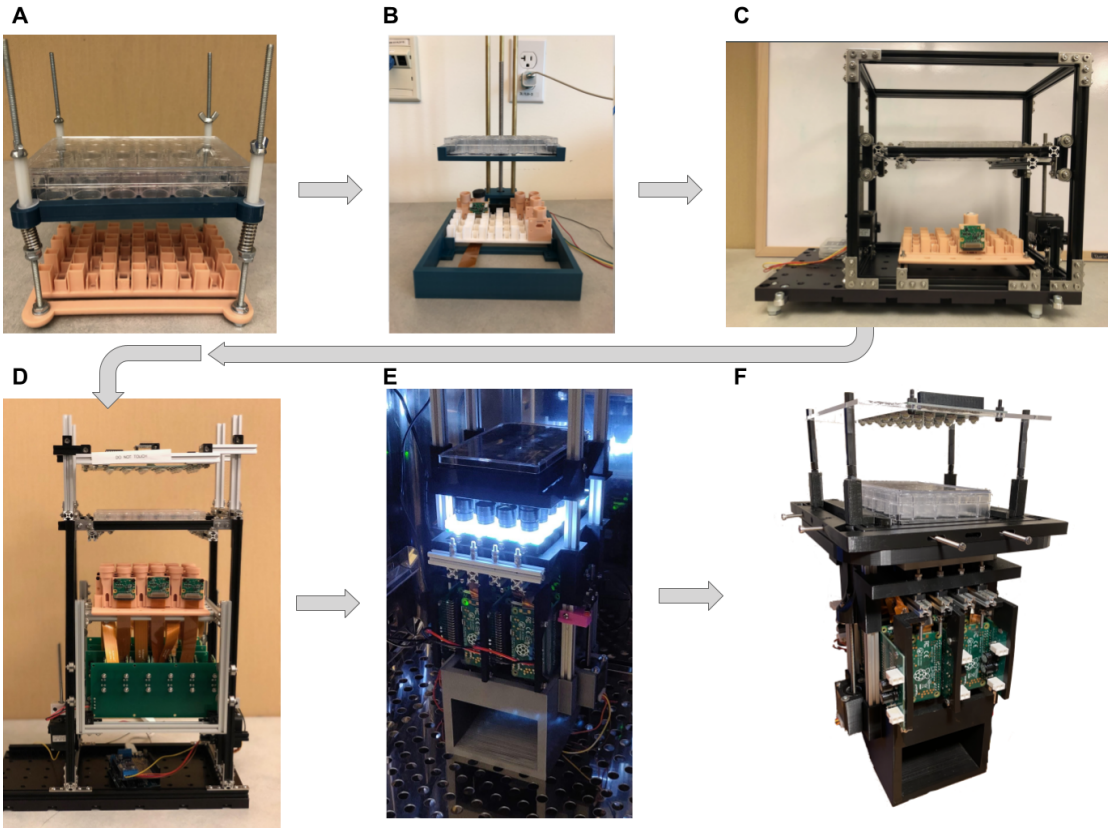


Figure 3.8: A. First-generation design with manual focus adjustment B.Second generation design with forklift motor focus adjustment C Third generation design with dual motor focus adjustment D.Fourth Generation Design Metal with camera array moving E. Fourth generation design with 3D printed components rather than aluminum extrusion bars F. Current design with the added feature of an XY stage.

3.2.5 Fourth Generation Design

In the summer of 2019, Science Internship Program at UCSC (SIP) interns embarked on a mission to refine the design, aiming to streamline it while minimizing the number of screws required. This endeavor led to the iteration depicted in Figure 3.8D, where the cell plate remains stationary, and the cameras are now adjustable along the Z-axis to facilitate focus adjustment.

However, a notable challenge encountered with this revised design was the potential misalignment of the cameras due to variations in 3D printing tolerances.

Over time, the repeated clipping of 3D printed components together resulted in slight loosening, exacerbating this issue.

Initially, for imaging purposes, we employed the V2 Raspberry Pi 8-megapixel camera. However, we later transitioned to the Raspberry Pi spy camera (5 megapixels) due to its smaller size and the placement of the sensor's PCB on the ribbon cable, enabling a more compact camera array tailored to match a 24-well plate.

Regarding the lens selection, we opted for the Arducam 1/2" M12 Mount 16mm Focal Length Camera Lens M2016ZH01 to address distortion concerns observed in our images while also significantly reducing costs from $25 per lens to $8 per lens. To try to remedy the difficulty of building this device with hundreds of screws, we turned to 3D printing, shown in figure 3.8E. However, unlike the concept box, we broke the device down into three primary layers, the LED panel holder, the elevator, and the base. These pieces were able to be printed within 3 hours each. We opted to print all six camera holders connected in one go for this design for the camera holders. We were able to do this because we switched to a different Raspberry Pi camera that had the PCB on the ribbon cable rather than an extra electronics board. The new Rapsberry Pi camera is a raspbery pi spy camera. This design had some flaws because once placed in an incubator, we noticed that there was condensation on the incubator door, which was from the excess heat coming from the Picroscope. We also noticed some warping after long periods of time in the incubator. This is due to the material choice, PLA. However, we can slightly remedy this problem by increasing the infill density of the material when printing, which leads to longer printing times.

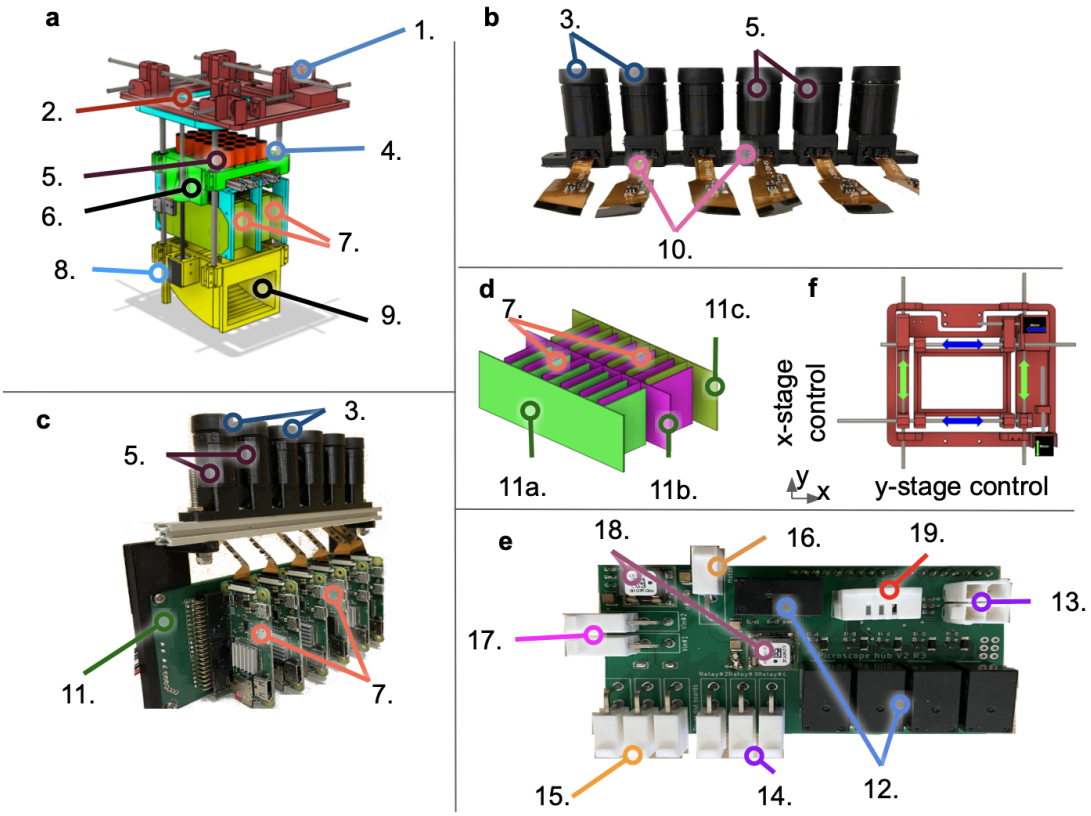


Figure 3.9: **The Picroscope. a** Physical representation of the proposed imaging system. **b** one line of independent cameras. **c** An integrated rack of cameras and Raspberry Pi board computers. **d** The interlacing strategy of four independent racks of power distribution boards. **e** The Raspberry pi Hub and Arduino Uno, Motor driver and custom relay board **f**The XY adjustment stage. 1 = XY stage, 2 = 3D printed Cell Culture Plate Holder & XY stage, 3 = Lenses, 4 = Illumination Board from below, 5 = 3D Printed Camera Bodies, 6 = 3D Printed Elevator, 7 = Raspberry Pi 0Ws, 8 = Motors, 9 = Base, 10 = Raspberry Spy Cameras, 11 = Interface Board a. row 1, b. rows 2 and 3 c. row 4, 12. Relays, 13. Power distribution board connectors, 14. Limit switches connectors, 15. Light board connectors, 16.Motor power connector, 17. 12 V power source, 18. Voltage regulators, 19. Temperature & Humidity sensor

3.2.6 Fifth Generation of the Picroscope

This design includes an air duct like design that pulls the heat from the Raspberry Pi's and blows it on the incubator door(figure 3.9). Thus far, our redesigned prototype has effectively addressed the condensation issue encountered in previ-

ous iterations. This latest design is crafted with 100% infill to mitigate warping caused by the high temperatures within the incubator. Additionally, it is printed using PCTG filament, renowned for its biocompatibility and superior temperature resistance compared to PLA. By changing the material from PLA to PCTG as well as increasing the infill when 3D printing the parts, has improved the design drastically.

To test the biocompatibility of PCTG, we conducted a series of experiments. We meticulously cleaned and disinfected two incubators, designating one as the control group and the other for experimentation. In the experimental group, we introduced both filament in its raw form and filament extruded from the 3D printer, positioning them in close proximity to the cells. It's worth noting that in the Picroscope design, the plastic never directly contacts the cells. To simulate a similar environment, we placed the plastic alongside the cells within the incubator.

Subsequently, we allowed the cells to incubate for a minimum of 14 days to monitor their growth. After the designated period, we compared the cell growth and any observed abnormalities between the control and experimental groups.

Our findings indicate no adverse effects associated with the presence of PCTG filament in close proximity to the cells. This conclusive evidence supports our decision to utilize PCTG for the Picroscope design.

The multi-well in-incubator imaging unit is mostly 3D printed and uses 4 linear rods. By using 4 linear rods and linear bearings, this allowed for a smoother translation in the Z direction. This device consists of 24 Raspberry Pi zeros, Raspberry Pi zero spy cameras, 24 white LEDs, optional blue LEDs for GFP, 2 stepper motors, and a fan. There are 3 layers: 1. Cell culture plate, which fits a standard 24 well plate 2. Elevator (which holds 24 cameras and Raspberry Pis) 3. The base which includes a fan.

The Picroscope has the ability to illuminate samples with lighting from above or below a standard 24 well cell culture plate. Currently we are only able to do brightfield microscopy. Illumination from above works well with translucent samples while illumination from below works best with opaque samples, best shown in Figure 3.10.

The 3D printed plate holder (2 in figure 3.9) supports the biological sample during an experiment. For easy alignment, the holder is attached to a XY sliding stage that consists of two interconnected linear stages (figure 3.9f). With this design of the XY stage, there are have 2 motors that are able to indepedently control the X stage (shown in blue in figure 3.9f) and Y stage movement control, (shown in green in figure 3.9f). The motor positions can be adjusted on the experiment UI page designed by my colleague Pierre Baudin. This particular piece is printed in a couple pieces with support and takes up the entire build plate on a MK3S Prusa 3D printer.

The imaging unit is comprised of 24 independent objectives attached to a vertical sliding stage ("elevator piece" in 3.9a) we constructed using four makerbean vertical columns and 2 Nema-11 stepper motors (3.175mum Travel/Step). The fine threads are necessary for focusing on specific biological features and collecting z-stack imaging (Figure3.13). The objectives are distributed on 4-rows and 6-columns to match a standard 24 well culture plate. Each objective consists of a 3D printed camera body that hosts a 5 MegaPixel (5MP) camera (Spy Camera for Raspberry Pi0W, with a 1.4 µm x 1.4 µm pixel pitch)[Fruit, 2015] and an off-the-shelf Arducam 1/2" M12 Mount 16 mm Focal Length. Each objective is controlled by a single board computer (Raspberry Pi 0Ws), which is connected to an individual slot on one of the three custom-made power distribution boards (3.9c and d). All 24 single-board computers (Raspberry Pi0W) communicate to a

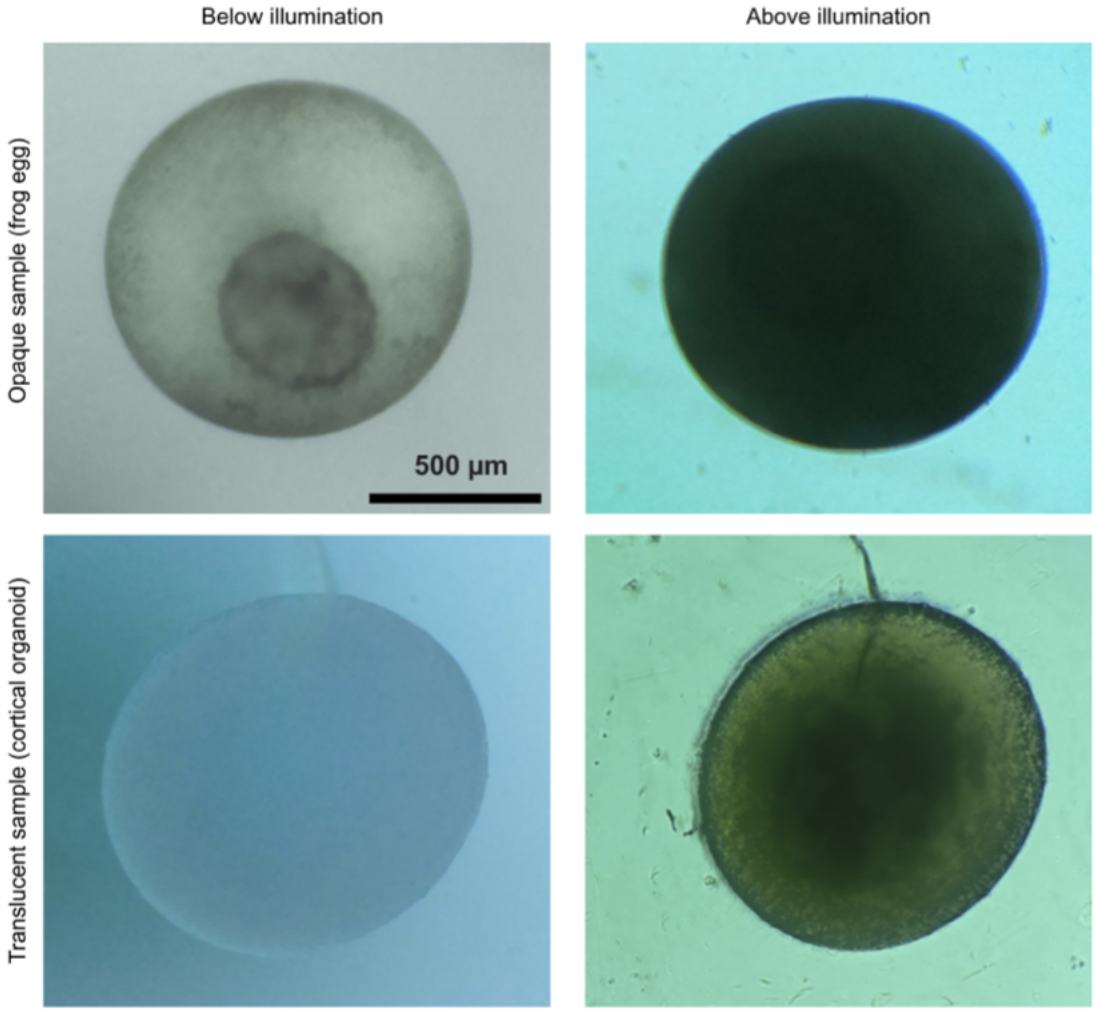


Figure 3.10: Comparison of illumination options in different sample types. Comparison between above and below illumination for imaging opaque samples (frog egg) and translucent samples (cortical organoid).

hub board computer (Raspberry Pi 4) that manages the images and autonomously uploads them to a remote server. The hub single board computer has the MIPI CSI-2 camera port and is connected to an Arduino Uno, which has a motor shield attachment, to controls the motors and lift the elevator piece (3.9). As a safety feature, the system also includes a custom-made Relay Board that is attached to the Arduino and motor driver stack. The relay board provides control of the LED boards and, in the event of an overheat, allows us to shut down the system, protecting the system and the biological sample. After each set of pictures, the imaging unit returns to the lowest ("park") position, determined by a limiting switch attached to the elevator unit. The entire system sits on a 3D printed base that includes a fan for heat dissipation.

During an experiment, the pictures are autonomously uploaded on a remote computer/server using the ethernet connection of the hub computer board, where they can be viewed or processed in near real-time.

As shown in figure 3.9 A, this device has 3 distinct layers: 1. Cell Culture Holder with XY Stage, 2. Elevator and 3. Base. All layers except layer 2, the elevator, is static. Layer 2, holds the cameras and microcontrollers, is able to move in the Z-direction. Each layer is 3D printed in PCTG Filament with an infill of 100% and can easily interface with a 300mm long makerbeam support beam. This material and infill choice complies with the criteria of withstanding an incubator's warm and humid environment.

Layer 1: Cell Plate Holder

Layer one is a rectangle with pegs that hold a standard 24 well plate. The cell plate holder interfaces with the makerbeams using 4 T-nut slots and screws. This allows for easy adjustment when setting the cell plate on the device.

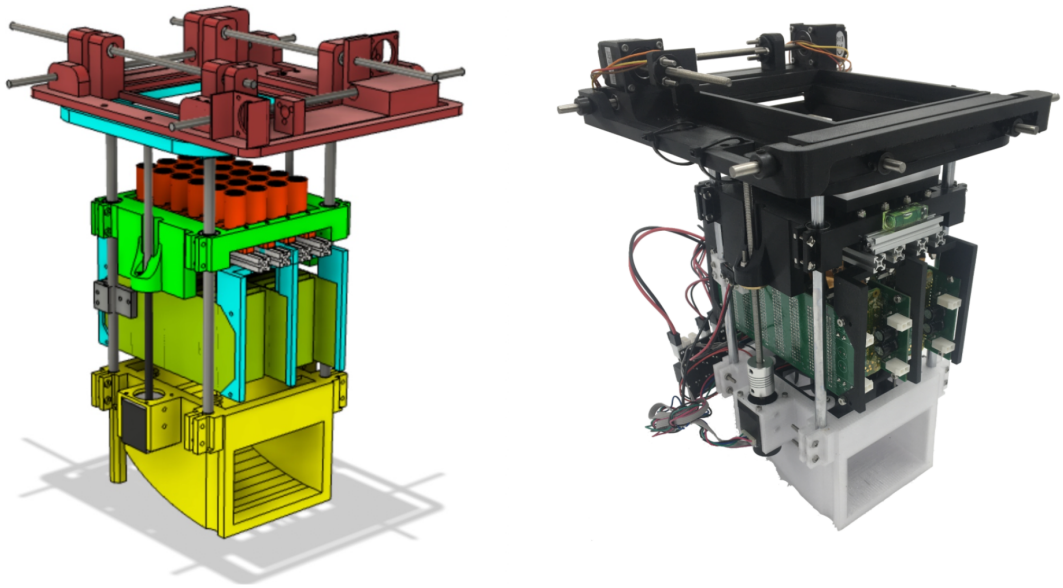


Figure 3.11: The latest Picroscope design, on the left is the CAD model on the right is a fully built and functional Picroscope

Layer 2: Elevator

The elevator level of the system consists of four camera holder units, as illustrated in Figure 3.9, accommodating a total of 24 cameras and 24 Raspberry Pi Zero Ws via a Printed Circuit Board (PCB) holder. Additionally, it interfaces with two stepper motors to facilitate movement.

Both motors collectively propel the elevator unit, enabling translation along the Y-axis. This design stands out for its ability to capture 24 images simultaneously, eliminating the need for a single camera to traverse each well individually. Furthermore, it supports z-stack imaging, allowing for image acquisition at various focal planes within the cell plate, as depicted in Figure 3.13.

The software facilitates easy adjustment of the number of images per z-stack, and the elevator automatically returns to the initial position (bottom of the z-stack) upon reaching a limit switch.

- Camera Holder Units: In order to fit the criteria of imaging a standard 24 well plate, we needed to design a camera holder to accommodate the pitch of a standard plate, which led us to the design in figure 3.15 for one row of a 24 well plate.
- LED Diffuser CNC out of frosted acrylic shown in figure 3.12
- PCB Holder: A 3D printed frame that holds the power distribution board for the microprocessors.

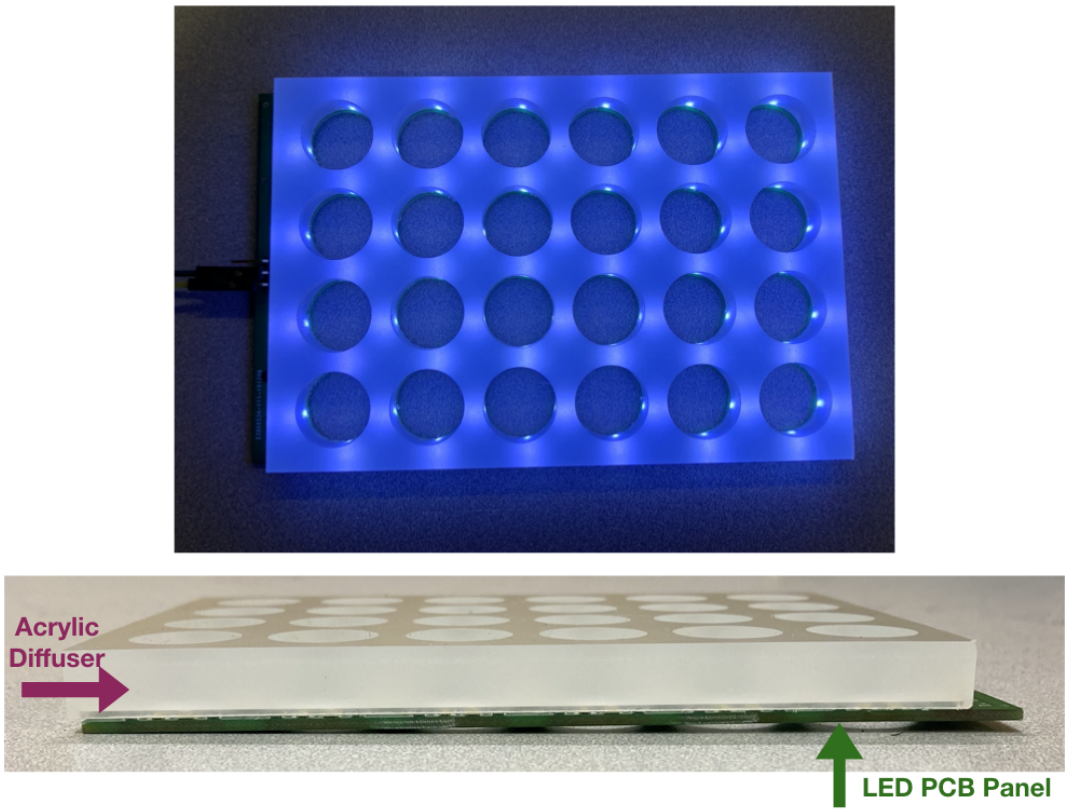


Figure 3.12: LED Diffuser made out of frosted acrylic, placed on top of LED panel for diffused light from below

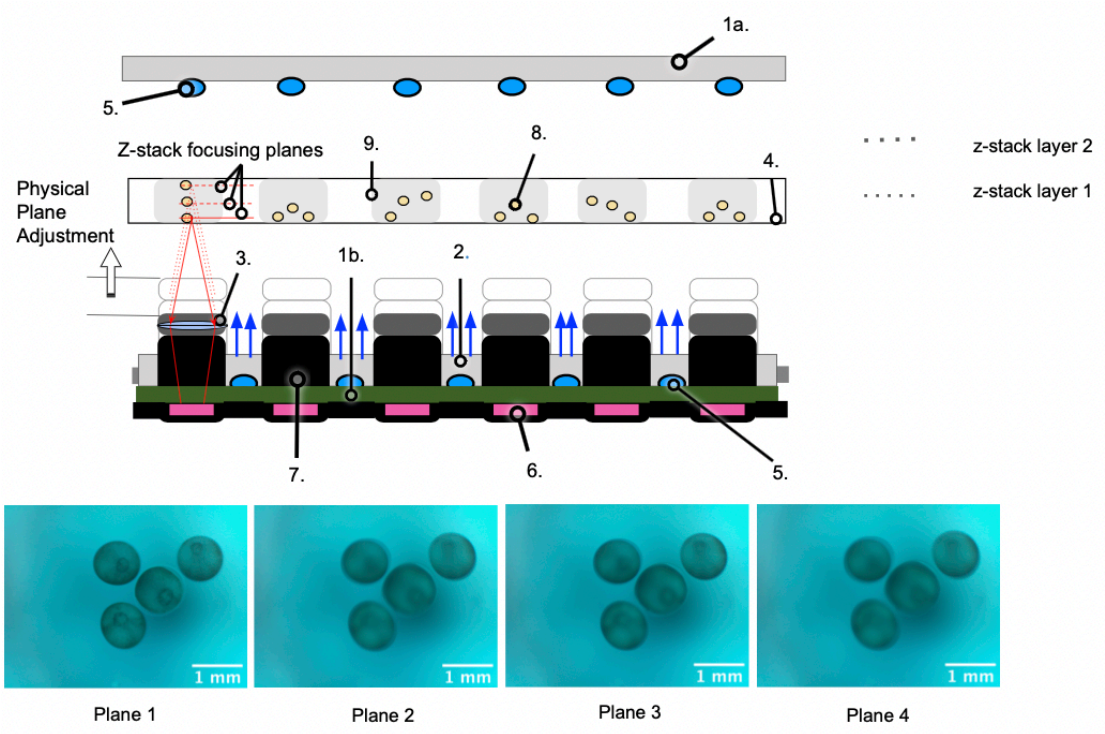


Figure 3.13: Z-stack imaging examples when cells are on different focal planes

Layer 3: Base

The Base is inspired by an air duct and also requires a fan to help distribute the heat from the microprocessors to the surrounding air in the incubator. While this piece is large, it is necessary to reduce condensation in the incubator. The particular layer holds the motors as well as the makerbeams through multiple screws.

3.2.7 Hardware

Camera Unit

Due to the constraint of the pitch of a 24 well plate, we needed a camera powerful enough to image cells but also small enough to fit into the compact space. The camera is a 5 megapixel Raspberry pi zero spy camera which has is

Figure 3.14: A.Shows the CAD rendering of our camera holder design and B. Shows the the camera holders when printed with cameras inserted into them

compact in size (8.6mm x 8.6mm) with PCB on the ribbon cable allowing for a more compact design than a standard Raspberry Pi camera. This camera produces a 2,560 (horizontal) by 1,920 (vertical) pixel image. We removed the stock lens and replaced it with a different off-the-shelf lens to increase the magnification and the field of view (FOV).

Microprocessor

In order to easily control the preferred camera, we naturally chose a Raspberry pi single-board computer (SBC) with a MIPI CSI-2 connected, a Raspberry Pi Zero W. There were not many off the self microcontrollers that easily interface

with the camera of our choice. This particular device has a small form factor of 65 x 30 mm, low power consumption and has wireless connectivity.

We require a microcontroller with a small form factor due to the spacing of a 24 well cell plate.

The device consists of two main microcontrollers, one Raspberry Pi 4 and 24 Raspberry Pi Zero W. This not only keeps the the device low cost but also is easily accessible to anyone that would like to replicate this device.

Motors

In order to move layer 3: the elevator, in the Y direction, we use 2 Nema 11 stepper motors with a shaft that has fine threads and allows 0.003175mm Lead Travel/Step(mm). This is about 3 microns per step. 100 steps is about 300 micron differences. This device requires fine movements to adjust and tune the focus of the image. When viewing cells, some cells are different focal planes and to remedy this to get clear images, we utilize z-stack images of each well.

Two Nema 11 steppers are controlled by ESP 32 and Adafruit Motor driver shield V2. On Top of Adafruit Motor driver shield V.2, a custom design Shield is attached on top. The custom design shield is features two debouching circuits for limit switches and four relays for controlling LEDs for illumination from below.

LEDs

This system is capable of bright field microscopy with white light from above or below the cell culture plate. For bright field microscopy, we have chosen MEI-HUA white LEDs with a brightness of 228 450MCD. For fluorescent microscopy, particularly green fluorescent protein (GFP), we picked an LED made by Chanzon with a wavelength of 450nm-455nm.

Custom Power Distribution PCBs

The Power distribution board is designed to power and provide structural support for the Raspberry Pi Zero W through their 5V GPIO pins. The design features a 0.1 uF bypass capacitor for every individual Raspberry Pi Zero and two 3.5 mm pitch screw terminal blocks for 5V Power input and output. The smallest trace for one Raspberry Pi Zero W had a width of 30 mil which allow to carry up to 2 A of current. In contrast, Raspberry Pi Zero W with spy cam have peak current consumption of 0.25 A.

Due to the simplicity of the design, the top layer is dedicated to +5V and bottom layer for Ground (GND). This eliminates the concern of trace width and copper thickness, thus the PCB is manufactured using standard 1.6 mm FR4 with 1 oz of copper thickness.

This particular feature is what allows us to have a double-sided PCB without actually creating a new design in addition to the single-sided PCBs.

There are three power distribution boards on the multi-well in-incubator imaging system. Two one-sided PCBs (each holding 6 Raspberry Pi Zero W's) and one double-sided PCB (that holds 12 Raspberry Pi Zero W's) that is sandwiched between the two one-sided PCBs show in figure 3.15. This is the only way we could accommodate for all 24 Raspberry Pi Zero W's and their associated camera component which is all held on layer 2, the elevator.

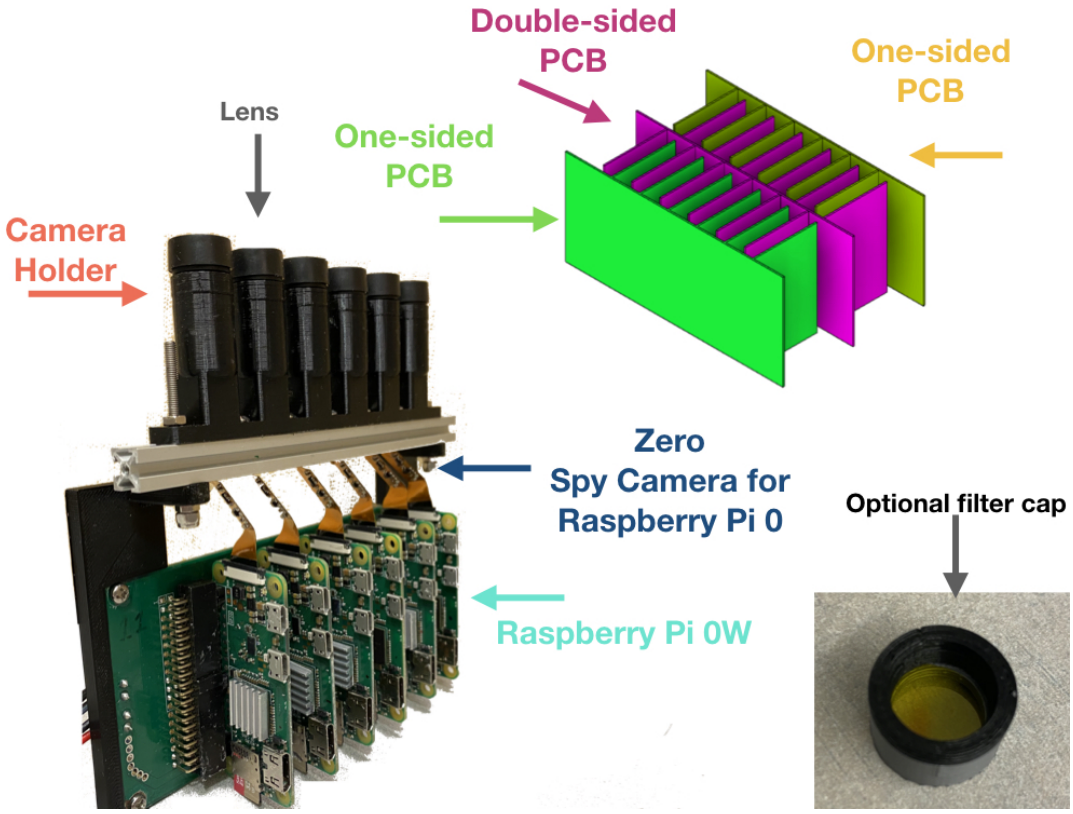


Figure 3.15: Shows the interlocking design as a well as one row of cameras fully assembled and conncected to the Raspberry Pi Ows on a custom pcb board.

Arduino Uno/Arduino Motor Driver Shield

The Arduino Uno and Motor Driver shield is an over the counter microcontroller that allows us to easily control the motors for fine focus adjustment. As an upgraded feature, motors with encoders have been added in order to

3.2.8 Power Consumption

Power consumption of the system consists of 24 Raspberry Pi Zero W, Camera, ESP32, two motors and LED panel. Raspberry Pi Zero W with Raspberry Pi camera have idle power consumption at 120 mA and peak at 230 mA (5.19V). Thus, at peak one Raspberry Pi Zero W consume 1.2 Watt and 0.63 Watt during

idle. ESP32, motor driver shield and limit switch consume roughly 4 Watt when motors when operate at full-speed and 0.5 Watt when idle. LED panel consists of 252 LEDs (63 of each color), and each LED consumed 20 mA per LED at 5V, thus each LED consume 0.1 Watt. When operate LED panel will consume 6.3 Watt. Thus, peak power consumption of the system is 1.2*24 + 4 + 6.3 = 39.1 Watt and 0.63*24 + 0.5 + 0 = 15.62 Watt.

3.2.9 Software

Experiments are initiated using our web-based control console, as illustrated in Figure 3. Within the console, users define various parameters crucial to the experiment, including the experiment ID, the number of pictures in the z-stack, the distance between layers, the initial offset distance, the type of lighting (Over-the-plate or Under-the-plate), and any additional camera control parameters facilitated by the raspistill library [ras,]. These specified parameters are then transmitted to the Picroscope via a cloud-based messaging service utilizing the Message Queue Telemetry Transport protocol (MQTT) [Locke, 2010]. Moreover, the control console enables dynamic adjustments to experiment parameters in real-time during the course of an ongoing experiment. In our system, each imaging event comprises a single z-stack per active camera. Upon the completion of each captured event, the Picroscope uploads the results to an S3 Object Store [ama,] hosted on a server. Subsequently, the captured pictures are made accessible through our image viewer website.

Through the image viewer, using proper credentials, we are able to view the progress of the cells through the images as they are uploaded. By clicking next timestamp you can view photos at different times and you can adjust the focal view and view the images in the specified z-stack height.

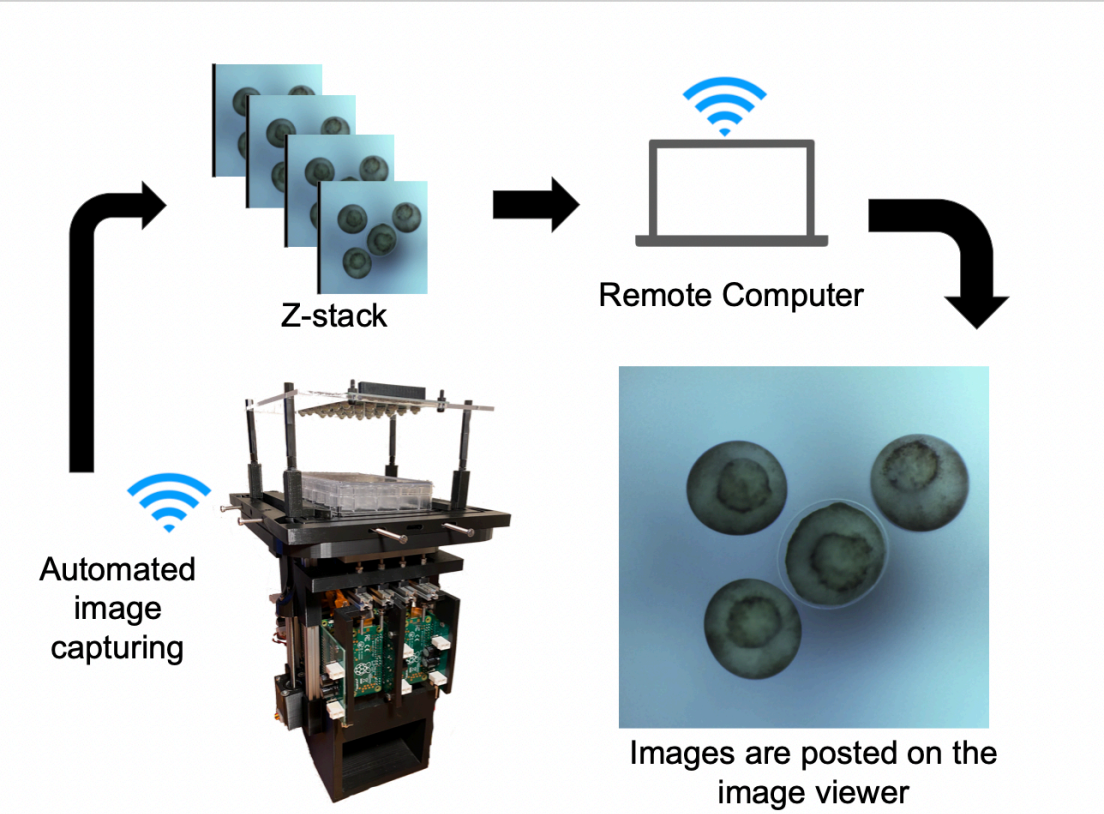


Figure 3.17: The images are autonomously collected and wirelessly transferred to a remote computer for viewing or post processing

3.2.10 Heat Distribution

From Figure 3.19, we can also determine if the heat from the electronics is impacting the biological samples. Due to the design of the distance between layers 1 and 2, we do not believe that the heat will impact the biological samples while imaging with the Picroscope.

In addition to mitigating condensation with the base design shown in figure 3.9a 9, we have discovered that lowering the set point temperature of the incubator is an effective strategy to prevent overheating of biological samples. Specifically, we will decrease the incubator set point by 2 degrees Celsius. This adjustment ensures that the temperature within the incubator remains within an optimal range

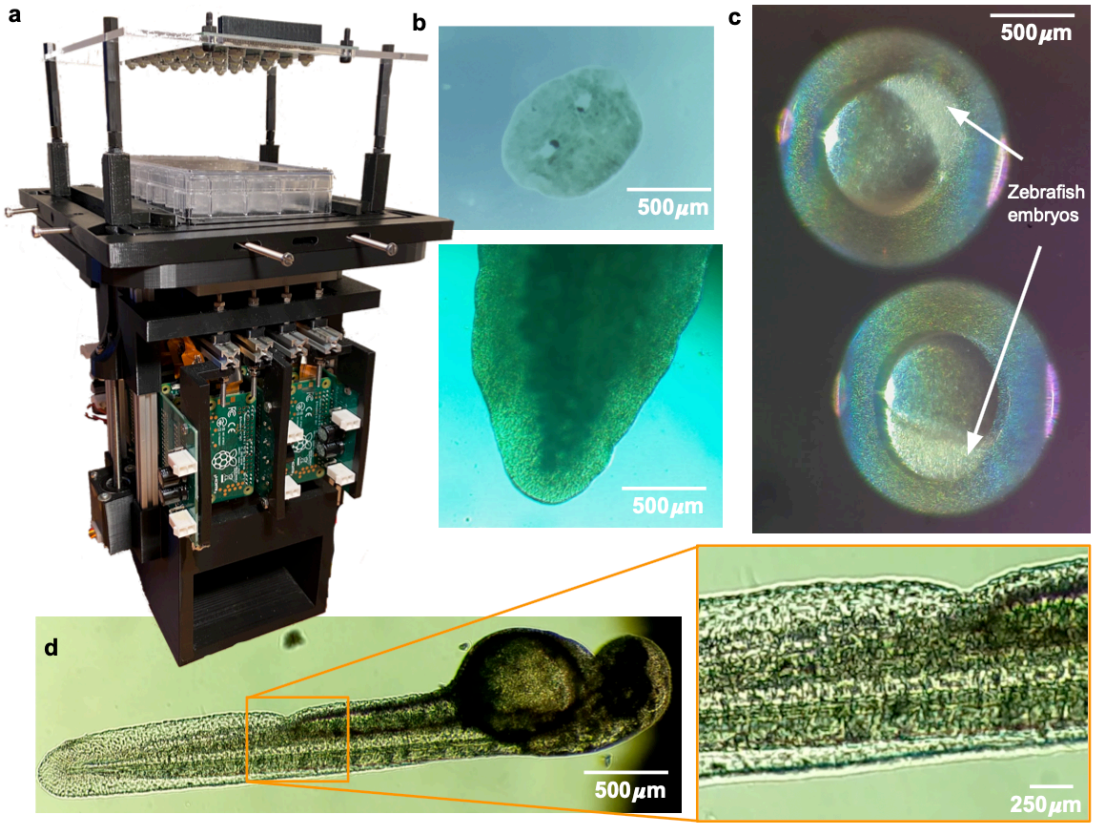


Figure 3.18: **Development of a low-cost system for simultaneous longitudinal biological imaging.** **a** The Picroscope fits a standard 24 well plate, it is controlled remotely and images can be accessed through a web browser. **b-d** Applications of the Picroscope to longitudinal imaging of developmental biology and regeneration. **b** Regeneration of planaria worms. **c** Zebrafish embryonic development at oblong stage. **d**.Zebrafish embryo at 48 hours post fertilization.

for the preservation and growth of biological samples without risking excessive heat.

3.3 Conclusion

The journey from conceptualization to realization of the Picroscope involved a series of iterative steps, each contributing to the refinement and enhancement of the device. With each iteration, we meticulously scrutinized the design, identify-

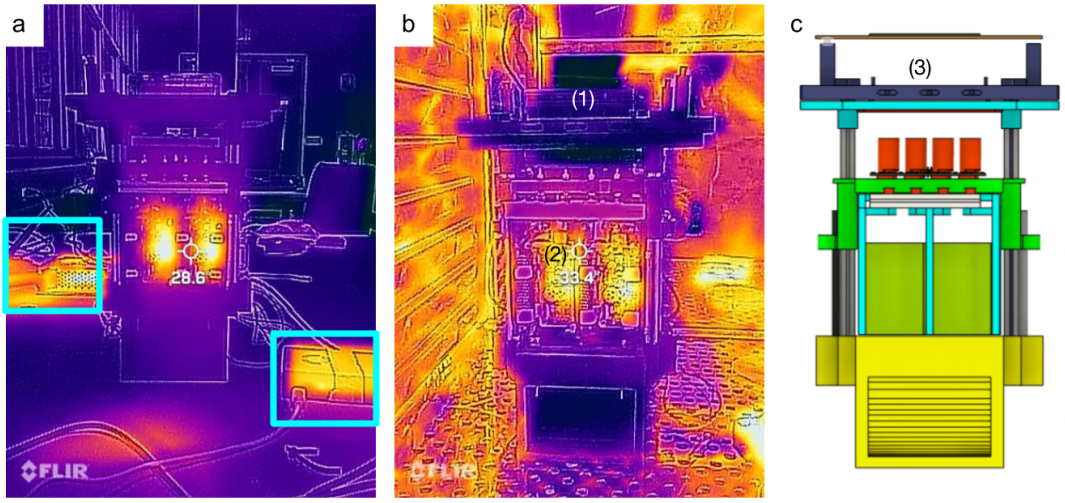


Figure 3.19: Using a thermal camera, we are able to determine where the hottest heat points are of the Picroscope.

ing areas for improvement and implementing modifications to address them. The iterative process enabled us to leverage the flexibility and adaptability afforded by 3D printing technology, allowing for rapid prototyping and testing of various design iterations. Through this iterative approach, we were able to systematically iterate on the design, fine-tuning its functionality and performance to meet the rigorous design criteria we had established at the outset. This iterative refinement process not only facilitated the development of a fully functional device but also fostered a deeper understanding of the design challenges and considerations inherent in creating an open-source multiwell longitudinal imaging system.

- Made from off the shelf components for easy replicability

- Automatically capture images for longitudinal studies on cells
- Withstand the environment of an incubator
- Bright-field microscopy
- Image a standard 24 well plate

From these iterations, we have also tested the Picroscope, which will be discussed in section 4.

Chapter 4

Testing and Applications

4.1 Introduction

To test the functionality of the Picroscope, we initiated experiments utilizing resilient biological samples, specifically Planaria worms and Xenopus tropicalis, capable of thriving at room temperature. This strategic choice not only mitigated the risk of contamination within the laboratory but also afforded us the opportunity to assess the performance of both the software and hardware components of the Picroscope within a controlled environment.

By selecting biological samples that posed minimal environmental challenges, we could observe the experiments with heightened precision and confidence. This preliminary phase served as a crucial validation step, ensuring the Picroscope's abilities before advancing to more intricate biological samples tailored for its intended use.

Having successfully demonstrated the Picroscope's ability to capture longitudinal imaging of these initial samples, we established a solid foundation to progress to the next phase of experimentation. This transition marked a pivotal moment, as we seamlessly transitioned from the validation stage to the targeted biological

samples, aligning with the Picroscope's intended applications.

4.2 Applications

4.2.1 Experiment 1: Planaria Worms

Planarian worms, a type of flatworm, possess remarkable regenerative capabilities, enabling them to regenerate portions of their body. As such, they represent an exemplary specimen for evaluating the stability and functionality of our device, particularly amidst the mandated shelter-in-place measures enforced due to the COVID-19 pandemic. However, our imaging efforts encountered several challenges. Notably, planarian worms exhibit photophobic behavior, swiftly evading areas illuminated by light, thereby complicating image acquisition. Furthermore, they tend to adhere to the sides of the experimental well, rendering them inaccessible to the camera's view.

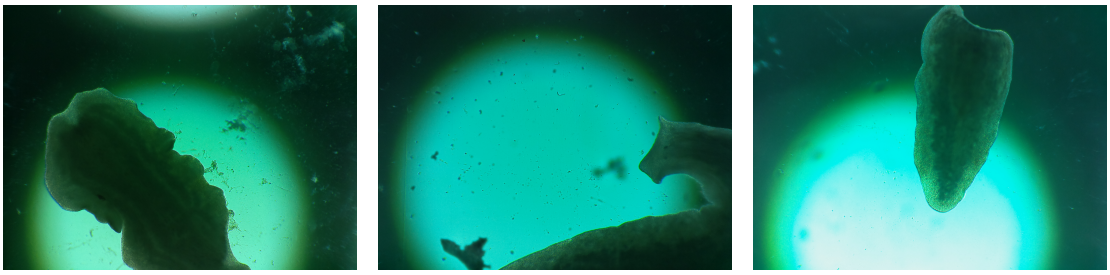


Figure 4.1: Images of Planaria that happened to drift over the view of the camera of the Picroscope

Using Shen et. al's protocol [Shen et al., 2018] we were able to immobilize, with agar, the planarian worms to stay in place to be imaged over a long duration of time. A two headed planaria worm was created by carefully slicing the worm in the head right between its "eyes" shown in the left images of figure 4.2. Due to the photophobia that planaria worms have, many perished during our experiment.

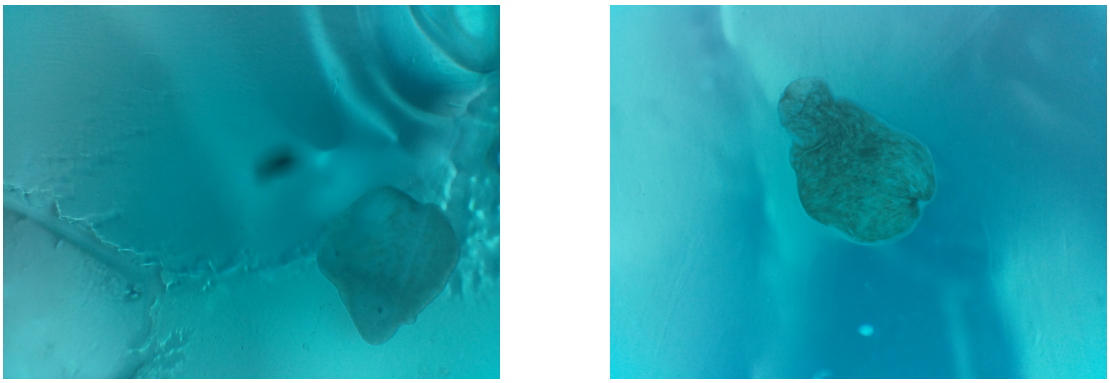


Figure 4.2: Images of immobilized Planaria taken with the Picroscope, One day post slice

4.2.2 Experiment 2: Live imaging of model organisms

Model organisms

Longitudinal imaging of Xenopus tropicalis embryonic development

We imaged the development of *Xenopus tropicalis* embryos from the onset of gastrulation through organogenesis (Figure 4.3 and 4.5).

Xenopus serves as a model organism for studying developmental processes and early-onset diseases affecting the nervous system [Borodinsky, 2017]. While various species of *Xenopus* are employed in research globally, *Xenopus tropicalis* is favored due to its diploid genomic constitution and rapid sexual maturation [Olmstead et al., 2009, Hirsch et al., 2002]. The optimal conditions for the normal development and husbandry of *Xenopus tropicalis* range between 25-27°C, akin to standard room temperature, negating the need for specialized incubation equipment [Mcnamara et al., 2018, Khokha et al., 2002].

This characteristic factored significantly into our decision to employ *Xenopus tropicalis* for an imaging experiment during the pandemic shutdown. Over a 28-hour duration, we imaged *Xenopus tropicalis* embryos, with four embryos per well in a 24-well plate configuration, reserving one well for calibration purposes

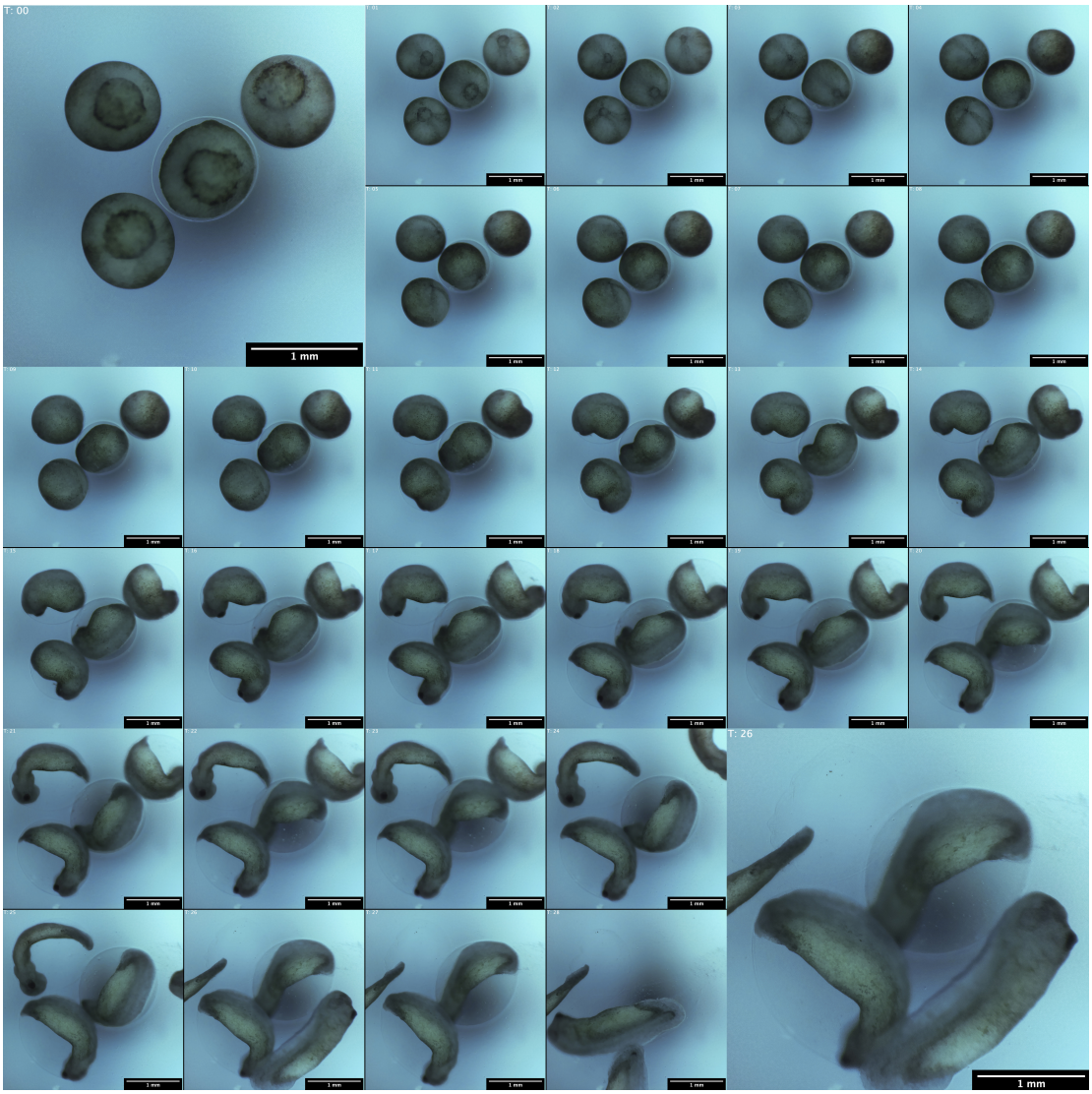


Figure 4.3: **Longitudinal imaging of *Xenopus tropicalis* development.** Images of a representative well in which 4 frog embryos developed over a 28 hours period. Images were taken hourly

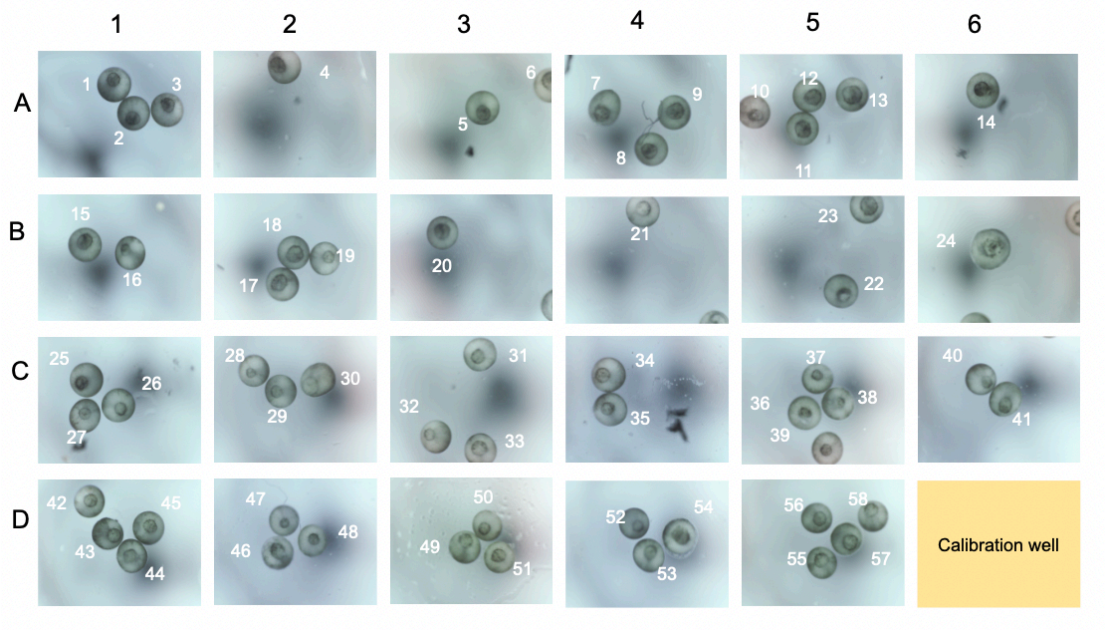


Figure 4.4: Image of 23 wells observing 57 frog embryos

(see figure 4.3). The embryos were cultured in a simple saline solution at room temperature. Imaging commenced hourly from gastrulation onwards (see Figure 4.5). Subsequently, each image was visually scrutinized, and the embryos were categorized into standard stages of frog development, encompassing gastrulation, neurulation, and organogenesis (see Figure 4.5a). Additionally, a subset of 27 embryos had their blastopore diameter measured during gastrulation, revealing a progressive reduction over a six-hour period consistent with developmental milestones. This experiment underscores the utility of the Picroscope for longitudinal sequential imaging and tracking of biological processes.

4.2.3 Experiment 3: Cortical organoids

Cortical Organoids

Human Embryonic Stem Cells and Brain Organoids

Not only we were able to image biological samples outside of an incubator,

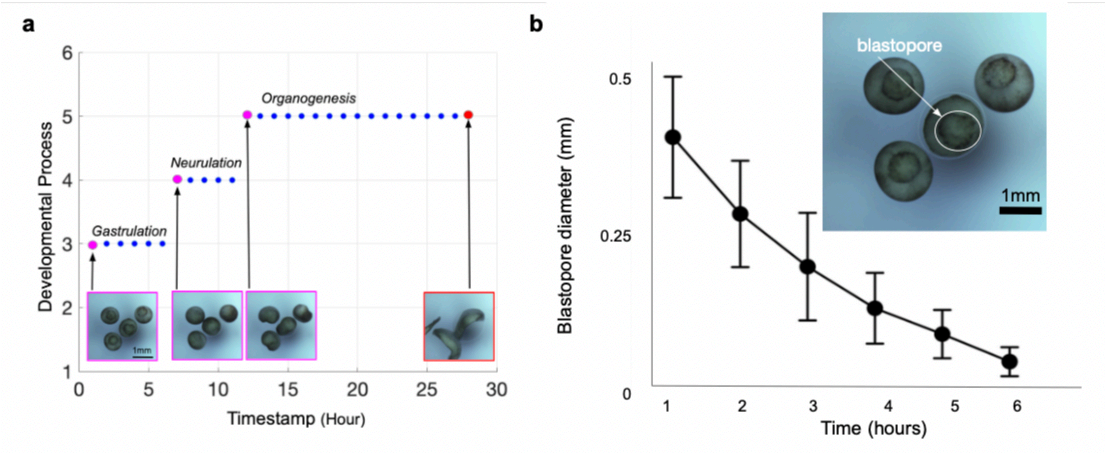


Figure 4.5: **Longitudinal imaging allows the tracking of individual developmental processes: a** The images shown in figure 4.3 were taken hourly over a 28 hours period and encompass 3 developmental stages: Gastrulation, neurulation and organogenesis. Y-Axis represents the stages of frog embryonic development: 1 = Fertilization , 2 = Cleavage, 3 = Gastrulation, 4 = Neurulation, 5 = Organogenesis, 6 = Metamorphosis. X-Axis represents the time point at which it occurs. Each dot in the plot represents a time point in which the images were taken. Magenta = the beginning of each developmental process. Red = the end of the experiment at 28 hours. Blue = intermediate timepoints. **b** Diameter of the blastopore is reduced over time from gastrulation to neurulation. The top right panel shows an example of an individual blastopore. A total of 27 embryos were considered for the analysis.

we could also image from within the incubator. This allows us to image 2D and 3D mammalian models or other biological samples that require special conditions requiring an incubator enclosure for warmth as well as the proper mixed gases.

However, enabling imaging within a warm and humid environment using 3D printed components presents inherent challenges. Typically, 3D printed components, depending on the material, are prone to deformation over time in such conditions. Integrating electronics into the Picroscope introduces another obstacle due to the compatibility issues between electronics and humidity. To address this, we applied a protective coating of inert substance, specifically Parylene C, to the electronics. However, the coating process requires access to a costly clean-

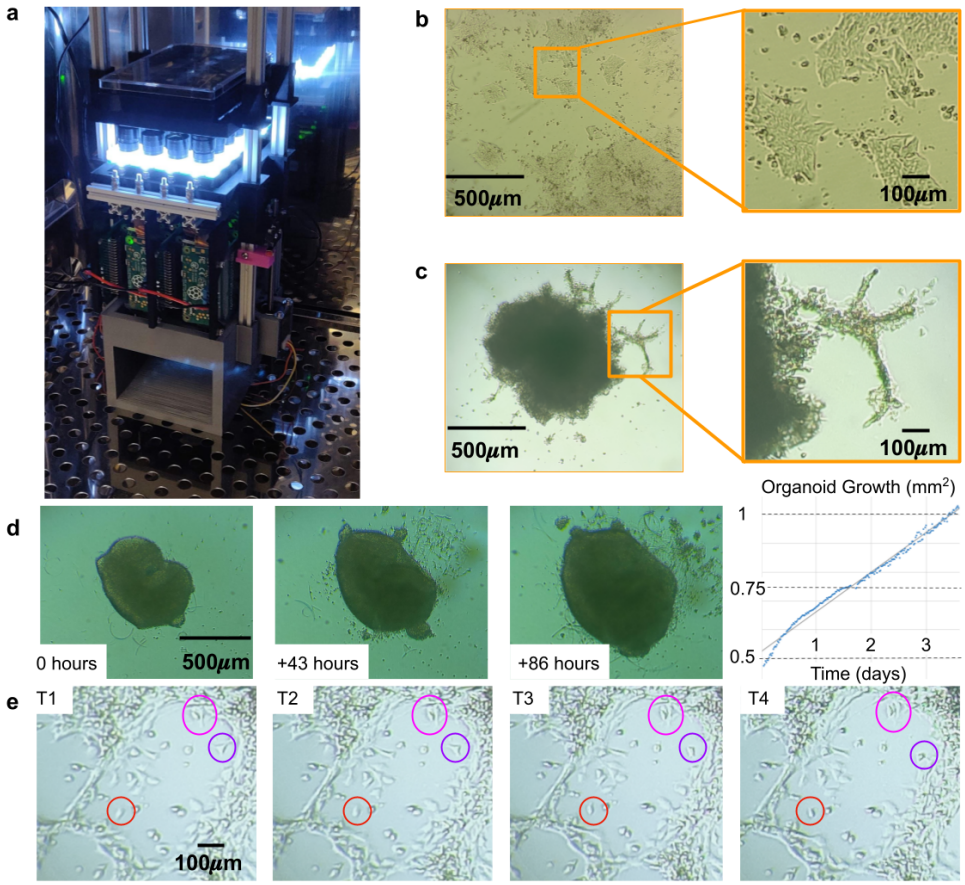


Figure 4.6: **In-incubator imaging of mammalian cell and cortical organoid models**. **a)** The Picroscope inside a standard tissue culture incubator. **b)** Imaging of human embryonic stem cells as a model of 2D-monolayer cell cultures. **c)** Longitudinal imaging of human cortical organoids embedded in Matrigel. Zoomed images show cellular outgrowths originating in the organoids. **d)** Tracking of cortical organoid development over 86 hours. Images were taken hourly. On left. Images of the tracked organoid at timepoints 0, 43 and 86. On right. Measurement of organoid area at each time point analyzed. **e)** Manual Longitudinal tracking of individual cells in embedded cortical organoids over 40 minutes. Images were taken every 10 minutes. Magenta = example of cell division, Red = example of cell migration, Purple = example of morphological changes.

room facility. To counter these challenges, we constructed the Picroscope using PCTG, a non-toxic and biodegradable material, with 100% infill, and reinforced vulnerable components with maker beams. Additionally, all electronic parts were coated with Corona Super Dope Coating to safeguard against heat and humidity

encountered within an incubator.

The use of Corona Super Dope Coating offers a cost-effective solution that can be easily procured online, facilitating the replication of the Picroscope by others.

The functionality of the Picroscope within a standard tissue culture incubator was validated by imaging 2D-monolayers of human embryonic stem cells (hESCs) (see figures 4.6a-b). Additionally, the system's capability for longitudinal imaging across the z-axis was demonstrated by imaging human cerebral cortex organoids embedded in Matrigel (see figure 4.6c). This platform enabled monitoring of organoid growth and neuronal process outgrowth (see figure 4.6d), as well as tracking the migration patterns and behavior of individual cells within organoid outgrowths. In this particular experiment, the Picroscope was oper-

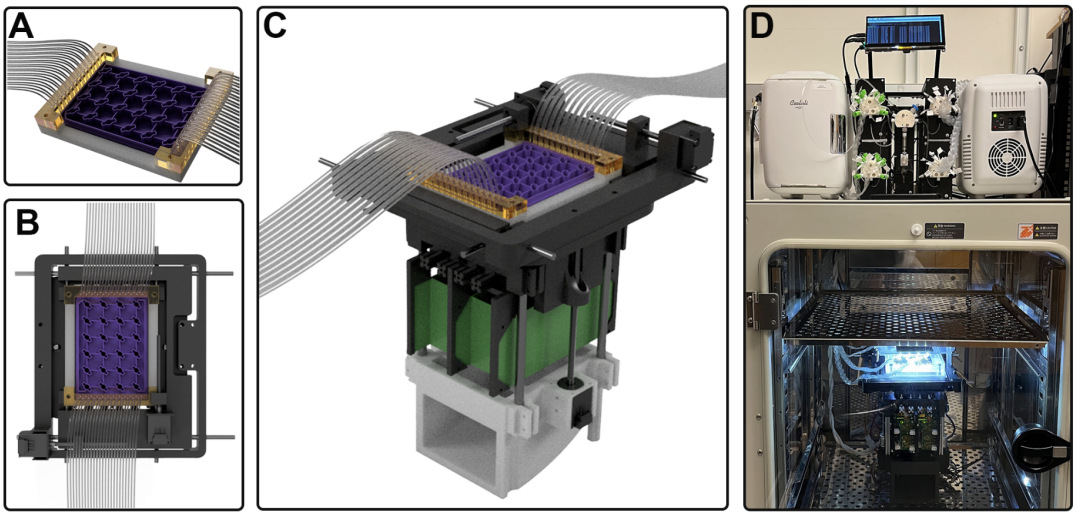


Figure 4.7: The Picroscope integrated with the modular automated microfluidics cell culture platform [Seiler et al., 2022]. A. The fluidic Plate in a CAD model. B. CAD model of the fluidic plate and the XY stage from the picroscope. C. CAD model of the Picroscope and the fluidic plate assembled together. D. Real life setup of the fluidics system on top of the incubator, and the Picroscope and modular microfluidic cell culture platform in the incubator.

ational for a continuous period of 12 days, effectively capturing the dynamic growth of organoids within the incubator, as illustrated in figure 4.9. The Pi-

croscope seamlessly integrates with a modular automated microfluidic cell culture platform developed by our colleagues at UCSC. By connecting these two devices, the process of feeding and imaging can be automated, facilitating the observation of robust organoid growth within the Autoculture wells. This growth pattern remained consistent with the observed increase in size for organoids cultivated under control conditions. Notably, organoids grown in the automated system exhibited a less dense perimeter compared to controls, suggesting that the reduction in velocities and shear forces may facilitate growth and migration that would otherwise be impeded.

From: Modular automated microfluidic cell culture platform reduces glycolytic stress in cerebral cortex organoids

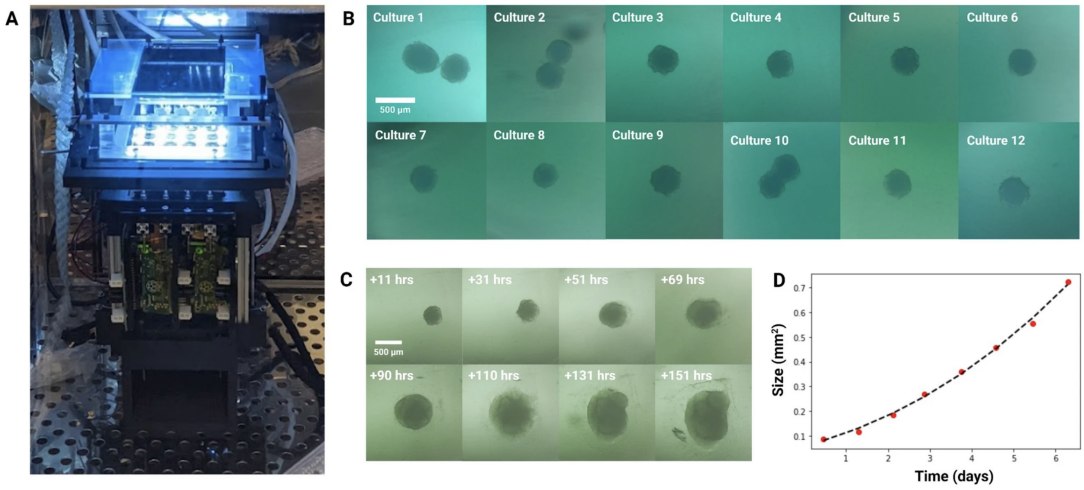


Figure 4.8: Longitudinal monitoring of organoid development. (A) The Autoculture microfluidic chip sits on a remote-controlled, IoT-enabled, 24-well automated imaging system. (B) Bright-field images of twelve individual 12-day-old cerebral cortex cultures at day 1 of automated feeding. (C) Longitudinal imaging of "Culture 4" during the experiment. (D) Projected area expansion of "Culture 4" during the experiment. This was obtained using a computer vision algorithm.

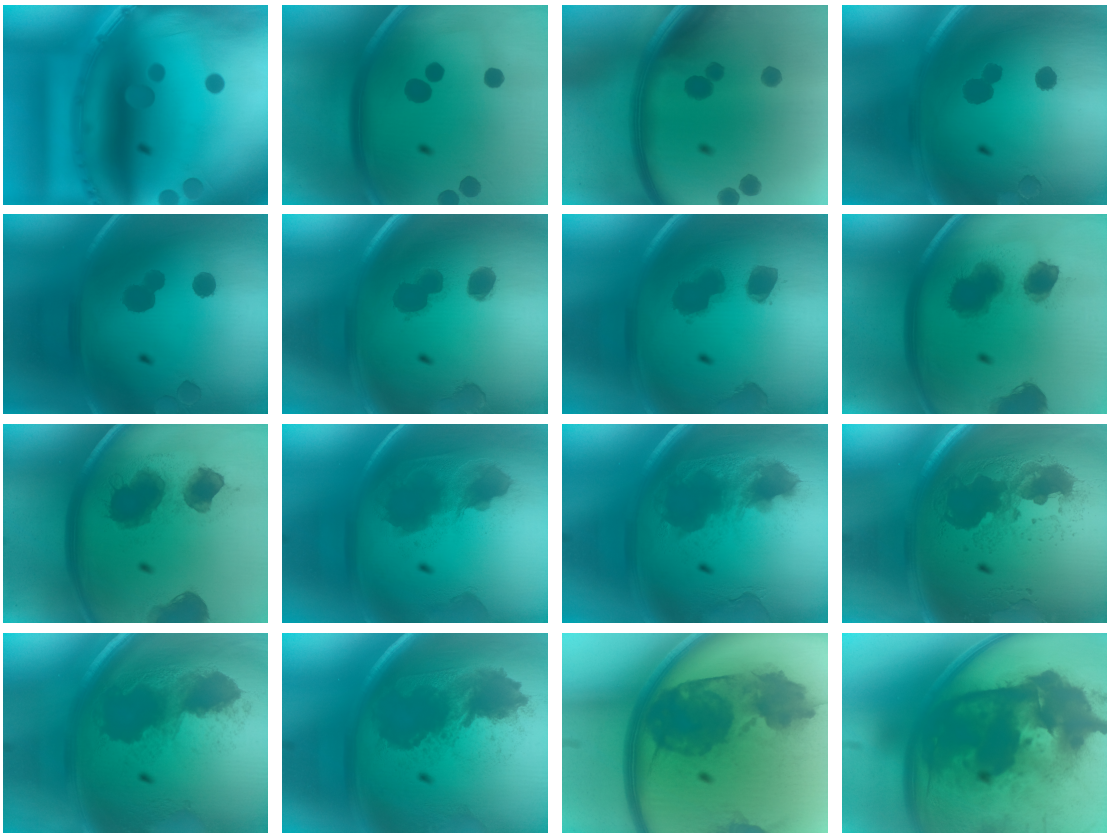


Figure 4.9: These 16 photos are from a 12 day experiment with automated feeding and image capturing. Timestamps: 1, 10, 15, 27, 40, 49, 59, 71, 85, 107, 117, 121, 134, 156, 201, 272

4.3 Conclusion

The Picroscope demonstrates vast potential applications, highlighting its versatility as an open-source longitudinal imaging system. While only a subset of these applications is discussed here, they illustrate the system's adaptability across various fields such as developmental biology, neuroscience, and tissue engineering. Each application experiment also serves as a platform for identifying necessary improvements, whether in mechanical, hardware, or software aspects. These iterative refinements aim to enhance stability, imaging quality, and data analysis efficiency. The collaborative and open-source nature of the Picroscope project fosters ongoing community contributions and innovations, ensuring its continual growth and relevance in scientific research. Ultimately, the Picroscope not only serves as a powerful imaging tool but also drives advancements in longitudinal imaging and applications in education which will be discussed in section 6 of this thesis.

Chapter 5

Image Analysis: Growth Rates

5.1 Introduction

In the realm of organoid research, a common challenge is the lack of real-time insights into organoid development during ongoing experiments. Traditionally, researchers analyze organoids post-experiment, providing retrospective information on their characteristics. This is one issue the Picroscope helps with by providing longitudinal images during an ongoing experiment.

To address this limitation, the incorporation of an algorithm for continuous image analysis during experiments emerges as a useful solution. This approach empowers researchers to obtain immediate estimates of organoid size as they grow. By analyzing data while the experiment is on going, it allows scientists to assess the normalcy of sample growth while the experiment is still in progress. Which could lead to a more efficient system, if organoids are not growing typically, researcher have the option to start their experiment at an earlier timepoint rather than continuing an experiment with organoids that aren't growing based on how researchers expect them to behave.

The significance of this early analysis is underscored by its potential to save

valuable time for biologists. Early detection of deviations from expected growth rates enables proactive intervention, potentially saving time and resources in cases of abnormal organoid development. This methodology not only enhances the efficiency of experimental workflows but also elevates the precision and impact of organoid studies.

5.2 Organoid size algorithm

Within the MATLAB environment, I developed a script with the aim of refining original images through the implementation of brightness and sharpening adjustments. Subsequently, the script intricately dissects the color images into their red, green, and blue (RGB) channels, allowing for an evaluation of each channel's impact on the overall image.

Given that the original images are in color and composed of pixels, each pixel representing primary colors, the script effectively separates the image into distinct color channels—namely, red, green, and blue. While the name suggests red, green and blue channels the images are greyscaled images of those distinct channels. Greyscale images are characterized by pixels from a single channel, resulting in varying shades of grey for each pixel. Notably, grayscale images differ from binary images, which exclusively feature black and white pixels.

Upon analyzing images acquired through the Picroscope, the investigation pinpointed the green channel as optimal for subsequent image processing. Its inherent brightness contributes to maximal contrast among the three channels. Conversely, images obtained from a biologist, via a cellphone through a microscope eyepiece, demonstrated that the blue channel was the most suitable for processing. By visual inspection, the user can pick the RGB channel of interest and then process the images from there to see which will yield the best filtered result in the

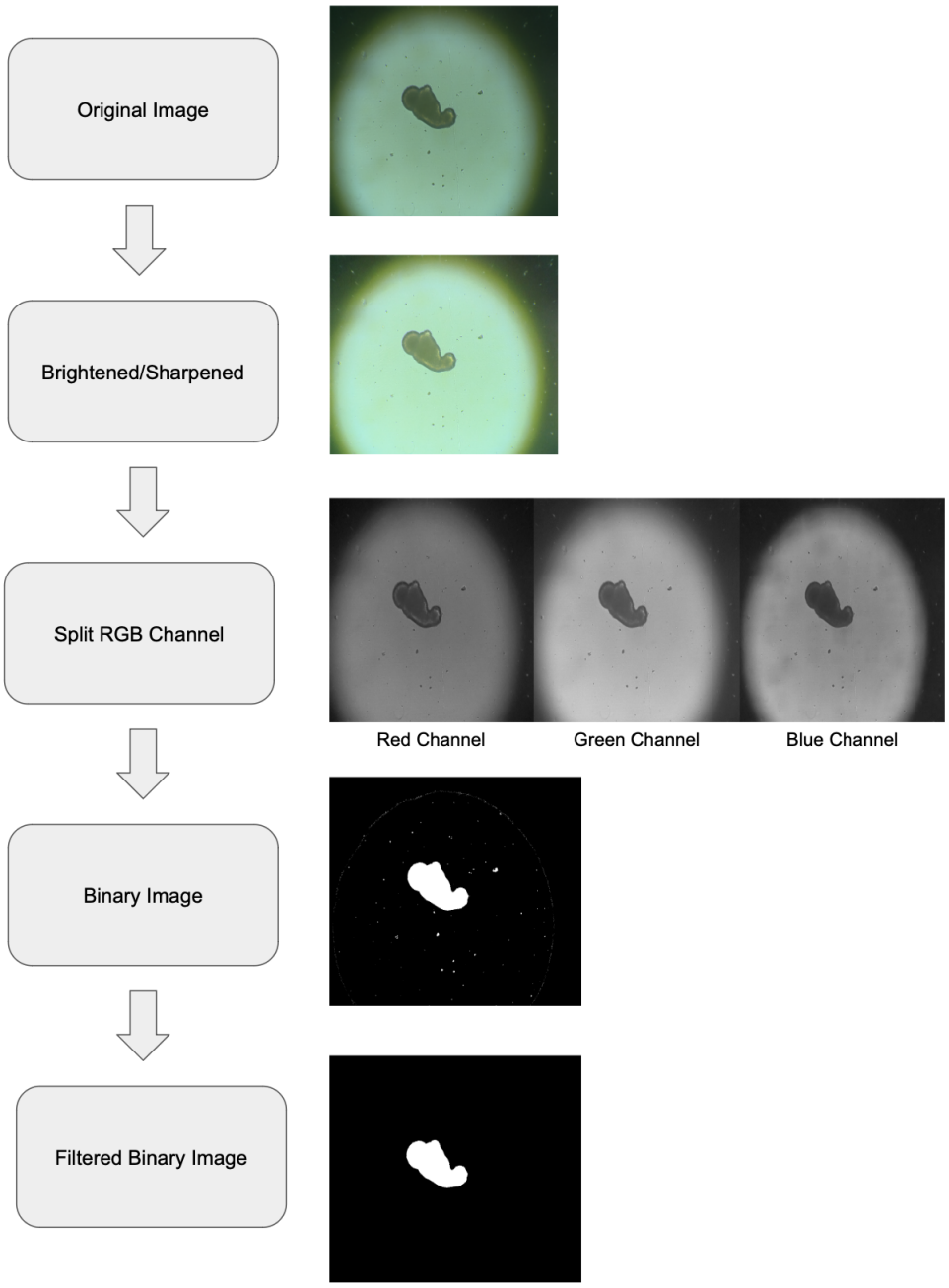


Figure 5.1: In the original image, there is an organoid (demonstrating not all organoids are circular), and these are the imaging processing steps to obtain information about a specific organoid at one time point, which is then processed into growth rate over time. The original images goes through many layers of filtering before coming to a point where we only have the organoid and a black background. This is just one organoid of the 11 organoids that we analyzed over the period of 6 days.

end.

The chosen channel undergoes transformation into an inverted binary image, a pivotal step that accentuates the organoid—the focal point of interest—against the background. Inverting the binary image prioritizes the visualization of the organoid, and the subsequent background fill operation results in black pixels, leaving only the organoid represented in white pixels. An extra step criteria to the "fill" step of the binary image, is the call for "hole", which is to tell the program to fill in a "hole" in the background, reducing the white specks around the organoid. As an added precaution, there is a call for the function "imclearborders" which ensures that if a part of an organoid is cut off by the edge of the image, it will remove it therefore providing a solid border on the perimeter of the image.

After this step, the image should show the organoid in white and the background should be completely black. From there we can find area, of the organoid, in pixels by counting the number of white pixels in the image. With this number, we can convert it from pixels to area in millimeters squared. This is done by using a calibration slide taken with the same camera set up. For the same camera set up, this requires the camera and the zoom/magnification to be constant. This ensures that we have an accurate real world measurement to pixel quantity.

Depending on the number of images taken during that time point, we can measure the size of multiple organoids and take the standard deviation which will give us an average size of organoids during the same time points. With this information we can compare different organoid protocols and potentially see how different steps within that protocol can cause the organoid to grow or shrink.

This intricate image processing workflow not only facilitates the accurate calculation of the organoid's area but also enables continuous tracking and plotting of its development over time (refer to figure 5.1).

5.3 Applications of the Organoid Size Algorithm

5.3.1 Trial 1: Tracking growth over the span of 5 days

After developing a functional algorithm, it was assessed using a limited set of images from a previous experiment conducted with the Picroscope. In this dataset, the green channel exhibited the highest contrast, leading us to prioritize the use of the green channel image when initializing the algorithm. Figure 5.2 displays the tracking of four organoids over a span of five days. Notably, the highlighted yellow and green organoids gradually converge and eventually merge, resulting in their identical size in the grab.

From here, we decided to look at a larger image data set from images also taken from the Picroscope.

5.3.2 Trial 2: Images taken with the Picroscope

In a previous experimental trial, longitudinal images were captured over a period of 9 days to monitor the growth rate of 11 organoids. Utilizing this dataset, the organoid sizes at each time point were plotted to visualize the growth progression on a daily basis. This experiment was conducted in collaboration with the Picroscope and a microfluidics system. The microfluidics system aims to demonstrate the reduction of glycolytic stress in cerebral cortex organoids [Seiler et al., 2022].

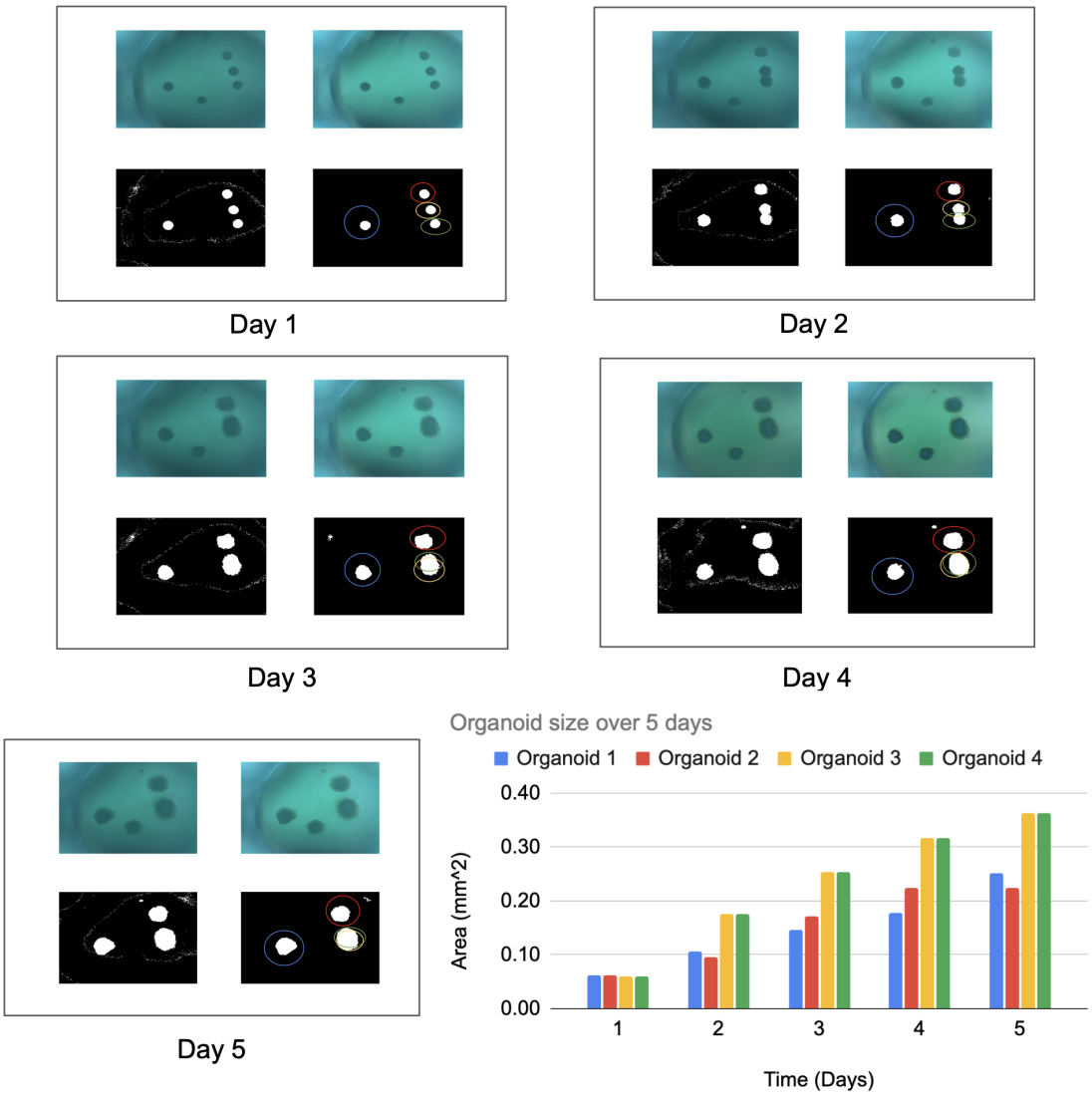


Figure 5.2: Images taken from the Picroscope, We selected one well and tracked these 4 organoids over the span of 5 days.

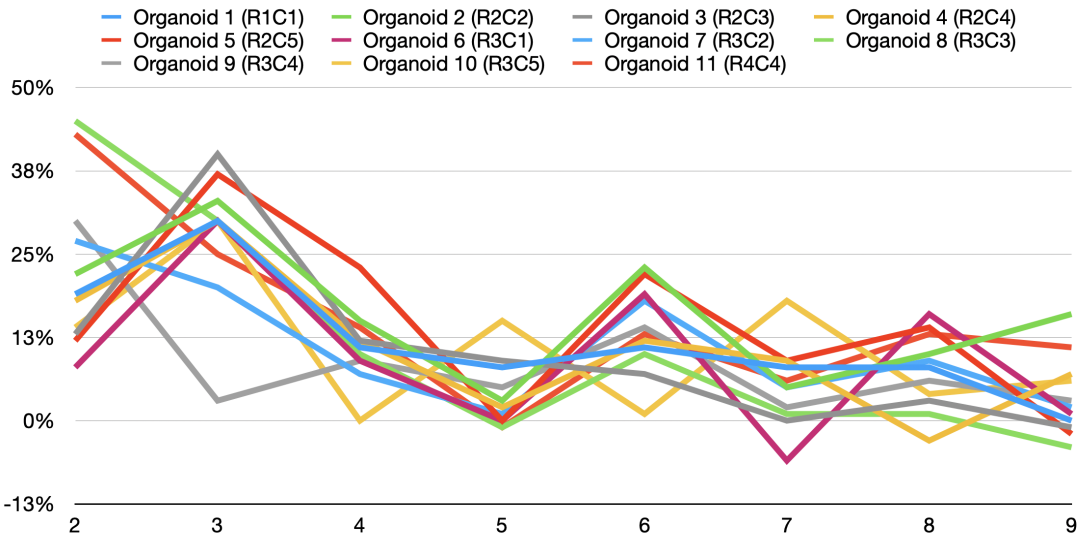


Figure 5.3: In this plot, we tracked the growth rate of 11 different organoids (labled as "R" and "C" which stands for the rows and columns of the wells the images were taken from since these images were taken with the Picroscope.

With this data set, we decided to look at the growth rate, using the equation:

$$growthRate = \frac{presentSize - previousSize}{previousSize} \tag{5.1}$$

One the growth rate is calculated, we can simply multiple it by 100% to find the growth rate percentage per day, which can been seen for 11 organoids in figure 5.3.

5.3.3 Trial 3: Images taken with a cell phone through a microscope eye piece

Organoid images were captured at specified time intervals: days 0, 7, 9, 14, 18, 28, 35, 42, 45, 56, 68, 70, and 73. Each time point typically featured a minimum of four images, with some containing up to 26 images. Initially, fewer organoids were visible, gradually increasing in number and size over time. The primary objec-

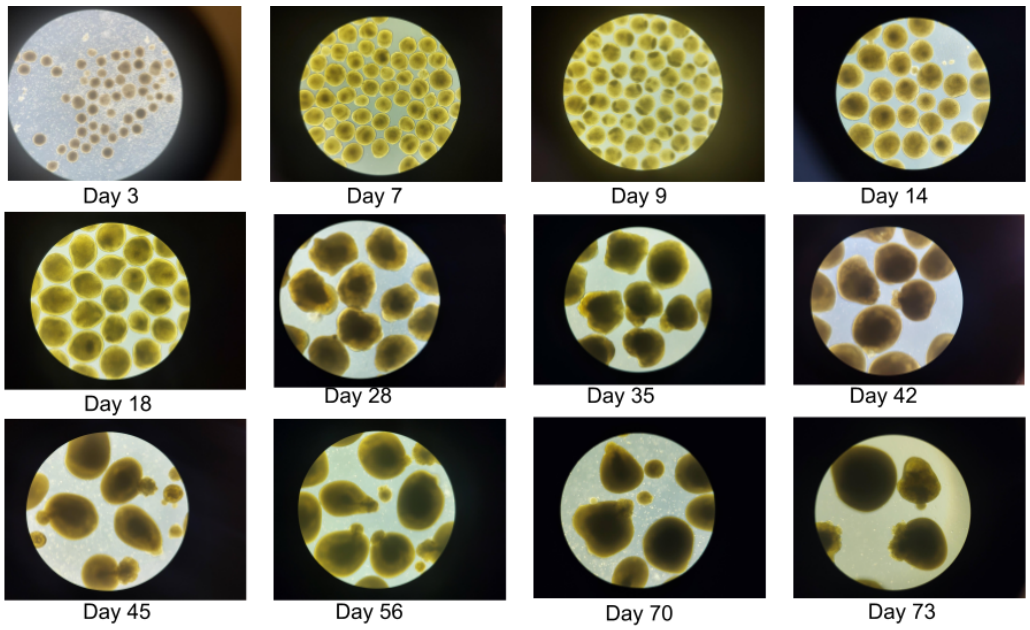


Figure 5.4: Images of organoids grown in matrigelfrom day 3 to 73

tive was to assess organoid growth across four distinct protocols simultaneously. Utilizing a consistent approach, each image was captured through a microscope eyepiece using the same cellphone camera, ensuring uniform magnification and field of view. To establish an accurate pixel-to-millimeter ratio, a calibration slide was also photographed under the same conditions.

The algorithm developed facilitated the analysis of organoid maximal projections over time, focusing on H9 stem cells human hESC (H9p31+6) organoids subjected to four experimental conditions. These conditions included variations in hydrogel coating (Matrigel vs. Collagen IV) and patterning protocol (Cerebral - CO vs. Choroid Plexus - ChPO) at each time point. Standard deviation calculations were performed for each set of timepoints to assess variability. By comparing the impact of patterning protocols with different hydrogels, insights into their respective effects were gleaned.

Illustrated in Figure 5.5 for the Cortical + Choroid patterning protocol, the

organoids cultivated in Matrigel exhibited larger average sizes compared to those grown in Collagen IV. Similarly, in figure 5.6 for the Cerebral CO patterning protocol, organoids cultured in Matrigel displayed larger average sizes than those in Collagen IV. While this investigation represents a single trial under these conditions, future endeavors may involve conducting additional trials to validate observed trends.

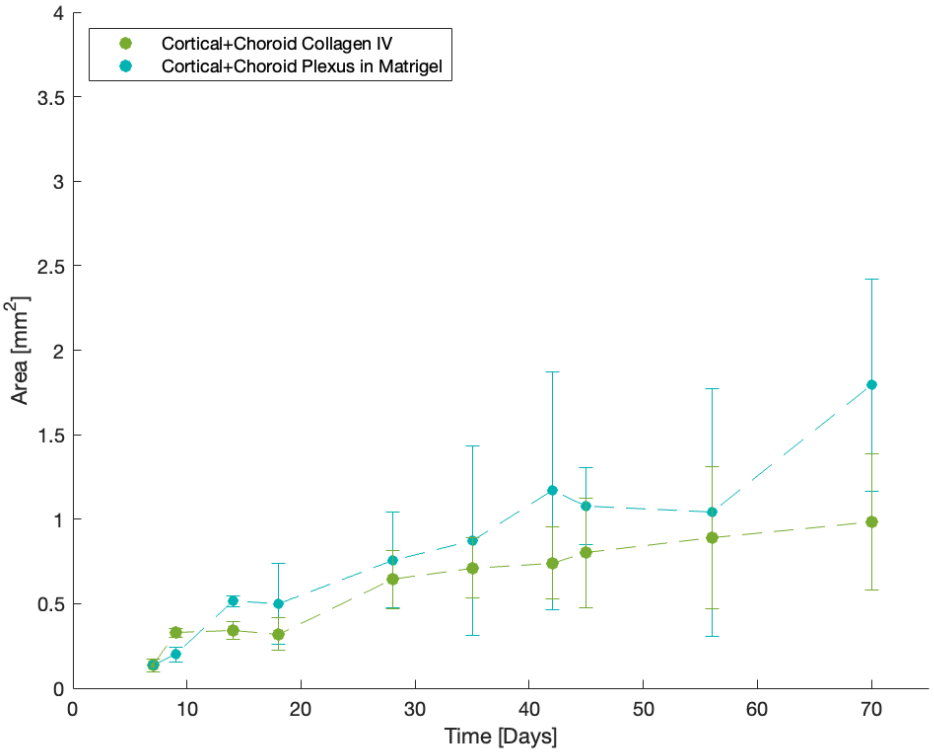


Figure 5.5: Growth data for 2 different batches of cortical plus Choroid organoids grown in collagen IV vs Matrigel from days 3 to 73. The experiment was to compare the different protocols to see which yielded the most organoid growth.

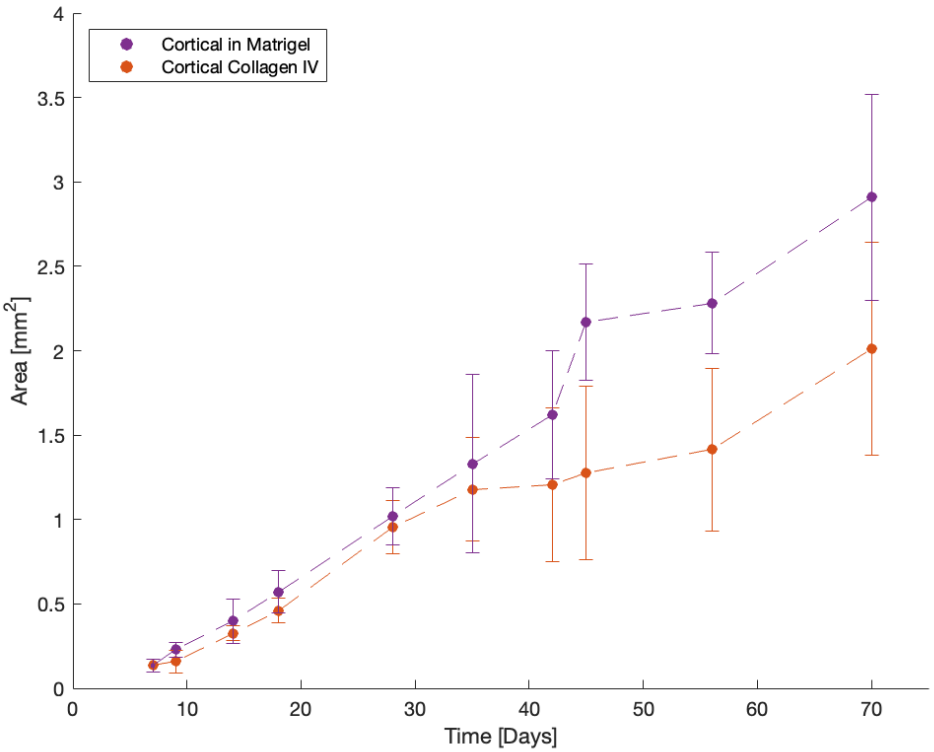


Figure 5.6: Growth data for cortical organoids grown in matrigel vs collagen IV of organoids from days 7 to 73. The experiment was to compare the different protocols to see which yielded the most organoid growth.

5.4 Conclusion

In conclusion, while organoid growth is just one facet in assessing their health, the developed script offers a valuable tool for researchers to investigate the factors influencing organoid growth or shrinkage. By aligning time points with steps in experimental protocols, this script can assist in elucidating the impact of various growth factors and inhibitors on organoid development, as well as aid in determining the optimal timing for signaling production.

Furthermore, the integration of this algorithm into ongoing experiments presents an opportunity to explore the effects of different protocol steps in real-time. Given

the absence of a universal protocol for organoid cultivation, this approach holds promise in identifying optimal conditions for organoid growth across diverse research settings. By capturing organoid measurements at critical time points, particularly during protocol transitions, this script has the potential to uncover the underlying mechanisms driving organoid size variations.

Beyond its utility for researchers, the data generated through this script has educational value, serving as a valuable resource for individuals interested in learning about organoids. Additionally, this data contributes to the development of educational tools like Seru-Otchi, a gamified approach aimed at enhancing undergraduate understanding of 3D cell culture concepts and protocols. By leveraging real-world organoid data, Seru-Otchi offers realistic organoid models to facilitate immersive learning experiences, as discussed further in Chapter 6.

Chapter 6

User Studies and Validation

6.1 Introduction

In contemporary education, project-based learning (PBL) stands as a recognized methodology for effectively imparting complex biology concepts. However, the implementation of PBL in educational institutions is often hindered by resource constraints. In response to this challenge, we have developed a pioneering framework for facilitating PBL using remote-controlled internet-connected microscopes. This innovative approach enables a single laboratory facility to simultaneously host experiments for students worldwide, transcending geographical barriers. By leveraging this platform, experiments can be conducted over extended periods and with materials typically inaccessible in traditional high school classrooms. Consequently, students are empowered to engage in novel research endeavors, moving beyond conventional classroom experiments.

Furthermore, this chapter explores the intersection of serious games in biology and gamified learning, augmenting traditional pedagogical approaches with interactive learning experiences. By integrating elements of gamification into biology and laboratory education, we aim to enhance student engagement and foster

a deeper understanding of biological, in this case, one organoid growting protocol and laboratory concepts. Through the gamification of learning, students are presented with interactive challenges and simulations that emulate real-world biological phenomena, thereby promoting active learning and problem-solving skills development.

To evaluate the efficacy of the Picroscope used in PBL and the impact of serious games and gamified learning in biology education, we conducted a series of user studies involving students from diverse geographical locations. Some of these studies, conducted remotely from Santa Cruz and San Francisco, California, while some were conducted in person at California State University Monterey Bay, engaged participants in hands-on experimentation, reading papers, playing a game and observation using their personal computers and cellphones. Subsequent surveys revealed a notable increase in students' enthusiasm for science and a heightened inclination towards pursuing careers in STEM fields as well as pursuing a position in a a laboratory.

6.2 Remote project based learning for Biology classes and clubs

6.2.1 Introduction

Pilot Study 1- ALISAL

Given the higher prevalence of caffeine consumption in student groups as opposed to the general population [Mahoney et al., 2019], our initial study concentrated on exploring the impacts of caffeine exposure on organism development. In this study, students enrolled in the AP Biology course at Alisal High School in Salinas, CA, conducted observations on the effects of three distinct caffeine

concentrations in developing zebrafish embryos. The observations encompassed overall effects on the organism, such as movement and twitching, as well as specific organ impacts, including effects on the heart. This inaugural user study played a pivotal role in gauging the program's feasibility under relatively straightforward experimental conditions.

The experiment extended over a period of one week, during which students were afforded the opportunity to remotely observe and analyze the outcomes in near-real-time from their respective locations.

User study 2 Second Round at ALISAL

Due to the successes from the pilot program, we started the collaboration again however instead of an after school program, we aligned the program with an AP biology course, at the same local high school ALISAL. Here, the students listened to graduate student led lectures, then designed their own experiment, observed the experiment, analyzed the data and presented the data. For this particular experiment, the students chose to see how ammonium nitrate and chlorine dioxide (common chemicals found in fertilizers and bleach) impacts the development of zebra fish. Overall, the students had a great learning experience, which can be shown in figure 6.1.

User Study 3

While the United States exhibits variable degrees of Internet reliability, the availability of high-speed internet is notably extensive compared to global standards [Tang and Ho, 2019]. To assess the feasibility of adopting Internet of Things (IoT)-enabled Project-Based Learning (PBL) in regions with less reliable internet infrastructure, we conducted a user study involving 20 students across five countries: Brazil, Bolivia, Spain, Mexico, and Peru. This study was conducted in conjunction with an online outreach initiative led by the United States-based

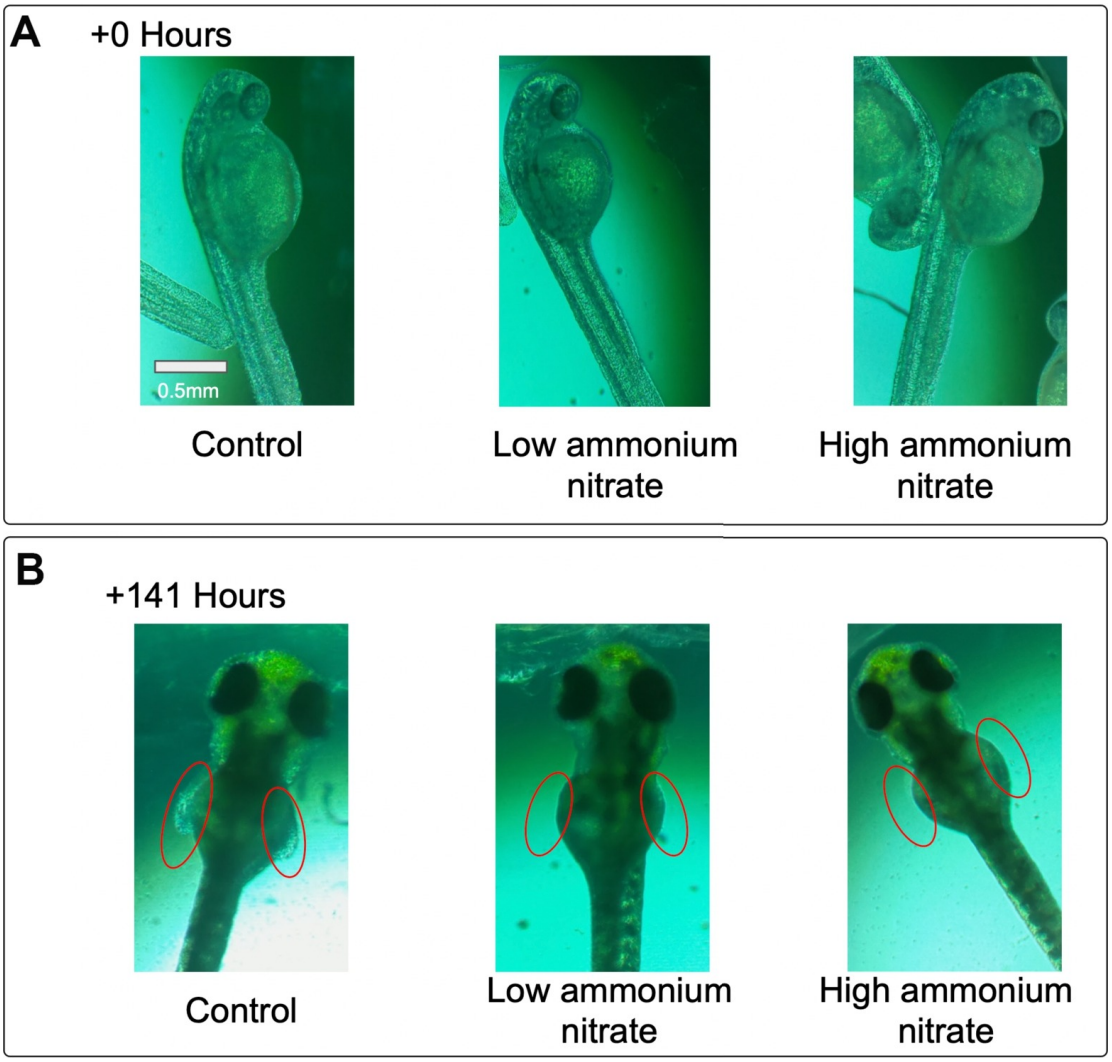


Figure 6.1: Undergraduate students monitored the impact of various chemicals, including ammonium nitrate, on the developmental progression of zebrafish embryos. Illustrative images spanning 141 hours reveal discernible effects on fin development due to ammonium nitrate exposure. A) Depicts an instance of zebrafish embryos at the experiment's initiation. B) Demonstrates zebrafish embryos after 141 hours, highlighting a noticeable delay in fin development observed in both low and high concentrations of ammonium nitrate.

non-profit organization Science Clubs International. The initiative targets high school and early college students in the aforementioned countries. During the study, students utilized remote microscopy to conduct biocompatibility studies on custom-made gold and graphene nanoparticles, aiming to determine the opti-

mal concentration for use in a bioengineering context. The outcomes of this study underscored the adaptability of our program and its capacity to cater to students across diverse geographical locations, accommodating varying levels of internet connectivity reliability.

User Study 4

In our fourth user study, our focus centered on scalability. We conducted this study in conjunction with a college-level pharmacology course in Bolivia, which boasted an enrollment of over 130 students spread across four cities: Santa Cruz de la Sierra, La Paz, El Alto, and Cochabamba. Notably, Bolivia faces challenges in internet connectivity, possessing the slowest connection in South America. To address the constraints of remote education, particularly during events like the COVID-19 pandemic, the government and education sector often resort to television and radio as alternative means for large-scale remote education [49].

In this study, students explored the toxic effects of chlorine dioxide on animal physiology. Chlorine dioxide, an industrial bleach, gained widespread popularity in Latin America during the COVID-19 pandemic, as several political figures endorsed its use for preventing SARS-CoV-2 infection [50]. The experiment demonstrated the robust performance of our system, accommodating a significantly larger user base than in our previous studies. The system's capability to allow students to access and review prior data served as an effective compensation strategy for any temporary gaps in Internet coverage experienced by users.

Table 6.1, summarizes the first four pilot and user studies with high school and undergraduate students. Which ultimately lead to our largest user study where we want to see how effect remote PBL is in comparison to in person learning.

User Study 5: Remote PBL

Table 6.1: Summary of user studies performed.

Experiment Theme	**Biological Sample**	**Experimental Condition**	**Number of Users**	**Geographical Area**
Effects of caffeine on development	Zebrafish embryos	Caffeine	12	Salinas, CA USA
Effects of agriculture byproducts on development and physiology	Zebrafish embryos	Ammonium nitrate, nicotine and caffeine	20	Salinas, CA USA
Biocompatibility of nanoparticles	Zebrafish embryos	Gold and Graphene Nanoparticles	20	Bolivia, Mexico, Peru, Brazil, and Spain
Toxicity of chlorine dioxide	Zebrafish embryos	Chlorine dioxide	131	Bolivia: Santa Cruz de la Sierra, Cochabamba, La Paz, and El Alto

The first four user studies validated our technological and educational approach. A subsequent study with first-year General Biology students in Bolivia focused on the impact of remote PBL education on attitudes toward the sciences. The students investigated the effects of three drugs on neuroblastoma cell lines, figure 6.2, presenting their findings at a university science fair.

Upon program completion, we conducted a survey to gauge the students' attitudes towards STEM and our remote Project-Based Learning (PBL) initiative. The survey questions were previously employed to evaluate STEM enthusiasm after an in-person biology PBL course with a distinct group of Bolivian students [Ferreira et al., 2019], facilitating comparisons between the remote and in-person PBL courses conducted with these two cohorts.

Our initial focus was on assessing their attitudes towards science, with survey questions outlined in Supplemental Table 1. In responses related to overall enthusiasm for science and interest in science careers, the students universally expressed

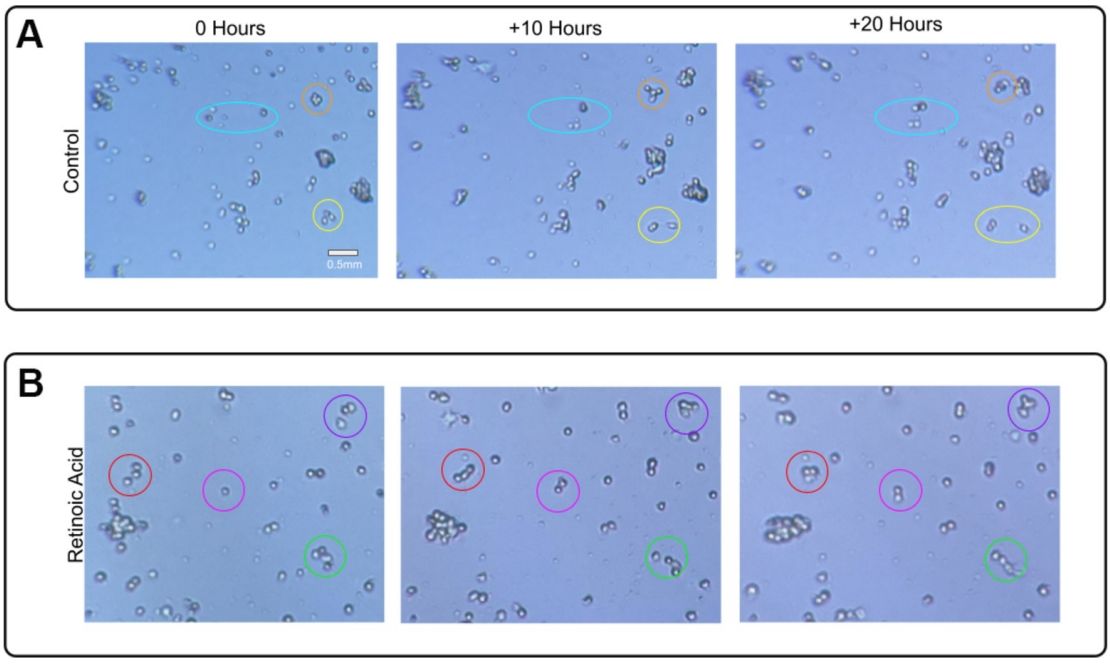


Figure 6.2: Exploring neuroblastoma cells within an educational setting, students utilized IoT-enabled microscopy to investigate the impact of drugs, including retinoic acid, on these cells. Illustrative images depict the monitoring of individual cells and cell clusters over a span of 20 hours. A) Presents an example of control cells at 0, 10, and 20 hours. B) Showcases cells subjected to retinoic acid treatment at 0, 10, and 20 hours.

positivity. However, a noticeable distinction emerged in responses to the question "hard work will help me be successful in science," with 67% of the remote cohort strongly agreeing, as opposed to 90% in the in-person cohort (Table 6.2 and figure 6.3). When probing feelings towards the program, both cohorts exhibited similar responses, uniformly expressing positive sentiments (Table 6.2 and figure 6.3).

> “The imaging viewer was very easy to access and after being taught on how to understand it, it was very easy to look for the data we wanted and find clear pictures to present.”
>
> [California Student]

> “The imaging viewer was very user-friendly. I had no trouble clicking back and forth through pictures.”
>
> [California Student]

> "It was very easy to use the website to access the images."
>
> [Bolivia student, translated from Spanish]

We inquired with the students about their enjoyment of observing a live experiment in near real-time and, if affirmative, the aspects they found appealing. The students predominantly expressed appreciation for the live nature of the experiment:

> "Yes, I did enjoy watching a live experiment in almost real time because it felt like it was in a real lab and I was able to see how it was that an experiment is set up."
>
> [California Student]

> "It was really interesting being able to come back a day later to see how much the cells had changed and making discoveries in real time rather than looking at something that's already been observed in the past."
>
> [California Student]

> "I loved it! It was very interesting to run a long-term project, as we had the opportunity to constantly collect data and progressively get new results that we could see in real time."
>
> [Bolivia student, translated from Spanish]

> "I liked being able to work with samples that are thousands of kilometers away, I feel that it is something that opens the doors for us to have a more entertaining and broader education."
>
> [Bolivia student, translated from Spanish]

The outcomes of this investigation underscore a significant revelation: within cohorts of students exhibiting analogous attitudes towards scientific inquiry, remote Problem-Based Learning (PBL) demonstrates efficacy equivalent to traditional in-person methodologies in nurturing heightened enthusiasm for science. Furthermore, it is noteworthy that participants expressed a palpable sense of enjoyment and fulfillment in their engagement with the remote project.

Table 6.2: Comparison of students' level of agreement with questions and statements used to assess enthusiasm for STEM after in-person and remote PBL courses. In person PBL data is from Ferreira et al., 2019 [1]. Cohort sizes: In person PBL n = 92, Remote PBL n = 18. Mann Whitney test. Comparison of the mean score received on a test related to the scientific method, administered following the remote or in-person PBL course at the Catholic University of Bolivia. Cohort sizes: In-person PBL n = 24, Remote PBL n = 17. Wilcoxon Signed Rank test. All significance tests were two-tailed.

Statement/Question	**Remote Versus In-Person p-value**
Science is exciting.	>0.05
I enjoy participating in science projects.	>0.05
I would like to pursue a career in science.	>0.05
I enjoy solving scientific problems.	>0.05
Hard work will help be successful in science.	0.0186
Do you want to become a scientist?	>0.05
Do you want to keep learning more about science?	>0.05
Did our program increase your interst in science?	>0.05
Would you participate in similar programs?	>0.05

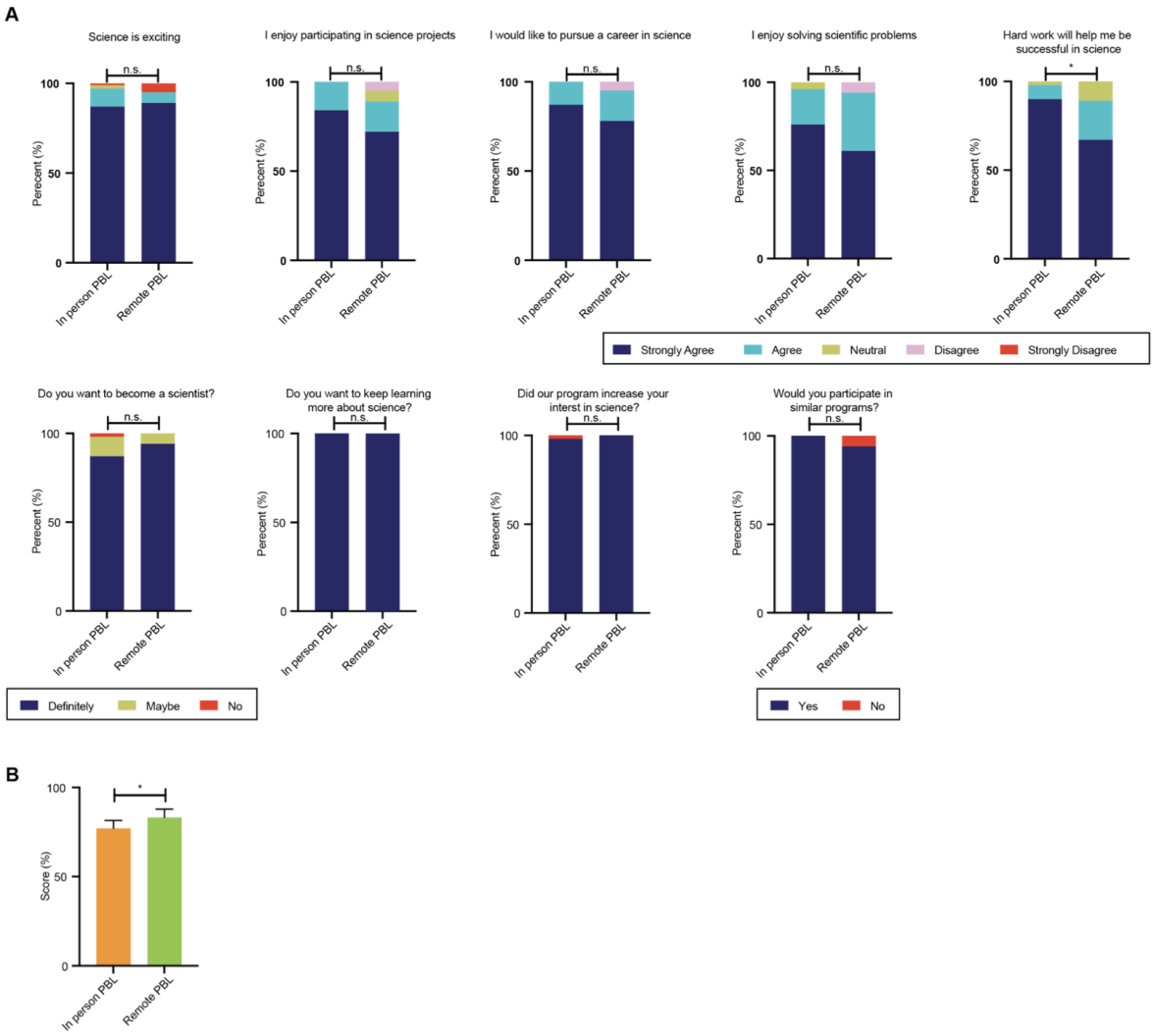


Figure 6.3: **Comparison of students' agreement levels with questions and statements assessing STEM enthusiasm after in-person and remote Project-Based Learning (PBL) courses.** (A) Illustrates student agreement levels with survey questions post in-person and remote PBL courses, with in-person PBL data sourced from Ferreira et al., 2019 [Ferreira et al., 2019]. Cohort sizes: In-person PBL n = 92, Remote PBL n = 18. Mann-Whitney test. * = $p < 0.5$, n.s. = not significant. (B) Depicts the mean score achieved on a scientific method-related test following the remote or in-person PBL course at the Catholic University of Bolivia. Cohort sizes: In-person PBL n = 24, Remote PBL n = 17. Student's t-test. n.s. = not significant. Error bars represent SEM. (D).

In soliciting feedback from students, our aim was to glean insights into their encounters with PBL initiatives, with a specific focus on their interaction with the Picroscope image viewer. The responses received overwhelmingly reflected positivity, indicating a favorable reception to the platform and its functionalities. This attests to the potential for remote learning modalities, particularly those integrating innovative technological tools, to elicit satisfaction and enthusiasm among participants.

6.3 Seru-Otchi: An Interactive Virtual Lab to Enhancing Undergraduate Understanding of 3D Cell Culture Concepts and Protocols

Culturing human stem cells requires many hours in the lab and thorough training in both procedure and safety protocols to properly maintain cell lines for biological experiments.

6.3.1 Introduction

The intricate and technical nature of cell culturing processes presents a formidable barrier to entry for undergraduate students aspiring to engage in research within this domain. Training procedures for entry into laboratories maintaining such cells vary widely across research groups, typically involving the daunting task of digesting complex journal articles, which often prove overwhelming and incomprehensible to novices. While these papers may impart cell culturing protocols, they often overlook crucial aspects such as laboratory safety and interpersonal skills, essential for students to transition into effective laboratory scientists. Each

laboratory group adheres to distinct training methodologies and specific etiquette protocols, complicating the process of bringing new researchers up to speed. Moreover, the shared lab space necessitates adherence to common practices, further complicating the integration process for newcomers.

Notably, the responsibility of initiating undergraduate researchers into laboratory practices often falls upon graduate and postdoctoral students, a task that demands significant time and effort. However, the inherent challenges of mentoring individuals unversed in pedagogy exacerbate this endeavor. Recognizing these obstacles, we endeavored to develop an interactive virtual lab aimed at imparting fundamental cell culture techniques *in vitro*, supplemented by protocol-specific safety measures and guidelines for effective laboratory citizenship. Collaborating closely with a postdoctoral researcher overseeing a laboratory specializing in the growth of human brain organoids, our game focuses on modeling the specific cell growth protocols and organizational techniques employed in this specialized research.

Furthermore, recognizing the myriad knowledge areas essential for success in laboratory environments, including nuanced lab etiquette, we aimed to consolidate these lessons within our game. By combining procedural knowledge and essential etiquette guidelines, we seek to provide students with a comprehensive preparation for laboratory engagement. Through close collaboration with a postdoctoral researcher, our game is tailored to facilitate students' readiness to contribute effectively to ongoing research endeavors within the laboratory. Moreover, our standardized approach to training within the laboratory group eliminates the need for Principal Investigators to validate different training methodologies, ensuring consistency and efficiency in researcher preparation.

6.3.2 Design Principles

Three main design principles were kept in mind during the development of this interactive virtual lab:

- Communicating the relevant information from the papers in an easy to understand.
- Showing the overall process of culturing cells and following a specified protocol.
- Providing additional information about laboratory safety and citizenship.

This interactive virtual lab takes inspiration from Tamagotchi, a virtual pet that lives in a portal device[Hellings et al., 2019], games like "Magic Flowerpot" [Zarraonandia et al., 2019], and StudyGotchi [Hellings et al., 2019]. The goal of the interactive virtual lab is to nurture a living creature as it grows and matures while also being motivated to learn a specified topic. Like with Tamagotchi, human intervention and care are absolutely critical for culturing cells, stem or otherwise. Taking care of cell culture is a hands-on process, and the cell culture growing healthily relies on being consistent with feeding and waste removal. For example, mistakes as simple as missing days of feeding or not checking if the incubator is set to the correct temperature can cause abnormal growth patterns or even kill the cells. The steps in cell culture care are repetitive which is why we rely to spaced-repetition learning convey the concepts set forth in our organoid interactive virtual lab[Schimanke et al., 2017]. Spaced-repetition learning has been shown to be effective as well as incorporating quiz or test questions into the repeated actions of the interactive virtual lab[Kang, 2016].

The interactive virtual lab's overall objective is to teach an experimental procedure while reminding student researchers to be mindful of their actions because

the laboratory is a shared space filled with expensive equipment and hazardous chemicals. In the game, success is measured by keeping the organoid alive for 77 days worth of feeding, which mimics the amount of time that the organoids grown using this protocol take to mature. Players start with an organoid that is freshly generated, or 0 days old, and they are given a schedule that contains a list of substances needed to create the particular cell culturing media (the 'food' for the growing organoid') depending on how old the organoid is. The schedule follows the experimental procedure our postdoctoral laboratory collaborator uses. In addition to the schedule, players have the ability to explore the purposes and effects of the various substances used in the medias by clicking on the name of the substance they want to learn more about, as seen in Figure 6.5. The game centralizes a lot of information to allow students to "ask questions" about reagents without worrying about seeming uniformed about the information before talking to senior laboratory personnel.

6.3.3 Interactive Virtual Lab Mechanics

The primary mode of interaction for players is adding the specified chemicals or reagents to the mixture column, which visually changes to reflect what has been added. Once the proper mixture has been fed, the organoid's age advances by two days since cell culture is typically fed every couple of days in this protocol. If a player makes a mistake and catches it before feeding the organoid, they can empty the mixture they have created to try again.

The interactive virtual lab occurs on the left half of the computer monitor, Figure 6.4. On this screen there is 1 modeled organoid that will "grow" as the user progresses in experimental procedure. This growth is modeled on data from previous experiments. The image of the organoid changes as the days go on,

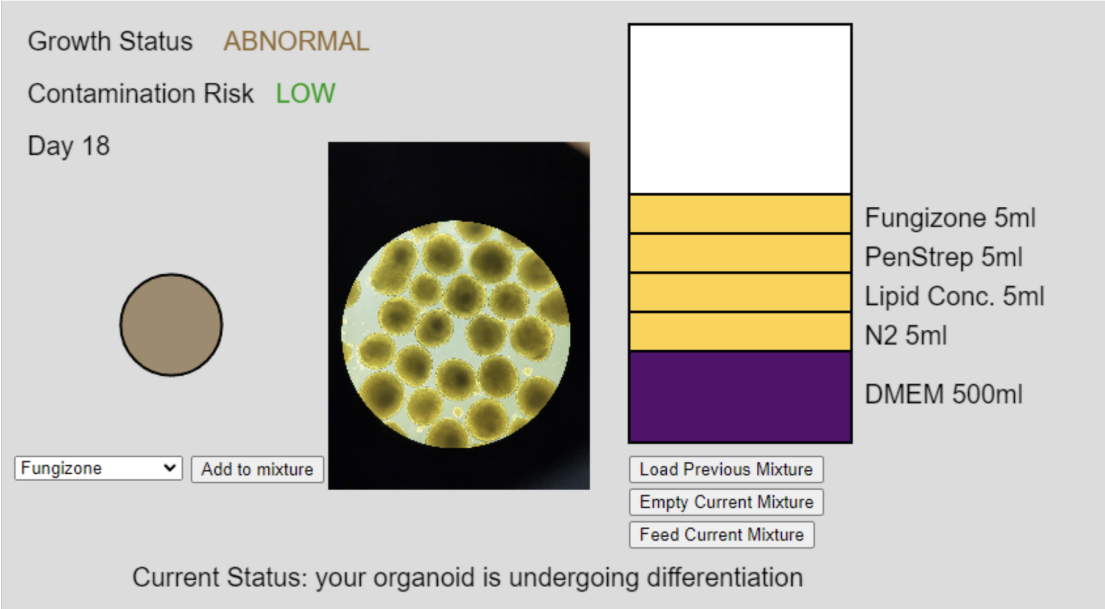


Figure 6.4: A screen capture of an even during the interactive virtual lab where the organoid is undergoing differentiation as well as an example of the mix-ins that the user has added to make the media of their choosing.

and a status message updates the player on what the organoid is typically going through at the current stage of development. The player then has the option to create a new mixture or use the mixture from the previous feeding to give to the organoid, which is typical in a laboratory setting since media is often prepared in advance and reused. This is a small detail, however it accurately reflects a normal laboratory task. As shown in Figure 6.4, there are two metrics used to evaluate how well a player is doing in the interactive virutal laboratory. Shown in the top left, the Growth Status measurement, which can be healthy, abnormal, or dying, is meant to show how well the organoid grows over time. If the media mixture differs from what is prescribed on the schedule, the health of the organoid will be downgraded. If a mistake is made while the Growth Status is Dying, the organoid will die, and the game will need to be restarted. Below Growth Status is the Contamination Risk metric, which measures how sterile the environment the

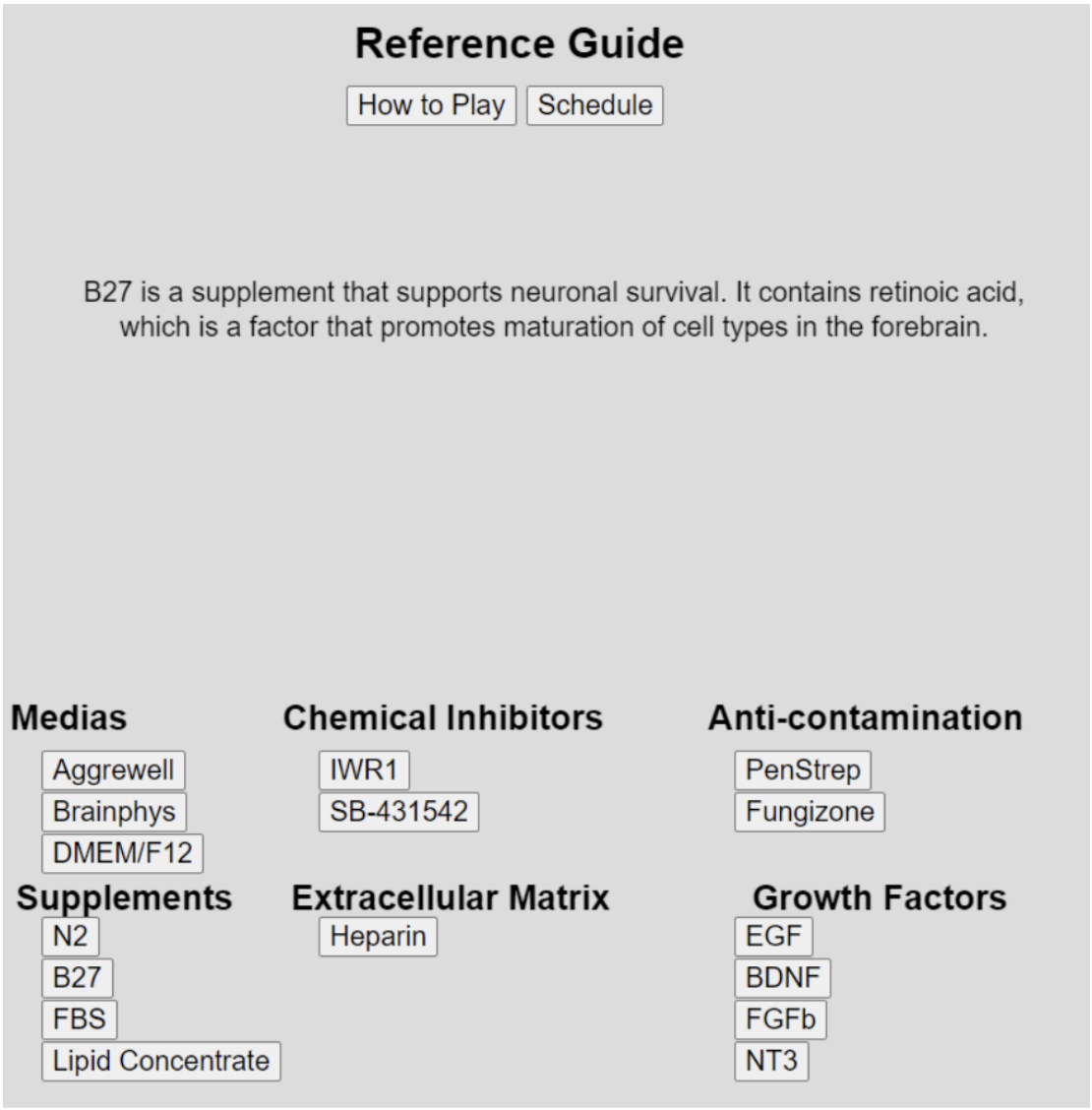


Figure 6.5: An example, if the user clicks on a button to learn about what B27 is and how it is helpful for organoids

organoid is being kept in. The Contamination Risk can be low, high, or extreme, and like with the Growth Status, if the risk increases past extreme, the organoid dies, and the game needs to be restarted. To control the contamination risk, a popup will randomly appear after a feeding asking a question about laboratory safety or laboratory etiquette tailored to our postdoctoral collaborator's group in mind. A correct response will maintain the level of sterility, while an incorrect

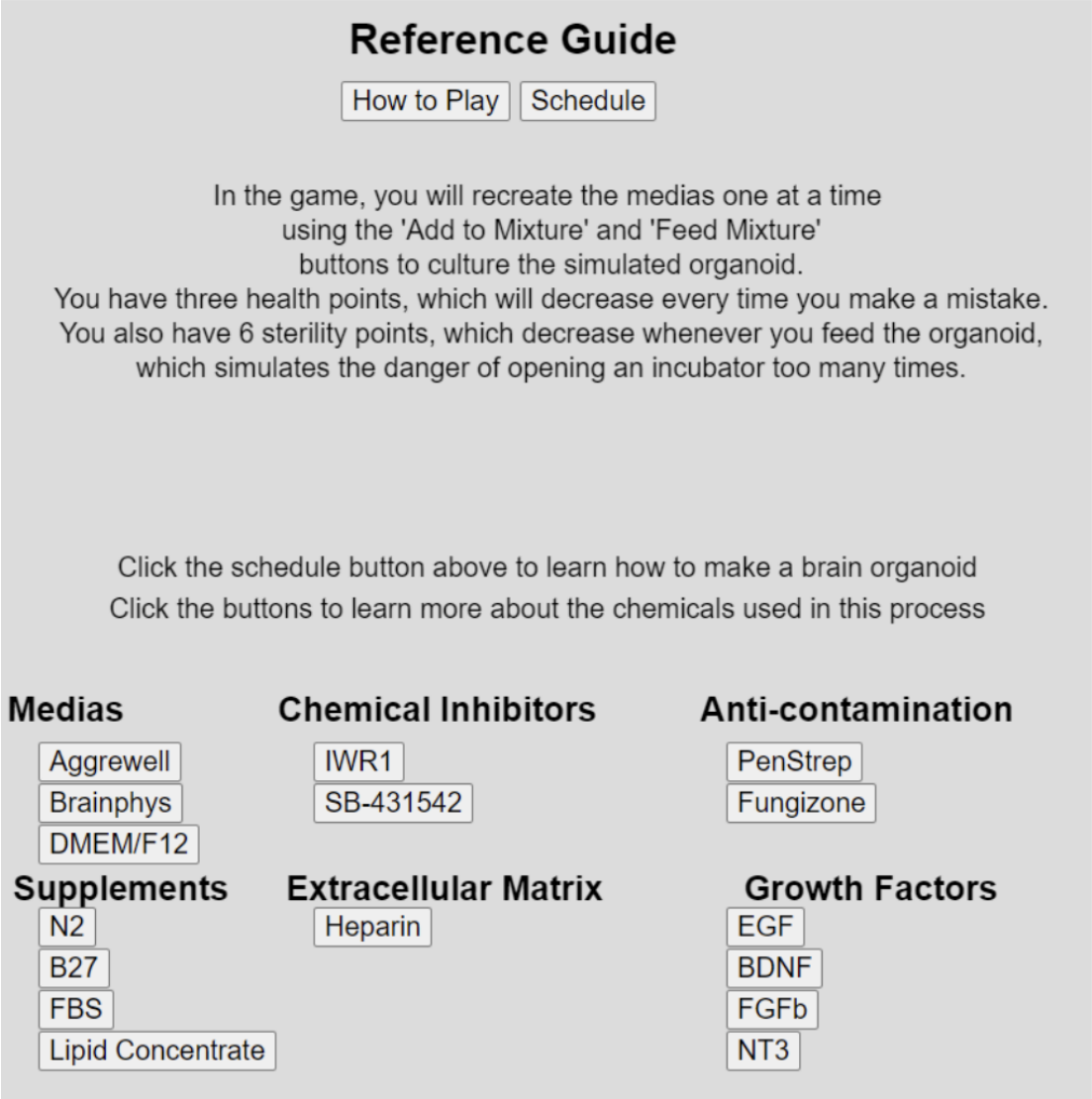


Figure 6.6: This is an interactive window for the user to 1. get instructions on how to interact with this activity, 2, learn what each of the components of the media is as well as have the schedule to follow for each day of the protocol that is prescribed for this learning activity.

response will upgrade the Contamination Risk. These systems are modeled off of experiments captured with devices used in the laboratory to monitor cell health.

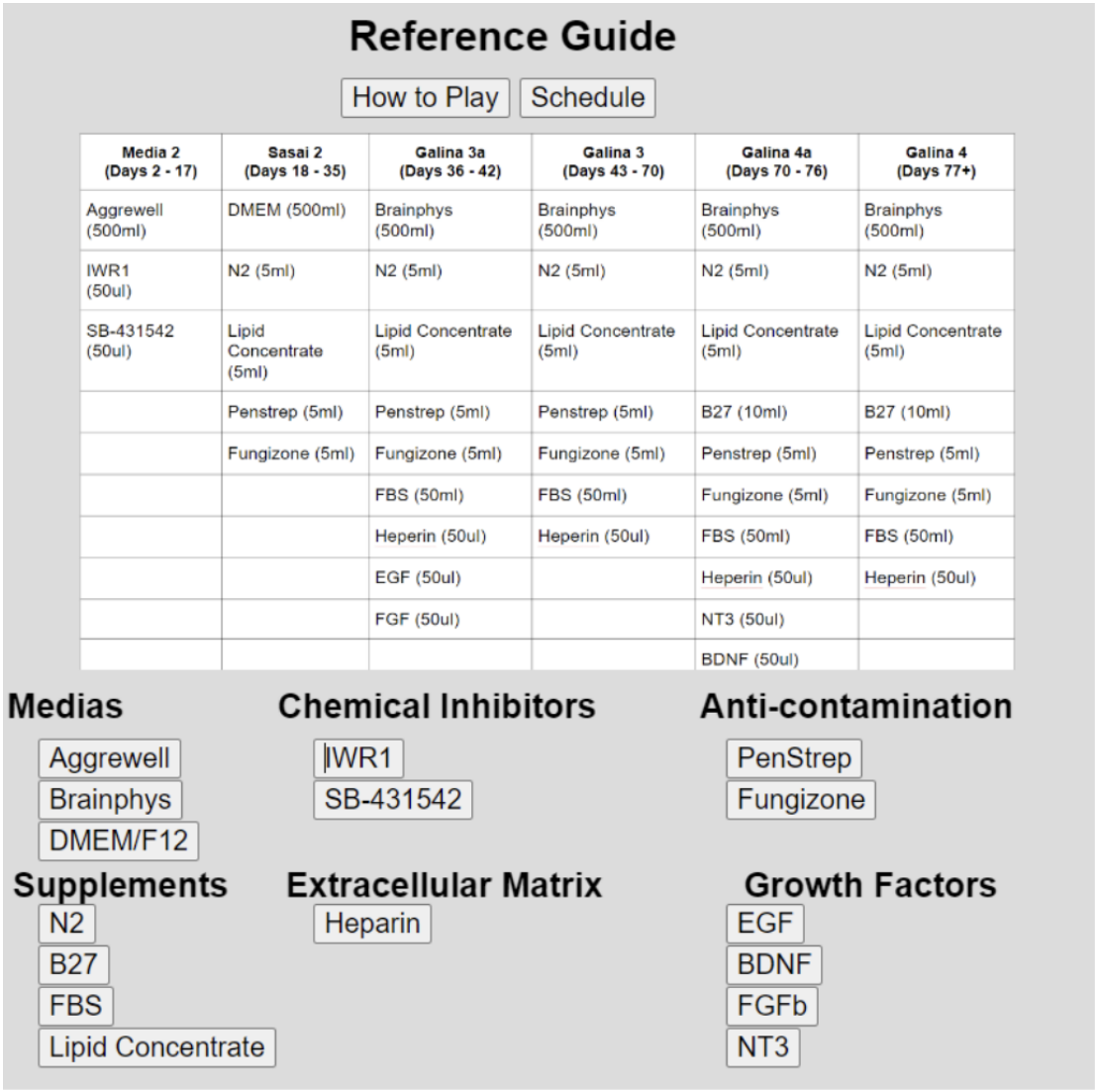


Figure 6.7: The schedule and recipes the user should follow in order to successfully learn the protocol and get an organoid that is 77+ days old

6.3.4 Organoid Growth Model

The organoid growth is modeled after data acquired from our image database from experiments with cortical organoids using the Picroscope [Ly et al., 2021, Baudin et al., 2022a] as well as images provided from a fellow collaborator Ryan Hoffman. The script used to build the organoid growth model was detailed in the previous section, section 5. We chose to base the growth of the organoid in the

interactive virtual lab is based off of real image data to give a sense of how an organoid can look and act while undergoing a real protocol that is used in our laboratory.

6.3.5 User Studies

The objective of this interactive virtual lab is to offer a gentle introduction for students who wish to join a laboratory but are unsure of how to begin. It serves as a supplementary tool intended to aid both student learning and the training of new undergraduate researchers by researchers. To evaluate the effectiveness in easing the transition for students entering a research lab setting, we conducted numerous pilot studies in conjunction with a thorough user study.

Pilot study 1

In this investigation, our participant pool comprised five individuals who do not currently a background in biology, however they are interested in biology. We designed a two-part activity sequence: firstly, participants engaged in reading a scientific paper (Activity 1), followed by completion of a confidence-measuring survey, shown in figure 6.8. Subsequently, participants proceeded to play our supplementary interacive virtual lab and revisit the same paper (Activity 2). Following completion of Activity 2, participants underwent interviews with a biologist, ensuring anonymity. Post-interview, participants filled out a brief survey to gauge changes in confidence levels.

Our hypothesis posited that engagement with our game would lead to enhanced confidence levels among participants regarding organoids and their care, alongside general laboratory etiquette. Feedback from participants regarding the game was overwhelmingly positive, corroborated by increases in confidence levels observed

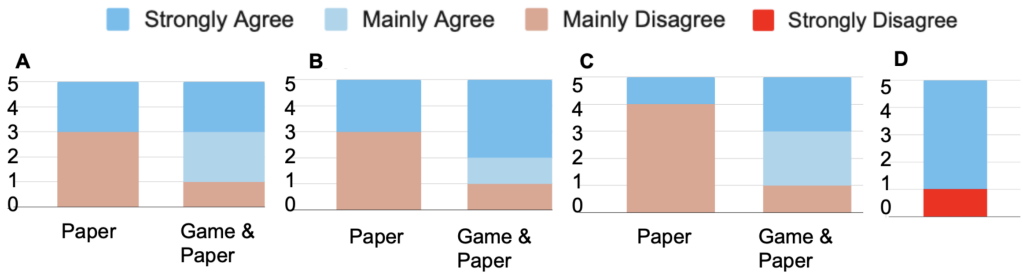


Figure 6.8: Charts measuring the confidence of participants before and after using the interactive virtual laboratory. Responses were rated on the Likert Scale. A. I feel confident in my answers to the interview questions. B. I feel like I have a better understanding of the process of creating organoids. C. I feel comfortable joining a cell culture laboratory after doing both activities. D. The second activity increased my confidence in my answers during the second interview.

in survey responses shown in figure 6.8.

From the findings of this first study, users provided feedback that can be summarized as follows:

- The interactive virtual lab, characterized by its low-stakes environment, was perceived as a valuable tool. It effectively simulated the challenges encountered in laboratory cell culturing, where the delicate nature of cells poses significant difficulties even with advanced laboratory equipment. Experts noted that the interactive virtual laboratory accurately reflected the intricacies of cell culturing procedures, allowing for experimentation and learning from mistakes without real-world consequences.

- Many participants emphasized the utility of a dedicated section within the interactive virtual lab providing explanations of processes and definitions. A substantial portion of the materials utilized in brain organoid culturing involves highly specialized substances not typically covered in undergraduate biology courses. Moreover, these definitions are often absent from scientific papers, which typically target an audience of graduate-level biologists

and beyond. Participants reported feeling more confident and at ease with the presented cell culturing protocols when provided with easily accessible definitions tailored to their level of comprehension.

- Additionally, participants highlighted the benefit of utilizing the training in the interactive virtual lab as a supplementary tool alongside the scientific paper. Papers such as the one utilized in this study are known for their density and reliance on domain-specific terminology, which can be daunting and inaccessible to individuals lacking specialization in the field. One participant, in particular, noted that their ADHD condition made reading the paper challenging, whereas the interactive alternative offered a more accessible and engaging learning experience.

Even though the results were positive for this study, given the limited size of our participant cohort, we opted to conduct an additional user study to validate the observed trends in subsequent survey responses.

Pilot Study 2

In this investigation, our participant cohort consisted of 11 individuals with backgrounds in psychology, concurrently enrolled in a neuroscience course. These undergraduate students were tasked with reading a scholarly paper on organoids, followed by responding to queries assessing their comprehension of the paper's content. Subsequent to their engagement with the paper, participants proceeded to interact with the organoid training game and subsequently completed a survey. This survey mirrored the initial set of questions but additionally incorporated inquiries regarding participants' confidence levels in their comprehension of both the paper's content and the concepts presented within the interactive virtual lab. The study yielded results similar to our previous research endeavors, shown in figure

6.9. However, a notable limitation emerged concerning the absence of qualitative insights elucidating the rationale behind participants' responses to the interactive virtual lab.

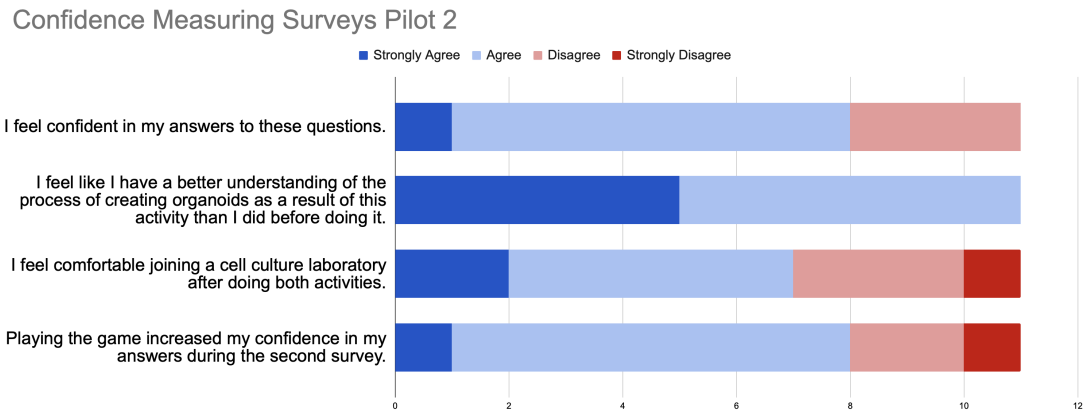


Figure 6.9: Post activity survey results measuring the confidence of the users after reading the paper and playing the second activity.

However, a survey prior to participants engaging with both the interactive virtual lab and the paper was not administered, we aimed to replicate the study with a larger sample size to address this limitation.

User Study

Based on the insights gained from two pilot studies, a more extensive user study was established, involving 29 students from a distinct course section compared to the previous pilot. A different paper was chosen for this study, taking into account feedback received earlier. Surveys were adjusted to offer greater chances for participants to provide qualitative feedback on both the game and their overall experience. Findings from our study encompass both quantitative and qualitative data analysis.

In the conducted studies, primary quantitative data were derived from surveys assessing participants' confidence in understanding the presented material. Ad-

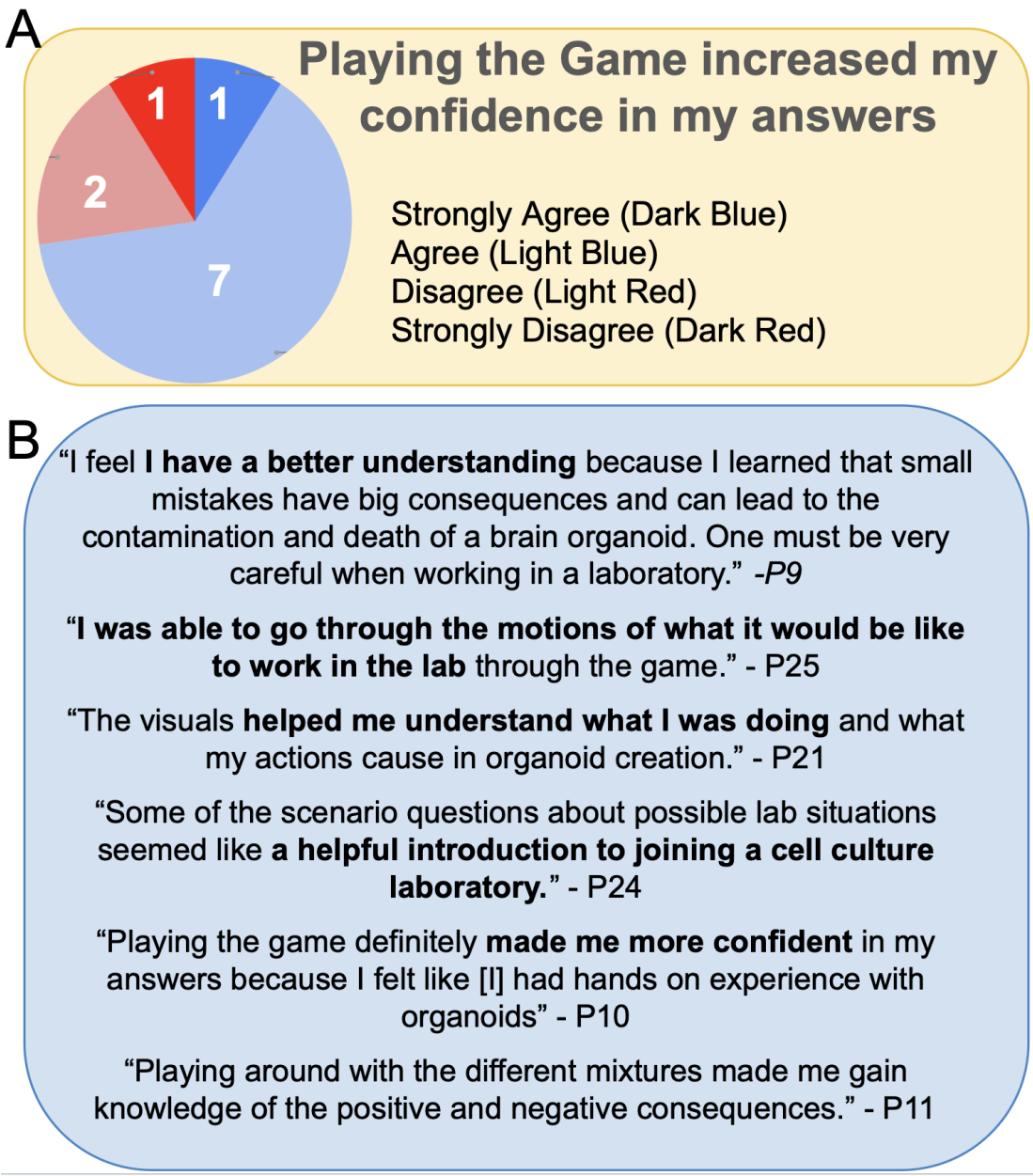


Figure 6.10: Finding 1A: The interactive virtual lab is a low stakes environment for practice and learning. A) Survey of participants from second pilot study (n=11) completed after reading paper and using the interactive virtual alb. B) Selected quotes from participants from user study (n=28) on how they thought the interactive virtual lab was beneficial to them.

ministered via Google Form, these surveys occurred initially after the participants read the selected paper and subsequently after completing both the reading and

Table 6.3: Pre & Post Responses for the statement, "I feel like I have a better understanding of the process of creating organoids as a result of this activity than I did before doing it."

Participant	Paper Only	Paper + interactive virtual lab
Participant 1	Disagree	Strongly Agree
Participant 2	Disagree	Agree
Participant 3	Disagree	Agree
Participant 4	Disagree	Disagree
Participant 5	Disagree	Strongly Agree
Participant 6	Disagree	Agree

the game. The surveys employed a Likert Scale to gauge participants' agreement with statements regarding their confidence in understanding the material, with key findings visually represented in figure 6.10.

A significant observation from the survey results was a discernible increase in participant confidence between the two surveys. This was particularly evident in aspects such as self-assessed understanding of the paper's topics, perceived ability to respond to interview questions, and confidence in joining a laboratory engaged in related research. Heightened confidence during the second survey suggests a positive impact of experiential learning through the interactive virtual lab, although repeated exposure to the material may have also contributed.

6 users that had noted in the pre-survey they disagreed or strongly disagreed with the statement, "I feel like I have a better understanding of the process of creating organoids as a result of this activity than I did before doing it."

While analyzing the changes in responses for the second statement "I feel comfortable joining a cell culture laboratory after reading only the paper" and the statement "I feel comfortable joining a cell culture laboratory after reading the paper and the second activity" participants that answered disagree in the pre-survey also answered disagree in the second survey. In the responses to why the participants choose their response, many said because they lacked the background

and experience of working in a laboratory which made them not comfortable joining a cell culture lab. Which is a valid response, because the users do not have experience in biology, and they have just read one paper and did one supplementary activity.

In summary, while the study's sample size did not allow for statistically significant conclusions, the observed trend in participant responses indicates a positive impact of the experimental intervention. Detailed qualitative feedback from participants, discussed in the following section, further elucidates the perceived value of the intervention in enhancing understanding and confidence.

Qualitative data collected from user feedback highlighted how the training game rendered the material in the paper more comprehensible and demystified associated laboratory methods. Thematic analysis focused on two aspects:

The interactive virtual lab can be described as a Low-Stakes Learning Environment: Participants described the interactive virtual lab as a 'low-stakes arena for practice', facilitating exploration and learning without typical fears associated with making mistakes. The interactive virtual lab helps users follow an organoid procedure, and make errors without real-life consequences. Feedback suggested that novices in cell culturing could indirectly learn about potential lab mistakes. Notably, 30% of respondents expressed interest in joining a laboratory to learn cell culturing after engaging with both the paper and the game. Enhanced Comprehension through Game-Paper Integration: Combining the interactive virtual lab with the paper facilitated a deeper engagement with the material, encouraging the use of scientific terminology. A majority of students reported increased confidence or improved understanding of the material after participating in the second activity. Extracts from post-game surveys detailed how the game aided understanding.

"Prior to this activity, I had no knowledge or experience working with

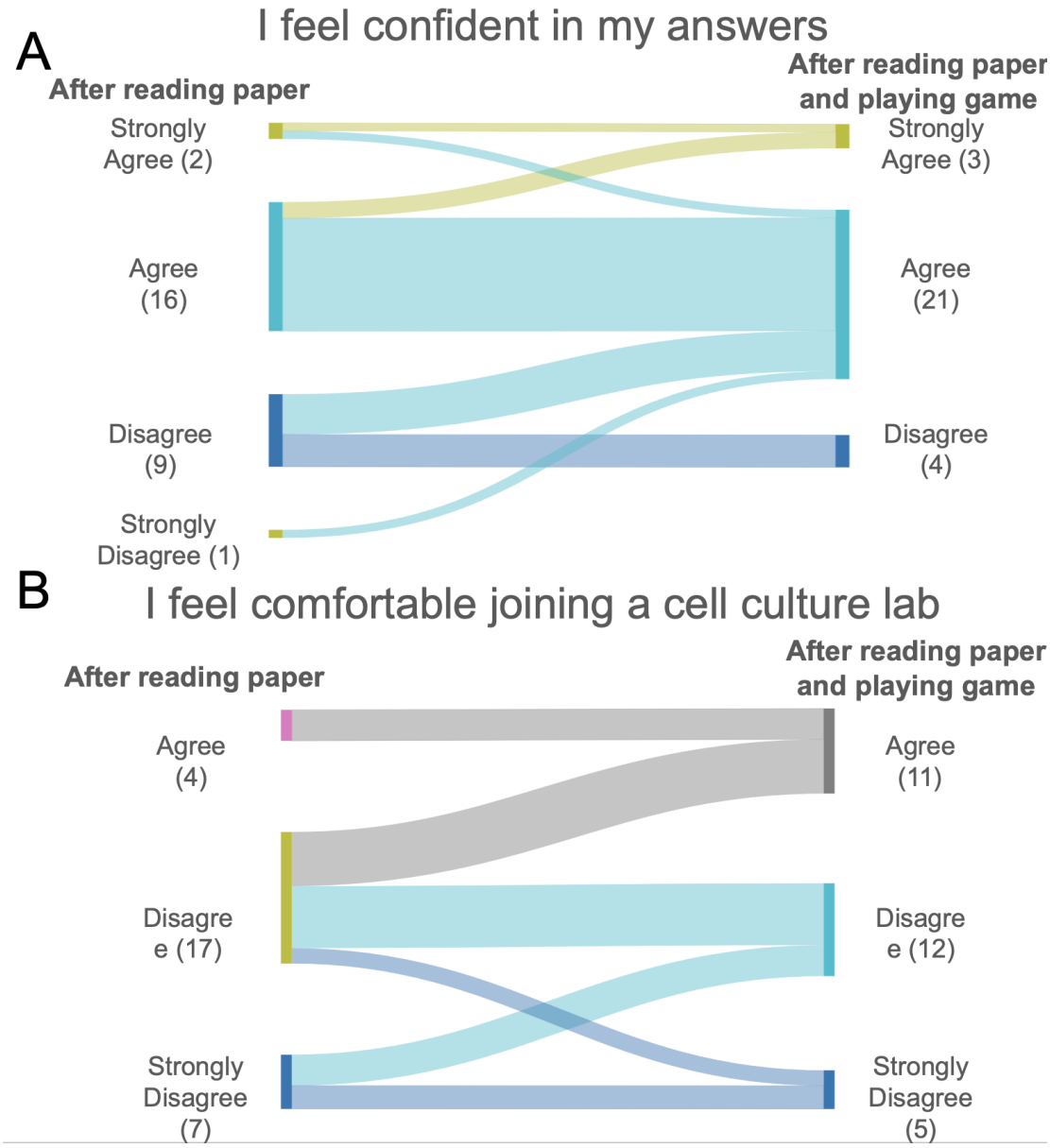


Figure 6.11: Finding 1B: A) Sankey diagram showing changes in participant responses for user study (n=28) on how they rated their confidence in their answers before and after playing the game. B) Sankey diagram showing changes in participant responses for user study (n=28) on how they rated their comfort in joining a cell culture laboratory before and after playing with the interactive virtual lab.

organoids. However, this game had a schedule to follow that was easy to understand, it was rewarding to see how actual organoids looked when growing." -P24

"Having the chance to play the game provides a better understanding then just the reading as you are involved is seeing what your [sic] reading about." -P7

"I feel now that I have done the game and steps were explained to me, it is easier for me to see what the authors were talking about in the paper." -P25

"I think after playing the game I have a more clear visual understanding of what organoids are." -P21

"Following my experience with the game, I believe that I understand the process of creating organoids even more than I did prior to the game." -P14

"The game helped me visual[ize] what I couldn't understand in the article." -P8

Enhancing Accessibility in Scientific Education

Mastering the skill of engaging with domain-specific literature is crucial for undergraduate researchers. However, the inherently complex nature of these texts often leads to reduced accessibility for novice readers. Educational games, like the one, can interact with is a pivotal role in democratizing scientific research, providing accessible learning tools to grasp foundational concepts. This interactive virtual alb offer a holistic view of working in a laboratory, incorporating elements like lab safety and ethical practices, enhancing overall preparedness for students' initial forays into research laboratories.

6.4 Conclusion

In our comprehensive endeavor spanning various regions including the United States, Bolivia, Brazil, Spain, Colombia, and Mexico, we integrated IoT-enabled

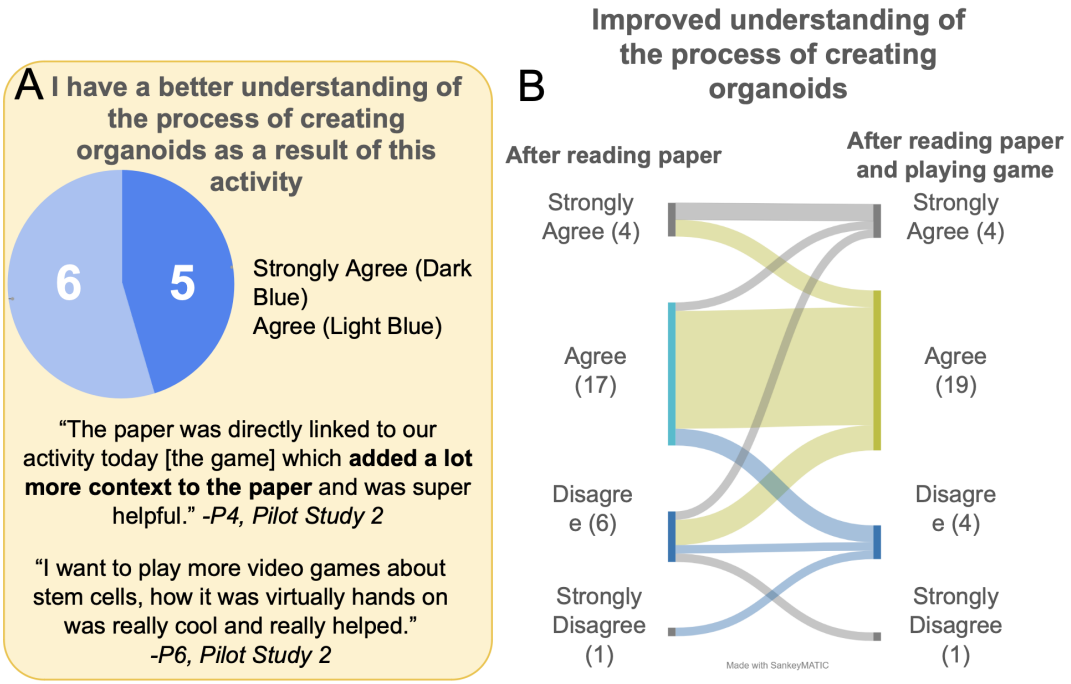


Figure 6.12: Finding 2: Having the training game as a supplement to the paper made the concepts and methods presented in the paper easier to understand. A) Survey of participants from second pilot study (n=11) completed after reading paper and playing game with selected quotes from participant responses (emphasis ours). B) Sankey diagram showing changes in participant responses for user study (n=28) on how they rated their understanding of the process of creating organoids before and after playing the game.

microscopy to enhance high school and college biology courses. Our adaptable framework, facilitated by Problem-Based Learning (PBL) and offered in multiple languages, catered to diverse student populations and facilitated engagement in real-world projects encompassing topics such as caffeine consumption, agricultural chemical exposure, and pharmacology.

A distinctive feature of our remote PBL approach is its capacity to enable students to conduct experiments involving materials typically deemed hazardous or logistically challenging to access in remote settings. For instance, while advanced placement (AP) and college biology curricula cover mammalian cellular biology, practical experiments in these areas have traditionally been constrained, primarily focusing on microbiology or plant biology. Access to experimental mammalian cell and tissue culture techniques typically occurs at the upper division college level.

Furthermore, our research introduces an innovative educational tool in the form of a cell culture training game, designed to enhance understanding and proficiency in maintaining in vitro brain organoids. This interactive platform translates complex scientific concepts and protocols into an engaging format, complemented by authentic images of cortical organoids to underscore the importance of proper care and safety measures—an aspect often overlooked in traditional scientific literature.

The study outcomes underscore the efficacy of interactive learning tools, even those with modest visuals, in augmenting comprehension of scientific content and bolstering confidence in mastering intricate subject matter. This approach not only deepens understanding but also broadens access to scientific knowledge, thereby making the initial forays into scientific research more accessible to a wider audience. In conclusion, our cell culture training game represents a compelling illustration of the potential of interactive learning tools to complement traditional

educational methods, thereby fostering inclusivity and efficacy in scientific education.

Chapter 7

Conclusion

7.1 Summary

The development of the Picroscope stemmed from trying to build a tool to help researchers save time manually imaging and provide an automated way to collect longitudinal imaging for extended amounts of time at a low-cost.

Remote learning applications came out when the challenges posed by the Covid-19 pandemic, which reshaped our work landscape and prompted the need for innovative solutions to overcome access limitations for students.

Through the development of a cost-effective multi-well imaging device and an open-source pump and stimulation system, we have endeavored to streamline the process of cell imaging, thereby enhancing efficiency for biologists and lab technicians. By leveraging off-the-shelf components and internet connectivity, our proposed solution holds promise in expanding the scope of experiments conducted in biological labs and enabling longitudinal cell studies with minimal time investment.

Moreover, beyond its immediate benefits for researchers, our internet-connected imaging system stands poised to revolutionize biology education. Through the

implementation of remote project-based learning activities and engaging serious games, we have demonstrated the potential to make complex topics more accessible to students, thereby fostering a smoother transition into the intricacies of joining a laboratory and learning about one organoid protocol for new scientists. Our cell culture training game exemplifies the potential of interactive learning tools in augmenting traditional methods of scientific education, underscoring their value in fostering a more inclusive and effective learning environment.

In essence, this thesis represents a step forward in democratizing access to microscopy resources, fostering collaboration and innovation, and inspiring the next generation of biologists. As we continue to explore the intersections of technology and education in the biological sciences, the potential for transformative impact remains boundless, promising a future where scientific discovery is within reach for all.

7.2 Future Work

There are many avenues on which we can improve on the Picroscope, size algorithm as well as the interactive virtual lab approach to enhancing undergraduate understanding of 3D cell culture concepts and protocols can be expanded to other biological protocols and laboratory safety protocols.

The Picroscope could benefit from more feedback and communication between other opensource devices, like the microfluidics system. It could be helpful to create a more useful UI for biologists to use.

We were unable to add florescence microscopy into the current design of the Picroscope, due to the size constraint and the current imaging sensor, however we can build a modified model with fewer cameras and provide a better camera sensors in order to try to build a modified Picroscope that is capable of florescence

microscopy.

Our interactive virtual lab approach, Seru-Otchi, designed to enhance undergraduate comprehension of 3D cell culture concepts and protocols, possesses the adaptability to be extended and customized for various other cell culture protocols and standard laboratory practices across different research laboratories.

Future developments based on this study could proceed in three directions:

Firstly, by adding challenges and unpredictable scenarios we can modify the interactive virtual lab and add a gamified learning component. For example, if we add some critical thinking questions, like "what should you do if we run out of a specific reagent, what could be a replacement?"

Another question could be, "if the electricity goes down, how would you recover the experiment?" By providing unexpected challenges and the interactive virtual lab becomes gamified by incorporating challenges for the user.

Secondly, updating the survey questions, particularly ”I feel comfortable joining a cell culture laboratory" to a different question would help improve the survey data, due to the user study pool, users without any biology experience do not really change their answer from the pre and post survey if they already marked that they do no have any experience in biology. Also conducting the study with a significantly larger sample size would enable the application of statistical tests with confidence measures. This would strengthen the claims regarding the interactive virtual lab's impact and help identify which student populations benefit most from serious games in STEM education. One intriguing avenue, suggested by a participant with ADHD, is to investigate the effectiveness of this approach for neurodivergent students.

Lastly, continuous development of the interactive virtual lab, including improvements to visuals and interface, as well as broadening the content to cover

areas such as neural development and metabolism, would enhance its educational value. The game's adaptable design lends itself to customization for various cell culturing protocols, presenting an opportunity to evaluate its applicability and efficacy in diverse scientific disciplines.

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