





HARDWARE PROTOTYPING AND INSTRUMENT DESIGN OF RASPBERRY PI-BASED MICROSCOPY FOR ELECTRONICS AND 3D PRINT INSPECTION

O.P.S. (Olivier) Donker

BSC ASSIGNMENT

Committee: dr. ir. J. Canyelles Pericàs dr. ir. E. Dertien

July, 2024

040RaM2024 **Robotics and Mechatronics** EEMCS University of Twente P.O. Box 217 7500 AE Enschede The Netherlands





UNIVERSITY | DIGITAL SOCIETY OF TWENTE. INSTITUTE

Abstract

Microscopy, especially the kind that uses specialised imaging techniques, is commonly an expensive endeavour, making its accessibility problematic. This motivates the development of an open-source microscope that incorporates open-source technologies like Arduino, 3D printing, and Raspberry Pi to reduce this cost and barrier to entry. Additionally, using such technologies yields significant freedom for the end user to customise designs and empowers them to facilitate their own needs. The Creative Technology design cycle was used to guide the design of microscopy hardware and evaluate it with an end user, resulting in a fully functional prototype for the inspection of 3D prints and electronic boards.

Contents

1	Introduction											
2	Background	5										
	2.1 Microscopy hardware	5										
	2.2 RTI	15										
	2.3 Conclusion	16										
	2.4 Discussion	16										
3	Design methodology	17										
	3.1 Ideation	17										
	3.2 Specification	18										
	3.3 Realisation	18										
	3.4 Evaluation	18										
	3.5 Execution	18										
4	Concept generation	19										
	4.1 Alternatives	21										
5	Specification & Realisation	22										
	5.1 XYZ-stage	23										
	5.2 Arm	24										
	5.3 RTI	25										
	5.4 Integration	26										
6	Evaluation											
7	Conclusion	27										
	7.1 Future work	30										
A	Interview questions	33										
в	End user evaluation form	34										
С	B End user evaluation formC Client evaluation form											

1 Introduction

Microscopy is truly one of the cornerstones of modern science: What we cannot observe, we cannot reason about or manipulate, after all. Antonie van Leeuwenhoek's pioneering work in microscopy directly led to the development of microbiology [1], allowing humanity to become more educated on the foundations of life itself. Today, microscopes are commonly used in educational contexts such as schools and universities, where they are especially indispensable in the life sciences. These devices come at a cost, however, quickly ranging from hundreds to thousands of EUR[2]. Cheaper microscopes exist, but these tend not to allow for imaging techniques beyond simple bright-field microscopy, and are closer to toys than scientific instruments in nature^[3]. In tandem with this last concern, a client in the Robotics and Mechatronics (RaM) laboratory of the University of Twente has a need for a Raspberry-Pi-based microscope using open-source hardware to inspect 3D prints and electronics. This client organised the project and supervised the work. The end user of the aforementioned device shall be a person working in the RaM lab, who has a specific desire to inspect 3D prints or electronic boards. By developing an open-source microscope for this client, accessibility to science due to the cost of microscopy could be improved, and a contribution to the open-source community could be made. A final stakeholder in this project is fellow student Matei Obrocea, who shall be responsible for the software of the microscope.

Undertaking the task above is no trivial feat, as it involves several challenges. These challenges include

- Performing a literature review that outlines the state of the art in open-source microscopy.
- Designing functional, modular microscope hardware.
- End user feedback collection for design specifications.
- Ensuring clear, stable optics with adequate magnification and other optical operations.
- Implementing a proper focus mechanism for the optics.
- Controlling the X-Y position of the sample relative to the optics with PID control.
- Ensuring proper data acquisition from the camera module to an input into software on the Raspberry Pi.

and can be distilled into a set of three research questions:

- 1. Can a high-performing microscope with open-source electronics be implemented?
- 2. What specific microscopy needs does the end user have?
- **3**. How can an easy-to-use microscope addressing these needs be designed and built for the Raspberry Pi and its camera module?

Attempting to answer these research questions yields an organised, focused approach to developing the microscope.

2 Background

Designing microscopy hardware is a challenging task. One needs to take into account myriad technical requirements and combine them all into a reliable device that is user-friendly. Pitfalls may be easily overlooked in the design of such a complicated machine, making it essential to be well-informed about what "good design" entails before embarking. This quest for knowledge also provides an overview of what has already been done in the past, putting tremendous amounts of trial-and-error on our side and preventing reinvention of that which already exists, thereby clearing the way for innovation. Additionally, an early encounter with the supervisor of the overarching graduation project - to which this literature review belongs - revealed a need to use a specific imaging technique named *Reflectance Transformation Imaging* (RTI) to image surface details in a 3D printed membrane. An understanding of the working of RTI and technical details of current implementations of it should be gained for this purpose. Literature review is an efficient way to address the above, from which the aim is to discover the answer to a series of particular questions.

- 1. What is the current state of the art in Raspberry Pi microscopy?
 - (a) What lighting systems are used in the state of the art of Raspberry Pi microscopy?
 - (b) What optical systems are used in the state of the art of Raspberry Pi microscopy?
- 2. How can modularity be introduced into microscopy design?
- 3. How can Reflectance Transformation Imaging (RTI) be used in microscopy?

The first section of this review will concern itself with answering questions 1 to 3, while the second section shall discuss question 4. Finally, a conclusion to briefly sum up the most relevant findings will be presented, along with a discussion of the review.

2.1 Microscopy hardware

At the heart of every microscope lies a lens, and no lens is perfect: in 2020, Bowman et al.[4] showed that when its stock lens is replaced¹, the Raspberry Pi camera module suffers from *vignetting*, a phenomenon wherein the saturation of colour diminishes towards the edge of the image, and *colour crosstalk*, where multiple filtered lightrays may activate the same light sensor. Concretely, this means an image that is darker and distorted in colour towards its edges. To counteract this, the authors propose a calibration setup that can easily be replicated with 3D prints in order to obtain calibration data to correct the image using two software solutions of their invention. One of these software solutions works in real-time, while the other features post-processing of images[4].

The problem above could well turn out to be significant for the quality of images obtained from the microscope, but is less crucial to early prototypes. To produce these prototypes, let us now consider an overview of the state of the art. Deglint et al.[5] designed a compact, low-cost multispectral microscope² in 2016 that features a stack of 3D printed parts that fit together. Interestingly, the lens and camera of the microscope inspect the sample from the bottom, which might make the design more stable due to a low centre of mass. The

¹This is relevant for this project, as the stock lens has no magnification and is thus not suited for microscopy, meaning an off-the-shelf lens with magnification will be used.

²That is to say, a microscope that can detect multiple intervals in wavelength of light.

design has some modularity in the sense that a certain component can be used to house optional additional electronics, and that all components except two have the same hardware "connector" to each other.

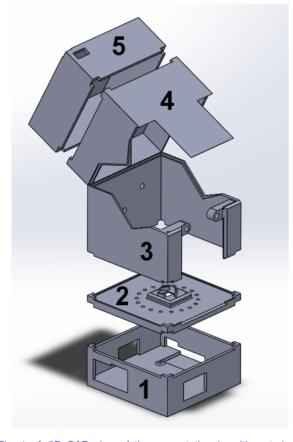


Fig. 1: A 3D CAD view of the computational multispectral microscopy system with five components. The Raspberry Pi computer is housed in component 1, which is the base of the microscope chassis while the camera module and lens is housed in component 2. Component 3 is used to move the microscope sample vertically to ensure the specimen being imaged is in focus. Finally, component 4 is used to house additional electronics.

Figure 1: Exploded-view image of the 2016 Deglint et al. microscope[5] with description of parts.

More use of modularity presents itself with Nuñez and Matute et al. (2017)[6], who produced a fully modular 3D printed design. Different kinds of lenses and light filters can be swapped in and out, provided they fit within the detachable camera holder, and the focus of the lens is adjusted manually by turning a gear. The overall distance between the camera and the sample can be changed using a simple friction mechanism consisting of a handle that screws in and out, which gives some (likely crude) control over magnification. When in use, a front panel is attached to block ambient light.

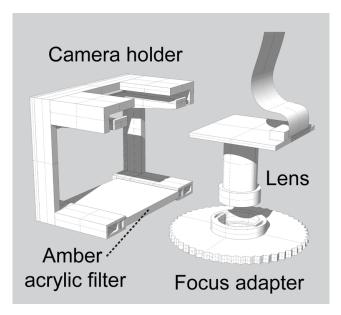


Figure 2: Close-up view of the camera holder and lens focusing system in the Nuñez and Matute et al.[6] microscope.

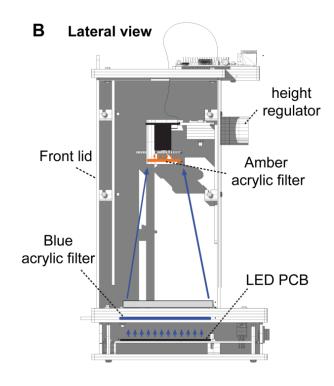


Figure 3: Lateral view of the Nuñez and Matute et al.[6] microscope design.

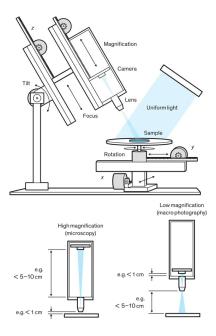


Figure 4: Low-detail overview of the Temiz microscope[7]. Note the mechanism to change magnifications.

Briefly moving away from only 3D prints, in 2020 Temiz[7] produced a design consisting largely of LEGO, allowing for very significant customisation and expansion by the end user without any fabrication equipment. Immediately obvious is the lack of any sort of enclosure, meaning ambient light is not blocked at all. The design features several linear actuators driven by electric motors, and uses one of these actuators to change the magnification of the microscope by changing the distance between the light sensor and the lens.

In an effort adjacent to conventional microscopy, Vernon et al. (2022)[8] constructed a setup to monitor and control the position of droplets on a Surface Acoustic Wave (SAW) device. The hardware surrounding the Raspberry Pi and its camera module is basic in this design, consisting of a rod the camera is mounted to. Vernon et al. provide a diagram of the control scheme used, which may prove valuable as inspiration for the overarching project. Additionally, fluorescent measurements were demonstrated using this setup. Also on the side of unconventionality is a design by Wang et al. (2018)[9], who produced a highly compact microscope designed to be placed inside cell incubators to wirelessly monitor growth. Its simplicity is remarkable, and it features a dual lens setup to magnify the object and bring the focal plane to the light sensor. However, no consideration has been put into modularity.

A simple improvement to a standard lab microscope was presented by Steiner and Rooney[10] in 2021. The group developed a 3D-printable stage that allows for automatic movement of samples in a a conventional, commercial microscope. The design uses two servos that drive linear actuators to facilitate X,Y movement of the sample, similar to the aforementioned LEGO design by Temiz[7]. Although modularity is not explicitly mentioned, the "arm" that holds the sample seems to be fairly straightforward to swap out to hold samples of different sizes.

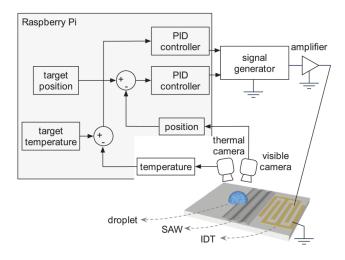


Figure 5: Control scheme used in the Vernon et al.[8] opto-acoustofluidic system.

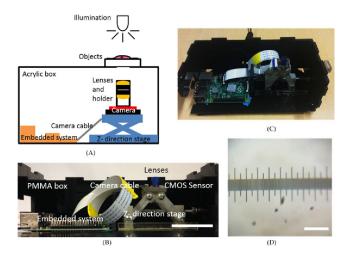


Figure 6: Lateral- (top right) and schematic (top left) views of the Wang et al. microscope[9].

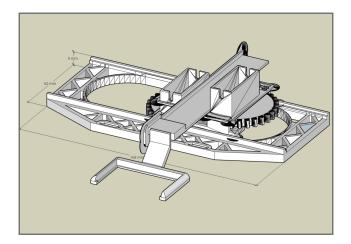


Figure 7: CAD model of the Steiner and Rooney microscope stage[10].



Figure 8: Labelled CAD model of the OpenFlexure design by Collins et al.[11].

The Raspberry Pi is linked to open-source projects: Collins et al.[11] provided a very significant contribution to open source microscopy in 2020: The *OpenFlexure microscope*. The design is highly modular, even allowing for several different microscopy techniques, and is easily configurable for automated imaging. Additional features like autofocus are also discussed. There exists another design in the OpenFlexure line: The *OpenFlexure Delta stage* by McDermott et al. (2022)[12]. It is similar to the OpenFlexure microscope decribed above, except that its optics module is utterly stationary, allowing for the implementation of even more microscopy methods than the original.

Onwards in this "microscopy platform" line of thinking, we also encounter UC2, opensourced by Diederich et al.[13] in 2020. Another highly modular system presents itself here, this time incorporating each functional part of the microscope into identical cubes that lock together magnetically. The customisability is truly unmatched, with a proposed workflow of imagining functionality, then building the microscope according to it.

In a more specific case, Watanabe et al. (2020)[14] implemented Raspberry Pi microsocopy using its camera module, standard laboratory hardware, and a square LED matrix to



Figure 9: A 3D printed OpenFlexure Delta stage by McDermott et al. [12].

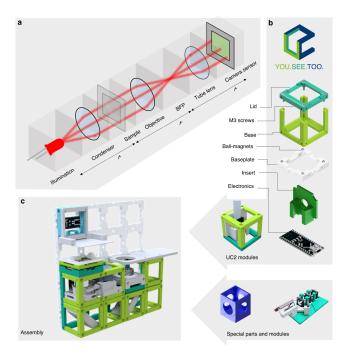


Figure 10: Example of manufacture of a microscope using UC2 by Diederich et al.[13].

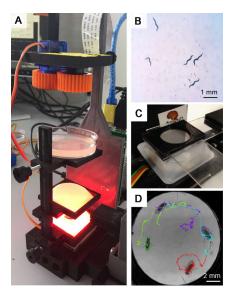


Figure 11: Example setup of the Maia Chagas et al.[15] microscope used for insect tracking.

show that different lighting patterns on said matrix allowed for different imaging methods. In particular differential phase-contrast imaging³ was shown to be possible by subtracting images taken under symmetrically different lighting conditions.

Finally, in 2017, Maia Chagas et al.[15] produced another modular, open-source microscope using Raspberry Pi and its camera module, basing the design around a vertical rod that a series of trays can be mounted to. An interesting feature is that the camera module can be mounted either above *or* under the sample, allowing for a choice in magnification and potential easy access to the top of the sample.

In order to gain a more obvious overview of the information gained above and expand on it, tables 1 and 2 were drafted to compare several aspects of the designs mentioned thus far. Something that becomes immediately clear from this overview is the prevalence of components that move either the sample or the optics of the microscope. It is also obvious that having optics either directly above or below the sample is common, likely due to the simplicity of these two setups for the holding of the sample. Another noteworthy point is the lack of mention of magnification values in the designs consider in this review, explaining its absence as a column in table 2.

 $^{^{3}}$ An imaging technique wherein differences in the refractive indices of materials are used to identify structures in a sample.

Author	Lighting setup	LED voltage	LED control	Sample movement			
Deglint et al. $(2016)[5]$	Directly above sample	Not specified	Not specified	Not specified			
Nuñez and Matute et al. (2017)[6]	Array of 100 470nm LEDs (5mm) below sample, with light filters.	12-24V	Custom PCB with transistor.	Optics mod- ule moves in z-direction.			
Temiz (2020)[7]	Tiltable back- light module	3V	None	XY-stage with linear actua- tors, sample can rotate. Optics can also tilt.			
Vernon et al. (2022)[8]	Ambient light/angled backlight with LED array or single LED. Fluorescent measurements.	4V (single LED) or 5V (array).	None	XY-stage using acoustic waves.			
Wang et al. (2018)[9]	LED array directly above sample	5V	None	Z-stage under optics module.			
Steiner and Rooney[10]	Not applicable	Not applicable	Not applicable	XY-stage with linear actuators.			
Collins et al.[11]	Directly above sample or be- hind optics (re- flection mode)	3.2V	None	Optics module moves on XYZ- stage.			
McDermott et al. (2022)[12]	Directly above sample, behind optics (reflec- tion mode) or array directly above.	3.2V	None	XYZ-stage			
Diederich et al. (2022)[13]	Highly config- urable	Various	Various	Various			
Watanabe et al. (2020)[14]	UNICORNHAT LED array be- hind sample horizontally	5V	Individual con- trol of LEDs built into array.	Manual using standard lab equipment.			
Maia Chagas et al. (2017)[15]	LED ring in diffuser module under sample	5V	Individual con- trol of LEDs built into ring.	None			

Table 1: Comparison of different aspects of microscope designs regarding lighting and sample movement. A PCB is a Printed Circuit Board, and the Z-direction is commonly "up and down". The X- and Y-directions are lef/right and forwards/backwards, respectively.

Author	Optics configuration	Focus mechanism							
Deglint et al. $(2016)[5]$	Directly below sample	Sample moves in z-direction.							
Nuñez and Matute et al.	Directly above sample	Manual focus with a 3D-							
(2017)[6]		printed ring							
Temiz $(2020)[7]$	At a variable angle above	Linear actuator moves optics							
	sample	in direction of sample.							
Vernon et al. $(2022)[8]$	Directly above sample	Manual movement of optics							
		in z-direction							
Wang et al. $(2018)[9]$	Directly below sample	Scissor mechanism moves op-							
		tics in z-direction							
Steiner and Rooney[10]	Not applicable	Not applicable							
Collins et al.[11]	Directly below sample	Flexure mechanism moves							
		optics in z-direction.							
McDermott et al. (2022)	Directly below sample	Flexure mechanism moves							
		sample in z-direction.[12]							
Diederich et al. $(2022)[13]$	Various	Various, based on distance							
		between optics and sample.							
Watanabe et al. $(2020)[14]$	In front of sample horizon-	Manually set distance be-							
	tally	tween optics and sample.							
Maia Chagas et al. $(2017)[15]$	Directly above sample or di-	Automated focus using a gear							
	rectly below sample	ring.							

Table 2: Comparison of different aspects of microscope designs regarding optics. A PCB is a Printed Circuit Board, and z-direction is commonly "up and down".

2.2 RTI

As mentioned in the introduction, early conversations with the end user of the microscope revealed a need for the inclusion of *Reflectance Transformation Imaging* (RTI), an imaging technique that uses lighting from different angles to gain more information about the sample. Specifically, subtle surface textures can be revealed [16]. In 2020, Hughes-Hallett et al. [16] presented a design for *micro-RTI* - RTI for physically small samples, that is - that features a dome with LEDs that can be addressed individually. The optics of the microscope insert through the top of the dome, creating a space inside that allows for illumination of the sample from different angles by selecting an LED to turn on. An image is captured for every illumination, following which software is used to combine all images into an interactive 2D view of the sample that allows the user to drag the mouse to highlight subtle details in the surface. The Rijksmuseum in Amsterdam, The Netherlands has exactly this device (shown in figure 12), and a session with a technician there revealed an interesting limitation: The small size of the dome may lead to discolouration in the lighting of the sample, perhaps due to the yellow lenses on the LEDs. It also became clear that the Rijksmuseum uses RTI for qualitative inspection of surfaces. This session, and the trip to the Rijksmuseum in order to have it, were valuable field work in the investigation of the state of the art, and provided a rich, direct experience of what RTI is used for in practice. Giachietti et al. (2018)[17] took a different approach in using a DSLR camera with a handheld light source to get different illumination angles. Black, glossy spheres were used for calibration of the system.



Figure 12: RTI device at the Rijksmuseum in Amsterdam.

In the bigger picture, in 2011 Earl et al. [18] produced a small overview of different methods to achieve RTI: *Lighting rings* and *highlight systems*. The former includes systems like the dome from above: Any kind of setup with multiple lights around the sample falls into this category. Complete spheres around the sample, arcs, and arms with lights on them

were also mentioned. The latter works on a similar principle of differing lighting conditions, but uses changes in highlights on a black sphere close to the object to perform the imaging in software.

More directly in line with the overarching project, Wilk et al. (2024)[19] used a Raspberry Pi to conduct automated RTI, with the Pi controlling lighting, the camera, and processing of images. The lighting system is based on specially designed integrated circuits to address LEDs individually, and consists of several arcs. Commercial LED strips were attached to the insides of these arcs. Also significant is that several lighting patterns were analysed in order to understand which pattern best preserves the imaged shape of the sample compared to its real counterpart. Removing lights that were angled more horizontally proved to have bigger impacts on shape preservation than removing more vertically oriented lights.

2.3 Conclusion

Literature review of instrument design in Raspberry Pi microscopy showed that there are a significant number of diverse designs to be found in the state of the art. These designs are largely 3D printed, although LEGO is also used once. A variety of different LED-based lighting systems in different positions around the sample are used in these designs, sometimes also utilising LED arrays. Similar in variety were the implementations of sample movement; Some designs moved the sample itself, while others moved the optics, all in a combination of the X-, Y-, or Z-directions. Complete manual movement of both the sample and the optics was uncommon. Regarding optics, the majority of designs featured an optics module either directly above or directly below the sample. These optics modules were focused on the sample by either adjusting the lenses themselves or by varying the distance between the sample and the optics.

Most of the designs included some degree of modularity, ranging from vertical stacks of customisable parts to interchangeable optics modules and a cube-based system that allows for myriad setups using the same parts. This modularity was achieved by a splitting of the design into its functional parts, then attaching these parts in a manner that allows for easy interchange with other, similar parts.

Finally, Reflectance Transformation Imaging (RTI) in microscopy was reviewed. It was found to consist of an imaging technique that uses lighting from different angles to gain more information about a sample than directly inspecting it in on lighting condition allows for. Ways to implement this technique include spheres, semi-spheres, arcs, or arms with individually controllable LEDs on them. Automated RTI using Raspberry Pi exists in the state of the art, and in this system lights that are angled more horizontally with respect to the sample have a higher impact than more vertically angled lights on the preservation of the shape of the imaged sample.

2.4 Discussion

First and foremost, this review shows there is a significant amount of information already available on instrument design in Raspberry Pi microscopy. However, one should keep in mind that all the work referenced is a *sample* of the total body of work available, given that there was limited time to perform this review. Also, there was no systematic filtering of papers as in a proper systematic literature review, which might result in bias towards the

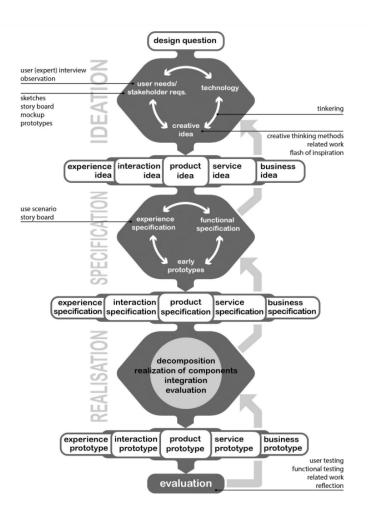


Figure 13: The Creative Technology design process, as specified by Mader and Eggink[20].

use of papers the author prefers for personal reasons. It must be noted that some papers left out technical details of designs, making for missing information.

3 Design methodology

Given that the device this project concerns itself with is directly related to a human end user, communicating with this end user and addressing their needs is by definition paramount to the success of the project. From this perspective, let us detail the use of the Creative Technology design process^[20], as it was specifically invented to aid projects of this kind.

3.1 Ideation

As is visible in figure 13, the ideation phase commences with the consideration of design questions. From these, user needs and the requirements of stakeholders of the project are

analysed and considered. Creative thinking techniques such as brainstorming may be applied in order to conceive of technological solutions that may satisfy all stakeholders. The result of this phase is an idea or a concept for the final solution.

3.2 Specification

In the specification phase, the concept from the ideation phase is expanded upon with lowfidelity prototypes and further specifications. This phase may relate back to the ideation phase, as new insights are gathered.

3.3 Realisation

Lower in figure 13, the realisation phase features tangible prototypes, and may see components of a system being produced and integrated together. This phase may also see early evaluations of the produced prototypes. Again, this phase may reroute those partaking in it to he specification phase, as evaluation may reveal new knowledge.

3.4 Evaluation

In the final evaluation phase, the prototype from the realisation phase is evaluated with its target users for the last time. Data on this evaluation may be collected, and the working of the prototype will be reflected on.

3.5 Execution

In this first phase of the project, the *Ideation* cycle of figure 13 was carried out. Important to note is that the *design question* refers to research questions 1., 2., and 3. of this paper. These design questions were arrived at in a particular way: At the start of the project, my collaborator Matei Obrocea and myself were introduced to the client. It became clear that the project would need to be split in two to clearly define tasks and roles. A brief brainstorm session was conducted, the result of which being my involvement with the hardware, and my collaborator's work on software. After this, the first step to take was to gain a better understanding of the needs of the end user to address these design questions, and thus an interview was carried out, in which a multitude of questions about the user's desires concerning inspection of 3D prints using microscopy were asked⁴. Important conclusions from this interview concerning hardware development were that the microscope should

- (a) Fit on a desk to allow for desktop use, where it needs not be moved.
- (b) Be able to capture video files and store them.
- (c) Have an optical magnification factor of at least 20^5 .
- (d) Have a display to show the outputs of the optics system.
- (e) Be able to be modified in the future, acting as a "platform" for different imaging techniques.

⁴These questions can be found in appendix A.

 $^{{}^{5}}$ That is to say, that lengths observed using the optics will appear 20 times larger than when observed without the optics.

ID	Name	Mar, 2024					Apr, 2024				Мау	May, 2024				Jun, 2024				Jul, 2024	
		26 Feb	03 Mar	10 Mar	17 Mar	24 Mar	31 Mar	07 Apr	14 Apr	21 Apr	28 Apr	05 May	12 May	19 May	26 May	02 Jun	09 Jun	16 Jun	23 Jun	30 Jun	07 Jul
1	✓ Module 11																				
12	Finalise proposal																				
3	Reflectance Transformation Imaging (RTI) lit																				
4	Instrument design literature																				
5	Optics/data acquisition prototype																				
6	RTI prototype	:																			
13	Write paper																				
2	▼ Module 12																				
7	RTI prototype																				
8	Instrument prototype																				
9	Control prototype																				
10	Write paper																				

Figure 14: Gantt chart concerning project timing.

- (f) Have manual overrides for automated features that control the device.
- (g) Be modular, such that is easy to replace or repair parts.
- (h) Be able to inspect a thin sample up to 10 cm in size.
- (i) Allow for movement of the sample in X- and Y-directions.

Given the engineering nature of this project, these user needs were already quite specific and technical. Indeed, there was little need for further investigation into the problems the user faced, as they were quite clear from the beginning and the first interview. Therefore, the ideation phase became more technical in finding technology that would satisfy the user's microscopy needs; It could also be interpreted as a lower-detail version of the specification phase.

In order to keep the project on track in terms of timing, a Gantt chart was developed as shown in figure 14. The chart serves as a general roadmap of the project, and prevents sidetracks. Note that by "write paper", a weekly moment to write is implied, not the entire week as shown.

4 Concept generation

With these needs in mind, concept generation commenced, meaning a certain preliminary hardware design would be conceived of, and evaluated with the end user. An initial priority in this phase was to incorporate results from the state-of-the-art review in section 2 in order to contribute to the already existing body of work. The first approach was to attempt to take desirable features from microscope designs in the state of the art, then compile them into a new design that would match the user's needs. However, conversations with the client suggested that this would be far beyond the scope of this project in terms of timing: As a result of this, a more pragmatic approach was chosen in taking a promising, existing open-source design and modifying it to suit the end user.

Continuing, the state of the art was first considered for materials selection. All opensource microscope designs discovered in the literature are 3D printed, or have 3D printed parts, and for good reason: 3D printing is a fast, automated type of manufacturing that lends itself well to prototyping, especially in comparison with techniques like wood- or

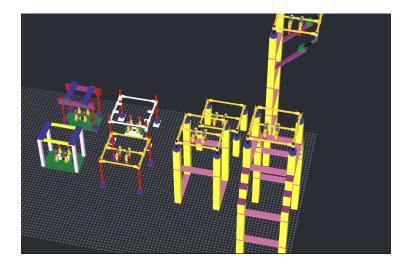


Figure 15: Several iterations of optics mount in BrickLink Studio.

metalworking. One author, Temiz[7] (2020), used LEGO pieces in combination with 3D printing. LEGO could be argued to be even faster in prototyping than 3D printing as human hands can work faster than a 3D printer, but has lower versatility as specialised parts may turn out to be missing in the prototyping process, requiring a wait for them to arrive in a new order.

Further communication with the end user revealed another need they had been hinting at in the first interview; A need to gather more information from the sample than simple brightfield microscopy⁶ with one set of optics and one light source. In discussion beyond this, the user revealed that Reflectance Transformation Imaging (RTI), as also introduced in section 2 would be a solution.

Considering the above, a first concept of the microscope design was established: 3D printed hardware that incorporates RTI, and could also include LEGO parts. To further ideate on this concept, the popular LEGO CAD^7 software *BrickLink Studio* was used to generate some first sketches of the concept. BrickLink Studio was chosen for the immediate, intuitive building it allows for, as opposed to other CAD software that might have a steeper learning curve. The result of this sketching can be seen in figure 15: Beginning with a mount for the optics, and ending at a structure around it. A similar structure was also physically built using readily available LEGO parts of the RaM lab, as shown in figure 16.

Regarding optics, for the concept a Raspberry Pi HQ camera module was used since it was already available from the client. The module featured a lens with 10x magnification, which is below the user's desired minimum of 20x, but suffices for the ideation phase. The camera module sent data to a Raspberry Pi 5, and a Raspberry Pi touchscreen display was used for experimentation. The former was also readily available, whereas the latter was bought.

An initial conclusion from this work was that positioning the optics module above the sample would be preferred to placing the module below it, as this removed the need for

 $^{^{6}\}mathrm{Meaning}$ microscopy that operates under background light, without any dark chambers or other obfuscation.

⁷Computer Aided Design.

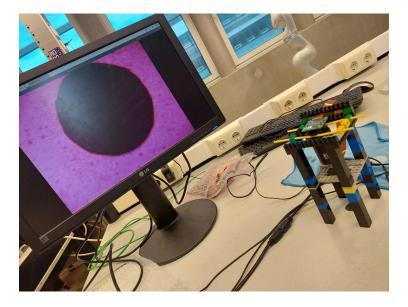


Figure 16: Physical version of LEGO sketch, including camera module and Raspberry Pi. The output of the camera module is shown on screen.

transparent materials due to the sample not having to be supported above the optics, saving cost and complication. As seen in the state of the art, glass microscope slides could be a cost-effective method to solve this problem, but these limit the dimensions of the sample to be inspected, reducing the flexibility of the design. Also, including the pragmatic approach of modifying an existing design described above implied a choice of a design from the state of the art was to be made. The best candidate was the OpenFlexure Delta stage, being a well-documented design featuring assembly instructions, unlike others found in the state of the art. The design does feature optics below the sample, although it seemed feasible to modify it in such a way that the optics are placed above the sample. Also relevant, and in contrast to the other OpenFlexure design found in the literature, is that the Delta stage moves its sample holder as part of the XY-stage, not the optics module. OpenFlexure claim this is a better design[21], as it allows for variation in imaging technique, which is relevant to the implementation of RTI.

With the new information gained in this iterative step, the current version of the concept can be formulated: A 3D-printed modification of the OpenFlexure Delta stage with optics above the sample to include RTI, the Raspberry Pi 5, and its HQ camera module. The Delta stage is shown in figure 17, and an experimental modification is shown in figure 18. This concept was positively evaluated with the end user.

4.1 Alternatives

Although the concept above is argued for, it can still be valuable to consider alternatives. For instance, it could turn out that the OpenFlexure Delta stage is infeasible to manufacture with 3D printing equipment in the RaM lab, making the concept infeasible. In such a case, the design by Maia Chagas et al. (2017)[15] could prove more realistic, as it is simpler in construction than the Delta stage, and needs no modification to inspect the sample from



Figure 17: A 3D-printed OpenFlexure Delta stage, image from McDermott et al.[12].

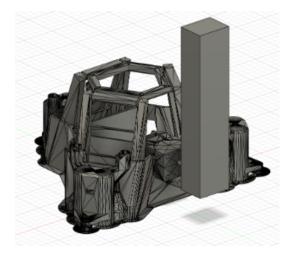


Figure 18: A concept modification to the OpenFlexure Delta stage.

above. However, an XY-stage would have to be designed and implemented, which is likely beyond the scope of this project. Another alternative is provided by the OpenFlexure Microscope, which is similarly well-documented to the Delta stage, being from the same authors. However, if the Delta stage is infeasible to manufacture, this model might also be, as their complexities are similar. Also, a modification would have to be made to allow the optics module to move the sample.

5 Specification & Realisation

In order to progress to a final design, it is necessary to go into more detail and enter the specification phase as described in figure 13. Note that, due to the engineering nature of this project and the fact that high-fidelity 3D printed prototypes were the most convenient way to prototype an intricate design like this one, the specification and realisation phases

unavoidably became highly intertwined, with 3D designs made in Fusion 360 immediately being manufactured and evaluated for feasibility. Considering this, it is detailed below how each element of the concept established in the previous section was developed further.

5.1 XYZ-stage

Given the clear choice for the OpenFlexure Delta stage, it was critical to establish its functionality early on. For this purpose, a 75%-scale model was 3D printed. Using the original authors' 3D printer settings⁸, this model was successfully produced, and was positively evaluated with the end user. It also proved that the actuation of the stage would indeed be functional. Some doubts did exist about the range of motion of the XYZ-stage, but these ended up not being a concern in the final, full-scale version. For the full-scale 3D print of the Delta stage, it was decided that, in order to meet the end user's need of inspecting a sample up to 10 cm in size, the stage would be printed at 128% of the original author's scale. This posed an uncertainty as to whether or not overhanging parts of the design would sag too significantly. In order to limit material use as much as possible, it was decided to print two necessary modifications to the stage (a holder for the arm, and some supports for an RTI dome⁹) in the same batch. This print (figure 19) was highly successful, with minimal sag, and provided a solid foundation for the continuation of the specification and realisation phase. The final design of the XYZ-stage is shown in figure 17.

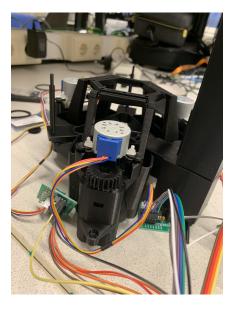


Figure 19: Successful full-scale print of the Delta stage being tested using a 28BYJ-48 stepper motor.

With this major print a success, the next aim was to establish actuation of the XYZstage with stepper motors - again in line with the original authors of the Delta stage. Given the now larger scale of the design, pieces were made to modify the stepper motor

 $^{^{8}\}mathrm{A}$ layer thickness of 0.15 mm and an infill of 20% - no support material used.

 $^{^9\}mathrm{The}\ \mathrm{RTI}$ dome is detailed below.

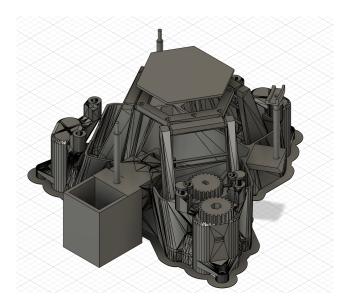


Figure 20: CAD design of the modified OpenFlexure Delta stage used as XYZ-stage. Note that only one the three sets of gears is shown, and only one of three dome holders is shown. The sample holder platform "hovers" over the stage.

bracket such that standard 28BYJ-48 stepper motors would fit. The gears that would link the actuators and the motors were also printed at a larger scale. However, it turned out that the gear ratio (2:1) was such that the motion of the actuators was too slow, causing the stage to be frustrating to use in a test run with the final lens to be used. Therefore, new gears were printed at a 1:1 gear ratio, which yielded an adequate result in terms of speed. Additionally, the motors were controlled using an Arduino Uno: Controlling LEDs in another part of the microscope proved highly complicated using the Pi directly, so this was offloaded to the Arduino. It was trivial to also control the motors using the Arduino, making it the preferred option. The motors were controlled using 4 Arduino digital pins each, connected to the respective controller PCBs that were shipped with the motors.

5.2 Arm

In line with the optics being positioned above the sample as specified in concept generation, it was necessary to design an arm with some kind of attachment to hold the optics. Manufacturing a lens was clearly beyond the scope of the project, so several commercial options were considered, before settling on the Seeed studio "300X Microscope Lens for Raspberry Pi High Quality Camera with C-Mount". The magnification factor of 300 is well above the end user requirement of 20, and this lens has the correct mount to fit the HQ camera module. Finally, the lens was easily available at several online retailers. The choice for this lens shaped the design of the arm that would support it. Conveniently, an open-source design for a ring-shaped holder for the lens was found[22], and could be easily printed using the facilities of the RaM lab. In CAD, a basic arm design was then made for this ring to be attached to, featuring an angled upper section to centre the lens over the XYZ-stage. To iterate on this basic digital protoype, it was quickly imagined that the end

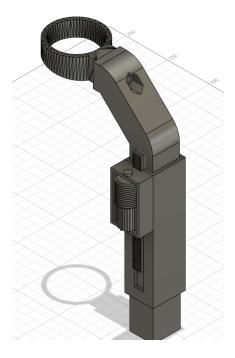


Figure 21: CAD design of the microscope arm, including worm gear mechanism.

user would need to be able to move the lens in the Z direction in order to get the sample in focus. The XYZ-stage could provide some part of this motion with a range of 5 mm in Z direction. However, for samples with greater dimensions in the Z direction this could prove insufficient. Therefore, it was decided to add a mechanism to the arm in order to allow for a greater range in Z direction. Firstly, a simple holder (now to be referred to as the *lower arm*) for the arm with a slot for a screw was considered, wherein the arm (now to be referred to as the *lower arm*) would be held up by friction between the screw and the lower arm. Valuable input from a technician in the lab yielded the idea of using a worm gear drive for this purpose, as the very nature of such a drive would prevent the upper arm from falling down due to a lack of friction. This is a feature of the final design of the arm, as presented in figure 21. The container for the worm gear was glued to the lower arm using epoxy after printing both parts, as printing the entire lower arm at once would have required a large amount of support material. Of note is that the worm gear drive was quite effective, so that in the interest of time the capability of the Delta stage to move in Z direction remained unused.

5.3 RTI

In order to implement RTI as established in the design concept, several considerations had to be made: In the first place, a choice for a light source. Digitally addressable LEDs were an obvious option, given their versatility and ubiquity in today's technological landscape. In particular the ability to individually turn LEDs on and off using a single control signal was attractive, given the nature of RTI where images are taken with sequential LEDs illuminated.



Figure 22: LED strips in the process of being connected using soldering.

Further investigation yielded that WS2812 LEDs were ideal, given their availability in strip format, allowing for flexibility in installation, along with the presence of libraries to interface these LEDs. Specifically, the WS2812b variant was chosen, as it needs 5 V, which happens to be the output voltage of both the Raspberry Pi and Arduino microcontrollers. The strips were connected by sets of wires, as shown in figure 22.

Moving on from this, first attempts were made to control the LEDs by means of Raspberry Pi. However, it soon surfaced that this was challenging due to software issues. Thus, the decision was made to offload LED control to an Arduino Uno as mentioned above.

Next, different options to arrange the LEDs around the sample bed of the Delta stage had to be considered, as discussed in the conclusion of the literature review. A matte black dome was chosen for this, given the combination of its ambient light-blocking properties and its ease of manufacture. This light-blocking did indeed prove useful, since it provided greater contrast as shown in tests of the RTI system.

In order to accommodate the optics, the 3D printed dome features a hole with an additional upstanding cylinder to block out additional ambient light. Beyond this, six protrusions were added to the inside of the dome in order to guide as a visual aid in attaching the LED strips with double-adhesive tape. The dome is shown in figure 23.

5.4 Integration

The development of the parts above was closely linked to the process of integrating them, as they could quickly be combined given the manufacturing method of 3D printing and the small scale of the project. Additionally, the modularity of the design meant that parts functioned separately from each other, reducing the need for integration significantly. There was significant integration in the communication between the Arduino and the Raspberry Pi, as the Pi sent commands to the Arduino to control the motors of the stage or control the LEDs according to user inputs. The full realisation of the microscope hardware can be



Figure 23: Most informative perspective of the RTI dome, including the protrusions for the LED strips.

seen in figures 24 and 25.

6 Evaluation

For the final evaluation of the hardware, the end user was brought in for a session to experiment with it, and attempt to use it for its intended purpose; The inspection of 3D prints. The session started off with a brief demonstration of the features of the prototype. and the user interface as developed by my collaborator was introduced. Following this, the user inspected several of his own samples using both the RTI and non-RTI modes. The result of this was remarkably succesful, as the user was able to effectively start his research right away, noting details and different visible layers in the sample when subjecting them to different lighting angles using the RTI dome. Images were also captured as part of this phase, two of which are shown in figures 26 and 27. Physically handling samples, bringing them under the optics, then using the worm gear to bring them into focus went well, and the motorised movement of the stage was highly functional. One remark the user did make concerned the alignment of the dome: There being no indicator for how it should be aligned meant it could end up in any rotation, potentially misaligning it with an element of the user interface. Additionally, the optics had to be taken out of its holding ring when switching samples, which the user felt could be streamlined. Overall, the hardware more than exceeded the user's expectation, being ready to use for research purposes. Desires for modularity were also met, as the end user reported the design could be used as a "platform" to expand upon in the future. The hardware was also evaluated with the client, who responded in a similarly positive manner, mainly remarking the hardware being a good prototype that could be further developed. Both the client and the end user received an evaluation form after the session, which can be found in appendices B and

7 Conclusion

As is apparent from the evaluation, 3D printed microscopy hardware using Raspberry Pi and Arduino was successfully developed into a working prototype to be used for 3D print

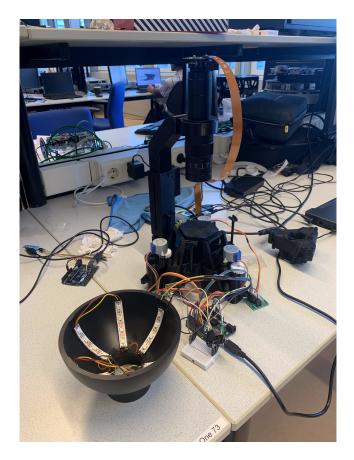


Figure 24: Realisation of the microcope hardware in non-RTI configuration. The RTI dome is shown in front.

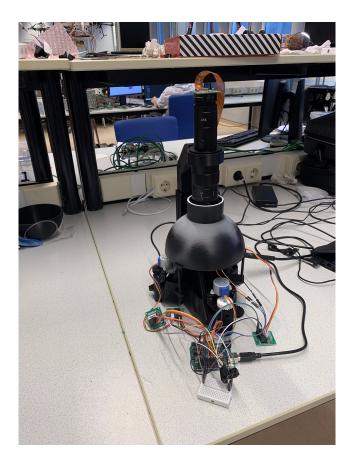


Figure 25: Realisation of the micrscope hardware in RTI configuration.

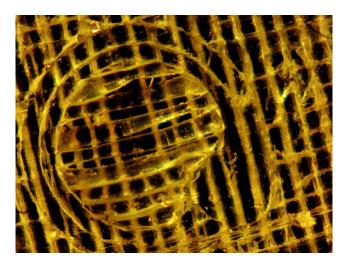


Figure 26: End user sample inspected under the microscope in RTI mode, from one lighting angle.

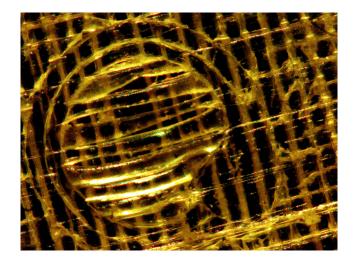


Figure 27: End user sample inspected under the microscope in RTI mode, from another lighting angle. The difference with figure 26 is striking, given the difference in lighting highlights.

and electronics inspection. The concept chosen early on in the project was indeed realised, thereby answering all three research questions. The expectations and needs of the end user were exceeded, and the design is promising for future development. The design is modular, features a motorised XY-stage, and captures detailed live images. Furthermore, it puts RTI into a new context, which is in line with the Creative Technology philosophy of bringing existing technology to novel applications. Additionally, the Delta stage was printed at 128% scale, validating the original design when printed at larger scales.

7.1 Future work

Given more development time, several parts of the design could be improved. These improvements are largely based on experience from working on the hardware, along with input from the end user. To start off, a software feature could be implemented on the Arduino to allow the XY-stage to be returned to its centre position. This would be useful to always allow full range of motion when inspecting a sample, as the sample can be aligned manually. Next, the movement of the stage could be limited according to the limits of the flexure mechanism, to prevent unnecessary motor wear at the edge of the range of motion. Another stepper motor could be included in the worm gear drive, such that autofocus may be implemented in the future. Also, as mentioned above, parts of the design were glued using epoxy. Slight redesigns could be made to allow for assembly without it. Finally, the design could be further polished to elevate it from a prototype to a proper device by hiding wires and further refining its aesthetics. Part of this future work is likely to be implemented, as a tentative agreement with the supervisor was reached on continuing the project part-time.

References

- T. Pettinger, Antony van leeuwenhoek biography: Jan. 2020. [Online]. Available: https: //www.biographyonline.net/scientists/antony-van-leeuwenhoek-biography. html.
- [2] Laboratorium discounter. [Online]. Available: https://www.laboratoriumdiscounter. nl/nl/laboratoriumbenodigdheden/optische-instrumenten-en-lampen/microscopenen-toebehoren/alle-microscopen/page2.html.
- [3] Bol. [Online]. Available: https://www.bol.com/nl/nl/l/microscopen/55090/.
- [4] R. Bowman, B. Vodenicharski, J. Collins, and J. Stirling, "Flat-field and colour correction for the raspberry pi camera module," *Journal of Open Hardware*, vol. 4, Apr. 2020. DOI: 10.5334/joh.20.
- [5] J. L. Deglint, K. Schoneveld, F. Kazemzadeh, and A. Wong, "A compact field-portable computational multispectral microscope using integrated raspberry pi," *Journal of Computational Vision and Imaging Systems*, vol. 2, no. 1, Oct. 2016, ISSN: 2562-0444. DOI: 10.15353/vsnl.v2i1.91.
- [6] I. Nuñez, T. Matute, R. Herrera, et al., "Low cost and open source multi-fluorescence imaging system for teaching and research in biology and bioengineering," PLOS ONE, vol. 12, no. 11, pp. 1–21, Nov. 2017. DOI: 10.1371/journal.pone.0187163.
- [7] Y. Temiz. "Build a sophisticated microscope using lego, 3d printing, arduinos, and a raspberry pi." (2020), [Online]. Available: https://spectrum.ieee.org/builda-sophisticated-microscope-using-lego-3d-printing-arduinos-and-araspberry-pi (visited on 03/13/2024).
- [8] J. Vernon, P. Canyelles-Pericas, H. Torun, et al., "Acousto-pi: An opto-acoustofluidic system using surface acoustic waves controlled with open-source electronics for integrated in-field diagnostics," *IEEE Trans. Ultrason. Ferroelectr. Freq. Control*, vol. 69, no. 1, pp. 411–422, Jan. 2022. DOI: 10.1109/TUFFC.2021.3113173.
- [9] Z. Wang, A. Boddeda, B. Parker, et al., "A high-resolution minimicroscope system for wireless real-time monitoring," *IEEE Transactions on Biomedical Engineering*, vol. 65, no. 7, pp. 1524–1531, Jul. 2018, ISSN: 1558-2531. DOI: 10.1109/tbme.2017.2749040.
- [10] R. A. Steiner and T. O. Rooney, "Piautostage: An open-source 3d printed tool for the automatic collection of high-resolution microscope imagery," *Geochemistry, Geophysics, Geosystems*, vol. 22, no. 5, May 2021, ISSN: 1525-2027. DOI: 10.1029/ 2021gc009693.
- [11] J. T. Collins, J. Knapper, J. Stirling, et al., "Robotic microscopy for everyone: The openflexure microscope," *Biomed. Opt. Express*, vol. 11, no. 5, pp. 2447–2460, May 2020. DOI: 10.1364/BOE.385729. [Online]. Available: https://opg.optica.org/ boe/abstract.cfm?URI=boe-11-5-2447.
- [12] S. McDermott, F. Ayazi, J. Collins, et al., "Multi-modal microscopy imaging with the openflexure delta stage," Opt. Express, vol. 30, no. 15, pp. 26377-26395, Jul. 2022. DOI: 10.1364/0E.450211. [Online]. Available: https://opg.optica.org/oe/ abstract.cfm?URI=oe-30-15-26377.
- [13] B. Diederich, R. Lachmann, S. Carlstedt, et al., "A versatile and customizable low-cost 3d-printed open standard for microscopic imaging," *Nature Communications*, vol. 11, no. 1, Nov. 2020, ISSN: 2041-1723. DOI: 10.1038/s41467-020-19447-9.

- W. Watanabe, R. Maruyama, H. Arimoto, and Y. Tamada, "Low-cost multi-modal microscope using raspberry pi," *Optik*, vol. 212, p. 164 713, Jun. 2020, ISSN: 0030-4026.
 DOI: 10.1016/j.ijleo.2020.164713.
- [15] A. Maia Chagas, L. L. Prieto-Godino, A. B. Arrenberg, and T. Baden, "The €100 lab: A 3d-printable open-source platform for fluorescence microscopy, optogenetics, and accurate temperature control during behaviour of zebrafish, drosophila, and caenorhabditis elegans," *PLOS Biology*, vol. 15, no. 7, e2002702, Jul. 2017, ISSN: 1545-7885. DOI: 10.1371/journal.pbio.2002702.
- [16] M. Hughes-Hallett, C. Young, and P. Messier, "A review of rti and an investigation into the applicability of micro-rti as a tool for the documentation and conservation of modern and contemporary paintings," *Journal of the American Institute for Conservation*, vol. 60, no. 1, pp. 18–31, Mar. 2020, ISSN: 1945-2330. DOI: 10.1080/01971360. 2019.1700724.
- [17] A. Giachetti, I. M. Ciortan, C. Daffara, G. Marchioro, R. Pintus, and E. Gobbetti, "A novel framework for highlight reflectance transformation imaging," *Computer Vision* and Image Understanding, vol. 168, pp. 118–131, Mar. 2018, ISSN: 1077-3142. DOI: 10.1016/j.cviu.2017.05.014.
- [18] G. Earl, P. Basford, A. Bischoff, et al., "Reflectance transformation imaging systems for ancient documentary artefacts," in *Electronic Workshops in Computing*, BCS Learning & Development, 2011. DOI: 10.14236/ewic/eva2011.27.
- [19] L. Wilk, P. Lech, M. Klebowski, M. Beldyga, and W. Ostrowski, "Application of a stand-alone rti measuring system with an integrated camera in cultural heritage digitisation," *Journal of Archaeological Science: Reports*, vol. 53, p. 104318, Feb. 2024, ISSN: 2352-409X. DOI: 10.1016/j.jasrep.2023.104318.
- [20] A. Mader and W. Eggink, "A design process for creative technology," Sep. 2014.
- [21] [Online]. Available: https://openflexure.org/projects/deltastage/.
- [22] canned_sardine. "Tripod mount for seeedstudio microscope 300x lens for rpi hq cam." (2020), [Online]. Available: https://www.thingiverse.com/thing:4651433/files (visited on 06/15/2024).

A Interview questions

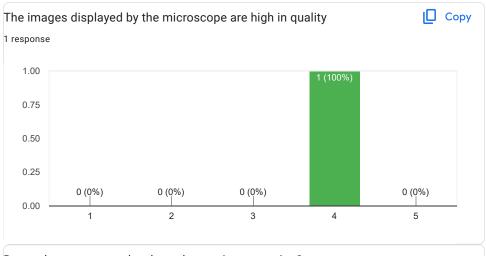
Below is a transcript of the notes used to help guide the first interview with the end user, which includes questions.

- Give context: Working together, separate parts
- What samples will you use?
- How big should the device be?
- What would you do with data from the microscope?
- Storing data?
- If you could any features yourself, what would those be?
- Display: dedicated display vs. hanging HDMI cable
- What would you dislike in a microscope?
- What sensor resolution would you need (level of detail)?
- What magnification would you need?
- What lighting or lighting method would you need?
- Does the device need to portable (with a battery and Wi-Fi)?
- Different people have different levels of knowledge on microscopy. On a spectrum from noob to expert, what information should the microscope provide? Noob would mean no prior knowledge required, expert means using microscopes professionally.
- Do you have any thoughts on maintenance and repair of the device?
- Do you have any other software this microscope must be compatible with?
- Do you have any other equipment this microscope must be compatible with?
- How important would the ability to customise the microscope to your needs be?

B End user evaluation form



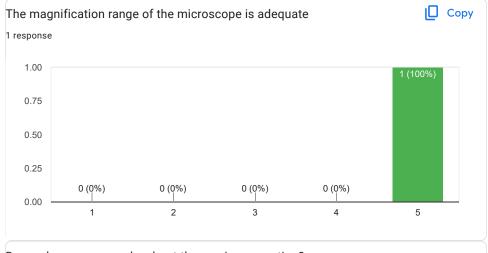
 $https://docs.google.com/forms/d/1V_Atw4xJdlbZEpeiQW1pLlcpnTSocTaXa8d9RllgbBc/viewanalytics$



Do you have any remarks about the previous question?

1 response

Arm for camera and lens could have had better connection for improved stability (makes it easier to adjust focal length without waiting too much for the image to stabilize)
Lens needs opened and cleaned of debris



Do you have any remarks about the previous question?

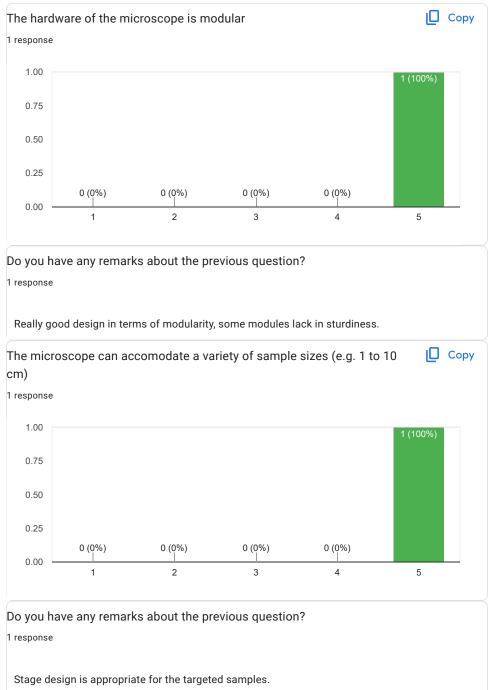
1 response

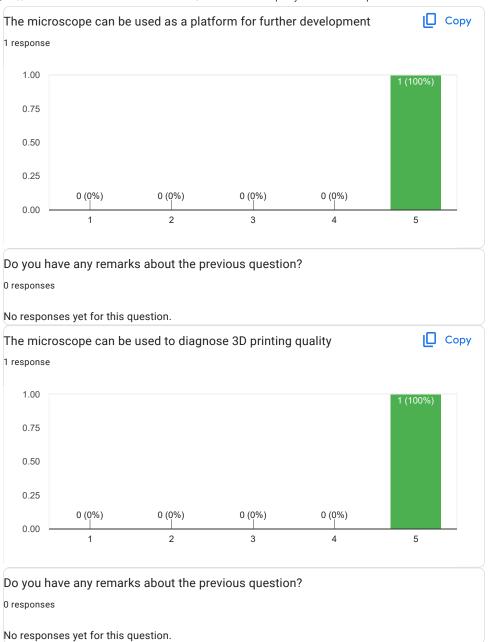
The chosen lens system is adequate for the purpose of the microscope.

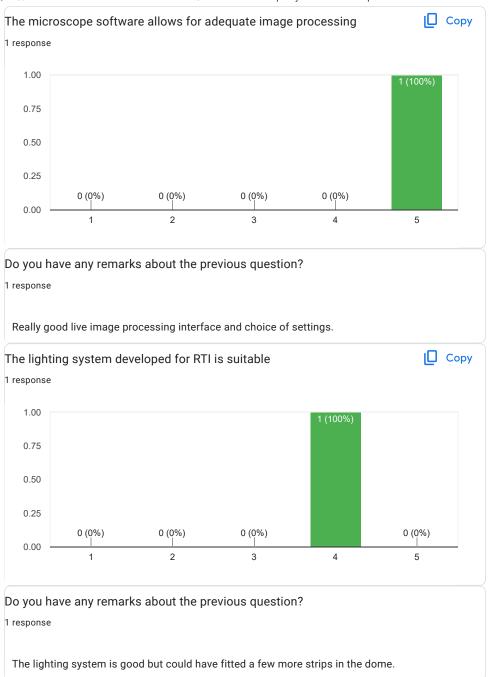
 $https://docs.google.com/forms/d/1V_Atw4xJdlbZEpeiQW1pLlcpnTSocTaXa8d9RllgbBc/viewanalytics$



User evaluation of Raspberry-Pi based microscope.

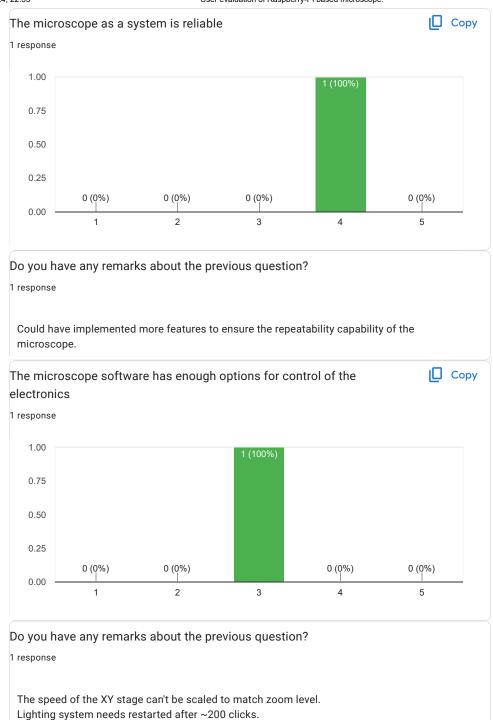


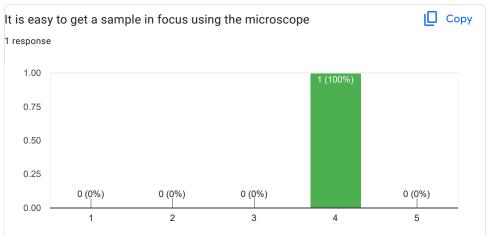






User evaluation of Raspberry-Pi based microscope.

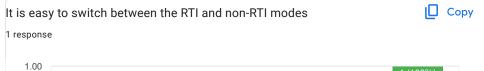


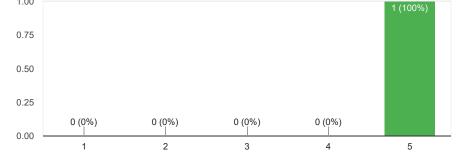




1 response

After some learning it gets easier. It's lack of sturdiness hinders the process of getting the sample in focus.

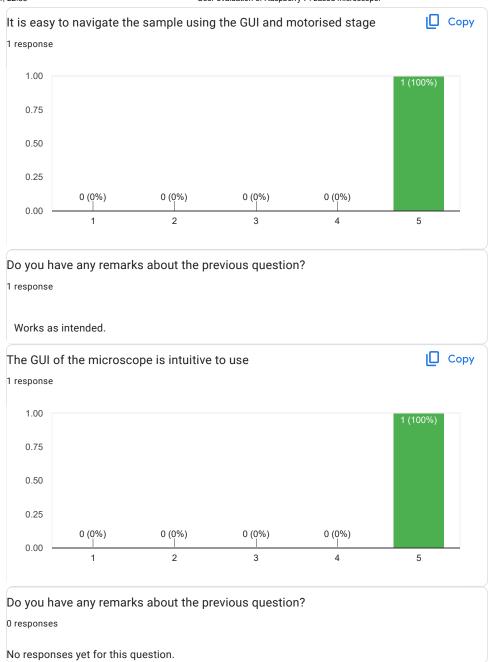




Do you have any remarks about the previous question?

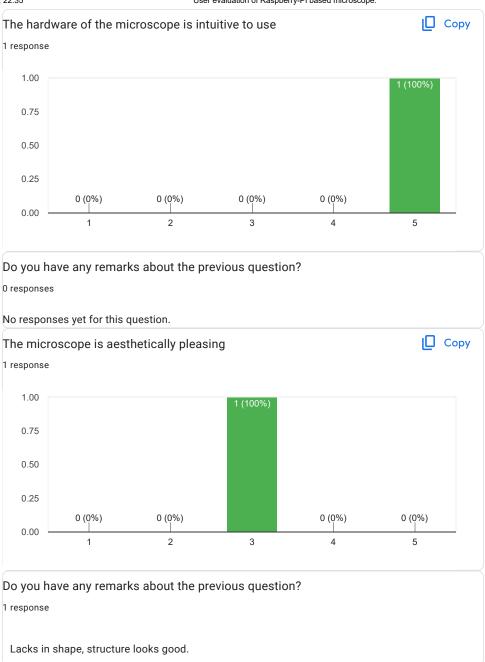
1 response

Restart button very handy.



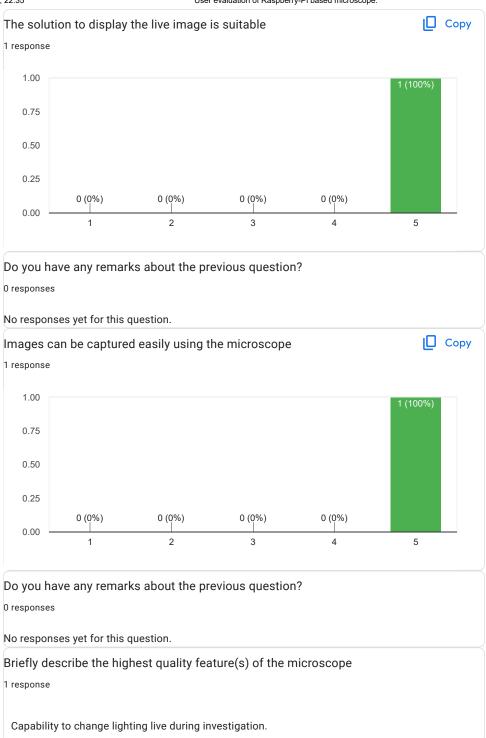


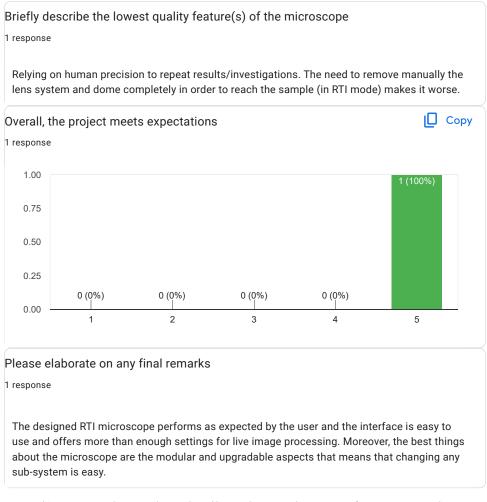












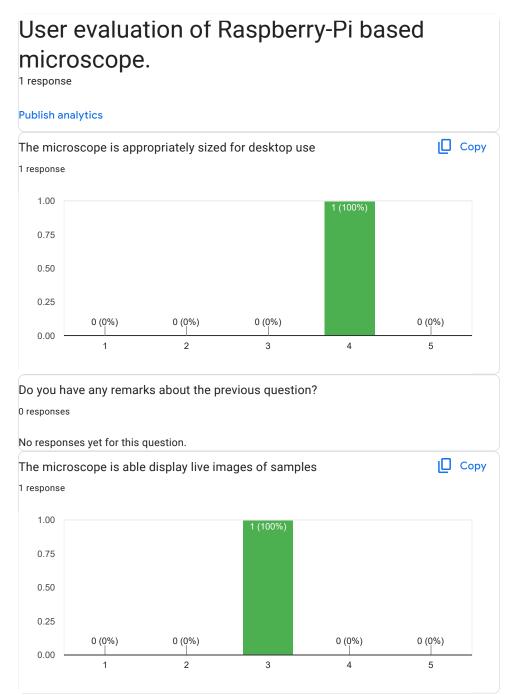
This content is neither created nor endorsed by Google. Report Abuse - Terms of Service - Privacy Policy.

Google Forms

User evaluation of Raspberry-Pi based microscope.

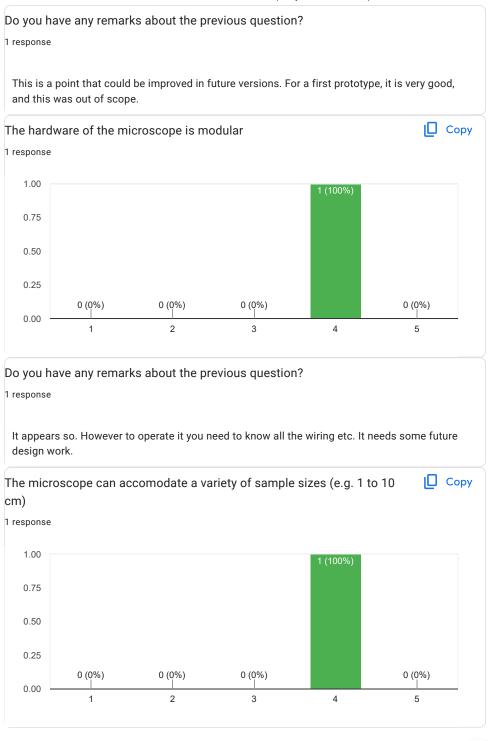
 $https://docs.google.com/forms/d/1V_Atw4xJdlbZEpeiQW1pLlcpnTSocTaXa8d9RllgbBc/viewanalytics$

C Client evaluation form





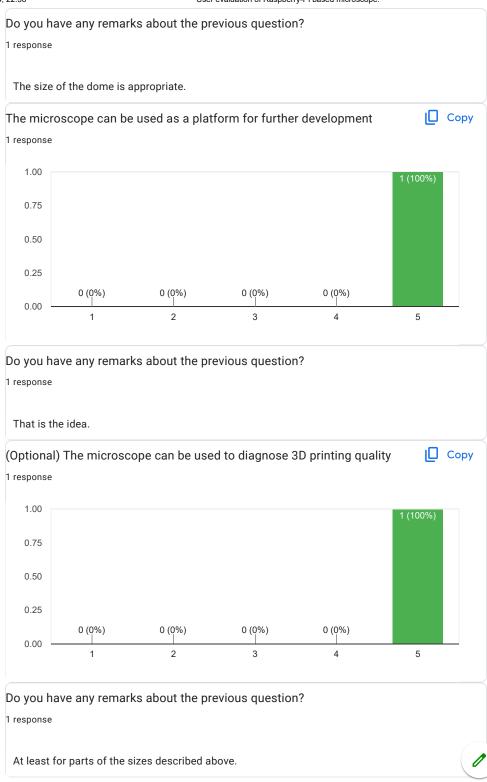


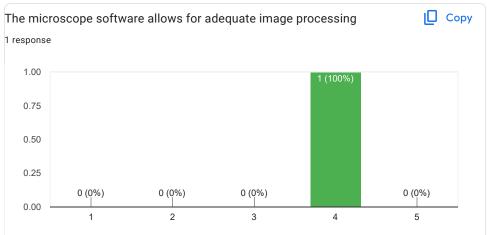


 $https://docs.google.com/forms/d/1p-WIDmTKqYiRXOkGAAhtwTFQV0rAs_liUoMsBG42zlk/viewanalytics$

1

User evaluation of Raspberry-Pi based microscope.

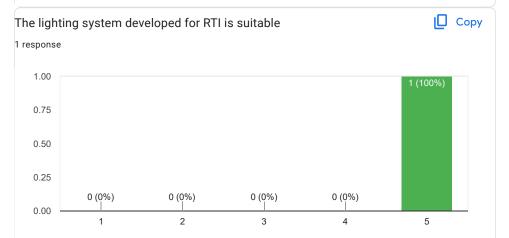






1 response

The user interface looks a bit under development but again for a first version and for the scope of the project this is ok.



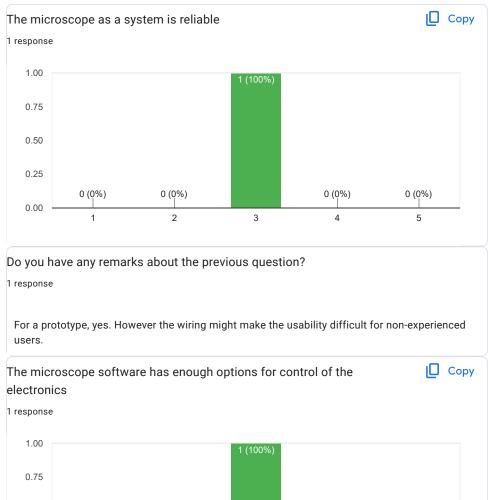
Do you have any remarks about the previous question? 1 response

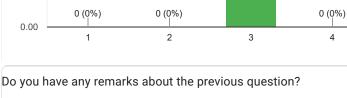
This I found it to be very good.





User evaluation of Raspberry-Pi based microscope.





1 response

0.50

0.25

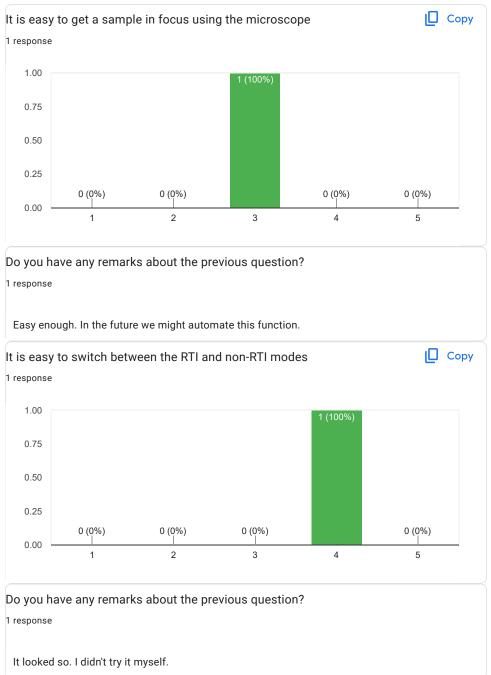
For now yes.

1

0 (0%)

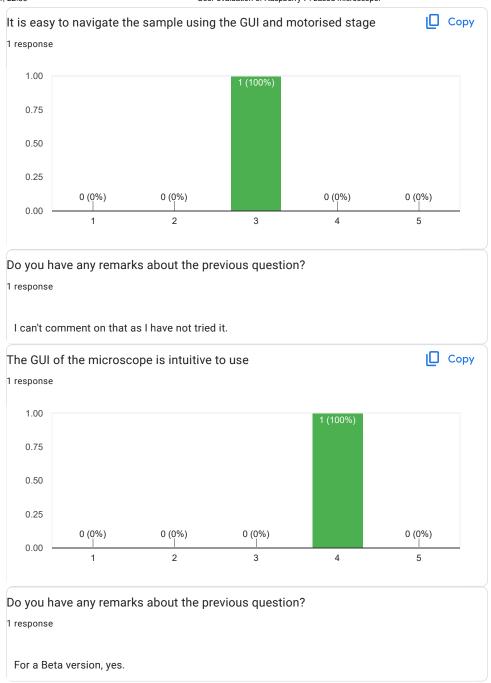
5

4







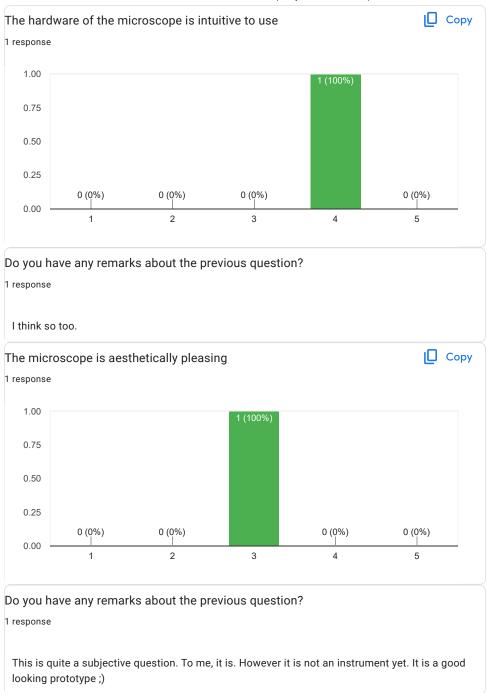




https://docs.google.com/forms/d/1p-WIDmTKqYiRXOkGAAhtwTFQV0rAs_liUoMsBG42zlk/viewanalytics



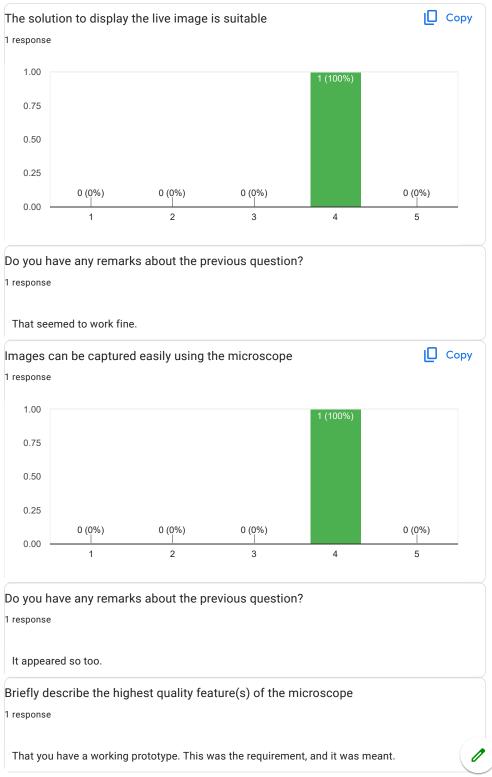
User evaluation of Raspberry-Pi based microscope.

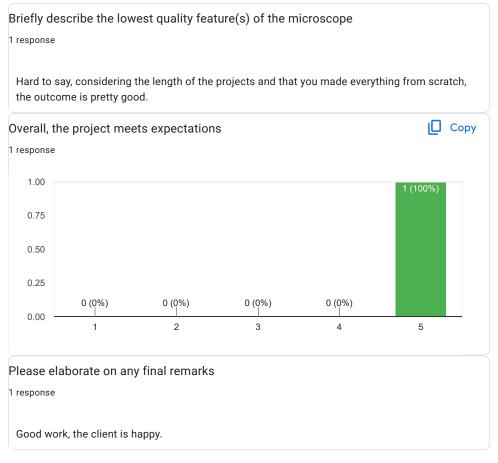


https://docs.google.com/forms/d/1p-WIDmTKqYiRXOkGAAhtwTFQV0rAs_liUoMsBG42zlk/viewanalytics



User evaluation of Raspberry-Pi based microscope.





This content is neither created nor endorsed by Google. Report Abuse - Terms of Service - Privacy Policy.

Google Forms



User evaluation of Raspberry-Pi based microscope.



 $https://docs.google.com/forms/d/1p-WIDmTKqYiRXOkGAAhtwTFQV0rAs_liUoMsBG42zlk/viewanalytics$