Imperial College London

MENG FINAL REPORT

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Advanced Microscopy for Everyone - Creating a Low-cost Confocal Microscope

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Abstract - **Confocal Microscopy is an advanced microscopy technique that provides high-contrast imaging and allows imaging at a chosen depth within a tissue and of 3D volumes. It has many useful applications but the high cost of commercial systems limits its universal use. This project uses re-scan confocal microscopy as a means to create a low-cost confocal microscope. By use of cheaper off-the-shelf parts and 3D printing a microscope was created for £1570. Testing revealed the microscopes ability to scan, re-scan and acquire images from the focal plane. However, difficulty aligning the pinhole limited testing of the complete system as well as testing with a microscope stage and objective lens. The results provide a promising look at what could be achieved when pinhole alignment is complete.**

1. Introduction

Confocal microscopy (CM) is an advanced microscopy technique that can improve resolution and contrast and allows image acquisition at a depth within a specimen [1]. These benefits come from the use of a pinhole within its optical setup. This pinhole only allows light from a focal point to pass through it. This is because light from the focal point is collected and refocused onto the pinhole by a pair of lenses as demonstrated in Figure 1. This focal point is then scanned across the specimen and the light intensity from each point is detected and constructed into an image. There are a few different variations in CM but this project is focused on laser scanning confocal microscopy (LSCM) where a laser is scanned across a specimen to create an image.

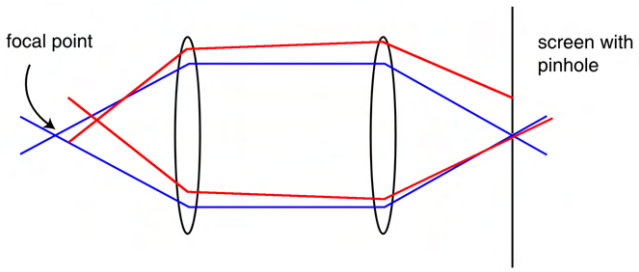

Figure 1: *Demonstration of principle behind CM. Exclusion of all light not from focal point is achieved by use of a pinhole. A pair of lenses collect light from a focal point and focus the light onto a pinhole, therefore rejecting light from outside the focal point. Figure adapted from [2]*

CM improves the contrast of acquired images because the light surrounding the focal point, which would usually be picked up as noise in wide-field microscopy, is rejected by the pinhole, increasing the signal-to-noise ratio. The same principle allows imaging of a virtual plane at a depth within a specimen [3], called optical sectioning, as light from above and below image plane cannot pass the pinhole as shown in Figure 2. Optical sectioning allows *in-vivo* imaging since specimens do not need to be prepared as a thin slice like normal microscopy. Optical sectioning also gives the microscope axial resolution - meaning that depth at which an image is taken in a specimen can be chosen by changing the depth of the focal point. This facilitates 3D imaging as many 2D images at different depths can be acquired and constructed into a volume [3].

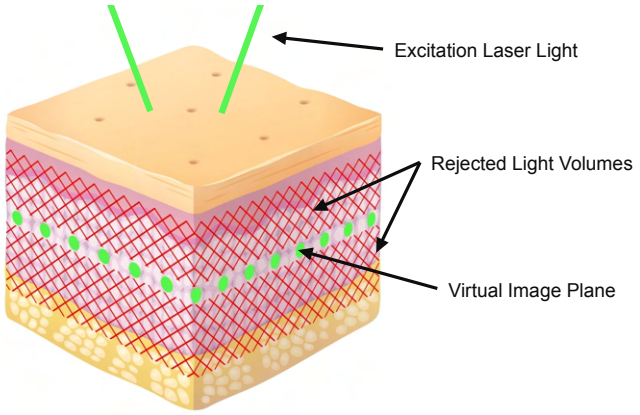

Figure 2: *Visualisation of optical sectioning. The excitation laser light is focused upon each of the focal points in the virtual image plane. The light from the tissue above and below the image plane (here marked in red mesh) will be rejected at the pinhole. This virtual image plane can then be moved up and down to image a volume.*

Re-scan Confocal Microscopy (RCM) is a variation of LSCM where the light returning from the focal point is re-scanned onto a camera sensor. This creates an image of the specimen on the image plane as opposed to collecting the intensities from the scanning focal point one by one and using software to construct them into and image. RCM is useful for this project because it allows the image acquisition to be done with a camera as opposed to and expensive and less sensitive photo-multiplier tube (PMT) [4].

The benefits of CM have led it to become a useful tool in many applications in biology, medicine and material science [5], specifically where optical sectioning is used for 3D imaging or *in vivo* investigations. One good example is its use in skin oncology where *in-vivo* imaging can provide an excellent assessment of tumour characteristics in real-time. The non-invasive nature also allows the same skin area to be repeatedly examined which is a significant advantage for following tumour progression over time [6].

While CM has many useful applications, its main drawback

is its high cost. Commerical CM systems usually cost upwards of $100,000 and can cost over $500,000 [7] making them very inaccessible for labs with smaller budgets including those in developing countries.

The general inaccessibility of microscopy due to the high-cost of commercial systems has led to the creation of many open-source, collaborative budget microscopy projects. This has been assisted by the vast improvement in both the quality and availability of 3D printing, which allows rapid prototyping and development as well as promoting collaboration between researchers all across the world. It has been reported in literature that 3D printing can reduce microscope part costs between 50-90% [8]. However, there have been very few attempts to create a budget CM.

Project Aims

The goal of this project is to build a re-scan confocal microscope module for a fraction of the price of a commercial system to make CM more accessible. The cost aim of this project is to create the microscope for £1000, meaning that low-cost manufacturing techniques (such as 3D printing) and off-the-shelf parts must be taken advantage of. The CM module will attach to the camera mount of a normal microscope, using its objective lens and stage and adding confocal functionality to it.

The CM system created in this project should be:

- **High-Performance** - Achieves the high-resolution of CM systems with a low signal-to-noise ratio.
- **Reproducible** - Manufactured using off-the-shelf parts and accessible techniques.
- **Adjustable** - Position of optics should be adjustable to account for poorer manufacturing tolerances.
- **Customizable** - Use of different optics should be possible with little modification.

2. Background

This background will provide a more in-depth explanation of normal and re-scan CM including the benefits and drawback of each as well as how the performance of a CM microscope can be quantified. The current progress of budget microscopy and past attempts at creating more budget oriented CM systems will be reviewed to highlight the importance and context of this project.

2.1. Intro to Confocal Microscopy (CM)

To create an image using LSCM, point-by-point illumination is used to image a specimen. The basic setup can be seen in Figure 3. LSCM works as follows:

- Laser light is focused onto a single point of a specimen which is adjusted by rotating a pair of galvo mirrors (computer controlled mirrors).
- The returning light (which is either fluorescence or reflected) from the specimen travels back along the same path as the original laser light.
- This light then hits a dichroic mirror or beam-splitter which separates the returning light's path from the laser light's.
- The returning light then passes through a pinhole. This pinhole only allows light from the focal point to pass through.
- This spatially filtered light then hits a detector, usually a Photo-multiplier Tube (PMT), which records the intensity of light from that point.
- This focal point is scanned across a plane of the specimen to gather a group of points. All the points from this slice are used to construct the image.

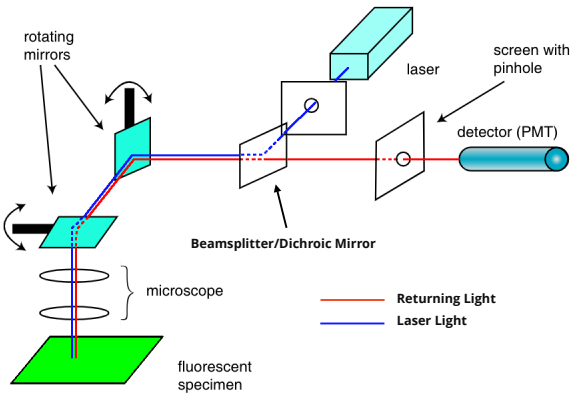

Figure 3: *A basic setup for a confocal microscope. The green line is the path of the excitation light. The blue line is the path of returning reflected or fluorescence light. Figure adapted from [2]*

2.2. Performance of a Confocal Microscope

The performance of any particular CM system can be simplified down to a few different characteristics. The most important of these characteristics are:

- **Penetration depth** - How deep the microscope can image into the specimen before the light scattering of the returning light is too great. This also limits the volume of 3D images that can be collected. The practical limit of this is usually around $200\mu m$ [1].

- **Image acquisition speed** - How quickly the system can collect an image. This is an important factor because the microscopes will often have to take numerous pictures, especially if a volume is being imaged. The speed of this also affects how well the microscope can capture dynamics. Commercial systems usually run at 6-9fps [9].

- **Resolution** - This can be quantified in many ways. A common way is with the full width at half maximum (FWHM) which describes how close two points can get to each other before they are indistinguishable.

- **Light efficiency** - The light efficiency of a CM is very important as it will affect the signal-to-noise ratio which affects the contrast of the microscope.

2.3. Re-scan Confocal Microscopy (RCM)

RCM is the variation of CM that is being used in this project. The RCM setup can be seen in Figure 2. A RCM setup consists of a normal CM setup alongside a re-scanner. The re-scanning unit scans the image from the pinhole onto the camera detector. This decouples the scanning magnification of the object (M_{obj}) and the scanning spot (M_{spot}) which can lead to a higher lateral resolution [10].

The improvement in lateral resolution is also independent of pinhole diameter [4]. This means that a greater resolution can be achieved at higher light collection efficiencies [11]. In standard CM as you increase the size of the pinhole the lateral resolution gets worse, leading to a trade-off between light collection efficiency and resolution. Because of its benefits, RCM can be configured for use for a number of specific biological and biomedical applications [12].

In RCM a camera is used instead of a Photo-multiplier Tube (PMT) since the image is re-scanned onto the sensor. Cameras are less expensive than PMTs and can also have a much higher collection efficiency. This also negates the need for computer processing and software to compile the point intensities picked up by the PMT into an image.

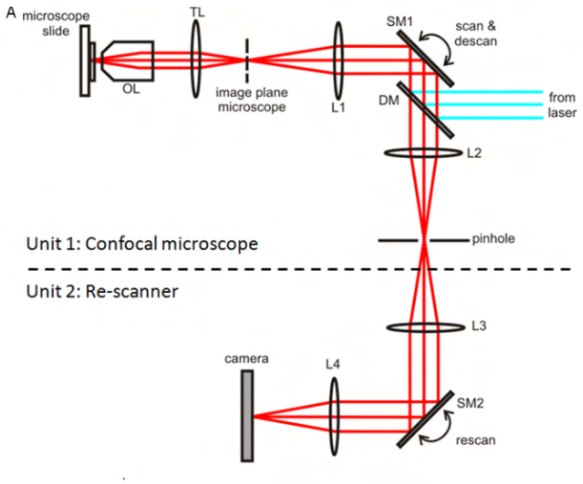

Figure 4: *Configuration of a re-scan confocal microscope. Consists of a normal confocal microscope and a re-scanning system to scan the image onto a camera sensor. Figure adapted from [10]*

2.4. Budget Microscopy

Microscopy is a crucial tool in many settings, but the prohibitive cost of hardware can make it completely inaccessible to some. Even labs that have the equipment may not have enough to meet the demand, necessitating the rationing of resources. For this reason, there have been many attempts at creating microscope hardware at a reduced price.

3D printing has greatly pushed forward the field of budget microscopy and is central to all the most popular projects. It has allowed global collaboration as well as the adoption of an iterative design approach. There are now numerous open-source microscopy projects which provide microscopes across a range of uses, quality and price. One of the most successful of these is the OpenFlexure project [13]. It is completely open-source with CAD files, build guides and software all readily available and easy to use. They use 3D printed flexure elements to allow sub-micron adjustment of the stage position, allowing extremely high precision. The optics are configurable depending on whether you want a cheap microscope or lab-quality optics. The OpenFlexure microscope can be seen in Figure 5. Other projects include Chea(i)p, FlyPi, OpenSPIM and UC2. All these projects are compared in Table 2 in Appendix B.

There are no CM open-source projects similar to those just discussed. There are, however, attempts at creating a CM system at a lower cost than commercially available systems. These systems tend to still cost much more than what is desired for this system and use existing parts such as ThorLabs cage system as opposed to using 3D printing to

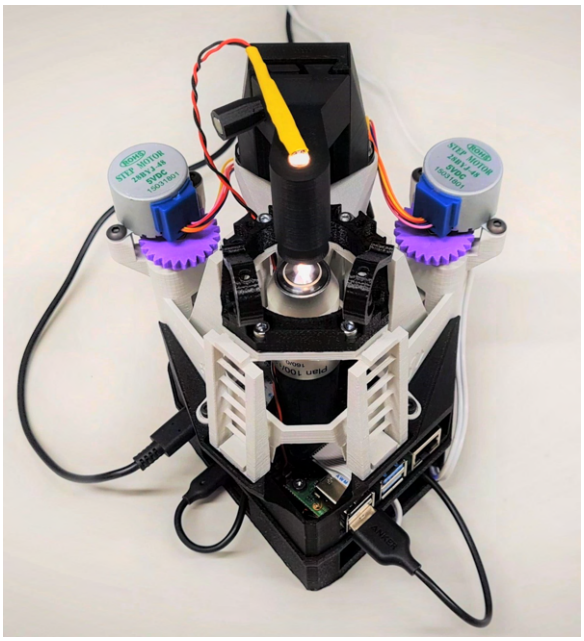

Figure 5: *The OpenFlexure microscope: A budget microscope project which is open-source, configurable and makes use of 3D printed flexures to allow precise positioning of the stage. Image from ref [13]*

create parts where possible. Two examples of such attempts are described below:

In ref [7], A laser scanning confocal microscopy (LSCM) module was created to overcome the prohibitive costs and the either superfluous or unspecific applications of commercial systems. The paper aimed to create a modular system which could easily be adjusted to very specific applications. Their system used the ThorLabs cage system to hold the optics in place so it did not have a permanent enclosure. The scanning system used allowed image acquisition at 12fps. This is excellent speed but the scanners alone cost $2500. Other expensive parts are the $1100 PMT to detect the light and the $1100 framegrabber to facilitate image construction, highlighting the high costs associated with conventional CM image acquisition. Overall they created a modular system capable of reflectance or fluorescence CM. The cost of the project came to over $12000.

In ref [14], a simplified LSCM was created with the goal of facilitating its use for education and in labs with a moderate budget. They set their system up on a breadboard, connecting to the side-imaging port of the microscope they were using for its objective lens and stage. Interestingly, they replaced the pinhole with a $9\mu m$ core optical fibre which collected the light and conducted it to a PMT for detection. This helped them simplify the alignment of the system. Their scanning system allowed them to acquire a 256x256 pixel image at 4fps. They concluded the system would be an excellent affordable option for giving students hands-on experience building and understanding CM systems.

There are a few projects that have attempted to create a budget confocal microscope that does not use laser scanning at all. In ref [15] they attempted to adapt more common and available total internal reflection fluorescence (TIRF) and epifluorescence microscopes to allow confocal microscopy with the use of a spinning disk. They did not specify the exact cost of their project. Their microscope performed well in terms of spatial resolution and utility. In ref [9] a low-cost smartphone CM was created for *in-vivo* skin imaging. In this project they replaced the pinhole with a slit aperture and diffraction grating. The whole system cost $4200 and they achieved comparable resolution to a commercial CM. Its limitation was its possible depth of imaging and low frame rate for image acquisition. They suggested it would be useful as a tool for widespread screening for early disease detection in resource-poor environments and as an educational tool. They created a new and improved microscope in ref [16] which they improved the depth and speed of their system. They replaced the smartphone with a camera with a CMOS sensor. They managed to increase their image acquisition speed by 50x. The whole price of the system did increase to $5200.

These ”budget” confocal systems provide some interesting insight for this project. Firstly, they are multiple times more expensive than budget for this project. The bulk of the costs comes from PMTs, expensive scanning units, image acquisition systems and off the shelf mounting hardware. This clearly highlights the areas where this project can use alternative methods and parts to cut costs. Secondly, both of these systems use temporary mounting hardware such as the ThorLabs cage systems. While this allows a lot of flexibility in configuration and hot-swapping optical components, it is a very different design philosophy from the open-source budget microscope projects which have been discussed. These projects extensively use 3D printing to create more permanent mounting which is easier for a 3rd party to manufacture and set-up. This project aims to create a system more aligned to ideas behind the open-source projects than these budget CM systems.

3. Methods

This section covers the design and testing procedures of the whole CM system. All CAD files, code and links to all components are available on GitHub (**Here**)

3.1. Design and Construction

The finished system can be seen in Figure 6

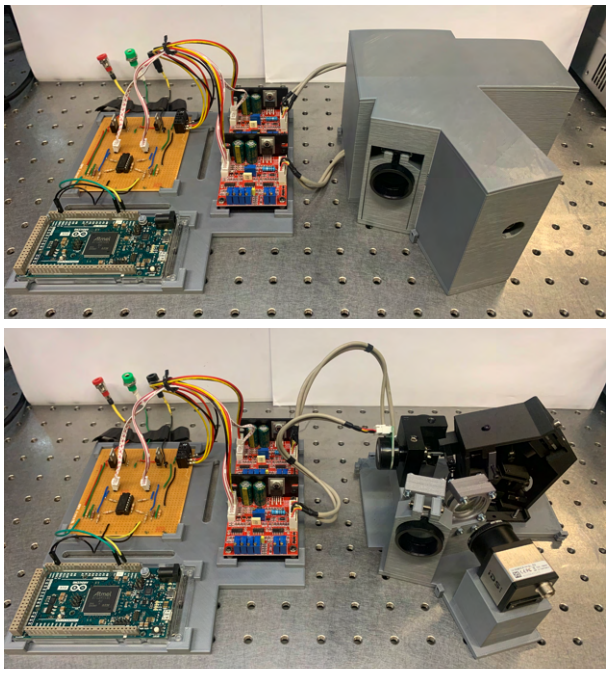

Figure 6: *The complete confocal microscopy system created in this project. On the left is the control circuit and on the right are the optics within the enclosure.*

3.1.1 Optics

The optical set-up is a re-scan confocal microscopy configuration which can be seen in Figure 7. The input laser from an optical fibre is collimated with a fibre collimation package (ThorLabs F220FC-B). This collimated light then passes through the beamsplitter (ThorLabs BS070). Half the light passes through and hits the galvo mirrors for the first time. They direct this light through a Plossl pair of lenses (2x ThorLabs AC254-100) which focus the light onto the specimen on the microscope stage. The confocal system is attached to the external microscope's camera port with a c-mount adaptor (Thorlabs CML10). The light returning from the specimen follows the same path to the mirrors where it is de-scanned back into one path. This light hits the beam-splitter again and half of it is reflected at a right angle through the a fluorescence filter (Edmund 67-027) which discards light of unwanted frequencies. The light is then reflected off a mirror (ThorLabs BB05-E02) in a mirror holder (ThorLabs MH12). The light passes through a small lens (ThorLabs AC080-16) which focuses it onto a 10µm pinhole (ThorLabs P10HD). The light that passes through the pinhole is then focused back on to the mirrors by another small lens (ThorLabs AC080-30). The mirrors scan this light through an Plossl pair (identical to the previous) to focus the light onto the sensor of an camera (IDS-UI-3880CP-M-GL).

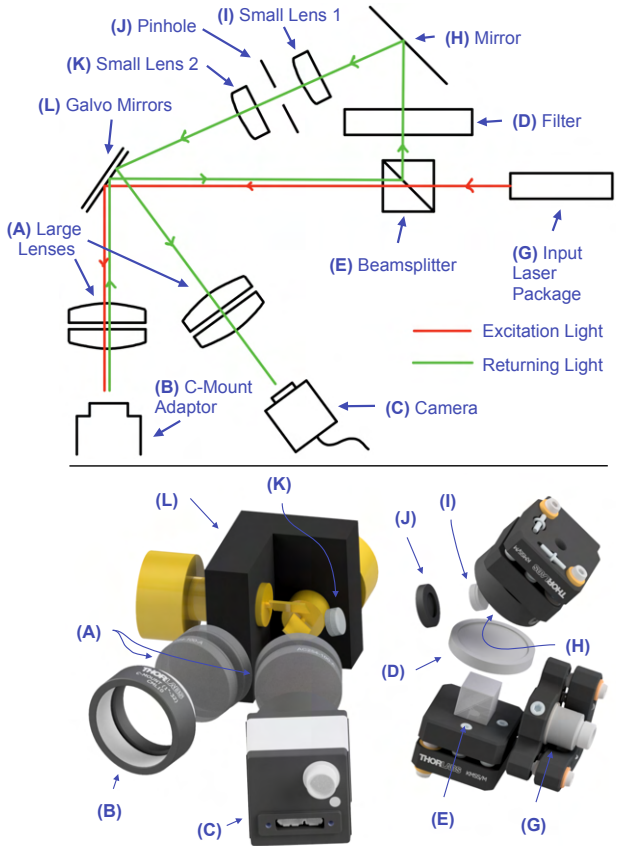

Figure 7: *Optical setup of the microscope.* ***Top:*** *Layout schematic of the optics showing the path of light through the microscope,* ***Bottom:*** *Positional layout of optical components from CAD model.*

3.1.2 Flexure Stages & Kinematic Mounts

The fine-tuning of XY pinhole position was controlled using a custom flexure stage. The flexure stage, which can be seen in Figure x, is also 3D printed. Screws are then used to adjust pinhole in the plane of the flexure stage. Since the pitch of the screws is 0.45mm, the X and Y position of the pinhole should be adjustable to 45um accuracy with a $\frac{1}{10}$ turn of a screw.

The beamsplitter cube, mirror and fibre collimation package were attached to ThorLabs kinematic mounts which can control the rotation of the optics in two axes. The beamsplitter and mirror were mounted on ThorLabs

KMSS/M and the fibre collimation packed was mounted on a ThorLabs KM05 with a ThorLabs AD11BA adaptor.

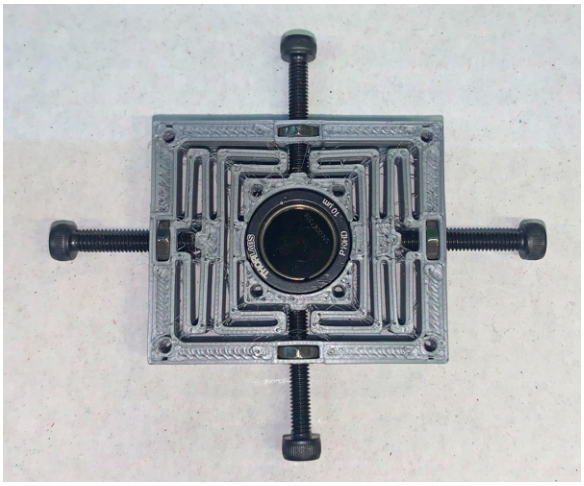

Figure 8: *The flexure stage used to adjust the position of the pinhole in the optical setup*

3.1.3 Enclosure

The role of the enclosure is to hold all the optics in position while shielding the setup from any external light pollution and fully enclosing the laser.

The enclosure was 3D printed in several different parts which are then connected using fixtures. The parts were printed using a Markforged Onyx and Prusa i3 printers. There are two mains 3D-printed parts to which the galvo mirror, kinematic mounts, fluorescent filter, camera and c-mount are connected to directly. All the lenses are secured in individual 3D-printed holders which are then attached to the main parts. Each of the individual parts as well as the whole enclosure system can be seen in Figure 9.

3.1.4 Mirror Control

The galvo mirrors used are an unbranded two-mirror system rated at 20K points-per-second (PPS) with a maximum range of +/-30 degrees (available from: **eBay Link**). They come with associated driver boards for each mirror which provides closed loop control. They require +/-15V power and the control signal to drive the mirror movement is an analog differential signal of +/-5V.

To provide this control signal an Arduino Due was used. It has two Digital to Analog Converters (DACs) built into its board which provides a simple way to drive the mirrors. The output range of the DACs is between

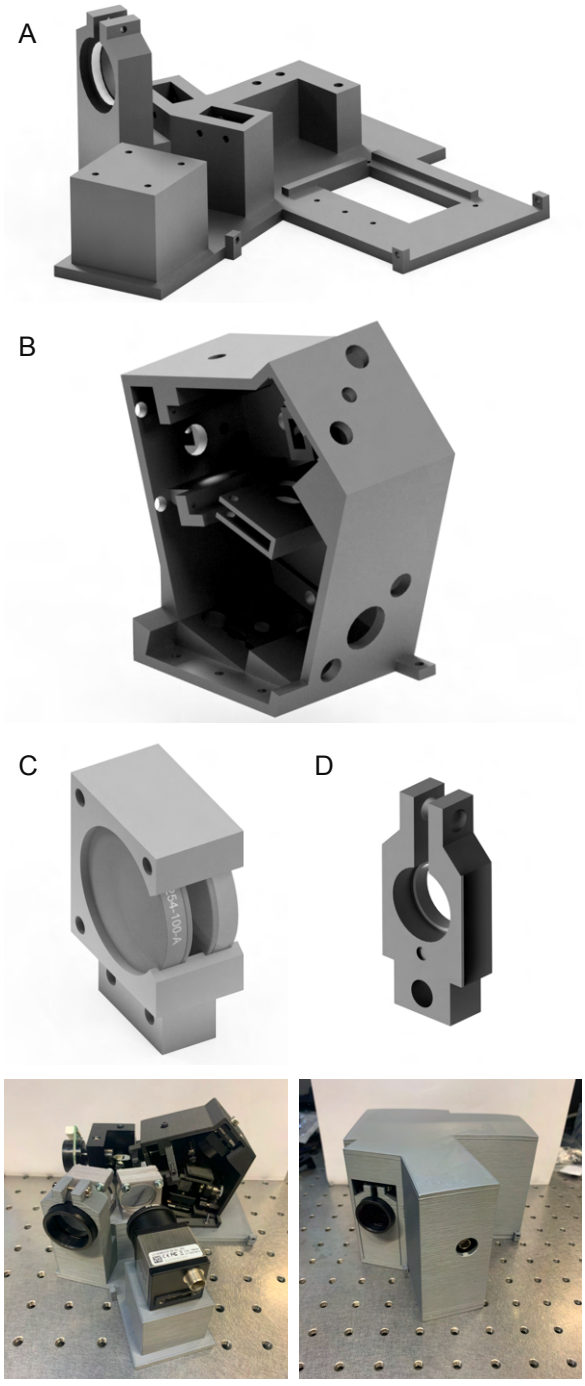

Figure 9: *A and B are the two main pieces. C and D are lens holders. The completed optical system can be seen on the bottom with and without the enclosure lid.*

0.55V-2.75V. An inverting op-amp amplifier circuit was used to centre and stretch the DAC output to a -5V-5V range. Another unity-gain inverting op-amp amplifier is then used

to create the other half of the differential signal. The op-amp used was a TL084CN. Each mirror requires one of these circuits to drive it. A schematic of the whole circuit can be seen in Figure 10. The complete board can be seen in Figure 11.

A bench power supply set to +/- 15V was used to power the whole system. This is fed directly to the galvo mirror driver boards and op-amps as well as to a voltage regulator (LN7805) to create a +5V supply to drive the Arduino.

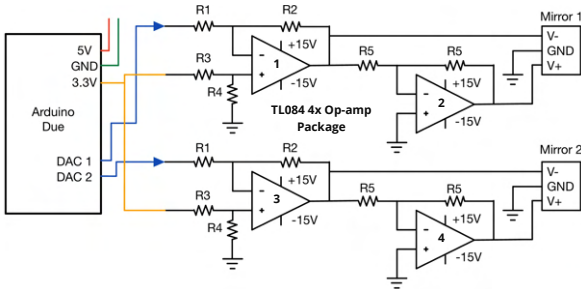

Figure 10: *Schematic for the mirror control circuit. R1 =* 300Ω*, R2 =* 1200Ω*, R3 =* 150Ω*, R4 =* 100Ω*, R5 =* $1K\Omega$

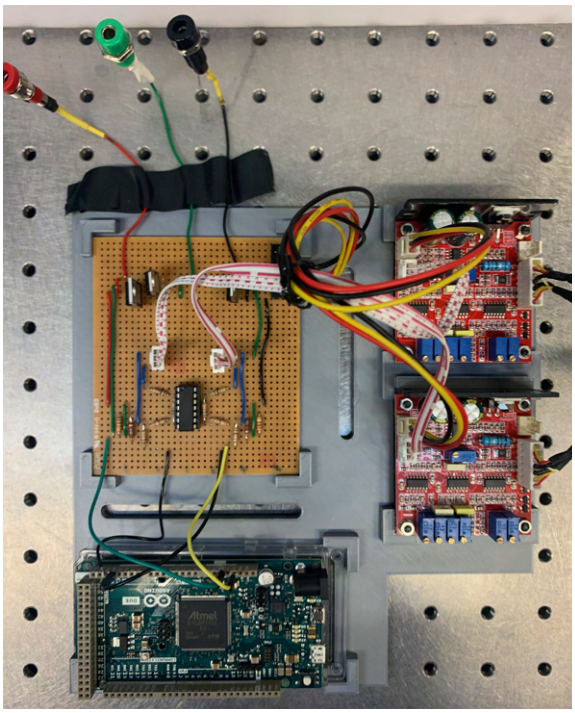

Figure 11: *The control circuit used to drive the galvo mirror pair. It consists of an Arduino Due, an op-amp amplifier circuit and a driver board for each mirror.*

The code to drive the control signal creates a 'fast' triangular wave output on one of the Arduino's DACs to scan horizontally and a 'slow' sawtooth wave output on the other DAC to change the vertical position of the fast scanning horizontal line. Syncing these two outputs will scan a raster pattern. This was done using the `AnalogWrite()` function which is built into the base Arduino library. The code for the system can be found **Here**.

3.1.5 Alignment

The alignment of the microscope was done in steps. Firstly, the galvo mirrors were centred independently of the rest of the system. A small 3D printed piece was used to shine a laser on the mirrors. Their zero position was adjusted until the laser light came out of the mirror horizontally at a right angle to the incoming light. Perfect alignment here is not required as there the zero position can be adjusted in software.

The mirrors were then attached to the system and the whole system was roughly aligned without the pinhole. Adjustments were possible with the kinematic mounts on which the beamsplitter, fibre collimation package and mirror were mounted.

Then the pinhole in the flexure stage was added to the system. A few techniques were used to aid alignment such as back-lighting the pinhole.

3.2. Testing

Mirror Scanning - The scanning of the mirrors were tested by attempting to scan a raster pattern and a square pattern. The raster pattern was tested with a number of speeds and sizes. The square pattern ran at a frequency of 30Hz.

Re-scanning - The re-scanning capability was tested by placing a mirror at the focal plane of the microscope to reflect back the light scanned onto it. This light should then pass through the whole system and get scanned onto the camera plane. The square pattern was scanned onto this mirror. The pinhole was not placed in the system as it is not needed to test re-scanning functionality.

Flexure Stages - The flexure stages movement accuracy (independence between X and Y movement) and drift over time were tested using a microscope. The flexure stage with pinhole inserted was securely fixed to the stage of a microscope as shown in Figure 12. A light source was then shone through the pinhole so that a dot of light could be seen on the microscope. The pinhole position was changed with the adjustment screws and the flexure was left in this position for

30 minutes. The change in pinhole position was then measured and the drift calculated.

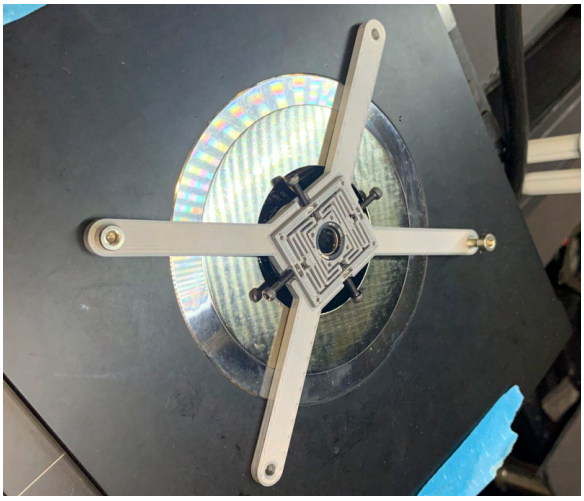

Figure 12: *Flexure stage fixed to another microscope for testing*

Image Acquisition - All the aforementioned scanning tests were done without using the system camera. With the camera placed in the optical system similar test were carried out. The re-scanning of a square pattern using, firstly, the same mirror and then a light scattering sample made with acetate sheet and correction fluid (seen in Figure 13). All images were acquired using uEye Cockpit software and the exposure and frame rate was adjusted as appropriate for each test. These tests were completed without a pinhole and then with a paper pinhole (around 0.7mm diameter) to provide some pinhole functionality without intensive alignment.

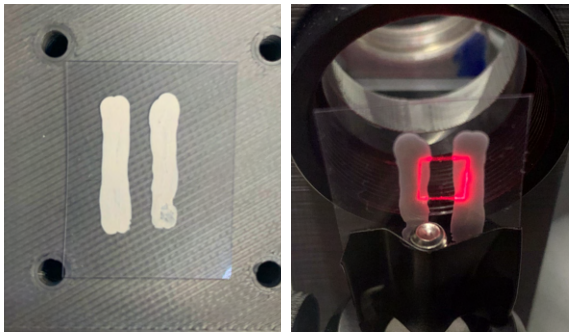

Figure 13: *Scanned square onto the scattering target place at the focal plane of the microscope to test the performance of the microscope at collecting back-scattered light.*

4. Results & Discussion

4.1. Test Results

The galvo mirrors were able to scan a raster pattern and a square. The scanned square can be seen below in Figure 14A. Running at the rated 20K pps the image acquisition speeds and associated fps for different resolution scans can be seen in Table 1.

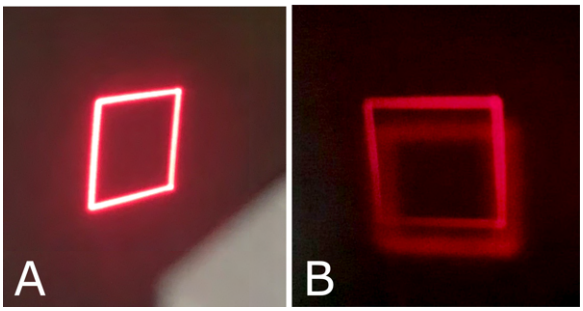

Figure 14: ***(A)*** *Square scanned onto the focal plane at 30fps,* ***(B)*** *Re-scanned square on image plane as a result of the mirror placed in the focal plane. System noise is also scanned onto the image plane and is seen as the second, out-of-focus square.*

Resolution	Frame Time (s)	FPS
128x128	0.872	1.147
256x256	3.488	0.287
512x512	13.952	0.072
4096x4096	892.928	0.001

Table 1: *Some example resolutions and associated frame time and fps running the galvo mirrors at their rated 20kpps*

The re-scanned square onto to the image plane can be seen in Figure 14B. It is less intense some light will be lost when passing through the optics, especially the beamsplitter. There is clearly some noise in the system at this point which is also scanned by the mirrors and is manifested as the second out of focus square that can be seen. This noise originates from the beamsplitter which seems to scatter a lot of light. This is only a problem because the pinhole was not being used in this test. With the pinhole inserted, this background noise should be rejected. A second source of noise discovered in this test which the pinhole cannot reject is scattered light from the mirrors. There is no way to avoid this but it should not affect the image acquisition apart from adding a small amount of evenly distributed light.

The results of the image acquisition tests can be seen in Figure 15. A and B were collected with a mirror in

the focal plane while C and D used the scattering target previously shown in Figure 13. For images B and D, the paper pinhole was placed in the system. This clearly shows the principle behind the pinhole as even a temporary one, around 70 times larger than the actual, can exclude a huge amount of noise. Image B is overexposed and it was not possible to tweak the camera settings to improve it any further. This is not a problem as having a mirror in the focal plane will provide much more returning light than would be seen in normal use. In D, the pattern from the scattering target can clearly be seen. There is also a very faint outline of the parts of the square that hit the acetate sheet as it is slightly reflective.

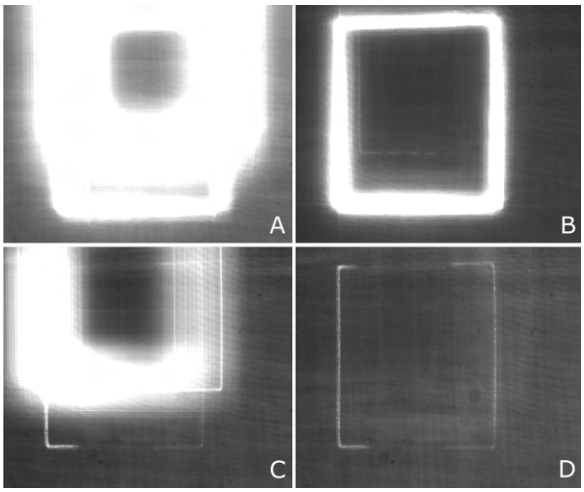

Figure 15: *Images acquired using the microscope while scanning a square. (A) No pinhole with mirror in focal plane, (B) Pinhole with mirror in focal plane, (C) No pinhole with scattering target in focal plane, (D) Pinhole with scattering target in focal plane*

The results of the flexure stage microscope test can be seen in Figure 16. A is the starting position. B and C are one full screw turn in the Y and X directions respectively. Unfortunately since it was not possible to focus on the pinhole it is hard to derive qualitative results from the test as the size of the pinhole cannot be used as a reference for how far it has moved. What this test did show is that flexure stage can provide reasonably independent X and Y positioning of the pinhole. The calculated value for positional change from the pitch of the screw should be quite accurate.

Alignment with the pinhole in the system has not yet been successful meaning that final testing of the system cannot be done. The achieved results have so far demonstrated that scanning, re-scanning and image acquisition are all possible with this optical setup and that the flexure stage should provide precision enough X-Y positioning of the pinhole.

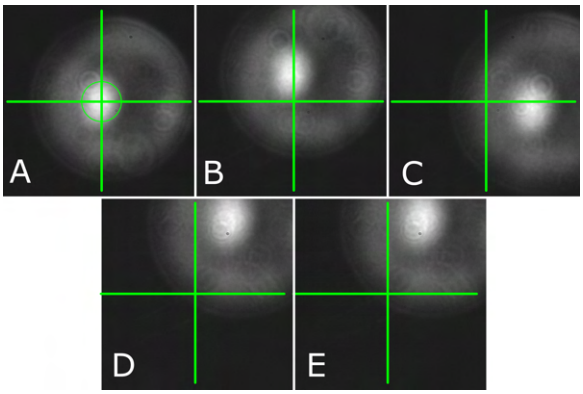

Figure 16: *Microscope images from testing the flexure stage. (A) Centre reference, (B) One screw turn in Y direction, (C) One screw turn in X direction, (D) Drift test: Flexure stage under tension in X and Y directions at 0 minutes, (E) Drift test: At 30 minutes*

4.2. Cost

Cost was the main driving point behind a lot of the design decisions for this microscope. To bring the costs down many compromises had to be made in manufacturing and choice of optical components to find the right balance between cost and functionality. The final cost of the system was £1570, placing it slightly over budget but still far cheaper than any attempt at budget CM seen in literature. The system is also very flexible, with little adjustment the performance of the microscope can be fitted to a user's needs depending on their budget.

4.2.1 3D Printing

One of the main cost-saving measures was the extensive use of 3D printing. The trade-off is that, while 3D printing has improved massively in recent years, it is still not going to be as accurate or provide the stiffness of machined parts. The possible inaccuracy of 3D printing is what led to the necessity for adjustable parts such as the flexure stage, ensuring that a perfect print was not necessary to have a functioning microscope. The flexure stage was a success since it provide precise XY position at a cost of around £0.10.

The varying orientation of the optics required for this CM system made the design of the enclosure for 3D printing a challenge. Ideally the whole enclosure would be printed as one, ensuring that the relative positioning of parts would be as accurate as possible. Given all the different orientations of optics there was no single print orientation that would be best for all the components. To overcome this the enclosure was split into multiple pieces to print separate parts in the

best possible orientation. While the enclosure was split into two main pieces to keep the number of parts at a minimum, initial testing showed that each lens would need it's own holder with the print layers in the optical plane to avoid layer effects in the circular profile of the lens holders. A comparison between a lens holder printed as part of a larger piece and separately can be seen in Figure 17. It also allowed the strain relief to be built into each lens holder, allowing the lens to be held securely without having to use any pressure to place in them in the holder.

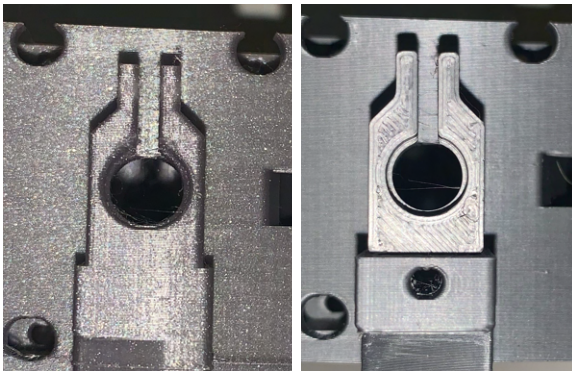

Figure 17: *Comparison between small lens holder printed as part of another piece (left) and separately so layers are perpendicular to the optical axis (right). Improved quality of the right is clear, allowing more secure and accurate positioning.*

Another benefit of using 3D printing is that it allowed a rapid development approach through lots of iterations. It is hard to judge from the CAD model how well any printing approach will work with the optics so the opportunity to test many different approaches helped converge to the best final design. The total cost of the 3D printed parts was £23.11.

One possible argument is that a good 3D printer is necessary to complete this project and that it should be included in the cost. However, 3D printers are widely available and in the worst case there are many online services which will print parts for a fee. Even with the price of a Prusa i3 printer included the system costs under £2500, still much cheaper than any confocal system previously proposed.

The only other parts of the microscope that could have been 3D printed were the kinematic mounts. The total cost of these off-the-shelf mounts is £90 meaning that a custom designed mount could be 3D printed for a fraction of this cost.

4.2.2 Optics

None of the optics chosen for this system are of the highest quality because of cost constraints but they are very functional. The limiting pieces in the system are the beamsplitter and the galvo mirrors.

A beamsplitter was used as opposed to a dichroic mirror as they are significantly cheaper. Dichroics will transmit or reflect light based on its wavelength, providing a more that 99% light efficient way of separating the excitation light path from the path returning from the specimen. A beamsplitter simply transmits half the light while reflecting the other half meaning it is at most 50% efficient. From testing, a lot of light scattering was seen from the beamsplitter reducing its efficiency even further and creating some background noise. Despite this, the beamsplitter did function as it was supposed to and background noise from it is negated by the pinhole.

The galvo mirrors are by far where most budget has been saved. With the unit costing under £100. Despite this, its scanning range and consistency were sufficient and did not limit any of the testing. The main drawback with the mirrors is their speed. Running at their rated 20K pps it takes around 3.5s to acquire a single frame at 256x256 resolution, amounting to 0.29fps. This is about 20 times slower than commercial systems and around 10 times slower than the budget confocal system in ref [14] running at the same resolution. Although 3.5s does not seem long, the number of frames taken multiplies when taking 3D images. This will also affect its use in imaging dynamics.

Cost-saving did not only come from compromise on the quality of optical components used. The use of the same galvo mirrors to re-scan the light onto the camera sensor means that adding re-scan functionality required almost no extra optics. This provided an RCM system with the only trade off being a more complex optical arrangement to design the enclosure around. This implementation of re-scan CM simultaneously saved budget in multiple areas while also providing multiple benefits to the performance of the microscope.

As previously mentioned, RCM allows a camera to be used instead of a PMT. This is better in three ways. Firstly, cameras are cheaper than PMTs so it reduces the cost of the whole system. Secondly, since the image is scanned directly onto the camera sensor, no image construction software is necessary. This software can be complex and expensive and would be hard to sync with the nonstandard galvo mirrors. Thirdly, cameras have close to single-photon sensitivity, making them more light efficient that a PMT which is important given the system lacks some light efficiency.

4.3. Accessibility

One of the central aims of this project is to make CM accessible. To do this a GitHub page and repository has been made to provide everything necessary to replicate the system with some additional guidance. The page is available **Here**.

4.4. Future Work

Future work on this project will aim to make it more functional, cheaper and more accessible.

Functionality – The main functionality upgrade to the system will be to allow axial adjustment of the pinhole. The flexure stage used allows the precise positioning of the pinhole in the XY plane but lack of axial adjustment, affecting the success of system alignment. There are many other functionality improvements that can be made but most come at a higher cost making them difficult to justify for a budget-orientated microscope. However, the flexibility of the microscope will be maintained to allow such improvements to be made by users with a larger budget.

Cost – As previously mentioned, the next cost reduction step is to design 3D printable kinematic mounts to replace the off-the-shelf ones currently in use - making everything but the optical components 3D printed.

Accessibility – With all the project resources already available on the GitHub, a future goal is to improve the documentation on the page making replication of the system as simple as possible. Keeping the GitHub updated with any progress is also important.

5. Conclusion

The results from this project have been very promising for the future of budget CM. While there are some issues with pinhole alignment and the speed of the galvo mirrors, these are not insurmountable problems. This project has created a system which is easily reproducible, allows adjustment of optics for alignment, is compatible with any external microscope with a C-mount and should be easily customisable for a user's needs. Further testing is required once fully aligned to quantify system performance but despite this, the project has achieved most of the aims laid out in the introduction.

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Manual Links

If the links embedded in the report do not work, everything can be accessed at the links below.

Repository: https://github.com/CallanTME/LowCostConfocal

Page: https://callantme.github.io/LowCostConfocal/

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Appendix A - Bill of Materials

Name	Quantity	Description	Price
		Circuit Components	
Arduino Due	1		£35.00
TL084CN	1	Quad-Channel op-amp	£0.65
LN7805	1	5V voltage Regulator	£0.50
0.1u Capacitor	2	To smooth voltage regulator	£0.10
100 Resistor	2		£0.10
150 Resistor	2		£0.10
300 Resistor	2		£0.10
1k Resistor	4		£0.20
1.2k Resistor	2		£0.10
		Optics	
XY Galvo System	1	Link from eBay - Similar to one already acquired	£72.77
Thorlabs KMSS/M	2	Kinematic mounts	£29.12
Thorlabs KM05	1	Kinematic mirror mount for input laser	£30.13
Thorlabs BS070	1	Beamsplitter cube	£143.91
Edmund Filter	1	Fluorescence Filter	£213.00
ThorLabs BB05-E02	1	Broadband Mirror	£39.80
Thorlabs MH12	1	Mirror Holder	£12.89
Thorlabs AC080-16	1	Achromatic Doublet, f = 16mm	£35.98
Thorlabs AC080-30	1	Achromatic Doublet, f = 30mm	£36.91
Thorlabs P10HD	1	Mounted Pinhole, 10um	£60.77
Thorlabs AC254-100	4	Achromatic Doublet, f = 100mm	£62.39
IDS-UI-3880CP-M-GL	1	Camera	£400.00
Thorlabs CML10	1	C-Mount Extension Tube	£13.79
Thorlabs AD11BA	1	Adaptor	£16.13
Thorlabs F220FC-B	1	Fiber Collimation Package	£126.75
		CAD Parts	
MainPartA	1		£9.00
MainPartB	1		£3.52
PinholeFlexMain	1		£0.10
PinholeFlexFixture	1		£0.01
LargeLensHolderS1P1	2		£0.14
LargeLensHolderS1P2	2		£0.14
LargeLensHolderS2P1	2		£0.14
LargeLensHolderS2P2	2		£0.14
SmallLensHolderInt	1		£0.15
SmallLensHolderExt	1		£0.15
Enclosure	1	Lid for the optics	£7.35
ElectronicsHolder	1	Holds electronic components in place	£2.27
		Total	**£1,572.25**

Appendix B - Comparison of Budget Microscope Projects

Name	Technique	Comments	Ref
OpenFlexure	Light or Fluorescence Microscopy	· Costs between $20-200 · Configurable for desired performance · Backed by documentation and software · Fully 3D printable · High Performance	[13]
Chea(i)p	Super-resolution Microscopy	· Costs less than $1000 · Super-resolution · Uses mobile phone for image acquisition & processing · 100nm optical resolution	[17]
FlyPi	Light or Fluorescence Microscopy	· Simple version costs less than €100 · Costs €200 with all modules · Designed for work with fruit flies, zebrafish or C. elegans · Optional thermogenic and optogenic stimulation	[18]
OpenSPIM	SPIM	· Almost costs £50,000 · Uses 3D printed and OFS part · Modular and customizable · Optical Sectioning from SPIM	[19],[20]
UC2	Multiple	· Setup costs range from €100-1000 · Modular frame that supports microscope projects · Extremely flexible · Full example microscope project available · Possible uses include Light, Fluorescence, SIM, ISM	[21]

Table 2: A comparison of five different open-source, budget microscopy projects and their properties.